Adsorptive fractionation of dissolved organic matter (DOM)
by mineral soil: Macroscale approach and molecular
insight

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ABSTRACT

Adsorption of dissolved organic matter (DOM) to mineral surfaces is an important process determining DOM bioavailability and carbon sequestration in soils. However, little is known about preferential adsorption of DOM at the molecular level. In this study, DOM originating from composted biosolids was analyzed in order to elucidate DOM adsorptive fractionation by clay soil. Structural changes in DOM due to adsorption to soil were studied using two complementary approaches: (i) macroscale analysis including resin separation, and (ii) molecular characterization using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Both approaches demonstrated consistency regarding the DOM adsorptive fractionation. Resin separation showed preferential adsorption of the hydrophobic acid (HoA) fraction by soil surfaces, with up to 70% of total adsorbed carbon; this fraction was apparently responsible for low DOM desorption. FT-ICR MS data demonstrated preferential adsorption of polyphenols, which are components of the HoA fraction. Adsorption of highly oxidized, saturated “carbohydrate-like” molecules was also observed, which might be a result of adsorption of the hydrophilic neutral (HiN) fraction. DOM exhibited concentration-dependent fractionation: enhanced adsorption of highly oxidized compounds at low DOM concentrations, and selective adsorption of less oxidized components at higher DOM concentrations, suggesting that adsorptive fractionation of DOM depended on the extent of its loading.

Our findings suggest that a significant amount of carbon originating from the applied DOM was irreversibly stabilized by mineral surfaces. The study demonstrates that both DOM chemical heterogeneity and DOM concentration need to be considered in order to predict DOM reactivity and carbon stabilization in soils.

Keywords: adsorption, desorption, hysteresis, FT-ICR MS, hydrophobic, hydrophilic, preferential adsorption, oxidized compounds

1. Introduction

Soil organic matter (SOM) is the largest carbon pool in terrestrial ecosystems (Cotrufo et al., 2015). Numerous studies have focused on carbon cycle modeling in order to improve understanding of carbon stabilization in soils (von Lützow et al., 2008; Kleber et al., 2011; Cotrufo et al., 2015). This knowledge is essential for predicting the global carbon balance as a response to climate change.

DOM represents a small but highly important fraction of the SOM carbon pool due to its high reactivity (Bolan et al., 2011). It is operationally defined as a soluble SOM fraction that is smaller than a specified size, usually 0.45 μm (Zsolnay, 2003). It is a complex heterogeneous mixture of water-soluble natural organic compounds, comprising individual compounds and supramolecular assemblies stabilized by hydrophobic effects, H bonding, and non-covalent interactions (Nebbioso and Piccolo, 2013). As one of the sources of SOM, DOM plays an important role in carbon cycling and distribution in the environment, including processes governing SOM accumulation and stabilization (Schmidt et al., 2011; Kaiser and Kalbitz, 2012). DOM plays an important role in biological activity in soils and water bodies (Bolan et al., 2011), and affects various physicochemical processes in soils, including binding of trace elements and organic pollutants to soil particles (Müller et al., 2007; Barriuso et al., 2010) and co-precipitation of metal oxides (Shimizu et al., 2013). In addition, DOM affects the transformation of redox-active elements (Borch et al., 2010) and organic and inorganic contaminants by mineral surfaces (Polubesova and Chefetz, 2014).

A major process that governs the fate of DOM and the stability of carbon in soils is its adsorption to soil mineral surfaces. The reactivity of DOM is related to the type and number of the functional groups, which determine specific interactions with soil minerals, including ligand exchange with OH groups on metal oxide surfaces (Gu et al., 1994; Wang et al., 1997). Inner-sphere complexation of metal oxides with DOM carboxylic moieties contributes to DOM accumulation in soils and impedes its desorption (Kaiser et al., 1997). Since DOM consists of a mixture of components, it may undergo fractionation upon sorption (Guo and Chorover, 2003; Kothawala et al., 2012), resulting in compositional changes that depend on the chemical and physical properties of DOM and the properties of the adsorbent.

Organic carbon (OC) analysis can be used to quantify the amount of DOM adsorbed to soil minerals, while UV-visible (UV-vis) absorption spectroscopy provides useful information about the character of the adsorbed DOM (e.g. by comparing specific UV absorbance of the DOM solution before and after adsorption; Weishaar et al., 2003). Techniques commonly applied to characterize the complexity of DOM include nuclear magnetic resonance (NMR) spectroscopy (Kaiser et al., 2003; McKee et al., 2016), scanning transmission X-ray microscopy (STXM) and near-edge X-ray absorption fine structure (NEXAFS) spectroscopy (Kinyangi et al., 2006; Geesey et al., 2008), size exclusion chromatography (Her et al., 2003), fluorescence spectroscopy (Borisover et al., 2012) and infrared spectroscopy (Oren and Chefetz, 2012). These techniques provide detailed information about DOM as a bulk mixture (e.g. functional group content), but little information about specific DOM components at the molecular level.

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is a technique that can simultaneously resolve and determine, with ultrahigh mass resolving power and mass accuracy, the molecular formulae of thousands of compounds in complex mixtures such as DOM (Sleighter and Hatcher, 2007; D'Andrilli et al., 2013). Without chemical standards, electrospray ionization (ESI) FT-ICR MS is semi-quantitative since different compounds have different ionization efficiency in complex mixtures (Nguyen et al., 2012). However, changes in signal intensity do follow changes in relative concentration (Dvorski et al., 2016). In addition, no other technique can provide comparable molecular level information for complex, highly oxygenated organic mixtures like DOM (Hertkorn et al., 2007; Minor et al., 2012). When combined with macroscale bulk analyses, the information obtained using FT-ICR MS, can provide new insights into geochemical processes involving DOM, which has been described as “one of the most complex mixtures known” (Simpson et al., 2012).

