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Microbial uptake and utilization of low molecular weight organic substrates in soil depend on carbon oxidation state

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Abstract The fate of low molecular weight organic substances (LMWOSs) in soil is regulated by microbial uptake. However, C oxidation state, the number of C atoms and –COOH groups in the LMWOS can affect their microbial utilization. Thus, the aim of this study was to reveal the effects of substance chemical properties on initial uptake and utilization of sugars, carboxylic and amino acids by microorganisms. Soil solution, spiked with ^{14}C-labelled glucose, fructose, malate, succinate, formate, alanine or glycine, was added to the soil and ^{14}C was traced in the soil solution, CO_2, cytosol, and soil organic carbon (SOC) over 24 h. The half-life time of all LMWOS in the soil solution varied between 0.6 min (formic acid) and 5.0 min (sugars), indicating its dependence on C oxidation state of the substances. The half-life time of ^{14}C in the fast mineralized pool in microorganisms, ranged between 30 (malic acid) and 80 (glycine) min and was independent on either C oxidation state, the number of C atoms, or number of –COOH groups. This suggests that intercellular metabolic pathways are more important for LMWOS transformation in soil than their basic chemical properties. The portion of mineralized LMWOS increased with their C oxidation state (20% for sugars vs. 90% for formic acid) corresponding to the decrease of C incorporated into the cytosol and SOC pools. Concluding, the physicochemical properties of the common LMWOS allow predicting their microbial uptake from soil solution and subsequent partitioning of C within microbial biomass.

Keywords Carbon use efficiency · CUE · Decomposition kinetics · Dissolved organic nitrogen · Organic acids

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Introduction

Low molecular weight organic substances (LMWOSs) in soil originate from a wide range of sources, including root and microbial exudation, animal

wastes, canopy throughfall, and the decomposition of plant and microbial necromass. Although LMWOS typically represent a small proportion of the total dissolved organic carbon (DOC) pool in soil, they play a critical role in many soil processes, including complexation of metal ions which increases their mobilization (e.g., carboxylic acids), as an important N source (e.g., amino acids) for plants and microorganisms, and as a source of C and energy for microorganisms (e.g., sugars) (Blagodatskaya and Kuzyakov 2013; Grayston et al. 1997; Hill et al. 2012; Kuzyakov and Blagodatskaya 2015). From a global perspective, LMWOS contribute significantly to total soil CO_2 flux (up to 30%) (van Hees et al. 2005) and thus represent an important parameter for modeling of soil organic carbon (SOC) dynamics.

Although LMWOS may be leached, become sorbed to the solid phase, abiotically mineralized or used by plants, their uptake by the microbial communities dominates their longevity in soil solution and represents the first step of their utilization (Glanville et al. 2016). The uptake of LMWOS from solution depends on their properties, namely broad substrate class (e.g., sugars, phenolics, etc.), which determines its subsequent use within cell metabolism (Gunina et al. 2014; Apostel et al. 2013), and concentration, which determines the transport systems used by microorganisms for taking up LMWOS (Hill et al. 2008). In addition, for amino acids it has been shown that substances with low C oxidation states (e.g., lysine) are taken up by microorganisms slower than ones having higher C oxidation states (e.g., glycine and glutamate) (Jones and Hodge 1999), while the fate of carboxylic acids in soil is dependent on their solubility and association with the soil's solid phase (Gunina et al. 2014). Thus, even if the general substance class plays a major role in the fate of LMWOS in soils, the physico-chemical properties of the individual compound are also highly important.

The second step of LMWOS utilization by microorganisms is their incorporation into metabolic cycles and subsequent mineralization to CO_2 or immobilization within cellular components (Apostel et al. 2013). It has also been shown that intercellular metabolism affects the fate of amino and carboxylic acid derived-C in soils (Gunina et al. 2014), as each compound class enters distinct metabolic cycles within the cell. The proportion of each mineralized LMWOS is also linked to the C oxidation state of the substrate. Carboxyl groups (–COOH; C oxidation state = +3.0) are mineralized to CO_2 at a higher amount than methyl groups ($–CH_3$; C oxidation state = −3.0) (Fischer and Kuzyakov 2010). So, the presence of a high number of reduced C atoms in LMWOS molecules can lead to low mineralization and high LMWOS-C incorporation into structural elements of the cell. At the same time, a higher proportion of mineralized C should be observed for substances with high number of oxidized C atoms (e.g., substrates rich in –COOH groups). Additionally, the standard enthalpy of combustion of organic compounds seems to be dependent on substance C oxidation state: for substances with "0"' C oxidation state (e.g., glucose, alanine) the values of standard enthalpy of combustion are in the range 1600–2800 kJ mol^{-1}, whereas for oxidized substances (C oxidation state +1 or +2) the values are lower: 280–1300 kJ mol^{-1} (Roels 1980). Thus, substance physico-chemical properties can directly impact the utilization processes of LMWOS within the microorganisms. In contrast, further fate of C contained within LMWOS may be closely related to cell metabolite turnover, where this C was incorporated during intercellular metabolisation (Glanville et al. 2016).

The aim of the study was to estimate the initial utilization (within 24 h of LMWOS application) of three main LMWOS classes (sugars, carboxylic and amino acids) and to reveal the effect of substance properties on their fate within soil. We hypothesized that: (i) LMWOS half-life times in soil solution will depend on substance properties, namely C oxidation state, number of –COOH groups and size of the molecules, (ii) the half-life of LMWOS-C in microbial biomass pool will depend on the properties of LMWOS and the pathway taken when entering into intercellular metabolic cycles, and (iii) substances with a high C oxidation state will be mineralized to a larger extent than substances with a low C oxidation state.

