



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

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27 **Abstract**

28 The fate of low molecular weight organic substances (LMWOS) in soil is regulated by microbial  
29 uptake. However, C oxidation state, the number of C atoms and -COOH groups in the LMWOS can  
30 affect their microbial utilization. Thus, the aim of this study was to reveal the effects of substance  
31 chemical properties on initial uptake and utilization of sugars, carboxylic and amino acids by  
32 microorganisms.

33 Soil solution, spiked with <sup>14</sup>C-labelled glucose, fructose, malate, succinate, formate, alanine or  
34 glycine, was added to the soil and <sup>14</sup>C was traced in the soil solution, CO<sub>2</sub>, cytosol, and soil organic  
35 carbon (SOC) over 24 hours.

36 The half-life time of all LMWOS in the soil solution varied between 0.6 min (formic acid) and  
37 5.0 min (sugars), indicating its dependence on C oxidation state of the substances. The half-life time  
38 of <sup>14</sup>C in the fast mineralized pool in microorganisms, ranged between 30 (malic acid) and 80  
39 (glycine) min and was independent on either C oxidation state, the number of C atoms, or number of  
40 -COOH groups. This suggests that intercellular metabolic pathways are more important for LMWOS  
41 transformation in soil than their basic chemical properties. The portion of mineralized LMWOS  
42 increased with their C oxidation state (20 % for sugars vs. 90% for formic acid) corresponding to the  
43 decrease of C incorporated into the cytosol and SOC pools.

44 Concluding, the physicochemical properties of the common LMWOS allow predicting their  
45 microbial uptake from soil solution and subsequent partitioning of C within microbial biomass.

46

47

48 **Key words:** carbon use efficiency, CUE, decomposition kinetics, dissolved organic nitrogen, organic  
49 acids.

50

## 51 **1. Introduction**

52 Low molecular weight organic substances (LMWOS) in soil originate from a wide range of  
53 sources, including root and microbial exudation, animal wastes, canopy throughfall, and the  
54 decomposition of plant and microbial necromass. Although LMWOS typically represent a small  
55 proportion of the total dissolved organic carbon (DOC) pool in soil, they play a critical role in many  
56 soil processes, including complexation of metal ions which increases their mobilization (e.g.  
57 carboxylic acids), as an important N source (e.g. amino acids) for plants and microorganisms, and as  
58 a source of C and energy for microorganisms (e.g. sugars) (Blagodatskaya and Kuzyakov, 2013;  
59 Grayston et al., 1997; Hill et al., 2012). From a global perspective, LMWOS contribute significantly  
60 to total soil CO<sub>2</sub> flux (up to 30%) (van Hees et al., 2005) and thus represent an important parameter  
61 for modeling of soil organic carbon (SOC) dynamics.

62 Although LMWOS may be leached, become sorbed to the solid phase, abiotically mineralized  
63 or used by plants, their uptake by the microbial communities dominates their longevity in soil  
64 solution and represents the first step of their utilization (Glanville et al., 2016). The uptake of  
65 LMWOS from solution depends on their properties, namely broad substrate class (e.g. sugars,  
66 phenolics etc), which determines its subsequent use within cell metabolism (Gunina et al., 2014;  
67 Apostel et al., 2013), and concentration, which determines the transport systems used by  
68 microorganisms for taking up LMWOS (Hill et al., 2008). In addition, for amino acids it has been  
69 shown that substances with low C oxidation states (e.g. lysine) are taken up by microorganisms  
70 slower than ones having higher C oxidation states (e.g. glycine and glutamate) (Jones and Hodge,  
71 1999), while the fate of carboxylic acids in soil is dependent on their solubility and association with  
72 the soil's solid phase (Gunina et al., 2014). Thus, even if the general substance class plays a major  
73 role in the fate of LMWOS in soils, the physico-chemical properties of the individual compound are  
74 also highly important.

