Incorporation of ^{13}C labelled shoot residues in *Lumbricus terrestris* casts

A combination of **Transmission Electron Microscopy** (TEM) and **Nanoscale Secondary Ion Mass Spectrometry** (NanoSIMS)

Alix Vidal, Laurent Rémusat, Françoise Watteau, Sylvie Derenne, Katell Quénéa

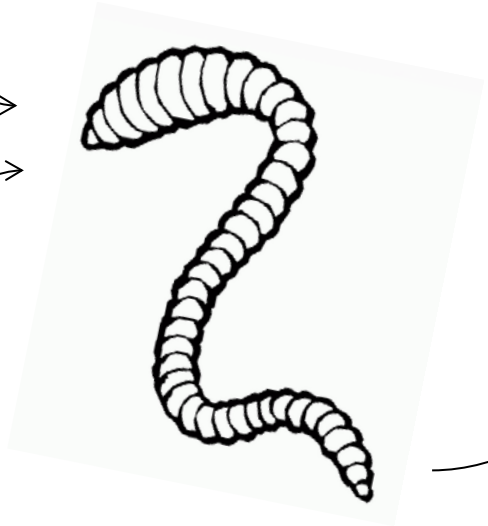
The 21st of September

Earthworms: essential soil engineers

Organic (plant residues and microorganisms)

Mineral (soil particles)

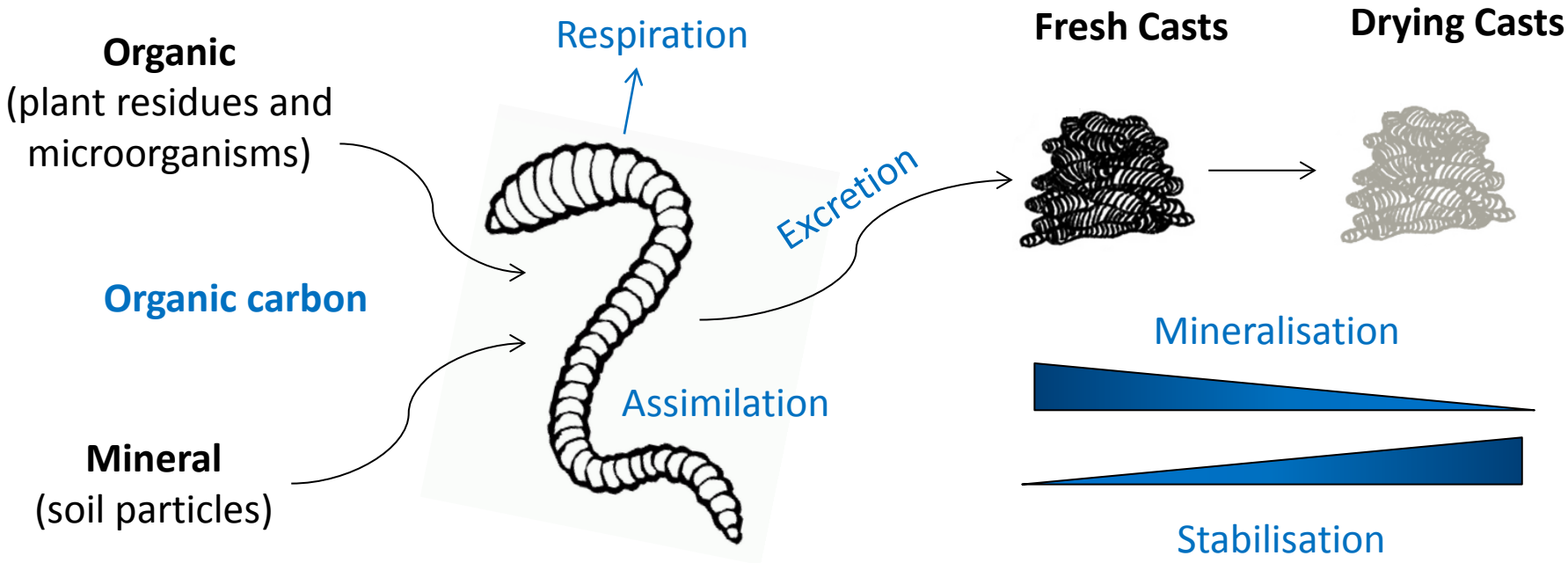
Soil and plant residues **mixed** and intimately associated with **mucus**



Casts

Intimate **organo-mineral association**

Earthworms: Impact on **carbon transfert** and **stock** in casts



Fate of carbon incorporated inside casts and its interaction with microorganisms at the **microscale** are still poorly understood

What methods to study casts at the microscale?

Transmission Electron Microscopy (**TEM**)

Identify microstructures
nm-scale resolution

Origin of organic matter ?

+

Nano-scale secondary ion mass spectrometry (**NanoSIMS**)

Elemental (**carbon**) and isotopic maps (δ^{13}**C**) of organic compounds

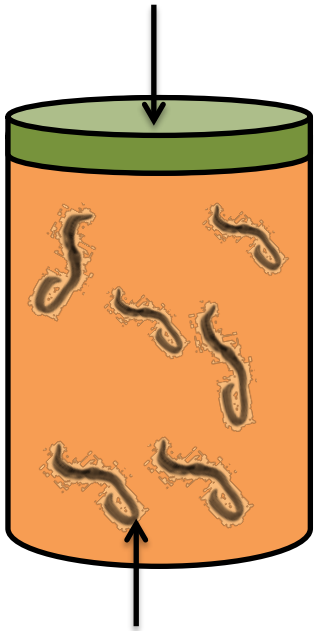
Nature of organic matter ?