Though DOM adsorption to mineral surfaces has been widely studied (Hur and Schlautman, 2003; Kothawala et al., 2012), comprehensive understanding of the qualitative and quantitative changes that occur at the molecular level during adsorption is lacking. Recent studies have used FT-ICR MS to examine adsorptive fractionation of fulvic acids, humic acids and peat DOM on metal oxides (Galindo and Del Nero, 2014, 2015; Lv et al., 2016). However, no other study has examined adsorptive fractionation of DOM by mineral soils at the molecular level.

In this study, we combined macroscale analysis and molecular level analysis to examine the adsorption of DOM on mineral soil. The main

objectives were to: (i) elucidate and quantify changes in the fractional composition and physicochemical properties of the bulk DOM sample due to its adsorptive fractionation by mineral soil (macroscale approach), and (ii) identify at the molecular level the DOM components that were selectively adsorbed by the mineral soil. The information should provide new insights into the accumulation and stabilization of all water soluble OM by mineral soils.

We used biosolid-derived DOM. Biosolids and stabilized biosolids (e.g. composted or digested biosolids) are widely used in agricultural systems as an organic soil amendment. Biosolids are ubiquitous sources of exogenous DOM entering agricultural soils, which have the potential to enter surface water through runoff or drainage (Topp et al., 2008; Yang et al., 2012). Hence, a detailed investigation of the adsorptive behavior of biosolid-derived DOM in agricultural soils is important.

2. Material and methods

2.1. Soil and DOM characterization

Soil (an Entisol) from 90-120 cm depth was collected from Akko, Israel. The soil, rich in clay ($67.5 \pm 1.4\%$) and poor in SOM ($0.15 \pm 0.02\%$ OC), was used as representative of soils from arid regions. Montmorillonite is the major clay mineral in the soil. The pH of soil suspension was 8.5 ± 0.1 . The concentration of Fe (6.5 ± 0.2 g/kg), Al (1.4 ± 0.2 g/kg) and Mn (0.9 ± 0.1 g/kg) were obtained using dithionite extraction. The specific surface area of soil particles (327 ± 2 m²/g) was measured from ethylene glycol monoethyl ether (EGME) adsorption (Carter et al., 1965). The measurements of soil properties were performed in triplicate, and the data represent average values with

standard deviation. Detailed characterization of the soil is described by Oren and Chefetz (2012).

DOM was extracted from composted biosolids by way of overnight agitation (200 rpm) at 25 °C with deionized water at a 1:10 solid/water ratio. The suspension was centrifuged (17000 g, 20 min) and filtered through a 0.45 µm Acrodisc filter with Supor membrane (PALL Corporation, Ann Arbor, MI, USA), producing the DOM solution that was used. The pH of the solution was 7.9, the electrical conductivity (EC) was 3.7 dS/m and the dissolved OC (DOC) content was ca. 800 mg C/l.

DOC concentration was measured with a VCPH model TOC analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). UV–Vis measurements were performed for diluted DOM samples (DOC < 30 mg C/l) with a Thermo Scientific Evolution 300 spectrophotometer. Based on the UV–Vis measurements, the following indices were calculated: SUVA₂₅₄ (ratio between absorption intensity at 254 nm and the DOC concentration; Weishaar et al., 2003), and E₂/E₃ (the ratio of absorption at 250 and 365 nm; Peuravuori and Pihlaja, 1997). NaN₃ (1.5 mM) was added to DOM solutions to inhibit microbial activity (Chefetz et al., 2006). The UV absorption of NaN₃ was not significant under the experimental conditions. The ionic composition of the DOM solution was measured with an Acros-EOP ICP/AES instrument (Spectro, Kleve, Germany).

2.2. DOM adsorption–desorption in soil

Adsorption and desorption of DOM were measured using the batch equilibrium technique, at a solid/water ratio of 1:10 (w/v) at 25 °C. Exogenous DOM was diluted to the desirable concentration values with a background

solution containing 1.5 mM NaN_3 and the cations (added as chloride salts) previously detected in DOM extracts (Ca^{2+} , 0.11 mM; Mg^{2+} , 0.16 mM; Na^+ , 0.57 mM and K^+ , 0.78 mM). This provided a constant ionic strength (3.7 mM) in all batch-equilibrium experiments at all DOM concentration values. Prior to the adsorption trials, soil aliquots (2-3 g) were agitated for 2 h with deionized water (1:10) to reduce the level of native DOM released from the soil. The solution was decanted and an elevated concentration of exogenous DOM (5 to 115 mg C/l) added to the pre-washed samples. After pre-washing, the concentration of native DOM released from the soil was ca. 2 mg C/l as measured for control soil suspensions (no exogenous DOM added). Native DOM consisted of 16% HoA (hydrophobic acid), 7% HoN (hydrophobic neutral), 1% HiB (hydrophilic base), 70% HiN (hydrophilic neutral) and 6% HiA (hydrophilic acid, C-based fraction). Separation procedures for these fractions are described in Section 2.3. Soil suspensions (no DOM added) and DOM solutions (no soil added) were used separately as controls for monitoring native DOM release and to examine DOM biodegradation.

All experiments were conducted in triplicate. The pH was 7–8 for all adsorption experiments. After 4 days agitation (the equilibrium time was determined by Oren and Chefetz, 2012), the tubes were centrifuged (12,000 g, 10 min), and 50% of the supernatant was decanted and replaced with fresh DOM-free background solution for the desorption experiments (for an additional 4 days). Three sequential desorption cycles were performed. Supernatants obtained from all sorption and desorption stages were filtered (0.45 μm) and analyzed for DOC concentration. Desorbed solutions were not chemically characterized due to minor DOM release during desorption.

2.3. DOM separation by polymeric resins

DOM resin separation experiments were conducted to elucidate and quantify changes in the fractional composition of DOM due to adsorptive fractionation by the soil. DOM samples collected before or after adsorption to the soil were fractionated using the method developed by Leenheer (1981) and modified by Chefetz et al. (1998). The polymeric resins (Supelite™ DAX-8, Amberlyst 15 and Amberlyst A-21) were from Sigma-Aldrich (St. Louis, MO, USA). Resin separation was performed for three DOM initial concentration values and for three DOM equilibrium concentration values: Low (C_e 23 - 27 mg C/l) Medium (C_e 57 - 65 mg C/l) and High (C_e 94 - 95 mg C/l).

