MICROBIAL ECOLOGY AND C AND N DYNAMICS IN AGROECOSYSTEMS

by

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

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Manhattan, Kansas

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Abstract

Soil C sequestration in agricultural ecosystems is an immediate and significant option to mitigate the increase in the atmospheric CO_2 concentration. The objectives of this study were to determine 1) the influence of crop and soil management practices applicable to Kansas (i.e., tillage, N fertilization, and crop rotations) on soil C and N, C sequestration rates, soil aggregation and aggregate-associated C and N; and 2) the influence of long-term tillage practices on SOC and total N, soil aggregation and aggregate-associated C and N in three soil types: an Oxisol (Brazil), a Vertisol (Argentina), and a Mollisol (Kansas, USA). The Kansas experiments included: tillage (conventional tillage (CT), reduced tillage (RT) and no-tillage (NT)) and native prairie; nitrogen (N) fertilization rates; and crop rotations comprising various combinations of winter wheat (*Triticum aestivum L.*), grain sorghum (*Sorghum bicolor L. Moench*), and soybean (*Glycine max L. Merrill*). The presence of a fallow period negatively affected C sequestration rates even under NT systems. Nitrogen fertilization generally increased C sequestration rates. Rotations that contained wheat or sorghum had the greatest C sequestration rates while continuous soybean had the lowest rates. Cultivation decreased the amount of macroaggregates with a concomitment increased in the amount of microaggregates. Wheat and sorghum increased total C in the macroaggregate fraction (>250 μm) under NT while soybean had the lowest C concentration. Cultivation reduced microbial biomass C and N and potentially mineralizable C and N. The combination of conservation tillage and rotations that

produced a greater amount of residue had greater C sequestration rates. In the Oxisol, NT had greater amounts of large macroaggregates (>2000 μm) than CT, however no differences between tillage practices were detected in the Vertisol and Mollisol. Cultivation of native grassland reduced the amount of macroaggregates and the associated C and N concentration; however NT tended to be more similar to the native grassland. Overall, our results indicated that the use of conservation tillage could be an important strategy to sequester C in these agroecosystems. The adoption of management practices that enhance C sequestration would be important for reducing GHGs emissions and maintaining the sustainability of agricultural systems.

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Table of Contents

List of Figures

List of Tables

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Dedication

To my husband, Guillermo for his love,
continuous support and encouragement
to inspire me to complete my dreams.
To my loved son Juan,
for his patient and continuous love

CHAPTER 1 - GENERAL INTRODUCTION

In the global C cycle, the main C pools include C buried in sedimentary rocks (8 x 10^7 Pg C), active C pools (40 x 10^3 Pg C; atmospheric CO_2, biota, soil organic matter, and oceans), and extractable fossil fuel (4 x 10^3 Pg C) (Schlesinger, 1997; Janzen, 2004). In the active C pools, oceans contain the largest C reserves (38,000 Pg C), 56 times more than the atmospheric pool (750 Pg C) (Schlesinger, 1997). Soils are the largest active C pool in terrestrial ecosystems (Lal and Kimble, 1997; Janzen, 2004); it is estimated that soils contain 1500 Pg C (Schlesinger, 1997) in organic and inorganic forms.

These active pools are connected through fluxes between atmospheric CO_2 and the ocean, and atmospheric CO_2 and land (Schlesinger, 1997). These fluxes have been relatively stable until recent decades, and have now been altered by anthropogenic activities (Janzen, 2004).

The greenhouse gases

The greenhouse effect is a natural process that has made the earth an inhabitable planet. Short-wave radiation coming from the sun arrives at the top of the atmosphere. Some of the energy is reflected and the rest absorbed by the earth's atmosphere and surface. The earth's surface is warmed and reemits energy as longer wavelength radiant energy, which is absorbed by radiatively active trace gases or greenhouse gases (GHGs) which heat the atmosphere. This process warms the atmosphere more than that from light energy alone (CAST, 2004). This warming effect maintains the earth temperature around 15ºC rather than –18ºC without the greenhouse effect (Schlesinger, 1995).

The greenhouse gases include water vapor (H_2O), carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) and can be found in the atmosphere at relatively small concentrations. Their concentrations have changed naturally through time. Nevertheless, in the last decades there has been increasing concern related to an increase in the emissions of the GHGs from anthropogenic activities, thus enhancing climate change and global warming (Lal, 2004). Even though there is uncertainity on the extent of warming and the contribution of anthropogenic sources to GHGs, most scientists agree on the influence of GHGs on global warming in the recent past (IPCC, 2001 cited by CAST, 2004).

The concentration of CO_2 in the atmosphere has increased from 280 μmol CO_2 mol^{-1} in 1850 to current levels greater than 370 μmol CO_2 mol^{-1} (USEPA, 2006). The CH_4 concentration in 2004 was about 1.76 μmol CH_4 mol^{-1}, twice that of the pre-industrial values (0.72 μmol CH_4 mol^{-1}) (USEPA, 2006). Also, the N_2O concentration has increased from 270 to present values of 319 nmol N_2O mol^{-1} (USEPA, 2006). The anthropogenic sources of GHGs include fossil fuel combustion and transport, the chemical industry, and agricultural and land use changes including deforestation, rice production, livestock and fertilizer use (Lal et al., 1998).

Agricultural and Land Use Changes

The agriculture sector worldwide produces about 47 and 84% of the anthropogenic CH_4 and N_2O emissions, respectively, and about 5% of CO_2 emissions (Cole et al., 1997; Rice, 2006). Deforestation, biomass burning, and other land use changes account for an additional 14%. Sources of CH_4 from the agricultural sector include enteric fermentation in ruminant animals, rice cultivation, and biomass burning. Sources of N_2O from agriculture include soils, fertilizers, manures, and biomass burning (Lal et al., 1998; Rice, 2006; Lokupitiya and Paustian, 2006).

The expansion of agriculture has transformed forests, grasslands and wetlands into agroecosystems. Native ecosystems have been cleared and the native perennial vegetation replaced with annual crops resulting in large losses of biomass and soil C stocks. In agroecosystems, CO_2 emissions are related to energy used for production and application of fertilizers, lime, and pesticides, as well as the use of tillage practices that accelerates the oxidation of soil organic matter (SOM) (CAST, 2004). Agricultural management influences soil organic C (SOC) stocks by increasing the rate of decomposition and, often, by changing the quantity, quality, and location of plant inputs (Lal and Kimble, 1997).

Mitigation strategies

The atmospheric concentration of CO_2 can be reduced by 1) decreasing emissions or 2) sequestering C within ecosystems. Carbon sequestration can be defined as the net removal of CO_2 from the atmosphere and its storage in long-lived pools of C, such as terrestrial and geologic systems (Lal, 2004). Several management options to reduce C emissions have been proposed to reduce C sources to the atmosphere, and/or increase C sinks from the atmosphere (Caldeira et al., 2004).

Some of the available near-term options include changes in agricultural management practices, and improved efficiency of appliances, lighting, motors, buildings, industrial processes, and vehicles (Caldeira et al., 2004). Long-term options include C storage in geologic reservoirs or oceans, large-scale development of solar and wind resources, cessation of deforestation, development of energy-efficient transportation systems, development of highly efficient coal technologies, and generation of electricity from biomass (Caldeira et al., 2004).

Throughout this dissertation, we will focus on the processes of C sequestration in terrestrial ecosystems, and specifically to land use and management in agricultural systems.

Carbon sequestration

Some options for C sequestration in agricultural ecosystems include 1) the improved management of permanent agricultural land; 2) conversion and/or restoration of marginal and degraded lands; and 3) use of biofuels for offsetting fossil fuel combustion. There is a diversity of options that can enhance C sequestration such as reduced tillage intensity, use of alternative crop rotations, and fertility and water management (Lal et al., 1998; Paustian et al., 1998). The second option includes reforestation and afforestation, conversion of cropland to pastures or to grassland set-asides (Conservation Reserve Program), restoration of soils affected by salt content or chemical problems, and desertification control (Gupta and Rao 1994; Lal et al., 1999, cited by CAST, 2004).

The adoption of best management practices (BMPs) can increase the sink capacity of croplands by reducing C losses through oxidization, methanogenesis and erosion. Best management practices need to account for differences in soil type, regions, and climate (Lal et al., 1998). Soil organic C in croplands can be increased through management practices that yield greater returns of organic material to the soil, decrease fallow periods, increase use of perennial and winter cover crops, recycle organic wastes, reduce tillage intensity, control erosion, and implement agroforestry practices (Cole et al., 1997; Lal et al., 1998; Paustian et al., 2000; West and Post, 2002; Lal, 2004; Post et al., 2004).

The United Nations Framework Convention on Climate Change (UNFCCC) is concerned with the increase of GHG concentrations in the atmosphere from anthropogenic sources and the resulting impact on the climate. The Kyoto Protocol was created in 1997 to provide obligatory limits on GHG emissions. The Kyoto Protocol allows credits for sinks for a limited list of activities. Those activities related to agricultural soils are treated in Article 3.4 as a possible future activity (Marland et al., 2001). Although the U.S. government has chosen not to participate in the Kyoto Protocol, the current U.S. policy is based on voluntary measures of GHG mitigation which could contribute to any future mandatory emission reduction targets in the U.S. and could contributed to international agreements (CAST, 2004).

Management Practices

Management practices that tend to minimize soil disturbance, maximize the amount of crop residue return to the soil, and improve water and nutrient use efficiency could favor C sequestration in soils. The use of no-tillage or reduced tillage has been widespread around the world, although the reasons for adoption have varied in different countries. In the U.S. the main reasons for the adoption of NT were greater water retention that favors crop intensification in semiarid regions in the Great Plains, and the reduction in soil erosion in more humid regions (Six et al., 2002). However, in South America, especially Argentina and Brazil, the main reasons for adoption of NT were to control erosion, reduce costs from fuel use and labor, and the option for early planting. The first experiences in no-tillage in Argentina and Brazil were in 1974 and 1971, respectively. Recent and projected expansion of cropland under no-tillage in Brazil provides a great opportunity for C sequestration (Cerri et al., 2004). In Argentina, Diaz-Zorita and Buschiazzo (2006) estimated that 22 Mhas of the Argentine Pampas are degraded, representing an opportunity to sequester C with the implementation of best management practices. In general, no-tillage through less disturbance promotes an increase in C stocks under different soil and climate conditions (Sá et al. 2001; Amado et al. 2006; Fabrizzi et al., 2003; Diaz-Zorita, 2002; Cambardella and Elliot, 1994; Six et al., 1999).

Intensification of cropping systems is another way to increase C sequestration through the increase in biomass and a change in residue quality. Elimination of the fallow period, use of high-yielding crop varieties and the adequate use of nutrients can increase organic matter inputs (Post et al., 2004; Lal et al., 1998, Peterson et al., 1998). Several studies have shown an increase in soil C with an intensification of crop rotations and a reduction of bare fallow (Havlin et al., 1990; West and Post, 2002; Sherrod et al., 2003; Peterson et al., 1998; Amado et al., 2006).

Most studies to evaluate the effect of N fertilization on SOC have reported a positive effect of N application on the level of SOC (Rasmussen and Rohde, 1988; Nyborg et al., 1995; Bowman and Halvorson, 1998). However, Halvorson et al. (2002) and Russell et al (2005) found that N application did not always increase SOC.

Carbon Stabilization and Aggregation

Carbon stabilization can be achieved through different mechanisms 1) biochemical recalcitrance, 2) chemical stabilization, and 3) physical protection (Christensen, 1996 cited by Jastrow and Miller, 1997). Biochemical recalcitrance depends on substrate characteristics and is the resistance to degradation of compounds such lignin or melanins produced by fungi or other organisms, or compounds produced by decomposition processes. Chemical stabilization implies the chemical bonding between organic compounds and mineral components. Finally, soil structure plays a key role in the physical protection of the SOM by controlling microbial access to substrate, microbial turnover processes, and food web interactions (Elliot and Coleman, 1988 cited by Jastrow and Miller, 1997).

The type of soil also influences the stabilization of C through relationships to the clay quantity and type. Six et al. (2002) reported lower C stabilization in tropical soils than temperate soils, which is partly attributed to differences in clay type. Oxisols are characterized by 1:1 clays with low specific surface and cation exchange capacity (CEC). These soil characteristics combined with high temperature and precipitation of tropical areas induce rapid decomposition rates and lower C stabilization. Mollisols with a predominance of 2:1 clays with higher CEC, have greater potential for C stabilization (Six et al., 2002). In Vertisols, the formation of clay-organic complexes has been suggested as the main mechanisms for C stabilization (Dalal and Bridge, 1996). Leinweber et al. (1999) found that most of the C was associated with the clay fraction. They concluded that faster decomposition and the shrink-swell pedoturbation influenced SOM composition and distribution in Vertisols.

