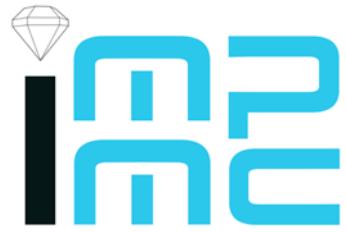




MUSÉUM
NATIONAL D'HISTOIRE NATURELLE



NanoSIMS: Principe et applications à l'étude de la matière organique

Nano scale Secondary Ion Mass Spectrometry

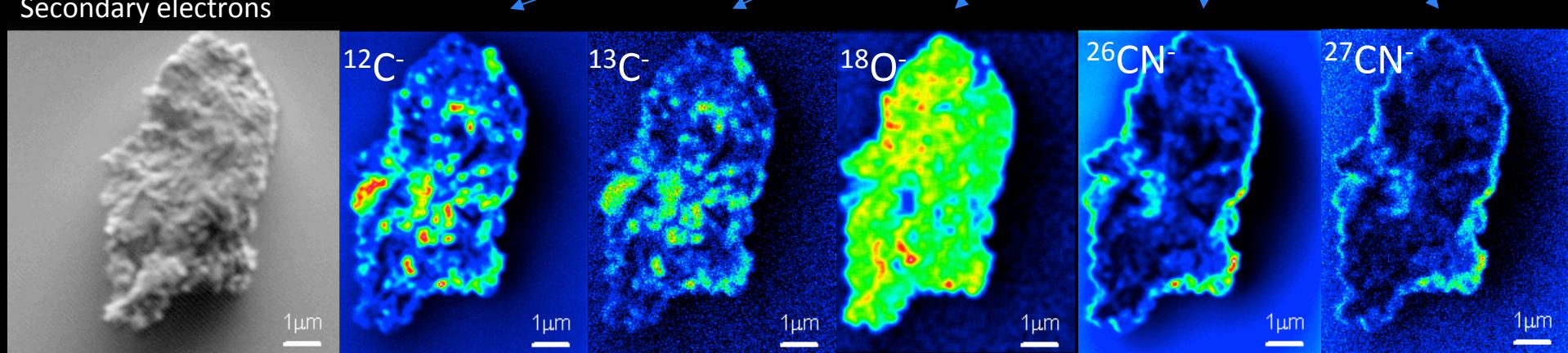
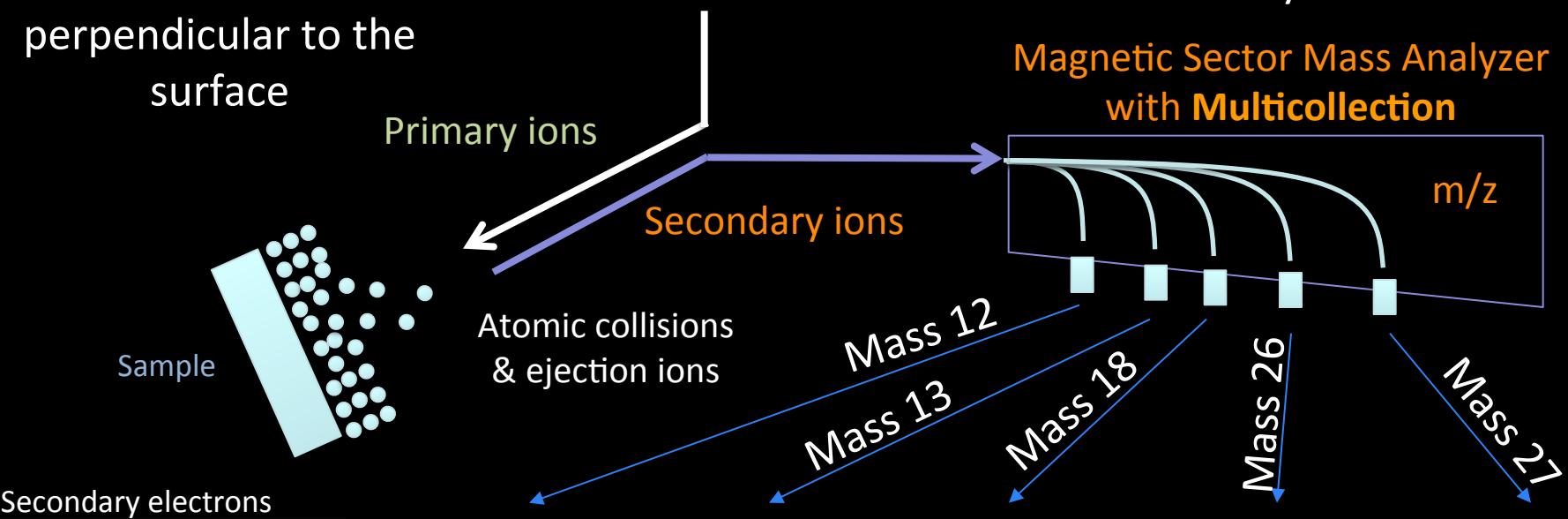
- Can detect any isotope of any element of the periodic table
- High sensitivity: from ppm to ppb detection limits
- Surface analytical tool: sampling around 5-10nm in depth
- High spatial resolution and high mass resolution
 - Cs beam: 50-100 nm (C, N, O, H isotopes in organic materials)
 - O beam: 200 nm (for cations like Fe, Al, Mg, etc...)
- multicollection mode: imaging 5 simultaneous masses

The incident beam is perpendicular to the surface

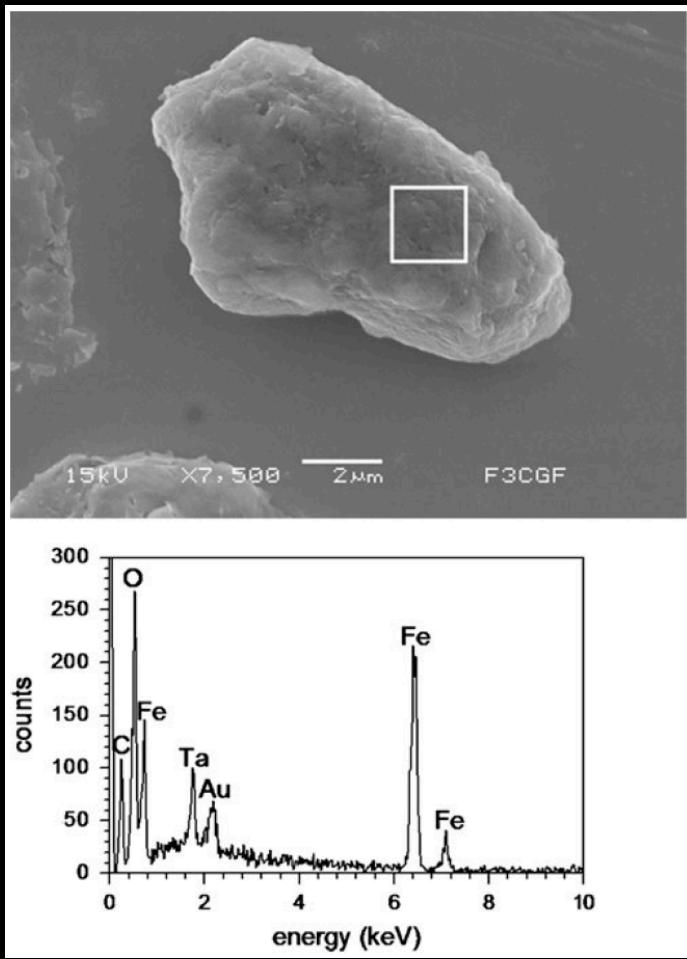
Primary source (Cs^+)

High-resolution isotopic analysis

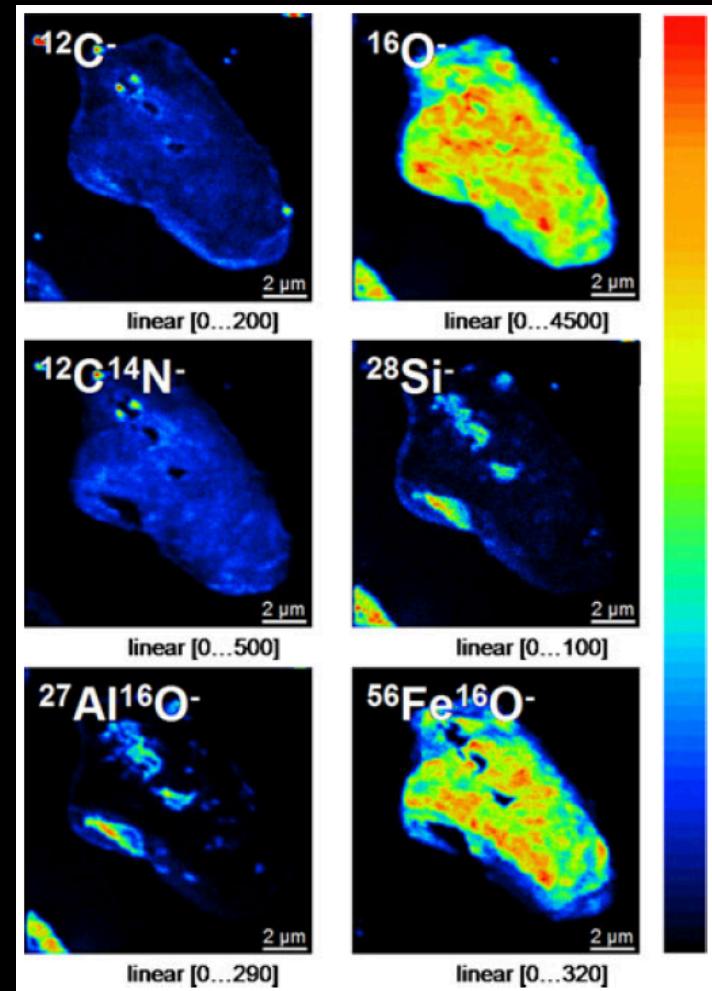
Magnetic Sector Mass Analyzer with Multicollection



SEM+EDS



NanoSIMS images



Ferrihydrite particle in a ferrihydrite+illite soil (Heister et al. 2012)

Sample requirements

- Analysis under ultra high vacuum (10^{-10} - 10^{-9} Torr).
- Sample has to be **dry!**
- Sample has to be as flat as possible
- Sample should be conductive
- Must fit the sample holders

Applications

- Métabolisme cellulaire
- Biominéralisation
- Relation symbiotiques
- Diffusion des éléments traces
- Cosmochimie
- Imagerie d'aérosols
- Et bien d'autres !

Composition isotopique de la MO et des phases carbonées des chondrites

Comment le NanoSIMS nous
aide à comprendre la
formation du système solaire

Carbonaceous chondrites

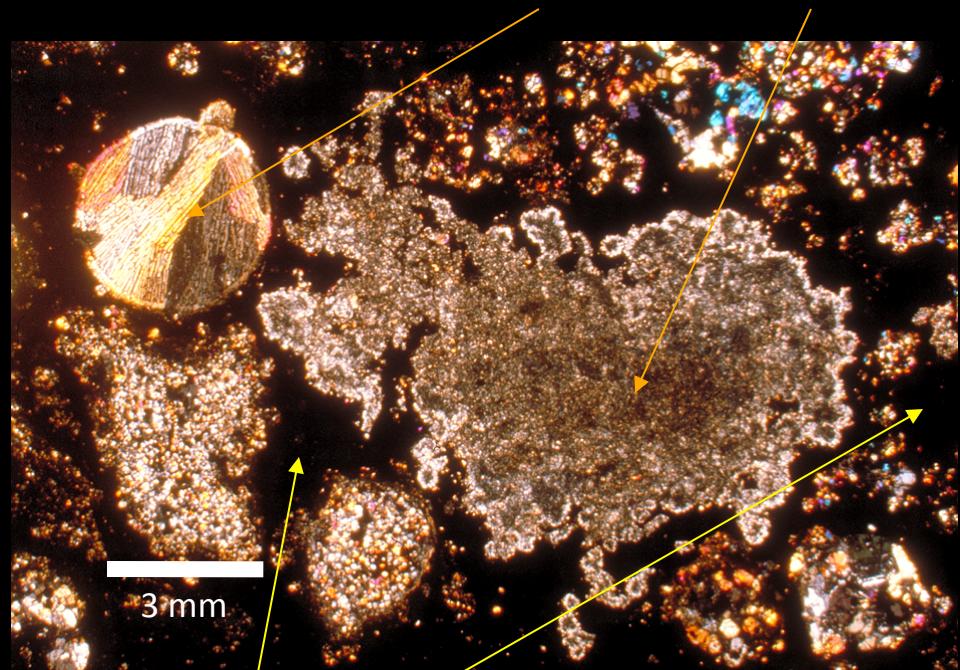
are undifferentiated meteorites,
formed during the early stages of
the solar system



Murchison

1 cm

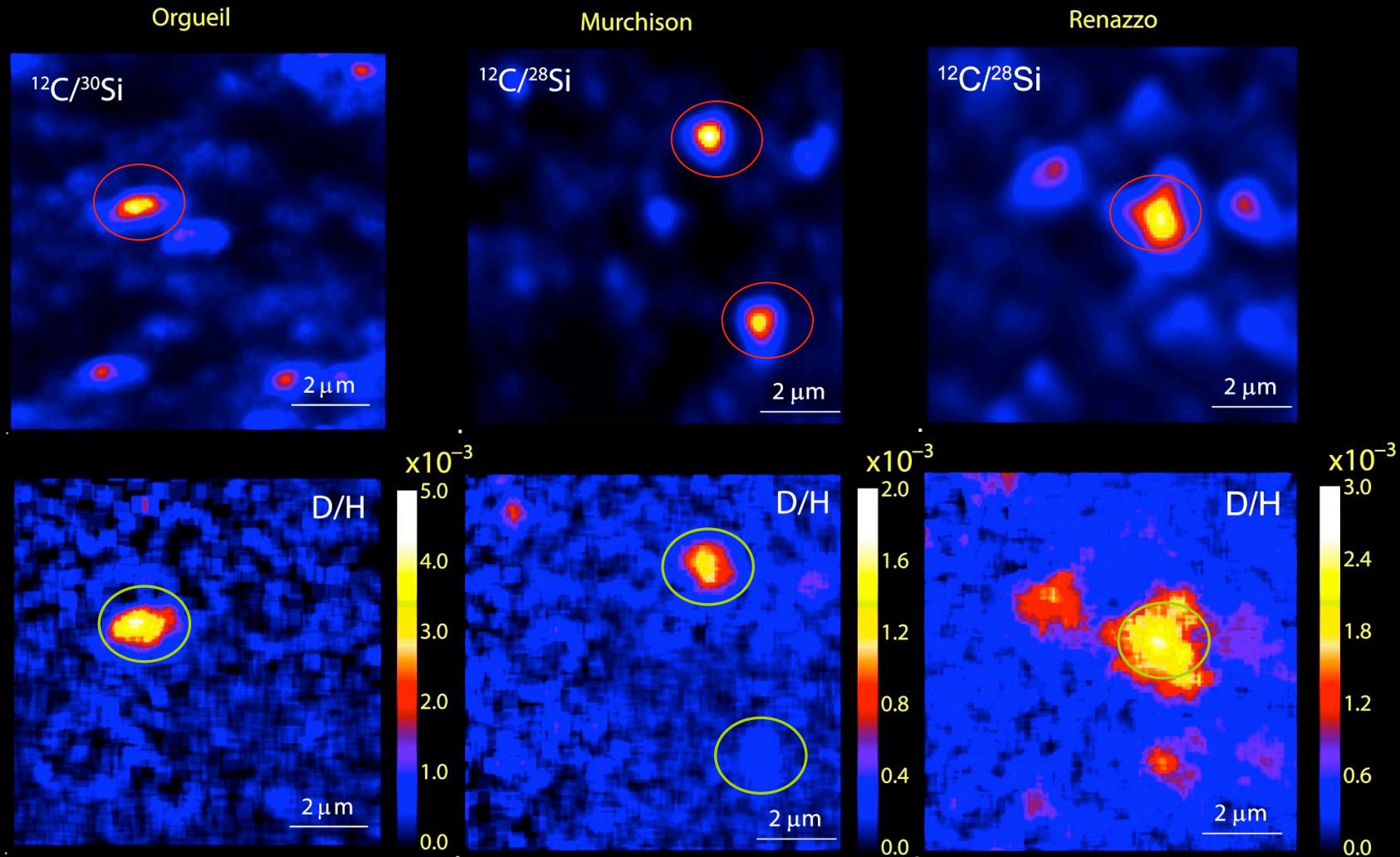
They contain high temperature
components like **chondrules** and **CAIs**