Each DOM sample was separated into five fractions: HoA, HoN, HiA, HiB and HiN. In brief, each sample (ca. 6 mg C) was acidified to pH ca. 3 and loaded onto a DAX-8 resin column. Hydrophobic fractions were retained whereas hydrophilic fractions were eluted. The HoA fraction adsorbed to the DAX-8 resin was eluted by washing the resin with 0.1 N NaOH (0.25 pore volumes) followed by 1 pore volume of distilled water. At this stage, the HoN fraction was retained by the resin. The pH of the hydrophilic fraction which was not adsorbed to the DAX-8 resin was adjusted to ca. 3, and loaded onto a cation-exchange resin (Amberlyst 15). The HiB fraction was retained on the resin and the eluted fractions (HiA and HiN) were loaded onto an anion-exchange resin (Amberlyst A-21). At this stage, the HiA fraction was adsorbed to the resin, and the HiN fraction was eluted from the column. The content of HoN fraction was calculated based on C mass differences. All samples were loaded at a flow rate that did not exceed 15 pore volumes/h. DOC concentration was measured for all eluted fractions.

The DOM fractional distribution among the five fractions was determined by calculating the **proportion** (%) of C of each obtained fraction relative to the total C amount initially loaded onto the resin. Adsorbed DOM fraction distribution was calculated based on differences in C mass measured in the bulk (initial) and unbound (equilibrium) solutions.

2.4. FT-ICR MS

DOM samples were analyzed with FT-ICR MS to identify and monitor compositional changes in the initial DOM solution due to the adsorptive fractionation by mineral surfaces. Four samples were analyzed: bulk (untreated DOM sample) and unbound DOM from batch equilibrium experiments at **Low**, **Medium** and **High** concentrations (C_e 27, 65 and 95 mg C/l, respectively). The unbound DOM samples for FT-ICR MS analysis at each C_e were prepared as **a** composite of 12 replicates. Replicates were not analyzed **with** FT-ICR MS separately since little experimental variability was observed in the adsorption experiments. In addition, **peak detection and relative peak magnitude** are highly reproducible in FT-ICR MS data when conservative **signal/noise (S/N)** thresholds (e.g. $S/N \geq 6$) are used (Sleighter et al., 2012). To remove salts (Kujawinski, 2002), each sample was dialyzed against deionized water (Spectra/Por cellulose ester dialysis membranes, molecular weight cut off 100-500 Da), freeze-dried and stored in a desiccator. Freeze-dried samples were dissolved in molecular grade water (JT Baker) to yield a stock solution of 1 mg/ml, and further diluted (1:1, by volume) to yield 50:50 (v/v) MeOH:double distilled water (DDW) with 1% (by volume) NH_4OH to increase deprotonation of acidic species for negative-ion electrospray ionization. All samples were prepared in borosilicate glass vials with PTFE-lined caps.

The samples were analyzed with a custom-built FT-ICR mass spectrometer (Kaiser et al., 2011a) equipped with a 9.4 T, horizontal 200 mm bore diameter superconducting solenoid magnet (Oxford Corp., Oxford Mead, UK) operated at room temperature. A modular ICR data station facilitated instrument control, data acquisition and data analysis (Blakney et al., 2011). Ions generated at atmospheric pressure entered the skimmer region (2 Torr) through a heated metal capillary and were accumulated for 500 to 1000 ms in a quadrupole, passed through an rf-only quadrupole to an octopole equipped with tilted wire extraction electrodes (Wilcox et al., 2002) for collisional cooling (ca. 10 ms) with He gas, and transferred through rf-only quadrupoles (total length 127 cm) into the ICR cell. ICR time-domain transients were collected from a 7 segment open cylindrical cell with capacitively-coupled excitation electrodes based on the Tolmachev configuration (Kaiser et al., 2011b). Multiple (150) individual time domain transients (6.1s) were co-added, apodized with a half-Hanning (absorption spectrum) weight function, and zero-filled once prior to fast Fourier transformation. ICR frequencies were converted to ion masses based on the quadrupolar trapping potential approximation (Shi et al., 2000), and broadband phase correction with baseline correction was applied to all spectra (Xian et al., 2010, 2012). Each spectrum was scaled according to its most abundant peak, internally calibrated with a “walking” calibration equation (Savory et al., 2011) based on a highly abundant homologous series (mass of $-\text{CH}_2-$ repeating unit 14.01565 Da), and confirmed with isotopic fine structures.

Experimentally measured masses were converted from the International Union of Pure and Applied Chemistry (IUPAC) mass scale to the Kendrick

mass scale in order to rapidly identify homologous series (mass of $-\text{CH}_2-$ repeating unit 14.01565 Da) for each heteroatom class (Kendrick, 1963). Mass spectral peaks with signal magnitude greater than 6x the baseline root-mean-square noise ($S/N \geq 6$) were assigned elemental compositions ($\text{C}_{0-100}\text{H}_{0-200}\text{N}_{0-2}\text{O}_{0-25}\text{S}_{0-2}$) using PetroOrg software (Y.E. Corilo; Florida State University; all rights reserved. <http://www.petroorg.com>) and criteria described in the Supporting Information.

2.5. Data processing and statistics

DOM adsorption coefficient values were calculated using the initial mass approach (Nodvin et al., 1986). The initial mass approach is described in Eq. S1 and S2 in the Supporting Information. K_d values were calculated directly from the adsorption isotherm for each data point by dividing the amount of adsorbed DOM by the DOM equilibrium concentration (C_e) in the supernatant. Statistical analysis was performed using JMP software (v. 7.0.1. SAS Institute Inc., Cary, NC, USA). Nonparametric Mann-Whitney U test was used to determine differences between bulk and unbound DOM fraction and to determine the differences between adsorbed DOM fractions at three equilibrium concentrations tested ($P < 0.05$).

