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Sorption, microbial uptake and decomposition of acetate in soil: Transformations revealed by position-specific ^{14}C labeling

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ABSTRACT

Many previous studies on transformation of low molecular weight organic substances (LMWOS) in soil were based on applying ^{14}C and/or ^{13}C labeled substances. Nearly all these studies used uniformly labeled substances, i.e. all C atoms in the molecule were labeled. The underlying premise is that LMWOS transformation involves the whole molecule and it is not possible to distinguish between 1) the flux of the molecule as a whole between pools (i.e. microbial biomass, CO_2, DOM, SOM, etc.) and 2) the splitting of the substance into metabolites and tracing those metabolites within the pools.

Based on *position-specific* ^{14}C labeling, we introduce a new approach for investigating LMWOS transformation in soil: using Na-acetate labeled with ^{14}C either in the 1st position (carboxyl group, $-COOH$) or in the 2nd position (methyl group, $-CH_3$), we evaluated sorption by the soil matrix, decomposition to CO_2, and microbial uptake as related to both C atoms in the acetate. We showed that sorption of acetate occurred as a whole molecule. After microbial uptake, however, the acetate is split, and C from the $-COOH$ group is converted to CO_2 more completely and faster than C from the $-CH_3$ group. Correspondingly, C from the $-CH_3$ group of acetate is mainly incorporated into microbial cells, compared to C from the $-COOH$ group. Thus, the rates of C utilization by microorganisms of C from both positions in the acetate were independently calculated. At concentrations of 10 $\mu mol\ l^{-1}$, microbial uptake from soil solution was very fast (half-life time about 3 min) for both C atoms. At concentrations $<100\ \mu mol\ l^{-1}$ the oxidation to CO_2 was similar for C atoms of both groups (about 55% of added substance). However, at acetate concentrations $>100\ \mu mol\ l^{-1}$, the decomposition to CO_2 for C from $-CH_3$ decreased more strongly than for C from $-COOH$.

We conclude that the application of position-specifically labeled substances opens new ways to investigate not only the general fluxes, but also transformations of individual C atoms from molecules. This, in turn, allows conclusions to be drawn about the steps of individual transformation processes on the submolecular level and the rates of these processes.

1. Introduction

Low molecular weight organic substances in soil consist, beyond carbohydrates and amino acids, mainly of low molecular weight organic acids (LMWOA) (Jones et al., 2004). LMWOA in soil originate from root exudation (Farrar et al., 2003; Kraffczyk et al., 1984; Fischer et al., in press) or decomposition of plant and microorganism residues, as well as, humified organics. Plants release LMWOA actively to increase the concentrations of dissolved ions such as Fe^{2+}, Cu^{2+}, Mn^{2+} and PO_4^{3-} and to detoxify high concentrations of Al^{3+} (Dakora and Phillips, 2002; Jones et al., 2003). Therefore, low nutrient supply (especially P) frequently boosts root exudation of LMWOA (Neumann and Römheld, 2002; Gerke et al., 1994).

Once in soil solution, LMWOA face a variety of fates: microbial utilization and decomposition to CO_2, sorption by mineral particles and soil organic matter, or leaching. Some studies (e.g. Jones and Darrah, 1995, 1996; Jones, 2004) indicate that LMWOA can even be re-incorporated by plants. Biernath et al. (2008), however, found that direct uptake of acetate (as representative for LMWOA) by maize plants was very low (<1% of added). Rapid mineralization of LMWOA leads to short half lives of a few min to few hours in soil solution (Boddy et al., 2007; Ryan et al., 2001). The mean residence time of C from LMWOA in microorganisms varies between 1 h and a few days (e.g. Kuzyakov and Demin, 1998). Aside from incorporation and sorption, LMWOA are subject to leaching. All these

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concurrent processes lead to removal of LMWOA from the soil solution and therefore to low concentrations of about 0.1–1000 μmol l^{-1} (Strobel, 2001; van Hees et al., 2002).

Due to the great variety of pathways by which LMWOA are released to and taken up from soil solution by all life forms in soil, and owing to the high turnover rates, shown by the short half-life times, they are important in the overall soil C cycle to a much greater extent than their concentrations would suggest. The investigation of the fate of C from LMWOA elucidates carbon transformations in a very sensitive transitional pool which is fast susceptible to small ecological changes. Understanding the dynamics of LMWOA transformation is the key for understanding carbon turnover in soil as a whole, which influences our conception on carbon sequestration in soil in general, and the interactions between soil, plants and soil microorganisms in particular.