Allende

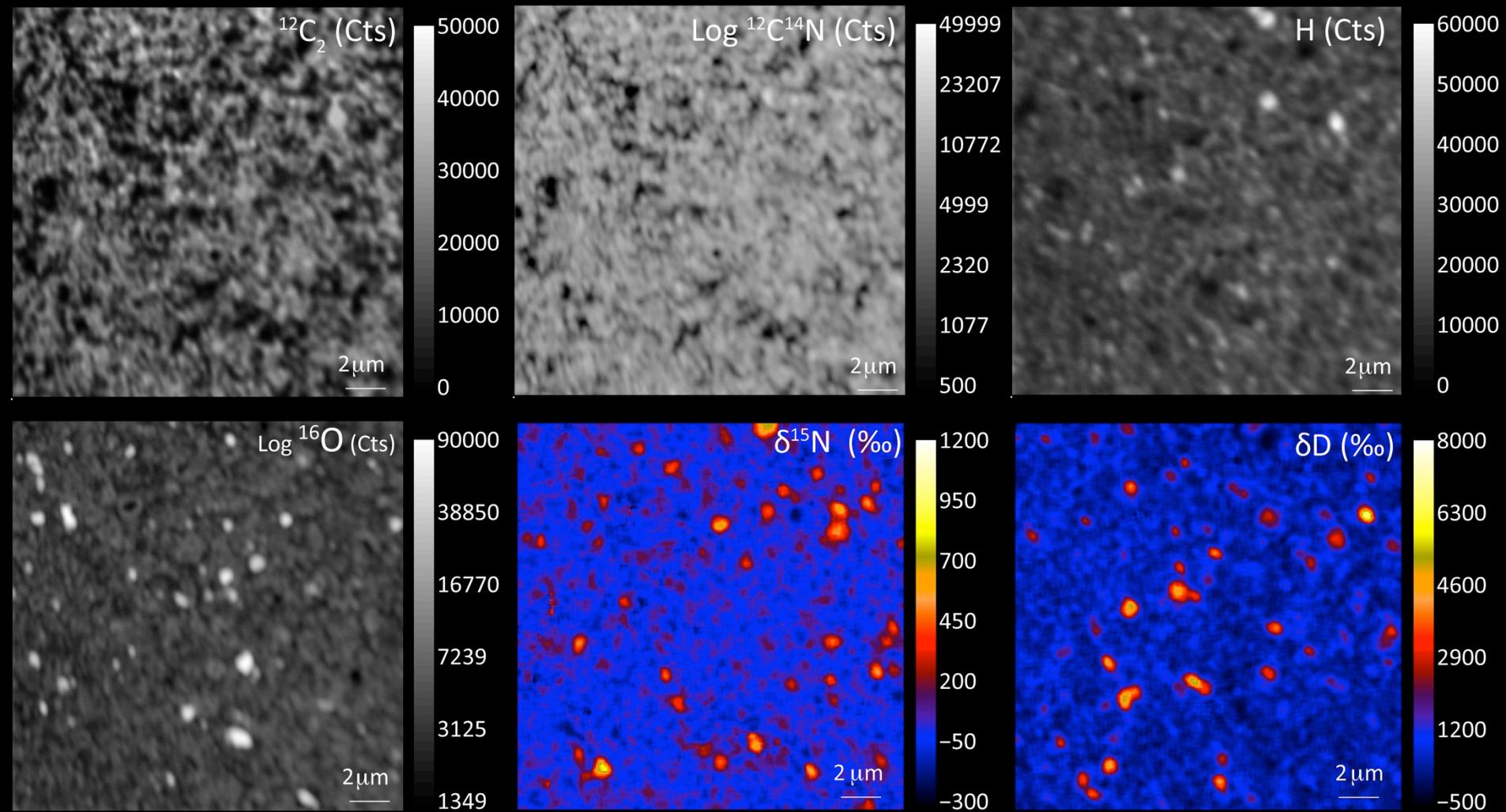
These components are associated with a **matrix** containing low temperature
constituents like organic matter, clay minerals or noble gases

Isolated organic particles in the matrices of carbonaceous chondrites



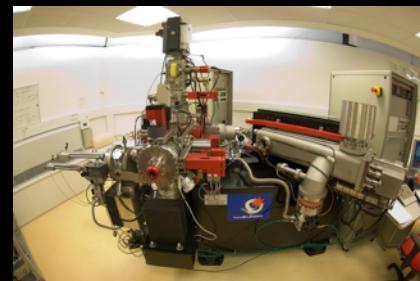
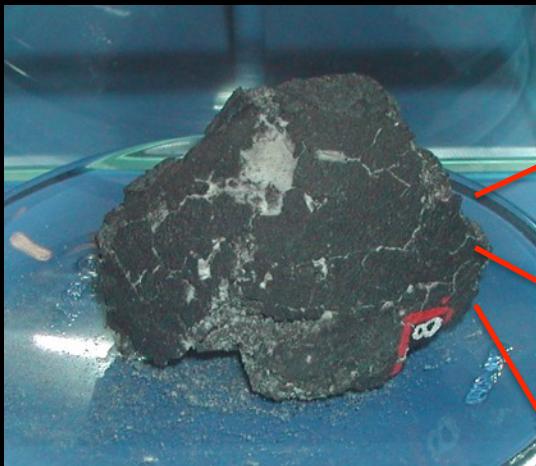
They exhibit a significant heterogeneity in D/H, but the textural context is similar

NanoSIMS images – Paris IOM

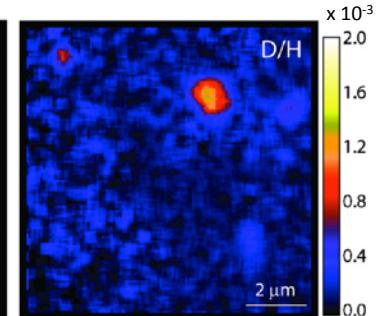
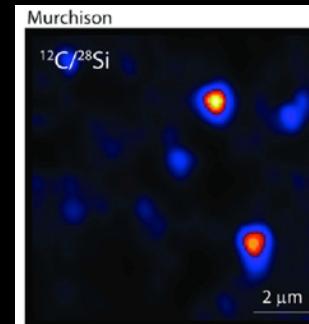


NanoSIMS

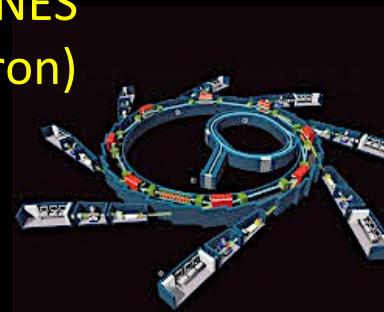
Orgueil (CI chondrite)



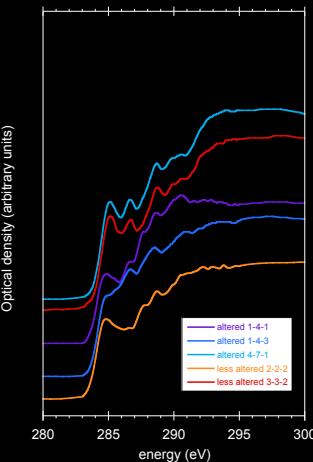
Isotopic and elemental maps



STXM/XANES (synchrotron)



Molecular characterization



Organic matter
Up to 4wt%

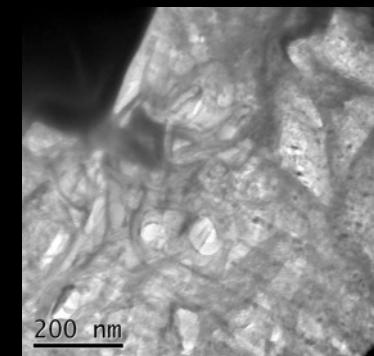
HF/HCl leaching

Solvent extraction

Insoluble macromolecule
 $(\text{C}_{100}\text{H}_{68}\text{O}_{18}\text{N}_{3.5}\text{S}_3)$

Complex mixing
of soluble
compounds

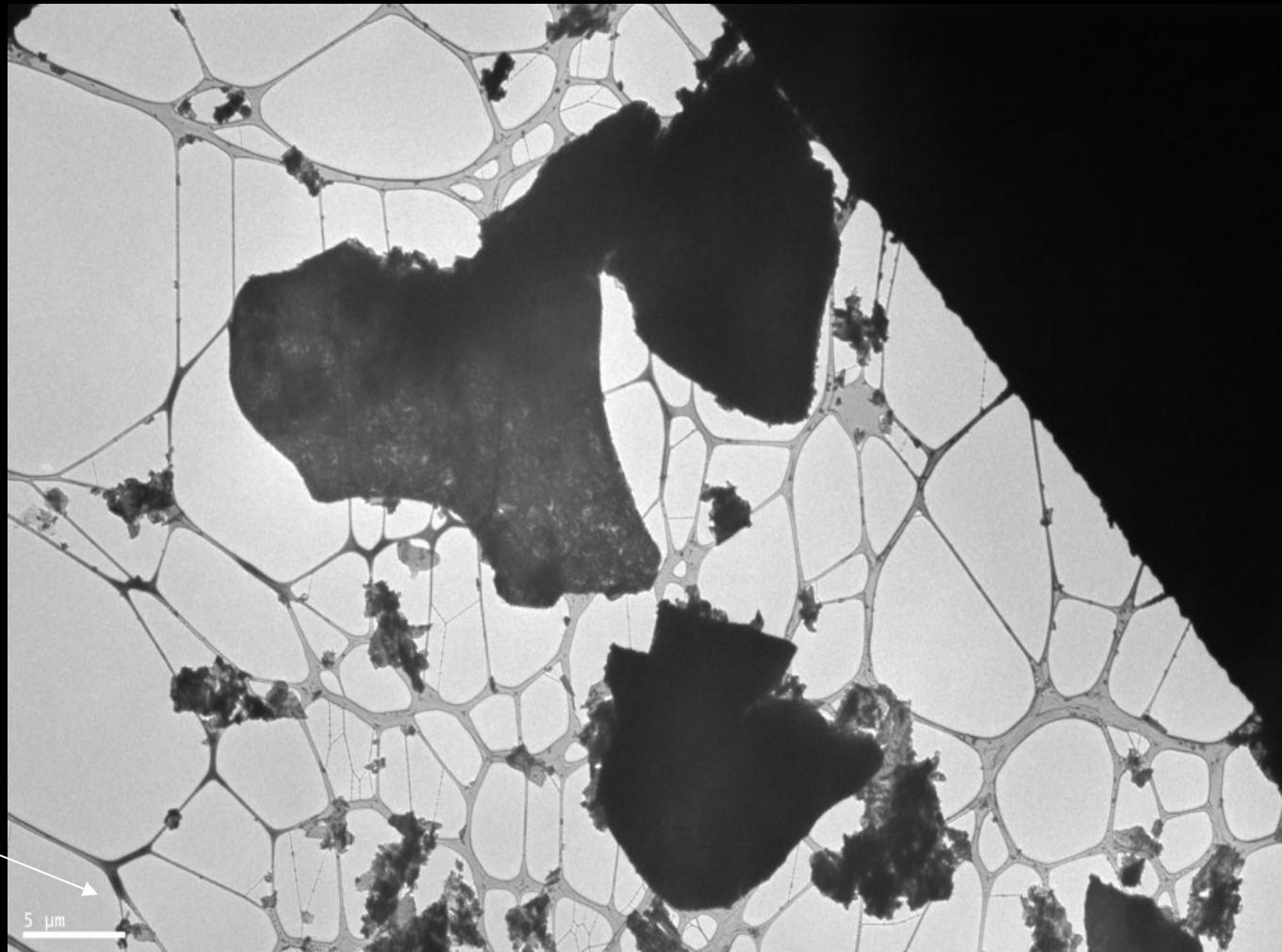
TEM Mineralogy & petrography



Abee (meteorite) acid residue

TEM

5 μm



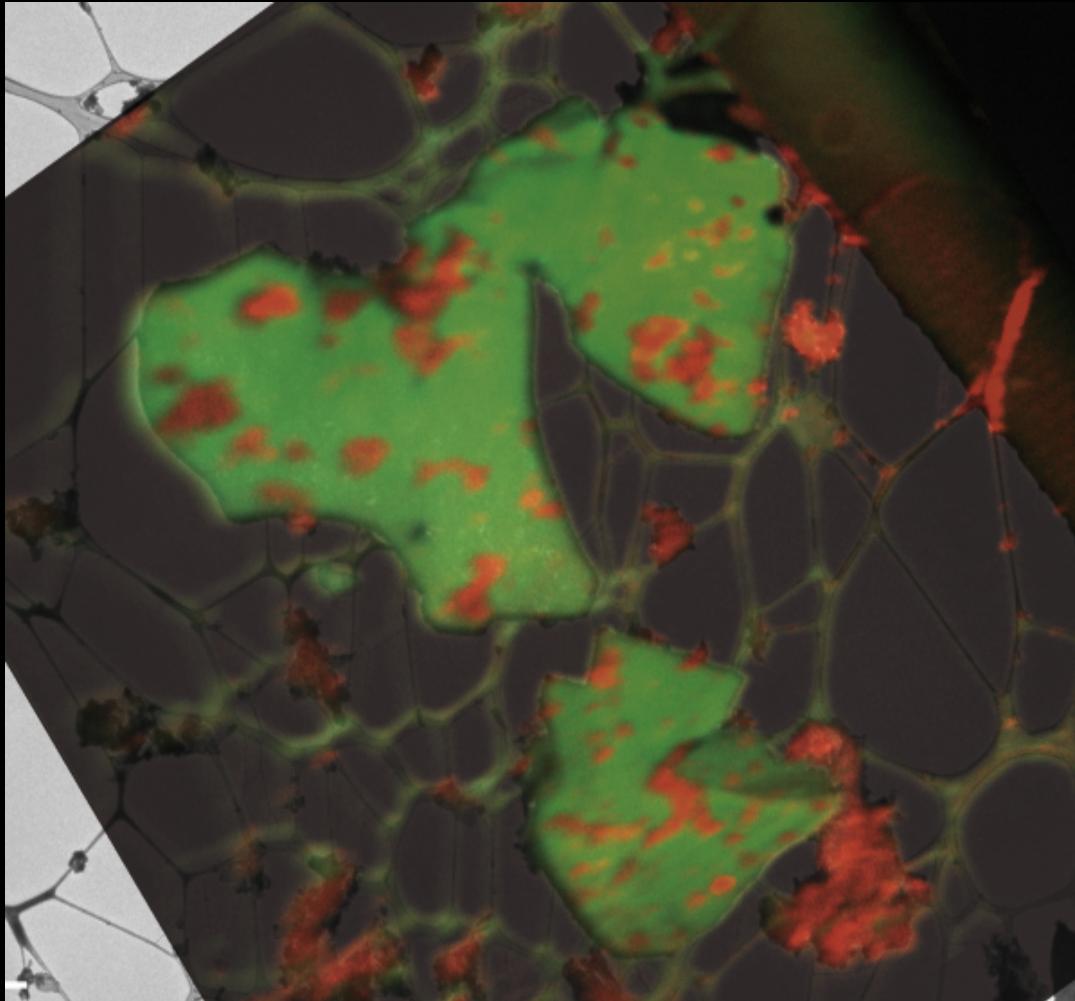
Remusat et al., GCA 2012

NanoSIMS elemental map

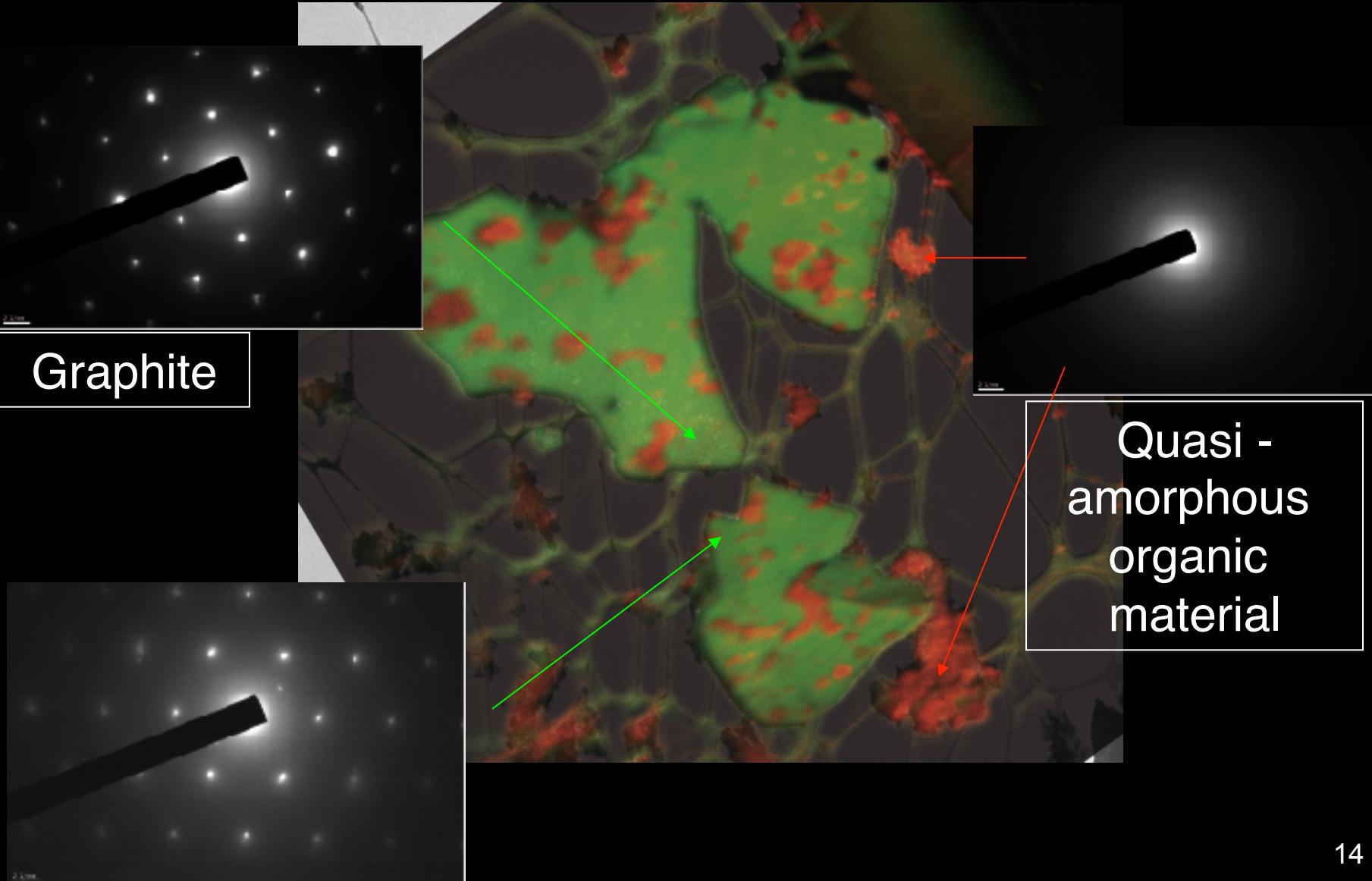
TEM +
NanoSIMS
images :

