Reprint Article

Importance of microbial soil organic matter processing in dissolved organic carbon production

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Abstract

Soil dissolved organic carbon (DOC) sources and its seasonal dynamics are poorly known. We aimed to determine the contribution of plant and soil organic matter (SOM) to size classes of DOC in a field experiment with C3 to C4 vegetation change on two soil types through different seasons. Stable isotope ratios of DOC size classes were measured using size exclusion chromatography (SEC) coupled online to liquid chromatography–isotope ratio mass spectrometry (LC-IRMS). SEC resolved DOC into three size classes: very high molecular weight/vHMW (> 10 kDa), high molecular weight/HMW (0.4–10 kDa), and low molecular weight/LMW (< 0.4 kDa). HMW DOC was most abundant in all seasons, soil types, and depths. In contrast, vHMW DOC was only seen postsnowmelt in upper 20 cm and was mainly (87 ± 9%) plant-derived. Through all seasons, HMW and LMW DOC had less than 30% recent plant contribution. Similar size range and source of DOC size classes and soil chloroform fumigation extracts suggest microbial origin of DOC. Thus, microbial SOM recycling is an important process in DOC production. We suggest that DOC molecules get partitioned manifold between soil solution and the mineral matrix (chromatography), thereby getting constantly decomposed, altered, or produced anew by soil microorganisms (reactive transport).

Introduction

Dissolved organic matter (DOM) is a small and reactive fraction of total organic matter in soil and is important in various biogeochemical processes (Battin et al., 2009; Kindler et al., 2011). Its movement through soil pores and interaction with solid organic matter make it a highly dynamic carbon pool subject to physical, chemical, and biological alteration. It primarily originates either from recent plant biomass (litter, root litter, and/or root exudates) or from soil organic matter (SOM) (Kalbitz et al., 2000). Besides the substrate type/amount, microbial activity, and community composition, abiotic factors such as hydrologic variability and temperature also influence DOM production and flux (McDowell, 2003). The current understanding of DOM sources suggests that carbon derived from SOM, not from newly introduced plant organic matter, is the primary source of DOM in mineral soil (Froeberg et al., 2007; Steinbeiss et al., 2008). Other studies demonstrate that plant residues or residues of microbial biomass feeding on the former are short-lived, and beyond this pulse, most of the DOM is derived from SOM (Gregorich et al., 2000; De Troyer et al., 2011). Thus, there is an increasing evidence to suggest that dissolved organic carbon (DOC) at depths originates primarily from within mineral soils being derived from highly altered SOM and is not merely a fraction of the fresh plant leachates transported in the soil solution (Karltn et al., 2005; Sanderman et al., 2008). There are other studies demonstrating the significance of abiotic processes such as exchange, sorption, or dissolution reactions in DOC production (Guggenberger & Kaiser, 2003; Toosi et al., 2012). In a conceptual model of the vertical movement of DOC, Kaiser & Kalbitz (2012) propose that organic compounds in soil water get precipitated or sorbed followed by its microbial processing and re-release by desorption or dissolution.

While most investigations on DOM relied on its bulk measurements to study its biogeochemical evolution, several other studies involving compositional and isotopic analysis of DOM suggest that different fractions have variable biodegradability or persistence (Qualls, 2005;
Kiikkila et al., 2012; Landry & Tremblay, 2012). By virtue of being persistent in the environment to a varying degree, DOM fractions could contribute differentially to SOM cycling. We thus aimed at delineating the sources of DOM at molecular level to quantify the relative contribution of fresh litter, SOM, and microbial biomass to size classes of DOM. We hypothesized that different molecular size classes of DOM collected from a long-term vegetation change field experiment originate from different sources. Stable carbon isotope ratios (δ13C) and its natural variability in plants with different photosynthetic pathways (C3 and C4) allow tracking of recent plant carbon into different soil carbon pools (Balesdent & Mariotti, 1996; Froeberg et al., 2007; Kramer & Gleixner, 2008).

