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# Testing the Soil Carbon Saturation Theory: Maximal Carbon Stabilization and Soil Organic Matter Stability as a Function of Organic Carbon Inputs

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# Testing the Soil Carbon Saturation Theory: Maximal Carbon Stabilization and Soil Organic Matter Stability as a Function of Organic Carbon Inputs

## **Abstract**

Soil carbon (C) stocks and fluxes represent significant components of the global C cycle. Application of the soil C saturation theory can help identify soils with large C storage potentials and estimate rates and durations needed to reach maximal soil C storage. The goal of my dissertation was to test the soil C saturation theory by estimating C saturation levels of fine soil particles and quantifying changes in soil organic matter (SOM) stability as fine soil particles approach C saturation. Current model using least-squares linear regression generally underestimates C the maximal amount of soil C stabilization in fine soil particles. Using an analysis of published data, I proposed two alternative methods (boundary line analysis and the organic C loading method) to improve estimates, and found that while the organic C loading method is better since it incorporated mineral specific surface areas which would influence C saturation, it requires information about soil mineralogy and further tests to determine whether the monolayer-equivalent C loading indeed represents a maximal C stabilization potential. Laboratory batch sorption experiment of dissolved organic matter onto soil minerals generated organo-mineral complexes with a range of organic C loadings. These organo-mineral complexes, as well as silt+clay fractions physically isolated from soil samples from three long-term agroecosystem field experiments with differing fertilizer and manure addition treatments, were used to test for differences in SOM stability as a function of organic C loading. Biological, chemical, and thermal test of SOM stability showed little change or the increase trend of SOM stability with increasing organic C inputs, which do not support the notion of the soil C saturation theory that SOM stability decreases as organic C inputs increase. This observation of SOM stability is likely due to the fact that most samples did not exhibit C saturation behavior. The results show that most soils are likely well below C saturation, and further studies of the driving factors (e.g., chemical composition of organic C inputs, mineralogy, and organo-mineral binding types and strength) is needed to determine maximal C loadings and estimate the maximal soil C storage potentials.

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Alain F. Plante

## **Second Advisor**

Arthur H. Johnson

---

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**TESTING THE SOIL CARBON SATURATION THEORY:  
MAXIMAL CARBON STABILIZATION AND SOIL  
ORGANIC MATTER STABILITY AS A FUNCTION OF  
ORGANIC CARBON INPUTS**

Wenting Feng

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Degree of Doctor of Philosophy

2012

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TESTING THE SOIL CARBON SATURATION THEORY: MAXIMAL CARBON  
STABILIZATION AND SOIL ORGANIC MATTER STABILITY AS A FUNCTION OF  
ORGANIC CARBON INPUTS

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WENTING FENG

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# **ABSTRACT**

## **TESTING THE SOIL CARBON SATURATION THEORY: MAXIMAL CARBON STABILIZATION AND SOIL ORGANIC MATTER STABILITY AS A FUNCTION OF ORGANIC CARBON INPUTS**

Wenting Feng

Advisor: Alain F. Plante

Soil carbon (C) stocks and fluxes represent significant components of the global C cycle. Application of the soil C saturation theory can help identify soils with large C storage potentials and estimate rates and durations needed to reach maximal soil C storage. The goal of my dissertation was to test the soil C saturation theory by estimating C saturation levels of fine soil particles and quantifying changes in soil organic matter (SOM) stability as fine soil particles approach C saturation. Current model using least-squares linear regression generally underestimates C the maximal amount of soil C stabilization in fine soil particles. Using an analysis of published data, I proposed two alternative methods (boundary line analysis and the organic C loading method) to improve estimates, and found that while the organic C loading method is better since it incorporated mineral specific surface areas which would influence C saturation, it requires information about soil mineralogy and further tests to determine whether the monolayer-equivalent C loading indeed represents a maximal C stabilization potential. Laboratory batch sorption experiment of dissolved organic matter onto soil minerals



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# **BACKGROUND**

## **1.1 Soil organic matter in the global carbon cycle**

Soil organic matter (SOM) is an important component of the global carbon (C) cycle, containing more C (3150 Pg C) than plant biomass (650 Pg C) and the atmosphere (750 Pg C) combined (Field and Raupach, 2004; Houghton, 2005), making soils the third largest C pool after the oceanic and geologic pools. The global soil C stock of 3150 Pg C includes 450 Pg C in wetlands, 400 Pg C in permafrost soils, and 2300 Pg C in upland soils, which is further subdivided into 1500 Pg C in top 1 meter and 800 Pg C in the deeper layers up to 3 meter (Jobbagy and Jackson, 2000; Sabine et al., 2003). Fluxes of C into and out of soils are also large. Raich and Schlesinger (1992) estimated the global respiratory flux of CO<sub>2</sub> from soils to be 68 Pg C yr<sup>-1</sup>, of which 50 Pg C yr<sup>-1</sup> results from the decomposition of litter and SOM. Hence, any change in the balance of soil C stocks, sources or sinks can significantly affect the global C cycle.

## **1.2 Soil organic matter stabilization**

Soil organic matter storage is the net balance of organic C inputs and outputs. Organic matter inputs are affected by plant productivity related to nutrient supply, temperature, and moisture (Chapin and Eviner, 2005) or by land use management (e.g.: use of organic amendments). Organic matter output is mainly controlled by microbial decomposition, which is greatly affected by moisture and temperature (Kirschbaum, 1995). In addition to environmental factors, it is widely accepted that SOM can be protected from microbial decomposition in three ways – biochemical protection, physical protection, and chemical protection (Baldock and Skjemstad, 2000; Six et al., 2002;



Sollins et al., 1996; von Lützow et al., 2006).

Biochemical protection refers to chemical recalcitrance as a mechanism that stabilizes SOM against microbial decomposition. Soil organic matter is a heterogeneous, composite material that includes components differently susceptible to degradation such as litter, microbial biomass, water soluble organics, and humus (Stevenson, 1994).

Although some organic substances decompose rapidly (e.g. starch and cellulose) (Martin and Haider, 1986), some decompose slowly due to complex chemical structure (e.g., lignin contains no hydrolytic bonds and black C comprises aromatic groups), and is therefore considered biochemically recalcitrant (Berg et al., 1982; Schmidt and Noack, 2000; Stevenson, 1994). Recalcitrance may be inherited from the original substrate or generated through the partial decomposition and formation of metabolites. Recently, SOM recalcitrance as a primary stabilization mechanism has been called into question using evidence from compound-specific isotopic analyses (Schmidt et al., 2011; Thevenot et al., 2010), and hence it has been proposed that no organic substance can escape microbial attack under optimal conditions (Hamer and Marschner, 2002; Hamer and Marschner, 2005; Hamer et al., 2004; Marschner et al., 2008). Instead, there is increasing appreciation for the stabilization potential afforded by the soil matrix itself.

Soil organic matter can be physically protected within soil aggregates (Christensen, 1996; Oades and Waters, 1991; Tisdall and Oades, 1982). Physical protection is the result of isolating organic matter from microbes or enzymes (Killham et al., 1993) or limiting O<sub>2</sub> diffusion (Sexstone et al., 1985). Soil aggregates are agglomerated by fungal hyphae and polysaccharides derived from root exudates or microbes (Tisdall and Oades, 1982). Soil organic matter within soil aggregates is affected

by soil aggregates distribution and stability (Six et al., 1999; Six et al., 2000), and it becomes easily decomposable when fungal hyphae or polysaccharides are disturbed. Turnover time of SOM within soil aggregates was found to be shorter than that associated with silt and clay particles (Balesdent et al., 1987; Balesdent et al., 1988), suggesting that while aggregates afford some protection in the short- to medium-term, long-term stabilization is achieved through direct mineral association (Balabane and Plante, 2004).

Soil organic matter can be protected by physical-chemical interactions between organic matter and soil minerals (Kaiser and Guggenberger, 2003; Sollins et al., 1996; von Lützow et al., 2006). Organic matter can react with soil minerals through ligand exchange, cation bridging, H-bonding, or van der Waals forces (Arnarson and Keil, 2000; Feng et al., 2005; Gu et al., 1994; Stevenson, 1994). Strong chemical bonds between organic matter and soil minerals make organic matter less susceptible to desorption or more resistant against biological mineralization (Gu et al., 1994; Kaiser and Guggenberger, 2007; Keil et al., 1994; Mikutta et al., 2007). Through these interactions, organic matter is associated with silt and clay particles to form organo-mineral complexes. Organic matter in organo-mineral complexes constitutes more than 50% of total SOM (Christensen, 1996; Kahle et al., 2002a). Organic matter associated with soil minerals has long turnover times and is therefore considered stable (Balesdent et al., 1987; Balesdent et al., 1988; Trumbore, 2000).

### **1.3 Soil C saturation theory**

Many soils, especially those with low soil organic carbon (SOC) levels due to intensive cultivation practices, show a linear response of SOC amount to increasing organic C inputs (Kong et al., 2005). Current models of SOC dynamics also show that

SOC amount rises linearly with increasing organic C inputs, which is attributable in part to their underlying first-order kinetics (Manzoni and Porporato, 2009). However, some long-term experiments show little or no response of SOC amount to long-term organic C additions when initial SOC amount is high (Campbell et al., 1991; Huggins et al., 1998; Huggins and Fuchs, 1997; Soon, 1998). In native grassland and cultivated cropland with the same type of soil, Hassink (1997) observed no difference in the amount of organic C associated with fine soil particles ( $< 20 \mu\text{m}$ ) but significant difference in organic C amount in bulk soil, implying a maximal organic C amount associated with fine soil particles. These observations of a potential maximal SOC amount, led to the development of the soil C saturation theory (Campbell et al., 1991; Hassink 1997; Six et al. 2002).

There is an important distinction to be made between an equilibrium soil C content and the soil C saturation level. Soil organic matter dynamics are typically viewed in terms of changes in stocks over time. If changes in management practices or land cover increase C input levels, then over time, soil C content will increase until a new equilibrium soil C content is reached (Figure 1). Multiple increases in long-term C inputs will produce multiple equilibrium SOC levels because equilibrium soil C levels are achieved where C additions are balanced by C losses. However, these steady-state levels may still be below the soil C saturation level. With no saturation, SOC content can continue rising with increasing C inputs (Figure 1, upper right). The soil C saturation theory predicts that there is a maximum equilibrium C level that will be attained, and as equilibrium SOC increases with greater C input rates, the incremental increase in SOC declines as the rate of C addition increases (Figure 1, lower right). Hence the storage efficiency decreases towards zero as the soil C content approaches the saturation level. It

is therefore essential to have a wide range of steady-state organic C inputs to test for the presence of a soil C saturation level.

In the conceptual soil C saturation model constructed by Six et al. (2002), organic C associated with silt and clay particles, microaggregate-protected, biochemically protected, and unprotected will all reach their own saturated levels. Considering large pool size and long turnover time of organic C associated with silt and clay particles, it is more meaningful to understand organic C saturation in this pool. Moreover, chemically protected organic matter is associated with soil minerals, and therefore the amounts of chemically protected organic C would be limited by finite mineral surface areas. Hassink (1997) observed that fine soil particles, rather than bulk soil, reached organic C stabilization potentials since C amounts of fine soil particles did not change when comparing them under agricultural practice and grassland. Gulde et al. (2008) also found an asymptotic relationship between organic C concentrations and manure inputs for silt and clay particles, suggesting organic C amount associated with silt and clay particles was approaching a saturated level. Stewart et al. (2008) reported that the relationship between concentrations of organic C associated with silt and clay particles and a wide range of steady organic C inputs well fitted soil C saturation model.

However, none of these studies specifically address the mechanism responsible for the saturation of organic C associated with silt and clay particles. Based on physical-chemical reactions between organic matter and soil minerals, mineral specific surface area (SSA) and soil charge characteristics likely affect organo-mineral interactions and may generate physical limits to C stabilization. Finite soil mineral SSA is considered as a potential factor limiting organic C association with soil minerals, and it was observed to

be positively related to organic C associated with silt and clay particles (Kahle et al., 2002b; Wiseman and Püttmann, 2006). After measuring organic C loadings (*i.e.*, organic C concentration normalized by SSA) for a variety of soil and sediment particles, Mayer (1994) proposed using  $1 \text{ mg C m}^{-2}$  (the so-called monolayer-equivalent) as the maximal C loading. Additionally, soil cations, ionic strength, and soil pH could regulate organic matter adsorption and precipitation (Feng et al., 2005; Jardine et al., 1989; Schneider et al., 2010). Soil organic matter associated with silt and clay particles was found to be correlated with CEC (Guibert et al., 1999; Leinweber et al., 1993). Amounts of Fe/Al oxyhydroxides and oxides were also found to be intimately related to organic C concentration (Kaiser and Guggenberger, 2003; Kleber et al., 2005; Soares and Alleoni, 2008; Zinn et al., 2007). Higher hydroxyl density on Fe/Al oxides than on phyllosilicates enables them to adsorb more organic matter (Gu et al., 1994; Murphy et al., 1990). Soil mineralogical properties would greatly influence C saturation behaviors of silt+clay sized fine soil particles.

## RESEARCH QUESTIONS AND HYPOTHESES

The soil C saturation theory is important to identify soils with large, unmet SOC storage potentials and to predict rates and durations needed for soils to reach the maximal SOC storage. However, details are still unknown about soil C saturation in specific soil-climate and management conditions, which can eventually be applied for SOC stock management. **Critical questions remain unanswered, such as: how can the soil C saturation level be determined and quantified, and how does SOM stability change as soils approach C saturation?** The main objective of this dissertation was to further test the soil C saturation theory by estimating the soil C saturation level of fine soil

particles and quantifying organic matter stability of soil minerals at different degrees of soil C saturation. My dissertation is structured into four research chapters seeking to answer the following questions:

Chapter 1: Improving the estimate of maximal soil C stabilization by fine soil particles. How can the current method to estimate maximal organic C stabilization by fine soil particles (*i.e.*, least square linear regression) be improved?

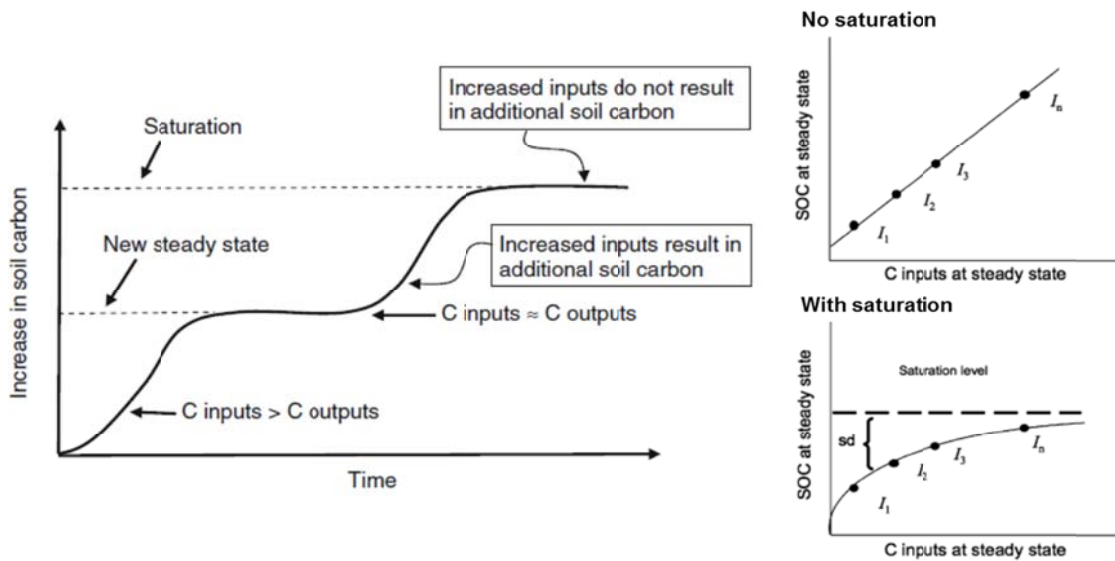
Chapter 2: Examining changes in soil organic matter stability in organo-mineral complexes with increasing organic C loading. How does SOM stability change as soils approach C saturation with increasing organic C inputs?

Chapter 3: Seeking carbon saturation behavior in agricultural soils receiving long-term manure amendments. What are C saturation behaviors of fine soil particles from different soils receiving long-term multi-level organic C inputs? How do soil mineralogical properties influence soil C saturation behaviors of fine soil particles?

Chapter 4: Quantifying soil organic matter stability in agricultural soils receiving long-term manure amendments. How does the biological, chemical, and thermal stability of SOM change as a function of differing amounts of long-term organic C inputs?

## Figure 1

Relationship between C inputs and soil organic C content at steady-state with and without soil C saturation. After a change in management or land-use a new steady state in soil C is achieved. If soil is C saturated, no increase in steady-state C content will occur after an increase in C inputs. (Adapted from West & Six, 2007 and Stewart et al., 2007).



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# CHAPTER ONE

Improving estimates of maximal organic carbon stabilization by fine soil particles

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## Abstract

Organic carbon (C) associated with fine soil particles ( $< 20 \mu\text{m}$ ) is relatively stable and accounts for a large proportion of total soil organic C (SOC). The soil C saturation concept proposes a maximal amount of SOC that can be stabilized in the fine soil fraction, and the soil C saturation deficit (i.e., the difference between current SOC and the maximal amount) is presumed to affect the capacity, magnitude, and rate of SOC storage. In this study, we argue that predictions using current models underestimate maximal organic C stabilization of fine soil particles due to fundamental limitations of using least-squares linear regression. The objective was to improve predictions of maximal organic C stabilization by using two alternative approaches; one mechanistic, based on organic C loadings, and one statistical, based on boundary line analysis. We collected 342 data points on the organic C content of fine soil particles, fine particle mass proportions in bulk soil, dominant soil mineral types, and land use types from 32 studies. Predictions of maximal organic C stabilization using linear regression models are questionable because of the use of data from soils that may not be saturated in SOC and because of the nature of regression itself, resulting in a high proportion of presumed over-saturated samples. Predictions of maximal organic C stabilization using the organic C loading approach fit the data for soils dominated by 2:1 minerals well, but not soils dominated by 1:1 minerals; suggesting that the use of a single value for specific surface area, and therefore a single organic C loading, to represent a large dataset is problematic. In boundary line analysis, only data representing soils having reached the maximal amount (upper 10% percentile) were used. The boundary line analysis estimate of maximal organic C stabilization ( $78 \pm 4 \text{ g C kg}^{-1}$  fraction) was more than double the

estimate by the linear regression approach ( $33 \pm 1 \text{ g C kg}^{-1}$  fraction). These results show that linear regression models do not adequately predict maximal organic C stabilization. Soil properties associated with soil mineralogy, such as specific surface area and organic C loading, should be incorporated to generate more mechanistic models for predicting soil C saturation, but in their absence, statistical models should represent the upper envelope rather than the average value.

## 1.1. Introduction

Land use management practices, such as no-till, manure and compost additions, and enhanced residue return are used to increase soil organic carbon (SOC) content. Current models of soil C dynamics assume that SOC content rises linearly with increasing organic C inputs, which is attributable in part to their underlying first-order kinetics (Lugato et al., 2007). However, several long-term field experiments have found that SOC, especially organic C associated with fine soil particles, did not always increase with increasing organic C inputs (e.g., Campbell et al., 1991; Chung et al., 2008; Gulde et al., 2008).

Organic matter can be stabilized in soils by selective recalcitrance, spatial inaccessibility, and organo-mineral interactions (Six et al., 2002; Sollins et al., 1996; von Lützow et al., 2006). Organo-mineral complexes result from the association of organic matter with soil minerals, which is largely controlled by soil texture (Christensen, 1998). Organic C retained in association with fine soil particles has relatively longer turnover times (Balesdent et al., 1998) and accounts for a large proportion of total SOC (Christensen, 1998; Kahle et al., 2002b), thus making this C pool of particular interest in the context of the broader global C cycle. The amount of organic C associated with fine soil particles is correlated to their mass proportions in bulk soil (Hassink, 1997; Liang et al., 2009; Six et al., 2002; Zhao et al., 2006), and are affected by soil mineral types (Kaiser and Guggenberger, 2003; Mikutta et al., 2007; Schulten and Leinweber, 2000; Six et al., 2002) and composition of organic C inputs (Oades, 1988). Soil specific surface area, which is closely associated with mineralogy, has also been found to be positively correlated to SOC (Kahle et al., 2002a; Mayer, 1994; Wiseman and Püttmann, 2005).

In paired cultivated cropland and undisturbed grassland of the same soil, Hassink (1997) observed considerable differences in the organic C content of bulk soils, but no significant differences in the organic C content of fine soil particles ( $< 20 \mu\text{m}$ ), which he attributed to the existence of a limit of organic C content retained on fine soil particles. Using soils with a wide range of mass proportions of fine soil particles in bulk soil, he hence proposed using the mass proportion of fine soil particles in bulk soil to predict the maximal organic C content associated with fine soil particles. In his least-squares linear regression model,  $y = 0.37x + 4.07$ , the dependent variable,  $y$ , is the organic C content of fine soil particles on a bulk soil basis ( $\text{mg C g}^{-1}$  soil), and the independent variable,  $x$ , is the mass proportion of fine soil particles in bulk soil ( $\text{g fraction } 100 \text{ g}^{-1}$  soil). Hassink (1997) used the slope of the regression model to predict the maximal organic C content of fine soil particles, which he termed the protective capacity.

Six et al. (2002) reviewed additional studies of organic C contents of fine soil particles under different land uses and soil mineralogy, and found different regression parameters for different size cut-offs for the fine fraction (i.e.,  $< 20 \mu\text{m}$  versus  $< 50 \mu\text{m}$ ), for soils dominated by 1:1 versus 2:1 minerals, and for soils from cropland, forest, versus grassland. The slopes of the least-squares linear regression lines generated by Hassink (1997) and Six et al. (2002) represent the organic C concentrations of fine soil particles ( $\text{mg C g}^{-1}$  fraction) at the theoretical protective capacity. The values of these slopes (0.37 for Hassink (1997) and 0.21-0.41 for Six et al. (2002)) are relatively low compared to observed C concentrations of fine soil particles published in a large number of studies, suggesting the theorized maximal organic C stabilization by fine soil particles may be underestimated. The objectives of the current study were: 1) to evaluate the limitations of

a least-squares linear regression approach to estimating maximal C stabilization, 2) to test two alternative methods (i.e., organic C loadings and boundary line analysis) to estimate maximal organic C stabilization, and 3) to compare organic C stabilized on fine soil particles in soils dominated by 1:1 and 2:1 minerals in cropland, forest, and grassland.

## **1.2. Materials and methods**

### *1.2.1 Data collection*

Analyses in the current study were performed on data collected from previously published studies. Publications reporting data on organic C concentration ( $\text{mg C g}^{-1}$  fraction) or C content ( $\text{mg C g}^{-1}$  soil) of fine soil particle fractions, and mass proportion of fine soil particles in bulk soil were selected for this analysis. In addition, publications required information concerning soil mineralogy or taxonomic classification, as well as land use. The selected studies were largely focused on organic matter associated with primary particle size fractions, but used a variety of methods for soil dispersion. This variation is known to impact the abundance of the fine soil fraction as well as the amount of organic matter associated with this fraction. Unfortunately, too few studies have used consistent methods or methods known to achieve complete dispersion of the silt- and clay-sized fraction. As such, it was not possible to determine the degree of full dispersion and hence the amount of potential microaggregation. We also selected studies using  $< 20 \mu\text{m}$  as the criterion for fine soil particles composed of silt and clay because organic C contents of fine soil particles compiled in this study were compared with those of Hassink (1997), in which  $20 \mu\text{m}$  was used to fractionate silt and sand. The criterion of  $50 \mu\text{m}$  as the boundary between silt and sand was not used because Six et al. (2002) found significantly different regression parameters of organic C contents of fine soil particles

with their mass proportions for fine soil particles less than 20  $\mu\text{m}$  and for those less than 50  $\mu\text{m}$ . In addition, the use of the smaller size for silt fraction (20  $\mu\text{m}$ ) can alleviate effects of organic C associated with the large silt fraction (20 – 50  $\mu\text{m}$ ) that may exist in the form of silt-sized microaggregates (Balabane and Plante, 2004; Virto et al., 2008). We found 32 suitable publications, 18 of which have previously been compiled in Six et al. (2002). Soil organic C content of fine soil particles ( $\text{mg C g}^{-1}$  soil) was calculated by multiplying organic C concentration of each fraction ( $\text{mg C g}^{-1}$  fraction) by the corresponding mass proportion of the fraction in bulk soil ( $\text{g fraction } 100 \text{ g}^{-1}$  soil). Only three out of 32 studies did not further separate fine soil particles (i.e., Barthes et al., 2008; Hassink, 1997; Liang et al., 2009), and the remainder of the compiled studies reported fine soil particles separated into multiple fractions (see Appendix). For studies with further fractionation of fine soil particles, mass proportions and organic C contents of the sub-fractions were summed. In total, we collected 342 data points of organic C contents of fine soil particles and mass proportions in bulk soil from 32 studies.

Interactions between fine soil particles and organic C are likely affected by soil mineralogical properties such as specific surface area and charge density (Schulten and Leinweber, 2000; Six et al., 2002). Therefore, we divided the dataset into two sub-groups consisting of soils dominated by 1:1 and 2:1 minerals based on the identification of clay mineralogy in the original publication or by inference from the soil classification. Soils dominated by 1:1 minerals were mainly kaolinitic, while soils dominated by 2:1 minerals were mainly illitic, montmorillonitic, or vermiculitic. The sub-group of soils dominated by 1:1 minerals consisted of 101 data points, and the 2:1 minerals sub-group consisted of 241 data points (see Appendix). After separation by mineral type, the dataset was further

sub-divided by land use: cropland, forest, and grassland. Cropland consisted of cultivated land under different management practices such as no tillage, conventional tillage, and organic and inorganic fertilizer application. Forest land uses included natural forest and plantation. Grassland included pasture, savanna, and grazed and non-grazed grassland.

### *1.2.2 Least-squares linear regressions*

The method used by Hassink (1997) to determine what he termed the protective capacity of a soil is least-squares linear regression, using mass proportion of fine soil particles ( $\text{g fraction } 100 \text{ g}^{-1} \text{ soil}$ ) as the independent variable and soil organic C content of the fine soil particles ( $\text{mg C g}^{-1} \text{ soil}$ ) as the dependent variable. We performed similar regression analyses on collected data for all 32 studies collectively, on sub-groups based on mineral type, and on each of the 28 individual studies that did not previously report regression results. Regression parameters and their uncertainties determined for the two sub-groups based on mineralogy were compared to those previously reported by Hassink (1997), a previous meta-analysis by Six et al. (2002), and other individual studies reporting such relationships (i.e., Liang et al., 2009; Zhao et al., 2006). In a second set of regression analyses, we forced intercepts to be zero because any positive intercept would represent organic C not directly associated with fine soil particles and thus not stabilized. Correlation coefficients ( $r$ ) and probabilities ( $P$ -values) were reported as measures of goodness of fit for both the slopes and intercepts.

### *1.2.3 Over-saturation calculations*

The regression model published by Hassink (1997; i.e.,  $y = 0.37x + 4.07$ ) and the mass proportions of fine soil particles in bulk soil from the compiled dataset from 32 studies were used to calculate maximal organic C contents of fine soil particles. These

predictions were then compared to the measured organic C contents reported in the studies. The saturation ratio for each data point was calculated as the measured organic C content of fine soil particles divided by the predicted organic C content using the Hassink regression model; soils with a saturation ratio larger than one were considered over-saturated.

#### *1.2.4 Organic C loading method*

Soil physical-chemical properties such as specific surface area are positively correlated with soil organic C of bulk soil and soil fractions (Kahle et al., 2002a; 2002b; Mayer, 1994; Wiseman and Püttmann, 2005; Zinn et al., 2007). Different soil minerals have different specific surface areas (Mitchell and Soga, 2005), and based on data from marine sediments and upland soils, the monolayer-equivalent C loading of  $\sim 1 \text{ mg C m}^{-2}$  was proposed as a potential maximal organic C associated with fine particles (Keil et al., 1994; Mayer, 1994). Subsequent studies (e.g., Mayer and Xing, 2001; Wagai et al., 2009) indicated that organic matter is not distributed as an actual monolayer on the mineral surface, but is instead patchy. However, the organic C loading of  $\sim 1 \text{ mg C m}^{-2}$  remains a reasonable estimate for maximal organic C stabilization because organic C loadings greater than this value have generally been observed in low density fractions with rapid C turnover time (Kahle et al., 2002a; Mayer, 1994; Wiseman and Püttmann, 2005), therefore not likely representing a stabilized pool. Soils with organic C loading less than  $\sim 1 \text{ mg C m}^{-2}$  are more likely to be found where C inputs are low, such as agricultural soils or in deep soil horizons (Wagai et al., 2009; Wiseman and Püttmann, 2005).

We used an organic C loading of  $1 \text{ mg C m}^{-2}$  and estimated soil specific surface area values as an alternative approach to predicting maximal organic C stabilization in



fine soil particles. Soil mineral specific surface area (SSA) of  $15 \text{ m}^2 \text{ g}^{-1}$  was assigned to soils dominated by 1:1 minerals, and SSA of  $80 \text{ m}^2 \text{ g}^{-1}$  for soils dominated by 2:1 minerals. These values were the averages of soil mineral specific surface areas of 1:1 minerals (i.e., kaolinite) and of 2:1 minerals (i.e., montmorillonite, vermiculite, and illite) (Mitchell and Soga, 2005). The SSA of the fine soil fractions will clearly vary with mineralogical composition, but the values stated above were used to represent overall mean estimates. Organic C content of fine soil particles was calculated by multiplying the organic C loading by soil mineral SSA, which was then normalized by mass proportion of fine soil particles in bulk soil. These estimates of the maximal organic C contents generated using the organic C loading were compared with lines of maximal organic C contents using the linear regression models.

#### *1.2.5 Boundary line analysis*

Boundary line analysis is a technique used to estimate the upper or lower limit of a response to independent variables. It was first introduced by Webb (1972) to estimate maximal plant productivity, and then widely used to assess maximal responses relative to limited and independent factors in other studies (e.g., Elliott and Dejong, 1993; Schmidt et al., 2000). Generally, a boundary line is made by fitting a line or curve to points identified on a boundary (Milne et al., 2006). A subset of data points along the upper boundary in the current study were assumed to represent soils having reached the maximal organic C content, and would be more appropriately used to predict the maximal organic C content of fine soil particles with mass proportion in bulk soil. We used boundary line analysis as an alternative statistical tool to least-squares regression to predict maximal organic C stabilization by fine soil particles for the entire data set and for

sub-groups based on clay mineral type and land use.

First, data were sorted by mass proportions of fine soil particles in bulk soil (g fraction  $100 \text{ g}^{-1}$  soil), then separated into groups with intervals of 10 g fraction  $100 \text{ g}^{-1}$  soil. For the entire data set, soils dominated by 1:1 minerals, and soils dominated by 2:1 minerals, data were separated into nine groups from  $< 10.00$  to  $80.00 - 89.99$  g fraction  $100 \text{ g}^{-1}$  soil. The upper tenth percentile of organic C content of fine soil particles in each group was then identified, and finally the upper tenth percentile of organic C contents of fine soil particles and the corresponding mass proportions from each group were combined together and used in a regression analysis where the intercept is forced through zero. While the selection of the upper tenth percentile is somewhat arbitrary, we believe it accounts for a reasonable amount of variability without selecting too many soils that are not at saturation. We reported  $r$  and  $P$  values for the boundary lines of the entire dataset and sub-group datasets based on mineralogy and land use as measures of goodness of fit.

### *1.2.6 Estimates of maximal organic C stabilization*

Estimates of the maximal amount of organic C that can be stabilized by fine soil particles are represented by the slope of the regressions, which represents the organic C concentration in the fine soil particle fraction on a fraction mass basis. Estimates of maximal organic C stabilization from least-squares linear regressions and boundary line analyses were determined for the entire dataset, and mineral type and land use sub-groups. Conversely, estimates of maximal organic C stabilization from the use of the organic C loading approach is dictated by the selected specific surface area. That is, the organic C loading ( $1 \text{ mg C m}^{-2}$ ) multiplied by soil specific surface area (in units of  $\text{m}^2 \text{ g}^{-1}$ ) yields a value with units of  $\text{mg C g}^{-1}$  fraction.

Soil C saturation deficit can be determined by comparison of the maximal versus measured organic C content once an estimate of the maximal soil organic C content is generated. Different land use types have different organic C inputs, typically resulting in different degrees of soil C saturation deficit. Thus, for soils dominated by 1:1 minerals and 2:1 minerals in cropland, forest, and grassland individually, measured and the maximal organic C contents of fine soil particles ( $< 20 \mu\text{m}$ ) were plotted against mass proportions in bulk soil to demonstrate C saturation deficit.

