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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

solutions with equal activity to the respective sorbed samples were added to the soil for rewetting instead of pure Millipore water. For each of the three sampling times, four replicates of each of the four labeled substances (^{14}C-1 Ala, ^{14}C-2 Ala, ^{14}C-3 Ala and U-^{14}C Ala (Table 1)) were prepared, as were three replicates of non-labeled backgrounds. Due to the sorption-desorption equilibrium, it is possible that a certain portion of the Alanine tracer that is added in solution will sorb to the soil particles after addition. The sorption pre-experiment suggests that this portion is lower than 10%. Vice versa, despite intensive washing of the sterilized soil with the adsorbed alanine, we cannot fully exclude that a very small portion of the sorbed tracer desorb abiotically in the first phase of incubation.

2.4. Analytical methods

2.4.1. $^{14}CO_2$-measurements

Cups with NaOH were placed inside the screw-cap glasses to trap CO_2. NaOH was replaced at 15, 30, 45 and 60 min and 2, 3, 4 and 6 h. After that, the NaOH cups were replaced daily. NaOH with trapped CO_2 was mixed 1:5 with scintillation cocktail (Rotiszint® eco plus, Roth, Karlsruhe, Germany) and stored in the dark for 24 h to exclude chemoluminescence. ^{14}C in CO_2 was determined by scintillation counting on a multipurpose scintillation counter (LS 6500, Beckman Coulter, Krefeld, Germany).

2.4.2. Bulk soil measurements

^{14}C remaining in soil after one, three and ten days was oxidized by combustion on an Oxidizer (Ox500, Zinsser Analytic, Frankfurt, Germany) and the resulting $^{14}CO_2$ was trapped in Oxysolve C400 (Zinsser Analytic, Frankfurt, Germany) and measured on a liquid scintillation counter (Packard-TriCarb low level, Perkin Elmer, Waltham, MA, USA). From the three C positions and the two treatments, different proportions of the applied ^{14}C were recovered in soil and CO_2 combined. It is possible that a very small part of the $^{14}CO_2$ was lost during the replacement of the NaOH traps. For this reason and to compensate for uncertainties in the amount of added tracer, we did the not refer the tracer recoveries of days 1, 3 and 10 to the added amount but to the recovered tracer amount, which is the sum of the cumulatively evolved $^{14}CO_2$ and the bulk soil ^{14}C at the respective time point.

2.4.3. Chloroform fumigation extraction

Incorporation of ^{14}C into microbial biomass was determined by chloroform fumigation extraction (Wu et al., 1994). Two subsets of 15 g of soil were extracted with 45 ml of 0.05 M K_2SO_4. The first subset was fumigated with chloroform for 3 days prior to extraction. The K_2SO_4 extracts of fumigated and non-fumigated soil were mixed 1:5 with scintillation cocktail (Rotiszint® eco plus, Roth, Karlsruhe, Germany) and stored in the dark for 24 h to exclude chemoluminescence, before being measured on a scintillation counter (LS 6500, Beckman Coulter, Krefeld, Germany). The ^{14}C contents of the non-fumigated samples were defined as dissolved organic ^{14}C (DO^{14}C). The ^{14}C content of the fumigated samples minus DO^{14}C corrected by a factor of 0.45 for less extractable cell components was defined as ^{14}C in microbial biomass.

2.5. Carbon use efficiency

Carbon use efficiency (CUE) describes how much of the assimilated C is used by the microbial community for growth (del Giorgio and Cole, 1998). CUE can be determined by comparing catabolic to anabolic C fluxes (Dijkstra et al., 2011, Dijkstra et al., 2015) or catabolic to anabolic energy fluxes (Herrmann et al., 2014; Herrmann and Bölscher, 2015). In tracer experiments, CUE can be approximated according to Spohn and Chodak (2015) as:

$$\mathrm{CUE} = \frac{C_B}{C_U} = \frac{C_B}{CO_2 + C_B} \quad (1)$$

where C_B is tracer C incorporated in microbial biomass C and C_U is tracer C taken up by microorganisms. Plants and therefore root respiration was excluded. Accordingly, all CO_2 was released by microbial respiration, and C_U was defined as the sum of $^{14}CO_2$ and microbial biomass ^{14}C.