In soils undergoing a vegetation change from continuous C3 to C4 plants, SOM is naturally labeled with C3 plant isotopic signature (δ13C = −27 ‰), and the recently fixed carbon introduced into the soil in the form of shoot litter or root litter/exudates is labeled with C4 plant signature (δ13C = −12 ‰). Comparing the isotopic composition in a plot continuously under C3 vegetation with that after vegetation change allows the quantification of plant carbon (both aboveground litter and roots) in soil DOM.

Online SEC liquid chromatography–isotope ratio mass spectrometry (SEC-LC-IRMS) is a novel technique for δ13C measurement of DOC molecular size classes (Malik et al., 2012). It couples the resolution of compounds in aqueous mixtures through a chromatographic column with the accurate isotopic measurement potential of an IRMS. DOC samples can be analyzed rapidly without sample processing or preconcentration (Scheibe et al., 2012). Isotopic ratios of DOC size classes give additional insights into DOC cycling because components in this complex mixture show differential chemical reactivity and bioavailability (Qualls, 2005; Kiikkila et al., 2012).

We analyzed soil water from a C3 to C4 vegetation change experiment in two soil types at three depths to quantify the relative contribution of recent plant- and old SOM-derived carbon in different size classes of DOC. To find systematic differences in the proportion of recently photosynthesized carbon in soil DOM, the measurements were taken for different seasons representing snowmelt, early and later vegetation periods, and winter. We also linked DOC sources to sources of microbial biomass carbon to discern the microbial involvement in DOM production and belowground carbon cycling.

Materials and methods

Experimental site

The experimental design consisted of two soil plots with an area of 48 m² each that were established at the Max Planck Institute for Biogeochemistry, Jena, Germany, in 2006. The plots were established using homogenized soil from different sites with varying properties and texture (Table 1); they were referred to as ‘sandy’ and ‘clayey’. Both soils had previous continuous C3 vegetation. A half of each soil plot was subjected to vegetation change from C3 to C4 plants since 2007 for 5 years. The other half was maintained in C3 cultivation as control for comparison of δ13C values. The annual cycle began after every winter when the soil was surficially tilled. This resulted in mixing and homogenization of the top 5-cm layer, but a soil profile developed below with time. Phacelia (scorpion weed), Helianthus annus (sunflower), and Triticum spp. (wheat) were grown in the C3 control plots, and Zea mays (maize), Amaranthus, and Sorghum were grown in the experimental C4 plots. Plant type over the years was rotated or changed to prevent monoculture effect in the fields. Seeds were sown in spring and plants allowed to grow normally until harvest in autumn. Care was taken to remove unwanted plants and weeds growing in the plots as this could affect the isotopic signal. Postharvest, the entire plant biomass was weighed, shredded, and returned to the respective soil plots. Equal amount of plant biomass was returned to C3 and C4 plots. Plastic sheets then covered the plots until the next spring to prevent pollen and seed dissemination onto the plots.

Sample collection and preparation

Soil water samples were collected from the four plots using borosilicate glass suction plates (thickness – 9 mm, diameter – 120 mm, pore size – 1 μm; UMS, Germany) located at 10, 20, and 30 cm depth. One glass suction plate per depth per soil type was used in the experiments. Moreover, the plates have a large diameter that allows obtaining a representative sample. A vacuum of 200 mbar was applied to suck soil solution into 2-L borosilicate

