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**ESTOQUES E MECANISMOS DE ESTABILIZAÇÃO  
DO CARBONO ORGÂNICO DO SOLO EM  
AGROECOSSISTEMAS DE CLIMA TEMPERADO E  
SUB-TROPICAL**

**TESE DE DOUTORADO**

**Rodrigo da Silveira Nicoloso**

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CARBONO ORGÂNICO DO SOLO EM AGROECOS-  
SISTEMAS DE CLIMA TEMPERADO E SUB-TROPICAL**

**por**

**Rodrigo da Silveira Nicoloso**

Dissertação apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Engenharia Agrícola, Área de Concentração em Mecanização Agrícola, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Engenharia Agrícola.**

**Orientador: Prof. Telmo Jorge Carneiro Amado**

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A Comissão Examinadora, abaixo assinada,  
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como requisito parcial para a obtenção do grau de  
**Doutor em Engenharia Agrícola**

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Santa Maria, 21 de Junho de 2009.

*O, God, thy sea is so great and my boat is so small...*

*Para minha filha, Ana Clara. Com amor!*

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A todos vocês, MUITO OBRIGADO!

## **RESUMO**

Tese de Doutorado  
Programa de Pós-Graduação em Engenharia Agrícola  
Universidade Federal de Santa Maria

### **ESTOQUES E MECANISMOS DE ESTABILIZAÇÃO DO CARBONO ORGÂNICO DO SOLO EM AGROECOSSISTEMAS DE CLIMA TEMPERADO E SUB-TROPICAL**

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O seqüestro de carbono (C) em solos agrícolas é uma opção de baixo custo para mitigação das mudanças climáticas globais. O plantio direto (PD), associado a boas práticas agronômicas, pode compensar até 15% das emissões antrópicas de CO<sub>2</sub> ao armazenar o C drenado da atmosfera na forma de carbono orgânico (CO) do solo. Para ser amplamente aceito como alternativa de mitigação, pesquisas devem ser conduzidas a fim de melhorar a precisão das estimativas de taxas de seqüestro de C em experimentos de campo, assim como as previsões feitas por modelos matemáticos em escalas regionais e locais. Complementarmente, é necessário aprimorar o conhecimento sobre os mecanismos de estabilização do CO, delimitando a capacidade real do solo em acumular C e quantificando quanto do C acumulado no solo pode ser re-emitido para atmosfera por mudança no manejo do solo. Desta maneira, o presente trabalho se divide em quatro capítulos com o objetivo de abordar estas questões. O primeiro capítulo tem por objetivo discutir a importância da profundidade de amostragem (0-0,30; 0-0,60; 0-0,90 m) e da definição de situações de “linhas-base” confiáveis a adequadas para o cálculo das taxas de seqüestro de C. Para isto, foram utilizados dois experimentos de longa duração sobre um solo de clima temperado (Mollisol) e outro de clima sub-tropical (Oxisol). Os experimentos testaram efeitos de sistema de preparo do solo (preparo convencional (PC) e PD) (Mollisol e Oxisol) e fontes e doses de nitrogênio para o milho no Mollisol (testemunha (T), 168 kg N ha<sup>-1</sup> na forma de sulfato de amônia (AM) e 168 kg N ha<sup>-1</sup> na forma de adubo orgânico (AO)) e diferentes sistemas de rotação de culturas no Oxisol (R0:soja-trigo, R1:soja-trigo-soja-aveia e R2:soja-aveia-soja-aveia+ervilhaca-milho-nabotrigo). O aumento da profundidade de amostragem não contribuiu com a melhoria das estimativas de taxas de seqüestro de C devido ao aumento do erro nas estimativas dos estoques de CO nas camadas mais profundas de solo. Para melhoria das estimativas das taxas de seqüestro de C devem-se preferir análises temporais da dinâmica do CO no solo ao invés da comparação de estoques de CO em um único momento. O segundo capítulo tem por objetivo aplicar equações matemáticas simples para descrever a dinâmica do CO e melhorar as estimativas taxas de seqüestro de C e também entender o papel da formação de macroagregados no acúmulo e saturação de CO no solo. O uso de equações lineares e cinéticas (crescimento exponencial) foi adequado para descrever a dinâmica do CO, aumentando a precisão das estimativas de taxas de seqüestro de C ao reduzir os erros de estimativa por variabilidade espacial do solo. O acúmulo de CO no solo mostrou-se uma função da quantidade de C aportada ao solo por resíduos vegetais e a formação de macroagregados no solo para proteção do CO. O processo de saturação do solo ocorreu das menores para as maiores frações de agregados do solo, limitando a capacidade de uma

determinada camada de solo em acumular CO. No entanto, verificou-se que a saturação de camadas superficiais de solo não indica o fim do seqüestro de C neste solo, visto que o acúmulo de CO passa a ocorrer em camadas sub-superficiais. No terceiro capítulo, a aproximação matemática para determinar mudanças nos estoques de CO e a limitação na capacidade do solo em acumular CO promovida pelo processo de saturação dos agregados do solo foram aplicados para melhorar a precisão do modelo matemático uni-compartimental de Hénin e Dupuis (1945) em prever futuras taxas de seqüestro de C. O Mollisol foi escolhido para este estudo em função da maior disponibilidade de dados (anos de amostragem) e também pela presença de dois tratamentos em PC e PD com camada de solo saturada por CO. O ajuste matemático (por equações lineares) dos coeficientes da dinâmica do CO melhorou o ajuste das previsões do modelo com os dados observados. A restrição do modelo matemático quanto à capacidade do solo em acumular CO (saturação de CO) evitou a superestimação do potencial de seqüestro de C deste solo. As previsões do modelo matemático indicam que a camada superficial (0-0,05 m) do solo sob PD pode apresentar taxas significativas de seqüestro de C por até 50 anos, em função da quantidade de C adicionada ao solo. No quarto capítulo, foi realizado um estudo detalhado dos compartimentos do CO em função da sua distribuição em classes de tamanhos de agregados estáveis em água e o fracionamento granulométrico e densimétrico do CO. O objetivo foi identificar em quais compartimentos está ocorrendo o acúmulo de CO no solo, os mecanismos de estabilização do CO, estimando o potencial do PD em promover seqüestro de C de longa duração. Verificou-se que o acúmulo de C ocorre preferencialmente em frações mais estáveis e recalcitrantes do CO (Mollisol e Oxisol) ou em frações protegidas fisicamente por micro e macroagregados (Mollisol). No Oxisol, o enriquecimento de CO ocorre principalmente nas frações de CO associadas aos minerais extra-microagregados oclusas em meso e macroagregados de solo, enquanto que no Mollisol, o acúmulo de CO ocorre tanto na fração intra como extra microagregados. Mais de 78 e 92% do seqüestro de C verificado no Mollisol e Oxisol, respectivamente, foi considerado de longa duração por ocorrer em frações estáveis do CO.

Palavras-chave: seqüestro de carbono; agregados; carbono orgânico particulado.

## **ABSTRACT**

Doctorate Thesis  
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Santa Maria Federal University

### **SOIL ORGANIC CARBON STOCKS AND STABILIZATION MECHANISMS ON TEMPERATE AND SUB-TROPICAL CLIMATE AGROECOSYSTEMS**

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Soil carbon (C) sequestration in agriculture soil is a low cost option to mitigate global climatic change. No-till (NT) associated with good husbandry practices could compensate up to 15% of the anthropogenic CO<sub>2</sub> emissions by storing the C from atmosphere as soil organic carbon (SOC). To be fully accepted as a mitigation alternative, research must be conducted to improve the accuracy of soil C sequestration estimates on field experiments as well as those made by mathematical models at regional and local scales. Complementarily, is necessary to improve the knowledge about the SOC stabilization mechanisms, delimiting the real soil's capacity into accumulate C, quantifying how much of the stored C could be re-emitted to the atmosphere by changes in soil management. The present work is divided in four chapters with the objective to answer these questions. The first chapter has the objective to discuss the importance of sampling depth (0-0.30, 0-0.60, and 0-0.90 m) and the definition of a reliable and adequate baseline for the calculation of the C sequestration rates. Two long-term field experiments from a temperate (Mollisol) and a sub-tropical (Oxisol) climate soil were selected for this research. The experiments tested soil tillage systems (conventional tillage (CT) and NT) (Mollisol and Oxisol) and sources and rates of nitrogen amendment on corn in the Mollisol (control without N, 168 kg N ha<sup>-1</sup> as ammonium sulfate, and 168 kg N ha<sup>-1</sup> as organic fertilizer) and different crop rotation systems in the Oxisol (R0:soybean-wheat, R1:soybean-wheat-soybean-oat, and R2:soybean-oat-soybean-oat+vetch-corn-radish-wheat). The increase of sampling depth provided limited contribution to the estimates of C sequestration rates due to the increase of the error on SOC stocks estimates at deeper soil depths. To improve the C sequestration rate estimates, SOC temporal dynamic analysis should be preferred rather than the comparison of the SOC stocks of paired plots at a unique time point. The second chapter had the objective to apply simple mathematical equations to describe the SOC dynamics and improve the estimates of C sequestration rates and also to understand the role of the macroaggregate formation on SOC accumulation and saturation. The use of linear and kinetic (exponential growth) equation was adequate to describe the SOC dynamics increasing the accuracy of the C sequestration rate estimates by reducing errors promoted by soil spatial variability. The SOC accumulation was a function of the amount of C input to the soil and the macroaggregate formation to protect SOC. The SOC saturation process occurred from the smaller to the larger aggregate size fraction, limiting the capacity of a given superficial soil layer to accumulate SOC. However, the SOC saturation at superficial soil layers did not indicate the end of C sequestration in the soil, since the SOC accumulation occurred at sub-superficial soil layers. In the third chapter, the mathematical approach to determine changes on SOC stocks and the SOC saturation-induced limitation for C sequestration were applied to improve the accuracy of the Hénin e Dupuís (1945) one-



compartmental mathematical model into predict future soil C sequestration rates. The long-term field experiment from the Mollisol was selected for this research because of the better data availability (sampling years) and also by the presence of two treatments under CT and NT with SOC saturated soil layers. The mathematical adjustment (by linear equations) of the SOC dynamic coefficients improved the adjustment of the model's predictions. The SOC saturation-induced restriction for SOC accumulation on the mathematical model avoided the overestimation of the soil's potential for C sequestration. The predictions of the mathematical models indicate that the Mollisol's superficial layer (0-0.05 m) under NT could maintain significant C sequestration rates for up to 50 years as a function of the amount of C input to the soil. For the fourth chapter, a detailed study of the SOC pools in water-stable aggregate size fractions by granulometric and densimetric fractionation was carried out. The objective was to identify the pools where SOC accumulation was occurring and what SOC stabilization mechanisms were present. This will provide estimates of the NT potential to promote long-term C sequestration. SOC accumulation occurred preferentially in the more stable and recalcitrant SOC fractions (Mollisol and Oxisol) or in microaggregate and macroaggregate physically protected fractions (Mollisol). In the Oxisol, the SOC enrichment occurred mostly in the mineral associated-SOC fractions extra-microaggregates occluded within meso- and macroaggregates, while in the Mollisol, the SOC accumulation occurred in both intra- and extra-microaggregate mineral associated-SOC fractions. More than 78 and 92% of the C sequestration verified in the Mollisol and Oxisol, respectively, were considered as long-term by occurring in stable SOC fractions.

Key-words: carbon sequestration, aggregates, particulate organic carbon.

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## GENERAL INTRODUCTION

Carbon sequestration has been a major subject due to the increasing carbon dioxide (CO<sub>2</sub>) emissions and the drastic climatic and global changes that could result from this CO<sub>2</sub>-enriched atmosphere. Agriculture could act as source or sink of CO<sub>2</sub> to the atmosphere. While source, it represents about 5% of the total anthropogenic CO<sub>2</sub> emissions (Rice, 2006; Cerri and Cerri, 2007). While sink, has been estimated a potential to remove 0.4 to 1.2 Gt C year<sup>-1</sup> from the atmosphere, offsetting up to 15% of the global fossil-fuel emissions (Lal, 2004; Smith et al., 2007). Among mitigation possibilities in the agriculture, soil C sequestration is the most cost effective and feasible short-term option (IPCC, 2007; Reed and Rice, 2007) accounting for 89% of the agricultural technical potential (Smith et al., 2007). In this process, C is stored in the soil mainly as soil organic matter.

The main strategies to increase soil organic carbon (SOC) are reduction of soil tillage intensity (e.g. no-tillage (NT)) and increase of C input through vegetal biomass or other organic residues. NT occupies about 7% of world's cropland area reaching 95.5 million ha (Huggins and Reganold, 2008) with a world-wide estimated potential to sink  $0.57 \pm 0.14$  Mg C ha<sup>-1</sup> year<sup>-1</sup> (West and Post, 2002). NT protects more efficiently the SOC and the new added C from microbial decomposition, reducing the SOC mineralization rates when compared to conventional tillage (Bayer et al., 2006). Therefore, NT is considered more carbon conservative than many tillage systems.

Most of the SOC accumulation verified in NT soil occurs at the soil surface (0-0.05-m) in a decreasing gradient to the sub-superficial soil layers (Sá et al., 2001; Bayer et al., 2001). This situation preconditioned that the majority of researches about soil C sequestration limited the sampling depth to the first 0.30-m soil layer (Baker et al., 2007). Recent works that conducted sampling at greater depths found contradictory results (VandenBygaart et al., 2003; Carter, 2005; Dolan et al., 2006; Blanco-Canqui and Lal, 2008): higher SOC stocks in NT than in conventional tillage (CT) in the top soil, but the inverse trend in deeper layers resulting in no difference among tillage systems or even more SOC in the CT if a deeper soil profile is considered. These results have lead to the first objective of the present research: conduct a critical investigation of soil C sequestration in deeper soil profiles (0-0.30-, 0-0.60-, and 0-0.90-m of a Mollisol and an Oxisol), considering the importance of reliable baselines to accurately estimate the C sequestration rates (chapter 1).

Additionally, to be widely accepted as mitigation alternative to CO<sub>2</sub> mitigation, further research must be conducted to improve the accuracy of soil C sequestration estimates in field experiments, and the predictions of soil C sequestration rates by mathematical models in regional and local scales, delimiting the soil's capacity to accumulate SOC. The most common methodology to estimate soil C sequestration rates is compare SOC stocks in paired NT and CT plots, considering the time of adoption of the management practices (West and Post, 2002). However, the soil C sequestration rates calculated by this methodology could have a standard error so large (introduced by the soil spatial variability) that makes the rate statistically insignificant (VandenBygaart et al., 2003; Baker et al., 2007). The alternative presented in the chapter 2 is to perform temporal SOC dynamic analysis by fitting mathematical equations to estimate soil C sequestration rates. The use of simple equations (linear, kinetic) could increase the accuracy of these estimates by eliminating the soil spatial variability present in punctual extemporal analysis.

The SOC dynamics in both CT and NT have direct relationship with the C input to the soil through vegetal or other organic residues, but for the same amount of C input it is expected a greater soil C recovery in NT than CT (Bayer et al., 2006). This difference in SOC stocks between tillage systems should be linked to SOC stabilization mechanisms, since the humification rate (conversion of the added C into SOC) does not seem to change significantly among tillage systems (Bayer et al., 2006; Lovato et al., 2004). The amount of C added to the soil and the SOC stabilization within aggregates can determine the NT soil carbon sequestration potential (Six et al., 2000) with implications on the time period the soil could act as a C sink. The soil has a limited capacity to store SOC within aggregates, in a process of saturation that occurs from the smaller to larger aggregate size fractions (Gulde et al., 2008). Therefore, the capacity of build-up and reduce the turnover of soil macroaggregates could determine how much SOC could be stored. Complementing the mathematical approach to estimate soil C sequestration rates in the chapter 2, an aggregate stability analysis and SOC stocks associated with the aggregate size fractions measurements were performed to better understand the process of SOC accumulation in NT soils and how the process of SOC saturation could limit the soil potential to act as a C sink.

In the chapter 3, the mathematical approach to determinate changes in SOC stocks and the limitation promoted by the process of SOC saturation to the soil's capacity to store SOC was applied to improve the accuracy of a one-compartmental model to predict soil carbon sequestration in the long-term. The one-compartmental model proposed by Hénin and Dupuis (1945) has been widely used with satisfactory accuracy between predict and measured SOC

stocks (Dalal and Mayer, 1986; Bayer et al., 2006, Vieira et al., in press). Although simpler than the more complex Century (Parton et al., 1987) and RothC (Jenkinson et al., 1987) models, it can be used to estimate the dynamics coefficients ( $k_1$  and  $k_2$ ), the SOC turnover and the minimum C input to maintain the original SOC stocks based on long-term experiments (Bayer et al., 2006). Although accurate in a short time, the one-compartmental model may be inadequate for long-term projections. The SOC at the new equilibrium is calculated by the model as a function of C input and its  $k_1$  coefficient divided by the SOC  $k_2$  coefficient (Bayer et al., 2006). This approach does not take in account other processes that regulate SOC stabilization. The increase of SOC stocks is followed by a concomitant increase of soil aggregation in larger size classes (Fabrizzi et al., 2009). The processes of SOC accumulation and saturation in a given soil layer are both regulated by the amount of macroaggregates (>2000- $\mu\text{m}$ ) (Nicoloso, chapter 2). This limitation must be considered in the predictions of the mathematical models to avoid an overestimation of the soil's capacity to accumulate SOC.

As the soil accumulates SOC, there is a concern about the permanence of this process. In other words, the SOC stored in the soil could be re-emitted to the atmosphere if the soil is tilled again? To answer that question a detailed SOC fractionation was performed assessing the mechanisms of C stabilization in the soil (chapter 4). There are three basic mechanisms: bio-physical protection, organo-mineral interaction, and biochemical recalcitrance (Christensen, 1996); and their interactions with the soil biota (Six et al., 2002). The bio-physical protection (i.e. aggregation) is the key factor controlling SOC stabilization in soils from temperate climate regions, where the increase of SOC is associated with an increase in the amount of macroaggregates (soil aggregates >2000  $\mu\text{m}$  diam.) (Fabrizzi et al., 2009). However, in weathered tropical and sub-tropical soils, it has been suggested that SOC and biological processes play a secondary role in the binding of aggregates (Six et al., 1999; Denef et al., 2002; Denef et al., 2004). In these aluminum and iron oxides rich soils, the organo-mineral interaction is a particularly important mechanism, where the interaction of the SOC with the soil oxides provides extensive stability (Bayer et al., 2001; Razafimbelo et al., 2008). The intensity how each stabilization mechanism acts protecting SOC from microbial decomposition will determine the permanence of the C storage in the soil and the resilience of this soil for degradation. Contrasting tropical and temperate soils is an opportunity to further understand the complex interactions of SOC stabilization mechanisms, and C sequestration in agricultural soils.

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# CHAPTER 1

## DEEP ORGANIC CARBON STOCKS IN MOLLISOL AND OXISOL: LONG-TERM EFFECT OF TILLAGE, NITROGEN SOURCES AND CROP ROTATION

### 1.1. Introduction

The agricultural potential to sequester atmospheric carbon dioxide (CO<sub>2</sub>) has been estimated to range from 5 to 15% of the global fossil-fuel emission or 0.4 to 1.2 Gt C year<sup>-1</sup> (Lal, 2004). Among carbon (C) sink options in agriculture the soil carbon sequestration accounts for 89% of the agricultural technical potential (Smith et al., 2007). In this process, the C is temporarily stored in soil mainly as soil organic matter. The crop and soil management practices recommended to increase the soil organic carbon stocks (SOC) are: minimum soil disturbance (e.g. no-till (NT)), reduction or elimination of fallow, control of soil erosion, use of cover crops and crop rotation, increase of fertilizer use efficiency, use of better crop varieties, among other agronomical and soil husbandry practices that lead to increase C inputs to the soil and stabilization of SOC (Smith et al., 2007). World-wide NT has been on 95.5 million hectares, less than 7% of world's cropland area (Huggins and Reganold, 2008). This conservation tillage system, regarding the carbon cycle, is especially important due to erosion control, reduction of SOC mineralization (Bayer et al., 2006) and increase stabilization of SOC by improvement in soil aggregation providing physical SOC protection (Six et al., 2002). An accurate knowledge of NT environmental benefits to mitigate global climate change could create a scenario to increase its world-wide adoption.

Change in SOC associated with different crop and soil management could be predicted in the long-term by mathematical models such as Century, C-Roth and others. The use of these models should be supported by long-term field experiments in order to ensure the accuracy of the carbon sequestration estimates. Also, there is a common sense that field data are preferable to modeled data. In this context, temporal changes in SOC stocks measured in long-term experiments have been the main tool to estimate carbon sequestration rates of different soil management options. In a global review of 67 long-term experiments, the average soil carbon sequestration rate achieved by the change from conventional tillage (CT) to NT was estimated to be  $0.57 \pm 0.14$  Mg C ha<sup>-1</sup> year<sup>-1</sup> (West and Post, 2002). Agriculture soils are considered a finite biological C sink, thus NT and other crop management practices

could lead the soil to sequester carbon for a 20 to 50 year time horizon when a higher equilibrium is reached (Lal, 2004).

Frequent critics to current methodology to estimate carbon sequestration rates are related to the fact that most of them were based only in the top 0.30 m of soil or less (Baker et al., 2007). The option to evaluate a shallow layer relies that the effect of tillage systems over the SOC stocks are restricted mainly to the soil surface (Six et al., 2002). However, the crop's roots system can grow in deeper layers and it is an important source of C totaling as much as 30 to 50% of the total C inputs by annual crops to the soil (Bolinder et al., 2007). In addition, the soil biological activity could transport C through the soil profile as well a soluble C transport (Wolters, 2000; Ekschmitt et al., 2008). These facts are argued as the driving reasons to evaluate SOC deeper in the soil profile.

Recently, there are an increasing number of studies that have assessed change in soil carbon stock in layers greater than 0.30 m but the results of carbon sequestration are quite contradictory. This way, some studies show higher SOC in NT than in conventional tillage (CT) in the top soil, but the inverse trend in deeper layers resulting in no difference among tillage systems or even more SOC in the CT if a deeper soil profile is considered (VandenBygaart et al., 2003; Carter, 2005; Dolan et al., 2006; Blanco-Canqui and Lal, 2008). On the other hand, some studies show that the NT soil carbon sequestration rate at the soil surface in relation to CT could exceed SOC increases at depth in CT (Six et al., 2002; VandenBygaart et al., 2003). The similarity in these studies is the lack of a reliable baseline at beginning of experiments. Almost all comparisons are made between the SOC of NT and CT in a specific year of evaluation, despite of the real SOC temporal dynamics per plot and the initial SOC stocks in each plot. This way, the high SOC in CT in some works does not necessarily mean that the NT is losing SOC or the CT is sequestering carbon in deep layers. Actually the opposite conclusion could be drawn if a reliable baseline were available. The approach to measure the difference between NT and CT stocks regardless the temporal dynamics of SOC has been the basis to assess the NT environmental service carbon sequestration and could lead to a misinterpretation of the results. Among the main reasons for this misinterpretation are the soil variability in deep layers that commonly are greater than the treatments effects (VandenBygaart et al., 2003; Carter, 2005) and the original variability in SOC stocks among plots at the beginning of the experiments. These factors could introduce an important error in the estimative of carbon sequestration rates.

In order to address the importance of the baseline and sampling depth to accurately estimate soil carbon sequestration rates, this research was carried out analyzing the data from

two long-term experiments contrasting tillage systems and nitrogen sources for corn crop in a Mollisol from Manhattan, KS, USA and tillage systems and crop rotations in an Oxisol from Cruz Alta, RS, Brazil.

## 1.2. Hypothesis

The full environmental benefit of the NT soil as a C sink only can be accurately assessed when a reliable baseline is available for temporal SOC stocks comparisons.

The SOC accumulation in NT is limited to the superficial soil layers (0-0.30-m) and compensates eventual losses of SOC in sub-superficial soil layers (0.30-0.90-m).

## 1.3. Material and Methods

### 1.3.1. Site Description

Two long-term experimental sites were selected to compose this research. The Mollisol was located at the North Agronomy Farm of Kansas State University in Manhattan – KS, United States of America (39° 12' 42" N, 96° 35' 39" W). The local average annual precipitation is 800-mm and the annual mean temperature is 11.4°C. The Oxisol was located at the Center of Experimentation and Research FUNDACEP in Cruz Alta – RS, Brazil (28° 36' 00" S, 53° 40' 21" W). The local average annual precipitation is 1727-mm and the annual mean temperature is 19.2°C. The table 1.1 shows the general soil characteristics of these sites.

Table 1.1. Main soils chemical characteristics of 0-5 cm soil layer of CT and NT plots in the Mollisol and Oxisol sites<sup>(1)</sup>.

Soil	Tillage	pH 1:1 H <sub>2</sub> O	Bray-P ----- mg kg <sup>-1</sup> -----	K -----	CEC cmol <sub>c</sub> kg <sup>-1</sup>	Sand -----	Silt g kg <sup>-1</sup> -----	Clay
Mollisol	CT	6.2	54.9	371	17.1	100	700	200
	NT	5.8	55.0	318	18.4	120	680	200
Oxisol	CT	5.1	18.4	146	16.2	250	230	520
	NT	5.1	26.8	261	17.1	250	240	510

<sup>(1)</sup> Adapted data from Fabrizzi et al. (2009).

#### 1.3.1.1. Mollisol

The soil was a moderately well-drained Kennebec silt loam (fine-silty, mixed, superactive mesic Cumulic Hapludoll) and is referred in the text as Mollisol. The experiment, initiated in 1990, was arranged in a split-plot randomized blocks with four replications in plots with continuous corn (*Zea mays* L.). The tillage systems were the main plots and

nitrogen sources were the sub-plots. The tillage systems were (1) Chisel Tillage (CT) and (2) No Tillage (NT). The CT consisted of chisel plow and offset disk and the NT consisted of planting directly into the residues. The N treatments consisted of different sources of the nutrient: (a) 168-kg N ha<sup>-1</sup> as ammonium nitrate (MF), (b) 168-kg N ha<sup>-1</sup> as cattle manure (OF), and (c) a control without nitrogen fertilization (CO). Rates of manure application were calculated assuming that 100% of the NH<sub>4</sub><sup>+</sup>-N was available immediately after applied and approximately 35% of the organic N was mineralized the first year following application. The plots were amended with lime and fertilized with P and K following soil analysis.

#### 1.3.1.2. Oxisol

The soil was a clayey Hapludox and is referred in the text as Oxisol. The experiment, established in 1985, had a split-plot randomized blocks design without replications. The tillage systems were the main plots and crop rotations were the sub-plots. The tillage systems were (1) conventional tillage (CT) and (2) no-tillage (NT). The tillage operation comprised of disk tandem and disk plow in the spring and autumn while the NT consisted of planting directly into the residues. The crop rotations were: (a) soybean (*Glycine max* (L) Merr.) – wheat (*Triticum aestivum* L.) (R0), (b) soybean – wheat – soybean – black oat (*Avena strigosa* Schreber) (R1) and (c) soybean – black oat – soybean – black oat + vetch (*Vicia sativa* (L.) Walp.) – corn – forage radish (*Raphanus sativus* L.) – wheat (R2). The plots were amended with lime and fertilized with N, P, and K following soil analysis. Details for the experiment were reported by Campos et al. (1995) and Campos (2006).

#### 1.3.2. Carbon inputs

The aboveground carbon input by vegetal residues to the soil was calculated by quantifying the aboveground biomass production by each crop and considering a carbon content of 40% for all crops (Lovato et al., 2004). The carbon input due to manure amendment in the Mollisol site was estimated by the manure C/N ratio (Eghball and Power, 1999) considering its total nitrogen content.

#### 1.3.3. Soil Sampling

The soil samples were collected in January of 2008 in the Oxisol site and in April of 2008 in the Mollisol site. The samples were taken at the 0-0.05-, 0.05-0.15-, 0.15-0.30-, 0.30-0.45-, 0.45-0.60-, and 0.60-0.90-m soil depth. The data from the Mollisol site in 1992 was extracted from Harris (1993).