Red: N rich areas

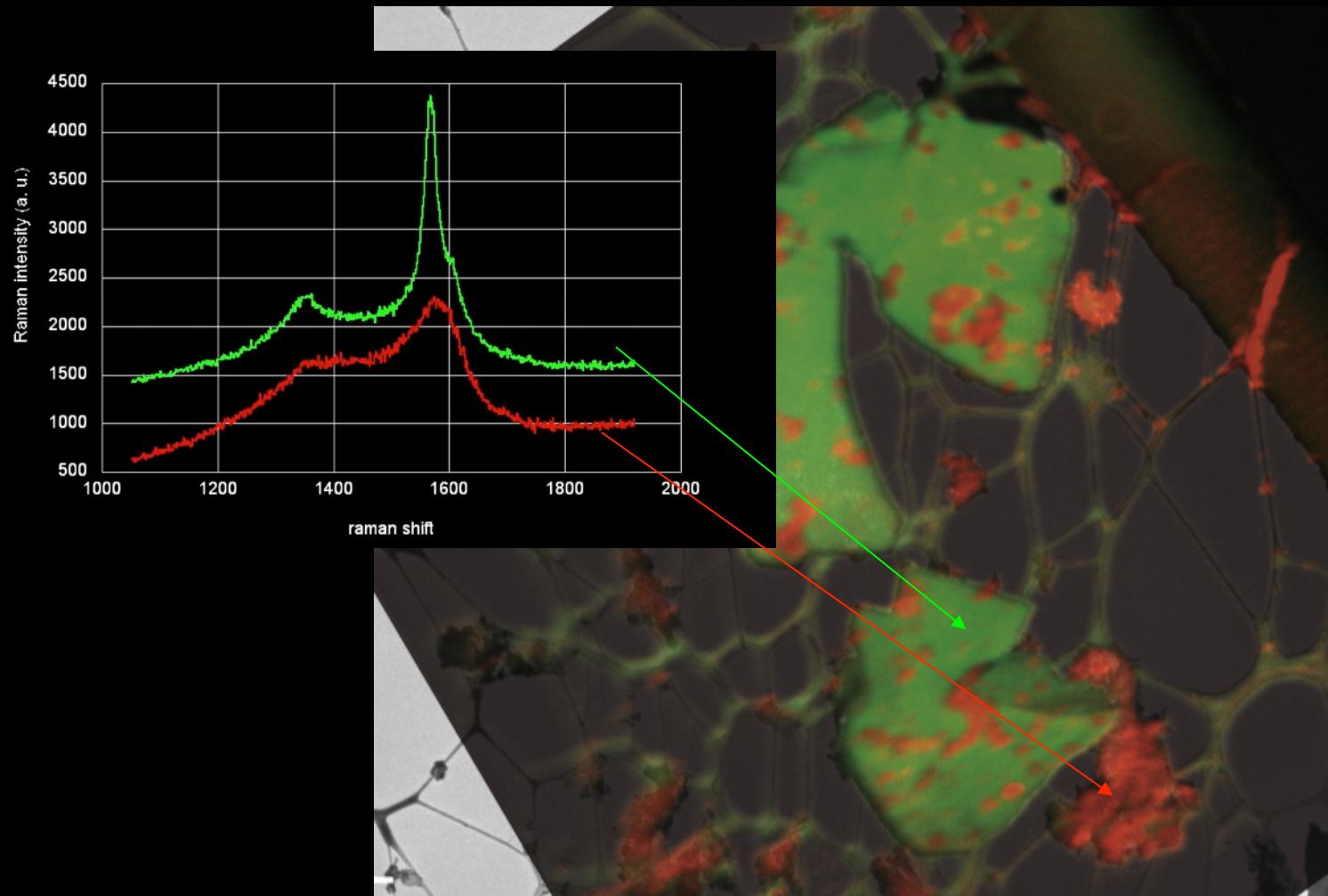
Green: C rich areas

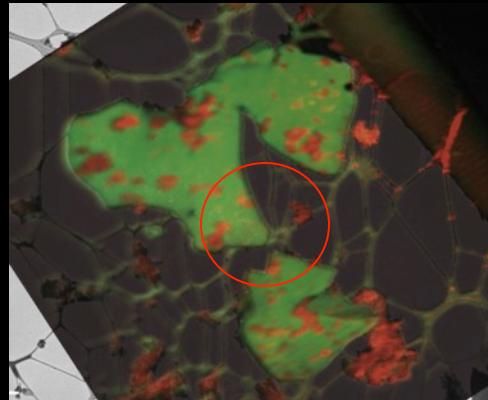


e- diffraction

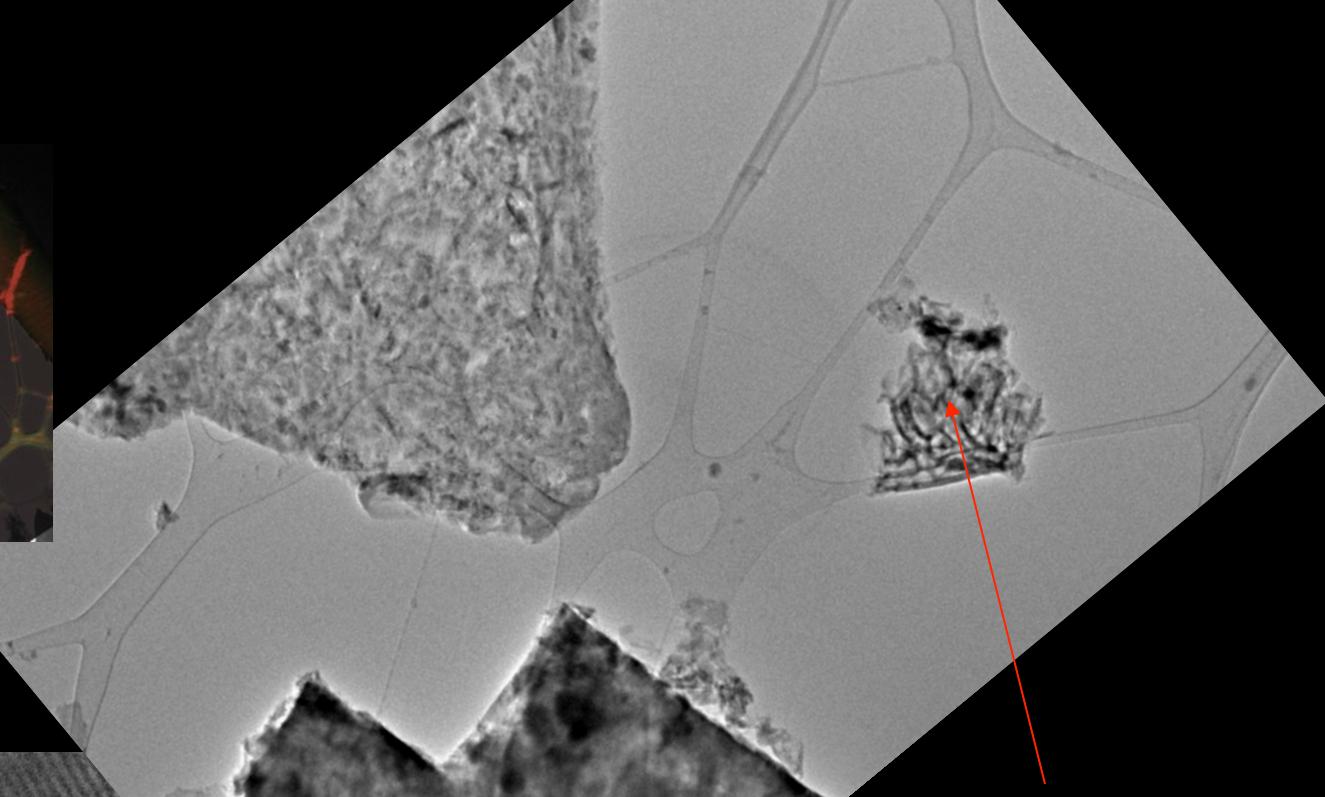


Raman

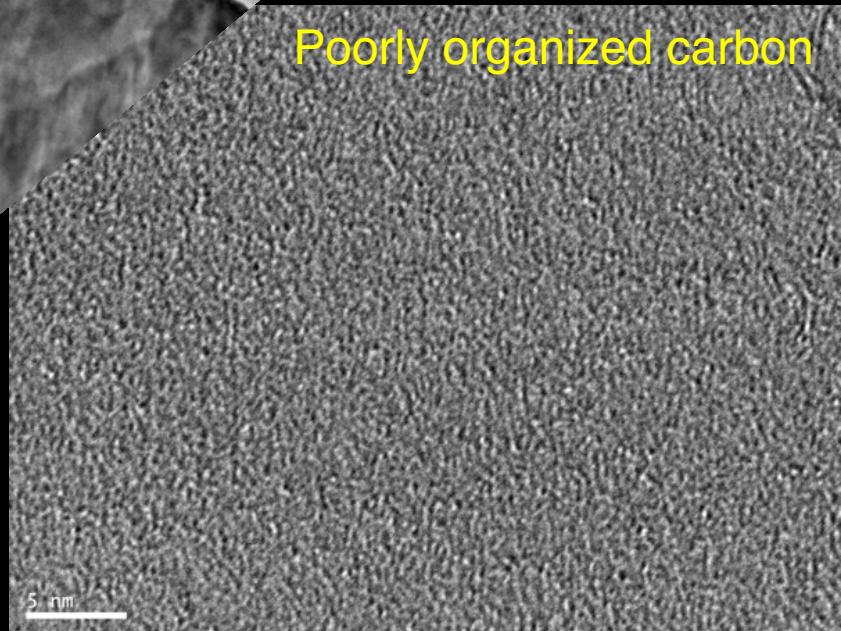
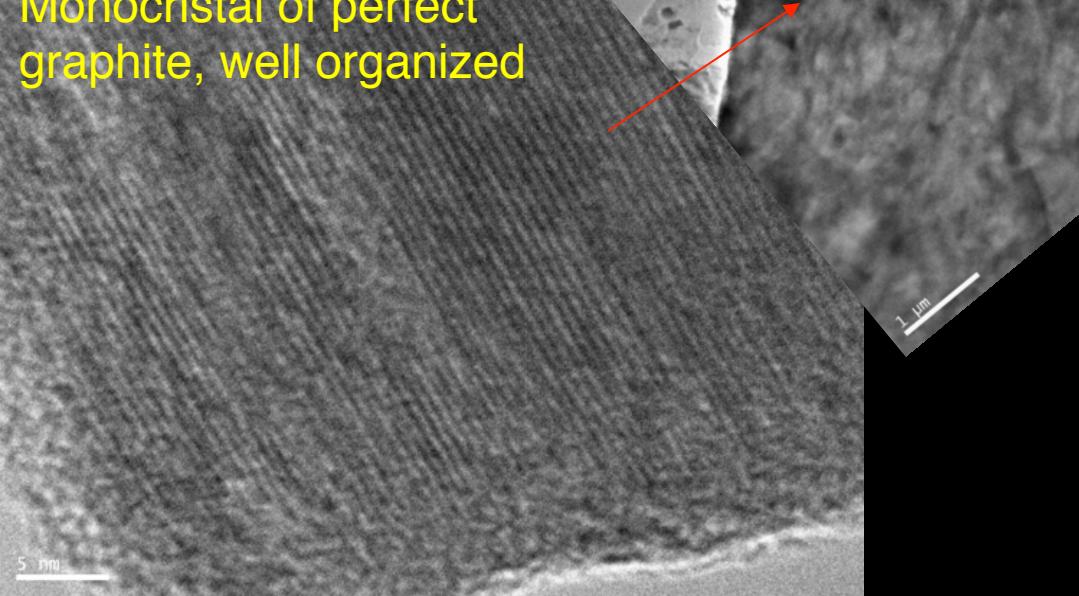




Monocrystal of perfect graphite, well organized



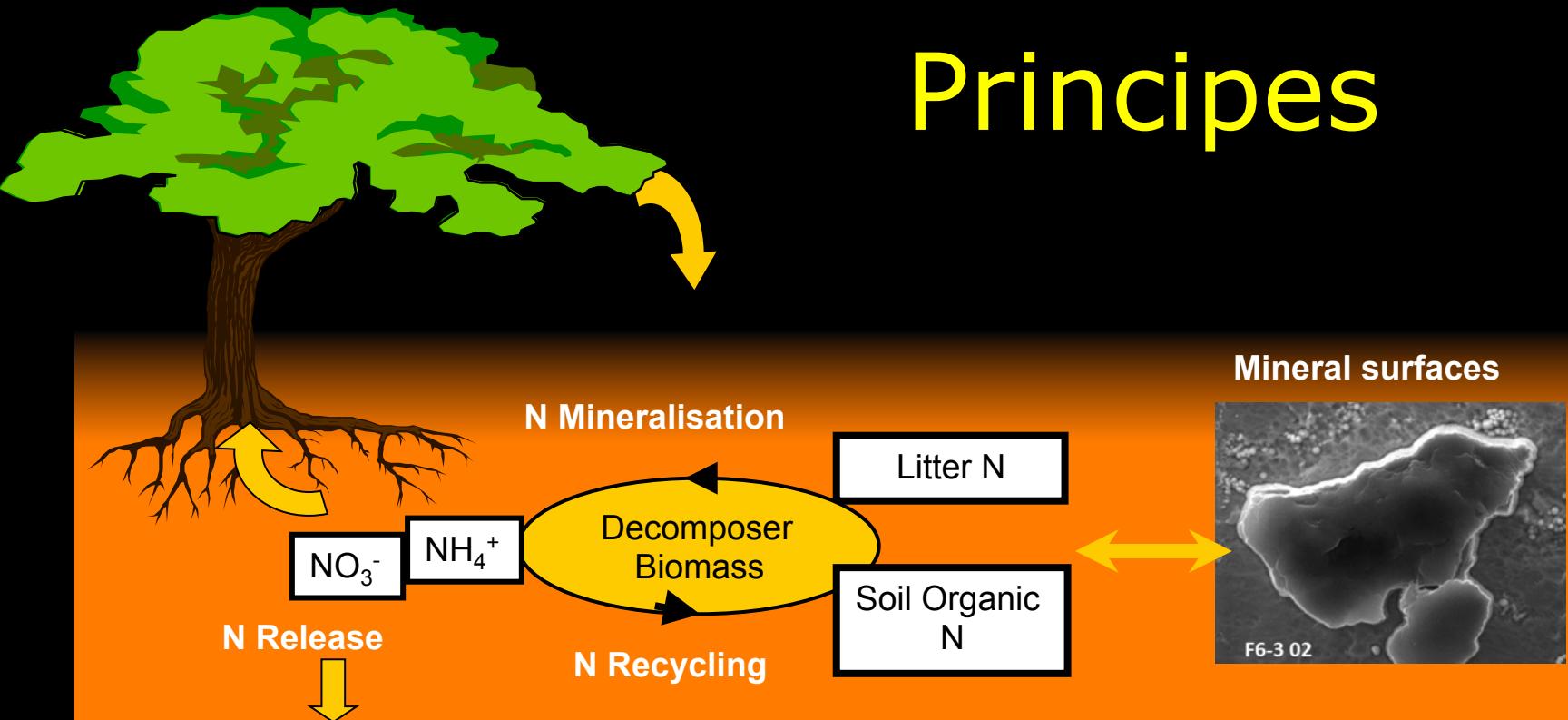
Poorly organized carbon



Cycles de la MO dans les sols

Comment on peut comprendre le fonctionnement d'un écosystème complexe en étudiant des processus à l'échelle du micron

