



## **Initial association of fresh microbial products to soil particles: a joint density fractionation and NanoSIMS study**

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While soil microorganisms are increasingly seen as shaping stable soil organic matter (OM) formation, the mechanisms controlling the attachment of microbial metabolites to soil particles are not fully understood yet. We investigate the spatial distribution of freshly produced microbial products among density-isolated fractions of soil using stable C and N isotopes and Nano-scale secondary ion mass spectrometry (NanoSIMS). A surface forest soil was amended with uniformly  $^{13}\text{C}/^{15}\text{N}$  labeled glycine and incubated for 8 hours in gamma-irradiated and non-sterile soils. Sequential density fractionation was then performed to isolate various classes of aggregates and of single mineral particles. Eight hours after the labeled glycine addition, 7 % of the  $^{13}\text{C}$  and  $^{15}\text{N}$  was tightly bound to soil assemblages. Comparison of sterile and non-sterile treatments revealed that microbial activity was almost completely responsible for this rapid association (>85 %). The distributions of glycine-derived  $^{13}\text{C}$  and  $^{15}\text{N}$ , considered as markers of new microbial products, were mapped on particles of the non-sterile treatment using NanoSIMS.

New microbial products were heterogeneously distributed and spatially decoupled at the surface of on soil particles.  $^{13}\text{C}$  microbial products were scarce and presumably within or in the vicinity of microbial cells. In contrast,  $^{15}\text{N}$  microbial products seemed evenly spread at the surface of soil particles, likely as soluble exoenzymes diffusing away from their parent cell. Macroscopic measurements among density fractions suggested that the diffusion of such  $^{15}\text{N}$  microbial products was spatially limited yet, because of pore space architecture. NanoSIMS images further allowed gaining insight into the attachment of the new microbial products on particle surfaces already covered by OM, in a multilayer fashion. Using an internal calibration method to determine C/N ratios of NanoSIMS images, we showed the preferential attachment of soluble microbial N-metabolites to N-rich mineral-attached OM (C/N ratios mostly < 16). Exceptions were found in dense particles, supposed to contained aluminium and iron (hydr)oxides, with the microbial N-metabolites apparently preferentially attached to C-rich mineral-attached OM (C/N ratios > 80).

This work provided visual evidences that the attachment of new microbial products to the soil matrix is mediated by distinct processes for N-rich and C-rich metabolites. It also demonstrated that the pore space architecture has impact on the formation of stable OM by limiting the diffusion of soluble microbial metabolites and their access to reactive and stabilising surfaces.