Materials and methods

Site description and soil sampling

Soil was collected from the BangorDIVERSE long-term forest diversity experiment, located in Abergwyngregyn, North Wales, UK (53°14′16″N, 4°1′1″W)

(Smith et al. 2013; Ahmed et al. 2016). Within this experiment, soil was collected from the replicated Silver birch (*Betula pendula* Roth.) plots. The soil is classified as a fine loamy textured Dystric Fluvic Cambisol (WRB 2006) and has a mixed glacial till parent material. The site has a mean annual soil temperature of 10.6 °C and an annual rainfall of ca. 950 mm. The basic properties of the soil are presented in Table 1 and in Ahmed et al. (2016). At each sampling site, surface litter (ca. 1–2 cm) was removed and the top 10 cm of the mineral soil (A horizon) was collected from four independent locations within each of four replicate plots and combined to make a composite soil sample. Soil samples were stored in gas-permeable plastic bags at 5 °C until extraction of soil solution, which was conducted within 24 h of sample collection. Substrate uptake and mineralization experiments were conducted within 1 week of soil sample collection.

Extraction of soil solution

Soil solution was obtained by centrifugation following the technique of Glanville et al. (2012). Briefly, 100 g of fresh soil was placed into a polypropylene centrifuge tube with a perforated bottom and covered by a fine mesh (pore size 50 µm). This was attached to a base unit which collects soil solution during centrifugation. This construction was centrifuged at $3500\times g$ for 15 min. The extracted soil solution was subsequently passed through a 0.2 µm cellulose acetate filter to remove microbial contaminants and stored at −20 °C prior to use in subsequent experiments.

LMWOS uptake from soil solution

The uptake of LMWOS by the soil microbial community was measured over 24 h for sugars (glucose and fructose), carboxylic acids (malic, succinic and formic acids) and amino acids (alanine and glycine). These substrates were chosen as they are either commonly found in root exudates/lysates or they represents the breakdown products arising from the main organic polymers entering soil (i.e., cellulose/protein). The C oxidation state of each LMWOS was calculated as sum of all C oxidation states divided by the amount of C atoms in the substance (Table 2).

The ^{14}C radiolabeled substances (<10 nM) were added separately to the extracted soil solution (see "Extraction of soil solution" sect) to obtain a total ^{14}C specific activity of 0.83 kBq ml^{-1} for each compound. No additional non-labeled substances were added so that we did not want to change the intrinsic concentrations of the compounds naturally present in soil solution. All LMWOS were uniformly labeled and ^{14}C specific activities of the each initial substances were: ^{14}C-glucose 7.4 MBq ml^{-1}, ^{14}C-fructose 37 MBq ml^{-1}, ^{14}C-malic acid 3.7 MBq ml^{-1}, ^{14}C-succinic acid 3.7 MBq ml^{-1}, ^{14}C-formic acid 35.6 MBq ml^{-1}, ^{14}C-alanine 3.7 MBq ml^{-1}, ^{14}C-glycine 1.8 MBq ml^{-1}.

To measure the depletion of the LMWOS from soil solution, fresh field-moist soil (1.2 g) was placed into a 1.5 cm^3 polypropylene microcentrifuge tube and 0.3 ml of ^{14}C-labelled soil solution was added to the soil surface. The solution immediately infiltrated into the soil. The microcentrifuge tubes were perforated at the bottom and the holes were covered with a small piece of Whatman GF/A glass fiber filter paper (pore size 1.6 µm). These soil-filled microcentrifuge tubes was then placed on top of another empty microcentrifuge tube and the dual-tube array was centrifuged ($14{,}000\times g$, 1 min). The soil solution from the upper tube passed to the lower tube where it was recovered for analysis. Soil solution was obtained 1, 4, 8, 10, 20, 30, 60, 240, 960 and 1440 min after addition of the ^{14}C-labelled solution to the surface of the soil in the upper microcentrifuge tube. ^{14}C activity of the recovered soil solution was measured

Table 1 Selected soil properties

Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})	Moisture content (%)	pH	Total C (g kg^{-1})	Total N (g kg^{-1})	C-to-N ratio
48.2 ± 1.3	33.6 ± 0.9	18.2 ± 2.1	32.9 ± 0.5	5.40 ± 0.03	32.2 ± 1.2	3.2 ± 0.3	10.6 ± 0.3

Soil texture was determined by laser diffraction. pH was measured in 1:2.5 (w/v) soil:distilled water extracts. Total C and N were determined by dry combustion. Values represent the mean ± SE ($n = 4$)

Table 2 Single first order kinetic coefficients describing the depletion of individual carbon substrates from soil solution over time

Substrates	C oxidation state	Pool a_1 (%)	Pool a_2 (%)	k (min^{-1})	Half-life, $T_{1/2}$ (min)
Glucose	0	11.9 ± 4.3	91.6 ± 7.0	0.18 ± 0.03^{d}	3.85 ± 0.12^{a}
Fructose	0	13.9 ± 4.4	91.1 ± 6.9	0.18 ± 0.01^{d}	3.76 ± 0.12^{a}
Formic acid	+2	8.9 ± 0.9	91.0 ± 2.1	1.12 ± 0.11^{a}	0.62 ± 0.08^{e}
Malic acid	+1	12.3 ± 1.6	87.4 ± 3.8	0.68 ± 0.08^{b}	1.03 ± 0.08^{d}
Succinic acid	+0.5	10.9 ± 2.2	88.5 ± 4.9	0.49 ± 0.06^{bc}	1.42 ± 0.09^{c}
Alanine	0	7.7 ± 1.9	93.6 ± 4.1	0.46 ± 0.05^{bc}	1.51 ± 0.07^{c}
Glycine	+1	12.0 ± 2.4	86.8 ± 5.1	0.35 ± 0.05^{c}	1.97 ± 0.09^{b}

