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### Interactive adsorption of phenolic acids and amino acids on soil minerals

*Implications for the formation and properties of soil mineral–organic associations*

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## **Chapter 2**

**Ten-year anniversary of the “multilayer model” — a mini-review**

## **Abstract**

Association with soil mineral phases enhances greatly the resistance of soil organic matter (SOM) to microbial decomposition. In addition, the traditional soil humic substances theory is increasingly challenged, whereas the importance of mineral–organic associations (MOAs) in regulating soil carbon cycling is now rigorously demonstrated by empirical evidence. Based on the substantial knowledge of the formation, composition and structure of MOAs, a multilayer conceptual model was proposed ten years ago. According to the model, the associated OM on mineral surfaces is discrete and self-organized into a multilayer structure. In this review paper, we aim to collect and summarize the existing studies that used this model to explain and understand natural observations, and based on this assess the state-of-the-art insights with respect to the performance and applicability of the model. The multilayer model has seen extensive adoption within the soil science and related fields. In general, existing studies strongly support the concept of a patchy distribution of adsorbed OM on the mineral surface. In addition, enrichment of N-rich OM is found to be controlled by mineral surfaces, and the interplay of different classes of organic compounds is found to be an essential factor for the overall adsorptive storage of SOM. Nevertheless, large uncertainty remains with respect to the multilayer organization of associated OM as rigorous testing of the model at the molecular level is still lacking after ten years of using.

## 2.1 Introduction

Soils play a crucial role in global carbon cycling by storing around two times as much carbon as the global vegetation and atmosphere combined (Lal, 2004). However, the carbon cycling process and the underlying mechanisms for the long-term storage of soil organic carbon (SOC) are still under debate (Schmidt *et al.*, 2011; Lehmann & Kleber, 2015). Considerable efforts have been devoted to understanding the interaction of soil organic matter (SOM) with minerals and demonstrated that the formed mineral–organic associations (MOAs) possess great contribution to the stabilization of SOM (Lützow *et al.*, 2006; Mikutta *et al.*, 2006; Kleber *et al.*, 2014; Paul, 2016).

Based on the initial layer structure ideas for MOAs from previous researchers (Schmidt *et al.*, 1990; Wershaw, 1993; Horne & McIntosh, 2000; Sollins *et al.*, 2006) and the research findings in soil science and related fields, Kleber *et al.* (2007) proposed a complex conceptual model for MOAs. This new model integrated the potential mechanisms involved in the formation of MOAs and visualized the possible structure of associated OM on mineral surfaces. Although the model refers to the experimental paper of Sollins *et al.* (2006), the experimental basis of the model is weak. Poor relationship exists between the findings of Sollins *et al.* (2006) and the multilayer model for MOAs because the data for the calculation of the organic layer thickness across various soil fractions is missing. The model is based on the assumption that SOM consists largely of amphiphilic molecular fragments with the ability to self-assemble into micellar structure on mineral surfaces in aqueous solution. Consequently, the adsorption of SOM on mineral surfaces is arranged in a discrete zonal structure (Figure 1.1, **chapter 1**) consisting of the following three zones: (1) the polar functional groups of organic compounds interact with mineral hydroxyls via ligand exchange to form the contact zone, directing the hydrophobic portions outwards towards aqueous solution. Proteinaceous material can also accumulate on charged mineral surfaces via electrostatic bonding and the process of structural unfolding that takes place upon adsorption increases their bonding strength via additional hydrophobic interactions (Quiquampoix *et al.*, 1995). In addition, some hydrophobic organic compounds may interact with non-charged mineral surfaces; (2) an entropically driven membrane-like bilayer structure is developed via hydrophobic interacting with other amphiphilic compounds, forming the so-called hydrophobic zone and exposing the hydrophilic portions towards aqueous solution. The strong adsorption of proteinaceous molecules with unfolding results in discontinuous distribution of the membrane-like structure and (3) more organic compounds possibly interact

with the hydrophilic moieties in the hydrophobic zone and the adsorbed proteinaceous compounds via cation bridging, hydrogen bonding and other interactions to form an outer region (kinetic zone). The weakly retained compounds in the kinetic zone may depend more on input than the available binding sites and possess high kinetic exchange rates with the surrounding compounds in aqueous solution. The significance of N-containing compounds is highlighted in this model because of the relatively high abundance of such compounds in soils and the ability of these compounds to adsorb irreversibly on mineral surfaces (Kleber *et al.*, 2007).

