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Short communication

Soil microbial carbon turnover decreases with increasing molecular size

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ABSTRACT

It is well established that soil microorganisms play an important role in respiration of newly fixed plant carbon. Recent results show that they also contribute significantly to soil organic matter (SOM) formation. We hypothesized that different molecular size classes of compounds in soil microbial biomass (SMB) have variable turnover time and in consequence influence SOM formation differentially. Here we used natural differences in carbon stable isotope signatures ($\delta^{13}C$ values) after C3–C4 vegetation change to track newly fixed C4 plant carbon into SMB molecular size classes. SMB was obtained by chloroform fumigation extraction (SFE) and $\delta^{13}C$ values of its size classes were measured using size exclusion chromatography coupled online to liquid chromatography–isotope ratio mass spectrometry (SEC–LC–IRMS). Resolved SMB was assigned to 5 size classes of 1800–9800, 800–1800, 380–800, 180–380 and 50–180 Da respectively. The contribution of recent C4 plant carbon to size classes of SMB decreased with increasing molecular weight (MW). It ranged from 77 ± 19% in the lowest MW size class size class to 41 ± 14% in the highest MW size class in a sandy soil and from 59 ± 18% in the lowest MW size class to 8 ± 15% in the highest MW size class in a clayey soil. A decreasing carbon turnover of compounds in SMB extracts along a continuum of molecular size from small to large implies that low molecular weight microbial compounds are rapidly metabolized products that link to fast respiratory carbon fluxes, whereas high molecular weight ones could be products of microbial synthesis like structural compounds that have slower turnover rates and link to slower SOM formation. Our methods help avoid contamination of CFE extracts and the results help explain why SMB turnover is faster in CFE extracts when compared to calculations using membrane lipids (e.g. PLFA-based).

Microorganisms play a central role in many soil processes, including decomposition and mineralization of organic matter. There is growing evidence that they are also responsible for carbon sequestration by production of compounds that persist in soil and form part of the stabilized soil organic matter (SOM) (Gleixner et al., 2002; Liang and Balser, 2011; Benner, 2011; Schimel and Schaeffer, 2012). It has been demonstrated that SOM is predominantly of microbial origin (sometimes up to 80%) as either living cells, their products or non-living biomass (Kindler et al., 2006; Simpson et al., 2007; Kramer et al., 2010; Miltner et al., 2011). The macromolecular structure and biochemical composition of the microbially sourced organic matter varies with microbial species and cell type and largely controls the stability and persistence of SOM (Simpson, 2002; Kiikkilae et al., 2012; Gleixner, 2013). It is however unknown whether different molecular size classes of soil microbial biomass show differential reactivity, origin and turnover time.

Since the most of SOM is of microbial origin and/or has been microbially processed, insights into the turnover of microbial biomass would help gain a better understanding of carbon cycling in soils. Stable isotope analysis provides a useful tool to track the flow of carbon in environments. Soils that have undergone vegetation change from C3 to C4 or *vice versa* are ideally suited to track carbon flow into different soil compartments at natural isotope abundance levels. In such a system with C3–C4 vegetation change, recent C4 plant carbon can be traced into the microbial biomass and be differentiated from SOM-derived carbon which has a C3 plant signature (Blagodatskaya et al., 2011; Kramer and Gleixner, 2008). An easy way to obtain microbial biomass residues from soil is via biocidal fumigation which lyses microbial cells and releases their contents (Vance et al., 1987; Tate et al., 1988). Chloroform fumigation-extraction (CFE) is widely used to estimate the amount of soil microbial biomass carbon (Franzluebbers, 1999; Philippot

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et al., 2012) and in combination with stable isotope analysis has been used to identify the source of microbial biomass carbon as well as to measure its turnover rate (Ryan and Aravena, 1994; Dijkstra et al., 2006).

This study aims to relate the molecular weight of compounds extracted from soil microbial biomass (SMB) to their turnover. To achieve this we used a long term field experiment with C3–C4 vegetation change performed on two soil types and used stable isotope signatures to track photosynthetically fixed carbon into SMB. Here we demonstrate that compounds in SMB have decreasing carbon turnover along a continuum of molecular size from small to large.