#### 1.3.3.1. Mollisol

The samples were collected using a 0.05-m diameter soil probe, placed in plastic bags and weighed. A sub sample was dried at 105°C to determine the soil bulk density considering the probe volume. The samples were air dried at 30±5°C. After they were passed through a 2-mm sieve, large roots and plant residues removed, and stored until use.

#### 1.3.3.2. Oxisol

The samples were collected manually with a shovel. At the 0-0.30-m soil layer four sub-samples per plot were collected and at the 0.30-0.90-m soil layer two sub-samples per plot were collected. The samples were air dried at 30±5°C. After they were passed through a 2-mm sieve, large roots and plant residues removed, and stored until use. To determine the soil bulk density, undisturbed samples were taken at the 0-0.05-, 0.05-0.10-, 0.10-0.15-, 0.15-0.20-, 0.20-0.25-, 0.25-0.30-, 0.30-0.35-, 0.35-0.40-, 0.40-0.45-, 0.45-0.50-, 0.50-0.55-, 0.55-0.60-, 0.60-0.65-, 0.65-0.70-, 0.70-0.75-, 0.75-0.80-, 0.80-0.85-, 0.85-0.90-m soil depth with 0.05-m diameter and 0.04-m height iron cylinders. The soil bulk density was calculated by dividing the dry-weight of the samples by the cylinder volume.

#### 1.3.4. Soil Organic Carbon Stocks

Soil samples were finely ground using a mortar and pestle. The soil organic carbon (SOC) contents are determined by dry combustion using a C/N Elemental Analyzer (Flash EA 1112 Series ThermoFinnigan Italia S.p.A., MI, Italy). The SOC stocks were calculated according to the methodology described by Ellert and Bettany (1995) to compare treatments in an equivalent soil mass basis. In the Mollisol site, the soil bulk density at the beginning of the experiment in 1990 (Harris, 1993) was used as reference, while in the Oxisol site, the soil bulk density in the treatment CT R0 was the reference.

#### 1.3.5. Soil Carbon Sequestration Rates

The soil organic carbon sequestration rates were calculated by three different methods considering the conceptual model shown in the figure 1.1 and the data availability of each experimental site.

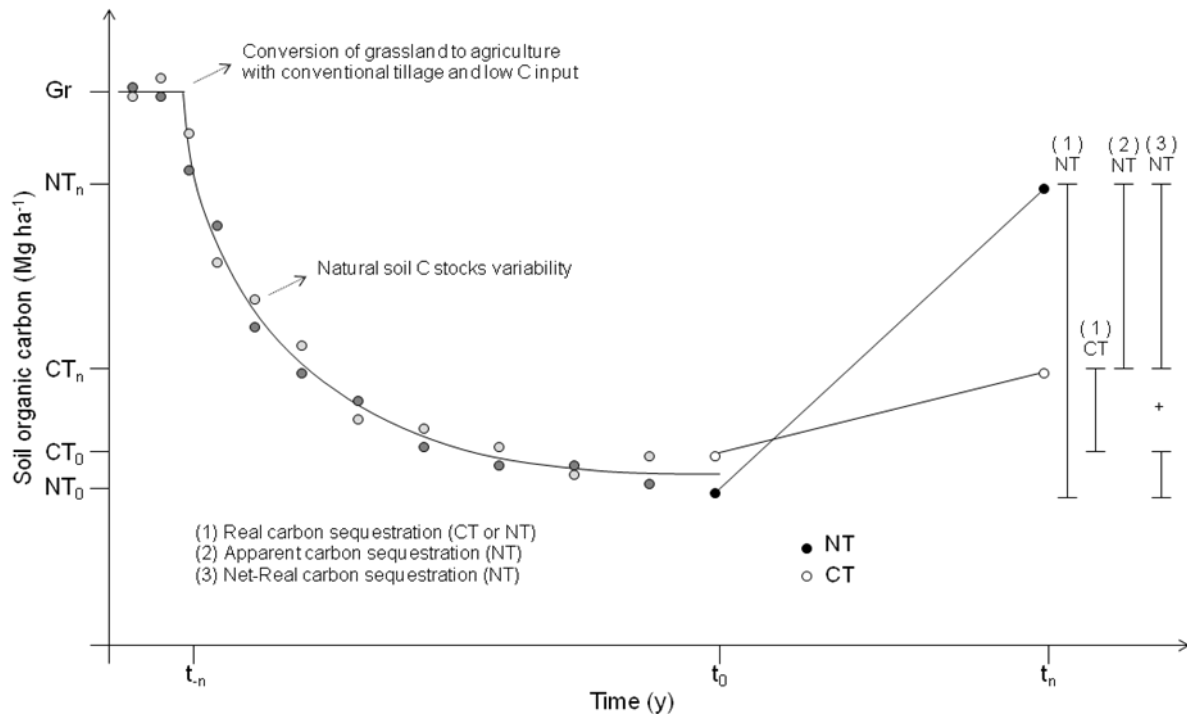


Figure 1.1. Conceptual model of C dynamics and calculation of soil carbon sequestration rates. NT= no-till, CT=conventional tillage.

The ‘real’, ‘apparent’ and ‘net-real’ soil organic carbon sequestration rates were calculated as following:

(1) Real carbon sequestration rate: 
$$SOC_R = \frac{C_{tr}t_n - C_{tr}t_0}{t_n - t_0}$$

(2) Apparent carbon sequestration rate: 
$$SOC_R = \frac{C_{NT}t_n - C_{T}t_n}{t_n - t_0}$$

(3) Net-real carbon sequestration rate: 
$$SOC_R = \frac{(C_{NT}t_n - C_{NT}t_0) - (C_{T}t_n - C_{T}t_0)}{t_n - t_0}$$

where, ‘ $SOC_R$ ’ is the soil organic carbon sequestration rate ( $Mg\ ha^{-1}\ y^{-1}$ ), ‘ $t_n$ ’ is the time at the end of the evaluation period (y), ‘ $t_0$ ’ is the time at the beginning of evaluation period (y), ‘ $C_{tr}$ ’ is the carbon stock in the treatment ( $Mg\ ha^{-1}$ ) at ‘ $t_n$ ’ ( $C_{tr}t_n$ ) or ‘ $t_0$ ’ ( $C_{tr}t_0$ ), ‘ $C_{NT}$ ’ is the carbon stock in the no-tillage treatment ( $Mg\ ha^{-1}$ ) at ‘ $t_n$ ’ ( $C_{NT}t_n$ ) or ‘ $t_0$ ’ ( $C_{NT}t_0$ ) and ‘ $C_T$ ’ is the carbon stock in the tillage treatment ( $Mg\ ha^{-1}$ ) at ‘ $t_n$ ’ ( $C_Tt_n$ ) or ‘ $t_0$ ’ ( $C_Tt_0$ ).

### 1.3.6. Statistical Analysis

The results were submitted to analysis of variance and the means were compared by the difference of LS means (C input, SOC stocks in 2007, C sequestration rates) or orthogonal contrast test (SOC content and stocks in 2007 compared to 1992 in the Mollisol site) using

SAS PROC MIXED software (SAS institute, 2002). The results were considered significantly different at  $P < 0.05$ , unless noted otherwise.

## 1.4. Results and Discussion

### 1.4.1. Mollisol

In the CT treatments, there was a significant increase of SOC content only at the soil surface (0-0.05 m) in the treatment CT OF. This increase was due to the high C input to the soil by manure. In the other CT treatments no significant differences were found in 2007 compared with the 1992 baseline. In contrast, for NT treatments in 2007 showed an increase of SOC content at the soil surface compared with the NT baseline. These results are similar to those in literature that show consistently increases of SOC at the soil surface with NT (Six et al., 2002; VandenBygaart et al., 2003). As the depth increase, the NT treatments in 2007 showed no differences to the NT baseline, with the exception of the treatment NT MF which had higher SOC content at 0.45-0.60 m soil layer and the treatment NT CO which had lower SOC content at 0.60-0.90 m soil layer. However, those results are probably due to the increase of soil variability than to treatments effects as noticed by the increasing error bars at the lower sampling depths.

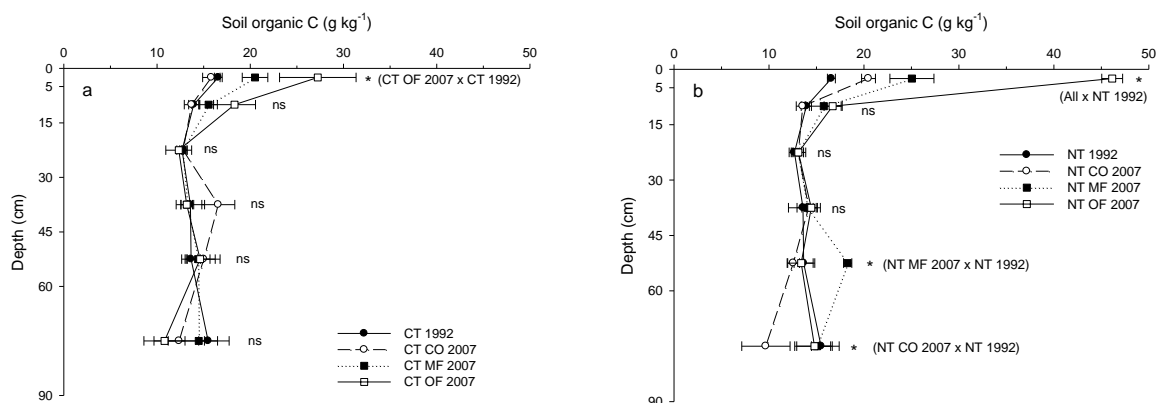


Figure 1.2. Soil organic carbon content in the 0-0.05-, 0.05-0.15-, 0.15-0.30-, 0.30-0.45-, 0.45-0.60-, and 0.60-0.90-m soil layers in the chisel tillage (CT) (a) and no tillage (NT) (b) treatments at 1992 (baselines) and 2007 as affected by mineral fertilization (MF), organic fertilization (OF) and a control without nitrogen fertilization (CO). The horizontal bars around the means represent the means' standard error (n=4). ns: means not significantly different by the ANOVA analysis ( $P > 0.05$ ). \*: means significantly different by the orthogonal contrast test ( $P < 0.05$ ).

The increase of SOC at soil surface was related with the different C inputs to the soil drove by rates and sources of nitrogen amendment and mostly to the better protection of the new added C to the soil by the NT, once the differences in C inputs between NT and CT were not significant (Table 1.2). The treatments with nitrogen amendment (MF and OF) had significant higher C input by corn residues than the CO treatment, but did not differ from each other. When the C input from manure is taken in account, the treatment OF had significantly more input than the other treatments. At this time the differences between total C input between CO and MF treatments become not significant. It is important to remind that in the OF treatment, about 60% of the added C was imported through the manure input to the system and not produced in situ. However, the manure amendment (OF) promoted 2.5 and 3.4 times more total C input than the MF and CO treatments.

Table 1.2. Average annual aboveground carbon input to the soil between 1990 and 2007 as affected by no-tillage (NT), conventional tillage (CT), control without nitrogen (CO), mineral fertilizer (MF), and organic fertilizer (OF) management practices.

Source	CT CO	CT MF	CT OF	NT CO	NT MF	NT OF
----- Mg ha <sup>-1</sup> yr <sup>-1</sup> -----						
Corn	1.71	2.36	2.39	1.75	2.45	2.32
Manure	-	-	3.58	-	-	3.58
Total	1.71	2.36	5.97	1.75	2.45	5.91
ANOVA						
	Tillage	N source		Interaction		
	P>F	P>F		P>F		
Corn	0.860	<0.001		0.895		
Total	0.921	<0.001		0.966		
N sources (mean)						
	CO	MF		OF		
Corn	1.73 B <sup>1</sup>	2.40 A		2.36 A		
Total	1.73 B	2.40 B		5.94 A		

<sup>1</sup> Means with different letters between nitrogen sources within corn or total C inputs are significantly different (P<0.05).



Table 1.3. Soil organic carbon stocks in 1992 and 2007 as affected by no-tillage (NT), conventional tillage (CT), control without nitrogen (CO), mineral fertilizer (MF), and organic fertilizer (OF) management practices.

Depth	1992		2007					
	CT	NT	CT CO	CT MF	CT OF	NT CO	NT MF	NT OF
cm	----- Mg C ha <sup>-1</sup> -----							
0-5	9.78	9.31	9.34 b†	12.10 b	16.08 b	12.09 b	14.78 b	27.22§ a
5-15	16.45	16.44	16.19	18.33	21.61	16.01	18.61	19.71
15-30	22.83	22.58	23.06	22.91	22.19	23.60	23.45	23.58
30-45	24.24	22.37	29.58	23.64	23.54	25.32	25.09	25.80
45-60	22.93	23.10	25.22	24.20	24.58	21.11	30.72	22.51
60-90	49.24	49.24	39.36 ab	46.02 a	34.35‡ b	30.78§ b	47.89 a	47.05 a
0-30	49.05	48.34	48.59	53.35	59.88	51.69	56.84	70.51§
0-60	96.23	93.80	103.39 bc	101.20 bc	108.00 abc	98.12 c	112.66§ ab	118.82§ a
0-90	145.47	143.04	142.75	147.22	142.35	128.90§	160.55§	165.87§
	CT	NT	CT	NT	CO	MF	OF	
0-30	49.05	48.34	53.94 B	59.68 A	50.14 b	55.10 b	65.20 a	
0-60	96.23	93.80	104.20	109.87	100.76	106.93	113.41	
0-90	145.47	143.04	144.11	151.77	135.83 b	153.89 a	154.11 a	

†Means followed by the same lower or upper case letter at the same depth are not significantly different ( $P < 0.05$ ).

‡ Significantly different from 1992 CT baseline at the same depth by orthogonal contrast test ( $P < 0.05$ ).

§ Significantly different from 1992 NT baseline at the same depth by orthogonal contrast test ( $P < 0.05$ ).

The effect of treatments over the SOC stocks were more clear in the soil surface (0-5 cm), but only the treatment NT OF had increase of SOC stock over its baseline. Again, the C footprint of treatments based in SOC stocks changes were easily distinguished in the soil surface (0-5 cm). At the depth 60-90 cm of soil depth, the treatments CT OF and NT CO showed decrease of SOC stocks in relation to their respective baselines. Comparing only the 2007 data, in both 0-5 and 60-90 cm soil layers there was an interaction of tillage systems and nitrogen sources ( $P = 0.0131$  and  $P = 0.0018$ , respectively). At the surface, the NT OF treatment had significantly more SOC than all other treatments, which did not differ statistically from each other. At the last sampled soil layer, the treatments NT MF, NT OF and CT MF, had the larger SOC stocks, while the treatments CT OF and NT CO had the lowest with the treatment CT CO presenting intermediate values. Considering cumulative soil layers (0-30, 0-60 and 0-90 cm) the effect of treatments were more clear. Compared to the NT baseline, the NT OF treatment showed significant increase of SOC stocks for all considered depths, while the treatment NT MF had significant increase only at the 0-60 and 0-90 cm soil depth and the treatment NT CO showed significant decrease of SOC stocks at 0-90 cm. However, neither of the CT treatments at 2007 showed significant differences to the CT baseline. Comparing only the 2007 data at these cumulative soil layers, there was a significant effect of tillage system

and nitrogen sources without interaction at the 0-30 cm soil depth, where on average the NT treatments had higher SOC stocks than CT treatments, and also in the OF treatments the soil had higher SOC stocks than MF and CO treatments. At the 0-60 cm there was interactive effect between tillage systems and nitrogen sources, with the treatments NT OF, NT MF, and CT OF presenting the larger SOC stocks. Finally, considering the whole soil profile (0-90 cm), there was noted only significantly effect of nitrogen sources in the ANOVA test ( $P=0.0444$ ), where the OF and MF treatments had significantly larger SOC stocks than CO treatment.

Given the SOC stocks in the baselines (1992) and at the last available data (2007), there was three possible soil carbon sequestration rates calculation methods, the real, net-real and apparent rates (Figure 1.1). The real soil carbon sequestration rate (RR), express the really measured SOC sequestration observed per plot and this is the real contribution of the agricultural soil system and practices to mitigate global warming. This calculation method is more appropriate for carbon budget purposes. The following apparent soil carbon sequestration rate (AR) and net-real soil carbon sequestration rate (NR) methods are more appropriate for carbon market purposes, since they account only the difference of change in SOC stocks between tillage systems and other agricultural practices. In that way, the AR is the option to calculate soil sequestration rates when the baseline is not available and is an estimate of the C impact for the option for one tillage system (NT) compared to another (CT). This calculation method is actually the standard for carbon market purposes, however could introduce an error by the lack of baselines for comparison. The NR express the real measured difference in SOC sequestration rates between tillage systems or other agricultural practices and indicates the actual net benefit for the option for one tillage system (NT) compared to another (CT). This calculation method reduces experimental errors of soil carbon sequestration rates for carbon market purposes, since eventual differences in SOC stocks at the baselines among tillage systems are computed in the equation.

Table 1.4. Real soil carbon sequestration rates as affected by no-till (NT), conventional tillage (CT), control without nitrogen (CO), mineral fertilizer (MF), and organic fertilizer (OF) management practices.

Tillage system	Nitrogen Source			Mean
	CO	MF	OF	
----- Mg C ha <sup>-1</sup> year <sup>-1</sup> -----				
Depth 0-30 cm				
CT	-0.03±0.14	0.29±0.17	0.72±0.43	0.33±0.17
NT	0.22±0.11	0.57±0.16	1.48±0.10	0.76±0.17
Mean	0.10±0.10 b†	0.43±0.12 ab	1.10±0.25 a	0.54±0.12
Depth 0-60 cm				
CT	0.48±0.57	0.33±0.70	0.78±0.78	0.53±0.33
NT	0.29±0.11	1.26±0.26	1.67±0.19	1.07±0.20
Mean	0.38±0.27	0.79±0.31	1.23±0.41	0.80±0.20
Depth 0-90 cm				
CT	-0.18±1.08 c	0.12±0.53 bc	-0.21±1.24 c	-0.09±0.52
NT	-0.94±0.63 c	1.17±0.36 ab	1.52±0.36 a	0.58±0.41
Mean	-0.56±0.60	0.64±0.36	0.66±0.68	0.24±0.33

†Means followed by the same letter at the same depth are not significantly different ( $P < 0.05$ ). The value after the  $\pm$  symbol represents the means' standard error ( $n=4$ ).

The Table 1.4 shows the observed RR after 15 years of tillage systems and nitrogen sources treatments. Opposing to the current method to calculate soil carbon sequestration rates – by the difference between NT and CT using an unique and punctual time data – that assumes that SOC stocks in CT doesn't change with time, the CT treatments showed positive or negative RR over this 15 years time period of management, according with nitrogen treatment and sampling depth. The increase in SOC stocks in CT treatments were probably related to the improvement of others agricultural techniques, such as use of better crop varieties, better weed control and other good soil and crop husbandry practices that lead to an increase of C inputs to the soil. The CT used in this experiment had no soil erosion due gentle slope and limited soil disturbance by chisel tillage. The statistical differences among treatments were noted at the 0-30 and 0-90 cm of soil depths. In the soil surface, there was no significant tillage effect of RR, but for nitrogen amendment effect the OF treatment had higher RR than CO treatment, while the MF treatment had intermediate values that are not different from the other treatments. In the 0-90 cm soil layer there was an interaction of tillage system and nitrogen treatments, where the treatments NT MF and NT OF presented the larger positive RR. The other treatments (CT CO, CT OF, and NT CO) presented negative RR and the treatment CT MF had intermediate but positive RR values. There were no differences on

the comparison of sampling depths mostly because of the increase of the soil variability when thicker layer is considered (0-90 cm).

Comparing the three soil sequestration rates calculation methods, the NR and AR appears to be comparable when the difference between the CT and NT baselines weren't significantly distinct as happens to be in this research's case (Table 1.5). However, the AR method underestimates the RR in 50% approximately on average. This underestimation is due to the fact that the CT also increases in SOC stocks over time. As proposed, the NR and AR methods were efficient to estimate only the gain in soil carbon sequestration when the farmer opts for NT replacing CT. The real NT environmental benefit only can be accurately estimated when at least two time points spaced sufficient time to discriminate treatments or a baseline at beginning of the experiment is available to calculate the RR.

On the average of all calculation methods, there was an interaction of nitrogen treatments and sampling depth. The increasing of sampling depth promoted distinct conclusions about the NT potential to mitigate CO<sub>2</sub> according with the amount of C input to the soil as represented by the nitrogen rates and sources treatments (CO, MF, and OF). Under high C input the NT soil had positive C sequestration rates, not mattering the calculation method, in all sampling depths (there was no difference among sampling depths under OF). In the MF treatment, NT soil also had positive C sequestration rates in all sampled depths, however, with significant higher rate at the 0-90 cm than the 0-30 cm soil layer. However, under low C input as verified in the CO treatment, the NT soil promoted C sequestration only when the superficial soil layer was considered. At higher depths, the CO treatment promoted C emission with increasing rates as a thicker soil layer was considered (0-90 cm). These results could indicate that in CO treatment under NT, the nitrogen restriction could be reducing the C input by corn roots below 30 cm of soil depth compared to the CT treatments. Under CT, SOC mineralization after the tillage operation could be supplying enough nitrogen to the corn plants to grow their root system at higher depths than the plants under NT. This process could explain why the CT CO treatment had higher SOC stocks than NT CO treatment at the 60-90 cm (Table 1.3) and lower C loss rates at the 0-90 cm (Table 1.4).

Table 1.5. Apparent, net-real and real no-till soil carbon sequestration rates as affected by the control without nitrogen (CO), mineral fertilizer (MF) and organic fertilizer (OF).

Nitrogen Source	Apparent	Net-Real	Real	Mean
----- Mg C ha <sup>-1</sup> year <sup>-1</sup> -----				
Depth 0-30 cm				
CO	0.21±0.13	0.25±0.10	0.22±0.11	0.23±0.06 CD
MF	0.23±0.22	0.28±0.29	0.57±0.16	0.36±0.13 BC
OF	0.71±0.35	0.76±0.38	1.48±0.10	0.98±0.19 AB
Depth 0-60 cm				
CO	-0.35±0.45 c†	-0.19±0.58 c	0.29±0.11 bc	-0.08±0.24 CD
MF	0.76±0.19 abc	0.93±0.47 abc	1.26±0.26 ab	0.98±0.18 AB
OF	0.72±0.55 abc	0.88±0.53 abc	1.67±0.19 a	1.09±0.31 A
Depth 0-90 cm				
CO	-0.92±0.48 b	-0.76±0.49 b	-0.94±0.63 b	-0.87±0.28 E
MF	0.89±0.78 a	1.05±0.65 a	1.17±0.36 a	1.03±0.33 A
OF	1.57±1.21 a	1.73±1.16 a	1.52±0.36 a	1.60±0.52 A

†Means followed by the same lower case letter at the same depth or the same uppercase letter at the same column are not significantly different ( $P < 0.05$ ). The value after the  $\pm$  symbol represents the means' standard error ( $n=4$ ).

Actually, the soil carbon sequestration rate adopted by the Chicago Climate Exchange (CCX) carbon offset market for the cropland areas of central Kansas, region where this research was carried out, is  $0.40 \text{ Mg C ha}^{-1} \text{ year}^{-1}$  (CCX, 2006). Considering that the treatment MF represents the majority of the cropland areas of Kansas, the AR estimated by our data for the 0-30 cm soil layer ( $0.23 \pm 0.22 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ) is inferior to the currently accepted rate by CCX. However, the real no-till potential to mitigate global warming should be re-evaluated by the RR that range from  $0.57 \pm 0.16$  to  $1.17 \pm 0.36 \text{ Mg C ha}^{-1} \text{ year}^{-1}$  according to the considered soil sampling layer (0-30 and 0.90 cm, respectively).

#### 1.4.2. Oxisol

The Table 1.6 shows the annual aboveground C input by crop residues to the soil. The increase of the crop rotation use intensity promoted the increase of C input both in CT or NT, crop rotation R2 promoted higher C input, followed by R1 and R0, respectively. The inclusion of corn in the crop rotation R2 and the better biomass production by wheat and oat in the rotation R1 were the causes of the increase of C input in those cropping systems. NT also promoted significant more C input than CT on the average of crop rotation. Under NT, all crops had better biomass production than under CT, specially the corn which promoted about  $0.62 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  more C input in NT than in CT.

Table 1.6. Average annual aboveground carbon input to the soil between 1985 and 2007 as affected by no-tillage (NT), conventional tillage (CT), and crop rotations (R0 and R2).

Source	CT R0	CT R1	CT R2	NT R0	NT R1	NT R2
----- Mg C ha <sup>-1</sup> y <sup>-1</sup> -----						
Soybean	1.72	1.65	1.78	1.85	1.87	2.01
Wheat	0.95	1.35	1.07	1.06	1.50	1.26
Oat	-	1.79	1.84	-	2.21	1.92
Corn	-	-	2.87	-	-	3.49
Oat+Vetch	-	-	2.04	-	-	2.33
Radish	-	-	1.16	-	-	1.16
Total	2.67	3.24	4.01	2.91	3.74	4.57
Tillage systems (mean)						
Total	CT 3.30 b <sup>1</sup>			NT 3.74 a		
Crop rotation (mean)						
Total	R0 2.79 c		R1 3.49 b		R2 4.29 a	

<sup>1</sup>Means with the same letters in the same line are not significantly different ( $p < 0.05$ ). R0:soybean/wheat; R1:soybean/wheat/soybean/oat; R2:soybean/oat/soybean/oat+vetch/corn/ radish/wheat.

The treatments effects over the SOC content were noticed until 0.30 m soil depth (Figure 1.3). In those soil layers there were significant interactions of tillage systems and crop rotations. At the surface (0-5 cm), the NT R2 treatment had a higher SOC content, followed by NT R1, then NT R0, and then by all CT treatment which had no differences among crop rotations. In the 5-15 cm layer, the treatments with CT had significantly more SOC content than NT treatments, with the exception of CT R0 treatment that did not differ from NT treatments. These results are well documented in the literature and in previous research over the same experimental area (Jantalia, 2005; Campos, 2006) and it are attributed to the inversion of the superficial soil layer and incorporation of crop residues due to the tillage operation (Allmaras et al., 1996). In the 0.15-0.30-m, the treatments under NT had significantly higher SOC content than the CT treatments, with the exception of the treatment NT R0 which was similar to the CT treatments. Normally, the increase of SOC in sub-superficial soil layers is attributed to root-derived C input (Puget and Drinkwater, 2001; Allmaras et al., 2004), soil biological activity (Wolters, 2000; Ekschmitt et al., 2008) or translocation of soluble SOC from superficial layers.

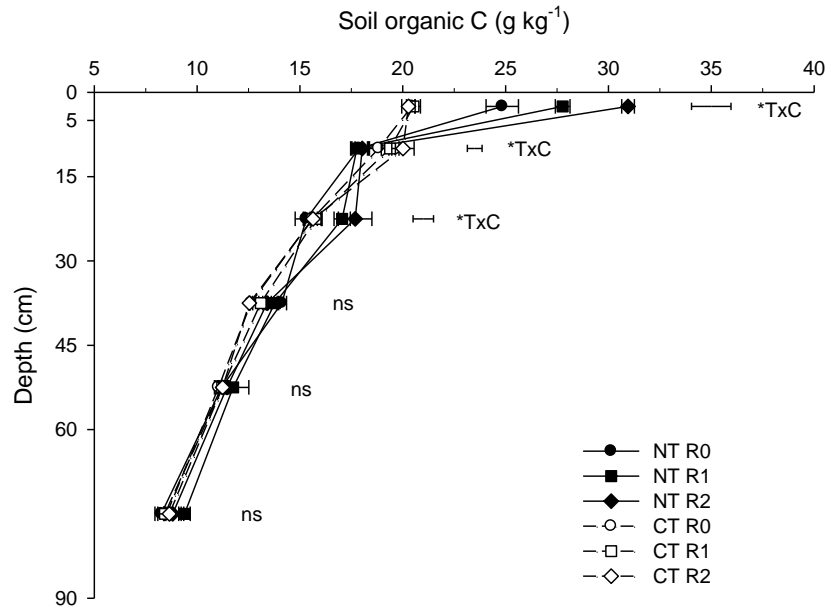


Figure 1.3. Soil organic carbon content in the 0-0.05-, 0.05-0.15-, 0.15-0.30-, 0.30-0.45-, 0.45-0.60-, and 0.60-0.90-m soil layers in the conventional tillage (CT) and no tillage (NT) treatments at 2007 as affected by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/radish/wheat. The horizontal bars around the means represent the means' standard error (n=4 at 0-0.30-m and n=2 at 0.30-0.90-m) ns: means not significantly different by the ANOVA analysis ( $P > 0.05$ ). \*: means significantly different for the interaction of tillage system and crop rotation (TxC) ( $P < 0.05$ ), the horizontal bars represent the least significant difference.

