Characterization of natural organic matter in low-carbon sediments: Extraction and analytical approaches

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ABSTRACT

Organic carbon (OC) concentrations in subsurface sediments are typically 10 to 200 times lower than in surface soils, posing a distinct challenge for characterization. In this study, a range of chemical extractions were evaluated for extraction of natural organic matter (NOM) from two low-carbon (< 0.2%) alluvial sediments. The OC extraction efficiency followed the order pyrophosphate (PP) > NaOH > HCl, hydroxylamine hydrochloride > dithionite, water. A NOM extraction and purification scheme was developed using sequential extraction with water (MQ) and sodium pyrophosphate at pH 10 (PP), combined with purification by dialysis and solid phase extraction in order to isolate different fractions of sediment-associated NOM. Characterization of these pools of NOM for metal content and by Fourier transform infrared spectroscopy (FITR) showed that the water soluble fraction (MQ-SPE) had a higher fraction of aliphatic and carboxylic groups, while the PP-extractable NOM (PP-SPE and PP > 1kD) had higher fractions of C=C groups and higher residual metals. This trend from aliphatic to more aromatic is also supported by the specific UV absorbance at 254 nm ($SUVA_{254}$) (3.5 vs 5.4 for MQ-SPE and PP-SPE, respectively) and electrospray ionization Fourier transform ion cyclotron resonance spectrometry (ESI-FTICR-MS) data which showed a greater abundance of peaks in the low O/C and high H/C region (0–0.4 O/C, 0.8–2.0 H/C) for the MQ-SPE fraction of NOM. Radiocarbon measurements yielded standard radiocarbon ages of 1020, 3095, and 9360 years BP for PP-SPE, PP > 1kD, and residual (non-extractable) OC fractions, indicating an increase in NOM stability correlated with greater metal complexation, apparent molecular weight, and aromaticity.

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1. Introduction

Belowground stocks of carbon (*i.e.*, in soils & sediments) represent a large fraction of the total earth carbon stocks, storing more than twice the carbon than the atmosphere and living plants combined (Fischlin et al., 2007). Despite decreasing organic carbon (OC) concentrations with depth in soils, approximately half of the soil carbon may be stored in the deeper subsurface (> 1 m) (Trumbore et al., 1995). It is largely unknown how subsurface carbon will respond to climate change (Conen et al., 2006; Smith et al., 2008; Conant et al., 2011; Rumpel and Kögel-Knabner, 2011;). Given the large size of this pool of carbon, research on the chemical nature of soil or sediment organic carbon and the factors controlling its stability is critical in order to understand and predict the dynamics of this pool.

For surface soils containing relatively high (5–10%) levels of OC, direct *in situ* analysis of natural organic matter (NOM) by FTIR (*e.g.*, Kaiser et al., 2016), synchrotron-based techniques (STXM, NEXAFS, FTIR) (*e.g.*, Lehmann et al., 2007; Remusat et al., 2012), and CPMAS NMR (*e.g.*, Kögel-Knabner, 2000; Knicker et al., 2006) are possible. Destructive techniques, such as pyrolysis GC–MS and thermal analysis are also applied to soils (*e.g.*, Plante et al., 2009; de la Rosa et al., 2011; González-Pérez et al., 2014), although interpretation of these data sets are more complex due to the alterations to OC structure during analysis. However, in low OC (< 1%) soils or sediments, such as those in the deeper subsurface (> 1 m), many of these techniques are not sensitive enough to be applied directly to the bulk material, necessitating the use of extractions to separate NOM from the mineral components. Extraction allows for the characterization of different pools of NOM with presumably different mobilization potentials and reactivities, potentially

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providing more information on NOM association and stability compared to bulk analytical techniques. For example, many studies have characterized the water-soluble NOM fraction of soils (*e.g.,* Ellerbrock and Kaiser, 2005; Kaiser and Ellerbrock, 2005; Heckman et al., 2011; Ohno et al., 2014; Kaiser et al., 2016). Separation and characterization of so-called "humic" and "fulvic" acid fractions is also common (*e.g.,* Solomon et al., 2005; Ikeya et al., 2012, 2015; DiDonato et al., 2016), based on their solubility in acidic and alkaline solutions (Stevenson, 1994). Recently, Tfaily et al. (2015) investigated a range of organic solvents to extract soil OM and characterized the extracted fractions using ESI-FTICR-MS. Due to observations of the correlation between metals (Fe, Al) and OC in natural waters and soils (Kaiser and Guggenberger, 2000; Knorr, 2013), a number of extractions have been employed to extract "Fe- and Al-associated" soil OM. These include alkaline sodium pyrophosphate (*e.g.,* Ellerbrock and Kaiser, 2005; Kaiser and Ellerbrock, 2005; Kaiser et al., 2016), which targets OM bound to mineral surfaces via ligand exchange and cation bridging as well as OM in metal-OM complexes, and dithionite (Wagai and Mayer, 2007; Lalonde et al., 2012; Wagai et al., 2013), which targets OM bound to crystalline Fe-oxides and oxyhydroxides. Sequential extraction schemes of varying degrees of complexity which include many of the extractants discussed above have also been developed to extract different pools of mineral-bound NOM (*e.g.,* Posner, 1966; Lopez-Sangil and Rovira, 2013).