Aggregation may afford physical protection of organic C. It has been reported that soil organic matter is the primary binding agent for soil aggregates in Mollisols and Alfisols, dominated by 2:1 clays (Six et al., 1999). Even in tropical soils dominated by 1:1 clays and Al and Fe oxides, SOM plays a partial role in aggregation (Six et al., 1999; Denef et al., 2002; Denef and Six, 2005). For Vertisols, studies have shown no correlation between SOC and aggregate stability (Bravo-Garza and Bryan, 2005; Whitbread et al., 1998; Blair and Crocker, 2000). The lack of correlation is explained by the greater importance of clay mineralogy and shrink-swell processes on the formation of soil structure (Bravo-Garza and Bryan, 2005).

Summary of Chapters 2-5

Chapter 2 – Soil Carbon Sequestration in Kansas: Long-Term Effect of Tillage, N Fertilization, and Crop Rotation

Soil C sequestration in agricultural ecosystems is a near-term option to mitigate the increase in the atmospheric CO_2 concentration. There are several management practices to reduce C loss from agricultural soils including reduced tillage intensity, a reduction in bare fallow period, and enhanced rotations. However, the SOC response will vary with soil type and climate. The objectives of this study were to:

1) Determine the influence of different long-term management practices of tillage, N fertilization, and crop rotations on soil C storage, and

2) Estimate the C sequestration rates under these management practices in different locations in Kansas.

Chapter 3 – Long-Term Effect of Crop and Soil Management on Aggregate-Associated C and N

Management practices can affect soil aggregation and thus SOC storage. Thus, the objective of this study was to:

1) Determine the influence of different long-term management practices (tillage, N fertilization, and crop rotations) on soil aggregation and aggregate associated C and N.

Chapter 4 – Soil Organic Carbon and Nitrogen Pools in Agricultural Management Systems

Management practices can influence soil biological activities through their effects on the quantity, structure, and distribution of SOC. The objective of this study was to:

1) Determine the effect of soil management including the interactions tillage, N fertilization and crop rotation on soil C and N pools.

Chapter 5 – Soil Organic Matter and Microbial Ecology of Mollisols, Vertisols and Oxisols: Effect of Native and Agroecosystems

Changes in management practices can influence the dynamics of C in soils affecting the quantity and quality of SOM, soil aggregation, and microbial populations; however, this influence will vary according to soil type and climate. Thus, the objective of this study was to:

1) Evaluate the effect of agricultural and native ecosystems on SOC, aggregation, and microbial community structure in the three soil types.

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CHAPTER 2 - SOIL CARBON SEQUESTRATION IN KANSAS: LONG-TERM EFFECTS OF TILLAGE, N FERTILIZATION, AND CROP ROTATION.

ABSTRACT

Soil C sequestration in agricultural ecosystems is a near-term option to mitigate the increase in the atmospheric CO_2 concentration. Some of the management practices to reduce C loss from agricultural soils include reduced tillage intensity, a reduction in bare fallow period, enhanced crop rotations, and the use of winter cover crops. The objectives of our study was to determine the influence of long-term management practices such as tillage, N fertilization, and crop rotations on soil C content, and to estimate the C sequestration rates of different cropping systems in Kansas. Four long-term experiments covering a range of climate conditions and management systems were sampled for soil organic carbon (SOC). All the sites evaluated (Tribune, Manhattan, Parsons, Hays) included three tillage systems as a variable: conventional tillage (CT), reduced tillage (RT) and no-tillage (NT). Nitrogen (N) fertilization was evaluated in studies at Parsons (0 and 140 kg N ha^{-1}) and at Hays (0, 22.45, and 67 kg N ha^{-1}). Crop rotation effects were studied at Manhattan, including continuous winter wheat (*Triticum aestivum L.*) (W-W), continuous sorghum (*Sorghum bicolor L. Moench*) (S-S), continuous soybean (*Glycine max L. Merrill*) (B-B), wheat-soybean (W-B), and sorghum-soybean (S-B). Soil samples were taken at depths of 0-5, 5-15 and 15-30 cm. Total C contents were determined by dry combustion. Soil organic C contents were significantly greater under NT, compared with CT, at 0-5 cm; differences were less pronounced at deeper depths. At 0-30 cm, SOC tended to be greater under NT than under CT. The presence of a fallow period in the rotations negatively affected C sequestration rates even under NT systems. Nitrogen fertilization increased C sequestration rates. Rotations that had wheat or sorghum had the greatest C sequestration rates, and continuous soybean had the lowest rates. The combination of conservation tillage and crop rotations that produce a greater amount of residue showed greater C sequestration rates.

Keys words: carbon sequestration, tillage, fertilization, crop rotation

INTRODUCTION

Over the past 150 years, an increase in atmospheric CO_2 has been attributed to an increase in fossil fuel combustion, deforestation, and land use change (Lal, 2004). Several strategies have been presented to reduce CO_2 emissions over both near- and long-term. Caldeira et al. (2004) summarized a range of management options to reduce C emissions: those that attempt to reduce C sources to the atmosphere, and those that tend to increase C sinks from the atmosphere. Some of the options available in the near-term include changes in agricultural management practices, and improved efficiency of appliances, lighting, motors, buildings, industrial processes, and vehicles (Calderia et al., 2004). Long-term options include C storage in geologic reservoirs or oceans, large-scale development of solar and wind resources, cessation of deforestation, development of energy-efficient transportation systems, development of highly efficient coal technologies, and generation of electricity from biomass (Caldeira et al., 2004).

Soil C sequestration is a viable short-term option to mitigate increased atmospheric CO_2 because it is relatively low cost and can be rapidly deployed across large areas (Post et al., 2004; Caldeira et al., 2004). Soils can be managed to maintain, restore, and/or enhance SOC content (Johnson, 1995). Enhanced soil C also can improve soil quality, productivity, water infiltration, and fertility, and reduce soil erosion (Halvorson et al., 2000).

Accumulation of soil organic C (SOC) is influenced by several factors, including climate, soil properties, vegetation, time, and management (Johnson, 1995). Soil C is a balance between inputs and outputs. Management practices that reduce C loss from agricultural soils include reduced tillage intensity, a reduction in bare fallow period, and those that enhance inputs of crop residues, such as rotations, winter cover crops, and water management (Lal et al., 1998; Paustian et al., 2000; West and Post, 2002; Lal, 2004; Post et al., 2004).

In the U.S. Great Plains, several years of cultivation under crop-fallow rotation have led to a significant loss of soil C (Peterson et al., 1998). The intent of fallow was to accumulate water, but fallowing results in no crop residue additions, whereas microbial activity and organic matter decomposition continue (Halvorson et al., 2002; Campbell et al., 2005). The introduction of conservation tillage systems such as reduced and no-tillage systems allow better retention of water, and can allow an intensification of the cropping system and reducing the fallow period. As a result, soil C content may increase, as noted by Peterson et al. (1998). Sherrod et al. (2003) also found greater SOC under continuous cropping than in a wheat-fallow system in the central Great Plains. In annual cropping systems in the northern Great Plains, Halvorson et al. (2002) found that soil C increased by 0.23 Mg C ha^{-1} yr^{-1} and 0.025 Mg C ha^{-1} yr^{-1} for no-till and minimum tillage, respectively, but decreased by 0.141 Mg C ha^{-1} yr^{-1} under CT. Conversely, Sainju et al. (2006) reported that tillage and crop rotation did not influence SOC in a 6-yr experiment conducted in the northern Great Plains, which may be due to the time needed to detect changes in SOC.

Adequate N fertilization is needed to ensure optimum productivity and crop residue returns to the soil. Some studies have reported a positive effect of N application on SOC content (Rasmussen and Rohde, 1988; Campbell et al., 1997; Bowman and

Halvorson, 1998; Halvorson et al., 1999), but others have reported little or no effect of N fertilizer on SOC stocks (Halvorson et al., 2002; Russell et al., 2005).

A reduction in tillage can reduce C losses and even increase soil C content. West and Post (2002) analyzed data from numerous long-term studies across the world and reported an average C gain of 0.57 Mg C ha^{-1} yr^{-1} when changing from CT to NT systems and 0.20 Mg C ha^{-1} yr^{-1} by increasing the crop rotation intensity. West and Marland (2002), analyzing 76 long-term experiments in the USA, reported a potential rate of C sequestration of 0.337 Mg C ha^{-1} yr^{-1} for the conversion of CT to NT in agricultural soils.

Evaluation of the changes in SOC due to land use, agricultural practices, and climate will be necessary, and the use of models will play a key role in determining regional estimates of C sequestration under these practices. These estimates are important to policy makers who will use these management practices as strategies to reduce greenhouse gases (GHG) emissions (Falloon et al., 1998). Models have been used to determine the impact of management practices on soil C storage; however, SOC measurements from long-term experiments are needed for model validation. Campbell et al. (2005) compared the rates of change in SOC using the Century model and Campbell et al. (2000) model. Both models effectively simulated the effect of cropping frequency, but the values were lower that those reported from the experiment. The degree of soil C change will vary according to the crop, crop rotation, soil type, and climate (Donigian et al., 1997). Donigian et al. (1997) reported that SOC could increase 10 to 15% for reduced tillage and up to 50% for NT compared with CT, but further model testing and validation was needed.

Long-term studies are needed to validate model estimations of the effects of management practices on C sequestration (Izaurralde et al., 2001). It is necessary to collect information on the amount of C sequestered for a specific soil and duration (Post et al., 2004). Understanding the effect of management practices on biological and edaphic processes will help identify the best management options to offset increased atmospheric CO_2.

The objectives of our study was to determine the influence of different long-term management practices of tillage, N fertilization, and crop rotations on soil C storage, and to estimate the C sequestration rates under these management practices in different locations in Kansas.

MATERIALS AND METHODS

Site description

Four long-term experiments were selected covering a range of climate conditions, soils and management systems in Kansas (Table 2.1).

The Hays experiment is located in central Kansas (38º 51'N, 99º 20'W). This experiment was initiated in 1965 on a Harney silt loam soil (fine, smectitic, mesic Typic Argiustoll). The 30-yr average annual precipitation was 533 mm with an annual mean temperature of 11.9 ºC. The crop rotation was wheat-grain sorghum-fallow, with three tillage systems: conventional tillage (CT), reduced tillage (RT), and no-tillage (NT). Conventional tillage consisted of using tools such as disk, one-way plow, and mulch treader. Reduced tillage included V-blade, sweeps or rod weeder. No-tillage consisted of planting directly into the residue. From the beginning of the experiment to 1975, N rates were 0 and 45 kg N ha^{-1}. Since 1975 four N rates were evaluated, 0 (0-N), 22 (22-

N), 45 (45-N), and 67 (67-N) kg N ha^{-1} yr^{-1}. Nitrogen fertilizer was applied as ammonium nitrate in the previous fall for June sorghum planting and in August prior to September wheat planting. The experimental design was split-plot with four replications, with tillage as the main plot and N as sub-plot.

The Tribune experiment, initiated in 1988, was located in western Kansas (38º 30'N, 101º 41'W). The 30-yr average annual precipitation is 422 mm, with an annual mean temperature of 11.3 ºC. The soil was classified as Richfield silt loam (fine-smectitc, mesic Aridic Argiustolls). The crop rotation was wheat-grain sorghum-fallow, with three tillage systems: CT, RT, and NT. Conventional tillage consisted of three or four operations per year with a sweep plow between crop harvest and planting the next crop. The RT system used a combination of tillage (primarily sweep plow) and herbicides for weed control during fallow. The number of tillage operations with RT was approximately 50% of CT. No-tillage consisted of planting directly into the residue. Nitrogen fertilizer as urea ammonium nitrate (UAN) was broadcast applied at 67 kg N ha^{-1} yr^{-1} to 112 kg N ha^{-1} yr^{-1}, depending on the year or crop. Native sod was included as part of the experimental design, which represented the natural vegetation types of C_3 and C_4 grass, with the dominant species being buffalograss (*Buchloe dactyloides*). The treatments were arranged in a randomized complete block design with four replications.

The Parsons experiment is located in southeastern Kansas (37º 21.02'N, 95º 17.13'W). The 30-yr average annual precipitation is 1016 mm, with an annual mean temperature of 13.7ºC. This experiment was initiated in 1983 on a Parsons silt loam soil (fine, mixed, active, thermic Mollic Albaqualfs). The crop rotation was grain sorghum-soybean, with three tillage systems (conventional tillage, reduced tillage, and no-tillage), and two N rates, 0 (0-N) and 140 kg N ha^{-1} yr^{-1} (140-N). Nitrogen fertilizer was urea-ammonium nitrate (UAN) solution. Conventional tillage included chisel, disk, and field cultivator. Reduced tillage included disk and field cultivator. No-tillage consisted of planting directly into the residue and using chemical weed control. The experimental design was split-plot with four replications, with tillage as the main plot and N as sub-plot.