| Table 1. Characteristics of soil used for the vegetation change experiment |
|------------------|------------------|------------------|
| Soil type/ NOMINATION | ‘Sandy’ | ‘Clayey’ |
| Soil parent material | Forest A horizon soil | B horizon of a calcareous soil |
| Soil texture | 50% sand, 44% silt, 6% clay | 9% sand, 75% silt, 16% clay |
| Soil pH | 6.9 | 7.8 |
| Organic C concentration | 4.20 ± 0.19% | 1.96 ± 0.27% |
| Inorganic C concentration | ND | 0.78 ± 0.15% |
| pH of soil water | 7.3 (± 0.2) | 8.0 (± 0.1) |
| SOM δ13C in C3 plots | −27.8 ± 0.1 ‰ | −29.0 ± 0.5 ‰ |
| SOM δ13C in C4 plots | −27.1 ± 0.2 ‰ | −27.9 ± 0.5 ‰ |
flasks. Soil water was collected every fortnight. Suction plates were chosen over zero-tension lysimeters as this allows soil water sampling almost throughout the year, not merely during storm events. Moreover, fortnight vacuum application that fades away successively implies that the soil solution collected corresponds to both slow flow path that is more in contact with the soil matrix and fast flow path that may represent fresh DOM. Samples for SEC-LC-IRMS analysis were collected in January, June, September, and December 2011; and March 2012. Soil abiotic parameters such as moisture (Theta-Probe, ML2X, Delta-T, UK) and temperature (NTC 107; Campbell Scientific, Australia) were obtained from continuous measurements using sensors fitted at different depths (Table 2). March 2012 sampling was chosen to compare the effect of litter quantity on abundances of DOC size classes and their sources. This period also represents snowmelt or thawing; however, the litter input from the preceding harvest was lower (32.6 g plant litter C kg$^{-1}$ SOC) compared with the harvest preceding January 2011 sampling (74.7 g plant litter C kg$^{-1}$ SOC). Soil water samples were immediately frozen (−20 °C) until analysis. All samples were acidified and purged to remove the dissolved inorganic carbon. One mL of sample was placed in 1.5-mL brown glass vials (silanized); 20 μL of 8.5% phosphoric acid (Merck, Germany) was added and vortexed for a minute. Samples were purged with a gentle stream of nitrogen (99.99% N$_2$) for 10 min using stainless steel syringe needles fitted to an automated 12-port chamber (VisisprepTM, Visidy TM, Supelco, Sigma-Aldrich). Acidified soil water samples were then analyzed by LC-IRMS in the column mode (HPLC mode). The soil solutions were further filtered through a 0.45-μm filter in the LC-IRMS interface to meet the operational description of DOM.

**DOC fractionation and δ$^{13}$C measurement**

Stable isotope analysis of DOC size fractions was carried out using an HPLC system coupled to a Delta$^{+}$ XP IRMS through an LC-ISO-LINK interface (Thermo Fisher Scientific, Germany). Details of the LC-IRMS system and modifications included are given elsewhere (Malik et al., 2012; Scheibe et al., 2012). SEC was performed on a mixed bed analytical column (TSK-GEL GMPW$_{XL}$– 7.8 mm × 30 cm; Tosoh Bioscience, Germany) with a guard column (TSKgel PW$_{XL}$), both maintained at a temperature of 25 °C. Using an autosampler (Surveyor autosampler; Thermo Fisher Scientific), 100 μL of analyte was injected into the mobile phase consisting of phosphate buffer 20 mM (pH 6.2) at a constant flow rate of 500 μL min$^{-1}$. Mobile phase solution and other reagents were degassed under vacuum (20 mbar) in an ultrasonic bath for 30 min, and to prevent regassing, a constant helium stream was maintained in solutions during analysis. Chromatographic runs were made for 45 min each and always duplicated. Isodat 2.0 SP 2.67 software (Thermo Fischer Scientific, Germany) was used to run the HPLC-IRMS system. After assessing the chromatograms, different size fractions as recurring peaks were assigned to 2 or 3 retention time intervals. Chromatographic resolution of the column was evaluated using polyethylene glycol and polyethylene oxide size standards. Molecular weight of DOC size classes thus achieved is only an apparent value, because the matrices of test substances used match only moderately to that of the DOC compounds. Linearity of the coupled system was ascertained using varying concentrations of different organic compounds. Additional information about the method and standardization is provided elsewhere (Malik et al., 2012; Scheibe et al., 2012).