Most previous studies on the transformation of LMWOA in soil used uniformly ^{14}C or ^{13}C labeled substances i.e. all C atoms in the molecule were labeled. The ^{14}C or ^{13}C served as a parameter of substance distribution between solution, absorbed amount, and decomposition to CO_2. Such labeling also helps distinguish the fate of C from LMWOA versus that from other (unlabeled) organic compounds present in soil in orders of magnitude higher than the LMWOA. The premise of using uniformly labeled substances is that LMWOA is transformed as a whole. However, Hobbie and Werner (2004) report that the $\delta^{13}C$ values of glucose from C3 plants vary within the same molecule by 11.2‰ according to the C atom's position. This hints that C atoms from different positions of one molecule origin from different pathways. A deeper understanding of the pathways of individual C atoms therefore contributes to a more comprehensive elucidation of the isotopic composition of soil organic carbon, C in microbial biomass and CO_2 evolved by decomposition from these pools. Applying uniformly ^{14}C or ^{13}C labeled substances cannot clarify which functional group (in the case of acetate $-CH_3$ or $-COOH$) controlled the processes and whether it was the whole molecule or one of its metabolites.

We overcome the shortcoming of the methods described above and trace C from individual positions in the molecule by using 1-^{14}C-acetate and 2-^{14}C-acetate. This *position-specific labeling* allowed us to distinguish between C from the $-CH_3$ group and C from the $-COOH$ group of the molecule contributing to all processes. In experiments with position-specific labeled amino acids, Fokin et al. (1993, 1994) and Kuzyakov (1997a) found that C from the $-COOH$ group was oxidized to CO_2 faster than C from the alkyl-amino group. Based on these results, we hypothize that microbial utilization of acetate does not occur as a whole, but that more C from the $-COOH$ group will be transferred to CO_2 than C from the $-CH_3$ group. The aims of this study were to determine differences according to the C atom from both functional groups in

- the rate of removal from soil solution by sorption or microbial uptake
- the distribution in the pools "decomposed to CO_2" and "incorporated into microbial biomass", i.e. preferred use of C from both positions for anabolism or catabolism.

Sorption of low molecular organic compounds to the soil matrix can protect these substances from decomposition by microorganisms (e.g. Jones and Edwards, 1998; Kalbitz et al., 2000; van Hees et al., 2002). In order to divide ^{14}C recovery in soil between these two pools (physicochemically adsorbed C and C incorporated into microorganisms) we also investigated the extent of physicochemical sorption in sterile soils. If the results for the two ^{14}C positions differ, then the acetate molecule is split and the C atoms from two functional groups are subject to different fates in the respective processes.

The first experiment yielded information on the rate at which the two functional groups were taken up from soil solution and utilized by microorganisms, and so to assess the time necessary to reach equilibrium between the pools. The second experiment provided information on how the distribution of the two groups in the pools (soil, solution, and CO_2) changed with varying concentrations between 0.01 and 1000 μmol l^{-1} (concentrations relevant for soil solutions).

2. Materials and methods

2.1. Soil

Soil samples were taken from the Ap horizon (upper 10 cm) of a Haplic Luvisol (IUSS Working Group WRB, 2007) from Heidfeldhof near Hohenheim University, Germany. On this site stood fruit trees 12 years prior to sampling time then a continuous rotation of vegetables, legumes, and wheat was established. The soil had the following characteristics: pH 6.9, total C content 1.5%, CEC_{pot} 210 $mmol_c$ kg^{-1}. The texture was 22.6% clay, 62.9% silt, and 14.5% sand (silt loam). The soil was air dried (25 °C, 96 h) and sieved (<2 mm) to remove roots and to homogenize the sample.

2.2. Experimental setup

The study consisted of two experiments. The first experiment was designed to describe the dynamics of acetate removal from soil solution and determine the time needed to reach steady state of the C fluxes between the pools dissolved (measured in soil solution), adsorbed, and incorporated into microbial biomass (measured in soil). The second experiment assessed the distribution of acetate in these pools depending on the initial solution concentration.

For both experiments, 2.5 ml of deionized water containing the respective concentration of acetate and 1.0 g soil were filled into a 15 ml centrifuge tube (VWR, Bruchsal, Germany). Three treatments were tested within both experiments: (1) soil without sterilization, (2) soil sterilized with 25 μl $CHCl_3$, and (3) soil sterilized with 50 μmol l^{-1} NaN_3 and 50 μmol l^{-1} $HgCl_2$. The sterilizing agents were mixed with the dry soil 24 h prior to acetate addition. At the same time, the same amount of deionized water (25 μl corresponding to 7% of WHC) was added to the not sterilized soil (Treatment 1) to initiate microbial growth prior to acetate addition.

For the first experiment, acetate concentration was 10 μmol l^{-1}. The tubes were shaken by hand for 1 min. Then, they were centrifuged for 10 min at 1500 *g*. Samples of 50 μl were taken from the supernatant. Subsequently, the tubes were shaken at 150 rpm on a reciprocating shaker (KS 10 Bühler, Tübingen, Germany) until the next sampling time, when the separation of soil solution by centrifugation was repeated. Samples were taken from soil solution at 1, 28, 85, 145, 220, 276, and 336 min after acetate addition to the soil and represented the amount that was not adsorbed to the soil and not incorporated into microbial biomass. Centrifugation time was not accounted as mixing time. The whole experiment was conducted in triplicates at temperatures ranging from 20 to 25 °C.