The Manhattan experiment, initiated in 1974, was located on the Kansas State University Agronomy Farm, Manhattan (Riley County; 39º 07'N, 96º 37'W). Soils were Muir silt loam (fine-silty, mixed, mesic Cumulic Haplustoll) and Reading silt loam (fine, mixed, mesic Typic Argiudoll). The 30-yr average annual precipitation was 813 mm, which was mainly concentrated in the spring-summer period, with an annual mean temperature of 11.3 ºC. Crop rotation and tillage systems were evaluated in this experiment. The experimental design was split-plot with four replications, with rotation as the main plot and tillage as sub-plot. The three crops, soybean (B) *(Glycine max (L.) Merrill)*, grain sorghum (S) *(Sorghum bicolor (L.) Moench)*, and winter wheat (W) *(Triticum aestivum L.),* were combined in five rotations: continuous sorghum (S-S), sorghum-soybean (S-B), continuous soybean (B-B), wheat-soybean (W-B), and continuous soybean (B-B). The three tillage treatments were CT, RT, and NT systems. Conventional tillage included chisel, disk, and field cultivator. Reduced tillage included disk and field cultivator. No-tillage consisted of planting directly into the residue and chemical weed control. A blend of urea and diammonium phosphate fertilizer providing 112 kg N ha^{-1} and 11.3 kg P ha^{-1} was broadcast applied prior to the last tillage operation before planting of each crop and year.

Soil Sampling

Soil samples were taken from each plot at 0- to 5, 5- to 15 and 15- to 30-cm depth increments. Sterile polypropylene bags (3.78 L) were filled with soil collected randomly from each plot using a 2-cm diam. Oakfield soil-probe (Forestry Suppliers, Inc., Jackson, MS). Samples were collected in March 2003 (before planting) on the sorghum rotation phase of the Hays experiment. For Parsons, samples were taken in December 2003 after sorghum harvesting. Tribune samples were collected in April 2004 in each phase of rotation (planted wheat, harvested sorghum, and fallow) and native sod. Soil samples from Manhattan were taken in May 2004, after soybean and sorghum planting and before wheat harvesting. Soil samples were passed through a 4-mm sieve, roots were removed, and samples were stored at 4ºC until use.

Total C

Soil samples were dried and ground to a fine powder with a mortar and pestle. Total C contents were determined by dry combustion using a C/N Elemental Analyzer (Flash EA1112, Carlo Erba Instruments, Milano, Italy).

C sequestration rates: Calculations

Carbon sequestration rates were calculated using two approaches:

1) Baseline data: Rates were determined as the difference between the C values in 2003-2004 and the original values at the beginning of each experiment (37, 16, 20, and 29 yr for Hays, Tribune, Parsons, and Manhattan, respectively).

$$\textbf{C rate (Mg C ha}^{-1}\textbf{ yr}^{-1}\textbf{)} = \textbf{(SOC}_x - \textbf{SOC}_0\textbf{) / years}$$

where

SOC_X= soil organic C content at time x

SOC_0= soil organic C content at initial point

years= number of years under the experiment

2) As a difference between NT or RT with respect to CT treatments in 2003-2004.

$$\textbf{C rate (Mg C ha}^{-1}\textbf{ yr}^{-1}\textbf{) = (SOC}_{RT\ or\ NT} - \textbf{SOC}_{CT}\textbf{)/ years}$$

where

SOC_{RT}= soil organic C content under reduced tillage

SOC_{NT}= soil organic C content under no-tillage.

SOC_{CT}= soil organic C content under conventional tillage.

years= number of years under the experiment

Carbon values at the beginning of the experiment (1965) in Hays were obtained from the sorghum phase. Bulk density values of 1.30 and 1.40 Mg m^{-3} for 0-5 and 5-15 cm were assumed to express the baseline data in mass of C. These values were close to the average bulk density data obtained in 2004.

For Tribune, it was assumed that the native prairie C values obtained in 2004 were the initial values because the experiment was established under native prairie soil in 1988. Bulk density was measured in 2004 to calculate C mass.

For Parsons, C concentrations were obtained in 1983 at the beginning of the experiment. Bulk density was assumed to be 1.35 Mg m^{-3} for 0-15 and 0-30 cm; these values were the average bulk density data obtained in 1983 at 0-5 cm.

For Manhattan, the initial values of 1975 were estimated by considering the C values from 1981 (Peterson, 1983). Soil C was measured 6 yr after implementation of the treatments. We assumed that there was a linear increase in C from 1975 to 1981. The rate of change between 2004 and 1981 (23 yr) for continuous wheat under CT was used to extrapolate estimated C values in 1975. Carbon values used as a reference were from plots under CT in the continuous wheat rotation, which were representative of the traditional management of the area before the experiment was started. Bulk density values used were those reported by Havlin and Kissel (1997), 1.36 Mg m^{-3} for 0-15 cm, and by Budde (2004) 1.46 Mg m^{-3} for 15-30 cm.

Statistical Analysis

Analysis of variance was performed by using SAS PROC MIXED (SAS Institute, 2002) to assess treatment differences on soil C and C sequestration rates. Results were considered statistically significant at $P < 0.05$, except where noted. Means were compared using LSD values.

RESULTS

Soil Organic Carbon

Tillage Effects

Soil organic C for Hays was significantly affected by tillage at the 0-5 and 15-30 cm depths (Table 2.2). No-tillage had greater amounts of SOC than RT and CT at the soil surface, but SOC was greater under CT than under RT or NT at 15-30 cm. No differences between tillage systems were observed when SOC was calculated for 0-15 and 0-30 cm.

At Tribune, SOC was similar between NT and RT, which were significantly greater than CT at 0-5 cm. There were no significant differences between tillage systems at the other depths, although NT had 2.1 Mg C ha^{-1} more C than CT for 0-30 cm (Table 2.2).

At Parsons, tillage significantly affected SOC values at 0-5 cm with no-tillage averaging 3 Mg C ha^{-1} more SOC than RT or CT (Table 2.2). No differences were observed at 5-15, 15-30 cm and 0-30 cm. Although, for 0-15 cm, NT had significantly greater amounts of SOC than RT and CT at *P*=0.08.

At Manhattan, a significant effect of rotation and tillage on SOC was observed at all depths. There was no significant interaction between tillage and rotation. At 0-5 cm, NT resulted in the highest SOC values; however, RT resulted in greater SOC at 5-15 and 15-30 cm, compared with NT and CT (Table 2.2). Soil organic carbon was similar between the NT and RT treatments, which were significantly greater than CT at 0-15 cm and 0-30 cm.

Nitrogen effects

Nitrogen application at Hays significantly affected SOC values for 0-5 cm and 0-15 cm. The higher N rates, 45-N and 67-N, resulted in greater SOC (8 and 8.4 Mg C ha^{-1}, respectively) than the 0-N and 22-N rates (7.3 and 7.4 Mg C ha^{-1}, respectively). For 0-15 cm, N application significantly affected SOC (*P*=0.058) where the 67-N rate (22.9 Mg C ha^{-1}) resulted in similar SOC to that for 47-N (22.2 Mg C ha^{-1}), but was greater than the SOC at the lower N rates (21.4 Mg C ha^{-1}).

At Parsons, there was a significant effect of N application at 0-30 cm where the 140-N rate (39.2 Mg C ha^{-1}) had 1.9 Mg C ha^{-1} more C than the 0-N rate (37.3 Mg C ha^{-1}). Also, differences in SOC between N rates were observed at 0-5 cm (*P*=0.0953) and 0-15 cm (*P*=0.0695), 0.5 Mg C ha^{-1} and 1.3 Mg C ha^{-1}, respectively.

Rotation effects

At Manhattan, continuous wheat resulted in the greatest amount of SOC at 0-5 cm, and continuous soybean resulted in the least SOC (Table 2.3). Continuous sorghum and S-B had similar SOC content, but less than W-W. At 5-15, 0-15 and 0-30 cm, SOC was greater under continuous wheat than under wheat in rotation (W-B). Also, SOC was greater where sorghum was in the rotation (S-S and S-B). Continuous soybean resulted in the lowest SOC (Table 2.3). At 15-30 cm, rotations with wheat had significantly greater SOC than those rotations that had sorghum or continuous soybean.

Carbon sequestration rates

Carbon sequestration rates for 0-15 cm at Hays were positive and significantly greater with NT systems, compared with CT and RT systems, which loss C (Table 2.4). Nitrogen application increased C sequestration rates ($p<0.10$). Across tillage, there was a reduction in the loss of C with the increase in the rate of N application, with no gain or loss in the 67-N treatment (Table 2.4). It should be noted that N application is considered sub-optimal over the course of the experiment as yield potential of the varieties changed. The N response would be expected to be greater under NT.

At Tribune, soil C sequestration rates were negative for all tillage systems at 0-15 and 0-30 cm, indicating a net loss of C from the system. This was not surprising given that this experiment was initiated in native prairie; however, NT lost the least amount of C (Table 2.5).

At Parsons, C sequestration rates were not significantly different among treatments, except at 0-30 cm where the 140-N rate resulted in a greater C sequestration rate (0.32 Mg C ha^{-1} yr^{-1}) than the 0-N rate (0.21 Mg C ha^{-1} yr^{-1}) (Table

2.6). There was an increasing trend for greater C sequestration rate as tillage intensity decreased.

At Manhattan, a significant rotation and tillage effect on C sequestration rates at the 0-15 and 0-30 cm depths. NT had similar rates of C sequestration as RT, but they were significantly greater than those under CT systems (Table 2.7). For 0-15 and 0-30 cm, the rates of C sequestration had the following order: W-W > W-B > S-B ~ S-S > B-B. Soybean monoculture had the lowest and negative C rate, which indicates a significant reduction in the SOC stocks after 29 yr of cultivation (Table 2.3).

Relative differences with respect to CT (Avoided C loss)

At Hays, the differences between NT or RT and CT were not significantly affected by tillage or N application. At 0-15 cm, the negative rates indicate a net C loss in both tillage systems, but this decrease tended to be greater under RT than under NT (Table 2.4). After 37 yr, NT lost 0.78 Mg C ha^{-1} and RT lost 2.04 Mg C ha^{-1} compared with CT.

At Tribune, there were no significant differences among tillage systems, but NT tended to have a greater C sequestration rate compared with RT at both depths (Table 2.5). After 16 yr, NT retained 1.59 Mg C ha^{-1} and RT 0.83 Mg C ha^{-1} compared with CT at 0-15 cm. At 0-30 cm, NT had 2.16 Mg C ha^{-1} and RT had 0.38 Mg C ha^{-1} compared with CT after 16 yr.

Similar to Tribune, there were no significant differences with respect to CT at the Parsons site (Table 2.6). On average, after 20 yr, NT had a positive increase in TOC of 1.76 Mg C ha^{-1}, compared with CT, at 0-15 cm and an increase of 2.37 Mg C ha^{-1} at 0-30 cm. The RT systems resulted in positive values only at 0-30 cm (Table 2.6).

At the Manhattan site, tillage was not significant, but C sequestration rates tended to be greater under NT systems than under RT at both depths (Table 2.7). At 0-30 cm, relative C sequestration rates tended to be greater under S-S, S-B, and B-B rotation (Table 2.7). These results reflect that a change to conservation tillage had a greater impact on C sequestration rates in continuous soybean or sorghum rotations.

DISCUSSION

Our results present differences in response to management practices, according to site location and previous and current management. The impact of tillage was reflected at the soil surface (0-5 cm) where SOC was greater under NT than under CT at all sites evaluated (Table 2.2, Fig. 2.1a). The differences between NT and CT were less at deeper depths (5-15 and 15-30 cm) (Fig 2.1b, c). Several authors have found that the tillage impact is confined to the soil surface (Six et al.,1999; West and Post, 2002; Deen and Kataki, 2003; Fabrizzi et al., 2003; Mikha and Rice, 2004; Wright and Hons, 2004, 2005a,b). Results from our research showed the positive impact that reduced tillage and no-tillage systems have on SOC accumulation. The limited soil disturbance and better aggregation (McVay et al., 2006) under these systems could explain the greater C storage with respect to CT systems.

When all the soil layers were combined (0-15 or 0-30 cm), there were no significant differences in SOC contents between tillage systems, except at the Manhattan site, although in most cases NT tended to result in greater SOC content (Fig. 2.2 and 2.3). At the Manhattan site, NT and RT had similar SOC contents, but values were significantly greater than those of CT at both depths. Previous researchers have reported similar positive gains with no-tillage management (Cambardella and Elliot, 1994; Six et al., 1999; Fabrizzi et al., 2003). However other studies found no increase in SOC contents under NT systems (Angers et al., 1997; Franzluebbers et al., 1999; Needelman et al., 1999; Puget and Lal, 2005; Sainju et al., 2006). Explanations for the lack of NT response for SOC are high initial content of SOC, high clay content, fine-textured and poorly drained soils, less crop residue returns to the soil, and reduced decomposition in cold-wet soils. Time is another factor for SOC (Needelman et al., 1999), however our studies were conducted at long-term sites that had been managed for 16 yr or more.