2.6. Statistical analysis

For the replicates, a Nalimov outlier test with significance levels of 95% (when four replicates were available) or 99% (when three replicates were available) was performed. Incorporation of ^{14}C from the three positions of free and sorbed Alanine into CO_2, microbial biomass, DOC and soil was tested with a two-way analysis of variance (ANOVA). Significant differences between positions and treatments on each day were determined with the Tukey Honest Significance Difference (Tukey HSD) post-hoc test with $p < 0.05$. Differences between incorporation of individual positions on successive days were tested with a Student's *t*-test. All statistical tests were done with RStudio version 0.98.1103.

3. Results

3.1. Initial respiration rates

The maximum respiration rates for all three Alanine positions (C-1 = —COOH, C-2 = —$CHNH_2$, C-3 = —CH_3) were 4 to 5 times higher from free Alanine than from sorbed Alanine (max. respiration rates: C-1_{free} ~ 22, C-1_{sorb} ~ 4, C-2_{free} ~ 4.5, C-2_{sorb} < 1, C-3_{free} ~ 4, C-3_{sorb} < 1% · h^{-1}) (Fig. 1). The mineralization peak of ^{14}C-1 from free Alanine occurred within the first 45 min after applying the tracer solutions. In contrast, mineralization of ^{14}C-1 from sorbed Alanine displayed two peaks: the first, lower peak also within the first 45 min, the second within 2 h after application. The two peaks indicate that mineralized, sorbed Alanine was derived from two pools.

In general, the position-specific pattern for both free and sorbed Alanine in CO_2 was: C-1 > C-2 > C-3. This reflects a microbial use by similar

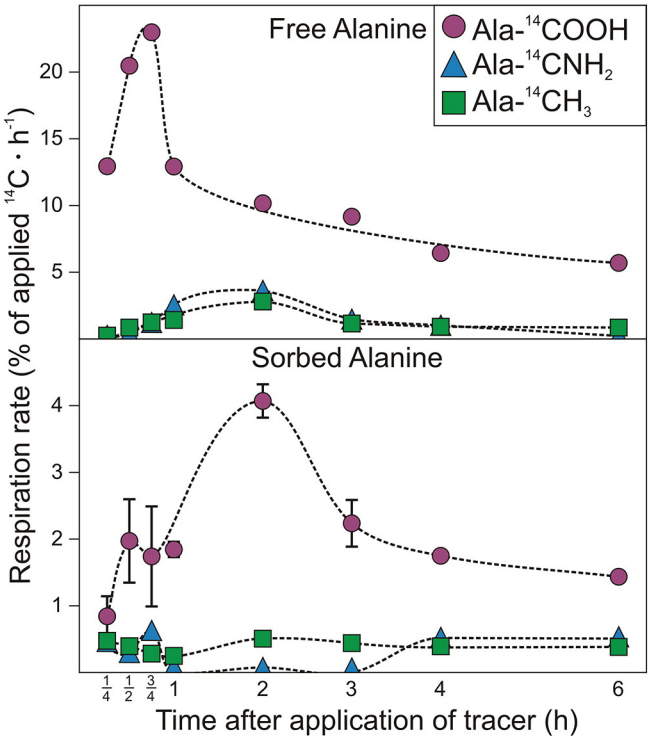

Fig. 1. Respiration rate in % of applied tracer per hour of free (top) and sorbed (bottom) Alanine C-1 (Ala-COOH), C-2 (Ala-$CHNH_2$) and C-3 (Ala-CH_3) up to 6 h after application.

metabolic pathways, but the utilization of the sorbed Alanine was delayed.

3.2. Alanine mineralization to CO_2 and stabilization in soil

The higher initial CO_2 release from all positions of free Alanine observed in the first 6 h was almost equal after one day (Fig. 2). Only cumulated $^{14}CO_2$ from sorbed ^{14}C-1 was 10% lower than from free ^{14}C-1, and cumulated mineralization from positions C-2 and C-3 was equal in both treatments. After 3 days, the position-specific pattern of Alanine-^{14}C in cumulated CO_2 was the same for sorbed and free C: ~100% of ^{14}C-1, ~45% of ^{14}C-2 and ~30% of ^{14}C-3 were mineralized. Accordingly, Alanine-derived ^{14}C remaining in soil after three days had an identical position-specific pattern, regardless of whether Alanine had been added free or sorbed.

The general pattern of sorbed and free Alanine-^{14}C positions incorporated in soil was complimentary to the pattern in CO_2: C-1 < C-2 < C-3. However, already on the first day after tracer application, less C-2 than C-3 from free Alanine was remaining in soil ($p < 0.05$). From sorbed Alanine, C-2 and C-3 remained in soil equally on day 1, and only after day 3C-2 was recovered less than C-3 ($p < 0.05$). Although sorption did not prevent the uptake by microorganisms, sorption altered Alanine transformation pathways at least over the first days.

