Nitrogen-rich compounds constitute an increasing proportion of organic matter with depth in O_i-O_e-O_a-A horizons of temperate forests

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ABSTRACT

Soil organic matter (SOM) in O horizons of temperate forests forms a natural gradient of increasing decomposition, as operationally separated into the Oi (leaf litter), Oe (partially decomposed), and Oa (highly decomposed) sub-horizons. This study determines the contribution of extractable nitrogen (N)-rich biomolecules and characterizes changes in SOM composition within O horizon decomposition gradients and in underlying A horizons of three temperate forest stands. Four biomolecules were chosen for this investigation: chlorophyll, protein, DNA, and chitin; combined, they represent the majority of identifiable N-rich organic compound classes present in soils. Selective chemical extractions and purification methods provide estimates of concentrations of each distinct biomolecule class, while short-term respiration measurements provide insight into possible relationships with microbial activity. Changes in the molecular character of SOM are probed using Fourier Transform Infrared (FTIR) and Carbon K-edge X-ray Absorption Near Edge Structure (C-XANES) spectroscopies. Differences in biomolecule concentrations and respiration by horizon reveal two contrasting trends with increasing decomposition and depth: chlorophyll and respiration decrease, whereas protein, DNA and chitin increase. Increasing contribution of these three extractable N-rich biomolecules with progressive decomposition and depth at all sites correspond to decreasing concentrations of C and N and to increases in C:N ratio. These trends are consistent with FTIR spectral results, which indicate a relative increase of N—H bending and aromatic C═N vibrations, and with C-XANES spectral results, which show a relative increase of aromatic C═N transitions. These multiple lines of evidence demonstrate that extractable N-rich biomolecules constitute a growing proportion of the organic C and N content within an organic matter decomposition gradient and that this trend continues into the underlying mineral soil. We posit that the persistence of these intact N-containing biomolecules represents a growing contribution from the microbial biomass pool, but also from non-specific interactions among biomolecules in the Oe and Oa horizons and from biomolecule-mineral interactions in mineral soils. The results of this investigation (increases in the proportion of extractable N-rich biomolecules) also provide valuable insight into the potential bioavailability and biochemical structure of SOM in relation to degree of decomposition.

1. Introduction

Temperate forests cover approximately $7.7 \times 10^6 \, \text{km}^2$ of Earth's surface area and contain an estimated 14% of the total global stock of terrestrial carbon (C) (Lal and Lorenz, 2012; Pan et al., 2011). The majority of these reserves are associated with the soil organic matter (SOM) pool, which includes living microorganisms and plant roots as well as their non-living residues and exudates (Stevenson, 1994). In these ecosystems, the periodic deposition of leaf litter to the forest floor is a natural mechanism which returns C and nitrogen (N) to the soil surface in organic forms (Schlesinger and Bernhardt, 2013). The

recurring inputs of fresh litter, combined with slow decomposition rates of older residues, facilitates the accumulation of an organic, or O, horizon directly overlying the mineral soil (A horizon).

Organic matter (OM) in the O and A horizons forms a natural gradient, encompassing the complete range of plant residues along a continuum of increasing degree of decomposition (i.e., oxidation) with depth. In the U.S. Soil Taxonomy system, three O sub-horizons have been operationally defined to represent this change in OM character, including i) the Oi horizon, which forms at the forest soil surface and consists of relatively fresh and undecomposed plant litter, ii) the Oe horizon, including partly decomposed (yet still somewhat recognizable)

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plant residues, and iii) the Oa horizon, which represents the highly decomposed, unrecognizable OM having a peat-like consistency (Schaetzl and Anderson, 2005). The C:N ratio of SOM progressively narrows with increasing depth and degree of decomposition, from 30-to-40 in leaf litter (Oi) to an average value of ~20 in the A horizon (Weil and Brady, 2017).

The progressive decrease in C:N during decomposition occurs as a function of time that can be separated into two broad stages. Early stages (i.e., weeks-to-months) are characterized by high initial mass loss of litter residues associated with microbial mineralization of un-protected non-structural (i.e., metabolic), cellulose, and hemicellulose fractions (McKee et al., 2016; Cotrufo et al., 2015; Soong et al., 2015; Wieder et al., 2014). These residue components are generally the most spatially accessible and nutrient-dense, so are associated with higher microbial carbon use efficiency (CUE) such that losses of carbon dioxide (CO_2) during respiration are lower compared to the amount of C allocated to new microbial biomass (Cotrufo et al., 2013, 2015; Manzoni et al., 2012). In later stages (i.e., years) of residue decomposition, there is a gradual decline in plant structural biomolecules (i.e., lignin and lignin-protected cellulose) and an overall net accumulation of microbial products (e.g., exudates, necromass) (Liang et al., 2017; Soong et al., 2015; Wieder et al., 2014). Within the context of an undisturbed temperate forest soil, the Oi (litter) horizon would be associated with early stage, whereas the SOM in the Oe, and particularly Oa, horizons would be characterized as later stage of decomposition. At the molecular scale, all OM inputs and pools of living and non-living SOM are ultimately composed of biomolecules and their derivatives. Biomolecules serve as a long-term source of nutrition to soil microorganisms when degraded by extracellular enzymes, a prerequisite for microbial assimilation and immobilization within biomass (Liang et al., 2017). In undisturbed ecosystems, SOM is the predominant source of N to microbes because most soil N occurs in organic (~90–95%) rather than inorganic forms (Kögel-Knabner, 2006; Bremner, 1965). The chemical character of the soil organic N (SON) pool has been evaluated using ^{15}N Nuclear Magnetic Resonance (NMR) spectroscopy, providing consistent evidence of a prevalence of amide-N (i.e., proteinaceous-N) forms (Schulten and Schnitzer, 1998; Preston, 1996). Data from wet chemical approaches, however, estimate that the SON fraction contains 30–40% hydrolysable amino acids (e.g., from proteins) (Roberts and Jones, 2008; Stevenson, 1982; Sowden et al., 1977), 5–10% amino sugar-N (e.g., from chitin degradation) (Zhang and Amelung, 1996; Sowden et al., 1977), and < 1% nucleic acid-N (e.g., from DNA and RNA) (Cortez and Schnitzer, 1979). Other known contributors to the SON pool include fungal melanins, various alkaloids, chlorophyll-derivatives (i.e., tetra-pyrroles), and a large amount of 'unclassifiable N' (Knicker, 2011; Kögel-Knabner, 2002; Schulten and Schnitzer, 1998). Many chemical derivatives of these N-biomolecules (e.g., amino sugars, amino acid enantiomers) are used as biomarkers of plant and microbial activity in soils and geologic sediments (Amelung, 2003; Hedges et al., 2000), but knowledge of the amounts of intact N-containing biomolecules in such samples remains limited. This information has the potential to provide valuable insight into the bioavailability and biochemical structure of SOM in relation to degree of decomposition.