δ$^{13}$C measurement of SOM, plant, and microbial biomass

Soil samples for SOC measurement were collected in October 2010 and September 2011 using a 5-cm-diameter stainless steel corer for different depth intervals: 5–10, 10–20, 20–30 cm. Samples were sieved < 2 mm, dried, ground, and measured for C using an elemental analyzer (Elementar analysator vario Max CN; Elementar Analysensysteme GmbH, Germany) and for δ$^{13}$C using an EA-IRMS (CE 1100 coupled via Con Flo III with a Delta$^{+}$; Thermo Fischer, Germany). δ$^{13}$C measurements of aboveground plant biomass collected following harvest every year were also taken after similar treatment on the

| Sampling date               | January 2011 | June 2011 | September 2011 | December 2011 | March 2012 |
|-----------------------------|--------------|-----------|----------------|---------------|------------|
| Description                 | Snowmelt, post-high litter input | Dry, early vegetation period | Moist, late vegetation period | Mild winter, post-low litter input | Snowmelt, post-low litter input |
| Soil moisture (Vol.%)        | 38.1 ± 0.8   | 21.8 ± 3.7 | 32.6 ± 5.3     | 33.3 ± 2.6    | 33.7 ± 1.5 |
| Soil temperature (°C)        | 5.1 ± 0.1    | 18.8 ± 0.8 | 18.5 ± 0.2     | 3.4 ± 0.3     | 7.4 ± 0.8  |

Harvest of aboveground plant biomass was carried out in November 2010 and October 2011.
EA-IRMS. Soil samples for microbial biomass extraction were collected in September 2011 using the same corer from the top 10 cm of all soil plots (n = 3). Soils were sieved < 2 mm, and chloroform fumigation extraction was performed immediately. The fumigation procedure was based on the method by Vance et al. (1987) with slight modifications (Malik et al., 2013). 7-g wet soil was fumigated with chloroform gas for 24 h followed by repeated evacuation with vacuum to remove chloroform vapors from the soils. A nonfumigated control was maintained with the same amount of soil. Following fumigation, DOC was extracted from all soils with 0.05 M K₂SO₄ solution in a ratio of 1 : 3. This mixture was homogenized on an orbital shaker (250 r.p.m. min⁻¹, 1 h), centrifuged for 10 min at 12 000 g, and then filtered using prewashed Whatman filter paper. Soil extracts were treated in the same way as soil water samples before measurement on the HPLC-IRMS system (Malik et al., 2013).

**Calculation of plant- and SOM-derived carbon**

We used a two-source model to calculate the contribution of differentially labeled substrates to DOC size classes and microbial biomass (Phillips & Gregg, 2001). The differences in δ¹³C (Δδ¹³C) of each size class in C3 and C4 cultivated soils were compared with the difference in the δ¹³C of plant biomass and SOM from the C3 and C4 cultivated soils (Kramer & Gleixner, 2008). The average δ¹³C of C3 and C4 vegetation was −28.3 ± 1.1 ‰ and −13.4 ± 1.4 ‰, respectively. The average δ¹³C of soil organic carbon from the plots without and with vegetation change was −28.1 ± 0.5 ‰ and −27.2 ± 0.8 ‰, respectively. Thus, the Δδ¹³C for plant biomass and SOM was calculated as 15 ‰ and 1 ‰, respectively. The contribution of plant-derived carbon in different fractions of DOC was calculated using Eqn (1). This equation not only considers the differences in the δ¹³C values, but also takes into account the differences in δ¹³C values of SOM that has a small value (~28 ‰) as a result of the slow incorporation of new plant carbon into the SOM.

\[
\text{Plant derived carbon (‰)} = \frac{(\delta^{13}C_{C4,DOC} - \delta^{13}C_{C3,DOC}) - (\delta^{13}C_{C4,SOM} - \delta^{13}C_{C3,SOM})}{(\delta^{13}C_{C4,Plant} - \delta^{13}C_{C3,Plant}) - (\delta^{13}C_{C4,SOM} - \delta^{13}C_{C3,SOM})} \times 100
\]