3.3. Tracer Alanine ^{14}C in dissolved organic matter and microbial biomass

Only 2–1% of the applied free and 1% of sorbed Alanine ^{14}C remained in the DOC pool after 10 days (Fig. 3). The position-specific pattern of ^{14}C from free Alanine in DOC after 3 days corresponded to the pattern in CO_2 (highest incorporation of C-1, followed by C-2 and C-3), suggesting similar sources of both, CO_2 and DOC. In contrast, the three positions from sorbed Alanine were equal in DOC through day 10, indicating non-metabolized Alanine. Both the activity and the position-specific pattern of ^{14}C from sorbed Alanine in DOC remained constant over 10 days.

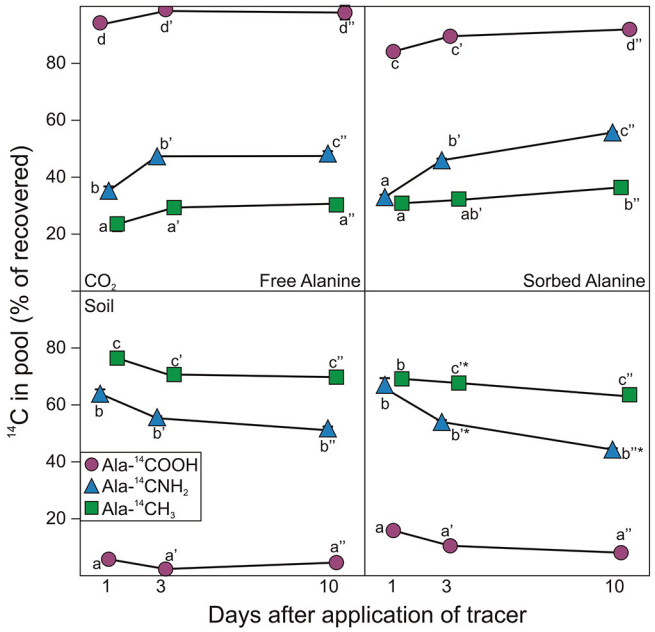

Fig. 2. Tracer ^{14}C from free (left) and sorbed (right) Alanine C-1 (Ala-COOH), C-2 (Ala-CNH$_2$) and C-3 (Ala-CH$_3$) recovered in CO_2 (top) and bulk soil (bottom). Amounts are given in % of recovered ^{14}C, which is the sum of cumulative $^{14}CO_2$ and bulk soil ^{14}C. Letters (a, a', a") indicate significant differences between positions in one pool on one day ($p > 0.05$); * indicates that the incorporation of a certain position is significantly different to the incorporation of the previous sampling day ($p > 0.05$).

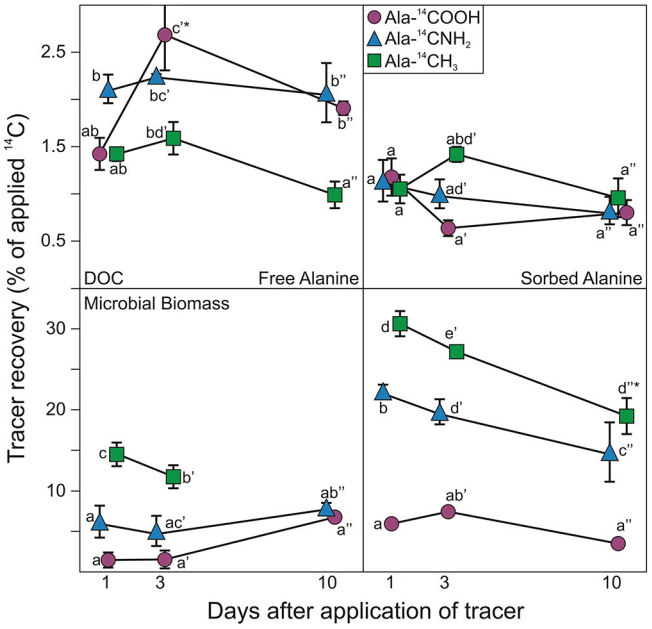

Fig. 3. Tracer ^{14}C from free (left) and sorbed (right) Alanine C-1 (Ala-COOH), C-2 (Ala-CNH$_2$) and C-3 (Ala-CH$_3$) recovered in DOC (top) and microbial biomass (bottom). Letters (a, a', a") indicate significant differences between positions in one pool on one day ($p > 0.05$); * indicates that the incorporation of a certain position is significantly different to the incorporation of the previous sampling day ($p > 0.05$). Incorporation of free C-3 on day 10 could not be reliably measured.