Because of its complex, supramolecular nature, traditional chemical characterization of SOM has relied upon chemo-lytic (e.g., acid hydrolysis, CuO oxidation, base extraction) and/or thermo-lytic (e.g., pyrolysis) degradation techniques followed by various downstream applications (e.g., chromatography, mass spectrometry) (Kögel-Knabner, 2000). These destructive approaches have provided much of the current information about the amount of different organic compound classes in soils (e.g., lignin, cellulose, lipids), but they do have some important limitations such as instrumentation availability and risks of secondary reactions and artifact formation (Kögel-Knabner, 2000). Furthermore, such techniques rarely make distinctions between biomolecule building blocks (e.g., amino acids, amino sugars) and larger biomacromolecules (e.g., protein, chitin) in the same samples,

which makes interpretation within an ecological context challenging. For example, the total amount of amino acids determined by any of the aforementioned techniques theoretically includes amino acids in protein, protein-humic complexes, peptides, and free amino acids (Roberts and Jones, 2008; Stevenson, 1982) that have a wide range of bioavailability (e.g., before cellular uptake can be achieved, a greater number of enzymatic steps are required to breakdown a protein molecule compared to a free amino acid) and stability within the soil matrix (e.g., mineral-associated OM is generally considered more stable against oxidation). These N pools have proven difficult to isolate in the laboratory, but they are biogeochemically distinct.

The application of spectroscopic techniques for characterizing SOM is increasingly common because they provide information about the molecular functional group composition of undisturbed samples (Chenu et al., 2015). Fourier Transform Infrared (FTIR) and Carbon K-edge X-ray Absorption Near Edge Structure (XANES) spectroscopies are two techniques commonly used to characterize SOM. FTIR probes molecular vibrations and can identify specific functional groups of SOM based on sample vibrational frequency, identifying groups containing C, N and oxygen. Due to the high-throughput nature of data collection and the chemical detail yielded by FTIR spectroscopy, it has been applied to the study of SOM character in many contexts, including changes in SOM with decomposition, adsorption, land management, and depth (Dhillon et al., 2017; Tivet et al., 2013; Tatzber et al., 2007; Chorover and Amistadi, 2001; Haberhauer et al., 1998; Gu et al., 1995). Carbon K-edge XANES spectroscopy detects core-level electronic transitions of C atoms, providing elementally specific and detailed insight into the local bonding environment of C, as well as information about the identity of other elements present in functional groups (e.g., phenolic, heterocyclic and carboxylic functionalities). Carbon K-edge XANES has been previously applied to study SOM characteristics with respect to different land uses, landscape position, adsorption, and co-precipitation (Dhillon et al., 2017; Chen et al., 2014; Gillespie et al., 2010; Jokic et al., 2003). FTIR and XANES have been applied in conjunction to evaluate SOM functional group composition (Dhillon et al., 2017; Chen et al., 2014; Solomon et al., 2005). Combined, these two approaches provide a profile of the functional group composition of the bulk of soil organic matter, albeit through two physically distinct means.

Leaf litter decomposition is a long-standing focus of soil biogeochemistry, but there remains great uncertainty related to the characterization of progressive changes in OM chemistry. While a wide variety of techniques are routinely used to characterize SOM, a major limitation is that they are not typically applied as complementary approaches. Furthermore, more traditional wet chemical approaches require advanced instrumentation (e.g., GC/MS, LC/MS, pyrolyzers), which is not as widely available to researchers as UV–Vis spectrophotometers. With the growing application of advanced spectroscopic techniques, such as FTIR and C-XANES spectroscopies, it is necessary to establish relationships to traditional wet chemical characterization techniques to improve data interpretation. For example, the combination of multiple analytical techniques has been recently demonstrated to provide valuable insight into OM transformations associated with grass litter decomposition (McKee et al., 2016). The present investigation was designed with a broad goal of placing concentration measurements from wet chemistry methods within the context of bulk spectroscopic data (and vice versa) to evaluate decomposition in temperate forest soils. We chose targeted, compound-specific extraction protocols now routinely used with '-omics' work over more traditional wet chemical approaches (e.g., acid hydrolysis) to achieve higher quality end-products with enhanced purity that could be easily measured using UV–Vis. The combined application of these modern methods has not been previously reported for the same set of samples, or for estimating major N-containing biomolecules as an extractable fraction of the SOM pool from the standpoint of forest leaf litter decomposition.

In this study, we used three complementary approaches to