In the second experiment the mixing time was fixed at 200 min (as obtained steady state time reached in the first experiment, see below) and acetate additions were varied from 0.01 μmol l^{-1} (typical for root free soil) up to 1000 μmol l^{-1} (upper end for rhizosphere conditions). These additions correspond to 0.025 μmol kg^{-1} soil to 2.5 mmol kg^{-1} soil. As the C of microbial biomass in this soil was about 0.021–0.031% of soil dry matter (Werth et al., 2006), the acetate additions of 1000 μmol l^{-1} correspond to about 20–28% of microbial C.

2.3. Chemicals and radiochemicals

Acetate was added to 1 g of dried, sieved, and re-moistened loamy soil as 2.5 ml aqueous solution and gently shaken for periods between 10 s and 1 d. Concentrations in the soil solution varied from 0.01 to 1000 μmol l^{-1}. ^{14}C activity in the supernatant was measured after centrifugation at 1500 *g* for 10 min and represented not absorbed acetate. Sterilization by $CHCl_3$ or $HgCl_2 + NaN_3$ allowed separating the removal from solution by microbial uptake and decomposition or sorption to the soil matrix.

^{14}C position-specific labeled Na-acetate was purchased from Sigma–Aldrich, Taufkirchen, Germany. 1-^{14}C Na-acetate is $H_3^{12}C-{}^{14}COOH$ and 2-^{14}C Na-acetate is $H_3^{14}C-{}^{12}COOH$. Both substances were mixed with the unlabeled Na-acetate to obtain the following concentrations in soil solutions: 0.01, 10, 100, 500, 1000 μmol l^{-1}. Regardless of the total amount of acetate addition, in each treatment the ^{14}C activity of 5 kBq was used.

2.4. Chemical and radiochemical analyses

^{14}C activity in soil solution was determined by adding 1 ml to the scintillation cocktail (4 ml, EcoPlus, Roth Company, Germany) and measuring the activity of the sample with a scintillation counter (Wallac 1409; EG&G Ltd, Milton Keynes, UK). ^{14}C activity in the soil matrix, i.e. ^{14}C adsorbed to the soil matrix as well as ^{14}C incorporated in microorganisms, was measured after discarding the supernatant and drying the soil (80 °C, 24 h). After drying, total carbon in the soil was converted into CO_2 via an OX 400 Biological Oxidizer (Harvey Instruments Corp., Hillsdale, NJ). The evolved CO_2 was trapped in Carbosorb and subsequently analyzed for ^{14}C activity by liquid scintillation counting.

2.5. Calculation of acetate distribution between the pools

Acetate added to the soil solution can have the following fates: In the sterilized soil, it may remain in the solution or be absorbed to the soil matrix. In the non-sterilized soil, it can additionally be taken up by microorganisms and incorporated in the cells, or be decomposed to CO_2. Methanogenesis, i.e. the decomposition of acetate to CH_4 can be excluded, as this process only runs in completely oxygen-free environments. In the centrifuge tubes more free oxygen was dissolved in the suspension and available in the headspace than could have been consumed due to decomposition.

Utilization by microorganisms can "break" the C–C bond of acetate, making the transformation different for C atoms from both positions in the molecule. Therefore, ^{14}C activity in non-sterilized samples refers only to the respective labeled group rather than the whole acetate molecule.

2.5.1. Description of distribution in pools

The ^{14}C from acetate was chased in soil and soil solution of sterilized and non-sterilized samples. The distribution of the labeled acetate is presented in ^{14}C activity [% of added ^{14}C]. Under sterile conditions, total applied ^{14}C activity (C_T = 100%) was distributed between the 2 phases: in soil solution (C_L) and in phase adsorbed to the soil matrix (C_A):

$$C_T = C_L + C_A \quad (1)$$

with C_L and C_A calculated in percentage of added amount. The relation between C_L [μmol l^{-1}] and C_A [μmol kg^{-1}] as dependent on concentration of added acetate was used to calculate sorption also in the non-sterilized variants.

In non-sterilized soil, ^{14}C in the soil or solution does not refer to the total acetate molecule. Due to microbial decomposition, the bond between the two C atoms can be broken. Thus, ^{14}C in the non-sterilized samples can be either one or a combination of the following: a) (still) complete acetate, b) the sole $-CH_3$ or $-COOH$ group, or c) new substances built by microorganisms that contain previously incorporated ^{14}C from one of the labeled functional groups.

