

# **Effect of Coating Phyllosilicate Clays with Hydrrous Oxides on Organic Carbon Stabilisation**

A thesis submitted to the University of Adelaide in fulfilment of the requirements for  
the degree of Doctor of Philosophy

**Akhmad Rizalli Saidy**

**Soils**

**School of Agriculture, Food and Wine**

**The University of Adelaide**

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## Table of Contents

Table of Contents.....	ii
Acknowledgements .....	iv
Abstract.....	vi
Declaration.....	x
Publications Arising from this Thesis .....	xi
Structure of this Thesis .....	xii
Chapter 1. Review of the Literature .....	1
1.1. Introduction .....	2
1.2. Global Carbon Pools and the Carbon Cycle in Terrestrial Systems.....	4
1.3. Mechanisms of Organic Carbon Stabilisation in Soils .....	6
1.3.1. Biochemical Stabilisation .....	8
1.3.2. Physical Protection.....	8
1.3.3. Chemical Stabilisation .....	9
1.4. Importance of Clay Minerals on the Chemical Soil Organic Carbon Stabilisation .....	12
1.5. Importance of Oxides on the Chemical Soil Organic Carbon Stabilisation .....	14
1.6. Factors Influencing the Sorption of Organic Carbon.....	16
1.6.1. Chemical composition of organic matter.....	16
1.6.2. Properties of minerals.....	18
1.6.3. Properties of aqueous phase .....	19
1.7. Stability of Sorbed Organic Carbon against Microbial Decomposition.....	22
1.8. Chemistry and Mineralisation of Sorbed Organic Carbon .....	24
1.9. Priming Effects .....	26
1.9.1. Effects of substrates and soil properties on the priming effect.....	26
1.9.2. Priming effects and soil organic carbon stabilisation .....	28
1.10. Objectives of this Research.....	30
1.11. References.....	31
Chapter 2. Effects of Clay Mineralogy and Hydrous Iron Oxides on Labile Organic Carbon Stabilisation.....	42
Chapter 3. The Sorption of Organic Carbon onto Differing Clay Minerals in the Presence and Absence of Hydrous Iron Oxide .....	52

Chapter 4. Microbial Degradation of Organic Carbon Sorbed onto Phyllosilicate Clays with and without Hydrous Iron Oxide Coating.....	83
Chapter 5. Mineralisation of Organic Carbon Sorbed to Phyllosilicate Clays is not Influenced by the Addition of Glucose.....	122
Chapter 6. Summary and Recommended Future Research .....	139
6.1. Summary and Conclusions.....	140
6.2. Recommendations for Future Work .....	148
6.3. References.....	151

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## Abstract

Phyllosilicate clays and hydrous oxides are recognised as important minerals in soils for organic carbon (OC) stabilisation. Most studies on the influence that hydrous oxides have on OC stabilisation have been carried out using different soils that contain different amounts of Fe or Al oxides or in experiments where OC stabilisation was measured separately on phyllosilicate clays and oxides. Consequently, the interactive effect of different types of phyllosilicate clays and hydrous oxides on OC stabilisation remains poorly understood. In this work, a series of experiments was carried out to obtain a better understanding of the effect of different phyllosilicate clays on OC stabilisation in the presence and absence of different hydrous oxides.

In the first set of experiments, stabilisation of plant-derived OC by three different phyllosilicate clays (kaolinite, illite, smectite) with and without goethite coating and illitic clay coated with different oxides (goethite, haematite, ferrihydrite, imogolite) was quantified by measuring mineralisation of added OC to these clays or clay-oxide associations in a model system consisting of sand mixed with clay. The amount of OC added to mineral was the same for all treatments ( $5 \text{ mg C g}^{-1}$  sand-clay mixture). These conditions correspond to a relatively high OC loading, such as may be experienced in top soils in highly productive systems (i.e. lots of vegetation). Experiments were carried out under moist soil conditions of  $\sim 70\%$  water-filled pore space; under these conditions OC mineralisation should not be limited by availability of water or oxygen. For uncoated clays, OC stabilisation increased in order kaolinite < illite < smectite. It was found that the stabilisation of OC for kaolinite increased with goethite coating; this effect was not observed for illite- and smectite-oxide associations. For illite coated with different oxides, only ferrihydrite increased OC stabilisation over the illitic clay alone. Increasing OC stabilisation for these oxide-coated clays was related closely with increasing specific surface area (SSA). These results demonstrate that there is a clear effect of oxide coating on OC stabilisation that varies with the mineralogy of phyllosilicate clays and type of hydrous oxides and that

under the conditions of this set of experiments, mineral surface area is the dominant factor.

In these experiments the progress of mineralisation over time was closely monitored. Fitting cumulative C mineralisation data to a two-pool C mineralisation model revealed that where the OC stabilisation increased in the presence of oxide-hydroxides, there were reductions in the size of both slowly and rapidly mineralisable C pools. This demonstrates that OC stabilisation was brought about by an increase in the size of undecomposable pool rather than changes in the rates of decomposition of either pool.

It has been suggested that the interactive effect of phyllosilicate clays and oxides on OC stabilisation is controlled by the capacity of clay-oxide associations to sorb dissolved OC. Therefore, a second set of experiments was carried out to assess the effect clay-oxide associations on the sorption of plant derived-OC. The sorption capacity of clays increased in the order kaolinite < illite < smectite on a mass basis or illite < smectite < kaolinite on a surface area basis. Goethite coating on kaolinite increased the sorption of dissolved OC while the sorption capacity of illitic and smectitic clays was not influenced by goethite coating. For illitic clay coated with three different hydrous iron oxides (goethite, haematite, ferrihydrite), an increase in the sorption capacity of dissolved OC was observed only for illite-ferrihydrite associations. Increases in the sorption capacity of dissolved OC were most evident for clay-oxide associations involving either a low-charge clay or high surface area oxide. Desorption experiments showed that only 6-14% of the initially sorbed OC by mineral associations was released by a single extraction step. Coating phyllosilicate clays with hydrous iron oxides reduced desorption of clays, but the effect was most evident for the kaolinite-goethite and illite-ferrihydrite associations. It is likely that the net charge of oxide-coated clays, which is influenced by the balance between the negative charge of phyllosilicate clays and the positive charge of hydrous iron oxides, was crucial for performance of clay-oxide associations in the sorption and desorption of dissolved OC. These experiments showed that differences in OC stabilisation observed in the first set

of experiments were closely related to the sorption capacity of the clays and oxide-coated clays. However, these experiments also showed that the strength of sorption of OC to the minerals, as reflected in the amount of DOC that could be desorbed in a single extraction, varied in a different order among the mineral assemblages and appeared to be related to surface charge rather than specific surface area. This suggests that the degree of OC stabilisation afforded by the assemblages may be quite different at low OC loadings than at high OC loadings.

A third set of experiments was conducted to investigate OC stabilisation at low OC loadings, such as may be experienced in many sub-soils. In these experiments, OC was pre-sorbed onto clays and oxide-coated clays under batch sorption conditions of equal initial solution OC concentration. This resulted in systems with different OC loading on a mass basis, but loadings that were approximately proportional to the sorption capacity of mineral assemblages. In these experiments, incubations were performed “wet”, with 0.5 – 1.g solid in 20 mL nutrient solution; treatments were kept oxic though intermittent shaking. Goethite coating increased the stability of OC sorbed to kaolinite and smectite against microbial decomposition, while the stability of OC sorbed to illite did not change with goethite coating. Among the three hydrous iron oxides tested, only ferrihydrite coating increased the stability of illite-associated OC against microbial degradation. These result showed that under these conditions of low OC loading, the biological stability of OC sorbed to clay-oxide associations again varied with the mineralogy of phyllosilicate clays and the different hydrous iron oxides. In this case, however, the bioavailability of mineral-associated OC was significantly correlated with the reversibility of OC sorption and with the affinity of dissolved OC for clay-oxide associations, as measured in the batch sorption experiments of the second set of experiments on these systems. This suggests the degree of OC stabilisation at low OC loading is controlled by the strength of mineral-organo associations rather the surface area of the minerals.

Under conditions of low OC availability, microbial activity can be limited by energy availability to microorganisms. Under such conditions, the addition of a small amount



of readily available organic matter can increase the decomposition of the pre-existing but less available OC. This is known as a priming effect. In a fourth set of experiments, the stability of OC sorbed onto minerals against microbial degradation was determined in the presence of glucose at C levels equivalent to 1% and 10% of sorbed OC over a 120-day incubation. It was found that the total amount of C mineralised from clay–OC associations for all phyllosilicate clays increased with glucose addition. However, the net amount of C mineralised from clay–associated OC with glucose, which was determined by subtracting the amount of CO<sub>2</sub> produced in the corresponding glucose-only treatment, was not significantly different from that mineralised from clay–associated OC without glucose addition. This result suggests the absence of a priming effect of easily decomposable C source on the mineralisation of OC sorbed to phyllosilicate clays. Therefore, it can be concluded that mechanisms other than energy availability control the stability of OC sorbed to phyllosilicate clays against microbial decomposition.

## Declaration

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Date : 15/05/2013

## **Publications Arising from this Thesis**

Saidy, A.R., Smernik, R.J., Baldock, J.A., Kaiser, K., Sanderman, J. and Macdonald, L.M. 2012. Effect of clay mineralogy and hydrous iron oxides on labile organic carbon stabilisation. *Geoderma* 173-174, 104-110.

Saidy, A.R., Smernik, R.J., Baldock, J.A., Kaiser, K., Sanderman, J. 2012. The sorption of organic carbon onto differing clay minerals in the presence and absence of hydrous iron oxide. Submitted.

## Structure of this Thesis

This thesis is presented as a combination of papers that have been published, submitted for publication and chapters that have not been submitted for publication.

Chapter 1 provides an overview of the literature on the stabilisation of soil organic carbon (OC), the importance of clays and oxides for chemical OC stabilisation and the stability of sorbed OC against biodegradation. This chapter also includes the proposed objectives of this study.

Chapter 2 consists of a paper published in *Geoderma* (Saidy, A.R., Smernik, R.J., Baldock, J.A., Kaiser, K., Sanderman, J. and Macdonald, L.M. (2012) Effect of clay mineralogy and hydrous iron oxides on labile organic carbon stabilisation. *Geoderma*, 173-174, 104-110). It describes an incubation experiment used to determine OC stabilisation at a relatively high OC loading, such as may be experienced in top soils, by different phyllosilicate clays with and without oxide coatings.

Chapter 3 describes a second experiment that follows on from the incubation experiment described in Chapter 2. It describes the effects of coating clay mineral with hydrous iron oxides on the sorption of dissolved OC. These results have been submitted to *Geoderma*.

Chapter 4 describes experiments to determine the effects of the presence of hydrous iron oxides on the mineralisation of OC sorbed to phyllosilicate clays. It provides approach to investigate the stabilisation of OC at low OC loading, such as may be found in many sub-soils. These results have been prepared but not yet submitted for publication.

Chapter 5 comprises an incubation experiment used to provide additional explanation of relatively low C mineralisation of sorbed OC found in Chapter 4. It describes the mineralisation of OC sorbed to clay minerals in the presence and absence of easily decomposable C source. These results have been prepared but not yet submitted for publication.

Chapter 2, 3, 4 and 5 were prepared as standalone for publication. Therefore, this style of presentation results in some areas of repetition, particularly in the introductions, methods and reference lists.

Chapter 6 provides a summary of the findings contained in this thesis and includes recommendations for future work.

## **Chapter 1. Review of the Literature**

## 1.1. Introduction

The global soil carbon (C) pool of 2500 gigatons (Gt) consists of about 1550 Gt of soil organic carbon (SOC) and 950 Gt of soil inorganic carbon (SIC) (Lal, 2004). The addition of organic matter associated with long-term agricultural practices may increase SOC and to a lesser extent lead to a change in the size of soil C pool. Increasing organic carbon in soils can be achieved by increasing C inputs (amount and quality of plant residues) and/or decreasing carbon losses through stabilising SOC against decomposition and mineralisation.

The fine particle size fraction of soils (< 20 µm) plays an important role in stabilisation of organic carbon. This can be attributed to the fact that soil silt and clay fractions contain higher amounts organic carbon (Homann et al., 2007; Lorenz et al., 2008) and show a lower loss of organic carbon after cultivation (Jolivet et al., 2003) or have a longer turnover time (Kalbitz et al., 2005; Ludwig et al., 2005) compared with larger particle size fractions (sand and gravel particles). Most studies on the relationship between clay and SOC contents have been conducted using different soils that may have different characteristics. Therefore, the amount of carbon adsorbed to the silt and clay fractions may also be influenced by the characteristics of soils.

Sorption of OC to iron (Fe) and aluminium (Al) oxide surfaces appears to contribute to a decreased SOC turnover (Schneider et al., 2010; Wiseman and Püttmann, 2006), and may therefore increase the content of organic carbon in soils. The organic carbon concentration in mineral horizons in some soils has been reported to be closely related

to extractable Fe and Al (Kaiser and Guggenberger, 2000; Kleber et al., 2005; Percival et al., 2000). However, other studies have reported DOC sorption was not to be related Fe oxides (Riffaldi et al., 1998) and Kiem and Kögel-Knabber (2002) reported SOC contents in a loamy soil did not correlate with iron oxide contents. Thus contributions of hydrous iron and aluminium oxides to SOC stabilisation remain uncertain.

Hydrous oxides may attach to both clay minerals and organic compounds (Ohtsubo, 1989; Tombácz et al., 2004; Zhuang and Yu, 2002) to form clay-mineral-organic associations, which influence important processes in soils because they alter the physicochemical properties of the minerals (Angove et al., 2002; Wang and Xing, 2005). Hydrous Fe and Al oxides generally sorb more OC than clay minerals (Chorover and Amistadi, 2001; Kaiser and Guggenberger, 2003; Meier et al., 1999), and it may be expected that the presence of oxide coating on different clay minerals would significantly influence the capacity of soils for SOC stabilisation. Therefore, it is necessary to understand the interactive effect of clay mineral and oxide on the several aspects of SOC stabilisation. These aspects of SOC stabilisation include the capacity of clay-associated oxide for OC sorption and the relative stability of OC sorbed to clays and hydrous oxides against microbial degradation.

The following review will focus on the effect of clay and iron oxides on the stabilisation of carbon in soils. The review begins by discussing the global carbon pool, the carbon cycle in terrestrial systems and mechanisms of SOC stabilisation. The effect that clay and iron oxides have on OC stabilisation through sorption and factors influencing OC

sorption will then be reviewed. Finally, an extensive discussion of the stability of sorbed OC against biodegradation and the importance of priming effects are presented.

## **1.2. Global Carbon Pools and the Carbon Cycle in Terrestrial Systems**

The principal global carbon (C) pools are the oceanic, geologic, pedologic, atmospheric and biotic pools (Figure 1). These are all interconnected through sizeable fluxes. The pedologic or soil C pool comprises two components: soil organic carbon (SOC) and soil inorganic carbon (SIC). The SIC pool is especially important in soils of the dry regions (Lal, 2004). Histosols, because of their very high C contents, are major contributors to the total soil C, although they occupy a relatively small proportion of the world's surface area. The carbon in Histosols, which globally occupy about 1.3% of the land surface, is estimated to account for 23% of the total soil C pool (Lal, 2004). The soil C pool is 3.3 times larger than the atmospheric pool and 4.5 times larger than the biotic pool (Lal, 2004). Changes in the amount of organic C in soil could lead to a considerable alteration of CO<sub>2</sub> concentration in the atmosphere. A 5% increase in the amount of organic C stored in the top 0-2 m of the world's soils would theoretically result in 16% less CO<sub>2</sub> in the atmosphere (Baldock, 2007).



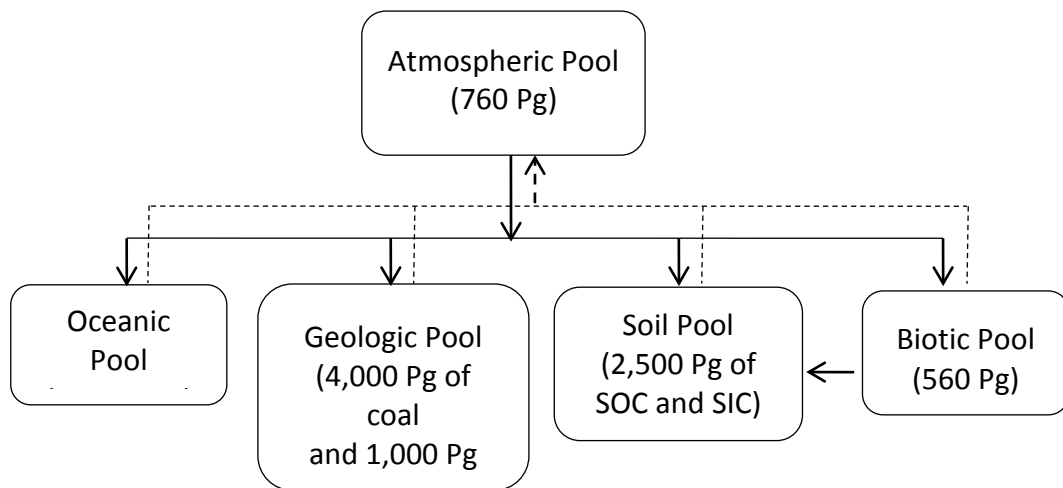


Figure 1. Global carbon pools (adapted from Lal, 2004)

The terrestrial biosphere is recognised as an important component for carbon sequestration as it is assumed to be a significant sink for carbon dioxide, with C fixation balancing or exceeding emissions brought about by changing land use (Schimel, 1995). The carbon cycle in terrestrial systems encompasses a number processes with two predominant components of terrestrial C sequestration: soil and biota (plant) (Figure 2). Carbon present in the atmospheric pool in the form carbon dioxide ( $\text{CO}_2$ ) is fixed into organic structures via photosynthetic organisms, mainly plants. When these biota die, organic carbon remaining in their residues is decomposed. During decomposition, a portion of the carbon is converted into the cellular structures of the decomposer organisms, a portion is mineralised directly to carbon dioxide ( $\text{CO}_2$ ) which re-enters the atmosphere, and a portion may be converted to a more biologically stable fraction and stabilised in soils. The amount of C stabilised in soils is controlled by the physical and chemical environment of the soil, the chemical structure of SOC, and the physical

accessibility of the organic carbon to microbes and enzymes (Krull et al., 2003). Studies using  $^{14}\text{C}$ -labeled plant residues have indicated that 12 – 25 % of the applied C remains in soils after a 5-year period, with a greater portion of the  $^{14}\text{C}$ -residues was retained by clay soils than by silt loam soils (Saggar et al., 1999).

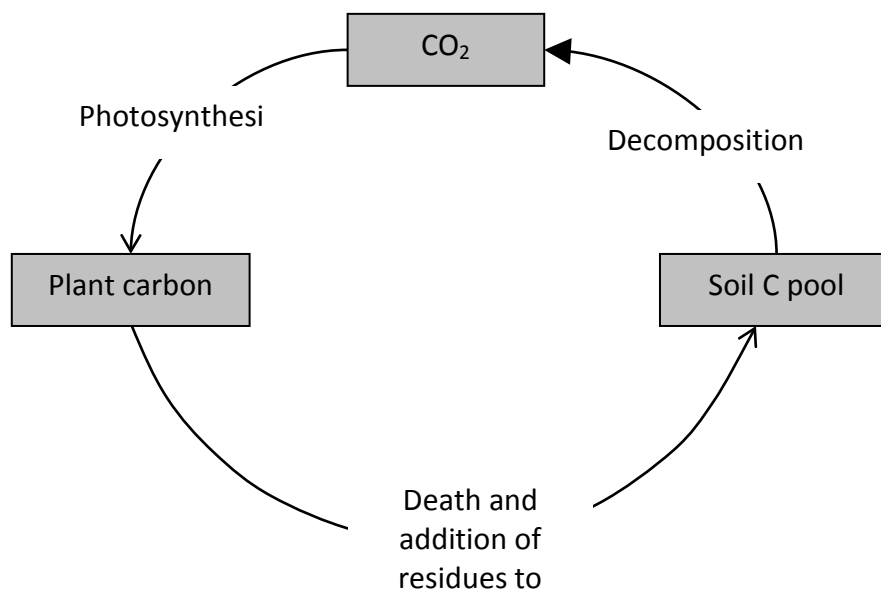


Figure 2. Carbon cycle in the terrestrial system (adapted from (Tate, 2000))

### 1.3. Mechanisms of Organic Carbon Stabilisation in Soils

Three main mechanisms of organic carbon stabilisation have been identified, namely biochemical stabilisation, physical protection, and chemical stabilisation (Six et al., 2002). Krull et al. (2003) reviewed mechanisms and processes of stabilisation of organic matter and emphasized that chemical recalcitrance appears to be the only mechanism by which soil organic matter can be stabilised for a long period of time.

