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# Physical protection of lignin by organic matter and clay minerals from chemical oxidation

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ABSTRACT

The role of organic matter (OM) concentration, structure and composition and how these relate to mineral protection is important for the understanding of long term soil OM dynamics. Various OM–clay complexes were constructed by sequential sorption of lignin and dodecanoic acid to montmorillonite. Humic acid–montmorillonite complexes were prepared at pH 4 and 7 to vary OM conformation prior to sorption. Results obtained with constructed OM–clay complexes were tested with isolated mineral fractions from two soils. Oxidation with an acidic $NaClO_2$ solution was used to chemically oxidize lignin in the OM–clay complexes, sand-, silt- and clay-size soil fractions to test whether or not it can be protected from chemical attack. Gas chromatography–mass spectrometry was used to analyze lignin-derived phenols, cutin OH–acid (after CuO oxidation), fatty acid and *n*-alkanol concentrations and composition. We found that carbon content was not solely responsible for lignin stability against chemical oxidation. Lignin was protected from chemical oxidation through coating with dodecanoic acid and sorption of humic acid to clay minerals in a stretched conformation at pH 7. Therefore, interactions between OM constituents as well as OM conformation are important factors that protect lignin from chemical oxidation. Lignin-derived phenol dimers in the Grassland-Forest Transition soil fractions were protected from chemical oxidation to a greater extent compared to those in Grassland soil fractions. Therefore, although lignin was protected from degradation through mineral association, the extent of this protection was also related to OM content and the specific stability of lignin components.

## 1. Introduction

The preservation of soil organic matter (OM) is important for maintaining soil quality and productivity (Janzen et al., 1998). The level of OM decomposition is higher in sand-size fractions which has led to the hypothesis that OM in fine sized soil fractions is protected from biodegradation through associations with mineral surfaces (Baldock et al., 1992; Guggenberger et al., 1995; Christensen, 2001; Quideau et al., 2001; Six et al., 2002). Furthermore, radiocarbon data ($^{14}C$) used to estimate OM age coupled with stable isotope ($\delta^{13}C$) turnover studies, suggest that silt- and clay-size fractions contain older and more slowly degraded OM (von Lutzow et al., 2007) and long term preservation of OM is attributed to its association with fine soil fractions (Christensen, 2001; Six et al., 2002; Kaiser and Guggenberger, 2003; Mikutta et al., 2007). Of particular interest is the stabilization of OM in clay-size fractions, because this fraction may contain as much as 50–75% of total soil OM (Christensen, 2001). However, clay surfaces have a finite amount of interaction sites, which can be saturated at high organic carbon concentrations (Six et al., 2002). Mikutta et al. (2006b) reported that OM was protected from degradation, through association with clay minerals. Sorption studies also suggest that clay mineralogy, structure and composition influenced the structural composition of OM sorbed to clay surfaces (Asselman and Garnier, 2000; Feng et al., 2005; Simpson et al., 2006; Ghosh et al., 2009). It is therefore important to understand factors that may contribute to the stability of OM associated with clay-size fractions to better manage OM input and organic carbon sequestration in soils (Christensen, 2001; Six et al., 2002).

OM associated with clay-size fractions may be stabilized through a number of physical and chemical interactions (Christensen, 2001; Six et al., 2002; Kögel-Knabner et al., 2008). One of which is the formation of microaggregates, where clay particles are coated with OM, which then subsequently limits access of degrading enzymes resulting in OM protection (Six, 2004; Chenu and Plante, 2006). Sorptive interactions between OM and clay mineral surfaces, which are governed by van der Waals interactions, ligand exchange, divalent cation bridging, electrostatic or hydrophobic bonding (Feng et al., 2005; Mikutta et al., 2007), also contribute to OM stabilization by mineral surfaces. The dominant mode of bonding is determined by solution properties (pH, ionic strength, presence of cations), valence of cations in solution, presence of competing ions (such as polyphosphate), properties of minerals and composition of the OM sorbate (Asselman and Garnier, 2000; Chi and Amy, 2004; Feng et al., 2005; Mikutta et al., 2007;

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Ghosh et al., 2009). For example, ligand exchange was found to contribute to the sorption of peat humic acid to montmorillonite clay at weakly acidic pH values (Feng et al., 2005). OM was also found to sorb to goethite clay mainly through ligand exchange, while $Ca^{2+}$ mediated cation bridging was found to be the dominant mechanism in OM sorption to vermiculite clay (Mikutta et al., 2007; Ghosh et al., 2009). The difference in strength and relative contribution of these sorption mechanisms was found to also play a role in the desorption of OM from clay and subsequent biodegradability (Mikutta et al., 2007).

The fate of lignin in soil environments is also hypothesized to be regulated by interactions with mineral surfaces (Heim and Schmidt, 2007a,b). Model sorption experiments have observed higher concentrations of aromatic structures sorbed to montmorillonite as compared to kaolinite (Feng et al., 2005) and suggests that lignin stabilization in soil may also be tied to interactions with clay minerals. Recent studies have reported that lignin-derived phenols are at a more advanced oxidation stage in clay-size fractions as compared to those in sand- and silt-size fractions (Kiem and Kögel-Knabner, 2003; Thevenot et al., 2010; Clemente et al., 2011). Recent studies also suggest that lignin phenols may be sequestered in silt-size fractions and may be older and less oxidized compared to those in clay-size fractions (Heim and Schmidt, 2007a). In contrast, Feng et al. (2008) reported accelerated lignin oxidation with 14 months of soil warming. The authors hypothesized that the soil type (sandy loam) provided little physical protection from enhanced microbial activity that was observed using phospholipid fatty acid concentrations. Therefore, it is important to ascertain whether lignin in clay-size fractions is preferentially degraded by microbes in sites with high clay content, or if oxidized lignin becomes sorbed to clay minerals and subsequently leads to longer environmental persistence. It is also necessary to determine whether lignin associated with clay is protected from degradation and to characterize the factors that influence this protection because lignin associated with clay minerals represent an important part of stabilized OM (Thevenot et al., 2010).

