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Short communication

Biodegradation of crop residue-derived organic matter is influenced by its heteroatomic stoichiometry and molecular composition

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ABSTRACT

Plant biomass is the primary source material for soil organic matter formation. Soil organic matter comprises the largest terrestrial pool of the global C cycle and provides critical ecosystem services. The objective of this study was to document microbially-driven molecular-level changes to the water-soluble organic matter (WSOM) fraction extracted from crop residues as it undergoes biodegradation in laboratory microcosms using ultrahigh resolution mass spectrometry. Crop residues from corn and soybean fields after harvest and a leguminous, hairy vetch green manure were the sources of the WSOM studied. The results show that, at the individual molecule-level, the biodegradation process does not follow the expected trends, i.e. that higher N and lower lignin concentrations in plant biomass should favor its decomposition compared to material with lower N and higher lignin. We also show that S- and P-containing molecules are consistently more biodegradable, as compared to N-containing molecules. In addition, the results suggest that carbohydrate molecules may be less biodegradable, and that aromatic compounds may be more biodegradable, than conventionally understood. Future studies are suggested to increase our knowledge of microbially-driven biodegradation of plant biomass. Understanding plant biomass decomposition at the molecular level is fundamental to understanding how soil organic matter forms and is stabilized. This knowledge is necessary to design management practices to preserve soil organic matter and the ecosystem services it provides.

1. Introduction

The annual input of plant biomass is the primary source material for soil organic matter formation (Kaiser and Guggenberger, 2000; Kögel-Knaber, 2002, 2017). Conservation tillage, typically defined as retention of crop residue to provide > 30% soil surface coverage, is used on about 70.1 million ha as compared to 42.8 million ha for convention tillage (USDA-NASS, 2014). An ecological impact assessment of crop residue management showed that greater quantities of residues retained in the fields led to improvements in soil quality, erosion control, and soil organic carbon pool (Stavi et al., 2016). The decomposition of plant biomass in the environment, although fundamental to preserving ecosystem services, is not well understood. It is believed to be a stepwise process with rain and soil moisture initially dissolving soluble components from plant residues. The leached water soluble organic matter (WSOM) is subject to oxidative biodegradation resulting in the formation of molecules such as altered water-soluble lignin products and microbial metabolites including amino acids and polysaccharides (Guggenberger et al., 1994; Kalbitz et al., 2003; Fellman et al., 2008). As soluble components biodegrade, WSOM declines due to mineralization of C to CO_2, the assimilation of C into soil microbial biomass and leaching through the profile (Kalbitz et al., 2003; Bowen et al., 2009). Resistance to WSOM biodegradation has been associated with low carbohydrate content and high aromatic content since carbohydrates, lipids, phospholipids, and amino acids are preferentially consumed by microorganisms, as compared to aromatic compounds (Marschner and Kalbitz, 2003).

Both ecological and agronomic studies have shown plant litter and crop residue breakdown is related to its nutrient, especially N, and lignin contents, with higher N and lower lignin generally favoring decomposition (Cabrera et al., 2005; Thuriès et al., 2002; Taylor et al., 1989; Stewart et al., 2015). The average lignin content of crop residues is 82 g kg^{-1} and its fate in soils has been enigmatic (Redin et al., 2014; Thevenot et al., 2010). Classically, lignin has been viewed as a highly recalcitrant component of plant biomass due to its high aromaticity. Lignin was thought to be transformed stepwise in microbially-driven processes to phenolic aldehydes and then to quinones, followed by polymerization to high molecular weight soil organic matter (Sparks,

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2003). However, recent studies have shown that plant biomass lignin undergoes rapid decomposition in the presence of available C energy sources, suggesting that lignin does not have inherent chemical recalcitrance (Dignac et al., 2005; Heim and Schmidt, 2007; Klotzbücher et al., 2011). Recent work suggests lignin is initially microbially depolymerized releasing aromatic WSOM and these molecules are repeatedly transformed and incorporated into microbial biomass, or mineralized to CO_2 (Klotzbücher et al., 2016).

In the past decade, the characterization of WSOM has been advanced through the use of ultrahigh resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) (Sleighter and Hatcher, 2007). With ESI-FTICR-MS, it is possible to assign unique elemental formulas to the thousands of peaks typically observed in the mass spectra of WSOM allowing chemical characterization through the use van Krevelen diagrams (VKD) (Hockaday et al., 2009; Kim et al., 2003). The primary objective of this study was to examine the biodegradation dynamics of WSOM using ESI-FTICR-MS to assess whether controls on biodegradation suggested by macro-level studies are valid at the molecular-level. We used field crop residues [corn (*Zea mays* L.) and soybean (*Glycine* max (L.) Merr.)] and a leguminous cover crop [hairy vetch (*Vicia villosa* L.)] to investigate biodegradation dynamics in a controlled laboratory microcosm incubation study. Understanding how soil WSOM biodegradation processes affect its molecular composition will hopefully lead to improving the management practices to better address a broad suite of environmental and ecological issues such as soil carbon sequestration and the sustainability of soil quality and health.