Investigate the **incorporation** and **decomposition** of **labelled litter** in earthworm **casts**

Sampling

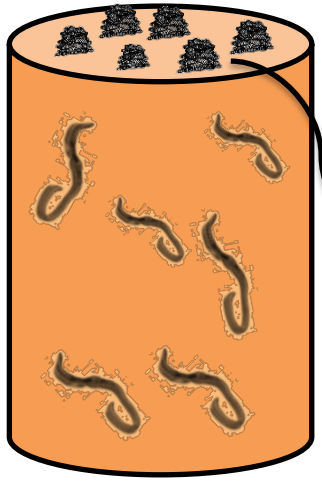
250 g of artificially labelled in **^{13}C Italian Ryegrass** shoots (*Lolium multiflorum*)

~ 75 L of a loamy-sand soil



Earthworms
Lumbricus terrestris

6 months



Sample of initial litter

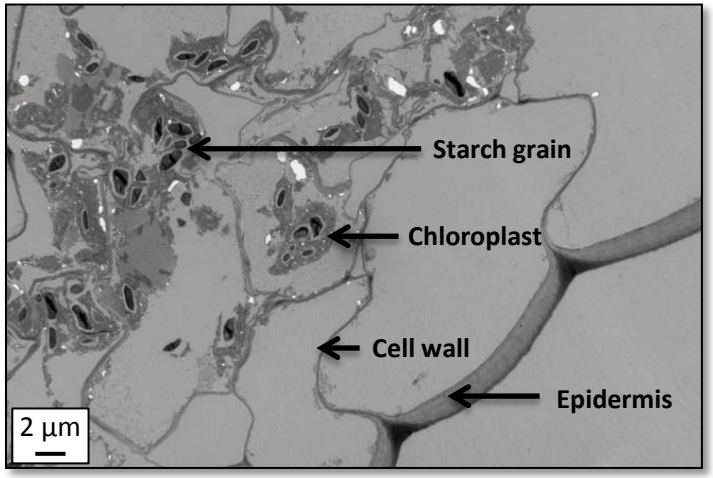
Samples for TEM and NanoSIMS analyses

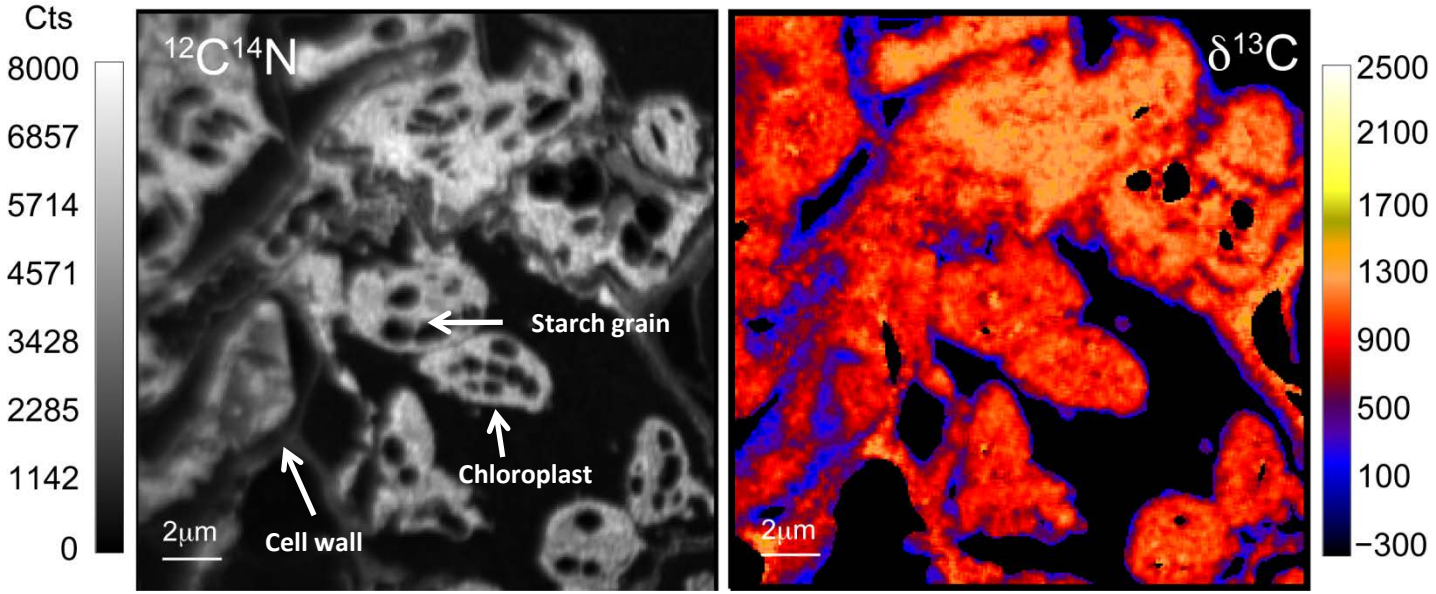
Composite sample of surface casts

Labelled litter characterization

Plant tissues

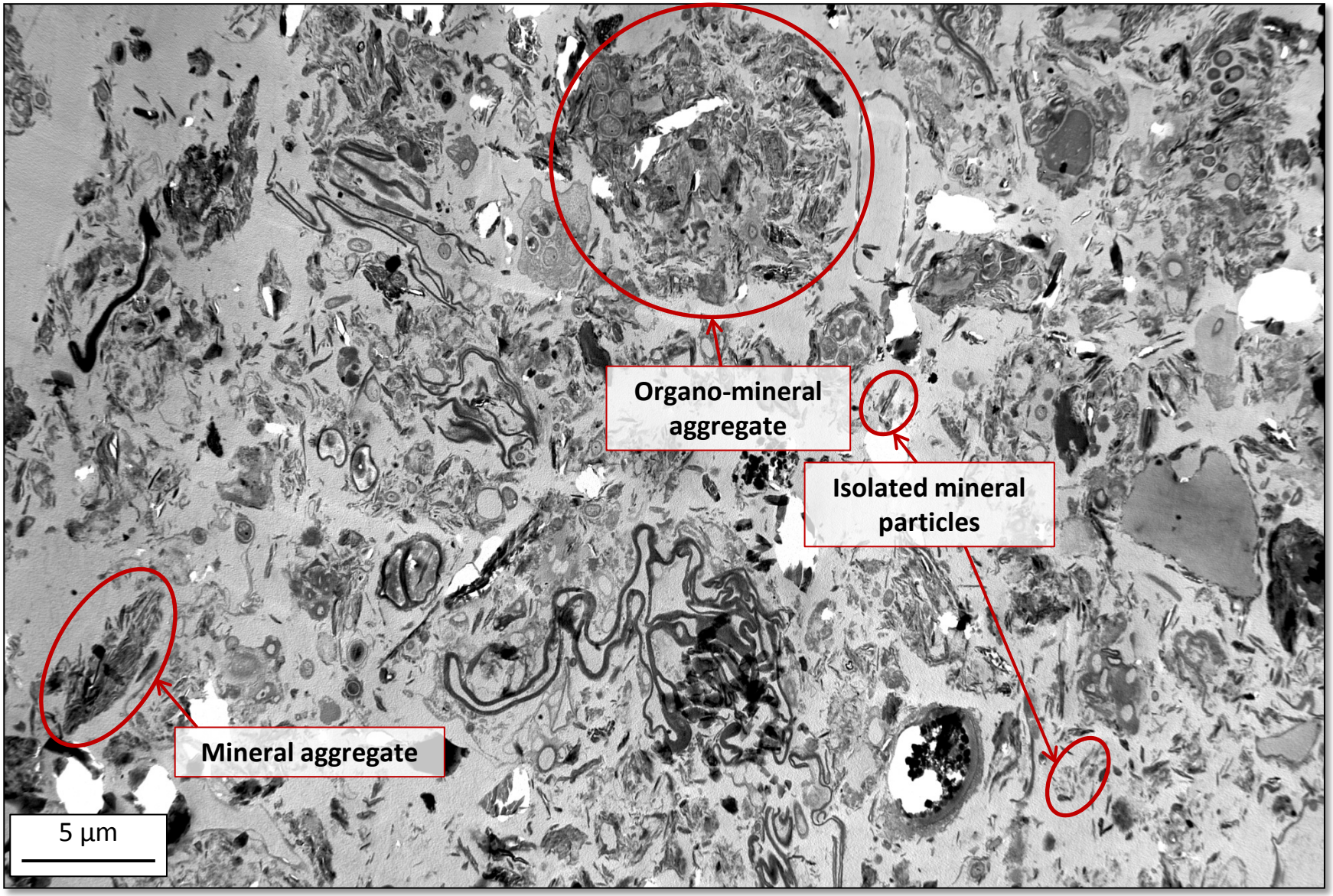
Mean $\delta^{13}C$: **1004 ‰**
→ Cell walls : 808 ‰
→ Chloroplasts : 1041‰