The SOC stocks calculated in an equivalent soil mass basis followed the same pattern observed with the SOC contents (Table 1.7). The main increase of SOC stocks were noticed at the 0-5 cm soil layer, where the treatment NT R2 had the larger SOC stock ( $15.96 \text{ Mg C ha}^{-1}$ ), similar to the treatment NT R1 ( $14.32 \text{ Mg C ha}^{-1}$ ) and superior to the treatment NT R0 ( $12.81 \text{ Mg C ha}^{-1}$ ). On the other hand, all treatments with CT maintained about  $10.5 \text{ Mg C ha}^{-1}$  of SOC in the superficial soil layer not differing among them but inferior that NT treatments. The 30-45 cm soil layer presented significantly larger SOC stocks in NT than in CT but not differences among crop rotations. In the cumulative soil layers (0-30, 0-60, and 0-90 cm), the treatments NT R2 and NT R1 had consistently larger SOC than NT R0 and CT treatments. Under low C input (NT R0) the soil had SOC stocks similar to CT treatments. The net SOC gain with NT over CT ranged between  $3.87$  to  $7.08 \text{ Mg C ha}^{-1}$  depending of the considered soil layer.

Table 1.7. Soil organic carbon stocks in 2007 as affected by no-tillage (NT), conventional tillage (CT), and crop rotations (R0, R1, and R2).

Depth	CT R0	CT R1	CT R2	NT R0	NT R1	NT R2
cm	----- Mg ha <sup>-1</sup> -----					
0-5	10.54 c†	10.58 c	10.45 c	12.81 b	14.32 ab	15.96 a
5-15	23.86 abc	24.56 ab	25.36 a	22.64 c	22.52 c	22.85 bc
15-30	30.35 b	30.70 b	30.41 b	29.76 b	33.16 a	34.39 a
30-45	22.84	23.70	22.70	25.55	24.95	24.25
45-60	18.85	19.15	19.19	19.10	20.06	19.54
60-90	25.66	25.38	26.04	24.87	28.25	26.53
0-30	64.75 c	65.84 c	66.22 c	65.21 c	70.00 b	73.20 a
0-60	106.44 c	108.69 bc	108.11 bc	109.86 b	115.01 a	116.99 a
0-90	132.10 b	134.07 b	134.15 b	134.73 b	143.26 a	143.52 a
	CT	NT	R0	R1	R2	
	----- Mg ha <sup>-1</sup> -----					
0-5	10.52	14.36	11.68	12.45	13.21	
5-15	24.59	22.67	23.25	23.54	24.11	
15-30	30.49	32.44	30.06	31.93	32.40	
30-45	23.08 B	24.92 A	24.20	24.33	23.48	
45-60	19.06	19.57	18.98	19.61	19.37	
60-90	25.69	26.55	25.27	26.82	26.29	

R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/radish/wheat. † Means followed by the same letter at the same depth are not significantly different (P<0.05).

Considering that in the Oxisol site there was no available temporal baseline for SOC stocks comparisons, the treatment CT R0 was taken as baseline for the calculation of the apparent soil carbon sequestration rates (Table 1.8). There was no difference of soil carbon sequestration rates among the cumulative soil layers. In all considered depths, the NT treatments had significantly higher soil carbon sequestration rates than CT treatments. As noticed in the Mollisol site, the increase of sampling depth resulted in the increase of the mean's standard error of the SOC stocks estimative and the soil carbon sequestration rates at higher depths. At the average of crop rotations under NT, the soil carbon sequestration rates observed in the 0-0.30-m soil layer represented 61.7 and 55.3 % of the soil carbon sequestration rates verified in the 0-0.60- and 0-0.90-m soil layers.



Table 1.8. Apparent soil carbon sequestration rates as affected by no-till (NT), conventional tillage (CT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/radish/wheat.

Tillage system	Nitrogen Source			Mean
	R0	R1	R2	
----- Mg ha <sup>-1</sup> year <sup>-1</sup> -----				
Depth 0-30 cm				
CT	-	0.05±0.01	0.07±0.02	0.04±0.01 b
NT	0.02±0.13	0.24±0.00	0.38±0.13	0.21±0.09 a
Mean	0.01±0.07	0.14±0.01	0.22±0.07	0.13±0.05
Depth 0-60 cm				
CT	-	0.10±0.00	0.08±0.03	0.06±0.01 b
NT	0.16±0.08	0.39±0.05	0.48±0.12	0.34±0.09 a
Mean	0.08±0.04 b	0.25±0.03 a	0.28±0.08 a	0.20±0.05
Depth 0-90 cm				
CT	-	0.09±0.03	0.09±0.11	0.06±0.05 b
NT	0.12±0.13	0.51±0.01	0.52±0.19	0.38±0.11 a
Mean	0.06±0.07	0.30±0.02	0.31±0.15	0.22±0.08

†Means followed by the same lower case letter in the rows or upper case letter in the columns at the same depth are not significantly different ( $P < 0.05$ ). The value after the  $\pm$  symbol represents the means' standard error ( $n=4$ ).

## 1.5. Conclusions

The real environmental benefit of NT could be currently underestimated by soil carbon sequestration rates based only in extemporal comparisons between paired NT and CT plots. In situations like those verified in the Mollisol site, when the CT plots had a slight concomitant increase of SOC stocks due to better agricultural management practices others than tillage system, the real accumulation rate of SOC in the NT was partially neglected by the calculation method of comparison of paired plots (AR and NR). The NT MF treatment in the Mollisol site had AR ranging between 0.23 and 0.89 Mg C ha<sup>-1</sup> year<sup>-1</sup>, while the RR ranged between 0.57 and 1.17 Mg C ha<sup>-1</sup> year<sup>-1</sup>, according with the sampling depth. In the Oxisol site, the AR of the NT treatments ranged from 0.02 to 0.52 Mg C ha<sup>-1</sup> year<sup>-1</sup> according with crop rotation and sampling depth. The AR and NR methods could lead to discrepancies on the evaluation of SOC dynamics by changes in SOC stocks or by real-time CO<sub>2</sub> exchange methods (eddy covariance). However, when the objective is to determinate soil carbon sequestration rates for carbon market purposes, the AR and NR methods are more appropriate, since is possible to determinate how much an specific management practice is contributing to mitigation of CO<sub>2</sub>. For experimental analysis, the NR method is more accurate than AR method by including in the calculation eventual differences in SOC stocks at the baselines. To

accurately evaluate the NT potential to mitigate CO<sub>2</sub> future research should include control plots and a minimum of two spaced time points to determine the full value of soil carbon sequestration.

The deeper soil sampling has provided limited contribution to the calculation of the carbon sequestration rates. The increasing soil variability in the Mollisol site at deeper soil layers increased the mean standard error of the measured carbon sequestration rates. On the other hand, in the Oxisol site, the increasing sampling depth didn't change significantly the carbon sequestration rates measured at the surface (0-30 cm), since most of the carbon accumulation occurred in that soil layer. On the other way, the evaluation of the SOC at deeper soil layers could enhance the knowledge of SOC dynamics in depth which could be considerably distinct from the soil surface.

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## **CHAPTER 2**

# **TEMPORAL SOIL ORGANIC CARBON DYNAMICS AND AGGREGATE STABILITY OF AN MOLLISOL AND OXISOL: LONG- TERM EFFECT OF TILLAGE, NITROGEN SOURCES AND CROP ROTATION**

### **2.1. Introduction**

The reduction or elimination of soil tillage (e.g. NT) is the main strategy to increase soil organic carbon (SOC) stocks, contributing to mitigate increased atmospheric CO<sub>2</sub> (Lal, 2004; Smith et al., 2007). This tillage system protects more efficiently the SOC and the new added C from microbial decomposition, reducing the SOC mineralization rates when compared to conventional tillage (Bayer et al., 2006). Therefore, NT is considered more carbon conservative than any other options of tillage systems.

The SOC stabilization mechanisms are biochemical recalcitrance, organo-mineral interaction and bio-physical protection (Christensen, 1996), processes mediated by their interactions with soil biota (Six et al., 2002). The importance of each mechanism of SOC stabilization depends of soil and climate characteristics. The bio-physical protection (i.e. aggregation) is the key factor controlling SOC stabilization in soils from temperate climate regions, as the Mollisols, where the increase of SOC is associated with the increase of the amount of macroaggregates (soil aggregates >2000- $\mu$ m diam.) (Fabrizzi et al., 2008).

The C input has direct relationship with changes in either NT or CT SOC stocks, but for the same amount of C input it is expected a higher soil C recovery in NT than CT (Bayer et al. 2006). This difference in SOC stocks should be linked to SOC stabilization mechanisms, since the humification rate (conversion of the added C into SOC) does not seem to change significantly among tillage systems (Bayer et al., 2006; Lovato et al., 2004). The amount of C added to the soil and the SOC stabilization within aggregates can determine the carbon sequestration potential of NT soil (Six et al., 2000) with implications about how long the soil could act as C sink. The soil has a limited capacity to store SOC within aggregates, in a process of saturation that occurs from the smaller to larger aggregate size fractions (Gulde et al., 2008). Therefore, the capacity of buildup and reduced turnover of soil macroaggregates could determinate how much SOC could be stored.

The accumulation of SOC under NT is often observed only in the first 0.10-m of soil depth (Amado et al., 2006; Blanco-Canqui and Lal, 2008) and mainly in the first 0.05-m (Sá et al., 2001). The saturation of this superficial soil layer could implicate that the soil is no longer able to sequester SOC, since the changes in SOC decrease with depth (Sá et al., 2001; Bayer et al., 2001). Basically, the increase of SOC in sub-superficial soil layers is attributed to root-derived C input (Puget and Drinkwater, 2001; Allmaras et al., 2004), soil biological activity (Wolters, 2000; Ekschmitt et al., 2008) or by translocation of soluble carbon from upper soil layers. However, the intensity of this process is not known especially when an immediate upper soil layer achieves SOC saturation.

In this study, based in two long-term experiments in central Kansas (USA) and southern Brazil, tillage systems, nitrogen sources and crop rotations were investigated over 15 years. The objective of this work was evaluate the long-term SOC dynamics of a Mollisol under different tillage systems and nitrogen sources in temperate climate conditions and of a Oxisol under different tillage systems and crop rotations in sub-tropical climate conditions, discussing the implications of SOC stabilization mechanisms over soil carbon sequestration rates, SOC saturation and transfer of SOC among soil layers.

## **2.2. Hypothesis**

The use of mathematical equations that represent the temporal SOC dynamics improve the accuracy of the SOC sequestration rates.

The SOC accumulation in NT soils is a function of C input to the soil and the protection of the new added C through aggregation.

The SOC saturation process is controlled by the soil's capacity to form stable large aggregates to protected the SOC.

The SOC saturation at superficial soil layers limit the soil's capacity into sequester atmospheric carbon, but this process is compensated by the translocation of SOC to sub-superficial layers.

## **2.3. Material and Methods**

In the Chapter 1, detailed explanation was made about experimental sites location and characteristics (soil , climate, and treatments description), procedures for aboveground carbon input estimates, and SOC content and stocks determination. In this chapter, only the specific methodology used to approach this chapter's topics will be further detailed.

### 2.3.1. Soil Sampling

Soil samples were collected in 1992, 2002, 2004, and 2007 in the Oxisol site and in 1992, 1995, 1999, 2002, 2003, 2004, and 2007 in the Mollisol site. The samples were taken at the 0-0.05, 0.05-0.15, and 0.15-0.30m soil depth. For the aggregate stability analysis, soil samples were collected at 0.05 m soil depth in January of 2008 in Oxisol site and in April of 2008 in the Mollisol site.

#### 2.3.1.1. Mollisol

For SOC determination in the bulk soil, the samples were collected using a 0.05 m diameter soil probe, placed in plastic bags and weighed. A sub sample was dried at 105°C to determine the soil bulk density considering the probe volume. After they were passed through a 2 mm sieve, large roots and plants residues removed, and stored until use.

For the aggregate stability analysis, one sample per plot was taken at the 0-0.05 m soil depth manually with a shovel. The samples were stored in a walk-in cooler with temperature at 4°C. Then the samples were passed through an 8 mm sieve and the aggregates retained on a 4 mm sieve were collected, large roots and plants residues removed, and dried in an oven at 40°C and stored until use.

#### 2.3.1.2. Oxisol

For SOC determination in the bulk soil, four samples per plot were collected manually with a shovel. The samples were air dried at 30±5°C. After they were passed through a 2 mm sieve, large roots and plants residues removed, and stored until use. To determine the soil bulk density, undeformed samples were taken at the 0-0.05, 0.05-0.10, 0.10-0.15, 0.15-0.20, 0.20-0.25, 0.25-0.30 m soil depth with 0.05 m diameter and 0.04 m height iron cylinders. The soil bulk density was calculated by dividing the dry-weight of the samples by the cylinder volume.

For the aggregate stability analysis, four samples per plot were taken at the 0-0.05 m soil depth manually with a shovel. The samples were air dried at 30±5°C and stored until use. The dry samples were placed on a plastic tray with a 50 mm sponge absorbed with water and stored at 4°C overnight to wet up the samples to separate the soil aggregates. The samples were passed through an 8 mm sieve and the aggregates retained on a 4 mm sieve were collected, large roots and plants residues removed, and dried in an oven at 40°C and stored until use.

### 2.3.2. Soil Carbon Sequestration Rates

The soil organic carbon sequestration rates were determined by the angular coefficient of the fitted linear equation in each soil layer of the treatments.

### 2.3.3. Water-Stable Aggregates

Water-stable aggregates (WSA) were separated using the procedure modified by Gulde et al. (2008) and the apparatus described by Mikha and Rice (2004). Briefly, 80 g of 4-8 mm soil aggregates was placed on the top of a 4 mm sieve mounted in the aggregate-sieving machine. To slake the air-dried soil, about one liter of water was rapidly added until the aggregates were submersed. The aggregates stood submerged for 5 minutes following 2 minutes of wet sieving. The soil that passed through the 4-mm sieve was collected in a bucket and transferred to a 2 mm sieve. The procedure was repeated until five aggregates size classes were collected from each treatment (>4000 , 2000-4000 , 250-2000 , 53-250 , and <53  $\mu\text{m}$  diameter). The water stable aggregates were dried, weighed, and subsampled to determine sand content of each fraction (Mikha and Rice, 2004). As the fraction 2000-4000  $\mu\text{m}$  resulted in a very small amount of soil for further analysis, the fractions >4000 and 2000-4000  $\mu\text{m}$  were considered the fraction >2000  $\mu\text{m}$ . Macroaggregates were defined as >2000  $\mu\text{m}$ , mesoaggregates 250-2000  $\mu\text{m}$ , microaggregates 250-53  $\mu\text{m}$ , and silt plus clay fraction by <53  $\mu\text{m}$  size fraction. Sub-samples of the isolated fractions were used for TOC analysis as described previously. Calculations for TOC in different aggregate-size fraction were adjusted for sand-free water stable aggregates.

### 2.3.4. Statistical Analysis

The results were submitted to analysis of variance and the means were separated by the Tukey test using SAS PROC MIXED software (SAS institute, 2002). The relationships between variables were evaluated by the significance of the determination coefficient ( $r^2$ ) of the fitted equations using linear regression analysis or forward stepwise regression analysis performed by the software SigmaStat v3.5 (SYSTAT, 2006). The results were considered significantly different at  $P < 0.05$ .

## **2.4. Results and discussion**

### 2.4.1. Mollisol



During the evaluated period, no increase in SOC stocks were noticed in CT CO and CT MF in any of the evaluated soil layers (Figure 2.1). Also, no differences in SOC stocks were noticed in deeper layers. However, the treatments NT CO and NT MF showed a significant soil carbon sequestration rate of  $0.23 \text{ Mg ha}^{-1} \text{ year}^{-1}$  ( $P=0.00639$ ) and  $0.32 \text{ Mg ha}^{-1} \text{ year}^{-1}$  ( $P=0.00477$ ) in the 0-0.05 m soil layers, respectively. When taken in account the cumulative 0-0.30 m soil layer the SOC increase rate was even higher ( $0.63 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ,  $P=0.00831$ ) in the treatment NT MF. The higher soil carbon sequestration rate in the cumulative 0-0.30 m than in the 0-0.05 m soil layer was due to the slightly and not significant increases observed in the 0.05-0.15 and 0.15-0.30 m soil layers. In this treatment, the increase of SOC stocks in the 0-0.05 m soil layer represents more than 50% of the total increase of 0-0.30 m soil layer.

The treatments CT OF and NT OF showed a distinct SOC accumulation pattern according to the kind of manure applied. On average, the composted manure was twice the C input of the fresh manure (Table 2.3). In the first 10 years of fresh manure amendment, both CT OF and NT OF presented a linear SOC increase in the 0-0.05 and in the cumulative 0-0.30 m soil layers, where the soil carbon sequestration rates were  $0.48$  and  $0.91 \text{ Mg ha}^{-1} \text{ year}^{-1}$  ( $P=0.04351$  and  $P=0.00881$ ), respectively at the superficial layer, and  $1.33$  and  $0.72 \text{ Mg ha}^{-1} \text{ year}^{-1}$  ( $P=0.01310$  and  $P=0.00536$ ), respectively at the cumulative layer. The higher increase in CT OF at the cumulative layer was due to the SOC increase at the 0.05-0.15 m soil layer with a rate of  $0.63 \text{ Mg ha}^{-1} \text{ year}^{-1}$  ( $P=0.00785$ ), while the NT OF showed a slight but significant decrease of  $-0.11 \text{ Mg ha}^{-1} \text{ year}^{-1}$  ( $P=0.01362$ ) in this soil layer. The increase of SOC stocks at the 0.05-0.15 -m soil layer in treatment CT OF can be attributed to the redistribution of the SOC and added C with the chisel plow operation, mixing the SOC of the 0-0.05 and 0.05-0.15 m soil layers and moving the C added at the soil surface to sub-superficial soil layers (Franzluebbers, 2002; Allmaras et al., 2004). On the other hand, the decrease of SOC at this depth in the treatment NT OF could be related to the increase of SOC mineralization promoted by the manure amendment (Nicoloso, chapter 3) and the placement of the added C broadcasted on soil surface, thus having limited effect in the 0.05-0.15-m soil layer.

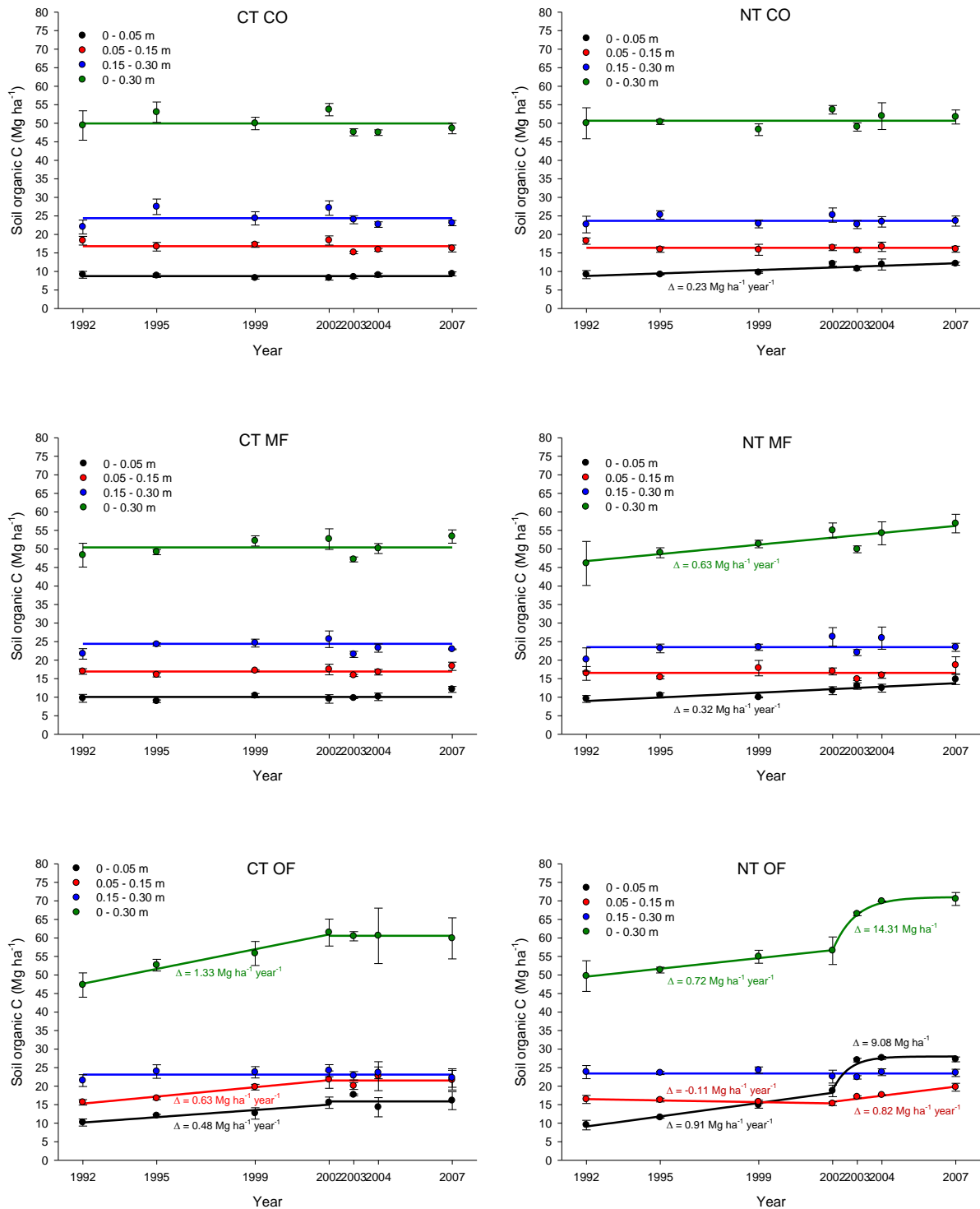


Figure 2.1. Temporal dynamics of SOC in the 0-0.05, 0.05-0.15, 0.15-0.30 and 0-0.30m soil layer estimated fitted linear or first-order kinetic equations as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment. The vertical bars represent the observed mean's standard errors (n=4).

Table 2.2. Regressions of soil organic carbon dynamics at different soil layers as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment.

Soil depth	Period	Fitted Equation	r <sup>2</sup>	P>F
CT CO				
0-5	1992-2007	SOC = 8.74	0.0000	0.94109
5-15	1992-2007	SOC = 16.81	0.0000	0.18807
15-30	1992-2007	SOC = 24.37	0.0000	0.79654
0-30	1992-2007	SOC = 49.95	0.0000	0.40265
CT MF				
0-5	1992-2007	SOC = 10.07	0.1341	0.11386
5-15	1992-2007	SOC = 16.93	0.0000	0.34711
15-30	1992-2007	SOC = 24.41	0.0000	0.88613
0-30	1992-2007	SOC = 50.41	0.0000	0.28182
CT OF				
0-5	1992-2002	SOC = 9.21 + 0.48 t	0.7446	0.04351
0-5	2002-2007	SOC = 15.88	0.0000	0.94953
5-15	1992-2002	SOC = 14.02 + 0.63 t	0.9531	0.00785
5-15	2002-2007	SOC = 21.52	0.0000	0.82011
15-30	1992-2007	SOC = 23.13	0.0000	0.74597
0-30	1992-2002	SOC = 44.99 + 1.33 t	0.9219	0.01310
0-30	2002-2007	SOC = 60.59	0.3360	0.11759
NT CO				
0-5	1992-2007	SOC = 8.32 + 0.23 t	0.7031	0.00639
5-15	1992-2007	SOC = 16.37	0.0000	0.19156
15-30	1992-2007	SOC = 23.66	0.0000	0.97187
0-30	1992-2007	SOC = 50.69	0.0000	0.39880
NT MF				
0-5	1992-2007	SOC = 8.40 + 0.32 t	0.7350	0.00477
5-15	1992-2007	SOC = 16.55	0.0000	0.50031
15-30	1992-2007	SOC = 23.50	0.0021	0.17356
0-30	1992-2007	SOC = 45.25 + 0.63 t	0.6715	0.00831
NT OF				
0-5	1992-2002	SOC = 7.28 + 0.91 t	0.9474	0.00881
0-5	2002-2007	SOC = -7.48 x 10 <sup>8</sup> + 7.48 x 10 <sup>8</sup> (1 - e <sup>-1.54 t</sup> )	0.9105	0.02984
5-15	1992-2002	SOC = 16.72 - 0.11 t	0.9189	0.01362
5-15	2002-2007	SOC = 5.94 + 0.82 t	0.8455	0.02775
15-30	1992-2007	SOC = 23.42	0.0447	0.15210
0-30	1992-2002	SOC = 48.13 + 0.72 t	0.9679	0.00536
0-30	2002-2007	SOC = -8.43 x 10 <sup>6</sup> + 8.43 x 10 <sup>6</sup> (1 - e <sup>-1.11 t</sup> )	0.9895	0.00348

SOC = soil organic carbon stock, t = duration of experiment (years)

Table 2.3. Annual average aboveground carbon addition to the soil between 1990 and 2007 as affected by no-tillage (NT), conventional tillage (CT), control without nitrogen (CO), mineral fertilizer (MF), and organic fertilizer (OF) management practices.

Source	CT CO	CT MF	CT OF	NT CO	NT MF	NT OF
----- Mg ha <sup>-1</sup> year <sup>-1</sup> -----						
1992-2001						
Corn	1.7	2.3	2.3	1.8	2.5	2.2
Manure	-	-	2.6	-	-	2.6
Total	1.7	2.3	4.9	1.8	2.5	4.8
2002-2007						
Corn	1.5	2.5	2.5	1.3	2.3	2.3
Manure	-	-	5.2	-	-	5.2
Total	1.5	2.5	7.7	1.3	2.3	7.5
1992-2007						
Corn	1.6	2.4	2.4	1.6	2.4	2.3
Manure	-	-	3.6	-	-	3.6
Total	1.6	2.4	5.9	1.6	2.4	5.8

After 2002, when the manure was changed to composted manure, the SOC stocks in CT OF treatments stabilized at 15.88 and 21.52 Mg ha<sup>-1</sup> at the 0-0.05 and 0.05-0.15 m soil layers respectively, while the treatment NT OF presented a first-order kinetic (P=0.02984) and linear (P=0.02775) SOC increase at the same depths, respectively. The soil under CT had reached SOC saturation after an increase of about 56 and 41 % of the SOC stocks in the 0-0.05 and 0.05-0.15 m soil layer, respectively. On the other hand, after 5 years of compost manure amendment, the SOC at the 0-0.05 m soil layer of the treatment NT OF increased kinetically 9.08 Mg ha<sup>-1</sup>, stabilizing at 28.03 Mg ha<sup>-1</sup>, while the 0.05-0.15 m soil layer showed a linear soil carbon sequestration rate of 0.82 Mg ha<sup>-1</sup> year<sup>-1</sup>. In this case, the soil under NT had reached SOC saturation after an increase of about 308 % of SOC stocks in the 0-0.05 m soil layer. In the cumulative 0-0.30 m soil layer, the SOC increase presented the same pattern of the superficial layer, either in CT OF or NT OF treatments due to the already related higher contribution of the 0-0.05 m soil layer to the soil carbon sequestration rates, since about 44 and 88 % of the total SOC increase was promoted by the superficial soil layer in CT OF and NT OF treatments, respectively. However, the fitted kinetic equation (P=0.00348), does not mean that the soil achieved saturation in the whole 0-0.30 m layer, since the 0.05-0.15 m layer keeps accumulating SOC.

