Incorporation of ^{13}C labelled shoot residues in *Lumbricus terrestris* casts: A combination of Transmission Electron Microscopy and Nanoscale Secondary Ion Mass Spectrometry

Alix Vidal [1], Laurent Remusat [2], Françoise Watteau [3], Sylvie Derenne [1], Katell Quenea [1]

[1]UMR METIS UPMC/CNRS, Paris, France
[2]UMR LMCM Muséum national d'Histoire naturelle/CNRS, Paris, France
[3]LSE ENSAIA/Université de Lorraine/CNRS/INRA, Vandoeuvre- lès-Nancy, France
Contact: alix.vidal@upmc.fr

Résumé:

Earthworms play a central role in litter decomposition, soil structuration and carbon cycling. They ingest both organic and mineral compounds which are mixed, complexed with mucus and dejected in form of casts at the soil surface and along burrows. Bulk isotopic or biochemical technics have often been used to study the incorporation of litter in soil and casts, but they could not reflect its complexity at the microscale. Nano-scale secondary ion mass (NanoSIMS), which is a high spatial resolution method providing elemental and isotopic maps of organic and mineral materials, has recently been applied in soil science (Vogel et al., 2014). It appeared a promising tool combined with other microscopic techniques . Transmission Electron Microscopy (TEM) has proven its efficiency to investigate organo-mineral associations in soil and earthworm casts (Pey et al., 2014). For the first time in this field, we combined NanoSIMS and TEM to investigate the dynamics of incorporation and decomposition of labelled litter in earthworm casts.

This study aimed to (**1**) determine the nature of the organic matter incorporated by earthworms in casts and its association with mineral particles and (**2**) identify the organic constituents coming from labelled residues. A one year mesocosm experiment was set up to study the incorporation of ^{13}C labelled Ryegrass (*Lolium multiflorum)* litter in a soil in presence of anecic earthworms (*Lumbricus terrestris*). After six months of incubation, surface casts were sampled, embedded in epoxy resin and cut into ultra-thin sections. Samples were analyzed with a JEOL EMXII TEM and a Cameca NanoSIMS 50, obtaining secondary ion images of ^{12}C, ^{16}O, $^{12}C^{14}N$, $^{13}C^{14}N$ and ^{28}Si. The $\delta^{13}C$ maps were obtained using the $^{13}C^{14}N^-/^{12}C^{14}N^-$ ratio.

NanoSIMS images highlighted the labelled organic matter incorporated in casts. Using TEM images, the organic material was recognized and characterized with high degree of confidence. Thus, the combination of these two methods participated to clarify the complex microscale organization in earthworm casts. The various stages of decomposition of plant residues suggest that earthworms incorporated fresh plant residues both by ingesting and ploughing them inside casts. Casts are populated with abundant and diverse microorganisms which participate actively to litter decomposition. The $\delta^{13}C$ values of plant residues and microorganisms are highly variable (from 14 to 943 ‰), underlying the complexity of organic matter dynamics and the importance of microscale analyses to identify and explain this variability. Ongoing investigations are performed to address the effect of earthworms on litter incorporation in soil.

Pey, B., Cortet, J., Capowiez, Y., Nahmani, J., Watteau, F., Schwartz, C., 2014. Technosol composition affects Lumbricus terrestris surface cast composition and production. Ecological Engineering. 67, 238–247.
Poch, R., Virto, I., 2014. Micromorphology Techniques for Soil Organic Carbon Studies, in: Hartemink, A.E., McSweeney, K. (Eds.), Soil Carbon, Progress in Soil Science. Springer International Publishing, 17–26.
Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schloter, M., Kögel-Knabner, I., 2014. Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. Nature Communications 5.