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Linking molecular size, composition and carbon turnover of extractable soil microbial compounds

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ABSTRACT

Microbial contribution to the maintenance and turnover of soil organic matter is significant. Yet, we do not have a thorough understanding of how biochemical composition of soil microbial biomass is related to carbon turnover and persistence of different microbial components. Using a suite of state-of-the-art analytical techniques, we investigated the molecular characteristics of extractable microbial biomass and linked it to its carbon turnover time. A $^{13}CO_2$ plant pulse labelling experiment was used to trace plant carbon into rhizosphere soil microbial biomass, which was obtained by chloroform fumigation extraction (CFE). ^{13}C content in molecular size classes of extracted microbial compounds was analysed using size exclusion chromatography (SEC) coupled online to high performance liquid chromatography–isotope ratio mass spectrometry (SEC-HPLC-IRMS). Molecular characterization of microbial compounds was performed using complementary approaches, namely SEC-HPLC coupled to Fourier transform infrared spectroscopy (SEC-HPLC-FTIR) and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR-MS). SEC-HPLC-FTIR suggests that mid to high molecular weight (MW) microbial compounds were richer in aliphatic CH bonds, carbohydrate-like compounds and possibly P=O derivatives from phospholipids. On the contrary, the lower size range was characterized by more oxidised compounds with hydroxyl, carbonyl, ether and/or carboxyl groups. ESI-FT-ICR-MS suggests that microbial compounds were largely aliphatic and richer in N than the background detrital material. Both molecular characterization tools suggest that CFE derived microbial biomass was largely lipid, carbohydrate and protein derived. SEC-HPLC-IRMS analysis revealed that ^{13}C enrichment decreased with increasing MW of microbial compounds and the turnover time was deduced as 12.8 ± 0.6, 18.5 ± 0.6 and 22.9 ± 0.7 days for low, mid and high MW size classes, respectively. We conclude that low MW compounds represent the rapidly turned-over metabolite fraction of extractable soil microbial biomass consisting of organic acids, alcohols, amino acids and sugars; whereas, larger structural compounds are part of the cell envelope (likely membrane lipids, proteins or polysaccharides) with a much lower renewal rate. This relation of microbial carbon turnover to its molecular size, structure and composition thus highlights the significance of cellular biochemistry in determining the microbial contribution to soil carbon cycling and specifically soil organic matter formation.

1. Introduction

Microbial growth and activity largely control soil carbon cycling (Liang and Balser, 2011; Schimel and Schaeffer, 2012). It is readily accepted that the majority of plant organic carbon passes through the soil microorganisms, a fraction of which is used for cellular energy needs and the rest for biomass build-up; and that microbial

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biomass forms soil organic matter (SOM) mostly from cell fragments (Gleixner, 2013; Kögel-Knabner, 2002; Miltner et al., 2012). The contribution of microbial biomass to maintenance and accumulation of SOM is significant, some estimates suggest it could be as high as 80% of organic carbon in soil (Kindler et al., 2009; Liang et al., 2011; Simpson et al., 2007; Six et al., 2006). The residence time of microbial compounds in soil has been attributed to its molecular structure and biochemical composition as well as ecosystem specific effects (Simpson et al., 2007; Throckmorton et al., 2012; Tremblay and Benner, 2006). However, we do not have a thorough understanding of the dependencies of C turnover and persistence of microbial compounds on their molecular size and composition.

A widely used method to obtain microbial biomass from soil is biocidal fumigation using chloroform that lyses microbial cells and releases their contents which is followed by its extraction using K_2SO_4 solution (Tate et al., 1988; Vance et al., 1987). It is likely that chloroform fumigation does not lyse certain microbial groups with tougher cell envelopes and that the K_2SO_4 extraction selectively extracts only specific molecular compounds out of the lysed cellular products (Malik et al., 2013). Notwithstanding these shortcomings, chloroform fumigation extraction (CFE) has been extensively used to estimate soil microbial biomass carbon (Franzluebbers, 1999; Philippot et al., 2012) and its source and turnover time when coupled with stable isotope analysis (Dijkstra et al., 2006; Ryan and Aravena, 1994). However, in spite of its popularity in soil biology there is still no knowledge of the molecular structure and composition of the CFE microbial fraction. One of the aims of this report is to provide this understanding that is essential in making reliable ecological interpretations from CFE results. We applied two state-of-the-art molecular profiling tools to characterize the extracted microbial compounds: size exclusion chromatography (SEC) high performance liquid chromatography coupled with Fourier transform infrared spectroscopy (SEC-HPLC-FTIR) and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR-MS). While SEC-HPLC-FTIR quantifies different functional groups in size classes of the dissolved natural organic matter (Landry and Tremblay, 2012); ESI-FT-ICR-MS is able to identify the elemental formulae of thousands of molecular ions over a wide mass range (Reemtsma, 2009; Sleighter and Hatcher, 2007). A combination of these complementary tools allows detailed molecular profiling investigations by revealing different information. Relating this information to stable carbon isotope ratios ($^{13}C/^{12}C$) of size separated compounds, when a ^{13}C tracer is applied in an experimental system, allows one to associate the molecular fingerprints to inherent carbon turnover rates. SEC-HPLC-IRMS involves size exclusion chromatography coupled online with an SEC-HPLC-IRMS interface that enables molecular size-dependent separation of organic compounds followed by direct online stable carbon isotope analysis of the eluted size fractions (Malik et al., 2012).