The increase of SOC stocks, regardless the tillage system, occurred only in the 250-2000, and >2000  $\mu\text{m}$  fractions, accounting for 31 and 69% of the increase in SOC stocks,

respectively (Figure 2.2). The SOC within the smaller aggregate size fractions (<53, and 53-250  $\mu\text{m}$ ) were not affected significantly by treatments, remaining stable among treatments. The non-response of the smaller fractions to the treatments confirms that the SOC within those fractions are highly stable in the soil (Six et al., 2002). Further fractionation of the larger aggregate size fractions should indicate how much of the accumulated SOC within those fractions are susceptible to be re-emitted to the atmosphere if the NT soil will be converted to CT by isolating the SOC protected only by the mesoaggregates (250-2000  $\mu\text{m}$ ) or macroaggregates (>2000  $\mu\text{m}$ ) or protected by the microaggregates (53-250  $\mu\text{m}$ ) within mesoaggregates or macroaggregates (Six et al., 2000; Gulde et al., 2008).

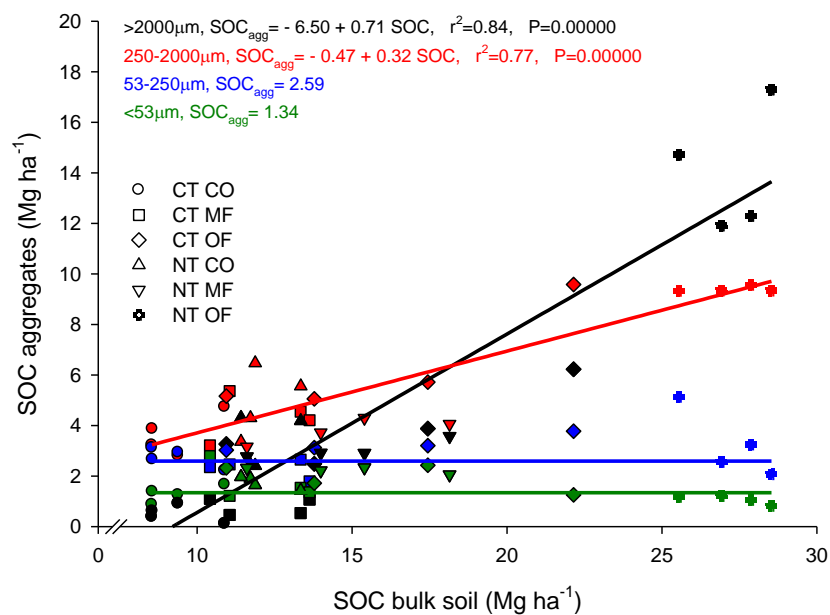


Figure 2.2. Relationship between SOC stocks in the bulk soil and SOC stocks within sand-free water-stable aggregates fractions of the 0-0.05 m soil layer (<53, 53-250, 250-2000, and >2000  $\mu\text{m}$ ) as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and control (CO) without nitrogen.

Considering that the SOC saturation occurs from the smaller to the larger soil aggregates fractions (Gulde et al, 2008) the relationship between the SOC stocks in the bulk soil and the SOC content within each aggregate size fractions isolated by wet-sieving (<53, 53-250, 250-2000, and >2000  $\mu\text{m}$ ) is presented in Figure 2.3. The larger aggregates size fractions had a larger capacity to accumulate SOC. However, all fractions reach SOC

saturation at about the same SOC content of the grassland aggregates, as showed by the fitted first-order kinetic equations.

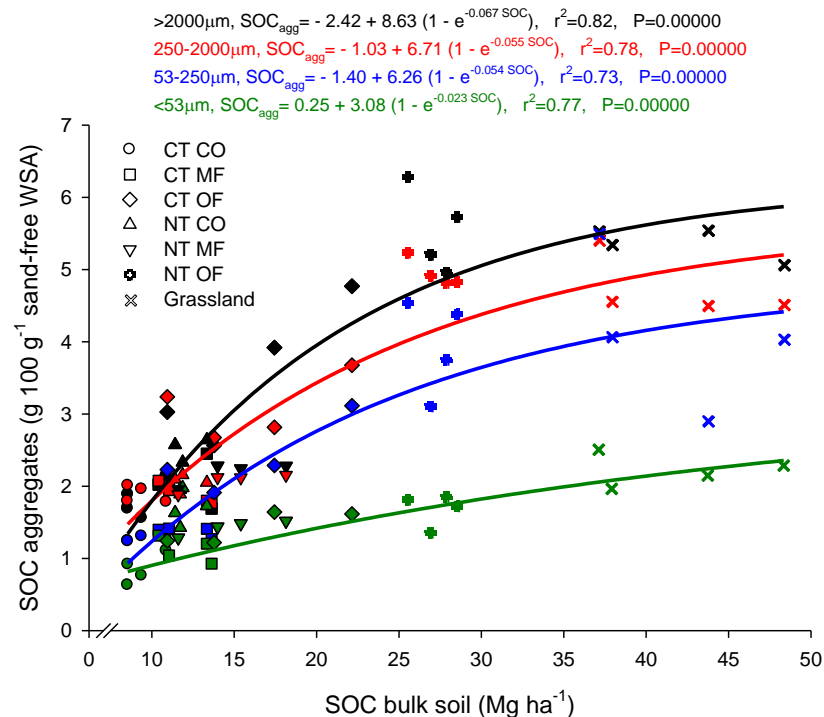


Figure 2.3. Relationship between SOC stocks in the bulk soil and SOC content of the sand-free water-stable aggregates fractions of the 0-0.05 m soil layer (<53, 53-250, 250-2000, and >2000  $\mu\text{m}$ ) as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment and the Grassland.

The relationship between the amount of sand-free water-stable macroaggregates (>2000- $\mu\text{m}$ ) and the SOC stocks associated with all aggregate size fractions (sum of SOC stocks within <53, 530-250, 250-2000, and >2000  $\mu\text{m}$  fractions) is presented in Figure 2.4. The intersection of the two linear equations that describe the increase of SOC stocks within aggregate size fractions in CT and NT treatments as the amount of macroaggregates increases in the soil with the kinetic curve fitted among those treatments with SOC stocks at saturation point the maximum SOC stocks possible to be achieved within the respective level of aggregation in the 0-0.05-m soil layer under CT and NT (14.2 and 28.0  $\text{Mg ha}^{-1}$ , respectively). These estimates are within the mean's standard error of the SOC stocks in the bulk soil for the treatments CT OF and NT OF ( $16.07 \pm 2.42$  and  $27.22 \pm 1.30 \text{ Mg ha}^{-1}$ , respectively). In the treatments CT OF and NT OF the SOC stock estimated within the aggregate fractions represents 99.3 and 102.2 % of the SOC stocks in the bulk soil (Annex 1, 2, and 3). The

estimates of the SOC stocks within the aggregate size fractions varied among treatments, with the treatments CO, MF and OF averaging 89.0, 73.6, and 100.7 % of the SOC stocks in the bulk soil, while in the grassland the SOC within the aggregate fractions was only 70.5 % of the measured in the bulk soil. The smaller SOC stocks within the aggregate size fractions than in the bulk soil within the aggregate fraction indicate that this unrecovered SOC was not associated with soil aggregates isolated for the fractionation, and probably was free and unprotected in the soil.

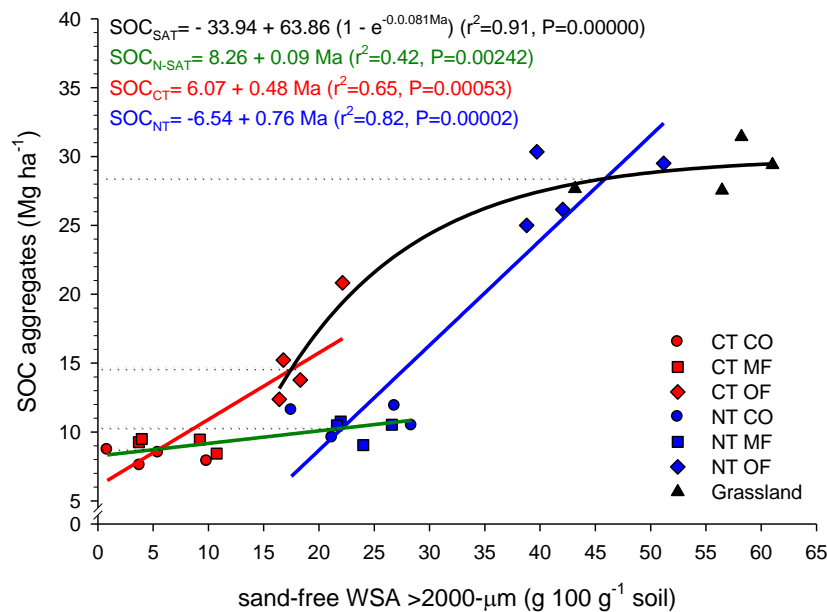


Figure 2.4. Relationship between sand-free water-stable macroaggregates >2000-μm and the total SOC stock within soil aggregates of the 0-0.05 m soil layer (sum of <53, 53-250, 250-2000, and >2000 μm fractions) as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment and the Grassland.

Additionally, the ratio of the SOC stocks within aggregates size fractions at the intersection of the same curves (CT and NT) with the linear equation fitted among those treatments that had not achieved saturation indicates that the NT aggregate fractions had, at average, 17% more SOC than the CT fractions. This ratio is similar to those proposed for the

NT bulk soil by West and Post (2002) and Fabrizzi (2006) which are 16 and 14%, respectively, and superior to the ratio of 10% proposed by Houghton (1997).

The relationship between the amount of macroaggregates and the SOC stocks in the bulk soil is presented in the figure 2.5. The macroaggregates have a more linear relationship with the SOC stocks under saturated ( $r^2=0.79$ ,  $P=0.00004$ ) than in non-saturated conditions ( $r^2=0.23$ ,  $P=0.01975$ ). These results confirm the importance of the macroaggregates to achieve high SOC stocks. Considering both saturated and non-saturated treatments, the relationship shows an exponential pattern ( $r^2=0.86$ ,  $P=0.00009$ ), due to the increasing participation of the macroaggregates to the SOC stocks as the soil becomes richer in SOC.

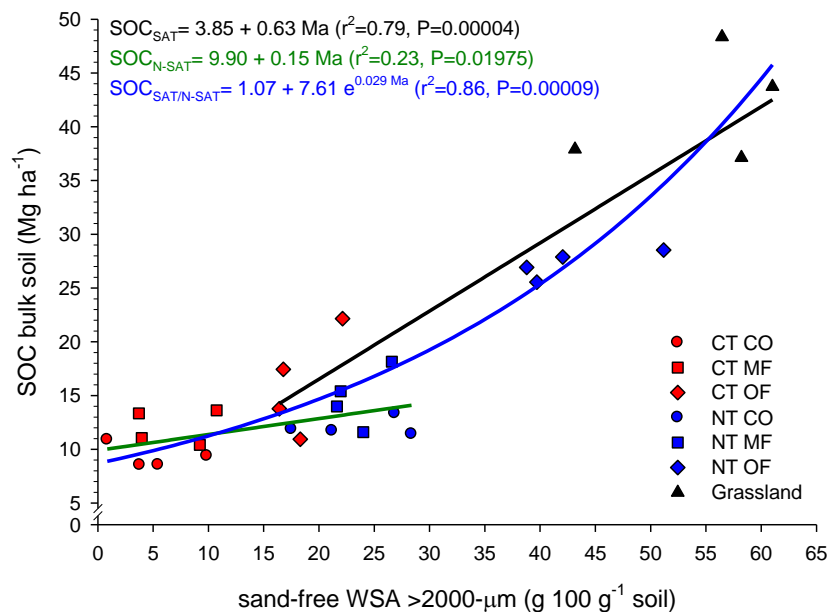


Figure 2.5. Relationship between sand-free water-stable macroaggregates >2000 μm and the SOC stock of the 0-0.05 m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment and the Grassland.

To accumulate more SOC, approaching the grassland SOC stocks, the agricultural soil should increase the proportion of the larger aggregate size fractions with larger storage capacity, substituting the SOC poorer <53 and 53-250 μm fractions for the SOC richer 250-2000 and >2000 μm fractions. However, larger proportions of macroaggregates seems to be



unlikely, since even under NT the soil has a minimum level of disturbance by agricultural practices (e.g. sowing, heavy machinery traffic) or constant growth and decomposition of the root system, while in the grassland there is no mechanical soil disturbance, the grassland root system is a majority perennial providing a more stable soil structure, and is permanently covered by vegetation which promotes less exposure to splash of rain drops, and more stable soil moisture and temperature conditions.

The saturation of the superficial soil layer does not indicate the capacity of the soil to store carbon, since the subsurface soil layer (0.05-0.15 m) also showed a linear increase in SOC stocks at a rate similar to that previously noticed in the 0-0.05 m soil layer (1992-2002). In this case when the soil reaches SOC saturation at the surface, the SOC transfer to adjacent soil layers probably is promoted by the translocation of soluble organic compounds from the manure recently applied or mineralized from the SOC of the upper soil layer by prime effect promoted by the continuous manure amendment.

Since most of the change in SOC stocks was due to the increase of the SOC within the macroaggregates fractions, the Figure 2.6 represents the combined effect of C inputs and soil macroaggregation over the SOC changes between 1992 and 2007 at the 0-0.05 m soil layer of all treatments. As already stressed in recent papers, both parameters show linear relationship with SOC stocks in temperate soils, but the same C input leads to distinct SOC stocks in CT and NT (Bayer et al., 2006; Vieira et al., in press; Fabrizzi et al., 2009). The fitted equation shows that the difference was due to the distinct capacity of buildup and maintain higher orders of soil aggregates in CT and NT, and both parameters can better explain together the changes in SOC stocks ( $r^2=0.82$ ,  $P=0.00000$ ).

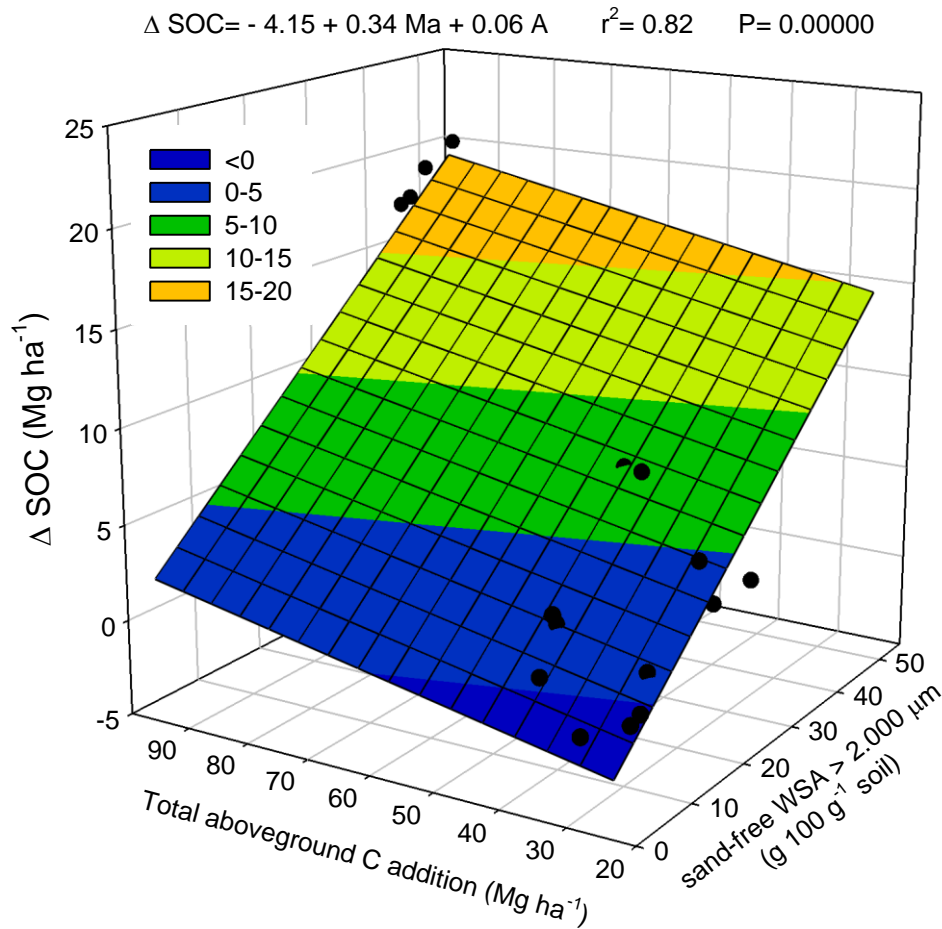


Figure 2.6. Relationship between sand-free water-stable macroaggregates >2.000 µm at 2008, total C inputs and the change in SOC stocks between 1992-2007 at the 0-0.05 m soil layer.

The evaluation of soil carbon sequestration rates by the temporal dynamics of the SOC and by regression analysis seems to be more appropriate than just the comparison of the stocks differences between NT and CT at the same time point (apparent soil carbon sequestration rate) or the stocks difference in the same treatment between two time points (real soil carbon sequestration rate) (Nicoloso, chapter 1). This alternative reduces the uncertainty associated with C estimates due to either soil spatial variability or methodological errors.

The CT did not show significant increases in SOC stocks when the source of carbon was only the corn residues. The accumulation of SOC under CT was only possible when an additional and imported C was applied to the soil as manure, however the soil had a limited capacity to store SOC. On the other hand, NT showed significant soil carbon sequestration

rates even with low C inputs as in treatments with lack of nitrogen fertilization. Under manure amendment, the soil under NT presented a larger capacity to store SOC. These results confirm that the tillage effect was dominant in relation to C inputs by crops systems or organic fertilizers in the SOC stock (Amado et al., 2006).

#### 2.4.2. Oxisol

Table 2.4 shows the annual C input by crop residues to the soil. The increase of crop rotation use intensity promoted increase of C input both in CT or NT. The NT did not promote significantly more C input than CT in the R0 and R1 crop rotations, but in the crop rotation R2, the NT treatment had an annual C input of 6.05 Mg ha<sup>-1</sup> year<sup>-1</sup> while the CT had 5.31 Mg ha<sup>-1</sup> year<sup>-1</sup>. This higher input was due mostly to the corn, which promoted 0.84 Mg ha<sup>-1</sup> year<sup>-1</sup> more C input in NT than in CT.

Table 2.4. Average annual aboveground carbon input to the soil between 1985 and 2007 as affected by no-tillage (NT), conventional tillage (CT), and crop rotations (R0 and R2).

Source	CT R0	CT R1	CT R2	NT R0	NT R1	NT R2
----- Mg ha <sup>-1</sup> y <sup>-1</sup> -----						
Soybean	2.36	2.87	2.53	2.57	2.57	2.80
Wheat	1.23	1.73	1.43	1.34	2.00	1.58
Oat	-	2.21	2.12	-	2.63	2.46
Corn	-	-	3.84	-	-	4.68
Oat+Vetch	-	-	2.61	-	-	2.94
Radish	-	-	1.51	-	-	1.51
Total	3.59	4.84	5.31	3.91	4.88	6.05

<sup>1</sup> Means with different letters between nitrogen sources within corn or total C inputs are significantly different (Tukey test, P<0.05). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/radish/wheat.

In the Oxisol site, there was only 4 years of available data of SOC stocks at the 0-0.05-m soil layer and 3 years of data at the 0-0.30 m soil layers. This situation limited the evaluation of the SOC temporal dynamics in this site (Figure 2.7). In the 0-0.30 m soil layer, no linear equation fitted to the data, however, in the 0-0.05 m soil layer, there was a significant adjustment of the linear equations to the treatments NT R1 and NT R2. In both treatments the soil carbon sequestration rate estimated by the angular coefficient of the linear equation was 0.26 Mg C ha<sup>-1</sup> year<sup>-1</sup>.

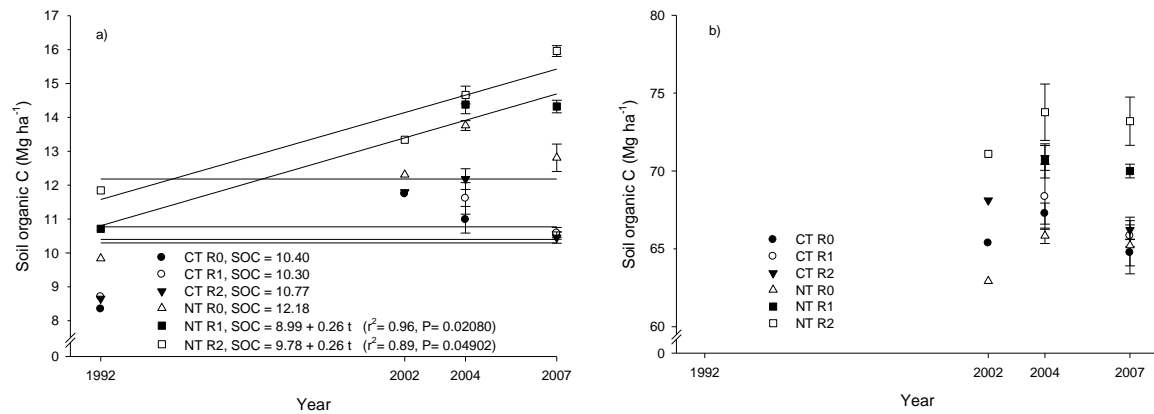


Figure 2.7. Temporal dynamics of SOC in the 0-0.05 (a) and 0-0.30m (b) soil layers estimated by the fitted linear equations as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/radish/wheat. The vertical bars represent the observed mean's standard errors (n=4).

The increase of SOC stocks verified in the NT R1 and NT R2 treatments was related to the increase of SOC stock associated with the soil macroaggregates (>2000- $\mu\text{m}$ ). The other aggregate size fractions did not change in the SOC stocks associated within (Figure 2.8). The increase of SOC stocks associated with the macroaggregates was better explained by the increase of the mean geometric diameter of the soil's aggregates (increase of the amount of macroaggregates) (Annex 4, 5, and 6) than by the increase of SOC content within macroaggregates, since all aggregate size fractions had increase of SOC content with the soil management improvement (Figure 2.9).

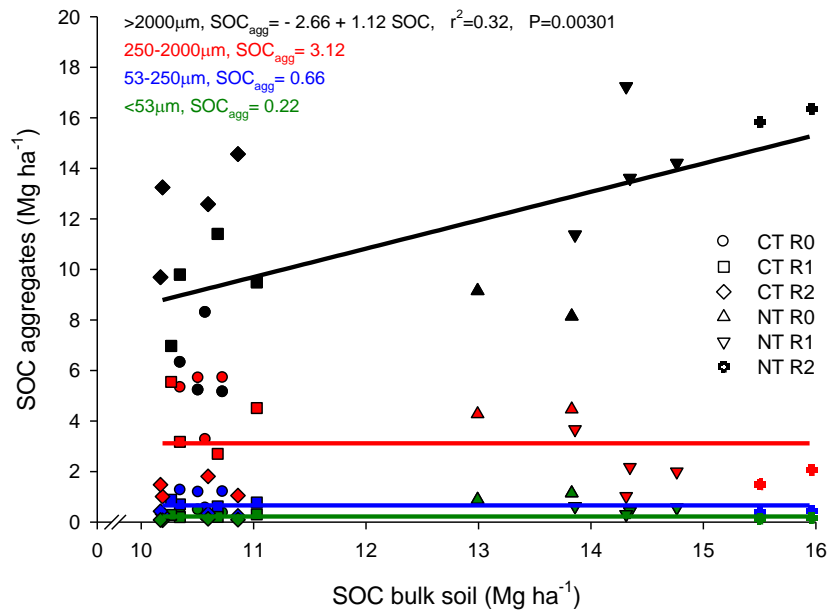


Figure 2.8. Relationship between SOC stocks in the bulk soil and SOC stocks within sand-free water-stable aggregates fractions of the 0-0.05 m soil layer (<53, 53-250, 250-2000, and >2000 µm) as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/ radish/wheat.

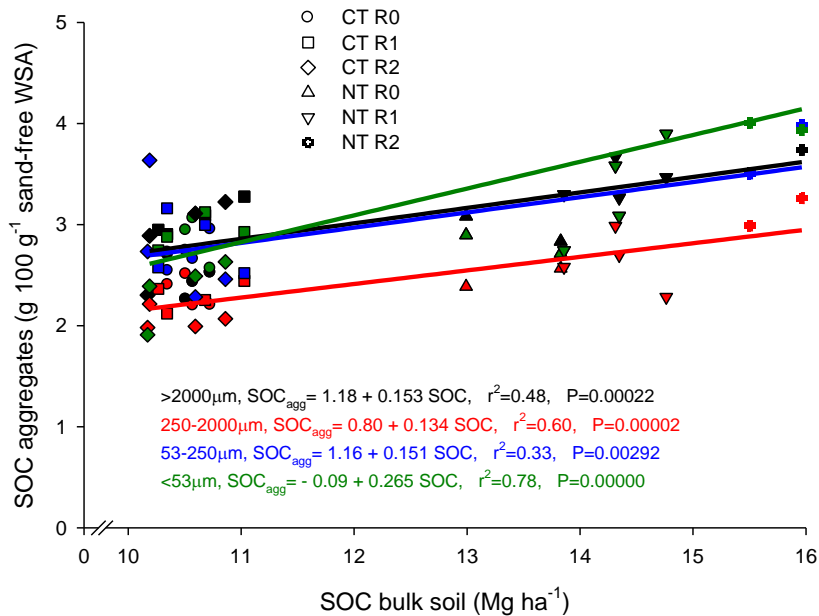


Figure 2.9. Relationship between SOC stocks in the bulk soil and SOC content of the sand-free water-stable aggregates fractions of the 0-0.05 m soil layer (<53, 53-250, 250-2000, and >2000 µm) as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/ radish/wheat.

Figure 2.10 shows the direct relationship verified between the amount of macroaggregates (>2000  $\mu\text{m}$ ) and the increase of SOC stocks in the bulk soil. There was no relationship between macroaggregates and SOC stocks in the CT treatments. In that case the effect crop rotation to increasing the soil macroaggregation was negated by the soil tillage (CT). This result seems to confirm that the fast macroaggregate turnover in CT promotes depletion or no gain of SOC stocks (Six et al., 1998, 1999). In the NT treatments, the increase of soil macroaggregates was related to the increase of SOC stocks in the bulk soil. It demonstrates that the increase of the amount of macroaggregates is the mechanism to the increase of SOC stocks even in soils where the mean geometric diameter of soil aggregates is naturally higher (Oxisol) than soils with lower clay content (Mollisol) (Annexes).

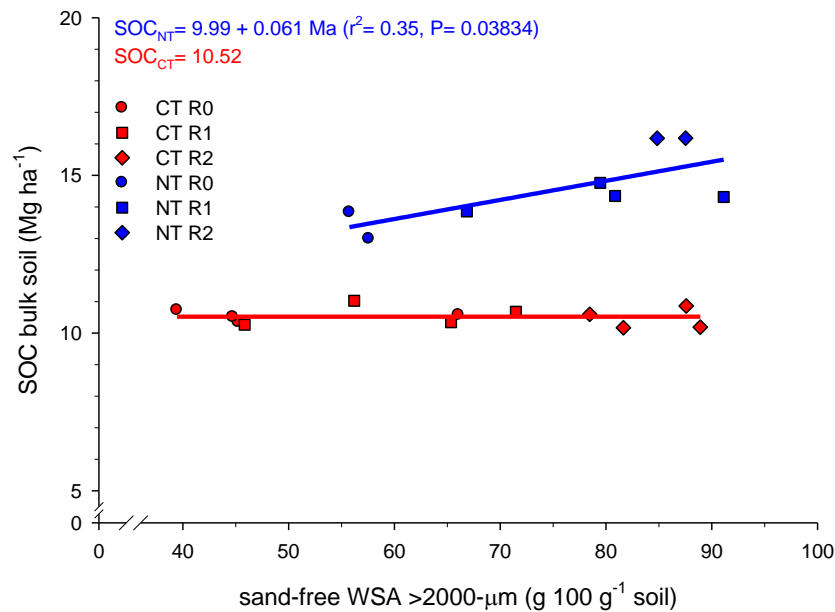


Figure 2.10. Relationship between sand-free water-stable macroaggregates >2000  $\mu\text{m}$  and the SOC stock of the 0-0.05 m soil layer soil as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/ radish/wheat.