Crop yields under NT were similar or lower than under CT or RT except at the Tribune and Manhattan site (Table 2.8). The lower yields might explain the lower response in SOC for NT at the Hays and Parsons sites. The increase in SOC seems to be related to less decomposition and greater physical protection of the C under NT systems (Mikha and Rice, 2004) since C inputs were generally the same or less with no-tillage systems.

One concern comparing different tillage systems is redistribution of SOC to shallower depths in NT with greater SOC in CT systems at deeper depths. Our results indicated that there was no redistribution of C among tillage systems. At 0-30 cm NT had a greater, but not significant C mass than CT, which is mainly due to the increase of 2 to 6% C under NT with respect to CT at 0-5 cm. There were no differences in SOC mass at 15-30 cm except at Hays. Our results are in accord with those reported by Frye and Blevins (1997), who found greater SOC under NT systems at 0-30 cm after 20 yr, with most of this C increase observed at 0-5 cm, however they observed greater SOC in NT at 5-15 and 15-30 cm. The authors mentioned that the increase in SOC at depth under NT could be related to inputs from crop roots. Olson et al. (2005) reported greater SOC under NT than under CT at down to 75 cm; all depths evaluated showed an increase of SOC compared with CT.

The negative C sequestration rates under CT and RT at Hays indicate a loss of C from the system, whereas the rate under NT was positive (0.020 Mg C ha^{-1} yr^{-1}). Thompson and Whitney (2000) for the same experiment reported no significant change in SOM after 30 yr, suggesting that the buildup or depletion was very slow because of the rotation and low annual precipitation for this area. The lower rates of C sequestration observed under NT might be explained by the presence of bare fallow in the rotation and the sub-optimal N fertilization (67 kg N ha^{-1}), which probably reduced the potential crop yield, and therefore the amount of residue returned to the soil.

Land use history is important when evaluating the effect of management on soil C (Paustian et al., 1997). At the Tribune site, all tillage systems had a negative C sequestration rate, but the loss of C was less under NT (4%) than under RT (7%) or CT (9%). This experiment was initiated in native prairie sod, thus having a better initial soil C condition than the other experiments, which had been under cultivation before the establishment of the tillage systems. Similar results were reported for a long-term experiment in Kentucky initiated in a native sod, in which the loss of C in the early years was less under no-tillage management (9%) than under conventional tillage (15%) for a continuous corn (*Zea mays*)-winter cover crop rotation (Frye and Blevins, 1997). In the experiment at Kentucky, SOC content after 20 yr was similar for CT, and greater under NT compared with SOC of the native sod. Our results showed that, even with the introduction of NT, after 16 yr it was not possible to reach the same C values present in the native prairie. Other authors also found similar results (Olson et al., 2005; Lyon et al., 1996).

Inclusion of bare fallow in the rotation had a negative effect on the buildup of SOC. The intent of the fallow is to accumulate soil water for plant growth, but during this period, increased soil water supports, microbial activity and decomposition of soil organic matter while no plant material is added to the soil (Halvorson et al., 2002). This scenario results in a net loss of soil C. West and Post (2002) reported no significant increase in SOC for a change from CT to NT under wheat-fallow rotations. In the northern Great Plains, a change from crop-fallow to more intensive systems can have a positive impact on C sequestration and farm profitability (Peterson et al., 1998; Halvorson et al., 2002; Sherrod et al., 2003). Several studies have mentioned that an intensification of the rotation by including more crops are needed to increase and maintain SOC stocks (Russell et al., 2005; Varvel, 2006; Sherrod et al., 2003; Halvorson et al., 2002; Campbell et al., 2005; Machado et al., 2006).

Nitrogen fertilization significantly affected C sequestration rates where the highest rate of N application had the greatest C sequestration rates, which can be attributed to greater amount of residues produced with increased N. These results are in accord with those reported by Nyborg et al. (1995) and Halvorson et al. (1999), however Halvorson et al. (2002) reported that N fertilization had little effect on C sequestration, even when the amount of residue returned increased with N fertilization.

Crop rotations that included wheat or sorghum had the greatest C sequestration rates. Soybean monoculture had a net loss of C from the system. The rotation effect could be related to residue quality. Wheat residues have a higher C/N ratio and lower turnover rates compared with sorghum and soybean residues (Wright and Hons, 2005a). Continuous soybean generally results in less SOC (Studdert and Echeverría, 2000; Wright and Hons, 2004). Carbon sequestration rates under W-B and W-W were similar or greater than those reported by Lal et al. (1998, 1999), which averaged 0.20 Mg C ha^{-1} yr^{-1} for an improvement in crop rotation management, and by West and Post (2002), who also reported an average mean C rate of 0.20 Mg C ha^{-1} yr^{-1}, excluding changes from continuous corn to a corn-soybean rotation.

The Intergovernmental Panel of Climate Change (IPCC) has developed guidelines to determine the National Greenhouse Gas Inventory through the estimation of emissions and sinks of GHG. They have recommended coefficients to estimate soil C stocks by different agricultural land-use and management practices. The IPCC suggests a tillage factor of 1.10 for NT and 1.05 for RT compared to CT, at 0-30 cm, to estimate the potential to sequester C. Our factor was 1.14 for NT, and 1.08 for RT at 0-30 cm (Fig. 2.3). Adopting reduced tillage, results in an increase in SOC about half of that obtained under NT systems. West and Post (2002) found values similar to our results (1.16 for CT to NT).

To determine temporal changes in SOC stocks, Izaurralde et al. (2001) described two alternatives for selecting the control; one considers SOC at time-zero, followed by sampling of SOC at another time, and the second is to measure SOC at the same time between the new practices with respect to the conventional management. Our data reflect the variability in response to management on the calculation of the C sequestration rates (Izaurralde and Rice, 2006); thus, both the baseline and the change in practices should be reported. McGill et al. (1996) concluded that both ways to calculate C were important to monitor soil C sequestration for determining soil C sinks.

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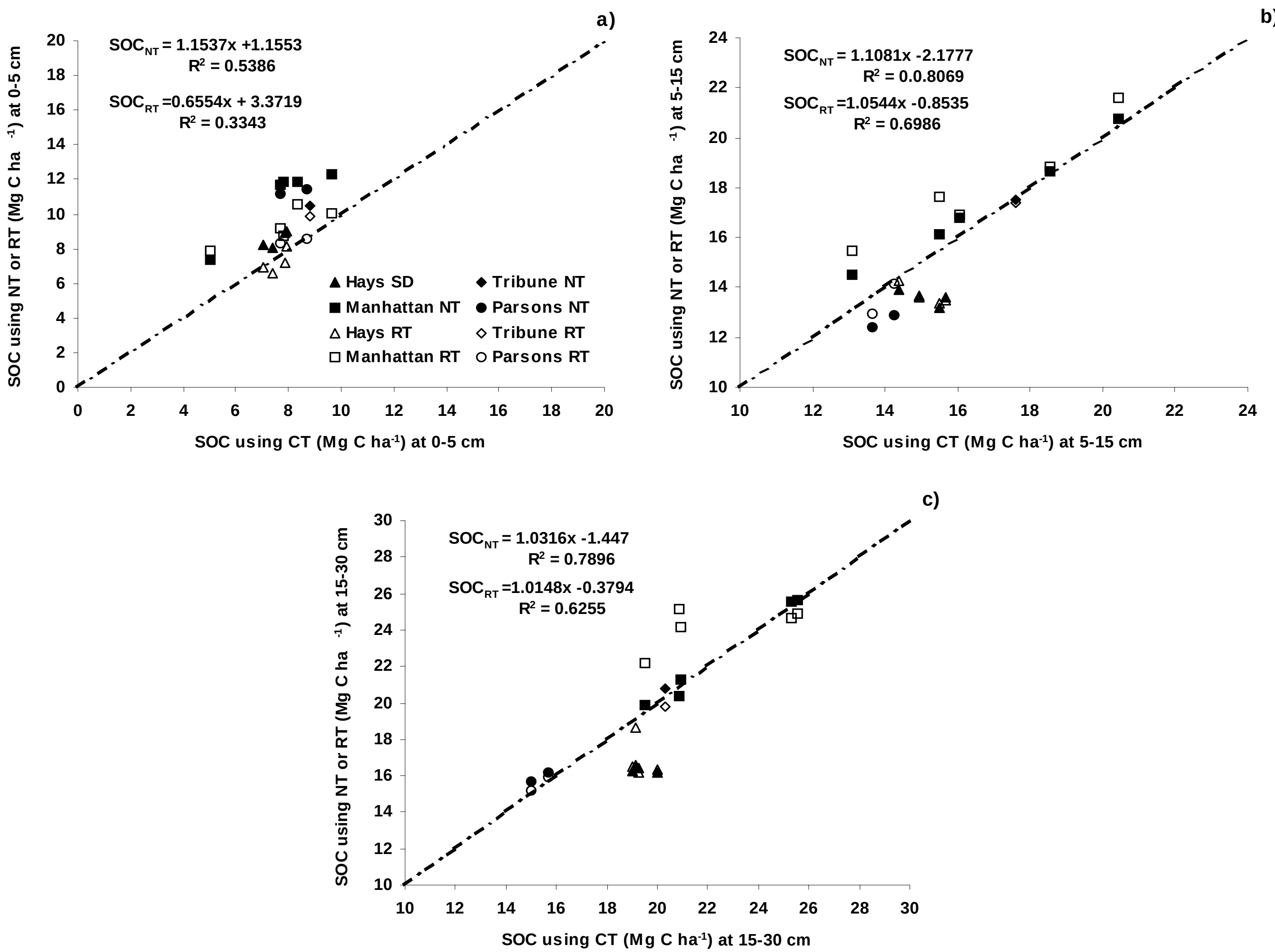

Figure 2-1. Soil organic carbon (SOC) at 0-5, 5-15, and 15-30 cm as a result of changing from conventional tillage (CT) to reduced tillage (RT) or no-tillage. Dashed line indicates 1:1 relationship.

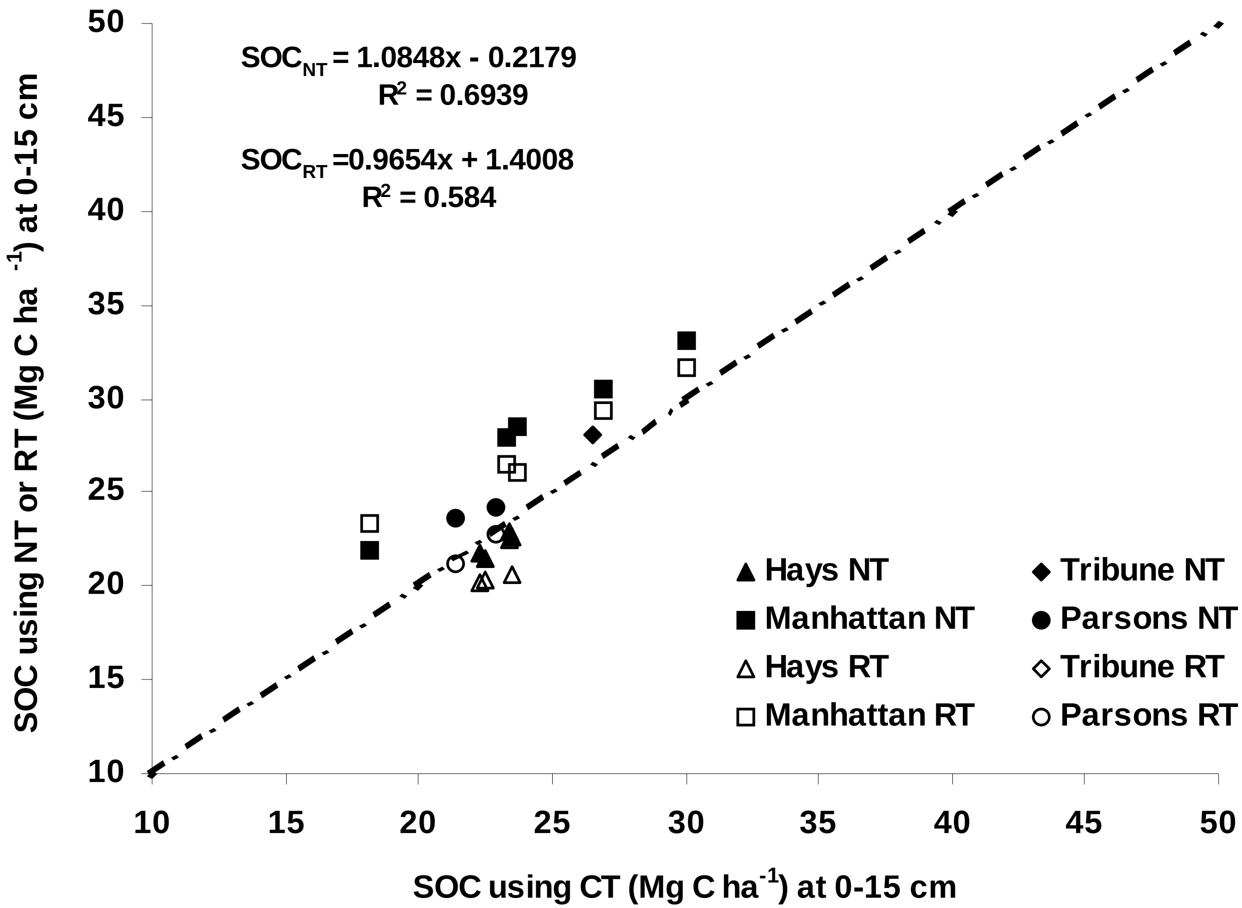

Figure 2-2. Soil organic carbon (SOC) at 0-15 cm as a result of changing from conventional tillage (CT) to reduced tillage (RT) or no-tillage.(NT). Dashed line indicates 1:1 relationship.