Microorganisms affected C_L and the amount of ^{14}C measured in non-sterilized soil (^NC_S). Therefore, in equilibrium ^NC_S is the sum of LMWOS adsorbed to the soil (^NC_A) and incorporated into microbial biomass (^NC_B):

$$^NC_S = {}^NC_A + {}^NC_B \quad (2)$$

^NC_A depends on the solution concentration in non-sterilized samples (^NC_L). It was calculated from a Freundlich isotherm on the data from the sterilized variants:

$$C_A = k_f * C_L^m \quad (3)$$

With C_L in [μmol l^{-1}] and C_A [μmol kg^{-1}], k_f = Freundlich coefficient [mg^{1-m} l^m kg^{-1}], m = Freundlich exponent [$^-$]. After determination of k_f and m, the solution concentration for the non-sterilized variants was inserted in Eq. (3) to yield ^NC_A. By subtracting ^NC_A from ^NC_S, the fraction incorporated to the microbial biomass (^NC_B) was calculated. ^{14}C not recovered in non-sterilized soil was assigned to a loss of CO_2 from complete microbial oxidation of the respective group ($-CH_3$ or $-COOH$) of the added acetate (Respir, [%]):

$$\text{Respir} = C_T - {}^NC_S - {}^NC_L \quad (4)$$

For clear demonstration of differences in the distribution of the C atoms from both groups we used the preference index (PI). The PI for the compartment x is defined as measured ^{14}C from $-COOH$ in x divided by the respective amount from $-CH_3$ minus 1, see Eq. (5). A positive preference index means that more C from $-COOH$ versus from $-CH_3$ was incorporated in the respective compartment. When PI is 1 for a particular pool, then 100% more C was recovered from $-COOH$ than from $-CH_3$ in that pool.

$$PI_x = \frac{^{14}C \text{ from} -COOH \text{ in x}}{^{14}C \text{ from} -CH_3 \text{ in x}} - 1 \quad (5)$$

2.5.2. Description of dynamics

Parameters of a single first order kinetics equation with horizontal asymptote were fitted to experimental data of the time experiment to determine the rate of removal from the solution up to the steady state:

$$^NC_L(t) = {}^NC_L + (C_T - {}^NC_L)e^{-kt} \quad (6)$$

with ^NC_L (t) = ^{14}C in soil solution at time t [%], ^NC_L = dissolved ^{14}C at equilibrium [%], C_T = ^{14}C added to soil samples (=100%), k = removal rate [min^{-1}], t = time [min].

The fitting procedure was done using SigmaPlot2000 for Windows (SPSS Inc, Chicago, USA) by minimization of least squares. The half-life time of acetate in the soil solution was calculated by

$$t_{0.5} = \frac{\ln 2}{k} \quad (7)$$

2.5.3. Statistics

The experiment was conducted in triplicates. All displayed data are means ± standard errors. Significance of difference between different concentrations was tested by one-way ANOVA. When the test for homogeneity of variances was positive, the post hoc test LSD was used. In the other case, Tamhane's T2 was applied. These tests were performed with SPSS 10.0.1 for Windows, SPSS Inc. Chicago, USA. If not stated otherwise the data are presented as % of ^{14}C added.

3. Results

3.1. Estimation of equilibration period

In sterilized soil, no significant differences between 1-^{14}C and 2-^{14}C were observed. This clearly indicates that such processes as sorption on soil matrix do not destroy the acetate's C–C bond. Acetate sorption was "instantaneous". At the first sampling time after 1 min after acetate addition, sorption accounted for 18% (corresponds to 82% of added ^{14}C remaining in the solution, see Fig. 1a and b) and remained unchanged during the total duration of the experiment.

Irrespective of the ^{14}C position, microbial uptake was completed until the second sampling (after 28 min). The first order decay fit (Eq. (6)) led to half-life times between 3 and 4 min in non-sterile soil solution (Table 1). Within 28 min, ^{14}C from $-CH_3$ decreased in solution more strongly (to 6.3 ± 0.2% of added ^{14}C) than ^{14}C from $-COOH$ (12.9 ± 0.6%).

3.2. Effect of acetate concentration on sorption and microbial utilization

As the transformation of substances in soil depends on their concentrations, we investigated the range between 0.01 μmol l^{-1} (typical for root free soil) up to 1000 μmol l^{-1} (upper end for rhizosphere conditions). The amount of adsorbed acetate peaked at ~40 μmol kg^{-1} soil at the concentration of 100 μmol l^{-1} and did not further increase at higher acetate concentrations. In Fig. 2, the distribution of ^{14}C from the respective groups in the different pools is illustrated for the addition of 100 μmol l^{-1}. At this concentration, CO_2 – production from both groups was similar, while difference in the incorporation into microbial biomass can be observed (19.0 versus 27.8%). The different values for sorption originated from the different solution concentrations. Fig. 3 shows that increased concentration of added acetate led to an increase of ^{14}C recovered in soil solution and a decrease of losses due to decomposition to CO_2, irrespective of the position of ^{14}C in one of both functional groups. Recovery of ^{14}C from $-CH_3$ in solution was significantly lower and recovery in soil significantly higher than for C from $-COOH$ over the whole concentration range. CO_2 losses were similar for C from both functional groups up to 100 μmol l^{-1}. At 500 and 1000 μmol l^{-1}, more CO_2 was produced from C from $-COOH$ than from $-CH_3$.