75 The second step of LMWOS utilization by microorganisms is their incorporation into  
76 metabolic cycles and subsequent mineralization to CO<sub>2</sub> or immobilization within cellular components

77 (Apostel et al., 2013). It has also been shown that intercellular metabolism affects the fate of amino  
78 and carboxylic acid derived-C in soils (Gunina et al., 2014), as each compound class enters distinct  
79 metabolic cycles within the cell. The proportion of each mineralized LMWOS is also linked to the C  
80 oxidation state of the substrate. Carboxyl groups (-COOH) (C oxidation state = +3.0) are mineralized  
81 to CO<sub>2</sub> at a higher amount than methyl groups (-CH<sub>3</sub>) (C oxidation state = -3.0) (Fischer and  
82 Kuzyakov, 2010). So, the presence of a high number of reduced C atoms in LMWOS molecules can  
83 lead to low mineralization and high LMWOS-C incorporation into structural elements of the cell. At  
84 the same time, a higher proportion of mineralized C should be observed for substances with high  
85 number of oxidized C atoms (e.g. substrates rich in -COOH groups). Additionally, the standard  
86 enthalpy of combustion of organic compounds seems to be dependent on substance C oxidation state:  
87 for substances with "0" C oxidation state (e.g. glucose, alanine) the values of standard enthalpy of  
88 combustion are in the range 1600-2800 kJ/mol, whereas for oxidized substances (C oxidation state +1  
89 or +2) the values are lower: 280-1300 kJ/mol. Thus, substance physico-chemical properties can  
90 directly impact the utilization processes of LMWOS within the microorganisms. In contrast, further  
91 fate of C contained within LMWOS may be closely related to cell metabolite turnover, where this C  
92 was incorporated during intercellular metabolisation (Glanville et al., 2016).

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

101

102

## 103 **2. Materials and methods**

### 104 *2.1. Site description and soil sampling*

105 Soil was collected from the BangorDIVERSE long-term forest diversity experiment, located  
106 in Abergwyngregyn, North Wales, UK (53°14'16" N, 4°1'1" W) (Smith et al., 2013; Ahmed et al.,  
107 2016). Within this experiment, soil was collected from the replicated Silver birch (*Betula pendula*  
108 Roth.) plots. The soil is classified as a fine loamy textured Dystric Fluvic Cambisol (WRB, 2006) and  
109 has a mixed glacial till parent material. The site has a mean annual soil temperature of 10.6 °C and an  
110 annual rainfall of ca. 950 mm. The basic properties of the soil are presented in Table 1 and in Ahmed  
111 et al. (2016). At each sampling site, surface litter (ca. 1-2 cm) was removed and the top 10 cm of the  
112 mineral soil (A horizon) was collected from four independent locations within each of four replicate  
113 plots and combined to make a composite soil sample. Soil samples were stored in gas-permeable  
114 plastic bags at 5 °C until extraction of soil solution, which was conducted within 24 h of sample  
115 collection. Substrate uptake and mineralization experiments were conducted within one week of soil  
116 sample collection.

117

### 118 *2.2. Extraction of soil solution*

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

125

### 126 *2.3. LMWOS uptake from soil solution*

127 The uptake of LMWOS by the soil microbial community was measured over 24 h for sugars  
128 (glucose and fructose), carboxylic acids (malic, succinic and formic acids) and amino acids (alanine

129 and glycine). These substrates were chosen as they are either commonly found in root  
130 exudates/lysates or they represents the breakdown products arising from the main organic polymers  
131 entering soil (i.e. cellulose/protein). The C oxidation state of each LMWOS was calculated as sum of  
132 all C oxidation states divided by the amount of C atoms in the substance (Table 2).

133 The  $^{14}\text{C}$  radiolabeled substances (<10 nM) were added separately to the extracted soil solution  
134 (see section 2.2) to obtain a total  $^{14}\text{C}$  specific activity of  $0.83 \text{ kBq ml}^{-1}$  for each compound. No  
135 additional non-labeled substances were added so that we did not want to change the intrinsic  
136 concentrations of the compounds naturally present in soil solution. All LMWOS were uniformly  
137 labeled and  $^{14}\text{C}$  specific activities of the each initial substances were:  $^{14}\text{C}$ -glucose  $7.4 \text{ MBq ml}^{-1}$ ,  $^{14}\text{C}$ -  
138 fructose  $37 \text{ MBq ml}^{-1}$ ,  $^{14}\text{C}$ -malic acid  $3.7 \text{ MBq ml}^{-1}$ ,  $^{14}\text{C}$ -succinic acid  $3.7 \text{ MBq ml}^{-1}$ ,  $^{14}\text{C}$ - formic acid  
139  $35.6 \text{ MBq ml}^{-1}$ ,  $^{14}\text{C}$ -alanine  $3.7 \text{ MBq ml}^{-1}$ ,  $^{14}\text{C}$ -glycine  $1.8 \text{ MBq ml}^{-1}$ .