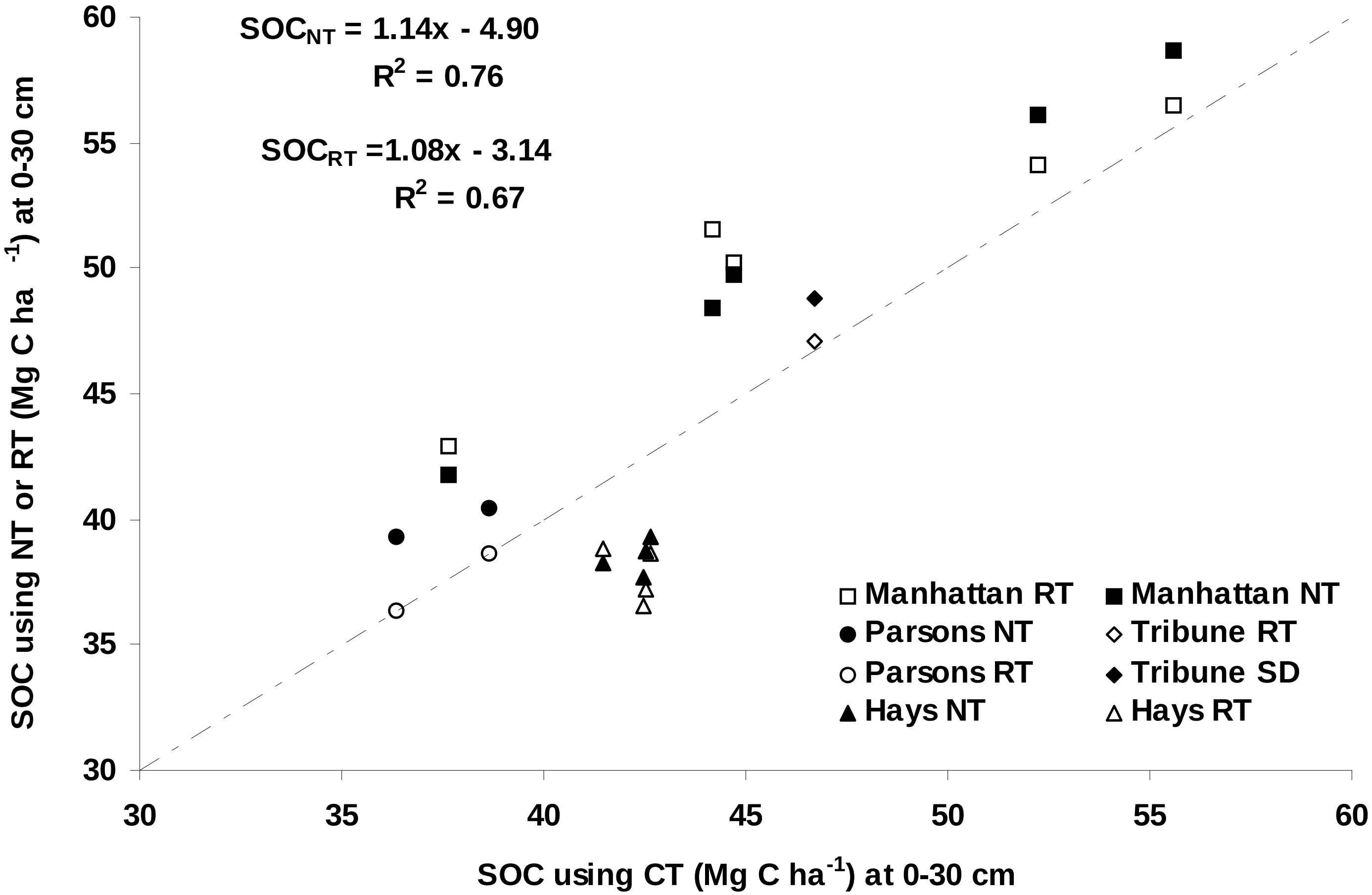

Figure 2-3. Soil organic carbon (SOC) at 0-30 cm as a result of changing from conventional tillage (CT) to reduced tillage (RT) or no-tillage (NT). Dashed line indicates 1:1 relationship.

Table 2-1. Description of the experimental sites.

Site	Kansas region	Soil Type	Yr. after initiation	Precipitation	Clay	Silt	Sand
			years	mm	………....%	……......	
Tribune ‡	Southwest	Aridic Argiustolls	16	421	24	60	16
Parsons†	Southeast	Mollic Albaqualfs	20	1014	13	68	19
Manhattan†	Northeast	Cumulic Haplustolls	29	813	20	71	9
Hays†	North Central	Typic Argiustolls	37	578	27	63	10

† Data for particle size distribution were obtained from McVay et al. (2006).

‡ Data for particle size distribution were obtained from Espinoza (2000).

Table 2-2. Soil organic carbon (SOC) under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT), and native prairie for each experiment.

Site	**Depth**	**SOC**			
	……cm….	………………..Mg C ha^{-1}………….			
		CT	**RT**	**NT**	**Native Prairie**
Tribune	0-5	8.8 **b**†	9.9 **a**	10.5 **a**	11.5
	5-15	17.6	17.4	17.5	17.1
	15-30	20.3	19.8	20.8	22.5
	0-15	26.5	27.2	28.0	28.6
	0-30	46.7	47.1	48.8	51.1
Hays	0-5	7.6 **b**	7.2 **b**	8.6 **a**	
	5-15	15.4	13.7	13.5	
	15-30	19.4 **a**	16.9 **b**	16.3 **b**	
	0-15	22.9	20.9	22.2	
	0-30	42.3	37.8	38.5	
Parsons	0-5	8.2 **b**	8.4 **b**	11.3 **a**	
	5-15	14.0	13.6	12.7	
	15-30	15.3	15.5	15.9	
	0-15	22.2	22.0	23.9	
	0-30	37.5	37.5	39.9	
Manhattan	0-5	7.7 **c**	9.3 **b**	11 **a**	
	5-15	16.7 **b**	18.1 **a**	17.4 **b**	
	15-30	22.4 **b**	24.2 **a**	22.5 **b**	
	0-15	24.4 **b**	27.4 **a**	28.4 **a**	
	0-30	46.8 **b**	51.6 **a**	50.9 **a**	

† Different letters represent significant differences between tillage systems at each depth ($P<0.05$).

Table 2-3. Effect of different crop rotations, continuous soybean (B-B), continuous sorghum (S-S), sorghum-soybean (S-B), wheat-soybean (W-B), and continuous wheat (W-W), on soil organic carbon (SOC) in Manhattan experiment.

Site	**Depth**	**SOC**				
	……cm…….	**………………..Mg C ha^{-1}…………**				
		B-B	**S-S**	**S-B**	**W-B**	**W-W**
Manhattan	0-5	6.7 **d**†	9.5 **b**	9.5 **b**	10.3 **ab**	10.6 **a**
	5-15	14.4 **d**	16.4 **c**	16.6 **c**	18.7 **b**	20.9 **a**
	15-30	20.5 **b**	22.1 **b**	22.1 **b**	25.2 **a**	25.3 **a**
	0-15	21.1 **d**	25.9 **c**	26.1 **c**	29.0 **b**	31.5 **a**
	0-30	41.6 **d**	48.0 **c**	48.2 **c**	54.2 **b**	56.8 **a**

† Different letters represent significant differences between crop rotations at each depth (P<0.05).

Table 2-4. Carbon sequestration rate (C rate) and C sequestration rate as difference with CT, under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT); with 0, 22, 45, or 67 kg N ha^{-1} at 0-15 cm soil depth in Hays experiment.

Treatments	C rate	(NT or RT) - CT
	.........Mg C ha^{-1} yr^{-1}........	
CT	-0.055 **b**†	
RT	-0.036 **b**	-0.055
NT	0.020 **a**	-0.021
0-N	-0.039 **b**‡	-0.038
22-N	-0.039 **b**	-0.045
45-N	-0.017 **ab**	-0.051
67-N	0.001 **a**	-0.019
Source	………..….*P* values……..…….	
Tillage (T)	0.0175	0.3496
Nitrogen (N)	0.0596	0.7475
T x N	0.9108	0.9362

† Different letters represent significant differences between tillage systems (P<0.05).
‡ Different letters represent significant differences among N rates (P<0.05).

Table 2-5. Carbon sequestration rate (C rate) and C rate as difference with CT, under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) at 0-15 and 0-30 cm soil depth in Tribune experiment.

Treatments	**C rate**	**(RT orNT) -CT**	**C rate**	**(RT or NT) -CT**
	.............Mg C ha^{-1} yr^{-1}..........		.............Mg C ha^{-1} yr^{-1}...........	
	.... 0-15 cm........		.... 0-30 cm........	
CT	-0.135		-0.273	
RT	-0.084	0.052	-0.250	0.024
NT	-0.036	0.100	-0.138	0.135
Source	……………………….*P* values……..……………………			
Tillage (T)	0.3478	0.4162	0.2569	0.1308

Table 2-6. Carbon sequestration rate (C rate) and C rate as difference with CT, under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT), with 0, or 140 kg N ha^{-1} at 0-15 and 0-30 cm soil depth in Parsons experiment.

Treatments	**C rate**	**(RT or NT) -CT**	**C rate**	**(RT or NT) -CT**
	............Mg C ha^{-1} yr^{-1}...........		...........Mg C ha^{-1} yr^{-1}.........	
	.... 0-15 cm........		.... 0-30 cm........	
CT	0.098		0.184	
RT	0.166	-0.007	0.277	0.002
NT	0.230	0.088	0.327	0.118
0-N	0.130	0.052	0.210 **b†**	0.075
140-N	0.199	0.029	0.315 **a**	0.046
Source	……………………….*P* values…….……………………			
Tillage (T)	0.1400	0.2443	0.1619	0.2422
Nitrogen (N)	0.1029	0.7327	0.0138	0.7267
T x N	0.4498	0.7544	0.1125	0.7356

† Different letters represent significant differences between N rates ($P<0.05$).

Table 2-7. Carbon sequestration rate (C rate) and C rate as differences with CT, under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) under continuous sorghum (S-S), sorghum-soybean (S-B), continuous soybean (B-B), wheat-soybean (W-B), and continuous wheat (W-W) rotation at 0-15 and 15-30 cm soil depth in Manhattan experiment.

Treatments	**C rate**	**(RT or NT) -CT**	**C rate**	**(RT or NT) -CT**
	Mg C ha^{-1} yr^{-1}	Mg C ha^{-1} yr^{-1}	Mg C ha^{-1} yr^{-1}	Mg C ha^{-1} yr^{-1}
	.... 0-15 cm........		.... 0-30 cm........	
CT	0.008 **b†**		0.044 **b†**	
RT	0.109 **a**	0.101	0.188 **a**	0.145
NT	0.143 **a**	0.136	0.183 **a**	0.139
B-B	-0.107 **d‡**	0.154	-0.166 **d‡**	0.162
S-S	0.059 **c**	0.134	0.084 **c**	0.201
S-B	0.064 **c**	0.121	0.089 **c**	0.181
W-B	0.163 **b**	0.106	0.294 **b**	0.099
W-W	0.254 **a**	0.078	0.389 **a**	0.068
Source	……………………….*P* values…….…………………….			
Rotation (R)	<.0001	0.3544	<.0001	0.4382
Tillage (T)	<.0001	0.1586	<.0001	0.8801
R x T	0.4847	0.4593	0.4800	0.4702

† Different letters represent significant differences among tillage at each depth (P<0.05).
‡ Different letters represent significant differences among crop rotations at each depth (P<0.05).