Baldock et al. (2004) have suggested a scheme for biological stabilisation based on three mechanisms, namely biochemical recalcitrance, the biological capability and capacity of the decomposer community, and physical protection. This concept suggests that stability can result from the presence of biochemically recalcitrant molecules (often rich in alkyl/aryl C) and that the other mechanisms are responsible for protection of potentially labile molecules and lead to the variable chemical structure observed for soil organic matter.

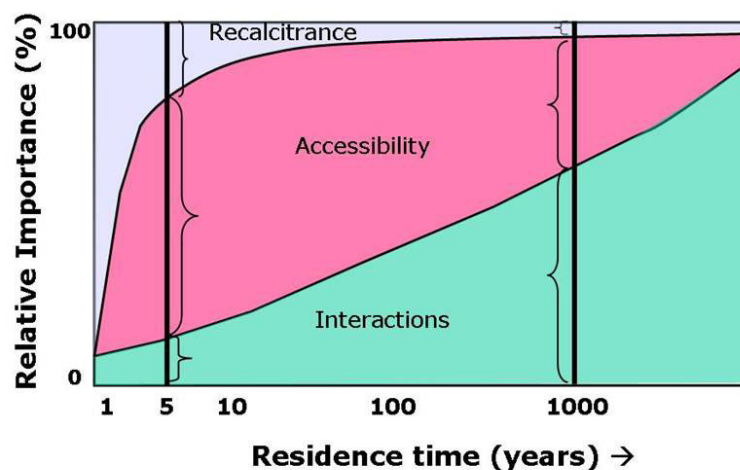


Figure 3. Importance of three main mechanisms of SOC stabilisation is a function of time (Kögel-Knabber and Kleber, 2011).

von Lützow et al. (2006) differentiated the mechanisms of organic carbon stabilisation as selective preservation, spatial inaccessibility, and interaction with surfaces and metal ions. Although von Lützow et al. (2006) named the three main mechanisms of soil organic carbon differently, the processes involved in each mechanism are similar to those identified by Baldock et al. (2004). These three mechanisms of SOC

stabilisation vary in importance with the residence time (Figure 3). Organic matter bound to mineral surfaces or physically separated from decomposing biota achieves protection for a long period of time (Kögel-Knabber and Kleber, 2011).

### **1.3.1. Biochemical Stabilisation**

Biochemical stabilisation is understood as the stabilisation of SOC due to its own chemical composition (e.g. recalcitrant compounds such as lignin and polyphenols) and through chemical complexing processes (e.g. condensation reactions) in soil. Alkyl structures are considered to be chemically more stable because of their highly aliphatic nature (Derenne and Largeau, 2001). Lignin, with its aromatic ring structures, is recognised to be more resistant to decomposition than carbohydrates, and together with alkyl carbon are considered to account for a biochemically stable component of SOC. Charcoal (or black carbon) derived from incomplete combustion of organic material is considered the most recalcitrant component of SOC due to its high degree of aromaticity and highly condensed chemical structure (Krull et al., 2003).

### **1.3.2. Physical Protection**

Physical protection refers to the localisation of organic matter in soils in positions that cannot be accessed by microorganisms or their enzymes. Inaccessibility of organic matter is caused by occlusion of organic matter through aggregation, intercalation within phyllosilicates, and encapsulation in organic macromolecules (von Lützow et al., 2006).

Aggregate formation occurs when soil particles are bound together into larger secondary entities. Silt-sized microaggregates (2-20  $\mu\text{m}$ ) in non-sodic soils are stable small particles that are bound together by bacterial and fungal debris, which in turn can be bound into larger microaggregates (20-250  $\mu\text{m}$ ) (Krull et al., 2003). Microaggregates can be combined into macroaggregates (> 250  $\mu\text{m}$ ) by chemical (e.g. microbial and plant-derived polysaccharides) or structural binding agents (e.g. fine roots and fungal hyphae), with their respective strengths becoming more important with increasing aggregate diameter (Jastrow and Miller, 1998). Microaggregates are considered to be more stable and less easily disrupted by mechanical disturbance (Balesdent et al., 2000). The stability of macroaggregates is strongly influenced by management practices (e.g. soil tillage), and therefore provides less protection than microaggregate formation (Beare et al., 1994).

### **1.3.3. Chemical Stabilisation**

The chemical stabilisation of organic matter in soils is based on the adsorption and chemical binding of soil organic matter onto mineral surfaces. In soils, layer silicates and sesquioxides constitute the majority of material capable of providing reactive sites to which organic materials can be adsorbed. Formation of mineral-organic matter association occurs via a variety of mechanisms including columbic (hydrogen bonding, anion and cation exchange, ligand exchange, cation bridges) and non-columbic (van der Waals) interaction (Arnarson and Keil, 2000; Sutton and Sposito, 2006). The most relevant mechanisms for organic matter bonding to mineral surface in natural environments are: (i) displacement of surfacial hydroxyl/water groups of minerals by

organic functional groups (i.e., ligand exchange), (ii) cation-mediated bridging of organic matter to permanently negative-charge siloxane surfaces or to hydroxyl of phyllosilicates and oxides (cation bridging), and (iii) van der Waals interactions (Table 1). Although under certain environmental conditions specific binding mechanisms may be dominant, in most conditions several mechanisms are involved simultaneously (Mikutta et al., 2007).

Table 1. Interaction of anion organic and mineral surfaces (von Lützow et al., 2006).

Mechanisms		Compounds/surfaces
Ligand exchange	Anion exchange	OH groups on Fe, Al and Mn oxides OH groups at edge sites of phyllosilicates Allophane, imogolite OM with aliphatic or phenolic OH-groups Aliphatic acids (citric acid, malic acid) Amines, ring-NH, heterocyclic-N
Cation bridges	Electrostatic cation bridges ( $\text{Fe}^{3+} < \text{Al}^{3+} < \text{Pb}^{2+} < \text{Ca}^{2+} < \text{Mn}^{2+} < \text{Mg}^{2+}$ )	Negatively charged functional groups: $\text{OH}^-$ , $\text{COO}^-$ Expandable layer silicates, e.g. smectite, vermiculite, illite OM functional groups: carboxyl, carbonyl, alcoholic $\text{OH}^-$ microbial polysaccharides with glucuronic-, galacturonic-, mannuronic-, pyruvic-, succinic-acid groups
Weak interactions	Hydrophobic interactions Van der Waals interactions  Hydrogen bonding	Non-polar, uncharged surfaces  Non-expandable layer silicates (kaolinite), neutral microsites on smectites Quartz sand OM: uncharged, non-polar groups (aromatic, alkyl-C) Any mineral with oxygen surfaces, e.g. kaolinite OM functional groups: carboxyl, carbonyl, phenolic $\text{OH}^-$ , amines, heterocyclic-N

Organic anions of carbon can be sorbed to mineral surfaces through the mechanism of ligand exchange. Anion exchange between simple coordinated OH groups at edge

sites of phyllosilicates and carboxyl groups and phenolic OH groups of organic matter forms a strong organo-mineral association (Gu et al., 1994). Formation of organo-mineral association via ligand exchange is considered to be dependent on pH. Maximal sorption of organic matter to soil minerals through ligand exchange is reported between pH 4.3 and 4.7, corresponding to  $pK_a$  values of the most abundant carboxylic acids in soils (Gu et al., 1994). As this mechanism occurs in acidic conditions, ligand exchange between reactive sites (OH group at edge sites of phyllosilicates) and organic carboxyl and phenolic OH groups is considered to be an important mechanism of carbon stabilisation in acidic soils (Kleber et al., 2005).

Another mechanism involved in the sorption of OC onto mineral particles is cation bridging. Multivalent cations such as  $Al^{3+}$ ,  $Fe^{3+}$ ,  $Ca^{2+}$  and  $Mg^{2+}$  function as a bridge between negatively charged surfaces of clay particles and organic matter. One positive charge of the di- or tri-valent cation bonds the negative charge of the organic anion and another charge bonds the negatively charged clay mineral surface, thereby serving as a bridging mechanism. The major polyvalent cations present in soil are  $Ca^{2+}$  and  $Mg^{2+}$  in neutral and alkaline soils and hydroxyl polycations of  $Fe^{3+}$  and  $Al^{3+}$  in acidic soils (von Lützow et al., 2006). The strong bonds between organic anions and clay minerals due to the action of the polyvalent cations protect the organic materials from microbial decomposition. The strength of bonds depends on the valence of the bridging metal cation following this order:  $Al^{3+} > Fe^{3+} > Ca^{2+}$  (Bohn et al., 2001).

Van der Waals interaction is the attractive or repulsive forces between molecules with one another or with neutral molecules and includes forces between two permanent dipoles (Keesom force), forces between a permanent dipole and a corresponding induced dipole (Debye force) and forces between two instantaneously induced dipoles (London dispersion force) (Schwarzenbach et al., 2003). Van der Waals interactions of OM with mineral surfaces are especially relevant in acidic and high-ionic strength environments (e.g., saline soils or marine system) where electrostatic repulsion forces between negatively charge organic polymers and negatively charged clay surface are weak (Arnarson and Keil, 2000; Sutton and Sposito, 2006). Adsorption energies of van der Waals interaction are smaller than those of chemical adsorption (Gu et al., 1994), suggesting a stronger desorbability and thus larger bioavailability of OM bound by non-columbic interaction.

#### **1.4. Importance of Clay Minerals on the Chemical Soil Organic Carbon Stabilisation**

Silt and clay contents are recognised as an important determinant in controlling the amount of organic carbon in soils. This premise originates from the observed relation between the percentage of fine silt and clay fractions and the amount of organic carbon present in those soil fractions. Fine-textured soils usually contain more organic matter than coarse-textured soils that have received the same input of organic matter. Using data collected from the surface 10 cm of soils from both temperate and tropical regions, Hassink (1997) observed that the amount of organic carbon in soil particles < 20  $\mu\text{m}$  was significantly correlated to the percentage of the soil particles < 20  $\mu\text{m}$ ; suggesting an importance of clay content on carbon stabilisation.



The important contribution of clay particles to the stabilisation of carbon in soils is also evidenced by the fact that fine silt and clay fractions contain older and higher carbon contents larger soil size fractions. Using radio carbon measurement, Quideau et al. (2001) reported that carbon associated with the clay fraction was older than that in fine silt, coarse silt and sand fractions. Ludwig et al. (2005) found that the storage of maize-derived C in particle size fractions of the Ap horizon decreased in the order clay ( $0.65 \text{ kg C m}^{-2}$ ) > fine and medium silt ( $0.43 \text{ kg C m}^{-2}$ ) > coarse silt ( $0.33 \text{ kg C m}^{-2}$ ) > fine sand ( $0.13 \text{ kg C m}^{-2}$ ) > medium sand ( $0.12 \text{ kg C m}^{-2}$ ) > coarse sand ( $0.06 \text{ kg C m}^{-2}$ ). Results of these studies emphasise that carbon in the clay fraction is more resistant to microbial decomposition compared to that in other soil particle size fractions.

Sorption of OC onto phyllosilicate clays generally occur through non-columbic mechanisms. Organic anions are repulsive of negatively charged clay minerals in soils, and the binding occurs when polyvalent cations are present on the exchange complex. Polyvalent cations such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$  are able to maintain neutrality at the surface by neutralising both the charge on the negatively charged surface (e.g. in clay minerals) and the acidic functional group of the OM (e.g.  $\text{COO}^-$ ) and thus act as a bridge between two charged sites (Kögel-Knabber and Kleber, 2011). Polysaccharides secreted from microorganisms bind strongly to negatively charged clay minerals through cation bridging due to the negative charge of uronic acids attached to the polysaccharides (Chenu, 1995). Organic anions also attach to phyllosilicate clays through hydrogen bonding, Van der Waals interaction and hydrophobic interactions (Kögel-Knabber and Kleber, 2011).

Differences in carbon storage and the quality of organic matter associated with clay are thought to be controlled by the specific surface areas (SSA) provided by clay minerals. Different clay types have different specific surface areas; therefore, it is expected that clay type will influence the capacity of soils to protect and store organic carbon. Specific surface areas vary from 2–4 m<sup>2</sup> g<sup>-1</sup> for quartz (Wilding et al., 1977), 10–70 m<sup>2</sup> g<sup>-1</sup> for kaolinite, chlorite and mica (Huang, 1990), 50–100 m<sup>2</sup> g<sup>-1</sup> for illite to 800 m<sup>2</sup> g<sup>-1</sup> for smectite and vermiculite (Robert and Chenu, 1992). Soils dominated by clays with a high specific surface area are expected to sorb more organic matter than soils dominated by clays with a low specific surface area.

#### **1.5. Importance of Oxides on the Chemical Soil Organic Carbon Stabilisation**

In addition to clay minerals, secondary minerals, in particular those with abundant hydroxyl groups, also provide significant surface areas to which organic matter can adsorb (von Lützow et al., 2006). These include Fe oxides (Kaiser and Guggenberger, 2007; Kögel-Knabner et al., 2008; Wagai and Mayer, 2007), Al-rich imogolite type materials (Basile-Doelsch et al., 2007; Percival et al., 2000; Scheel et al., 2007) and poorly crystalline minerals in general (Egli et al., 2008; Kleber et al., 2005; Mikutta et al., 2006; Rasmussen et al., 2007). The presence of these minerals in different amounts in soils influences the capacity of soils for OC stabilisation through ligand exchange between positive charge of oxides and negative charge of OC. Eusterhues et al. (2005) studied the role of Fe oxides and Al silicates on the formation of organo-mineral associations using soil particles < 6.3 µm extracted from acid soils (Dystric Cambisol and Haplic Podzol). The amount of Fe in the soil particle was determined using dithionate-

citrate-bicarbonate extraction ( $Fe_{DCB}$ ) and ammonium-oxalate extraction ( $Fe_{Ox}$ ). Eusterheus et al. (2005) found that the amount of carbon in the soil particles was positively correlated with the  $Fe_{DCB}$  and  $Fe_{Ox}$ . Eusterheus et al. (2005) also reported that the specific surface area of particle-size fractions reduced significantly from 4-145  $m^2 g^{-1}$  to 2.4-54  $m^2 g^{-1}$  after extraction of total Fe oxides by dithionite-citrate-bicarbonate (DCB). The results of this study emphasise the importance of Fe oxides in providing specific surface areas for organic matter sorption in organo-mineral associations in acid soils. Positive correlations between total iron oxides and organic carbon content in topsoil and illuvial subsoil (Kaiser and Guggenberger, 2000) as well as for surface horizons of agricultural soils (Kiem and Kögel-Knabber, 2002) have also been noted.

Phyllosilicate clays are often considered to be weaker sorbents for organic matter than Fe oxides, especially under acidic conditions. This suggests that it is not the size of mineral surfaces that is decisive for carbon stabilisation, but rather their reactivity and ability to interact with organic compounds. Working on the premise that the organic carbon remaining after chemical removal with NaOCl solution represents a stable organic carbon pool, Kleber et al. (2005) reported that clay content does not correlate with organic carbon in both untreated and NaOCl-treated soils. Carbon in the treated-soils had a positive correlation with the amount of Fe and Al extracted by ammonium oxalate. Other studies conducted by Kaiser and Zech (2000) and Chorover and Amistadi (2001) reported that subsoil clay fractions exhibited a weak adsorption of organic carbon when Fe oxides were removed from soils. Several sorption experiments

also showed that the capability of oxides to sorb OM is generally higher than that of phyllosilicate clays (Kaiser and Guggenberger, 2003; Meier et al., 1999; Tombácz et al., 2004).

In a study of carbon storage in coarse and fine clay fractions of soils exhibiting a similar clay mineralogy, Kahle et al. (2003) calculated the loading of mineral surface area with C and Fe by dividing C content or Fe content of the untreated fraction by the specific surface areas. C and Fe loadings in all fine clay fractions were reported to be in the same range, while in the coarse clay C loading exceeded Fe loading (Kahle et al., 2003). The authors suggested that Fe oxides predominantly provided important surface area for association with organic matter in fine clay, while silicate mineral surfaces were more important for C storage than Fe oxides in coarse clay fractions.

## **1.6. Factors Influencing the Sorption of Organic Carbon**

The extent of SOC stabilisation through chemical stabilisation is dependent on the several factors controlling in the sorption of dissolved OC to soil minerals. These factors can be grouped into three categories: properties of organic matter (chemical composition), properties of minerals and properties of the aqueous phase.

### **1.6.1. Chemical composition of organic matter**

Sorption of OM onto mineral surfaces primarily involves interaction of negative charge on organic matter and negative charge of clays or positive charge of oxides; therefore, the number of functional groups on organic matter providing sorptive sites determines

the the degree of OM sorption onto mineral surfaces. It was found found that humic acids extracted from B horizons of forest soils sorbed much more strongly onto goethite than fulvic acids (Weng et al., 2006) . This difference is related to a preference of higher molecular weight OM in the sorption processes (Davis and Gloor, 1981; Gu et al., 1995). Using liquid-state  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy, Kaiser and Zech (1997) reported that sorption causes a preferential removal of aromatic and carboxyl C from solution while alkyl C accumulates in solution. It has been suggested that the number of acidic groups attached to aromatic compounds control the interaction of organic matter and mineral surfaces as indicated by an inverse relationship between the ratio of aromatic to carboxyl C and the sorption capacity (Kaiser, 2003). Specific ultraviolet absorbance at 280 nm normalised to DOC concentration (SUVA) has a strong positive correlation with the proportion of aromatic compounds in DOC (Chorover and Amistadi, 2001; Kalbitz et al., 2003a; Scheel et al., 2007). A significant decrease in the SUVA of dissolved OC following sorption to mineral surfaces has been reported in several studies (Kalbitz et al., 2005; Mikutta et al., 2007), indicating a preferential sorption of aromatic-rich OC fractions. The effect of structural properties on OC sorption was also examined by Schneider et al. (2010) who reported that OC featuring more aromatic structures and carboxylic groups had a higher affinity to amorphous Al hydroxide than that with lower aromatic and carboxyl C contents. Results of these study demonstrate the importance of aromatic and carboxyl C for OM sorption to mineral surfaces.

## 1.6.2. Properties of minerals

### a. Mineral surface properties

The surface properties of phyllosilicate clays and oxides play the major role in the sorption of organic matter. Two types of charge can be identified in phyllosilicate clays, permanent or constant charge and variable or pH-dependent charge. Permanent negative charges of clays on the basal planes result from isomorphic substitution of the central Si and Al-ions in the crystal lattice for lower positive valence ions. Variable charge is a result of protonation and deprotonation of surface hydroxyls (Al-OH, Si-OH) situated at the broken edges, and thus it varies with pH (Johnston and Tombácz, 2002; Sposito, 1984). Edge sites and corners of phyllosilicates are considered to be high energy sites which are preferentially occupied by soil organic matter (Cornell and Schwertmann, 2003). Surface ionisation (protonation and deprotonation) reactions could also occur at the surface hydroxyl groups (S-OH) of metal oxides. Protonation of surface hydroxyls ( $\text{SOH} + \text{H}^+ \rightleftharpoons \text{S-OH}_2^+$ ) is promoted in acidic condition, whilst deprotonation ( $\text{SOH} + \text{OH}^- \rightleftharpoons \text{S-O}^- + \text{H}_2\text{O}$ ) is enhanced under alkaline conditions (Sposito, 1984). This charge heterogeneity influences the interaction of mineral surfaces and organic matter in soils. Due to the differences in the surface structures of phyllosilicate clays and oxides, the sorbed amount of soil organic matter can differ significantly (Chorover and Amistadi, 2001; Feng et al., 2005; Meier et al., 1999).

