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Sorption of Alanine changes microbial metabolism in addition to availability

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ABSTRACT

Sorption is one of the main processes stabilizing organic matter in soil against microbial mineralization. We hypothesize that besides reduced accessibility for microorganisms and enzymes, changes in microbial metabolism additionally intensify this organic matter stabilization effect of sorption.

Position-specifically ^{14}C labeled Alanine was applied to soil as solution or sorbed on sterilized soil to investigate the mechanisms underlying this metabolism related stabilization effect. Sorption decreased initial mineralization of Alanine by ~80% and doubled the duration until the mineralization maxima ($^{14}CO_2$ peak). Almost all Alanine was taken up by microorganisms independent on sorption, and *C*-1 was completely (>99%) decarboxylated during glycolysis after one day. Sorption could not prevent microbial utilization of Alanine, but increased the carbon use efficiency (CUE) of sorbed Alanine for 60% compared to Alanine in solution and increased C incorporation in microbial biomass up to four times. The position-specific pattern of ^{14}C in soil and in microbial biomass showed that oxidation of C-2 from sorbed Alanine was strongly lowered compared to free Alanine. Both higher CUE and delayed C-2 mineralization were achieved by a higher C flux towards efficient anabolism, or/and to slower cycling cell components.

Limitation of accessibility for microorganisms alone does not explain the stabilizing effect of sorption on organic substances like amino acids and the observed changed position specific pattern. Even though all sorbed Alanine was taken up by microorganisms within 3 days, C partitioning towards anabolism, slower microbial turnover and increased CUE increased C retention from sorbed compounds in soil even after microbial uptake. Position-specific labeling clearly showed that LMWOS are stabilized by sorption not as intact molecules, but after microbial metabolization – as released metabolites or microbial biomass. We conclude that the indirect effects of sorption, namely 1) more C partitioned to anabolism, 2) slower decomposition, 3) higher incorporation into microbial biomass and 4) increased carbon use efficiency promote C retention in soil and may be even more important than the direct effect, namely inaccessibility. The finding that stabilization did not significantly impede microbial utilization, but sorption greatly increased carbon use efficiency has major implications for conceptual and numerical representation of organic matter stabilization and losses in soils.

1. Introduction

Sorption of organic molecules to soil particles is a key mechanism of soil organic matter (SOM) stabilization (Guggenberger and Kaiser, 2003): Although those mineral-organic associations only consist to 0.2–20% of carbon, 70–100% of SOM is stabilized within those complexes (Christensen, 2001; Lützow et al., 2006; Schmidt et al., 2011; Sollins et al., 2007, Sollins et al., 1996) and turnover of mineral-associated C is on average four times slower than that of non-associated OM (Baisden et al., 2002; Kögel-Knabner et al., 2008). The mechanisms behind the stabilization effects of sorption, however, are not yet fully understood (Kleber et al., 2015). The majority of studies analyzing the effects of sorption on SOM retention were carried out in simplified systems, either: a) in suspension, b) with the addition of pure minerals or c) by inoculating with individual bacterial strains or a combination thereof (Barré et al., 2014). Even with the application of simplified systems, results on SOM retention varied from no effect on biodegradation to a total stop of biodegradation (Barré et al., 2014). It is therefore necessary to measure not only the effect of sorption on SOM mineralization, but also to identify the mechanisms behind this effect. The most common explanation is that sorption of SOM decreases availability to microorganisms and enzymes (Vieublé Gonod et al., 2006), which are the most important drivers of C dynamics in soil (Kögel-Knabner, 2002). Indeed, sorption strength negatively correlates with microbial

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metabolization of amino acids (Jones and Hodge, 1999), but this effect varies strongly with soil type, minerology and experimental approach (Barré et al., 2014). On the other hand, microorganisms facilitate sorption. To interact with mineral surfaces, OM needs to be water-soluble and therefore requires chargeable functional groups (Kleber et al., 2015). Plant-derived primary OM from litter or root exudates often does not possess these traits before being microbially metabolized to low molecular weight organic substances (Oades, 1989). The application of position-specifically labeled Alanine also showed that stronger sorption to mineral particles not only decreased microbial C uptake, but also shifted metabolic pathways towards a higher flux through the anabolism (Dippold et al., 2014). If substrates are used to a higher portion in anabolic pathways, the carbon use efficiency (CUE) increases (Dijkstra et al., 2011; del Giorgio and Cole, 1998), while CO_2 production decreases. To understand how sorption stabilizes OM, it is therefore necessary not only to quantify changes of C-fluxes from SOM to CO_2 or microbial biomass, but also to identify changes in microbial metabolism induced by sorption. To achieve this, free and sorbed tracers that are position-specifically ^{14}C labeled need to be applied. In contrast to uniformly labeled tracers, position-specific labeling allows the reconstruction of metabolic pathways. The allocation of C from individual molecule positions towards CO_2, i.e. mineralization, can be compared with the known network of metabolic pathways and the fate of individual molecule positions therein. From such a comparison, metabolic pathways can be identified based on the position-specific fingerprint in individual C pools like CO_2.