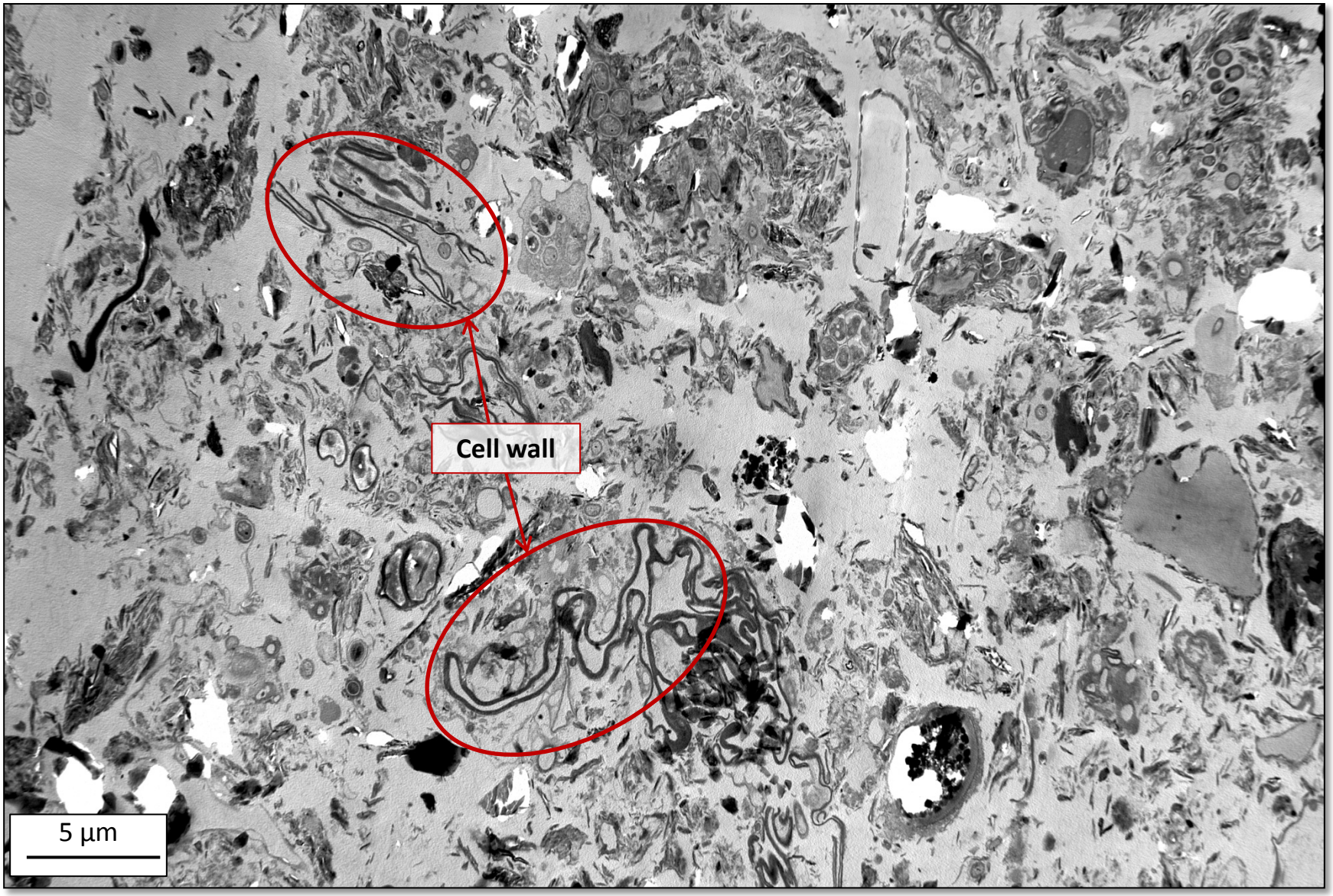


Earthworm cast characterization

Organic and mineral compounds isolated or forming aggregates

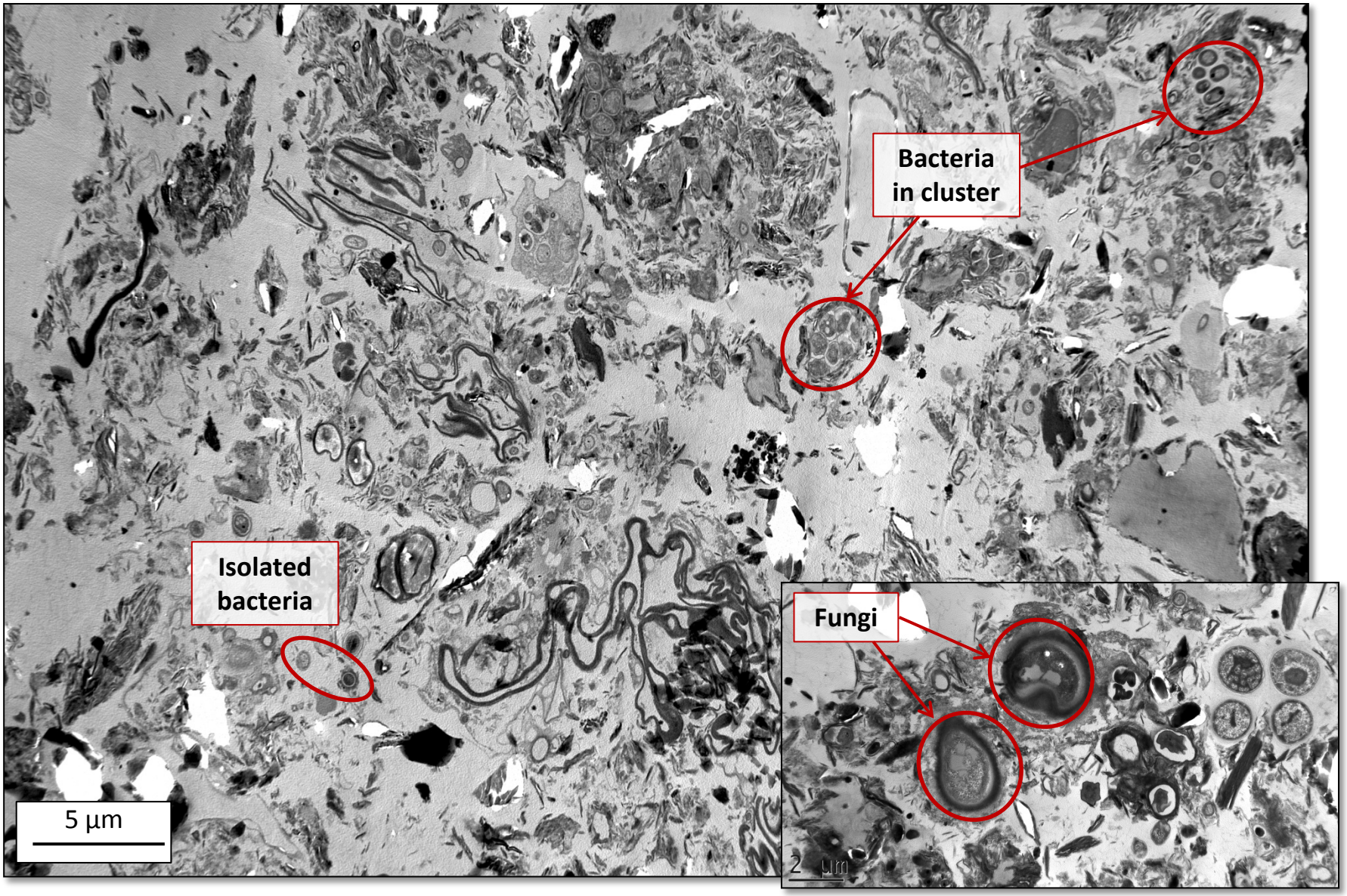


Earthworm cast characterization

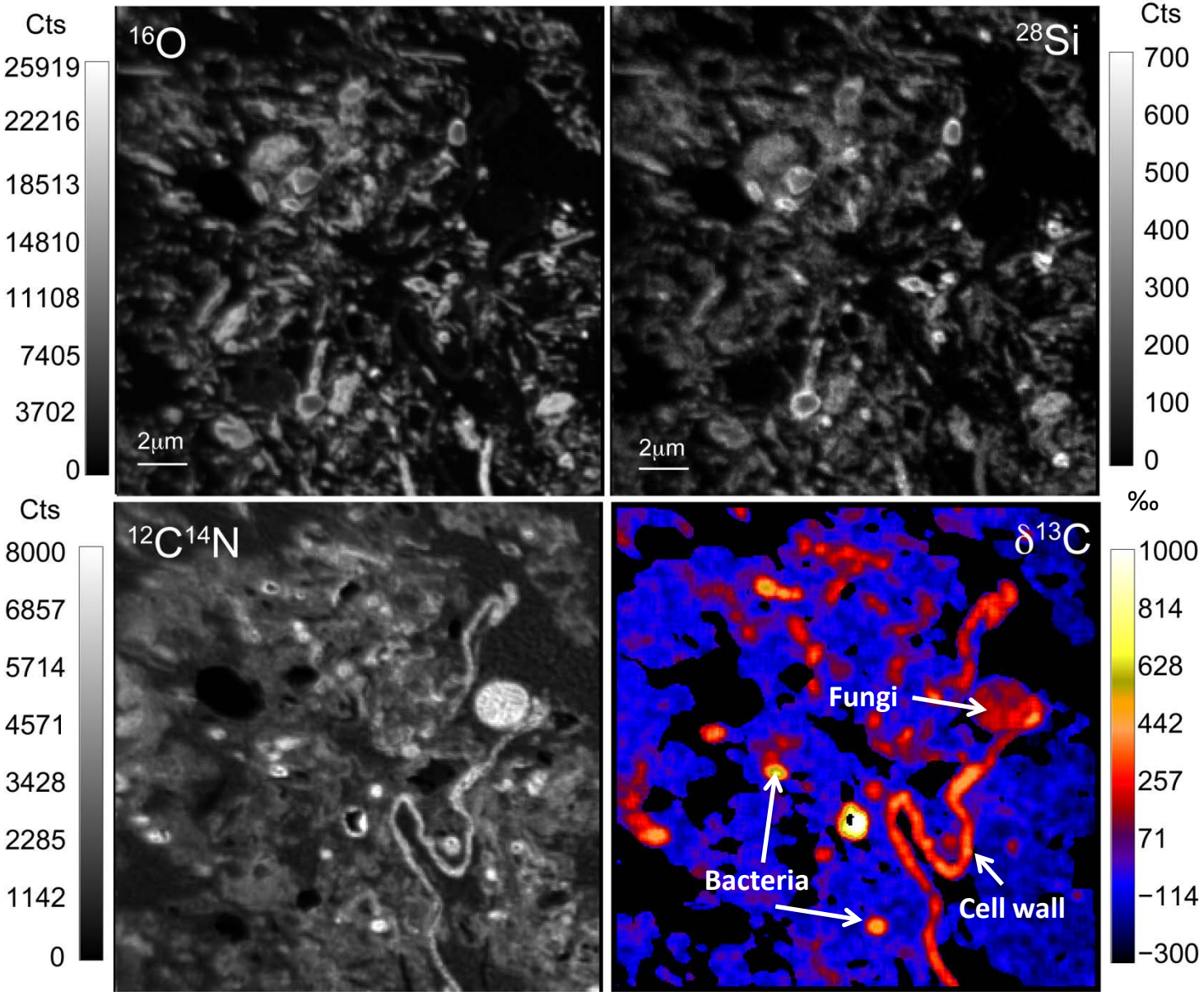


Earthworm cast characterization

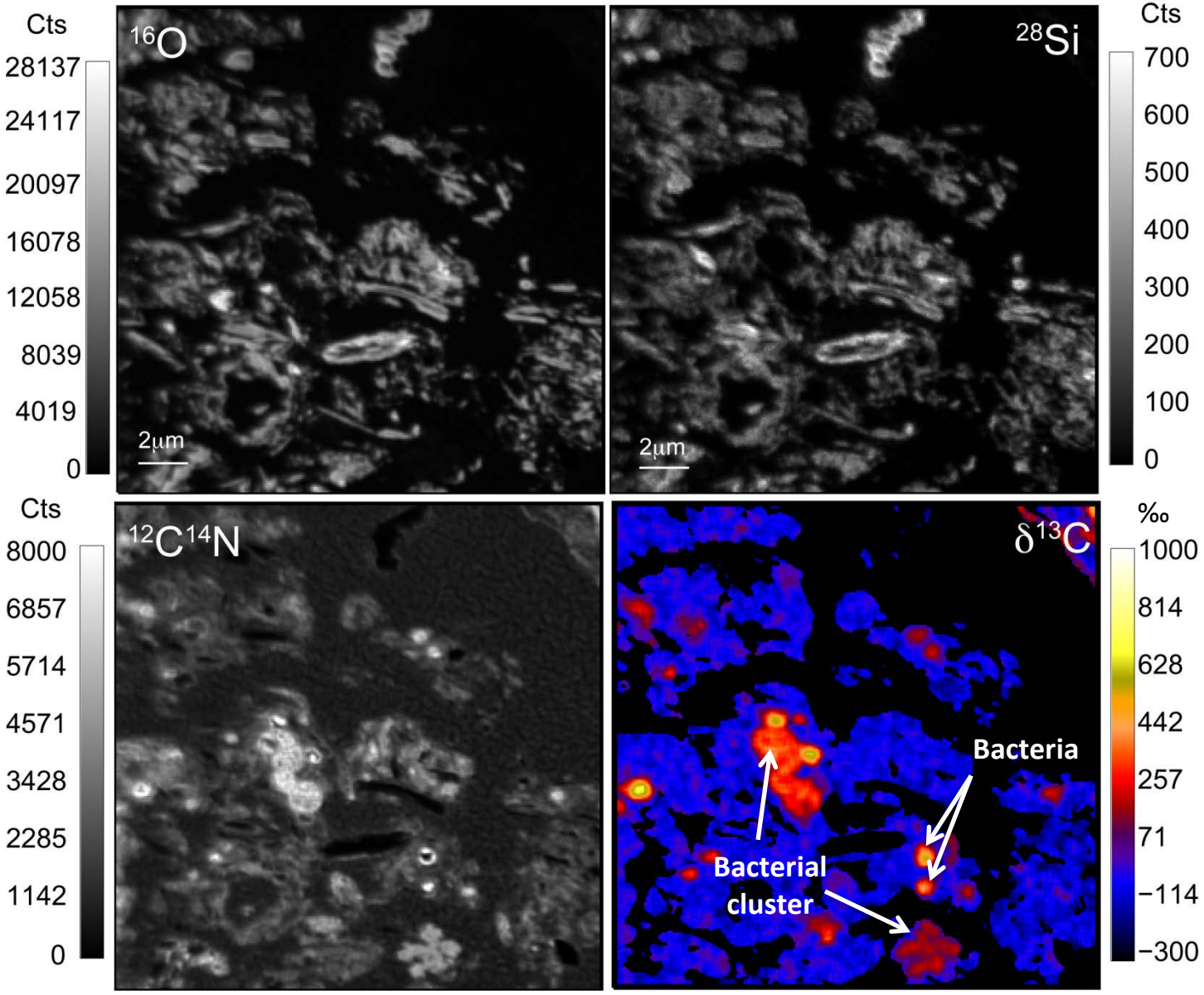
Microorganisms



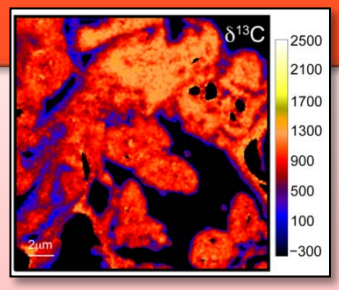
Earthworm cast characterization



Earthworm cast characterization



Incorporation and **decomposition**



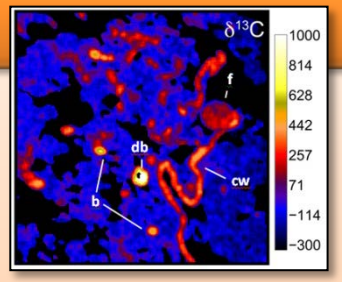
Initial litter : **1004 ‰**

Non-structural compounds

Structural compounds

^{13}C

^{13}C



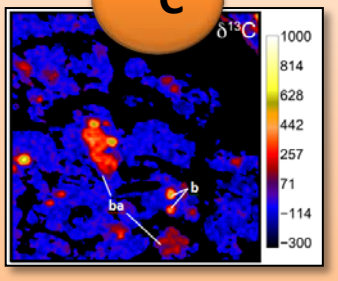