#### b. Specific surface area

When sorption is dominated by adsorption (surface sorption) rather than absorption, one would expect sorption to be influenced by surface areas. Specific surface area (SSA) describes the surface area that is potentially available for organic matter sorption. Soil organic matter sorption seems to increase with increasing SSA of soil minerals (Kahle et al., 2004; Kaiser and Guggenberger, 2003). A higher OC sorption of 2:1 layer silicate than that of 1:1 layer silicate has been found in several studies (e.g., (Dontsova and Bigam, 2005; Kahle et al., 2004; Wang and Xing, 2005), indicating the sorption capacity depends on the mineralogy and, more specifically, on the surface area of mineral constituents. Sorption capacity for several untreated surface and subsoil horizons was correlated significantly with SSA of those soils (Nelson et al., 1992). Despite their potential sites for sorption, soil surface areas are often considered a poor predictor of OC sorption. Kaiser and Guggenberger (2000) found no relationship between OC contents and SSA of sediments. A “masking” of mineral surfaces by adsorbed OM and clustering of OM patches at highly reactive sites of metal hydroxides were supposed as probable reasons for this finding.

### **1.6.3. Properties of aqueous phase**

#### a. pH

Charges of functional groups of organic matter vary with pH, with pKa of functional groups ranging from 4.2–4.9 (carboxyl groups) to 8.5–9.9 (phenolic groups) (Thurman, 1986). As discussed above, mineral surfaces as a sorbent phases also have pH-

dependent charge; thus, the charge of both organic matter and mineral are dependent on pH. Because the charge of sorbate (organic matter) and sorbent (mineral phase) affect sorption affinity, the pH of the solution phase plays a significant role in sorption of OC onto mineral surfaces.

It is generally well accepted that the sorption capacities of several mineral phases for organic material increase with decreasing pH, as Tipping (1981) showed for iron oxides sorbing DOC and Varadachari (1994) showed for clay minerals interacting with fulvic acids. At lower pH values, humic acids (HA) become less negatively charged (Arnarson and Keil, 2000; Majzik and Tombácz, 2007). This leads to decreased electrostatic repulsion between HA and clay surfaces and in turn increases the possibility of non-coulombic interactions (van Der Waals force), which is a key mechanism in HA sorption to clay surfaces. For oxides, for which the dominant mechanism of organic matter sorption is ligand exchange, maximum OM sorption occurred at pH 4.3–4.7, corresponding to  $pK_a$  values of the most abundant carboxylic acidic groups in soils (Gu et al., 1994).

#### b. Ionic strength

The influence of ionic strength on the sorption of organic matter has been studied comprehensively for clay minerals (Arnarson and Keil, 2000; Baham and Sposito, 1994; Feng et al., 2005) and oxides (Antelo et al., 2007; Weng et al., 2006). It is well known that OM sorption increases with increasing ionic strength. At higher ionic strength, OM molecules are closer to mineral surfaces and enhance OM sorption to minerals



(Weng et al., 2006). Jones and O'Melia (2000) suggested that the effect of ionic strength on OM sorption is mainly due to changes in lateral electrostatic repulsion among adsorbed humic acid molecules. Repulsion between adsorbed molecules increases with decreasing ionic strength and this results in a reduction in OM sorption. The double-layer of clay minerals is compressed at higher ionic strength and leads to increasing OM sorption via van der Waals interaction (Arnarson and Keil, 2000).

### c. Composition of cations

The presence of cations in solution leads to differential OM sorption behavior through the involvement of the cation bridging mechanism (Droge and Goss, 2012; Feng et al., 2005; Majzik and Tombácz, 2007). Droge and Goss (2012) studied sorption of peat-soluble OC onto montmorillonite with different  $\text{Ca}^{2+}$  loadings and found increasing total concentration of  $\text{Ca}^{2+}$  from 0.0013 to 0.0055 M, corresponding to 25–100% of cation exchange capacity (CEC) of montmorillonite, significantly increased OC sorption. It has been shown that the presence of  $\text{Ca}^{2+}$  significantly enhanced OC sorption on clay particles in comparison to  $\text{Na}^+$  (Feng et al., 2005), where a significant contribution of cation bridging was identified alongside ligand exchange and van der Waals interaction when calcium salt was the electrolyte. Calcium is more effective than monovalent sodium for bridging the repulsive charges between negatively charged clay minerals and anionic functional groups of OM (Theng, 1982).

### **1.7. Stability of Sorbed Organic Carbon against Microbial Decomposition**

It is not only sorption of dissolved OC onto mineral surfaces that influences stabilisation of OC in soils; the rate mineralisation of sorbed OC is also an important factor that affects organic matter preservation in soils. Organic carbon sorbed to phyllosilicate clays and oxides is more stable against microbial decomposition than OC either dissolved or not attached to mineral surfaces (Kalbitz et al., 2005; Mikutta et al., 2007; Schneider et al., 2010). Biological resistance of sorbed OC to decomposition is likely related to the strong chemical bonds formed between organic molecules and minerals (Kaiser and Guggenberger, 2007) and inaccessibility of OC sorbed in molecular-size mineral pores ( $< \sim 10$  nm) to microorganisms and enzymes (Baldock and Skjemstad, 2000; Kaiser and Guggenberger, 2003; Mayer, 1994). Strong chemical bonds of organic molecules to mineral surfaces reduce the reversibility of OC sorption (Kaiser and Guggenberger, 2007) and lead to higher stability of mineral-associated OC against microbial decay. It has been suggested that that small molecules sorbed to mineral surfaces cannot be utilised by microorganisms unless they are desorbed so that they can be transported into the cell (Chenu and Stotzky, 2002). In addition, Schneider et al. (2010) suggested that the availability of mineral surfaces during sorption, the structural properties of dissolved OC and the presence of inorganic solutes competing with OC for sorption sites determine the degree of stability of mineral-attached OC against microbial decomposition.

It has been suggested that the stability of sorbed OC against microbial degradation is controlled by reversible mineral-associated OC. There is ample evidence that the microbes are able to degrade sorbed OC following desorption of mineral-attached OC. For example, Keil et al. (1994) desorbed OC from sediments using sea water, 2 N KCl and distilled water and then the desorbed OC was exposed to natural microbes. It was found that between 70% and 95% of desorbed OC could be mineralised within 7 days while the mineralisation of sediment-attached OM without desorption (control) was only < 6% of sorbed OC (Keil et al., 1994). Results of this study highlight the importance of reversibly sorbed OC for mineralisation of mineral-organic associations, and are in agreement with Nelson et al. (1994) and Jones and Edwards (1998), who suggested that desorption is essential for the commencement of microbial degradation of organic matter sorbed to pure minerals and clays.

The importance of desorption to the stability against microbial degradation of sorbed OC is also addressed by Mikutta et al. (2007), who studied mineralisation of OC bound to minerals in experiments consisting of solid of mineral-OC associations in 35 mL nutrient solutions (pH 4.0). They found a significant correlation between the amount of OC (<0.45  $\mu\text{m}$ ) present in the solution at the end of 90-day incubation experiment and that released during desorption experiments, indicating a proportional amount of weakly bound OC was released from mineral-OC associations in the course of incubation. The amount C mineralised from mineral-associated OC in this study was significantly correlated with the amount of OC from the desorption experiment. It has

been suggested that during the incubation period, the mineralisable OC was supplied by OC reversibly bound to mineral surfaces (Mikutta et al., 2007).

### **1.8. Chemistry and Mineralisation of Sorbed Organic Carbon**

Stabilisation of SOC increases with decreasing particle size; therefore, study of the chemistry of carbon in soil fine particles gives an indication of the chemistry of sorbed organic carbon. Using solid-state  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy, Baldock et al. (1992) observed a decrease in the relative intensity of O-alkyl-C and an increase in alkyl-C with increasing size of soil particles. The changes associated with carboxyl-C and aromatic-C were variable and much smaller than those associated with alkyl-C and O-alkyl carbon. Laird et al. (2001) found differences in the chemical composition of OM in mineral–organic associations were associated a shift in mineral composition from coarse to fine clay. The coarse clay fraction had stronger carboxyl and O-alkyl  $^{13}\text{C}$ -NMR peaks and smaller concentrations of extractable amino acids, fatty acids, monosaccharides and amino sugars than OM associated with the fine clay fraction (Kahle et al., 2003; Laird et al., 2001). Other studies also revealed that OM in organo-mineral associations of fine fractions was mostly composed of aliphatic C structures (Cuyppers et al., 2002; Eusterhues et al., 2005; Leifeld and Kögel-Knabber, 2001; Rumpel et al., 2004). Several studies showed evidence for selective stabilisation of O-alkyl C especially by interactions with pedogenic oxides on mineral surfaces within the clay fractions (Grandy and Neff, 2008; Schöening et al., 2005; Spielvogel et al., 2008). However, in another study the absolute amounts of alkyl C and aromatic C chemistry in fine (< 0.2  $\mu\text{m}$ ) and coarse clay (0.2-2  $\mu\text{m}$ ) subfractions of illitic clay were

similar, suggesting that changes in chemical composition of OM in this case were independent of interactions with mineral surfaces (Kleber et al., 2004).

The chemical composition of mineral-associated OC seems to influence the mineralisation rate of sorbed OC. It was found that there was a higher rate mineralisation for sorbed OC derived from dissolved OC with low carboxyl groups and aromatic structures than from dissolved OC featuring more carboxyl groups and aromatic structures (Schneider et al., 2010). Mikutta et al. (2007) also observed decreases in mineralisation where sorbed OC was dominated by aromatic moieties. Such compounds are relatively resistant against microbial decay compared to polysaccharide-dominated components (Kalbitz et al., 2003a; Kalbitz et al., 2003b; Marschner and Kalbitz, 2003). Kalbitz et al. (2005) found that mineralisation of C after sorption of OC from less decomposed organic material was faster and more complete than for OC from highly decomposed organic matter. However, in another study OC sorbed from highly decomposed material with relatively high nitrogen content was more readily mineralised than OC from less decomposed in particular at high OC loading (Schneider et al., 2010), indicating the importance of N compounds in mineralisation of sorbed OC. Generally the OM associated with soil minerals has a low C:N ratio (often around 8-12) (Kögel-Knabber and Kleber, 2011). Nitrogen compounds such as proteins, peptides and DNA are likely to be associated with mineral surfaces (Kleber et al., 2007; Knicker, 2004; Pietramellara et al., 2009; Rillig et al., 2007).

## **1.9. Priming Effects**

The priming effect (PE) describes changes in the turnover of native soil organic matter (SOM) induced by the addition of organic or mineral substances (Jenkinson et al., 1985; Kuzyakov et al., 2000). Changes in the amounts and availability of C by freshly added organic substances result in changes in microbial activity, which eventually alter the decomposition rate of SOM. SOM decomposition can increase on the addition of another C source, in which case the PE is said to be positive, or it can decrease, resulting in a negative PE. A positive PE may result from increases in the activity of microorganisms already present and/or the activation of previously dormant microorganisms which are capable of utilising C from native organic matter after the exhaustion of added substrates (Blagodatskaya and Kuzyakov, 2008; Guenet et al., 2010a; Kuzyakov et al., 2000). On the other hand, a preferential utilisation of added substrates by microorganisms originally growing on native SOM will lead to a negative PE (Bremer and Vankessel, 1990; Guenet et al., 2010b; Kuzyakov, 2002; Wu et al., 1993; Zimmerman et al., 2011).

### **1.9.1. Effects of substrates and soil properties on the priming effect**

#### **a. Initial nitrogen availability**

Many studies have been reported differences in priming effects as a result of different initial nitrogen availability (e.g., Hartley et al., 2010; Zhang and Wang, 2012). It was found that the presence of easily decomposable C on soils or systems with a relatively low nitrogen availability resulted in a positive PE (Conde et al., 2005; Fontaine et al.,

2004; Hamer and Marschner, 2005). One of most common explanations is that soil microorganisms are activated to decompose SOM to acquire nitrogen, which in turn increases decomposition of SOM. The nutrients released during SOM decomposition are used by microorganisms and later by roots. On the other hand, the addition of nitrogen to soil organic C has been reported to decrease SOC mineralisation (Blagodatskaya et al., 2007; Fontaine et al., 2004). This decrease is related to preferential utilisation of the added substrate over SOM in the presence of nutrients, such as N.

#### b. Substrate properties

The magnitude and type of PE (real or apparent) is influenced by availability, composition and amount of substrate. The presence of glucose, fructose and alanine (easily available C sources) was found to result in a greater PE than the addition of less readily decomposed substrates such as catechol, oxalic acid, plant residues, manure, or slurry to soil (Conde et al., 2005; Hamer and Marschner, 2005). Alanine led to a higher decomposition of lignin and peat than glucose, fructose, glycine and oxalic acid (Hamer and Marschner, 2002). Even though glucose is monomer of plant-originated organic polymers, glucose addition caused a lower PE than L-glutamic acid (Mondini et al., 2006) and complex substrate mixtures such as root extract and rhizosphere soil extract (De Nobili et al., 2001).

### c. Soil pH

Priming effects of the addition of easily decomposable substances and plant residues occur more often in neutral soils. Luo et al. (2011) found that biochar addition to soils at pH 7.6 resulted in 28% higher PE than that at pH 3.7. Blagodatskaya and Kuzyakov (2008) reviewed studies of priming effects conducted at different pH values and found that the amount of primed CO<sub>2</sub> increased with increasing pH. Increases in microbial activity (Bergman et al., 1999; Curtin et al., 1998) and changes community structure of microorganisms (Rousk et al., 2011) are likely causes.

#### **1.9.2. Priming effects and soil organic carbon stabilisation**

Organic matter is protected from biodegradation in soils by various mechanisms (Baldock and Skjemstad, 2000; von Lützow et al., 2006). Interactions between organic carbon and mineral surfaces decrease the availability of the organic substrates to microorganisms, which results in increases in the stability of OC against biodegradation (Guggenberger and Kaiser, 2003). Many studies provide evidence that carbon mineralisation decreases significantly after sorption. For example, the work of Jones and Edwards (1998) on the sorption of simple organic compounds (citrate and glucose) onto clay minerals and oxides, studies by Kalbitz et al. (2005) who found a decrease in C mineralisation of different OM upon sorption onto soils, and the work of Scheel et al. (2007) on the impact of precipitation of dissolved OC by dissolved aluminium on the mineralisation of Al-associated OC. Up to now, however, only a few studies have related the contribution of the individual protection mechanisms to the extra CO<sub>2</sub>



released during PE (Rasmussen et al., 2007; Salome et al., 2010). Hamer and Marschner (2002) observed a priming effect on the decomposition of lignin, a compound that is thought not to be easily degradable due its aromatic structures, with the addition of fructose, glycine and alanine. Hamer et al. (2004) attributed an increase in the growth of microbial biomass and the accompanying increased enzyme production to explain the acceleration of black carbon mineralisation on glucose addition.

In addition to the priming effect on the mineralisation of recalcitrant compounds, the addition of easily decomposable C sources may also influence the decomposition of OC bound to mineral surfaces. Fontaine et al. (2007) studied mineralisation of  $2,567 \pm 226$ -year-old carbon in a subsoil (0.6-0.8 m depth), for which 58% of total carbon was bound to minerals, and found an acceleration of mineralisation with the addition of cellulose. It is likely OC from this sub-soil does not provide enough energy to sustain an active microbial population and thereby the production of sufficient enzyme. The study of Ohm et al. (2007) also reported a strong priming effect for three soil fractions: sand (63-2000  $\mu\text{m}$ ), silt (2-63  $\mu\text{m}$ ) and clay (<2  $\mu\text{m}$ ) on the addition of fructose and alanine. It was presumed that the stability of the OM in the clay fraction was not only due to recalcitrance or to interactions with the minerals, but that it may also be caused by a substrate limitation of the degrading microorganisms.

### **1.10. Objectives of this Research**

Increasing SOC can potentially reduce the amount of atmospheric carbon. Changes in organic carbon in soils depend on carbon inputs and the stabilisation of organic carbon in soils. A considerable body of evidence indicates that the stabilisation of organic carbon in soils is the result of complex physical and biochemical processes that are primarily controlled by the inorganic soil components, including clay minerals and oxide-hydroxides of iron and aluminium. Reactive sites provided by phyllosilicate clays and hydrous oxides are considered to be crucial for OC stabilisation in soils. Increases in the amount of clay and Fe oxide in soils appear to increase the number of reactive sites within the inorganic matrix and result in an increase in SOC stabilisation.

Most studies of the effects of hydrous oxides on the SOC stabilisation have been carried out using soil samples containing different amounts oxides or in experiments where the SOC stabilisation was measured separately on phyllosilicate clays and oxides. These approaches result in little information on the interactive effects of different types of phyllosilicate clays and hydrous oxides on SOC stabilisation. This information is essential as phyllosilicate clays and hydrous oxides co-occur in soils and they may interact with each other to form clay-oxide associations, which may influence the capacity of soil for OC stabilisation. Therefore, the general objective of this research is to obtain a better understanding of the stabilisation of organic carbon by phyllosilicate clays in the presence and absence of hydrous oxides. The specific objectives are to:

1. quantify the capacity of phyllosilicate clays in stabilising organic carbon with and without the presence of hydrous-oxides;
2. quantify the capacity of phyllosilicate clays with and without hydrous iron oxide coating to sorb dissolved organic carbon;
3. define the effect of phyllosilicate clays coating with hydrous iron oxides on the stability of mineral-organic associations against microbial decomposition, and;
4. determine changes in mineralisation of OC sorbed to phyllosilicates clays in response to the addition of easily decomposable C source.

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## **Chapter 2. Effects of Clay Mineralogy and Hydrous Iron Oxides on Labile Organic Carbon Stabilisation**

A. R. Saidy<sup>a,d</sup>, R. J. Smernik<sup>a</sup>, J. A. Baldock<sup>b</sup>, K. Kaiser<sup>c</sup>, J. Sanderman<sup>b</sup>, L. M. Macdonald<sup>b</sup>

<sup>a</sup> School of Agriculture, Food & Wine and Waite Research Institute, Waite Campus, The University of Adelaide, Urrbrae SA 5064, Australia

<sup>b</sup> CSIRO Land and Water, Private Bag 2, Glen Osmond SA 5064, Australia

<sup>c</sup> Soil Sciences, Martin Luther University Halle-Wittenberg, von-Seckendorff-Platz 3, 06120 Halle, Germany

<sup>d</sup> Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru 70714, Indonesia

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stabilisation (Geoderma 173-174, 104-110)**

**SAIDY, AKHMAD R. (Candidate)**

Performed analysis on soil samples, data analysis and interpretation, wrote  
manuscript, acted as a corresponding author.

I hereby certify that the statement of contribution is accurate.

Signed

Date: 15/05/2013

**SMERNIK, RON J.**

Contributed to planning of experiment, supervised development of work and data  
interpretation, provided critical evaluation and revision of manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**BALDOCK, JEFF A.**

Contributed to planning of experiment, supervised development of work and data  
interpretation, manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**KAISER, KLAUS**

Contributed to sample preparation, data interpretation, reviewed and edited  
manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**SANDERMAN, JON**

Contributed to planning of experiment, manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**MACDONALD, LYNNE M.**

Contributed to planning of experiment, manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013



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### **Chapter 3. The Sorption of Organic Carbon onto Differing Clay Minerals in the Presence and Absence of Hydrous Iron Oxide**

A. R. Saidy<sup>a,d</sup>, R. J. Smernik<sup>a</sup>, J. A. Baldock<sup>b</sup>, K. Kaiser<sup>c</sup>, J. Sanderman<sup>b</sup>

<sup>a</sup> School of Agriculture, Food & Wine and Waite Research Institute, Waite Campus, The University of Adelaide, Urrbrae SA 5064, Australia

<sup>b</sup> CSIRO Land and Water, Private Bag 2, Glen Osmond SA 5064, Australia

<sup>c</sup> Soil Sciences, Martin Luther University Halle-Wittenberg, von-Seckendorff-Platz 3, 06120 Halle, Germany

<sup>d</sup> Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru 70714, Indonesia

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**The sorption of organic carbon onto differing clay minerals in the presence and  
absence of hydrous iron oxide (submitted)**

**SAIDY, AKHMAD R. (Candidate)**

Performed analysis on soil samples, data analysis and interpretation, wrote  
manuscript, acted as a corresponding author.

I hereby certify that the statement of contribution is accurate.

Signed

Date: 15/05/2013

**SMERNIK, RON J.**

Contributed to planning of experiment, supervised development of work and data  
interpretation, provided critical evaluation and revision of manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**BALDOCK, JEFF A.**

Contributed to planning of experiment, supervised development of work and data  
interpretation, manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**KAISER, KLAUS**

Contributed to sample preparation, data interpretation, reviewed and edited  
manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**SANDERMAN, JON**

Contributed to planning of experiment, manuscript evaluation.

