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Fate of low molecular weight organic substances in an arable soil: From microbial uptake to utilisation and stabilisation

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ABSTRACT

Microbial uptake and utilisation are the main transformation pathways of low molecular weight organic substances (LMWOS) in soil, but details on transformations are strongly limited. As various LMWOS classes enter biochemical cycles at different steps, we hypothesize that the percentage of their carbon (C) incorporation into microbial biomass and consequently stabilisation in soil are different.

Representatives of the three main groups of LMWOS: amino acids (alanine, glutamate), sugars (glucose, ribose) and carboxylic acids (acetate, palmitate) – were applied at naturally-occurring concentrations into a loamy arable Luvisol in a field experiment. Incorporation of ^{13}C from these LMWOS into extractable microbial biomass (EMB) and into phospholipid fatty acids (PLFAs) was investigated 3 d and 10 d after application. The microbial utilisation of LMWOS for cell membrane construction was estimated by replacement of PLFA-C with ^{13}C.

35–80% of initially applied LMWOS-^{13}C was still present in the composition of soil organic matter after 10 days of experiment, with 10–24% of ^{13}C incorporation into EMB at day three and 1–15% at day 10. Maximal incorporation of ^{13}C into EMB was observed from sugars and the least from amino acids. Strong differences in microbial utilisation between LMWOS were observed mainly at day 10. Thus, despite similar initial rapid uptake by microorganisms, further metabolism within microbial cells accounts for the specific fate of C from various LMWOS in soils.

^{13}C from each LMWOS was incorporated into each PLFA. This reflects the ubiquitous utilisation of all LMWOS by all functional microbial groups. The preferential incorporation of palmitate into PLFAs reflects its role as a direct precursor for fatty acids. Higher ^{13}C incorporation from alanine and glucose into specific PLFAs compared to glutamate, ribose and acetate reflects the preferential use of glycolysis-derived substances in the fatty acids synthesis.

Gram-negative bacteria (16:1ω7c and 18:1ω7c) were the most abundant and active in LMWOS utilisation. Their high activity corresponds to a high demand for anabolic products, e.g. to dominance of pentose-phosphate pathway, i.e. incorporation of ribose-C into PLFAs. The ^{13}C incorporation from sugars and amino acids into filamentous microorganisms was lower than into all prokaryotic groups. However, for carboxylic acids, the incorporation was in the same range (0.1–0.2% of the applied carboxylic acid ^{13}C) as that of gram-positive bacteria. This may reflect the dominance of fungi and other filamentous microorganisms for utilisation of acidic and complex organics.

Thus, we showed that despite similar initial uptake, C from individual LMWOS follows deviating metabolic pathways which accounts for the individual fate of LMWOS-C over 10 days. Consequently, stabilisation of C in soil is mainly connected with its incorporation into microbial compounds of various stability and not with its initial microbial uptake.

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1. Introduction

Low molecular weight organic substances (LMWOS) comprise 5–10% of dissolved organic carbon (DOC) in soils (Ryan et al., 2001) and are products of rhizodeposition, above and belowground litter and microbial residue degradation. The main compound classes within the LMWOS are amino acids, sugars (mainly monosaccharides) and carboxylic acids (Fischer et al., 2010).

A main process removing LMWOS from soil solution is microbial uptake, which out-compete physicochemical sorption of LMWOS at mineral surfaces and their leaching from the soil profile, probably by orders of magnitude (Fischer et al., 2010). Microbial removal of LMWOS from solution appears within minutes in the upper soil horizons (Jones et al., 2004). In contrast, the half-life time of C from such LMWOS in the soil is much longer – from several hours to months or even decades (van Hees et al., 2005), because after incorporation in microbial biomass it can further be stabilised in soils. Thus, utilisation within the microbial biomass is assumed to be one of the main factors controlling the LMWOS-C stabilisation in soil on the short- and long-term scales.

To evaluate the contribution of functional microbial groups to LMWOS utilisation ^{13}C or ^{14}C-labelling, coupled with analysis of microbial biomarkers such as amino sugars (Amelung et al., 2001; Engelking et al., 2007; Glaser et al., 2004), phospholipid-derived fatty acids (PLFA) (Frostegard et al., 2011; Zelles, 1997) or DNA-based methods (Ibekwe et al., 2002; Radajewski et al., 2003) can be applied. Coupling PLFA analysis with ^{13}C-labelling has shown that gram-negative (G−) bacteria are more active in the utilisation of plant C (low or high molecular weight) than gram-positive (G+) bacteria, even if the latter group has a higher PLFA content in soil (Garcia-Pausas and Paterson, 2011; Waldrop and Firestone, 2004). Fungi contribute less to the utilisation of plant-derived C than bacteria (Waldrop and Firestone, 2004). In contrast, the use of ^{13}C pulse-labelling of plants to trace ^{13}C in PLFAs has shown that either fungi (Butler et al., 2003) or G− bacteria (Tian et al., 2013) are the most active consumers of rhizodeposits. Incorporation of ^{13}C from labelled straw into PLFAs has shown that fatty acids such as 16:0 (universal fatty acid), 18:1ω9 (bacterial or fungal biomarker), 18:2ω6,9 (typical fungal biomarker) were more ^{13}C-enriched, whereas other 16:1ω5 (fungal or G− bacteria biomarker) or 10Me17:0 (typical actinomycetes biomarker) fatty acids contained negligible amounts of ^{13}C (Williams et al., 2006). Consequently, members of the microbial community are differentially involved in the assimilation of litter- or root-derived C (Williams et al., 2006) and the activity of individual microbial groups appears to depend on the quality of substrate and on environmental conditions such as soil type, season and climate (Bray et al., 2012). Thus, general principles of LMWOS utilisation by individual groups of bacteria and fungi still remain open.

