



Assessing microbial utilization of free versus sorbed Alanine by using position-specific ^{13}C labeling and ^{13}C -PLFA analysis

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Microbial utilization is a key transformation process of soil organic matter (SOM). Sorption of low molecular weight organic substances (LMWOS) to soil mineral surfaces blocks or delays microbial uptake and therefore mineralization of LMWOS to CO_2 , as well as all other biochemical transformations. We used position-specific labeling, a tool of isotope applications novel to soil science, combined with ^{13}C -phospholipid fatty acid (PLFA) analysis, to assess microbial utilization of sorbed and non-sorbed Alanine in soil. Alanine has various functional groups enabling different sorption mechanisms via its positive charge (e.g. to clay minerals by cation exchange), as well as via its negative charge (e.g. to iron oxides by ligand exchange).

To assess changes in the transformation pathways caused by sorption, we added uniformly and position-specifically ^{13}C and ^{14}C labeled Alanine to the Ap of a loamy Luvisol in a short-term (10 days) incubation experiment. To allow for sorption of the tracer solution to an aliquot of this soil, microbial activity was minimized in this subsample by sterilizing the soil by γ -radiation. After shaking, the remaining solutions were filtered and the non-sorbed Alanine was removed with Millipore water and then added to non-sterilized soil. For the free Alanine treatment, solutions with Alanine of similar amount and isotopic composition were prepared, added to the soil and incubated as well. The respired CO_2 was trapped in NaOH and its ^{14}C -activity was determined at increasing times intervals. Microbial utilization of Alanine's individual C positions was evaluated in distinct microbial groups classified by ^{13}C -PLFA analysis.

Sorption to soil minerals delayed respiration to CO_2 and reduced initial respiration rate by 80%. Irrespective of sorption, the highest amount was respired from the carboxylic position (C-1), whereas the amino-bound (C-2) and the methylic position (C-3) were preferentially incorporated into PLFA of microorganisms due to the basic microbial metabolism of C_3 molecules in glycolysis. Reconstruction of microbial transformation pathways showed that the C-2 position of Alanine was lost as CO_2 faster than its C-3 position regardless of whether the molecule was used ana- or catabolically. The highest incorporations of all positions in PLFA were accomplished by Gram negatives. Free Alanine was preferentially used by highly competitive prokaryotes, while sorbed Alanine was preferred by filamentous microorganisms. In detail, the free living osmotrophic Gram negative bacteria utilize more easily accessible dissolved substances. The utilization of sorbed substances are achieved by less mobile microorganisms, e.g. eukaryotic fungi and Actinomycetes, which form biofilms.

None of these findings could have been achieved without the position-specific labeling approach, therefore this method will strongly improve our understanding of stabilization processes and soil C fluxes.