Labelled plant residues in casts : **279 ‰**

Decomposition

- ↓ **δ^{13}C** of **plant residues** in casts
- Labelling of **microorganisms**

Assimilation + Respiration

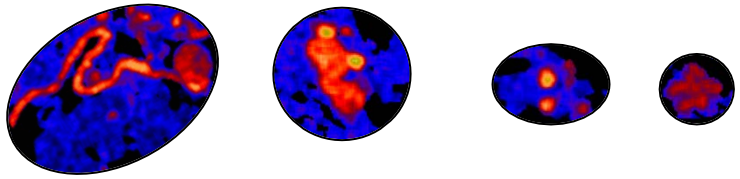
^{13}C

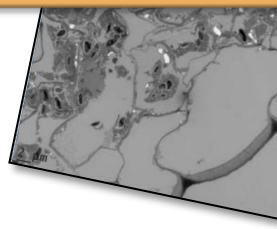


Microorganisms **: 236 ‰**

^{13}C

Structural compounds



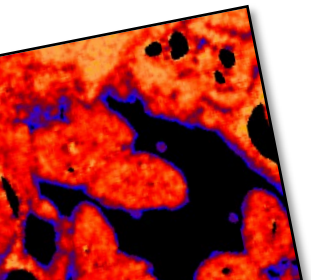


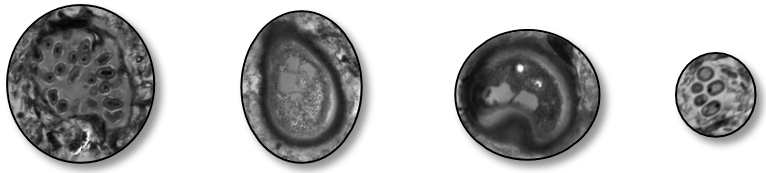
Investigate the **incorporation** and **decomposition** of **labelled litter** in earthworm **casts**

NanoSIMS reveals the incorporation in casts of plant litter deposited on the soil surface

NanoSIMS and TEM images : **organic components identified** within the casts