### **1.3. Results and discussion**

#### *1.3.1 Regressions of organic C contents of fine soil particles with mass proportions*

A number of previously published studies have reported least-squares linear regressions similar to that developed by Hassink (1997) to estimate maximal C stabilization (third group in Table 1). Slopes of regression lines from these studies ranged from 0.22 to 0.41. We found statistically significant regressions for data from seven out of 32 individual studies (e.g., Amelung et al., 1998; Barthes et al., 2008; Feller and Beare, 1997; Guibert et al., 1999; Steffens et al., 2009; Thuriès et al., 2000; Zinn et al., 2007; fourth group in Table 1). Using data collected from these studies, regression slopes showed a wider range: from 0.08 to 0.64 (IPCC et al.). The slope of the regression line based on the entire dataset of the current meta-analysis was 0.30 with standard error of 0.02 (solid line in Figure 1), which is relatively close to the value reported by Hassink (1997). This similarity is somewhat surprising because Hassink (1997) used only uncultivated and grassland soils, while a large number of soils included in the current analysis were cultivated soils that would presumably decrease the slope.

The intercepts in five of the seven studies found to have statistically significant

relationships were not found to be statistically different from zero (third group, Table 1). Only the data from Guibert et al. (1999) and Zinn et al. (2007) were found to have a positive intercept. Intercepts were also found to be zero for the composite analyses of subsets of data for 1:1 and 2:1 minerals (IPCC et al.). Comparison of the slopes generated in regressions with and without intercepts found that the slopes did not significantly differ. In their analysis, Liang et al. (2009) found the intercept for their regression was not significant and therefore used a reduced model without intercept as their final regression model.

### *1.3.2 Drawbacks of the least-squares linear regression approach*

We propose that the least-squares linear regression approach used by Hassink (1997) and others to estimate the protective capacity for soil C stabilization suffers from several significant limitations. One such limitation is the implicit assumption that soil mineralogy does not affect organic C stabilization on fine soil particles. This was previously addressed by Six et al. (2002), who generated separate regressions based on mineral type (IPCC et al.). Our own meta-analysis also showed significantly different regression parameters: a slope of  $0.23 \pm 0.02$  for 1:1 minerals and a slope of  $0.33 \pm 0.03$  for 2:1 minerals (Table 3; dashed lines in Figure 1). Direct experimental evidence for the influence of soil mineralogy on organic C stabilization is, however, sparse. Wattel-Koekkoek et al. (2001) found that the organic C concentration of smectite did not differ significantly from that of kaolinite, and concluded that soil mineral types did not affect organic C concentrations. Conversely, Schulten and Leinweber (2000) reviewed studies of organo-mineral complexes and concluded organic C capacity of different soil minerals may vary. Ngole and Ekosse (2009) reported that Vertisols dominated by smectitic

minerals contained greater amounts of organic matter than Luvisols dominated by kaolinite, feldspars, and quartz, and Deneff et al. (2004) reported differing degrees of C stabilization in soils of contrasting mineralogy through the effect on aggregation. One difficulty in establishing a strong link between mineralogy and C stabilization is that the dominance of specific mineral species is frequently associated with climate, which in turn alters soil C dynamics.

Perhaps a more important drawback lies in the application of least-squares linear regression itself. Assuming the regression line represents the maximum protective capacity implies that all samples used to generate the line are near their maximum C stabilization, with the difference between observed and predicted organic C contents (i.e., the dependent variable in the regression) being due to random error. If the organic C contents of fine soil particles have not reached their maximum, then it is unreasonable to use such soils to develop a model to predict maximal organic C stabilization. In spite of the use of only uncultivated and grassland soils in the analysis, there was no information regarding the degree of C saturation in the soils used to develop the regression in the original Hassink (1997) study. Given the range of organic C concentrations, land uses, and soil mineral types in our broader analysis, we suppose that the probability that all samples were near the protective capacity is quite low.

Additionally, an inherent outcome of a good regression is that the total squared error above and below the regression line is approximately equal. In essence, the regression line is designed to pass through the middle of the data points and therefore represents the mean value. This is contrary to the desire to estimate the maximum SOC associated with fine soil particles, where one might expect that measured SOC contents

should generally be lower than the predicted maximal stabilization value (i.e., exhibiting a C saturation deficit), with few and predictable exceptions. We found that a significant proportion (54 out of 342, 15.8%) of soils to be over-saturated based on predictions using the Hassink (1997) regression (Table 2). Few soils dominated by 1:1 minerals were found to be over-saturated, and these were only found in forest and grassland land uses where C inputs are relatively high. Conversely, for soils dominated by 2:1 minerals, the proportion of over-saturated soils was much higher: 21.2% overall, 84.6% in forest, 33.0% in grassland, and 6.1% in cropland.

Lastly, in the case of the compiled data of this analysis, the least-squares regression between organic C content stabilized on fine soil particles and their mass proportions in bulk soil violates the assumption of equal variance of the dependent variable. That is, there is greater variation in values of organic C content as the mass proportion of fine soil particles in bulk soil increases (Figure. 1).

More generally, a least-squares linear regression approach allows the data to determine the model. We would argue that an alternative approach that will test the data against a given, more mechanistic, model is more valuable. The difference between these approaches is most easily illustrated by the use of an intercept in the regression model. While an uninformed linear regression approach would allow a non-significant intercept to remain in the model, a mechanistic approach would provide the rationale for using a reduced model with no intercept.

### *1.3.3 Organic C loading method*

One alternative approach to estimating maximum soil C stabilization was to use an apparent maximum amount of C that can sorb to mineral surfaces based on the

monolayer-equivalent C loading ( $\sim 1 \text{ mg C m}^{-2}$ ). Using this method, the predicted maximal organic C content of fine soil particles in soils dominated by 1:1 minerals was generally less than measured organic C contents (Figure 2). Conversely, in soils dominated by 2:1 minerals, predictions using the organic C loading were higher than most observed data (Figure 3). There are two ways to explain these observations: an organic C loading of  $1 \text{ mg C m}^{-2}$  does not represent a correct maximal value, or the assumed value of SSA for 1:1 mineral-dominated soils was incorrect.

The assumption in using this method to predict the maximal organic C content of fine soil particles was that the organic C loading value of  $1 \text{ mg C m}^{-2}$  represents the maximal amount of C that can be stabilized by fine soil particles. Previous studies have demonstrated positive relationships between organic C concentration and specific surface area for both marine sediments and soil particles (Kahle et al., 2003; Keil et al., 1994; Mayer, 1994; Zinn et al., 2007). Organic C loadings vary greatly, from less than  $1 \text{ mg C m}^{-2}$  often observed in deep soil horizons or with low organic C input (Kahle et al., 2002a; Mayer, 1994) to those larger than  $1 \text{ mg C m}^{-2}$  found in low density fraction samples or soils dominated by labile organic C (Kahle et al., 2002a; Kögel-Knabner et al., 2008; Mayer, 1994). Though their intent was not to test for maximal C sorption, Schneider et al. (2010) generated organo-mineral complexes by mixing dissolved organic matter with amorphous Al hydroxides, and the resultant C loadings were all below  $1.2 \text{ mg C m}^{-2}$ . In another mixing experiment using dissolved organic C and goethite, pyrophyllite, and vermiculite, the maximal organic C loadings were all less than  $1 \text{ mg C m}^{-2}$  (Mikutta et al., 2007). Several studies have demonstrated the organic matter is not uniformly distributed on mineral surfaces as a monolayer (e.g., Mayer and Xing, 2001; Wagai et al., 2009), but

using the proposed monolayer-equivalent organic C loading of  $1 \text{ mg C m}^{-2}$  as a potential maximum appears to be the best current estimate, until further experimental evidence is provided.

An alternate source of error in the organic C loading approach is the estimate of the mineral SSA of fine soil particles from soils dominated by 1:1 minerals. Soils dominated by 1:1 minerals such as kaolinite have low specific surface area (Kaiser and Guggenberger, 2003; Mitchell and Soga, 2005), but typically have a large amount of Fe oxides compared to other soils, especially in tropical areas (Mitchell and Soga, 2005; Wiseman and Püttmann, 2006). Iron oxides and hydroxides have high specific surface areas (Kaiser and Guggenberger, 2003; Yong et al., 1992), and positive relations have been observed between organic C and Fe oxides content (Kaiser and Guggenberger, 2003; Kleber et al., 2005). It is therefore possible that the value of  $15 \text{ m}^2 \text{ g}^{-1}$  for the mineral SSA of the fine soil particles from soils dominated by 1:1 minerals is a significant underestimate because it does not account for the full range of mineralogical constituents. Unfortunately, iron oxide concentrations or mineral specific surface area data are not routinely reported and therefore could not be included in the current study. Improved estimates of SSA of fine soil particles would improve the estimation of maximal organic C content stabilization in fine soil particles for soils dominated by 1:1 minerals, but more generally, the use of a single value for SSA to represent a large dataset is problematic. The organic C loading approach will likely yield better estimates for maximal C stabilization only when SSA is measured for each sample in the data set. However, even if the specific surface area were measured for each sample in the dataset, the organic C loading approach may still be subject to variability in the values of organic C loading that



represent potential maximal stabilization.

#### *1.3.4 Boundary line analysis*

In the boundary line analysis, only the upper tenth percentile of organic C contents in fine soil particles were considered as samples having reached maximal stabilization, and thus were used to develop the regression to estimate upper boundary of organic C stabilization potentials of fine soil particles. The remaining data points are assumed to exhibit some C saturation deficit and are therefore excluded from the boundary line analysis. The results show markedly greater slopes generated by boundary line analysis than by least-squares regression. For all data combined, the boundary line analysis generated an estimate of maximal organic C stabilization ( $78 \pm 4 \text{ g C kg}^{-1}$  fraction) that was more than double the estimate generated by the linear regression approach ( $33 \pm 1 \text{ g C kg}^{-1}$  fraction) (Table 3).

Similar to least-squares regression, soils dominated by 1:1 and 2:1 mineral types had significantly different regression parameters (Figures 2 and 3). The slope of the boundary line regression between organic C contents and mass proportions of fine soil particles in bulk soil was  $0.84 \pm 0.01$  for soils dominated by 2:1 minerals (Table 3). This value was relatively similar to the estimate based on the organic C loading approach, suggesting that a C loading of  $\sim 1 \text{ mg C m}^2$  of 2:1 mineral with a SSA of approximately  $80 \text{ m}^2 \text{ g}^{-1}$  represents a reasonable estimate of maximal organic C stabilization. In soils dominated by 1:1 minerals, the slope of the boundary line regression was  $0.43 \pm 0.01$ . This was significantly greater than the estimate based on the organic C loading approach, and suggests that the SSA estimate of  $15 \text{ m}^2 \text{ g}^{-1}$  used in this approach underestimates the true SSA of soils dominated by 1:1 minerals. The boundary line analysis suggests that a

mean SSA of  $\sim 40 \text{ m}^2 \text{ g}^{-1}$  might be more appropriate, though this result needs to be verified experimentally. Although the boundary line analysis alleviates some of the limitations identified in the use of conventional least-squares regression, it remains essentially a statistical approach, depends on the dataset compiled, and lacks a mechanistic underpinning. Soil physical and chemical properties associated with soil clay mineralogy, such as specific surface area and cation exchange capacity, incorporated into a mechanistic model for prediction of maximal organic C stabilization on fine soil particles is preferable over a strictly deterministic model.

#### *1.3.5 Effect of land use on estimates of maximal C stabilization*

In developing the concept of protective capacity, Hassink (1997) used only uncultivated and grassland soils in the regression analysis. In the current study, data from cultivated, forest and grassland land uses were analyzed separately to generate estimates of maximal C stabilization by least-squares regression with forced-zero intercept and boundary line analysis. As expected, estimates of maximal C stabilization were greater for forest and grassland land uses compared to cultivated land use (Table 3). Least-squares regression estimates for the cultivated and grassland land uses were similar to those reported previously by Six et al. (2002), but our estimate for forest land use was significantly lower. Testing the effect of mineral type on estimates of maximal C stabilization within each land use type was not possible using boundary line analysis because of low sample numbers. However, estimates from least-squares regression differed in forest ( $0.22 \pm 0.02 \text{ mg C g}^{-1}$  for 1:1 minerals and  $0.74 \pm 0.06 \text{ mg C g}^{-1}$  for 2:1 minerals) and grassland ( $0.32 \pm 0.02 \text{ mg C g}^{-1}$  for 1:1 minerals and  $0.45 \pm 0.02 \text{ mg C g}^{-1}$  for 2:1 minerals), but not in cultivated land use ( $0.24 \pm 0.01 \text{ mg C g}^{-1}$  for 1:1 minerals

and  $0.26 \pm 0.01 \text{ mg C g}^{-1}$  for 2:1 minerals). The lack of differences in the cultivated land use is attributable to the likely presence of too many samples that were not actually at saturation.

Larger organic C saturation deficits (i.e., differences between measured organic C and saturated levels) of fine soil particles were observed in cropland compared to forest and grassland (Figures 4 and 5). The large organic C saturation deficit in cropland soils suggests that croplands are potentially important SOC sinks that could be exploited with proper land use management. As noted above, estimates of maximal C stabilization were greater using boundary line analysis compared to estimates using least-squares regression. These differences in forest and grassland land uses (Table 3) suggest that even though these soils were under native vegetation and likely at equilibrium in terms of C inputs and outputs, they have not necessarily reached their maximal C stabilization potential. The data points representing forests with large organic C saturation deficit were from an afforested plantation (Zinn et al., 2007), and also have the potential to store additional organic C inputs. In grasslands, some soils were near their organic C saturation level while others were below it, which is perhaps due to differing land management practices such as grazing intensity or the application of fertilizers. Organic C deficits of fine soil particles in cropland, forest, and grassland indicate how much additional organic C can be stabilized in the fine soil fraction. In reality, soils in these land uses can store more organic C inputs than the saturated level of fine soil particles. This additional organic C is stored in forms that are not directly associated with the minerals of fine soil particles; i.e., fine particulate organic matter. This non-mineral-associated organic matter is likely to have more rapid turnover times and not be stabilized for the long-term (Gregorich et al.,

2006). Estimates of the maximal organic C stabilized on fine soil particles must therefore take into account the physical or biochemical protection mechanisms acting on the stored C when organic C concentrations of fine soil particles are high. Few studies to date, however, have reported on the relative proportions of mineral-associated and non-mineral associated organic matter in fine soil fractions (e.g., Chenu and Plante, 2006; Virto et al., 2010).

#### **1.4. Conclusions**

The use of soils not having reached maximal organic C in a linear regression approach will cause unreliable underestimates of maximal organic C stabilization on fine soil particles, and the neglect of soil mineralogical properties will make estimates inaccurate. Methods for improving estimates of maximal organic C stabilization should represent our best current understanding of the mechanisms acting to stabilize SOC in fine soil particles. The alternative organic C loading method is proposed as a preliminary concept based on organic C stabilization on soil mineral SSA with the maximal organic C loading of  $1 \text{ mg C m}^{-2}$ . The approach is, however, not without its own problems. Ideally, SSA measurements for all samples would be necessary to more accurately determine maximal C stabilized based on organic C loadings, and the maximal loading value itself is subject to further examination particularly in regards to the effect of potential microaggregation in the fine soil fraction. The boundary line assessment used only the upper tenth percentile of samples rather than the entire data to formulate the regression to predict the maximal organic C stabilized on fine soil particles, which is presumed to better represent which samples have actually reached the maximal level of organic C stabilization. Predicted maximal organic C contents stabilized on fine soil particles using

boundary line analysis were similar to that by using organic C loading method for soils dominated by 2:1 minerals but not for soils dominated by 1:1 minerals. Although this alternative method provides a less mechanistic underpinning for improving estimates, it represents a significant improvement over the use of least-squares linear regression. The use of an organic C loading of  $1 \text{ mg C g}^{-1}$  and the role of soil mineral SSA in organic matter stabilization need further validation before they can be used to better estimate maximal organic C stabilization in soils.

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**Table 1.1**

Least-squares linear regression between organic C contents of fine soil particles (< 20  $\mu\text{m}$ , mg C  $\text{g}^{-1}$  soil) and their mass proportions (g fraction 100  $\text{g}^{-1}$  soil). Values in the third group are previously published regression parameters, and values in the fourth group were determined in the current meta-analysis using data from cited papers. Slopes and intercepts are reported as mean  $\pm$  SE. Intercept values found to be not statistically different from zero are marked with  $^{\S}$ .

Slope	Intercept	r	P	Sample size	Data source
0.37	4.07	0.89	0.001	40	Hassink (1997)
$0.32 \pm 0.03$	$1.68^{\S} \pm 1.77$	0.52	<0.01	241	Current study (2:1)
$0.23 \pm 0.02$	$1.04^{\S} \pm 1.07$	0.72	<0.01	101	Current study (1:1)
$0.41 \pm 0.01$	$3.86 \pm 0.49$	0.62	<0.05	95	Six et al. (2002) (2:1)
$0.30 \pm 0.01$	$1.22 \pm 0.37$	0.86	<0.05	45	Six et al. (2002) (1:1)
0.36	NA	0.51	<0.05	27	Liang et al. (2009)
$0.22 \pm 0.04$	$-0.52^{\S} \pm 2.13$	0.89	<0.05	12	Zhao et al. (2006)
$0.64 \pm 0.12$	$-7.63^{\S} \pm 5.58$	0.76	<0.01	21	Amelung et al. (1998)
$0.22 \pm 0.04$	$1.74^{\S} \pm 2.26$	0.81	<0.01	18	Barthès et al. (2008)
$0.31 \pm 0.03$	$0.64^{\S} \pm 1.55$	0.85	<0.01	37	Feller and Beare (1997)
$0.11 \pm 0.03$	$2.05 \pm 0.78$	0.77	<0.05	8	Guibert et al. (1999)
$0.39 \pm 0.03$	$1.39^{\S} \pm 0.82$	0.97	<0.01	12	Steffen et al. (2009)
$0.12 \pm 0.01$	$-0.34^{\S} \pm 0.40$	0.90	<0.01	16	Thuriès et al. (2000)
$0.08 \pm 0.03$	$3.62 \pm 1.42$	0.38	<0.05	36	Zinn et al. (2007)

**Table 1.2**

Over-saturation of organic C content of fine soil particles (< 20 µm) by comparison of measured organic C contents to predicted maximal organic C estimated using the Hassink (1997) regression model for soils with dominant minerals of 1:1 and 2:1 in cropland, forest, and grassland.

Mineral type	Land use	Total sample size	Over-saturated sample size	Over-saturation ratio	
				Mean	Maximum
1:1 mineral	Cropland	33	0	NA	NA
	Forest	46	2	1.14	1.22
	Grassland	22	1	1.04	1.04
	All	101	3	1.10	1.22
2:1 mineral	Cropland	131	8	1.54	2.46
	Forest	13	11	1.64	2.61
	Grassland	97	32	1.42	2.35
	All	241	51	1.49	2.61
All		342	54	1.47	2.61



**Table 1.3**

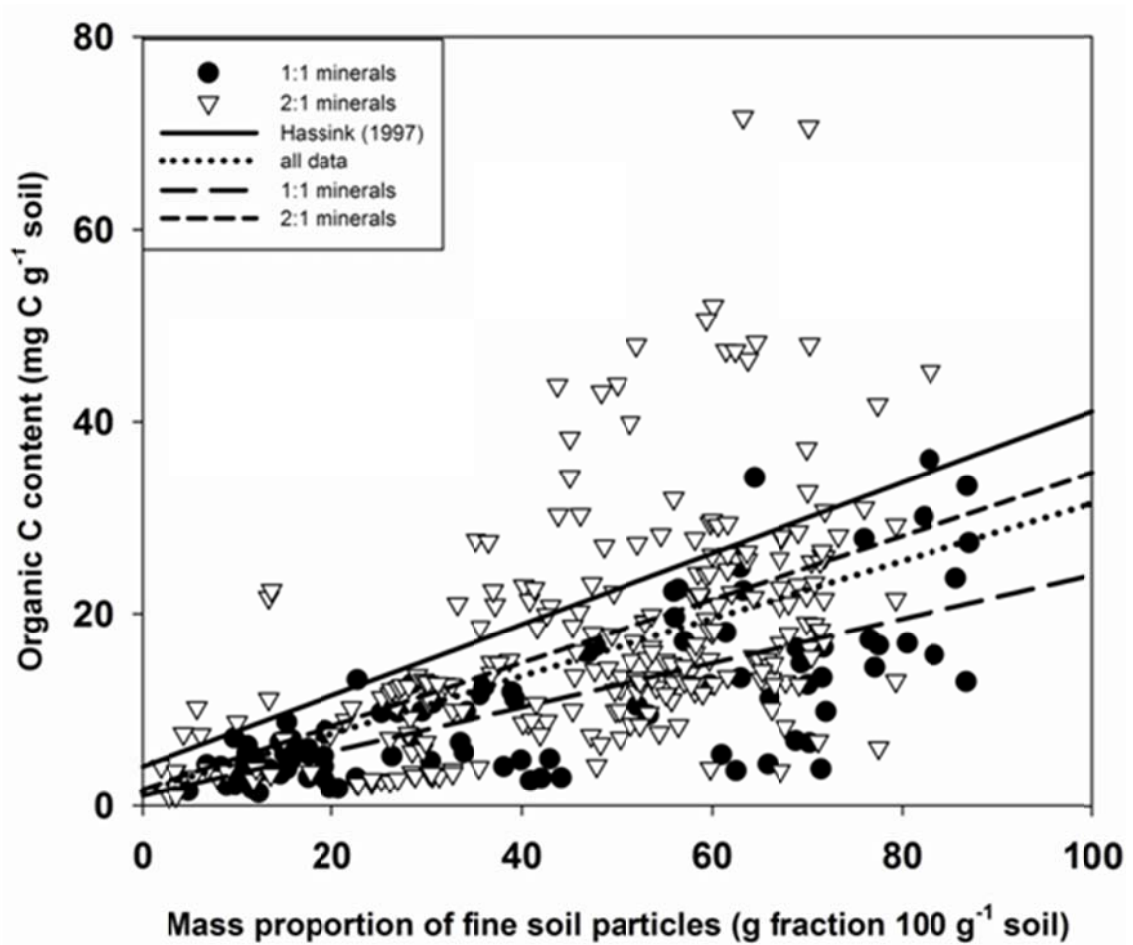
Estimated maximal organic C stabilized by fine soil particles (< 20  $\mu\text{m}$ ) as determined by the slope of least-squares linear regression (LSQ) with forced-zero intercept, organic C loading method (OCL), and boundary line analysis (BL). Slopes are reported as mean  $\pm$  SE.

Method	Dataset	Slope	RMSE	P	n <sup>†</sup>
LSQ	All	0.33 $\pm$ 0.01	9.42	< 0.0001	342
	1:1 mineral	0.25 $\pm$ 0.01	5.70	< 0.0001	101
	2:1 mineral	0.36 $\pm$ 0.01	10.23	< 0.0001	241
	Cultivated	0.25 $\pm$ 0.01	5.77	< 0.0001	164
	Forest	0.34 $\pm$ 0.04	13.04	< 0.0001	59
	Grassland	0.42 $\pm$ 0.02	9.18	< 0.0001	119
OCL	1:1 mineral	0.15			
	2:1 mineral	0.80			
BL	All	0.78 $\pm$ 0.04	9.12	< 0.0001	30
	1:1 mineral	0.43 $\pm$ 0.04	4.49	< 0.0001	5
	2:1 mineral	0.84 $\pm$ 0.04	8.57	< 0.0001	20
	Cultivated	0.45 $\pm$ 0.05	8.13	< 0.0001	12
	Forest	1.07 $\pm$ 0.08	6.44	0.0058	3
	Grassland	0.89 $\pm$ 0.05	6.32	< 0.0001	7

<sup>†</sup> Only data points from the top tenth percentile of organic C content were included in the boundary line analysis.

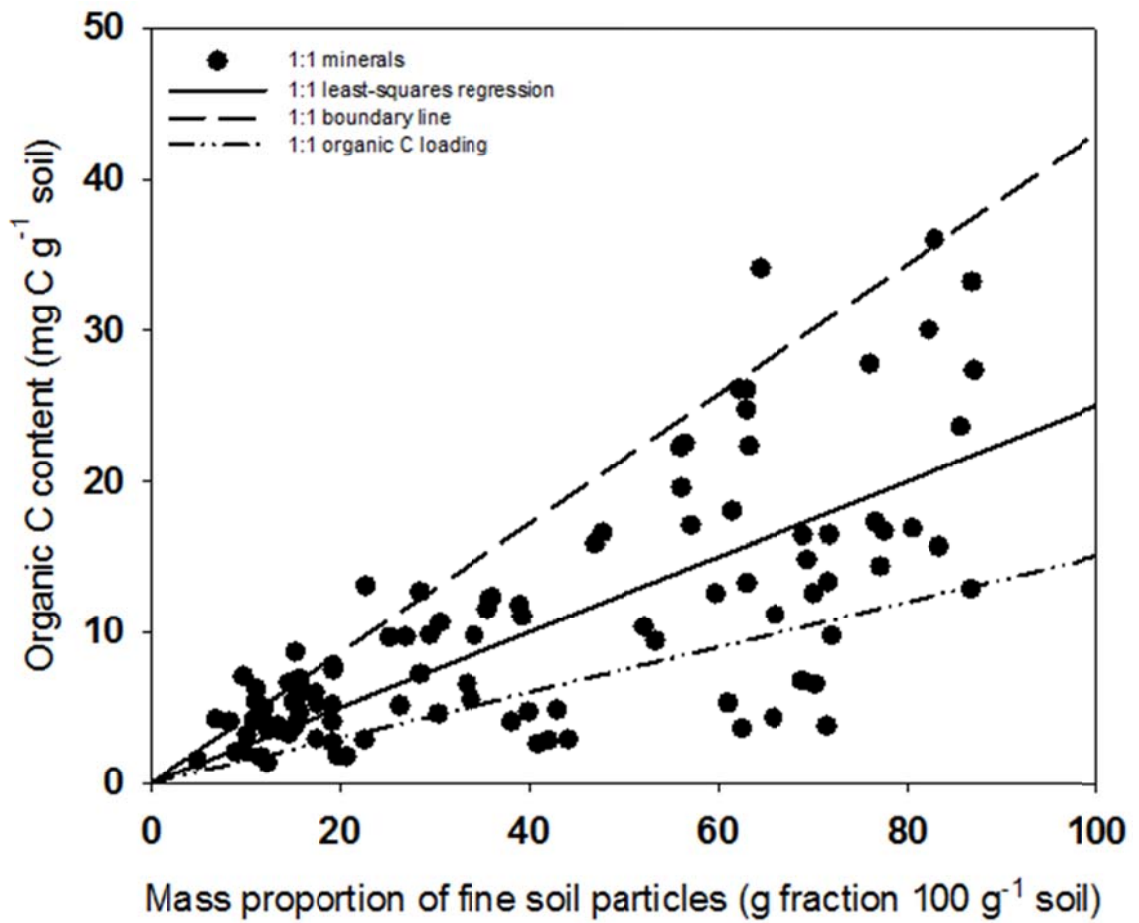
**Figure 1.1**

Least-squares linear regression of organic C content ( $\text{mg C g}^{-1}$  soil) of fine soil particles with their mass proportions ( $\text{g fraction } 100 \text{ g}^{-1}$  soil) in all bulk soils, soils dominated by 1:1 minerals, soils dominated by 2:1 minerals, and the Hassink (1997) model (1997) for all soils.



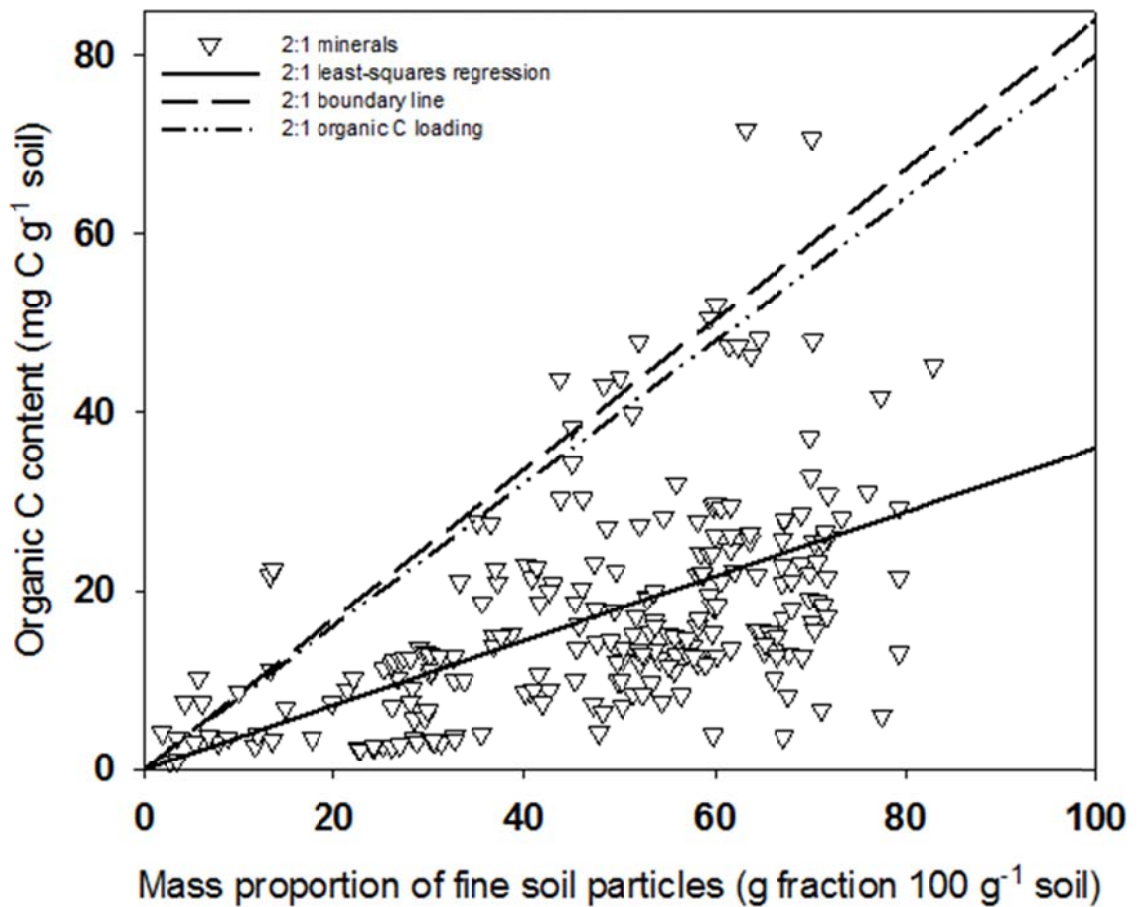
**Figure 1.2**

Predictions of the maximal organic C content of fine soil particles for soils dominated by 1:1 minerals by using the boundary line analysis, organic C loading method, and the least-squares linear regression with forced-zero intercept.



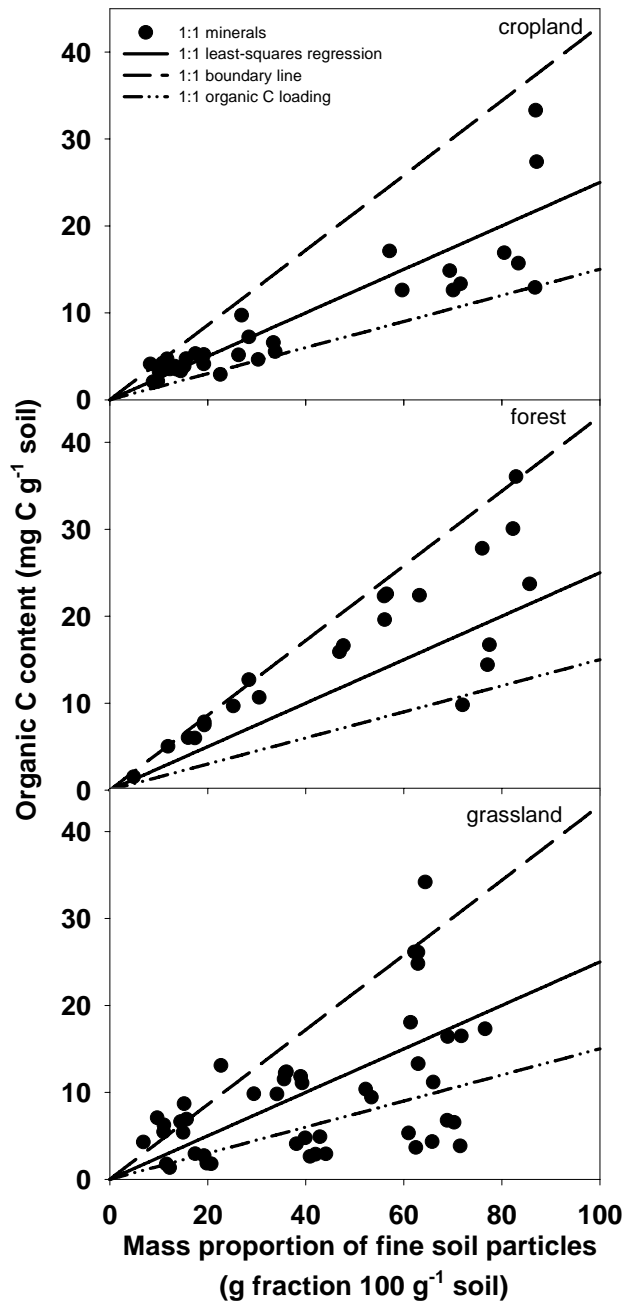
**Figure 1.3**

Predictions of the maximal organic C content of fine soil particles for soils dominated by 2:1 minerals by using the boundary line analysis, organic C loading method, and the least-squares linear regression with forced-zero intercept.



