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Sorption affects amino acid pathways in soil: Implications from position-specific labeling of alanine

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ABSTRACT

Organo-mineral interactions are the most important mechanisms of long-term C stabilization in soils. Nevertheless, a part of the sorbed low molecular weight organic substances (LMWOS) remains bioavailable. Uniformly labeling of substances by ^{14}C or ^{13}C reflects only the average fate of C atoms of a LMWOS molecule. The submolecular tool of position-specific labeling allows to analyze metabolic pathways of individual functional groups and thus reveals deeper insight into mechanisms of sorption and microbial utilization.

Alanine labeled with ^{14}C in the 1st, 2nd or 3rd position was adsorbed to five sorbents: two iron oxides with different crystalline structure: goethite and haematite; two clay minerals with 2:1 layers – smectite, and 1:1 layers – kaolinite; and activated charcoal. After subsequent addition of these sorbents to a loamy haplic Luvisol, we analyzed ^{14}C release into the soil solution, its microbial utilization and $^{14}CO_2$ efflux from individual C positions of alanine.

All sorbents bound alanine as an intact molecule (identical sorption of 1st, 2nd or 3rd positions). The bioavailability of sorbed alanine and its microbial transformation pathways depended strongly on the sorbent. Goethite and activated charcoal sorbed the highest amount of alanine (~45% of the input), and the lowest portion of the sorbed alanine C was microbially utilized (26 and 22%, respectively). Mineralization of the desorbed alanine peaked within the first 5 h and was most pronounced for alanine bound to clay minerals. The initial mineralization to CO_2 of bound alanine was always highest for the C-1 position (–COOH group). Mineralization rates of C-2 and C-3 exceeded the C-1 oxidation after 10–50 h, reflecting the classical biochemical pathways: 1) deamination, 2) decarboxylation of C-1 within glycolysis, and further 3) oxidation of C-2 and C-3 in the citric acid cycle. The ratio between two metabolic pathways – glycolysis (C-1 oxidation) versus citric-acid cycle (oxidation of C-2 and C-3) – was dependent on the microbial availability of sorbed alanine. High availability causes a peak in glycolysis C-1 oxidation followed by an abrupt shift to oxidation via the citric acid cycle. Low microbial availability of sorbed alanine, in turn, leads to a less pronounced, parallel oxidation of all three positions and to a higher relative incorporation of alanine C into microbial compounds. Modeling of C fluxes revealed that a significant portion of the sorbed alanine was incorporated in microbial biomass after 78 h and was further stabilized at the sorbents' surfaces.

Position-specific labeling enabled determination of pathways and rates of C utilization from individual molecule positions and its dependence on various sorption mechanisms. We conclude that position-specific labeling is a unique tool for detailed insights into the submolecular transformation processes, mechanisms and rates of C stabilization in soil.

1. Introduction

Soil organic carbon (SOC) represents a major terrestrial carbon (C) sink. This makes studies on the transformation of organic substances in soils important for understanding the C cycle in terrestrial ecosystems. Plant residues, rhizodeposits and pyrogenic

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organic matter are the main sources of organic matter in soils (Knicker, 2011a; Kuzyakov and Domanski, 2000; Rasse et al., 2005). Accordingly, many studies have focused on decomposition, microbial utilization and stabilization of C from these sources in soils (Rasse et al., 2005; von Luetzow et al., 2006; Dungait et al., 2012).

During litter decomposition, all macromolecular compounds are split by enzymes into low molecular weight organic substances (Cadisch and Giller, 1996). Thus, transformation of LMWOS is a key step in biogeochemical processes in soils: all high molecular substances pass this stage during their degradation. Microorganisms determine the fate of LMWOS in soil because they either decompose them to CO_2 (catabolism) or incorporate them into cellular compounds (anabolism). Both main branches of metabolism occur in parallel and C partitioning between them depends on environmental conditions.

Within the LMWOS, amino acids play an important role because they are the quantitatively most important compound class coupling the C and N cycles. In topsoils, amino acid N – mainly bound in proteins – constitutes 7–50% of the total organic N (Knicker, 2011b; Stevenson, 1982), and concentrations of free amino acids range from 0.5 μM in root-free bulk soil up to 5 mM directly next to bursting cells (Jones and Hodge, 1999; Fischer et al., 2007). Thus, many recent studies focused on the fate of N-containing LMWOS (Kuzyakov, 1996, 1997; Lipson et al., 2001) and investigated the three major pathways of amino acid in soil: 1) sorption (Jones, 1999), 2) microbial utilization (Vinolas et al., 2001a, 2001b), and 3) plant uptake (Nasholm et al., 1998; Lipson and Nasholm, 2001). Whereas the importance of plant uptake strongly depends on the type of ecosystem (especially its N limitation) (Lipson and Nasholm, 2001; Jones et al., 2005; Sauheitl et al., 2009), sorption and microbial utilization are significant in all ecosystems and are competing processes. They lead either to a stabilization of amino acid C and N in soils or to their decomposition.

Many studies on the microbial utilization of free amino acids showed that their metabolization occurs mainly intracellularly after uptake by transport systems (Anraku, 1980; Hediger, 1994; Hosie and Poole, 2001; Dippold and Kuzyakov, 2013). Based on the uptake kinetics of some single amino acids, microbial uptake outcompetes sorption in soils (Jones and Hodge, 1999; Vinolas et al., 2001a; Fischer et al., 2010). Nevertheless, sorption is thought to be the most relevant long-term stabilization mechanism for amino acids in soils. This is even more relevant for their amino acid polymers – the proteins – which accumulate at mineral surfaces (Duemig et al., 2012; Spence and Kelleher, 2012). However, neither the exact processes nor the relevance of amino acid sorption compared to stabilization by inaccessibility in micropores or aggregates has been analyzed (Sollins et al., 1996; von Luetzow et al., 2006).

The lack of accumulation of strongly sorbed amino acids in ecosystems (Stevenson, 1982) supports the idea that sorption does not completely protect amino acids from biodegradation. They do, however, remain at least partially bioavailable (Gonod et al., 2006). Jones and Hodge (1999) demonstrated that sorption strength of three amino acids (lysine > glycine > glutamic acid) behaves contrary to microbial utilization (glutamic acid > glycine > lysine). These results support the idea that the presence of substrate in solution and consequently sorption is key driver regulating the fate of amino acids. Furthermore, both the quantity of microbial uptake and the metabolization pathways of LMWOS are affected by sorption (Schneckenberger et al., 2008; Fischer and Kuzyakov, 2010; Dijkstra et al., 2011a).

Broadly, amino acids can be adsorbed by three types of functional groups (Jones and Hodge, 1999): 1) ion exchange by the positively charged amino groups, 2) ligand exchange by the carboxyl groups and 3) hydrophobic interactions by the alkyl groups. In addition, weak electrostatic interactions (H-bondings, dipole-dipole-interactions, van-der-Waals bondings) are possible by the C skeleton (Brigatti et al., 1999) and intercalation into the clay mineral interlayer has also been discussed (Wattel-Koekkoek et al., 2003). There is strong evidence that soil properties (Kemmitt et al., 2008) such as soil mineralogy (Strahm and Harrison, 2008) influence the microbial utilization of LMWOS, but detailed studies comparing sorption on various mineral phases and their effect on microbial utilization of amino acids are rare (Dashman and Stotzky, 1982).

Here, we use the approach of position-specific labeling to elucidate the shifts in microbial amino acid transformation pathways caused by sorption. This tool, commonly used in biochemistry to reconstruct metabolic pathways, has increasingly been applied in soil science in recent years (Dijkstra et al., 2011a, 2011b, 2011c; Fischer and Kuzyakov, 2010). It overcomes the limitations of uniform labeling because it helps differentiate between cleavage of a molecule vs. utilization of the entire molecules.

Our model amino acid – alanine (one of the most abundant amino acids in soil) – occurs under soil conditions as a dipolar ion: it has a positive charge, a negative charge and a hydrophobic methyl group – enabling different sorption mechanisms. As a representative subset of sorbents common in soils and representative for three basic sorption mechanisms, we chose a three- and a two-layer clay mineral (smectite and kaolinite), two iron oxides (goethite and haematite) and activated charcoal. Thus, we present here a submolecular approach to elucidate LMWOS stabilization on sorbents and effects of sorption on amino acid transformations in soil. We assume that alanine interacts differently with various mineral phases and that interaction strength and mechanism strongly affects its subsequent microbial utilization, e.g. we assume a preferred allocation of LMWOS-C into the anabolic pathways of maintenance metabolism, the lower the availability of a substrate is. We hypothesize that various sorption mechanisms affect the stabilization of amino acid C on mineral surfaces not only by direct interaction of LMWOS-C with the mineral but also by changing C allocation: The stronger a substrate is sorbed, the more its C is transformed to products, which are prone to be stabilized on the sorbents. In the case of polymeric microbial products (proteins, cell walls,…), this process could be identified by an equal incorporation of all three alanine C positions.