a_1 is an asymptote to which ^{14}C activity fells in single exponential curves, a_2 is an estimated pool size for uptake, and k is an uptake rate constant. Half-life ($T_{1/2}$) values are derived from the parameter values for k. Values represent mean ± SE ($n = 4$). Letters reflect significant differences between the substances, confidential interval = 84%. For a_1 and a_2 no significant differences were found

by liquid scintillation counting (Wallac 1409 scintillation counter, Wallac EG&G Ltd., Milton Keynes, UK) using Wallac Optiphase 3 scintillation cocktail (Wallac EG&G Ltd., Milton Keynes, UK). This procedure was also done with sterile soil (autoclaved, 121 °C, 30 min) to determine the importance of abiotic losses of LMWOS from soil solution (i.e., sorption to the solid phase) in the absence of the microbial activity (Hill et al. 2008). Each component of the experiment was replicated four times. The uptake rate of ^{14}C-labelled LMWOS from soil solution was calculated as follows:

$$R = a_1 + a_2 \exp^{-kt},$$

where R is the percent of applied ^{14}C remaining in soil solution, a_1 is an asymptote to which ^{14}C activity fells in single exponential curves, a_2 is an estimated pool size for uptake, t is time and k is an uptake rate constant. The half-life times of LMWOS in soil solution ($T_{1/2\ solution}$) were calculated as ln(2)/k. As the main portion (>80%) of the applied tracer was taken up from soil solution within 60 min, only this period of time is presented, whereas the single first order kinetic equation was fitted to all the data collected over the experimental period (24 h).

LMWOS mineralization in soil

To estimate the mineralization rate of each LMWOS, a similar procedure to that described above was employed except that we measured the rate of $^{14}CO_2$ evolution from the soil. Briefly, fresh soil (1.2 g) was placed into a 1.5 ml microcentrifuge tube and 0.3 ml of each ^{14}C-labeled solution added (according to procedure described above). The microcentrifuge tubes were placed into a larger 50 ml polypropylene container and a 1 M NaOH trap (1 ml) added to capture evolved CO_2 in the closed system. The NaOH traps were changed at 1.5, 3.5, 5.5, 8.5, 13, 22, 24, 25.5 and 27.5 h after LMWOS addition. ^{14}C activity of the NaOH solutions was measured by liquid scintillation counting as described above. To describe mineralization rate of each LMWOS, a double first order kinetic equation was applied to the portion of ^{14}C remaining in the soil [$^{14}C_{SOC}$; calculated as 100 − $^{14}C_{CO_2}$ (%)]:

$$^{14}C_{SOC} = a \cdot \exp^{-k_a t} + b \cdot \exp^{-k_b t},$$

where a and b are pool sizes for the fast and slow mineralization phases, t is time and k_a and k_b are the rate constants for the fast and slow mineralization phases (Glanville et al. 2016). The $T_{1/2}$ for LMWOS-C of the fast and slow phases of C mineralization within the microbial community were calculated as ln(2)/k_a or ln(2)/k_b and will subsequently be referred to as $T_{1/2\text{-}fast}$ and $T_{1/2\text{-}slow}$, respectively.

At the end of the experiment (27.5 h), ^{14}C activity was measured in the microbial cytosol pool using the chloroform fumigation–extraction procedure of Wu et al. (1990). As no extraction efficiency correction factor was applied to the extracted dissolved organic C pool after fumigation (Glanville et al. 2016), this pool was referred to "cytosol" rather than microbial biomass. The amount of ^{14}C remaining in the bulk soil at the end was also measured by combusting the soil at 800 °C in a OX400 biological oxidiser (R.J. Harvey Instrument Corp., USA) and $^{14}CO_2$ measured by scintillation counting after capture in Oxosol

scintillant (National Diagnostics, Atlanta, GA, USA). To obtain ^{14}C in SOC pool (further referred to as ^{14}C-SOC) the ^{14}C portions in CO_2 and cytosol pools were subtracted from ^{14}C in bulk soil, and present the pool containing non-extractable microbial biomass and microbial metabolites. Tracer incorporation into cytosol and SOC pools was presented as a percent of the total applied ^{14}C.

Based on the calculated ^{14}C incorporation into CO_2 and microbial cytosol pools (for the last measurement point: 27.5 h), the anabolism–catabolism ratio was calculated as:

$$\frac{Anabolism}{Catabolism} = \frac{^{14}C_{cytosol}}{^{14}C_{CO_2}},$$

which shows the proportion of ^{14}C used for energy production relative to that incorporated into cell components.

Statistics

Data on ^{14}C in CO_2, cytosol and SOC as well as pool sizes, rate constants and $T_{1/2}$ were subjected to ANOVA and significant differences between the various LMWOS were tested with LSD post hoc test with $P < 0.05$. Exponential equations were fitted to the experimental results using a least squares iteration routine in Statistica 10.0 (Dell Statistica, Inc., Tulsa, OK). The simple regression analysis was performed in Statistica 10.0 (Dell Statistica, Inc., Tulsa, OK) with data on C oxidation state, number of C atoms, number of COOH groups versus LMWOS $T_{1/2\ solution}$, $T_{1/2\text{-fast}}$, $T_{1/2\text{-slow}}$, portion of ^{14}C in SOC, cytosol and CO_2 pools.