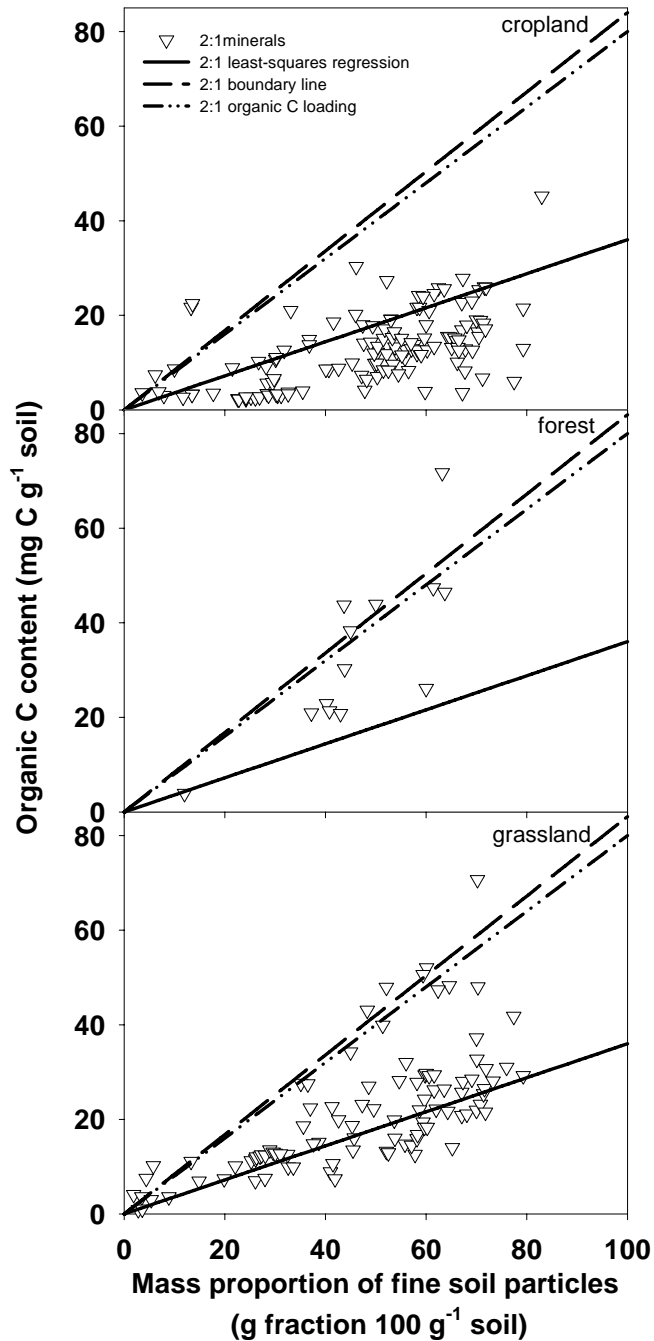
**Figure 1.4**

Current organic C content and predicted maximal organic C content of fine soil particles by using the boundary line, organic C loading method, and the least-squares linear regression with forced-zero intercept in cropland, forest, and grassland soils dominated by 1:1 minerals.



**Figure 1.5**

Current organic C content and predicted maximal organic C content of fine soil particles by using the boundary line, organic C loading method, and the least-squares linear regression with forced-zero intercept in cropland, forest, and grassland soils dominated by 2:1 minerals.



## CHAPTER TWO

Changes in organic matter stability in organo-mineral complexes with increasing C  
loading

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## **Abstract**

The soil carbon (C) saturation theory predicts that soil C storage efficiency decreases as a soil approaches C saturation, which may be due to a decline in soil organic matter (SOM) stability as the result of changes in the types, strengths, and turnover times of organo-mineral interactions with increasing organic C input. The goal of this study was to test whether the stability of organic matter associated with soil minerals decreases as organo-mineral complexes approach C saturation with increasing C loading. A series of batch sorption experiments with natural dissolved organic matter (DOM) and soil mineral components were conducted to obtain organo-mineral complexes with a range of organic C loadings. The relative stability of the C in the organo-mineral complexes generated from batch DOM sorption was subsequently assessed using laboratory incubation and evolved CO<sub>2</sub> gas analysis during thermal analyses. Values of C/N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  of organo-mineral complexes generated by DOM sorption were significantly smaller or more negative than initial soil material subjected only to water and the initial stock DOM solution, suggesting that the composition of the organic matter in the organo-mineral complexes may have changed as the amount of sorbed organic matter increased. The amount of organic C sorption determined by differences in DOM concentrations before and after sorption overestimated the actual amount of organic C sorbed on soils, which was more accurately determined using solid phase measurements. The observed linear relations between DOM sorption and initial DOM for the four types of soils suggest that these soils may not have reached C saturation under the conditions of batch sorption experiments in this study. Incubation results suggest that the relative biological stability of sorbed C increased as organic C loading increased. The size of labile C pool relative to



the total sorbed C decreased with increasing C loading, even though increases in the value of the decomposition constant indicated that the labile pool of sorbed C was being degraded more rapidly. Thermal analyses showed thermal stability of sorbed C increased as organic C loadings increased, since the temperature at which half of the CO<sub>2</sub> had evolved increased with increasing C loading for the four types of soils. Overall, the results did not support the hypothesis that SOM stability decreases with increasing C loading. This may be partly attributable to the fact that organic matter sorption on soil minerals enhanced organic matter stabilization especially when C inputs were well below the C inputs level sufficient for organo-mineral complexes to reach C saturation. The conditions of the DOM sorption experiments conducted in this study were unable to achieve C loadings greater than 1 mg C m<sup>-2</sup>, and thus further work is needed to seek either natural or laboratory-generated organo-mineral complexes to test for changes in sorbed SOM stability.

## 2.1 Introduction

Most current models of soil organic matter (SOM) dynamics assume that soil organic carbon (C) stocks increase linearly with increasing organic C inputs without an upper limit (Lugato et al., 2007; Paustian et al., 1997). In a few cases, however, soil C stocks have showed no significant increase in response to increasing long-term organic C inputs, reaching a maximal soil C level (Campbell et al., 1991; Gulde et al., 2008; Huggins et al., 1998; Soon, 1998). This phenomenon has been proposed as the soil C saturation theory (Six et al., 2002; Stewart et al., 2007; West and Six, 2007), and the maximal soil C stock as the soil C saturation level. Subsequent studies demonstrated that chemically protected organic matter associated with soil minerals as organo-mineral complexes are more likely to reach C saturation than other physical or chemical fractions or the bulk soil (Chung et al., 2008; Gulde et al., 2008). The C saturation of mineral-associated organic matter may be attributable to the finite amount of mineral specific surface area (SSA) onto which organic matter may be stabilized. The amount of organic C stabilized on soil minerals can be expressed as soil C loading, which is the mass of mineral associated C per unit of mineral surface area (i.e.,  $\text{mg C m}^{-2}$ ).

Organic C inputs are stabilized onto soil mineral surfaces through various organo-mineral bonding reactions such as ligand exchange, cation bridging, H-bonding, and van der Waal forces, depending on the composition of the organic inputs, soil mineralogy, and environmental factors (Arnarson and Keil, 2000; Gu et al., 1994; Stevenson, 1994). Organic C stabilization on soil minerals accounts for more than 50% of total soil organic C (Christensen, 1996; Kahle et al., 2002), and mineral-associated C has longer turnover times than other fractions such as particulate organic matter or physically protected

organic C in aggregates (Balesdent et al., 1987; Balesdent et al., 1988; Trumbore, 2000). Changes in the amount or stability of organic C inputs stabilized on soil minerals will therefore greatly affect bulk soil C storage.

According to the soil C saturation theory, soil C storage efficiency decreases as a soil approaches C saturation (Stewart et al., 2007; Stewart et al., 2008). A decrease in soil C storage efficiency may be due to a decline in SOM stability as soils approach C saturation, which could be the result of changes in the types, strengths, and turnover times of organo-mineral interactions with increasing organic C inputs (Kleber et al., 2007; Sollins et al., 2007). We therefore hypothesize that the stability of organic C associated with soil minerals decreases with increasing soil C loadings as the organic-mineral bonding strength changes from strong to weak. It is difficult to sample natural organo-mineral complexes from field soil samples with well constrained mineralogy but a wide range of organic C loadings reaching high values ( $>1 \text{ mg C m}^{-2}$ ). We therefore conducted a series of laboratory experiments with natural dissolved organic matter and soil mineral components to obtain organo-mineral complexes with a range of organic C loadings. The relative biological stability of the C in the organo-mineral complexes generated from DOM sorption was subsequently assessed using a laboratory incubation experiment. The goal was to test whether the stability of OM decreases as organic C loading increases.

## **2.2 Materials and Methods**

The current study is constructed as a two-step experimental design, with the first step consisting of several batch sorption experiments designed to create organo-mineral complexes with differing organic C loadings, and to determine whether these complexes exhibit organic C saturation behavior, and the second step consisting of laboratory

incubation and thermal analysis to assess the relative stability of the C sorbed on the organo-mineral complexes.

### *2.1.1 Batch sorption experiment*

The selection of the mineral and organic materials used in the batch sorption experiments was made with the goal of simulating phenomena observed in the field as directly as possible, while increasing the likelihood of observing C saturation. For this reason, soil samples from sub-surface horizons were selected instead of neat and pure minerals present in soils (*e.g.*, kaolinite, illite, goethite and gibbsite), recognizing that soil samples contain a significant amount heterogeneity in composition. Sub-surface soils were selected rather than surface soils because of low initial C concentrations that enable them to sorb greater amounts of organic C inputs as well as avoiding issues of distinguishing newly sorbed versus exchanged organic matter.

Four soils (referred to here by their soil series names: Edgemont, Drummer, San Ysidro and Towaliga) were selected for use in the batch sorption experiments (Table 2.1). The Edgemont soil was collected from the Stroud Water Research Center. Three other soils were selected from a set of 213 subsurface samples used in a previous study on DOM sorption (Mayes et al., 2012). These soils represent three soil orders, and were selected on the basis of having a maximum DOC sorption capacity ( $Q_{\max}$ ) in the upper 25<sup>th</sup> percentile of their respective soil order. While clay and Fe oxide contents have been found to be the strongest predictors of  $Q_{\max}$  (Kothawala et al., 2009; Mayes et al., 2012), the selected samples used in the current study varied in Fe oxide content and mineralogy, but did not vary widely in clay content (Table 2.1). Based on Natural Resources Conservation Service data for the soil series (NRCS-USDA, Official Soil Series

Descriptions), the dominant clay mineralogy was smectite for the Drummer and San Ysidro soils, and kaolinite for the Towaliga and Edgemont soils.

Soils were ground to pass through a 500- $\mu\text{m}$  sieve and stored air-dry prior to analysis and use in the batch sorption experiments. Soil mineral specific surface area was measured using the  $\text{N}_2$ -BET method after removing organic matter by muffling at 350 °C for 18 h. Samples were de-gassed at 325 °C for 4 h with  $\text{N}_2$  and He to remove adsorbed water. Nitrogen was dosed on the surfaces at 77 K with different gas pressures in a Tristar 300 surface area and porosity analyzer (Micromeritics, Norcross, GA). The multi-point Brunauer-Emmett-Teller (BET) method was used to calculate SSA values under the relative pressure between 0.05 and 0.3 atm.

Similar to the approach used to select the mineral component, a high C concentration, chemically complex DOM solution derived from leaf litter was selected instead of commercial humic acids or single, model compounds. A stock DOM solution was prepared by mixing surface leaf litter with water (10:1 water volume to litter mass ratio) in a 40 L plastic carboy. The litter was collected from a mixed oak-maple stand, with dominant species consisting of *Quercus palustris*, *Quercus rubra*, *Quercus alba*, *Acer rubrum*, *Liriodendron tulipifera*, and *Fagus grandifolia* at the Stroud Water Research Center, in the same area from where the Edgemont soil sample was collected. The litter was kept field moist, cut into small pieces (1-3 cm), added to the water, and allowed to soak for 48 hours at room temperature with occasional stirring to invert the mixture. The resulting suspension was decanted, passed through glass fiber filter (GF/F, 0.45  $\mu\text{m}$  diameter), and the DOM filtrate was stored at 4 °C until subsequent use. The resulting stock DOM solution was found to have a dissolved organic carbon (DOC)

concentration of  $614 \pm 7.6 \text{ mg C L}^{-1}$ .

The batch sorption experiments were conducted by mixing 0.25 g of soil with 100 mL of DOM in a 250 mL Erlenmeyer flask. DOM of differing concentrations was prepared by diluting the stock DOM solution with deionized water to target DOC concentrations of 50, 100, 150, 200, 300, 350, 400, 450, 500, 600  $\text{mg C L}^{-1}$ . The batch sorption experiment for each soil included one blank sample consisting of DOM ( $\sim 600 \text{ mg C L}^{-1}$ ) without soil to determine DOM degradation during the experiment, and a second blank composed of 0.25 g soil mixed with deionized water ( $0 \text{ mg C L}^{-1}$ ). Thus, a total of 12 Erlenmeyer flasks for each experiment were shaken for 24 h on a reciprocal shaker (180 rpm) placed inside a constant temperature chamber set to  $20 (\pm 0.2) \text{ }^\circ\text{C}$ . To maximize DOM sorption, we did not use a biocide and used room temperature instead of the more typical temperature of  $4 \text{ }^\circ\text{C}$  during DOM sorption. Previous experiments showed that microbial activity actually enhanced sorption (A. K. Aufdenkampe, unpublished work). After 24 h, the suspensions were filtered through a  $0.45 \text{ }\mu\text{m}$  cellulosic membrane (GE Osmonics, Minnetonka, MN). The organo-mineral complexes retained on the filters were washed into aluminum pans and oven-dried at  $50 \text{ }^\circ\text{C}$ . The filtrates were stored at  $4 \text{ }^\circ\text{C}$  in glass vials for a maximum of 24 hours before DOC determination. Batch sorption experiments were replicated three times for each soil, and each replicate set of experiments was run independently.

The original stock DOM solution, the initial dilution series and the filtrates after the batch sorption experiments were analyzed for DOC using a persulfate digestion and spectrophotometric method (Method 10173, Hach Company, Loveland CO). Carbon and nitrogen concentrations,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  of the organo-mineral complexes retrieved after

DOM sorption were analyzed using an elemental analyzer coupled to a stable isotope ratio mass spectrometer. Organic C loadings of the organo-mineral complexes were then determined by dividing the solid-phase C concentrations ( $\text{mg C g}^{-1}$  sample) after DOM sorption by mineral SSA ( $\text{m}^2 \text{g}^{-1}$  sample).

### *2.2.2 Laboratory incubation*

A laboratory incubation experiment was used to assess the relative biological stability of the organic matter associated with the organo-mineral complexes generated in the DOM sorption experiments. Approximately 0.2 g of organo-mineral complex was mixed with 0.2 g of acid-washed sand in 4 mL Exetainer glass vials (Labco Limited, UK), and mixed for 10 seconds on a vortex shaker. The sand was added to increase the porosity of the incubation matrix and improve aeration. Deionized water was added to the glass vials to 55% water-filled pore space to ensure optimal moisture for microbial activity. Vials were kept open with the septa caps placed loosely on the vials, and placed in a glass desiccator with the bottom filled with water to keep humidity constant. Samples were incubated for 60 days at  $25 (\pm 0.2) ^\circ\text{C}$ . Respiration rates were determined at incubation days 1, 4, 7, 10, 15, 20, 30, 40, 50 and 60. Prior to  $\text{CO}_2$  analysis, each vial was capped and flushed with 20 mL  $\text{CO}_2$ -free air, then returned to the incubator for 24 h early in the incubation experiment and for 48 h later in the incubation, to allow sufficient  $\text{CO}_2$  to accumulate in the headspace of the vial. The small headspace volume in the vials increased the potential for measurable changes in  $\text{CO}_2$  concentration generated by the small mass of samples available, but required a small sample aliquot volume for analysis.  $\text{CO}_2$  concentrations were analyzed using gas chromatography-mass spectrometry (GC-MS). Ninety  $\mu\text{L}$  of headspace sample were extracted from each vial by syringe and

injected into a Trace GC containing a 25 m Porabond-Q capillary column at 45°C (Thermo Scientific, Asheville, NC). The eluted gases were then introduced into a Delta+ stable isotope mass spectrometer (Finnigan MAT, Bremen, Germany) via an open split. Carbon dioxide concentrations were calculated by calibration of peak areas using CO<sub>2</sub>/air formulations of known concentration, and isotope data was discarded.

### *2.2.3 Thermal analysis*

The stability of sorbed organic matter was also characterized using evolved CO<sub>2</sub> gas analysis during thermal analyses. Aliquots from a subset of samples generated in the DOM sorption experiments were analyzed using a Netzsch simultaneous heat flux thermal analyzer (STA 409PC Luxx) coupled to a LI-840 CO<sub>2</sub>/H<sub>2</sub>O infrared gas analyzer (IRGA; LI-COR Biosciences, Lincoln NE). Samples were heated from ambient (~25 °C) to 105 °C at 10 °C min<sup>-1</sup>, held at this temperature for 15 minutes to remove sample moisture, then heated to 800 °C at 10 °C min<sup>-1</sup>. CO<sub>2</sub> concentrations in the evolved gas were recorded as partial pressure (ppm CO<sub>2</sub>), and the temperature at which one-half of the CO<sub>2</sub> had evolved (CO<sub>2</sub>-T<sub>50</sub>) was determined as an index of the stability of the sorbed organic matter, with the underlying assumption that thermal stability is related to biological stability (Plante et al., 2011).

### *2.2.4 Data analysis*

The amount of DOM sorbed onto the soils to generate organo-mineral complexes with differing organic C loadings was determined in three ways. The first method was to calculate the difference in DOC concentrations of the solution phase before and after the batch sorption experiment, without accounting for C losses due to decomposition (referred to as ΔDOC). The second method was to calculate corrected differences in DOC



concentrations before and after sorption by subtracting C losses attributable to decomposition (referred to as  $\Delta\text{DOC}_{\text{corr}}$ ). Decomposition C losses were estimated from decreases in the DOC concentration of the soil-free blank sample during each set of 24-h batch experiments. The third method was to calculate the difference in solid phase (soil) C concentrations before and after the DOM sorption experiment (referred to as  $\Delta\text{SOC}$ ).

The Langmuir isotherm is frequently used to relate the amount of a species adsorbed to a solid surface, to the solution concentration. We compared the amounts of DOM sorption on soils as a function of initial C concentrations of DOM to linear and Langmuir regression models to test whether or not the organo-mineral complexes were potentially exhibiting C saturation behavior. Model fitting comparisons were performed using the Akaike's Information Criterion approach with CurveExpert Professional v.1.5 (Daniel G. Hyams, [www.curveexpert.net](http://www.curveexpert.net)). In addition, soil C loading values of  $1 \text{ mg C m}^{-2}$  have been proposed as a potential maximal amount of organic C that can be stabilized on mineral particles (Feng et al., 2012; Mayer, 1994). Carbon loadings greater than  $1 \text{ mg C m}^{-2}$  have previously been observed, but are generally found in low density fractions that may include a significant contribution from particulate organic matter (Mayer and Xing, 2001; Wagai et al., 2009). The organo-mineral complexes generated in the current study are predominantly mineral with coatings of sorbed DOM, and thus the so-called monolayer equivalent ( $\sim 1 \text{ mg C m}^{-2}$ ) may be an appropriate maximum. Soils may therefore exhibit C saturation behavior if the pattern of DOM sorption with increasing initial DOM fits the asymptotic regression, or if the maximum organic C loading is close to, but does not significantly exceed  $1 \text{ mg C m}^{-2}$ .

Cumulative  $\text{CO}_2$  respired during the 60-day incubation was determined using

respiration rates measured periodically during the incubation. Values were normalized to the amount of sorbed C (*i.e.*, g CO<sub>2</sub>-C (100 g sorbed-C)<sup>-1</sup>), which was determined by subtracting the initial solid-phase C concentration from the solid-phase C concentration measured after sorption. Relative biological stability indices were determined by fitting cumulative CO<sub>2</sub> respiration data to the first-order exponential equation  $C_t = C_o(1 - e^{-kt})$ , where  $C_t$  is cumulative CO<sub>2</sub> respiration,  $C_o$  is the potentially mineralizable C, and  $k$  is the first-order decomposition constant (day<sup>-1</sup>). Data from each of the three incubated replicates from each soil-DOM concentration combination were individually fit using non-linear regression (Sigmaplot 11.0, Systat Software Inc.).

## 2.3 Results and Discussion

### 2.3.1 DOM sorption

Values of C/N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  of organo-mineral complexes generated by DOM sorption were significantly smaller or more negative ( $P < 0.01$ ) than values of initial soil material subjected only to water, and were also different from the values of these variables of initial stock DOM (Table 2.1). Values of  $\delta^{15}\text{N}$  and C/N of organo-mineral complexes obtained from DOM sorption and were smaller compared to  $\delta^{15}\text{N}$  values (5.8‰ for Edgemont soil, 4.5‰ for Drummer soil, 5.8‰ for San Ysidro soil, and 2.0‰ for Towaliga soil) and C/N ratios (8.9 for Edgemont soil, 11.4 for Drummer soil, 8.2 for San Ysidro soil, and 14.1 for Towaliga soil) of stock soils used for sorption experiment. C/N ratios of the organo-mineral complexes remained relatively constant as a function of initial DOC concentration (and therefore of final C loading), where  $P$ -values for regression slopes were generally non-significant (*i.e.*,  $P > 0.05$  for Edgemont, Drummer, and Towaliga). However,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values generally decreased as C loading

increased, with the exception of San Ysidro, which showed no statistically significant trends for either  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ . Overall, these results suggest that the composition of the organic matter in the organo-mineral complexes may have changed as the amount of sorbed organic matter increased, which is consistent with the concept of molecular fractionation during DOM sorption (Kaiser et al., 2001; Oren and Chefetz, 2012).

The amount of DOM sorbed onto the four soils differed significantly based on how it was calculated, particularly at greater initial DOM concentrations (Figure 2.1). Negative amounts of C sorption observed at initial DOM concentrations less than 100 mg C L<sup>-1</sup> suggest that C from the original soil material was being desorbed. At larger initial DOM concentrations,  $\Delta\text{DOC}$  and  $\Delta\text{DOC}_{\text{corr}}$  estimates of C sorption were significantly greater than  $\Delta\text{SOC}$  estimates. These larger differences may be because DOM degradation rates were relatively slow when initial DOM concentrations were small, and increased as initial DOM concentrations increased. Larger estimates of DOM sorption by the  $\Delta\text{DOC}$  and  $\Delta\text{DOC}_{\text{corr}}$  methods compared to the  $\Delta\text{SOC}$  method may also be due to enhanced decomposition in the presence of the mineral phase, which was not sterile, remembering that the correction for DOM decomposition was done using a soil-free blank. The results demonstrate that the  $\Delta\text{DOC}$  methods commonly used to determine organic C sorption may overestimate the actual amount of organic C sorbed on soils. The amount of C sorbed based on the solid phase ( $\Delta\text{SOC}$ ) measurements is used in our subsequent analyses and discussion because it is a direct measurement of C in the organo-mineral complexes generated, and therefore judged to be a better estimate of the amount of C being assessed for stability in the incubation experiment.

The amount of C sorbed on the soils increased with increasing initial DOM

concentrations, up to  $30.4 \pm 0.1 \text{ mg C g}^{-1}$  in the Drummer soil,  $28.1 \pm 1.1 \text{ mg C g}^{-1}$  in Edgemont,  $25.1 \pm 1.1 \text{ mg C g}^{-1}$  in Towaliga and  $24.8 \pm 0.6 \text{ mg g}^{-1}$  in San Ysidro (Figure 2.1). These sorbed C concentrations were ten times greater than the  $Q_{\text{max}}$  estimates generated by Mayes et al. (2012), which is likely attributable to differing experimental conditions. For instance, the maximal C to mineral ratio in Mayes et al. (2012) was  $6 \text{ mg C g}^{-1}$  soil versus  $24 \text{ mg C g}^{-1}$  soil in the current study. Amounts of C sorbed on all soils showed linear relationships with initial DOC concentration, and did not exhibit the same degree of asymptotic behavior as previously observed by Mayes et al. (2012). Model comparisons indicated that the likelihood that the linear model was the better model was  $>70 \%$  for each soil. The observed linear relationships indicate that these soils may not have reached C saturation under the conditions of our batch sorption experiments.

The observed increases in C concentrations resulted in increased C loadings of organo-mineral complexes. Carbon loadings increased up to  $0.75 \text{ mg C m}^{-2}$  in the San Ysidro soil,  $0.86 \text{ mg C m}^{-2}$  in Drummer,  $1.07 \text{ mg C m}^{-2}$  in Towaliga, and  $1.19 \text{ mg C m}^{-2}$  in Edgemont. The C loadings calculated using the  $Q_{\text{max}}$  estimates from Mayes et al. (2012) were significantly lower than our observations, ranging from  $0.08$  to  $0.18 \text{ mg C m}^{-2}$ . The so-called monolayer equivalent C loading of  $1 \text{ mg C m}^{-2}$  has been proposed as a maximal C loading (Feng et al., 2012; Mayer, 1994). The highest C loadings of the San Ysidro and Drummer soils were less than  $1 \text{ mg C m}^{-2}$ , suggesting that these two soils have not reached C saturation, while the highest C loading of the Towaliga and Edgemont soils were greater than  $1 \text{ mg C m}^{-2}$ , suggesting these soils may have reached C saturation.

While the evidence for C saturation behavior was inconclusive, the organic C loadings of the organo-mineral complexes spanned a sufficiently wide range, from  $0.2$  to

1.2 mg C m<sup>-2</sup> overall, to test for differences in SOM stability as a function of C loading.

### 2.3.2 Stability of C sorbed in organo-mineral complexes

Total CO<sub>2</sub> respiration (μg CO<sub>2</sub>-C g<sup>-1</sup> soil) over the 60-day incubation increased with increasing C loading for all four soils (Figure 2.2, left column). However, when normalized for the proportion of sorbed C, total CO<sub>2</sub> respiration (g CO<sub>2</sub>-C 100g<sup>-1</sup> sorbed-C) decreased with increasing C loadings initially, then reached a relatively stable value (Figure 2.2, right column). Results of the exponential model fitting found that estimates of the proportion of potentially mineralized C ( $C_0$ ) were similar to measured values, and that  $C_0$  exhibited the same patterns with C loadings (data not shown). The results suggest that the 60-day incubation was sufficiently long to allow the respiration of the mineralizable or labile organic matter pool in all four soils. Modeled organic matter decomposition rate constants of organic-mineral complexes ( $k$ ) initially increased with increasing soil C loadings, then appeared to level off in three of the four soils, with Drummer being the exception (Figure 2.3). The incubation results suggest that the relative biological stability of sorbed C decreases as organic C loading increases. While increases in the decomposition constant indicate that the labile pool of sorbed C is being degraded more rapidly, the size of this labile C pool relative to the total sorbed C (*i.e.*,  $C_0$ ) decreases with increasing C loading.

Results of CO<sub>2</sub>-EGA analyses generally support the notion that sorbed C stability increases with increasing C loading. Values of CO<sub>2</sub>-T<sub>50</sub> generally increased with increasing C loading for all four soils (Figure 2.4), though the regressions for the Drummer and San Ysidro soils were found to be non-significant due to a combination of large scatter and small slope. Greater CO<sub>2</sub>-T<sub>50</sub> values indicate a greater energy input

required to combust the sorbed organic matter, which may be attributable to either a shift in greater chemical complexity as noted above in reference to shifts in isotopic signature, or to an increase in the sorption bond strength. Patterns of CO<sub>2</sub>-EGA did not show large shifts indicative of major changes in chemical composition (Figure 2.5). Instead, increasing C loadings resulted in small accumulations of organic matter combusted at ~400-500 °C, as indicated by the growing shoulder on the main peak of CO<sub>2</sub> evolution.

## 2.4 Conclusions

A series of batch DOM sorption experiments generated organo-mineral complexes with a wide range of C loadings that did not exhibit asymptotic behavior, but also did not significantly exceed the monolayer-equivalent C loading thought to represent a potential maximum to C stabilization. With increasing C loadings, organo-mineral complexes from all four soils (Edgemont, Drummer, San Ysidro, and Towaliga) showed increasing decomposition rate constants and decreasing proportions of respired C during 60-day laboratory incubations and increasing CO<sub>2</sub>-T<sub>50</sub> during thermal analysis.

Overall, these results do not strongly support our hypothesis that SOM stability decreases with increasing C loading. This may be partly attributable to the fact that observed C loadings were generally < 1 mg C m<sup>-2</sup>. It is possible that organo-mineral associations are relatively strong and consistent until the threshold of C saturation is reached, and not until then are increases in lability observed. In addition, the maximal C loading of organo-mineral complexes at C saturation might not be 1 mg C m<sup>-2</sup> as assumed. Unfortunately, the conditions of the DOM sorption experiments were unable to achieve larger C loadings, and thus further work is needed to seek either natural or laboratory-generated organo-mineral complexes to test for changes in sorbed SOM stability.

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**Table 2.1**

Characteristic properties of four soils used for dissolved organic matter batch sorption experiments.

Site	Soil Taxonomy	Depth (cm)	Clay (%)	Silt (%)	Sand (%)	pH <sub>H2O</sub>	Total Fe (g kg <sup>-1</sup> )	Mineral specific surface area (SSA, m <sup>2</sup> g <sup>-1</sup> )	Initial C (mg g <sup>-1</sup> )
Edgemont	Hapludults	54-67			43.4	6.26	23.8	24.9	3.30
Drummer	Endoaquolls	50-75	32.3	52.7	15.0	7.51	45.03	35.3	3.12
San Ysidro	Palexeralfs	36-76	33.2	31.0	35.8	6.99	11.40	33.2	3.86
Towaliga	Hapludults	10-21	33.0	24.5	42.6	7.70	21.97	23.5	2.85

**Table 2.2**

Soil C loading ( $\text{mg C m}^{-2}$ ), soil C/N and stable isotopic signature ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , ‰) of organo-mineral complexes after 24 h batch sorption of four soils with differing initial dissolved organic C concentrations ( $\text{DOC}_i$ ,  $\text{mg C L}^{-1}$ ).