## 2.5. Conclusions

The soil's capacity to accumulate SOC was limited by its capacity into protect and stabilize the SOC within aggregates. The soil structure organization into higher levels of macroaggregation is the mechanism that permits the soil to accumulate SOC and give its potential to mitigate global warming even in soils with naturally higher levels of macroaggregation (Oxisol). However, in the NT system the SOC saturation of the superficial soil layer does not necessarily determine the capacity of the soil to accumulate carbon, since the soil carbon sequestration occurred in adjacent soil layers. Future field or mathematically simulated assessments of SOC dynamics should include parameters such as SOC saturation promoted by macroaggregation, analysis of C inputs and macroaggregation, and SOC accumulation in sub-superficial soil layers under either saturated or unsaturated soil layers conditions.

## 2.6. References

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# CHAPTER 3

## PREDICTING SOIL CARBON SEQUESTRATION RATES OF AN MOLLISOL BY ONE-COMPARTMENTAL MODEL RESTRICTED BY AGGREGATE SATURATION

### 3.1. Introduction

Agriculture can provide a low cost competitive option to mitigate climate change with the reduction or elimination of soil tillage and increasing SOC (Smith et al., 2007). The SOC increase follows a sigmoid pattern, achieving the maximum rates within 5 to 20 years after the adoption of the conservative soil tillage system (Lal, 2004). However, the soil is considered a finite C sink, with a 20 to 50 years utile time span according with the amount of C inputted to the system (West and Post, 2002).

The use of mathematical models to simulate the SOC stocks dynamics is an useful tool to predict soil carbon sequestration rates along time and estimate the SOC storage capacity. The one-compartmental model proposed by Hénin and Dupuis (1945) has been widely used with satisfactory accuracy between predict and measured SOC stocks (Dalal and Mayer, 1986; Bayer et al., 2006, Vieira et al., in press). Although simpler than the more complexes Century (Parton et al., 1987) and RothC (Jenkinson et al., 1987) models, it can be used to estimate the dynamic coefficients ( $k_1$  and  $k_2$ ), the SOC turnover and the minimum C input to maintain the originals SOC stocks based on long-term experiments (Bayer et al., 2006).

The  $k_2$  is strongly affected by climate and soil characteristics as well as tillage system, while the  $k_1$  is mainly affected by the quality of added C (Bayer et al., 2006). Using isotopic techniques, Huggins et al. (2008) determined the  $k_2$  of a Mollisol (0-0.45-m) from Minnesota under continuous corn as 0.030, 0.017 and 0.020 year<sup>-1</sup> for the moldboard-plow, chisel-plow and no-tillage systems, respectively, and the  $k_1$  coefficient as 0.17, 0.20 and 0.23 year<sup>-1</sup> for the same treatments, respectively. In sub-tropical climate conditions, Bayer et al. (2006) estimated the  $k_2$  of an Alfisol (0-0.175-m) under CT and NT as 0.040 and 0.019 year<sup>-1</sup>, respectively, while the  $k_1$  were about the same (0.148 and 0.146 year<sup>-1</sup> for CT and NT as well). In a re-evaluation in the same site, Vieira et al. (in press) found a decrease of 34% in the  $k_1$  (0.0964) and a  $k_2$  of and 0.0124 year<sup>-1</sup> for the soil under NT, respectively. The wide variation among assessments made in the same experiment along time could indicate that the

determined coefficients could have been strongly affected by discrepancies in each re-evaluation due to soil spatial variability or methodological errors.

Six and Jastrow (2002) comparing the SOC MRT of soils from temperate climate conditions found the turnover estimated by first-order kinetic models were comparable to those values estimated by isotopic techniques, with NT system presenting a MRT usually higher than the CT (80 and 52 years, respectively) in a range from 14 to 127 years. Under sub-tropical environment, Bayer et al. (2006) found that the SOC under NT had a  $t_{1/2}$  about twice than under CT (36 and 17 years, respectively).

The minimum C input requirement to maintain the SOC stocks is dependent as well of the climate, quality of the added of C, contribution of root-system and tillage system (Johnson et al, 2006). These authors made a compilation of the minimum C input requirement determined by long-term experiments carried out in US, Sweden, and Mexico. On average, to maintain the SOC under moldboard-plow system there was necessary the annual input of  $2.5 \pm 1.0 \text{ Mg C ha}^{-1}$ , while under NT or chisel-plow system the requirement was  $1.8 \pm 0.4 \text{ Mg C ha}^{-1}$ . Specifically in temperate climate conditions, Havlin and Kissel (1997) determined the minimum C requirement to maintain the SOC stocks of a Mollisol from the east central plains of Kansas as 1.7 and 1.2  $\text{Mg C ha}^{-1}$  of a soybean-sorghum rotation under chisel-plow and NT, respectively. Lovato et al. (2004) determined the annual C input requirement of 4.2, and 8.9  $\text{Mg C ha}^{-1}$  to maintain the SOC stocks of a Alfisol in sub-tropical climate under NT and CT, respectively.

Although accurate in the short-time, the one-compartmental model may be inadequate for long-term projections. The SOC at the new equilibrium is calculated by the model as a function of C input and its  $k_1$  coefficient divided by the SOC  $k_2$  coefficient (Bayer et al., 2006). This approach does not take in account any other process that regulates the SOC stabilization. The increase of SOC stocks is followed by a concomitant increase of soil aggregation in larger size classes (Fabrizzi et al., 2009). However, soil aggregates could be saturated with SOC in a process from the smaller to the larger aggregate size fractions (Gulde et al., 2008). The processes of SOC accumulation and saturation in a given soil layer are both regulated by the amount of macroaggregates ( $>2000\text{-}\mu\text{m}$ ) (Nicoloso, chapter 2). This limitation must be considered in the predictions of the mathematical models to avoid an overestimation of the soil's capacity into accumulate SOC.

The objectives of this study were to estimate the SOC dynamics coefficients, SOC turnover and C input requirement to maintain the original SOC stocks of the 0-0.05-m soil layer of a silt loam Hapludoll from a temperate climate region, predicting in the long-term soil

carbon sequestration rates and the capacity of this soil to store SOC. We evaluated just the superficial soil layer because it was the only soil layer with an established SOC saturation point that could be applied to restrict the predictions of SOC accumulation, and also because this soil layer represents the majority of the SOC accumulation of the 0-0.30-m soil layer (Nicoloso, chapters 1 and 2).

### **3.2. Hypothesis**

The predictions of SOC dynamics by one-compartmental model should be restricted by the saturation of SOC in the superficial soil layers in order to avoid overestimations of the soil's potential into accumulate SOC and mitigate global warming.

The estimation of the humification and mineralization coefficients by the linear equation that describe the dynamics of the SOC over a given time period is more accurate than the estimation of these same coefficients in an specific year.

### **3.3. Material and Methods**

#### 3.3.1. Site Description

This research is based on a long-term experiment established in 1990 at the North Farm of Kansas State University in Manhattan, KS (39° 12' 42" N, 96° 35' 39" W). The local average annual precipitation is 800 mm and the annual mean temperature is 11.4°C. The soil is a moderately well-drained Kennebec silt loam (fine-silty, mixed, superactive mesic Cumulic Hapludoll). The experiment was arranged in a split-plot randomized blocks with four replications in plots with corn (*Zea mays* L.). The tillage systems were the main plots and nitrogen sources were the sub-plots. The tillage systems were CT with chisel plow and offset disk pre-planting, and NT by planting directly into the residues with minimum soil disturbance. The nitrogen treatments were different sources of this nutrient: 168 kg N ha<sup>-1</sup> as ammonium nitrate (MF), 168 kg N ha<sup>-1</sup> as cattle manure (OF), and a control (CO) without nitrogen amendment. Until 2001, the cattle manure was fresh and after 2002 the manure was composted.

#### 3.3.2. Soil Sampling

The soil samples were taken in 1992, 1995, 1999, 2002, 2003, 2004 and 2007 at the 0-0.05 m soil layer using a 0.05 m diameter soil probe. The samples were air-dried, ground and sieved (<2 mm) and sub-samples were finely ground for SOC content analysis.

### 3.3.3. Carbon inputs

The aboveground carbon inputs by corn residues were calculated by determining the corn stover dry biomass, and assuming a carbon content of 40 % (Bayer et al., 2006). We assumed that the corn root-system contributed 10 % of the aboveground carbon inputs to account for its contribution to the total carbon addition to the 0-0.05 m soil layer. Usually, it is estimated that the root system contribution to the C input is around of 30% of aboveground biomass (Bolinder et al, 2006), although as the carbon dynamic is evaluated only in 0-0.05 m soil layer we used 1/3 of that value. The value of 10% was chosen because it is 1/3 of the 30% of root system contribution to the C input used by Vieira et al. (2008) to the 0-0.175 m soil layer. The carbon input from manure was estimated by its C/N ratio (Eghball and Power, 1999) considering its total nitrogen content. The Table 3.1 reports the mean total carbon input by corn and manure to the soil between 1992 and 2002, and between 2002 and 2007.

Table 3.1. Annual average aboveground carbon addition to the soil between 1990 and 2007 as affected by no-tillage (NT), conventional tillage (CT), control without nitrogen (CO), mineral fertilizer (MF), and organic fertilizer (OF) management practices.

Source	CT CO	CT MF	CT OF	NT CO	NT MF	NT OF
----- Mg ha <sup>-1</sup> year <sup>-1</sup> -----						
1992-2001						
Corn	1.7	2.3	2.3	1.8	2.5	2.2
Manure	-	-	2.6	-	-	2.6
Total	1.7	2.3	4.9	1.8	2.5	4.8
2002-2007						
Corn	1.5	2.5	2.5	1.3	2.3	2.3
Manure	-	-	5.2	-	-	5.2
Total	1.5	2.5	7.7	1.3	2.3	7.5
1992-2007						
Corn	1.6	2.4	2.4	1.6	2.4	2.3
Manure	-	-	3.6	-	-	3.6
Total	1.6	2.4	5.9	1.6	2.4	5.8

### 3.3.4. Soil organic carbon analysis and stocks calculation

Soil samples were analyzed for SOC content by dry combustion in a C/N elemental analyzer (Flash EA 1112 Series, Thermo Scientific, Waltham, MA). The stocks were calculated considering the soil bulk density determined of the collected soil cores. The stocks

were adjusted to the soil bulk density determined in 1992, using the equivalent soil mass method (Ellert and Bettany, 1995).

### 3.3.5. Mathematical model adjustment

The one-compartmental model was adjusted to the 0-0.05 m soil layer according to the method described by Bayer et al. (2006) according to the following first-order kinetic equation (Dalal and Mayer, 1986):

$$C_t = C_0 e^{-k_2 t} + \frac{A k_1}{k_2} (1 - e^{-k_2 t}) \quad (\text{Eqn. 1})$$

where,  $C_t$  is the SOC stocks at time  $t$ ,  $C_0$  is the SOC stocks at time zero,  $k_2$  is the SOC annual mineralization rate (SOC decay rate by microbial decomposition),  $k_1$  is the added C annual humification rate (recovery of added C into SOC) and  $A$  is the annual C input.

The  $k_1$  and  $k_2$  coefficients were estimated by two different approaches: with the relationship between the measured SOC stocks in 1995, 1999 and 2002 and the annual C input from 1992 to the end of each considered period, and the relationship between the SOC stocks in 2002 estimated by the linear equation that described the SOC evolution from 1992 to 2002 and the annual C input of the period. In the first approach, the  $C_0$  was the measured SOC stocks in 1992, while in the second approach, the  $C_0$  in 1992 was estimated by linear equation. The significance of the linear equations was disregarded at this point, because in those treatments where the change in SOC was too small, the variance between samples could be higher than the effects of the treatments, inducing the lack of fit of the linear equation, but it does not mean that the SOC were not changing. The SOC stocks estimates by linear equation approach aims to improve the accuracy of the  $k_1$  and  $k_2$  coefficients estimates, by reducing possible errors due to the SOC spatial variability or methodological errors among the years evaluated. The 1992 to 2002 period was chosen because that was the last year of fresh manure application and the increase of SOC in the treatments CT OF and NT OF was still linear. After the initial procedure, the  $k_1$  and  $k_2$  were calculated with the following equations:  $k_2 = (\ln(C_0) - \ln(a))/t$  (Eqn. 2) and  $k_1 = k_2 b / (1 - e^{-k_2 t})$  (Eqn. 3), where  $a$  is the angular coefficient of the fitted linear equation between C input and SOC stocks in the considered period and  $b$  is the remaining  $C_0$  at time  $t$ .

Additionally, the SOC stocks mean residency time (MRT) was calculated as:  $MRT = 1/k_2$  (Eqn. 4); and the SOC stocks half-life ( $t_{1/2}$ ) as:  $t_{1/2} = \ln 2/k_2$  (Eqn. 5). The minimum C input ( $A_0$ ) to maintain the original SOC stocks (1992) were calculated as:  $A_0 = C_0 k_2/k_1$  (Eqn. 6) and the SOC stocks at the new equilibrium ( $C_e$ ) were calculated as:  $C_e = A k_1/k_2$  (Eqn. 7). The

SOC stocks were predicted for a 100 years period after the beginning of the experiment (1990), starting at the second year. The SOC accumulation predicted by the model was restricted by the SOC saturation of the 0-0.05-m soil layer determined in the treatments CT OF and NT OF with composted manure amendment. The model was adjusted to the CT and NT treatments with CO, MF and fresh cattle manure amendment only (OF 1992-2001).

### 3.3.6. Statistical analysis

The results were submitted for analysis of variance (ANOVA) and the means were separated by the Tukey test ( $P < 0.05$ ) using SAS PROC MIXED (SAS Institute, 2002). The adjustment of mathematical model and its estimated parameters  $k_1$  and  $k_2$ , and the relationships between variables were evaluated by the significance of the determination coefficient ( $r^2$ ) of the fitted linear regressions using regression analysis performed by the software TableCurve 2d v.5.01 (Systat, 2002). Complimentarily, the mathematical model was submitted to the Lack-of-fit (LOFIT) and root mean square errors (RMSE) tests to assess if the predicted SOC stocks were comparable to those actually measured. The LOFIT and RMSE test were performed by the program MODEVAL 1.1 for Excel in accordance with Smith et al. (1997).

## **3.4. Results and discussion**

### 3.4.1. Temporal dynamics of SOC stocks

No significant increase of SOC stocks were observed in the 0-0.05 m soil layer of the treatments CT CO and CT MF (Figure 3.1), though there was a slight but not significant tendency of increase of the SOC stocks of the treatment CT MF was noticed ( $\Delta \text{SOC} = 0.12 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ,  $P = 0.11386$ ). However, the treatments NT CO and NT MF showed a significant soil carbon sequestration rate of  $0.23 \text{ Mg ha}^{-1} \text{ year}^{-1}$  ( $P = 0.00693$ ) and  $0.32 \text{ Mg ha}^{-1} \text{ year}^{-1}$  ( $P = 0.00477$ ), respectively.



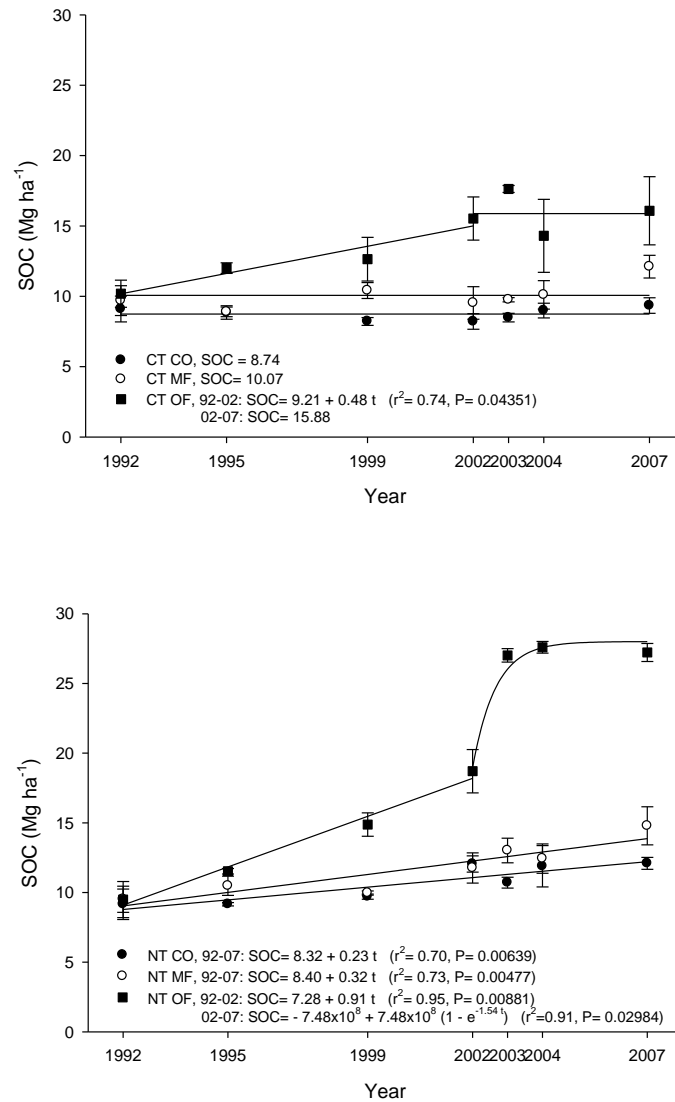


Figure 3.1. Temporal dynamics of SOC in the 0-0.05-m soil layer estimated by the one-compartmental model or by fitted linear or first-order kinetic equations. The vertical bars represents the observed mean's standard errors ( $n=4$ ).

The treatments CT OF and NT OF showed a distinct pattern of SOC dynamic according to the kind of manure applied. At average, the composted manure promoted twice the C input of the fresh manure (Table 3.1). In the first 10 years of fresh manure amendment, both CT OF and NT OF experienced a linear SOC increase ( $0.74$  and  $0.91 \text{ Mg ha}^{-1} \text{ year}^{-1}$ , respectively). After 2002, when the kind of manure was changed to composted manure, the SOC stocks in CT OF treatments stabilized at  $15.88 \text{ Mg ha}^{-1}$ , while the NT OF treatment presented a first-order kinetic pattern SOC increase. After 5 years of compost manure amendment, the SOC stock of the treatment NT OF increased kinetically  $9.08 \text{ Mg ha}^{-1}$ ,

stabilizing at  $28.03 \text{ Mg ha}^{-1}$ . The total change in SOC stocks was promoted by the increase of SOC associated to the larger aggregate size fraction (250-2000, and  $>2000 \mu\text{m diam.}$ ), which reached SOC saturation in the treatments CT OF and NT OF (Nicoloso, chapter 2). The SOC stocks in the treatment CT OF and NT OF at saturation ( $15.88$  and  $28.03 \text{ Mg ha}^{-1}$ , respectively) were used to restrict the SOC storage capacity predicted by the one-compartmental mathematical model.

#### 3.4.2. Estimative of $k_1$ and $k_2$ coefficients

The coefficients of the SOC were determined by the relationship between C inputs between 1992 and the considered period and the SOC stocks measured in 1995, 1999, and 2002 and estimated in 2002 by linear equation (Figure 3.2). There was a wide variation of the relationship among the evaluated years. Part of this variability is due to the variation of C input among years and partly due to the soil spatial variability in the measurements of SOC stocks. The linear relationship between C inputs and the SOC in 2002 estimated by linear equation had larger determination coefficients than the relationships determined in the other years with the actual measured SOC stocks. The estimates of the SOC stocks by linear equation was efficient to reduce errors due to high soil spatial variability.

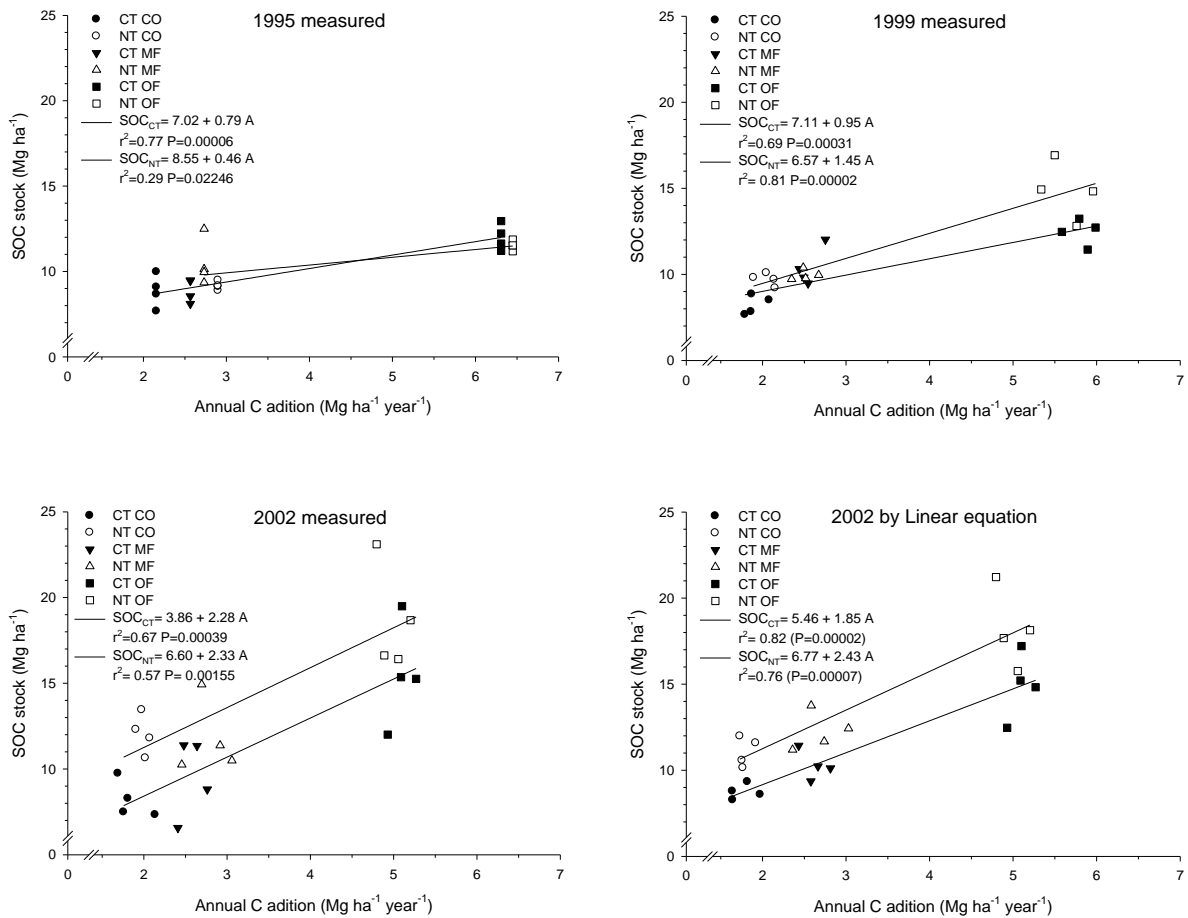


Figure 3.2. Relationship between annual carbon input between 1992 and the considered period and the SOC stocks measured in 1995, 1999, and 2002 and estimated in 2002 by linear equation at the 0-0.05-m soil layer estimated by linear regression as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment.

The  $k_1$  and  $k_2$  coefficients estimated by the two different mathematical approaches is presented in the Table 3.2. There was large variability of the adjusted  $k_1$  and  $k_2$  coefficients when they were estimated using the actual measurements of the SOC stocks in 1995, 1999, and 2002. The estimates were affected by the variability of C inputs among years, since the experiment was rain-feed, the spatial soil variability, and eventual SOC determination methodological errors among samplings. The high variability of the adjusted coefficients by this approach is also noticed in the literature, where Bayer et al. (2006) and Vieira et al. (in press) estimated a  $k_1$  of 0.146 and 0.096 year<sup>-1</sup> and a  $k_2$  of 0.019 and 0.012 year<sup>-1</sup> for the soil under NT in the 13<sup>a</sup> and 19<sup>a</sup> year of conduction of the same experiment, respectively.

Table 3.2. Humification coefficient ( $k_1$ ), mineralization coefficient ( $k_2$ ) as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment determinate in 1995, 1999 and 2002 with actual SOC measurements and with the 2002 SOC stocks estimated by linear equation.

Year	CT			NT		
	CO	MF	OF	CO	MF	OF
----- year <sup>-1</sup> -----						
$k_1$						
1992	0.298	0.307	0.315	0.157	0.160	0.160
1999	0.153	0.158	0.161	0.244	0.249	0.249
2002	0.339	0.349	0.356	0.273	0.278	0.278
Mean	0.263	0.271	0.277	0.225	0.229	0.229
Linear Eqn.	0.232	0.236	0.249	0.255	0.258	0.259
$k_2$						
Mean	CO 0.244 b		MF 0.247 ab		OF 0.254 a	
Mean	CT 0.239 b			NT 0.257 a		
$k_1$						
1992	0.086	0.108	0.124	0.023	0.036	0.035
1999	0.035	0.044	0.051	0.047	0.053	0.053
2002	0.086	0.092	0.097	0.033	0.037	0.036
Mean	0.069	0.081	0.091	0.034	0.042	0.041
Linear Eqn.	0.047	0.050	0.062	0.026	0.029	0.029
Mean	CO 0.036 b		MF 0.039 ab		OF 0.046 a	
Mean	CT 0.053 a			NT 0.028 b		

The  $k_1$  and  $k_2$  coefficients estimated with the actual measured SOC stocks also had uncommon values, out of the range of the coefficients determinate by isotopic techniques. The  $k_1$  determined with the measured SOC stocks ranged from 0.157 to 0.278 year<sup>-1</sup> for the NT treatments, and from 0.153 to 0.356 year<sup>-1</sup> for the CT treatments. In a review of the humification coefficient of the C added by corn residues to the soil, Bolinder et al. (1999) report  $k_1$  coefficients ranging from 0.077 (shoots) to 0.30 (roots) year<sup>-1</sup> for the 0-0.30-m soil layer. When the corn shoot and roots were evaluated together, the  $k_1$  ranged from 0.163 to 0.23 year<sup>-1</sup>. Allmaras et al (2004) reported a humification coefficient of the corn residues of 0.26 and 0.11 year<sup>-1</sup> for NT and CT, respectively. The  $k_1$  determined with the SOC stocks estimated by linear equation averaged 0.239 and 0.257 year<sup>-1</sup> for the 0-0.05-m soil layer under CT and NT, respectively. The more superficial soil layer evaluated in this study could

promote higher  $k_1$  coefficients, since this layer is directly affected by close contact with the aboveground C inputs.

In the same way, the  $k_2$  coefficient showed a wide variability when determined with the measured SOC stocks. For the CT treatments, the  $k_2$  values ranged from 0.035 to 0.124 year<sup>-1</sup>, and for the NT treatments, from 0.023 to 0.053 year<sup>-1</sup>. This wide variation is unlikely to happen among years, and could be overestimating the mineralization rate of the SOC. Even the option to use the average of the  $k_1$  and  $k_2$  coefficients estimated in different years with the measured SOC stocks seems to be not the best alternative, since the out-of-range values results in equally uncommon averages. The average  $k_2$  coefficient determined with the SOC estimated by linear equation verified in the NT and CT treatments (0.028 and 0.053 year<sup>-1</sup>) is similar to the 0.022 year<sup>-1</sup> coefficient for NT, and superior to the 0.030 year<sup>-1</sup> coefficient for CT observed by Huggins et al. (2007) using isotopic techniques. The CT increased in about two-fold the SOC mineralization rates, confirming the effect of the NT into stabilize the SOC into soil aggregates (Nicoloso, chapter 2).

To assess the adjustment of the mathematical model and the coherence of the determined  $k_1$  and  $k_2$  coefficients, the SOC stocks predicted by the one-compartmental model were compared to those SOC actually measured over the experimental period (1992 to 2007 for the CO and MF treatments and 1992 to 2002 for the OF treatments under CT and NT) (Figure 3.3). All predicted SOC stocks showed correlation with the measured SOC stocks, as noticed by the significance of the  $r^2$  determination coefficient of the fitted linear equation among parameters. However, the lower angular coefficient of the linear equations of the SOC predicted with the  $k_1$  and  $k_2$  coefficients determined in 1995, 1999 and by the average of the  $k_1$  and  $k_2$  coefficients determined in 1995, 1999 and 2002, shows that the models adjusted with those coefficients were underestimating the SOC stocks and therefore they were not the best option to simulated the SOC dynamics.

