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)

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

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

- **Organic** (plant residues and microorganisms)
- **Mineral** (soil particles)

**Context**

**Material - Methods**

**Results - Discussion**

**Conclusion**
What methods to study casts at the microscale?

- **Transmission Electron Microscopy (TEM)**
  - **Identify microstructures**
  - **nm-scale resolution**

- **Nano-scale secondary ion mass spectrometry (NanoSIMS)**
  - **Elemental** (carbon) and isotopic maps ($\delta^{13}C$) of organic compounds

**Origin** of organic matter?

**Nature** of organic matter?

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

Context | Material - Methods | Results - Discussion | Conclusion
250 g of artificially labelled in $^{13}$C Italian Ryegrass shoots (*Lolium multiflorum*)

~75 L of a loamy-sand soil

Earthworms *Lumbricus terrestris*

Sample of initial litter

Samples for TEM and NanoSIMS analyses

Composite sample of surface casts
Plant tissues

Mean $\delta^{13}C: 1004\ \%$

$\rightarrow$ Cell walls: 808\ \%

$\rightarrow$ Chloroplasts: 1041\ \%
Earthworm cast characterization

Organic and mineral compounds isolated or forming aggregates
Earthworm cast characterization

Plant residues

Cell wall

5 µm
Earthworm cast characterization

Microorganisms

- Bacteria in cluster
- Isolated bacteria
- Fungi

Material - Methods

Results - Discussion

Context

Conclusion
Earthworm cast characterization

- **Material - Methods**
- **Context**
- **Results - Discussion**
- **Conclusion**
Earthworm cast characterization

- Bacterial cluster
- Bacteria

Results - Discussion
Initial litter: 1004 ‰

- Non-structural compounds
- Structural compounds

Labelled plant residues in casts: 279 ‰

Assimilation + Respiration

Microorganisms: 236 ‰

Decomposition:
- \( \delta^{13}C \) of plant residues in casts
- Labelling of microorganisms
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.
Thank you
**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

**TEM JEOL EXII (80kV)**
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 $^{12}\text{C}$, $^{12}\text{C}^{14}\text{N}$, $^{13}\text{C}^{14}\text{N}$, $^{16}\text{O}$ and $^{28}\text{Si}$

L’IMAGE® software

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

Regions of interest (ROI): manually drawn

Kerogen standard to check for instrumental stability

Instrumental fractionation < 5‰ neglected for the $\delta^{13}\text{C}$ correction

Resin masked on the $\delta^{13}\text{C}$ maps ($^{12}\text{C}^{14}\text{N}$ pixel distribution)

NanoSIMS 50 (Cameca, France) at Museum National d’Histoire Naturelle in Paris
Various stages of plant residues degradation
Earthworm casts characterization

12C 14N

$\delta^{13}C_{14N}/12C_{14N}$

Results - Discussion
### Earthworm casts characterization

<table>
<thead>
<tr>
<th>Images</th>
<th>Total</th>
<th>Plant residues</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Units</strong></td>
<td>%</td>
<td>%</td>
<td>(min ; max)</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>72</td>
<td>250 (n=4)</td>
<td>186 (n=13)</td>
<td>(14 ; 497)</td>
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<tr>
<td>2</td>
<td>104</td>
<td>253 (n=12)</td>
<td>395 (n=4)</td>
<td>(122 ; 597)</td>
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<tr>
<td>3</td>
<td>-5</td>
<td>129 (n=1)</td>
<td>408 (n=7)</td>
<td>(109 ; 943)</td>
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<tr>
<td>4</td>
<td>47</td>
<td>176 (n=6)</td>
<td>483 (n=2)</td>
<td>(258 ; 707)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>279 (n=3)</td>
<td>304 (n=17)</td>
<td>(109 ; 723)</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>158 (n=4)</td>
<td>236 (n=12)</td>
<td>(144 ; 489)</td>
</tr>
<tr>
<td>7</td>
<td>148</td>
<td>421 (n=8)</td>
<td>326 (n=11)</td>
<td>(115 ; 665)</td>
</tr>
</tbody>
</table>