2. Materials and methods

2.1. Soil

Soil samples were taken from an Ap horizon of a loamy silt, haplic Luvisol (WRB 2006) from a long-term cultivated field in Bavaria near Hohenpölz (49.907 N, 11.152 E, 501 m asl, mean annual temperature 6.7 °C, mean annual precipitation 874 mm), where a continuous rotation of corn, barley, wheat and triticale was established. The soil had the following characteristics: pH_{KCl} 4.9 and pH_{H2O} 6.5, TOC and TN content were 1.77% and 0.19%, respectively, and potential CEC was 174 $mmol_c\ kg^{-1}$. The soil was stored field-moist at 5 °C for less than 1 month, sieved to 2 mm, and all roots were removed manually before adding to the minerals. 800 mg of field-moist soil were used per replicate.

2.2. Sorbents

Minerals were ordered from Kremer pigments (Aichstetten/Allgäu, Germany): smectite-dominated Bentonite (58900), kaolinite-dominated Kaolin (58250), heamatite-dominated “Eisenoxid rot” (48600) and goethite-dominated “Eisenoxidocker” (40301). Activated charcoal was ordered from Sigma–Aldrich

(Taufkirchen, Germany) and ball-milled until the texture was comparable to the mineral phases. Specific surface area (SSA) was determined with a Quantachrome Nova 4000 surface analyzer (Quantachrome GmbH, Odelzhausen, Germany) by N_2 adsorption using the BET method (Mikhail and Brunauer, 1975). Effective cation exchange capacity (CEC_{eff}) was determined for all sorbents and the soil by exchange of the cations with Ba^{2+} according to NF ISO 11260 1994 (Rhoades, 1982) (Table 1). SSA and CEC_{eff} were determined with sterilized, heated minerals (300 °C over night) to have identical conditions than with the minerals used for the main experiment. Whereas loss of smectite crystal water is a reversible process, 300 °C heating might have caused some transformations in goethite structure lowering its adsorption capacity. However, values of SSA and CEC_{eff} shown in Table 1 characterize the sorbent properties of the main experiment.

2.3. Chemicals and radiochemicals

Sterile stock solutions with 50 μM alanine and an equal ^{14}C activity of 830 kBq ml^{-1} were prepared from U–^{14}C-labeled alanine and the position-specifically labeled isotopomeres 1-^{14}C-, 2-^{14}C- and 3-^{14}C-labeled alanine (American Radiolabeled Chemicals Inc, St. Louis, USA) as well as non-labeled alanine (Sigma–Aldrich, Taufkirchen, Germany).

The incubation of the sorbed alanine with soil was conducted in 24 well microtiter plates. The CO_2 efflux from the wells was trapped in 2.0 M NaOH-solution placed on a filter mat on top of the microtiter plates. The filter mat segments were separated by a thin line of silicone oil added on the well boarders which diffused into the mat. In addition 1 M NaOH solution was prepared to trap the CO_2 after combustion (Sigma–Aldrich, Taufkirchen, Germany).

2.4. Preliminary experiments

Two preliminary experiments were performed to evaluate parameters and optimize the experiment design: First, the time needed for the sorption experiment was determined by adding U–^{14}C-labeled alanine to the five sterile sorbents (five replications). 200 mg of each sorbent were added per well of a 24 deep-well plate, and 1.0 ml of the U–^{14}C-labeled alanine solution was added to each well. The plate was closed and shaken on a horizontal shaker with 120 rpm. An aliquot of 50 μl was taken after 0.5, 1, 1.5, 4, 10 and 24 h and the ^{14}C activity in the supernatant was determined.

Second, the efficiency of the CO_2-trap was tested. A glassfiber-filtermat was installed on top of the 24 well plate. The preprinted well borders on the filtermat were redrawn with silicon-oil to avoid diffusion of the NaOH-drops between the filter segments above each well. 70 μl of 2 M NaOH were added onto the segments above each well to capture the evolved CO_2. To test the capacity and efficiency of this trap, 500 μl of a 0.08 M ^{14}C-Na_2CO_3 solution with an activity of 0.083 kBq were transferred into the wells. The amount of CO_2 derived from the carbonate reflects the maximum of soil respiration expected during the 78 h of the experiment (approx. 3% of TC). The CO_2-trap was installed above these wells and the CO_2 volatilized completely by adding several drops of 2 M HCl by a syringe directly into the well. The ^{14}C activity trapped on the filter mat during incubation was compared with the added ^{14}C activity. Efficiency of the trap was around 90% and CO_2 efflux from the wells was corrected for this efficiency.

Table 1
Effective cation exchange capacity and specific surface area of the five sorbents and the soil used for this experiment.

	Soil	Goethite	Haematite	Smectite	Kaolinite	Activated charcoal
Effective CEC ($mmol_c\ kg^{-1}$)	119.5	133.0	84.5	208.5	54.0	50.0
Internal surface ($m^2\ g^{-1}$)	16.8	36.2	26.3	58.0	18.3	980

2.5. Experimental setup

The availability of absorbed alanine was analyzed in two steps: 1) Alanine was absorbed on sterilized sorbents (heated for 300 °C for 12 h) and the not absorbed alanine was removed by washing with distilled water. This step reflected the affinity of the sorbent to alanine. 2) Thereafter, the sorbed alanine was mixed with the soil and incubated for 3 days. This step showed the effect of sorption on microbial utilization and decomposition.

For the main experiment the sorbents were sterilized by heating overnight at 300 °C and afterward cooled down and stored in a dry air desiccator. 200 mg of sorbent were added in each of 20 wells of the 24 well plate. Four wells (one per line: A5, B5, C5 and D5) without mineral and ^{14}C addition were used as controls e.g. to check for diffusion from one filter segment to another through the silicon oil and to check for contamination by suspension drops into the wells. Solution in the well and on the filter segment was never contaminated by neighboring wells and was used to correct for the background ^{14}C activity. To the five remaining wells per row, 1 ml of a 50 μM alanine solution with a ^{14}C activity of 0.83 kBq was added. The four rows represented one treatment of U–^{14}C-labeled alanine (row A) and the three position-specifically labeled isotopomers 1-^{14}C- (row B), 2-^{14}C- (row C) and 3-^{14}C-labeled alanine (row D). After adding the sorbent the plate was shaken for 24 h on the horizontal shaker (120 rpm). The plate was then centrifuged at 4000 rpm for 10 min and all the supernatant was removed. The sorbent was washed 3 additional times with millipore water to remove all unbound alanine. Until the experiment start the prepared plates were stored frozen (−20 °C) to prevent microbial degradation of the sorbed alanine.

After defrosting the plate, the sorbent was resuspended in 3 ml millipore water and 800 mg of fresh soil were added. The plate was covered by the CO_2-trap (with filter segments separated by silicon oil as described above), which was fixed by a complete cover with parafilm. This was fixed under slight pressure on a metal construction and shaken at 120 rpm (shaking intensity was optimized that no cross-contamination between the wells occurred). Sampling took place after 1, 3, 6, 12, 24, 36, 52 and 78 h. The filtermat was removed and cut into segments. Simultaneously the plate was centrifuged for 5 min at 4000 rpm and a 70 μl aliquot of the supernatant was removed to measure the ^{14}C activity. Afterward, soil and sorbent were resuspended, a new CO_2-trap was installed and incubation continued as before. After the last sampling, the supernatant was removed, the plates were frozen, freeze-dried and the soil was combusted at 600 °C for 10 min under a constant O_2 stream with an HT 1300 solid combustion module of the multi N/C 2100 analyzer (Analytik Jena, Jena, Germany). $^{14}CO_2$ released by combustion was trapped in 10 ml of 1 M NaOH in a vigreux column.

2.6. Chemical and radiochemical analyses

^{14}C activity of the supernatants was determined on a scintillation counter (Wallac 1450, MicroBeta® TriLux, PerkinElmer, Wallham MA; USA) by adding the 70 μl aliquot to 0.6 ml scintillation cocktail in 24 well plates. Filter segments and NaOH-solution after combustion were measured in glass scintillation vials on the LS 6500 scintillation counter (LS 6500, Beckman–Coulter, Krefeld, Germany). 3 ml of the NaOH-solution was mixed with 6 ml of scintillation cocktail (EcoPlus, Roth Company, Germany). Filter segements were also added to 6 ml of scintillation cocktail,

preconditioned with 0.5 ml of 2 M NaOH. Each scintillation vial was stored for 24 h in dark until disappearance of chemiluminescence.

2.7. Calculations and modeling

The initially sorbed ^{14}C activity per well was calculated by the sum of 1) the captured CO_2 over 72 h, 2) the dissolved activity in the supernatant and 3) the activity of the combusted soil-sorbent mixture. All graphs and calculations were done in percent of the initially sorbed ^{14}C activity.