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## **Chapter 4. Microbial Degradation of Organic Carbon Sorbed onto Phyllosilicate Clays with and without Hydrous Iron Oxide Coating**

A. R. Saidy<sup>a,d</sup>, R. J. Smernik<sup>a</sup>, J. A. Baldock<sup>b</sup>, K. Kaiser<sup>c</sup>, J. Sanderman<sup>b</sup>

<sup>a</sup> School of Agriculture, Food & Wine and Waite Research Institute, Waite Campus, The University of Adelaide, Urrbrae SA 5064, Australia

<sup>b</sup> CSIRO Land and Water, Private Bag 2, Glen Osmond SA 5064, Australia

<sup>c</sup> Soil Sciences, Martin Luther University Halle-Wittenberg, von-Seckendorff-Platz 3, 06120 Halle, Germany

<sup>d</sup> Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru 70714, Indonesia

**The work contained in this chapter has been prepared for publication.**

**STATEMENT OF AUTHORSHIP**

**Microbial degradation of organic carbon sorbed onto phyllosilicate clays with and  
without hydrous iron oxide coating (manuscript)**

**SAIDY, AKHMAD R. (Candidate)**

Performed analysis on soil samples, data analysis and interpretation, wrote  
manuscript, acted as a corresponding author.

I hereby certify that the statement of contribution is accurate.

Signed

Date: 15/05/2013

**SMERNIK, RON J.**

Contributed to planning of experiment, supervised development of work and data  
interpretation, provided critical evaluation and revision of manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**BALDOCK, JEFF A.**

Contributed to planning of experiment, supervised development of work and data  
interpretation, manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**KAISER, KLAUS**

Contributed to sample preparation, data interpretation, reviewed and edited  
manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**SANDERMAN, JON**

Contributed to planning of experiment, manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

## **Microbial degradation of organic carbon sorbed onto phyllosilicate clays with and without hydrous iron oxide coating**

### **ABSTRACT**

Sorption of organic carbon (OC) onto phyllosilicate clays and hydrous iron oxides retards OC mineralisation, thus contributing to stabilisation of organic carbon in soils. Little is known about the interactive effects of hydrous iron oxides and phyllosilicate clays on microbial degradation of mineral-associated OC. We carried out an incubation experiment to examine the effect that goethite coating of kaolinitic, illitic and smectitic clays has on the mineralisation of sorbed OC. The effect of coating illitic clay with different hydrous iron oxides (haematite, goethite, ferrihydrite) on OC mineralisation was studied in a second experiment. Organic matter extracted from dried medic (*Medicago truncatula* cv. Praggio) shoot residue was sorbed onto minerals, and the stability of sorbed OC against microbial degradation was quantified by measuring mineralisation of sorbed OC during a 120-day “wet” incubation. The stability of sorbed OC decreased in the order kaolinite > illite > smectite amongst the uncoated clays. Goethite coating of kaolinite and smectite increased the stability of sorbed OM against microbial decomposition, while the stability of illite-associated OC did not change with goethite coating. For illite coated with different hydrous iron oxides, only ferrihydrite increased the stability of sorbed OC against microbial decomposition. These differences in the bioavailability of mineral-associated OM closely reflect differences in the strength and reversibility of OC sorption, as measured in previous batch sorption experiments on these systems. They also contrast with results from previous “moist”

incubation experiments on these systems, carried out at higher OC loadings, in which OC stabilisation was controlled by mineral surface area rather than sorption affinity. Together, these results demonstrate that the degree of protection of OC provided by mineral surfaces is a complex function mineral type, interactions among minerals and the level of OC loading.

Keywords: biodegradation, coated clays, electrostatic interaction, sorbed OM

## **1. Introduction**

Soil organic matter (OM) is an important component of the global carbon cycle. Dissolved organic matter (DOM) produced from plants, microbes and organic soil horizons contributes to the transfer of C within the soil. For example, it has been estimated that approximately 115–500 kg C ha<sup>-1</sup> passes into mineral subsoil as DOM annually, of which 40–370 kg C ha<sup>-1</sup> persists in organic–mineral associations through OM sorption onto soil minerals (Guggenberger and Kaiser, 2003; Michalzik et al., 2001). In general, the sorption of organic carbon (OC) to mineral surfaces is strong and only partially reversible, with only a small portion being extractable into fresh water, salt water or organic solvents (Butman et al., 2007; Kahle et al., 2004; Kaiser and Guggenberger, 2007). Strong chemisorption of OM onto minerals renders OM more resistant to microbial degradation (Jones and Edwards, 1998; Keil et al., 1994; Schneider et al., 2010). This suggests sorption processes contribute to the accumulation and stabilisation of OC in soils.



Phyllosilicate clays and hydrous iron oxides have been recognised as the minerals most relevant to OM stabilisation (Balcke et al., 2002; Kaiser et al., 2007; Meier et al., 1999; Tombácz et al., 2004). Phyllosilicate clays seem less capable than oxides of stabilising sorbed OM against microbial decomposition. For example, Mikutta et al. (2007) reported that 6–16% of OM bound to vermiculite was mineralised during 90 days of incubation while only 3–8% of OM sorbed onto goethite was mineralised, and Jones and Edwards (1998) reported simple carbon substrates (glucose and citrate) added to kaolinite and illite-mica were more decomposable than those added to ferric hydroxide.

It has been suggested that the differences in bioavailability of OM associated with different minerals reflect differences in sorption binding mechanisms, which in turn are influenced by the nature of mineral surfaces and the chemical composition of OM (Chorover and Amistadi, 2001; Feng et al., 2005; Kalbitz et al., 2005; Mikutta et al., 2007). Iron hydrous oxides have a point of zero charge (PZC) at between pH 7.9 and 8.6 (Kaiser and Guggenberger, 2003), whilst the PZC of phyllosilicate clays is generally below pH 3.7 (Chorover, 2005; Kosmulski, 2011). Therefore, at circumneutral soil reaction, such as found in many agricultural soils, iron hydrous oxides have a positive charge while phyllosilicate clays have a negative charge. The electrostatic interaction of negatively charged phyllosilicate clays with positively charged oxides is likely to modify the capability of clay–oxide associations to sorb DOC and thereby affect the stabilisation of sorbed OC against microbial decomposition.

Hydrous oxides are able to interact with both clay minerals and organic compounds (Ohtsubo, 1989; Tombácz et al., 2004) to form clay–mineral–organic associations, which may significantly influence the size of the mineral-associated OM fraction resistant to biodegradation. In our previous study (Saidy et al., 2012), goethite coating of kaolinite was shown to increase the stabilisation of plant residue-derived OC as compared to kaolinite without goethite coating. In a subsequent study, we found that coating kaolinite with goethite resulted in an increase in the capacity of coated-clay to adsorb DOC (Saidy et al., submitted). These results suggest that the increase in SOC stabilisation upon oxide-coating of clays may be associated with an increase in the capacity of coated-clays for DOC adsorption, presumably because the sorbed DOC is relatively resistant against microbial decomposition (Kalbitz et al., 2005; Mikutta et al., 2005; Schneider et al., 2010; van Hees et al., 2003). However, at present there is only scarce information on the interactive effects of phyllosilicate clays and hydrous iron oxides on the mineralisation of sorbed OC.

In a previous study (Saidy et al., 2012), we found that stabilisation of plant-derived OM varied significantly depending on the nature of both phyllosilicate clay and hydrous oxide. The degree of stabilisation closely reflected differences in the specific surface area (SSA) of the clay and clay–oxide assemblages. This was somewhat unexpected, given the different affinities and binding mechanisms of the different minerals. A likely explanation is that the OC was close to or even in excess of the minerals' sorption capacity. Under these conditions, the OC stabilisation might be controlled primarily by

the amount of mineral surface available rather than by the nature/strength of mineral–OM interactions.

The goal of the present study was to re-examine the effect of interactions between clay minerals and hydrous iron oxides on the stabilisation of plant-derived OC under different conditions to those used in our previous study (Saidy et al., 2012). In particular, the OC was pre-sorbed to the mineral phases rather than just added. As a result, the present study involves lower OC loadings that are proportional to and below the sorption capacities of minerals. We reacted plant-derived soluble OC with clays (kaolinite, illite and smectite) and oxide-coated clays (kaolinite, illite and smectite coated with goethite and illitic clay coated with goethite, haematite, and ferrihydrite), and then determined the stability of sorbed OC against biodegradation by measuring mineralisation during a 120-day incubation period. The objectives were to examine: (i) the effect of coating of different phyllosilicate clays with goethite, and (ii) the effect of coating of illitic clay with different hydrous iron oxides on the mineralisation of sorbed OC.

## **2. Materials and Methods**

### **2.1. Clay minerals, iron hydrous oxides and chemical characterisation**

Three different phyllosilicate clays (kaolinite, illite and smectite) were obtained from the collection of clay minerals at CSIRO Land and Water, Adelaide, Australia. Goethite ( $\alpha$ -FeOOH) was produced as described by Atkinson et al. (1967), by slowly neutralising

a  $\text{FeCl}_3$  solution with NaOH and aging the precipitate at 55 °C for 3 d. Ferrihydrite ( $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ ) was prepared by neutralising a 0.1 M  $\text{FeCl}_3$  solution with NaOH (Schwertmann and Cornell, 1991). Haematite ( $\alpha\text{-Fe}_2\text{O}_3$ ) was produced by aging a suspension of fresh ferrihydrite at pH 7 and a temperature of 90 °C (Schwertmann and Cornell, 1991). The nature of the three mineral phases was confirmed by X-ray diffraction (D5005, Siemens AG/Bruker AXS, Karlsruhe, Germany), and tests for solubility in dithionite-bicarbonate-citrate reagent (Mehra and Jackson, 1958) as well as in acid oxalate solution (Schwertmann, 1964). The specific surface areas (as determined by  $\text{N}_2$  adsorption-desorption using a Nova 4200 analyser, Quantachrome Corp., Boynton Beach, USA) were  $36 \text{ m}^2 \text{ g}^{-1}$  (haematite),  $73 \text{ m}^2 \text{ g}^{-1}$  (goethite), and  $212 \text{ m}^2 \text{ g}^{-1}$  (ferrihydrite).

Coated-clays (kaolinite, illite and smectite with goethite; illite with goethite, haematite and ferrihydrite) were prepared via the precipitation of suspensions of clays and hydrous iron oxides in 0.01 M  $\text{CaCl}_2$  at pH 6.0. Further details of coated-clay preparation can be found in Saidy et al. (2012). The clays without coating received similar treatments to clays with hydrous iron oxide coatings.

Clay mineralogy was confirmed by X-ray diffraction (Siemens AG/Bruker AXS D5000). The specific surface area (SSA) of clay minerals and coated-clay minerals was determined by nitrogen adsorption at 77 K and subsequent desorption of nitrogen with a Tristar 5-point BET-instrument on freeze-dried samples. The cation exchange capacity (CEC) of clays and coated-clays was determined by the ammonium acetate

(pH 7) method (Rhoades et al., 1982). The content of exchangeable bases (Na, K, Ca and Mg) was determined by atomic absorption spectroscopy (AAS) after extraction with ammonium acetate. The contents of dithionite-extractable iron ( $Fe_d$ ) were measured using the method of Blakemore et al. (1987). Briefly, 0.5 g of freeze-dried clay or coated-clay was shaken with 1 g sodium dithionite and 50 mL sodium citrate for 16 hours. Then 0.05 M  $MgSO_4$  was added as a flocculant, the samples were centrifuged and the supernatant decanted. The supernatant was made up to 100 mL with deionized water, and the concentration of Fe was determined by inductively coupled plasma spectroscopy.

## **2.2. Preparation of dissolved organic carbon (DOC)**

Water-soluble organic carbon was extracted from oven-dried medic (*Medicago truncatula* cv. Praggio) shoot residue by adding 2 L deionised water to 200 g of ground medic (<2 mm) as described in Saidy et al. (2012). After 10 minutes of gentle stirring, the suspension was allowed to settle for 40 hours at 22 °C and then filtered through a 0.45- $\mu$ m membrane filter (Millipore Corporation, USA). The filtrate contained 14.7 g C  $L^{-1}$  and 1.44 g N  $L^{-1}$  (determined by Thermalox total organic C – total N analyser; Analytical Sciences Limited, Cambridge). An organic matter solution containing 150 mg C  $L^{-1}$  was prepared by diluting the original DOC with an OC-free solution containing 10 mg NaCl  $L^{-1}$ , 20 mg  $CaCl_2 \cdot 2H_2O$   $L^{-1}$  and 24 mg  $K_2SO_4$   $L^{-1}$  and adjusting the pH of the solution to 6.0 by adding HCl or NaOH.

### 2.3. Preparation of inoculant

A mixture of 29 Australian soils from a range of different environments that therefore contain a diverse range of microorganisms adapted to different conditions was used as an inoculant for the incubations. Before extraction, air-dried soils were rewetted to 70% water holding capacity and incubated for 7 days at 22 °C to reactivate microorganisms. The soils were shaken for 30 minutes with a 4 mM CaCl<sub>2</sub> solution (1:2 soil:solution ratio) and filtered through a 5-µm filter (Millipore Corporation, USA).

### 2.4. Incubation experiments

Two incubation experiments were carried out: *Experiment 1* to determine mineralisation of OC sorbed onto different clays with and without goethite coating, and *Experiment 2* to examine mineralisation of OC sorbed onto illite with three different hydrous iron oxide coatings (haematite, goethite ferrihydrite). Microbial degradation of OC was measured over 120 days, with carbon mineralisation monitored using a Servomex 1450 infrared gas analyser (Servomex, UK). Samples of clays or clay-oxide assemblages with sorbed OC were prepared by shaking 200 mL of organic matter solution of 150 mg DOC L<sup>-1</sup> and 0.20 g of clay or clay-oxide for 12 hours at 22 °C. The 12-hour shaking was sufficient to establish quasi-equilibrium sorption conditions; longer incubation times were avoided to minimise possible microbial degradation of DOC. Thereafter, the suspensions were centrifuged for 30 minutes at 2000g, and the supernatants were filtered through 0.45-µm polycarbonate membrane filters (Millipore Corporation, USA). The concentration of DOC in the filtrate was measured

using a Thermalox TOC-TN analyser (Analytical Sciences Limited, Cambridge). The amount of OC sorbed was calculated as the difference between OC in the initial and equilibrium solutions. The calculated amounts of OC sorbed were verified by analysing an aliquot of reacted mineral phases (LECO CNS2000, LECO Corporation, MI, USA).

Fractionation of DOC during reaction with minerals was monitored by measuring the ultraviolet (UV) absorbance at 280 nm of solutions before and after sorption on a Shimadzu 1601 UV/Vis spectrophotometer, using a 1-cm quartz cell. Results are expressed as specific UV absorption (SUVA,  $\text{L mg C}^{-1} \text{ m}^{-1}$ ), i.e., absorption at 280 nm normalised to DOC concentration, which is an indicator of dissolved aromatic compounds (Chorover and Amistadi, 2001; Kalbitz et al., 2003; Scheel et al., 2007).

After washing with deionised water, the filters with the OC-loaded minerals were transferred into 50-mL incubation flasks. To ensure sufficiently high  $\text{CO}_2$  concentrations for measurements, we pooled several subsamples so that the total OC content per flask was approximately 10 mg C (this equated to 0.5–1.0 g of solid). An organic matter solution without minerals was prepared by diluting the original DOC and was used as a control treatment. A second control treatment of deionised water and inoculum was used to measure carbon mineralisation from the added inoculum. Nutrient solution (20 mL) containing  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{K}_2\text{HPO}_4$  (setting the initial C/N and C/P ratio at ~10 and ~25, respectively) and other nutrients ( $\text{CaSO}_4$ ,  $\text{MgSO}_4$ ,  $\text{K}_2\text{SO}_4$  and micronutrients) was added to the flasks, along with 1 mL of inoculum suspension. Following inoculation, the flasks were sealed and incubated in the dark at 22 °C for 120

days. There were three replicates of each control and each treatment. To avoid anoxic conditions, the samples were shaken manually once a day. The initial measurement of released CO<sub>2</sub>-C was four days after initiation of the incubation and subsequent measurements were carried out on a weekly basis.

## 2.5. Carbon mineralisation model

Incubation data was fitted to a two-pool C mineralisation model consisting of rapidly and slowly decomposing pools (Eq. 1):

$$C_t = C_s(1-e^{-st}) + C_f(1-e^{-ft}) \quad (1)$$

where  $C_t$  is the cumulative amount of C mineralised (% of sorbed C) by time  $t$ ;  $C_s$  and  $C_f$  are the sizes of slow and fast pools of mineralisable C (% of sorbed C), respectively; and  $s$  and  $f$  are the corresponding mineralisation rate constants for the slow and fast pools (day<sup>-1</sup>). The measured CO<sub>2</sub>-C mineralisation data were fitted to the model using the least-square non-linear curve fitting routine in Microsoft Excel<sup>®</sup> (de Levie, 2001). The values of  $C_s$ ,  $C_f$ ,  $s$ , and  $f$  that gave the smallest residual sums of squares (RSS) were retained.

## 2.6. Statistical analysis

The analysis of variance (ANOVA) procedure of GenStat 11<sup>th</sup> Edition (Payne, 2008) was used to analyse the effect of hydrous iron oxides on the microbial degradation of mineral-OM associations. In the case of significance in ANOVAs, means were compared by the least significant difference (LSD) multiple comparison procedure at P



<0.05. Correlation analyses between variables of sorption and desorption obtained from our previous study (Saidy et al., 2012 submitted – Chapter 3) and carbon mineralisation of sorbed OC were performed using GenStat 11.

### **3. Results**

#### **3.1. Characteristics of clays and coated-clays**

The characteristics of the clays used in this experiment have been reported previously (Saidy et al., 2012) and are summarised in Table 1. Phyllosilicate clays used in this study were almost pure materials. Trace quantities of muscovite were detected in the kaolinitic clay, while the illitic and smectitic clays contained traces of quartz. Carbon and nitrogen contents of all three clays were low ( $\leq 3.2 \text{ g kg}^{-1}$  and  $\leq 0.2 \text{ g kg}^{-1}$ , respectively). For uncoated clays, the smectitic clay had the highest CEC, while the kaolinitic clay had the lowest CEC (Table 1). Calcium (Ca) was the dominant exchangeable cation for all three phyllosilicate clays. The specific surface area (SSA) of the three uncoated clays increased in the order kaolinite < illite < smectite (Table 1).

Table 1. Properties of the clays and coated clays

Properties	Kaolinite	Illite	Smectite	Kaolinite- goethite	Illite- goethite	Illite- haematite	Illite- ferrihydrite	Smectite- goethite
Clay mineralogy								
Dominant	Kaolinite	Illite	Smectite	-	-	-	-	-
Accessory	Muscovite	Quartz	Quartz	-	-	-	-	-
Carbon (g kg <sup>-1</sup> )	0.3	3.2	1.3	-	-	-	-	-
Nitrogen (g kg <sup>-1</sup> )	<0.05	0.2	0.2	-	-	-	-	-
CEC (cmol kg <sup>-1</sup> )	8.9	23.0	86.9	7.5	21.4	20.8	21.3	73.6
Na <sup>+</sup> (cmol kg <sup>-1</sup> )	<0.10	0.14	0.91	0.16	0.30	0.11	0.13	0.70
K <sup>+</sup> (cmol kg <sup>-1</sup> )	0.2	1.5	1.9	<0.05	1.4	1.3	1.3	1.9
Mg <sup>+</sup> (cmol kg <sup>-1</sup> )	<0.2	2.1	10.5	<0.2	4.4	3.8	3.1	9.4
Ca <sup>+</sup> (cmol kg <sup>-1</sup> )	1.3	12.8	54.3	0.8	12.2	8.5	5.5	45.2
SSA (m <sup>2</sup> g <sup>-1</sup> )	6.01	108.0	169.8	11.48	98.8	95.9	115.6	157.8
Fe <sub>d</sub> (mg kg <sup>-1</sup> )	129	947	883	52800	55600	61900	51000	55700

Coating phyllosilicate clays with hydrous oxides resulted in changes in SSA and CEC. The SSA of coated clays increased where the SSA of the clay was less than that of the hydrous oxides (kaolinite–goethite, illite–ferrihydrite) and decreased where the clay's SSA exceeded that of the oxide (illite–goethite, illite–haematite and smectite–goethite) (Table 1). The changes in SSA were in line with added amounts of oxides. Decreases in CEC were also observed to result from the coating, suggesting that the coating involved electrostatic interactions between the negatively charged clays and the positively charged oxides. Decreases were smallest for kaolinite, moderate for illite, and largest for smectite (Table 1). The drop in CEC suggests that there were some irreversible binding, probably due to steric effects, especially for the smectite, which has a large accessible surface area. The drop in CEC was accompanied by a decrease in exchangeable  $\text{Ca}^{2+}$ , indicating replacement of  $\text{Ca}^{2+}$  by the positively charged hydrous iron oxides, probably at the cost of some or most of the hydrous oxides' positive charge. The smallest decrease in exchangeable  $\text{Ca}^{2+}$  occurred on coating smectite with goethite. That could be due to the concentration of smectite's charge in interlayer spaces, which goethite cannot enter, resulting in less replacement of  $\text{Ca}^{2+}$  and, likely, a lesser compensation of goethite's charge.