The objective of this study was to link the molecular size and structure of extractable microbial compounds to their carbon turnover time. CFE derived soil microbial biomass from a plant $^{13}CO_2$ pulse-labelling experiment over a time series was analysed to estimate the turnover time of its molecular size classes and to gain the molecular profiles of both bulk microbial fraction as well as its size classes. The combination of analytical tools used here allowed us to profile the CFE-derived microbial compounds and ascertain if different compound size classes have variable turnover time. The methods also provide valuable information on the molecular characteristics of compounds in soil organic matter, which has been discussed in relation to the microbial contribution to SOM formation.

2. Materials and methods

2.1. Soil sampling and experimental setup

Soil from an arable field at the Jena Biodiversity Experiment located in Jena, Germany was used in a greenhouse experiment. *Dysphania ambrosioides* (formerly *Chenopodium ambrosioides*), a temperate herb, was grown in soil mesocosms and after 3 months of plant growth; a $^{13}CO_2$ pulse labelling was performed for 10 h at a CO_2 concentration of 350–400 ppm in an airtight glass chamber. The plants were returned to the greenhouse at the end of the labelling period and destructive soil sampling was performed at 1, 3, 12 and 24 h, then at 2, 4, 7, 14, 21 and 28 days after the pulse labelling. Rhizosphere soil from 3 mesocosms was sampled at each time point. Soil was then sieved to <2 mm, fine roots were extensively removed (this excludes mesofauna and plant residues) and stored at −20 °C. Details about the experimental design and the sampling strategy are given elsewhere (Malik et al., 2015).

2.2. Microbial biomass extraction

Microbial biomass from soil was obtained using a slightly modified version of the CFE method (Vance et al., 1987; Malik et al., 2013). Soil (7 g wet weight) was fumigated in a desiccator with chloroform gas for 24 h followed by repeated (8 times) evacuation. Organic matter was extracted from fumigated and non-fumigated control soils with 0.05 M K_2SO_4 solution in a ratio of 1:4 (w/v). The 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 resultant dissolved organic matter (DOM) was acidified and purged with nitrogen gas in order to remove the dissolved inorganic carbon (Scheibe et al., 2012). Fumigated and non-fumigated K_2SO_4 extracts from all time points and replicates ($n = 30$) were measured for stable carbon isotope ratios using SEC-HPLC-IRMS, whereas only two composite samples from ten randomly pooled K_2SO_4 extracts were used for the other detailed molecular analyses because as expected the microbial biomass content and composition did not change in our steady state experimental system (Malik et al., 2015). The K_2SO_4 extracts were directly measured on SEC-HPLC-IRMS without any further treatment but for the other analyses a solid phase extraction (SPE) was performed in order to concentrate and desalt the DOM. Fumigated and non-fumigated DOM was acidified to pH 2 and applied to activated SPE cartridges (Bond Elut PPL cartridge; 1 g, Agilent Technologies; Dittmar et al., 2008). CFE protocol blank (K_2SO_4 extract without any soil sample) and SPE cartridge blank were also maintained throughout the molecular fingerprinting analyses. CFE microbial biomass and background non-fumigated DOM represented approximately 1.2 and 0.3% of total organic carbon, respectively, in the studied soil system.

2.3. SEC-HPLC-FTIR analysis

The SPE extracts were dissolved in methanol in order to obtain a DOC concentration of ~3 mg mL^{-1}. 40 μL of this solution was injected into an Agilent 1200 HPLC system equipped with the Polymer standards service (PSS) SUPREMA analytical Linear S (8 × 300 mm; 5 μm) SEC column. Otherwise, the SEC-HPLC-FTIR technique and calibration used were the same as previously described (Landry and Tremblay, 2012). Before deposition and FTIR analysis, UV detection was carried out at a wavelength of 254 nm. SEC separated DOM was deposited as tracks onto a rotating germanium disk where a background spectrum was taken on a portion of the track where no DOM was eluting. Each band of every FTIR spectrum was integrated, valley-to-valley. The absolute