Microbial decomposition of alanine was expressed in % of sorbed alanine ^{14}C per h and as cumulative respiration of all sampling times. A four pool model, based on first-order kinetics according to Kuzyakov and Demin (1998) – considering sorbed alanine C, microbially uptaken C, respired CO_2 and alanine C incorporated in microbial biomass (Fig. 1) – was adapted to these data. Curve fitting by simplex algorithm was done by Model Maker (Model Maker, Version 3.1 MMAN 1, CHEM Research GmbH, Hamburg) and delivered the following parameters: microbially available alanine C, the uptake constant k_{upt}, the incorporation constant k_{inc} and the respiration constant k_{resp}. The three constants reflect the kinetic constant of alanine transfer between the pools (see Fig. 1). The decrease in sorbed alanine d(sorbAlaC)/dt by time occurs by microbial uptake (-Uptake) (first equation). The incorporation rate into stable microbial biomass (Inc = d(stableMB)/dt) as well as the respiration rate (Resp = CO_2 (d(CO_2)/dt)) originates from C incorporated the pool of microbial metabolites (micMetab) (second and third equation). Consequently uptake is a source for microbial metabolites whereas respiration to CO_2 and stabilization in stable microbial biomass are C sinks for microbial metabolites (fourth equation).

$$\frac{d(\text{sorbAlaC})}{dt} = -\text{Uptake} = -k_{upt}\cdot\text{sorbAla}$$

$$\frac{d(\text{stableMB})}{dt} = \text{Inc} = k_{inc}\cdot\text{micMetab}$$

$$\frac{d(CO_2)}{dt} = \text{Resp} = k_{resp}\cdot\text{micMetab}$$

with

$$\frac{d(\text{micMetab})}{dt} = \text{Uptake} - \text{Resp} - \text{Inc} = k_{upt}\cdot\text{sorbAlaC} - (k_{inc} + k_{resp})\cdot\text{micMetab}$$

In this model we disregard the other fluxes (from stableMB to micMetabol and stableMB to sorbAlaC) because of their minor importance within the few days of the experiment.

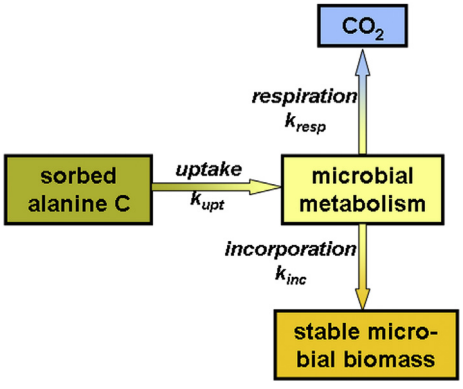

Fig. 1. Scheme of the adapted four-pool model to the measured $^{14}CO_2$ efflux.

2.8. Calculation of the C-1/C-2,3-ratio and the divergence index DI_i

According to Dijkstra et al. (2011a; 2011c) the C-1/C-2,3-ratio was calculated for 78 h of incubation from the fitted CO_2-release (equation (1)). Ala-C-j represents the percent of Ala-C incorporation from the position j of the molecule into that pool. The ratio reflects the relative activity of glycolysis (leading to C-1 respiration) compared to the citric acid cycle (causing C-2 and C-3 respiration):

$$\text{C}-1/\text{C}-2,3 = \frac{[\text{Ala}-\text{C}-1]}{\sum_{j=2}^{3}[\text{Ala}-\text{C}-j]/2} \quad (1)$$

After 78 h of incubation the alanine C remaining in the soil and that decomposed to CO_2 was determined as a relative percentage of the sum of total ^{14}C activity. According to Dippold and Kuzyakov (2013) the transformation of C from individual molecule positions was presented by the Divergence Index DI_i, which was calculated for each of the three labeled C positions of alanine, whereas Ala-C-i is accordingly the percentage of incorporation of the position i into the respective pool:

$$\text{DI}_i = \frac{n\cdot[\text{C}-\text{i}]}{\sum_{j=1}^{n}[\text{C}-\text{j}]} = \frac{3\cdot[\text{Ala}-\text{C}-i]}{\sum_{j=1}^{3}[\text{Ala}-\text{C}-\text{j}]} \quad (2)$$

This index reflects the fate of individual C atoms from the position i relative to the mean transformation of the total number of C atoms n within a transformation process. Thus, a DI_i of 1 means that the transformation of this position in the investigated pool corresponds to the transformation of uniformly labeled substance (average of all C atoms). DI_i ranges from 0 to n, and values between 0 and 1 reflect reduced incorporation of the C into the investigated pool, whereas values between 1 and n show increased incorporation of the C atom into this pool as compared to the average. As this index is independent of absolute amounts of the substance, it enables comparing the alanine C distribution in various pools.

2.9. Statistics

All experiments were done with five replications, and the values on figures present mean ± standard error of mean (±SEM). SEM of the divergence index was gained by Gaussian error propagation. Measured variables were screened for outliners using the Nalimov test (Gottwald, 2000), tested for normal distribution using the Kolmogorov Smirnoff test and for homogeneous variances using Levene's test. Nested ANOVA, with the factor C position being nested in the factor sorbent treatment, were done using Statistica (version 7.0, Statsoft GmbH, Hamburg, Germany). If assumptions such as normal distribution or homogeneous variances were not met, the result of the nested ANOVA was confirmed by non-parametric Kruskal–Wallis ANOVA before performing a Tukey HSD post-hoc test for unequal sample size.

3. Results

3.1. Sorption and microbial utilization of uniformly labeled alanine

In the first part of the experiment, sorption of alanine to various sorbents was compared. There were no differences in the sorption of the three alanine positions and uniformly labeled alanine onto the sterile sorbents. Thus, sorption affinity is represented by the results of the uniformly labeled treatment (Table 2): activated charcoal and goethite showed highest sorption of the added

Table 2
Initially sorbed alanine C and fitted parameters to the four-pool model for microbial utilization of sorbed alanine (Fig. 1) fitted to the data of uniform alanine labeling.

	Sorbed Ala C (% of added)	Available Ala C (% of sorbed)	Uptake rate k_{upt} (% h^{-1})	Incorporation rate k_{inc} (% h^{-1})	Mineralization rate k_{resp} (% h^{-1})	R^2 of model
Goethite	44.37	26.23 ± 0.39	0.1627 ± 0.0068	2.098 ± 0.105	4.852 ± 0.237	0.993
Haematite	18.50	43.61 ± 0.88	0.0841 ± 0.0041	1.923 ± 0.146	5.331 ± 0.401	0.983
Smectite	11.84	33.42 ± 0.61	0.6390 ± 0.0405	5.067 ± 0.411	17.486 ± 1.392	0.980
Kaolinite	26.08	32.69 ± 0.69	0.6268 ± 0.0497	6.584 ± 0.666	24.875 ± 2.497	0.990
Activated charcoal	45.41	22.31 ± 0.38	0.1585 ± 0.0066	8.330 ± 0.319	10.200 ± 0.390	0.991

alanine (45%), with lower values in kaolinite, haematite and smectite (26, 19 and 12%, respectively). This sorbed amount of alanine was set to 100% for all further calculations of microbial utilization in soil.

In the second part of the experiment the minerals with sorbed alanine were added to soil to prove the effects of an active microbial community. This experiment demonstrated that the sorption strength varied strongly between the minerals: Within the iron minerals, goethite sorbed most alanine, and the lowest portion – only 26% – of this U–^{14}C alanine was available to microorganisms. In contrast, haematite sorbed only 19% of the added alanine and 44% of this U–^{14}C alanine was microbially available. Within the clay minerals a quite similar percentage of 30–35% of the sorbed U–^{14}C alanine was usable by microorganisms (Table 2). Activated charcoal was the most efficient sorbent for alanine: it did not only adsorb the most of the added alanine (45%), but also the smallest portion (22%) was accessible.

The decomposition to CO_2 of the U–^{14}C labeled alanine bound to clay minerals peaked within the first 5 h, with subsequent fast decrease (Fig 2). In contrast, the peak of decomposed alanine bound to iron minerals and to activated charcoal was ca. 5 times lower, with a subsequent slow decrease. It has to be considered that the continued low mineralization of clay mineral bound alanine is not represented well by the model, which approaches an equilibrium state for the last hours of the experiment. However, the faster initial decomposition of clay mineral bound alanine is also reflected by the fitted microbial uptake rate for bound alanine k_{upt}, which was highest for the clay minerals (~0.6% h^{-1}) and at least 5 times lower for the iron minerals and activated charcoal (0.08–0.16% h^{-1}). This microbial availability and the respective uptake rate of sorbed alanine affected the metabolic utilization: the faster the desorption and uptake took place, the higher the portion of microbial mineralization versus incorporation. The mineralization rate of alanine sorbed on clay minerals was 3–4 higher than the incorporation rate, whereas for iron minerals this ratio lies between 2 and 3, with activated charcoal having the lowest value (1.2). Thus, the availability of alanine C and desorption kinetics of individual minerals had a distinct effect on C allocation between cata- and anabolism in microorganisms.

3.2. Kinetics of position-specific utilization of sorbed alanine C

In the first part of the experiment the sorption of individual alanine molecule positions was identical. However, microbial transformation of sorbed alanine in the second part of the experiment was strongly different for individual molecule positions. Therefore, a more detailed insight into the mechanisms of catabolic to anabolic alanine utilization can be gained by comparing the kinetics of the utilization of individual positions of alanine C: For each of the sorbents, the mineralization to CO_2 followed the order C-1 > C-2 > C-3 (Fig. 3 and Table 3). This is also reflected in the fitted amount of available C and uptake constants k_{upt} (Table 3). Thus, irrespective of the sorbent, the carboxyl group was less stabilized and the highest amount was respired. Consequently, the alanine was either 1) bound to all sorbents not by the carboxyl group, but by the methyl or alkyl-amino group and extracellularly cleaved or 2) the desorption of alanine occurs as the whole molecule, and microbial metabolism accounts for the preferential decarboxylation of C-1.