2. Material and methods

2.1. Experimental setup

Corn and soybean crop residues were obtained from fields in the fall after harvest. Hairy vetch was sampled at the full flowering stage when it is typically incorporated into soils. All plant samples were air-dried and ground to pass through a 1-mm sieve. Biodegradation of the plant samples were conducted in microcosms (Hunt and Ohno, 2007). Briefly, 2.0 g of sample and 10 mL of distilled-deionized water (DI-H_2O) were added to 36 g of acid-washed SiO_2 sea sand (Acros, New Jersey, USA) in 125-mL glass containers. A soil inoculum was prepared by adding 1.0 g of fresh field soil to 10 mL of DI-H_2O and kept in the dark for 24 h, and a 30 µL aliquot of this inoculum was added to each microcosm. Ammonium nitrate was also added attain a C/N ratio of about 20 to ensure that biodegradation was not N limited. Eight replicates of each treatment were prepared and kept in the dark at room temperature. Control microcosms consisted of acid-washed sand, DI-H_2O, and the soil inoculum. The microcosms were loosely capped to minimize CO_2 build-up and weighed daily with DI-H_2O additions as needed to maintain the initial moisture level.

Two replicate microcosms chosen at random of each treatment were destructively sampled at times 0 (i.e., immediately after microcosm preparation), 2, 7, and 14 days. The 14 day length was shown to be a sufficient period for biodegradation of plant biomass under controlled laboratory conditions (Ohno and Doolan, 2001). At each sampling date, 15.0 g sub-samples were mixed with 15 mL of DI-H_2O, shaken for 1 h, and centrifuged at 6000 rpm for 25 min. The supernatants were sequentially filtered through Whatman 1 paper filter, GF/C pre-combusted glass fiber filter, and a 0.45-µm cellulose-ester filter. All solutions were stored in a freezer until analysis. The concentration of C in the supernatant was determined by high temperature catalytic combustion (TOC-VCPH, Shimadzu).

2.2. ESI-FTICR-MS analysis

The WSOM was characterized using negative ion mode ESI with a 12 T Bruker Daltonics Apex Qe FT-ICR-MS instrument at the COSMIC facility at Old Dominion University. To increase the ionization efficiency, ammonium hydroxide was added immediately prior to ESI to raise the pH to 8. Samples were introduced by a syringe pump providing an infusion rate of 120 µL h^{-1}, with ESI voltages being optimized for each sample to maintain consistent and stable ion currents. Ions (in the range of 200–1200 *m/z*) were accumulated in a hexapole for 0.5–1.0 s before being transferred to the ICR cell. Exactly 300 transients, collected with a 4 MWord time domain, were added for a total run time of ~30 min. The summed free induction decay signal was zero-filled once and Sine-Bell apodized prior to fast Fourier transformation and magnitude calculation using the Bruker Daltonics Data Analysis software. Prior to data analysis, all samples were externally calibrated with a polyethylene glycol standard and internally calibrated with naturally present fatty acids and other compounds containing a CH_2 homologous series within the sample.

For molecular formula assignments, *m/z* values with a signal to noise (S/N) ratio above five were assigned using the formula extension approach (Kujawinski and Behn, 2006). A MATLAB script was used to generate empirical formula matches for using combinations of C (8–50 atoms), H (8–100 atoms), O (1–30 atoms), N (0–5 atoms), S (0–3 atoms), and P (0–2 atoms) as the constraining possible atomic number values and within a ± 1 ppm *m/z* error window. In the cases were multiple formulas were within the error window, the assignment was based on a hierarchy of: (1) Kendrick mass defect analysis, (2) least number of non-oxygen heteratoms, and (3) lowest ppm *m/z* deviation. The MATLAB script then parsed the assigned formulas into the appropriate VKD space, which consisted of six discrete regions: 1) condensed aromatic molecules ($AI_{mod} > 0.66$); 2) aromatic molecules ($0.66 \geq AI_{mod} > 0.50$); 3) highly unsaturated molecules ($AI_{mod} \leq 0.50$ and $H/C < 1.5$); 4) saturated molecules (Sat, $H/C \geq 2.0$ or $O/C \geq 0.9$.); 5) non-N containing aliphatic molecules ($2.0 > H/C \geq 1.5$); and 6) N containing aliphatic molecules ($2.0 > H/C \geq 1.5$) (Seidel et al., 2014). The modified aromaticity index (AI_{mod}) was calculated as (1 + C-½O-S-½H)/(C-½O-S-N-P) and the double bond equivalent (DBE) values were calculated as 1 + ½(2C-H + N + P) (Koch and Dittmar, 2006). The relative distribution of the six classification groups on an intensity-weighted (peak intensity/sum of all assigned peak intensities) basis were calculated for each of the chemical classes. Further post-processing details can be found elsewhere (Ohno and Ohno, 2013). Formulas present in both treatment replications were used in the subsequent data analysis steps.

2.3. Biodegradability classifications

A R script was used to merge the assigned formulas based on the number of C, H, O, N, S, and P atoms from the four sampling dates for each of the three plant species samples. The chemical factors affecting WSOM biodegradation were evaluated by focusing on the bioavailability/stability of the formulas present at day 0. These formulas were assessed by assigning the formulas into four biodegradiability classes: 1) high availability, present only at day 0; 2) intermediate availability, present at days 0 and 2; 3) low availability, present at days 0, 2, and 7; and 4) stable, present in all four sampling dates.

2.4. Statistical analysis

The DOC concentration data was analyzed by the *t*-test using Systat 12 at the $p = 0.05$ level. The decreasing WSOM heteroatomic content as a function of incubation time was fitted to the exponential equation $y = a * \exp(b * x)$ using the MATLAB Curve Fitting Tool, where y is the percent heteroatomic content, x is the incubation time, and a and b are fitting parameters.