### **3.2. Mineralisation of OC sorbed to different phyllosilicate clays with hydrous iron oxide coating**

Figure 1 shows the progress of mineralisation for the control DOC solution and all mineral–OC associations. For the control DOC solution, rapid mineralisation during the first 18 days was followed by a decrease in the rate of the mineralisation, with the rate

levelling off after 42 days. Approximately 51% of initial DOC was recovered as CO<sub>2</sub> by the end of the 120-day incubation period (Fig. 1).

Sorption to minerals reduced the rate and extent of mineralisation of OC and this varied among the different phyllosilicate clays and clay–oxide mixtures assemblages. For kaolinite, mineralisation of sorbed OC increased gradually during the first 35 days, and then levelled off for the remainder of the incubation period (Fig. 1a). The mineralisation of OC sorbed to illitic and smectitic clays with and without goethite coating was rapid during the first 18 days of incubation, and then levelled off after 42 days (Fig. 1b and 1c). Cumulative mineralisation of OC sorbed to uncoated clays over the 120-day incubation was smallest for kaolinite (13%), intermediate for illite (20%) and largest for smectite (24%). Coating clays with goethite decreased C mineralisation but the decrease was only significant ( $P < 0.01$ ) for the kaolinitic and smectitic clays (Fig. 1). The decrease in mineralisation of OC sorbed to goethite-coated illite was not significant ( $P > 0.05$ ).

The C mineralisation from DOC solution and OC sorbed to illitic clay coated with different hydrous iron oxides is shown in Fig. 2. The cumulative amount of C mineralised during the 120-day incubation ranged from 13 to 19% of sorbed OC, which was 32–38% less than for the DOC solution (control) (Fig. 2b). Ferrihydrite coating of illitic clay resulted in a statistically significant ( $P < 0.01$ ) decrease (5%) in cumulative C mineralisation (Fig. 2b). On the other hand, haematite and goethite coating of illitic clay did not significantly change C mineralisation ( $P > 0.05$ ).

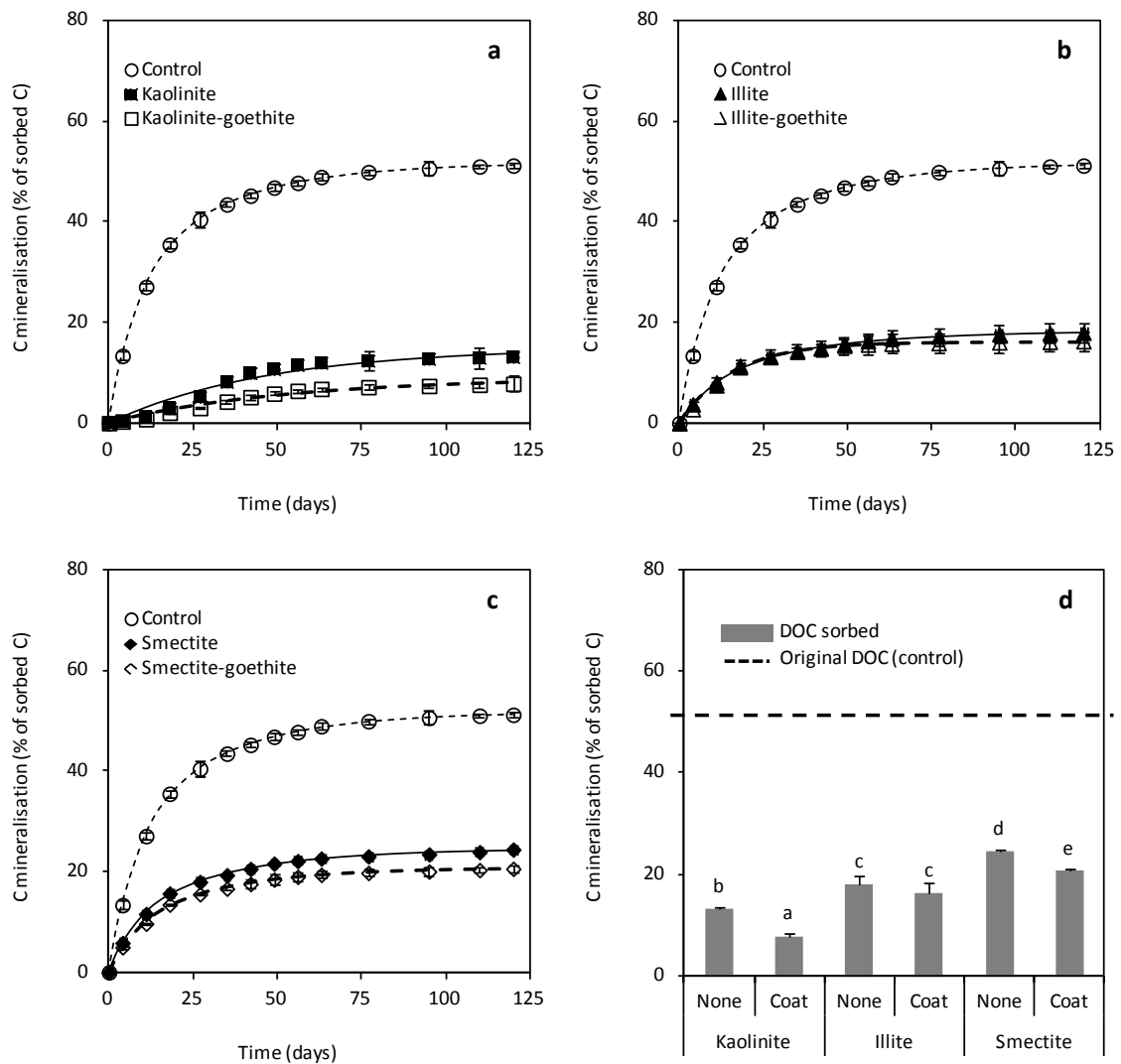


Fig. 1. Carbon mineralisation of DOC and DOC sorbed onto kaolinite (a), illite (b) and smectite (c) with and without goethite coating during the course of the 120-day incubation. The vertical bars represent standard error of the means ( $n=3$ ). The lines indicated are curves fitted to the two-pool decomposition model:  $C_t = C_a(1-e^{-kt}) + C_s(1-e^{-ht})$ ;  $R^2 > 0.99$  and  $P < 0.001$  for all curves. (d) Cumulative C mineralisation of DOC and DOC sorbed onto different clays with and without goethite coating at the end of the 120-day incubation. The vertical bars represent standard error of the means ( $n=3$ ). Similar letters above columns indicate no statistical difference between the treatments based on the LSD test at  $P < 0.05$ .

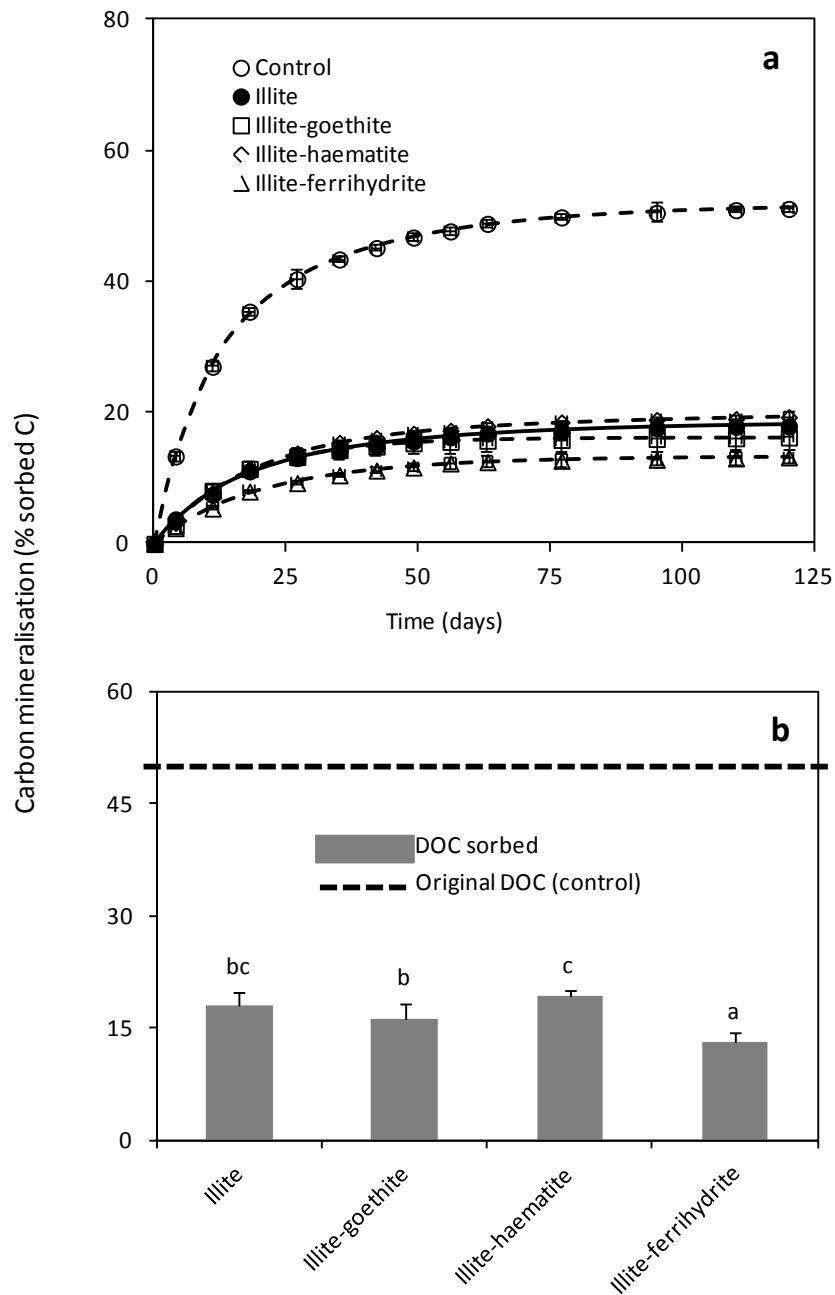


Fig. 2. (a) Carbon mineralisation of DOC sorbed onto illitic clay coated with different hydrous iron oxides. The vertical bars indicate standard error of the means (n=3). The lines indicated are curves fitted to the two-pool decomposition model:  $C_t = C_a(1-e^{-kt}) + C_s(1-e^{-ht})$ ;  $R^2 > 0.99$  and  $P < 0.001$  for all curves. (b) Cumulative C mineralisation after 120 days. The vertical bars represent standard error of the means (n=3). Similar letters above columns indicate no statistical difference between the treatments based on the LSD test at  $P < 0.05$ .

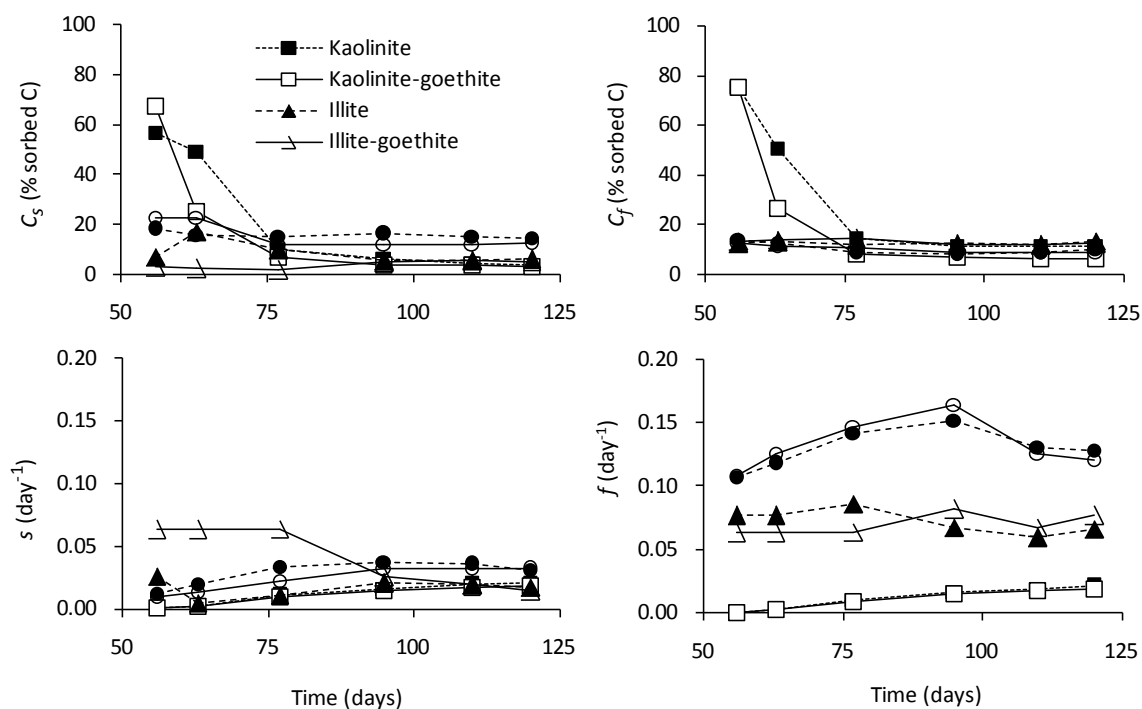


Fig. 3. Changes in the size of fitted slowly and rapidly decomposing pools of C and their mineralisation rates for three phyllosilicate clays with and without goethite coating.

### 3.3. Carbon mineralisation dynamics

The mineralisation data for OC sorbed onto different clays with and without goethite coating fitted well to the two-pool C mineralisation model (Eq. 1) (Fig. 1a, 1b, 1c). The magnitude of some of the parameters derived from the two-pool C mineralisation model ( $C_s$ ,  $C_f$ ,  $s$ , and  $f$ ) varied with the length of incubation (Fig. 3). In particular, the size of both slowly ( $C_s$ ) and rapidly ( $C_f$ ) decomposable pools varied considerably, whereas the mineralisation rate of both slowly ( $s$ ) and rapidly ( $f$ ) decomposable pools varied little. Both  $C_s$  and  $C_f$  values are much higher when only the data of shorter incubation periods is included in the fit (Fig. 3). It is only for the last two readings (for

data up to and including the 110- and 120-day incubation periods) that there is no significant difference in any of the variables of the two-pool C mineralisation model. This is the point at which we ended the incubation, as established in our previous study (Saidy et al., 2012). Only fits to the data obtained over 120 day-incubation period are considered in the discussion below.

Table 2. Results of the two-pool model fit to C mineralisation data. Similar letters in each column indicate no statistical difference between the treatments based on the LSD test at  $P < 0.05$ .

Treatment	$C_s^a$ (% sorbed C)	$s^b$ (day <sup>-1</sup> )	$C_f^c$ (% sorbed C)	$f^d$ (day <sup>-1</sup> )	R <sup>2</sup>
Control	24.20	0.033	27.50	0.120	0.99
Experiment 1.					
Kaolinite	3.80 a	0.021	11.29 cd	0.021	0.99
Kaolinite-goethite	2.93 a	0.019	6.14 a	0.019	0.99
Illite	5.98 b	0.014	13.54 e	0.067	0.99
Illite-goethite	5.21 b	0.015	12.37 de	0.077	0.99
Smectite	14.23 d	0.032	10.11 bc	0.129	0.99
Smectite-goethite	12.21 c	0.032	8.81 b	0.121	0.99
Experiment 2.					
Illite	5.98 b	0.014	13.54 b	0.067	0.99
Illite-goethite	5.21 ab	0.015	12.37 ab	0.077	0.99
Illite-haematite	5.30 ab	0.008	15.99 c	0.059	0.99
Illite-ferrihydrite	3.70 a	0.011	10.58 a	0.064	0.99

<sup>a</sup> The amount of slowly decomposing OC calculated using the two-pool model.

<sup>b</sup> Mineralisation rate constant of the slowly decomposing OC pool calculated using the two-pool model.

<sup>c</sup> The amount of rapidly decomposing OC calculated using the two-pool model.

<sup>d</sup> Mineralisation rate constant of the rapidly decomposing OC pool calculated using the two-pool model.



Results of fitting the full 120-day mineralisation data to the two-pool C mineralisation model are presented in Table 2. For untreated clays, the size of the slowly decomposing pool ( $C_s$ ) differed significantly with clay type, decreasing in the order smectite > illite > kaolinite; its mineralisation rate ( $s$ ) decreased in the order smectite > illite = kaolinite (Table 2). The size of the rapidly decomposing pool ( $C_f$ ) was significantly greater for illite than for kaolinite or smectite. The mineralisation rate of the rapidly decomposing C pool ( $f$ ) increased in the order kaolinite < illite < smectite (Table 2).

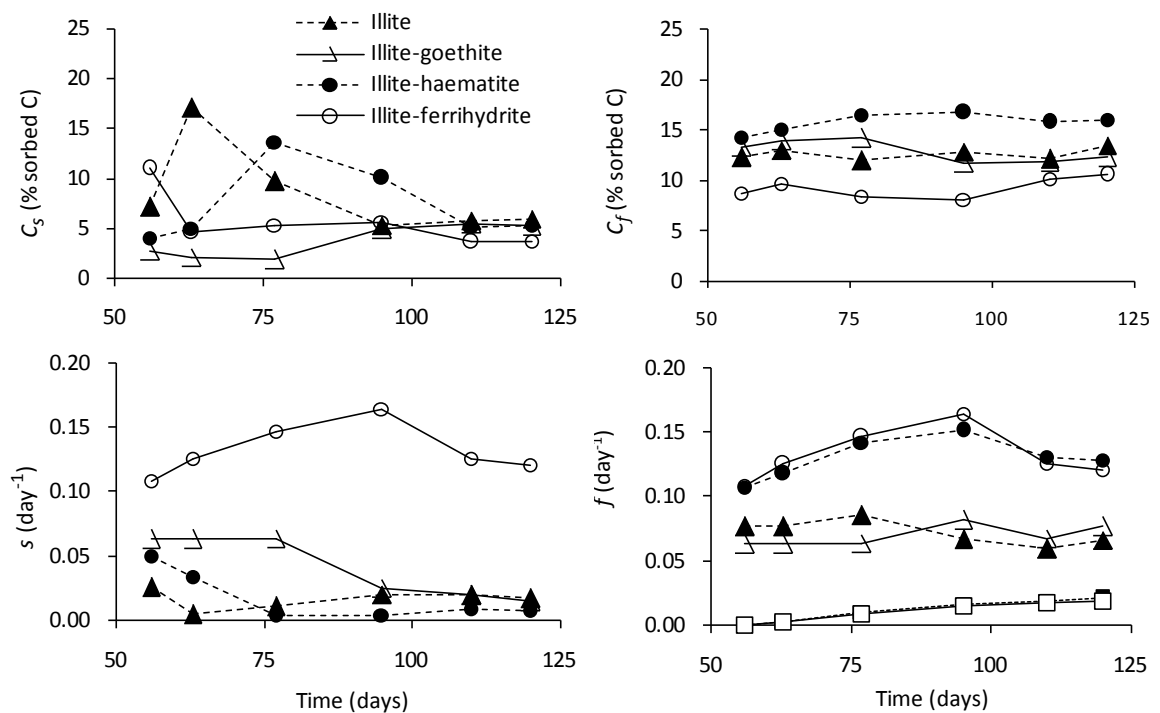


Fig. 4. Changes in the size of fitted slowly and rapidly decomposing pools of C and their mineralisation rates for illitic clay with and without different hydrous iron oxide coatings.