The initial mineralization peak was most pronounced for the alanine sorbed on clay minerals. During this peak, the mineralization rate was highest for C-1 and differences between C-1 and both other positions (C-2 + C-3) were most pronounced within the first hours (Fig. 4). Later, the C-1 mineralization rate was even lower than that of C-2- and C-3 mineralization (Fig. 4). This C-1 oxidation peak was less pronounced for alanine sorbed to iron minerals and

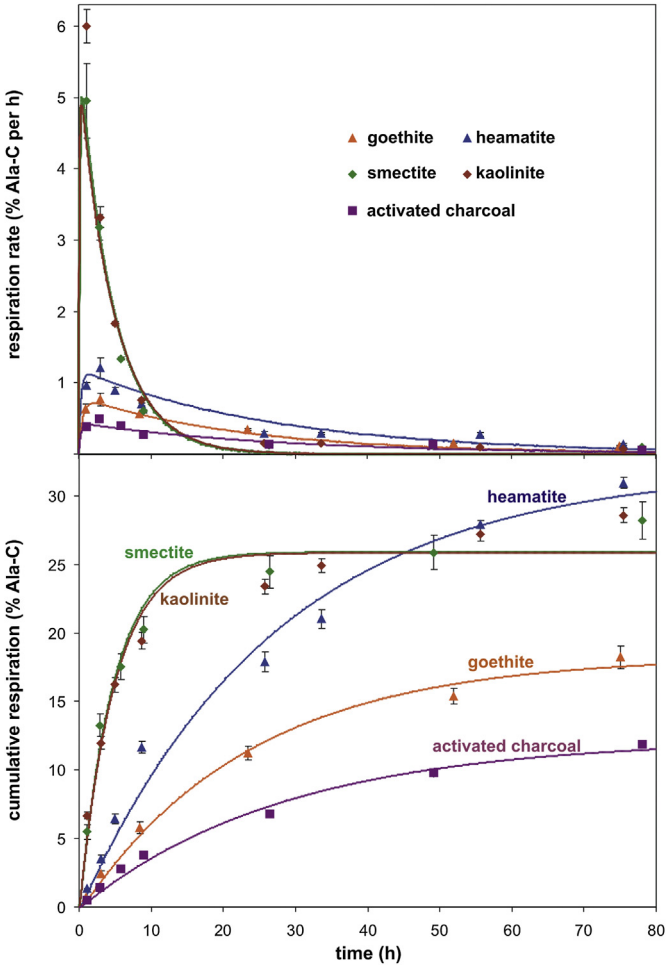

Fig. 2. Respiration rate (in % of sorbed alanine C per h) and cumulative $^{14}CO_2$ efflux from alanine adsorbed to various sorbents; Experimental points (means ± SEM, $N = 6$) and fitted curves based on the microbial utilization model (Fig. 1) are presented.

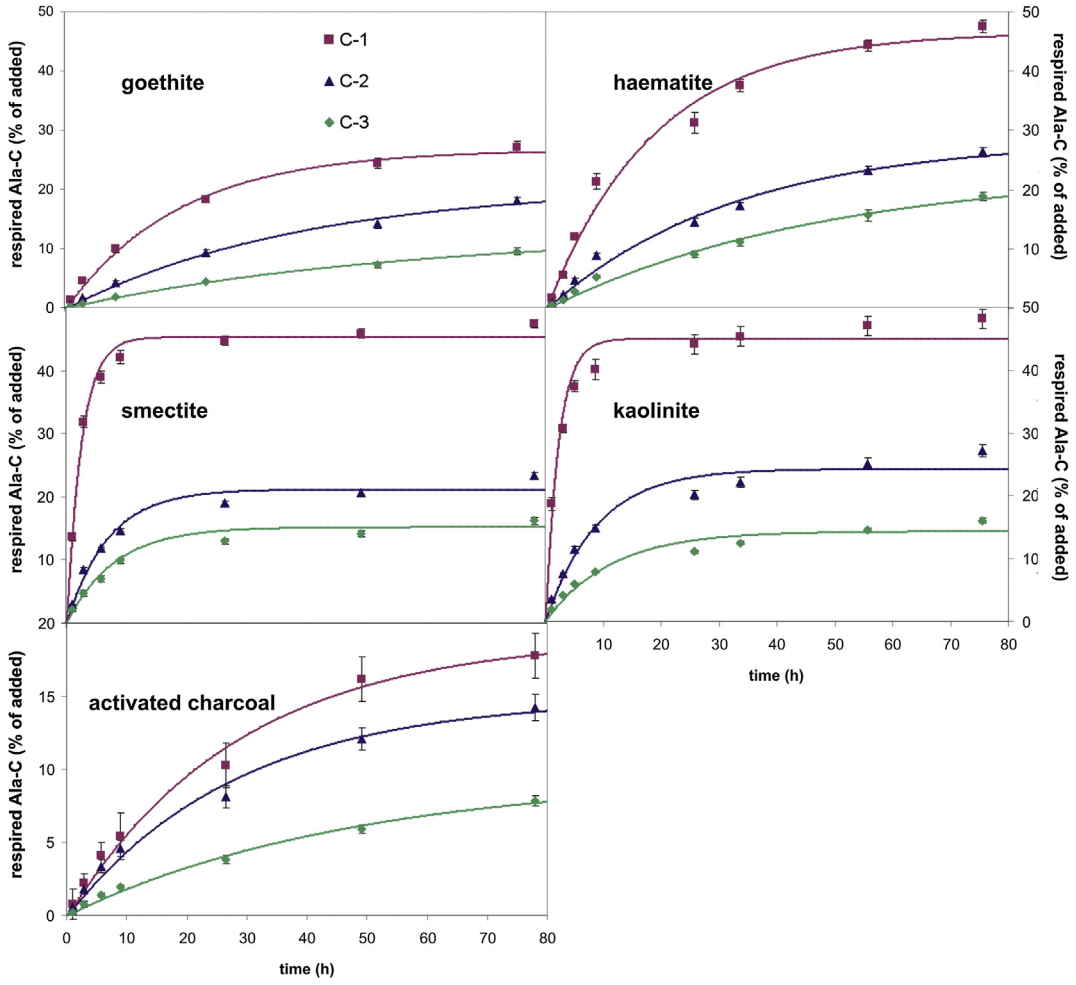

Fig. 3. Cumulative $^{14}CO_2$ efflux (in % of initially sorbed alanine C) from individual molecule positions of alanine; Experimental points (means ± SEM, $N = 6$) and fitted curves on the microbial utilization model (Fig. 1) are presented.

did not occur for activated charcoal sorbed alanine (Fig. 4). For this most efficient sorbent the general behavior of the positions changed. C-1 and C-2 behaved more similarly, whereas C-3 was best stabilized by activated charcoal (Figs. 3 and 4). This trend was also visible in the fitted microbially available C and the uptake constant k_{upt}: for all minerals, k_{upt} of C-1 was higher than the respective values for C-2 and C-3, whereas in the case of activated charcoal the C-3 and C-2 exceeded C-1 (Table 3). In addition, the C-1/C-2,3 ratio shown in Fig. 4 reflects a constant ratio of positon-specific respiration for the whole experiment. This is in contrast to the other four treatments with mineral-associated alanine, where always an initial peak of C-1 respiration was followed by a peak in C-2,3 oxidation for the late period of the experiments. These divergences in the curve shape (Fig. 4) as well as position-

Table 3
Fitted parameters of the four-pool model for microbial utilization of sorbed alanine (Fig. 1) for the individual alanine C positions.

		Available Ala C (% of sorbed)	Uptake const. k_{upt} (% h^{-1})	Incorporation const. k_{inc} (% h^{-1})	Mineralization const. k_{resp} (% h^{-1})	R^2 of model
Goethite	1-Ala	31.54 ± 0.40	0.168 ± 0.007	1.055 ± 0.089	5.869 ± 0.468	0.995
	2-Ala	28.76 ± 0.41	0.089 ± 0.003	1.427 ± 0.075	3.648 ± 0.183	0.994
	3-Ala	19.53 ± 0.25	0.091 ± 0.002	2.771 ± 0.098	5.186 ± 0.191	0.996
Haematite	1-Ala	56.56 ± 1.02	0.096 ± 0.045	0.949 ± 0.097	4.393 ± 0.441	0.985
	2-Ala	43.07 ± 0.75	0.071 ± 0.034	2.165 ± 0.112	4.257 ± 0.218	0.988
	3-Ala	36.53 ± 0.62	0.056 ± 0.023	2.243 ± 0.108	4.027 ± 0.191	0.989
Smectite	1-Ala	59.79 ± 0.62	0.685 ± 0.031	2.065 ± 0.090	6.449 ± 0.271	0.992
	2-Ala	28.28 ± 0.58	0.515 ± 0.035	5.738 ± 0.476	17.120 ± 1.353	0.977
	3-Ala	18.98 ± 0.38	0.636 ± 0.042	9.134 ± 0.810	31.090 ± 2.759	0.980
Kaolinite	1-Ala	55.83 ± 1.00	0.798 ± 0.067	2.537 ± 0.236	10.559 ± 0.965	0.969
	2-Ala	31.65 ± 0.72	0.359 ± 0.029	7.216 ± 0.707	23.860 ± 2.327	0.969
	3-Ala	18.48 ± 0.48	0.484 ± 0.044	14.855 ± 1.732	51.958 ± 5.977	0.962
Activated charcoal	1-Ala	36.20 ± 0.58	0.097 ± 0.004	3.740 ± 0.026	4.155 ± 0.141	0.992
	2-Ala	25.62 ± 0.45	0.138 ± 0.006	5.909 ± 0.032	8.184 ± 0.341	0.990
	3-Ala	16.35 ± 0.25	0.128 ± 0.004	12.832 ± 0.034	18.113 ± 0.664	0.993