140 To measure the depletion of the LMWOS from soil solution, fresh field-moist soil (1.2 g) was  
141 placed into a  $1.5 \text{ cm}^3$  polypropylene microcentrifuge tube and 0.3 ml of  $^{14}\text{C}$ -labelled soil solution was  
142 added to the soil surface. The solution immediately infiltrated into the soil. The microcentrifuge tubes  
143 were perforated at the bottom and the holes were covered with a small piece of Whatman GF/A glass  
144 fiber filter paper (pore size  $1.6 \mu\text{m}$ ). These soil-filled microcentrifuge tubes was then placed on top of  
145 another empty microcentrifuge tube and the dual-tube array was centrifuged ( $14,000 \text{ g}$ , 1 min). The  
146 soil solution from the upper tube passed to the lower tube where it was recovered for analysis. Soil  
147 solution was obtained 1, 4, 8, 10, 20, 30, 60, 240, 960 and 1440 min after addition of the  $^{14}\text{C}$ -labelled  
148 solution to the surface of the soil in the upper microcentrifuge tube.  $^{14}\text{C}$  activity of the recovered soil  
149 solution was measured by liquid scintillation counting (Wallac 1409 scintillation counter, Wallac  
150 EG&G Ltd, Milton Keynes, UK) using Wallac Optiphase 3 scintillation cocktail (Wallac EG&G Ltd,  
151 Milton Keynes, UK). This procedure was also done with sterile soil (autoclaved,  $121^\circ\text{C}$ , 30 min) to  
152 determine the importance of abiotic losses of LMWOS from soil solution (i.e. sorption to the solid  
153 phase) in the absence of the microbial activity (Hill et al., 2008). Each component of the experiment

154 was replicated four times. The uptake rate of  $^{14}\text{C}$ -labelled LMWOS from soil solution was calculated  
155 as follows:

$$156 \quad R = a_1 + a_2 \exp^{-kt},$$

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

163

#### 164 2.4. LMWOS mineralization in soil

165 To estimate the mineralization rate of each LMWOS, a similar procedure to that described  
166 above was employed except that we measured the rate of  $^{14}\text{CO}_2$  evolution from the soil. Briefly, fresh  
167 soil (1.2 g) was placed into a 1.5 ml microcentrifuge tube and 0.3 ml of each  $^{14}\text{C}$ -labeled solution  
168 added (according to procedure described above). The microcentrifuge tubes were placed into a larger  
169 50 ml polypropylene container and a 1 M NaOH trap (1 ml) added to capture evolved  $\text{CO}_2$  in the  
170 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  
171 LMWOS addition.  $^{14}\text{C}$  activity of the NaOH solutions was measured by liquid scintillation counting  
172 as described above. To describe mineralization rate of each LMWOS, a double first order kinetic  
173 equation was applied to the portion of  $^{14}\text{C}$  remaining in the soil ( $^{14}\text{C}_{\text{SOC}}$ ), (calculated as  
174  $100 - ^{14}\text{C}_{\text{CO}_2}$  (%)):

$$175 \quad ^{14}\text{C}_{\text{SOC}} = a \cdot \exp^{-k_a t} + b \cdot \exp^{-k_b t},$$

176 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  
177 rate constants for the fast and slow mineralization phases (Glanville et al., 2016). The  $T_{1/2}$  for  
178 LMWOS-C of the fast and slow phases of C mineralization within the microbial community were



179 calculated as  $\ln(2)/k_a$  or  $\ln(2)/k_b$  and will subsequently be referred to as  $T_{1/2\text{-fast}}$  and  $T_{1/2\text{-slow}}$   
180 respectively.