Results

Uptake of LMWOS from soil solution

The three classes of LMWOS showed a similar uptake pattern from soil solution based on the ^{14}C depletion from the DOC pool (Fig. 1). Calculated LMWOS-C $T_{1/2\text{-solution}}$ changed in the order: sugars > amino acids > carboxylic acids (Table 2). Glucose and fructose showed a similar $T_{1/2\text{-solution}}$ (3.8 min), which was 1.5–2 times longer than for other the substances. The lowest $T_{1/2\text{-solution}}$ (<1 min) was found for formic acid. Estimates of the total amount of LMWOS ascribed to modelled pool a_2 were similar for all substances (Table 2). There was a negative relationship between the $T_{1/2\text{-solution}}$ of each substrate and its C

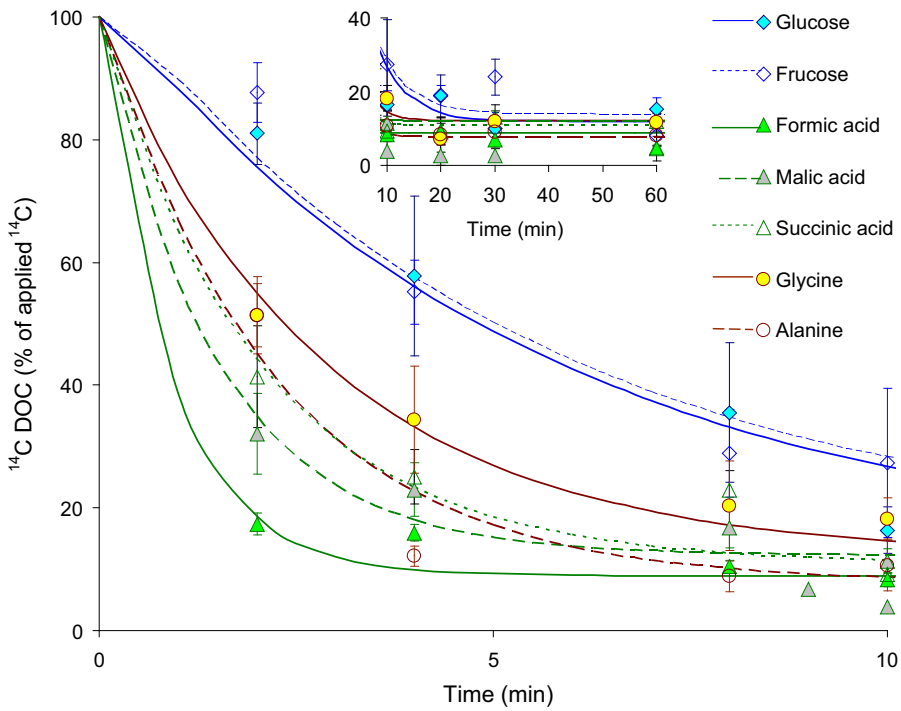

Fig. 1 Temporal dynamics of ^{14}C-labelled sugar, organic acid and amino acid disappearance from soil solution. Values represent mean ± SE ($n = 4$). *Lines* are the following: *blue*: *solid* glucose, *dotted* fructose, *green*: *solid* formic acid, *dashed* malic acid, *dotted* succinic acid, *brown*: *solid* glycine, *dashed* alanine

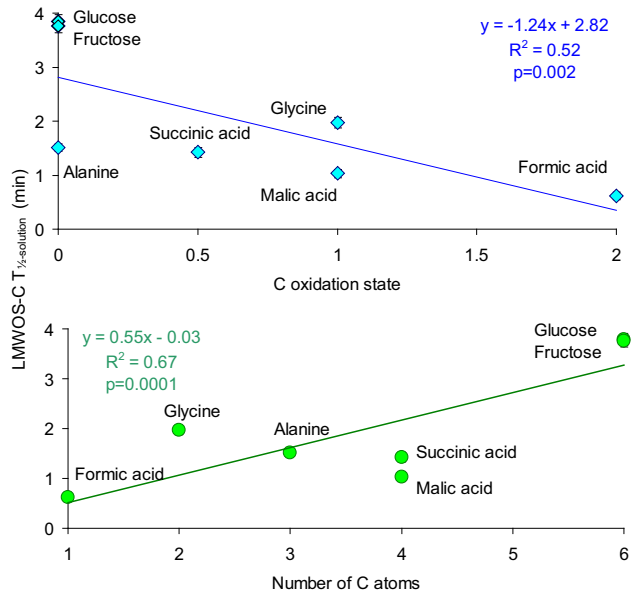

Fig. 2 Relationship between the half-life (min) of different LMWOS in soil solution and their C oxidation state (*top panel*) and number of C atoms in the molecule (*bottom panel*). Values represent mean ± SE ($n = 4$). The *error bars* for the half-life times of LMWOS in DOC are smaller than size of icon *symbols*

oxidation state (Fig. 2, top panel) and number of –COOH groups (Supplementary material; Fig. S1). Furthermore, there was a positive relationship between the $T_{1/2\text{-solution}}$ of all LMWOS and the number of C atoms within the individual substrates (Fig. 2, bottom panel). Results for the autoclaved soil (Supplementary material; Fig. S2) showed some dilution with the intrinsic soil solution and that sorption can occur for some substances (e.g., carboxylic acids and, glycine). However, as shown previously (Fischer et al. 2010), biotic uptake of LMWOS out-competes the abiotic sorption processes, from which we predict that sorption processes will not greatly influence the results in the non-autoclaved soil.