Assessment of the degree of unsaturation of molecules in DOM samples was performed by normalizing the calculated double bond equivalent (DBE; Eq. S3) to the total number of Cs in the molecule (i.e. DBE/C) cf. Koch and Dittmar (2006). Modified AI (ModAI) values were computed based on the assumption that 50% of the O in each formula is present in $\text{C}=\text{O}$ functional groups (Koch and Dittmar, 2006) and were used to identify aromatic ($\text{ModAI} > 0.5$) and condensed aromatic ($\text{ModAI} > 0.67$) structures (Eqs. S4 and S5;

Stubbins et al., 2010; Riedel et al., 2013). Average carbon oxidation state (AvgOS_C ; Eq. S6) is an indirect measure of the polarity of a compound and was calculated based on Kroll et al. (2011). The identified peaks were assigned to heteroatom classes, and to descriptive compound classes based on their compositional similarity to familiar natural organic compounds (e.g., lignin, lipids and carbohydrates). The descriptive compound classes included carboxyl-rich alicyclic molecules (CRAMs), since CRAMs are derived from biomolecules similar to sterols and hopanoids (Hertkorn et al., 2006), and because biosolids composted with tree clippings are likely to contain such molecules (Jardé et al., 2005). A detailed description of all calculations and assignment criteria are described in the Supporting Information (Table S1).

Finally, a procedure was created for determining which assigned elemental compositions were selectively removed as the result of adsorption. Only mass spectral peaks from the bulk DOM sample above 12σ of baseline rms noise (i.e. 2x the signal magnitude of the least abundant identified peak) were considered due to comparability problems associated with strict S/N ratio values (Sleighter et al. 2012). Detailed information about peak comparability can be found in the Supporting Information. Principal component analysis (PCA) was applied to the relative abundances (%) of N and O heteroatom classes (e.g. N_1O_4 represents the class of $\text{C}_c\text{H}_h\text{N}_{n=1}\text{O}_{o=4}$ formulae). The assignment was based on the nonlinear iterative partial least-squares (NIPALS) algorithm, and zero-filling to account for missing variables.

3. Results

3.1. Adsorption-desorption and adsorptive fractionation

DOM exhibited pseudo-linear adsorption isotherms (Fig. 1), which did not reach maximum adsorption capacity within the range of applied initial concentration values. DOM sorption affinity for the soil (m, Eq. S1) was 0.285 ± 0.002 (based on the initial mass approach), and the K_d value was 3.98 ± 0.03 l/kg (Fig. S2). This value is similar to the average values calculated directly for each concentration of the adsorption isotherm (3.50 ± 0.39 l/kg). Adsorption-desorption isotherms for DOM exhibited considerable hysteresis (Fig. 1). The adsorbed amount after adsorption was not significantly different ($p < 0.05$) from the adsorbed amount after three sequential desorption stages as calculated from differences in C concentrations of initial and equilibrium solutions.

For each of characterized equilibrium concentration values after adsorption, E_2/E_3 values for the unbound DOM increased, whereas $SUVA_{254}$ decreased, in comparison with the values obtained for initial bulk DOM (Table 1). Changes in structural composition of the DOM after adsorption were also evaluated with resin-based separation. The hydrophobic fractions (HoA and HoN) made up 37–48% of the bulk DOM (Fig. 2). After adsorption to the soil, the proportion of HoA in the unbound DOM was significantly lower (22-29%; Fig. 2) than that in the initial bulk DOM solution. HoA comprised a major fraction of the adsorbed phase with adsorbed amount of 40-182 mg C/kg soil (47-72% of total adsorbed C; Fig. 3). The increase in the initial DOM concentration from Low to Medium (32 to 81 mg C/l) resulted in enhanced HoA adsorption, from 47 to 72% of total adsorbed C. An increase from Medium to

High concentration did not result in an additional increase in HoA adsorbed proportion. At the High initial DOM concentration (128 mg C/l), HoA represented 70% of total adsorbed C (182 mg C/kg). At every concentration, the proportion of HoN was not significantly different after adsorption to the soil (Fig. 2), suggesting that this fraction was not selectively adsorbed. Indeed, HoN made up only a few percent of the total adsorbed C (Fig. 3). The hydrophilic fractions (HiN, HiA, and HiB) comprised 52–63% of the bulk DOM. In particular, the HiN content was 37–43%. After adsorption, the proportion of HiN was approximately unchanged at DOM Low concentration, and slightly greater at Medium concentration and High DOM concentration (Fig. 2). Nevertheless, the adsorbed HiN made up 17–40% of the total adsorbed C (30–60 mg C/kg; Fig. 3). For the Low concentration and Medium DOM concentration, there was a higher proportion of HiA in the unbound DOM than in the initial bulk DOM samples (Fig. 2). For the High DOM concentration, there was no substantial change (Fig. 2). The overall proportion of HiA was < 15% in all samples. As a result, it comprised a negligible proportion of the adsorbed phase (Fig. 3). Finally, the HiB fraction was present in the lowest amount in all samples. The low content as well as the small differences in DOM solutions before and after DOM adsorption, resulted in negligible HiB adsorption.

3.2. FT-ICR MS

Fig. S2 shows the negative-ion ESI broadband FT-ICR mass spectra derived from the bulk DOM and unbound DOM samples. Each sample contained > 7,000 mass spectral peaks that were assigned elemental compositions in the range $150 < m/z < 600$, highlighting the compositional diversity and heterogeneity of DOM (Kim et al., 2003; Sleighter et al., 2012;

Wagner et al., 2015). Since lignin, lipids, carbohydrates and other major biogeochemical classes of compounds have characteristic H/C ratio or O/C ratio (Kim et al., 2003), a van Krevelen diagram was used to associate the assigned formulae with similarly structured compound classes in the bulk DOM (Fig. S3). Based on ModAI values, 19% of the bulk DOM was composed of aromatic compounds (ModAI > 0.5) and 7% of condensed aromatic compounds (O/C 0.0-1.0; H/C 0.3-0.7; ModAI $\geq$ 0.67; Koch and Dittmar, 2006).