Table 2-8. Average grain yield for conventional tillage (CT), reduced tillage (RT) and no-tillage (NT) at Tribune, Hays, Parsons, and Manhattan.

Site	Yield		
	……………….Mg ha^{-1}…………		
	CT	RT	NT
Tribune			
W-S-F: sorghum	2.61	4.22	4.73
W-S-F: wheat	2.43	2.85	3.03
Hays			
W-S-F: sorghum	4.03	4.02	3.89
W-S-F: wheat	2.41	2.37	2.15
Parsons			
S-B: sorghum	3.62	3.46	2.71
S-B: soybean	1.49	1.51	1.48
Manhattan			
S-S	5.40	5.40	5.21
S-B- sorghum	5.85	5.98	6.15
S-B- soybean	2.15	2.22	2.49
B-B	1.75	1.75	1.95
W-B- wheat	3.16	3.23	3.09
W-B- soybean	2.42	2.49	2.62
W-W	3.03	2.89	2.29

*Data provided for A. Schlegel. Average 1991-2001
**Data provided for C. Thompson. Average 1975-2002
***Data provided for D. Sweneey. Average 1983-2001
****Data provided for D. Peterson. Average 1974-2003

CHAPTER 3 - LONG-TERM EFFECTS OF CROP AND SOIL MANAGEMENT ON AGGREGATE-ASSOCIATED C AND N

ABSTRACT

Management practices can affect soil aggregation and consequently, soil organic matter storage. The objective of our study was to determine the influence of different long-term management practices (tillage, N fertilization, and crop rotations) on soil aggregation and aggregate-associated C and N in different locations in Kansas. Four cropping long-term experiments covering a range of climate conditions and management systems were sampled for SOC. All the sites evaluated (Tribune, Manhattan, Parsons, Hays) included three different tillage systems: conventional tillage (CT), reduced tillage (RT) and no-tillage (NT). Nitrogen fertilization was evaluated at Parsons (0 and 140 kg N ha^{-1}) and at Hays (0, 22, 45, and 67 kg N ha^{-1}). Crop rotation effects were evaluated for continuous wheat (W-W), continuous sorghum (S-S), continuous soybean (B-B), wheat-soybean (W-B), and sorghum-soybean (S-B) at Manhattan. Soil samples were taken at 0-5 cm depth. Water-stable aggregates (WSA) were separated using a wet sieving method. Total C and N contents were determined by dry combustion. Cultivation decreased the amount of macroaggregates and increased the microaggregate fraction. Rotations also affected aggregation and C and N associated with aggregates. Total C was greater in the macroaggregate fraction (>250 μm) under NT continuous wheat and NT continuous sorghum compared to all other tillage and crop sequence combinations. Continuous soybean under CT had the lowest total C in the macroaggregate fraction. The increase in the C associated with macroaggregates reflects the increase in soil C related to management at each site.

Keys words: aggregates, tillage, fertilization, crop rotation

INTRODUCTION

Management practices that reduce C emissions to the atmosphere have been suggested as one alternative to reduce atmospheric levels of CO_2. Some of these practices include reduced tillage intensity, a reduction in bare fallow periods, intensive rotations, winter cover crops, and water management (Lal et al., 1998; Paustian et al., 2000, West and Post, 2002; Lal, 2004; Post et al., 2004). More knowledge is needed on the effect of these management practices on soil aggregation and its relationship with C sequestration across climate and soil types.

Soil structure influences the soil environment through its influence on soil water retention and movement, and aeration, which affects nutrient cycling, root penetration and ultimately crop yield. Soil structure also affects water quality due to its influence on soil erosion, crusting, and runoff (Bronick and Lal, 2005). Soil structure is also involved in the protection of soil organic C (SOC) and microorganisms (Six et al., 2004).

Aggregation is a key process defining soil structure. Oades (1984) modified a theory on soil aggregate formation, where temporary binding agents, such as roots and hyphae hold macroaggregates together, with microaggregates forming inside macroaggregates through the interaction of microbial mucilages and clay particles. This theory has been corroborated by others (Beare et al., 1994; Jastrow, 1996; Angers et al., 1997; Six et al., 1998) suggesting a redistribution of C, which is first incorporated in macroaggregates and then into new microaggregates. Bossuyt et al. (2002) found that short and long-term C stabilization was higher under NT systems with respect to CT, and that stabilization occurred at the microaggregate level.

Continuous cultivation and intensive tillage result in loss of aggregate stability and SOC (Tisdall and Oades, 1982; Elliott, 1986). Tillage exposes more SOC to microbial activity due to the disruption of soil aggregates (Beare et al., 1994b; Paustian et al., 1997) and leads to the loss of C-rich macroaggregates and an increase in C-depleted microaggregates (Six et al., 2000b). Tillage also results in soil mixing and fragmentation of the crop residue, thus, affecting soil structure and soil organic matter dynamics (Balesdent et al., 2000), resulting in a reduction of the stability and amount of macroaggregates (Tisdall and Oades, 1982; Elliott, 1986; Mikha and Rice, 2004, McVay et al., 2006). No-tillage results in greater amounts of macroaggregates, and also slower macroaggregate turnover, thus fostering greater stabilization of soil C (Six et al., 1998, 1999, 2000b; Denef et al., 2004). No-tillage also promotes fungal biomass, which contributes to the formation of macroaggregates (Beare et al., 1993; Frey et al., 1999; Watson and Rice, 2004).

Plant species can directly affect aggregation through differences in root structure and distribution (Angers and Caron, 1998). Roots can affect the proportion of macroaggregates (Materechera et al., 1994; Denef et al., 2002), where roots increase the fragmentation of the soil and the formation of failure zones within macroaggregates. Roots also compress the surrounding soil when they penetrate reducing the porosity and resulting in pore enlargement and formation (Angers and Caron, 1998). Root exudates act as binding agents through the production of various mucilages that enhance aggregation (Six et al., 2004). Associated with roots, vesicular-arbuscular mycorriza, present in many plants, enhance aggregate formation and stabilization (Miller and Jastrow, 1990; Jastrow et al., 1998).

Plant species can indirectly affect aggregation by the amount of plant residue returned to the soil, its biochemical composition, and C released from the growing roots, thus affecting microbial composition and activity (Bronick and Lal, 2005; Rillig et al., 2002; Rice and Angle, 2004). Martens (2000) found differences in aggregation after soybean, compared to corn and native prairie. After soybean, aggregation decreased compared with corn and native prairie which the author attributed to a lower phenolic acid content of soybean and a lower amount of residue returned to the soil.

Wright and Hons (2004, 2005a,b) reported the effect of different cropping systems on soil aggregation, where aggregation was greater under wheat than sorghum or soybean. The differences were attributed to differences in crop residue production and residue quality. For example, cropping sequences that included sorghum or soybean were more readily decomposed than wheat residue (Wright and Hons, 2004).

While tillage and crop rotations have often been examined for their effects on aggregation, there has not been a systematic analysis of the interactive effects of tillage, crop rotation, and N on aggregation. Thus, the objective of our study was to determine the influence of different long-term management practices of tillage, N fertilization, and crop rotations on soil aggregation and aggregate-associated C and N in different locations in Kansas.

MATERIALS AND METHODS

Site description

The Hays experiment is located in central Kansas (38º 51'N, 99º 20'W). This experiment was initiated in 1965 on a Harney silt loam soil (fine, smectitic, mesic Typic Argiustoll). The 30-yr average annual precipitation was 533 mm with an annual mean temperature of 11.9 ºC. The crop rotation was wheat *(Triticum aestivum L.)* -grain sorghum *(Sorghum bicolor (L.) Moench)*-fallow, with three tillage systems: conventional tillage (CT), reduced tillage (RT), and no-tillage (NT). Conventional tillage consisted of using tools such as disk, one-way plow, and mulch treader. Reduced tillage included V-blade, sweeps or a rod weeder. No-tillage consisted of planting directly into the residue. From the beginning of the experiment to 1975, N rates were 0 and 45 kg N ha^{-1} yr^{-1}. Since 1975 four N rates were evaluated, 0 (0-N), 22 (22-N), 45 (45-N), and 67 (67-N) kg N ha^{-1} yr^{-1}. Nitrogen fertilizer was applied as ammonium nitrate in the previous fall for June sorghum planting and in August prior to September wheat planting. The experimental design was split-plot with four replications, with tillage as the main plot and N as sub-plot

The Tribune experiment, initiated in 1988, was located in western Kansas (38º 30'N, 101º 41'W). The 30-yr average annual precipitation is 422 mm, with an annual mean temperature of 11.3 ºC. The soil was classified as Richfield silt loam (fine-smectitc, mesic Aridic Argiustolls). The crop rotation was wheat-grain sorghum-fallow, with three tillage systems: CT, RT, and NT. Conventional tillage consisted of three or four operations per year with a sweep plow between crop harvest and planting the next crop. The RT system used a combination of tillage (primarily sweep plow) and herbicides for weed control during fallow. The number of tillage operations with RT was approximately 50% of CT. No-tillage consisted of planting directly into the residue. Nitrogen fertilizer as urea ammonium nitrate (UAN) was broadcast applied at 67 kg N ha^{-1} yr^{-1} to 112 kg N ha^{-1} yr^{-1}, depending on the year or crop. Native sod was included as part of the experimental design, which represented the natural vegetation types of C_3 and C_4 grass, with the dominant species being buffalograss (*Buchloe dactyloides*). The treatments were arranged in a randomized complete block design with four replications.

The Parsons experiment is located in southeastern Kansas (37º 21.02’N, 95º 17.13’W). The 30-yr average annual precipitation is 1016 mm, with an annual mean temperature of 13.7ºC. This experiment was initiated in 1983 on a Parsons silt loam soil (fine, mixed, active, thermic Mollic Albaqualfs). The crop rotation was grain sorghum-soybean *(Glycine max (L.) Merrill)*, with three tillage systems (conventional tillage, reduced tillage, and no-tillage), and two N rates, 0 (0-N) and 140 kg N ha^{-1} yr^{-1} (140-N). Nitrogen fertilizer was urea-ammonium nitrate (UAN) solution. Conventional tillage included chisel, disk, and field cultivator. Reduced tillage included disk and field cultivator. No-tillage consisted of planting directly into the residue and using chemical weed control. The experimental design was split-plot with four replications, with tillage as the main plot and N as sub-plot.

The Manhattan experiment, initiated in 1974, was located on the Kansas State University Agronomy Farm, Manhattan (Riley County; 39º 07’N, 96º 37’W). Soils were Muir silt loam (fine-silty, mixed, mesic Cumulic Haplustoll) and Reading silt loam (fine, mixed, mesic Typic Argiudoll). The 30-yr average annual precipitation was 813 mm, which was mainly concentrated in the spring-summer period, with an annual mean temperature of 11.3 ºC. Crop rotation and tillage systems were evaluated in this experiment. The experimental design was split-plot with four replications, with rotation as the main plot and tillage as sub-plot. The three crops, soybean (B), grain sorghum (S), and winter wheat (W) were combined in five rotations: continuous sorghum (S-S), sorghum-soybean (S-B), continuous soybean (B-B), wheat-soybean (W-B), and continuous soybean (B-B). The three tillage treatments were CT, RT, and NT systems. Conventional tillage included chisel, disk, and field cultivator. Reduced tillage included disk and field cultivator. No-tillage consisted of planting directly into the residue and chemical weed control. A blend of urea and diammonium phosphate fertilizer providing 112 kg N ha^{-1} and 11.3 kg P ha^{-1} was broadcast applied prior to the last tillage operation before planting of each crop and year.

Soil Sampling

Soil samples were taken from each plot at 0- to 5 cm depth. A sterile polypropylene bags (3.78 L) were filled with soil samples collected randomly from each plot using a 2-cm diam. Oakfield soil-probe (Forestry Suppliers, Inc., Jackson, MS). Samples were collected in March 2003 (before planting) on the sorghum rotation phase for the Hays experiment for all tillage in the 0-N and 67-N treatments. For Parsons, samples were taken in December 2003 after sorghum harvest. Tribune samples were collected in April 2004 on the sorghum rotation phase and native sod. Soil samples from Manhattan were taken in May 2004, after soybean and sorghum planting and before wheat harvesting. Soil samples were passed through 4-mm sieve, roots removed, and stored at 4ºC until use.