Fig. 4 shows the preference (PI – preference index) of C from $-COOH$ compared to C from $-CH_3$ in the investigated pools: soil, solution, or CO_2. At low acetate concentrations, the PI for soil solution was about 1.25. Accordingly, in the soil solution more than twice as much C was recovered from $-COOH$ than from $-CH_3$. In the soil, more C from $-CH_3$ than from $-COOH$ was always found. For CO_2 the PI was close to zero up to 100 μmol l^{-1}. At higher concentrations, PI increased, indicating preferred decomposition to CO_2 of C from $-COOH$ than from $-CH_3$. At 1000 μmol l^{-1}, very low decomposition of C from $-CH_3$ to CO_2 caused an increase of the PI up to 165 (not pictured in Fig. 4).

Table 1
Parameters of the first order equation with horizontal asymptote (Eq. (6)) fitted according to removal of C from $-COOH$ and $-CH_3$ from solution due to microbial uptake and decomposition: $^{N}C_L$: ^{14}C in soil solution after 336 min after acetate addition [%], k removal rate [min^{-1}], $t_{0.5}$: C half life [min], calculated from k (Eq. (7)) describing removal from soil solution. Fitted parameters ± SE.

C position	Functional group	$^{N}C_L$ [%]	k	$t_{0.5}$ [min]
1-^{14}C	$-COOH$	12.9 ± 0.6	0.21 ± 0.1	3.75
2-^{14}C	$-CH_3$	06.3 ± 0.2	0.18 ± 0.1	3.23

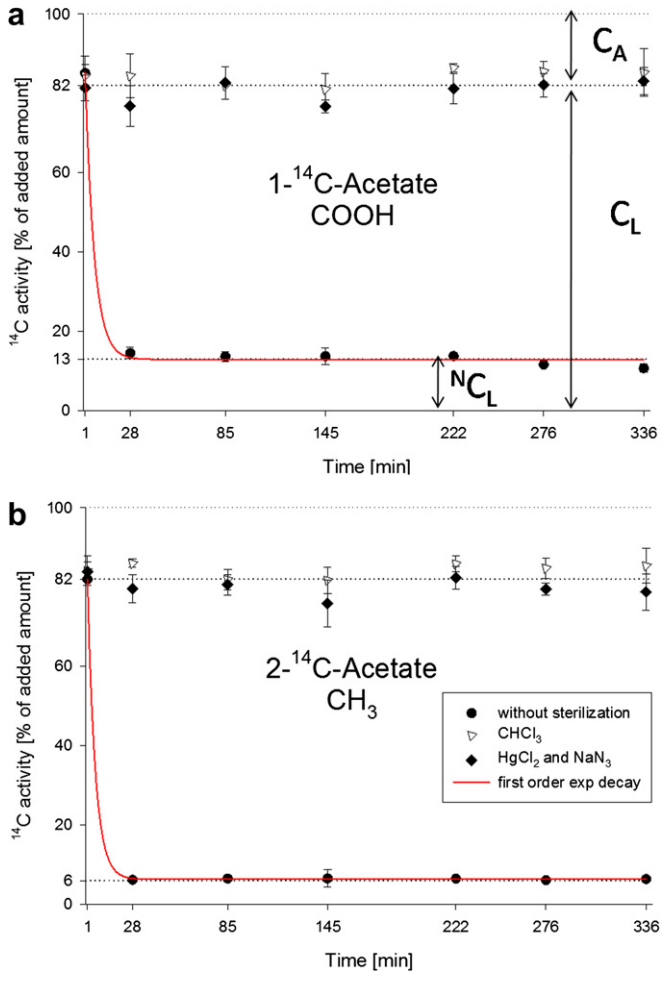

Fig. 1. Dynamics of soil solution concentration in sterile and non-sterilized samples: ^{14}C activity in % of added ^{14}C in soil solution of a) 1-^{14}C and b) 2-^{14}C-acetate at 10 μmol l^{-1} with sterilization (CL) and without (NCL). Experimental points (mean values ± SE, $n = 3$) and model lines fitted based on exponential decay ($^{N}C_L$) are presented. The legend applies to both subplots.

4. Discussion

4.1. Sorption of acetate

Based on similar results of the 1-^{14}C and 2-^{14}C labeling in the sterilized soil (see Fig. 1), we conclude that acetate sorption involves

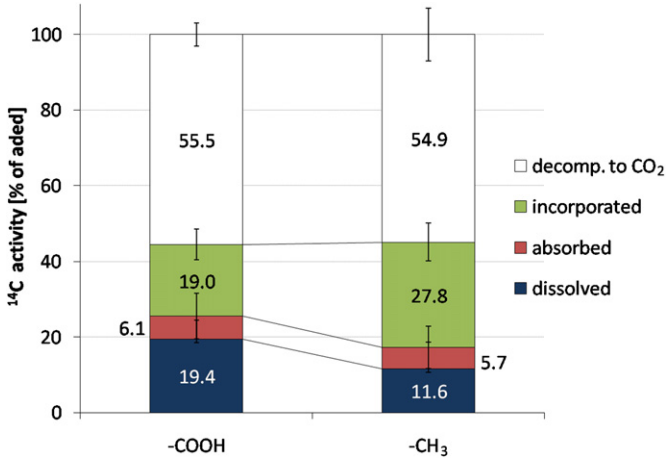

Fig. 2. ^{14}C amounts [% of ^{14}C added] in non-sterilized variants from 1-^{14}C acetate (left column) and 2-^{14}C-acetate (right column) at a concentration of 100 μmol l^{-1} after 300 min, $n = 3$. Means ± SE.