181 At the end of the experiment (27.5 h),  $^{14}\text{C}$  activity was measured in the microbial cytosol pool  
182 using the chloroform fumigation-extraction procedure of Wu et al. (1990). As no extraction  
183 efficiency correction factor was applied to the extracted dissolved organic C pool after fumigation  
184 (Glanville et al., 2016), this pool was referred to "cytosol" rather than microbial biomass. The amount  
185 of  $^{14}\text{C}$  remaining in the bulk soil at the end was also measured by combusting the soil at 800 °C in a  
186 OX400 biological oxidiser (R.J. Harvey Instrument Corp., USA) and  $^{14}\text{CO}_2$  measured by scintillation  
187 counting after capture in Oxosol scintillant (National Diagnostics, Atlanta, GA, USA). To obtain  $^{14}\text{C}$   
188 in SOC pool (further referred to as  $^{14}\text{C}\text{-SOC}$ ) the  $^{14}\text{C}$  portions in  $\text{CO}_2$  and cytosol pools were  
189 subtracted from  $^{14}\text{C}$  in bulk soil, and present the pool containing non-extractable microbial biomass  
190 and microbial metabolites. Tracer incorporation into cytosol and SOC pools was presented as a  
191 percent of the total applied  $^{14}\text{C}$ .

192 Based on the calculated  $^{14}\text{C}$  incorporation into  $\text{CO}_2$  and microbial cytosol pools (for the last  
193 measurement point - 27.5 h), the anabolism to catabolism ratio was calculated as:

194 
$$\frac{\text{anabolism}}{\text{catabolism}} = \frac{{}^{14}\text{C}_{\text{cytosol}}}{{}^{14}\text{C}_{\text{CO}_2}},$$

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

197

## 198 2.5. Statistics

199 Data on  $^{14}\text{C}$  in  $\text{CO}_2$ , cytosol and SOC as well as pool sizes, rate constants and  $T_{1/2}$  were  
200 subjected to ANOVA and significant differences between the various LMWOS were tested with LSD  
201 post hoc test with  $P < 0.05$ . Exponential equations were fitted to the experimental results using a least  
202 squares iteration routine in Statistica 10.0 (Dell Statistica Inc., Tulsa, OK). The simple regression  
203 analysis was performed in Statistica 10.0 (Dell Statistica Inc., Tulsa, OK) with data on C oxidation

204 state, number of C atoms, number of COOH groups vs. LMWOS  $T_{1/2}$  solution,  $T_{1/2}$ -fast,  $T_{1/2}$ -slow, portion of  
205  $^{14}\text{C}$  in SOC, cytosol and  $\text{CO}_2$  pools.

### 206 3. Results

#### 207 3.1. Uptake of LMWOS from soil solution

208 The three classes of LMWOS showed a similar uptake pattern from soil solution based on the  
209  $^{14}\text{C}$  depletion from the DOC pool (Fig. 1). Calculated LMWOS-C  $T_{1/2}$ -solution changed in the order:  
210 sugars > amino acids > carboxylic acids (Table 2). Glucose and fructose showed a similar  $T_{1/2}$ -solution  
211 (3.8 min), which was 1.5 - 2 times longer than for other the substances. The lowest  $T_{1/2}$ -solution (<1 min)  
212 was found for formic acid. Estimates of the total amount of LMWOS ascribed to modelled pool  $a_2$   
213 were similar for all substances (Table 2). There was a negative relationship between the  $T_{1/2}$ -solution of  
214 each substrate and its C oxidation state (Fig. 2 top panel) and number of -COOH groups  
215 (Supplementary material; Fig. S1). Furthermore, there was a positive relationship between the  $T_{1/2}$ -  
216 solution of all LMWOS and the number of C atoms within the individual substrates (Fig. 2, bottom  
217 panel). Results for the autoclaved soil (Supplementary material; Fig. S2) showed some dilution with  
218 the intrinsic soil solution and that sorption can occur for some substances (e.g. carboxylic acids and ,  
219 glycine). However, as shown previously (Fischer et al., 2010), biotic uptake of LMWOS out-  
220 competes the abiotic sorption processes, from which we predict that sorption processes will not  
221 greatly influence the results in the non-autoclaved soil.

