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Assimilation and accumulation of C by fungi and bacteria attached to soil density fractions

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ABSTRACT

Soil microorganisms play a key role in soil organic matter (SOM) dynamics, but little is known about the controls affecting the distribution of microbial biomass and their residues in soil. Here, a forested Cambisol topsoil was incubated with ^{13}C-labeled glycine or beech leaves for 12 weeks prior to sequential density fractionation. The incorporation of the ^{13}C label in amino sugars (AS) was used to gain insight into bacterial and fungal assimilation of the substrates. AS derived from glycine or leaves were compared to total AS to investigate how microbial residues and active communities were distributed among soil density fractions.

Bacteria slightly dominated leaf C assimilation, while a pronounced fungal dominance was observed for glycine. The glycine-derived AS and original AS were similarly distributed among the soil density fractions, both peaking in microbial aggregates (1.8–2.4 g cm^{-3}). Leaf-derived AS were mostly found in association with the plant debris (<1.65 g cm^{-3}). The ratios of substrate-derived AS C to substrate-derived C increased with soil fraction density for both glycine and leaves. The same pattern was observed with original AS C to soil fraction C ratios. We concluded that bacteria and fungi were most active where the resource was even though their residues accumulate mostly in microbial aggregates (1.8–2.4 g cm^{-3}). We suggest that such accumulation might be attributed to (1) an increasing stabilization efficiency of microbial residues and (2) the progressive SOM transfer, from plant debris to microbial aggregates (1.8–2.4 g cm^{-3}).

1. Introduction

Soil microorganisms are widely recognized as important controlling factors for soil organic matter (SOM) dynamics (Schmidt et al., 2011; Zhou et al., 2012), primarily because of their prominent role in SOM degradation and accumulation (Kindler et al., 2009; Miltner et al., 2012). Microbial residues constitute an important component of SOM (Koegel-Knabner, 2002; von Lützow et al., 2006; Koegel-Knabner et al., 2008) and their allocation might well play a key role long-term soil C sequestration (Schimel and Schaeffer, 2012). By jointly analyzing fumigation-extracts and biomarkers of total and living microbial biomasses, Appuhn and Joergensen (2006) showed that half of the SOM of rhizospheric soil might be present as dead microbial residues (necromass), against only 3% as living microorganisms (biomass). More recently, spectrometric and molecular approaches corroborated the essential role of microbial necromass to the maintenance and the accumulation of the SOM (Simpson et al., 2007; Clemente et al., 2011; Dumig et al., 2012; Miltner et al., 2012). However, estimations of the contribution of fungi and bacteria to SOM stabilization in temperate forest soils remain as scant as the understanding of their spatial distribution throughout the soil matrix.

Amino sugars (AS) are mostly found in cell walls and extracellular polysaccharides of fungi and bacteria, in the exoskeleton of

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arthropods and in the gut of some worms. The fraction of AS derived from macro and meso organisms is considered negligible relatively to that of microorganisms (Amelung, 2001; Simpson et al., 2004). While 26 AS are identified (Amelung et al., 2008), only glucosamine (GlcN), galactosamine (GalN), muramic acid (MurN) are found in quantifiable concentration and can be used as proxies for their bacterial or fungal origin. Bacterial cell walls are the unique source of MurN (Glaser et al., 2004; He et al., 2005; Liang et al., 2009). GlcN and GalN have much less specific origins and are both found in considerable amounts in both bacteria and fungi (Amelung et al., 1999; He et al., 2005; Engelking et al., 2007).

AS long-survive their producers and are thus used as proxies for microbial residues (Amelung, 2001). The introduction of isotopic labels permits to trace substrate-derived AS, hereafter reported as AS_S, to capture the response of soil microorganisms to substrate additions (Boschker and Middelburg, 2002; Glaser, 2005; Amelung et al., 2008). Some studies have focused on compound specific stable isotope techniques using ^{13}C and ^{15}N labels to quantify AS_S dynamics (Glaser and Gross, 2005; Liang et al., 2007; Hilscher and Knicker, 2011; He et al., 2011a, 2011b). Until recently, however, ^{13}C analysis of AS was hampered by undetermined biases on the isotopic signature of the studied AS resulting from a derivatization step for gas chromatography separation (Decock et al., 2009). To overcome that issue, Bodé et al. (2009) developed a new method for ^{13}C analysis of AS by liquid chromatography coupled with isotope ratio mass spectrometry (LC-IRMS) that provides more accurate and precise measurement. This method has been successfully used to trace AS_S during an incubation experiment of 21 days with ^{13}C labeled plant residues (Bai et al., 2013). This new analytical tool is now available to determine the effect of the chemical composition of substrates on the distribution of fungal and bacterial AS residues throughout distinct soil compartments.