Aggregate-Size Distribution

Water-stable aggregates (WSA) were separated using a wet sieve method described by Yoder (1936) with modifications by Mikha and Rice (2004). Soil was air-dried and 50 g placed on the top of the sieve of each nest. To slake the air-dried soil, 1 L of distilled water was rapidly added until the soil was covered with water. Soils were submerged in water for 10 min following by 10 min of wet sieving. Four aggregate size classes were collected from each treatment >2000 or 1000, 250-2000 or 250-1000, 53-250, and 53-20 μm diam. Large macroaggregates were defined as >2000 μm, small macroaggregates 250-2000, microaggregates 250-53 μm, and silt plus clay by 20-53 μm size fraction. For Manhattan site we used a 1000 μm sieve instead of 2000 μm sieve Sand-free WSA was measured using a subsample of intact aggregates (2-5g) and combined with fivefold volume (10-25 mL) of 5 g L^{-1} sodium hexametaphosphate, left overnight and shaken on an orbital shaker at 350 RPM for 4h. The dispersed organic matter and sand was collected on a 53 μm mesh sieve, washed with deionized water, and dried at 105ºC for 24 h, and the aggregate weights were recorded for estimating the sand-free correction.

Total C and N

Soil samples were dried and ground to a fine powder using a mortar and pestle. Total C and N contents were determined by dry combustion using a Carlo Erba C/N Analyzer (Carlo Erba Instruments, Milano, Italy). Calculations for total C and N in different aggregate-size fraction were adjusted for sand-free water stable aggregates.

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.

RESULTS

Sand-free water stable aggregates

Sand-free WSA were significantly affected by aggregate size class and the interaction of tillage x aggregate size (P<0.01) at the Tribune site (Table A.1). Native prairie had significantly greater amounts of macroaggregates compared with the cropped treatments. Of the cropped systems, NT and RT systems had greater macroaggregates than the CT system; indicating reduced tillage was able to maintain a higher amount of macroaggregates (Fig. 3.1). This increase in the largest size aggregates was followed by a corresponding decrease in the microaggregate fraction under the prairie, and NT and RT systems.

At the Parsons site, a significant Tillage x Aggregate size interaction (P<0.03) was found in the distribution of the aggregates. No-till had similar amounts of large macroaggregates than RT, but both NT and RT were greater than CT (Table A.2, Fig. 3.2). No-till had greater amounts of the small macroaggregates than RT and CT with no difference between RT and CT. In the microaggregate fraction, CT and RT had higher amounts than in the NT system (Fig. 3.2).

At the Hays site, a significant Tillage x N x Aggregate size (P<0.05) interaction was found in the distribution of the sand-free WSA (Table A.3). For the large macroaggregates and the silt plus clay size fraction no differences were found among treatments (Fig. 3.3). For the small macroaggregates, NT and RT with N fertilization had a greater amount of WSA than all other treatments (Fig. 3.3). The NT 67-N treatment had the least amount of microaggregates.

At the Manahattan site, aggregates were significantly affected by a Tillage x Rotation x Aggregate size interaction (P<0.01) (Table A.4). For the macroaggregate fraction (>250 μm) NT continuous wheat had the greatest amounts of sand-free WSA (Table A.5, Fig. 3.4), with the corresponding lowest values in the macroaggregate fraction.

Total C and N concentrations

At Tribune, the C concentration in the macroaggregates was greater with the CT system; however, the native prairie had a higher C concentration in the microaggregate fraction (Table A.6, Fig. 3.5). No differences between treatments were observed in the silt plus clay fraction. No significant differences were found in the N concentration of the aggregates at any size fraction (Table A.7, Fig. 3.6).

At Parsons, the C and N concentrations of the aggregates were significantly affected by the interactions of Tillage x Aggregate Size and N x Aggregate size (P<0.05) (Table A.8, A.9). For the large macroaggregates, the C concentration was greater under NT with respect to RT and CT, which did not differ from each other (Fig. 3.8). For the small macroaggregates, RT had greater concentrations of C than NT and CT. No differences among tillage were found in the microaggregate and silt plus clay fractions (Fig. 3.7). Nitrogen fertilizer significantly increased the C concentration of the macroaggregates, with no changes in the microaggregates (Table A.8. Fig. 3.8). No-tillage significantly increased the N concentration of the large macroaggregates, but there were no differences in the other size classes (Table A.9, Fig. 3.9). Similar to C, N fertilizer significantly increased the N concentration of the macroaggregates with no differences in microaggregates (Table A.9, Fig. 3.10).

At Hays, the concentration of C and N of the aggregates was similar among treatments (Table A.10, A.11).

At Manhattan, the C concentration was significantly affected by the interactions of Tillage x Aggregate size and Rotation x Aggregate size (P<0.05) (Table A.12). Wheat-soybean and continuous sorghum had higher C concentrations in the macroaggregates. No differences in C concentrations were found on the smallest size fraction (Fig. 3.11). Averaging across rotations, NT had higher C concentrations than CT in the large macroaggregate fraction (Fig. 3.12). The N concentrations were not significantly affected by treatments (Table A.13). The large macroaggregate fraction had the highest N concentrations (Table A.13).

Total C and N mass

For Tribune, total C mass was significantly greater under native prairie compared with NT and RT, which both were similar and greater than CT (Fig. 3.13). For the microaggregate fraction, the CT system had significantly more total C mass than RT, NT and native prairie (Table A.14, Fig. 3.13). A similar tendency was observed in total N mass in each size fraction (Table A.15). No differences were observed in the silt plus clay fraction for both C and N. Total C and N mass followed the same pattern of sand-free WSA distribution, which drives the response of the different tillage on total C and N mass.

At Parsons, total C and N mass were significantly affected by the interactions of Tillage x Aggregate size and Nitrogen x Aggregate size ($P<0.05$) (Table A.16, A.17). Total C and N mass in the large macroaggregates were significantly greater with NT compared with RT and CT (Fig. 3.14, 3.15). For the small macroaggregates, NT had similar total C mass than RT but significantly greater than CT; however, NT had greater total N mass than RT which was significantly greater than CT. Conventional tillage and RT had significantly greater total C mass in the macroaggregates than NT, with no differences at the silt plus clay fraction. Nitrogen fertilizer application only increased total C and N mass associated with the small macroaggregates (Fig. 3.16,3.17).

At Hays, total C and N mass was significantly affected by the interaction of Tillage x N x Aggregate size ($P<0.05$), where there were no differences in the large macroaggregates and silt plus clay fraction (Table A.18, A.19 ; Fig 3.18, 3.19). However, in the macroaggregates, NT 67-N had the greatest total C mass than the other treatments (Fig. 2.18).

At Manhattan, total C and N mass were significantly affected by the interaction of Tillage x R x Aggregate size ($P<0.05$) (Table A.20, A.21). Continuous wheat and continuous sorghum under NT systems had the greatest total C and N mass while continuous soybean showed the lowest values in the macroaggregates, with corresponding decrease in the microaggregates (Fig. 3.20, 3.21).

DISCUSSION

In general, our results showed that NT systems increased the proportion of macroaggregates (>250 μm). Several authors have also reported an increase in the proportion of macroaggregates under NT systems (Beare et al., 1994a; Mikha and Rice, 2004; Wright and Hons, 2005a).

Tillage can affect aggregation in different ways by: 1) exposing the aggregates to more frequent wet-dry cycles thereby increasing the susceptibility of aggregates to disruption; 2) increasing SOC decomposition; and 3) changing microbial communities, especially reducing fungal growth and proliferation that contribute to macroaggregate formation (Six et al., 1998).

Cultivation of native prairie reduced the mass of macroaggregates. Even no-tillage was unable to maintain the level of macroaggregates characteristic of that in native prairie. Similar to our results, Elliott (1986) reported greater amounts of macroaggregates in native prairie than in cultivated soils. Tillage can decrease the length of the roots, and reduce fungi resulting in a decrease in aggregation (Tisdall and Oades, 1980). Greater fungal to bacterial ratio has been reported under NT than under CT systems (Beare, 1997; Frey et al., 1999; Doyle et al., 2004).

A greater concentration of C and N associated with the macroaggregate fraction than in the microaggregate fraction was observed at all sites. In general, NT had greater C concentrations in the macroaggregate fraction, with no differences in the microaggregate and silt plus clay fraction. With tillage, soil aggregates are disrupted exposing SOC to microbial decomposition resulting in a loss of C-rich macroaggregates and an increase in C-depleted microaggregates (Six et al., 2000b). However, at Tribune, CT had greater C concentrations in the macroaggregates than the other cropped treatments and native prairie.

Nitrogen applications appear to increase the mass of macroaggregates and their C and N concentration. This response is likely due to the greater yields and biomass returned to the soil. Tillage seemed to negate the effect of N application.

Plant species may affect soil aggregation. At Manhattan, we were able to evaluate the effect of crop type on the distribution and C concentrations of aggregates in different size classes. Wheat and sorghum increased total C mass in the macroaggregate fraction under NT while soybean had the lowest. Tillage negated the effects of the crop type. Wright and Hons (2005a) reported greater aggregation with wheat than with sorghum and soybean, which they attributed to differences in the amount and quality of the residues. Wheat residue has a higher C/N ratio, and therefore lower decomposition rate than sorghum, and soybean (Franzluebbers et al., 1995b; Wright and Hons, 2005a). Wheat with a high C:N ratio can promote more fungi than soybean with a low C:N ratio. Martens (2000) suggested that the lower phenolic acid content of soybean might limit formation of macroaggregates.

Overall, the C and N associated with the macroaggregates (250-2000 and >2000 μm) tended to be most responsive to long-term management while the 20-53 μm fraction was the least affected by management. No-tillage systems increased the aggregate-associated C and N in the macroaggregates. Nitrogen application significantly increased C and N associated with the macroaggregates but this effect was reduced by tillage. Wheat and sorghum increased aggregate-associated C and N with differences more pronounced under no-tillage. The increase in C associated with macroaggregates reflects the increase in soil C related with different management practices. Thus, for these soils, buildup and maintenance of macroaggregates seems to be one of the primary mechanisms for C retention in these agroecosystems.

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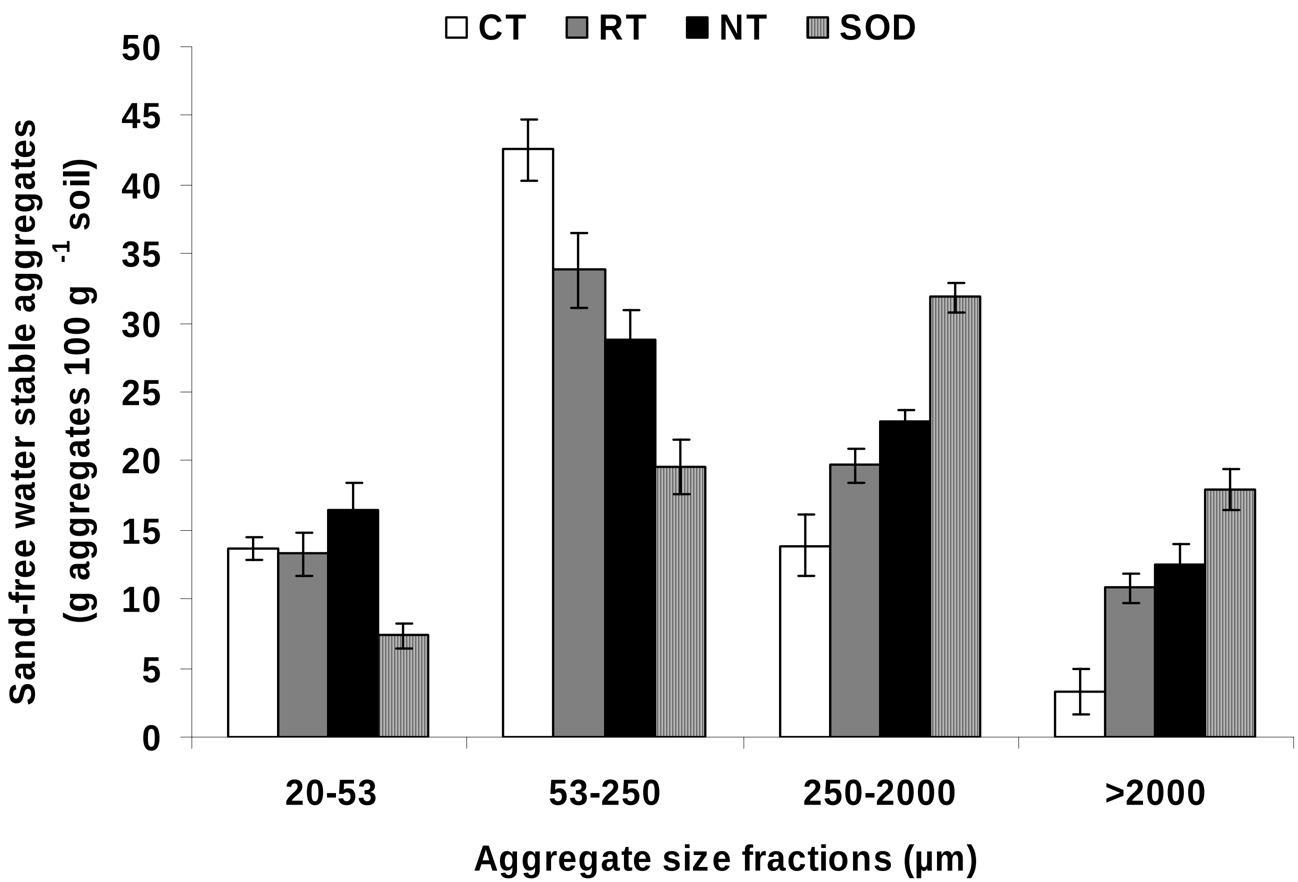

Figure 3-1. Distribution of sand-free water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment. Error bars represent the standard error of the mean (n=4).