Mineralization of LMWOS in soil

Mineralization patterns were similar for all three LMWOS classes, namely the highest portion of C was mineralized in the first 5 h, and later ^{14}C-CO_2 reached a plateau (Fig. 3). The maximum proportion of mineralized LMWOS was found for carboxylic acids, followed by amino acids and sugars (Fig. 3). Overall, 15–80% of the applied LMWOS were decomposed to CO_2 within the first mineralization phase (pool a, k_a) depending on substance class (Fig. 3). Constant rates for the first mineralization phase were between 0.5 and 1.3% h^{-1} and calculated $T_{1/2\text{-fast}}$ values for pool a for each LMWOS-C were in the range of 0.52–1.34 h (30–80 min; Table 3), with the shortest values observed for malic acid and the longest for glycine. The $T_{1/2\text{-fast}}$ values for each LMWOS-C were much longer than those calculated for their loss from soil solution, showing that mineralization does not occur immediately after LMWOS uptake. No significant correlation was found between the $T_{1/2\text{-solution}}$ values of each substrate and it subsequent mineralization during the fast utilization phase (Supplementary materials; Fig. S3).

Constant rates for the second mineralization phase (model pool b, k_b; Table 3), which describes the turnover of substrate-C immobilized in the microbial biomass, were up to three orders of magnitude lower than for the first modeled pool (a, k_a). Calculated LMWOS-C $T_{1/2\text{-slow}}$ ranged between 25 and 290 h, with the shortest values observed for formic acid and the longest for glucose. The $T_{1/2\text{-slow}}$ values for each LMWOS showed relationships with C oxidation state and number of C atoms (Supplementary material; Fig. S5).

The partitioning of LMWOS-C between CO_2, the microbial cytosol and that remaining in SOC is shown in Fig. 4. The maximum proportion of mineralized substances was observed for formic acid, which was followed by malic and succinic acid, amino acids and sugars. In contrast, the ^{14}C recovered in the cytosol and remaining in SOC followed the opposite trend. The proportion of mineralized LMWOS increased

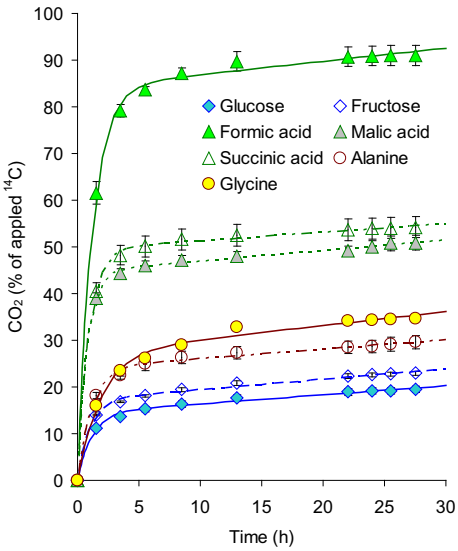

Fig. 3 Cumulative ^{14}C-CO_2 production from mineralization of ^{14}C-labelled LMWOS in soil. Values represent mean ± SE ($n = 4$). In case *error bars* are not present, they are smaller than size of icon *symbols*

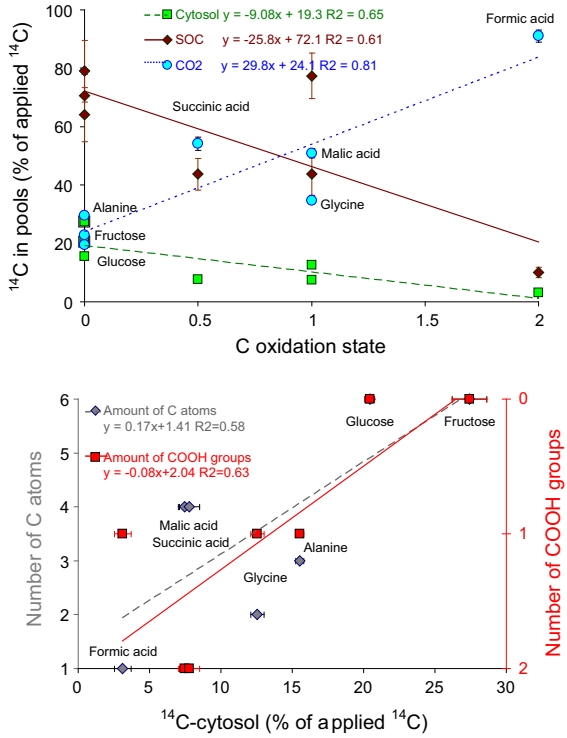

Fig. 4 Relationship between ^{14}C remaining in the cytosol, SOC and CO_2 pools and C oxidation state (*top panel*) and ^{14}C remaining in the cytosol and number of C atoms and –COOH groups (*bottom panel*) in different LMWOS. Values represent mean ± SE ($n = 4$). *P*-values for the regression lines on the *top panel* figure are less than 0.002; *p*-values for the regression lines on the *bottom panel* figure are less than 0.004. The substance names are shown only once

with substance C oxidation state, whereas the amount of ^{14}C incorporated into the cytosol and remaining in SOC (for all substances) followed the opposite trend (Fig. 4, top panel). Additionally, the proportion of LMWOS-C incorporated into the microbial cytosol increased with the number of C atoms present in the molecule and decreased with the number of –COOH groups (Fig. 4, bottom panel). Anabolism/catabolism ratio (Fig. 5) was the highest for the sugars (both glucose and fructose) and for alanine, having zero C substance oxidation states. The lowest value was found for formic acid.