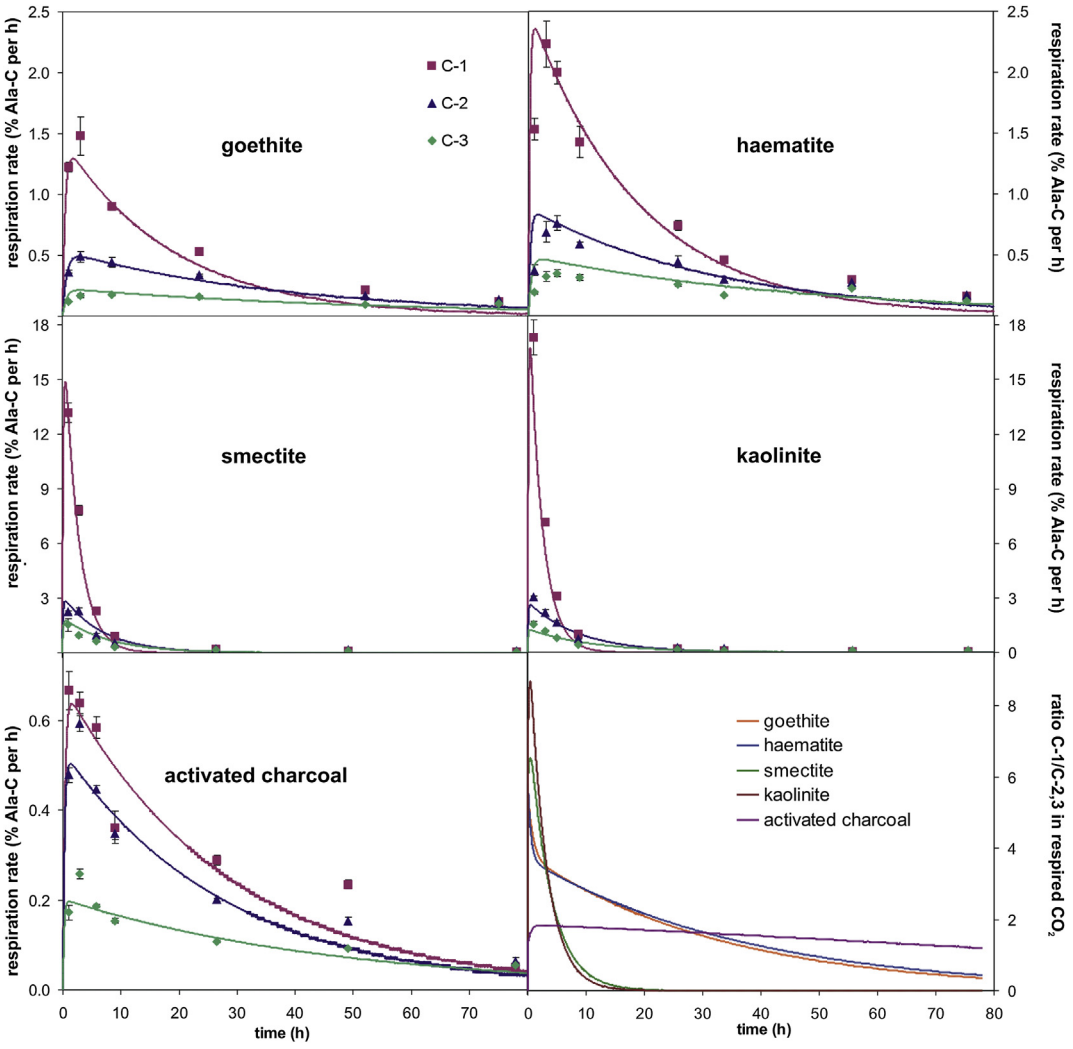

Fig. 4. Respiration rate (in % of initially sorbed alanine C per h) of individual molecule positions of alanine and ratio of C-1 to (C-2+C-3)/2 respiration of alanine C for the used sorbents calculated from the fitted position-specific oxidation rate; Experimental points (means ± SEM, $N = 6$) and fitted curves on the microbial utilization model (Fig. 1) are presented.

specific individualities (Fig. 4) suggest of special transformation pathways observed for alanine bound to activated charcoal.

The sorbent affected not only microbial uptake but also the intracellular metabolization of C from individual amino acid positions. Whereas mineralization and the incorporation constants of alanine bound on clay minerals and activated charcoal followed the order C-3 > C-2 > C-1, the alanine bound on iron oxides showed also the lowest incorporation rate for C-1, but similar mineralization rates for C-1, C-2 and C-3. Intracellular metabolization is also reflected by the C-1/C-2,3-ratio, which provides information about the relative intensity of glycolysis to citric acid cycle (Fig. 4). A clear peak in glycolysis intensity was observed for the mineralization of clay-mineral-bound alanine, but after an initial phase of C-1 oxidation the alanine metabolization changed to an increasing proportion of citric acid cycle activity. In contrast, this initial glycolysis activity was much less expressed for iron-oxide-bound alanine, and no change in the metabolization pathway over time was observed for the microbial utilization of active-coal-bound alanine.

Thus, the sorbed amount as well as sorption strength of the sorbents affected not only the availability and uptake rate of alanine, but apparently influenced the uptake processes. Subsequent splitting of the molecule, as well as intracellular utilization by cata- and anabolism of the microorganisms, are controlled by the way alanine is sorbed in soils.

3.3. Incorporation of C from alanine positions in stabilized pools and decomposition to CO_2

During the incubation of sorbed alanine with soil, the portion of dissolved and respired alanine C was measured. Whereas cumulative CO_2 efflux for each position and each sorbent showed a saturation curve (Fig. 4), the amount of dissolved alanine C was always very low, never exceeding 1% of the total alanine C in the system (Fig. 5). Thus, desorbed alanine or alanine fragments persisted only extremely briefly in the dissolved state or were even directly exchanged from the sorbed form. As there was no significant difference in the alanine C positions in solution (Suppl. Table 1), the measured ^{14}C in the supernatant is intact alanine or the deaminated product pyruvate.

After 72 h, between 8 and 48% of alanine C were decomposed to CO_2 and 52–92% were still bound to the sorbents or incorporated into microbial biomass (Fig. 5). The absolute portion of ^{14}C incorporation per pool (Fig. 5, Suppl. Table 1) as well as the divergence

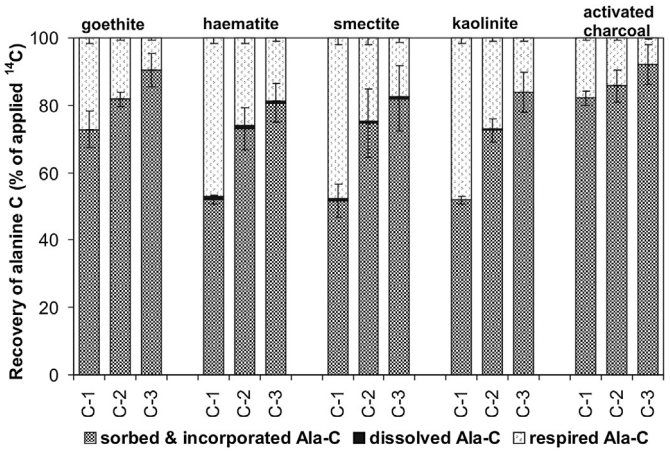

Fig. 5. Percentage of alanine C in bound fraction, dissolved fraction or respired to CO_2 after 78 h. Values show means ± SEM ($N = 5$) depending for the sorbents for the individual alanine C positions.

index (Fig. 6, Suppl. Table 2) revealed that the general trend is similar irrespective of the sorbents: C-1 is preferentially mineralized to CO_2, whereas C-3 either remains bound or is incorporated into microbial biomass.

Considering the relative C allocation by the DI, released CO_2 – the product of microbial catabolism – had much higher discrimination between the positions than bound alanine C (Fig. 6). Hence, the DI of respired alanine C has to reflect a value inverse to the incorporation into microbial biomass – the anabolic branch. Fig. 6, however, shows that DI of respired CO_2 and sorbed C are not inverse and highest DI was observed for smectite, kaolinite and haematite (Fig. 6). These were the minerals with the highest percentage of microbially available alanine. In contrast, activated charcoal and goethite showed the lowest range in DI for the sorbed alanine C (Fig. 6).