Bulk and unbound DOM samples were distinguished primarily by the first two PC (Fig. 4), which accounted for > 97% of the sample variance by heteroatom content. The loading plot (Fig. 4b) suggested that O₂ was the heteroatom class mainly responsible for the variation in PC1, as indicated by its separation along the x-axis. To a lesser extent, there was also a clear variation in the relative abundances for the classes O₄ and O₅ along PC2, as indicated by their separation along the y-axis. Since the PCA plots suggested that O content was an important source of variation between the bulk and unbound DOM, and because it is well known that the dominant fraction of NOM is composed primarily of C_cH_hO_o compounds (Witt et al., 2009) we focused the FT-ICR MS analysis of adsorptive fractionation exclusively on C_cH_hO_o compounds.

A van Krevelen diagram that illustrates the assigned C_cH_hO_o formulae from the bulk DOM sample as a function of their H/C ratio and O/C ratio, and as a function of their assigned descriptive compound classes is presented in Fig. 5. By relative abundance, lignin-like compounds (35%), CRAMs (33%), lipids (7%), and other aliphatic compounds (16%) comprised > 90% of the bulk DOM sample; carbohydrate-like compounds (5%) and polyphenols (3%)

comprised almost 9% (Table 2). Table 2 demonstrates characteristic differences between the bulk and the unbound DOM samples. At the Low equilibrium concentration, the most pronounced differences were the reduced proportion of carbohydrate-like compounds (5.4% in bulk vs. 2.9% in unbound) and polyphenolic compounds (3.2% in bulk vs. 1.5% unbound).

Each compound class was divided according to its O content, and the effect of adsorption on each subcategory determined by comparing its relative abundance (%) in the bulk DOM sample to its relative abundance (%) in each unbound DOM sample (Fig. 6). At the Low equilibrium concentration (Fig. 6a), when compared with the bulk DOM, the unbound DOM was enriched with lower order O-containing compounds (e.g. O₃ - O₆ CRAMs and lignin-like compounds and O₂ lipid-like and other aliphatic compounds), and depleted in higher order O-containing compounds (e.g. O₈ - O₁₅ polyphenols and carbohydrate-like compounds). Similar differences were evident at the Medium equilibrium concentration compared with the bulk DOM sample (Fig. 6b), except that the unbound DOM was also depleted in O₄ - O₈ lipid-like and other aliphatic compounds. In contrast, when the High equilibrium concentration was compared with the bulk DOM (Fig. 6c), the unbound DOM was enriched with higher order O containing compounds (O₇ - O₁₅), and depleted in lower order O-containing compounds (e.g. O₂ - O₅ CRAMs and lignin-like compounds).

The FT-ICR MS data were used to assign compounds that were selectively removed from the solution due to adsorptive fractionation of the bulk DOM (Fig. 7). We hypothesized that the selective removal of individual DOM components would be easiest to detect at the lowest equilibrium concentration because at low concentration the compound interacts directly with mineral

surfaces (Chassé et al., 2015; Kleber et al., 2007). Hence, close contact with iron-containing mineral surfaces might result in preferable adsorption of electron-rich DOM components. Therefore, a van Krevelen diagram (Kim et al., 2003, Fig. 7a) and a Kroll diagram (Kroll et al., 2011, Fig. 7b) were prepared to compare the chemical change between the bulk and the unbound DOM at the Low equilibrium concentration. AvgOS_C was used in order to characterize the DOM after adsorption to the soil. AvgOS_C values range from -4 for the most reduced form (CH₄) to +4 for the most oxidized form (CO₂), but many “highly oxidized” organic compound classes have AvgOS_C values below +1 (Kroll et al., 2011). The orange symbols represent the C_cH_hO_o formulae that were selectively removed (S/N ≥ 12 in the initial DOM solution and S/N < 6 in the unbound DOM solution). The green symbols represent the C_cH_hO_o formulae that were not selectively removed (S/N ≥ 12 in the initial DOM solution and S/N ≥ 6 in the unbound DOM solution). Finally, the gray symbols represent C_cH_hO_o formulae that were inconclusive (6 ≤ S/N < 12 in the initial DOM solution and S/N < 6 in the unbound DOM solution). Three distinct groups of selectively removed compounds were identified: (i) CRAMs and lignin-like compounds with 1.3 ≤ H/C ≤ 1.6 and O/C < 0.4; (ii) carbohydrate-like and other aliphatic compounds with H/C ca. 2 and 0.4 ≤ O/C ≤ 0.7; and (iii) carbohydrate-like and polyphenolic compounds with 1 ≤ H/C ≤ 2 and O/C > 0.8. The first group had AvgOS_C values ca. -1 and carbon numbers approximately equal to 30. The second group had AvgOS_C values ca. -1 and carbon numbers between 20 and 30. The third group was smallest (5 < C_n < 15) and the most highly oxidized (AvgOS_C generally > 0). Finally, the relative abundances of highly oxidized compounds (0 < AvgOS_C < 2) decreased from 6.1% in the bulk

sample to 2.8% in the unbound DOM at the Low equilibrium concentration (Table 2), indicating that the degree of oxidation correlated positively with the observed adsorptive fractionation.

4. Discussion

DOM adsorption distribution coefficient values are within the range reported for adsorption of other DOM types by soil (Kaiser et al., 1996). The increase in E_2/E_3 and decrease in $SUVA_{254}$ after DOM adsorption (Table 1) implies preferential adsorption of large molecular size and aromatic-rich moieties (Peuravuori and Pihlaja, 1997; Weishaar et al., 2003). This trend has been shown in studies of other DOM types (Chorover and Amistadi, 2001; Ohno et al., 2007). Here, preferential adsorption of aromatic-rich DOM moieties (i.e. decrease in $SUVA_{254}$ values) was observed. A similar trend has been reported for adsorption of aquatic NOM and soil DOM to soil (Kothawala et al., 2012).