Incorporation of ^{14}C from all positions of sorbed Alanine into microbial biomass was higher than from the same positions from free Alanine ($p < 0.05$) (except C-1 on day 10). Thus, sorption increased the C amount retained in microbial biomass. The preference of sorbed over free C was different for all three Alanine positions: C-2 from sorbed Alanine was incorporated nearly four times as much on days 1 and 3; on day 10 it was still twice as much as C-2 from free Alanine ($p < 0.05$). C-3 and C-1 from sorbed Alanine in microbial biomass were also more than twice as high as from free Alanine on days 1 and 3. The fact that C from all three positions of sorbed Alanine were incorporated more than their free counterparts, but each to a different extent, suggests that free and sorbed Alanine are used in different metabolic pathways.

3.4. Carbon use efficiency

As C-1 from free and sorbed Alanine was mineralized to CO_2 almost completely, its CUE was the same in both treatments (Fig. 4). CUE for C-2 and C-3, however, was about twice as high for sorbed than for free Alanine on day 1 and 3 ($p < 0.05$), indicating increased anabolic use of sorbed versus free C.

4. Discussion

4.1. Sorption delays Alanine uptake and mineralization to CO_2

During the initial 6 h, respiration rates from all positions (C-1 = —COOH, C-2 = —CHNH$_2$, C-3 = —CH$_3$) of sorbed Alanine were ~75% lower than from the corresponding positions of free Alanine (Fig. 1). Moreover, the time until respiration rates peaked from the sorbed Alanine was doubled compared to free Alanine. This decreased and delayed CO_2 release corresponds to decreased accessibility of Alanine due to sorption. In contrast, half-lives of free Alanine in DOC of <10 min have been observed (Jones et al., 2009). Sorbed, position-specifically labeled Acetate was taken up within minutes (Fischer and Kuzyakov, 2010), however, Alanine has a higher sorption potential due to its three possible sorption mechanisms. Small peaks in the

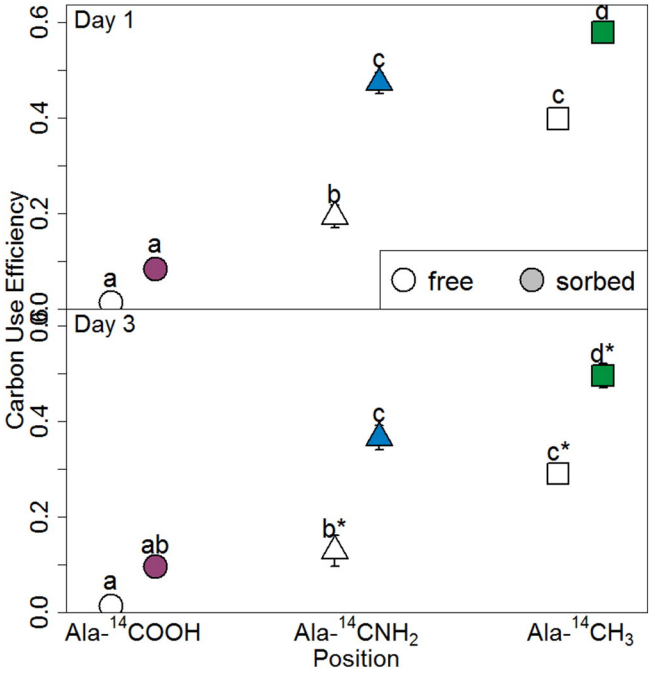

Fig. 4. Carbon use efficiency of free and sorbed Alanine 1 (top) and 3 (bottom) days after application.