The DI of CO_2, which represents a pool passed to 100% through microbial catabolism, reflected differences in metabolic utilization of C from the three positions of alanine. Alanine sorbed to clay minerals and haematite showed a clear preferential degradation of C-1 compared to C-2 and C-3. In goethite-sorbed alanine, the C-2

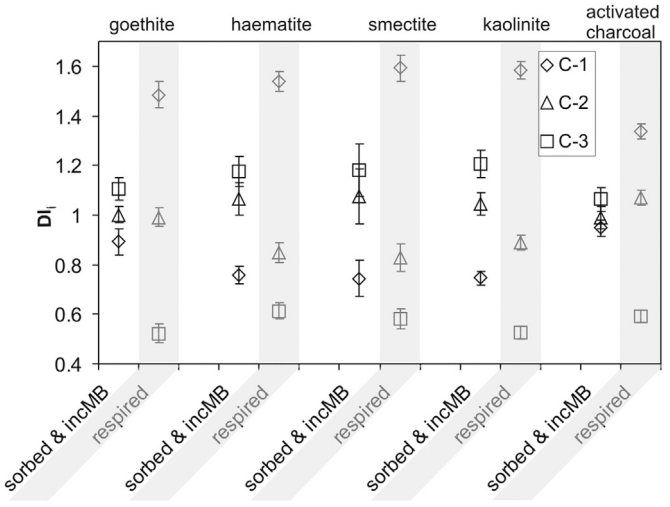

Fig. 6. Divergence Index (DI) of sorbed alanine and alanine incorporated into microbial biomass (sorbed & incMB) and respired alanine C for the five sorbents after 78 h; Values show means ± SEM ($N = 5$) calculated by Gaussian error propagation.

was not preferentially degraded or incorporated, but showed a DI of 1. In contrast, alanine bound on charcoal reflected a preferential degradation of C-1 and C-2, and only C-3 was preferentially protected from decomposition. Thus, sorption mechanisms as well as microbial availability affected alanine transformation (Suppl. Table 2), at least over the first 78 h.

4. Discussion

4.1. Sorption mechanisms of amino acids

Preliminary studies to this experiment with mineral phases (results not shown) as well as sorption studies with soil (Dippold and Kuzyakov, 2013) confirmed the hypothesis that sorption of alanine occurs as a whole molecule. Abiotic splitting as described for glycine by Wang and Huang (2003) seemed to be of minor importance for alanine sorption.

The high range of microbial uptake rates of alanine bound to various sorbents – differing by a factor of 6 – suggests various sorption strengths and presumably also sorption mechanisms. For a eutric Cambisol, Jones and Hodge (1999) found the highest sorption for positively charged lysine compared to the dipolar glycine and lowest sorption for the negatively charged glutamic acid. Similarly, the mineralization of cationic lysine was higher compared to the dipolar ion leucine in an eutric Cambisol (Gonod et al., 2006). These results clearly support the concept of cation exchange by positively charged amino groups as the main sorption mechanism for amino acids in soil especially for amino acids with a net positive charge.

Investigations of negatively charged LMWOS focused mainly on organic acids. Many studies have demonstrated the ability of LMWOS to sorb by their carboxyl group (Jones, 1998; Jones and Brassington, 1998; Strahm and Harrison, 2008) either by direct ligand-exchange or via cation bridges. Iron oxides are known to effectively bind and stabilize them (Jones and Edwards, 1998; Kaiser and Zech, 2000a). Nagaraja et al. (1970), however, showed that the ability of alanine as a dipolar ion for ligand-exchange mechanism is much lower than for most of the investigated organic acids, which were confirmed by Rothstein (2010). Accordingly, neutral amino acids such as alanine have no clear preference for a charge-dependant sorption mechanism and are either able to interact 1) by ligand exchange via their carboxyl group (Strahm and Harrison, 2008), 2) by cation- or anion exchange with the charged group (Rothstein, 2010) or 3) show non-specific weak interactions such as dipole–dipole and H-bondings, van-der-Waals or entropy-driven hydrophobic interactions (Brigatti et al., 1999).

Our results support diverse but specific sorption mechanisms for alanine: Amount of sorbed alanine is clearly related to the specific surface area of the sorbents, especially in the case of activated charcoal. Specific sorption mechanisms of the investigated sorbents can be concluded from differences in the sorbed amount as well as from differences in the percentage of microbially available alanine. Fast desorption and microbial uptake of alanine sorbed by clay minerals shows its weak bonding, which can either be cation exchange or non-specific weak interactions (Fig. 7). Both mechanisms show exchange reactions with competing molecules. Thus, when sorbent with alanine was added to the soil, cations as well as DOC compounds caused immediate exchange reactions, releasing bound alanine (Fig. 7). This mechanism explains the immediate and pronounced peak in respiration (Fig. 2). This initial $^{14}CO_2$ peak, however, was only a small portion of the totally sorbed alanine (33%). This indicates an additional binding mechanism causing the portion of non-bioavailable alanine in this study. Intercalation into clays is unlikely because this mechanism preferentially stabilizes hydrophobic, aromatic compounds and typically occurs with smectites rather than kaolinites (Wattel-

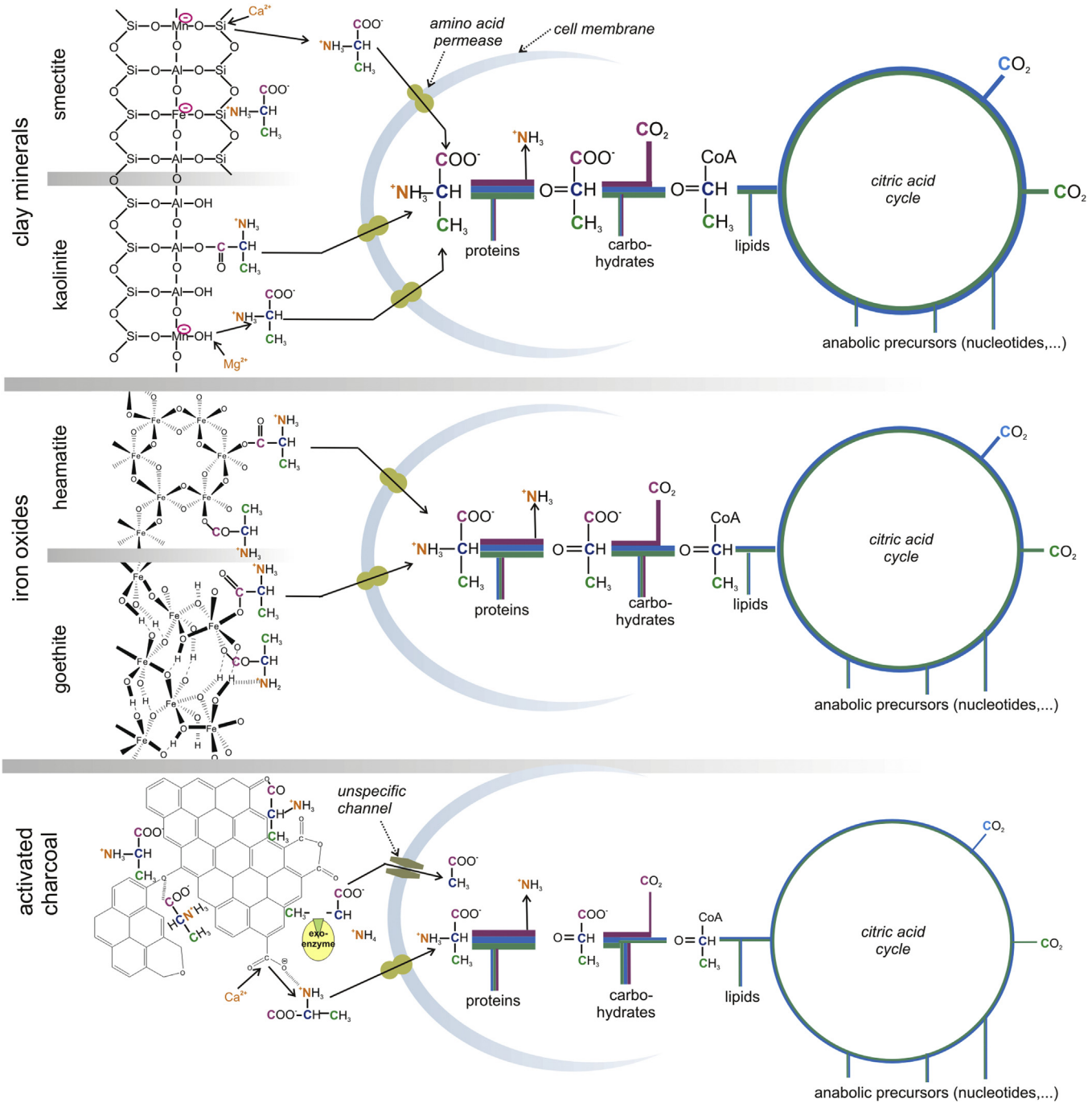

Fig. 7. Metabolic pathways of alanine sorbed on clay minerals (smectite and kaolinite), iron oxides (haematite and goethite) and activated charcoal. Detailed explanations in text. Various colors show the pathways of C from individual positions of alanine. Line width represents the qualitatively estimated relative shifts in the fate of alanine C positions between certain pathways dependent on the sorbent class. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Koekkoek et al., 2001). As the total amount of sorbed alanine is higher for kaolinite than for smectite, another mechanism has to dominate. Boudot (1992) observed that mainly surface $Al(OH)_3$-groups stabilize organic C, probably by interactions with the carboxyl groups of LMWOS. Only kaolinite (not smectite) possesses free $Al(OH)_3$ groups, explaining the higher sorption capacity of kaolinite in this experiment (Fig. 7).

