



Spatial and temporal distribution of ^{13}C labelled plant residues in soil aggregates and *Lumbricus terrestris* surface casts: A combination of Transmission Electron Microscopy and Nanoscale Secondary Ion Mass Spectrometry

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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 techniques have often been used to study the incorporation of litter in soil and casts, but they could not reflect the complex interaction between soil, plant and microorganisms at the microscale. However, the heterogeneous distribution of organic carbon in soil structures induces contrasted microbial activity areas. Nano-scale secondary ion mass spectrometry (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 (Herrmann et al., 2007; Vogel et al., 2014). The combination of Nano-scale secondary ion mass spectrometry (NanoSIMS) and Transmission Electron Microscopy (TEM) has proven its potential to investigate labelled residues incorporation in earthworm casts (Vidal et al., 2016). In line of this work, we studied the spatial and temporal distribution of plant residues in soil aggregates and earthworm surface casts. This study aimed to (1) identify the decomposition states of labelled plant residues incorporated at different time steps, in casts and soil, (2) identify the microorganisms implied in this decomposition (3) relate the organic matter states of decomposition with their ^{13}C signature. A one year mesocosm experiment was set up to follow the incorporation of ^{13}C labelled Ryegrass (*Lolium multiflorum*) litter in a soil in the presence of anecic earthworms (*Lumbricus terrestris*). Soil and surface cast samples were collected after 8 and 54 weeks, embedded in epoxy resin and cut into ultra-thin sections. Soil was fractionated and all and analyzed with TEM and NanoSIMS, obtaining secondary ion images of ^{12}C , ^{16}O , $^{12}\text{C}^{14}\text{N}$, $^{13}\text{C}^{14}\text{N}$ and ^{28}Si . The $\delta^{13}\text{C}$ maps were obtained using the $^{13}\text{C}^{14}\text{N}/^{12}\text{C}^{14}\text{N}$ -ratio. We identified various states of decomposition within a same sample, associated with a high heterogeneity of $\delta^{13}\text{C}$ values of plant residues. We also recognized various labelled microorganisms, mainly bacteria and fungi, underlining their participation in residues decomposition. $\delta^{13}\text{C}$ values were higher in casts than soil aggregates and decreased between 8 and 54 weeks for both samples.

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