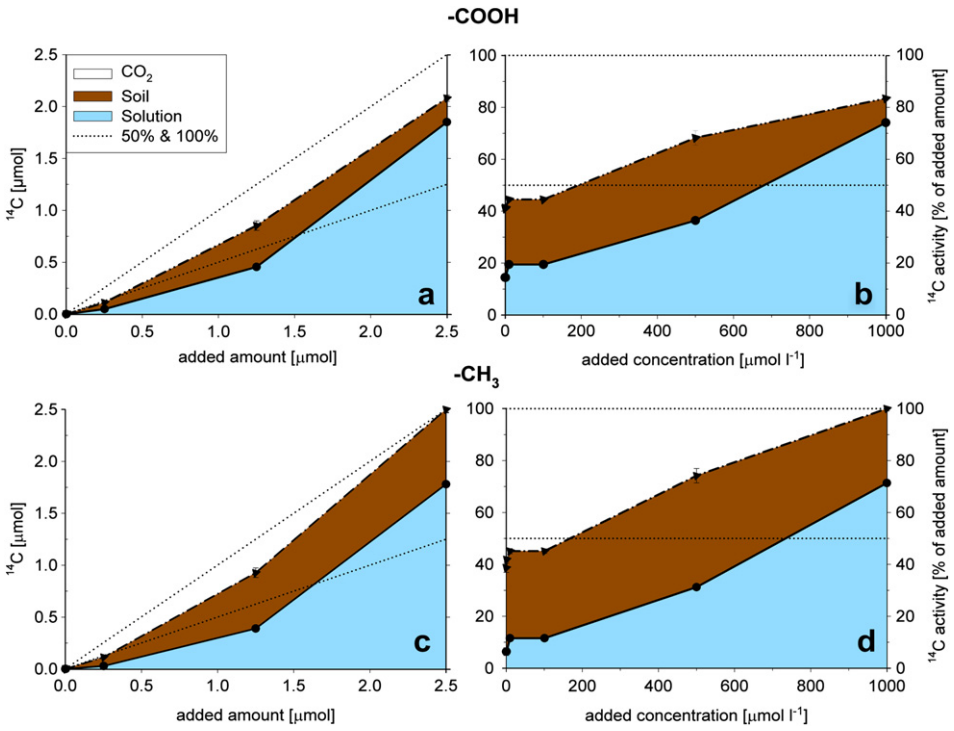

Fig. 3. ^{14}C measured in soil and solution, and calculated as loss due to decomposition to CO_2 from C of 1-^{14}C acetate (−COOH) (above, a) and b)) and 2-^{14}C acetate (−CH3) (below, c) and d)) in µmol (left, a) and c)) and in % of the added amount (right, b) and d)) ($n = 3$, standard errors). Dotted lines are 50% and 100% lines for orientation.

the whole molecule. Consequently, exoenzymes that were probably still active in the sterilized variants did not split acetate. The low sorption of acetate in sterilized soil, which had its maximum capacity at about 45 µmol kg^{-1} suggests that ^{14}C found in soil of non-sterilized variants was predominantly incorporated into microbial biomass and that sorption in non-sterilized soil is minimal. The very little sorption of acetate found by Angeles et al. (2006) for concentrations up to 1.05 mmol l^{-1} was due the monocarboxylic character of acetate. For di- and tricarboxylic acids, sorption >60% of added substance is described (e.g. Jones, 1998; Ström et al., 2001).

The slightly, but significantly higher ^{14}C of acetate in the solution from $CHCl_3$-sterilized soil than in the $NaN_3 + HgCl_2$-sterilized soil (paired samples t-test, $P < 0.05$) were probably due to release of unlabeled organic substances with properties similar to those of acetate from microbial cells whose membranes were dissolved by $CHCl_3$. After release into the soil solution, these unlabeled substances competed with the added labeled acetate for sorption places, slightly increasing the amount of the acetate in the solution.

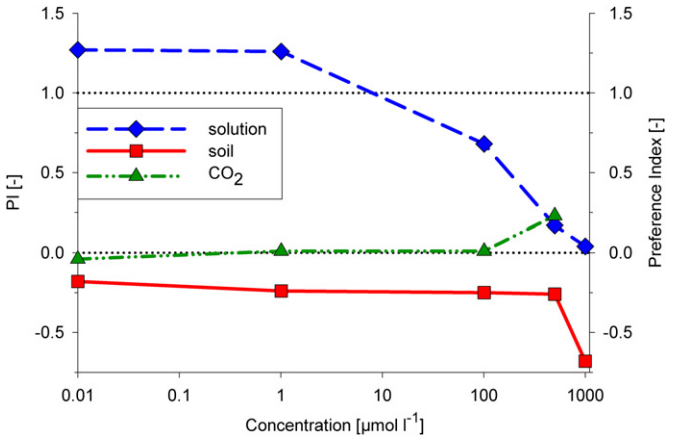

Fig. 4. Preference Index (PI). For explanation see Eq. (5). (^{14}C recovery from COOH/CH_3) − 1. Positive values mean that more ^{14}C from −COOH than from $-CH_3$ was found in the respective compartment: soil solution, soil matrix, or CO_2. A value of "0" means equal recovery of C from −COOH and of C from $-CH_3$ in the pool.