Principes

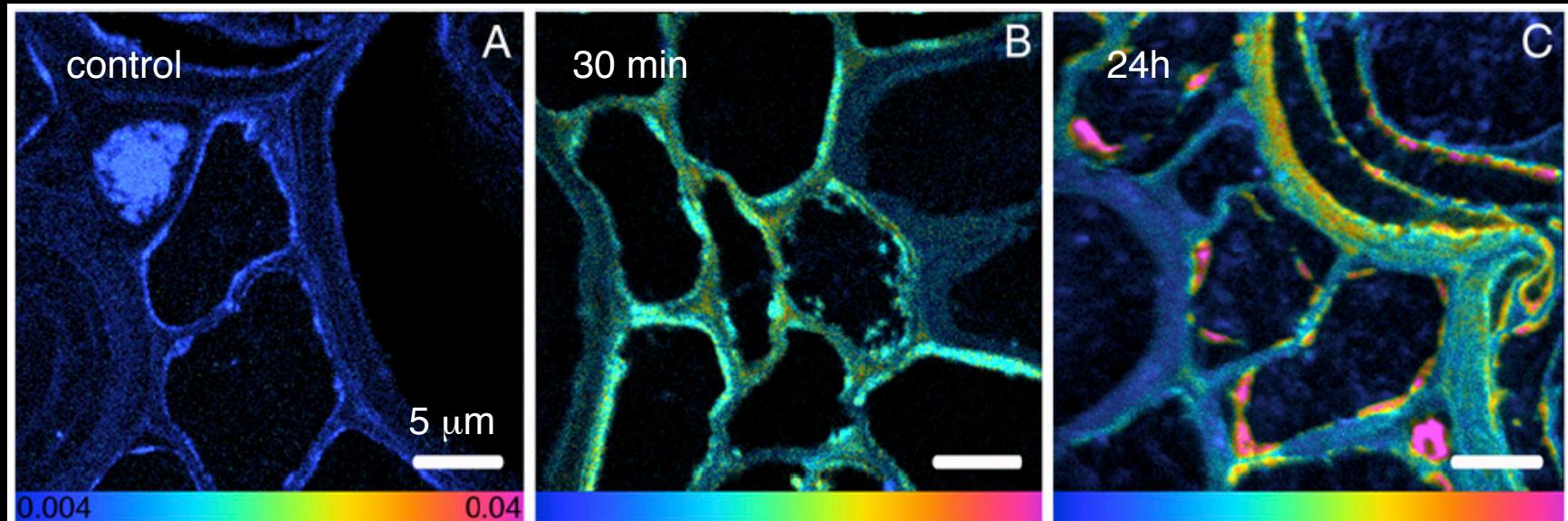
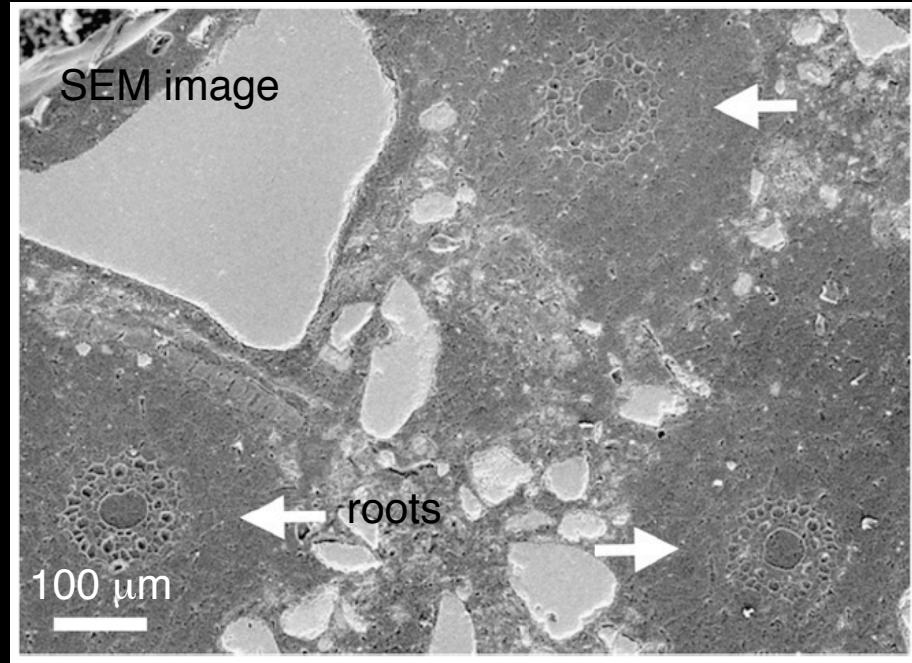


- Pour comprendre le recyclage et la préservation de la MO dans les sols, il faut s'intéresser aux relations MO-minéraux.
- Il faut donc utiliser des techniques d'étude à l'échelle du micron.
- Le marquage isotopique est un outil très puissant pour ce genre d'étude.

N uptake by roots

Injection of solutions with NH_4^+ with $^{15}\text{N}/^{14}\text{N} = 1$.
(Clode et al. 2009)

$^{15}\text{N}/^{14}\text{N}$ NanoSIMS images

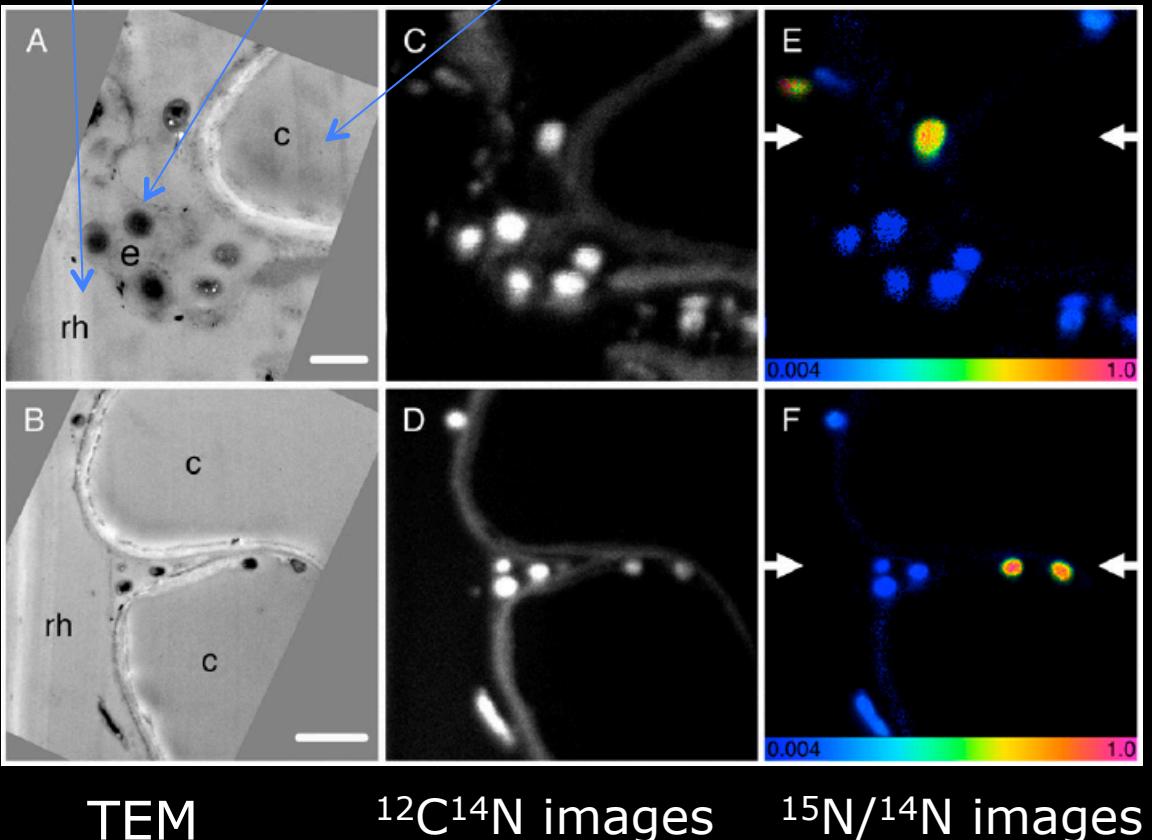


Competition plants/micro-organisms

rhizosphere

micro-organisms

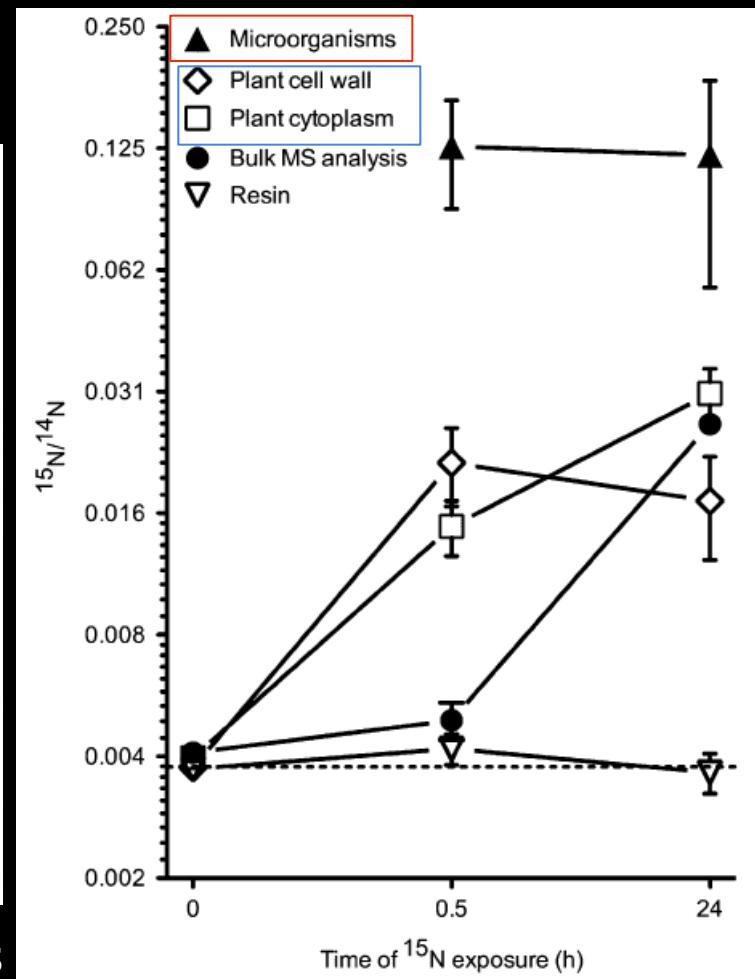
root cells



TEM

$^{12}\text{C}^{14}\text{N}$ images

$^{15}\text{N}/^{14}\text{N}$ images



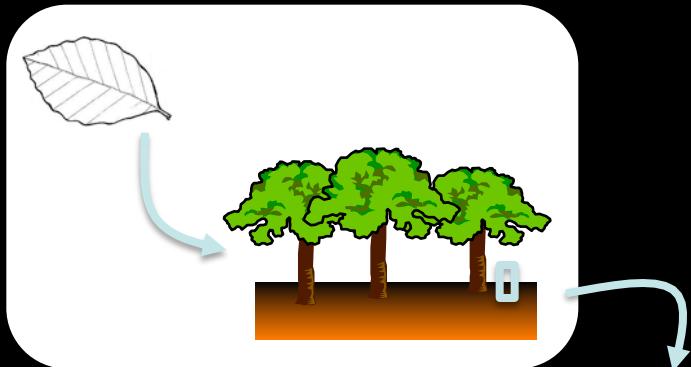
Clode et al. 2009

Isotope labelling experiment

1. Study site & soil sampling

German Beech forest with moder humus-type.

Acidic dystic Cambisol.

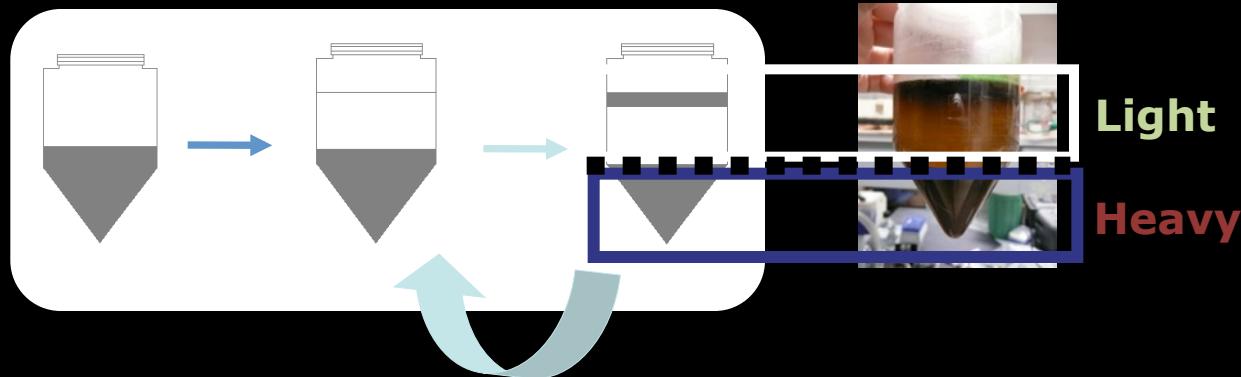


2. ^{15}N labeling

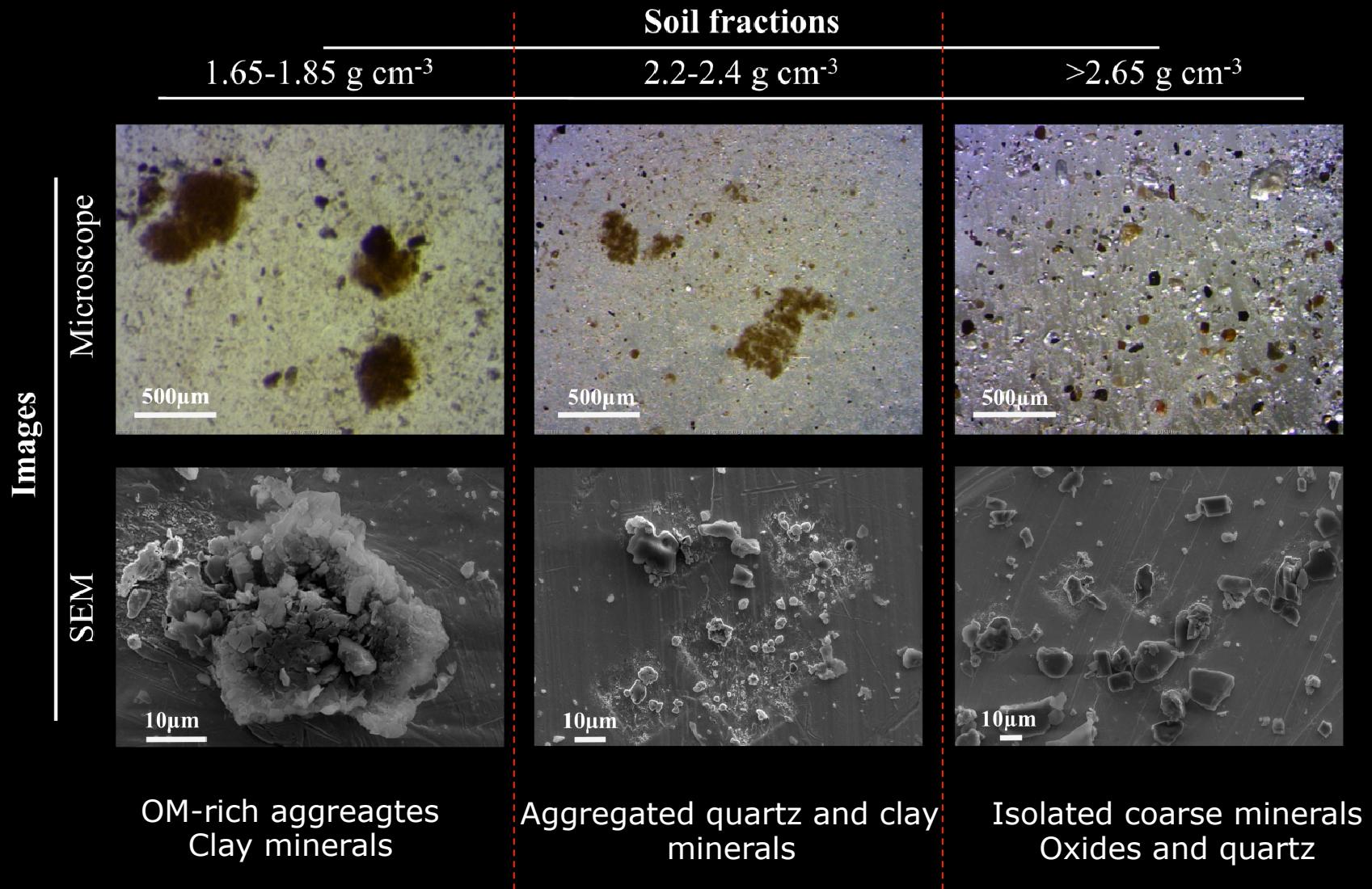
Beech leaves enriched in ^{15}N (2.5 At%) to follow degradation of complex OM (Zeller et al., 2000). Sampling after 12 years.