222

#### 223 3.2. Mineralization of LMWOS in soil

224 Mineralization patterns were similar for all three LMWOS classes, namely the highest portion  
225 of C was mineralized in the first 5 h, and later  $^{14}\text{C}$ - $\text{CO}_2$  reached a plateau (Fig. 3). The maximum  
226 proportion of mineralized LMWOS was found for carboxylic acids, followed by amino acids and  
227 sugars (Fig. 3). Overall, 15 to 80% of the applied LMWOS were decomposed to  $\text{CO}_2$  within the first  
228 mineralization phase (pool  $a$ ,  $k_a$ ) depending on substance class (Fig. 3). Constant rates for the first  
229 mineralization phase were between 0.5 and 1.3 %  $\text{h}^{-1}$  and calculated  $T_{1/2}$ -fast values for pool  $a$  for each

230 LMWOS-C were in the range of 0.52-1.34 h (30-80 min) (Table 3), with the shortest values observed  
231 for malic acid and the longest for glycine. The  $T_{1/2\text{-fast}}$  values for each LMWOS-C were much longer  
232 than those calculated for their loss from soil solution, showing that mineralization does not occur  
233 immediately after LMWOS uptake. No significant correlation was found between the  $T_{1/2\text{-solution}}$  values  
234 of each substrate and its subsequent mineralization during the fast utilization phase (Supplementary  
235 materials; Fig. S3).

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

242 The partitioning of LMWOS-C between  $\text{CO}_2$ , the microbial cytosol and that remaining in  
243 SOC is shown in Figure 4. The maximum proportion of mineralized substances was observed for  
244 formic acid, which was followed by malic and succinic acid, amino acids and sugars. In contrast, the  
245  $^{14}\text{C}$  recovered in the cytosol and remaining in SOC followed the opposite trend. The proportion of  
246 mineralized LMWOS increased with substance C oxidation state, whereas the amount of  $^{14}\text{C}$   
247 incorporated into the cytosol and remaining in SOC (for all substances) followed the opposite trend  
248 (Fig. 4, top panel). Additionally, the proportion of LMWOS-C incorporated into the microbial cytosol  
249 increased with the number of C atoms present in the molecule and decreased with the number of -  
250  $\text{COOH}$  groups (Fig. 4, bottom panel). Anabolism/catabolism ratio (Fig. 5) was the highest for the  
251 sugars (both glucose and fructose) and for alanine, having zero C substance oxidation states. The  
252 lowest value was found for formic acid.

253 Overall, initial utilization of LMWOS within the microbial biomass was not dependent on the  
254 substance properties. In contrast, the total amount of LMWOS-C which can be utilized (including

255 mineralization to CO<sub>2</sub> and incorporation in to cellular compounds) within the microbial biomass was  
256 clearly dependent on the physico-chemical properties of the individual substrates.

257

## 258 **4. Discussion**

259 In this study, the utilization of LMWOS in soil focused on: i) the initial rate of uptake from  
260 soil solution, ii) mineralization to CO<sub>2</sub>, and iii) subsequent utilization and partitioning of C within the  
261 microbial cells. These processes were studied within 24 h, to deduce the initial fate of LMWOS-C  
262 rather than the turnover of secondary metabolites within the microbial community or the turnover of  
263 the biomass itself (i.e. necromass). The fate and flux of LMWOS was studied at natural  
264 concentrations (soil solution was only labeled at trace levels for each <sup>14</sup>C-compound), to best reflect  
265 conditions which naturally exist in the field. This contrasts with almost all previous studies which  
266 have used high substrate addition rates to investigate LMWOS turnover in the soil. Although these  
267 former studies may reflect pulse additions of soluble C arising from root lysis or organic waste  
268 addition, they misrepresent the much lower concentrations of LMWOS produced by the slower  
269 turnover of more recalcitrant (and arguably more important) pools of soil organic matter.