<u>Edgemont</u>					<u>Drummer</u>				
$\text{DOC}_i$	C loading	C/N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\text{DOC}_i$	C loading	C/N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
0	0.11	9.0	-23.98	3.05	0	0.14	8.4	-14.15	3.22
50	0.27	8.8	-25.35	1.33	49	0.24	7.5	-20.39	-1.17
100	0.37	7.9	-25.72	0.51	99	0.33	6.9	-22.58	-0.98
150	0.51	7.6	-26.31	0.30	148	0.36	6.6	-22.33	1.04
200	0.50	7.9	-26.32	1.03	198	0.41	6.9	-22.75	0.83
300	0.53	7.9	-26.59	0.68	297	0.47	7.3	-22.47	1.2
350	0.53	8.2	-27.16	-0.85	346	0.55	7.1	-23.53	1.69
400	0.74	8.1	-26.22	-0.42	395	0.59	6.8	-23.65	0.1
450	0.74	7.9	-26.14	0.36	445	0.66	6.9	-24.49	0.51
500	0.78	8.0	-26.42	-0.03	494	0.76	6.9	-24.98	-0.73
600	1.13	8.0	-26.69	-0.18	566	0.86	6.9	-25.53	0.62
<u>San Ysidro</u>					<u>Towaliga</u>				
$\text{DOC}_i$	C loading	C/N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\text{DOC}_i$	C loading	C/N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
0	0.13	7.9	-24.17	4.18	0	0.15	12.4	-23.27	6.36
47	0.21	7.7	-25.26	0.31	46	0.30	8.6	-25.38	-0.10
95	0.26	7.3	-26.94	0.00	93	0.38	7.1	-27.22	-0.94
142	0.31	7.2	-26.64	2.83	139	0.43	7.5	-25.66	0.83
190	0.35	7.2	-27.47	0.45	185	0.56	6.8	-26.29	0.07

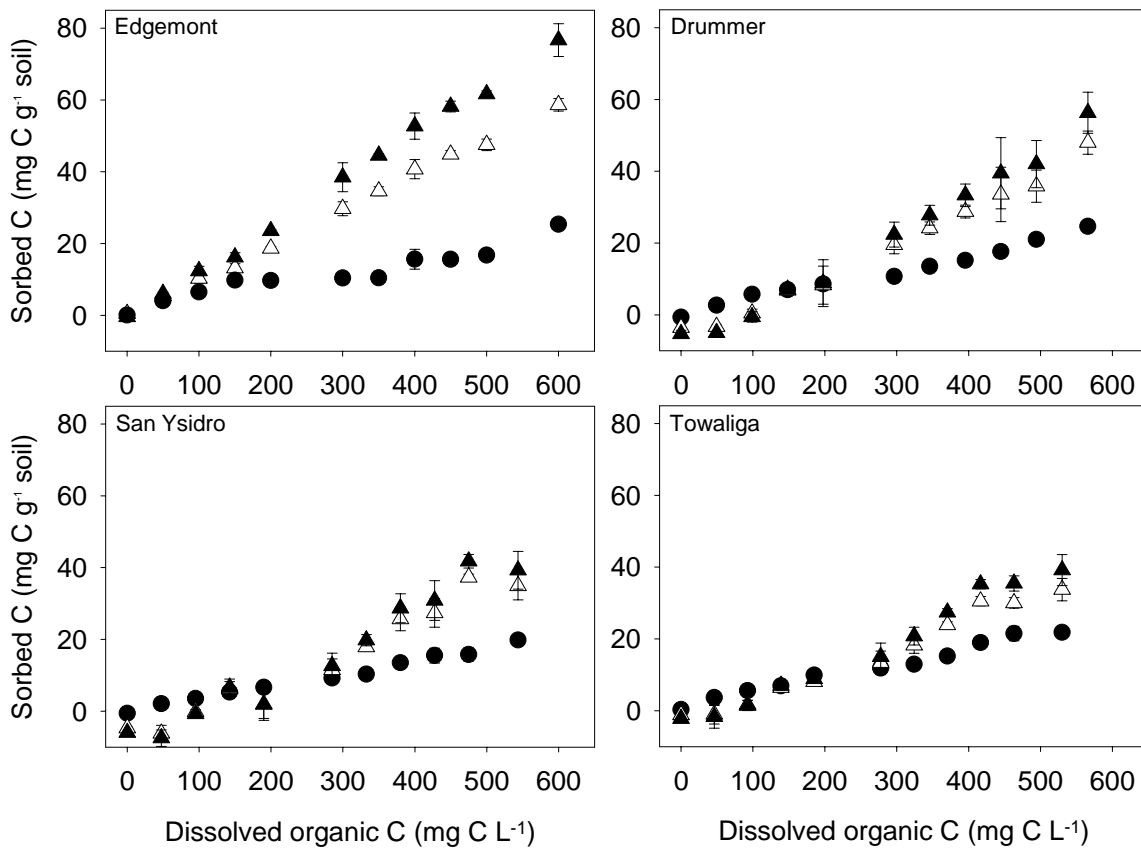
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285	0.43	7.3	-27.18	1.21	278	0.64	7.0	-26.97	-0.38
332	0.46	7.7	-26.69	1.09	324	0.69	7.4	-26.38	-1.14
380	0.56	7.8	-26.82	0.72	370	0.79	7.5	-26.71	-1.38
427	0.62	8.0	-26.71	-0.51	417	0.95	7.6	-26.82	-1.90
475	0.62	8.2	-27.18	-0.37	463	1.06	8.6	-26.86	-1.78
544	0.75	7.9	-27.28	-0.72	530	1.07	7.6	-26.65	-1.64

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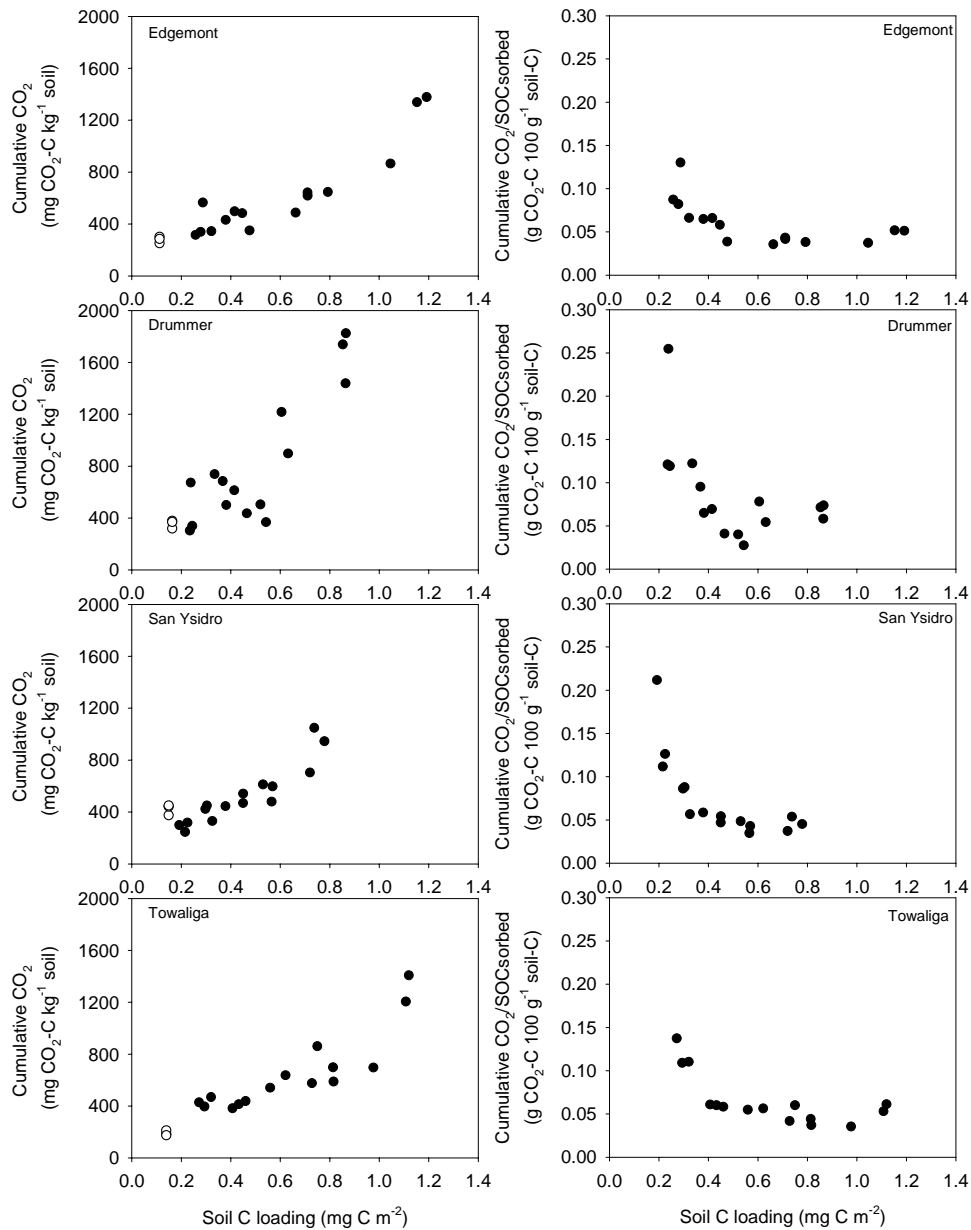
**Figure 2.1**

Comparison of amounts of sorbed dissolved organic matter in four soils (Edgemont, Drummer, San Ysidro, and Towaliga). Three methods to calculate DOM sorption are: direct measurement of soil C concentration after sorption experiment (closed circle, ●), difference of C concentrations of DOM before and after sorption experiment corrected for decomposition (open triangle, △), and uncorrected difference of C concentrations of DOM before and after sorption experiment (closed triangle, ▲). Data are mean ± SE (n=3).



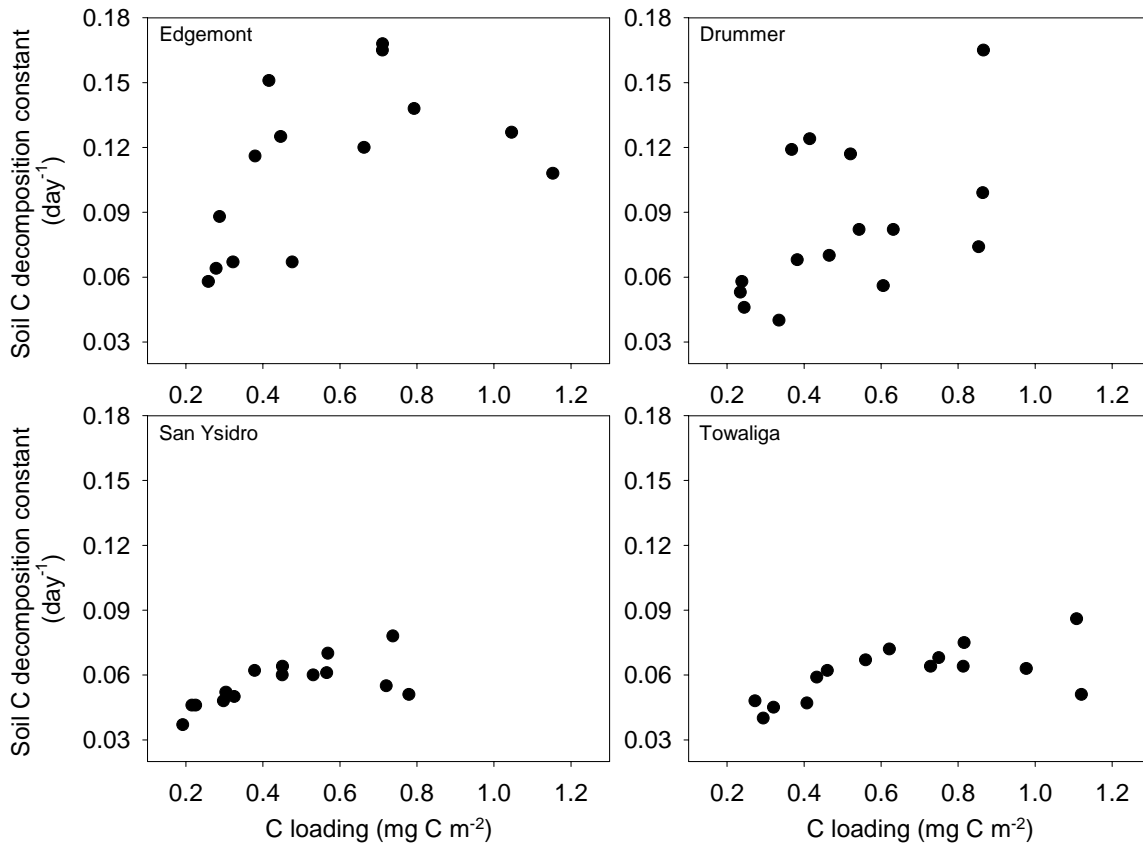
## Figure 2.2

Total ( $\text{mg CO}_2\text{-C kg}^{-1}$  soil, left column) and sorbed C normalized ( $\text{g CO}_2\text{-C } 100 \text{ g}^{-1}$  sorbed-C, right column) cumulative  $\text{CO}_2$  respired during a 60-day laboratory incubation of organo-mineral complexes from four soils (Edgemont, Drummer, San Ysidro, and Towaliga). Open symbols are control soils that did not undergo dissolved organic matter sorption.



**Figure 2.3**

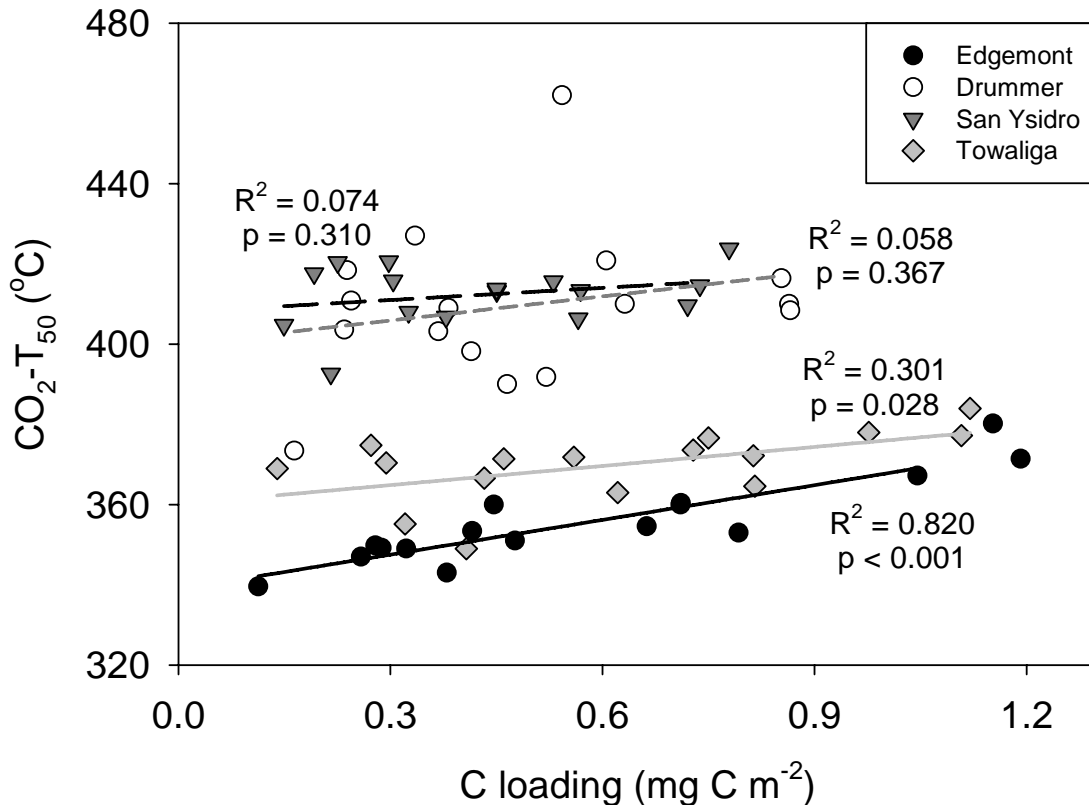
Soil organic C decomposition constants ( $\text{day}^{-1}$ ) from models of  $\text{CO}_2$  respiration during 60-day laboratory incubation of organo-mineral complexes from four soils (Edgemont, Drummer, San Ysidro, and Towaliga).





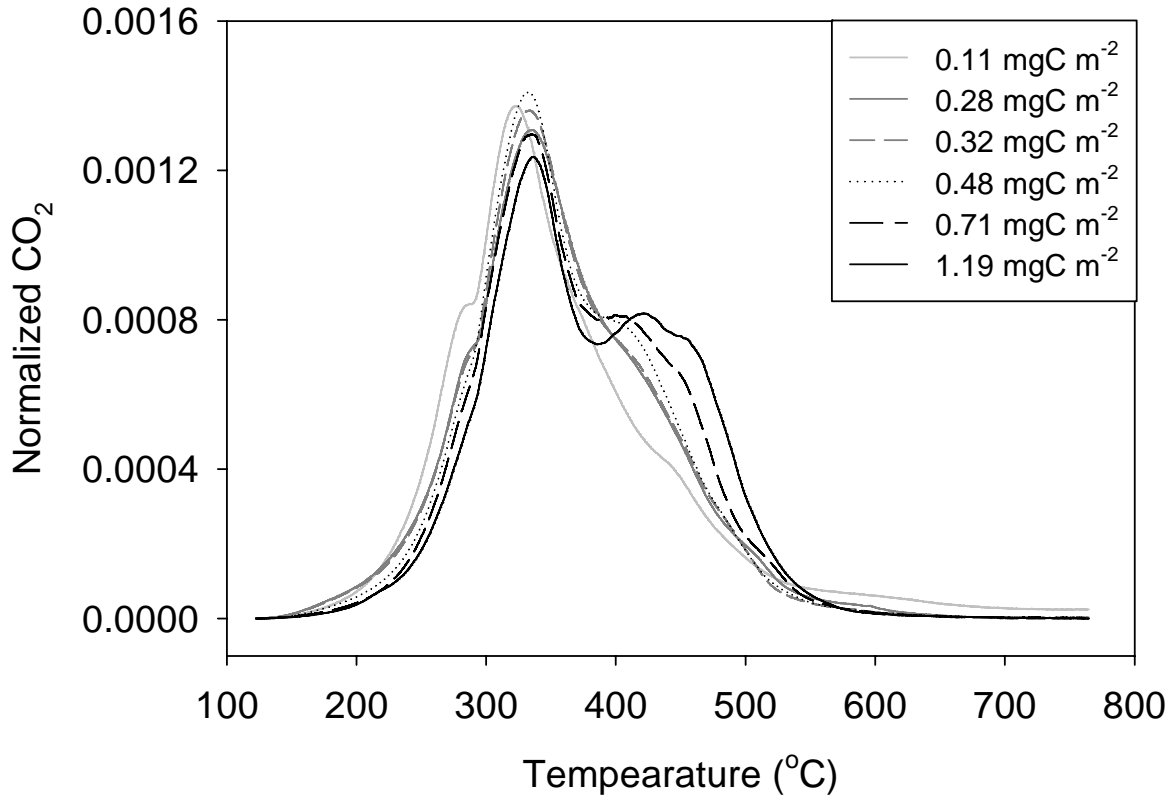
**Figure 2.4**

Temperature at which half of the evolved CO<sub>2</sub> is generated during thermal analysis (CO<sub>2</sub>-T<sub>50</sub>) as a function of organic C loading on organo-mineral complexes from four soils (Edgemont, Drummer, San Ysidro, and Towaliga).



**Figure 2.5**

Patterns of CO<sub>2</sub> evolution during thermal analysis of organo-mineral complexes from the Edgemont soil.



## CHAPTER THREE

Seeking carbon saturation behavior in agricultural soils receiving long-term manure  
amendments

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## **Abstract**

To maintain soil quality in agricultural ecosystems, it is important to restore soil carbon (C) stocks with organic C additions, and the application of the soil C saturation theory can help identify soils with large soil C storage potentials and estimate rates and durations to reach maximal soil C storage. The goal of this study was to test whether soil C saturation can be observed in a wide range of soil types in agricultural ecosystems, and to find soil mineralogical properties that could influence soil C saturation. Seven long-term agricultural field experiments at Gongzhuling, Zhengzhou, Qiyang and Laiyang, China, and at Melfort and Dixon (two experiments at this site), Canada were selected for this study. Soils from these seven experiments have different mineralogy, and represent typical soil types in the northern, middle, and southern regions in China and dominant croplands soils in the central prairies of Canada. Multiple levels of organic C inputs for 10-20 years at each experiment were used to test for soil C saturation behavior in each soil. Surface (0-20 cm) soils were sampled from the seven experiments in 2010 or 2011 were fractionated to obtain bulk soil (<2 mm), particulate organic matter (POM+sand, >53  $\mu\text{m}$ ), and silt+clay (<53  $\mu\text{m}$ ) fractions using the size and density fractionation. C concentrations of bulk soil, POM+sand, and silt+clay differed across experiments and among treatments within each experiment, and were generally higher in treatments with manure amendments. Although increases in C concentrations of POM+sand were 1.1-5.8 times greater than those of silt+clay, the proportion of C in the silt+clay fraction still accounted for 68-77% of the bulk soil C. Changes in C concentrations of silt+clay as a function of individual gross and net organic C inputs did not fit the asymptotic regression better than the linear regression, suggesting that silt+clay

fractions have not reached C saturation. However, organic C loading values for 39 out of 88 silt+clay samples were higher than presumed maximal C loading ( $1 \text{ mg C m}^{-2}$ ), suggesting that the maximal C loading may not be  $1 \text{ mg C m}^{-2}$  for all types of soils as previously suggested. In addition, soil mineralogical properties (*i.e.*, mineral specific surface area and Fe/Al oxides) were not well correlated with organic C concentrations in the silt+clay fraction from the seven experiments, indicating no significant influence of these soil mineralogical properties on the amount of organic matter bound on silt+clay in these soils. Overall, the lack of soil C saturation behavior in these agricultural soils suggests that the long-term organic C inputs from manure additions were not sufficient to fill the large C saturation deficits typical of intensely cultivated soil, but that these soils represent a significant and unmet C storage potential.

### 3.1. Introduction

Soil organic matter (SOM) is important to maintain soil quality (*e.g.*, water hold capacity and nutrient availability) for plant productivity sustainability, especially in agricultural ecosystems. Organic carbon (C) stored in croplands (111-170 Pg C) accounts for approximately 10% of total soil C up to 1 meter (1500 Pg C) on earth (Eswaran et al., 1993; Jobbagy and Jackson, 2000; Paustian et al., 1997). Soils in croplands are frequently C depleted compared to soil C level before cultivation, which enables croplands soils to serve as a potential C sink to slow down the increasing rate of the atmospheric CO<sub>2</sub>. It is estimated that 50 Pg C or more has been lost from agricultural soils and therefore are required to restore to the prior soil C storage level (Lal, 2004a; Lal, 2004b; Paustian et al., 1998).

In agricultural ecosystems, land use management practices, such as fertilizer application, crop rotation, plant residue return, and compost and manure inputs, are used to enhance soil quality, which can simultaneously increase soil organic C (SOC) stock.

Subjected to land use management practices, SOC stock are expected to linearly increase with increasing organic C inputs as suggested by most current SOC models. However, it has been found that SOC storage efficiency (*i.e.*,  $\Delta\text{SOC}/\Delta\text{organic C inputs}$ ) decreased even approached zero with increasing organic C inputs in some agricultural sites (Campbell et al., 1991; Gulde et al., 2008; Huggins et al., 1998), which suggests the existence of an upper limit of SOC storage. These observations have been proposed as the soil C saturation theory (Six et al., 2002). West and Six (2007) have proposed that soils firstly reach C steady state multiple times when organic C inputs equal outputs, and eventually achieve C saturated level. According to the soil C saturation theory, a soil has

the maximal SOC storage (*i.e.*, C saturated level), and SOC storage efficiency decreases as this soil approaches C saturated level. Therefore, the determination of soil C saturated level can help identify soils with great C storage potentials, and the quantification of SOC storage efficiency in the process of soils approaching C saturation can be used to estimate rates and durations needed to reach the maximal C storage in a specific management-soil-climate condition.

In the studies from which soil C saturation has been observed, fine soil particle were more likely to reach C saturation than coarse soil particles and bulk soil (Gulde et al., 2008; Hassink, 1997; Stewart et al., 2008). It may be because organic matter of fine soil particles are protected by sorption to soil minerals, whereas soil minerals have finite mineral surface areas. Thus, the amounts of organic C that can be stabilized on soil minerals would be limited by finite mineral surface areas. Amounts of organic C stabilization by soil minerals can also be indicated by organic C loading, showing amounts of organic C on per unit of mineral surface area (*e.g.*, mg C m<sup>-2</sup>). In addition, the maximal organic C loading has been proposed as 1 mg C m<sup>-2</sup> for solid particles by Mayer et al. (1994). Few studies have found organic C loadings higher than 1 mg C m<sup>-2</sup> for soils, except for top horizon soils or light fraction of soils which mainly consist of organic substances (Mayer and Xing, 2001; Wagai et al., 2009). Moreover, organic C that can be stabilized by fine soil particle accounts for a large percentage of entire SOC stock (Christensen, 1996; Kahle et al., 2002a), which makes the understanding of C saturation of soil minerals more meaningful.

Organic matter that can be stabilized on soil minerals would be affected by soil properties associated with soil mineralogy (*e.g.*, mineral specific surface area (SSA) and

charge characterization) and environmental factors (*e.g.*, temperature, moisture, and pH), which would, therefore, influence the processes of soil minerals approaching C saturation. In addition to influences of soil mineralogy and environmental factors on C saturation of soil minerals, organic C inputs would also affect C saturation of soil minerals. After examining the arrival of soil C saturation from agricultural sites in north America, Stewart et al. (2007) found that a wide range of organic inputs are needed for soils to approach C saturation, and pointed out that different mineralogy, climate, and local environmental factors across sites would complicate the interpretation of the arrival of soil C saturation with increasing organic C inputs.

To maintain soil quality in agricultural ecosystems, it is important to restore SOC stock with organic C additions, and the application of the soil C saturation theory can benefit the identification of soils with great SOC storage potentials and the estimates of rates and durations to reach maximal SOC storage. However, few studies have addressed the factors and mechanisms regulating C saturation behaviors with continuous organic C inputs for different types of soils, especially for fine soil particles. To achieve it, it is necessary to find different types of soils have reached C saturation. This chapter was to primarily test whether or not soil C saturation can be observed in a wide range of soil types in agricultural ecosystems, and to find soil mineralogical properties that could influence soil C saturation. This study also aimed to examine changes in organic C storage efficiency of bulk soil and fine and coarse soil particles at different levels of manure amendments. Seven agricultural experiments have been selected for this study. Soils from the seven experiments have different mineralogy, and represent typical soil types in the northern, middle, and southern regions in China and dominant croplands soils



in Canada. Meanwhile, each experiment has three or four levels of manure inputs for 10-20 years. The multiple levels of manure inputs in each experiment enable the examination of arrival of C saturation for each soil type without interference with soil mineralogy, climate, and local environmental factors across experiments. And the long duration of manure amendments may enable soils to pass C steady states to reach C saturation.

## **3.2. Methods**

### *3.2.1 Study sites and sampling*

The primary requirement for testing for soil C saturation behavior is a set of soils with differing rates of long-term C inputs, with all else being equal. We selected several long-term agroecosystem experiments performed at six sites receiving different forms and rates of organic C additions. The experiments and sites provided a range of site variables (*e.g.*, MAT, MAP, soil pH, texture, and mineralogy), as well as differing types and amounts of organic C inputs from manure and fertilizer applications, and crop rotations (Table 3.1).

#### *3.2.1.1 Gongzhuling, Zhengzhou and Qiyang*

The Gongzhuling, Zhengzhou, and Qiyang experimental sites are part of the National Soil Fertility and Fertilizer Effects Long-term Monitoring Network in China. Soils from these three sites were selected because they share the same experimental design, and represent typical soil types in the northern, central, and southern regions of China. Experimental plot sizes are 400 m<sup>2</sup> at Gongzhuling and Zhengzhou, and 200 m<sup>2</sup> at Qiyang (Xu et al., 2006). Although there is no field replication of experimental plots in these three sites, soils were randomly sampled from three different locations within each plot and kept separate for further analyses. The large plot sizes allowed the use of these

samples as pseudo-replicates to account for within plot variability. Surface (0-20 cm) soil samples were collected from Gongzhuling in October 2010, from Zhengzhou in May 2011, and from Qiyang in October 2010.

Prior to the initiation of the field experiments, the fields had been under continuous corn (Gongzhuling) or corn-wheat (Zhengzhou and Qiyang) cropping for at least ten years. The fertilizer experiments were initiated in 1990, when cropping practices were continued as before, and eleven fertilizer treatments were initiated at all three sites. Of those treatments, the following four treatments were selected for the current study: non-fertilized as the control (CK), mineral fertilizer additions (NPK), mineral fertilizer and livestock manure additions (NPKM), and 1.5 times both mineral fertilizer and manure inputs as those in the NPKM treatment (1.5NPKM). Mineral fertilizers were added as urea or diammonium phosphate for N, calcium superphosphate for P, and potassium chloride or potassium sulfate for K. Fertilizer addition rates varied from site to site, but were consistent through all years of the experiment (Table 3.2). Manure addition rates also varied from site to site, but were designed to ensure a fixed ratio of organic to inorganic N of 7:3 at all three sites. The overall mean C concentration of the manure applied over the duration of the field experiment was 169-388 g C kg<sup>-1</sup> dry matter and C/N ratio of 20 (Table 3.2). In Gongzhuling, manure and mineral fertilizers were applied before seeding (*i.e.*, 1/3 N, and all P and K) and the remaining 2/3 mineral N fertilizer was applied at the stem elongation stage. In Zhengzhou and Qiyang, manure and mineral fertilizers were applied before seeding (*i.e.*, 1/2 N, and all P and K) and the remaining N mineral fertilizer was applied at wheat elongation and flowering and corn tasseling. At the Zhengzhou and Qiyang sites, annual amounts of fertilizer applied for corn growth

were 7/3 of those applied for wheat growth.

### 3.2.1.2 Laiyang

Soils were collected from the Laiyang site in Shandong, China. This site is the first experimental site in China designed to monitor the long-term effects of mineral and organic fertilizers applied at different rates and combination ratios on crop yields, and has been in operation since 1978 (Yao et al., 1991). In Laiyang, the treatments selected were the control (CK), normal manure inputs (1×), and double manure inputs (2×). The experimental plot sizes are 33.3 m<sup>2</sup> for each treatment, and each treatment has three field replicates. The pig manure addition rate is 7.2 Mg (dry weight) ha<sup>-1</sup> in the (1×) treatment, and the C concentration of the manure is 133-333 g C kg<sup>-1</sup> dry matter, resulting in C addition rates of 2.06 Mg C ha<sup>-1</sup> yr<sup>-1</sup> and double this amount in the (2×) treatment. Soil samples were collected from the top 0-20 cm horizon in May 2010.

### 3.2.1.3 Dixon and Melfort

Samples were collected from two long-term manure management research sites in Saskatchewan, Canada. These sites were identified from a literature search for experiments using multiple addition rates of long-term manure additions. At each site, liquid swine manure has been applied at different rates and sequences for more than ten years. A similar experiment with solid cattle manure was present at the Dixon site only. The original objective of these experiments was to assess how different rates and sequences of application of liquid swine manure and solid cattle manure influence soil nutrient amounts and distribution, crop nutrition and yield (Stumborg et al., 2007). The Dixon site is located in east-central Saskatchewan, while the Melfort site is located in north-central Saskatchewan. Both sites are on loamy Mollisolic soils. Prior to the

initiation of manure inputs experiment (fall of 1996 at Dixon and fall of 1999 at Melfort), no manure had been applied. The experimental design at both sites is a randomized complete block design with four replicates for each treatment, consisting of 30 m × 6 m plots.

At the Dixon site, liquid swine and solid cattle manure have been applied at different rates and sequences: no fertilizer as the control (CK), one time manure inputs (1×), two times manure inputs (2×), and four times manure inputs (4×) (Table 3.2). Liquid swine manure effluent from a nearby earthen storage pit was injected 10-13 cm deep using a low disturbance injector each year. The cattle manure was broadcast and incorporated using a rotary tiller every year. Surface soil samples (0-20 cm) were collected in October 2010 from four treatments for the current study:

At the Melfort site, only liquid swine has been applied at different rates. Surface soil samples were collected in October 2010 from three manure input rate treatments: no fertilizer as the control (CK), a one-time manure application each year (1×), and a four-times manure application every third year (4×). Liquid swine manure was applied to soils at the Melfort site in the same way as the Dixon site.

### *3.2.2 Soil fractionation*

Soil samples were air-dried and passed through a 2-mm sieve prior to being shipped to the University of Pennsylvania. Samples were further separated using a combined size-density fractionation scheme. Size fractionation was used to obtain the coarse soil fraction (> 53 μm) consisting of particulate organic matter (POM) and sand as a relatively labile, non-saturatable soil C pool. The fine soil fraction (< 53 μm) consisted of silt and clay as a relatively stable, saturatable soil C pool. Subsequent density

fractionation was used to further separate the fine fraction into the *uncomplexed* pool consisting of free, particulate organic matter in the light fraction, and the *bound*, organo-mineral pool in the heavy fraction (Chenu and Plante, 2006) .

In the size fractionation, 30 g of air-dried soil sample were combined with 10 glass beads (6 mm diameter) and 100 mL deionized water in a 500 mL Nalgene bottle, and shaken for 18 hours on a reciprocal shaker (120 rpm). The suspension was poured onto a 53- $\mu\text{m}$  sieve and washed until the solution passing the sieve was clear. The fraction retained on the sieve (*i.e.*, POM+sand) was washed into a pre-weighed aluminum pan and oven dried at 50 °C. The fraction passing through the sieve (*i.e.*, silt+clay) was freeze-dried. In the subsequent density fractionation, 6 g of freeze-dried silt+clay fraction was vigorously mixed with 30 mL sodium polytungstate (SPT, density = 1.8 g cm<sup>-3</sup>) for 10 seconds on a vortex shaker and allowed to stand for 48 hours. The light fraction and SPT solution were siphoned, vacuum filtered and washed on a Whatman #42 filter, while the heavy fraction was washed with deionized water three times by centrifugation, then oven-dried.

### 3.2.3 Chemical analyses

Total C and N concentrations in the bulk soil, POM+sand and silt+clay fractions were analyzed by dry combustion using a Carlo-Erba NA 1500. Samples were ground to pass through a 500- $\mu\text{m}$  sieve prior to CN analysis. Soils from Gongzhuling and Zhengzhou were found to contain carbonate, and therefore organic C concentrations in these samples were determined after removing carbonates using acid fumigation (Harris et al., 2001). Samples were placed in silver capsules, moistened, fumigated with concentrated HCl for 48 h, oven-dried at 50 °C, then analyzed for C concentrations.