**Results**

**DOC size fractionation**

SEC of soil DOC resulted in chromatograms with 2 or 3 discrete peaks each representing a molecular size class (Fig. 1a–b). The earliest eluting peak between 20 and 27 min was assigned as the ‘very high molecular weight’ – vHMW size class (10–150 kDa). The following peak from 27 to 31 min was called as the ‘high molecular weight’ – HMW size class (0.4–10 kDa, peak maxima at 1.5–3 kDa). The late eluting peak with retention time between 31 and 35 min was assigned as the ‘low molecular weight’ – LMW fraction (0.05–0.4 kDa). The vHMW size class of DOC was rare and only seen in water samples from one season (snowmelt, higher litter input) and the lower depths, viz. 10 and 20 cm. Its average concentration across soil plots and depths was 2.3 ± 1.5 mg C L⁻¹. The HMW DOC size class was the most abundant and seen in all seasons and depths; its average concentration was 10.1 ± 7.6 mg C L⁻¹ (n = 30). The LMW size class of DOC was also present in soil water from all seasons. However, its concentration was low at 2.5 ± 1.3 mg C L⁻¹ (n = 30). Total DOC concentration in the two soil types ‘sandy’ and ‘clayey’ was 16.7 ± 11.5 mg C L⁻¹ and 7.9 ± 3.4 mg C L⁻¹, respectively (Supporting Information, Fig. S1). The apparent DOC export for each size class was calculated by multiplying the concentration of each size class with the total volume of soil water collected and dividing it by the area of the suction plate and the time period from suction to sample collection (Kindler et al., 2011). DOC export for individual size classes showed a seasonal pattern, it was lowest in June 2011 (early vegetation period) in both soil types (Fig. 2a–b). The yearly apparent flux across all depths sampled in the two soil plots ‘sandy’ and ‘clayey’ was 1.2–7 g C m⁻² year⁻¹ and 0.1–2.2 g C m⁻² year⁻¹, respectively. The absolute amount of plant carbon in total DOC export was highest in January 2011; it was 2.8 ± 2.4 mg C m⁻² day⁻¹ and 4 ± 2.6 mg C m⁻² day⁻¹ in ‘sandy’ and ‘clayey’ soil, respectively. It was lowest in June 2011 (early vegetation period, dry) at 0.1 mg C m⁻² day⁻¹ each in both soil types. In later sampling points, the apparent DOC fluxes remained mostly constant (0.3–0.9 mg C m⁻² day⁻¹) except in September 2011 (late vegetation period) in ‘sandy’ soil; here, the DOC concentration and export (3 ± 0.8 mg C m⁻² day⁻¹) were significantly higher than in ‘clayey’ soil and other seasons.

**δ¹³C of DOC size classes**

The δ¹³C of DOC size classes showed distinct patterns based not only on vegetation type, but also on sampling depth and molecular size of fractions. In the control plots without vegetation change, DOC sources (plant and SOM) have similar isotopic signature (~ −28 to −29 ‰).
Hence, these plots can be used to monitor the degree of isotopic fractionation in different DOC size classes. No isotopic fractionation was observed during HPLC separation in the SEC column as seen in the isotope ratio trace values of standard materials as well as environmental samples (Malik et al., 2012). In the C3 control plots, the vHMW and HMW DOC size classes were isotopically very similar to the substrate (average for the two soil types and three depths sampled: $\delta^{13}C_{vHMW} = -28.1 \pm 0.9 \%_{oo}$, $\delta^{13}C_{HMW} = -28.2 \pm 0.5 \%_{oo}$, $n = 30$), whereas the LMW size fraction was isotopically depleted compared with the substrates available ($\delta^{13}C_{LMW} = -29.3 \pm 0.7 \%_{oo}$). On the contrary, DOC from plots with C3/C4 vegetation change was isotopically enriched. Here, the isotope ratio of the vHMW size class was close to the C4 plant signature (average $\delta^{13}C_{vHMW} = -14.8 \pm 1.6 \%_{oo}$). The HMW and the LMW size classes had isotopic values in between that of C3 and C4 signatures ($\delta^{13}C_{HMW} = -23.9 \pm 1.7 \%_{oo}$, $\delta^{13}C_{LMW} = -24.6 \pm 1.6 \%_{oo}$, $n = 30$) and thus show contribution of both new C4 plant carbon and old C3-labeled SOM-derived carbon.