270

### 271 *4.1. Uptake of LMWOS from soil solution*

272 We found that up to 90% of the applied LMWOS were taken up from soil solution within the  
273 first 10 minutes (Fig. 1). Similar results have been found for glucose applied to soil in the  
274 concentration range from 1 μM to 10 mM (Hill et al., 2008). The rapid removal of substrates can be  
275 attributed to the rapid uptake of LMWOS by the C-limited soil microbial community, extracellular  
276 enzymatic decomposition or sorption on the mineral phases. For most neutral or monovalent  
277 LMWOS, microorganisms represent the dominate loss pathway from solution, particularly in  
278 comparison to sorption to mineral phases (Fischer et al., 2010). In the case of di- and tri-valent  
279 substrates, however, sorption can significantly suppress microbial uptake, especially in soils  
280 containing large amounts of Fe and Al oxyhydroxides (Jones and Edwards, 1998), however, it was

281 not the case in our study. We attempted here to estimate the effect of abiotic sorption processes by  
282 measuring the loss of LMWOS under sterile (autoclaved) and non-sterile soil. Sorption had low  
283 importance in the fate of LMWOS because larger percent of  $^{14}\text{C}$  was removed from soil solution in  
284 non-sterile soil compare to sterile for the same time interval. This is the consequence of neutral pH  
285 values and low contents of Fe and Al in the soil. Overall, our results are consistent with microbial  
286 transformation being the dominant process. Although extracellular enzymes may exist in soil solution  
287 and could extracellularly cleave our substrates (e.g. deaminases acting on alanine or glycine to  
288 produce pyruvate and lactate), we expect this transformation pathway to be insignificant in  
289 comparison to the direct uptake by microbial membrane transporters.

290         The fastest uptake rates from solution and subsequent  $T_{1/2\text{-solution}}$  values (0.6-1.5 min) were  
291 found for carboxylic acids while the slowest  $T_{1/2\text{-solution}}$  value was found for sugars (3.7 min) (Table 2).  
292 Although the rate of depletion was very rapid for all substrates, the variation in uptake rate can be  
293 attributed to differences in (i) relative diffusion speed of the substrates in solution, (ii) different  
294 affinities and expression of the various transport systems within the microbial community, and (iii)  
295 rate of intracellular processing of the various substrate classes which may feedback on transporter  
296 activity (Hill et al., 2008; Jones and Edwards, 1998). The  $T_{1/2\text{-solution}}$  of carboxylic and amino acids  
297 decreased with the C oxidation state of substances suggesting that LMWOS with low C oxidation  
298 states remain in soil solution longer than ones which are highly oxidized. At the same time, LMWOS  
299  $T_{1/2\text{-solution}}$  values increased with the number of C atoms indicating that substances with a lower  
300 molecular weight are taken up faster. For substances with a similar C oxidation state (both sugars and  
301 alanine), a longer  $T_{1/2\text{-solution}}$  was found for larger molecules although more substrates would need to be  
302 tested to confirm this. Overall, even if the substance class is one of the significant parameter  
303 determining the fate of LMWOS in soil solution (Gunina et al., 2014), we conclude that the  $T_{1/2\text{-solution}}$   
304 of LMWOS depends also on substance C oxidation state and on molecular size. Further, the very  
305 rapid uptake of all LMWOS classes from soil solution suggests that this is not the limiting step of  
306 their initial utilization by microorganisms.

307

#### 308 4.2. Mineralization of LMWOS

309 The  $T_{1/2\text{-fast}}$  values, describing the initial transformation of LMWOS-C within the microbial  
310 biomass, were 30-80 times higher than the  $T_{1/2\text{-solution}}$  values, indicating that mineralization may occur  
311 more slowly than cellular uptake. However, we added tracer amounts of substrate to extracted soil  
312 solution which was then injected to the soil to try and mimic natural C concentrations. Therefore, we  
313 would expect the system to be at quasi-steady state (i.e. a stable microbial biomass) and the rate of C  
314 influx into soil solution should be equal to the rate of C efflux from the microbial biomass. However,  
315 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  
316 due to i) dilution of the LMWOS in the labile metabolite pool within the cytosol (Hill et al., 2008),  
317 and ii) passage of LMWOS through contrasting metabolic pathways which enter aerobic or anaerobic  
318 respiratory cycles at different points. Additionally, natural artifacts such as release of  $\text{HCO}_3^-$  from the  
319 cell, its diffusion through extracellular water films and the subsequent degassing and diffusion of  $\text{CO}_2$   
320 through the pore network to the soil surface can effect on the temporal dynamic of captured  $\text{CO}_2$   
321 (Boddy et al., 2007). However, due to the small amount of soil, which was used in the present  
322 experiment, these artifacts should not strongly affect our results, but would need to be accounted for  
323 when working with large undisturbed field samples. This highlights the intrinsic problems associated  
324 with sole reliance on quantifying substrate turnover rates based on mineralization data alone,  
325 especially for short-lived substrates. It also indicates that previous studies may have vastly  
326 underestimated substrate turnover rates (van Hees et al., 2002).