A comparable initial $^{14}CO_2$ peak cannot be observed with alanine sorbed to iron oxides (Fig. 2) indicating other, stronger binding mechanisms. Many studies showed that iron oxides are very strong and efficient sorbents in soil exceeding the capacity of clay minerals (Jones and Edwards, 1998; Kaiser and Zech, 2000a, 2000b). These studies suggested a sorption mechanism based on the carboxyl group of the LMWOS – presumably ligand exchange (Jones and Edwards, 1998; Kaiser and Zech, 2000b; Strahm and Harrison, 2008). This helps explain the strong sorption and slow desorption and microbial utilization of iron-oxide-bound alanine (Fig. 7). Our result that goethite sorbed more alanine, along with the lower bioavailability of the sorbed alanine, supports the mechanistic view of ligand exchange: Goethite is known to have a

higher portion of OH-groups than haematite, which is the relevant functional group for ligand bonding. Nevertheless, a combination of binding mechanisms as well as multi-side coordinative bonds should be considered for interpretation (Kaiser and Zech, 1999).

Activated charcoal is a relevant sorbent in hydrophobic interactions (Choi et al., 2008), and sorption studies with organic molecules of various properties show that aromatic rings are not necessary for interaction with coal structures (Cornelissen et al., 2005b). Hydrophobic interactions by the methyl group of alanine as well as polar-π-interactions with COO^-, $-NH_2$ or NH_3^+ groups are possible (Keiluweit and Kleber, 2009) – all reflecting very strong binding mechanisms. Thus, theoretically each functional group of amino acids can interact with the activated charcoal (Fig. 7). In combination with the large surface of the activated charcoal, this explains its high amount of sorbed alanine. The strong binding is shown by the missing initial mineralization peak and activated charcoal showed the slowest respiration kinetics as well as the lowest proportion of bioavailable alanine (Fig. 2 and Table 2). This confirms the view of Keiluweit and Kleber (2009) and Cornelissen et al. (2005a) on coal's high sorption capacity for a broad range of LMWOS, which is presumably even higher for activated charcoal.

Caution should be exercised in transferring these results and supposed mechanisms directly to natural soil conditions, because we used systems showing no initial covering of the sorbents' surface at the experiment start. Under soil conditions, however, a high proportion of sorbent positions are likely to be occupied by a diverse spectrum of ions and organic compounds. In addition, the adsorption properties of sorbents may strongly change if multilayer sorption (as described by the zonal model of Sollins et al. (2007)) takes place in soils. Assuming this sorption model, the mechanisms investigated here reflect only contact zone sorption, and the situation under soil conditions becomes more complex. At the same time, only a small percentage of the sorbent's surface may be covered by organic films according to the multilayer model (Heister et al., 2012). Therefore, the relevance of complex multilayer sorption versus direct interaction with the mineral surfaces is not yet quantified.

4.2. Bioavailability of sorbed alanine

The available C in the soil as well as the bioavailable portion of the sorbed alanine are potential microbial C sources. The results showed that for each of the sorbents a distinct portion of the sorbed alanine is bioavailable and can be mineralized by microorganisms (Table 2). Dashman and Stotzky (1982) showed, for clay minerals, that permeases have a higher affinity for amino acids than their sorbents. Hence, from a kinetic and energetic point of view, microorganisms located close to a sorbent can take up at least parts of the sorbed amino acids. Thus, two mechanisms can explain the partial bioavailability of sorbed amino acids: 1) passive desorbing due to exchange with DOC and ions from the soil and 2) active desorbing by microorganisms that is followed by active uptake through their membranes (Fig. 7).

Protein expression of the respective transport proteins is a fast process (Jones et al., 1996). The respective amino acid transporters as well as degradation pathways are ubiquitous, enzymes and transporters constitutively expressed (Anraku, 1980; Gonod et al., 2006) and consequently it can be expected that they are already present in the microbial community living based on the available soil C sources when the experiment started. The establishment of microbial communities that live near or on the sorbents' surface is a more time consuming process (Chenu and Stotzky, 2002) and probably explains the observed delay in microbial utilization of sorbed amino acids (Jones and Hodge, 1999; Gonod et al., 2006). An additional explanation might be that alanine is slowly desorbed from inaccessible domains of the sorbents and time for desorption as well as diffusion delays the uptake of alanine by microorganisms. Nonetheless, after establishment of such a sorbent-associated microbial community, e.g. in biofilms, a positive feedback reaction can be expected: The sorbent might provide a co-location of various growth resources (sorbed DOC + nutrients from the soil), and biofilms themselves are able to establish synthrophies (Schink and Friedrich, 1994; Schink, 1997). This is consistent with our results, especially for the activated charcoal: The decomposition of alanine to CO_2 is still ongoing at 78 h. Accordingly, even after 78 h, microorganisms may still not have reached all parts of the high specific surface area of activated charcoal (Table 1). Continued mineralization of alanine in each of the treatments (but most pronounced for activated charcoal) can be explained by microorganisms gaining access and colonizing new surfaces. This process was also observed but less expressed for each of the mineral phases.

Conversely, inaccessibility of parts of the sorbent surfaces for microorganisms explains the portion of irreversible sorption and thus stabilization of LMWOS observed in this and other studies (Table 2 and Fig. 7). This inaccessibility is likely to occur in activated charcoal, where large DOC molecules can fill pores and block accessibility and exchange of LMWOS from these pores (Nguyen et al., 2007). Together with the strong sorption mechanism of activated charcoal, this explains the high portion of non-bioavailable amino acids (Table 2). In addition to inaccessibility of the LMWOS, microbial cells or exoenzymes can be immobilized by sorbents and reduce mineralization (Novick and Rozell, 2005; von Luetzow et al., 2006; Jin, 2010). The immobilization of microbial cells and exoenzymes by charcoal can strongly affect the subsequent transformation pathways of alanine.

Sorption occurs as a whole molecule, i.e. all three positions are sorbed together. Accordingly, an increase in inaccessible, not bioavailable alanine at the sorbent would cause an approximation of the DI of all three positions to 1.0. On contrast alanine incorporated into microbial biomass should have a DI complimentary to respired CO_2. This study showed that sorbents with a low portion of accessible alanine and thus a high portion of untransformed alanine bound to the sorbent (such as activated charcoal or goethite) have DIs closest to one (Fig. 6). On contrast, haematite and the clay minerals reveal significant differences between the DI of the individual positions. Hence, a DI close to one reflects a high proportion of untransformed alanine C bound to the sorbent whereas a DI close to the complimentary value of respired CO_2 reflects a dominance of microbial biomass C associated to the mineral surface (Fig. 6). The DI of alanine C, however, was never exactly 1.0 for any of the sorbents after 78 h (Fig. 6). This shows that at least some of the surface-associated alanine C was transformed to metabolites. This part shows the classical pattern of alanine-C utilization of microbial cells (Dippold and Kuzyakov, 2013): preferential incorporation of C-2 and C-3 in microbial biomass due to a preferential mineralization of the carboxyl group (C-1). This result supports the idea that cells that approach the sorbents or even establish as biofilms on their surfaces are mainly responsible for the utilization of sorbed LMWOS.

4.3. Pathways of microbial metabolization of sorbed alanine

The DI enables a better comparison between the sorbents. This is because DI is not overprinted by absolute differences in the uptake and utilization rates and amounts. The DIs of incorporation of single C positions into the decomposed and sorbed pool were calculated and revealed significant differences (Suppl. Table 1): DI

of the respired CO_2 showed, for each sorbent, a preferential respiration of the C-1 group and a preferential stabilization of the C-3 group (Fig. 3). Thus, the metabolization of bound alanine generally follows the same pathways as demonstrated for free alanine (Dippold and Kuzyakov, 2013): 1) deamination to pyruvate, 2) entering glycolysis and decarboxylation to acetyl-CoA and 3) successive oxidation in the citric acid cycle. This enables applying the C-1/C-2,3-ratio – used by Dijkstra et al. (2011a) for pyruvate metabolization in soil – to alanine metabolization to determine the ratio of glycolysis to citric-acid cycle oxidation. Both this ratio (Fig. 4) and the fitted kinetic constants for C metabolization via ana- and catabolism (k_{inc} and k_{resp} in Table 2) showed a clear effect of the sorbent, i.e. the sorption mechanism, on the metabolization of alanine. The uptake rate k_{upt} showed that, in contrast to sorption, desorption and microbial uptake do not necessarily occur as an intact molecule. This means that the alanine molecule will be split just before, during or immediately after microbial uptake. The exoenzymes using alanine exist in soil but play a minor role for the utilization of free alanine (Dippold and Kuzyakov, 2013). For strongly sorbed molecules the uptake of intact alanine by microbial cells may no longer be possible. Indeed, some functional groups of alanine (e.g. COOH groups or amino-bound C) might still be accessible for exoenzymes and consequently will be split before transformation products enter microbial cells (Fig. 7). This was already described for charcoal surfaces by the co-location model of Lehmann et al. (2011), which explains the accumulation of microbial cells as well as their enzymes on the sorbent surface. Thus, parts of the molecules might be taken up as split fragments after exoenzymatic cleavage. However, the kinetic constants k_{inc} and k_{resp} differed more strongly between the positions than the uptake constant k_{upt}. This indicates that the main splitting of the C skeleton of alanine occurred within the microbial cells (Fig. 7) and that further specific pathways of cells and enzymes located at charcoal surfaces occur. This cannot be resolved within the present study.