### Sources of DOC

To determine the carbon source of different DOC size classes, the differences in $\delta^{13}C$ ($\Delta\delta^{13}C$) of each fraction in C3 and C4 cultivated soils were calculated. The contribution of plant-derived carbon in different fractions of DOC was then calculated using Eqn (1). The vHMW DOC size class was almost entirely derived from recent plant material (Fig. 3a). The average plant contribution to this fraction of DOC was 87% ($\%_{oo}$). The other two size classes of DOC, viz. HMW and LMW, were mostly derived from SOM. The average plant biomass contribution to the HMW and LMW size classes across all seasons and soil types was 22–51% at 10 cm, 10–39% at 0–10 cm, 9–19% at 10–20 cm, and 8–15% at 20–40 cm (Fig. 3b). There was also a distinct depthwise trend; the percentage of plant-derived carbon decreased with increasing depth in both soils (Fig. 3b). It was 22–51% at 10 cm, 10–39% at 0–10 cm, 9–19% at 10–20 cm, and 8–15% at 20–40 cm (Fig. 3b).

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**Fig. 1.** Representative SEC-LC-IRMS chromatograms showing resolution of DOC into three size classes in soil water from ‘Clayey’ soil plot collected in January 2011 (a). Soil DOC from all other sampling points resolved into only 2 size classes. Shown here is a chromatogram of DOC from the same plot sampled in September 2011 (b). Apparent molecular weights of size classes are presented next to the peaks.

**Fig. 2.** Seasonality in apparent DOC flux for individual size classes in ‘Sandy’ (a) and ‘Clayey’ soil (b). Labels – LMW, low molecular weight; HMW, high molecular weight; vHMW, very high molecular weight.
20 cm, and 6–18% at 30 cm. The seasonal pattern was not very clear to elucidate (Figs 3c and 4a–b, Fig. S2). The vHMW size class was only seen in soil water from January 2011 (snowmelt, higher litter input) and was almost entirely plant-derived. The plant contribution in the HMW and LMW DOC size classes that were present in soil water from all seasons was relatively stable and did not vary much across seasons, although it was slightly, but not significantly higher during the vegetation period in June 2011 and September 2011.

Based on relative terms, the amount of aboveground plant litter C added compared with the amount of native SOC was low: 2.5% and 4.9% in ‘sandy’ and ‘clayey’ soil, respectively. This estimate is low because it does not include belowground plant C input (from root biomass and exudates) that is 1.3 times higher than aboveground plant C input (calculated with data from Steinbeiss et al., 2008; Nguyen, 2009; Gleixner, 2013). In comparison, the mean overall relative total plant C contribution to DOC was much higher (14.5% and 35.4%, respectively). This relation suggests that 1 g of aboveground plant litter C contributes approximately 6.5 (2.8 in case of total plant C input) times more to DOC compared with 1 g of SOC, which mirrors the faster decomposability of plant C relative to SOC.

**Sources of soil microbial biomass**

Microbial biomass extracts were obtained using chloroform fumigation extraction from the topsoil during the vegetation period to compare its molecular size distribution and isotopic variability with that of DOC. Microbial biomass was resolved by SEC into various size classes (Malik et al., 2013). It was a complex mixture with an
apparent molecular size ranging from 0.1 to 10 kDa with peak maxima between 0.2 and 1 kDa. $\delta^{13}C$ of microbial biomass in the C3 control plots was $-30.6 \pm 0.6 \%_{oo}$ and $-28.6 \pm 0.2 \%_{oo}$ in ‘sandy’ and ‘clayey’ soil, respectively. In the plots with vegetation change, the $\delta^{13}C$ of microbial biomass was $-22.2 \pm 1.1 \%_{oo}$ and $-22.7 \pm 0.1 \%_{oo}$ in the two soils. Plant contribution to microbial biomass carbon was estimated at 54 ± 5% and 30 ± 2% in ‘sandy’ and ‘clayey’ soil, respectively.