- **Decomposed plant residues** in the casts
- Abundant and diverse **microorganisms** highly labelled : actively participate in **litter decomposition**








ajes

metis
UMR 7619

UPMC
SORBONNE UNIVERSITÉS

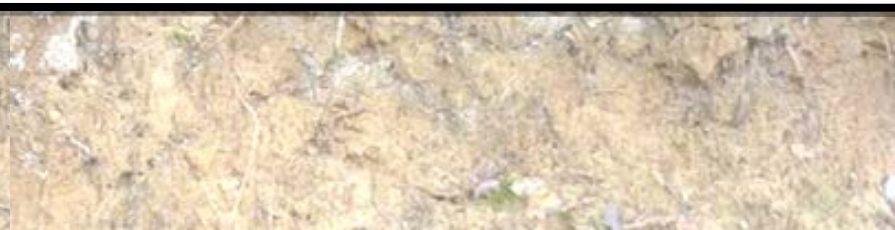
Ecosphère
Continentale et
Côtière

cnrs

UNIVERSITÉ
DE LORRAINE

MUSÉUM
NATIONAL
D'HISTOIRE
NATURELLE

Thank you

TEM

Initial litter : Chemically fixation - osmium tetroxide
Casts : Chemically fixation - osmium tetroxide
Physically preservation - agar

↓

Dehydrated in graded acetone series

↓

Embedded in epoxy resin (Epon 812)

↓

Ultrathin sections of 80-100 nm
(Leica Ultracut S ultramicrotome)