327 An absence of dependence between LMWOS-C  $T_{1/2\text{-fast}}$  and C oxidation state, number of C  
328 atoms, or number of -COOH groups of the substances (Supplementary material; Fig. S4) are likely  
329 due to incorporation of LMWOS into various metabolic pathways within the microorganisms  
330 (Gunina et al., 2014; Apostel et al., 2013; Apostel et al., 2015; Dippold and Kuzyakov, 2013; Dijkstra  
331 et al., 2011). So, calculated alanine C  $T_{1/2\text{-fast}}$  was 1.5 times faster than glycine (Table 3). This could be  
332 explained as alanine enters the citric acid cycle as pyruvate, whereas glycine is metabolized in the

333 cells via three different pathways: i) by glycine cleavage enzyme, ii) by conversion of glycine to  
334 pyruvate via serine and iii) by conversion of glycine to glyoxylate by L-amino acid oxidase or L-  
335 amino acid dehydrogenase (Keseler et al., 2009), thus, glycine-C can be metabolized slower than  
336 alanine. In contrast, LMWOS-C  $T_{\frac{1}{2}\text{-slow}}$  decreased with an increase in C oxidation state and increased  
337 with the amount of C atoms in the LMWOS molecule, showing that more time is needed to oxidize  
338 the LMWOS with a low C oxidation state. Thus, the initial mineralization processes of LMWOS by  
339 soil microorganisms are mainly connected with the point at which compounds enter into metabolic  
340 cycles, whereas subsequent utilization of LMWOS-derived C can be affected by properties of the  
341 substances.

342

#### 343 *4.3. Partitioning of LMWOS-C between the CO<sub>2</sub>, cytosol and SOC pools*

344 The amount of C mineralized followed the order: carboxylic acids > amino acids > sugars.  
345 This is in agreement with some previous laboratory and field studies (Jones and Edwards, 1998), but  
346 contrasts with others where no differences were observed (Gunina et al., 2014). Such contradictory  
347 results are connected with i) various observation periods used during the studies, ii) the amount of  
348 time elapsed between LMWOS application and the start of sampling, and iii) various half-life time of  
349 cell metabolites, where LMWOS-C was incorporated. Additionally, the total amount of LMWOS  
350 applied to the soil can affect the amount of substrate mineralized, especially if the amount added is  
351 sufficient to stimulate microbial growth. Typically, when concentrations of LMWOS exceed 10 mM  
352 the amount of C incorporated into microbial biomass compartments increases and less C is respired  
353 (Hill et al., 2008). In this study, the proportion of substrate-C mineralized increased with the C  
354 oxidation state of the substances (Fig. 4, top panel, Fig. 6), showing that oxidized compounds are  
355 used preferentially for respiration with less C incorporated into cell metabolites.

356 The highest portion of <sup>14</sup>C-LMWOS recovered from the cytosol pool was from sugars,  
357 suggesting that sugars are the universal compounds for construction of cell components (constituents  
358 of the bacterial and fungal cell membranes and cell walls, lipoteichoic and teichoic acids of Gram-