Such experiments require position-specific metabolic tracers, which enter the basic metabolism of glycolysis, pentose-phosphate pathway and citric acid cycle at a central, branching point. Pyruvate is one of these tracers entering the metabolism at the interface from glycolysis and pentose-phosphate pathway to citric acid cycle (Caspi et al., 2014). Its aminated form – Alanine – can easily be transferred by transamination to pyruvate and thus is an equivalent metabolic tracer. Furthermore, Alanine, is a neutrally charged amino acid, with a negatively chargeable carboxylic position C-1 (—COOH), a positively chargeable amino-bound position C-2 (—$CHNH_2$) and a methylic position C-3 (—CH_3). Therefore, Alanine can be sorbed to soil particles by 1) cation exchange, 2) anion exchange or 3) ligand exchange. Alanine is also the most abundant amino acid in dissolved organic matter (Fischer et al., 2007). In previous studies using position-specifically labeled Alanine, the microbial metabolization in soil could successfully be reconstructed (Apostel et al., 2013). The effect of sorption to various pure minerals in aquatic suspension on the Alanine metabolization could also be assessed by position-specifically labeled tracers (Dippold et al., 2014). Therefore, position-specifically labeled Alanine is a suitable tracer to disentangle effects and mechanisms of sorption on organic C stabilization at mineral surfaces in soil and validate existing studies on pure minerals.

We hypothesize that sorption affects microbial utilization and stabilization of Alanine C in two ways. The direct effect: Uptake of Alanine sorbed to mineral particles by microorganisms will be slower and decreased compared to free Alanine, due to stabilization by mineral surfaces and lower accessibility. The indirect effect: sorbed Alanine will be microbially metabolized to a larger extent by anabolic pathways because slow desorption and slow continuous uptake by microorganisms lead to more efficient C use compared to fast C utilization of Alanine from solution.

2. Materials and methods

2.1. Experimental design

The experiment consisted of two treatments – sorbed Alanine and free Alanine – in which tracers were added to soil from the same site (description see section 2.2). To produce soil with sorbed tracer, solutions of Alanine labeled with ^{14}C on each of the three positions (C-1 = —COOH, C-2 = —$CHNH_2$, C-3 = —CH_3) were added to soil sterilized by γ-radiation. The soil with sorbed Alanine was added to non-sterilized soil at the beginning of the incubation. In the free Alanine treatment, position-specifically labeled Alanine solutions were applied (Table 1). During the 10 days of incubation, incorporation of ^{14}C from Alanine into CO_2, extractable organic carbon, microbial biomass and soil was analyzed. This enabled to compare the dynamics of Alanine in solution and sorbed Alanine during the 10 days of the experiment and thus, to test hypothesis 1. As position-specifically labeled tracers were applied, the metabolic pathways utilized after the uptake could also be reconstructed, allowing to test hypothesis 2.

Table 1
Activities of the added free and sorbed tracers.

	Free			Sorbed		
	Ala-1	Ala-2	Ala-3	Ala-1	Ala-2	Ala-3
^{14}C-addition ($Bq \cdot g\ soil^{-1}$)	500	500	500	550 ± 0.4	450 ± 0.3	485 ± 0.4

All radiochemicals: American Radiolabeled Chemicals Inc., St. Louis, USA.

2.2. Preparation of sorbed tracer soil

The soil used throughout this experiment was taken from the Ap horizon of an agriculturally used loamy Luvisol (pH_{KCl} 4.88, pH_{H2O} 6.49, TOC 17.7 $g \cdot kg^{-1}$,TN of 1.9 $g \cdot kg^{-1}$, CEC 13 $cmolc \cdot kg^{-1}$) in northern Bavaria (49°54′ northern latitude; 11°08′ eastern longitude, 500 a.s.l.), sieved to 2 mm and dried. To prepare soil with sorbed Alanine, a subset of soil was sterilized by γ-radiation (10 h at 53 kGy) at Synergy Health (Radeberg, Germany). To remove cytosolic products of the microbial cells lysed by the radiation that would compete with the tracer for exchange places on the soil particles, we pre-extracted the sterilized soil with 1 M K_2SO_4 for 1 h on a horizontal shaker. Microbial extracts were removed by filtering on 300 °C pre-heated glass fiber filters. Then, uniformly and position-specifically labeled tracer solutions (^{14}C-1 Ala, ^{14}C-2 Ala, ^{14}C-3 Ala and U ^{14}C Ala) with an activity of ~50 $Bq \cdot g\ soil^{-1}$ were added to the soil. All substances were purchased from American Radiolabeled Chemicals Inc. (St. Louis, USA) with an activity of 3,7 $MBq \cdot ml^{-1}$. The soil-tracer-suspensions were shaken on a horizontal shaker for 2 h to enable the Alanine to sorb to the soil particles. The remaining solutions, containing the non-sorbed alanine, were removed from the soil by filtering. Additional non-sorbed Alanine was removed by repeated post-extraction with 100 ml Millipore water. After filtering, the soils with sorbed Alanine were freeze-dried and their ^{14}C activity was quantified: approximately 50% of the added ^{14}C remained sorbed to the soil. As the recovery from all C positions was the same, we conclude that a) Alanine sorbed to the soil as intact molecule and b) no microbial decomposition took place before the start of the experiment. Their ^{14}C activities were determined, and solutions with similar ^{14}C activity and Alanine contents were prepared for the treatment, where free Alanine was added in solution to soil.

2.3. Experimental setup

The incubations were conducted in screw-cap microcosms with a layer of quartz sand at the bottom. In the sorption treatments, each sample consisted of ~10 g of freeze-dried soil containing sorbed ^{14}C-Alanine and ~80 g of dry, non-labeled, non-sterilized soil. In the free Alanine treatments, each sample consisted of ~90 g of dry, non-labeled, non-sterilized soil. The soils were filled into soil sample rings installed on ceramic plates. To equalize bulk densities in all samples, a defined pressure was applied. Soils with the sorbed Alanine were rewetted by dripping 10 ml of Millipore water onto the soil surface. Another 10 ml were added to the sand, to be taken up into the soil through the ceramic plate up to field capacity. In the free treatment, the ^{14}C-Alanine