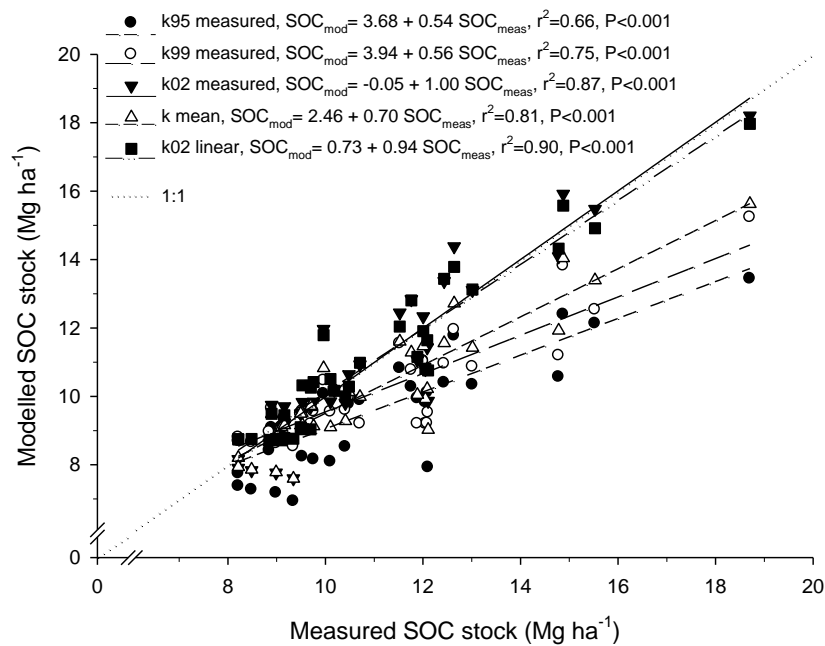


Figure 3.3. Relationship between SOC stocks measured and estimated by the one-compartmental model adjusted with the  $k_1$  and  $k_2$  coefficients in 1995 (k95), 1999 (k99), 2002 (k02), the mean of coefficients adjusted in 1992, 1999, and 2002 (k mean) and in 2002 by linear equation (k linear), in the 0-0.05-m soil layer.

On the other hand, the SOC stocks predicted with the  $k_1$  and  $k_2$  coefficients adjusted with the SOC stocks measured in 2002 and estimated by linear equation presented high coefficient of determination ( $r^2 = 0.87$  and  $r^2 = 0.90$ , respectively) and angular coefficients approaching 1.0, what indicates that the adjusted models are estimating correctly the SOC stocks. However, further assessment of the adjustment of the mathematical model was performed using the LOFIT and RMSE statistical analysis (Table 3.3).

Table 3.3. Statistical analysis of lack-of-fit (LOFIT) and root mean square error (RMSE) of the predicted and measured SOC stocks by different methodologies.

Treatment†	n‡	LOFIT (P>F) §	RMSE¶	RMSE 95%
<i>k<sub>1</sub> and k<sub>2</sub> estimated with 1995 data</i>				
CT CO	7	0.04796	7.76	16.20
CT MF	7	0.00003	21.17	20.69
CT OF	4	0.44139	6.50	32.37
NT CO	7	0.46783	6.02	18.49
NT MF	7	0.00102	17.70	20.84
NT OF	4	0.03269	12.61	34.55
<i>k<sub>1</sub> and k<sub>2</sub> estimated with 1999 data</i>				
CT CO	7	0.39071	5.16	16.20
CT MF	7	0.04301	11.26	20.69
CT OF	4	0.18689	8.77	32.37
NT CO	7	0.00118	14.10	18.49
NT MF	7	0.00147	17.16	20.84
NT OF	4	0.23126	8.27	34.55
<i>k<sub>1</sub> and k<sub>2</sub> estimated with 2002 data</i>				
CT CO	7	0.02789	8.32	16.20
CT MF	7	0.10317	9.90	20.69
CT OF	4	0.28240	7.75	32.37
NT CO	7	0.51069	5.82	18.49
NT MF	7	0.21630	9.26	20.84
NT OF	4	0.60899	5.41	34.55
<i>Mean k<sub>1</sub> and k<sub>2</sub></i>				
CT CO	7	0.02418	8.46	16.20
CT MF	7	0.00630	13.99	20.69
CT OF	4	0.18304	8.82	32.37
NT CO	7	0.04993	9.57	18.49
NT MF	7	0.12148	13.35	20.84
NT OF	4	0.05069	11.69	34.55
<i>k<sub>1</sub> and k<sub>2</sub> by linear equation</i>				
CT CO	7	0.50312	4.72	12.96
CT MF	7	0.37122	7.51	16.55
CT OF	4	0.54595	5.80	16.14
NT CO	7	0.47032	6.01	14.79
NT MF	7	0.40418	7.85	16.67
NT OF	4	0.76133	4.42	17.22

† CT=conventional tillage, NT=no-tillage, CO=control without N, MF=mineral N fertilizer, OF=organic N fertilizer. ‡ Number of sampling years used in the statistical analysis. § P value (F-test) of the Lack-of-fit test. P<0.05 indicates that the predicted SOC stocks does not represents the measured SOC stocks. ¶ Values of RMSE < RMSE 95% indicates that the predicted SOC stocks error is smaller than the measured SOC stock error.

The one-compartmental model adjusted with the  $k_1$  and  $k_2$  coefficients determined by the linear equation approach was the only one that passed both LOFIT and RMSE test for all treatments, representing consistently higher P values in the LOFIT (LOFIT P>0.05 indicates

that the predicted SOC stocks are comparable to the measured SOC stocks) test and smaller RMSE than the others models (RMSE smaller than RMSE 95% indicates that the predicted SOC stocks are within the 95 % confidence interval of the measured SOC in all years of comparison). The linear equation approach to estimate the SOC stocks for the determination of the  $k_1$  and  $k_2$  coefficients was an efficient alternative, reducing the error of the predictions of the model. From now on, all data showed was based in the  $k_1$  and  $k_2$  coefficients estimated by the linear equation approach.

### 3.4.3. SOC turnover and C input requirement

The  $k_2$  coefficients were used to estimate the SOC turnover for the 0-0.05-m soil layer by applying the equations 4 and 5 (Table 3.4). The NT showed MRT and  $t_{1/2}$  two-fold higher than CT. At the average of nitrogen fertilizer sources, the NT presented MRT and  $t_{1/2}$  of 37 and 26 years, respectively, while in the CT the MRT and  $t_{1/2}$  were only 19 and 13 years. These results are within the range presented by Six and Jastrow (2002) and similar to those reported by Huggins et al. (2007) for CT and NT systems when the C source was corn residues.

Table 3.4. Mean residency time (MRT) and half-life ( $t_{1/2}$ ) of SOC stocks of 0-0.05 m layer as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment.

Tillage	Nitrogen Source			Mean
	CO	MF	OF	
	----- years -----			
	MRT			
CT	21	20	17	19 b
NT	39	39	36	37 a
Mean	30	30	26	28
	$t_{1/2}$			
CT	15	14	11	13 b
NT	27	27	25	26 a
Mean	21	20	18	20

The slower turnover of the SOC in the NT indicates the higher level of stabilization of the SOC under this tillage system. However, a non-significant tendency of increase of the SOC turnover was notice in the treatment with manure amendment (OF). This tendency could be result of the different quality of the C added by the manure or an indication that the higher



proportion of macroaggregates founded in this treatment (Nicoloso, chapter 2) was contributing to the increase of the SOC turnover rate (Six et al., 2000, Six et al., 2004).

The ratio of the  $k_2$  and  $k_1$  coefficients multiplied by the  $C_0$  (Eqn. 6) were used to estimate the minimum C input required to maintain the original SOC stocks (Table 3.5). The soil under CT required at average twice the C input required under NT to maintain the SOC stocks. The higher  $k_2$  promoted by the manure amendment in the CT OF treatment increased the C input requirement to 2.54 Mg ha<sup>-1</sup> year<sup>-1</sup>, while in the treatments CT CO and CT MF, the C input requirement was 1.76 and 1.93 Mg ha<sup>-1</sup> year<sup>-1</sup>, respectively. On the other hand, there was no difference of C input requirement in the NT treatments, which ranged from 0.90 to 1.04 Mg ha<sup>-1</sup> year<sup>-1</sup>.

Table 3.5. Minimum C input to maintain original SOC stocks ( $A_0$ ) of the 0-0.05 m layer as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment.

Tillage	Nitrogen Source			Mean
	CO	MF	OF	
	----- Mg ha <sup>-1</sup> -----			
CT	1.76 b	1.93 b	2.54 a	2.07
NT	0.90 c	1.01 c	1.04 c	0.98
Mean	1.33	1.47	1.79	1.53

The estimated minimum C input requirement is in accordance with the experimental observations. Thus, the estimated C input requirement for the treatment CT CO was 1.76 Mg ha<sup>-1</sup> year<sup>-1</sup> while the average C input observed in that treatment was 1.6 Mg ha<sup>-1</sup> year<sup>-1</sup> and the SOC stocks were stable over the 15 years. In the treatment CT MF, the estimated C input requirement was 1.93 Mg ha<sup>-1</sup> year<sup>-1</sup>, and the observed average C input was 2.40 Mg ha<sup>-1</sup> year<sup>-1</sup>. The CT MF treatment did not show a significant increase of SOC stocks, but tended to increase 0.12 Mg ha<sup>-1</sup> year<sup>-1</sup> (P=0.11386). In the other treatments the observed C inputs were superior to the minimum C input requirement showing SOC increase rates compatible with the observed C inputs in each tillage system. The estimated minimum C input requirements are comparable to those reported by Johnson et al. (2006) and Havlin and Kissel (1997) for temperate climate conditions.

#### 3.4.4. Predicting soil carbon sequestration rates

Applying the equation 7, is possible to estimate the SOC stocks at the new equilibrium as a function of the annual C input and the ratio of  $k_1$  and  $k_2$  coefficients (Table 3.6). However, this calculation does not take in account the capacity of the soil into accumulate and stabilize the SOC, disregarding the process of SOC saturation within soil aggregates (Gulde et al., 2008; Nicoloso, chapter 2). This way,  $C_e$  could be overestimated.

Table 3.6. SOC stocks of the 0-0.05-m layer at stability (SOCs) restricted or not by aggregation as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment.

Tillage	Nitrogen Source			Mean
	CO	MF	OF	
----- Mg ha <sup>-1</sup> -----				
Cs model only				
CT	8.77 d	12.35 d	31.92 b	17.35
NT	17.63 cd	27.21 c	71.21 a	38.39
Mean	13.20	19.78	51.57	28.18
Cs restricted by aggregate saturation				
CT	8.77	12.35	15.88	12.33 b
NT	17.63	22.73	28.03	22.80 a
Mean	13.20 c	17.54 b	21.96 a	17.56

The predicted SOC stocks at  $C_e$  without the aggregate-induced saturation restriction were 8.77, 12.35, and 31.92 Mg ha<sup>-1</sup> for the treatments CT CO, CT MF and CT OF, respectively, and 17.63, 27.21, and 71.21 Mg ha<sup>-1</sup> for the treatments NT CO, NT MF and NT OF, respectively. However, the SOC stocks in CT and NT showed no further increase after 15.88 and 28.03 Mg ha<sup>-1</sup>, indicating SOC aggregate-induced saturation at this soil layer (Nicoloso, chapter 2). The SOC stocks in the treatments CO and MF in both CT and NT were predicted to reach the new equilibrium under the SOC saturation, while in the treatments CT OF and CT MF the models predictions indicates a new equilibrium with SOC stocks 2.0 and 2.5 times higher than the SOC saturation and 3.1 and 7.8 times higher than the initial SOC stocks, respectively. The predicted SOC stock at  $C_e$  for the treatment NT OF is also 1.7 times superior to the SOC stocks of the nearby grassland (41.78 Mg ha<sup>-1</sup>). These results indicate that to accurately predict the SOC stocks, the model should be restricted by SOC aggregate-induced saturation. The Figure 3.4 presents the evolution of the SOC stocks and soil carbon sequestration rates predicted by the one-compartmental model for a 50 year period. The model was restricted by the SOC stocks of the CT OF and NT OF treatments at saturation.

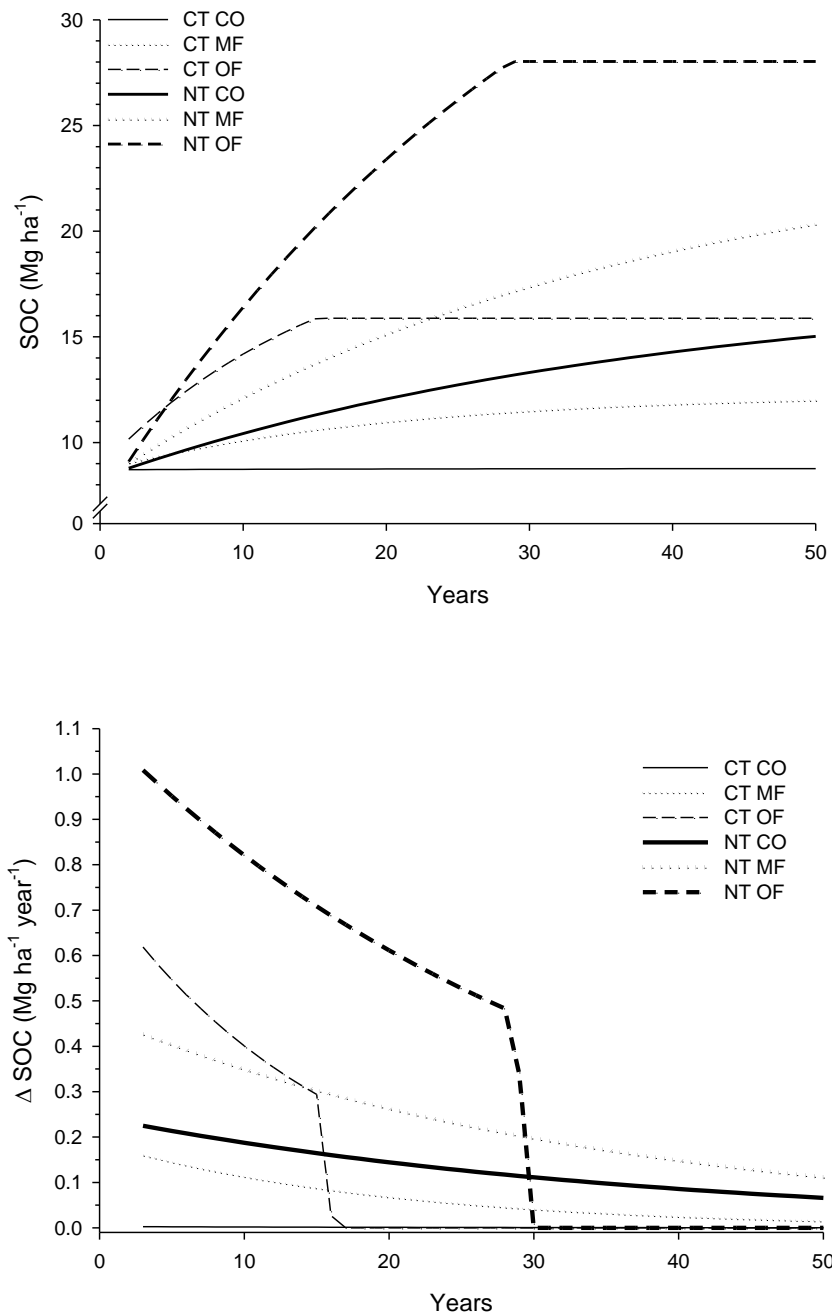


Figure 4. One hundred years simulation of SOC stocks and soil organic carbon sequestration rates by the one-compartmental model.

With fresh cattle manure amendment (OF), the soil under CT and NT was expected to accumulate SOC at larger rates than the treatments where the only source of C was the corn residues, reaching saturation after 15 and 29 years, respectively. After this point, this soil layer was no longer able to accumulate SOC. However, the transfer of SOC among adjacent

soil layers must be considered. This process was noticed in the soil under NT, where after reaching saturation at the 0-0.05-m soil layer, the adjacent 0.05-0.15-m soil layer started to accumulate SOC. The same process was not observed in the soil under CT (Nicoloso, chapter 2).

The predicted SOC dynamic for the treatment NT MF indicates that the soil should keep accumulation SOC at higher rates than the treatment CT MF, reaching after 50 years the SOC stock of 20.34 Mg ha<sup>-1</sup>, about 70% superior to the treatment CT MF (11.99 Mg ha<sup>-1</sup>) but 49% of the SOC stocks of the grassland. For the treatment NT CO and CT CO, the predictions are that their SOC stocks will rise to 15.08 Mg ha<sup>-1</sup> and maintain stable at 8.80 Mg ha<sup>-1</sup>, respectively, after 50 years of management. The SOC stocks of the treatment NT CO will approach the stocks of the treatment CT OF after 50 years and should reach  $C_e$  at a level even superior (17.63 Mg ha<sup>-1</sup>). The Table 3.7 summarizes the soil carbon sequestration rates predicted for the treatments in intervals of 0-10, 10-20, 20-30 and 30-50 years after the adoption of the management practices.

Table 3.7. Average soil carbon sequestration rates at 0-0.05 m soil layer according to management system adoption time as affected by conventional tillage (CT) and no-tillage (NT) and low, medium and high levels of C input.

Tillage	C input	Management system adoption time (years)			
		0-10	10-20	20-30	30-50
----- Mg ha <sup>-1</sup> year <sup>-1</sup> -----					
CT	CO	0.002	0.001	0.001	0.000
	MF	0.135	0.086	0.052	0.025
	OF	0.503	0.170	0.000	0.000
NT	CO	0.216	0.163	0.125	0.086
	MF	0.387	0.299	0.225	0.148
	OF	0.912	0.700	0.463	0.000

The soil carbon sequestration rates decreases with the time of management adoption in all treatments, following the kinetic pattern of the model. The NT OF was able to maintain high soil carbon sequestration rates (0.912 to 0.463 Mg C ha<sup>-1</sup> year<sup>-1</sup>) for 30 years, stabilizing after that when the soil saturates with SOC, with SOC stocks 307 % superior to the initial conditions. The NT CO and NT HF, however, keep accumulation SOC for a longer period, achieving after 50 years 85 and 89 % of the SOC stocks of the new equilibrium, respectively, and SOC stocks 71 and 124 % higher than the initial stocks. In the treatment CT OF the predictions indicates that the soil is able to accumulate SOC for no longer 20 years, presenting

soil carbon sequestration rates of  $0.503 \text{ Mg ha}^{-1} \text{ year}^{-1}$  for the first 10 years and  $0.170 \text{ Mg ha}^{-1} \text{ year}^{-1}$  until reach saturation, with SOC stocks 56 % superior to the initials. The treatment CT MF presents very limited potential to accumulate SOC, with soil carbon sequestration rates no higher than  $0.135 \text{ Mg ha}^{-1} \text{ year}^{-1}$  in the first 10 years of management adoption, and achieving 97 % of the SOC at the new equilibrium after 50 years, with a SOC stocks only 32 % superior of the initials SOC stocks. Considering only the 0-0.05-m soil layer, the utile time span of the NT into accumulate SOC ranged from 30 to 50 years according to the amount of C inputs, showing very discrete soil carbon sequestration rates after that. The NT useful time span was coincident with those proposed by West and Post (2002), Lal (2004), and West and Six (2007). Under even higher C inputs, as verified in the treatment NT OF with composted manure amendment (not used for model simulation) the time necessary for the soil reach saturation could be reduced (Figure 3.1).

### 3.5. Conclusions

This study indicates that the one-compartmental model is a very useful tool to simulate and predict the SOC dynamics, but requires adjustment of the humification and mineralization coefficients to be accurate. The estimation of the SOC stocks by linear equation for the determination of the dynamic coefficients was an efficient alternative to reduce the estimative errors induced by the variability inherent to the measurements of SOC stocks. Also, the predictions of the model should be restricted by the real capacity of the soil to accumulate SOC (aggregate-induced SOC saturation), avoiding overestimation of the potential of the management practices to sequester C.

The soil under NT showed slower turnover rates, demonstrating the higher level of stabilization of the SOC, with lower minimum C input requirement to maintain the original SOC stocks than the CT. Also, the capacity of the soil under NT to act as a carbon sink was superior to the CT, performing higher soil carbon sequestration rates and storage capacity. The model's predictions indicate that the 0-0.05 m soil layer of this Mollisol under NT is able to maintain significant soil carbon sequestration rates for up to 50 years, according to the amount of C inputs.

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# CHAPTER 4

## SOIL ORGANIC CARBON FRACTIONS IN WATER-STABLE AGGREGATES OF MOLLISOL AND OXISOL: LONG-TERM EFFECT OF TILLAGE, NITROGEN SOURCES AND CROP ROTATION

### 4.1. Introduction

The storage of atmospheric carbon in agricultural soil is often considered a limited and reversible strategy of carbon sequestration. The soil's capacity to accumulate soil organic carbon (SOC) is dependent of the amount of C input by crop residues to the soil and the SOC mineralization rate. The increase of aggregation and the inclusion of SOC within soil aggregates reduces its decomposition rate (Oades, 1984) and is considered as the main process of carbon stabilization in no-tillage (Six et al.; 2000, Fabrizzi et al., 2009).

SOC stabilization mechanisms are biochemical recalcitrance, organo-mineral interaction and bio-physical protection (Christensen, 1996), processes mediated by their interactions with soil biota (Six et al., 2002). The importance of each mechanism in SOC stabilization depends of soil and climate characteristics. The bio-physical protection (i.e. aggregation) is the key factor controlling SOC stabilization in soils from temperate climate regions, as the Mollisols, where the increase of SOC is associated to the increase of macroaggregates (soil aggregates >2000- $\mu\text{m}$  diam.) (Fabrizzi et al., 2009).

As the new fresh crop residue is added to soil it appears after a first process of decomposition as a free light particulate organic carbon (fl-POC). This fraction is associated with the aggregate size fractions >53- $\mu\text{m}$  but is not protected by aggregates (Spycher et al., 1983; Golchin et al., 1994, Puget et al., 1995; Six et al., 1998). After further decomposition, the fl-POC is reduced to extra-microaggregate coarse particulate organic carbon (ec-POC) and can be incorporated by the aggregates >250- $\mu\text{m}$ . When occluded within meso- and macroaggregates the ec-POC is less exposed to decomposition and has higher probability to be stabilized into finer SOC fractions (Golchin et al., 1994, Puget et al., 1995). Continuing the decomposition process the ec-POC is reduced to fine particulate organic carbon extra-microaggregate (e-POC) or intra-microaggregate (i-POC). The e-POC is only protected by meso- and macroaggregate while the i-POC is protected by microaggregate occluded or not in larger aggregate size fractions. The SOC pools protected by meso- and macroaggregates are a less labile pool, but easily decomposable if exposed by aggregate disruption (Gulde et al., 2008). The stabilization of these SOC pools is dependent of high aggregate stability and slow

aggregate turnover (Six et al., 1998). The inclusion of i-POC inside of microaggregate reduces even more the availability of this fraction to microbial decomposition. The i-POC protected inside of microaggregates could be considered as long-term physically protected (Skjemstad et al., 1990; Six et al., 2000) since the microaggregates are less affected by tillage, while the e-POC and ec-POC fractions could be exposed to microbial decomposition if the SOC enriched meso- and macroaggregates were disrupted.

Tropical soils dominated by 1:1 clay minerals and aluminum and iron oxides are less dependent of the SOC to build up meso- and macroaggregates and have a larger proportion of large aggregates even under low SOC content than temperate 2:1 soils (Denef et al., 2002; Fabrizzi et al., 2009). In those soils inorganic/chemical binding agents have a major role in macroaggregate formation and stabilization (Six et al., 2002). The increase of SOC stocks results in a substantial increase of macroaggregation in temperate soils, while in tropical soils the response is limited (Denef et al., 2002; Fabrizzi et al., 2009), suggesting that the tropical soils do not follow the classic aggregate hierarchy proposed by Tisdall and Oades (1982). This independence of SOC to build up large aggregates could provide less vulnerability in tropical soils (Fabrizzi et al., 2009) preventing SOC loss following tillage. Tropical soils also have a higher proportion of microaggregates occluded within meso- and macroaggregates than temperate soils increasing the possibility of long-term SOC physical protection (Denef et al., 2004).

With further decomposition, the i-POC and e-POC are reduced to mineral associated carbon (MAC) when the particle size is  $<53\text{-}\mu\text{m}$ . The MAC could be founded extra-microaggregates (e-MAC) and occluded in meso- and macroaggregate, intra-microaggregate (i-MAC) occluded or not in meso- or macroaggregate, or free in the soil. Most of the SOC is associated with the silt and clay fractions as MAC (Six et al., 2000; Bayer et al., 2001). Under temperate and semiarid climatic conditions, the MAC represents about 50 % of the total SOC ((Franzluebbers and Arshad, 1997; Chan, 1997), while in tropical environments the MAC fraction corresponds to 85 to 90 % (Bayer et al., 2001) or 65 to 92 % (Amado et al., 2006) of the total SOC, according to soil type, tillage and crop systems, and sampling depth. This fraction is highly stable in the soil due to its interaction with the silt and clay particles (organo-mineral interaction) (Bayer et al., 2001) or biochemical recalcitrance and has a higher residence time of all SOC fraction in the soil (Anderson & Paul, 1984; Six et al., 2000). The organo-mineral interaction is a particularly important mechanism in aluminum and iron oxides rich tropical soils, where the interaction of MAC and the soil oxides provides great stability to this pool (Bayer et al., 2001; Razafimbelo et al., 2008).

Part of the SOC is also formed of recalcitrant carbon, highly resistant to microbial decomposition due to the complexity of its molecules (biochemical recalcitrance). About 35 to 65% of the total SOC is recalcitrant, with 1300-1800 years older than the average SOC (Leavitt et al., 1996; Paul et al., 1997; Paul et al., 2001). The recalcitrant SOC proportion in the soil generally increases in SOC depleted soils due to its resistance to microbial decomposition (Plante et al., 2006). During the transformation of the fresh C added to the soil to the finer fractions of the SOC pools there is a selective consumption of easily decomposable carbohydrates (i.e. O-alkyl C) and preservation of more complex molecules (i.e. alkyl C) (Golchin et al., 1994). That way, the degree of molecular recalcitrance increases from the fl-POC to the MAC fractions. The recalcitrant MAC (MAC-r) is an even higher stable fraction in the soil due to its double protection through organo-mineral interaction and biochemical recalcitrance (Plante et al., 2006). SOC quality analysis (alkyl/alkyl-O ratio) comparing tropical and temperate soil are often contradictory but indicate a tendency of more recalcitrant SOC forms in the short-term stabilized SOC associated with sand and silt particles in tropical than temperate soils (Six et al., 2002). These results are credited to the faster SOC turnover in tropical soils (Six et al., 2002). However, the SOC associated with clay particles is more recalcitrant in temperate than in tropical soils (Six et al., 2002)

The objective of this study was to assess the different mechanisms of SOC stabilization in a Mollisol from central Kansas (EUA) and an Oxisol from southern Brazil evaluating the SOC fractions associated with soil aggregates affected by tillage systems, nitrogen sources and crop rotation, discussing if the soil carbon sequestration is a permanent or reversible process.

## **4.2. Hypothesis**

The increase of the amount of macroaggregates with NT promote the increase of physical protection and the SOC stabilization with the silt plus clay soil fraction inside the macroaggregates resulting in long-term carbon sequestration.

## **4.3. Material and Methods**

In the Chapter 1, detailed explanation was made about experimental sites location and characteristics (soil, climate, and treatments description), procedures for aboveground carbon input estimates, and SOC content and stocks determination. In this chapter, only the specific methodology used to approach this chapter's topics will be further detailed.

#### 4.3.1. Soil Sampling

Soil samples were collected in January of 2008 for the Oxisol site and in April of 2008 for the Mollisol site.