The C-1/C-2,3-ratio reveals that C-1-oxidation by glycolysis occurred faster and with higher intensity than the C-2 and C-3 oxidation by the citric acid cycle. The initially exchanged alanine is immediately taken up by the microorganisms (Fischer et al., 2010; Dippold and Kuzyakov, 2013) and the C-1 is metabolized very fast via glycolysis (Fig. 7). With a temporal delay the oxidation via the citric acid cycle starts. The glycolysis peaks much less for the alanine sorbed to iron minerals and does not occur for that sorbed on activated charcoal (Figs. 4 and 5). Thus, highly available free alanine is metabolized by a different intensity of metabolic pathways than sorbed alanine. Kinetics of desorption versus kinetics of microbial uptake determines the relative availability of alanine in soil solution, which determines the C allocation in microbial metabolism (Dippold and Kuzyakov, 2013). If alanine has to be removed from sorbents by microorganisms this occurs by time- and energy-consuming mechanisms. Two possible reasons might explain the shifts in alanine metabolization if it requires microbial induced desorption: Dashman and Stotzky (1982) already discussed that the more intensive a substrate is bound to a sorbent, the less attractive it is for catabolism because the energy efficiency of that substrate decreases. If, however, C- or N-demand for the anabolism exists, then these substrates are nevertheless desorbed and used, but mainly by anabolic pathways. It has to be considered that in addition to sorbed alanine further C sources were available from the added soil – which might be preferentially used the more inaccessible the alanine is. If we compare the ratio of the kinetic constants for mineralization and incorporation k_{resp}/k_{inc} of the five sorbents investigated in this study, clay minerals ($3.4 < k_{resp}/k_{inc} < 3.8$) exceed iron minerals ($2.3 < k_{resp}/k_{inc} < 2.8$), with the lowest value shown by activated charcoal (1.2). This is consistent with the concept of Dashman and Stotzky (1982) and reflects the increasing portion of anabolic C utilization with increasing sorption strength.

In contrast, Jones and Edwards (1998) argued that cell metabolism might change if cells are attached to surfaces: They have an increasing demand in structural cellular components needed to attach to the surface or for the formation of biofilms like extracellular polysaccharides (Chenu and Stotzky, 2002). This would cause a high demand for gluconeogenesis products and, in turn, dampen the opposite process – glycolysis (Fig. 4). This phenomenon was observed for charcoal for the entire 78 h; it was less expressed with the iron oxides and was not visible for microorganisms using alanine bound on clays.

Our approach cannot definitively distinguish between the potential explanations given by Dashman and Stotzky (1982) or Jones and Edwards (1998). Answering this question would require a metabolite tracing, i.e. characterization of the newly formed microbial products from alanine C. It has to be considered that data based on the modeling approach e.g. the kinetic constants k_{inc} and k_{resp} have to be considered carefully: the model strongly simplifies reality e.g. not considering backflux from stable microbial products towards fast cycling microbial metabolites. This simplification can cause a worse fit of the model especially for the last time points where slower processes, not considered here, become more relevant. Thus, slow processes (e.g. the further mineralization observed at late time points) might be underestimated by this approach. Nevertheless, DI and modeling revealed that with decreasing bioavailability of a substrate due to sorption, an increasing relative portion of this substrate is incorporated into microbial C and this microbial C remains partially associated with the sorbents' surface.

4.4. Stabilization of amino acid C by sorption

Clearly, interactions with sorbents stabilize DOC and also LMWOS compounds in soils (Kaiser and Kalbitz, 2012). In a similar experiment with lysine, 3.6% of the lysine sorbed on soil was respired (Gonod et al., 2006). This rate was 10 times lower than the decomposition of free lysine, and also 6.6 times lower than the average alanine mineralization observed in this experiment (23.7% of added U-alanine). This can be explained by the two-times-higher sorption of positively charged lysine (two NH_2^+ groups) compared to dipolar ions such as glycine or alanine (Jones and Hodge, 1999). Indeed, Jones and Hodge (1999) observed a maximally 3 times higher sorption of positively charged compared to dipolar ion amino acids. Thus, an additional mechanism preventing microbial utilization must have occurred in Gonod's experiment (2006), e.g. spatial inaccessibility within the intact aggregates or enhanced sorption due to a multilayer sorption with additional sorption sites and mechanisms present within soils. The pure minerals used in this study do not allow clear conclusion about sorption mechanisms on partially saturated soil surfaces. In addition, spatial inaccessibility in aggregates and micropores is of minor relevance for this experiment, as we used fine powdered minerals in a shaken soil suspension. However, binding of microbial cells or exoenzymes to strong sorbents may contribute to a reduced utilization of alanine in treatments with strong sorbents. Stabilization by sorption might even be more pronounced in natural soils than in individual clean sorbents because additional stabilization mechanisms like spatial inaccessibility might have synergistic effects there.

Our study shows that a remarkable percentage of sorbed alanine is still microbially available and that direct interactions are not the only mechanisms explaining how LMWOS-C can be stabilized by sorbents. The DI of the bound alanine after 78 h clearly demonstrates some of the sorbed alanine C is already microbially

transformed (Fig. 6). Presumably, anabolic cellular as well as extracellular components accumulate on the sorbents' surface and sorption sites (Dashman and Stotzky, 1982; Jones and Edwards, 1998; Miltner et al., 2012). This corresponds to an increase in microbial polysaccharides and proteins associated on the mineral surfaces with increasing soil development (Duemig et al., 2012). Similar results were observed for aging charcoal (Lehmann et al., 2011). Thus, the direct stabilization of LMWOS-C by sorbents is potentially less relevant for the stabilization of C by mineral interactions: Interestingly, the microbially desorbable LMWOS may contribute even more to C stabilization than the irreversibly bound ones if initiating the accumulation of microbial products on the mineral surfaces (Miltner et al., 2007). These products are probably more recalcitrant than the initial LMWOS because they are polymeric polysaccharides, proteins or larger lipids. Nonetheless, they exhibit multiple sorption sites which allow a more intensive binding to the mineral surfaces. They may also initiate a more diverse sorption surface (Sollins et al., 1996) that may have a self-enhancing effect on further stabilization by sorption.

5. Conclusion and outlook

This study demonstrates that stabilization of LMWOS-C by sorption is a complex process: amino acids will be sorbed as whole molecules, but by various sorption mechanisms to the individual sorbents. Clay minerals show a combination of exchangeable, weak binding of alanine as well as stronger interaction with Al–OH-groups, protecting alanine from microbial degradation. Iron oxides sorbed a higher amount of alanine presumably by ligand exchange with the carboxyl group. Especially for goethite, a low portion of sorbed alanine was available for microorganisms. Highest sorption capacity as well as sorption strength was measured for activated charcoal. Modeling reflected that between 22% (activated charcoal) and 44% of sorbed alanine C can be microbially used, but microbial transformation products can be further stabilized by the sorbents.

We conclude that the stronger the sorption by the individual sorbent, the lower the microbial utilization. The fate of individual molecule positions showed that, at least for the four mineral phases, the alanine is used by the classical biochemical pathways: deamination, decarboxylation of C-1 and further oxidation of C-2 and C-3 in the citric acid cycle. The ratio of C-1 oxidation in glycolysis versus oxidation of C-2 and C-3 in the citric acid cycle depends on the microbial availability of alanine: high availability of sorbed DOC and alanine due to fast cation exchange causes an initial peak in C-1 oxidation by glycolysis and an abrupt shift to oxidation via the citric acid cycle – i.e. high amount of energy production by catabolism. Low microbial availability of sorbed alanine, in contrast, leads to a slow, parallel oxidation of all three positions by glycolysis and the citric acid cycle and a large transfer of C toward maintenance anabolism. The DI of the alanine C remaining in soil after three days reflects a mixture of untransformed sorbed alanine ($DI_{1,2,3} = 1$) and microbial transformation products ($DI_1 < 1$ and $DI_{2,3} > 1$): The higher the microbial availability, the higher the portion of bound alanine C present as microbial transformation products.

Activated charcoal shows a deviating behavior, with preferential stabilization of C-3 and oxidation of C-1 and C-2. This indicates that the hydrophobic C-3 is preferentially stabilized by charcoal and that, in addition to basic microbial mechanisms, further pathways of alanine transformation occur (e.g. by exoenzymes).

Details on the pathways and the newly formed microbial products can be further deepend combining position-specific labeling with compound-specific isotope analysis of microbial products (Apostel et al., 2013). In addition, studies with a broader range of LMWOS with deviating sorption properties as well as sorption under natural soil conditions are needed. This will yield a more mechanistic understanding of the processes leading to a stabilization of C by sorption.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.01.015.

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