**Discussion**

The C3/C4 vegetation change experiment allowed us to quantify the relative contribution of plant-derived carbon and, from this, the SOM-derived carbon in DOM assuming two end members. Fractionation of DOM by SEC coupled to an LC-IRMS gave additional insights into the carbon sources and turnover of individual size classes of DOM. There are certain assumptions that are made in quantifying the contribution of recent C4 plant vs. old C3 SOM in a vegetation change experiment. We assume that same amount of DOM is produced and exported in both control and experimental plots. We also assume that isotopic fractionation, if any, during the production and degradation of DOM, is similar in both the fields. However, the differences in $\delta^{13}C$ values of DOM size classes from the C3 and C4 soils were used to calculate the contribution of different sources; hence, any isotopic
fractionation involved gets canceled. In addition, the C3 control plots were used to monitor the degree of isotopic fractionation in different size classes as both the recent plant organic matter and the old SOM have similar isotope signature.

The DOM concentration and flux varied depending on hydrologic conditions and other abiotic factors including soil properties. These factors in addition to the amount of litter added to the fields also determined the amount of plant carbon in DOM export. The advantage of the field experiment was that we could also consider the effects of abiotic factors in a natural environment on sources of DOM (Kalbitz et al., 2000), and thus, the quantification was more reliable than previous experiments with controlled environmental conditions. In January 2011 sampling, the DOM export was quite high because of snowmelt or thaw. After the harvest in November 2010, a higher litter amount was added to the fields, and because this was followed by a long and severe winter, most plant carbon leached into the DOM only during the snowmelt. June 2011 sampling period (early vegetation phase) was dry, and so there was very little leached soil water. In later sampling points, the DOM fluxes remained mostly constant. Soil solution collected using tension lysimeters primarily corresponds to slow flow path that is more in contact with the soil matrix and is different from the DOM collected using zero-tension lysimeters (Sanderman et al., 2008). However, application of vacuum that fades away during the course of DOM sampling allows collection of both fast and slow flow path soil solution. We used such a setup with large suction plates to collect soil water from different seasons.

Size fractionation resolved soil DOM into three size classes based on apparent molecular weights. The DOC detector also allows accurate estimation of carbon concentration of the size classes (Malik et al., 2012; Scheibe et al., 2012). The vHMW size class (10–150 kDa) was rare and less abundant. The HMW size class contained mid-sized to large molecules ranging from 0.4 to 10 kDa. Majority of DOM compounds have this molecular size range, and this fraction was found in all seasons. Very small molecules (< 0.4 kDa) that elute last were grouped into the LMW size class, and although this fraction was seen in all seasons, its proportion was very small. Such fractionation of DOM has been demonstrated in other studies that estimated the molecular weights of organic matter fractions in water and by combining SEC with UV detectors or total carbon analyzers, two to four fractions of DOC have been reported (Mueller et al., 2000; Her et al., 2002; Landry & Tremblay, 2012). However, it should be noted that it is difficult to obtain separation solely based on size as there can be other forms of non-specific interactions between certain compounds in the DOM and the column material. In addition, there is a lack of suitable size standards for DOC measurements. Molecular weights of DOC size classes thus determined by SEC are only apparent. These are the limitations to be considered in interpretation of results obtained by SEC-LC-IRMS.

DOC is a complex mixture of different components that vary in biodegradability and chemical reactivity and may have different sources (Kiikkila et al., 2005). SEC of DOC coupled to isotope analysis suggests that different molecular size classes of DOC indeed have variable sources. The vHMW DOC size class appeared only after a high litter application event and during snowmelt. Moreover, this fraction is almost entirely plant-derived. Under high moisture conditions such as snowmelt, DOC export is mostly by preferential pathways, leading to kinetic restriction of sorption processes, thus enabling young DOC to get transported to larger depths. Thus, we hypothesize that the vHMW size class of DOC consists of nondegraded plant biomass leaching downwards. This finding is supported by observations from SEC-FTIR (Fourier transform infrared spectroscopy) and SEC-NMR (nuclear magnetic resonance) measurements, which suggest that larger compounds in terrigenous DOC (> 5 kDa) are mostly carbohydrates, alkenes, and/or aliphatics (Woods et al., 2009; Landry & Tremblay, 2012). The presence of such compounds suggests more recent and reactive DOC. Our isotopic results prove that the vHMW fraction consists mostly of recent plant-derived carbon. The other two size classes, viz. HMW and LMW, were mostly derived from old SOM that suggests the presence of highly processed material in these size classes of DOC. This observation is further substantiated by compositional studies of DOC (Woods et al., 2009; Landry & Tremblay, 2012), which suggest that compounds in this molecular size range contained more hydroxyl- and carboxyl-rich alicyclic molecules as also hypothesized by Hertkorn et al. (2006).