Goethite coating decreased both  $C_s$  and  $C_f$ , but the decrease was only significant for smectite in the case of  $C_s$  and for kaolinite in the case of  $C_f$  (Table 2). ANOVA indicated that the mineralisation rate of neither slowly ( $s$ ) nor rapidly ( $f$ ) decomposing pools was significantly affected by goethite coating ( $P > 0.05$ ) (Table 2).

The mineralisation data for OC sorbed onto illite coated with different hydrous iron oxides also fitted well to the two-pool C mineralisation model (Eq. 1) (Fig. 2a). Figure 4 shows how the parameters derived from the two-pool C mineralisation model ( $C_s$ ,  $C_f$ ,  $s$ , and  $f$ ) vary with the length of incubation. Again, these values stabilised with increasing incubation time and there were no significant differences between parameters for 110- and 120-day incubation periods.

There was no statistically significant effect on  $C_s$  upon coating illite with goethite or haematite but there was a significant increase ( $P < 0.01$ ) upon coating with haematite (Table 2). Coating illite with ferrihydrite resulted in a significant reduction ( $P < 0.01$ ) in the size of both slowly ( $C_s$ ) and rapidly ( $C_f$ ) decomposable pools (Table 2). The mineralisation rate of neither slowly ( $s$ ) nor rapidly ( $f$ ) decomposing pools was affected by different hydrous iron oxide coatings ( $P > 0.05$ ).

#### **3.4. Changes of UV absorbance of dissolved organic carbon during sorption**

Specific UV absorbance (SUVA) of DOC at 280 nm (i.e., absorbance normalised to OC concentration) has been used to estimate the aromaticity of OC (Chorover and Amistadi, 2001; Kalbitz et al., 2005). Specific UV absorbance of OC remaining in

solution after sorption onto smectite, kaolinite both with and without goethite coating, and illite both with and without goethite coating, was not significantly different from that before sorption (Fig. 5). However, there was a statistically significant 17% decrease in SUVA for OC solution after sorption to goethite-coated smectite. For illitic clay coated with different hydrous iron oxides, SUVA of OC remaining in solution was significantly reduced after reaction with illite-haematite and illite-ferrihydrate associations.

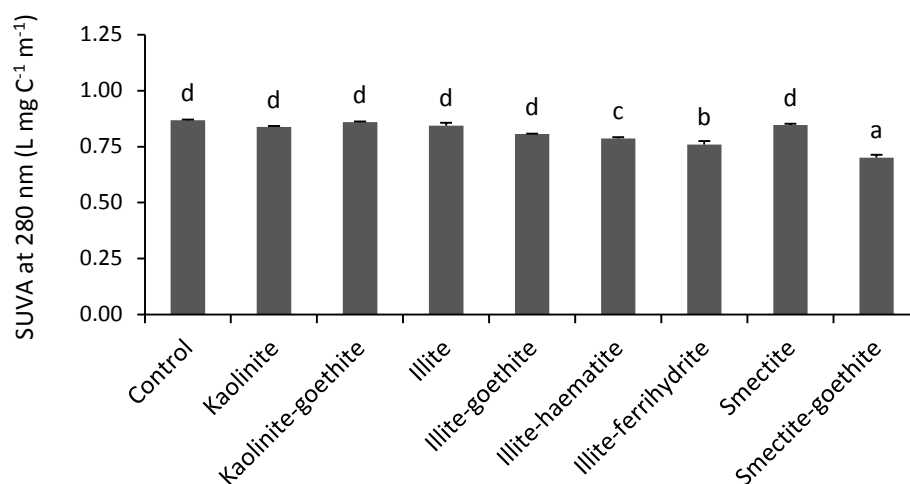


Fig. 5. Specific UV absorbance at 280 nm of DOC before and after sorption to different clays with and without oxide coatings. Solution after sorption was obtained by centrifugation and filtration. The vertical bars indicate standard error of mean (n=3) and similar letters above columns indicate no statistical difference between the treatments based on the LSD test at P<0.05.

## 4. Discussion

### 4.1. Mineralisation of sorbed OC is influenced by the nature of minerals

Mineralisation of OC sorbed onto clays and hydrous iron oxide coated clays over the 120-day incubation period was 27–43% lower than for the DOC solution (Fig. 1d). The amount of C mineralised from sorbed OC in this study is consistent with previous similar studies. For example, Schneider et al. (2010) reported sorption of OC onto amorphous Al hydroxide reduced mineralisation by 17–48% and Mikutta et al. (2007) reported that mineralisation of sorbed OC was 23–76 % lower than that of free DOC. Similar results were also obtained by Kalbitz et al. (2005), who found that mineralisation of OC after sorption onto minerals ranged from 13–27% while 28–91% of the DOC in free solution was mineralised in 365 days of incubation. Jones and Edwards (1998) demonstrated that mineralisation of simple compounds such glucose and citrate decreased significantly after the sorption of those compounds to minerals.

Mineralisation of sorbed OC decreased when kaolinitic and smectite clays were coated with goethite, but was not significantly different between illite and goethite-coated illite (Fig. 1). These results indicate that the effect of hydrous oxide coatings on the stability of sorbed OC against microbial degradation varies with the mineralogy of phyllosilicate clays. Different effects of goethite coating onto clays on the mineralisation of sorbed OC have been attributed to different binding mechanisms dominating for different clay–oxide associations. Mikutta et al. (2007) reported that OM bound to minerals via ligand exchange is more resistant against microbial

degradation than that held by non-columbic interactions (van der Waals forces) or via cation bridging. Goethite has a point of zero charge (PZC) of pH 8.1 (Kaiser and Guggenberger, 2003). In this study, in which the incubation was carried out at a pH of 6.0, goethite surfaces would have had a net positive charge. On the other hand, clay minerals have a negative charge at a pH of 6.0 (PZC of kaolinite at pH 3.7; PZC of illite at pH 2.5; PZC of smectite at pH < 2.3 – Zhuang and Yu, 2002; Chorover, 2005). Under these circumstances, the net charge depends on the relative magnitudes of the positively charged coatings on the clay surface and the negative layer charge of the clay (Roth et al., 1969; Zhuang and Yu, 2002). Since the CEC of kaolinite is low (Table 1), the negative charge of kaolinite would only partly balance the positive charge of goethite. The net positive charge on kaolinite–oxide surfaces allows for sorption of negatively-charge DOC via strong columbic mechanisms (e.g., ligand exchange). The net effect is an increase in binding strength and greater stability of sorbed OC against microbial decay for kaolinite-goethite associations than for to kaolinite.

Illite has a much higher CEC than kaolinite (Table 1), and goethite coating would have relatively little influence on charge balance, leaving the goethite-coated illite with an overall negative charge. The strong decrease in exchangeable  $\text{Ca}^{2+}$  (Table 1) upon coating with goethite clearly points to strong electrostatic interactions between the two minerals. A net negative charge on illite–oxide surfaces implies sorption of negatively charged OC primarily via either cation bridges (note the high  $\text{Ca}^{2+}$  saturation of the CEC) or non-columbic mechanisms (e.g., van der Waals forces). Consequently, sorption onto pure and coated illite should not differ much, and so it is not surprising

that there was no difference in the stability of sorbed OC between uncoated and goethite-coated illite.

Smectite has an even higher CEC than illite (Table 1), and therefore goethite-coated smectite should also have a net overall negative charge. Sorption of OC onto both smectite and goethite-coated smectite would be expected to be via non-columbic mechanisms or cation bridging, and thus, the stability of sorbed OC against biodegradation for smectite–organic and smectite–oxide–organic associations should theoretically be similar. However, goethite coating on smectite resulted in a decrease in mineralisation of sorbed DOC (Fig. 1d).

Besides the lower than expected rate of mineralisation of sorbed OC, goethite-coated smectite treatment was distinctive in that there was a significant decrease in the specific UV absorbance of DOC following sorption (Fig. 5). This change indicates a preferential sorption of aromatic-rich fractions to smectite–goethite associations. Aromatic-rich compounds are considered amongst the most resistant OC components to microbial decomposition (Kalbitz et al., 2003). This result is in agreement with Kalbitz et al. (2005), who reported that around half the measured decrease in mineralisation after sorption of DOC onto soils could be attributed to selective sorption of aromatic compounds, as indicated by a decrease in the specific UV absorbance of DOC after sorption. Soluble aromatic compounds tend to sorb more strongly to hydrous iron oxide than to phyllosilicates (e.g., Mikutta et al., 2007), suggesting goethite contributed to sorption and stabilisation of the sorbed OC.

Despite the larger overall CEC of smectite, the compensation of the positive charge of the goethite is far less complete than by the illite. The negative charge of smectites results mainly from isomorphic substitution in the octahedral planes and thus is rather diffuse and allows for hydrated cations to enter the interlayer. In contrast, the negative charge of illite comes most from strong isomorphic substitution in the tetrahedral planes, resulting in highly charged basal planes, which causes intercalation of  $K^+$  (or  $NH_4^+$ ), thus collapsing of the interlayer spaces. As a result, much of the charge of smectite is located in the internal surfaces while that of illite is largely confined to external surfaces. The external surfaces of illite are therefore more highly charged than those of smectites. Since goethite can only bind to external surfaces, complete balancing of its charge is more likely for illite than for smectite, leaving goethite at the surface of smectite more reactive than on the surface of illite. The drastic drop in exchangeable  $Ca^{2+}$  after coating illite with goethite and the rather moderate decrease upon coating smectite with goethite (Table 1) are consistent with this description.

In the second experiment, which compared the effect of different hydrous oxide coatings on illite, only coating with ferrihydrite increased the stability of sorbed OC against microbial degradation (Fig. 2). This is consistent with previous studies. Mineralisation of a low molecular weight organic acid (citrate) was observed to be lower upon sorption onto ferrihydrite compared to mineralisation of citrate alone (van Hees et al., 2003). Our previous experiment also showed that ferrihydrite addition to illitic clay reduced C mineralisation relative to illite alone, while addition of goethite and haematite did not change C mineralisation (Saidy et al., 2012). This stronger effect

of ferrihydrite than goethite or haematite implies that the different charge properties of the different hydrous iron oxides also play an important role in the sorptive stability of OC against microbial degradation. The PZC of ferrihydrite is higher than that of goethite or haematite (PZC of ferrihydrite at pH 8.6; PZC of goethite at pH 8.1; PZC of haematite at pH 7.9 – Kaiser and Guggenberger, 2003). Therefore, at pH 6.0, ferrihydrite would have higher positive charge than the other hydrous oxides, increasing the stability of sorbed OC against microbial decay through strong sorption of OC onto the illite–ferrihydrite system via a ligand exchange mechanism. Note that ferrihydrite coating caused the strongest reduction in exchangeable  $\text{Ca}^{2+}$  (Table 1), which underlines its larger charge. It is likely that the positive charge of ferrihydrite was large enough to not only replace most of the illite’s exchangeable  $\text{Ca}^{2+}$  but to leave the illite–ferrihydrite system with a net positive charge, resulting in strong OC binding and stabilisation.

The reduction in mineralisation of sorbed OC observed for kaolinite–goethite associations can be attributed to decreases in both slowly and rapidly decomposing pools compared to that without goethite coating (Table 2). For smectite, goethite coating resulted in a decrease in slowly decomposable C while rapidly decomposable C remained unchanged. It seems that goethite coating on kaolinite and smectite resulted in a larger proportion of OC being virtually undecomposable on the timeframe of the incubation. Changes in rapidly and slowly decomposing pools of mineralisable C were also observed for illitic clay coated with ferrihydrite (Table 2). This implies that ferrihydrite coating on illite caused protection of a larger proportion of sorbed OC.



Table 2 shows that the mineralisation rate constant of both slow and fast pools of mineralisable C derived from two-pool model did not change significantly when clays were coated with hydrous iron oxides. This result indicates that the stability of sorbed OC against microbial degradation occurred through changes in the size of both slowly and rapidly decomposing pools, thereby increasing the amount of non-decomposable C upon sorption. In our previous study (Saidy et al., 2012), we also found that where C mineralisation decreased for clays coated with aluminium and iron oxides, the size of slowly and rapidly decomposing pools changed significantly, while the mineralisation rate of these pools remained unchanged. It has been reported previously that the size of the stable C pool, derived from a two-pool C mineralisation model, increases considerably after the sorption of OC onto soils (Kalbitz et al., 2005) and phyllosilicates and goethite (Mikutta et al., 2007). That could result from either stronger binding of organic matter or selective sorption of already more resistant compounds, or both (e.g., Kaiser and Guggenberger, 2000).

#### **4.2. Carbon mineralisation determined in this study and in a previous incubation study on the same materials emphasise different aspects of stabilisation of OC by mineral surfaces**

In a previous study (Saidy et al., 2012), we also found that the presence of these clays and hydrous iron oxides resulted in stabilisation of a proportion of plant-derived OC against microbial degradation. The magnitude of this stabilisation varied among the clays and oxide-coated clays, but was positively correlated with increasing mineral surface area. This suggests that under the experimental conditions used in the

previous study, OC stabilisation may have been limited by the capacity of the mineral surfaces to sorb DOC. In this study, we re-examined the susceptibility the same OC to microbial degradation under different experimental conditions. We have used a 'wet incubation system' consisting of clays or oxide-coated clays suspended in 20 mL of nutrient solution whereas in the previous study (Saidy et al., 2012), we used a 'moist incubation system' consisting of 5% clay admixtures in a predominantly sand matrix at 70% water-filled pore space. In addition, the experimental conditions between these two studies differ in two further important aspects: (i) the ratio of DOC to clay was approximately an order of magnitude higher in the previous study (Saidy et al., 2012) than in this study; and (ii) in this study the OC was sorbed onto the mineral surfaces before incubation rather than added at the start of the incubation.

Comparison of C mineralisation between these two studies sheds light on how mineral surfaces stabilise OC against microbial degradation, because they emphasise different aspects of stabilisation. Under the experimental conditions used in Saidy et al (2012), OC is present in excess of the capacity of the minerals to stabilise it all. These conditions emphasise how much OC can be stabilised to any extent. In contrast, under the experimental conditions used in the present study, OC is present at a lower loading and in proportion to the capacities of the minerals to stabilise it. These conditions emphasise the degree of stabilisation provided to sorb OC. It should be noted that both of these aspect of stabilisation of OC have "real-world" importance: the former being important when DOC is present in excess of available mineral surfaces (e.g., at

the interface of organic and mineral topsoil horizons) and the latter when available reactive mineral surfaces are not limiting (e.g., in subsoil horizons).

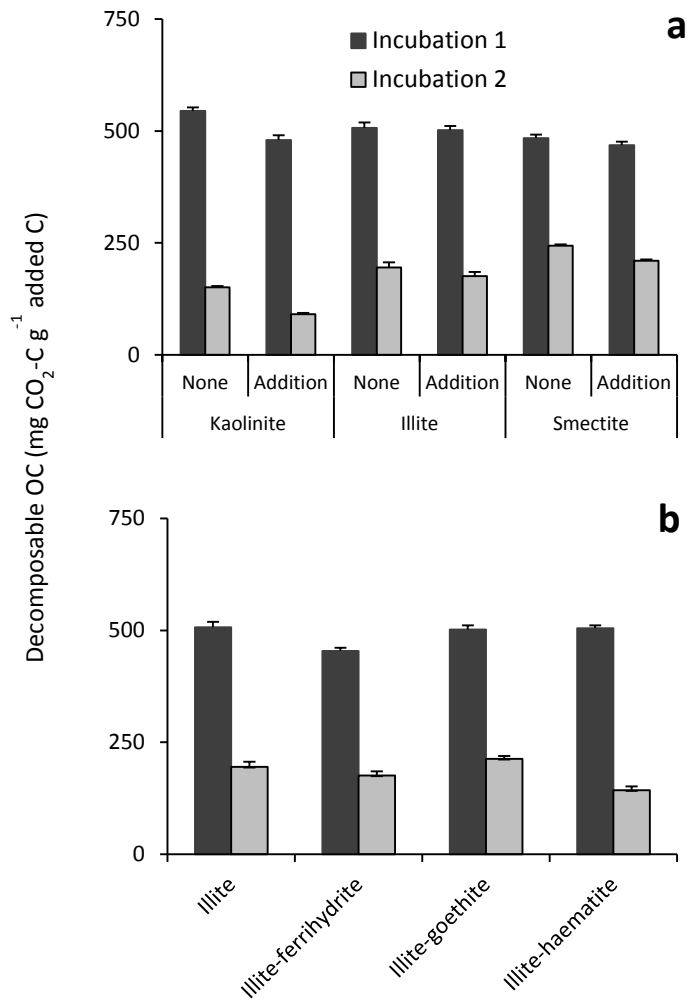


Fig. 6. Decomposable C pool (sum of slowly and rapidly mineralisable C pools) derived from the two-component C model of a previous study (Saidy et al., 2012 – incubation 1) and this study (incubation 2) for three different clays with and without goethite coating (a) and illitic clay coating with different hydrous oxide coating (b). The vertical bars indicate standard error of mean.

Mineralisation of OC was greater in the previous experiment (Saidy et al., 2012 - incubation 1) than this experiment (incubation 2) for all mineral assemblages (Fig. 6). In fact, the amount of mineralisable DOC in incubation 1 is not much less than for the incubation 2 “control sample” (517 mg/g), in which no mineral was present. This is consistent with the sorption capacities of the minerals in the previous experiment having been exceeded.

For clays without oxide coating, there were opposite trends between the two incubations in terms of total mineralisable DOC, i.e., it decreased in the order kaolinite > illite > smectite in incubation 1 and it increased in same order in incubation 2 (Fig. 6). This is again consistent with the DOC load in previous incubation being beyond the capacity of the mineral surfaces to sorb it; under these conditions, stabilisation is mainly controlled by how much DOC can “find” a mineral surface. Results of sorption experiments showed that the sorption capacity of smectite for DOC was ~14-fold higher than kaolinite and ~3-fold higher than illite (Saidy et al. submitted). On the other hand, in the present experiment where DOC was at levels proportional to the minerals’ sorption capacities, the stabilisation is mainly controlled by how strongly each surface holds the DOC it has sorbed. These results highlight that both the capacity and the affinity of mineral surfaces for DOC are important in OC stabilisation in soils. The effects of coating clays with hydrous iron oxide on total decomposable C in incubation 1 and incubation 2 were similar, with hydrous oxide coating generally decreasing total mineralisable C (Fig. 6).

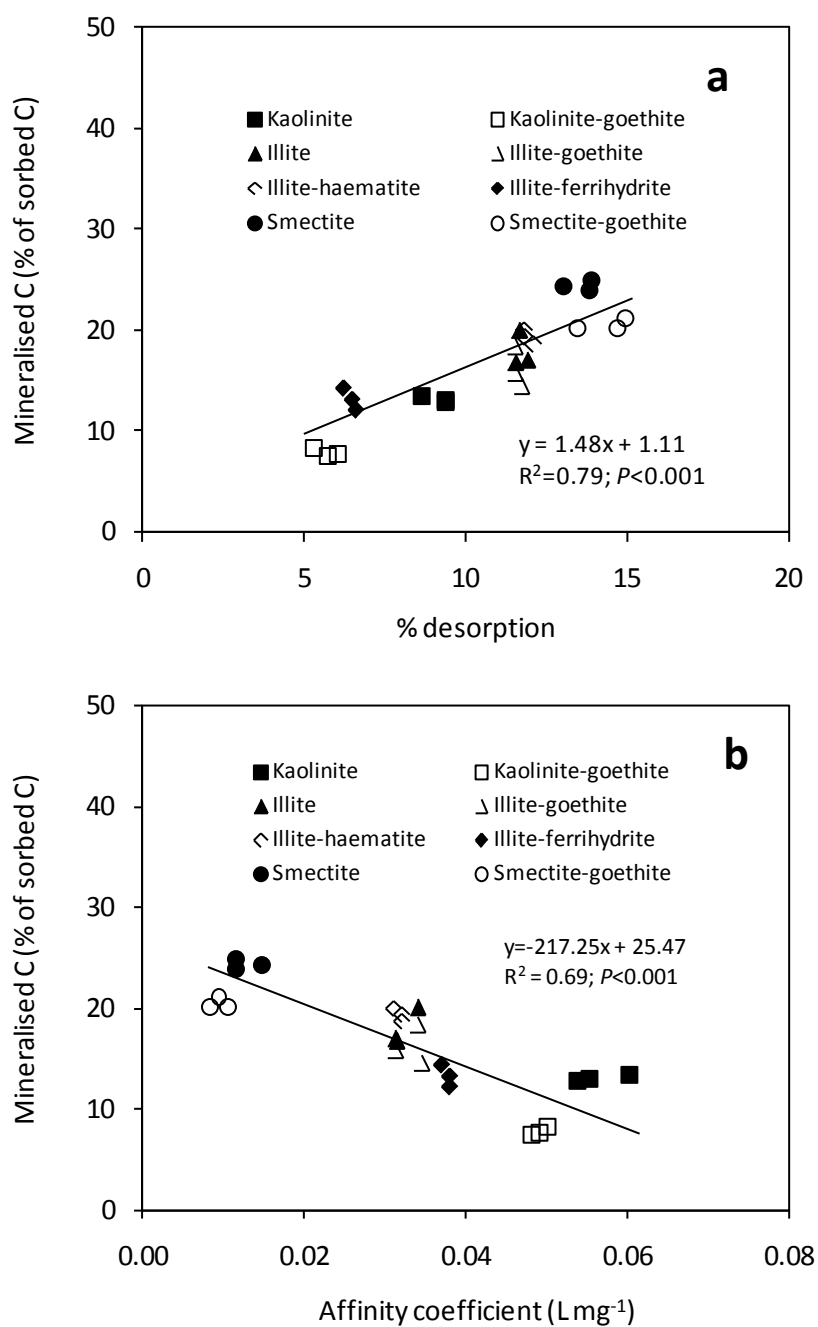


Fig. 7. Mineralisation of C sorbed to minerals after 120-days incubation versus (a) % desorption, and (b) affinity coefficient. Data for % desorption and affinity coefficients are taken from previous experiments (Saidy et al., 2012 submitted – Chapter 3).