As the relatively stable and saturatable soil C pool, and therefore the fraction of primary interest, the silt+clay fraction was subjected to several additional analyses to generate information about the nature of the organo-mineral complexes and potential mechanisms for C saturation. Mineral specific surface area of the silt+clay fraction was determined by the N<sub>2</sub> adsorption analysis. Before SSA analysis, organic matter in silt+clay fraction samples was removed by muffling in a normal atmosphere at 350 °C for 18 hours (Keil et al., 1997). In addition, all samples were de-gassed at 325 °C for 4 hours by N<sub>2</sub> and He to remove adsorbed water. Nitrogen was dosed on the surfaces at 77 K with different gas pressures in a series of batch adsorption experiments in a Tristar 300 surface area and porosity analyzer (Micromeritics, Norcross, GA). The multi-point Brunauer-Emmett-Teller (BET) method was used to calculate SSA values under the relative pressure between 0.05 and 0.3 (Brunauer et al., 1938). Mineral SSA was measured to determine C loading (mg C m<sup>-2</sup>), which is calculated by dividing C concentration (mg C g<sup>-1</sup>) by SSA (m<sup>2</sup> g<sup>-1</sup>), and can be used as an index of the degree of C saturation (Feng et al., 2012).

Cation exchange capacity of the silt+clay fraction after removing organic matter by muffling was determined by the silver-thiourea method (Dohrmann, 2006; Oorts et al., 2003). Half a gram of freeze-dried silt+clay were extracted by ethanol-glycol with a 50:1 solution-to-soil ratio on a reciprocal shaker for 1 hour. The suspensions were filtered (Whatman #42), washed with 15 mL ethanol-glycol, and air-dried on the filter papers. After air-drying, soils and filter papers were placed together in a 100-mL polyethylene bottle with 50 mL of 0.01 mol L<sup>-1</sup> silver-thiourea, and shaken for 4 hours. The extract was then filtered (Watman #42) and the filtrate was stored in glass containers at 4 °C. The

silver concentration of the filtrate was analyzed by inductively coupled plasma spectroscopy (Spectro Genesis, Mahwah, NJ). The amounts of silver sorbed to soils, indicated by the difference of silver concentrations before and after extraction, were used to calculate the cation exchange capacity.

Total Fe/Al concentrations in the silt+clay fractions were analyzed using the dithionite-citrate extraction method. Briefly, 0.25 g of sample were mixed with 0.5 g sodium dithionite and 25 mL sodium citrate (22%) in 50-mL centrifuge tubes and shaken for 16 hours. Five mL of 0.05 mol L<sup>-1</sup> MgSO<sub>4</sub> was added as flocculent to each sample. After centrifugation at 1520 × g for 15 min, the supernatant was appropriately diluted to determine Fe/Al concentrations using inductively coupled plasma spectroscopy (Spectro Genesis, Mahwah, NJ) (Kahle et al., 2002b). Amorphous Fe/Al concentrations were determined using acid ammonium oxalate extraction. Briefly, 0.25 g of silt+clay was mixed with 25 mL ammonium oxalate (0.2 mol L<sup>-1</sup>) and oxalic acid (0.2 mol L<sup>-1</sup>) at pH 3, and shaken for 4 hours in the dark. The extract was centrifuged at 1520× g for 30 min, and Fe/Al concentrations of the supernatant were measured by inductively coupled plasma spectroscopy (Spectro Genesis, Mahwah, NJ) (Blakemore et al., 1987).

#### *3.2.4 Estimation of organic C inputs*

Gross organic C inputs were estimated as the sum of manure C inputs and plant C inputs in the seven experiments. Manure C inputs were calculated using the frequency of manure application, annual manure application rates, and average C concentrations of manure applied in these experiments (Table 3.2). Plant C inputs were estimated by multiplying the sum of above-ground and below-ground biomass residues with C concentrations of plant residues at each experiment. Annual above-ground residues were

calculated from standard regression equations to convert measured grain yields to estimate above-ground residues, and below-ground residues were estimated using ratios of below-ground residues to above-ground biomass (IPCC et al., 2006 Guidelines Vol. 4) (Table 3.3). Grain yields for each crop in these seven experiments are from published technical reports or personal communications, and are relatively constant during the experimental period. At the Gongzhuling, Zhengzhou, Qiyang, and Laiyang sites in China, all above-ground plant material is removed from the field with the exception of short stubble (3-10 cm), and therefore plant C inputs consisted of below-ground plant C inputs only. In Gongzhuling, Zhengzhou, Qiyang, and Laiyang sites, plant C concentration used for corn was 44.4% and for wheat was 39.9% (NCATS, 1994). While at the Melfort and Dixon sites, a plant C concentration of 43.0% was used for plant residue of barley, oat, wheat, canola, and flax (Kong et al., 2005).

Net organic C inputs were C concentrations of bulk soil. It is known that C concentration of bulk soil is the results of manure and plant residue addition to soils and decomposition of these organic C inputs. Additionally, C concentrations of bulk soil were correlated with gross organic C inputs in these experiments, and have been proved to be a proxy of organic C inputs by Stewart et al. (2008).

### *3.2.5 Statistical analysis*

Treatment differences in the C concentrations of bulk soil, mass and C distributions between the size fractions, and enrichment factors of silt+clay fraction which were calculated by dividing organic C concentrations of silt+clay to organic C concentrations of bulk soil were determined using ANOVA and multiple comparisons using the Tukey HSD method in JMP7.0 (SAS Institute, Cary, NC).



Soil C saturation is characterized by an asymptotic relationship between soil C concentration and C inputs at steady state (Six et al., 2002; Stewart et al., 2007; Stewart et al., 2008). In the seven agroecosystem experiments used in this study, the fertilization, residue management, and manure application treatments have been in place for more than 10 years, and soil C concentrations were therefore considered to have approached steady state. Changes in C concentrations of silt+clay with increasing rates of C inputs have been used to fit the linear and the asymptotic regressions. Carbon inputs used in the regression fitting were gross and net organic C inputs. The linear and asymptotic regression fitting was done by using CurveExpert Pro v1.5 ([www.curveexpert.net](http://www.curveexpert.net)), and slopes from the linear regression and C saturated levels from the asymptotic regression as well as AIC values for both the linear and asymptotic regressions were reported for each experiment. A model with smaller AIC value is considered to be a better fit.

Linear regressions between C concentrations and mineralogical properties (*i.e.*, mineral SSA, CEC, Fe<sub>d</sub>, Fe<sub>o</sub>, and Al<sub>d</sub>) of silt+clay fraction were performed in JMP 7.0 (SAS Institute, Cary, NC) to determine the influence of various soil mineralogical properties on amounts of organic C associated with fine soil particles.

### **3.3. Results and discussion**

#### *3.3.1 Bulk soil C responses to long-term C additions*

The various long-term agronomic treatments in each of the experiments resulted in a range in gross C inputs, from the lowest (5.7 Mg C ha<sup>-1</sup>) in Melfort to the highest (184.7 Mg C ha<sup>-1</sup>) in Laiyang, with a small increase compared to the control from NPK addition and a large increase from the manure additions (Table 3.4). The resultant organic C concentrations of bulk soil differed across experiments (0.51-4.58%) and among

treatments within each experiment (Table 3.4). Except for samples from the Melfort and Dixon swine experiments, organic C concentrations of bulk soil were significantly higher in treatments with manure amendments compared to the control and the treatment with mineral fertilizer application alone.

Soil organic C storage efficiency is indicated by slopes of changes in organic C concentration as a function of organic C inputs. With increasing gross organic C inputs, organic C storage efficiency of bulk soil decreased only in the Melfort and Dixon cattle experiments and increased in the other five experiments (Figure 3.1). Decreased SOC storage efficiency of bulk soil with increasing organic C inputs in Melfort might be due to fluctuations within a narrow range since gross organic C inputs in this experiment were small and had a narrow range. Decreased SOC storage efficiency of bulk soil with increasing organic C inputs in Dixon cattle suggests increase rates of organic C concentrations of bulk soil would slow with additions of organic C inputs. Increases in SOC storage efficiency in other five experiments indicate that increased rates of organic C concentrations of bulk soil will still rise.

### *3.3.2 Distributions of soil mass and C in physically isolated fractions*

Soil mass recovery after the size fractionation to separate soils into POM+sand and silt+clay fractions ranged from 94.8 to 100.0% for all treatment-site combinations (Table 3.5). Mass proportions of POM+sand fraction differed from site to site (9.0-59.4%), and showed general increase patterns with increasing organic C inputs in all the sampling sites except the decrease for samples from Laiyang and no change for samples from Melfort (Table 3.5).

Carbon recoveries after the size fractionation were significantly less than 100%

(Table 3.5), indicating that an average of 5.9% of the initial soil C was solubilized during the fractionation. Carbon concentrations of POM+sand were different across sites and among different treatments within each of the seven experiments, and C concentrations of bulk soil had quite similar patterns in the seven experiments (Table 3.4). Carbon concentrations of POM+sand had greater variations (0.1-8.9%) relative to C concentrations of silt+clay both across experiments and with different treatments within each experiment. Significantly higher C concentrations of POM+sand were observed in treatments with manure amendments relative to the treatment of the control and the treatment of mineral fertilizer application alone.

Organic C concentrations of silt+clay were also different across experiments (0.8-4.2%) and among treatments within each experiment (Table 3.4). Organic C concentrations of silt+clay were significantly higher in treatments with manure amendments than in the control or mineral fertilizer treatments. Enrichment factors of silt+clay relative to bulk soil were approximately 1.00 in most samples, except values much higher than one (1.32-1.88) in Laiyang and much lower than one (0.68-0.93) in Gongzhuling. High enrichment factors of organic C concentration of silt+clay relative to bulk soil in Laiyang may be due to the sandy soil texture with the percentage of sand (>53  $\mu\text{m}$ , 55%), where silt+clay is more capable than sand to store organic C inputs. And the low enrichment factors of organic C concentrations of silt+clay relative to bulk soil in Gongzhuling suggest that organic C additions were preferably stored into soil fractions other than silt+clay in this experiment.

Carbon contents of POM+sand accounted for only 9-39% of the bulk SOC, and generally increased with fertilizer and manure treatments (Table 3.4). Proportions of C in

the silt+clay fractions differed from site to site, with the lowest percentages of C contents of silt+clay in Laiyang (64.5%). Moreover, C contents of silt+clay were significantly higher in treatments with manure amendments than treatments without manure amendments only in Gongzhuling and Qiyang, and Dixon cattle experiments. Organic C contents of silt+clay were significantly higher at treatment with manure application than those at treatments without manure inputs only in Gongzhuling and Qiyang. The results indicate that manure amendments to soil would more likely to cause increased organic C in POM+sand fraction than in silt+clay fraction, reflecting the large POM increase from the manure itself.

Sample mass recovery after the density fractionation to separate silt+clay into light and heavy fractions ranged from 95.0 to 101.9% (Table 3.5). Values above 100% suggest that the removal of polytungstate was incomplete in some samples. Density fractionation was not completed for the Dixon cattle, Dixon swine and Melfort samples because the mass proportion of the light fraction was found to account for an average of only 0.6% (Table 3.5). These results suggest that even in the large manure addition rate treatments, where a large amounts of uncomplexed organic matter might be expected, the amount of uncomplexed organic matter in the silt+clay fraction was negligible and organic matter in the silt+clay fraction can be considered to be bound to soil minerals.

### *3.3.3 Soil C saturation behavior*

Changes in organic C concentrations of silt+clay as a function of gross C inputs can fit both the linear and the asymptotic regressions, except for data from Melfort and Dixon swine (Table 3.6). Comparing the linear and the asymptotic fittings, the linear regression is a better fit for changes in organic C concentration of silt+clay as a function

of organic C inputs in the five experiments, since AIC values were lower for the linear regression than for the asymptotic regression. The results suggest that silt+clay fraction from the five experiments have not reached C saturation.

One reason why silt+clay fraction has not reached C saturation might be narrow ranges of gross organic C inputs. Ranges of gross organic C inputs from manure and plant residues are lower than  $6.0 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  in these experiments except in Qiyang and Laiyang. The organic C inputs in the study sites may be too low to exhibit C saturation dynamics (Stewart et al., 2007). Another reason why silt+clay fraction from the sites in China has not reached C saturation might be because of very low initial SOC before manure amendments. The initial soil C concentrations is 1.55% in Gongzhuling, 0.78% in Zhengzhou, and 0.78% in Qiyang (Xu et al., 2006). Carbon depleted soils are able to store large amounts of organic C inputs to restore and further to increase SOC and eventually reach C saturated level. The third reason is short durations of manure amendments in study sites. While the durations of manure amendments are 10 years in Melfort and 13 years in Dixon cattle and Dixon swine experiments, which may be not long enough for soil minerals to approach C saturation. West and Six (2007) estimated that at least 21 year are needed for cropland soils to reach C steady state, and longer durations are needed for soils to pass C steady states to reach C saturation.

Organic C concentrations of bulk soil were used as the net organic C inputs to test whether or not silt+clay has reached C saturation. Organic C concentrations of bulk soil function as a proxy of gross organic C inputs, since they were positively correlated with gross organic C inputs in all the experiments, except in Melfort (Figure 3.2c). The absence of correlation between organic C concentrations of bulk soil and gross organic C

inputs in Melfort is due to large variations of organic C concentrations of bulk soil at each treatment. While the slopes of linear regressions between C concentrations of bulk soil and gross organic C inputs indicate transformation rates of manure C and plant C inputs to into soil total C, following the order: Dixon swine (0.0238) > Gongzhuling (0.0169) > Laiyang (0.0093) > Qiyang (0.0065) > Dixon cattle (0.0062) > Zhengzhou (0.0038). The transformation rates of manure C and plant C inputs to into soil total C would be the results of chemical composition of manure and plant residues, local climate, soil texture and mineralogy, and initial SOC.

When fitting changes in organic C concentration of silt+clay as a function of net organic C inputs to the linear and the asymptotic regressions, the linear regression is generally a better fitting for samples from all the experiments except from Gongzhuling and Dixon cattle (Table 3.6). Values of AIC were only slightly higher for the linear regression than for the asymptotic regression in Gongzhuling and Dixon cattle experiments, therefore, it is difficult to conclude that the asymptotic regression is a better fitting for samples from these two experiments only according to AIC values. Why silt+clay have not reached C saturation would be due to small ranges of net organic C inputs and C depleted soils before manure amendments. Coefficients of variation of net organic C inputs among treatments within each experiment were 6.1% in Melfort, 28.0% in Dixon swine, and 29.2% in Dixon cattle. Although in Gongzhuling, Zhengzhou, and Qiyang, net organic C inputs among treatments within each experiment have relatively wide ranges with coefficients of variation of 71.7%-116.6%, soils from these three experiments are able to store large amounts of organic C inputs given low SOC before manure amendments.

Carbon loading higher than  $1 \text{ mg C m}^{-2}$  was mainly found for samples from Zhengzhou, Melfort, and Dixon cattle experiments and at treatments with high levels of organic C inputs in Laiyang and Dixon swine experiments (Table 3.7). Carbon loadings of silt+clay higher than  $1 \text{ mg C m}^{-2}$  in Melfort and Dixon cattle might be due to high SOC without manure amendments. While C loading of silt+clay higher than  $1 \text{ mg C m}^{-2}$  in Laiyang would be due to lower mineral SSA compared to those in other experiments (Table 3.7).

To seek evidence of the arrival of soil C saturation, C loadings of silt+clay in treatments with different levels of organic C inputs were compared to  $1 \text{ mg C m}^{-2}$ , which is presumed to be the maximal C loading for soils (Feng et al., 2012; Mayer, 1994). Soil with C loadings higher than the maximal C loading may have reached C saturation, and otherwise not. However, C loadings of silt+clay higher than  $1 \text{ mg C m}^{-2}$  accounted for 44% of entire silt+clay samples from the seven experiments, suggesting that the maximal C loading for soils would be higher than  $1 \text{ mg C m}^{-2}$ . Moreover, organic matter in silt+clay sized soil particles was bound to soil minerals since light fraction of silt+clay generated from the density fractionation was negligible (Table 3.5), which precludes the contribution of uncomplexed organic matter to C loading of silt+clay. Taken together C loadings of silt+clay higher than  $1 \text{ mg C m}^{-2}$  is inappropriate to be used as a criteria to conclude the arrival of soil C saturation without other evidence.

Since changes in organic C concentrations of silt+clay as a function of organic C inputs fit the linear regression better than the asymptotic regression in Laiyang, Melfort, and Dixon cattle experiments, C loadings of silt+clay fraction from the experiments would linearly increase with additions of organic C inputs, and therefore would lead to C

loadings of silt+clay much higher than  $1 \text{ mg C m}^{-2}$  as these samples have C loadings already higher than  $1 \text{ mg C m}^{-2}$ . Thus, silt+clay fractions with C loading higher than  $1 \text{ mg C m}^{-2}$  in these three experiments cannot show that silt+clay fractions have reached C saturation.

The slowdown of SOC storage efficiency of silt+clay in Gongzhuling and Dixon cattle (Figure 3.1) may be due to large gross organic C inputs and decreased soil C saturation deficits. Gross organic C inputs were as high as  $5.17 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  in Gongzhuling and  $12.11 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  in Dixon cattle. Large amounts of organic C inputs have occupied mineral surface areas of silt+clay fractions and left less reactive spots of mineral surface area to bind more additional C inputs in Gongzhuling and Dixon cattle. While as gross organic C inputs increase, organic C storage efficiency of POM+sand decreased in Qiyang, Laiyang, and Melfort, and increased in the other four experiments (Figure 3.1). The results demonstrate that less organic C inputs would be stored into POM+sand fraction in Qiyang, Laiyang, and Melfort than before, and more organic C inputs would be stored into POM+sand fraction in the other four experiments. Comparing organic C storage efficiency of POM+sand to this of silt+clay, the former was higher than the latter in the seven experiments except in Laiyang and Dixon swine (Figure 3.1), showing that POM+sand is the soil C pool that has stored more manure-C and plant-C inputs than silt+clay.

### *3.3.4 Regressions between C concentrations and soil mineral properties*

In the seven agricultural experiments, mineral SSA of silt+clay fraction did not vary greatly at different treatments within each experiment but differed between experiments (Table 3.7). Linear regressions between organic C concentrations of silt+clay



fraction and mineral SSA were only found in Zhengzhou and Laiyang (Table 3.8).

Organic C concentrations of silt+clay were not greatly influenced by mineral SSA. Since silt+clay fractionated from soils in these agricultural experiments have not reached C saturation, C concentration of silt+clay would be affected more by amounts of organic C inputs and decomposition rather than mineral SSA.

Across the seven experiments, CEC was much lower in Qiyang than in the other experiments, and was significantly higher in treatments with manure inputs than treatments without manure inputs in Qiyang, Laiyang, and Dixon swine (Table 3.7). Significantly higher CEC of silt+clay fraction in treatments with manure amendments than treatments without manure amendments suggests the influence of organic matter derived from manure may have affected CEC. In addition, C concentrations of silt+clay linearly increased with CEC in Qiyang, Laiyang, and Dixon swine (Table 3.8), indicating that cations derived from soil minerals may influence organic matter associated with silt+clay in these experiments.

Soil total and amorphous Fe oxides were much higher in Qiyang ( $36.2 \text{ g kg}^{-1}$ ) and Laiyang ( $11.6 \text{ g kg}^{-1}$ ) than in the other experiments ( $5.6\text{-}8.5 \text{ g kg}^{-1}$ ). Amorphous Fe oxides accounted for 15.5-46.5% of total Fe oxides in the seven experiments. While soil total Al oxides were high in Qiyang ( $12.1 \text{ mg g}^{-1}$ ) and Gongzhuling ( $6.2 \text{ mg g}^{-1}$ ), and low in the other five experiments. Soil total Al oxides did not vary greatly across experiments as total Fe oxides (Table 3.7). Organic C concentrations of silt+clay linearly decreased with total Fe oxides for samples from Gongzhuling, Laiyang, Dixon cattle, and Dixon swine experiments (Table 3.8). It is unknown what are the reasons for this observation. Linear regressions between organic C concentrations of silt+clay fraction and amorphous Fe

oxides and total Al oxides were generally not observed in these experiments, indicating no impact of amorphous Fe oxides and total Al oxide on amounts of organic C stabilized on silt+clay fraction. The lack of linear regression is possibly because of small amounts of amorphous Fe oxides and total Al oxides in soils from these experiments.

### **3.4. Summary and conclusions**

In the seven agricultural experiments with long-term manure amendments, C concentrations of bulk soil, POM+sand, and silt+clay were different across experiments and among treatments with different levels of organic C inputs within each of these experiments, and were generally significantly higher at treatments with manure amendments compared to the control and the treatment with mineral fertilizer application alone. Although increase in C concentrations of POM+sand were 1.1-5.8 times on average as those of silt+clay, C contents of silt+clay still accounted for a large proportion (68-77% on average) of entire SOC pool. The results show that manure inputs to soil would more likely to cause increase in POM+sand fraction than in silt+clay fraction. In addition, it was found that the mass proportions of the light fraction of silt+clay were found to account for only 0.1-3.5% with the average of 0.6% of the fraction, suggesting that even in the large manure addition rate treatments, organic matter in the silt+clay fraction can be considered to be bound to soil minerals with negligible proportion of uncomplexed organic matter associated with.

Changes in organic C concentrations of silt+clay as a function of individually gross and net organic C inputs did not fit the asymptotic regression better than the linear regression in the seven experiments, suggesting that silt+clay in these experiments have not reached C saturated level. The possible reason why silt+clay in these long-term

agricultural sites have not reached C saturation is that organic C inputs ranges are not wide to exhibit soil C saturation dynamics. Duration of annual organic C inputs in these agricultural sites may be not long enough for C inputs to accumulate sufficient amounts to allow silt+clay to approach C saturated level. considering that SOC were depleted before organic C inputs initiation and silt+clay fractions in these agricultural sites are able to stabilize large amounts of C inputs due to large mineral SSA.

Carbon loadings of silt+clay fractions higher than the presumed maximal C loading ( $1 \text{ mg C m}^{-2}$ ) of soils accounted for 44% of the entire silt+clay samples. These silt+clay fractions with C loadings higher than  $1 \text{ mg C m}^{-2}$  have not approached C saturation and showed linear increase trend with increasing C inputs. However, organic matter of silt+clay fractions were bound on silt+clay with negligible uncomplexed organic matter associated with. The results suggest that the maximal organic C loading for all types of soil minerals must be higher than  $1 \text{ mg C m}^{-2}$ .

Soil organic C storage efficiency, indicated by slopes of changes in C concentrations of bulk soil, POM+sand, and silt+clay against gross organic C inputs, generally increased with increasing C inputs, indicating that these soil fractions have potentials to store more organic C additions compared to current SOC storage rates.

Soil mineral properties, mineral SSA, CEC, and Fe/Al oxides, were significantly different across experiments and barely varied among treatments within each experiment. Organic C concentrations of silt+clay were not correlated with mineral SSA and Fe/Al oxides, but were correlated with CEC for samples from Gongzhuling, Qiyang, Laiyang, and Dixon swine experiments, suggesting cations derived from soil minerals may have influenced organic matter bounded on silt+clay in these experiments.

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**Table 3.1**

Geographic locations, soil properties, fertilizer application, and crop rotation in seven agricultural experiments with long-term manure amendments.

Site	Location	MAT (°C)	MAP (mm)	Soil pH	Soil taxonomy	Soil texture		Crop
						Silt (%)	Clay (%)	
Gongzhuling, China	43.50° N, 124.80° E	4.5	525	7.6	Typic Argiustolls	41	32	Corn
Zhengzhou, China	34.78° N, 113.67° E	14.0	645	8.3	Typic Eutropept	35	13	Wheat-corn
Qiyang, China	26.75° N, 111.87° E	18.0	1250	5.7	Typic Hapludult	32	44	Wheat-corn
Laiyang, China	36.90° N, 120.7° E	11.2	779.1	6.8	Typic Ustochrept	--	--	Wheat-corn
Dixon, Canada	52.10° N, 114.20° E	0.7	373	7.5	Udic Boroll	24	45	oilseed- cereal
Melfort, Canada	52.82° N, 104.60° E	0.6	410	6.0	Mollic Cryoboralf	40	44	multi-crop



**Table 3.2**

Annual mineral fertilizer and manure application rates in seven long-term agricultural experiments.

Experiment	<u>Mineral fertilizers</u>			<u>Manure</u>	
	N (kg ha <sup>-1</sup> )	P <sub>2</sub> O <sub>5</sub> (kg ha <sup>-1</sup> )	K <sub>2</sub> O (kg ha <sup>-1</sup> )	Application rate <sup>†</sup> (Mg dry matter ha <sup>-1</sup> )	Manure C concentration (g kg <sup>-1</sup> )
Gongzhuling	165	82.5	82.5	8.8	226.4
Zhengzhou	353	176.5	176.5	13.6	169.0
Qiyang	300	120	120	12.4	388.6
Laiyang	0	0	0	8.8	233.0
Melfort	0	0	0	36.0 <sup>ξ</sup>	350.0
Dixon cattle	0	0	0	14.0	368.4
Dixon swine	0	0	0	68.3 <sup>ξ</sup>	368.4

<sup>†</sup> Annual manure application rate is for the treatment with the lowest level at each experiment.

<sup>ξ</sup> Liquid manure application rate is in units of m<sup>3</sup> wet matter ha<sup>-1</sup>.

**Table 3.3**

Equations and parameters used to estimate plant C inputs to soils in seven long-term agricultural experiments in China and Canada. The regression equations estimate above-ground residue dry matter ( $y$ ,  $\text{Mg ha}^{-1}$ ) as a function of grain yield ( $x$ ,  $\text{Mg ha}^{-1}$ ).

Experiment	Crop	Regression equation	Ratio of below-ground to above-ground biomass
Gongzhuling, Zhneqzhou, Qiyang, Laiyang	corn	$y = 1.03x + 0.61$	0.21
	wheat	$y = 1.61 x + 0.38$	0.21
Melfort, Dixon cattle, Dixon swine	barley	$y = 0.87 x + 0.59$	0.22
	oat	$y = 0.81 x + 0.89$	0.25
	Wheat	$y = 1.34 x + 0.52$	0.24
	canola & flax	$y = 1.03 x + 0.85$	0.19

**Table 3.4**

Gross organic C inputs (Mg C ha<sup>-1</sup>), C concentrations of bulk soil (g C 100g<sup>-1</sup> (bulk soil)), enrichment factors of C concentrations of silt+clay to bulk soil, and proportions of C contents of silt+clay to soil total organic C for samples at different fertilizer application treatments in seven long-term agricultural experiments. Data are mean ± SE.

Experiment	Treatment	Gross C inputs	C concentration of bulk soil	Enrichment factor (silt+clay)	C proportion (silt+clay)
Gongzhuling	Check	15.3	1.29±0.10	0.93±0.05	80.3±4.6
	NPK	41.2	1.44±0.06	0.90±0.03	76.7±2.5
	NPKM	83.1	2.37±0.12	0.71±0.00	59.3±0.1
	1.5NPKM	103.4	2.68±0.21	0.68±0.01	56.3±0.6
Zhengzhou	Check	15.9	0.75±0.05	1.06±0.09	79.1±4.5
	NPK	65.5	0.78±0.07	1.00±0.01	76.7±1.1
	NPKM	111.1	1.11±0.07	1.00±0.02	78.0±2.1
	1.5NPKM	134.6	1.15±0.05	1.01±0.04	74.6±2.9
Qiyang	Check	3.8	0.83±0.04	0.96±0.04	84.1±3.4
	NPK	9.4	1.10±0.02	0.87±0.06	75.0±5.3
	NPKM	118.9	1.61±0.09	0.76±0.01	63.8±1.0
	1.5NPKM	169.5	2.05±0.13	0.77±0.04	62.0±3.4
Laiyang	Check	27.9	0.53±0.01	1.88±0.15	71.5±1.7
	1×	109.0	1.23±0.14	1.44±0.05	63.0±2.7
	2×	184.7	1.98±0.06	1.32±0.06	59.0±5.4
Melfort	Check	1.6	3.51±0.20	0.93±0.01	77.9±1.9
	1×	5.9	3.69±0.41	0.93±0.02	77.5±0.6
	4×	7.2	3.73±0.36	0.93±0.04	77.5±2.7
Dixon cattle	Check	1.4	2.98±0.05	1.06±0.08	82.5±5.0
	1×	42.6	3.57±0.22	1.05±0.03	76.6±3.7
	2×	80.9	3.73±0.16	0.97±0.03	71.2±3.8
	4×	157.5	4.02±0.09	0.98±0.04	72.7±4.4
Dixon swine	Check	1.4	2.45±0.20	1.13±0.06	78.5±1.1
	1×	10.5	2.49±0.10	1.02±0.06	75.2±4.8
	2×	16.7	2.40±0.27	1.11±0.07	77.7±2.1
	4×	29.1	3.13±0.26	1.19±0.08	78.5±3.6

**Table 3.5**

Mass and C recovery of the size fractionation and the density fractionation for soil samples from different fertilizer application treatments in seven long-term agricultural experiments. Data are mean  $\pm$  SE. NA: data are not available. ND: not determined.

Experiment	Treatment	Size fractionation			Density fractionation	
		Mass recovery (%)	POM mass proportion (%)	C recovery (%)	Mass recovery (%)	Light fraction mass proportion (%)
Gongzhuling	Check	96.8 $\pm$ 0.4	10.1 $\pm$ 0.2	96.0 $\pm$ 4.2	95.0 $\pm$ 1.7	0.1 $\pm$ 0.0
	NPK	96.3 $\pm$ 0.3	10.7 $\pm$ 0.3	95.1 $\pm$ 2.5	100.2 $\pm$ 1.4	0.4 $\pm$ 0.1
	NPKM	96.9 $\pm$ 0.3	13.3 $\pm$ 0.1	88.1 $\pm$ 0.5	95.4 $\pm$ 2.1	0.2 $\pm$ 0.0
	1.5NPKM	96.8 $\pm$ 0.2	14.3 $\pm$ 0.3	90.9 $\pm$ 1.5	98.8 $\pm$ 0.4	0.3 $\pm$ 0.1
Zhengzhou	Check	97.8 $\pm$ 1.3	23.1 $\pm$ 0.8	96.8 $\pm$ 5.9	98.1 $\pm$ 0.1	0.4 $\pm$ 0.2
	NPK	97.2 $\pm$ 0.1	20.6 $\pm$ 0.6	91.6 $\pm$ 1.3	97.6 $\pm$ 0.0	0.1 $\pm$ 0.0
	NPKM	98.9 $\pm$ 1.1	20.6 $\pm$ 0.2	98.7 $\pm$ 2.9	97.0 $\pm$ 0.3	0.2 $\pm$ 0.1
	1.5NPKM	96.9 $\pm$ 0.3	23.0 $\pm$ 0.2	104.4 $\pm$ 3.5	97.1 $\pm$ 0.7	0.2 $\pm$ 0.7
Qiyang	Check	96.9 $\pm$ 0.3	9.0 $\pm$ 0.1	99.1 $\pm$ 4.8	100.1 $\pm$ 0.2	0.2 $\pm$ 0.1
	NPK	96.3 $\pm$ 0.2	9.9 $\pm$ 0.4	94.4 $\pm$ 5.1	101.0 $\pm$ 0.3	0.2 $\pm$ 0.1
	NPKM	97.1 $\pm$ 0.1	13.1 $\pm$ 0.5	96.1 $\pm$ 3.8	101.9 $\pm$ 0.6	0.6 $\pm$ 0.3
	1.5NPKM	96.5 $\pm$ 0.2	15.9 $\pm$ 0.7	93.2 $\pm$ 2.0	101.2 $\pm$ 0.1	0.4 $\pm$ 0.1
Laiyang	Check	97.5 $\pm$ 0.2	59.4 $\pm$ 0.5	95.4 $\pm$ 4.7	99.7 $\pm$ 0.4	1.6 $\pm$ 1.3
	1 $\times$	98.0 $\pm$ 0.1	54.2 $\pm$ 0.9	96.0 $\pm$ 2.4	97.2 $\pm$ 0.5	0.5 $\pm$ 0.2
	2 $\times$	97.4 $\pm$ 0.0	52.7 $\pm$ 0.3	86.2 $\pm$ 1.6	101.1 $\pm$ 0.1	3.5 $\pm$ 0.3
Melfort	Check	95.5 $\pm$ 0.1	11.4 $\pm$ 1.1	89.6 $\pm$ 2.1	ND	ND
	1 $\times$	94.8 $\pm$ 0.3	11.1 $\pm$ 2.1	91.5 $\pm$ 1.7	ND	ND
	4 $\times$	95.5 $\pm$ 0.2	11.9 $\pm$ 0.9	90.9 $\pm$ 1.6	ND	ND
Dixon cattle	Check	NA	22.1 $\pm$ 1.4	94.6 $\pm$ 4.9	ND	ND
	1 $\times$	95.5 $\pm$ 0.2	22.8 $\pm$ 1.8	97.8 $\pm$ 2.6	ND	ND
	2 $\times$	98.0 $\pm$ 1.2	24.5 $\pm$ 0.6	89.4 $\pm$ 3.7	ND	ND
	4 $\times$	NA	26.1 $\pm$ 2.1	99.3 $\pm$ 2.1	ND	ND
Dixon swine	Check	96.3 $\pm$ 0.1	26.3 $\pm$ 2.8	93.8 $\pm$ 1.8	ND	ND
	1 $\times$	96.0 $\pm$ 0.2	22.6 $\pm$ 1.1	87.3 $\pm$ 4.1	ND	ND
	2 $\times$	95.8 $\pm$ 0.1	25.1 $\pm$ 2.4	91.9 $\pm$ 0.8	ND	ND
	4 $\times$	95.6 $\pm$ 0.3	29.1 $\pm$ 4.7	97.7 $\pm$ 4.1	ND	ND

**Table 3.6**

Linear and asymptotic regressions of C concentrations of silt+clay as a function of gross organic C inputs as the sum of plant C and manure C inputs and net organic C inputs in terms of C concentrations of bulk soil for soils in seven agricultural experiments with long-term manure amendments. “ns” means statistical insignificance.