This study aims at capturing the distribution of newly-produced fungal and bacterial biomasses derived from the added substrates and firmly attached to soil density fractions. We hypothesized that the contribution of AS C to the C content of density fraction (fraction C) increases with increasing fraction density. We expected the microbial assimilation of the substrate C among the soil density fractions to be dictated by substrate availability and thus to reveal preferential microbial habitats. To test these hypotheses, a forested topsoil amended with ^{13}C-labeled glycine (readily available to soil microorganisms) or finely ground ^{13}C-labeled beech leaves (more complex and recalcitrant substrate) were incubated in the laboratory for 92 days and sequentially separated by density. The formation of (^{13}C-labeled) AS_S was determined by LC-IRMS (Bodé et al., 2009). Finally, the formation of fungal and bacterial residues derived from the (^{13}C-labeled) substrates was estimated using established conversion factors.

2. Materials and methods

2.1. Lab incubation

The soil was collected in November 2009 from an even-aged beech forest of 23-year old at Ebrach, Germany (49°52′N, 10°27′E). Both site and soil have been described in a previous study (Hatton et al., 2012a). Briefly, the soil is an acidic dystric Cambisol (pH_{H_2O}: 3.9; sand: 80%; loam: 13%; clay: 7%). The first 2.5 cm of the A-horizon were sampled on 2 m^2. The entire volume of soil was thoroughly mixed and sieved to pass 2 mm. Observable roots and faunal organisms were removed. The soil was stored for 2 weeks at +4 °C prior pre-incubation.

The soil was pre-incubated for 15 days at 20 °C (dark; 1:3 soil to air ratio; 42% water holding capacity) prior substrate amendment. Two substrates were used: uniformly ^{13}C-labeled glycine (isotopic abundance = 98%; Sigma–Aldrich, France) and ^{13}C-labeled beech leaves (isotopic abundance = 4.6%). The ^{13}C-labeled leaves were obtained from 10 year-old beech trees grown in airtight chamber at the GRAP-CEA Cadarache (France) and ^{13}C-labeled twice a week using $^{13}CO_2$ over a vegetation season (from April to September). Senescent leaves were collected at fall, oven-dried at 25 °C and ball milled. Glycine and beech leaf fragments were added in an amount corresponding to the natural annual N input (Hatton et al., 2012a), i.e. 80 μg g^{-1}_{soil} for glycine and 2.5 mg g^{-1}_{soil} for the leaf fragments ($n = 4$ lab replicates).

The amended soils were thoroughly mixed by hand and sieved twice to ensure a homogeneous mixing. Both treatments were incubated at 20 °C (dark; 1:3 soil to air ratio; 42% water holding capacity), aerated once a day for 2 weeks and twice a week until collection. The moisture content was readjusted when necessary. The incubation time was set so that the respiration rate, which increased after substrate addition, came back to a level comparable to the respiration rate before substrate addition (Bai et al., 2013). The beech leaves treatment was sampled after 92 days. The glycine treatment was sampled after 7 and 92 days, because the microbial utilization of the glycine C is known to be much faster than for chemically more complex structures (Jones and Murphy, 2007; Rinnan and Baath, 2009). Soils were kept frozen at −20 °C until analysis. No macro or meso fauna was detected neither before nor after incubation.

2.2. Sequential density fractionation

Subsamples were sequentially separated by density to isolate structurally and functionally different soil fractions (Hatton et al., 2012a), shown to be relevant for studying mineral-attached OM dynamics (Moni et al., 2012). The sequential density fractionation was performed according to Hatton et al. (2012a) with a centrifugation kept <2000 *g* to preserve microbial cells intact. Soils were suspended in a solution of sodium polytungstate (SPT_0, TC-Tungsten Compounds, Germany). For the first separation, the solution density was set to 1.65 g cm^{-3} and centrifuged. The light floating fraction was aspirated, rinsed and frozen at −20 °C. The same procedure was repeated with solutions of increasing density to finally obtain five operationally-defined density fractions earlier described by Hatton et al. (2012a): plant debris with little minerals attached (<1.65 g cm^{-3}), plant aggregates dominated by poorly altered plant debris and phyllosilicates minerals (1.65–1.85 g cm^{-3}), microbial aggregates dominated by microbial-processed SOM and phyllosilicates minerals (1.85–2.4 g cm^{-3}) and mineral grains dominated by poorly reactive quartz and feldspar with little SOM attached (2.4–2.65 g cm^{-3}). The remaining mineral grains denser than 2.65 g cm^{-3} were not treated here because of the presence of pedogenic oxides, which interfere strongly with the chromatography. However this fraction accounted only for 0.9% and 0.2% of soil dry weight and C, respectively.

2.3. Analyses

Carbon content and isotopic composition were determined in three analytical replicates using an elemental analyzer (CE instruments, NA 1500 type II) coupled to an isotope ratio mass spectrometer (Finnigan, Delta S) at the Technical Platform of Functional Ecology (OC 081) at INRA Forest Ecology and Ecophysiology Unit, INRA Nancy, France.

AS concentration and isotopic composition were determined using liquid chromatography coupled to isotope ratio mass spectrometer (LC-IRMS) at the Isotope Bioscience Laboratory – ISOFYS, Ghent University, Belgium. We followed the method described by Bodé et al. (2009) and two replicates selected from composite