#### **4.3. Desorption and affinity coefficients are good predictors of differences in the degree of stabilisation afforded by the different mineral surfaces**

Mineralisation rates of sorbed OC measured in this study can also be compared against sorption-desorption parameters determined in previous experiments, in which the OC was sorbed to the clay mineral–iron oxide associations (Saidy et al., 2012 submitted – Chapter 3). As shown in Fig. 7a, there is a significant positive correlation between the amount of mineralised sorbed OC and the proportion of OC that can be desorbed. This is consistent with the findings of Mikutta et al. (2007), who observed that mineralisation of sorbed OC was greatest for the mineral–organic associations where proportional desorption was largest. It has also been suggested that desorption is essential for the commencement of microbial degradation of organic matter sorbed to pure minerals and clays (Jones and Edwards, 1998; Keil et al., 1994; Nelson et al., 1994).

Differences in the stability of sorbed OC against microbial decomposition may also be related to differences in the affinity of DOC for the clay mineral–iron oxide associations. Our previous sorption experiment showed that kaolinite had a relatively large affinity for DOC (Saidy et al., 2012 submitted – Chapter 3), and indeed this coincides with the smallest C mineralisation of any clay–OC associations. In addition, smectite–OC associations, which show a low affinity for OC, coincide with the greatest mineralisation of sorbed OC. Figure 7b reveals that the amount of mineralised sorbed OC decreases linearly with increasing affinity for DOC. This result is consistent with Mikutta et al. (2007) who observed an inverse relationship between the sorption

affinity of OM and the mineralisation of sorbed OM for OM–mineral associations. The inverse relationship between the affinity for DOC and the mineralisation of sorbed OC supports the concept of Henrichs (1995) that the strength of mineral–organic associations as indicated by adsorption constants are useful for predicting the stability of sorbed DOC against microbial decay.

## **5. Conclusions**

Coating clays with goethite reduced mineralisation of OC sorbed onto kaolinite and smectite, but the mineralisation did not change when illite was coated with goethite. Among three hydrous iron oxides tested, only ferrihydrite coating of illite increased the stability of sorbed OC against microbial degradation. In other words, a reduction in the mineralisation of sorbed OC was most evident where there was either a low-surface–low-charged clay or a high-surface–high-charge oxide (kaolinite–goethite and illite–ferrihydrite) involved in the clay–oxide assemblage. These results suggest that the biological stability of OC sorbed to clay–oxide associations depend on the net charge of clay-oxides assemblages, which is influenced by the balance between the negative charge of phyllosilicate clays and the positive charge of hydrous iron oxides. Note, the results on smectite, though apparently inconsistent with this explanation at the first glance, do conform when the localisation of charge is also considered. This implies that types of clays and of hydrous iron oxides interact differently, with resultant consequences on the sorptive stability of OC against microbial degradation.

In this study, in which DOC was present in quantities proportional to the sorption capacity of minerals, OC stabilisation was controlled by the strength of OC–mineral interactions. This contrasts with results from our previous study, in which DOC was present in excess of the sorption capacity of minerals and, therefore, OC stabilisation was governed by the available surface area of the minerals. Together, the two studies suggest that the strength of mineral–organic associations and the capacity of minerals for DOC sorption are both key aspects that determine the degree of OC stabilisation by clay-oxide associations.

Another key finding of this study is that mineral–organic associations increased the biological stability of sorbed OC by reducing the size of slowly and rapidly decomposable C pools, while mineralisation rate constants for these pools were relatively unaffected. This indicates that the stability of sorbed OC against microbial degradation occurs primarily through a reduction in the size of decomposable C pool, at least on the timescale measurable in incubations such as these experiments (i.e. several months). Possible reasons are either stronger mineral–organic interactions and/or selective sorption of more stable compounds.

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## **Chapter 5. Mineralisation of Organic Carbon Sorbed to Phyllosilicate Clays is not Influenced by the Addition of Glucose**

A. R. Saidy<sup>a,c</sup>, R. J. Smernik<sup>a</sup>, J. A. Baldock<sup>b</sup>

<sup>a</sup> School of Agriculture, Food & Wine and Waite Research Institute, Waite Campus, The University of Adelaide, Urrbrae SA 5064, Australia

<sup>b</sup> CSIRO Land and Water, Private Bag 2, Glen Osmond SA 5064, Australia

<sup>c</sup> Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru 70714, Indonesia

**The work contained in this chapter has been prepared for publication.**

**STATEMENT OF AUTHORSHIP**

**Mineralisation of organic carbon sorbed to phyllosilicate clays is not influenced by  
the addition of glucose (manuscript)**

**SAIDY, AKHMAD R. (Candidate)**

Performed analysis on soil samples, data analysis and interpretation, wrote  
manuscript.

I hereby certify that the statement of contribution is accurate.

Signed

Date: 15/05/2013

**SMERNIK, RON J.**

Contributed to planning of experiment, supervised development of work and data  
interpretation, provided critical evaluation and revision of manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for  
the inclusion of the paper in the thesis.

Signed

Date: 15/05/2013

**BALDOCK, JEFF A.**

Contributed to planning of experiment, supervised development of work and data  
interpretation, manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for  
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Date: 15/05/2013

## **Mineralisation of organic carbon sorbed to phyllosilicate clays is not influenced by the addition of glucose**

### **ABSTRACT**

It is well established that the addition of easily decomposable carbon sources can influence the mineralisation rate of native soil organic carbon (SOC), a phenomenon known as the priming effect. However, priming does not always occur and the specific case of addition of highly mineralisable C to organic carbon (OC) bound to clay minerals has not been addressed. An incubation experiment was carried out to examine the effect of glucose addition on the mineralisation of OC sorbed to clay minerals. Plant-derived soluble OC was pre-sorbed to kaolinite, illite and smectite at pH 6.0, and then 2 levels of glucose, representing C additions of 1% and 10% of sorbed OC present, were added. The stability of the sorbed OC against biodegradation was quantified by measuring mineralisation over a 120-day incubation period. Glucose addition increased significantly the total amount of C mineralised from clay–OC associations for all phyllosilicate clays. However, the increase in mineralised C equated almost exactly to the amount of glucose C added in every case, suggesting the absence of a net priming effect of easily decomposable C on the mineralisation of OC sorbed onto phyllosilicate clays. Results of this study demonstrate that mechanisms other than energy availability control the stability of sorbed OC to phyllosilicate clays against microbial decomposition.

Keywords: easily decomposable carbon, microbial stability, clay minerals, priming effect

## 1. Introduction

Natural organic matter (OM) and clay minerals are important components of soils and sediments. They can interact with each other to form organo–mineral associations, which influence important processes in soils because they alter the physicochemical properties of minerals (Angove et al., 2002; Wang and Xing, 2005) and affect the availability and mobility of nutrients and contaminants in soils and waters (Xing, 2001). Interactions between OM and soil minerals have also been recognised as one principal mechanism leading to the stabilisation of organic matter against microbial degradation in soils (Schneider et al., 2010; von Lützow et al., 2006).

In a previous study (Saidy et al., 2012 manuscript – Chapter 4), we reported that OC sorbed to minerals was 27-43% less mineralisable than “free” DOC in solution. In general terms, this can be explained by chemical bonding of OC to mineral surfaces that decreases the availability of the organic substrates to microorganisms (Guggenberger and Kaiser, 2003). Kalbitz et al. (2005) proposed that the formation of such strong chemical bonds, along with the selective sorption of intrinsically stable compounds to minerals, are the main factors that retard mineralisation of OC bound to minerals. It has been suggested that desorption of OC from mineral surface–OC associations controls the biodegradation of sorbed OC (Keil et al., 1994; Mikutta et al., 2007).

The input of easily available organic substances to soils can significantly change the degradability of otherwise resistant native soil organic matter. Fontaine et al. (2004)

demonstrated just such a “priming effect” in which the addition of fresh organic matter accelerated the decomposition of native SOC, thus resulting in decreases in SOC contents. In another study, Fontaine et al. (2007) showed that the stability of organic matter in deep soil layers (2500 year-old carbon), much of which is likely to be sorbed to minerals, is controlled by the supply of fresh plant-derived carbon. In addition, the mineralisation rate of very recalcitrant organic matter, such as found in biochar, has been reported to increase substantially on the addition of an easily decomposable C source (Hamer et al., 2004; Kuzyakov et al., 2009). Several mechanisms have been suggested for the enhancement of native SOC mineralisation, including cometabolism, competition or mutualism between various microbial communities (Blagodatskaya and Kuzyakov, 2008; Guenet et al., 2010). However, it remains unclear whether the presence of easily decomposable C influence the mineralisation of OC sorbed to phyllosilicate clays.

In this study, we examined the effect of glucose addition on the resistance of sorbed OC to microbial degradation. Our approach was to sorb plant-derived soluble OC to different phyllosilicate clays (kaolinite, illite and smectite), then add glucose at two levels (1% and 10% of C in clay-associated OC). The stability of sorbed OC against biodegradation was quantified by measuring mineralisation over a 120-day incubation period.



## 2. Materials and Methods

### 2.1. Clay minerals and preparation of clay-organic carbon associations

Three clays with different mineralogy (kaolinite, illite and smectite) were obtained from the collection of clay minerals at CSIRO Land and Water, Adelaide, Australia. Dissolved organic carbon (DOC) and OC bound to clays for this study were prepared as described by Saidy et al. (2012- manuscript Chapter 4). Briefly, 0.20 g of each clay was reacted with water-extractable OC (from dried-ground medic (*Medicago truncatula* cv. Praggio) shoot residue (< 2 mm)) at pH 6.0 and an initial DOC concentration of 150 mg C L<sup>-1</sup> with of 10 mg NaCl L<sup>-1</sup>, 20 mg CaCl<sub>2</sub>·2H<sub>2</sub>O L<sup>-1</sup> and 24 mg K<sub>2</sub>SO<sub>4</sub> L<sup>-1</sup> as background electrolytes. After shaking for 12 hours at 22 °C, the suspensions were centrifuged for 30 minutes at 2000g, and the supernatants were filtered through 0.45-µm polycarbonate membrane filters (Millipore Corporation, USA). The concentration of DOC in the filtrate was measured using Thermalox TOC-TN analyser (Analytical Sciences Limited, Cambridge). The amount of organic C sorbed was calculated as the difference between organic C in the initial and equilibrium solutions.

### 2.2. Incubation experiment

Following OM sorption, the stability of sorbed OC against biodegradation in the presence and absence of glucose was determined during an incubation over 120 days at 22 °C, using conditions identical to those used in Saidy et al. (3<sup>rd</sup> manuscript). In brief, fresh clay-organic associations were transferred into 50-mL flasks and 20 mL of a nutrient solution containing (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub> (setting the initial C/N and C/P ratio

at ~10 and ~25) and other nutrients ( $\text{CaSO}_4$ ,  $\text{MgSO}_4$ ,  $\text{K}_2\text{SO}_4$  and micronutrients) was added to the flasks. In order to ensure sufficiently high  $\text{CO}_2$  concentrations for measurements, several subsamples were combined so that the OC content per flask was approximately 10 mg C (0.5–1.0 g of solid). Treatments for each different clay-OC associations included a control (no glucose added) and two levels of glucose addition in which glucose C equated to 1% and 10% of OC sorbed onto the clay minerals. To each treatment, 1 mL of an inoculum (from a mixture of 29 Australian soils pre-equilibrated at 70% water holding capacity for 7 days at 22 °C) was added. An organic carbon solution containing of 10 mg C without minerals was prepared by diluting the original DOC and was included in the incubation, as was a treatment containing only nutrient solutions and inoculums, which was used to measure carbon mineralisation from the added inoculum. Finally, treatments containing glucose at the same concentrations used above, nutrient solution and inoculum were prepared to determine carbon mineralisation from glucose in the absence of clay minerals. There were three replicates of each treatment. To avoid anoxic conditions, the samples were shaken manually once a day. Released  $\text{CO}_2$ -C was measured by determining the headspace  $\text{CO}_2$  concentrations within each flask using a Servomex 1450 infra-red gas analyser (Servomex, UK).

Table 1. Total initial carbon (C), mineralised C (in g and percent of initial total C) and corrected C mineralised after 120 day-incubation. Means  $\pm$  SE of three replicates.

Treatment	Total initial C (mg C)	Mineralised C(mg C)	Corrected mineralised C (mg C) <sup>a</sup>	Percent of initial C mineralised (%)
Control	10.0	5.12 (0.03)	-	51.24 (0.26)
Glucose 1%	0.1	0.10 (0.01)	-	100.00 (3.08)
Glucose 10%	1.0	0.99 (0.01)	-	99.00 (1.42)
Kaolinite	10.0	1.31 (0.02) a <sup>b</sup>	1.31 (0.02)	13.11 (0.18) a
Kaolinite-glucose 1%	10.1	1.43 (0.04) a	1.32 (0.35)	14.12 (0.37) a
Kaolinite-glucose 10%	11.0	2.37 (0.02)d	1.32 (0.19)	21.52 (0.17) d
Illite	10.0	1.80(0.10) b	1.80 (0.10)	17.96 (1.05) b
Illite-glucose 1%	10.1	2.01 (0.06) c	1.91 (0.57)	19.91 (0.58) c
Illite-glucose 10%	11.0	2.81 (0.04) e	1.76 (0.25)	25.54 (0.36) e
Smectite	10.0	2.06 (0.03) c	2.06 (0.03)	20.56 (0.32) cd
Smectite-glucose 1%	10.1	2.15 (0.05) c	2.04 (0.15)	21.32 (0.45) cd
Smectite-glucose 10%	11.0	3.20 (0.05) f	2.15 (0.22)	29.10 (0.20) f

<sup>a</sup> Calculated as cumulative C mineralised from clay+glucose minus cumulative C mineralised from corresponding glucose only treatment

<sup>b</sup> Similar letters in each column indicate no statistical difference between the treatments based on the LSD test at P<0.05.

### *2.3. Statistical analysis*

Cumulative C mineralisation data were statistically determined by analysis of variance (ANOVA) (GenStat 12<sup>th</sup> Edition; (Payne, 2008)). The data were checked for normal distribution with the Shapiro–Wilk test. In the case of significance in ANOVAs, means were compared by the least significant difference (LSD) multiple comparison procedure at  $P < 0.05$ .

### **3. Results**

Cumulative C mineralisation at the end of the 120-day incubation was lower for DOC sorbed to each of the clay minerals (kaolinite, illite and smectite) than for DOC alone (Table 1). In the absence of added clay, 51% of DOC was mineralised to CO<sub>2</sub> during the 120-day incubation. On the same timeframe, 100% of glucose C was converted to CO<sub>2</sub> at both of the loadings tested. As reported previously (Saidy et al., 2012 manuscript Chapter 4) the extent of mineralisation of untreated clays increased in the order kaolinite (13% of DOC mineralised to CO<sub>2</sub>) < illite (18%) < smectite (21%).

Total CO<sub>2</sub> produced increased on addition of glucose, but in the case of 1% glucose addition, the increase was only significant for the illitic clay (Table 1). Total CO<sub>2</sub> produced was significantly higher for the 10% glucose treatments than for the 1% glucose and unamended treatments. Net mineralisation of DOC in the glucose-amended treatments was determined by subtracting the amount of CO<sub>2</sub> produced in the corresponding glucose-only treatment (i.e. 0.1 mg C or 1 mg C glucose C

treatments), and was found to be not significantly different from C mineralised in corresponding clays without glucose addition (Table 1).

For most of the treatments, the rate of mineralisation was greatest 4 days into the incubation and decreased thereafter (Fig. 1). The exceptions were the kaolinite and kaolinite + 1% glucose treatments, for which the rate of mineralisation increased until day 35 of the incubation and decreased thereafter. Although the pattern of mineralisation rates was similar among the treatments, the mineralisation rates of clay–OC was significantly higher in the presence of glucose for all phyllosilicate clays during the first 75 days of incubation. After 75 days of incubation, the mineralisation rates of clays with glucose addition in most cases were not significantly different from those without glucose addition (Fig. 1).

#### **4. Discussion**

Glucose is the monomer of abundant plant-originated organic polymers (e.g. cellulose, starch); therefore, all soil microorganisms are capable of metabolising glucose (Anderson and Domsch, 1978; Landi et al., 2006). Approximately 90% of glucose in the absence of clay minerals in our experiment was mineralised after 75 days (data not shown), and all the added glucose was completely utilised by microorganisms at the end of incubation (Table 1). This is consistent with glucose mineralisation rates reported in the literature. Hamer et al. (2004), for example, found that 60 – 82% of glucose added to different black carbons was mineralised after 60 days of incubation. Saggari et al. (1999) observed that the amount of C mineralised accounted for between

51 and 66% of glucose added to soils differing in C and clay contents. In addition, Martin and Haider (1986) observed a glucose decomposition of 82% after four weeks. Differences are likely due to different experimental designs, e.g. nutrient status, temperature, water content and incubation conditions (“wet” vs. “moist”).

The presence of fresh organic matter increases the activity of microorganisms and is generally accompanied by the activation of various previously dormant microorganisms, leading to the acceleration of microbially-mediated processes, potentially including the degradation of native organic matter (Blagodatskaya and Kuzyakov, 2008). Indeed, increased degradation of native organic matter in the presence of fresh organic matter has been reported in several studies (Blagodatskaya et al., 2007; Fontaine et al., 2007; Hamer and Marschner, 2005; Kuzyakov et al., 2009). In contrast to these studies, we found that mineralisation of OC sorbed to clays appeared to be unaffected by the addition of glucose at loadings equivalent to 1% and 10% of sorbed OC (Table 1). This suggests the absence of a priming effect of easily decomposable C on the biological degradation of OC sorbed onto phyllosilicate clays. The absence of a priming effect in the presence of easily decomposable substrate has also been reported in some incubation studies on soils (Hartley et al., 2009; Hoyle et al., 2008; Wu et al., 1993) and biochar (Zavalloni et al., 2011). In addition, Hamer and Marschner (2002) found that the addition of two different levels of glucose (80 and 400 mg C g<sup>-1</sup>) had no effect on the decomposition of a system that consisted of sand mixed with lignin.