respiration rates of C-1 and C-2 of sorbed Alanine in the first hour of the experiment correspond to the peaks of the same positions of free Alanine. This indicates that some of the sorbed Alanine desorbs very quickly after rewetting. Those molecules were sorbed by weak sorption mechanisms and were vulnerable to fast exchange reactions, e.g. with cations or other charged organic anions in the soil solution (Saidy et al., 2012). Dippold et al. (2014) compared the metabolization of position-specifically labeled Alanine sorbed to clay particles, iron oxides and activated charcoal: Alanine sorbed to clay minerals reached a maximum mineralization of 15–17%·h^{-1} for C-1 and was therefore only slightly more stabilized than the free Alanine in our experiment (maximum respiration rate: ~20%·h^{-1} for C-1). Respiration rates of Alanine sorbed to soil (maximum respiration rate ~ 4%·h^{-1} for C-1) lie between the maximum mineralization rates of Alanine sorbed to clay minerals and iron oxides (Dippold et al., 2014). As soils are heterogeneous mixtures of various minerals and organic matter, naturally more than one sorption mechanisms can be present. In contrast to the initial mineralization rates, the cumulative mineralization of Alanine C-1 within 10 days was the same for free and sorbed Alanine – almost 100% of added C. This means that none of the initial Alanine remained irreversibly sorbed to the soil (Fischer et al., 2010): everything was taken up by microorganisms and decarboxylated during glycolysis. Decreased accessibility (Dungait et al., 2012) due to sorption did not promote Alanine C stabilization. It is well known that sorption and desorption in the soil can occur very quickly and that a sorption-desorption equilibrium will be established very fast. Therefore, a part of the tracer added in solution will sorb to the soil particles before being taken up by microorganisms, while some of the Alanine from the sorption treatment will be released into the solution prior to microbial uptake. With our experimental approach, it is not possible to finally estimate the portion of Alanine that was exchanged by the abiotic processes of the sorption-desorption equilibrium. However, the difference in dynamics of Alanine mineralization to CO_2 between the two treatments can only be explained if microbial uptake is faster than the abiotic sorption-desorption kinetics (Fischer et al., 2010) – which would lead to an equal behaviour of ^{14}C in both treatments.

4.2. Sorption changes microbial metabolic pathways

While cumulative mineralization to CO_2 of free and sorbed Alanine was similar after 10 days, the position-specific ^{14}C patterns in soil and microbial biomass indicate a shift in metabolic pathways induced by sorption (illustrated in Fig. 5). Within the basic metabolization of Alanine in microbial cells, first the carboxylic C (C-1 position) is respired during glycolysis. The C-2 position is the second to be respired. This occurs almost instantly if C is used in the citric acid cycle (Caspi et al., 2014). If biomass produced from Acetyl-CoA is further metabolized, C-2 will also be lost before C-3. This process, however, takes several days and occurs in many anabolic pathways during recycling of anabolic products e.g. fatty acids (Apostel et al., 2013). More C-2 than C-3 of free Alanine is lost from soil already after one day (Fig. 2). In contrast, C-2 and C-3 of sorbed Alanine remain in soil in equal proportions until day 3. Therefore, C from sorbed Alanine was either used less for energy production in the citric acid cycle, or it was fed into slower cycling microbial pools. In both cases, the amount of C from sorbed Alanine that remains stabilized in soil is higher than from free Alanine. Cell residues from dead microorganisms contribute greatly to SOM (Miltner et al., 2012). Accordingly, if more C is partitioned to production of high molecular weight biomass from sorbed low molecular weight C sources, more C remains stabilized after cell death (Apostel et al., 2015).

The trends observed in bulk soil are even stronger in microbial biomass (Fig. 3): C from all positions of sorbed Alanine is incorporated more into microbial biomass than their free counterparts. Especially the incorporation of C-2 from sorbed versus free Alanine into microbial biomass is higher than in the bulk soil. This corresponds to a decrease of C fluxes through the citric acid cycle (Caspi et al., 2014) and/or incorporation into slower cycling microbial biomass pools. Microorganisms increase investment in microbial biomass formation pathways when C accessibility decreases (Dippold et al., 2014) by two potential mechanisms: a) uptake of sorbed C sources increases demand for anabolic C investment (e.g. biofilm formation on surfaces, …) (Dashman and Stotzky, 1982) and/or b) microbial specialists with a more efficient metabolism are taking up C from sorbed, but not from free Alanine. Which