Organic C inputs	Experiment	<u>Linear</u>			<u>Asymptotic</u>		
		Slope	r	AICC	C <sub>sat</sub>	r	AICC
Gross C inputs	Gongzhuling	0.0083	0.95	-54.2	1.98	0.82	-39.8
	Zhengzhou	0.0042	0.80	-44.0	1.78	0.57	-36.8
	Qiyang	0.0040	0.94	-51.8	1.42	0.87	-43.2
	Laiyang	0.0103	0.98	-32.0	3.85	0.94	-24.2
	Melfort	ns	ns	-16.3	ns	ns	-16.3
	Dixon cattle	0.0042	0.57	-31.6	3.78	0.60	-32.6
	Dixon swine	0.0351	0.62	-23.2	ns	ns	-16.1
Net C inputs	Gongzhuling	0.46	0.95	-54.0	3.66	0.96	-56.1
	Zhengzhou	0.84	0.71	-40.3	3.13	0.69	-39.7
	Qiyang	0.61	0.97	-61.7	4.87	0.97	-60.0
	Laiyang	1.10	0.99	-37.7	6.94	0.99	-36.4
	Melfort	0.78	0.95	-41.8	22.93	0.95	-41.5
	Dixon cattle	0.66	0.72	-36.9	9.81	0.73	-37.5
	Dixon swine	0.99	0.83	-33.9	44.67	0.82	-33.6

**Table 3.7**

Mean of cation exchange capacity (CEC, cmol kg<sup>-1</sup>), total and amorphous iron oxides (Fe<sub>d</sub>/Fe<sub>o</sub>, mg g<sup>-1</sup>), and total aluminum oxides (Al<sub>d</sub>, mg g<sup>-1</sup>), soil mineral specific surface area (SSA, m<sup>2</sup> g<sup>-1</sup>), and C loading (mg C m<sup>-2</sup>) of silt+clay fraction in seven agricultural experiments with long-term manure amendments.

Experiment	Treatment	CEC	Fe <sub>d</sub>	Fe <sub>o</sub>	Al <sub>d</sub>	SSA	C loading
Gongzhuling	Check	39.7	7.6	2.3	6.6	37.2	0.38
	NPK	31.5	8.1	2.7	7.1	42.3	0.32
	NPKM	37.2	6.8	2.4	5.6	32.6	0.60
	1.5NPKM	37.6	7.0	2.5	5.6	37.3	0.57
Zhengzhou	Check	38.9	5.1	1.3	3.6	13.4	1.18
	NPK	44.3	5.7	1.6	3.9	13.0	1.26
	NPKM	44.2	5.8	1.8	3.9	13.9	1.33
	1.5NPKM	40.3	5.7	1.7	3.8	14.4	1.39
Qiyang	Check	15.3	33.3	4.9	11.1	34.5	0.23
	NPK	14.4	38.1	5.4	12.6	35.1	0.27
	NPKM	19.0	38.9	5.8	12.9	36.1	0.34
	1.5NPKM	19.0	34.4	6.2	12.0	32.1	0.49
Laiyang	Check	28.0	12.9	4.3	5.4	24.5	0.40
	1×	31.5	11.2	3.9	4.8	20.9	0.85
	2×	37.0	10.6	4.0	4.9	21.6	1.21
Melfort	Check	31.1	8.0	4.2	4.7	28.8	1.15
	1×	34.0	8.5	3.7	5.0	30.0	1.13
	4×	34.5	9.0	3.9	5.3	30.7	1.17
Dixon cattle	Check	36.3	7.6	2.4	5.5	36.6	0.87
	1×	33.0	6.3	3.1	4.4	31.2	1.21
	2×	36.2	6.0	2.9	4.3	28.8	1.26
	4×	40.5	6.8	2.7	5.0	28.4	1.40
Dixon swine	Check	37.0	7.0	2.8	5.0	29.4	0.95
	1×	29.1	7.1	2.4	5.0	32.5	0.79
	2×	34.2	7.2	2.4	5.0	32.5	0.81
	4×	49.4	5.9	2.6	4.0	29.0	1.29

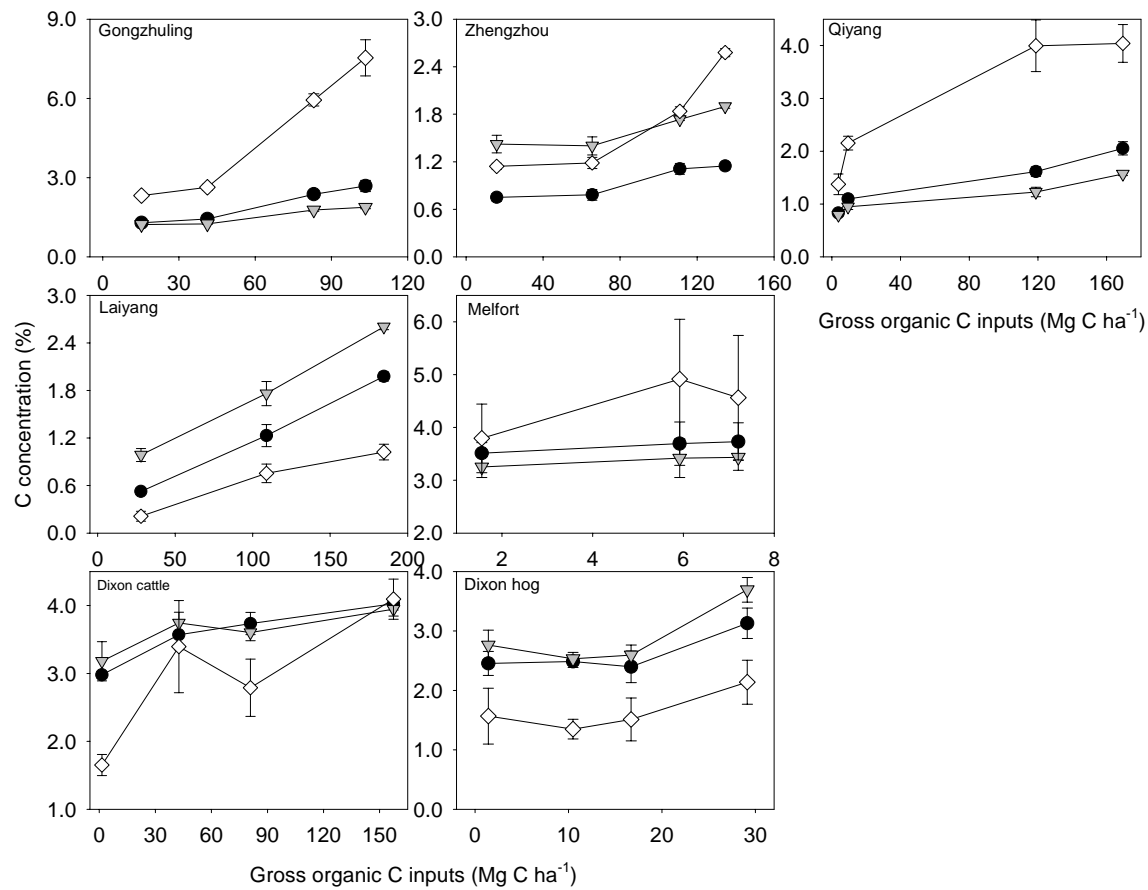
**Table 3.8**

Slopes of linear regressions between C concentration and soil mineral specific surface area (SSA,  $\text{m}^2 \text{g}^{-1}$ ), cation exchange capacity (CEC,  $\text{cmol kg}^{-1}$ ), total and amorphous iron oxides ( $\text{Fe}_d/\text{Fe}_o$ ,  $\text{mg g}^{-1}$ ), and total aluminum oxides ( $\text{Al}_d$ ,  $\text{mg g}^{-1}$ ) of silt+clay fraction in seven agricultural experiments with long-term manure amendments. “ns” means statistical insignificance.

Variable	Gongzhuling	Zhengzhou	Qiyang	Laiyang	Melfort	Dixon cattle	Dixon swine
SSA	ns	0.205	ns	-0.265	ns	ns	ns
CEC	0.018	ns	0.102	0.133	ns	ns	0.041
$\text{Fe}_d$	-0.327	ns	ns	-0.439	ns	-0.287	-0.424
$\text{Fe}_o$	ns	ns	0.439	ns	ns	ns	ns
$\text{Al}_d$	-0.322	ns	ns	ns	ns	ns	-0.671

**Figure 3.1**

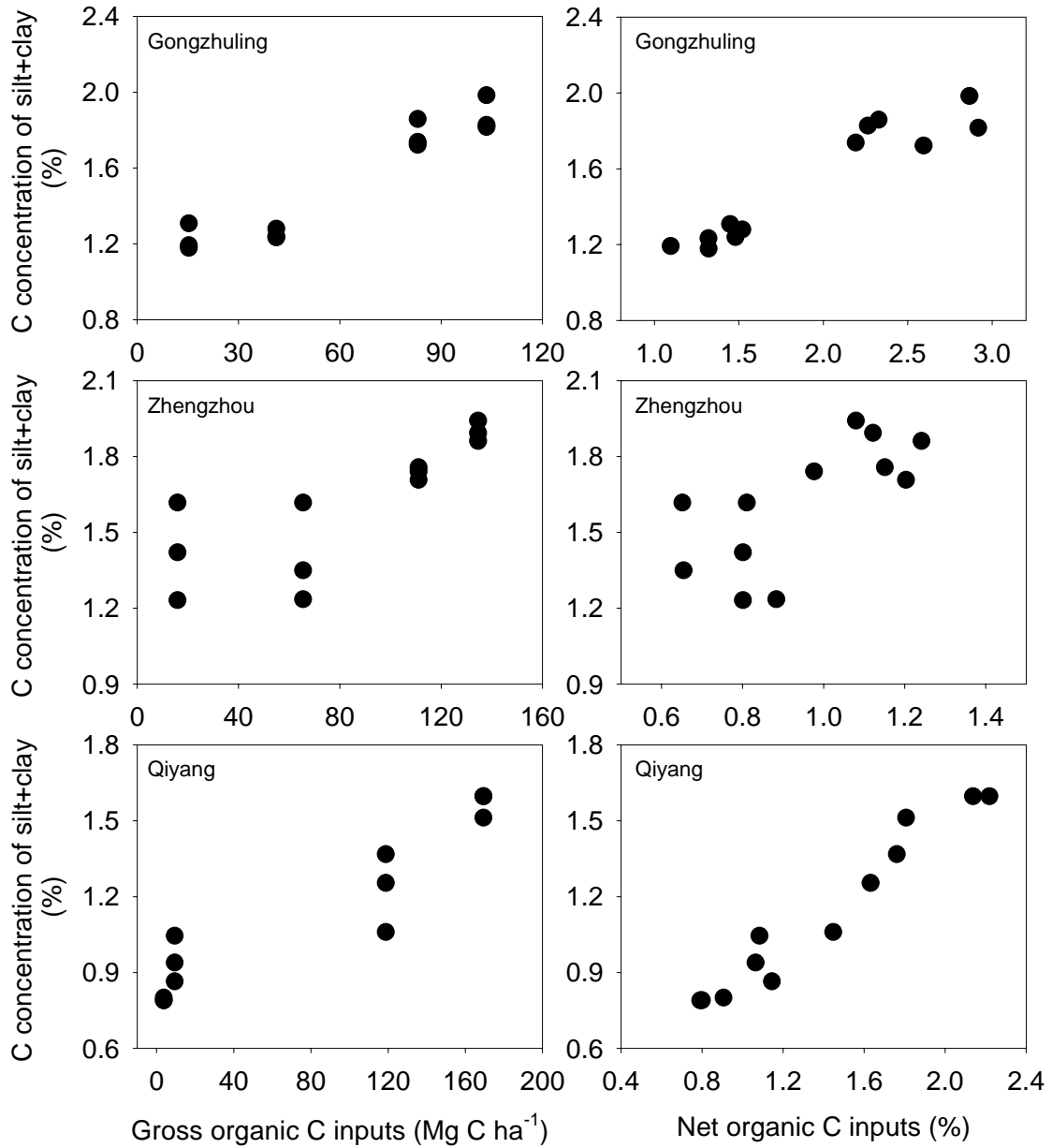
Carbon concentrations of bulk soil (closed circle), POM+sand (open diamond), and silt+clay (gray triangle) as a function of gross C inputs in seven agricultural experiments with long-term manure amendments.





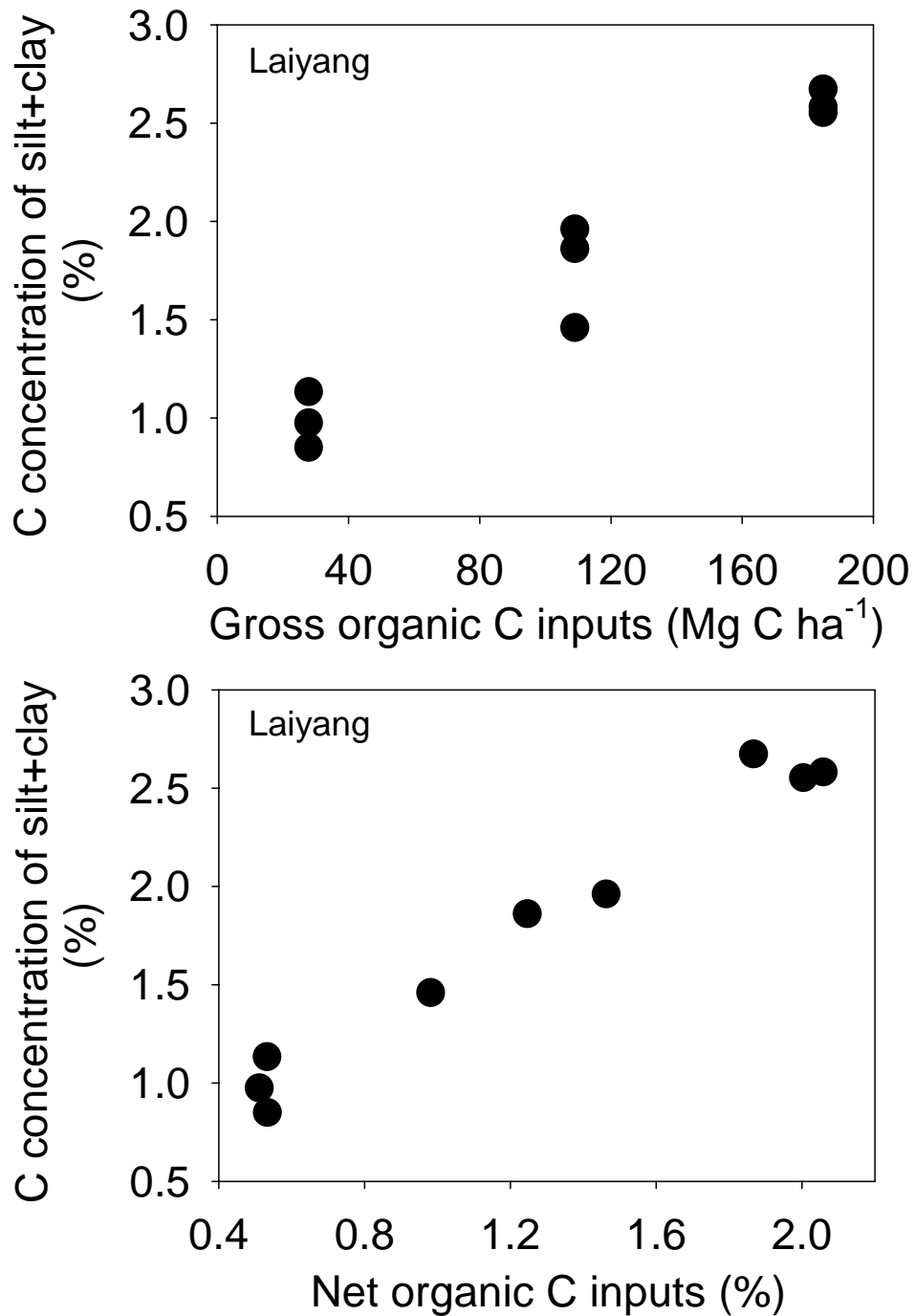
**Figure 3.2a**

Organic C concentrations of silt+clay as a function of gross organic C inputs as the sum of plant C and manure C inputs and net organic C inputs in terms of C concentrations of bulk soil in Gongzhuling, Zhengzhou, and Qiyang with long-term manure amendments.



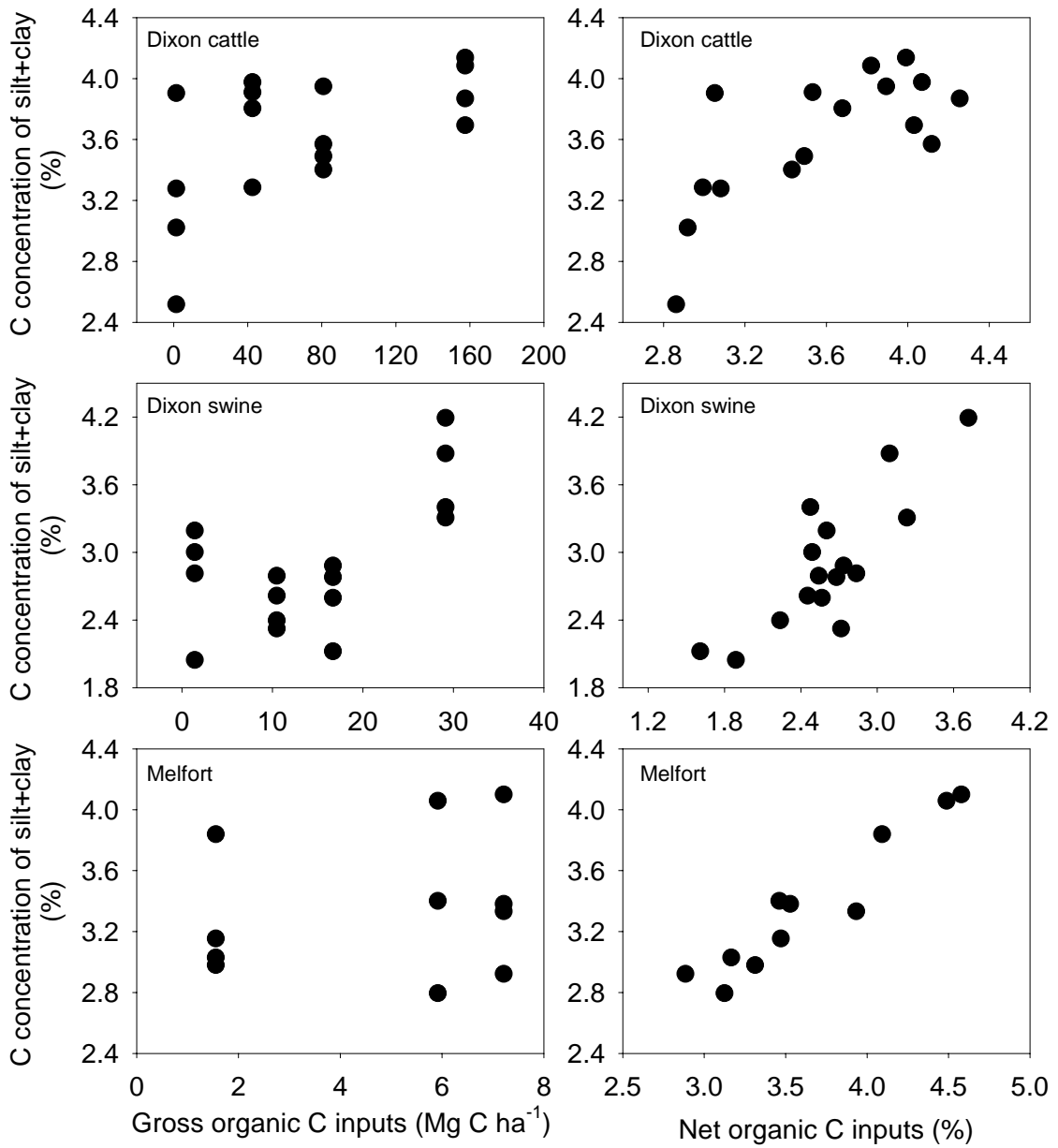
**Figure 3.2b**

Organic C concentrations of silt+clay as a function of gross organic C inputs as the sum of plant C and manure C inputs and net organic C inputs in terms of C concentrations of bulk soil in Laiyang with long-term manure amendments.



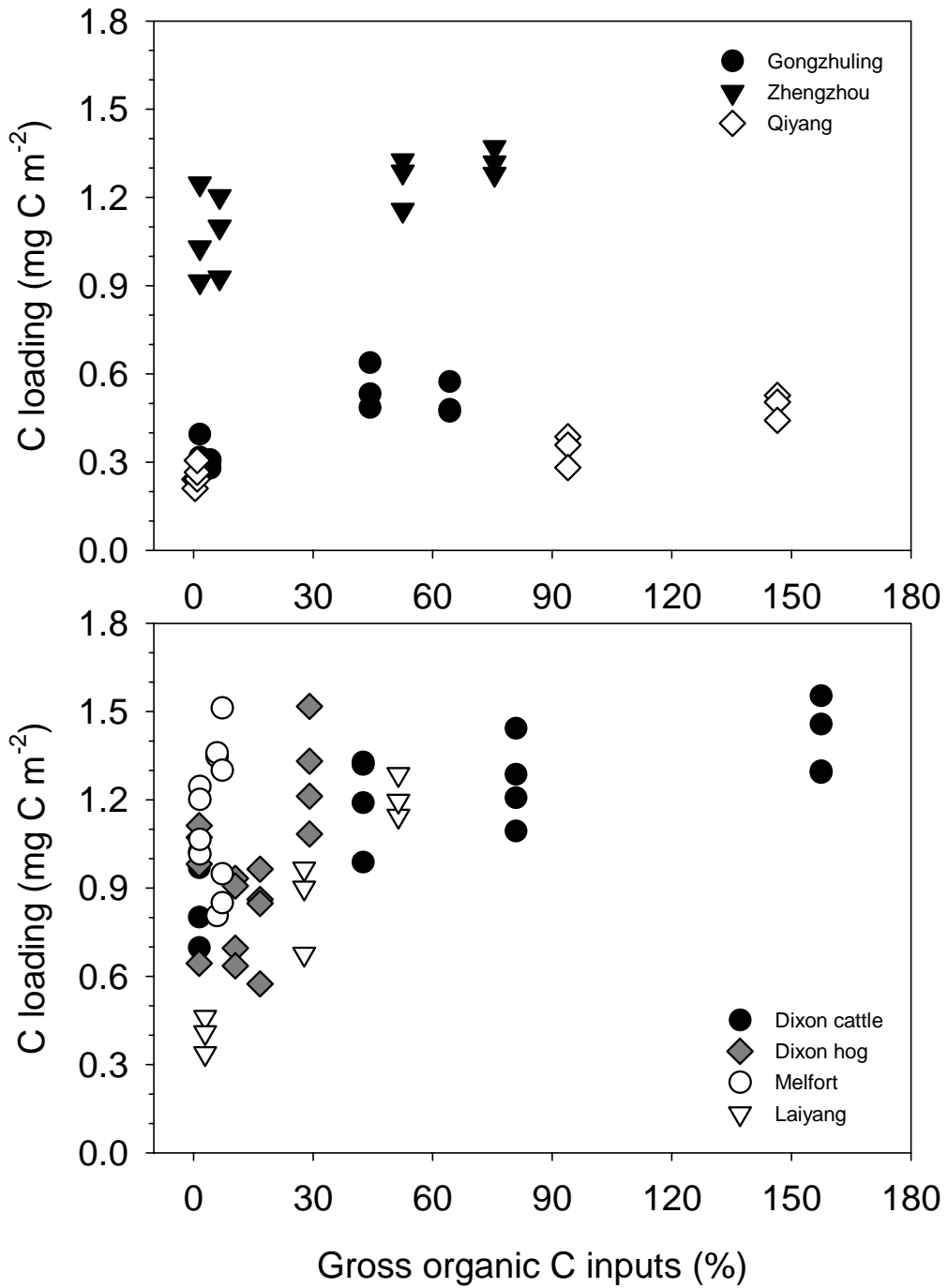
**Figure 3.2c**

Organic C concentrations of silt+clay as a function of gross organic C inputs as the sum of plant-C and manure-C inputs and net organic C inputs in terms of C concentrations of bulk soil in Melfort, Dixon cattle, and Dixon swine experiments with long-term manure amendments.



**Figure 3.3**

Carbon loadings of silt+clay as a function of gross organic C inputs in seven agricultural experiments with long-term manure amendments.



## CHAPTER FOUR

Soil organic matter stability in agricultural soils receiving long-term manure amendments

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## Abstract

Changes in soil organic matter (SOM) stability need to be determined for soils that are subjected to carbon (C) additions to predict soil organic C stock with organic C inputs. Soils from three agricultural sites (*i.e.*, Gongzhuling, Zhengzhou, and Qiyang) with long-term organic C inputs were selected to examine biological, chemical, and thermal SOM stability for silt+clay and bulk soil. The three sites have the same treatments of the control, mineral fertilizer application, and the combined manure and mineral fertilizer applications, which result in different levels of organic C inputs. Biological SOM stability was assessed by using laboratory incubation, chemical stability by using sodium pyrophosphate extraction, and thermal stability by using simultaneously thermal analysis coupled with evolved gas analysis. Biological SOM stability of silt+clay did not change with increasing organic C inputs in the three sites, which might be due to small changes in organo-mineral bindings since chemical composition of organic C inputs applied over years was similar. Chemical SOM stability of silt+clay showed no general change patterns with increasing organic C inputs either, with no changes for samples from Gongzhuling and Zhengzhou and decrease for samples from Qiyang, which was probably because chemical stability index (*i.e.*, ratios of organic matter soluble in pyrophosphate) has been affected by different fertilizations in each site and various mineralogy across sites. Thermal SOM stability of silt+clay fractions showed no consistent patterns with increasing C inputs in three study sites which might be due to different organo-mineral bindings across these three sites. In addition, biological SOM stability of bulk soil decreased in treatments with manure inputs than in the control, which was likely caused by increased contributions of particulate organic matter which is

easily decomposable. Stability of biologically labile organic matter pool of bulk soil showed no changes in treatments with manure inputs relative to the control, suggesting that the stability and the relative size of this organic matter pool are independent of each other. Thermal SOM stability of bulk soil in treatments with different levels of C inputs was site specific. Thermal SOM stability were not well correlated with biological SOM stability, suggesting that factors regulating thermal SOM stability would be different from those for biological SOM stability. Overall, in the three study sites, no changes of biological and chemical SOM stability and mixed change patterns of thermal SOM stability with increasing organic C inputs do not support the prediction that SOM stability decreases with increasing organic C inputs. Decreased biological SOM stability of bulk soil subjected to manure inputs suggests that larger amounts of organic C inputs than previous levels are needed to store the same amount of organic C inputs to bulk soil.

## 4.1. Introduction

In agricultural ecosystems, land management practices including mineral fertilizer application, plant residue return, and compost and manure additions are used to maintain or increase soil organic carbon (SOC) stocks. Organic carbon (C) stored in soils holds the promise to slow the increase rate of the atmospheric CO<sub>2</sub> and to sustain soil quality and productivity.

Changes in SOC stock as a function of organic C inputs can be defined by SOC storage efficiency (*i.e.*,  $\Delta\text{SOC}/\Delta\text{C}$  inputs), and it has been found to decrease with increasing organic C inputs (Campbell et al., 1991; Gulde et al., 2008; Huggins et al., 1998), while most current SOM models suggest constant increase of SOC storage as a function of organic C inputs. Decreased SOC storage efficiency with increasing organic C inputs suggests the existence of an upper limit of soil C storage, which has been used as the basis of the soil C saturation theory (Six et al., 2002b; Stewart et al., 2007; West and Six, 2007).

The soil C saturation theory predicts that SOC storage efficiency decreases as soils approach C saturation with increasing organic C inputs (Six et al., 2002a; Stewart et al., 2007). Decrease in SOC storage efficiency would be results of declined SOM stability. It is known that changes in SOC storage can be affected by organic C inputs and soil organic matter (SOM) stability. Given constant organic C inputs in a specific soil-climate condition, SOC storage efficiency is mainly determined by SOM stability and could eventually influence amounts and permanence of organic C inputs that can be stored into soils.

When soils are approaching C saturation with increasing organic C inputs directly



or indirectly from inorganic and organic fertilizer applications, changes in SOM stability in the processes may differ for fine soil particles (*e.g.*, silt+clay) and for bulk soil. Since fine soil particles have been found to be more likely to reach C saturation (Gulde et al., 2008; Hassink, 1997; Stewart et al., 2008), stability of organic matter associated with fine soil particles are expected to decrease with increasing organic C inputs. Organic matter stabilized by fine soil particles would be limited by finite mineral surface areas, and strength of bonding between organic matter and soil mineral may turn to be weaker as reactive spots on soil mineral surfaces become less with increasing organic C inputs (Kleber et al., 2007; Sollins et al., 2006). While bulk soil would show different response of SOM stability to increasing organic C inputs compared to those of fine soil particles, considering that organic matter in bulk soil consists of heterogeneous organic C pools include organic matter associated with soil minerals and uncomplexed particular organic matter, which have different turnover times (Balesdent et al., 1987; Flessa et al., 2008).

The goal of this study was to examine SOM stability of silt+clay and bulk soil fractions from agricultural sites with different levels of organic C inputs, either directly or indirectly resulted from inorganic and organic fertilizer applications. The objective was to test whether or not SOM stability decreases with organic C inputs, especially for silt+clay. Bulk soil and silt+clay were fractionated from soils in Gongzhuling, Zhengzhou, and Qiyang sites in China, which have been subjected to the same fertilizer application for 20 years. In addition to assess conventional biological and chemical SOM stability, and thermal techniques were used to examine thermal SOM stability. Thermal analysis for SOM has its advantages, considering that it is inexpensive and quick and requires little sample preparation and small sample size. And thermal SOM stability has been found to

be correlated with biological SOM stability (Plante et al., 2011).

## **4.2. Methods**

### *4.2.1 Study sites and sampling*

A subset of the soils from the agricultural sites with long-term manure amendments used in Chapter 3 was selected for the current study. The Gongzhuling, Zhengzhou, and Qiyang experimental sites are part of the National Soil Fertility and Fertilizer Effects Long-term Monitoring Network in China, and thus share the same experimental design. The same control (CK), mineral fertilizer (NPK), and combined NPK and manure (NPKM and 1.5NPKM) treatments were selected as in Chapter 3. Soils from these three sites represent typical soil types in the northern, central, and southern regions of China. Detailed information about geographic, climate and soil properties, and the crop, fertilizer applications, and experimental design in these three study sites can be found in Chapter 3.

### *4.2.2 Stability of soil organic matter*

Bulk soil samples as well as the previously isolated silt+clay fractions from Gongzhuling, Zhengzhou, and Qiyang were used to assess the relative biological, chemical, and thermal stability of organic matter as a function of organic C inputs. Organic matter stability in bulk soil (< 2 mm) samples was determined to assess changes in the decomposability of the entire SOC pool in response to differing fertilizer treatments, while organic matter stability of silt+clay (< 53  $\mu\text{m}$ ) samples was assessed to more specifically test whether or not organic matter stability decreases as soil minerals approach C saturation. Biological SOM stability was assessed using laboratory

incubation, chemical SOM stability was estimated as the proportion of sodium pyrophosphate extractable C pool, and thermal SOM stability was analyzed using simultaneous thermogravimetry (TG) and differential calorimetry scanning (DSC) coupled with evolved CO<sub>2</sub> gas analysis (CO<sub>2</sub>-EGA).