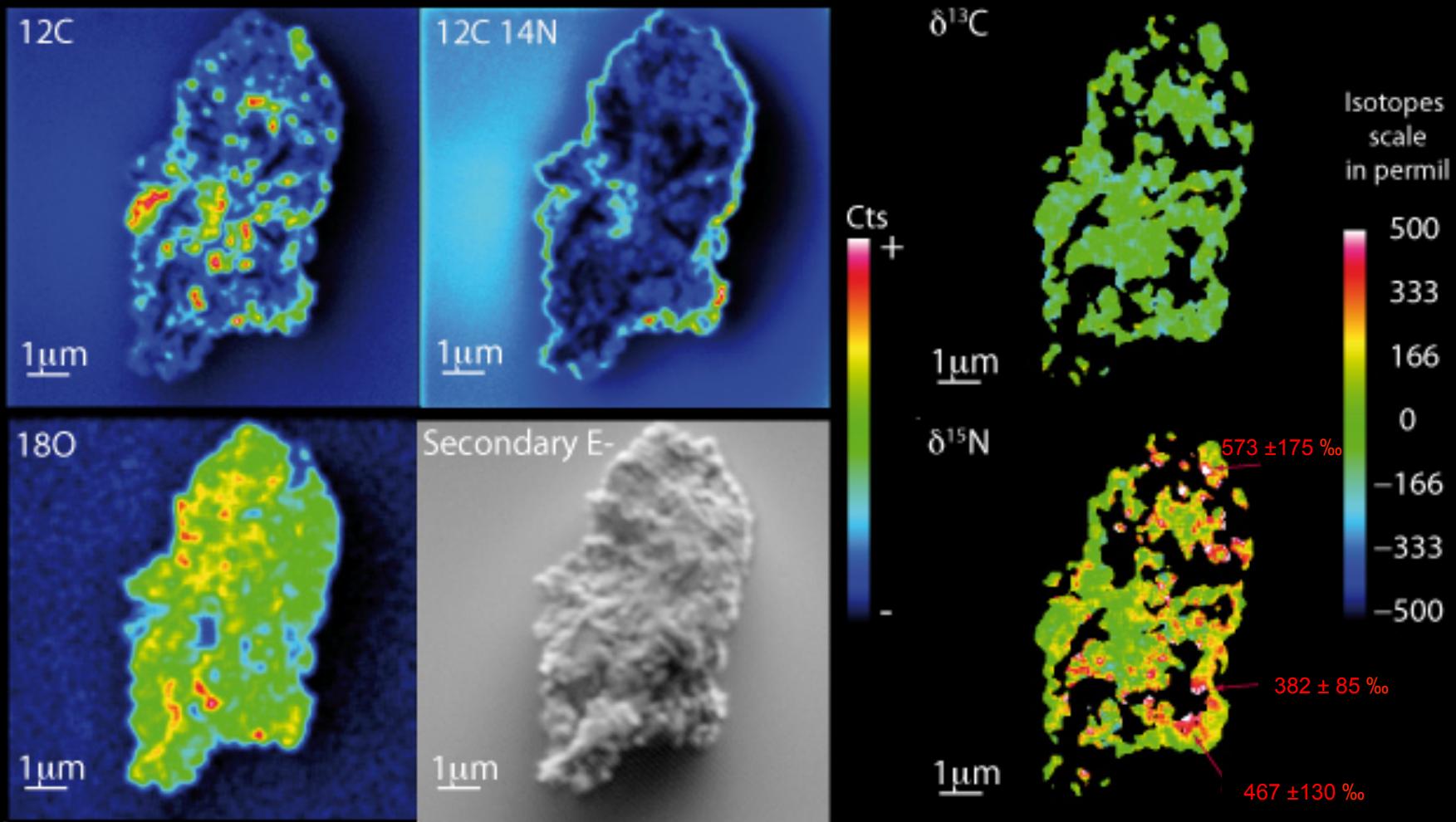
3. Isolation of soil microstructures

The sequential density fractionation isolates fractions that differ in their mineral and OM composition (Sollins et al., 2006).



Physical organization of soil fractions



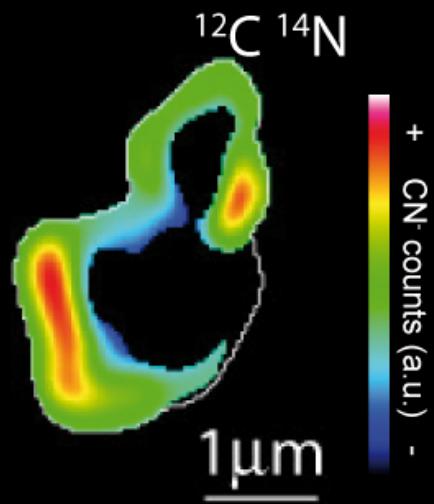


Remusat et al. ES&T 2012

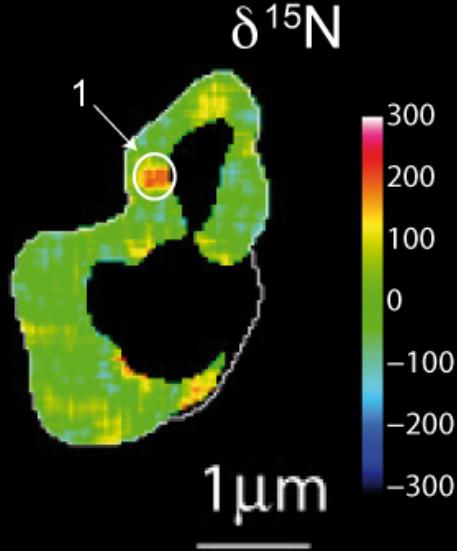
These spots could be preserved plant OM (not processed during the 12 years) or slightly recycled OM (now being microbial OM).

One example of combined characterization of a soil particle

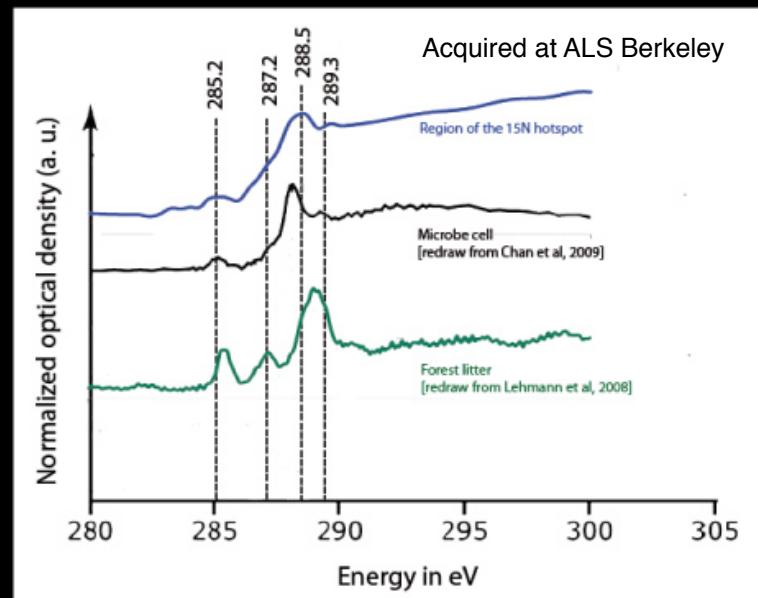
NanoSIMS N map



NanoSIMS isotopic map



C K-edge XANES



Remusat et al. ES&T 2012

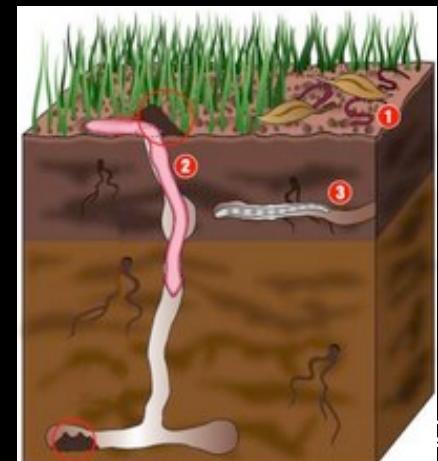
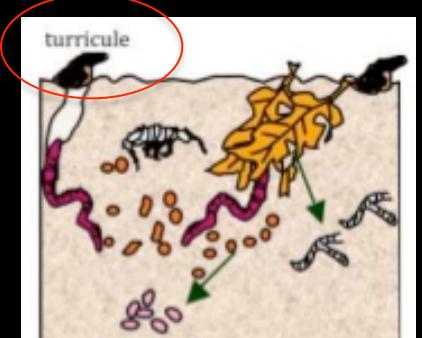
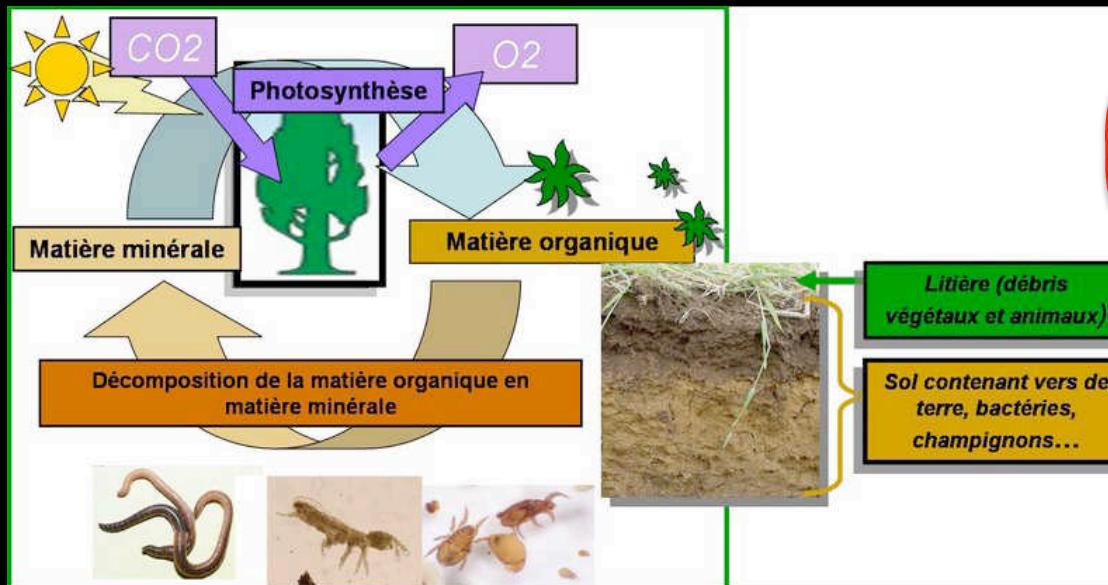
- The label seems related to microbial OM: the original organic label has been recycled but not diluted in a few spots.
- N can remain several years in the soil while being incorporated in the microbial pool.

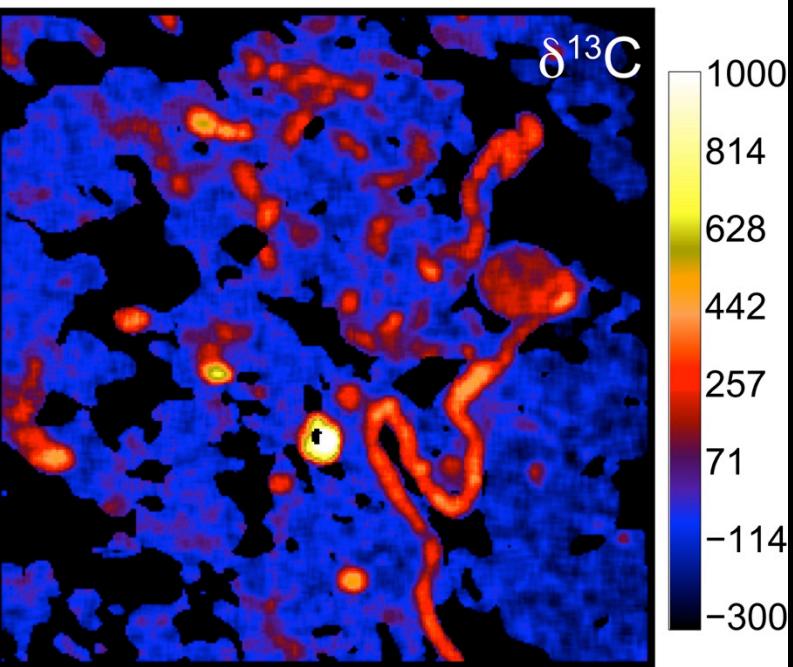
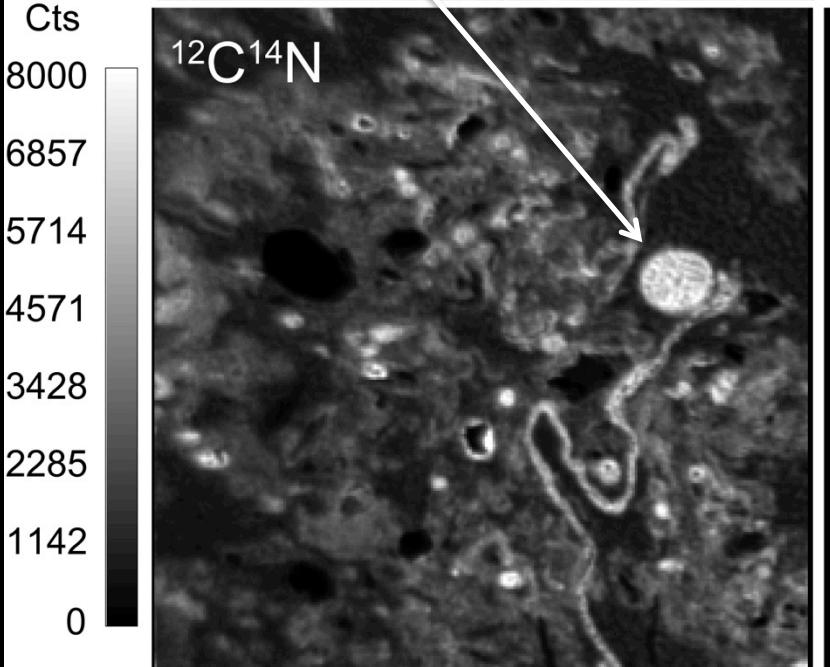
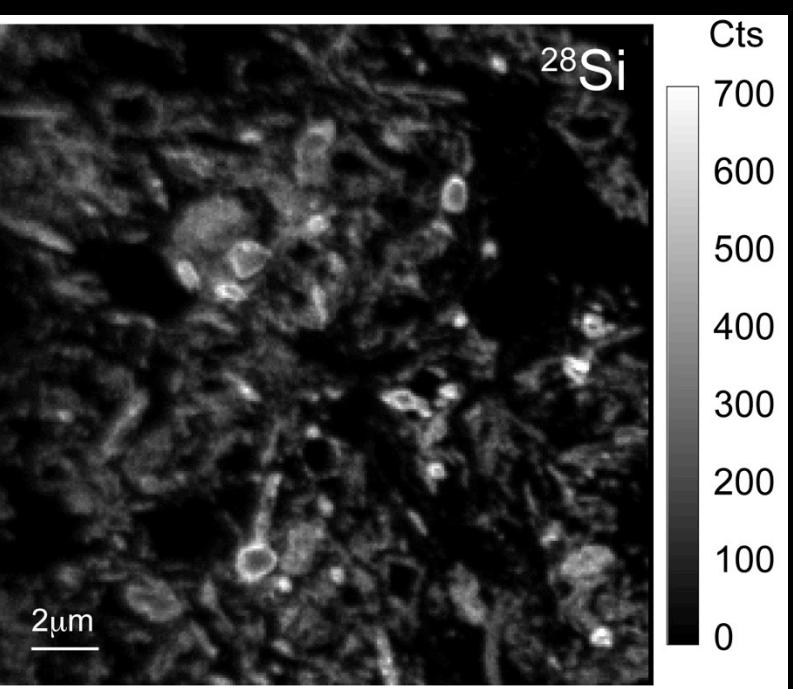
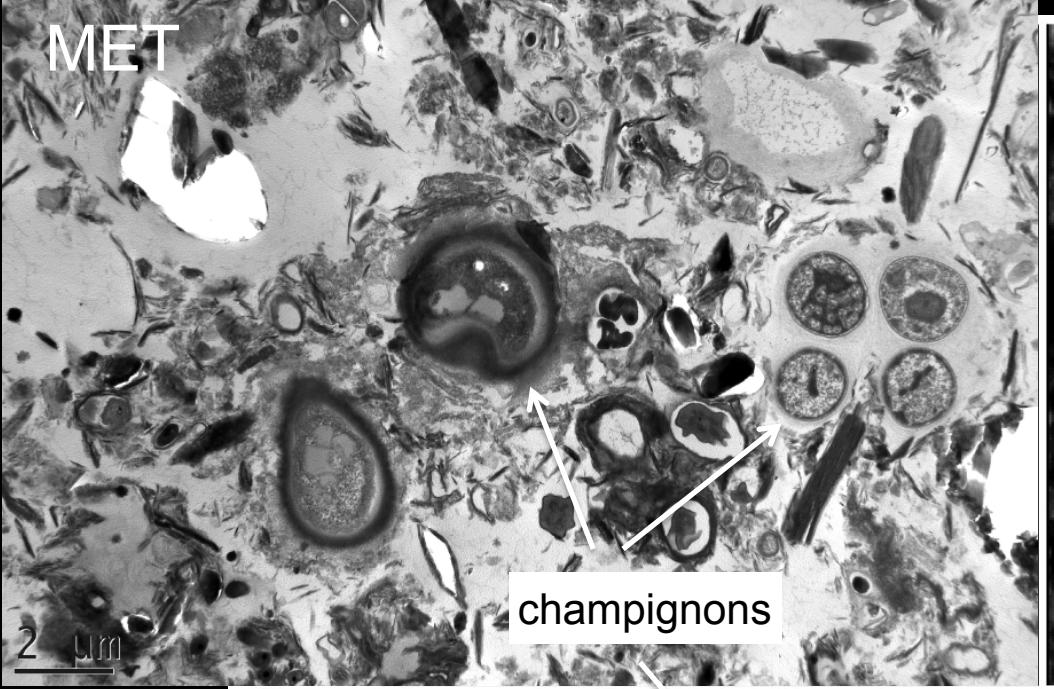
Influence des vers de terre sur le cycle de la MO dans les sols