This review aims to collect and summarize the existing studies that used this multilayer model to explain and understand natural observations, and based on this assess the state-of-the-art insights with respect to the performance, applicability and further development / improvement of the model.

## **2.2 Application in soil science and related fields**

### **2.2.1 Soil wettability**

Soil OM contributes greatly to soil water repellency (SWR), a widespread natural phenomenon (Goebel *et al.*, 2011; Achtenhagen *et al.*, 2015). The formation of SWR is closely related with associated OM on mineral surfaces and the surrounding environment. The multilayer model has been used successfully to explain the dynamic and reversible SWR under drying and wetting cycles (Goebel *et al.*, 2011; Kaiser *et al.*, 2015; Achtenhagen *et al.*, 2015). Other factors that may affect the SWR include soil microorganisms (Achtenhagen *et al.*, 2015) and H<sub>2</sub>O molecules remained in dry soils (Kaiser *et al.*, 2015). Similarly, Diehl *et al.* (2009) used the multilayer model to explain the increased normalized C-H peak area of soils with increasing dryness, which indicates an increase in the proportion of outward-oriented hydrophobic moieties in the outer layer of SOM. In addition, the multilayer model was adopted by Bachmann *et al.* (2008) to show the possible mechanisms involved in the development of soil wettability, i.e. the spatial structure of the OM molecules changing from a sparse flat to a dense upright orientation with increasing surface loading. This hypothesis was also made by Kaiser & Guggenberger (2003). At small OM loading, the hydrophobic groups are arranged at the outside of the particle, while at high OM loading, another layer of organic molecules can be attached to the adsorbed OM with upright orientation via hydrophilic functional groups, thus resulting hydrophobic groups

oriented towards outside (Bachmann *et al.*, 2008). However, the different orientation of organic molecules might be explained by their polyelectrolytic character as well. Such molecules can re-arrange under variable water contents. Moreover, Woche *et al.* (2017) reported that the intrinsic properties of surface OM, i.e. the proportion of non-polar C species, played a crucial role in the surface wettability.

### 2.2.2 SOM storage and dynamics

Many researchers presented the multilayer model merely to provide an overview of the stabilization processes and mechanisms of SOM and the importance of MOAs in storing SOM (e.g. Brüggemann *et al.*, 2011; Khalaf *et al.*, 2014; Swenson *et al.*, 2015; Kaiser *et al.*, 2016). In addition, the multilayer model was widely used to explain the high amount of OC in soils with limited reactive mineral surfaces (O'Brien & Jastrow, 2013; Araujo *et al.*, 2017; Souza *et al.*, 2017). Similarly, the continued accumulation of SOC with increasing C and nutrients inputs (Orgill *et al.*, 2017) and the increased adsorption capacity of dissolved organic matter (DOM) on subsurface soils with increasing original SOC content were also explained by the multilayer model (Mayes *et al.*, 2012). Based on the model, Lin & Simpson (2016) attributed the protection of cutin and suberin components in soils to the indirect interaction with mineral-bound SOM. However, these observations can also be explained by aggregation effects (O'Brien & Jastrow, 2013; Lin & Simpson, 2016).

Based on the multilayer model, Han *et al.* (2016) made a hypothesis that the adsorbed OC on mineral surfaces can be divided into two categories: directly and indirectly associated SOC. The former being in the "contact zone" has a stronger resistance to decomposition, thus slower turnover rate and longer residence time than the latter, i.e. SOC in the "hydrophobic and kinetic zone". A similar idea has been introduced before that and discussed in the following studies: Castanha *et al.* (2008) observed that isolated soil density fractions contained a mix of old and young carbon indicated by <sup>14</sup>C data. Using a modelling approach, Torn *et al.* (2013) reported the coexistence of strongly bound (old) and weakly bound, fast-cycling (young) OM in different soil density fractions. Zollinger *et al.* (2013) showed higher <sup>14</sup>C-ages for the stable soil C-fraction (with H<sub>2</sub>O<sub>2</sub> treatment) compared to the bulk soil. Because physical density fractionation makes only a rough separation between active and passive OC pool (Lützow *et al.*, 2006), the presence of occluded free OM in aggregates across all density fractions can also explain the mix of fast and slow cycling of