In this study, the factors that contribute to the stabilization of lignin on clay mineral surfaces were investigated using model OM compounds sorbed to montmorillonite and clay-size fractions isolated from soil. The influence of OM conformation was tested by creating humic acid–clay complexes at pH 4 and 7, since humic substances form coiled and compact aggregates at acidic pH and become stretched and disaggregated as pH increases (Chien and Bleam, 1998; Avena and Wilkinson, 2002). These OM–mineral compounds were subjected to chemical oxidation to test whether or not lignin is physically protected from enhanced chemical oxidation. We also examine the role of other OM compounds in the protection of lignin, as this was identified in our previous study as an important consideration of OM stabilization in soils (Clemente et al., 2011). The objectives of this study are to (1) determine whether the presence of other OM compounds can protect lignin from chemical oxidation, (2) determine the role of OM concentration in protection of lignin from chemical oxidation, (3) determine whether the sorption mechanism and OM conformation influences lignin protection at pH 4 and pH 7 and (4) test findings from model OM–clay complexes by examining lignin protection from chemical oxidation in the sand-, silt-, and clay-size fractions of two soils, which have similar clay mineralogy.

## 2. Materials and methods

### 2.1. Preparation of organic matter–clay complexes

We prepared four types of model OM–clay complexes: lignin–clay, lignin–clay coated with dodecanoic acid and peat humic acid sorbed to clay at pH 4 and 7. Control OM–clay complexes served as the reference point for ascertaining the relative changes to OM composition with chemical oxidation (see Section 2.5 for more information on data analysis and Fig. S1; Supplementary material). Sodium-rich montmorillonite (SWy-2) was purchased from the Source Clay Repository (Clay Minerals Society; Purdue University). The clay was suspended in a 3 mM NaCl + 2 mM $CaCl_2$ solution at a 1:500 weight ratio to maintain the concentrations of $Na^+$ and $Ca^{2+}$, which are the major exchangeable cations associated with this clay mineral. Dissolved lignin (alkali lignin; Sigma–Aldrich) was added to the suspension (pH = 7), using the following concentrations: 0.4, 4, 20 and 100 g lignin/100 g clay to reflect the varying amounts of OM found in soils. Preliminary experiments found that vanillyl monomers were the main lignin phenols extracted using CuO oxidation from alkali lignin (Fig. S1; Supplementary material) and have been observed to be the most environmentally persistent lignin-derived phenols (Ertel and Hedges, 1984; Kiem and Kögel-Knabner, 2003; Bahri et al., 2006). The base soluble OM (humic acid) was isolated from the Pahokee Peat soil (International Humic Substances Society) as described by Salloum et al. (2001). This peat humic acid sample has been previously characterized by our laboratory using solid state and solution state nuclear magnetic resonance methods (Salloum et al., 2001; Feng et al., 2005) and the CuO oxidation products are shown in Fig. S1 (Supplementary material). Three humic acid–clay complexes were prepared in a similar manner as those with alkali lignin, with the following concentrations: 0.4, 4 and 20 g humic acid/100 g clay. The pH was adjusted to either 7.0 or 4.0 using HCl and NaOH, which changes the conformation of humic substances, coiled at pH 4 and stretched at pH 7 (Chien and Bleam, 1998; Avena and Wilkinson, 2002). The suspensions were placed on a shaker for 20 h, and then centrifuged at 4500 rpm for 1 h to isolate the OM–clay complexes. Complexes were then washed 10 times with the 3 mM NaCl + 2 mM $CaCl_2$ salt solution to isolate the OM–clay complexes and avoid disturbing cation mediated associations. The complexes were freeze dried and ground to pass through a 106 μm sieve to reduce the contributions of any large aggregates. To create the lignin–clay–dodecanoic acid complexes, 1 g of the lignin–clay complexes (described as loadings 2, 3 and 4, which correspond to 4, 20 and 100 g lignin/g clay, respectively) were mixed in acetone with 200 mg dodecanoic acid overnight on a shaker. Preliminary tests showed that the alkali lignin chosen for this study was soluble in water, but not in acetone. Therefore, extraction of lignin from the lignin–clay complexes was minimized by using acetone to dissolve the dodecanoic acid. This was verified by CuO oxidation followed by gas chromatography–mass spectrometry (GC–MS) analysis of the acetone supernatant which did not detect any lignin-derived phenols (data not shown). The OM–clay complexes were collected by centrifugation at 9500 rpm, washed twice with 30 ml acetone, then five times with the 3 mM NaCl + 2 mM $CaCl_2$ salt solution. This was dried in the fume hood to remove residual acetone, then freeze dried and ground to pass through a 106 μm sieve.

### 2.2. Soil sampling and density fractionation

Two samples from the Alberta Prairie Ecozone were collected: Southern Grassland (SG; collected near Lethbridge, Alberta) and Grassland-Forest Transition (GFT; collected near Tofield, Alberta) soils. Detailed descriptions of the soils and sampling areas can be found in Dudas and Pawluk (1969) and Janzen et al. (1998) and characteristics of the sand-, silt- and clay-size fractions are described by Clemente et al. (2011). These soils contain high amounts of montmorillonite and illite clays as well as chlorite and kaolinite but to a lesser extent (Bentley, 1979). Overlying vegetation in the SG soil site is dominated by Western Wheatgrass, while the GFT soil site is dominated by both grasses and stands of Quaking Aspen