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Organic matter protection by kaolinite over bio-decomposition as suggested by lignin and solvent-extractable lipid molecular markers

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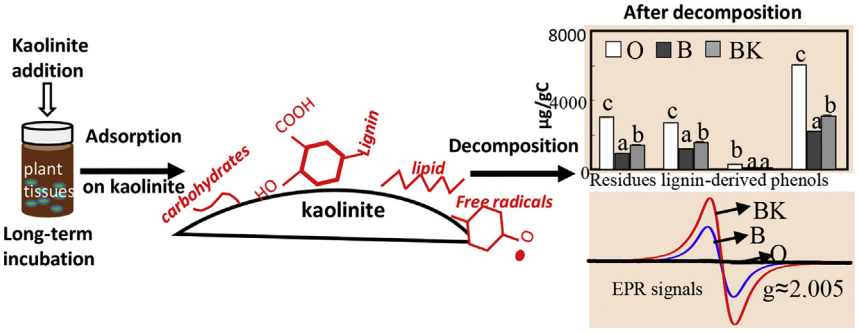
HIGHLIGHTS

- Kaolinite addition enriches lignin-derived phenols and n-alkanoic acids.
- Some carbohydrates are strongly protected by kaolinite addition.
- Kaolinite stabilizes free radicals as suggested by strong and stable EPR signals.

GRAPHICAL ABSTRACT



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ABSTRACT

The formation of organo-mineral complexes is essential in organic matter (OM) stabilization. However, limited studies have been conducted to systematically examine the mineral influence on the decomposition of plant residuals at a molecular level. In this study, pine needles and chestnut leaves were mixed with kaolinite at the weight ratio of 5:1. The controls were plant tissues without kaolinite. All the samples were incubated in the laboratory for one year. Molecular markers, including lignin-derived phenols (e.g. Vanilly units, syringyl units and cinnamyl units) and solvent-extractable lipids (e.g. n-alkanoic acid, n-alkanols and n-alkanes), were analyzed. The concentrations of lignin-derived phenols and lipid compounds were higher in the presence of kaolinite than without kaolinite. Lower degradation indexes, such as $(Ad/Al)_V$ (ratio of vanillic acid to vanillin) and CPI (carbon preference index of n-alkanoic acid and n-alkanes), were found in the kaolinite system. These results indicate that kaolinite reduced the OM decomposition. The addition of kaolinite also stabilized some carbohydrates from plants. Furthermore, the degradation of OM led to the generation of persistent free radicals, indicated by electron paramagnetic resonance (EPR) signals. The EPR signals were higher with than without kaolinite. We hypothesize that the adsorption of semiquinone or quinone radicals on kaolinite may limit their reaction with other OM moieties and thus extended their lifetimes. In addition to embedding OM in soil aggregates, our results provide direct evidence of another mineral protective mechanism of soil OM.

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1. Introduction

Plant residuals are the primary resources for organic carbon input into soils. Therefore, the decomposition of plant residuals is a key process for controlling the formation and alteration of soil organic matter (SOM) (Almeida et al., 2018). During the decomposition of plant tissues, various organic moieties are released into soils, such as carbohydrates, proteins, lignin compounds, and lipids (e.g. n-alkanoic acids, n-alkanols and n-alkanes) (Mueller et al., 2012). These organic compounds are subject to different turnover pathways due to their chemical structures. Generally, carbohydrates and proteins are susceptible to microbial degradation, whereas lignin and lipids are relatively resistant against degradation. Even during their decomposition, core structures are preserved and thus these structures can be used as molecular markers to trace source and degradation state of SOM (Mueller et al., 2012). In the past few decades, molecular markers received increasing scientific attention for global carbon cycling. However, recent studies found that the lignin and lipid signatures in plant tissue were not directly related to that of OM in soils (Moingt et al., 2016; Mueller et al., 2012). The factors that alter the distribution of lignin and lipids in plant and soil seem to be complex, which depend on multiple decomposition pathways of plant residual (Xu et al., 2017), soil components (e.g. minerals) (Miltner and Zech, 1998; Moingt et al., 2016), as well as climate and microbial activity (Osono, 2017). Most of these studies characterized the apparent mass loss or the changes of bulk chemical compositions of plant residuals, without a detailed look at the molecular level.