Overall, initial utilization of LMWOS within the microbial biomass was not dependent on the substance properties. In contrast, the total amount of LMWOS-C

Table 3 Double first order kinetic coefficients describing the depletion of individual carbon substrates from soil over time

Substrates	Pool *a* (%)	Pool *b* (%)	k_a (h^{-1})	k_b (h^{-1})	k_a $T_{1/2}$ (h)	k_b $T_{1/2}$ (h)
Glucose	14.3 ± 0.7^{d}	85.6 ± 0.5^{a}	0.88 ± 0.12^{ab}	0.0024 ± 0.0003^{c}	0.79 ± 0.10^{bc}	288.8 ± 0.09^{a}
Fructose	17.1 ± 0.5^{d}	82.9 ± 0.3^{b}	1.01 ± 0.08^{ab}	$0.0028 \pm 0.0002b^{c}$	0.68 ± 0.06^{bc}	247.6 ± 0.05^{b}
Formic acid	82.8 ± 2.5^{a}	17.2 ± 1.9^{g}	0.87 ± 0.07^{b}	0.0270 ± 0.0070^{a}	0.80 ± 0.05^{b}	25.7 ± 0.19^{e}
Malic acid	44.6 ± 1.4^{b}	55.4 ± 0.9^{d}	1.33 ± 0.14^{a}	0.0044 ± 0.0008^{b}	0.52 ± 0.08^{c}	157.5 ± 0.13^{d}
Succinic acid	49.4 ± 2.5^{b}	50.6 ± 1.6^{e}	1.11 ± 0.17^{ab}	0.0039 ± 0.0020^{bc}	0.63 ± 0.11^{bc}	177.7 ± 0.31^{c}
Alanine	23.9 ± 1.5^{c}	76.0 ± 1.0^{c}	0.86 ± 0.15^{ab}	0.0028 ± 0.0007^{bc}	0.81 ± 0.11^{bc}	247.6 ± 0.18^{b}
Glycine	26.7 ± 1.2^{c}	73.0 ± 1.0^{c}	0.52 ± 0.06^{c}	0.0044 ± 0.0007^{b}	1.34 ± 0.07^{a}	157.5 ± 0.11^{d}

Pool *a* and *b* are the estimated pool sizes for the fast and slow mineralization phases, respectively, while k_a and k_b are the rate constants describing the rate of turnover of these two pools. $T_{1/2}$ values are the half-times for pools *a* and *b* determined from k_a and k_b, respectively. Values represent mean ± SE ($n = 4$). Letters reflect significant differences between the substances, confidential interval = 84%

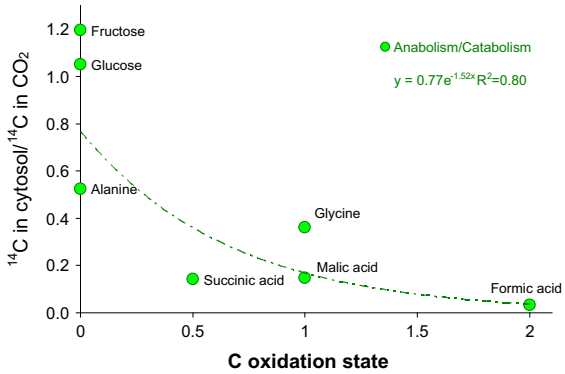

Fig. 5 Relationship between ^{14}C incorporated into cytosol (anabolism)/^{14}C incorporated into CO_2 (catabolism) and C oxidation state at the end of LMWOS mineralization experiment

which can be utilized (including mineralization to CO_2 and incorporation into cellular compounds) within the microbial biomass was clearly dependent on the physico-chemical properties of the individual substrates.

Discussion

In this study, the utilization of LMWOS in soil focused on: (i) the initial rate of uptake from soil solution, (ii) mineralization to CO_2, and (iii) subsequent utilization and partitioning of C within the microbial cells. These processes were studied within 24 h, to deduce the initial fate of LMWOS-C rather than the turnover of secondary metabolites within the microbial community or the turnover of the biomass itself (i.e., necromass). The fate and flux of LMWOS was studied at natural concentrations (soil solution was only labeled at trace levels for each ^{14}C-compound), to best reflect conditions which naturally exist in the field. This contrasts with almost all previous studies which have used high substrate addition rates to investigate LMWOS turnover in the soil. Although these former studies may reflect pulse additions of soluble C arising from root lysis or organic waste addition, they misrepresent the much lower concentrations of LMWOS produced by the slower turnover of more recalcitrant (and arguably more important) pools of soil organic matter.

Uptake of LMWOS from soil solution

We found that up to 90% of the applied LMWOS were taken up from soil solution within the first 10 min (Fig. 1). Similar results have been found for glucose applied to soil in the concentration range from 1 μM to 10 mM (Hill et al. 2008). The rapid removal of substrates can be attributed to the rapid uptake of LMWOS by the C-limited soil microbial community, extracellular enzymatic decomposition or sorption on the mineral phases. For most neutral or monovalent LMWOS, microorganisms represent the dominate loss pathway from solution, particularly in comparison to sorption to mineral phases (Fischer et al. 2010). In the case of di- and tri-valent substrates, however, sorption can significantly suppress microbial uptake, especially in soils containing large amounts of Fe and Al oxyhydroxides (Jones and Edwards 1998), however, it was not the case in our study. We attempted here to estimate the effect of abiotic sorption processes by measuring the loss of LMWOS under sterile (autoclaved) and non-sterile soil. Sorption had low importance in the fate of LMWOS because larger percent of ^{14}C was removed from soil solution in non-sterile soil compare to sterile for the same time interval. This is the consequence of neutral pH values and low contents of Fe and Al in the soil. Overall, our results are consistent with microbial transformation being the dominant process. Although extracellular enzymes may exist in soil solution and could extracellularly cleave our substrates (e.g., deaminases acting on alanine or glycine to produce pyruvate and lactate), we expect this transformation pathway to be insignificant in comparison to the direct uptake by microbial membrane transporters.