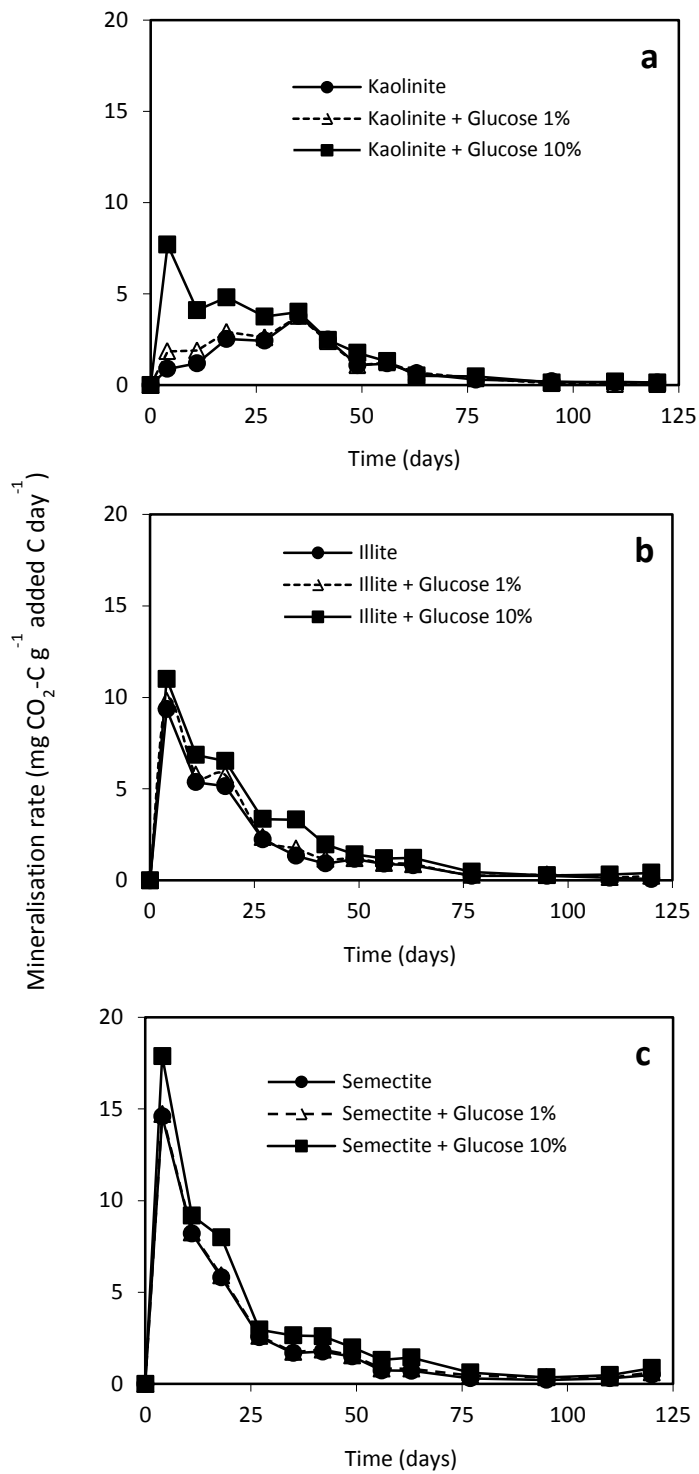


Fig. 1. Mineralisation rate of OC sorbed onto kaolinite (a), illite (b) and smectite (c) with and without glucose addition during the course of a 120-day incubation. Bars show a standard errors of the means (n=3). Standard errors are not shown when less than the symbol size.

There is some discussion in the literature regarding the conditions under which priming does or doesn't occur. In some studies where priming does occur, SOC mineralisation rates increased only while the easily decomposable substrate was present and then decreased after the added substrate was exhausted. The stability of SOC decomposition rates in other studies even after the exhaustion of added substrate indicates that microorganisms activated by the presence of added substrates were able to survive on SOC after the exhaustion of added substrates (Fontaine et al., 2007). In our study, mineralisation rates of OC sorbed to clay minerals in treatments with glucose was higher than those without glucose addition during the first 75 days of incubation (Fig. 1). As the added glucose started to exhaust after 75 days of incubation (see above), in most cases there was no significant difference in the mineralisation rates between OC sorbed to clays with and without glucose treatments (Fig 1). This suggests the microorganisms activated by the presence of glucose addition in this system are not able to use OC sorbed to clays as energy source after the exhaustion of added glucose. This is in agreement with the view that the presence of glucose in soils, especially at high levels, favours the development of fast growing microorganisms, which are not capable of utilising more resistant C pools as an energy source (Bremer and Vankessel, 1990; Wu et al., 1993).

The effect of easily decomposable C addition on the mineralisation of OC can also be dependent on the nutrient status of the system (Blagodatskaya et al., 2007; Fontain et al., 2007). In the case of an input of C-rich substrates without N, microorganisms can adapt by decomposing native OM to acquire N, with the result being an increase in the



decomposition of native OC (Blagodatskaya and Kuzyakov, 2008). The nutrient solution in this experiment was set to have an initial C-to-N ratio of 10, which is not considered N-limited for microbial decomposition. This may have contributed to the absence of a priming effect. However, we suggest the absence of an effect of glucose addition on the mineralisation of sorbed OC is most likely related to the strength of mineral-OC associations, which render OC bound to mineral surfaces unavailable as a source of C and energy. It has been suggested previously that the amount of C mineralised from mineral-organic associations is related to that which is reversibly bound to mineral surfaces (Jones and Edwards, 1998; Keil et al., 1994; Nelson et al., 1994). In support of this argument, Mikutta et al. (2007) found a significant correlation between the amount of OC desorbed from mineral surfaces and the mineralisation of mineral-associated OC.

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## **Chapter 6. Summary and Recommended Future Research**

## 6.1. Summary and Conclusions

The global soil carbon (C) pool, which is 3.3 times larger than the atmospheric carbon pool, plays an important role in counterbalancing rising atmospheric concentrations of CO<sub>2</sub> by acting as carbon store (Lal, 2004). Increases in the soil carbon pool through long-term agricultural practices also improve physical, chemical and biological soil properties (Robertson and Swinton, 2005). The size of the soil carbon pool is a function of the amount and quality of the C input and its subsequent rate of mineralisation, which can be reduced by stabilisation processes that protect soil organic carbon (SOC) against further decomposition. Association between soil minerals and SOC may lead to soil carbon stabilisation by entrapment in soil micropores (Baldock and Skjemstad, 2000; Bossuyt et al., 2002; Verchot et al., 2011; von Lützow et al., 2006) and by intermolecular interactions (sorption) between SOC and the surface of soil minerals (Basile-Doelsch et al., 2007; Kahle et al., 2004; Oades et al., 1989; Schneider et al., 2010).

Organic C may be stabilised and stored in soils through sorption of OC onto the surface of phyllosilicate clays (Jones and Edwards, 1998; Mikutta et al., 2007). Different clay minerals have different specific surface areas; therefore, it may be expected that the suite of clay minerals present will influence the capacity of soils to protect and store organic carbon. It was highlighted in the literature review that hydrous iron (Fe) and aluminium (Al) oxides are also capable of providing surfaces for soil organic carbon sorption. Therefore, it can be expected that the presence of hydrous oxides influences OC stabilisation in soils. Clay minerals and oxides coexist in the soils, and they can

interact with each other to form clay-oxide associations. Despite the fact that phyllosilicate clays and hydrous iron and aluminium oxides have been shown to stabilise SOC and to interact, little information is available on the effect of different phyllosilicate clays on SOC stabilisation in the presence of hydrous oxides. This issue was addressed in this study.

In the first part of this study, the effects of clay mineralogy and hydrous iron oxides on SOC stabilisation was investigated by incubation of added plant-derived OC to three different clays (kaolinite, illite and smectite) in the presence and absence of goethite for 144 days in a sand-dominated matrix. SOC stabilisation by illitic clay in the presence of four oxides (haematite, goethite, ferrihydrite, imogolite) was also studied in another experiment. Each clay or oxide-coated clay treatment received the same amount of OC ( $5 \text{ mg C g}^{-1}$  sand-clay mixture); this equated to a relatively high OC loading, such as may be experienced in top soils in highly productive systems (i.e. with lots of active vegetation). For clays without goethite coating, SOC stabilisation, as quantified by measuring C mineralisation of added OC, increased in the order kaolinite < illite < smectite. Goethite coating increased SOC stabilisation for kaolinite but did not change SOC stabilisation for illite or smectite; this is consistent with previous studies (Barthes et al., 2008; Bruun et al., 2010; Kleber et al., 2005; Wiseman and Püttmann, 2006). Among oxides tested in this study, only ferrihydrite coating on illitic clay resulted in an increase in SOC stabilisation.

The progress of mineralisation throughout the incubations was closely monitored. Fitting cumulative C mineralisation data to a two-component C mineralisation model revealed that in cases where the SOC stabilisation was increased by the presence of hydrous oxides, there was a reduction in the size of both slowly and rapidly mineralisable C pools. There were no significant differences in the mineralisation rates of each pool for clays with and without oxide coatings. Another important experimental issue for incubations such as this is the length of incubation used for determining C mineralisation dynamics. It was observed that variables derived from a two-component model varied significantly with the length of incubation period, and this could lead to serious errors in the interpretation of C mineralisation data from experiments that use inadequate incubation periods. For example, it was found that if only the first 60 days of incubation data was used, the size of the slowly decomposing pool ( $C_s$ ) was overestimated while its rate was underestimated. Therefore, in experiments where C mineralisation dynamics are measured over a relatively short incubation period, the derived parameters may not be comparable to those derived from incubations with either shorter or longer periods. Work completed in the first experiment in this thesis stresses the need to define the influence of incubation period on dynamic C mineralisation to ensure that acquired data are interpreted correctly.

Results of the first set of experiments (detailed in Chapter 2) suggested that the interaction of clay minerals and hydrous oxides results in changes in the specific surface areas of clays, which in turn influence the capability of clay minerals to adsorb organic carbon. However, the effect of the presence of hydrous iron oxides on the



capacity of clays to adsorb OC has not been fully established. In the next set of experiments, described in detail in Chapter 3, sorption of plant-derived organic matter onto different clays in the presence and absence of hydrous iron oxides was carried out to test whether the capacity of clay minerals to adsorb OC changes with oxide coating.

The capability of hydrous oxides to stabilise SOC through sorption is reportedly higher than that of clay minerals (Chorover and Amistadi, 2001; Kaiser and Guggenberger, 2003; Meier et al., 1999; Mikutta et al., 2007; Tombácz et al., 2004). However, hydrous oxides may interact with phyllosilicate clays to form clay-oxide associations (Fusi et al., 1989; Ohtsubo, 1989; Tombácz et al., 2004; Zhuang and Yu, 2002), which may significantly influence the sorption properties of both and consequently affect the capacity of soil for OC sorption. The effect of goethite coating onto three phyllosilicate clays and the effect of coating three hydrous iron oxides (haematite, goethite and ferrihydrite) onto illitic clays on the sorption of plant-derived OC were assessed in a set of batch sorption experiments. The sorption capacity (based on a fit to the Langmuir equation) of phyllosilicate clays was higher for smectite than illite and kaolinite when expressed on a mass basis. This reflects their specific of surface areas (SSA) and concentrations of exchangeable  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , which increase in the order kaolinite < illite < smectite. Goethite coating on phyllosilicate clays increased DOC sorption onto kaolinite but did not change the capacity of illite and smectite for DOC sorption. That the effect of goethite coating on DOC sorption was significant only for kaolinitic clay is consistent with the results of the previous experiment (Chapter 2) where it was found

that the presence of goethite increased SOC stabilisation only for kaolinite. Similar results were observed for ferrihydrite coating of illite, which increased its adsorption capacity ( $Q_{\max}$ ) above that of illite without coating; again this corresponded to the only instance where hydrous oxide coating of illite increased SOC stabilisation above that of illite alone as observed in Chapter 2. Results of this experiment suggest that the interactive effects of clays and oxides on SOC stabilisation are related primarily to the effects that these interactions have on the surface areas of the resulting assemblages, at least under conditions of high DOC loading.

The OC initially sorbed to clays or clay-oxide associations was found to be strongly held, with only 6 – 14 % of initially sorbed OC removed by a single extraction step. The amount of OC desorbed was influenced by the presence of hydrous iron oxides, but only when either a low-charge clay or high-surface area hydrous oxide (kaolinite-goethite or illite-ferrihydrite) was involved in the clay-oxide associations.

The high OC loadings used in the incubations described in Chapter 2 substantially exceeded the sorption capacity of clays or clay-oxide associations as determined in Chapter 3. Although this is representative of the situation in many soils, it is not universally the case. In particular, in many sub-soils, OC loadings are lower than or similar to the sorption capacity of mineral surfaces, and under these circumstances, microbial decomposition of sorbed DOC onto clay-oxide associations is critical for organic matter preservation. Therefore, SOC stabilisation by clays in the presence and

absence of hydrous iron oxides with OC loading equal to the sorption capacity of minerals was investigated in the next set of experiments (Chapter 4).

Organic carbon sorbed to mineral surfaces has been shown to be more resistant to microbial decomposition than OC either dissolved or not attached to mineral surfaces (Kalbitz et al., 2005; Schneider et al., 2010). Some studies suggest organic carbon bound to phyllosilicate clays is mineralised more slowly than OC sorbed to hydrous iron oxides (Jones and Edwards, 1998; Mikutta et al., 2007). The difference in the bioavailability of organo-mineral associations to microbes may relate to differences in binding mechanisms of OC sorption (Chorover and Amistadi, 2001; Feng et al., 2005; Kaiser and Guggenberger, 2003; Keil et al., 1994). In this experiment, plant-derived soluble OC was pre-sorbed to clays (kaolinite, illite and smectite) and oxide-coated clays (kaolinite, illite and smectite coated with goethite and illitic clay coated with goethite, haematite and ferrihydrite) prior to incubation. In contrast to the previous incubation experiments (detailed in Chapter 2), incubation was carried out in a system consisting of 0.5–1.0 g solid of clay- or clay-oxide-associated OC in 20 mL nutrient solution; the treatments were kept oxic by shaking once a day. Under these experimental conditions, mineralisation of OC bound to phyllosilicate clays increased in the order kaolinite < illite < smectite, i.e., in the opposite order to that observed in the previous incubations (Chapter 2). Coating of these clays with goethite increased the stability of sorbed OC against microbial decomposition in kaolinitic and smectitic clays, but did not change C mineralisation of sorbed OC for illitic clay. For illitic clay coated with different hydrous iron oxides, only ferrihydrite coating reduced C

mineralisation. The amount of C mineralised from OC–clay/clay-oxide associations was significantly correlated with the amount of readily desorbed C and with sorption affinity coefficients, as opposed to sorption capacity.

In common with the previous incubation experiments (Chapter 2), variables derived from a two-component C mineralisation model varied with the length of incubation period. Also, the dynamics of C mineralisation were such that reductions in C mineralised due to iron oxide coating occurred via reductions in the size of decomposable C pools rather than changes in their mineralisation rates.

The experiments described in Chapter 4 demonstrate that microorganisms are capable of using some OC sorbed to mineral surfaces as a source of energy but indicate that a large proportion of sorbed OC is strongly protected. However, it is possible that the low apparent availability of OC might in part be due to the lack of any easily decomposable OC. The final experiment in this study (detailed in Chapter 5) was designed to examine the effect of easily decomposable C addition on the mineralisation of clay-associated OC.

Several studies have shown that the presence of easily decomposable C increases mineralisation of native OC and very recalcitrant organic matter (Fontaine et al., 2004; Fontaine et al., 2007; Hamer et al., 2004; Kuzyakov et al., 2009). In this study, plant residue-derived OC was sorbed onto kaolonite, illite and smectite under similar conditions (pH, ionic strength, DOC concentration and solid:solution ratio). Two levels

of glucose (equal to 1% and 10% of C in clay-associated OC) were added to clay-OC associations and C mineralisation was measured throughout a 120-day incubation. Glucose addition significantly increased the total amount of C mineralised from clay-OC associations for all phyllosilicate clays. However, the net amount of C mineralised from clay-associated OC with glucose addition, which was determined by subtracting the amount of CO<sub>2</sub> produced in a corresponding glucose-only treatment, was not significantly different from that mineralised from clay-associated OC without glucose addition. Results of this study demonstrate that mechanisms other than energy availability control the stability of OC sorbed to phyllosilicate clays against microbial decomposition.

This study highlights that there are different factors that dominate SOC stabilisation for different levels of OC loading. In situations where OC is present at relatively high loadings, i.e. greatly in excess of the sorption capacity of mineral surfaces, most OC remains freely available for microorganisms. In such cases, the amount of OC stabilised by soil minerals is controlled by the sorption capacity of mineral assemblages. On the other hand, when OC loadings in soils are relatively low, i.e., below the sorption capacity of mineral surfaces, most OC will be sorbed to mineral surfaces. In this situation, the strength of OC binding to minerals and the reversibility of OC sorption determine the degree of SOC stabilisation. The presence of easily decomposable C did not influence the amount of C mineralised from mineral-associated OC, indicating this model system was not limited by energy availability. Results of this study emphasise that the capacity of minerals for OC sorption and the strength of mineral-organo

associations are both key aspects that determine the degree of OC stabilisation by clay-oxide associations.

This dissertation represents a significant advance in the fundamental understanding of the interactive effect phyllosilicate clays and hydrous iron oxides on the stabilisation of OC in soils. In experiments using both OC loadings, coatings of kaolinite with goethite and illite with ferrihydrite increased SOC stabilisation. This implies that the presence of hydrous iron oxides with those two clays not only increases their sorption capacity as observed in the sorption experiments, but it also improves the strength of mineral-OC associations as showed in the desorption experiments. These interactive effects between clays and oxides were evident where there was either a low-charged clay or a high-surface oxide (kaolinite-goethite and illite-ferrihydrite) involved in the clay-oxide assemblage. This suggests the net charge of clay-oxide associations, the balance between the negative charge of phyllosilicate clays and the positive charge of hydrous iron oxides, determines the effect of clay-oxide associations on SOC stabilisation. In other words, the effects of interactions between clays and oxides on the SOC stabilisation are dependent on the mineralogy of clays and types of hydrous iron oxides.

## **6.2. Recommendations for Future Work**

It is clear from the above discussion that many questions remain unanswered. To address some of these issues and in order to continue to develop our understanding of

the role of phyllosilicate clays and hydrous iron oxides in SOC stabilisation, further research is recommended in the following areas.

- 1) The interactive effects of clay minerals and hydrous iron oxides on SOC stabilisation in this study varied with clay mineralogy and types of hydrous iron oxides. This study was carried out using three clay minerals and three hydrous iron oxides. Other clays and iron oxides would have different chemical surface properties, and different combinations of clays and oxides are therefore likely to give different results. Further experiments using different combinations of clay and hydrous oxide would be instructive, including combinations of low negative charge clay with low positive charge oxide and high negative charge clay with high positive charge oxide.
- 2) Alkyl, O-alkyl and carboxyl C appear to be abundant carbon types in the plant residue-derived OC used in the experiments. It is generally accepted that other organic matter groups such as aromatic C play an important role in the sorption of dissolved OC onto mineral surfaces. Further experiments using different plant residue-derived material is required to determine the effects of chemical composition of OC on SOC stabilisation by clay-oxide associations.
- 3) The presence of an easily decomposable C source did not influence the stability of sorbed OC against microbial decomposition. This experiment was carried out in a nutrient solution prepared so that the C/N ratio was 10, which is considered ideal conditions for microbial decomposition. It has been well established that in the

condition of low availability nitrogen, the presence of fresh organic carbon can lead to the activation of soil microorganisms to decompose native SOM to acquire nitrogen, which in turn increases decomposition of SOM. Further investigation is required to determine whether the effect of the addition of easily decomposable C on the mineralisation of sorbed OC would vary with the different levels of nitrogen availability.

- 4) Glucose addition led to increases total C mineralisation in treatments where glucose was added to mineral-OC associations. These increases closely matched the amount of glucose C added but it could not be proven definitively that the released CO<sub>2</sub> was derived solely from glucose or whether there was some mineralisation of OC sorbed onto clay minerals that was balanced by stabilisation of an equal amount of glucose-derived C. Further experiments using <sup>13</sup>C- or <sup>14</sup>C-labelled glucose would clearly differentiate between these possibilities.
- 5) It has been suggested that soils have a limited capacity to stabilise and store OC and in a set of batch experiments described in Chapter 3 the capacity of mineral surfaces to sorb DOC had reached a saturation. A proportion of this sorbed OC was mineralised during the incubation (detailed in Chapter 4), and so by the end of the incubation there should be some capacity to sorb fresh DOC. It would be instructive to carry out further cycle of batch sorption experiments to test the capability of the mineral sites for sorbing additional OC to replace OC released during the incubation. This would test whether the saturation level of OC stabilisation changes over time.



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