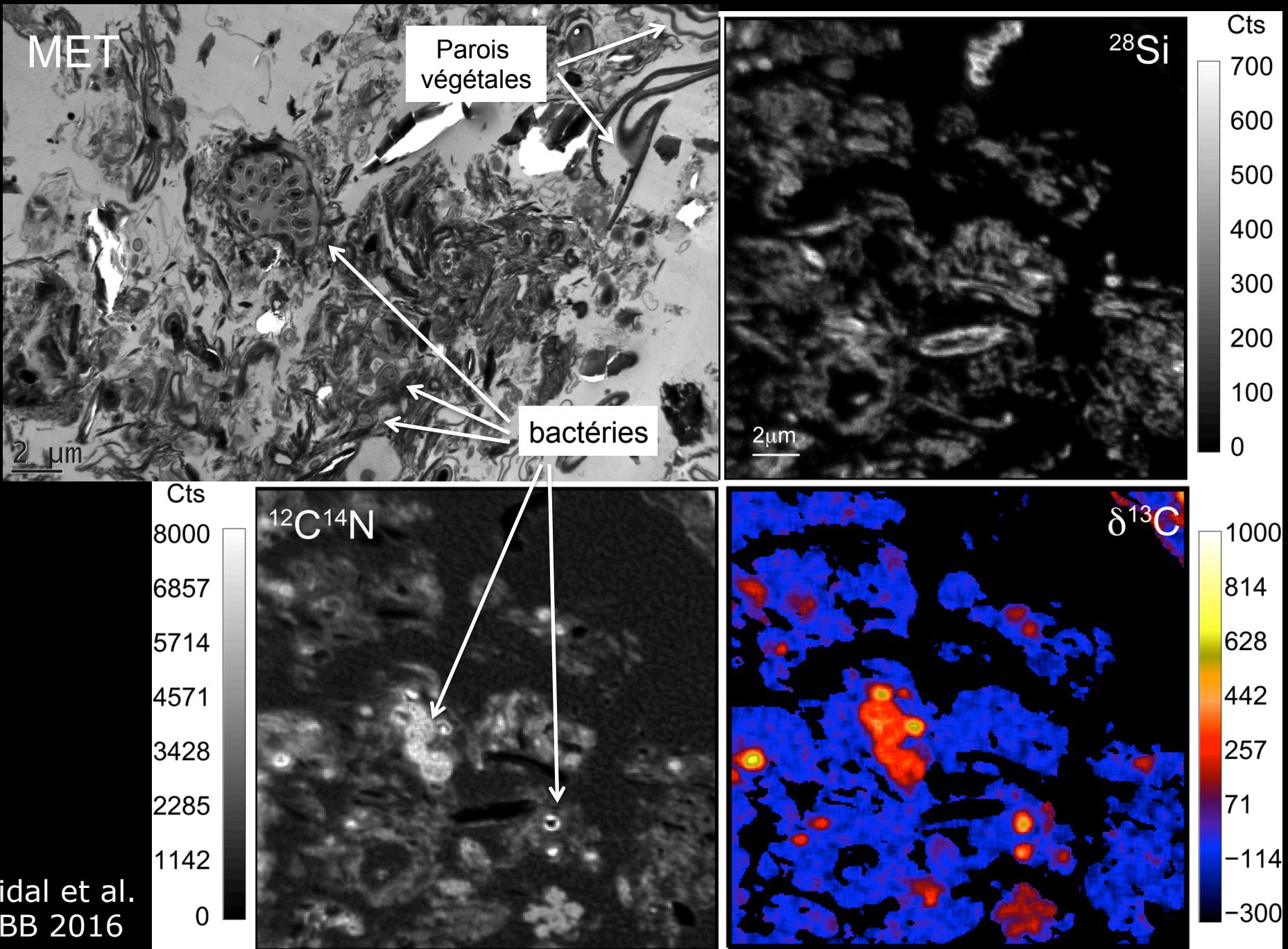
Etude d'un cycle complexe à l'échelle du micron. Combinaison NanoSIMS et MET

(Vidal et al. Soil Biology and Biochemistry 2016)

Les vers de terre participent au recyclage de la MO dans les sols







Biominéralisation des bactéries

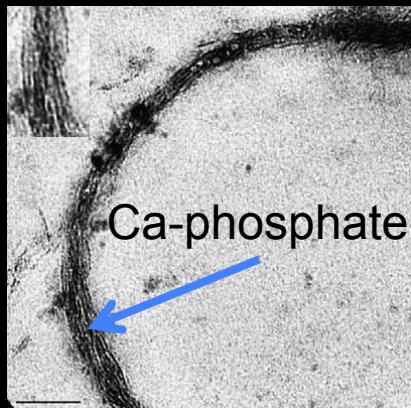
Comment le NanoSIMS permet d'étudier
les mécanismes de survie des bactéries
en milieu minéralisant.

Coll. J. Miot (IMPMC)

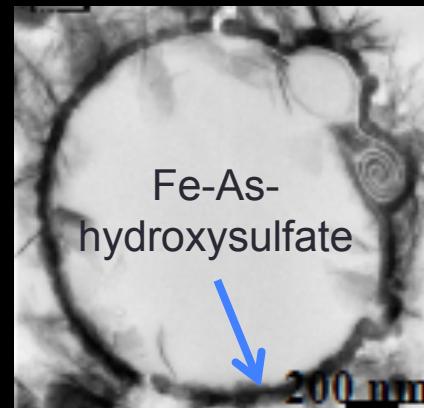
Periplasm Encrustation



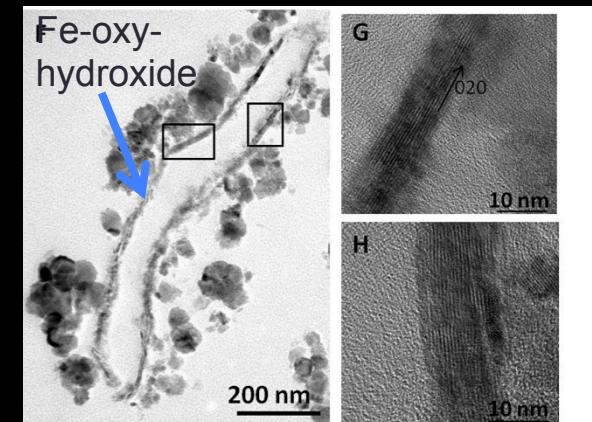
- A widespread pattern of biomineralization



Benzerara et al., *EPSL*, 2004

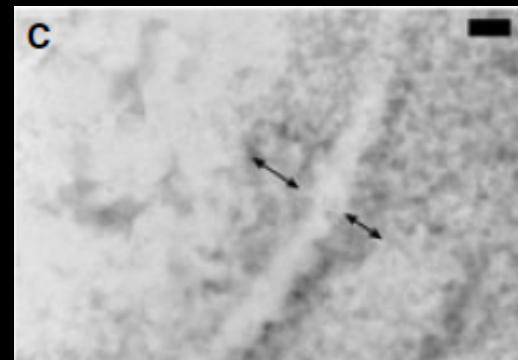
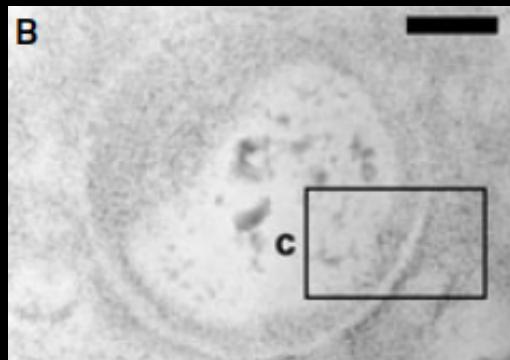


Benzerara et al., *GCA*, 2008



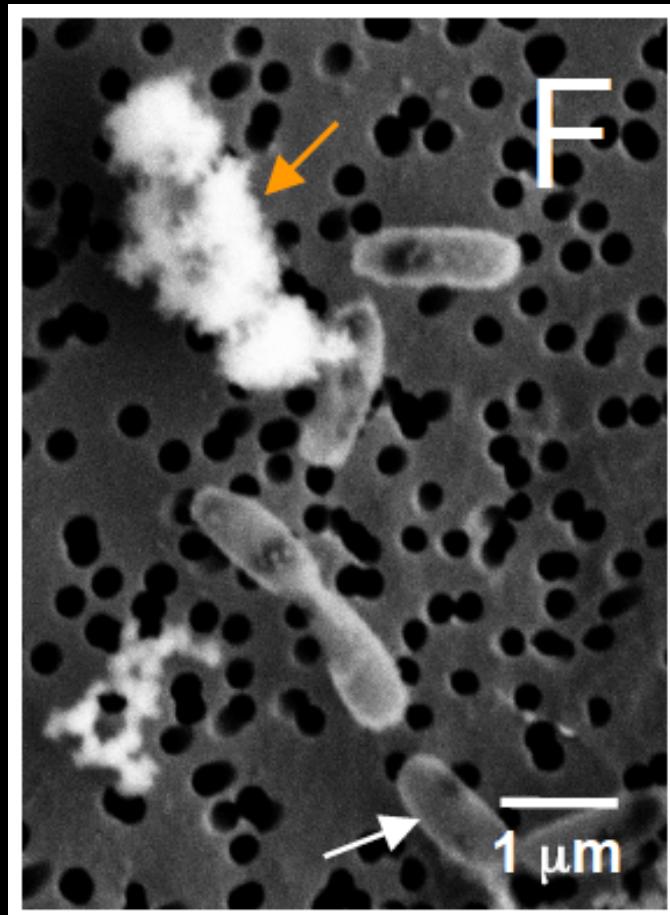
Miot et al., *GCA*, 2014

- Might enhance preservation in the fossil record



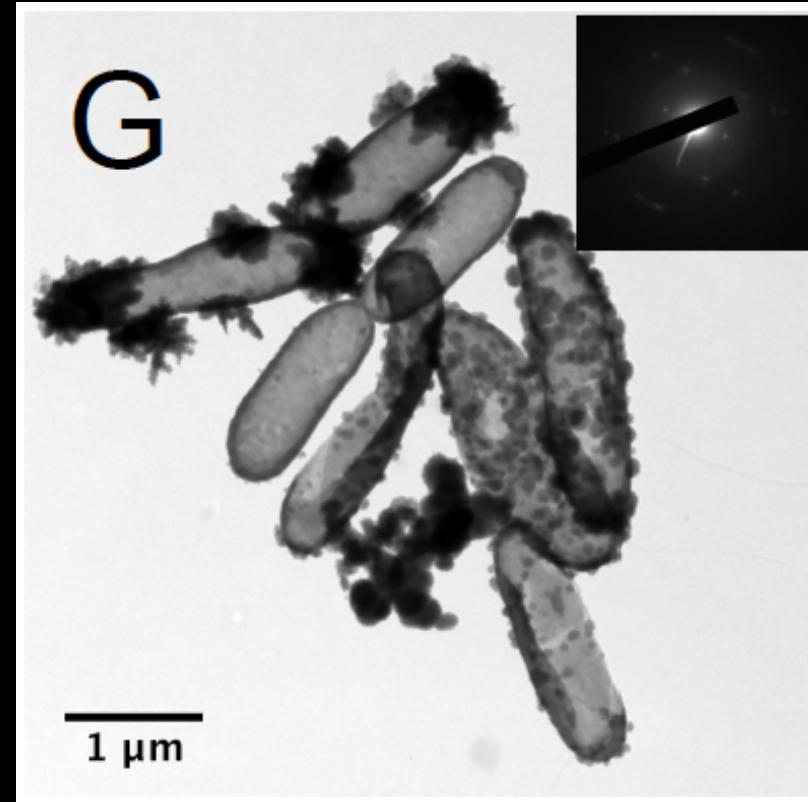
Cosmidis et al.,
Geobiology, 2013

Mineralized and non mineralized bacteria sitting on polycarbonate film



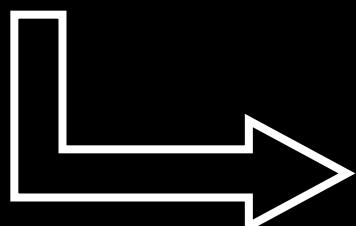
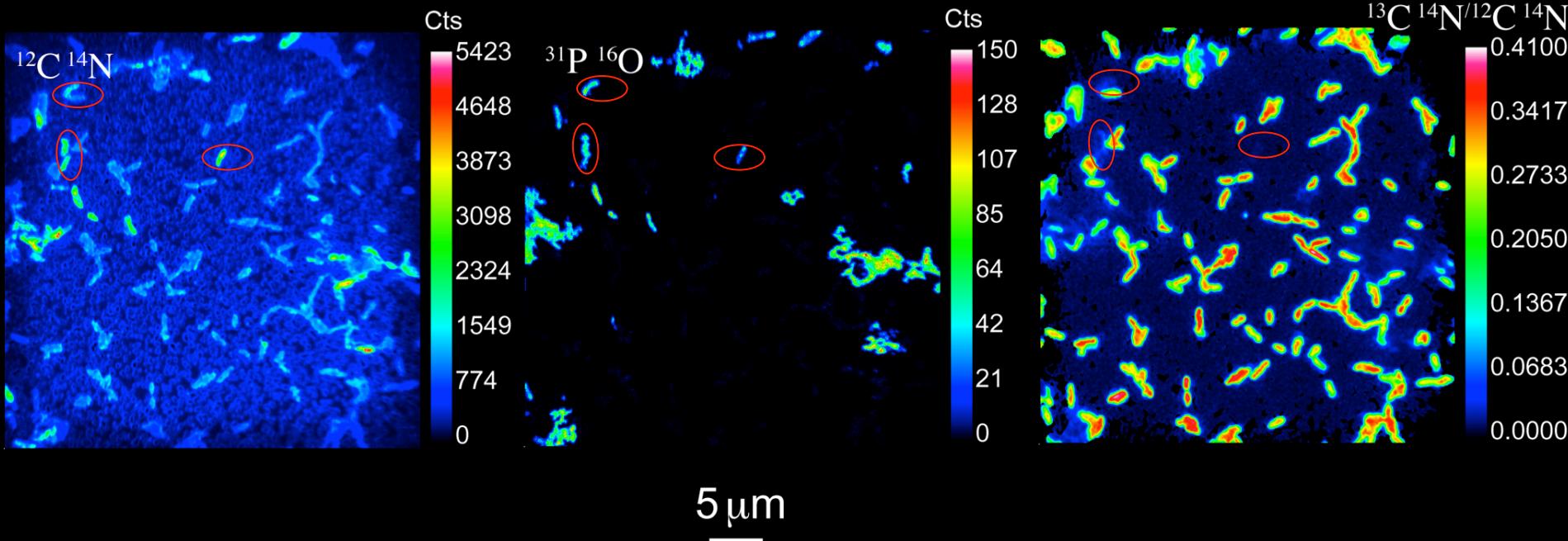
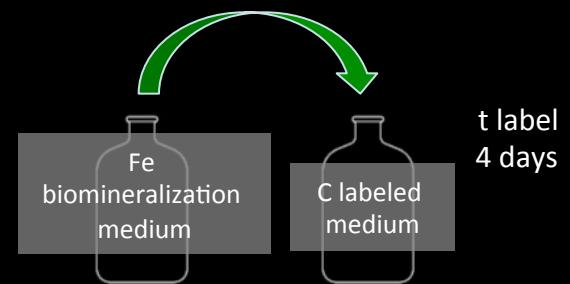
SEM

For higher resolution imaging, we used TEM grids



TEM

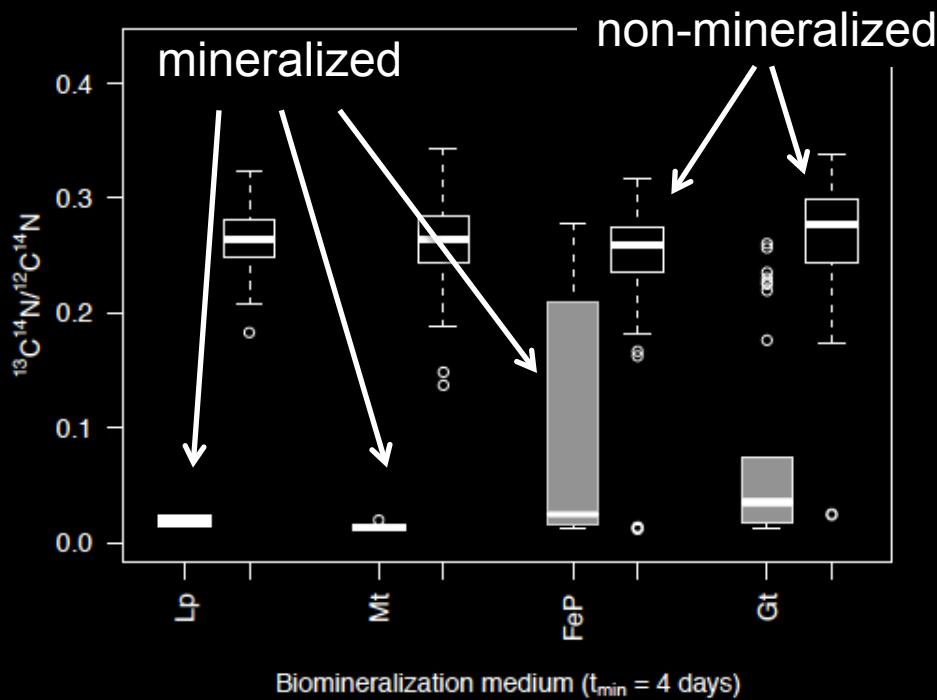
Heterogeneities of Carbon Incorporation



Non-Mineralized cells incorporate labeled acetate,
whereas
Mineralized cells DO NOT incorporate labeled acetate