The second factor controlling LMWOS fate is microbial metabolism: various classes of LMWOS enter different pathways and consequently are utilised differently (Blagodatskaya et al., 2011). Sugars are mainly used directly by the basic glycolysis pathway (Caspi et al., 2012; Keseler et al., 2009), carboxylic acids enter from side branches of the citric acid cycle (Caspi et al., 2012; Keseler et al., 2009), and amino acids enter glycolysis or the citric acid cycle from individual side branches at different steps (Apostel et al., 2013; Knowles et al., 2010). Thus, we assume that universal substances such as sugars, entering glycolysis directly, will be metabolised very rapidly in comparison to carboxylic acids and amino acids entering the citric acid cycle. However, glycolysis also enables entry into many anabolic pathways, i.e., we hypothesise that sugars are used more for anabolism than carboxylic acids, which enter the oxidising citric acid cycle and can be directly metabolised for energy production. The highest diversity in pathways can be expected for amino acids, because they enter basic metabolism at various steps (Apostel et al., 2013). Since carboxylic acid utilisation is substrate-controlled (van Hees et al., 2002), we expect divergence in the utilisation of short- and long-chain acids. Because three classes of LMWOS enter metabolic cycles at various points, we hypothesise that their role in the synthesis of cell components such as PLFAs should be different. Based on the various fates of LMWOS in biomass the conclusions about short-term LMWOS-C stabilisation in soil organic matter (SOM) can be done.

Thus, the overall aim of this study was to estimate the transformation of representatives of three main classes of LMWOS: monosaccharides (glucose and ribose), carboxylic acids (acetate and palmitate) and amino acids (alanine and glutamate) under field conditions, coupling ^{13}C substrate labelling with the analysis of specific cell PLFA biomarkers.

2. Materials and methods

2.1. Experimental design

The field experiment was carried out at an agricultural field trial in Hohenpölz (49°54′ N, 11°08′E, at 500 m a.s.l.). The mean annual temperature was +7 °C and mean annual precipitation was 870 mm. The site is cultivated by a rotation of triticale, wheat and barley. The soil is an arable loamy haplic Luvisol (IUSS Working group WRB, 2007) and had the following characteristics: pH 6.6, total C content 1.5%, C/N 10.7, CEC 13 $cmol_C\ kg^{-1}$, clay content 22%.

In August 2010, following harvest of the triticale and harrowing, columns (i.e. plastic tubes with 13 cm height and 10 cm diameter) were inserted to a depth of 10 cm and six, ^{13}C uniformly-labelled substances: alanine, glutamate, glucose, ribose, sodium acetate and palmitate were injected with a syringe into separate columns. The injection was done in 5 points, which allowed spreading the ^{13}C homogeneously inside the column. The injection was done as a single pulse-labelling. The amounts of applied tracer were: alanine 96.3, glutamate 91.6, glucose 93.4, ribose 91.8, acetate 95.8 and palmitate 49.5 μmol $^{13}C\ column^{-1}$. The amount of added C was kept as low as possible and constant for all columns, including the controls, where similar amounts of non-labelled C were applied (735 μmol C $column^{-1}$). Each column contained 1.5 kg soil. The field experiment had a randomised block design with four blocks, which represented four field replicates. Preventing rainfall by using a protective roof excluded leaching through the columns for the 10 days of the experiment. Due to the absence of leaching and uptake by plants, we assumed that all losses of ^{13}C from soil are connected to LMWOS mineralisation to CO_2. After day three and day 10, separate soil columns were destructively sampled. We assumed that on the day three the maximum incorporation of LMWOS-C into microbial biomass should occur, and on the day 10 the maximal differences in LMWOS-C utilisation within the microbial metabolism can be observed. The soil was removed from the column, weighed and the water content was determined in a subsample. The soil water content was between 22 and 25% for the both time points. Each soil sample was sieved to 2 mm and divided into two portions. One was cooled (+5 °C) for microbial biomass analysis and another was stored frozen (−20 °C) until PLFA analysis.

2.2. Bulk soil $\delta^{13}C$ analysis

The soil for the $\delta^{13}C$ analysis was freeze-dried, milled and $\delta^{13}C$ values of bulk SOM were determined using a Euro EA Elemental Analyser (Eurovector, Milan, Italy) unit coupled via a ConFlo III interface (Thermo-Fischer, Bremen, Germany) to a Delta V Advantage IRMS (Thermo Fischer, Bremen, Germany). The amount of applied ^{13}C in the soil was calculated based on the mixing model