The fastest uptake rates from solution and subsequent $T_{1/2\text{-solution}}$ values (0.6–1.5 min) were found for carboxylic acids while the slowest $T_{1/2\text{-solution}}$ value was found for sugars (3.7 min; Table 2). Although the rate of depletion was very rapid for all substrates, the variation in uptake rate can be attributed to differences in (i) relative diffusion speed of the substrates in solution, (ii) different affinities and expression of the various transport systems within the microbial community, and (iii) rate of intracellular processing of the various substrate classes which may feedback on transporter activity (Hill et al. 2008; Jones and Edwards 1998). The $T_{1/2\text{-solution}}$ of carboxylic and amino acids decreased with the C oxidation state of substances suggesting that LMWOS with low C oxidation states remain in soil solution longer than ones which are highly oxidized. At the same time, LMWOS $T_{1/2\text{-solution}}$ values increased with the number of C atoms indicating

that substances with a lower molecular weight are taken up faster. For substances with a similar C oxidation state (both sugars and alanine), a longer $T_{1/2\text{-solution}}$ was found for larger molecules although more substrates would need to be tested to confirm this. Overall, even if the substance class is one of the significant parameter determining the fate of LMWOS in soil solution (Gunina et al. 2014), we conclude that the $T_{1/2\text{-solution}}$ of LMWOS depends also on substance C oxidation state and on molecular size. Further, the very rapid uptake of all LMWOS classes from soil solution suggests that this is not the limiting step of their initial utilization by microorganisms.

Mineralization of LMWOS

The $T_{1/2\text{-fast}}$ values, describing the initial transformation of LMWOS-C within the microbial biomass, were 30–80 times higher than the $T_{1/2\text{-solution}}$ values, indicating that mineralization may occur more slowly than cellular uptake. However, we added tracer amounts of substrate to extracted soil solution which was then injected to the soil to try and mimic natural C concentrations. Therefore, we would expect the system to be at quasi-steady state (i.e., a stable microbial biomass) and the rate of C influx into soil solution should be equal to the rate of C efflux from the microbial biomass. However, it was not the case in our study and observed slow rate of C efflux and high values of $T_{1/2\text{-fast}}$ could be due to (i) dilution of the LMWOS in the labile metabolite pool within the cytosol (Hill et al. 2008), and (ii) passage of LMWOS through contrasting metabolic pathways which enter aerobic or anaerobic respiratory cycles at different points. Additionally, natural artifacts such as release of HCO_3^- from the cell, its diffusion through extracellular water films and the subsequent degassing and diffusion of CO_2 through the pore network to the soil surface can effect on the temporal dynamic of captured CO_2 (Boddy et al. 2007). However, due to the small amount of soil, which was used in the present experiment, these artifacts should not strongly affect our results, but would need to be accounted for when working with large undisturbed field samples. This highlights the intrinsic problems associated with sole reliance on quantifying substrate turnover rates based on mineralization data alone, especially for short-lived substrates. It also indicates that previous studies may have vastly underestimated substrate turnover rates (van Hees et al. 2002).

An absence of dependence between LMWOS-C $T_{1/2\text{-fast}}$ and C oxidation state, number of C atoms, or number of –COOH groups of the substances (Supplementary material; Fig. S4) are likely due to incorporation of LMWOS into various metabolic pathways within the microorganisms (Gunina et al. 2014; Apostel et al. 2013, 2015; Dippold and Kuzyakov 2013; Dijkstra et al. 2011). So, calculated alanine C $T_{1/2\text{-fast}}$ was 1.5 times faster than glycine (Table 3). This could be explained as alanine enters the citric acid cycle as pyruvate, whereas glycine is metabolized in the cells via three different pathways: (i) by glycine cleavage enzyme, (ii) by conversion of glycine to pyruvate via serine and (iii) by conversion of glycine to glyoxylate by L-amino acid oxidase or L-amino acid dehydrogenase (Keseler et al. 2009), thus, glycine-C can be metabolized slower than alanine. In contrast, LMWOS-C $T_{1/2\text{-slow}}$ decreased with an increase in C oxidation state and increased with the amount of C atoms in the LMWOS molecule, showing that more time is needed to oxidize the LMWOS with a low C oxidation state. Thus, the initial mineralization processes of LMWOS by soil microorganisms are mainly connected with the point at which compounds enter into metabolic cycles, whereas subsequent utilization of LMWOS-derived C can be affected by properties of the substances.

Partitioning of LMWOS-C between the CO_2, cytosol and SOC pools

The amount of C mineralized followed the order: carboxylic acids > amino acids > sugars. This is in agreement with some previous laboratory and field studies (Jones and Edwards 1998), but contrasts with others where no differences were observed (Gunina et al. 2014). Such contradictory results are connected with (i) various observation periods used during the studies, (ii) the amount of time elapsed between LMWOS application and the start of sampling, and (iii) various half-life time of cell metabolites, where LMWOS-C was incorporated. Additionally, the total amount of LMWOS applied to the soil can affect the amount of substrate mineralized, especially if the amount added is sufficient to stimulate microbial growth. Typically, when concentrations of LMWOS

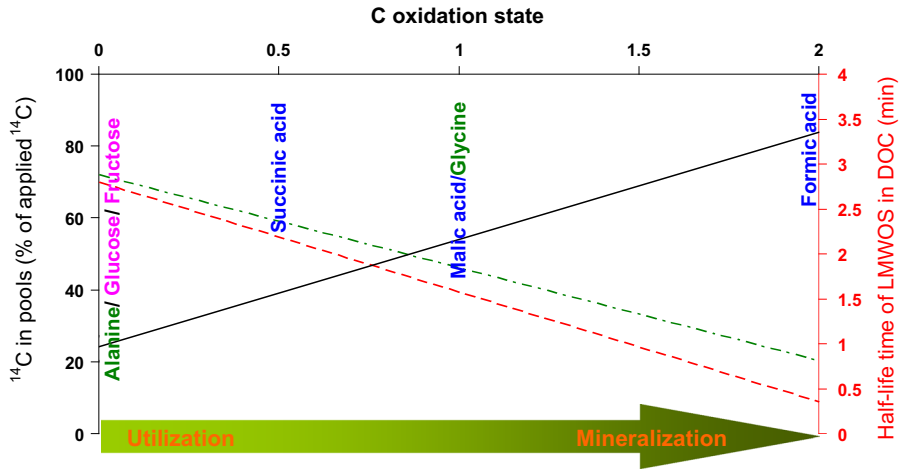

Fig. 6 Schematic representation showing the dependence of microbial uptake rate (*red*), utilization (*green*) and mineralization efficiency (*black*) of three distinct classes of LMWOS as a function of substrate C oxidation state