#### 4.2.2.1 Biological stability of soil organic matter

A laboratory incubation experiment was used to assess the relative biological stability of organic matter of both bulk soil and isolated silt+clay fraction samples. Two and one half grams of silt+clay were mixed with 2.5 g of acid-washed sand in 50 mL plastic conical centrifuge tubes, and mixed for 10 seconds on a vortex shaker. The sand was added to increase the porosity of the incubation matrix and to improve aeration. Deionized water was added to the samples to achieve 55% water-filled pore space to ensure optimal moisture for microbial activity. Tubes were kept open with the septa caps placed loosely on the tubes, and placed in an incubator with a pan of water to keep humidity constant inside the incubator. Samples were incubated for 40 days at 25 ( $\pm$  0.2) °C. All samples were pre-incubated at constant temperature and moisture for one week before the first determination of CO<sub>2</sub> respiration. Respiration rates were analyzed using a LI-7000 infrared gas analyzer (IRGA, LICOR Corp., Lincoln, NE) at incubation days 1, 4, 7, 10, 15, 20, 30, and 40. Prior to CO<sub>2</sub> analysis, each tube was capped and flushed with CO<sub>2</sub>-free air, then was returned to the incubator for 24 h early in the incubation experiment and for 48 h later in the incubation, to allow sufficient CO<sub>2</sub> to accumulate in the headspace of tubes. Four mL samples were drawn from the headspace of each tube and injected into the IRGA to determine CO<sub>2</sub> concentrations, and the tubes were then kept open until the next sampling.

#### 4.2.2.2 Chemical stability of soil organic matter

Chemical SOM stability can be assessed by the relative solubility of SOM, which depends on interactions between organic matter, polyvalent cations, and mineral surfaces (Stevenson, 1994). Pyrophosphate anions are able to remove cations that bridge organic matter to mineral surfaces, resulting in the solubilization of organic matter (Kaiser and Ellerbrock, 2005). A sodium pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7$ ) extraction was therefore used to remove organic matter associated with soil minerals that can dissolve in  $\text{Na}_4\text{P}_2\text{O}_7$  solution and to assess chemical stability. Two grams of silt+clay fraction were mixed with 200 mL of  $0.1 \text{ mol L}^{-1}$   $\text{Na}_4\text{P}_2\text{O}_7$  in a 500 mL centrifuge bottle and shaken for 6 h. The suspension was centrifuged at 10,000 rpm for 15 min. The supernatant was decanted and discarded, while the silt+clay residue was re-suspended in deionized water and transferred into an aluminum pan and oven-dried at  $50 \text{ }^\circ\text{C}$ . Oven-dried samples were analyzed for C concentration by dry combustion using a Carlo-Erba NA 1500. The difference of C concentration of silt+clay fraction before and after  $\text{Na}_4\text{P}_2\text{O}_7$  extraction was organic C solved in  $\text{Na}_4\text{P}_2\text{O}_7$  solution (OM(pyro)), and the ratio of OM(pyro) relative to the C concentrations before  $\text{Na}_4\text{P}_2\text{O}_7$  extraction indicates organic matter removal efficiency by  $\text{Na}_4\text{P}_2\text{O}_7$ .

#### 4.2.2.3 Thermal stability of soil organic matter

Thermal indices of SOM stability in silt+clay and bulk soil samples were determined by simultaneous TG-DSC coupled with  $\text{CO}_2$ -EGA. Thermal analyses were performed using a Netzsch STA 409PC Luxx simultaneous thermal analyzer equipped with a type-S (Pt/PtRh) TG-DSC sample carrier (Netzsch-Gerätebau GmbH, Selb, Germany) coupled to a LI-840  $\text{CO}_2/\text{H}_2\text{O}$  IRGA (LI-COR Biosciences, Lincoln NE). All

samples were ground to pass through a 500- $\mu\text{m}$  sieve and air-dried before thermal analysis. Samples (~30 mg) were placed in a Pt/Rh crucible (with an identical and empty crucible used as the reference) and heated from ambient to 700 °C at 10 °C min<sup>-1</sup> under an oxidizing atmosphere of 30 mL min<sup>-1</sup> of synthetic air (20% O<sub>2</sub> and 80% N<sub>2</sub>) and 10 mL min<sup>-1</sup> of N<sub>2</sub> as a protective gas. Further details on the thermal analysis procedure and data handling are described in Plante et al. (2011).

The exothermic region in which SOM is oxidized was defined as 190–600 °C based on CO<sub>2</sub>-EGA data. The net energy content (in mJ) of the organic matter of a sample was determined by integrating the DSC heat flux in this temperature range. In the exothermic region (190-600 °C), the temperature at which half of mass loss has occurred (TG-T<sub>50</sub>), the temperature at which half of net energy has been released (DSC-T<sub>50</sub>), and the temperature at which half of CO<sub>2</sub> has been evolved (CO<sub>2</sub>-T<sub>50</sub>) were calculated as indices of thermal SOM stability. In addition, ratios of relative mass loss at each of the three temperature ranges (Exo<sub>1</sub>, 200-380 °C; Exo<sub>2</sub>, 380-475 °C; Exo<sub>3</sub>, 475-600 °C) to relative mass loss at the entire temperature range (200-600 °C), and ratios of CO<sub>2</sub> evolved at each temperature range of Exo<sub>1</sub>, Exo<sub>2</sub>, and Exo<sub>3</sub> to CO<sub>2</sub> evolved at the entire temperature range (200-600 °C) were determined to fractionate thermally labile from thermally stable SOM (De la Rosa et al., 2008). The underlying assumption of the analysis is that SOM combusted at high temperature is more resistant to decomposition because of increased molecular complexity and greater activation energy requirements. Exo<sub>1</sub> is the temperature range considered for the combustion of thermally labile organic matter, Exo<sub>2</sub> is the temperature range for thermally stable organic matter, and Exo<sub>3</sub> is the temperature range for thermally recalcitrant organic matter.

#### 4.2.3 Statistical analysis

Cumulative CO<sub>2</sub> respired was determined using respiration rates measured periodically during the incubation. Values were normalized to sample C concentration (*i.e.*, g CO<sub>2</sub>-C (100 g sample-C)<sup>-1</sup>). Relative biological stability indices were determined by fitting cumulative CO<sub>2</sub> respiration data to the first-order exponential equation  $C_t = C_o(1 - e^{-kt})$ , where  $C_o$  is the potentially mineralizable C,  $k$  is the first-order decomposition constant (day<sup>-1</sup>), and  $C_t$  is cumulative CO<sub>2</sub> respiration. Data from each of the three incubated replicates from soil samples at each fertilizer treatment were individually fit using non-linear regression (Sigmaplot 11.0, Systat Software Inc.). Since it is a short-term laboratory incubation experiment, CO<sub>2</sub> respired over 40 days incubation is assumed to be from biologically labile organic matter pool of soil fractions. Therefore, normalized cumulative CO<sub>2</sub> and  $C_o$  represent the relative size of biologically labile organic matter pool and can indicate biological stability of entire organic matter pool of soil fractions, and SOM decomposition constant represents stability of biologically labile organic matter pool of soil fractions.

Regression analysis was used to examine the change trends of variables with increasing organic C inputs indicated by organic C concentrations of soil fractions. Changes in  $C_o$  and  $k$ , OM(pyro), TG-T<sub>50</sub>, CO<sub>2</sub>-T<sub>50</sub>, DSC-T<sub>50</sub>, ratios of relative mass loss at Exo<sub>1</sub>, Exo<sub>2</sub>, and Exo<sub>3</sub>, and ratios of CO<sub>2</sub> evolved at Exo<sub>1</sub>, Exo<sub>2</sub>, and Exo<sub>3</sub> with increasing organic C concentrations for bulk soil and silt+clay were subjected to the linear regression using JMP 7.0 (SAS Institute, Cary, NC).

In order to test differences of SOM stability among treatments with different levels of organic C inputs, multiple comparisons of  $C_o$  and  $k$  for both silt+clay and bulk

soil and OM(pyro) for silt+clay were analyzed by using Tukey HSD in JMP 7.0 (SAS Institute, Cary, NC).

Correlations between biological stability indices (normalized cumulative CO<sub>2</sub>, C<sub>0</sub>, and k), and thermal stability indices (TG-T<sub>50</sub>, CO<sub>2</sub>-T<sub>50</sub>, and DSC-T<sub>50</sub>) for bulk soil and silt+clay were examined using Pearson's method using JMP 7.0 (SAS Institute, Cary, NC).

### **4.3. Results and discussion**

#### *4.3.1 SOM stability of silt+clay as a function of C inputs*

##### 4.3.1.1 Biological SOM stability

The relative size of biologically labile organic matter pool (C<sub>0</sub>) did not increase or decrease with increasing SOC in Gongzhuling ( $R^2 = 0.007$ ,  $P = 0.793$ ), Zhengzhou ( $R^2 = 0.002$ ,  $P = 0.908$ ) or Qiyang ( $R^2 = 0.266$ ,  $P = 0.086$ ), showing that biological stability of entire organic matter associated with silt+clay sized soil particles did not change greatly with increasing organic C inputs (Figure 4.1, middle row). The results do not support the prediction that SOM stability decreases as organic C inputs increase. One reason that might explain this observation is that organic matter that has been stabilized on silt+clay sized soil particles and caused increase in organic C concentration, has similar chemical composition, since soils accepted similar pig manure inputs and plant residues of the same crops over years within each site. Moreover, the linear increase of organic C concentrations of silt+clay with organic C inputs in the three sites (Chapter 3) suggests the stability of organic matter cumulated on silt+clay was quite similar, unless organic C inputs are switched to others types with distinct chemical composition, or organic C inputs are sufficient for silt+clay to pass a C threshold which can indicate changes in

types or strengths of bindings between organic matter and soil minerals. Stability and turnover time of organic matter associated with silt+clay sized soil particles can be regulated by strengths and types of organo-mineral bonding (Kleber et al., 2007; Sollins et al., 2009), which would be influenced by chemical composition of organic matter associated with soil minerals.

The results show that  $C_0$  were not significantly higher in treatments with manure inputs than in the control for samples from all the three sites (Figure 4.1, middle row), confirming that manure inputs with current levels did not change the relative size of biologically labile organic matter pool. One exception is that  $C_0$  was significantly higher in the treatment with inorganic fertilizer application alone (NPK) than in the control for samples from Gongzhuling (Figure 4.1, middle row), which might be due to larger absolute biologically labile SOM pool. Demands for nutrients from the mineralization of SOM especially of the labile SOM pool were probably greater in the treatment (NPK) than in the control, since higher crop yields in the treatment (NPK) than in the control indicate greater nutrient demand for crop growth. Organic C concentrations of silt+clay were almost the same in the treatment (NPK, 1.27%) as in the control (1.25%), and therefore the greater absolute labile organic matter pool would enlarge the relative size of this pool to the entire soil C.

Changes in  $k$  with increasing SOC showed no patterns for samples from Gongzhuling ( $R^2 = 0.048$ ,  $P = 0.492$ ), Zhengzhou ( $R^2 = 0.006$ ,  $P = 0.816$ ), or Qiyang ( $R^2 = 0.232$ ,  $P = 0.112$ ) (Figure 4.1, bottom row). However, differences of  $k$  among treatments with different levels of organic C inputs were site specific. Multiple comparison results showed that SOM decomposition constant was significantly lower in



the treatment (NPK) than in the control in Gongzhuling, and were higher in treatments with manure inputs (NPKM and 1.5NPKM) than in the control in Qiyang (Figure 4.1, bottom row). The results suggest that organic C inputs could increase stability of biologically labile SOM without the influences from changes in the relative size of this pool.

#### 4.3.1.2 Chemical SOM stability

Mean values across treatments for the proportion of pyrophosphate soluble C (*i.e.*, OM(pyro)) were 23.0% for samples from Gongzhuling, 19.2% from Zhengzhou, and 30.6% from Qiyang (Figure 4.3), similar to the ratios (16.0-28.6%) for soils at treatments with inorganic and organic fertilizers applications from the long-term field experiment “Ewiger Roggen” in Halle (Kaiser and Ellerbrock, 2005).

Ratios of OM(pyro) decreased with increasing SOC for samples from Qiyang ( $R^2 = 0.415$ ,  $P = 0.024$ ), but no patterns were observed for samples from Gongzhuling ( $R^2 = 0.001$ ,  $P = 0.910$ ) or Zhengzhou ( $R^2 = 0.004$ ,  $P = 0.850$ ) (Figure 4.3). The OM(pyro) is highly dependent on the importance of cation bridging in organo-mineral interactions. Cation bridging is one of the mechanisms to form organo-mineral complexes (Stevenson, 1994) and has different contributions to organo-mineral binding in sites where soil mineralogy are different (Kaiser and Ellerbrock, 2005; Wattel-Koekkoek et al., 2001).

Ratios of OM(pyro) showed different change patterns among four treatments within each of the three study sites. Ratios of OM(pyro) did not significantly vary for samples from Gongzhuling, and were significantly lower in the treatment (NPK) than in the control for samples from Zhengzhou and also significantly lower in treatments with manure inputs (NPKM and 1.5NPKM) than in the treatment (NPK) for samples from

Qiyang. Inconsistent change patterns of chemical SOM stability among four treatments within each study site might be due to different contributions of plant C, manure C, and microbial derived C to organic matter that was stabilized on silt+clay fractions (Kaiser and Ellerbrock, 2005).

#### 4.3.1.3 Thermal stability

TG-T<sub>50</sub> decreased with increasing SOC for samples from all the three sites (Gongzhuling,  $R^2 = 0.99$ ,  $P = 0.004$ ; Zhengzhou,  $R^2 = 0.93$ ,  $P = 0.038$ ; Qiyang,  $R^2 = 0.96$ ,  $P = 0.018$ ) (Figure 4.4). The results suggest that organic matter turned thermally labile with increasing organic C inputs. And ratios of relative mass loss as a function of SOC increased at either Exo<sub>1</sub> or Exo<sub>2</sub> but decreased in Exo<sub>3</sub> in all the three sites (Table 4.2), suggesting that organic matter may have shifted from thermally stable to labile and thermal SOM stability decreased, which was in line with decrease in TG-T<sub>50</sub> with increasing SOC.

Changes in CO<sub>2</sub>-T<sub>50</sub> with increasing SOC were site specific. In Gongzhuling, although CO<sub>2</sub>-T<sub>50</sub> did not change with increasing SOC ( $R^2 = 0.03$ ,  $P = 0.624$ ), ratios of CO<sub>2</sub> evolved with increasing SOC decreased in Exo<sub>1</sub> and increased in Exo<sub>2</sub> (Table 4.2), suggesting that SOM may have shifted from thermally labile to stable as C inputs increased. In Zhengzhou, CO<sub>2</sub>-T<sub>50</sub> decreased with increasing SOC ( $R^2 = 0.63$ ,  $P = 0.002$ ), and ratios of CO<sub>2</sub> evolved with increasing SOC increased in Exo<sub>2</sub> and decreased in Exo<sub>3</sub> (Table 4.2), implying that thermal SOM decreased with increasing organic C inputs. In Qiyang, CO<sub>2</sub>-T<sub>50</sub> increased with increasing SOC ( $R^2 = 0.77$ ,  $P < 0.001$ ), and ratios of CO<sub>2</sub> evolved with increasing SOC increased in both Exo<sub>2</sub> and Exo<sub>3</sub> (Table 4.2), suggesting that thermal SOM stability increased with increasing C organic C inputs. Thermal SOM

stability based on CO<sub>2</sub> evolved during combustion would likely be affected by factors other than amounts of organic matter, such as chemical composition of organic matter in three soils and strengths and types of organo-mineral binding (Boily et al., 2007), and therefore could have caused different performance across the three sites.

Changes in DSC-T<sub>50</sub> with increasing SOC did not change for samples from Gongzhuling ( $R^2 = 0.02$ ,  $P = 0.878$ ) or Zhengzhou ( $R^2 = 0.39$ ,  $P = 0.378$ ), but increased for samples from Qiyang ( $R^2 = 0.97$ ,  $P = 0.017$ ). Since the heat flux detected during combustion is the result of energy needed to initiate combustion of samples and energy released from combustion of samples that includes organic matter and soil minerals, DSC-T<sub>50</sub> would be influenced by bindings between organic matter and soil minerals. The complicated organo-mineral assemblage may be responsible for different patterns of DSC-T<sub>50</sub> with increasing SOC, and decreases the power of using DSC-T<sub>50</sub> to indicate SOM stability.

#### *4.3.2 SOM stability of bulk soil with different levels of C inputs*

As silt+clay sized soil particles are more likely to reach C saturation than bulk soil, it is more meaningful to investigate changes in SOM stability for bulk soil at different levels of organic C inputs.

Multiple comparison showed that C<sub>0</sub> was significantly larger in the treatments with manure inputs than in the control for samples from Gongzhuling and Qiyang, and did not differ greatly among the three treatments with inorganic fertilizer inputs (NPK) and with manure inputs combined (NPKM and 1.5NPKM) (Figure 4.2, middle row). Increased C<sub>0</sub> in treatments with manure inputs relative to the control shows that the relative size of biologically labile organic C pool became larger after manure

amendments. It was indeed found that manure inputs have resulted in greater increase in particulate organic matter (POM) fraction than in silt+clay fraction in the three sites (Chapter 3).

SOM decomposition constants did not significantly vary among treatments of different levels of organic C inputs in Gongzhuling ( $P = 0.471$ ), Zhengzhou ( $P = 0.115$ ), and Qiyang ( $P = 0.314$ ) (Figure 4.2, bottom row), showing that the stability of biologically labile organic matter did not change among treatments with different levels of organic C inputs in the three sites. No changes in stability of biologically labile organic matter pool suggest that stability of this organic matter pool is hardly altered with C additions, since biologically labile organic matter pool is actively mineralized and primarily controlled by microbial community and environment (*e.g.*, temperature).

Change patterns in TG-T<sub>50</sub>, CO<sub>2</sub>-T<sub>50</sub>, and DSC-T<sub>50</sub> in treatments with different levels of organic C inputs for bulk soil were similar to those for silt+clay in all the three sites (Figures 4.4 and 4.5), but values of these thermal indices of bulk soil were different from values of silt+clay in each of the four treatments. TG-T<sub>50</sub> was lower and DSC-T<sub>50</sub> were higher for bulk soil than for silt+clay at four treatments in all the three sites (Figures 4 and 5). It is probably due to increased contributions of POM and decreased contributions of organic matter associated with soil minerals to organic matter in bulk soil (Chapter 3), and POM are considered to being more easily decomposable than organic matter associated with silt+clay.

#### 4.3.3 Correlations between biological and thermal stability indices

Quality of SOM can be indicated by the number of enzymatic step to mineralize SOM to CO<sub>2</sub> (Ågren and Bosatta, 1996), and the recalcitrance of SOM has been redefined

as the energy inputs required to mineralize a substrate (Rovira et al., 2008). Biological and thermal SOM stability has the potential to be linked (Plante et al., 2011).

Correlations between biological SOM stability indices and thermal stability indices were hardly found (Table 4.3). Exceptions were that  $C_0$  were negatively correlated with TG-T<sub>50</sub> for both bulk soil and silt+clay from Qiyang and for bulk soil from Zhengzhou (Table 4.3), and were positively correlated with CO<sub>2</sub>-T<sub>50</sub> for bulk soil from Qiyang and with DSC-T<sub>50</sub> for both bulk soil and silt+clay from Qiyang (Table 4.3). The k of silt+clay was negatively correlated with TG-T<sub>50</sub> and positively correlated with DSC-T<sub>50</sub> for samples from Qiyang (Table 4.3), and k of bulk soil showed no correlations with thermal SOM stability indices for samples from all the three sites (Table 4.3).

The lack of correlations between biological and thermal stability indices indicates that biological and thermal SOM stability were not well linked for samples from three study sites. It should be cautious to use thermal analysis to indicate SOM stability without other information, such as chemical composition and mineralogical properties.

#### **4.4. Summary and conclusions**

SOM stability of silt+clay and bulk soil was investigated in treatments with different levels of organic C inputs.

Biological SOM stability of silt+clay did not change with increasing organic C inputs, which might be due to unaltered types or strengths of organo-mineral bindings. By contrast, differences of stability of biologically labile organic matter pool of silt+clay among treatments with difference levels of organic C inputs were site specific. Chemical SOM stability of silt+clay did not change with increasing organic C inputs for samples from Gongzhuling and Zhengzhou and decreased for samples from Qiyang. Thermal

SOM stability, indicated by TG-T<sub>50</sub> decreased with increasing organic C inputs for samples from all the three sites.

Biological SOM stability of bulk soil became smaller in treatments with manure inputs than in the control, but stability of biologically labile organic matter pool did not change in treatments with different levels of manure inputs. The results suggest that the relative size and stability of biologically labile organic matter pool are independent of each other. Thermal SOM stability of bulk soil were also smaller in treatments with manure inputs than in the control, similar to the patterns of silt+clay.

However, correlations between biological and thermal stability indices were hardly found for samples from three study sites, showing that biological and thermal SOM stability were not well linked. Hence, other information of samples should be incorporated to interpret thermal data for SOM stability.

Overall, biological and chemical SOM stability of silt+clay showed no trends with increasing organic C inputs, which do not support the prediction of the soil C saturation theory that SOM stability decreases as soils approach C saturation with increasing organic C inputs. In the three study sites, biological SOM stability of bulk soil decreased when soils were subjected to manure amendments, suggesting that larger amounts of organic C inputs compared to previous level are needed to store the same amount of organic C inputs to bulk soil.

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**Table 4.1**

Organic C concentrations of silt+clay and bulk soil at different treatments in Gongzhuling, Zhengzhou, and Qiyang sites. Data are mean  $\pm$  SE.

Site	Treatment	C concentration (silt+clay, %)	C concentration (bulk soil, %)
Gongzhuling	Check	1.23 $\pm$ 0.04	1.29 $\pm$ 0.10
	NPK	1.25 $\pm$ 0.01	1.44 $\pm$ 0.06
	NPKM	1.77 $\pm$ 0.04	2.37 $\pm$ 0.12
	1.5NPKM	1.88 $\pm$ 0.05	2.68 $\pm$ 0.21
Zhengzhou	Check	1.42 $\pm$ 0.11	0.75 $\pm$ 0.05
	NPK	1.40 $\pm$ 0.11	0.78 $\pm$ 0.07
	NPKM	1.74 $\pm$ 0.01	1.11 $\pm$ 0.07
	1.5NPKM	1.90 $\pm$ 0.02	1.15 $\pm$ 0.05
Qiyang	Check	0.79 $\pm$ 0.00	0.83 $\pm$ 0.04
	NPK	0.95 $\pm$ 0.05	1.10 $\pm$ 0.02
	NPKM	1.23 $\pm$ 0.09	1.61 $\pm$ 0.09
	1.5NPKM	1.57 $\pm$ 0.03	2.05 $\pm$ 0.13

**Table 4.2**

Changes in ratios of relative mass loss and CO<sub>2</sub> evolved during combustion as a function of organic C concentration at three exothermic temperature ranges for silt+clay and bulk soil from Gongzhuling, Zhengzhou, and Qiyang sites. Exo<sub>1</sub>: 200-380 °C; Exo<sub>2</sub>: 380-475 °C; Exo<sub>3</sub>: 475-600 °C. “ns” means no statistically significant.

Fraction	Site	Temperature	Mass loss		CO <sub>2</sub> evolved	
			slope	p	slope	p
Silt+clay	Gongzhuling	Exo <sub>1</sub>	0.053	<0.001	--	ns
		Exo <sub>2</sub>	-0.017	0.005	-0.020	0.016
		Exo <sub>3</sub>	-0.036	0.004	--	ns
	Zhengzhou	Exo <sub>1</sub>	0.096	0.002	0.080	0.002
		Exo <sub>2</sub>	--	ns	0.021	0.000
		Exo <sub>3</sub>	-0.099	0.002	-0.101	0.001
	Qiyang	Exo <sub>1</sub>	0.080	<0.001	-0.080	0.004
		Exo <sub>2</sub>	--	ns	0.063	<0.001
		Exo <sub>3</sub>	-0.087	0.000	0.032	0.012
Bulk soil	Gongzhuling	Exo <sub>1</sub>	0.024	<0.001	-0.017	0.008
		Exo <sub>2</sub>	--	ns	0.013	0.007
		Exo <sub>3</sub>	-0.023	0.001	--	ns
	Zhengzhou	Exo <sub>1</sub>	0.118	0.002	--	ns
		Exo <sub>2</sub>	0.018	0.053	0.047	0.002
		Exo <sub>3</sub>	-0.135	0.002	-0.084	0.011
	Qiyang	Exo <sub>1</sub>	0.054	0.000	-0.058	0.013
		Exo <sub>2</sub>	--	ns	0.056	0.001
		Exo <sub>3</sub>	-0.069	<0.001	--	ns

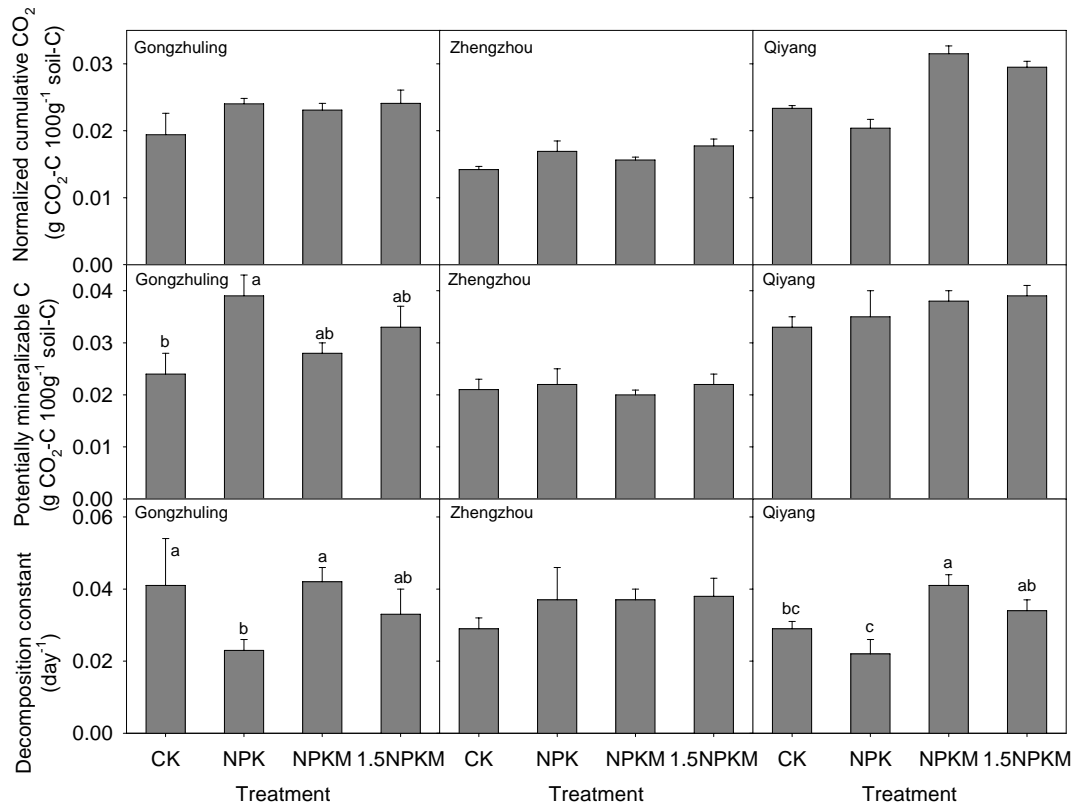
**Table 4.3**

Correlations biological SOM stability (normalized cumulative CO<sub>2</sub>, potentially mineralizable C (C<sub>0</sub>), and SOM decomposition constant (k)) and thermal SOM stability (TG-T<sub>50</sub>, CO<sub>2</sub>-T<sub>50</sub>, and DSC-T<sub>50</sub>) for silt+clay and bulk soil from Gongzhuling (GZ), Zhengzhou (ZZ), and Qiyang (QY) sites.

Variable	Variable	<u>Silt + clay</u>			<u>Bulk soil</u>		
		GZ	ZZ	QY	GZ	ZZ	QY
Cumulative CO <sub>2</sub>	TG-T <sub>50</sub>	--	--	--	--	--	-0.79
	CO <sub>2</sub> -T <sub>50</sub>	--	--	--	--	--	0.85
	DSC-T <sub>50</sub>	--	--	0.61	--	--	0.69
C <sub>0</sub>	TG-T <sub>50</sub>	--	--	-0.76	--	-0.61	-0.90
	CO <sub>2</sub> -T <sub>50</sub>	--	--	--	--	--	0.79
	DSC-T <sub>50</sub>	--	--	0.83	--	--	0.70
k	TG-T <sub>50</sub>	--	--	-0.59	0.58	--	--
	CO <sub>2</sub> -T <sub>50</sub>	--	--	--	--	--	--
	DSC-T <sub>50</sub>	--	--	0.64	--	--	--

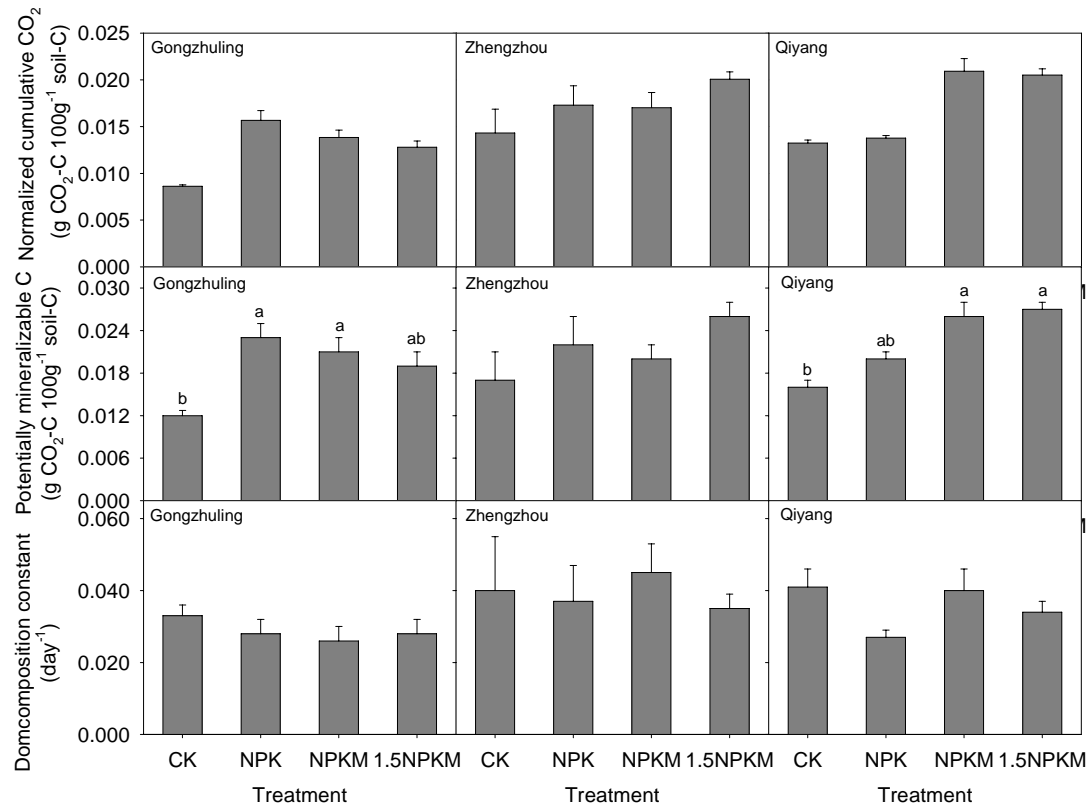
**Figure 4.1**

Biological stability (normalized cumulative CO<sub>2</sub>, potentially mineralizable C, and SOM decomposition constant) of organic matter in different treatments for silt+clay from Gongzhuling, Zhengzhou, and Qiyang with long-term manure amendments. Different letters indicate values are significantly different from each other.



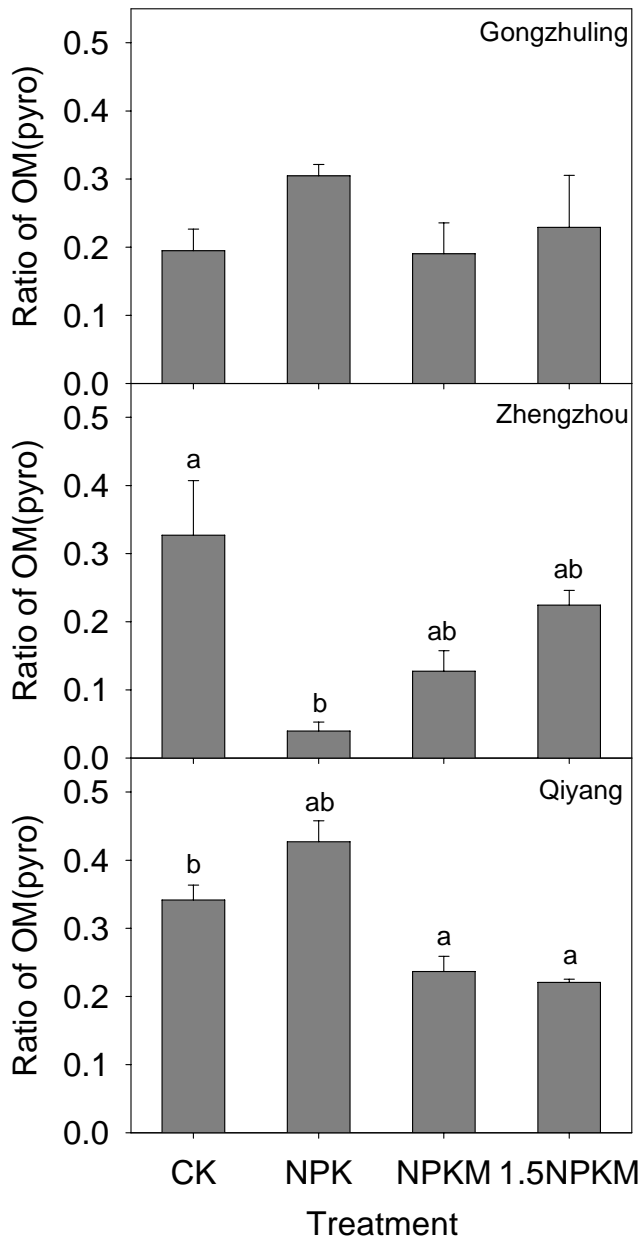
**Figure 4.2**

Biological stability (normalized cumulative CO<sub>2</sub>, potentially mineralizable C, and SOM decomposition constant) of organic matter in different treatments for bulk soil from Gongzhuling, Zhengzhou, and Qiyang with long-term manure amendments. Different letters indicate values are significantly different from each other.



