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From the litter layer to the saprolite: Chemical changes in water-soluble soil organic matter and their correlation to microbial community composition

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ABSTRACT

Organic matter content and chemistry is vital to the structure and function of soil systems, but while organic matter is recognized as biogeochemically important, its chemical interaction with soil processes is not well understood. In this study we used fluorescence spectroscopy, which has been used extensively for understanding the role of organic matter in aquatic systems, to identify chemical changes in organic matter with depth in a soil system. Soil was collected from nine different pits in a first-order montane catchment in the Colorado Front Range. The water-soluble soil organic matter was extracted from each sample and fluorescence and UV–vis spectroscopy was used to analyze its chemical character. While organic matter chemistry had little correlation with landscape location and local vegetation, there were noticeable consistent trends between soil horizon and organic matter chemistry in each pit. Total organic matter decreased with depth and became less aromatic with increasing depth. This less aromatic material in the saprolite also had a greater microbial signature. The redox character of the organic matter accompanied this change in source and molecular structure, with more oxidized character corresponding with organic matter with more microbial input and more reduced character corresponding to organic matter with more plant input. A concurrent investigation of the microbial population of the same soil samples also showed microbial population composition varying more with soil depth than landscape position, and depth changes in microbial diversity occurred concomitantly with depth changes in organic matter chemistry.

1. Introduction

The biogeochemical processes that drive the functioning of the Earth's critical zone are dependent on biotic and abiotic elements. Within a soil system, soil formation requires organic matter input that primarily originates at the top of the soil column and mineral input that primarily originates from the bottom, creating stratification (Kögel-Knabner, 2002). Near the surface, soil horizons (O, A) are defined by their rich organic matter content, but organic matter is still present in deeper horizons and this smaller pool of organic matter is still active in biogeochemical processes (Chorover et al., 2007; Fierer et al., 2009). In each soil horizon, production of new material, transformation of existing material, and transport of material from other soil layers determine organic matter chemistry. Organic matter transformation due to interactions with microbes, plants, minerals and fungi depends not only on the amount of organic matter present, but also on its structure and chemical character (Kalbitz et al., 2003; Marschner and Kalbitz, 2003; Six et al., 2004). Thus, the chemistry of organic matter is partially determined by its interactions with microbes but also defines the nature of those processes. However, much work is still needed to understand the biogeochemical relationship between organic matter and micro-organisms in the soil system (McDowell, 2003).

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One key role of organic matter in natural systems is the ability of humic substances, a subset of organic matter, to act as an electron shuttle for bioreduction processes (Peretyazhko and Sposito, 2006). Humic substances can accept electrons from micro-organisms and then shuttle those electrons to inorganic compounds such as iron or chromium, reducing those elements. Lovley et al. (1996) studied the reduction of iron by *Geobacter metallireducens* and found the rate greatly increased in the presence of humic acids from both soil and aquatic sources. These results showed that electron shuttling from humic materials had the potential to play a significant role in microbe-driven reduction processes in the natural environment. In laboratory settings, humic substances from a wide range of aquatic, terrestrial, and anthropogenic sources have been shown to act as electron shuttles in microbially-driven redox reactions and a variety of microbes have been shown to utilize humics as electron acceptors (Chen et al., 2003; Peretyazhko and Sposito, 2006). These humic-mediated oxidation–reduction processes have been observed in systems including freshwater sediments and both anoxic and aerobic soils (Kappler et al., 2004; Rakshit et al., 2009; Brose and James, 2010).

To investigate this electron shuttle behavior, Scott et al. (1998) used electron spin resonance spectroscopy to measure the electron accepting capacity of humics from a variety of sources. They were able to demonstrate that the quinone moieties found in humic material are the main drivers of its capacity to shuttle electrons. They found that humic material extracted from soils had a substantially higher electron accepting capacity, as well as a greater degree of aromaticity and organic radical content, than humic material extracted from aquatic sources. In addition, Roden et al. (2010) showed that humic substances in particulate form could shuttle electrons, and thus this function was not limited to dissolved humic material. While the particulate humics in this study showed only a modest electron accepting capacity compared to the dissolved species, they still significantly increased the rate of microbial reduction of iron. Overall these findings suggest that humics in soil systems have the potential to be even more involved in redox reactions than humics in aquatic systems.