Selective adsorption of aromatic DOM constituents was also demonstrated in the resin separation method and showed that the largest proportion of total adsorbed carbon was attributable to the HoA fraction (Fig. 3). HoA is known to contain aromatic units associated with aliphatic moieties (Chefetz et al., 1998). Thus, significant reduction in $SUVA_{254}$ values supports the enhanced HoA adsorption. HoA is additionally characterized by a high level of polar functional groups, such as OH and CO_2H groups (Qualls and Haines, 1991), enabling its adsorption to the mineral soil through specific interactions, mainly via ligand exchange with OHs of iron-containing minerals and H bonding (Kaiser et al., 1996; Shen, 1999). In particular, HoA is known to

contain tannins and other polyphenols and flavonoids (Qualls and Haines, 1991). The FT-ICR MS data demonstrated preferential adsorption of oxidized polyphenols for the Low equilibrium concentration (Table 2 and Fig. 7b), in agreement with enhanced HoA adsorption obtained using UV-Vis and resin separation methods.

Adsorption of HiN to the soil was less efficient than the HoA fraction (Fig 3). The HiN fraction, the least polar of the hydrophilic fractions, is known to contain simple sugars and poly- and oligosaccharides (Qualls and Haines, 1991; Chefetz et al., 1998). However, it might also contain glycosides with non-carbohydrate residues (Hockaday et al., 2009) and uronic acids from microbially-secreted polysaccharides (von Lutzow et al., 2006; Miltner et al., 2012). In fact, the FT-ICR MS analysis showed that > 70% of the “carbohydrate-like” compounds had DBE/C values greater than those of polysaccharides such as cellulose and amylose (DBE/C 0.1667), and almost 80% of the carbohydrate-like compounds had H/O values different from 2 (the H/O ratio of most many carbohydrates). Adsorption of the HiN fraction was more pronounced at the Low equilibrium concentration, where equal amounts of HoA and HiN were adsorbed (47 and 40% of total adsorbed C, respectively). The increase in DOM concentration resulted in preferable HoA adsorption, up to 4x greater than that of HiN (Fig. 3). The FT-ICR MS results demonstrated a decrease in highly oxidized carbohydrate-like molecules at the Low equilibrium concentration (Table 2; Fig. 7b). Carbohydrates are expected to be adsorbed to mineral surfaces mainly via H bonding and van der Waals interactions. However, the HiN fraction from compost DOM may also contain polysaccharides with uronic acid residues, which contain CO₂H groups

(Chefetz et al., 1998), enabling its adsorption via ligand exchange with OH groups on mineral surfaces. Furthermore, carbohydrate-like compounds can be present in the HoA fraction. Peuravuori (2005) reported that carbohydrates made up 21% of the HoA fraction isolated from DOM of a freshwater lake. The FT-ICR MS results are in agreement with the results obtained from resin separation, which revealed preferential adsorption of both HoA and HiN fractions at the Low equilibrium concentration (Fig. 3).

The HoN fraction demonstrated low affinity for the soil (Fig. 3). It consists of aliphatic and aromatic organic components (Polubesova et al., 2007) and can be adsorbed to mineral surface by non-specific interactions. When introduced separately to montmorillonite, HoN was adsorbed to a greater extent than HoA, due mainly to its hydrophobic character (Polubesova et al., 2008). Here, HoA and HoN fractions were simultaneously introduced to the soil as DOM components, and preferential adsorption of HoA was clearly observed. This highlights the impact of soil mineral composition, and metal oxide content in particular, on the selective adsorption of specific DOM components, and the types of specific interactions that occur between DOM and mineral surfaces. The HiA fraction consists of low molecular weight humic-like acids and oxidized organic compounds (Chefetz et al., 1998), and is therefore capable of being adsorbed through specific interactions with metal oxides. Due to the relative hydrophilicity of HiA and its low content in the bulk DOM (< 14%), adsorption of this fraction was negligible.

The heterogeneity of DOM makes determination of adsorption mechanisms to soil challenging. We suggest that different combinations of adsorption mechanisms occur at different initial concentration values of DOM.

It is interesting to note that, with increase in DOM concentration, a slight increase in the proportion of HoA in the bulk and unbound DOM was observed (Fig. 2). As the DOM concentration increases, larger and more hydrophobic DOM clusters are formed in solution (Zsolnay, 2003), demonstrating supramolecular association of DOM molecules. Thus, HoA adsorption was enhanced with increased DOM concentration (Fig. 3). Aggregation of hydrophobic clusters may result in the non-specific adsorption of various DOM components, together with the specific adsorption of CO_2H -containing and phenolic compounds in HoA through ligand exchange and H bonding. This appeared evident at the molecular level by the adsorption of less oxidized CRAMs and lignin-like molecules as the DOM concentration increased (Fig. 6). Enhanced HoA adsorption might occur according to the mechanism proposed as the “onion” model (Sollins et al., 2006; Chassé et al., 2015), suggesting that an initial layer is created by specific interactions with CO_2H - and NH_2 -containing compounds, and hydrophobic moieties sorb to the resulting “hydrophobic zones” in subsequent layers (Kleber et al., 2007). Another possibility is the development of “patchy structures” (Vogel et al., 2014), where primary HoA layers or patches interact with the surface through strong specific interactions, and provide additional sorption sites for molecules that can be adsorbed by weak interactions such as H bonding or van der Waals interactions (von Lutzow et al., 2006). This patchy structure model is consistent with results obtained from examining OM-clay mineral interactions using STXM-near edge X-ray absorption fine structure (STXM-NEXAFS) spectroscopy: though only 44-62% of the total mineral surface was covered by OM, the OC

loadings were greater than those estimated for monolayer coverage from N₂ adsorption measurements (Chen and Sparks, 2015).