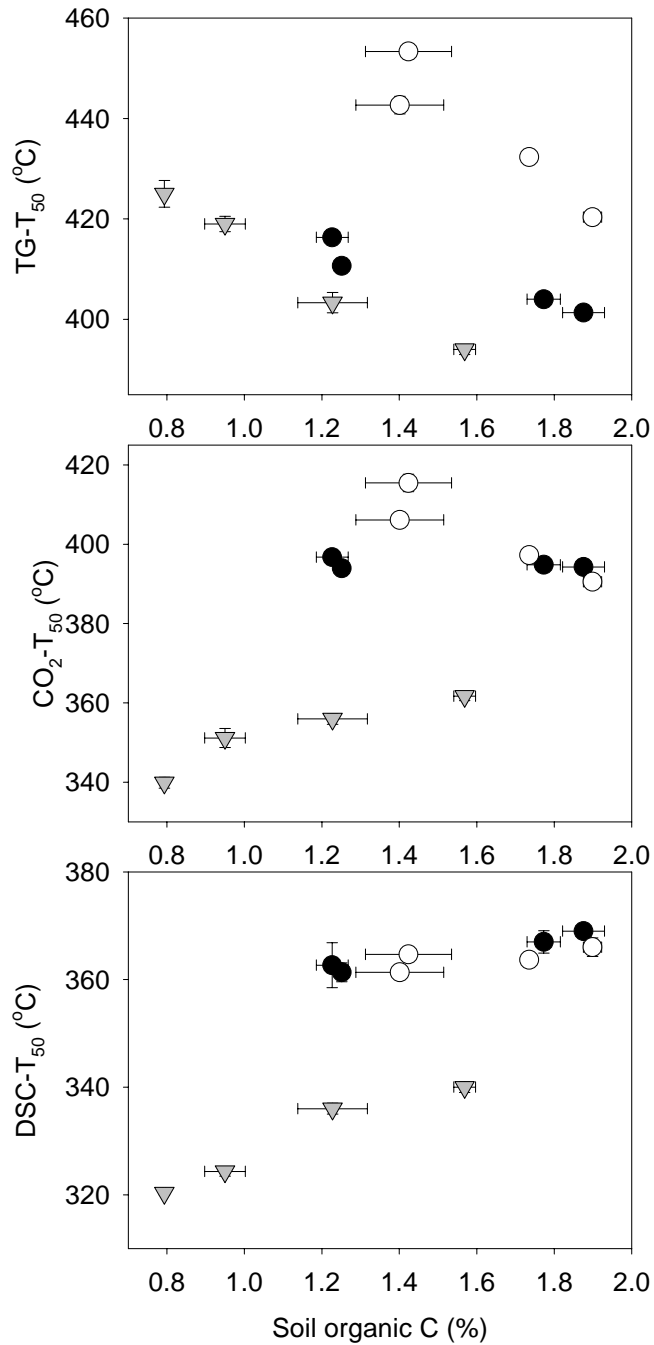
**Figure 4.3**

Ratios of organic matter solved in sodium pyrophosphate (OM(pyro)) in different treatments for silt+clay from Gongzhuling, Zhengzhou, and Qiyang with long-term manure amendments. Different letters indicate values are significantly different from each other.



**Figure 4.4**

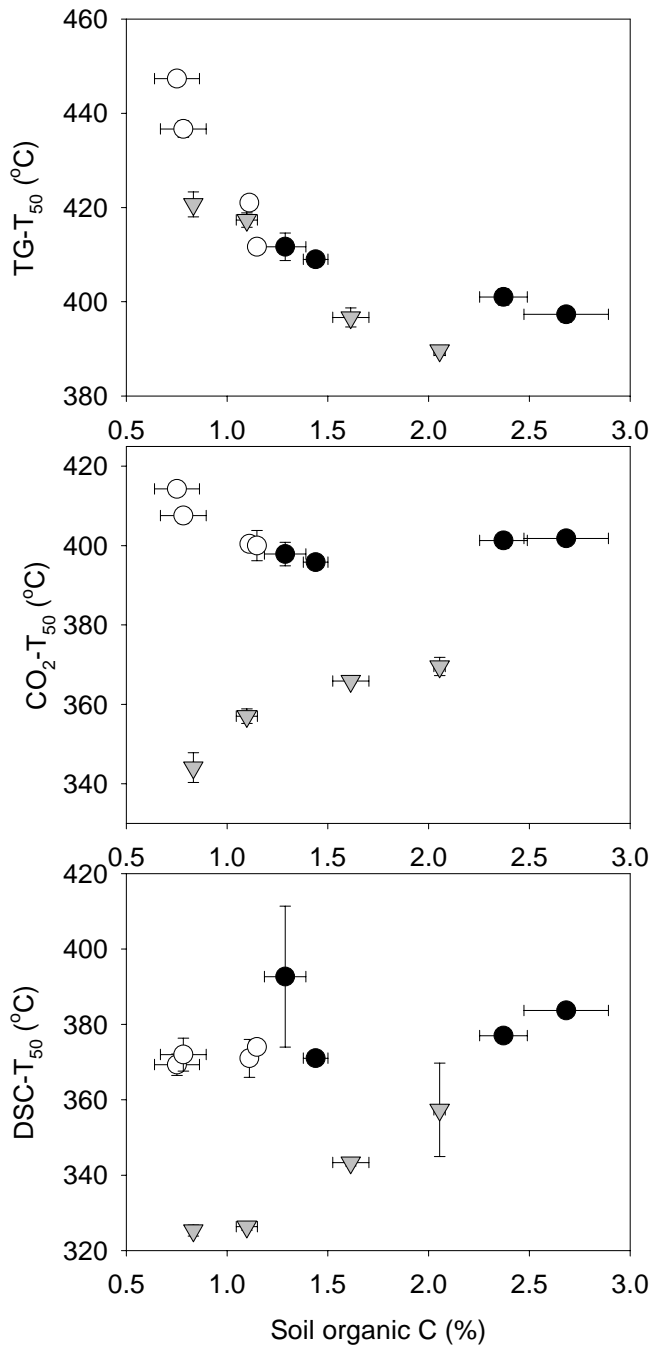
Thermal stability indices (TG-T<sub>50</sub>, CO<sub>2</sub>-T<sub>50</sub>, and DSC-T<sub>50</sub>) of soil organic matter as a function of organic C concentrations for silt+clay from Gongzhuling (closed circle), Zhengzhou (open circle), and Qiyang (gray triangle) with long-term manure amendments.





**Figure 4.5**

Thermal stability indices (TG-T<sub>50</sub>, CO<sub>2</sub>-T<sub>50</sub>, and DSC-T<sub>50</sub>) of soil organic matter as a function of organic C concentrations for bulk soil from Gongzhuling (closed circle), Zhengzhou (open circle), and Qiyang (gray triangle) with long-term manure amendments.



## SUMMARY AND CONCLUSION

The goal of this dissertation was to test the soil C saturation theory using two approaches, first to find ways to estimate C saturated levels of soil minerals, and second to quantify changes in the stability of organic matter associated with soil minerals as soils approach C saturation.

**Chapter 1:** The goal of this chapter was to improve predictions of maximal organic C stabilization by using two alternative approaches – organic C loadings and boundary line analysis. A meta-analysis shows that predictions of maximal SOC stabilization using least squares linear regression indeed does not work well. While predictions of maximal organic C stabilization using the organic C loading approach fit the data for soils dominated by 2:1 minerals well, such predictions do not fit the data for soils dominated by 1:1 minerals. The boundary line analysis estimate of maximal organic C stabilization was more than double the estimate by the linear regression approach. These results show that linear regression models do not adequately predict maximal organic C stabilization. Soil properties associated with soil mineralogy, such as specific surface area and organic C loading, should be incorporated to generate more mechanistic models for predicting soil C saturation, but in their absence, statistical models should represent the upper envelope rather than the average value.

**Chapter 2:** The goal of this study was test whether the stability of organic matter associated with soil minerals decreases with increasing C loading. Batch DOM sorption experiments were conducted to obtain organo-mineral complexes with a range of organic C loadings. The relative stability of organic matter sorbed on organo-mineral complexes was subsequently assessed using laboratory incubation and evolved CO<sub>2</sub> gas analysis

during thermal analyses. Amounts of organic C sorption determined by difference in DOM concentrations before and after sorption was found to overestimate the actual amount of organic C sorbed on soils, which was more accurately determined using solid phase measurements. Results also suggested that the composition of the organic matter in the organo-mineral complexes may have changed as the amount of sorbed organic matter increased. Incubation and thermal analysis results suggested that the relative biological and thermal stability of sorbed C increased as organic C loading increased, which do not support the hypothesis that SOM stability decreases with increasing C loading.

**Chapter 3:** This chapter primarily tested whether soil C saturation can be observed in a wide range of soil types in agricultural ecosystems, and to find soil mineralogical properties that could influence soil C saturation. Seven long-term agricultural field experiments (*i.e.*, Gongzhuling, Zhengzhou, Qiyang, Laiyang, Melfort, Dixon cattle, and Dixon swine), with each having multiple levels of manure inputs for 10-20 years, were selected for this study. In the seven sites, C concentrations of bulk soil, POM+sand, and silt+clay were higher in treatments with manure amendments than in treatments without manure amendments. However, changes in C concentrations of silt+clay as a function of gross and net organic C inputs did not fit the asymptotic regression better than the linear regression, suggesting that silt+clay has not reached C saturation in the study sites. In addition, C loadings of silt+clay were larger than the presumed maximal organic C loading ( $1 \text{ mg C m}^{-2}$ ) in some sites where silt+clay did not exhibit C saturation, suggesting that the maximal C loading may be greater than  $1 \text{ mg C m}^{-2}$ . Soil mineralogical properties (*i.e.*, mineral specific surface area and Fe/Al oxides) did not greatly influence the amount of organic matter bounded on silt+clay.

**Chapter 4:** This study examined SOM stability of silt+clay and bulk soil from agricultural sites with different levels of organic C inputs. Soils from three agricultural sites (*i.e.*, Gongzhuling, Zhengzhou, and Qiyang) with long-term multiple levels of organic carbon inputs were selected to examine biological, chemical, and thermal SOM stability for silt+clay and bulk soil. In the three study sites, with increasing organic inputs, biological SOM stability of silt+clay did not change greatly and chemical and thermal SOM stability showed no general change patterns. The results do not support the hypothesis that SOM stability decreases with increasing C inputs. Biological SOM stability of bulk soil was lower in treatments with manure inputs than in the control, and thermal SOM stability indicated by TG-T<sub>50</sub> decreased in treatments with manure inputs than in the control, both of which suggest that larger amounts of organic C inputs than previous levels are needed to store the same amount of organic C inputs to bulk soil.

## **Conclusions**

Results of studies that aimed to estimate soil C saturated level confirmed that the current model to estimate C saturated level by fine soil particles by using the linear regression does not adequately predict maximal organic C stabilization. Alternative methods, such as the organic C loading method which is more mechanistic and incorporates properties associated with soil mineralogy, should be used to estimate C saturated level of fine soil particles. Meanwhile, searching for factors that may regulate soil C saturated level by using soils have reached C saturation did not work either in a long-term manure amended field study or in a batch DOM sorption experiment. In the long-term manure amended field study, fine soil particles from the seven agricultural sites, each of which has experienced 10-20 years of multi-level manure inputs, have not

reached C saturation. It is likely that soils in agricultural ecosystems are far away from C saturated levels, and therefore are able to store large amounts of organic C inputs. In the batch DOM sorption experiment, four types of soils with low initial soil C and different mineralogy have not reached C saturation under the experimental conditions, suggesting sufficient amounts of organic C inputs are needed for soils to reach C saturated level.

It is still inconclusive to use presumed maximal C loading of soils ( $1 \text{ mg C m}^{-2}$ ) as a standard to determine whether soils have reached C saturation, considering that fine soil particles with organic C loading higher than  $1 \text{ mg C m}^{-2}$  were found to linearly increase with organic C inputs in both the field study with long-term manure amendments and the batch DOM sorption experiment.

In the studies that tested whether SOM stability decreases as soils approach C saturation, biological stability of organic matter sorbed on four types of soils increased as organic C loadings increased, and biological and chemical stability of organic matter associated with silt+clay from agricultural sites showed no change patterns with increasing organic C inputs. The results do not support the prediction of the soil C saturation. Inconsistent patterns of how SOM stability changes with increasing organic C inputs suggest that further studies on this topic should investigate the influences of chemical composition of organic C inputs and types and strengths of organo-mineral bindings on changes of SOM stability with increasing C inputs.

## **Future studies**

Although the soil C saturation theory was proposed in 2002 and has been studied since then for ten years, it is still not well known how to estimate C saturated levels for different types of soils, and how to predict rates and durations required to reach C

saturation by quantifying changes in SOM stability as soils approach C saturation.

After confirming that the current model underestimates the C saturated level of fine soil particles, two alternative methods (*i.e.*, organic C loading and boundary line analysis), improve the estimate to some extent, however, both methods require further studies. Organic C loading is the preferred method for estimating C saturated levels since mineral surface area can be easily measured, while the boundary line analysis is relatively subjective and lacks underlying mechanisms. However, it is critical to find what the maximal organic C loading is for different types of soils in order to use organic C loading method to estimate C saturated level of fine soil particles.

The results of the field study with manure amendments and batch DOM sorption suggest that maximal organic C loadings of certain types of soil minerals may be higher than presumed value ( $1 \text{ mg C m}^{-2}$ ). One way to find the maximal organic C loading is to examine C loadings of soil minerals that have reached C saturation. Another approach might be to identify the critical C loading value at which stability of organic matter associated with soil minerals dramatically decreases as organic C inputs increase.

To address the questions regarding the soil C saturation theory, one direct way is to study various soils that have reached C saturation and to find factors that regulate soil C saturated level. However, it is not easy to find soils that have approached C saturated levels in agricultural ecosystems. A promising alternative approach to produce C saturated soils with sufficient organic C inputs in laboratory experiments, such as batch DOM sorption. The results from the batch DOM sorption experiment in Chapter 2 suggest that soils are likely to approach C saturated levels when organic C input are sufficient. When conducting simulating experiments to study the soil C saturation,

organic C inputs, soil matrix properties, and environmental conditions should be close to conditions in field.

Even if organic C levels of most soils in the field, especially in agricultural ecosystems, are below C saturated levels with typical amounts of organic C inputs, we still need to better understand soil C saturated levels in order to identify soils that have the potential to store large amounts of organic C inputs. Additionally, in order to manage SOC stocks, it is necessary to quantify changes in SOM stability with increasing organic C inputs, which are critical to estimate rates and durations required for soils to approach maximal SOC storage. Changes in SOM stability of soil minerals with increasing organic C inputs from my experiments show mixed results, which might be caused by differences in chemical composition of organic C inputs and organo-mineral bindings. Studies are needed to address how chemical composition and organo-mineral interactions influence changes in SOM stability as organic C inputs increase.

It is important to know organo-mineral interactions well by using different analytical techniques, considering that the soil C saturation model may be only applied to organo-mineral complexes, which are influenced by organic and inorganic components as well as specific environmental conditions. Analyses using multiple techniques of organo-mineral complexes may illustrate how organic and inorganic components influence organo-mineral interactions under changing environment. In addition to the studies that focus on organo-mineral interactions to stabilize organic C inputs, it is also important to know more about nitrogen and phosphorus transformation and availability and enzymatic activity coupled with SOM stabilization and de-stabilization processes, Such studies will

improve our understanding of important functions of SOM. such as supplying nutrients for plant growth, that may occur in the SOM de-stabilization processes.



## APPENDIX

Soil mass proportion (g fraction 100 g<sup>-1</sup> soil) and organic C content (g C kg<sup>-1</sup> soil) of fine soil particles (< 20 µm), Hassink organic C (g C kg<sup>-1</sup> soil), saturation ratio, soil fractions (µm), land use type, and dominant soil mineral types of compiled publications.

Source	Mineral type	Land Use	Mass proportion	Organic C content	Hassink organic C	Saturation ratio
Amelung et al. (1998)	2:1	Grassland	19.90	7.41	11.45	0.65
			26.10	7.00	13.75	0.51
			28.00	7.51	14.45	0.52
			32.50	12.56	16.12	0.78
			32.60	9.92	16.15	0.61
			33.60	9.92	16.52	0.60
			35.10	27.63	17.08	1.62
			38.70	15.12	18.41	0.82
			41.30	22.64	19.37	1.17
			45.30	18.69	20.85	0.90
			45.70	16.06	21.00	0.76
			47.30	23.12	21.59	1.07
			48.60	27.01	22.07	1.22
			49.60	22.22	22.44	0.99
			51.40	39.88	23.11	1.73
			53.70	19.84	23.96	0.83
57.80	12.57	25.48	0.49			
59.70	24.24	26.18	0.93			
59.80	29.39	26.22	1.12			
64.60	48.23	27.99	1.72			
77.40	41.76	32.73	1.28			
Balabane and Plante (2004)	2:1	Cropland	17.70	3.52	10.62	0.33
			7.90	3.04	6.99	0.44
			28.40	5.63	14.58	0.39
			26.70	10.24	13.95	0.73
Balesdent et al. (1998)	2:1	Cropland	57.66	13.80	25.42	0.54
			62.46	25.80	27.20	0.95
			61.43	47.40	26.82	1.77
Barthès et al. (2008)	1:1	Cropland	8.30	4.10	7.16	0.57
			14.50	3.30	9.46	0.35

			17.50	5.30	10.57	0.50
			19.20	4.10	11.19	0.37
			19.20	5.20	11.19	0.46
			22.60	2.90	12.45	0.23
			57.10	17.10	25.22	0.68
			59.70	12.60	26.18	0.48
			83.40	15.70	34.95	0.45
			86.80	12.90	36.21	0.36
	1:1	Forest	76.60	17.30	32.43	0.53
	1:1	Grassland	46.90	15.90	21.44	0.74
			47.70	16.60	21.74	0.76
			63.20	22.40	27.47	0.82
			76.00	27.80	32.21	0.86
			77.10	14.40	32.62	0.44
			77.50	16.70	32.77	0.51
			85.70	23.70	35.80	0.66
Bates (1960)	1:1	Forest	9.70	7.06	7.68	0.92
Besnard et al. (2001)	2:1	Cropland	40.68	8.58	19.14	0.45
			50.30	12.16	22.70	0.54
			41.60	18.57	19.48	0.95
			54.20	12.30	24.14	0.51
Bonde et al. (1992)	2:1	Cropland	70.30	16.46	30.10	0.55
			70.50	15.49	30.18	0.51
	1:1	Forest	62.90	24.80	27.36	0.91
			62.20	26.14	27.10	0.96
Caravaca et al. (1999)	2:1	Cropland	29.50	5.76	15.01	0.38
			35.50	3.97	17.23	0.23
			45.30	9.92	20.85	0.48
			47.20	7.28	21.55	0.34
			47.80	4.16	21.78	0.19
			48.20	6.43	21.92	0.29
			59.80	3.89	26.22	0.15
			66.20	10.07	28.58	0.35
			67.20	3.64	28.95	0.13
			67.70	8.24	29.14	0.28
			67.80	12.89	29.18	0.44
			71.20	6.73	30.43	0.22
			77.50	6.02	32.77	0.18
			79.30	12.96	33.43	0.39

	2:1	Forest	37.20	20.90	17.85	1.17
			40.20	22.89	18.96	1.21
			40.80	21.38	19.19	1.11
			43.70	43.74	20.26	2.16
			45.00	38.28	20.74	1.85
			50.00	43.88	22.59	1.94
Christensen (1985)	2:1	Cropland	6.90	3.83	6.64	0.58
			10.00	8.67	7.79	1.11
			21.50	8.89	12.05	0.74
			28.20	9.15	14.52	0.63
			31.80	12.61	15.86	0.80
Christensen (1986)	2:1	Cropland	13.50	3.36	9.09	0.37
			13.50	3.26	9.09	0.36
			36.80	14.88	17.71	0.84
			36.80	13.75	17.71	0.78
Christensen (1987)	2:1	Cropland	13.30	21.69	9.01	2.41
			13.60	22.44	9.12	2.46
			30.10	10.70	15.23	0.70
			30.20	11.07	15.26	0.73
Dalal and Mayer (1986)	2:1	Grassland	52.10	13.23	23.37	0.57
			55.80	14.75	24.74	0.60
			65.20	14.01	28.21	0.50
Feller and Beare (1997)	1:1	Cropland	10.10	3.22	7.83	0.41
			12.30	3.53	8.64	0.41
			12.50	3.74	8.72	0.43
			13.30	3.85	9.01	0.43
			13.60	3.52	9.12	0.39
			15.20	3.86	9.71	0.40
			15.60	4.72	9.86	0.48
			26.90	9.71	14.04	0.69
			28.40	7.22	14.60	0.49
			69.40	14.84	29.77	0.50
			70.10	12.60	30.03	0.42
			71.60	13.34	30.58	0.44
			80.50	16.91	33.88	0.50
			86.90	33.29	36.24	0.92
			87.10	27.37	36.32	0.75
	1:1	Forest	11.00	5.45	8.16	0.67
			22.70	13.09	12.49	1.05

			29.40	9.82	14.97	0.66
			61.40	18.04	26.81	0.67
			62.90	26.11	27.36	0.95
			64.40	34.18	27.92	1.22
	1:1	Grassland	4.90	1.53	5.90	0.26
			11.90	5.02	8.49	0.59
			17.40	5.98	10.53	0.57
			19.30	7.48	11.23	0.67
			19.30	7.82	11.23	0.70
			25.20	9.67	13.41	0.72
			28.40	12.69	14.60	0.87
			30.50	10.65	15.38	0.69
			72.00	9.79	30.73	0.32
			82.30	30.07	34.54	0.87
			82.90	36.05	34.76	1.04
	2:1	Cropland	11.70	2.68	8.42	0.32
			70.50	25.40	30.18	0.84
	2:1	Forest	12.00	3.89	8.53	0.46
	2:1	Grassland	59.40	19.41	26.07	0.74
			70.10	32.70	30.03	1.09
Guggenberger et al. (1994)	2:1	Cropland	58.50	24.17	25.74	0.94
	2:1	Forest	63.20	71.70	27.47	2.61
			63.70	46.43	27.66	1.68
	2:1	Grassland	60.10	52.04	26.33	1.98
Guggenberger et al. (1995)	1:1	Grassland	56.00	22.31	24.81	0.90
			56.10	19.60	24.85	0.79
			56.50	22.56	25.00	0.90
Guibert et al. (1999)	1:1	Cropland	8.90	2.07	7.38	0.28
			9.80	2.12	7.72	0.28
			10.80	4.20	8.09	0.52
			11.70	4.70	8.42	0.56
			26.30	5.15	13.82	0.37
			30.30	4.62	15.30	0.30
			33.40	6.57	16.45	0.40
			33.80	5.54	16.60	0.33
Hassink (1997)	2:1	Cropland	3.60	3.60	5.42	0.66
			6.20	7.40	6.38	1.16
	2:1	Grassland	1.90	4.10	4.79	0.86
			2.80	0.90	5.13	0.18
			3.50	3.60	5.39	0.67

			3.60	1.00	5.42	0.18
			4.40	7.50	5.72	1.31
			5.40	3.00	6.09	0.49
			5.80	10.20	6.24	1.64
			8.90	3.60	7.38	0.49
			13.30	11.10	9.01	1.23
			35.60	18.60	17.26	1.08
			36.50	27.50	17.60	1.56
			42.60	19.90	19.85	1.00
			45.50	13.50	20.93	0.65
			76.00	31.00	32.21	0.96
Jandl et al. (2004)	2:1	Cropland	47.60	14.10	21.68	0.65
			47.40	17.96	21.61	0.83
Kahle et al. (2002a)	2:1	Cropland	49.40	17.78	22.37	0.79
			49.80	9.84	22.52	0.44
			50.20	9.88	22.66	0.44
			50.30	7.04	22.70	0.31
			51.50	8.45	23.15	0.37
			55.80	10.97	24.74	0.44
			56.00	12.76	24.81	0.51
Kölbl et al. (2006)	2:1	Cropland	52.20	11.85	23.40	0.51
			53.40	9.63	23.85	0.40
Larré-Larrouy et al. (2003)	2:1	Cropland	56.50	8.31	25.00	0.33
	2:1	Grassland	62.00	22.17	27.03	0.82
Leinweber and Reuter (1992)	2:1	Grassland	37.60	14.82	18.00	0.82
			41.00	8.88	19.26	0.46
			41.50	10.63	19.45	0.55
			41.90	7.41	19.59	0.38
Liang et al. (2009)	2:1	Cropland	52.50	8.40	23.52	0.36
			53.80	13.30	24.00	0.55
			57.10	12.90	25.22	0.51
			58.20	21.70	25.62	0.85
			58.20	11.90	25.62	0.46
			58.30	12.70	25.66	0.49
			58.70	21.70	25.81	0.84
			59.70	15.20	26.18	0.58
			60.00	18.00	26.29	0.68
			60.20	12.70	26.36	0.48
			60.60	21.00	26.51	0.79
			61.60	24.60	26.88	0.92

		61.60	13.40	26.88	0.50
		63.60	25.60	27.62	0.93
		64.30	15.50	27.88	0.56
		67.10	16.90	28.92	0.58
		67.10	22.70	28.92	0.79
		67.30	27.80	28.99	0.96
		68.10	17.90	29.29	0.61
		69.10	23.00	29.66	0.78
		70.00	19.00	29.99	0.63
		70.80	18.90	30.29	0.62
		71.40	18.30	30.51	0.60
		71.60	26.00	30.58	0.85
		71.80	17.10	30.66	0.56
		71.90	25.90	30.69	0.84
		79.30	21.50	33.43	0.64
2:1	Grassland	52.50	12.90	23.52	0.55
		53.80	16.00	24.00	0.67
		57.10	14.50	25.22	0.58
		58.20	27.80	25.62	1.08
		58.20	15.90	25.62	0.62
		58.30	16.80	25.66	0.65
		58.70	22.00	25.81	0.85
		59.70	18.30	26.18	0.70
		60.00	29.70	26.29	1.13
		60.20	18.30	26.36	0.69
		60.60	29.20	26.51	1.10
		61.60	29.40	26.88	1.09
		61.60	26.20	26.88	0.97
		63.60	26.40	27.62	0.96
		64.30	21.70	27.88	0.78
		67.10	20.80	28.92	0.72
		67.10	25.70	28.92	0.89
		67.30	28.00	28.99	0.97
		68.10	21.10	29.29	0.72
		69.10	28.50	29.66	0.96
		70.00	21.90	29.99	0.73
		70.80	23.20	30.29	0.77
		71.40	25.40	30.51	0.83
		71.60	26.50	30.58	0.87

			71.80	21.50	30.66	0.70
			71.90	30.70	30.69	1.00
			79.30	29.20	33.43	0.87
McKeague (1971)	2:1	Forest	43.00	20.78	20.00	1.04
			43.80	30.25	20.30	1.49
			60.00	26.08	26.29	0.99
	2:1	Grassland	37.00	22.40	17.78	1.26
			45.00	34.25	20.74	1.65
			48.30	43.07	21.96	1.96
			52.10	47.93	23.37	2.05
			59.40	50.60	26.07	1.94
			62.40	47.36	27.18	1.74
			70.00	37.20	29.99	1.24
			70.20	70.69	30.06	2.35
			70.30	48.01	30.10	1.59
			73.30	28.10	31.21	0.90
Nacro et al. (1996)	1:1	Grassland	16.02	6.03	10.02	0.60
Schulten et al. (1993)	2:1	Cropland	33.10	21.02	16.34	1.29
Steffens et al. (2009)	2:1	Grassland	14.89	6.94	9.60	0.72
			22.10	10.17	12.27	0.83
			25.28	11.24	13.44	0.84
			26.14	11.64	13.76	0.85
			26.28	12.05	13.81	0.87
			27.01	12.23	14.08	0.87
			27.33	12.48	14.20	0.88
			28.05	12.42	14.47	0.86
			28.95	13.54	14.80	0.91
			29.61	13.06	15.04	0.87
			30.02	12.82	15.20	0.84
			31.05	12.68	15.58	0.81
Thuriès et al. (2000)	2:1	Cropland	24.2	2.37	13.02	0.18
			25.2	2.70	13.39	0.20
			22.6	2.37	12.43	0.19
			22.8	2.26	12.51	0.18
			24.2	2.58	13.02	0.20
			26.1	2.53	13.73	0.18
			24.2	2.73	13.02	0.21

			26.9	2.76	14.02	0.20
			28.4	3.45	14.58	0.24
			28.7	3.10	14.69	0.21
			30.5	3.08	15.36	0.20
			31.2	3.08	15.61	0.20
			30.2	3.31	15.24	0.22
			30.4	3.35	15.32	0.22
			32.6	3.76	16.13	0.23
			32.6	3.39	16.13	0.21
Turchenek and Oades (1979)	2:1	Cropland	83.00	45.19	34.80	1.30
Zhang et al. (1988)	2:1	Cropland	49.00	14.29	22.22	0.64
			53.00	19.20	23.70	0.81
	2:1	Grassland	54.67	28.17	24.32	1.16
			56.00	32.00	24.81	1.29
Zhao et al. (2006)	2:1	Cropland	29.80	6.60	15.12	0.44
			40.10	8.60	18.93	0.45
			42.60	8.80	19.85	0.44
			46.00	20.20	21.11	0.96
			46.10	30.30	21.15	1.43
			49.70	12.00	22.48	0.53
			50.40	13.40	22.74	0.59
			51.50	15.10	23.15	0.65
			51.80	17.10	23.26	0.74
			52.20	27.30	23.40	1.17
			53.00	19.10	23.70	0.81
			53.30	15.30	23.81	0.64
			53.70	16.50	23.96	0.69
			54.50	7.60	24.26	0.31
			55.10	15.10	24.48	0.62
			55.20	11.60	24.51	0.47
			57.00	14.20	25.18	0.56
			59.00	11.70	25.92	0.45
			59.40	24.00	26.07	0.92
			64.70	15.40	28.03	0.55
			65.10	13.20	28.18	0.47
			65.70	15.20	28.40	0.54



			66.00	14.60	28.51	0.51
			66.30	13.20	28.62	0.46
			66.30	13.20	28.62	0.46
			66.50	14.80	28.70	0.52
			66.50	12.60	28.70	0.44
			69.20	12.60	29.69	0.42
Zinn et al. (2007)	1:1	Forest	6.86	4.27	6.63	0.64
			11.08	6.24	8.19	0.76
			11.57	1.74	8.37	0.21
			12.26	1.33	8.62	0.15
			14.50	6.62	9.45	0.70
			14.97	5.38	9.63	0.56
			15.19	8.68	9.71	0.89
			15.67	6.90	9.89	0.70
			17.44	2.94	10.54	0.28
			19.27	2.70	11.22	0.24
			19.82	1.83	11.42	0.16
			20.71	1.78	11.75	0.15
			34.13	9.79	16.72	0.59
			35.58	11.52	17.25	0.67
			35.85	12.22	17.35	0.70
			36.07	12.36	17.43	0.71
			38.11	4.08	18.19	0.22
			38.97	11.82	18.51	0.64
			39.27	11.07	18.62	0.59
			39.90	4.76	18.85	0.25
			40.88	2.62	19.22	0.14
			42.00	2.86	19.63	0.15
			42.91	4.88	19.97	0.24
			44.10	2.91	20.41	0.14
			52.22	10.36	23.41	0.44
			53.40	9.43	23.85	0.40
			60.98	5.31	26.65	0.20
			62.47	3.64	27.21	0.13
			62.96	13.28	27.38	0.49
			65.81	4.33	28.44	0.15
			66.00	11.17	28.51	0.39
			68.85	6.78	29.56	0.23
			68.97	16.41	29.61	0.55
			70.28	6.55	30.09	0.22

71.49	3.82	30.54	0.13
71.72	16.48	30.63	0.54

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<sup>a</sup>: Hassink organic C was calculated by using Hassink model.

<sup>b</sup>: Saturation ratio was calculated by dividing measured organic C of fine soil particles over organic C predicted by Hassink model.

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