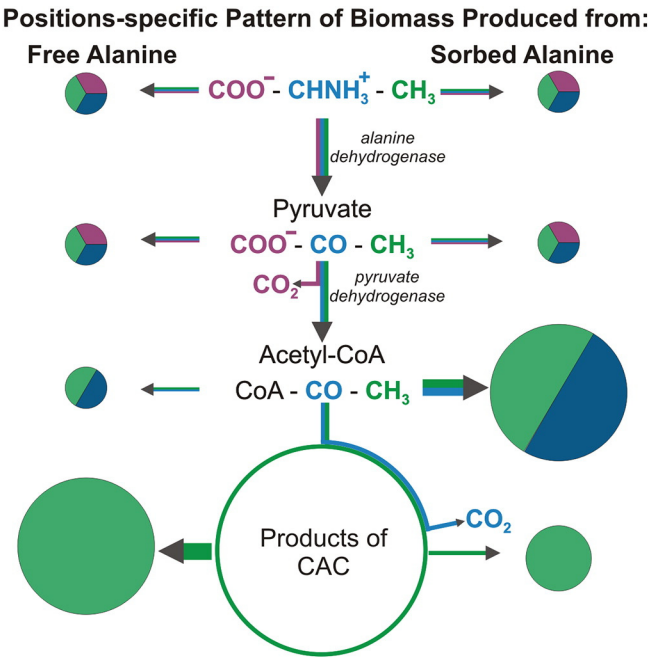

Fig. 5. Metabolic pathways of Alanine: expected C-fluxes (arrows) and position-specific pattern (pie charts) in microbial biomass from free and sorbed Alanine. Size of arrows/charts represents amount of C in the flux/pool, colors represent position-specific pattern (purple = COOH, blue = $CHNH^+_3$, green = CH_3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of these explanations is valid can be investigated in the future either 1) by tracing extracellular polysaccharide formation by compound-specific isotope analysis of microbial monosaccharides or 2) by tracing metabolic pathways of individual microbial groups based on compound-specific isotope analysis of microbial biomarkers.

4.3. Sorption increases carbon use efficiency

As the incorporation of the carboxylic group was close to 0 in both treatments, no difference between CUE of free and sorbed C-1 could be detected. For both C-2 and C-3, however, the CUE of sorbed Alanine was between 20 and 60% higher than that of free Alanine (Fig. 4). Several reasons why sorbed Alanine was processed more efficiently than free Alanine are possible: (Blagodatskaya et al., 2014) attributed high CUEs in soil to the formation of storage compounds, not actual microbial growth. Such a shift in C flux towards biosynthesis pathways – not necessarily solely for storage compounds but perhaps also for extracellular polysaccharides or other biofilm compounds (Gorbushina, 2007) – would also explain the delayed loss of C-2 both in bulk soil and microbial biomass (Fig. 2, Fig. 3). (Dijkstra et al., 2015), however, used metabolic flux modelling of position-specifically labeled Glucose and identified balanced growth with production of various metabolites, rather than only production of storage compounds, as the major process leading to high CUE.

Another explanation for the increased CUE of sorbed Alanine might be a preferential uptake and metabolization by microbial specialists, which possess a more efficient metabolism. Measuring incorporation of ^{14}C in microbial biomarkers and extracellular polysaccharides would reveal whether the higher CUE from sorbed Alanine occurs due to a metabolic shift towards anabolism within the whole microbial community or due to a stronger impact of microbial specialists' metabolism.

5. Conclusions

Sorption is one of the main factors stabilizing organic matter in soil and it is tacitly accepted that the spatial accessibility for microbial uptake and enzymatic decomposition is the solely mechanism responsible for stability of sorbed compounds. By using position-specifically labeled Alanine, the dominant amino acid in soil, we showed a new mechanism of organic matter stabilization by sorption: a shift in the microbial metabolism of compounds initially sorbed on minerals.

Due to reduced accessibility of Alanine sorbed to soil and its slow release into the solution, the initial CO_2 release is decreased by ~80% and the time until the mineralization peak is doubled compared to free alanine. After one day, however, the carboxylic C-1 is mineralized almost completely, in the sorbed and free treatment. This means that sorbed Alanine is taken up by microorganisms intact and transformed according to the basic metabolism of C_3-molecules (glycolysis). However, up to four times more C from sorbed than free Alanine was retained in microbial biomass. Although sorption did not stabilize the primary amino acid, more retention in microbial biomass facilitates the stabilization of secondary microbial products.

Although the direct effect of sorption – the inaccessibility – was of short-term importance for this amino acid, sorption of Alanine did increased the CUE by 20–60% and the C incorporation in microbial biomass up to four times. The position-specific pattern also revealed that sorption increased C-2 retention in soil and microbial biomass either due to a) a shift from C utilization in the catabolic citric acid cycle to anabolic production of biomass or/and b) a shift towards production of slower cycling cell components. Both mechanisms increase the metabolic stabilization of sorbed Alanine C, especially because close proximity to mineral phases facilitates sorption of the microbial transformation products, which might be more likely stabilized as they are in average of higher molecular weight than the initially added Alanine.

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