OC. Moreover, the residual OC after H<sub>2</sub>O<sub>2</sub> treatment often contains pyrogenic material (Mikutta *et al.*, 2005), which possible lead to high <sup>14</sup>C-ages. The higher bioavailability of newly synthesized amino acids (AAs) compared to the original AAs in soils was explained by their retention in the outer layer of MOAs (Zhang *et al.*, 2015). However, the protection of original soil AAs could be also attributed to entrapment in pores which are too small for enzyme access, interaction with and encapsulation in other OM components (Zhang *et al.*, 2015). Based on the multilayer model, Helassa *et al.* (2009) and Feng *et al.* (2014) assumed that adsorption and variation in loading of natural OM, possibly representing different bonding modes for different OM layer on mineral surfaces, alter the thermal and biological stability of natural OM. They found the thermal and biological stability of natural OM upon adsorption on minerals generally increased with C loading which is in contrast to the multilayer model. The reason was attributed to the unsaturated loading of OM on mineral surfaces and absence of an ageing process for the MOAs (Feng *et al.*, 2014).

Bingham & Cotrufo (2016) adopted the multilayer model to support a theory that fresh inputs of N-containing OM into the upper soil horizons are weakly retained on OM coating of mineral surfaces. This enables them to be readily displaced and migrate to deeper horizons, where strong adsorption on mineral surfaces occurs due to the abundant fresh mineral surfaces. Similarly, Macías & Arbestain (2010) used the multilayer model to explain why the importance of MOAs in SOM stabilization increases with soil depth (Von Lützow *et al.*, 2008). The low degree of oxidation of the fresh inputs of OM likely results in weak interaction with the mineral phase in the top soil horizons. With increasing soil depth oxidation of OM and the availability of mineral surfaces increase gradually and results in higher affinity between DOM and mineral phase in the deep horizons (Lehmann & Kleber, 2015). Avneri-Katz *et al.* (2017) used the multilayer model coupled with patchy structure theory to clarify the enhanced adsorption of hydrophobic DOM on mineral soils with increasing DOM concentration. Yet no experimental evidence of the model was included. Vázquez-Ortega *et al.* (2014) used the multilayer model to explain the displacement of weakly bound grassland soil DOM molecules by high-affinity organic molecules from forest soil DOM in soil column experiments. He *et al.* (2016) integrated the model to a theory to illustrate the dynamic exchanges between DOM and particulate organic matter (POM) in coastal and inland aquatic ecosystems. However, the inner- and outer-sphere complexes theory (Sposito, 2008) can be used as well to explain these observations and assumptions.

Some studies used the multilayer model to explain their findings but not necessarily referring to the notion of multilayer structure, such as higher stable C and N concentration in soils from the Alps than that from the Wind River Range (Egli *et al.*, 2012). Cause mentioned is a higher production of secondary minerals with strong adsorption capacity of SOM for the Alps soils (Egli *et al.*, 2012). Moreover, the multilayer model was used to support the positive relationship of SOC content with poorly crystalline oxyhydroxides (Egli *et al.*, 2008), and the close relationship between clay content, accumulated OC and proteinaceous substances during SOC accumulation (Siewert & Kučerík, 2015). Apparently, these experimental results do not require the multilayer model for interpretation but can be explained by a variable availability of reactive mineral surfaces and by the fact that minerals offer different adsorption sites for the various components of OM. Unfortunately, most of the citing studies take the validity of the multilayer model for granted, despite it has never been soundly proven. This is astonishing because the fundamental principle of science, to test ideas first, is thus often violated.

### **2.2.3 Strong adsorption potential of N-containing compounds and enrichment of N in the stable SOM pool**

The multilayer model proposes that an N-rich inner layer of OM exists and it is bound in a stable manner to specific mineral surfaces. This concept derived from the model is widely used to explain the lower C/N ratio of the subsoil than the topsoil (Favilli *et al.*, 2008), a marked decrease in the C/N ratios of the soil heavy fractions after sonication (Castanha *et al.*, 2008) and the enrichment of N in the stable SOC pool, i.e. H<sub>2</sub>O<sub>2</sub>-resistant fractions and the heavy density fractions (Barbera *et al.*, 2008; Sleutel *et al.*, 2011; Bonnard *et al.*, 2012; Dümig *et al.*, 2012; Zollinger *et al.*, 2013). However, all these studies ignored determining the fixed inorganic N (i.e. ammonium). Therefore, the soil N could be presented as fixed inorganic N too especially in deep mineral soils (Nieder *et al.*, 2011).