↓

Stained with uranyl acetate and lead citrate

NanoSIMS

Ultrathin sections of **200 nm**

↓

Coated with 10 nm of **gold**

↓

Sample surface sputtered by a 1.5 pA **Cs^+ beam**

↓

20×20 µm² images divided into **256×256 pixels**

↓

Secondary images of **$^{12}C^-$**, **$^{12}C^{14}N^-$**, **$^{13}C^{14}N^-$**, **$^{16}O^-$** and **$^{28}Si^-$**

↓

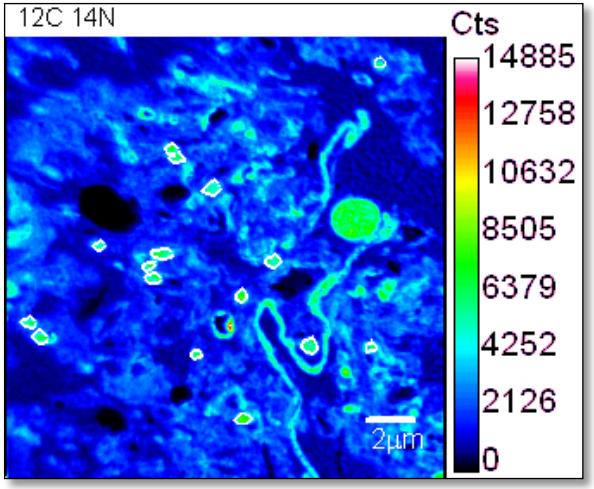
L'IMAGE® software

↓

$^{13}C^{14}N^-/^{12}C^{14}N^-$ ratio used to generate ^{13}C isotopic maps (/PDB standard)

↓

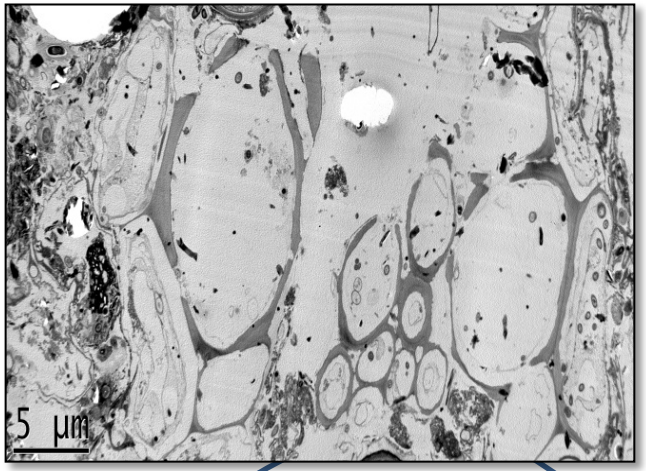
Regions of interest (ROI): manually drawn

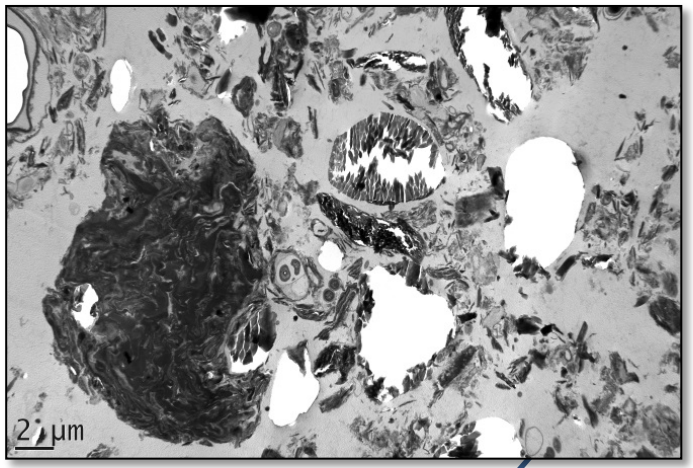


Kerogen standard to check for instrumental stability
Instrumental fractionation < 5‰ neglected for the $\delta^{13}C$ correction
Resin masked on the $\delta^{13}C$ maps ($^{12}C^{14}N$ pixel distribution)

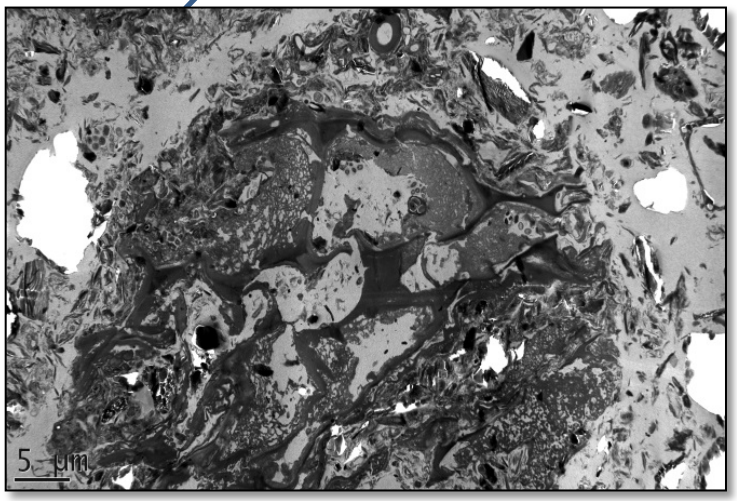
NanoSIMS 50 (Cameca, France) at Museum National d'Histoire Naturelle in Paris

