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Microbial transformations of free versus sorbed alanine analyzed by position-specific 13C and 14C labeling and 13C-PLFA analysis

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Sorption of charged or partially charged low molecular weight organic substances (LMWOS) to soil mineral surfaces delays microbial uptake and therefore mineralization of LMWOS to CO₂, as well as all other biochemical transformations. We used position-specific labeling, a tool of isotope applications novel to soil sciences, to compare the transformation mechanisms of sorbed and non-sorbed alanine in soil. Alanine as an amino acid links C- and N-cycles in soil and therefore is a model representative for the pool of LMWOS.

To assess transformations of sorbed alanine, we combined position-specifically and uniformly 13C and 14C labeled alanine tracer solution with a loamy haplic luvisol that had previously been sterilized by γ -radiation. After shaking the mixtures, the supernatant was removed, as was all non-sorbed alanine by repeated shaking with millipore water. The labeled soil was added to non-sterilized soil from the same site. To compare the effect of sorption, soil labeled with the same position-specifically labeled tracers without previous sorption was prepared and incubated as well. We captured the respired CO_2 and determined its 14C-activity at increasing time steps. The incorporation of 14C into microbial biomass was determined by CFE, and utilization of individual C positions by distinct microbial groups was evaluated by 13C-PLFA analysis.

A dual peak in the respired CO₂ revealed the influence of two sorption mechanisms. Microbial uptake and transformation of the sorbed alanine was 3 times slower compared to non-sorbed alanine. To compare the fate of individual C atoms independent of their concentration and pool size in soil, we introduced the divergence index (DI). The DI reveals the convergent or divergent behaviour of C from individual molecule positions during microbial utilization. The DI revealed, that alanines C-1 position was mainly oxidized to CO₂, while its C-2 and C-3 were preferentially incorporated in microbial biomass and PLFAs. This indicates that sorption by the COOH group does not protect this group from preferential oxidation. Microbial metabolism is determinative for the preferential oxidation of individual molecule positions. Uptake into the PLFA of gram negatives was highest for both the sorbed and non-sorbed treatment, showing that this group is not only highly competitive regarding the uptake of free LMWOS (like root exsudates) but are also able to desorb and utilize sorbed LMWOS.

The use of position-specific labeling revealed mechanisms and kinetics of microbial utilization of sorbed and non-sorbed alanine, as well as interactions between microbial groups, soils and LMWOS. None of these findings could have been achieved without the use of position-specific tracers, therefore this method will improve our understanding of stabilization processes and soil C fluxes.