exceed 10 mM the amount of C incorporated into microbial biomass compartments increases and less C is respired (Hill et al. 2008). In this study, the proportion of substrate-C mineralized increased with the C oxidation state of the substances (Fig. 4, top panel, Fig. 6), showing that oxidized compounds are used preferentially for respiration with less C incorporated into cell metabolites.

The highest portion of ^{14}C-LMWOS recovered from the cytosol pool was from sugars, suggesting that sugars are the universal compounds for construction of cell components (constituents of the bacterial and fungal cell membranes and cell walls, lipoteichoic and teichoic acids of Gram-positive bacteria, lipopolysaccharides of Gram-negative bacteria, polysaccharides, etc.) (Dippold et al. 2014; Gunina and Kuzyakov 2015; Lengeler et al. 1999). In contrast, the lowest incorporation of ^{14}C-LMWOS found in the cytosol was from carboxylic acids, with the lowest of that group being formic acid (Fig. 4; Supplementary material; Fig. S6). Reported ratios of mineralized-C to immobilized-C for carboxylic acids is 3:2 (Jones and Edwards 1998). A wider range of mineralized-to-immobilized C was reported for formic acid: 19:1 (Herlihy et al. 1987) and our results (Fig. 4; Supplementary material; Fig. S6) are in accordance with these findings. Such high mineralization can be explained by the fact that formic acid is a toxic substance (Herlihy et al. 1987), and thus, even if it is taken up by microorganisms it is mainly decomposed to CO_2 within the cells. The proportion of C incorporated into the cytosol decreased with the substance C oxidation state (Fig. 4, top panel), suggesting that more oxidized compounds are mainly used for respiration, whereas reduced compounds are utilized for cell biomass construction. Thus, despite the initial LMWOS mineralization dynamics being independent of substance properties, the final partitioning of the LMWOS-C between mineralized and immobilized pools is dependent on their physiochemical properties.

Anabolism/catabolism ratio (Fig. 5) declined as C oxidation state increased, suggesting that losses for respiration prevail during the assimilation of C from oxidized substances or functional groups (e.g., –COOH). This is directly connected with energy production, which microorganisms can obtain during utilization of LMWOS—with C oxidation state increases, energy content of the LMWOS decreases. Thus, it shows that substrates with high oxidation state are used primarily for energy, whereas substrates with low C oxidation state are primarily used for cell construction and maintenance.

Conclusions

Typically, the turnover of individual LMWOS in soil is estimated by measuring the rate of CO_2 appearance after substrate addition to soil (i.e., substrate-induced respiration). However, this approach fails to realistically capture the dynamics of LMWOS in soil. In this study, the uptake of three common classes of LMWOS (sugars, carboxylic and amino acids) from soil solution and their subsequent mineralization by the soil microbial community was studied over a 24 h period. While previous studies have mainly focused on the

effect of substance class or concentrations, in the present study the main focus was on the physico-chemical properties of substances, including substance C oxidation state, number of –COOH groups and C atoms. We combined the use of substrates at natural abundance with repeated measurements over short time scales. This allowed us to estimate actual rates of LMWOS loss from solution rather than the processing of C once it had already been incorporated into cell metabolites.

The half-life of the LMWOS in soil solution ranged from 0.5 to 3.8 min, with the shortest for carboxylic acids and the longest for sugars. Thus, the extremely fast microbial uptake of all LMWOS classes from solution suggests that this is not a rate-limiting step in the utilization of LMWOS by the microbial community. The $T_{1/2}$ of the LMWOS in solution decreased with C oxidation state. In contrast, the $T_{1/2}$ of LMWOS in soil solution increased with the number of C atoms showing that larger molecules persist longer, possibly due to their slower rate of diffusion in soil. Our data suggests that the uptake of common LMWOS from soil solution by microorganisms may be possible to predict from the physio-chemical properties of the substance.

The LMWOS-C $T_{1/2\text{-fast}}$ values ranged between 30 and 80 min and was lowest for amino acids and highest for carboxylic acids. Large differences between LMWOS $T_{1/2}$ values in solution and in soil shows that microbial uptake and subsequent mineralization of LMWOS are temporarily decoupled. The $T_{1/2\text{-fast}}$ of LMWOS-C in soil was not dependent on the properties of the substance, from which we infer that intercellular metabolism is the main factor determining initial mineralization of C derived from LMWOS.

The total proportion of C mineralized from each LMWOS increased with the substance's C oxidation state, suggesting that oxidized compounds are mineralized to a greater degree than more reduced compounds. To support this observation, the LMWOS-C $T_{1/2\text{-slow}}$ decreased with C oxidation state increase. The portion of LMWOS-C incorporated into the cytosol and remaining in SOC decreased with each substance's C oxidation state. Thus, substance properties directly affected the final partitioning of LMWOS-C between mineralized and microbially utilized pools. The anabolism/catabolism ratio decreased with compound C oxidation state, showing that more oxidized substances are mainly mineralized, whereas less oxidized LMWOS are primarily used by microorganisms for cell construction and maintenance.

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