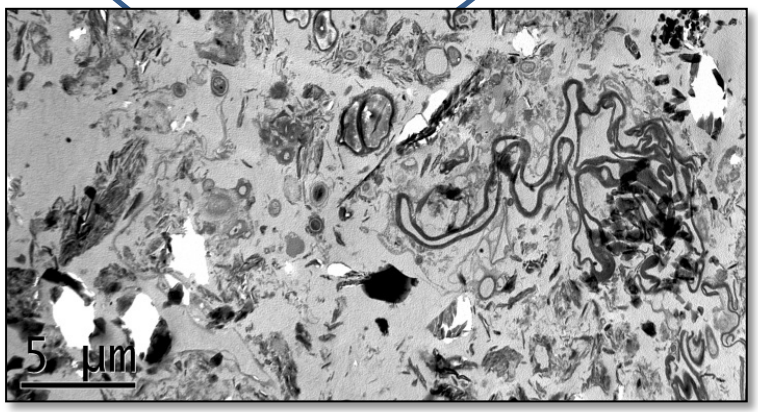
Earthworm casts characterization



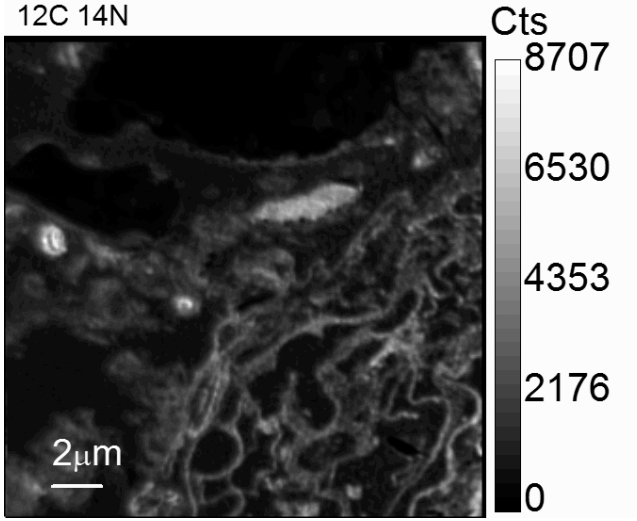


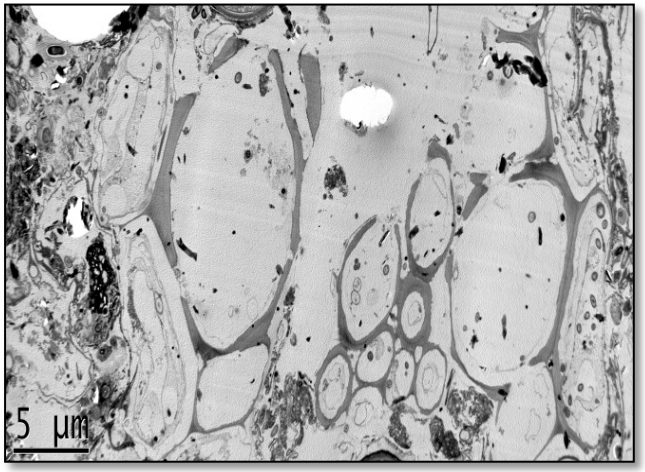
Various stages of plant residues degradation

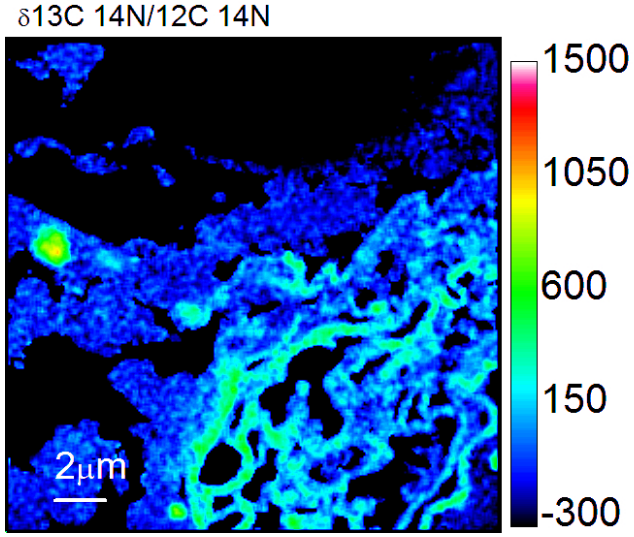


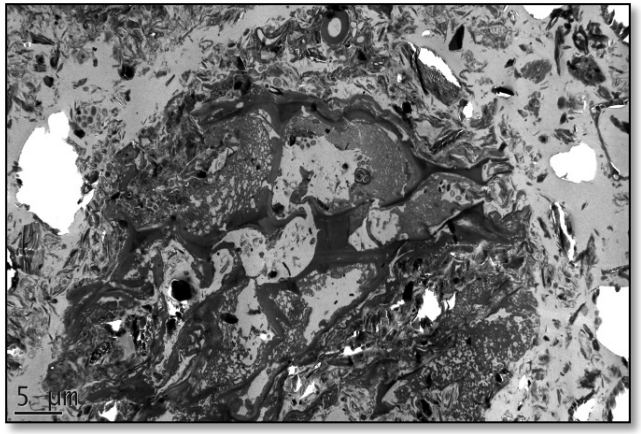


Earthworm casts characterization









Earthworm casts characterization

Images	Total	Plant residues		Bacteria		Fungi
Units	‰	‰	*(min ; max)*	‰	*(min ; max)*	‰
1	72	250 (n=4)	*(22 ; 401)*	186 (n=13)	*(14 ; 497)*	NA
2	104	253 (n=12)	*(20 ; 509)*	395 (n=4)	*(122 ; 597)*	NA
3	-5	129 (n=1)	-	408 (n=7)	*(109 ; 943)*	NA
4	47	176 (n=6)	*(45 ; 326)*	483 (n=2)	*(258 ; 707)*	NA
5	10	279 (n=3)	*(201 ; 358)*	304 (n=17)	*(109 ; 723)*	191 (n=1)
6	3	158 (n=4)	*(31 ; 270)*	236 (n=12)	*(144 ; 489)*	NA
7	148	421 (n=8)	*(281 ; 633)*	326 (n=11)	*(115 ; 665)*	NA