4.2. Rate of microbial uptake of acetate

Acetate sorption was faster than its microbial uptake and utilization (Fig. 1). Very fast (within few min) sorption was formerly described even for larger molecules, such as polycyclic aromatics (Celis et al., 2005). Thus, it is logical that the smaller acetate molecule may be sorbed at least at the same rate. The calculated half life of acetate in non-sterilized soil solution (a few minutes) was shorter than reported in the literature for LMWOA (hours, e.g. reviewed by Jones, 1998). This can be (1) due to the lower acetate concentrations used here than in the literature (10 µmol l^{-1} versus 5 mmol l^{-1}) and/or (2) due to increased contact between acetate and microorganisms during shaking compared to common incubation experiments as e.g. Kuzyakov and Demin (1998) or compared to experiments where soil solution was allowed to settle (Jones et al., 1996). In addition, acetate as very small LMWOA should be turned over faster; causing shorter half lives in soil solution compared to larger LMWOA. Another important reason for a shorter half life is connected with methodological differences in the approaches used: In our study, the half life of acetate refers to the soil solution. In contrast, most other publications did not measure the removal from soil solution; rather, they indirectly derived half lives from C completely oxidized to and trapped as CO_2 (e.g. Boddy et al., 2007). Hill et al. (2008), who measured removal from solution directly, report equally short half lives (about 30 s) for 54 µM glucose. Consequently, most previous studies, which were based on $^{14}CO_2$ or $^{13}CO_2$ release from soil, assessed the half life of C from the added substance in microbial biomass rather than in the soil solution.

Our approach used acetate in realistic concentrations; however the shaking caused an complete mixing of the suspension. In real soil, acetate will not be concentrated equally but will occur in very high spatial heterogeneity in different concentrations. Thus, our results refer to transformations of acetate under ideal, standardized homogenous conditions and should serve as potential values for conditions in the field.

4.3. Microbial utilization of C from the −COOH and the $-CH_3$ groups

As illustrated in Figs. 1, 2, and 4, microbial utilization of acetate's two C atoms points at least partly to different pathways of C from both functional groups. 33.5% of C from $-CH_3$ but only 25.1% of C from −COOH was found in soil after acetate addition of 10 μmol l^{-1}. Accordingly, at least 8.4% of the C from $-CH_3$ must have been incorporated into microbial biomass after breaking of the acetate's C–C bond and without the −COOH group which was released to soil solution. In soil solution, the C–C bond was split at least for 7.8% of the acetate at 10 μmol l^{-1}, with C from −COOH remaining in solution while C from $-CH_3$ was removed from the solution (either due to incorporation into microbial biomass or to decomposition to CO_2). Other authors also reported preferred incorporation into microbial biomass of certain C atoms (Hobbie et al., 2004; Kuzyakov and Demin, 1998; Kuzyakov, 1997a). Hobbie et al. (2004) measured that saprotrophic and ectomycorrhizal fungi preferentially incorporated the glucose C-6 atom (21%) instead of the C-4 atom (9%). Kuzyakov (1997a) found that twice as much C from $-CH_2NH_2$ than from −COOH of glycine and alanine remained in soil. Our results support this: incorporation of C from the terminal CH_3-group is preferred to C from other positions in the molecule. The continuous incorporation of C from $-CH_3$ indicates that the supply with other nutrients (especially N) was sufficient for the microorganisms even at the high acetate additions to grow (and incorporate continuously ^{14}C) at any time.

Apparently, at concentrations <10 μmol l^{-1} the need for acetate as an energy source was so high that no preference of the respiratory system for any C-atom of acetate took place: C from both positions was lost equally as $^{14}CO_2$ (indicated by the PI close to zero, Fig. 4). Based on the more complete and faster decomposition of acetate compared to glucose, we suppose that acetate is not stored in the so-called transitional pool (Hill et al., 2008) but is directly utilized for energy requirements.