The increase in HoA adsorption from the Low to Medium DOM concentration (47 to 72% of total adsorbed C) might indicate gradual covering of mineral surface sites due to specific and non-specific adsorption. However, weaker interactions of HoA with mineral surfaces at higher concentration values of HoA are unlikely to result in significant sorption-desorption hysteresis, which generally is explained by inner-sphere complexation in the contact zone with the mineral surface (Kaiser et al., 1997; Kleber et al., 2007). Furthermore, an increase in the applied DOM concentration did not affect the relative proportion of adsorbed HoN, suggesting that hydrophobic zone formation does not facilitate adsorption of the HoN fraction. Further investigation is required to examine the aggregation of hydrophobic clusters in solution, its effect on adsorptive fractionation and the potential for layer or patch formation during carbon stabilization at mineral surfaces.

As described above, oxygenated functionalities, including CO₂H groups, have been found to play a major role in DOM adsorption to soil and minerals. A flocculation study found that Fe and Al ions predominantly removed peat leachate substances with O/C $\geq$ 0.4 (Riedel et al., 2012). Galindo and Del Nero (2014) demonstrated fractionation of Suwannee River fulvic acid by alumina surfaces, and reported major removal of highly oxidized aromatic compounds with multiple oxygenated functionalities, unsaturated polycyclic aromatic compounds with fewer oxidized groups, and aliphatic compounds characterized by high O/C values. It was suggested that adsorption of these compounds was due mainly to the interaction of their oxygenated

functionalities with the particle surfaces (Galindo and Del Nero 2014). AvgOS_c affects various chemical and biological processes and properties including solubility, biogeochemical reactivity and bioavailability (Kroll et al., 2011; Riedel et al., 2012; Lavonen et al., 2013). AvgOS_c values might be particularly useful for describing DOM adsorptive fractionation in soils. Here, the relative abundance of compounds with $0 \leq \text{AvgOS}_c \leq 2$ decreased during adsorptive fractionation of the Low and Medium concentration DOM samples (Table 2; Fig. 7b) supports the importance of oxygenated functional groups in DOM adsorption to mineral surfaces, and the advantage of AvgOS_c values.

Adsorption of DOM to soil and mineral surfaces is commonly reported as irreversible, displaying major sorption–desorption hysteresis (Guggenberger and Kaiser, 2003) attributed mainly to DOM interactions with metal oxides (Kaiser and Zech, 1999). Like previous findings, our results show that DOM exhibited negligible desorption, even after three desorption cycles. At the lowest initial DOM concentration, adsorption would be more likely to reflect direct interaction with mineral surfaces (Chassé et al., 2015; Kleber et al., 2007), but selective adsorption might also vary with DOM concentration. At the Low DOM concentration, the supernatant was enriched with less oxidized CRAMs and lipid-like compounds, and highly oxidized polyphenols and carbohydrate-like compounds were depleted (Fig. 6a). This was supported by the resin separation analysis, which suggested that the adsorbed DOC at that concentration was composed of a higher proportion of HiN than the other two concentrations (Fig. 3). Less abundant polyphenols in unbound DOM (Table 2) are also in agreement with HoA adsorption (Fig. 3). At the High DOM concentration, less oxidized, more hydrophobic CRAMs and lignin-like

compounds were reduced in the supernatant, and most other compounds were enriched (Fig. 6c). This is in agreement with the enhanced HoA adsorption at elevated concentration values (Fig. 3), due to the increased concentration of hydrophobic components. Although all differences in the relative abundance of O classes at elevated concentration were relatively small, a clear concentration-dependent change in adsorptive selectivity of certain chemical groups was observed (Fig. 6).

Interestingly, despite the use of dialysis with molecular weight cut-off of 500 Da, most of the masses detected with FT-ICR MS analysis of the retentate were < 500 Da. This result is reported by Remucal et al. (2012), who examined the differences between the dialysate and retentate of Suwannee River fulvic acid after it was dialyzed through a 100-500 Da dialysis membrane. The authors reported that both the dialysate and the retentate had $m/z < 500$, and that the retentate was slightly enriched in the higher masses relative to the initial sample. As evidenced from the resin separation method, the FT-ICR MS results also support the hypothesis that DOM is composed of supramolecular associations of smaller MW molecules held together by weak bonds that are stable in water but may dissociate in the ESI process (Piccolo, 2002; Peuravuori et al., 2007). It can also be suggested that DOM consists of higher molecular weight molecules that are not ionized efficiently and are not detected under the current experimental setup, or higher molecular weight molecules that are fragmented to smaller compounds in the ion source despite the “soft” impact of ESI (Stenson et al., 2002). Resolution of this controversy is beyond the scope of this work.

5. Conclusions

A combination of macroscale (adsorption-desorption experiments and resin separation) analysis and molecular (ESI FT-ICR MS) analyses was used to investigate adsorption, desorption and fractionation behavior of DOM with a mineral soil. The combination of both approaches provided better understanding of the mechanisms of DOM adsorption and fractionation by soil mineral surfaces. The results suggest concentration-dependent adsorptive fractionation of DOM at environmentally relevant concentration values, and changes in selectivity of different functional groups at increasing DOM concentration.

The significant sorption-desorption hysteresis implies that the HoA fraction is most likely responsible for the irreversible adsorption behavior of DOM in mineral soils, regardless of DOM nature and origin. HoA fraction, and particularly the small and highly oxidized polyphenols and CO_2H -containing compounds are likely to be adsorbed by mineral surfaces via ligand-exchange, and therefore are retained in the soil solid phase. HiN fraction, containing CO_2H functional groups can also contribute to DOM sorption behavior. The low desorption potential of the adsorbed DOM suggests that constant input of exogenous DOM to agricultural soils (via biosolid amendments and/or irrigation with treated wastewater) would lead to gradual changes in SOM properties. This process would be significantly affected by the presence of metal oxides due to accumulation of oxygenated hydrophobic and hydrophilic fractions associated with oxides. Strongly adsorbed specific DOM components (HoA and HiN fractions) would be stabilized at mineral surfaces and additional carbon would be stabilized if layers or patchy

structures formed as a consequence of such adsorption. Carbon stabilization will be controlled by the strength of DOM adsorption and the ability of the mineral matrix to protect adsorbed carbon from chemical and biological degradation. However, it might be possible to create soil amendments that facilitate strong carbon adsorption and enhance carbon stabilization according to expected DOM concentration.