We used the online size exclusion chromatography–liquid chromatography–isotope ratio mass spectrometry (SEC–LC–IRMS) to measure the $\delta^{13}C$ values of different size classes of microbial biomass (Malik et al., 2012; Scheibe et al., 2012). Soil extracts were obtained from two soils with C3–C4 vegetation change which allowed us to estimate the contribution of newly fixed C4 plant carbon (<5 years) into different molecular size classes of microbial biomass. The experimental site, located at the Max Planck Institute for Biogeochemistry in Jena-Germany, consisted of two soil types that contrasted in soil texture and pH. The first, denoted "Sandy" (50% sand, 44% silt and 6% clay; pH 6.9) was originally derived from a forest A-horizon and the second denoted "Clayey" (9% sand, 75% silt and 16% clay; pH 7.8) from the B-horizon of a calcareous soil. Both soils originally had C3 vegetation; since 2006 they were cultivated with C4 crops. A part of each soil plot was continued in C3 vegetation for comparison of $\delta^{13}C$ values. More details about the experimental site are provided in Malik et al. (2012). Soil samples were collected in September 2011 (late vegetation period) from 0 to 10 cm depth using a 5 cm diameter stainless steel corer ($n = 3$). Soils were sieved <2 mm and chloroform fumigation extraction performed immediately based on the method by Vance et al. (1987) with slight modifications which were aimed at: 1) increasing the C concentration in the extracts (Needelman et al., 2001), 2) avoiding the necessity of dilution of salty extracts (Appel, 1998; Haney et al., 2001), 3) reducing the extraction time and preventing rapid microbial degradation of labile C compounds (Rousk and Jones, 2010). Aliquots (7 g) of fresh soil were fumigated with chloroform gas for 24 h followed by repeated (8 times) evacuation with vacuum. A non-fumigated control was maintained with the same amount of soil. Organic carbon was extracted from fumigated and non-fumigated soils with 0.05 M K_2SO_4 solution in a ratio of 1:4 (w/v). This mixture was homogenized on an orbital shaker (250 rev per min, 30 min), centrifuged for 5 min at 12,000 *g* and then filtered using prewashed Whatman filter paper. The whole extraction procedure was completed in 40 min. Soil extracts were acidified and purged with nitrogen gas in order to remove the dissolved inorganic carbon (DIC) and analyzed using SEC–HPLC–IRMS (Malik et al., 2012). Measurements were carried out using an HPLC system coupled to a Delta$^+$ XP IRMS through an LC IsoLink interface (Thermo Fisher Scientific, Germany). SEC was performed on a mixed bed analytical column (TSK-GEL GMPW$_{XL}$ – 7.8 mm × 30 cm; Tosoh Bioscience, Germany) maintained at a temperature of 25 °C using a column oven. 100 µL aliquot of soil extracts was injected using an autosampler (Surveyor autosampler, Thermo Fisher Scientific) into the mobile phase that consisted of phosphate buffer 20 mM (pH 6.2) maintained at a constant flow rate of 500 µL min^{-1} using a Surveyor MS pump. All reagents and the eluent were degassed under vacuum (20 mbar) in an ultrasonic bath for 30 min, and to prevent regassing a constant stream of helium was maintained in solutions during analysis. Chromatographic runs were performed for 45 min. Based on the calibration curves of polyethylene oxide and polyethylene glycol as molecular weight (MW) standards, the apparent MW of microbial biomass size classes was determined using their retention time (Malik et al., 2012).

LC–IRMS can be used for fast and reliable measurement of carbon stable isotope ratios in aqueous mixtures like dissolved or water extractable organic carbon (Scheibe et al., 2012). We used the system to measure $\delta^{13}C$ values of SMB obtained by the chloroform fumigation extraction method, and to get additional insights we performed its online size fractionation. SEC of OC in the fumigated extracts were assigned to 5 size classes (Fig. 1) eluting between 27 and 29 min (fraction 1/F1), 29–30 min (F2), 30–31 min (F3), 31–32 min (F4) and 32–33.5 min (F5). Apparent MW of size classes were estimated as 1800–9800 Da (F1), 800–1800 Da (F2), 380–800 Da (F3), 180–380 Da (F4) and 50–180 Da (F5) in the order of elution. In contrast, OC from non-fumigated soil extracts (often referred to as extractable organic carbon) appeared in only the two largest assigned size classes: F1 and F2 (Fig. 1). Traces of chloroform that might remain in the fumigated soils (Alessi et al., 2011) or other organic contaminants inadvertently added to the extracts did not elute with these size fractions (Supporting Information: Text S1; Fig. S1, S2). Thus, the SEC–LC–IRMS measurement of SMB from chloroform fumigation extraction can boost the precision of its isotopic measurement by excluding the late eluting contaminant fraction from soil extracts.

SEC–LC–IRMS results suggest that most of the SMB residues consisted of compounds in the apparent size range of 180–800 Da. The most abundant size classes F3 and F4 made up 27 and 33% of the total MB carbon (14.85 ± 2.1 mgC L^{-1}). Observations from SEC-Fourier transform infrared spectroscopy (SEC-FTIR) and SEC-nuclear magnetic resonance (SEC-NMR) measurements demonstrate that

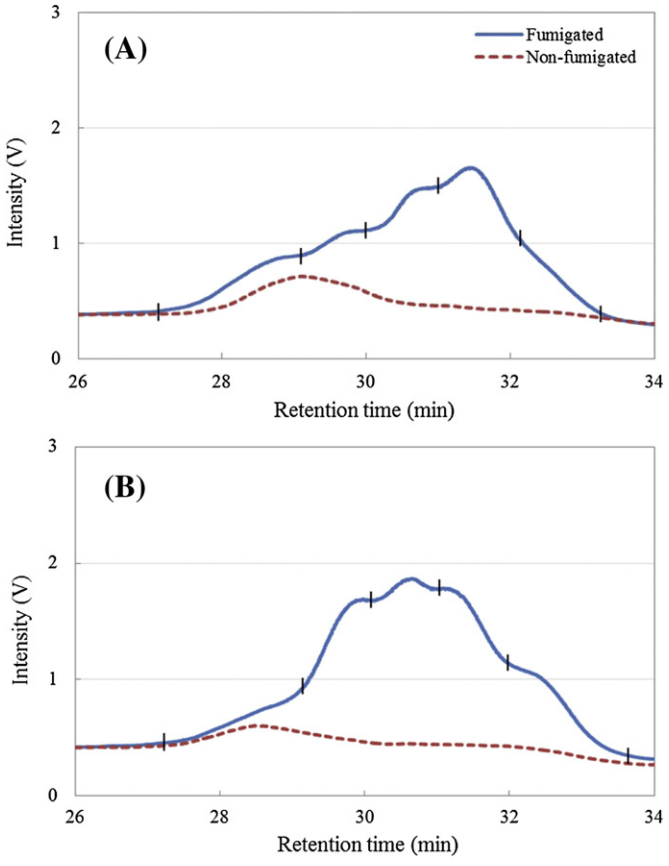

Fig. 1. Size exclusion chromatographic separation of extractable organic carbon from fumigated and non-fumigated soils: (A) 'Sandy'; (B) 'Clayey'.