The goals of this study were to (1) evaluate extraction approaches to separate soil NOM from mineral components and develop an extraction and isolation approach for understudied low-carbon sediments, (2) characterize the different extracted NOM pools using FTIR and ESI-FTICR-MS, and (3) provide insight into the stabilization mechanisms of NOM in low-carbon sediments. By developing this protocol, we hope to facilitate more investigations into the chemical nature of organic carbon in low carbon sediments and alleviate a potential bias that could result from only high-OC materials being considered for analysis.

2. Materials and methods

2.1. Site description and sediment samples

Two sediment samples [Little Rusty Composite (LRC) and Backhoe 2-1-13 (BH)] were collected from a subsurface alluvial aquifer in Rifle, Colorado hosted within a floodplain along the Colorado River. The shallow, unconfined aquifer has a saturated thickness of approximately 4 m, and is underlain by the impermeable Wasatch formation at approximately 8 m below ground surface (DOE, 1999; Yabusaki et al., 2007). The aquifer sediment consists of unconsolidated clay, silt, sand, gravel and cobbles, with approximately 30% of sediment in the < 2 mm fraction (DOE, 1999; Fox et al., 2012). Sediment mineralogy is primarily composed of quartz and feldspars, with minor amounts of calcite, chlorite, kaolinite and iron oxides (magnetite, hematite, and goethite) (Komlos et al., 2008; Campbell et al., 2012; Fox et al., 2013). A description of the LRC sediment sampling and processing is provided by Hyun et al. (2009). Briefly, the sample was collected by backhoe

from just below the water table (approximately 4.6 m below ground surface), air-dried for approximately 1 week, and sieved to < 2 mm. BH sediment was collected in a similar manner on February 1, 2013. However, the BH sample was not air-dried, but was sieved to < 2 mm under field-moist conditions and stored at 4 °C. Very little visible litter fragments (roots, twigs, etc.) were present in these samples, thus it is expected that the vast majority of the organic carbon in these sediments resides in the < 2 mm size fraction. The water content of BH was determined by drying a subsample at room temperature and all data are presented in terms of dry weight of sediment.

2.2. Extractions

2.2.1. Single extractions

Sediments were subjected to six different extractions, designed to target various mineralogical (*e.g.,* Fe-oxide/oxyhydroxide) and organic pools as shown in Table 1. All extractions were carbon-free in order to allow for analysis of extracted OC. The acidic extracts HCl and hydroxylamine hydrochloride (HH) are designed to dissolve amorphous or easily reducible Fe-oxides (Chao and Zhou, 1983) and NOM associated with those minerals. However, these extracts will only release NOM which is soluble at acidic pH. The inorganic dithionite extraction targets NOM associated with both crystalline and non-crystalline Fe-oxides (Wagai and Mayer, 2007), and includes a dilute HCl rinse to release any FeS precipitated in the dithionite solution. Alkaline extracts are often more effective at releasing NOM from soils, thus we also test NaOH, an extractant commonly used to extract NOM (Stevenson, 1994) and sodium pyrophosphate (PP), which in addition to the alkaline pH has metal-chelating properties which can solubilize NOM stabilized through metal complexation (Loeppert and Inskeep, 1996; Pansu and Gautheyrou, 2006).

Each extraction was performed at least in triplicate and results are presented as the average and standard deviation of the replicates. For each extraction, 5 g sediment was weighed into 50 mL polycarbonate centrifuge tubes, extractant was added, and tubes were capped and placed on an end-over-end sample rotator for the specified time, except for HH extractions, which were performed at 50 °C in a shaking water bath. Samples were then centrifuged at 25,000g for 30 min and the supernatant was filtered through a 0.45 μm (PVDF or Acrodisc Supor PES) syringe filter. Method blanks were produced for each extractant using only the extracting solution (*i.e.,* without solid); these blanks were processed and analyzed in the same manner as the samples. The pyrophosphate and dithionite extractions were tested at several different solid to liquid ratios (10–200 g/L for PP and 33–167 g/L for dithionite). Results from the solid to liquid ratio tests are shown in the supporting information (SI).

2.2.2. Sequential extractions

Based on the results of the single extractions, a sequential extraction protocol was developed using water (MQ) and sodium pyrophosphate (PP) on larger sediment samples in order to recover enough NOM for FTIR and ESI-FTICR-MS analysis. The extraction

Table 1
Chemical extractions applied to sediment samples.

Extraction	Target pool	Method	Reference
MQ	Water-soluble OM	MilliQ water, 20 h, 200 g/L	
PP	OM−complexed Fe	0.1 M sodium pyrophosphate, pH 10, 20 h, 200 g/L	Loeppert and Inskeep (1996)
HCl	Amorphous Fe-oxide bound OM	0.5 M HCl, 1 h, 200 g/L	
HH	Easily reducible Fe and Mn-bound OM	0.25 M hydroxylamine hydrochloride, 0.25 M HCl, 50 °C, 1 h, 200 g/L	Chao and Zhou (1983)
Dithionite	Iron-oxide bound OM	100 g/L dithionite, 16 h; followed by 0.05 M HCl rinse 1 h; 167 g/L	Wagai and Mayer (2007)
NaOH	Humic and fulvic acids	0.5 M NaOH, 20 h, 200 g/L	Stevenson (1994)