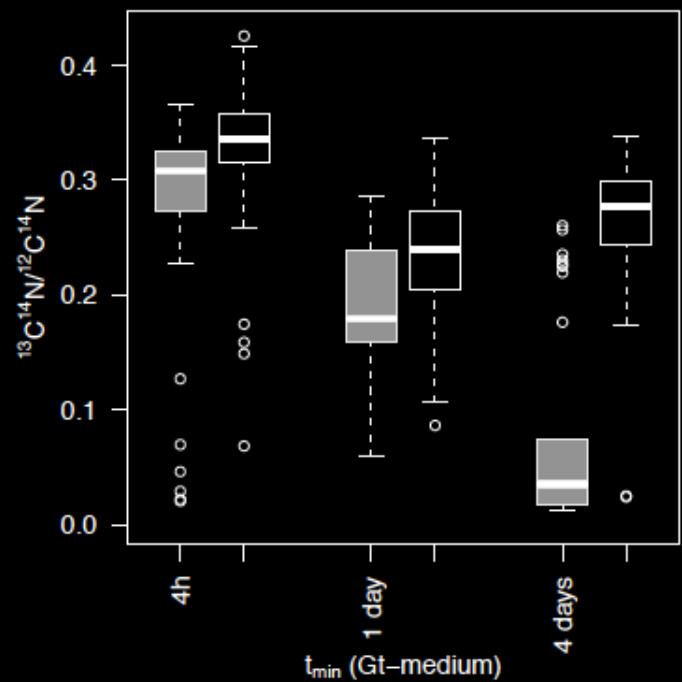
Carbon assimilation

Different mineralization conditions



No ^{13}C incorporation for mineralized cells

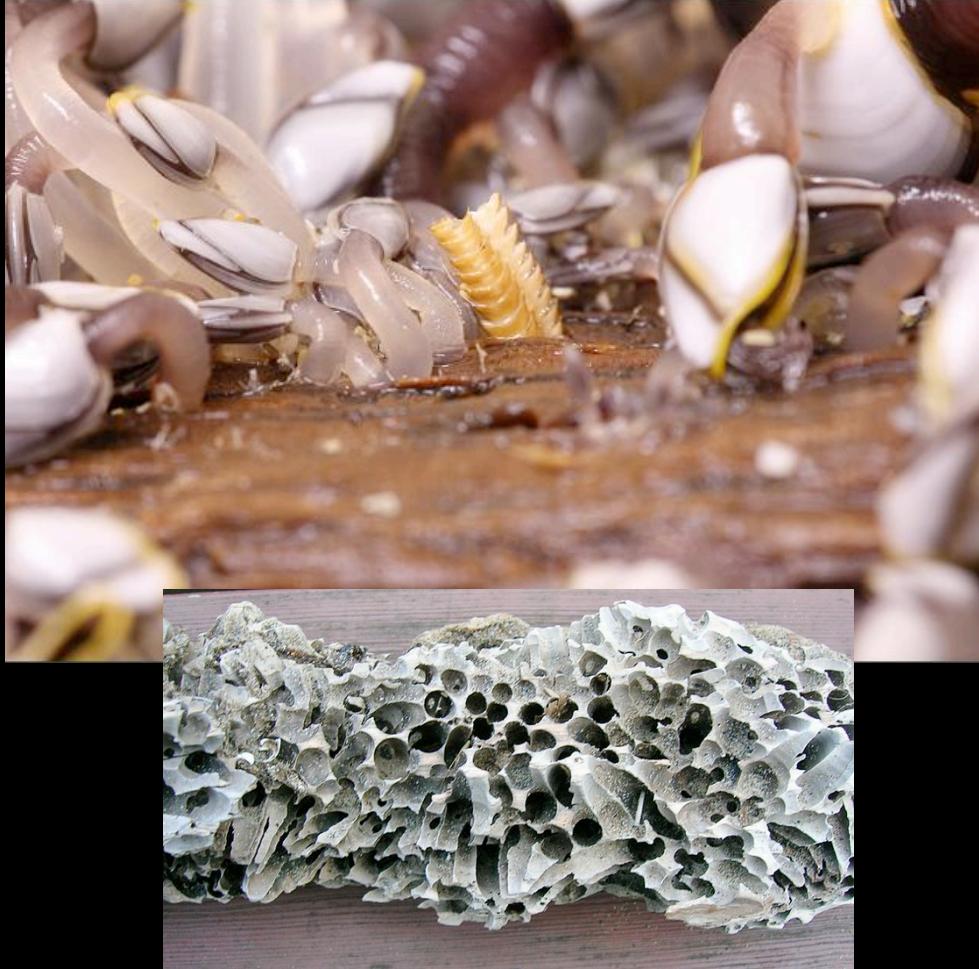
Different exposure to mineralization conditions



Ability to fix ^{13}C (\approx viability) decreases as exposure to mineralization increases.

Etude des métabolismes assimilations et biominéralisation

Fixation N₂ par un bivalve xyloophage : taret (Shipworm)



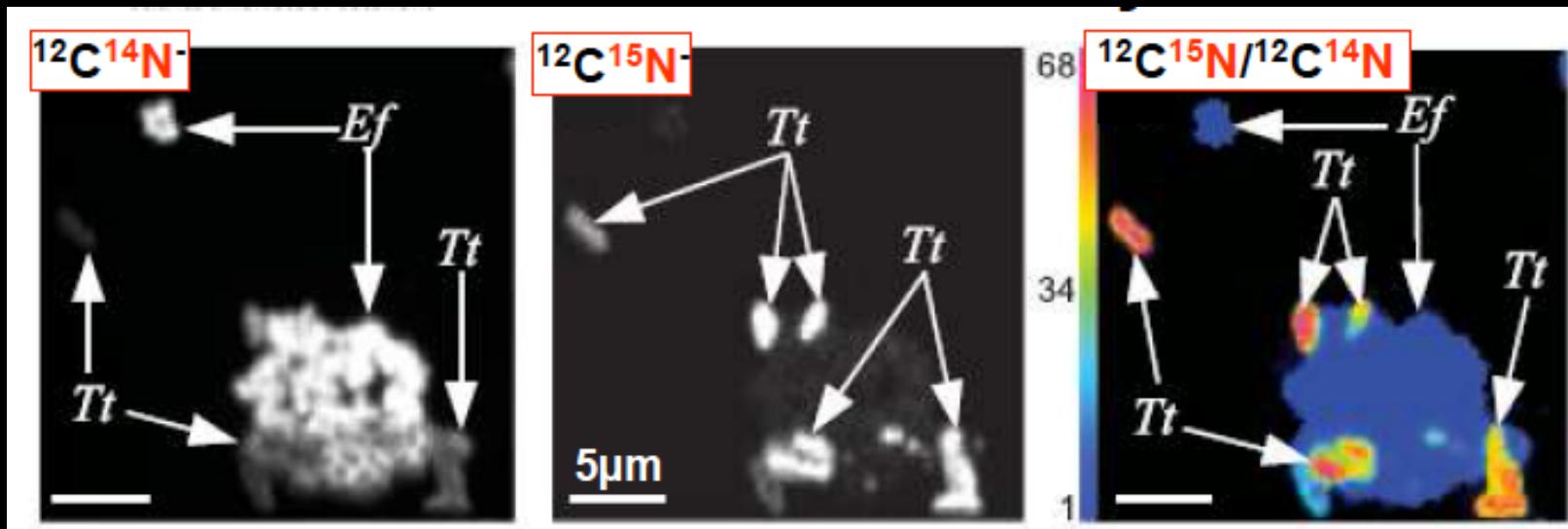
- “Quantitative imaging of Nitrogen fixation by individual bacteria within animal cells”.
C. Lechene, Y. Luyten, G. McMahon, D. Distel. (2007)
Science, 317 pp1563
- Measurement of N fixation with the help of labeled N₂.
- The study aimed at identifying the cells fixing the N and the trophic relations with the host.
- Test on 2 types of bacteria, with and without fixing capabilities.
- Then study of the symbiosis with the same protocol.



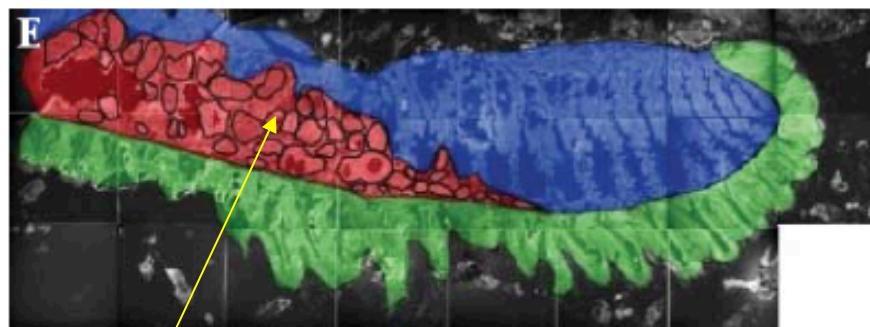
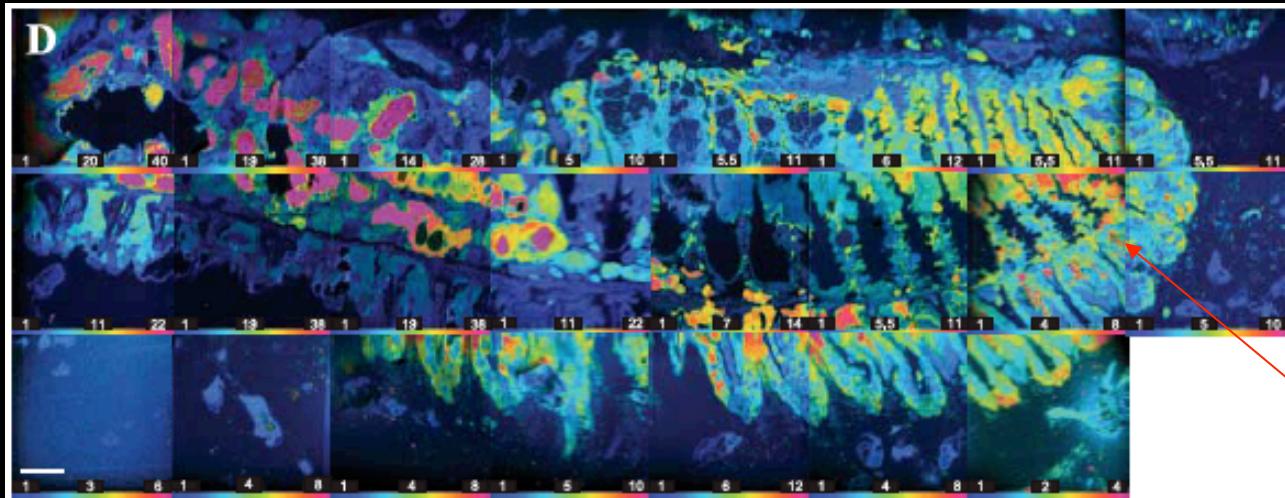
Bactéries + $^{15}\text{N}_2$

T. Turnarae bacteria (Tt): fixing ^{15}N .
E. Faecalis (Ef): not fixing ^{15}N .
HSI Color scale: Blue = terrestrial ratio

After 7 days, the $^{15}/^{14}\text{N}$ ratio
is increased by a factor of 68

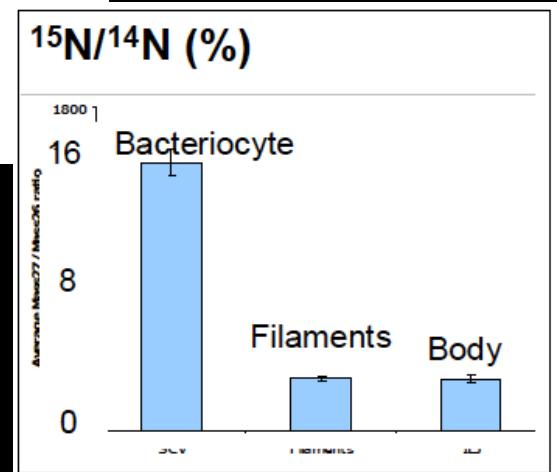


NanoSIMS images are compared to TEM images to identify structures



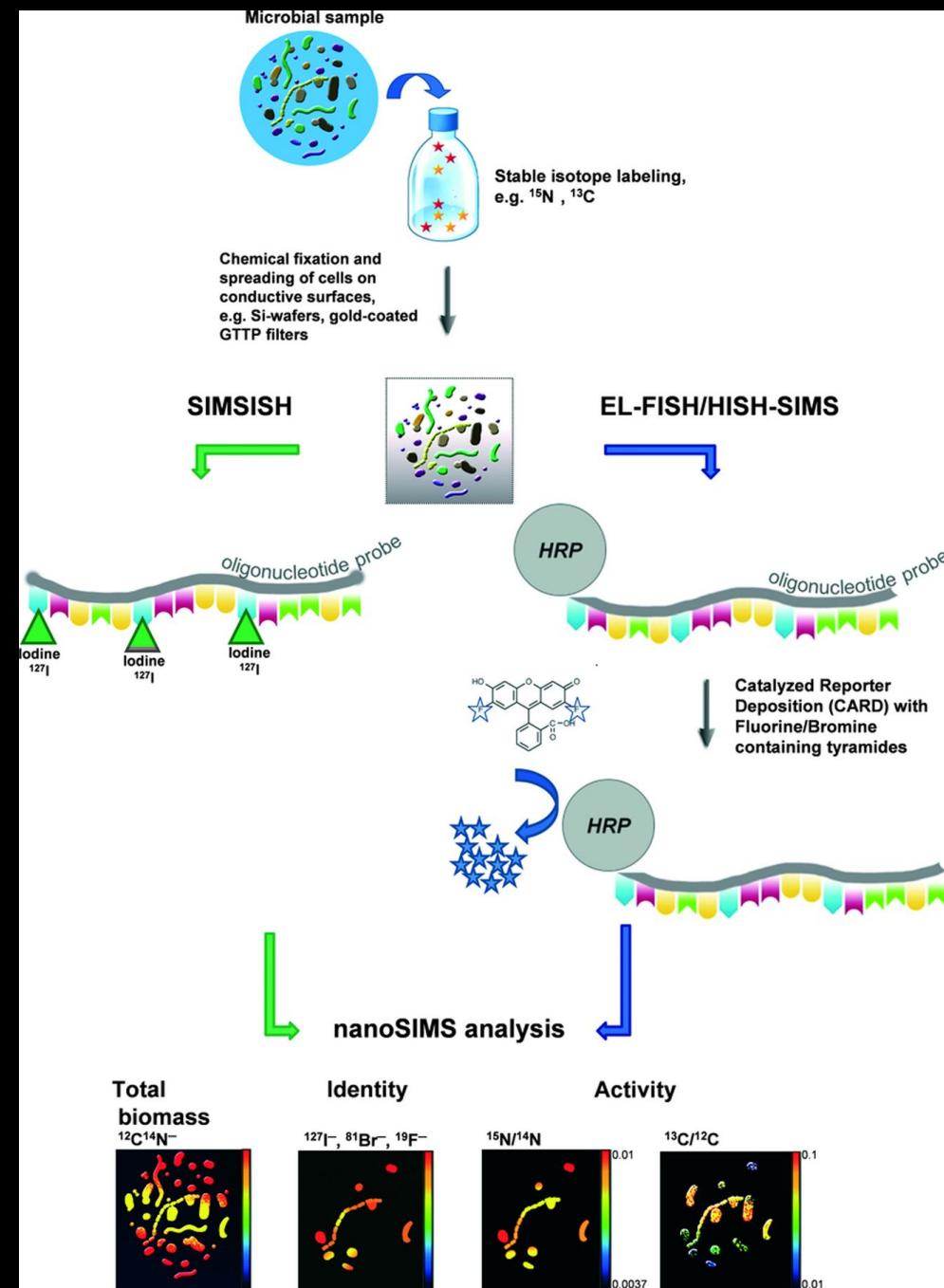
Most of the N fixation occurs in the gland where the symbiont is hosted

Late generation cells are also enriched in ^{15}N : N transfer from the gland to other places in the animal



- Identification des microorganismes, distinction des souches bactériennes
- Nécessité de combiner avec d'autres techniques d'imagerie
- MET, FISH (hybridation et fluorescence)

Combinaison avec hybridation in situ (ISH techniques)