359 positive bacteria, lipopolysaccharides of Gram-negative bacteria, polysaccharides, etc) (Dippold et  
360 al., 2014; Gunina and Kuzyakov, 2015; Lengeler et al., 1999). In contrast, the lowest incorporation of  
361  $^{14}\text{C}$ -LMWOS found in the cytosol was from carboxylic acids, with the lowest of that group being  
362 formic acid (Fig. 4, Supplementary material; Fig. S6). Reported ratios of mineralized-C to  
363 immobilized-C for carboxylic acids is 3:2 (Jones and Edwards, 1998). A wider range of mineralized-  
364 to-immobilized C was reported for formic acid - 19:1 (Herlihy et al., 1987) and our results (Fig. 4,  
365 Supplementary material; Fig. S6) are in accordance with these findings. Such high mineralization can  
366 be explained by the fact that formic acid is a toxic substance (Herlihy et al., 1987), and thus, even if it  
367 is taken up by microorganisms it is mainly decomposed to  $\text{CO}_2$  within the cells. The proportion of C  
368 incorporated into the cytosol decreased with the substance C oxidation state (Fig. 4, top panel),  
369 suggesting that more oxidized compounds are mainly used for respiration, whereas reduced  
370 compounds are utilized for cell biomass construction. Thus, despite the initial LMWOS  
371 mineralization dynamics being independent of substance properties, the final partitioning of the  
372 LMWOS-C between mineralized and immobilized pools is dependent on their physiochemical  
373 properties.

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

381

## 382 **5. Conclusions**

383 Typically, the turnover of individual LMWOS in soil is estimated by measuring the rate of  
384  $\text{CO}_2$  appearance after substrate addition to soil (i.e. substrate-induced respiration). However, this



385 approach fails to realistically capture the dynamics of LMWOS in soil. In this study, the uptake of  
386 three common classes of LMWOS (sugars, carboxylic and amino acids) from soil solution and their  
387 subsequent mineralization by the soil microbial community was studied over a 24 h period. While  
388 previous studies have mainly focused on the effect of substance class or concentrations, in the present  
389 study the main focus was on the physico-chemical properties of substances, including substance C  
390 oxidation state, number of -COOH groups and C atoms. We combined the use of substrates at natural  
391 abundance with repeated measurements over short time scales. This allowed us to estimate actual  
392 rates of LMWOS loss from solution rather than the processing of C once it had already been  
393 incorporated into cell metabolites.

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

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

408 The total proportion of C mineralized from each LMWOS increased with the substance's C  
409 oxidation state, suggesting that oxidized compounds are mineralized to a greater degree than more  
410 reduced compounds. To support this observation, the LMWOS-C  $T_{1/2\text{-slow}}$  decreased with C oxidation

411 state increase. The portion of LMWOS-C incorporated into the cytosol and remaining in SOC  
412 decreased with each substance's C oxidation state. Thus, substance properties directly affected the  
413 final partitioning of LMWOS-C between mineralized and microbially utilized pools. The  
414 anabolism/catabolism ratio decreased with compound C oxidation state, showing that more oxidized  
415 substances are mainly mineralized, whereas less oxidized LMWOS are primarily used by  
416 microorganisms for cell construction and maintenance.

417

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540 **Tables and Figures captions**

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542 **Table 1** Selected soil properties.

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

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

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551

552 **Figure 1.** Temporal dynamics of  $^{14}\text{C}$ -labelled sugar, organic acid and amino acid disappearance from  
553 soil solution. Values represent means  $\pm$  SE ( $n = 4$ ). Lines are the following: blue: solid - glucose,  
554 dotted - fructose; green: solid - formic acid, dashed - malic acid, dotted - succinic acid; brown: solid -  
555 glycine, dashed - alanine.

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

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563

564 **Figure 3.** Cumulative  $^{14}\text{C}$ - $\text{CO}_2$  production from mineralization of  $^{14}\text{C}$ -labelled substances in soil.  
565 Values represent means  $\pm$  SE ( $n = 4$ ).

566

567

568 **Figure 4.** Relationship between  $^{14}\text{C}$  remaining in the cytosol, SOC and  $\text{CO}_2$  pools and C oxidation  
569 state (top panel) and  $^{14}\text{C}$  remaining in the cytosol and number of C atoms and -COOH groups (bottom  
570 panel) in different LMWOS. Values represent means  $\pm$  SE ( $n = 4$ ).  $P$ -values for the regression lines  
571 on the top panel figure are less than 0.002;  $p$ -values for the regression lines on the bottom panel  
572 figure are less than 0.004. The substance names are shown only once.

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575 **Figure 5.** Relationship between  $^{14}\text{C}$  incorporated into cytosol (anabolism)/ $^{14}\text{C}$  incorporated into  $\text{CO}_2$   
576 (catabolism) and C oxidation state at the end of LMWOS mineralization experiment.

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

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