Alteration of DOM composition due to its interaction with soil mineral surfaces can also affect DOM reactivity towards both organic and inorganic pollutants, including pesticides and fertilizers. DOM impact on polar organic pollutants would be more pronounced at low DOM concentration values due to competition of DOM and polar O-containing pollutants for mineral surface sites. The process may be less significant at higher DOM concentration values because mineral surfaces become less selective towards oxygenated components. We emphasize the importance of both the DOM chemical heterogeneity and DOM concentration for its adsorptive fractionation, and stress that both of these aspects need to be considered in order to predict the mobility of organic pollutants and carbon stabilization in soils.

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Appendix A. Supplementary data

The following is the supplementary data related to this article:

Additional information and data on Initial mass model (Eq. S1, S2 and Fig. S1); Broadband mass spectra for bulk and unbound DOM samples (Fig. S2); van Krevelin diagram of the assigned compound classes in bulk DOM (Fig. S3); calculations used for FT-ICR MS data analysis; criteria for assignments of compound classes used in this study (Table S1).

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Figure Captions

Fig. 1. Adsorption and desorption isotherms of DOM extracted from composted biosolids by soil.

Fig. 2. Composition of bulk and unbound DOM (filled and open columns, respectively) at Low (a) Medium (b) and High (c) equilibrium concentration (C_e 23, 57 and 94 mg C/l). Asterisks indicate significant differences in proportions of C_i and C_e (Mann-Whitney, $P < 0.05$).

Fig. 3. Adsorbed DOM fractions at Low ($C_e = 23$ mg C/l), Medium (C_e 57 mg C/l) and High (C_e 94 mg C/l) equilibrium concentration.

Fig. 4. PCA of the elemental composition from negative mode ESI FT-ICR mass spectra for bulk DOM and three equilibrium concentration values (C_e 27, 65 and 95 mg C/l): (a) score plot of PC1 vs. PC2 for major classes (relative abundances $> 1\%$), (b) loading plot from the corresponding PCA.

Fig. 5. Van Krevelen diagram constructed for $C_cH_hO_o$ formulae assigned to mass spectral peaks from the bulk DOM sample.

Fig. 6. Change in proportional relative abundance of $C_cH_hO_o$ formulae in bulk vs. unbound DOM samples at Low (a), Medium (b) and High (c) concentrations (C_e 27, 65 and 95 mg C/l).

Fig. 7. Van Krevelen diagram illustrating the H/C and O/C ratios (a), and Kroll diagram plotting average carbon oxidation states (AvgOSc) vs. numbers of carbon (b) for $C_cH_hO_o$ formulae from the bulk DOM solution based on their selective removal during the Low concentration (C_e 27 mg C/l) batch equilibrium experiment (orange diamonds - selectively removed; green circles - not selectively removed; gray circles - inconclusive).

Table 1

UV parameters (mean $\pm$ standard error) for bulk and unbound DOM samples.

DOM type	Concentration (mg C/l)	SUVA ₂₅₄ (l mg/C m)	E ₂ /E ₃
Bulk	32	3.64 $\pm$ 0.03	3.16 $\pm$ 0.01
Unbound	23	1.97 $\pm$ 0.03	4.59 $\pm$ 0.01
Bulk	81	3.71 $\pm$ 0.03	3.10 $\pm$ 0.06
Unbound	57	1.97 $\pm$ 0.01	4.83 $\pm$ 0.07
Bulk	128	3.72 $\pm$ 0.02	3.16 $\pm$ 0.01
Unbound	94	2.11 $\pm$ 0.02	4.52 $\pm$ 0.03

Table 2

Characteristics of bulk and unbound DOM sample at Low (C_e 27 mg C/l), Medium (C_e 65 mg C/l) and High (C_e 95 mg C/l) equilibrium concentrations based on $C_cH_hO_o$ formula assignments (with signal magnitude $> 6\sigma$) derived from FT-ICR MS analysis.

	Bulk DOM	Low	Medium	High
# $C_cH_hO_o$ formulae	1889	1688	1524	2079
# $C_cH_hO_o$ of unbound DOM formulae shared with bulk DOM	–	1619	1450	1755
Compound classes (%)				
Lignin-like	35.30	36.95	35.92	31.09
CRAM	33.17	35.29	37.43	35.15
Carbohydrate-like	5.41	2.91	2.69	6.09
Polyphenols	3.16	1.47	2.09	4.86
Lipid-like	6.98	7.75	8.77	7.59
Condensed aromatic C	0.09	0.09	0.10	0.14
Other aliphatic C	15.64	15.39	12.93	14.92
Other aromatic C	0.19	0.13	0.06	0.13
Unclassified	0.04	0.02	0.02	0.03
Average carbon oxidation state (AvgOS _C , %)				
$1 \leq \text{AvgOS}_C < 2$	0.01	0.00	0.02	0.03
$0 \leq \text{AvgOS}_C < 1$	6.11	2.81	3.67	8.75
$-1 \leq \text{AvgOS}_C < 0$	58.47	59.88	60.76	59.85
$-2 \leq \text{AvgOS}_C < -1$	35.41	37.31	35.56	31.36

Figure 1

Fig. 1

Figure 2

Fig. 2

Figure 3

Fig. 3

Figure 4

Fig. 4

Figure 5

Fig. 5

Figure 6

Fig. 6

Figure 7

Fig. 7