##### 4.3.1.1. Mollisol

One sample per plot was taken at the 0-0.05 m soil depth manually with a shovel. The samples were store in a cooler with temperature at 4°C. Then the samples were passed through an 8 mm sieve and the aggregates retained on a 4 mm sieve were collected, large roots and plants residues removed, and dried in an oven at 40°C and stored until use.

##### 4.3.1.2. Oxisol

Four samples per plot were taken at the 0-0.05 m soil depth manually with a shovel. The samples were air dried at 30±5°C and stored until use. The dry samples were placed on a plastic tray with a 50 mm sponge absorbed with water and stored at a cooler with temperature at 4°C overnight to wet up the samples to separate the soil aggregates. Te samples were then passed through an 8 mm sieve and the aggregates retained on a 4 mm sieve were collected, large roots and plants residues removed, and dried in an oven at 40°C and stored until use.

#### 4.3.2. Water-stable aggregates

Water-stable aggregates (WSA) were separated using the procedure modified by Gulde et al. (2008) and the apparatus described by Mikha and Rice (2004). Briefly, 80 g of 4-8 mm soil aggregates was placed on the top of a 4 mm sieve mounted in the aggregate-sieving machine. To slake the air-dried soil, about one liter of water was rapidly added until the aggregates were submersed. The aggregates stood submerged for 5 minutes following 2 minutes of wet sieving. The soil that passed through the 4-mm sieve was collected in a bucket and transferred to a 2 mm sieve. The procedure was repeated until five aggregates size classes were collected from each treatment (>4000, 2000-4000, 250-2000, 53-250, and <53 µm diameter). The water stable aggregates were dried, weighed, and a subsample was used to determine sand content of each fraction (Mikha and Rice, 2004). As the fraction 2000-4000 µm resulted in a very small amount of soil for further analysis, the fractions >4000 and 2000-4000 µm where considered the fraction >2000 µm. Macroaggregates were defined as >2000 µm, mesoaggregates 250-2000 µm, microaggregates 250-53 µm, and silt plus clay fraction by <53 µm size fraction. Sub-samples of the isolated fractions were used for TOC analysis as

described previously. Calculations for TOC in different aggregate-size fraction were adjusted for sand-free water stable aggregates.

#### 4.3.2.1. Occluded microaggregates isolation

The microaggregates occluded within meso and macroaggregates were isolated by placing 10-g subsamples on the top of a 250  $\mu\text{m}$  mesh sieve. The samples were slaked with deionized water and manually disrupted until all material  $< 250 \mu\text{m}$  were flushed onto a 53  $\mu\text{m}$  sieve. The material remained on the top of the 250  $\mu\text{m}$  sieve consisted of sand and extra-microaggregate coarse particulate organic matter (ec-POC). The material retained on the top of the 53  $\mu\text{m}$  sieve was wet-sieved to separate the microaggregates from the silt plus clay fraction (extra-microaggregates mineral associated carbon (e-MAC)). All fractions were collected in an aluminum pan and oven dried at 60°C.

#### 4.3.2.2. Free-light fraction and particulate organic matter isolation

About 3 g subsamples of each fraction of the WSA were used for density flotation of free-light fraction (fl-POC) and of the occluded microaggregates for density flotation of extra-microaggregates particulate organic carbon isolation (e-POC). The samples were suspended in 20 mL of sodium polytungstate ( $1.85 \text{ g cm}^{-3}$ ) in a plastic tube and shaken slowly by hand to mix it without breaking up the aggregates. After, they were placed under vacuum of 10 minutes to evacuate the air entrapped within aggregates. Subsequently, the samples were centrifuged for 1 hour at 20°C at approximately  $1250 \times g$ . The suspended material was aspirated onto a pre-weighed Whatman GC/B filter and rinsed with deionized water to remove the sodium polytungstate. The filters with the fl-POC or e-POC fractions were oven dried at 60°C, weighed and grounded for TOC and TN analysis.

#### 4.3.2.3. Microaggregates dispersion and intra-microaggregate organic fractions isolation

About 3 g of microaggregates were dispersed by shaking with 20 mL of  $5 \text{ g L}^{-1}$  sodium hexametaphosphate for 18 h and passed through a 53  $\mu\text{m}$  sieve. The material retained on the sieve was collected, dried and weighed and consists of the intra-microaggregate fine-particulate organic matter fraction (i-POC) plus sand. The material that passed through the sieve was collected in a sieve pan, dried and weighed and consists of the intra-microaggregate silt plus clay fraction (i-MAC).

#### 4.3.2.4. Flowchart

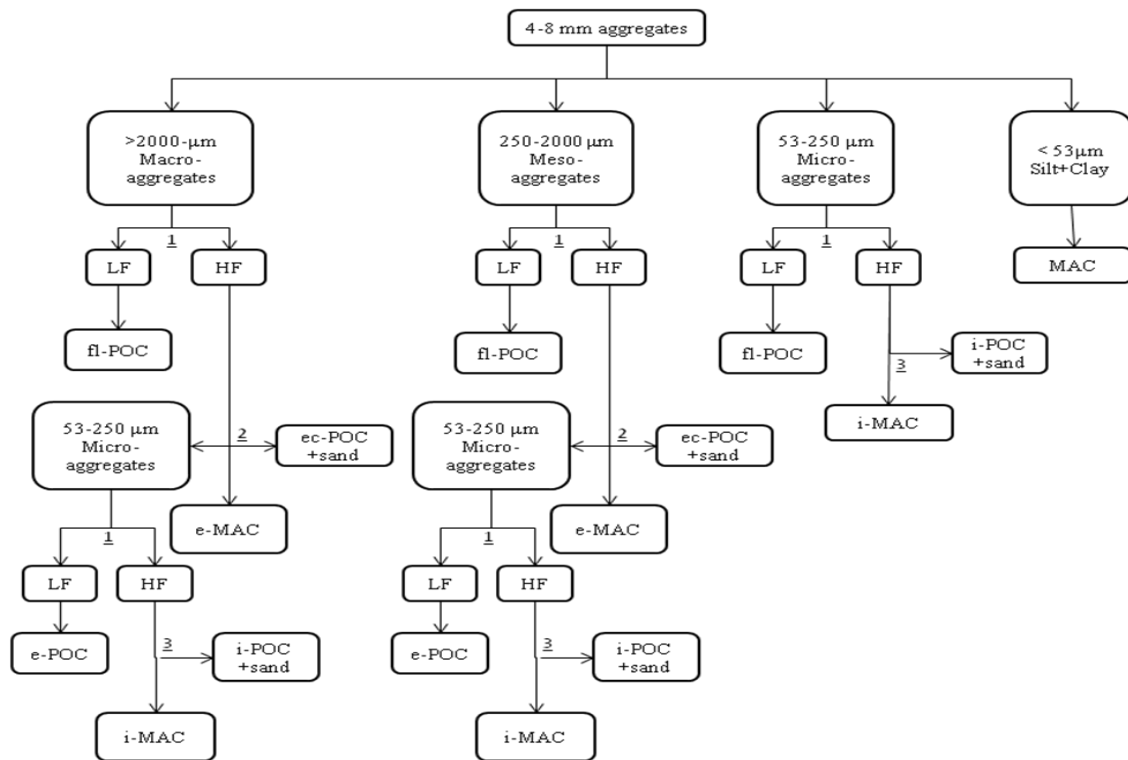


Figure 4.1. Physical fractionation scheme: LF=light fraction, HF=heavy fraction, fl-POC=free light particulate organic carbon, MAC= mineral associated carbon, ec-POC=extra-microaggregates coarse particulate organic carbon, e-MAC=extra-microaggregates mineral associated carbon, e-POC=extra-microaggregates particulate organic carbon, i-POC=intra-microaggregates particulate organic carbon, i-MAC=intra-microaggregates mineral associated carbon. 1=flotation in sodium polytungstate, 2= wet-sieving (250- and 53 µm sieves), 3= wet sieving (53 µm sieve).

#### 4.3.3. Non-hydrolysable Carbon

The MAC, i-MAC and the e-MAC fractions were subjected to acid hydrolysis with a method described by Plante et al. (2006). Briefly, 0.3–0.5 g of sample was refluxed at 95°C for 16 hours in 25 ml of 6 M HCl. After refluxing, the suspension was filtered and washed with deionized water over a glass-fibre filter. The residue was oven-dried at 60°C, and weighed. The hydrolysability of samples is expressed as the percentage of nonhydrolysable C (%NHC), and was calculated using the following equation, which accounts for mass loss of the sample during hydrolysis and incomplete recovery during filtration:

$$\% \text{ NHC} = \text{g C kg}^{-1} \text{ sample}_{\text{after}} \times (\text{mass}_{\text{after}} / \text{mass}_{\text{before}}) / \text{g C kg}^{-1} \text{ sample}_{\text{before}}$$

where the terms represent the sample organic C concentrations and masses before and after acid hydrolysis. The recalcitrant portion of the MACs fractions was identified as MAC-r, e-MAR-r or i-MAC-r.

#### 4.3.4. Total Organic Carbon

The isolated fractions were finely ground using a mortar and pestle. Total organic carbon (TOC) contents were determined by dry combustion using a C/N Elemental Analyzer (Flash EA 1112 Series ThermoFinnigan Italia S.p.A., MI, Italy).

#### 4.3.5. Statistical Analysis

Analysis of variance was performed using SAS PROC MIXED (SAS Institute, 2002) to assess differences between treatments. Results were considered statistically significant at  $P < 0.05$  unless noted otherwise. Means were compared using the Tukey test ( $P < 0.05$ ).

### **4.4. Results**

#### 4.4.1. Mollisol

Aggregate size distribution and its mean geometric diameter in the 0-0.05 m soil layer of the Mollisol is presented in the Table 4.1. The reduction of tillage intensity (NT) and organic fertilization (OF) promoted the increase of the mean geometric diameter of the soil aggregates. In the NT treatments, a larger proportion macroaggregates was formed mainly by reducing the 53-250 and  $<53 \mu\text{m}$  fractions. There were significant effects of both tillage and fertilization treatments over the aggregate size distribution but no interaction between factors. The soil under NT and high C input (NT OF) had more than twice the MGD of the other treatments. Concomitantly, the treatment NT OF increased the proportion of microaggregates occluded within meso- and macroaggregates (Table 4.2). In that treatment the microaggregates corresponded to 39.2 and 36.2% of the weight of the macro- and mesoaggregates, respectively. In the other treatments, the proportion of microaggregates occluded within larger aggregate fractions averaged about 30 and 32 % of the weight of meso- and macroaggregates, respectively. These values are within those verified by Six et al. (2000) in a Pachic Haplustoll at the superficial soil layer where the soil under CT presented 27% of microaggregates occluded within mesoaggregates and the soil under NT presented 47.1% of occluded microaggregates. A larger proportion of microaggregates occluded within larger

aggregate fractions is desirable since the microaggregates are more stable in the soil resulting in long-term SOC protection.

Table 4.1. Sand-free water stable aggregates in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization.

Tillage	N source	Sand-free water-stable aggregates ( $\mu\text{m}$ )				Recovery	MGD
		< 53	53 - 250	250 - 2000	> 2.000		
		----- g 100 g <sup>-1</sup> soil -----				---- % ----	--- $\mu\text{m}$ ---
CT	MF	25.6	28.5	39.1	6.9	97.9	206 b†
	OF	23.4	23.7	34.4	18.4	99.7	274 b
NT	MF	18.8	26.6	31.1	23.5	99.9	305 b
	OF	10.9	13.9	32.2	42.9	99.8	625 a
Mean	MF	22.18 aB	27.52 aB	35.07 aA	15.23 bC	-	256
	OF	17.17 bB	18.94 bB	33.32 aA	30.67 aA	-	450
Mean	CT	24.50 aB	26.10 aB	36.74 aA	12.66 bC	-	216
	NT	14.85 bB	20.26 bB	31.66 bA	33.24 aA	-	439

† Means followed by the same lowercase letter in the same column or the same uppercase letter in the same line are not significantly different by the Tukey test ( $P < 0.05$ ).

Table 4.2. Microaggregates occluded within meso- and macroaggregates in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization.

Tillage	N source	Sand-free water-stable aggregates ( $\mu\text{m}$ )	
		250 - 2000	> 2.000
		----- g 100g <sup>-1</sup> fraction -----	
CT	MF	29.1 b†	32.3 b
	OF	29.2 b	31.6 b
NT	MF	30.8 b	32.3 b
	OF	36.2 a	39.3 a

† Means followed by the same letter in the same column are not significantly different by the Tukey test ( $P < 0.05$ ).

Table 4.3 shows the weight proportion of the fl-POC related to each aggregate size fraction. As the fl-POC is formed from crop and manure residues recently added to the soil, the size of this pool is dependent on a continuous input of fresh residues. Six et al. (1999) observed that the fl-POC fraction is more affected by C inputs than soil disturbance. In that way, the use of manure amendment increased the proportion of fl-POC in the aggregate size fractions, as the manure was rich in fl-POC. The proportions of fl-POC also increase from the microaggregate to meso- and macroaggregate fractions in all treatments. However, in the MF treatments, the higher proportion of fl-POC was found in the macroaggregate fraction, while in the OF treatments, it was found in the mesoaggregate fraction. These results could be



explained, besides the differences of aggregate size distribution among treatments, by the fact that the manure applied in the OF treatments was previously grounded and partially decomposed in the composting process. The effect of manure amendment on fl-POC was previously reported by Gulde et al. (2008) in a Typic Haplustoll from Canada, where the use of 60 Mg ha<sup>-1</sup> year<sup>-1</sup> of manure increased the proportion of fl-POC in the total soil from 1.3 to 6.2 %.

Table 4.3. Weight proportion of fl-POC with aggregate size fractions of the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization.

Tillage	N source	Sand-free water-stable aggregates (µm)			Total soil
		53 - 250	250 - 2000	> 2.000	
fl-POC proportion in soil					
----- % (w/w) -----					
CT	MF	0.29±0.04	0.95±0.17	2.64±0.27	0.64±0.15
	OF	0.63±0.11	2.43±0.73	1.29±0.58	1.22±0.45
NT	MF	0.24±0.03	0.44±0.09	1.27±0.23	0.50±0.11
	OF	1.26±0.18	2.93±0.26	1.60±0.35	1.80±0.16

The value after the ± symbol represents the means' standard error (n=4).

The C content occluded within soil aggregates presented the same pattern of the C content of the full aggregate fraction (fl-POC + occluded-C) (Table 4.3). In the >2000 µm fraction, the reduction of soil disturbance (NT) and increase of C input (OF) increased the proportion of SOC occluded inside macroaggregates. In the 250-200 -µm fraction, the higher weight proportion of fl-POC in the OF treatments (Table 4.2) reduced the percentage of C occluded within aggregates. Of total soil, about 95% of the SOC was occluded within aggregates with no significant differences among treatments, meaning that most of the SOC has some degree of protection by aggregation alone or by aggregation and other stabilization mechanisms (organo-mineral interaction or recalcitrance) and is stable or partially stabilized in the soil.

Table 4.4. Total and fl-POC C content of the aggregate size fraction in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization.

Tillage	N source	Sand-free water-stable aggregates (µm)				Total soil
		< 53	53 - 250	250 - 2000	> 2.000	

		Aggregate fractions C content					
		----- g 100g <sup>-1</sup> fraction -----				g 100g <sup>-1</sup> soil	
CT	MF	1.12 <sup>ns</sup>	1.37 c†	1.89 c	2.03 c	1.55 c	
	OF	1.43	2.38 b	3.10 b	3.57 b	2.62 b	
NT	MF	1.01	1.43 c	2.07 c	2.19 c	1.72 c	
	OF	1.68	3.94 a	4.94 a	5.54 a	4.69 a	
		fl-POC free Aggregate fractions C content					
		----- g 100g <sup>-1</sup> fraction -----				g 100g <sup>-1</sup> soil	
CT	MF	1.12 <sup>ns</sup>	1.32 c	1.79 c	1.81 c	1.48 c	
	OF	1.43	2.28 b	2.86 b	3.40 b	2.48 b	
NT	MF	1.01	1.39 c	2.00 c	2.03 c	1.66 c	
	OF	1.68	3.71 a	4.53 a	5.34 a	4.45 a	
		Occluded					
		----- g 100g <sup>-1</sup> fraction -----				g 100g <sup>-1</sup> soil	
CT	MF	100.0 <sup>ns</sup>	96.3 <sup>ns</sup>	94.7 a	89.6 b	95.5 <sup>ns</sup>	
	OF	100.0	95.8	92.2 ab	95.2 a	94.6	
NT	MF	100.0	97.2	96.6 a	92.7 ab	96.5	
	OF	100.0	94.2	91.7 b	96.4 a	94.9	

† Means followed by the same letter in the same line are not significantly different by the Tukey test (P<0.05).

Figure 4.2 shows the C content of each SOC fractions in the four isolated aggregate size fractions (> 2.000, 250-2000, 53-250, and < 53  $\mu\text{m}$ ). In the macroaggregate fraction, there was no difference among C contents of the fl-POC fraction among treatments. All other fractions were increased by a reduction of tillage intensity (NT) and increasing C input (OF). The same pattern was observed in the other aggregate size fractions where all aggregate size fractions were affected by tillage systems and C input, including the fl-POC in the meso- and microaggregate fractions. The sensibility (proportional change of C content) of the isolates SOC fraction to management (tillage and C input) decreased from the larger aggregate size classes to the smaller ones. The POC fractions (fl-POC, e-POC, ec-POC, and i-POC) were also more sensitive to the treatments than the MAC fractions (e-MAC, i-MAC,

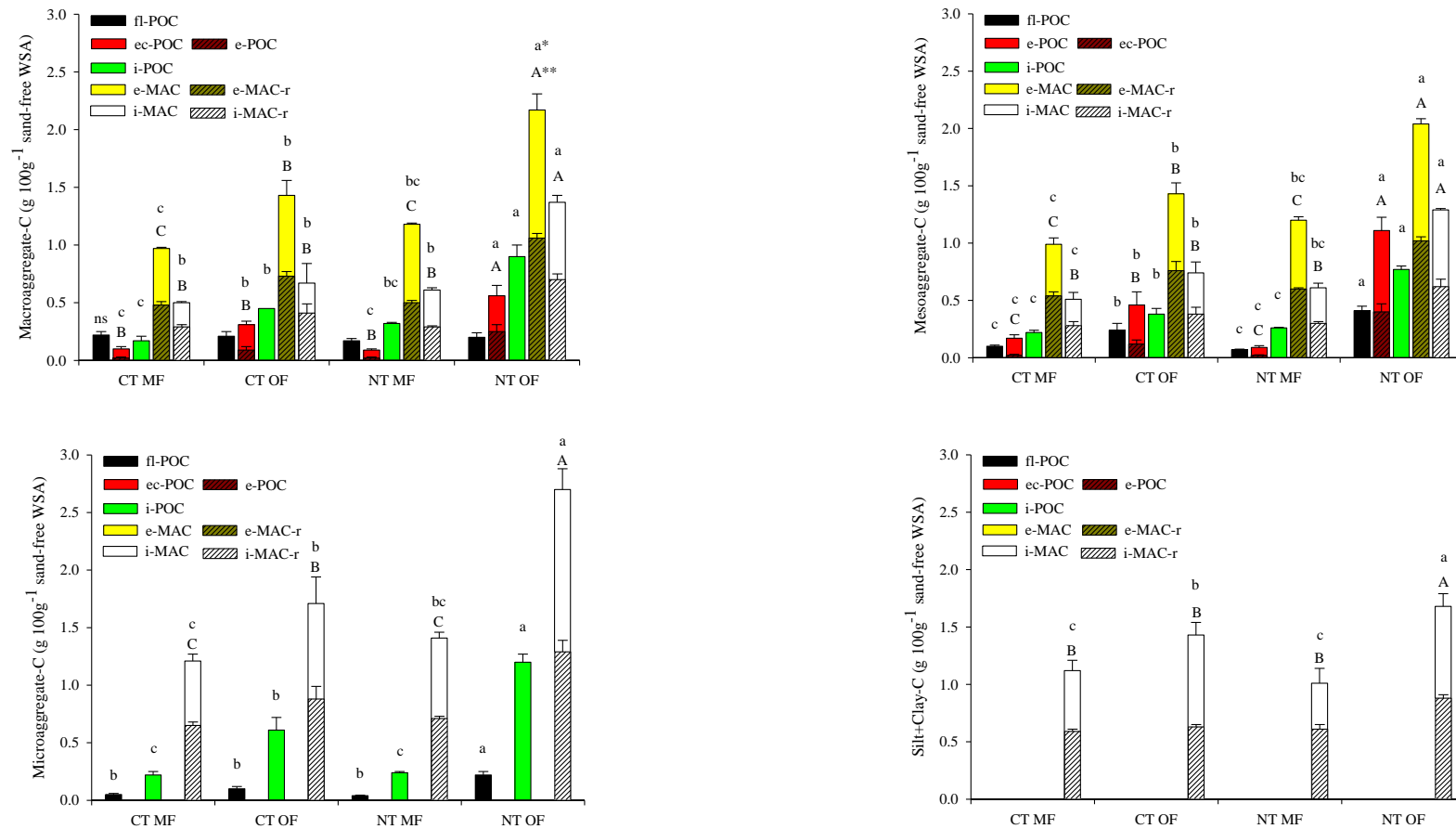


Figure 4.2. SOC fractions content in aggregate size classes (macro-, meso-, micro-aggregates, and silt + clay) as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization. fl-POC= free-light particulate organic carbon, e-POC= extra-microaggregate POC, ec-POC= extra-microaggregate coarse POC, i-POC= intra-microaggregate POC, e-MAC= extra-microaggregate mineral associated carbon, e-MAC-r= recalcitrant e-MAC, i-MAC, intra-microaggregate MAC, i-MAC-r= recalcitrant i-MAC, ns= not significant by the ANOVA analysis ( $P > 0.05$ ), \* Bars under the same lowercase letter in the same SOC fraction are not significantly different by the Tukey test ( $P < 0.05$ ). \*\* Bars under the same uppercase letter in the same SOC recalcitrant fraction are not significantly different by the Tukey test ( $P < 0.05$ ). The vertical line above bars represents the mean's standard error ( $n=4$ ).

and MAC), which are in turn more sensitive than the recalcitrant fractions (e-MAC-r, i-MAC-r, and MAC-r).

In absolute numbers the changes in SOC fractions occurred in the opposite way, from the smaller to the larger aggregate size fractions. Among SOC fractions, the MAC fractions (e-MAC, i-MAC, and MAC) had together with the i-POC fraction the highest absolute increment, followed by the MAC-r fractions (e-MAC-r, i-MAC-r, and MAC-r) and then by the others POC fractions (fl-POC, e-POC and ec-POC). These results are similar to those reported by Bayer et al. (2001) where the POC fractions were more sensitive to management but the major changes in SOC stocks were due to the MAC fractions. The combination of sensitivity and absolute change of the i-POC fraction could be useful to the use of this fraction as a soil quality indicator.

Considering that all aggregate size fractions had the same bulk density of the bulk soil of  $1.18 \text{ Mg m}^{-3}$  (equivalent soil mass basis, bulk density at the beginning of the experiment), table 4.5 shows the SOC fractions stocks. The increase of SOC stocks was result of the increase of SOC fractions protected inside of soil aggregates, rather than by the increase of fl-POC and MAC or MAC-r fractions. Most of the SOC stocks increase was verified in the occluded i/e-MAC or i/e-MAC-r and i-POC fractions, indicating that this SOC accumulation could be very stable in the soil and hardly reversible by the change of soil management or tillage practices. The reductions of SOC stocks within MAC and MAC-r fractions are due mostly be the inclusion of this fractions within other e-MAC or i-MAC fractions, since there was a reduction of the proportion of the silt+clay fraction in the aggregate distribution.

To discriminate the participation of each mechanism of C stabilization in the soil, the SOC fractions were grouped according to the mechanism involved in the stabilization of that fraction (Table 4.5). The fractions fl-POC from all aggregate size fractions were considered unprotected in the soil. The e-POC and ec-POC fractions were protected by aggregation only and since the meso- and macroaggregates have a faster turnover in the soil and are more vulnerable to soil tillage, these fractions were considered as short-term physically protected. The i-POC fraction that was protected by microaggregates (occluded or not within larger aggregates) was considered as long-term physically protected, since those aggregates are less vulnerable to soil tillage and have a slower turnover in the soil (Denef et al., 2004). The MAC fractions found in all aggregate size fractions were considered protected by organo-mineral interaction with the soil matrix while the recalcitrant MAC fractions (MAC-r) was considered biochemically stabilized (Six et al., 2002).

Table 4.5. Distribution of SOC fractions stocks as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization.

Fraction	CT		NT		Mean		Mean	
	MF	OF	MF	OF	MF	OF	CT	NT
	----- Mg ha <sup>-1</sup> -----							
fl-POC	0.41 c†	0.82 b	0.42 c	1.48 a	0.42	1.17	0.64	0.95
ma/me e/ec-POC	0.42 c	1.27 b	0.30 c	3.54 a	0.36	2.40	0.84	1.92
ma/me e-MAC	1.24 c	2.13 b	2.04 b	4.76 a	1.64	3.44	1.68	3.40
ma/me e-MAC-r	1.44 d	2.32 b	1.80 c	4.61 a	1.62	3.47	1.88	3.20
i-POC	0.94 c	2.12 b	1.29 c	4.72 a	1.12	3.42	1.53	3.01
i-MAC	1.55 c	2.16 b	2.11 b	4.10 a	1.83	3.13	1.86	3.11
i-MAC-r	1.86 c	2.46 b	2.07 c	4.02 a	1.97	3.24	2.16	3.05
MAC	0.81	1.10	0.44	0.51	0.62	0.81	0.95 a	0.48 b
MAC-r	0.89	0.87	0.68	0.57	0.78	0.72	0.88 a	0.62 b
Sum	9.56 c	15.25 b	11.15 c	28.31 a	10.35	21.80	12.42	19.73
Unprotected	0.41 c	0.82 b	0.42 c	1.48 a	0.42	1.17	0.64	0.95
Short-term physical protection	0.42 c	1.27 b	0.30 c	3.54 a	0.36	2.40	0.84	1.92
Long-term physical protection	0.94 c	2.12 b	1.29 c	4.72 a	1.12	3.42	1.53	3.01
Organo-mineral stabilization	3.59 d	5.40 b	4.59 c	9.37 a	4.09	7.38	4.49	6.98
Biochemical stabilization	4.19 c	5.66 b	4.55 c	9.20 a	4.37	7.43	4.92	6.87

† Means followed by the same letter in the same line are not significantly different by the Tukey test ( $P < 0.05$ ).

Considering the treatment CT MF as the baseline, the accumulation of SOC due to organo-mineral interaction and stabilization ranged from 30.8 to 62.5% of the total SOC increase. The biochemical stabilization was responsible for 22.5 to 26.7% of the total SOC accumulation. Besides the importance of these two mechanisms to C sequestration and stabilization in the soil, it is important to remind that this process is only possible through aggregation. Deneff et al. (2004) proposed that the increase of macroaggregation results in greater formation of microaggregates occluded within those macroaggregates, and higher concentrations of i-POC and i-MAC. The increase of the e-MAC fractions protected only by macroaggregates could indicate that the macroaggregation also could lead to long-term C sequestration outside the microaggregates by enhancing organo-mineral interaction and biochemical stabilization in that interface.

Table 4.6 shows the soil carbon sequestration rates calculated by the change in SOC fractions stocks and the degree of stabilization of each fraction in the soil, considering the treatment CT MF as a baseline. The increase of SOC stocks in the MACs and i-POC pools was considered as a long-term soil carbon sequestration, since these fractions are stabilized in the soil by biochemical recalcitrance or organo-mineral interaction (MAC) or occlusion inside of microaggregates (i-POC) which are not affected by tillage practices. The soil carbon sequestration was considered management dependent when occurred in the e-POC or ec-POC

fractions, since these SOC fractions are protected only by meso- and macroaggregates, which are feasible to be disrupted by change of tillage system. The short-term soil carbon sequestration was verified by the change of fl-POC stocks, since this fraction is not protected in the soil and in easily decomposable.

Table 4.6. Short-term, management dependent and long-term soil carbon sequestration as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization.