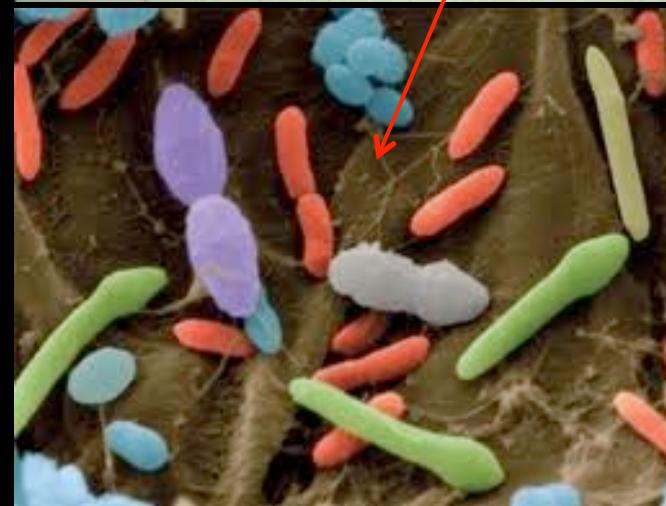
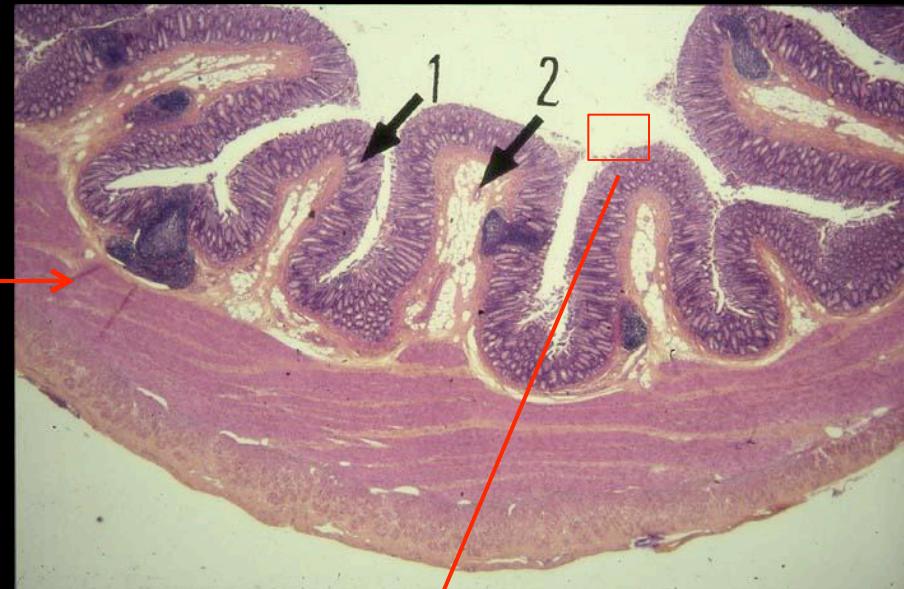
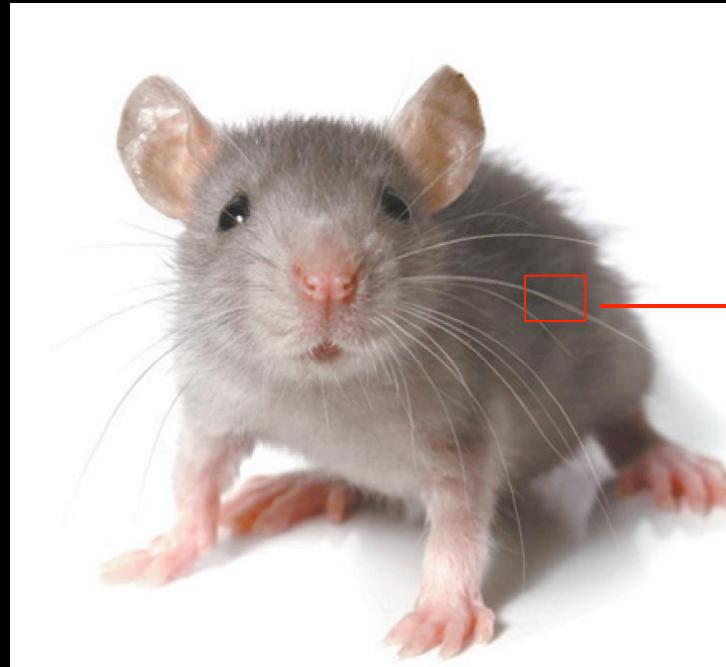


Niculina Musat et al. (2012)
FEMS Microbiol Rev

FEMS

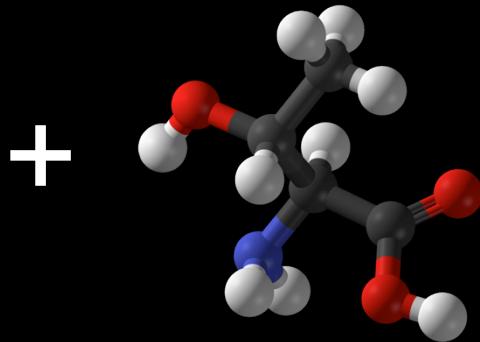
MICROBIOLOGY
REVIEWS

Interactions trophiques dans les intestins des animaux



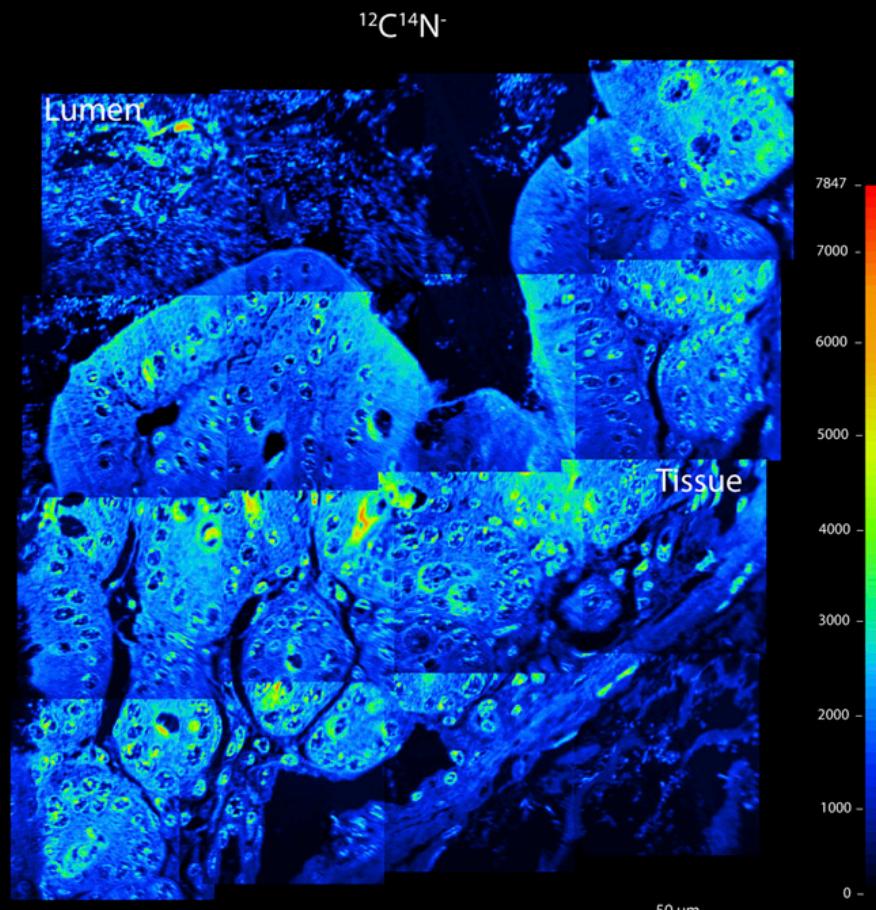
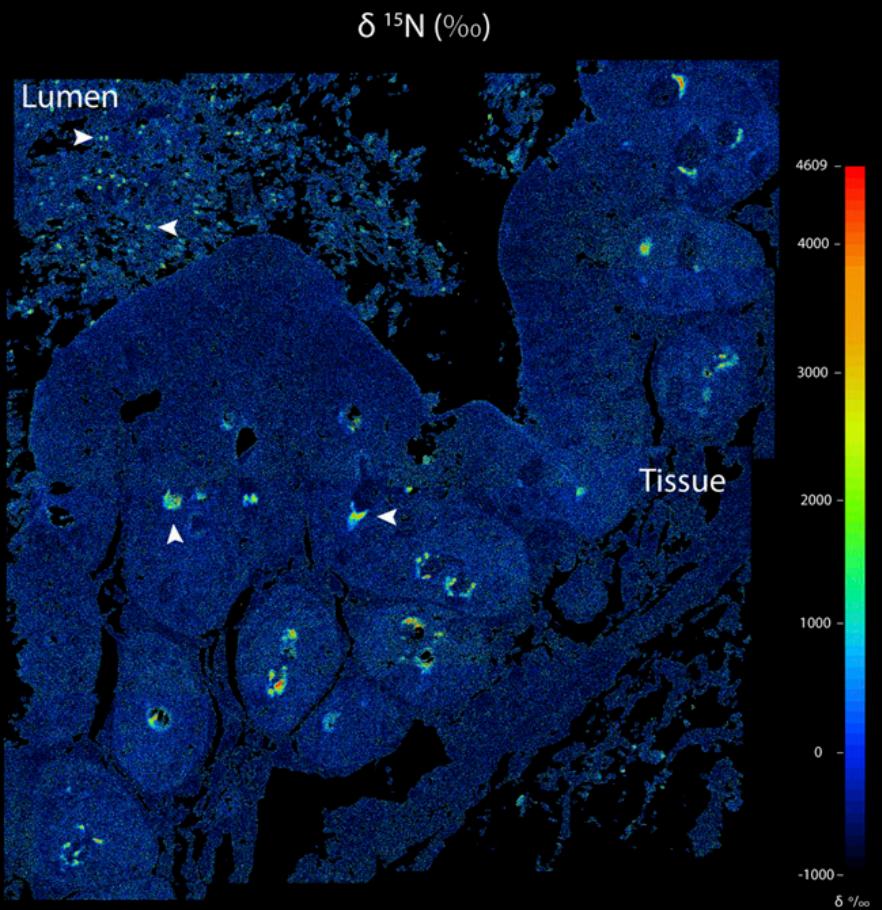
?

Interactions trophiques
dans l'intestin?

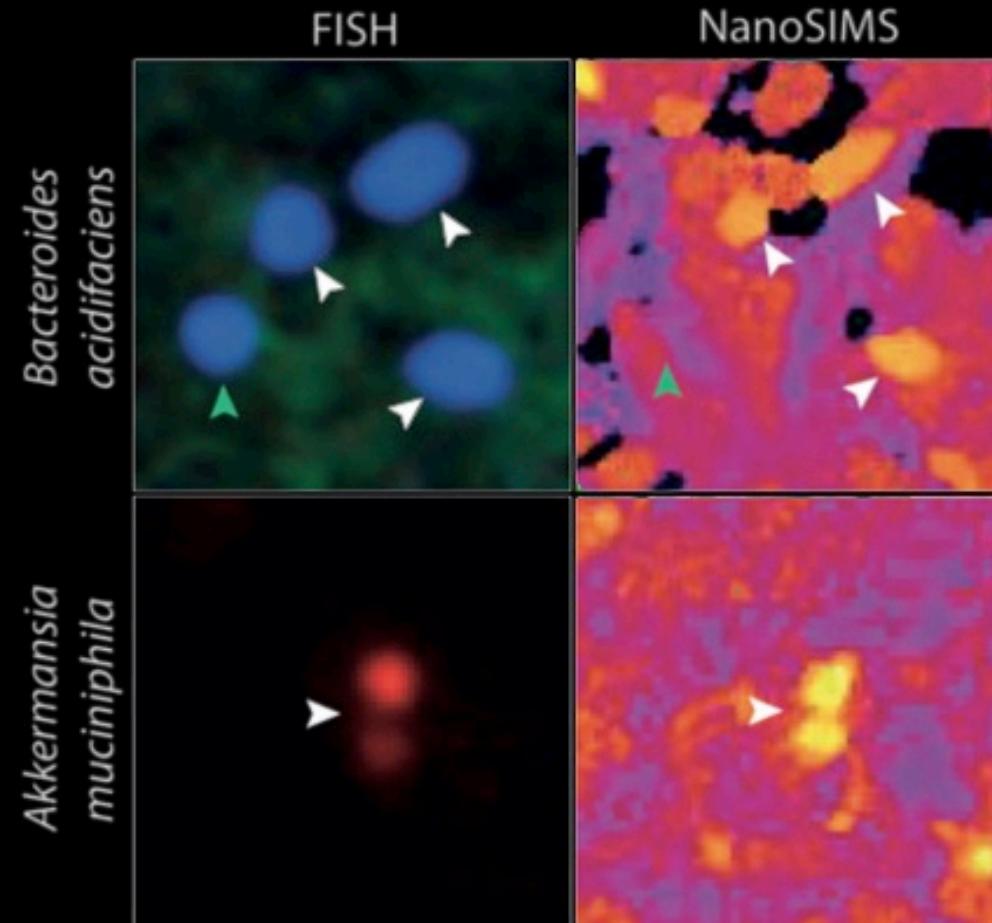


Thréonine marquée
au ^{13}C et ^{15}N

Berry et al. 2013 PNAS



Interactions trophiques dans les intestins des animaux

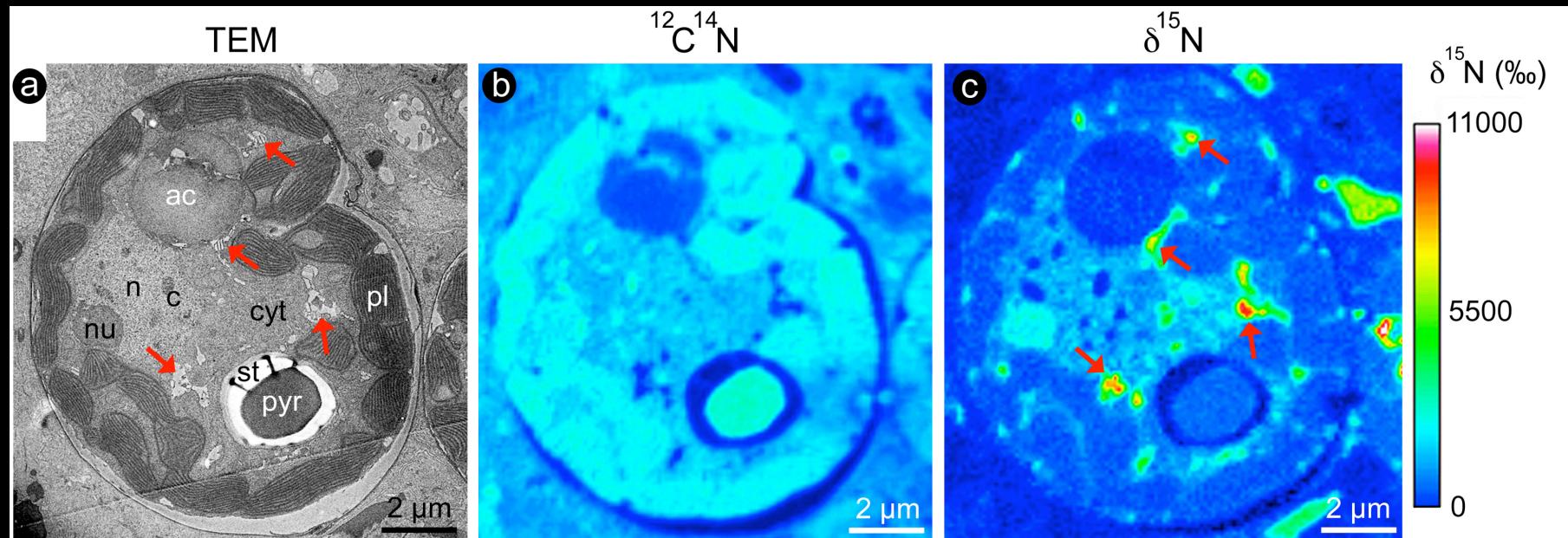


Berry et al. 2013 PNAS

Deux souches sont les principales consommatrices de protéines dans l'intestin.

Meilleure compréhension de l'écosystème dans l'intestin.

Symbiosis between zooxanthellae and reef-building corals



(Pernice et al. ISME 2012)

- ^{15}N -rich ammonia was used in the experiment
- the algae is responsible for ammonium assimilation
- algae cell present N-storage structures

L'imagerie NanoSIMS pour étudier le vivant

- Marquage isotopique indolore pour l'organisme
- Les marqueurs isotopiques sont révélés par NanoSIMS
- Etude des processus à l'échelle du micron, donc à l'échelle des compartiments cellulaires

Pourquoi NanoSIMS?

- NanoSIMS : technique dédiée à l'imagerie isotopique à l'échelle de la 100^{aïne} de nm.
- Le NanoSIMS est le complément idéal d'une étude de marquage isotopique (processus métaboliques, migrations de fluides, étude des corrosions, etc...).
- Permet d'étudier les processus à l'échelle du micron.
- Stabilité est suffisante pour atteindre une précision autour du pour mile.
- Combinaison avec d'autres méthodes d'imagerie pour identifier les structures et les microorganismes

Conclusions

- NanoSIMS= microscope chimique permettant d'étudier les éléments léger et les traces à haute résolution spatiale.
- Technique quantitative, précisions de l'ordre du %.
- Complexe, sensible aux effets de matrices, nécessite des standards et à combiner à d'autres techniques de caractérisation fine.
- Applications très variées en geosciences, sciences de l'environnement, microbiologie...