A growing number of studies highlighted that the formation of organo-mineral associates is crucial in controlling the turnover and decomposition of SOM (Barré et al., 2014; Han et al., 2016; Thevenot et al., 2010). There are some apparent inconsistencies, or even oxymorons, in the significance of individual processes. For instance, the chemical resistance of the hardly degradable lignin seemed to be overestimated in the long-term stabilization of SOM (Kiem and Kögel-Knabner, 2003; Thevenot et al., 2010); or as an easily degradable compound, polysaccharides are well preserved in fine soil particles (Cotrufo et al., 2013; Liang and Balser, 2008). Based on these statements, the turnover and stabilization of SOM need to be considered in depth on both chemical structures and their interaction with minerals. Likewise, minerals will also be a key determinant affecting the rate of plant residual decomposition. Miltner and Zech (1998) noticed that the presence of Fe oxides and Al hydroxides inhibited lignin decomposition by adsorbing lignin or their oxidized byproducts and thus limiting their availability to microorganisms. Clearly, mineral particles also played an important role in stabilizing organic matter. However, the effect of minerals in OM stabilization was unclear. In general, montmorillonite with large specific surface area showed higher sorption to aromatic structures than kaolinite (Feng et al., 2005), while recent studies found that the sorption of lignin on kaolinite was stronger than that on montmorillonite (Hernes et al., 2013). Compared with lignin, the alkyl structures such as n-alkanoic acids generally preserved better to the clay fraction in all soils, however, recent studies also reported that lignin was well-preserved in the silt-size fractions (Heim and Schmidt, 2007). On the other hand, the presence of mineral accelerating degradation of some OM compounds is also reported. For example, microorganisms may be enriched on kaolinite surface (Gong et al., 2016; Gong et al., 2018). The microbial derived sugars and hyphae could facilitate the aggregation of soil particles (Cosentino et al., 2006), which may protect organic matter from degradation. We hypothesize that these apparent contradictions can be, at least in part, solved by monitoring adsorption and decompositions processes of plant organic matter at the molecular level.

Kaolinite is a dominant clay mineral in most red soils, accounting for up to 50% in clay minerals (Hong et al., 2010). We therefore applied kaolinite in the system of plant material transformation during one-year period of biodegradation. The molecular markers, include lignin and solvent-extractable lipid markers, are measured to provide molecular-level insight for OM protection or degradation.

2. Materials and methods

2.1. Sampling site, material, collection and preparation

Pine needles (*Pinus yunnanensis*, Franch.) and chestnut leaves (*Castanea henryi* (Skan) Rehd. et Wils.) were sampled for the laboratory biodegradation. The sampling site is shown in Fig. S1. The fresh plant leaves were sampled in the same region and thus no climate difference was involved. Plant tissues were freezed-dried. The plants tissues were cut into pieces smaller than 5 mm, and then stored at 4 °C.

2.2. Biodegradation experiments

For biodegradation, the experiments were conducted according to previous studies (Feng et al., 2011; Miltner and Zech, 1998). In brief, plant leaves were mixed with kaolinite (5:1, w:w). The control samples were plants tissues without kaolinite. The mixed inoculum which contained microorganisms was obtained from fresh soil. This inoculum soil solution was made from100 g fresh soil which was mixed with 200 mL of deionized water, and was shaken at 120 rpm for 2 h. After shaking, the mixture was settled for 30 min. Then 10 g of plant sample were added in 100 mL brown glass jar, wetted with 2 mL of the inoculum of soil (containing microorganisms) and 15 mL of deionized water, mixed and all jars were capped with air-tight lids. Each plant sample has three replicates for microbial degradation experiment. All samples were incubated at 20 °C in the dark. To ensure the given water holding capacity, jars were opened for 10 min and deionized water was added every week. After one year, all samples were freeze dried and ground to below 20 mesh.

2.3. The analysis of molecular markers

Organic solvent extraction and CuO oxidation were used successively to extract the solvent extractable lipid and lignin-derived phenols, respectively. Meanwhile, some carbohydrates were identified in the solvent extracts of plant samples. The detailed method has been reported in previous studies (Li et al., 2015; Otto et al., 2005). For organic solvent extraction, 2 g of samples were sonicated in 30 mL dichloromethane for 15 min in a bath sonicator at 520 W. The mixture was centrifuged at 3000 rpm for 30 min. The solid particles were then successively extracted using 30 mL dichloromethane: methanol (1:1; v/v) and 30 mL methanol. All the extracts were mixed, concentrated and completely dried under pure N_2.

During CuO oxidation, the detected phenolic products include vanilly units (noted as V, such as vanillin, vanillic acid, and acetovanillone), syringyl units (noted as S, such as syringaldehyde, syringic acid, and acetosyringone), cinnamyl units (noted as C, such as ferulic acid and *p*-coumaric acid), and *p*-hydroxyl phenols (noted as P, such as *p*-hydroxyacetophenone (PON), *p*-hydroxybenzoic acid (POD), and *p*-hydroxybenzaldehyde (POV)). Briefly, 0.2 g plant tissues after the removal of organic solvent extractable lipids were mixed with 1 g CuO (pre-extracted with dichloromethane), 100 mg ammonium iron sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2.6H_2O$] and 15 mL of 2 M NaOH in Teflon-lined bombs at 170 °C for 4.5 h. After the reaction, the extracts were acidified using HCl and centrifuged at 3000 rpm for 30 min. The supernatants were liquid–liquid extracted with diethyl ether; then concentrated by rotary evaporation, transferred to 2 mL glass vials, and dried under the purge of pure N_2.

The extracts were converted to trimethysilyl (TMS) derivatives by reacting with 90 μL *N, O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) and10 μL pyridine for 3 h at 70 °C. After cooling, the derivatized solutions were analyzed by gas chromatography–mass spectrometry (GC–MS) (Agilent, 7890A GC equipped with a DB-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and a 5975C quadrupole mass selective detector). The operating condition of GC was adopted from previous studies (Otto et al., 2005): initial temperature