At the same concentrations (<10 μmol l^{-1}), microorganisms preferentially used $-CH_3$ for incorporation in biomass (anabolism). During their growth, the uptake of C from $-CH_3$ instead of from −COOH is energetically preferred because the latter C is almost completely oxidized. The energy investment for the reduction of C from −COOH stands in contrast to the general tendency of organisms to utilize C by oxidation. The preferential incorporation of C from $-CH_3$ resulted in an up to two-fold higher ^{14}C activity for C from −COOH than from $-CH_3$ in soil solution. At 500 and 1000 μmol l^{-1}, the preferred incorporation of C from $-CH_3$ versus −COOH even increased. At these concentrations, C from −COOH was preferentially decomposed to CO_2. Yan et al. (1996) showed that adding organic acids such as malate, glycine and acetate increased the pH of the soil solution. That can be explained by the following decarboxylation reaction:

$$R{-}CO{-}COO^- + H^+ \rightarrow R{-}CHO + CO_2 \quad (8)$$

Loll and Bollag (1983) showed that microbial decarboxylation is performed by endoenzymes, i.e. inside the microorganisms. Our results support this finding: In the sterilized treatments, where exoenzymes were still active, the transformations of C from −COOH and from $-CH_3$ were similar. In contrast, in non-sterilized variants (with active endoenzymes) significant differences in the transformations of C from −COOH versus $-CH_3$ were observed. Note that ^{14}C in soil solution can also originate from dissolved $H^{14}CO_3^-$, which is in dynamic equilibrium with the CO_2 of the headspace (Rasmussen and Kuzyakov, 2009). These natural constants (Henry-coefficient, dissociation constants) yield carbonate concentrations in the range of 10^{-5} mol l^{-1}. That means about 1% of the highest acetate addition and a 100-fold of the lowest acetate addition could be dissolved as carbonate in water.

The high percentage of both 1-^{14}C and 2-^{14}C acetate remaining in soil solution at 1000 μmol l^{-1} (see Fig. 3b and d) indicates that 200 min was insufficient to reach equilibrium or that the acetate concentration was too high for complete uptake by microorganisms. After the end of the experiment (200 min) a considerable part of acetate was incorporated but not (yet) decomposed to CO_2. Such an arrest of the substance in microbial cells before actual utilization has been demonstrated for glucose (Nguyen and Guckert, 2001; Hill et al., 2008). Concordantly, Jones and Hodge (1999) found that CO_2 evolution from the decomposition of three amino acids in concentrations from 100 μmol l^{-1} to 1000 μmol l^{-1} continued for 24 h.

As the C from the $-CH_3$ group will be more strongly incorporated into microorganisms than C from −COOH (and thus remains longer in soil), it is explainable that C from the $-CH_3$ group (and other low oxidized C atoms) contributes more to formation of soil organic matter that C from the −COOH group (Kuzyakov, 1996, 1997b).

4.4. Summary of acetate transformation in soil

At least up to 8.4% of added acetate was split, followed by different pathways for C from −COOH and from $-CH_3$. At low concentrations (<100 μmol l^{-1}), 15 ($-CH_3$)–20% (−COOH) of added acetate C remained in solution. About 26 (−COOH)–36% ($-CH_3$) was found in soil (incorporated into microbial biomass or adsorbed to the soil matrix). Consequently, acetate C was mainly decomposed to CO_2 (>50%), but C from the −COOH group was decarboxylated much faster and more completely than oxidation to CO_2 of C from the $-CH_3$ group.

At 500 μmol l^{-1}, incorporation into microorganisms increased (in % of added substance): 32% for C from −COOH and 43% for C from $-CH_3$. These numbers indicate that microorganisms preferentially used C from $-CH_3$ for growth. Percentages of decomposition to CO_2 decreased for C from both labeled groups, but especially for C from $-CH_3$.

Increasing acetate concentrations led to increasing percentages of ^{14}C in soil solution after the end of the experiment. This may be due to 1) insufficient time for the microorganisms to take up the acetate in such high quantities 2) saturation of acetate uptake due to surplus acetate supply.

4.5. Conclusions and outlook

Irrespective of labeled C atom, the distribution between the three pools – incorporated into microbial biomass, decomposed to $^{14}CO_2$ and dissolved ^{14}C – was finished after 3 min. This shows extremely fast transformation of the acetate in soil solution. Such high rates have never been reported before because most studies measured not the disappearance from soil solution, but the release as CO_2 from soil. The simultaneous assessment of ^{14}C distribution between soil matrix, soil solution, and that lost as $^{14}CO_2$ revealed that:

1. The fate of the C atoms of the two functional groups of acetate is controlled by microbial utilization and depends on the acetate concentration.
2. Acetate sorption plays a minor role compared to incorporation into microbial biomass or decomposition to CO_2.

Position-specific labeling of acetate with ^{14}C allowed us to trace the different pathways of C from $-CH_3$ and $-COOH$ groups in soil and to show that:

1. In general, C from $-CH_3$ was preferentially incorporated in microbial biomass and C from $-COOH$ was preferentially decomposed to CO_2.
2. Acetate will be split for metabolites.
3. Exoenzymes do not break down acetate. Therefore, we hypothesize that acetate will be split after uptake into microorganisms by endoenzymes.

We conclude that applying position-specifically labeled substances opens new ways for investigating not only the general fluxes of substances, but also the transformations of individual C atoms from molecules. This allows conclusions to be drawn about the steps of individual transformations processes on the submolecular level and about the rates of these processes.

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