The stable binding of N-rich OM on mineral surfaces guarantees their strong resistance to decomposition. Therefore, Barbera *et al.* (2012) used the multilayer model to support the explanation that the stronger resistance of silt-clay associated OM to mineralization induced by agricultural practices was due to the higher total N concentration compared to the bulk soil. However, the high concentration of N should be the result of interactions between SOM and minerals rather than the trigger for the slow turnover of silt-clay associated OM.

Nitrogen depleted compounds can also form stable associations with minerals. Cusack *et al.* (2013) used the multilayer model to explain the higher abundance of proteinaceous C in the soils with agricultural disturbance. This was also attributed to the higher microbial activity in cultivated soils (Cusack *et al.*, 2013). Hobbie & Hobbie (2012) used the multilayer model to explain the stronger protection of native soil AAs from microbial uptake compared to the freshly added AAs. However, the native soil AAs could be hidden in microaggregates or located in pores inaccessible to microbes (Hobbie & Hobbie, 2012). Bimüller *et al.* (2014) compared N mineralization of particle size fractions and bulk soil and interpreted the smaller total N mineralization of different size fractions by an additional binding of N-rich compounds on the increased surfaces after fractionation. However, this conclusion is questionable because the loss of N during fractionation is unknown. With a high affinity between N-containing compounds and mineral surfaces, the multilayer model was adopted to explain the relative increase in amide+carboxyl functional groups adsorbed on Fe-containing precipitates in ground water over an eight-month aging and reductive transformation process (Cismasu *et al.*, 2016) and the possible long-term effects of N inputs on the increase of forest SOM storage by forming stable MOAs (Nave *et al.*, 2009). Although these studies focused on interaction of N-rich organic compounds with specific mineral surfaces, the role of N-rich OM in the formation of MOAs was not investigated.

## **2.3 Experimental evidence of the multilayer model?**

### **2.3.1 Patchy distribution of adsorbed OM**

In theory, a certain amount of OM must be clustered in small patches with a certain vertical extension if they are not evenly distributed on available mineral surfaces. This may support indirectly the multilayer model (Kleber *et al.*, 2007). In addition to the previous evidence for the patchy distribution of OM on mineral surfaces (Kleber *et al.*, 2007 and references cited therein), visual and direct evidence was obtained recently by state-of-the-art techniques, such as near-edge X-ray absorption fine structure spectroscopy (NEXAFS) and nano-scale secondary ion mass spectrometry (NanoSIMS) (Lehmann *et al.*, 2008; Solomon *et al.*, 2012; Vogel *et al.*, 2014; Hatton *et al.*, 2015). Vogel *et al.* (2014) observed that only 1–2% of the total area for the non-aggregated particles from clay-sized fractions was covered by OM, whereas 15–21% was covered by OM for the aggregated particles. Moreover, Hatton *et al.* (2015) reported that the particle area covered by OM across all density fractions was 42.4 and 8.3% on

average for aggregated and non-aggregated particles, respectively. To date, the evidence for the patchy distribution of associated OM cannot support the multilayer model on its own.

### 2.3.2 The role of proteinaceous compounds

Recently the enrichment of N-rich compounds on mineral surfaces was observed using NanoSIMS (Hatton *et al.*, 2012; Hatton *et al.*, 2015). However, NanoSIMS images also displayed C-rich organic compounds (C/N ratios > 80) in the densest soil fractions (> 2.65 g cm<sup>-3</sup>; Hatton *et al.*, 2015). Solomon *et al.* (2012) demonstrated numerous N-containing compounds located nearby mineral-organic interface by imaging N maps of the ultrathin section (approximately 150 µm diameter and 400 nm thick) of soil microaggregates using scanning transmission X-ray microscopy (STXM) coupled with NEXAFS spectroscopy. In fact, these interesting results showed different places with different N enrichment in microaggregates but cannot be used as an experimental proof of the multilayer model. No enrichment of N at natural soil mineral-organic interfaces was found by Mikutta *et al.* (2009), who observed a parallel decrease in C and N intensity during X-ray photoelectron spectroscopy (XPS) depth profiling analysis and an increasing portion of aromatic C. This finding was in line with other studies showing aromatic compounds possess a high affinity for metal oxides (Kaiser & Guggenberger, 2000; Chorover & Amistadi, 2001; Mikutta *et al.*, 2007). In contrast to the preferential adsorption of proteinaceous compounds from extracellular polymeric substances on goethite (Omoike & Chorover, 2006), the preferential adsorption of N was not observed on Al(OH)<sub>3</sub> (Mikutta *et al.*, 2011). Organic phosphorus containing compounds possibly play an important role in the formation of MOAs because these materials were adsorbed preferentially on both goethite and Al(OH)<sub>3</sub> (Omoike & Chorover, 2006; Mikutta *et al.*, 2011). Accordingly, the current evidence does not support the idea that N-rich organic compounds are generally enriched on mineral surfaces by preferential adsorption. The enrichment of N could be dependent on mineral surfaces as illustrated in the diagram of the multilayer model (Figure 1.1, **chapter 1**) that N-containing OM only serves as a surface conditioner on siloxane surfaces. Meanwhile, more attention should be paid on the application of surface analytical techniques to ensure the analysis of real mineral-organic interface. Furthermore, just the enrichment of N-rich organic compounds on mineral surfaces cannot be used as direct evidence of the multilayer model.