Carbon sequestration	CT OF	NT MF	NT OF
	----- Mg ha <sup>-1</sup> year <sup>-1</sup> -----		
Short-term	0.024	0.001	0.063
Management dependent	0.050	-0.007	0.183
Long-term	0.261	0.100	0.857
Sum	0.335	0.094	1.103
	----- Mg ha <sup>-1</sup> -----		
Short-term	0.407	0.011	1.067
Management dependent	0.846	-0.122	3.119
Long-term	4.444	1.705	14.571
Sum	5.698	1.594	18.757

The long-term soil carbon sequestration accounted for all C sequestration rate verified in the NT MF treatment, since the management dependent fractions had a slight reduction compared to the baseline. In the CT OF and NT OF treatments, the long-term sequestration accounted for 78 % of the total soil carbon sequestration.

#### 4.4.2. Oxisol

Aggregate size distribution and its mean geometric diameter in the 0-0.05-m soil layer of the Oxisol is presented in the table 4.7. Besides the naturally higher MGD of the Oxisol aggregates when compared to the Mollisol, the increase of C input by the R2 treatment promoted the increase of macroaggregates in both CT and NT treatments. The higher proportion of macroaggregates in the R2 treatment in both CT and NT treatments was mostly due to the reduction of the mesoaggregates fractions. No significant effect of tillage system was notice on the aggregate distribution or MGD. This result could indicate that the macroaggregates in this soil are less vulnerable to soil tillage than the macroaggregates from the Mollisol, which had significantly less macroaggregates in CT treatments. This lower vulnerability could be explained by the lower dependency of biotic factors for aggregate

formation in the Oxisol (Six et al., 2004). The similar increase of macroaggregation in the NT and CT treatments under the crop rotation R2 could also indicate that another biologic factor, other than SOC stock and C input, is driving macroaggregate formation in this soil. The more diverse root system present in the treatment R2 (soybean, corn, black-oat, vetch, forage radish, wheat) could be enhancing macroaggregate formation. Bearden and Petersen (2000) concluded that plant roots and fungi hyphae are especially important to formation of aggregates larger than 2.000  $\mu\text{m}$ . Jastrow et al. (1998) also found a direct relationship of fine plant roots and the formation of macroaggregates. There was no significant effect of both tillage system and crop rotation over the proportion of microaggregates occluded within larger aggregates (Table 4.8). The proportion of microaggregates occluded within meso- and macroaggregates averaged about 53 and 55% among treatments. These values are higher than those verified by Deneff et al. (2004) in an Oxisol from southern Brazil (about 40%). However, that author also did not find any differences in the proportion of microaggregates occluded within larger aggregate fraction among tillage systems. In that case, the very stable microaggregates are even less dependent of C input or tillage system for their permanence in the soil.

Table 4.7. Sand-free water stable aggregates in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/radish/wheat.

Tillage	Crop rotation	Sand-free water-stable aggregates ( $\mu\text{m}$ )				Recovery	MGD
		< 53	53 - 250	250 - 2000	> 2.000		
		----- g 100 g <sup>-1</sup> soil -----				---- % ----	--- $\mu\text{m}$ ---
CT	R0	2.3	7.4	41.4	48.9	92.9	1011
	R2	0.8	2.4	12.7	84.2	95.1	1609
NT	R0	2.0	7.1	34.2	56.7	98.5	1089
	R2	0.7	2.2	10.9	86.2	98.4	1651
Mean	R0	2.2 cA†	7.2 cA	37.8 bA	52.8 aB	-	1050 b
	R2	0.7 cA	2.3 cA	11.8 bB	85.2 aA	-	1630 a
Mean	CT	1.6	4.9	27.0	66.5	-	1310
	NT	1.4	4.6	22.6	71.4	-	1370

† Means followed by the same lowercase letter in the same column or the same uppercase letter in the same line are not significantly different by the Tukey test ( $P < 0.05$ ).

Table 4.8. Microaggregates occluded within meso- and macroaggregates in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/ radish/wheat.

Tillage	Crop rotation	Sand-free water-stable aggregates ( $\mu\text{m}$ )	
		250 - 2000	> 2.000
----- g 100g <sup>-1</sup> fraction -----			
CT	R0	56.8 <sup>ns</sup>	54.7 <sup>ns</sup>
	R2	53.1	55.8
NT	R0	53.4	55.7
	R2	52.1	56.7

<sup>ns</sup> Means not significantly different by ANOVA analysis (P<0.05).

The weight proportion of the fl-POC in the Oxisol site is very lower than that verified in the Mollisol site (Table 4.9). Probably the higher temperatures and precipitation in this location promoted higher decomposition rates of the C added to the soil and this fraction is rapidly reduced to smaller SOC fractions. Also, the higher level of macroaggregation of this soil could result that the fl-POC fraction is rapidly incorporated by the soil aggregates. Razafimbelo et al. (2008) also found a very small pool of fl-POC in an Oxisol from Madagascar (0.6 and 1.0 g of fl-POC fraction 100 g<sup>-1</sup> soil in CT and NT treatments, respectively). In the same work, the authors reported that the POC fractions occluded within soil aggregates were surrounded by clay particles. This close interaction of clay particles with the POC fractions could reduce the amount of fl-POC in the samples, since most of the POC could stay attached to the aggregates.

Table 4.9. Weight proportion of fl-POC with aggregate size fractions of the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/ radish/wheat.

Tillage	Crop rotation	Sand-free water-stable aggregates ( $\mu\text{m}$ )			Total soil
		53 - 250	250 - 2000	> 2.000	
free LF proportion in soil					
----- % (w/w) -----					
CT	R0	0.07±0.02	0.24±0.07	0.04±0.07	0.12±0.09
	R2	0.06±0.02	0.22±0.13	0.09±0.08	0.10±0.05
NT	R0	0.05±0.07	0.19±0.05	0.06±0.08	0.10±0.06
	R2	0.19±0.04	0.14±0.03	0.08±0.06	0.08±0.05

The value after the ± symbol represents the means' standard error (n=4).



As a result of the small fl-POC fraction presence in the total soil, about 99% of the SOC in the NT treatments was found occluded within soil aggregates (Table 4.10). The proportion of occluded SOC increases from the micro- to the macroaggregate fractions in both R0 and R2 treatments under NT. In the CT treatments, no significant amount of fl-POC was founded and virtually 100% of the SOC was occluded in soil aggregates. These results indicate that in this Oxisol, almost all SOC has some degree of protection by aggregation alone or by aggregation and other stabilization mechanism (organo-mineral interaction or recalcitrance) and is stable or partially stabilized in the soil.

Table 4.10. Occluded SOC content of the aggregate size fraction in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/radish/wheat.

Tillage	Crop rotation	Sand-free water-stable aggregates ( $\mu\text{m}$ )				Total soil
		< 53	53 - 250	250 - 2000	> 2.000	
Aggregate fractions C content						
----- g 100g <sup>-1</sup> fraction -----						g 100g <sup>-1</sup> soil
CT	R0	2.82	2.72	2.33	2.48	2.44
	R2	2.35	2.78	2.06	2.88	2.77
NT	R0	3.50	2.81	2.48	2.96	2.80
	R2	3.97	3.74	3.12	3.62	3.57
LF free Aggregate fractions carbon content						
----- g 100g <sup>-1</sup> fraction -----						g 100g <sup>-1</sup> soil
CT	R0	2.82	2.72	2.33	2.48	2.44
	R2	2.35	2.78	2.06	2.88	2.77
NT	R0	3.50	2.73	2.44	2.90	2.76
	R2	3.97	3.62	3.06	3.59	3.55
Occluded						
----- g 100g <sup>-1</sup> fraction -----						g 100g <sup>-1</sup> soil
CT	R0	100.0	100.0	100.0	100.0	100.0
	R2	100.0	100.0	100.0	100.0	100.0
NT	R0	100.0	97.1	98.4	98.0	98.6
	R2	100.0	96.8	98.1	99.2	99.4

Figure 4.3 shows the C content of each SOC fractions in the four isolated aggregate size fractions (> 2.000, 250-2000, 53-250, and < 53  $\mu\text{m}$ ) from the Oxisol. In the macroaggregate fraction, the reduction of tillage intensity and increase of C input by crop rotation increased the C content in all SOC pools, with the exception of the e-POC and ec-POC fractions, and the i-MAC-r and e-MAC-r fractions. The fl-POC, i-POC, and i-MAC fractions were higher

in the NT than CT treatments. The e-MAC fraction was higher in the NT R2 treatment, followed by both NT R0 and CT R2 treatments, and lower in the CT R0 treatment. In the mesoaggregate fraction, only the fractions fl-POC, i-POC and e-MAC had differences among treatments. The higher concentrations of these fractions were found in the NT R2 treatment, decreasing to the CT R0 treatment. All other fractions were similar among treatments. In the microaggregate size fraction, the fl-POC and i-POC fractions were higher in the NT than in the CT treatments, while the fraction i-MAC-r had no significant differences among treatments. However, the i-MAC fractions had a higher C content in the NT R2 treatment, followed by NT R0 and CT R0 treatments. The CT R2 treatment had the lowest C concentration in the i-MAC fraction not differing from the CT R0 fraction. This result is due to the inclusion of this fraction within larger aggregate size fractions. In the silt+clay fraction, the MAC and MAC-r fractions had similar patterns to the i-MAC fraction in the microaggregate size fraction. The sensitivity (proportional change of C content) of the isolated SOC fraction to management (tillage and C input) followed a similar pattern to that verified in the Mollisol site, decreasing from the coarser to the finer SOC fractions, but had no clear differences among aggregate size classes. In absolute numbers, the changes in SOC fractions occurred from the smaller to the larger aggregate size fractions, as verified in the Mollisol site. The MAC and MAC-r fractions had the highest absolute changes with treatments, followed by the others e-MAC and i-MAC fractions, and later by the POC fractions, results similar to those reported by Bayer et al. (2001). As verified in the Mollisol site, the i-POC fraction had both sensitivity and significant absolute change of its C content, and could be useful as a soil quality indicator.

Table 4.11 shows the distribution of SOC fractions stocks considering that all aggregate size classes have the same bulk density of the bulk soil of  $1.03 \text{ Mg m}^{-3}$  (equivalent soil mass basis, treatment CT R0 is the reference). Most of the increase of SOC stocks was observed in the e-MAC fractions (recalcitrant or not) occluded within meso- and macroaggregates, where the difference of the treatments NT R2 and CT R0 was 1.49 and 1.04  $\text{Mg C ha}^{-1}$  for the e-MAC and e-MAC-r fractions, respectively. The i-MAC fractions also contributed for C accumulation in the soil, but in a lower proportion than the e-MAC fractions. Contrasting the NT R2 and CT R0 treatments, the differences of SOC stocks of the i-MAC and i-MAC-r fractions were of 0.61 and 0.54  $\text{Mg C ha}^{-1}$ , respectively. The i-POC fraction contributed for C sequestration with 0.60  $\text{Mg C ha}^{-1}$  (difference of NT R2 and CT R0 treatment), while the others POC fraction had limited contribution since its low proportion in the soil.

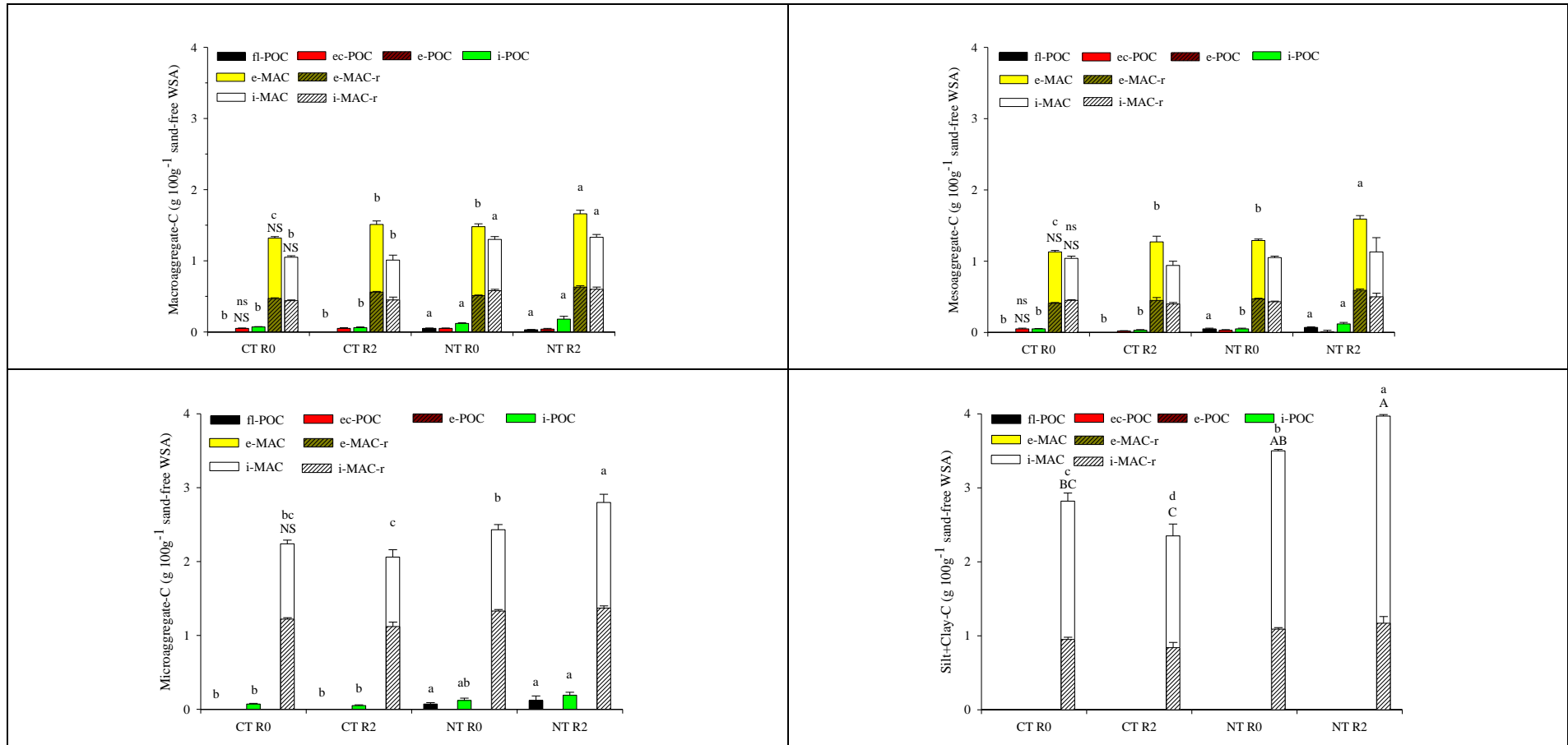


Figure 4.3. SOC fractions content in each aggregate size classes (macro-, meso-, micro-aggregates, and silt + clay) as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/ radish/wheat. fl-POC= free-light particulate organic carbon, e-POC= extra-microaggregate POC, ec-POC= extra-microaggregate coarse POC, i-POC= intra-microaggregate POC, e-MAC= extra-microaggregate mineral associated carbon, e-MAC-r= recalcitrant e-MAC, i-MAC, intra-microaggregate MAC, i-MAC-r= recalcitrant i-MAC, ns= not significant by the ANOVA analysis ( $P>0.05$ ), \* Bars under the same lowercase letter in the same SOC fraction are not significantly different by the Tukey test ( $P<0.05$ ). \*\* Bars under the same uppercase letter in the same SOC recalcitrant fraction are not significantly different by the Tukey test ( $P<0.05$ ). The vertical line above bars represents the mean's standard error ( $n=4$ ).

Table 4.11. Distribution of SOC fractions stocks as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/ radish/wheat.

Fraction	CT		NT		Mean		Mean	
	R0	R2	R0	R2	R0	R2	CT	NT
	----- Mg ha <sup>-1</sup> -----							
If-POC	0.00 c†	0.00 c	0.27 a	0.18 b	0.13	0.09	0.00	0.22
ma/me e/ec-POC	0.22	0.31	0.20	0.19	0.21	0.25	0.27 a	0.19 b
ma/me e-MAC	3.66	4.69	4.29	5.15	3.98 b	4.92 a	4.17 b	4.72 a
ma/me e-MAC-r	2.07	2.70	2.31	3.11	2.19 b	2.91 a	2.39 b	2.71 a
i-POC	0.29	0.26	0.49	0.89	0.39	0.58	0.28 b	0.69 a
i-MAC	3.18 b	2.89 b	3.62 a	3.79 a	3.40	3.34	3.04	3.70
i-MAC-r	2.54	2.37	2.94	3.08	2.74	2.73	2.45 b	3.01 a
MAC	0.22	0.06	0.25	0.10	0.24 a	0.08 b	0.14	0.17
MAC-r	0.11	0.03	0.11	0.04	0.11 a	0.04 b	0.07	0.08
Sum	12.30 d	13.33 c	14.47 b	16.53 a	13.39	14.93	12.82	15.50
Unprotected	0.00 c	0.00 c	0.27 a	0.18 b	0.13	0.09	0.00	0.22
Short-term physical protection	0.22	0.31	0.20	0.19	0.21	0.25	0.27 a	0.19 b
Long-term physical protection	0.29	0.26	0.49	0.89	0.39	0.58	0.28 b	0.69 a
Organo-mineral stabilization	7.07	7.64	8.16	9.04	7.61 b	8.34 a	7.35 b	8.60 a
Biochemical stabilization	4.71 c	5.11 b	5.35 b	6.24 a	5.03	5.67	4.91	5.80

† Means followed by the same lowercase letter in the same line are not significantly different by the Tukey test (P<0.05).

Considering the treatment CT R0 as the baseline, the accumulation of SOC due to organo-mineral interaction and stabilization ranged from 43.7 to 55.3% of the total SOC increase. The biochemical stabilization was responsible in turn by 21.0 to 43.2% of the total SOC accumulation. However, while in the Mollisol, both i-MAC and e-MAC fractions had similar contribution to C accumulation in the soil, in the Oxisol, the e-MAC fraction was more important. These results indicate that in the Oxisol, there is a limited redistribution of C among SOC fractions. In this case, from the e-MAC to the i-MAC fractions. The conceptual model for aggregate formation by Six et al. (2000) demonstrates that as the new microaggregate is formed in the soil it encrusts the i-POC with minerals to form a stable aggregate. In that way, the source for i-MAC accumulation in the microaggregate is the decomposition of the i-POC fractions. However, another possibility for the increase of i-MAC fraction is the inclusion by the microaggregate of the enriched e-MAC pool as new microaggregates form in the soil. This later process does not seem to occur in the Oxisol, since there was no increase of the proportion of microaggregates occluded within larger aggregate fractions. Again, as observed in the Mollisol, the C sequestration and SOC stabilization in the Oxisol is a process of interaction of the three stabilization mechanisms, where the increase of macroaggregation also promotes the increase of the organo-mineral interaction and biochemical stabilization. However, in the Oxisol, the maintenance of larger

aggregates in the soil in even more important, since most of the C sequestered in the soil was protected and stabilized outside the microaggregates.

Table 4.12 shows the soil carbon sequestration rates calculated by the change in SOC fractions stocks and the degree of stabilization of each fraction in the soil, considering the treatment CT R0 as a baseline. The increase of SOC stocks in the MACs and i-POC pools was considered as a long-term soil carbon sequestration, since these fractions are stabilized in the soil by biochemical recalcitrance or organo-mineral interaction (MAC) or occlusion inside of microaggregates (i-POC) which are not affected by tillage practices. The soil carbon sequestration was considered management dependent when occurred in the e-POC or ec-POC fractions, since these SOC fractions are protected only by meso- and macroaggregates, which are feasible to be disrupted by change of tillage system. The short-term soil carbon sequestration was verified by the change of fl-POC stocks, since this fraction is not protected in the soil and is easily decomposable.

Table 4.12. Short-term, management dependent and long-term soil carbon sequestration as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/radish/wheat.

Carbon sequestration	CT R2	NT R0	NT R2
	----- Mg ha <sup>-1</sup> year <sup>-1</sup> -----		
Short-term	0.005	0.012	0.007
Management dependent	-0.002	0.010	0.032
Long-term	0.051	0.091	0.184
Sum	0.047	0.098	0.192
	----- Mg ha <sup>-1</sup> -----		
Short-term	0.089	0.238	0.141
Management dependent	-0.032	0.195	0.596
Long-term	0.969	1.734	3.493
Sum	1.026	2.167	4.230

The long-term soil carbon sequestration accounted for all C sequestration rate verified in the CT R2 treatment, since the management dependent fractions had a slight reduction compared to the baseline. In the NT R0 and NT R2 treatments, the long-term sequestration accounted for about 92 and 95 % of the total soil carbon sequestration.

#### 4.5. Conclusions

The increase of macroaggregation improves the stabilization of the SOC at smaller fractions associated to the silt and clay soil fractions promoting a long-term carbon sequestration in soil. The organo-mineral interaction and biochemical recalcitrance are key mechanisms for carbon stabilization and sequestration in a processes mediated by the biophysical protection (i.e. macroaggregation) in both Mollisol and Oxisol. In the Mollisol, the formation of microaggregates occluded within meso- and macroaggregates permits the stabilization of SOC within microaggregates, while in the Oxisol, the SOC stabilization occurs mostly at the meso- and macroaggregate level.

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## ANNEXES

Table 1. Sand-free water stable aggregates in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment.

Tillage	N source	Sand-free water-stable aggregates ( $\mu\text{m}$ )				Recovery	MGD
		< 53	53 - 250	250 - 2000	> 2.000		
		----- g 100 g <sup>-1</sup> soil -----				---- % ----	--- $\mu\text{m}$ ---
CT	CO	25.5	36.4 a	33.1	5.0	98.8	169
	MF	25.6	28.5 a	39.1	6.9	97.9	206
	OF	23.4	23.7 b	34.4	18.4	99.7	273
NT	CO	18.1	17.8 b	40.6	23.5	99.4	385
	MF	18.8	26.6 a	31.1	23.5	99.9	305
	OF	10.9	13.9 b	32.2	42.9	99.8	625
Mean	CO	21.8	27.1	36.9	14.2 b	-	277
	MF	22.2	27.5	35.1	15.2 b	-	256
	OF	17.2	18.8	33.3	30.7 a	-	450
Mean	CT	24.8 a	29.5	35.5	10.1 b	-	216
	NT	15.9 b	19.4	34.7	30.0 a	-	439
Grassland		5.4	7.4	32.5	54.7	100.0	327

Table 2. Soil organic carbon content within sand-free water stable aggregates in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment.

Tillage	N source	Sand-free water-stable aggregates ( $\mu\text{m}$ )			
		< 53	53 - 250	250 - 2000	> 2.000
		----- g 100 g <sup>-1</sup> sand free fractin <sup>-1</sup> -----			
CT	CO	0.85	1.27 c	1.88 c	1.82 c
	MF	1.12	1.37 c	1.89 c	2.03 c
	OF	1.43	2.38 b	3.10 b	3.57 b
NT	CO	0.86	1.69 c	2.05 c	2.37 c
	MF	1.01	1.43 c	2.07 c	2.19 c
	OF	1.68	3.94 a	4.94 a	5.54 a
Mean	CO	0.86 b	1.48	1.97	2.10
	MF	1.06 b	1.40	1.98	2.11
	OF	1.55 a	3.16	4.02	4.56
Mean	CT	1.13	1.68	2.29	2.47
	NT	1.18	2.35	3.02	3.37
Grassland		2.25	4.13	4.75	5.37

Table 3. Soil organic carbon stocks within sand-free water stable aggregates in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and mineral (MF) or organic (OF) fertilization and a control (CO) without nitrogen amendment.

Tillage	N source	Sand-free water-stable aggregates ( $\mu\text{m}$ )				Agg/bulk <sup>1</sup>
		< 53	53 - 250	250 - 2000	> 2.000	
		----- Mg ha <sup>-1</sup> -----				---- % ----
CT	CO	1.29	2.73	3.65 c	0.50 c	88.0
	MF	1.72	2.31	4.33 c	0.79 c	77.0
	OF	1.93	3.28	6.38 b	3.96 b	99.3
NT	CO	0.89	1.74	4.92 c	3.33 b	90.1
	MF	1.13	2.23	3.80 c	3.04 b	70.2
	OF	1.06	3.25	9.39 a	14.05 a	102.2
Mean	CO	1.09	2.23	4.29	1.92	89.0 a
	MF	1.43	2.27	4.07	1.92	73.6 b
	OF	1.49	3.26	7.88	9.01	100.7 a
Mean	CT	1.65	2.77	4.79	1.75	88.1
	NT	1.03	2.40	6.04	6.81	87.5
Grassland		0.70	1.88	9.05	17.38	70.5

<sup>1</sup>Proportion of the SOC stocks in the bulk soil recovered within the aggregate size fractions.

Table 4. Sand-free water stable aggregates in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/radish/wheat.

Tillage	Crop	Sand-free water-stable aggregates ( $\mu\text{m}$ )				Recovery	MGD
		< 53	53 - 250	250 - 2000	> 2.000		
		----- g 100 g <sup>-1</sup> soil -----				---- % ----	--- $\mu\text{m}$ ---
CT	R0	2.3	7.4	41.4	48.9	92.9	1011
	R1	1.7	5.2	33.4	59.7	93.5	1184
	R2	0.8	2.4	12.7	84.2	95.1	1609
NT	R0	2.0	7.1	34.2	56.7	98.5	1089
	R1	0.9	2.9	16.7	79.6	97.6	1525
	R2	0.7	2.2	10.9	86.2	98.4	1651
Mean	R0	2.2	7.2	37.8 a	52.8 c	-	1050
	R1	1.2	4.1	25.0 b	69.7 b	-	1355
	R2	0.7	2.3	11.8 c	85.2 a	-	1630
Mean	CT	1.6	5.0	29.2 a	64.2 b	-	1268
	NT	1.2	4.0	20.6 b	74.1 a	-	1421

Table 5. Soil organic carbon content within sand-free water stable aggregates in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/ radish/wheat.

Tillage	Crop	Sand-free water-stable aggregates ( $\mu\text{m}$ )			
		< 53	53 - 250	250 - 2000	> 2.000
----- g 100 g <sup>-1</sup> sand free fraction <sup>-1</sup> -----					
CT	R0	2.82	2.72	2.33	2.48
	R1	2.92	2.81	2.29	3.06
	R2	2.35	2.78	2.06	2.88
NT	R0	3.50	2.81	2.48	2.96
	R1	3.75	3.33	2.64	3.42
	R2	3.97	3.74	3.12	3.62
Mean	R0	3.16 b	2.76	2.40 b	2.72
	R1	3.33 a	3.07	2.47 b	3.24
	R2	3.16 b	3.26	2.59 a	3.25
Mean	CT	2.70 b	2.77 b	2.23	2.80 b
	NT	3.74 a	3.29 a	2.75	3.34 a

Table 6. Soil organic carbon stocks within sand-free water stable aggregates in the 0-0.05-m soil layer as affected by conventional tillage (CT) and no-tillage (NT) and by crop rotations (R0, R1, and R2). R0: soybean/wheat; R1: soybean/wheat/soybean/oat; R2: soybean/oat/soybean/oat+vetch/corn/ radish/wheat.

Tillage	Crop	Sand-free water-stable aggregates ( $\mu\text{m}$ )				Agg/bulk <sup>1</sup>
		< 53	53 - 250	250 - 2000	> 2.000	
----- Mg ha <sup>-1</sup> -----						
CT	R0	0.34	1.04	4.99	6.23	119.4
	R1	0.25	0.74	3.98	9.41	136.0
	R2	0.09	0.33	1.33	12.52	136.6
NT	R0	0.36	1.02	4.37	8.65	112.4
	R1	0.16	0.48	2.21	14.10	118.4
	R2	0.15	0.42	1.77	16.09	115.5
Mean	R0	0.35	1.03 a	4.68 a	7.44 c	115.9 b
	R1	0.20	0.61 b	3.10 b	11.76 b	127.2 a
	R2	0.12	0.37 b	1.55 c	14.30 a	126.0 ab
Mean	CT	0.23	0.70	3.43 a	9.39 b	130.7 a
	NT	0.22	0.64	2.78 b	12.95 a	115.5 b

<sup>1</sup>Proportion of the SOC stocks in the bulk soil recovered within the aggregate size fractions.