We also aimed to link DOM to microbial biomass residues in soil, because there is growing evidence that microorganisms are responsible for formation, stabilization, and processing of SOM (Liang & Balser, 2011; Miltner et al., 2012; Gleixner, 2013). SEC of microbial biomass from these soils obtained by chloroform fumigation extraction suggests that microbial residues or metabolic products fall mostly in the 0.3–1 kDa range. From its isotope analysis, it was clear that microorganisms use plant carbon in the form of root exudates or soluble compounds from litter for respiration or energy metabolism, and very little is used for biosynthesis; therefore, these fresh compounds are not seen in the DOC. Moreover, high contribution of old soil organic carbon to microbial biomass, particularly the HMW size classes.
(Malik et al., 2013), indicates a slow renewal rate of microbial biosynthetic compound. The similarities in molecular size ranges and isotope values of HMW and LMW DOC size classes with those of microbial biomass suggest that DOC is a footprint of microbial activity. Evidently, only when microbial activity and decomposition rates are lower, plant-derived compounds like those in the vHMW size class appear in the DOC. On the contrary, when conditions are favorable for microbial growth and activity, more SOM-related compounds are seen in the DOC. These compounds are most likely a result of microbial processing and alteration in SOM (Steinbeiss et al., 2008; Kindler et al., 2009; Miltner et al., 2012).

The depthwise trend in plant contribution to DOC suggests that plant carbon is lost in the upper 20 cm. The DOC in higher depths has less than 18% plant contribution. It is also important to note that the vHMW size class that consists of nondegraded plant biomass was seen only in the upper 20 cm. There was also a clear distinction between the soil types; DOC from ‘sandy’ soil had a higher contribution of SOM than that from ‘clayey’ soil. This may be because of the higher soil organic carbon concentration in ‘sandy’ soil (Table 1).

The plant contribution to all DOC size classes except the vHMW fraction was consistently low across all seasons. Even after a very high biomass input, plant-derived carbon in DOC was less than 40%. The vHMW size class was absent in March 2012 soil water, which was also a snowmelt period. This could be because the litter amount added in the preceding harvest was much lower compared with the earlier harvest. Consequently, the plant contribution to HMW and LMW size classes was also much lower. Similar results demonstrating low input of plant biomass into DOC through litter decomposition experiments have been reported (Cleveland et al., 2004; Froeberg et al., 2007; Sanderman et al., 2008; De Troyer et al., 2011). We show in this seasonal monitoring experiment that plant contribution through both litterfall and root litter/exudation is a less significant source of DOC in mineral soil. Even in the active vegetation period, the contribution of fresh plant biomass to DOC was low, which suggests that incorporation of plant residues remobilizes some soil organic carbon that is leached into the dissolved phase (Marx et al., 2007). These results thus confirm that across all seasons and soil types, majority of the compounds in the DOC are derived from SOM. Our observations thus lead to the conclusion that decomposition and remobilization of SOM and its processing by microorganisms are the most important processes in DOC production. In consequence, we suggest that DOC is reactivity transported downward in soil whereby DOC molecules are partitioned manifold between soil solution and the mineral matrix (chromatography), thereby getting constantly decomposed, altered, or produced anew by soil microorganisms (reactive transport; see Data S1).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Seasonal trend in total DOC concentration in the two soil types expressed as a mean of all depths and vegetation types.

Fig. S2. Seasonal variation in contribution of recent plant and old soil organic matter (SOM) to total DOC in the two soil types.

Data S1. Chromatographic behavior of soil: a historical perspective.