### 2.3.3 Multilayer structure

To date, it is still challenging to directly visualise the spatial organization of associated OM on mineral surfaces in a molecular scale and unravel the involved mechanisms as suggested in the multilayer model due to the heterogeneous SOC forms and mineral composition and the lack of suitable analytical techniques (Lehmann *et al.*, 2008; Liu *et al.*, 2013). However, some advanced techniques have showed potential to address these issues. A general increasing trend of aromatic C towards the mineral-organic interface was found via XPS depth profiling analysis (Mikutta *et al.*, 2009). This may indicate the vertical heterogeneity of the associated OM. Du *et al.* (2014) identified three different layers (ca. 100  $\mu\text{m}$  thick) for the associated OM on soil particles using Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) with enrichment of heterocyclic N moieties in the inner layer (ca. 60  $\mu\text{m}$  deep). But the spatial resolution in this study does not allow any conclusion about the validity of a molecular-based multilayer model. By integrating NanoSIMS with isotopic tracing, Vogel *et al.* (2014) and Hatton *et al.* (2015) observed new OM was preferentially attached to the OM-conditioned surface sites rather than bare mineral surfaces. However, any conclusion about a multilayer structure has to consider that thin coatings cannot be detected by NanoSIMS. A combination of laser desorption synchrotron postionization (synchrotron-LDPI) mass spectrometry and secondary ion mass spectrometry (SIMS) was adopted by Liu *et al.* (2013) to investigate the molecular properties of soil MOAs and they found lignin compounds was enriched in light soil fractions while aliphatic hydrocarbons was enriched in dense fractions. Moreover, the power of STXM, combined with NEXAFS, in identifying C functional groups in nanoscale was tested by Lehmann *et al.* (2008), Solomon *et al.* (2012) and Chen *et al.* (2014). They demonstrated complex C forms (aromatic, polysaccharides, aliphatic, amide and nucleic acids) in mineral-organic interface and correlate them to the surface Ca, Fe, Al and Si ions, Fe and Al hydr(oxides) and phyllosilicates. Furthermore, Remusat *et al.* (2012) showed the potential capabilities of NanoSIMS coupled with STXM-NEXAFS in analysing the chemical compositions of MOAs. It is clear that more research focussing on the molecular interactions of SOM and specific fractions thereof and soil minerals is needed to truly validate the multilayer model. Meanwhile, it is worthwhile to improve the feasibility and reliability of the applied techniques regarding the spatial resolution, sample preparation, control of radiation damage and spectral quantification (Lehmann & Solomon, 2010).

## 2.4 Conclusion

The conceptual multilayer model of MOAs has been widely used to underpin experimental observations and develop new theory in soil science and related fields across various spatial and temporal scales. However, to date, only very few studies have investigated directly its validity at a molecular level. This highlights a fundamental weakness of nowadays science in a way that popular, edge-cutting ideas become extensively used without profound testing beforehand. In particular the hypothesized multilayer structure and its coupled interaction between different types and classes of molecules that together constitute SOM still lacks rigorous testing. Many of the experimental findings can be explained by simpler explanations instead of the conceptual multilayer model, like aggregation effects for the storage of SOM and differences in the availability of different reactive mineral surfaces for the positive relationship between SOC content and the content of specific minerals. In other words, many studies violate the Occam's razor principle: the simplest explanation will be the most likely one. Nevertheless, given the success of the model in explaining important aspects of the dynamics of SOM, there is an urgent need for further validation of the model, particularly through studies that focus on specific classes of organic molecules and their interaction with well-defined soil mineral phases.