The optical properties of organic matter have been utilized to understand the chemical nature of organic matter in both soil and aquatic systems (Fellman et al., 2010). Organic matter can be separated into chromophoric and non-chromophoric fractions, and studies have shown that the fluorescently-active chromophoric portion of organic matter is predominately composed of humic material. While only a fraction of organic matter is composed of fluorescently-active material, evidence suggests that a substantial portion of that fraction contains the quinone-like moieties (Cory and McKnight, 2005) which have been shown to serve as electron shuttles within humic material. Early work on soil systems found that fluorescence spectroscopy could differentiate between soil organic matter from different sources, and could detect chemical changes in organic matter exposed to various physical and biogeochemical processing (Senesi et al., 1991; Zsolnay et al., 1999; Kalbitz et al., 2003). More detailed exploration of soil at depth has shown that deeper organic matter is less aromatic and from a more microbial origin (Hassouna et al., 2010). Another study of soil depth profiles found the degree of humification drops along with the aromaticity, suggesting smaller molecules may be transported to these deeper environments (Corvasce et al., 2006). Work in aquatic systems has demonstrated the ability of fluorescence to distinguish between organic matter from microbial and plant sources (Parlanti et al., 2000; McKnight et al., 2001) and the redox state of the organic matter (Fulton et al., 2004; Miller et al., 2006; Mladenov et al., 2010). These techniques have been used extensively in aquatic systems, and they are also likely to identify important processes in the soil, where they have not been as commonly used.

While many studies of humics as electron shuttles have occurred in controlled laboratory settings, there have been studies in lakes, streams, and groundwater systems that provide evidence of similar reactions happening in natural landscapes. Fluorescence techniques, which require a sample size and concentration that can be easily obtained in the field, as opposed to NMR or ESR, which require concentrations of organic matter much higher than is found in natural settings, have been utilized in many of these studies. Because it is a spectroscopic technique, fluorescence is also highly sensitive to the electronic state of the molecules, and thus the reduced or oxidized state of the quinone-like moieties thought to drive humic electron shuttling. Examples of systems where a change in humic redox character has been identified with fluorescence include sediment interstitial waters (Klapper et al., 2002), an Antarctic lake (Fulton et al., 2004), the hyporheic zone of a mountain stream (Miller et al., 2006) and Bangladesh groundwater (Mladenov et al., 2010).

The electron shuttling capacity of humic material from aquatic, terrestrial, and anthropogenic sources has been established in both dissolved and particulate forms in laboratory studies, and evidence for this biogeochemical shuttling process has been found in natural aquatic systems. However, fluorescence evidence for similar redox properties in soil systems has not been investigated. Thus, a logical next step would be to look for evidence of humic-mediated electron transfer within soil horizons. In this study, we investigated the use of fluorescence spectroscopy to identify changes in the chemical character of organic matter in soil at various depths with the goal of improving our understanding of the role it plays in biogeochemical processes and saprolite formation. We extracted organic matter from the profiles of nine soil pits dug within a first-order montane catchment of the Colorado Front Range. The water-soluble organic matter was characterized using UV–vis and fluorescence spectroscopy to identify changes in the chemical character of organic matter with soil depth. Building on the approach of Eilers et al. (2012), a PCA analysis of the microbial community was utilized to explore whether these changes in organic matter chemistry corresponded with changes in the soil microbial community. This allowed us to evaluate the hypothesis that changes in soil organic matter chemistry correspond to changes in the microbial community.

2. Materials and methods

2.1. Site description and sampling

All samples were collected from Gordon Gulch (105.47 W, 40.04 N), a first-order catchment in a montane ecosystem, located within the Boulder Creek watershed in the Colorado Front Range. The catchment has an area of 2.75 km^2 and average elevation of 2627 m. The upper portion of the watershed is a bowl-shaped catchment with lodgepole pines (*Pinus contorta*) and ponderosa pines (*Pinus ponderosa*) sparsely distributed in the forest and a stream originating in the middle of a meadow characterized by grasses and shrubs. Fuel reduction logging, or thinning, has been done in parts of the catchment, most noticeable along the western edge that was thinned approximately one year before this study. The stream continues to the lower portion of the watershed and runs west to east between steep north- and south-facing slopes. The north-facing slope is characterized by dense lodgepole pines with minimal understory vegetation and ground covered by pine-needle duff. A winter snowpack is usually present from late October through April or May, depending on the year, and the soil is typically moist. The south-facing slope is a meadow with sparse ponderosa pines and many bedrock outcrops, which are less common on the north-facing slope. Winter snowpack on the