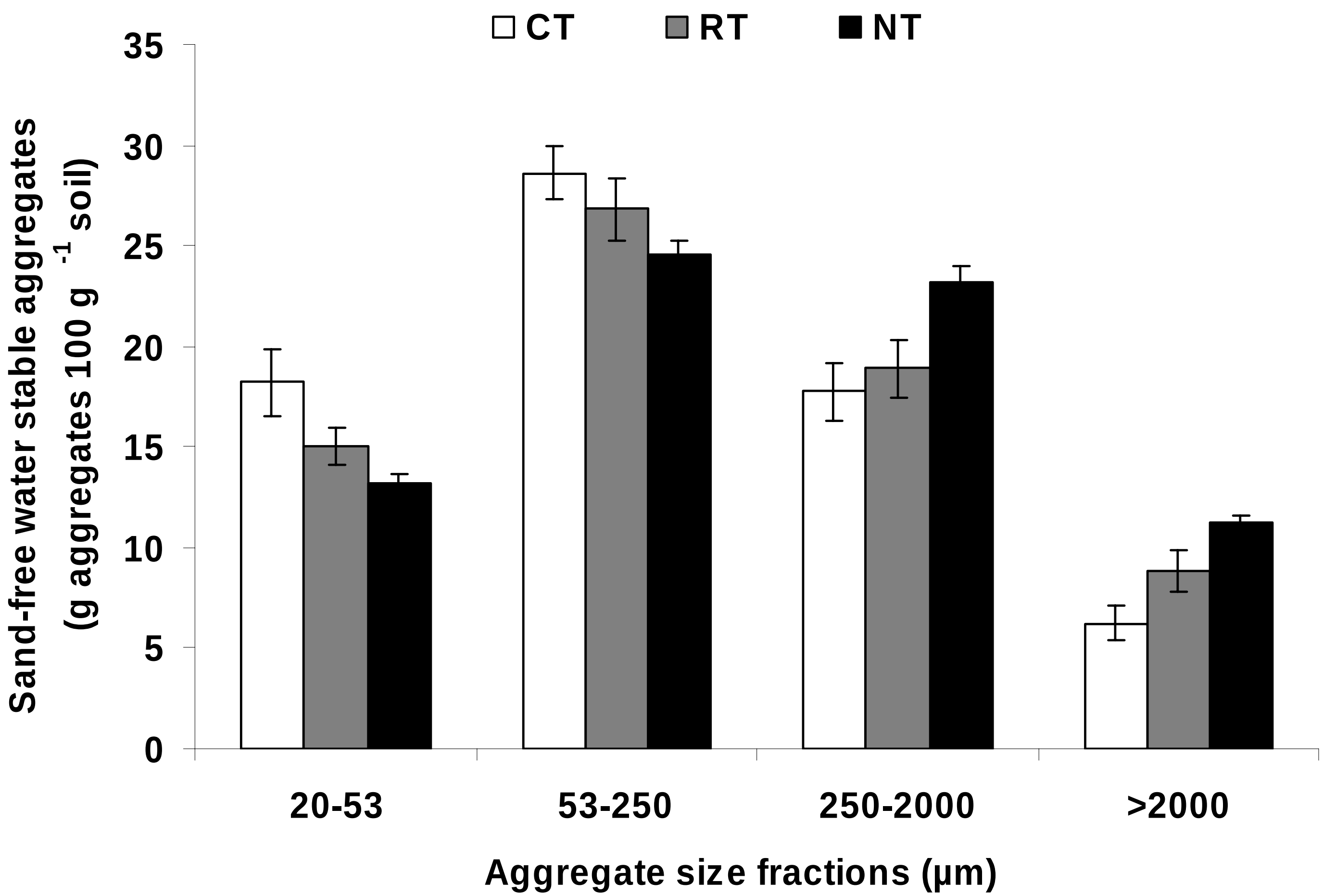

Figure 3-2. Distribution of sand-free water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), for Parsons experiment. Error bars represent the standard error of the mean (n=4).

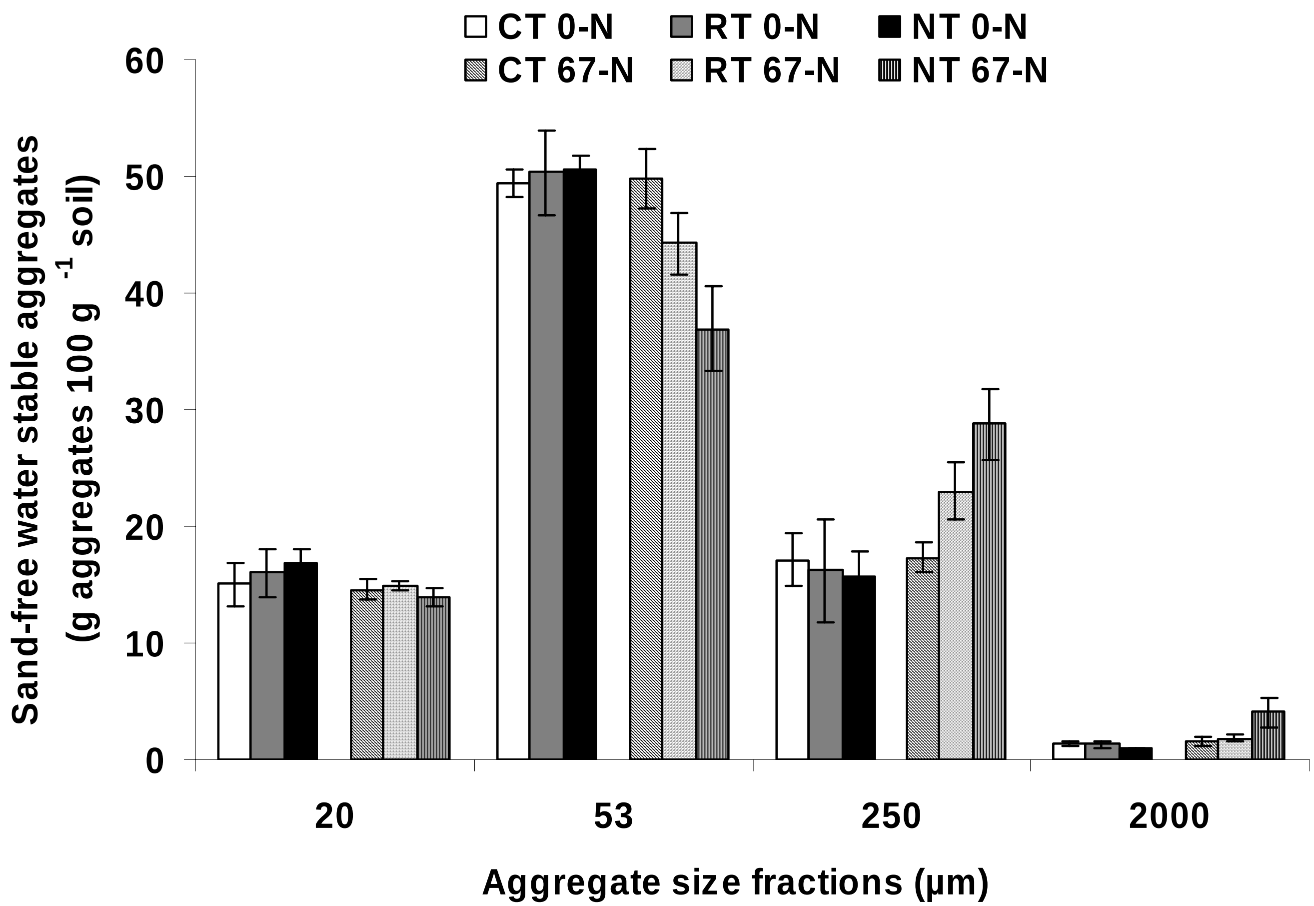

Figure 3-3. Distribution of sand-free water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} in Hays experiment. Error bars represent the standard error of the mean (n=4).

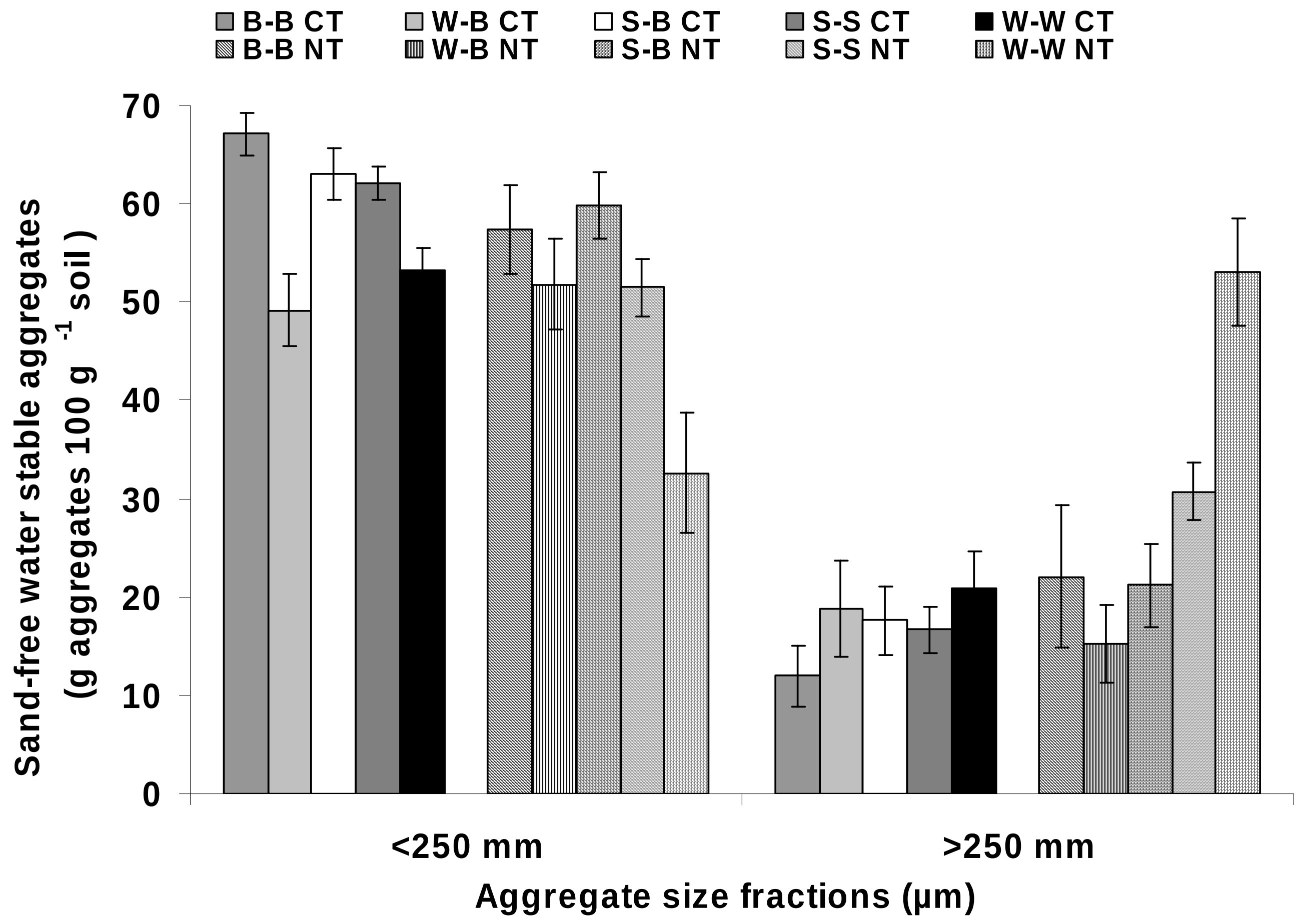

Figure 3-4. Distribution of sand-free water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), in five crop rotations: continuous wheat (W-W), wheat-soybean (W-B), continuous sorghum (S-S), sorghum-soybean (S-B), and continuous soybean (B-B) for Manhattan experiment. Error bars represent the standard error of the mean (n=4).

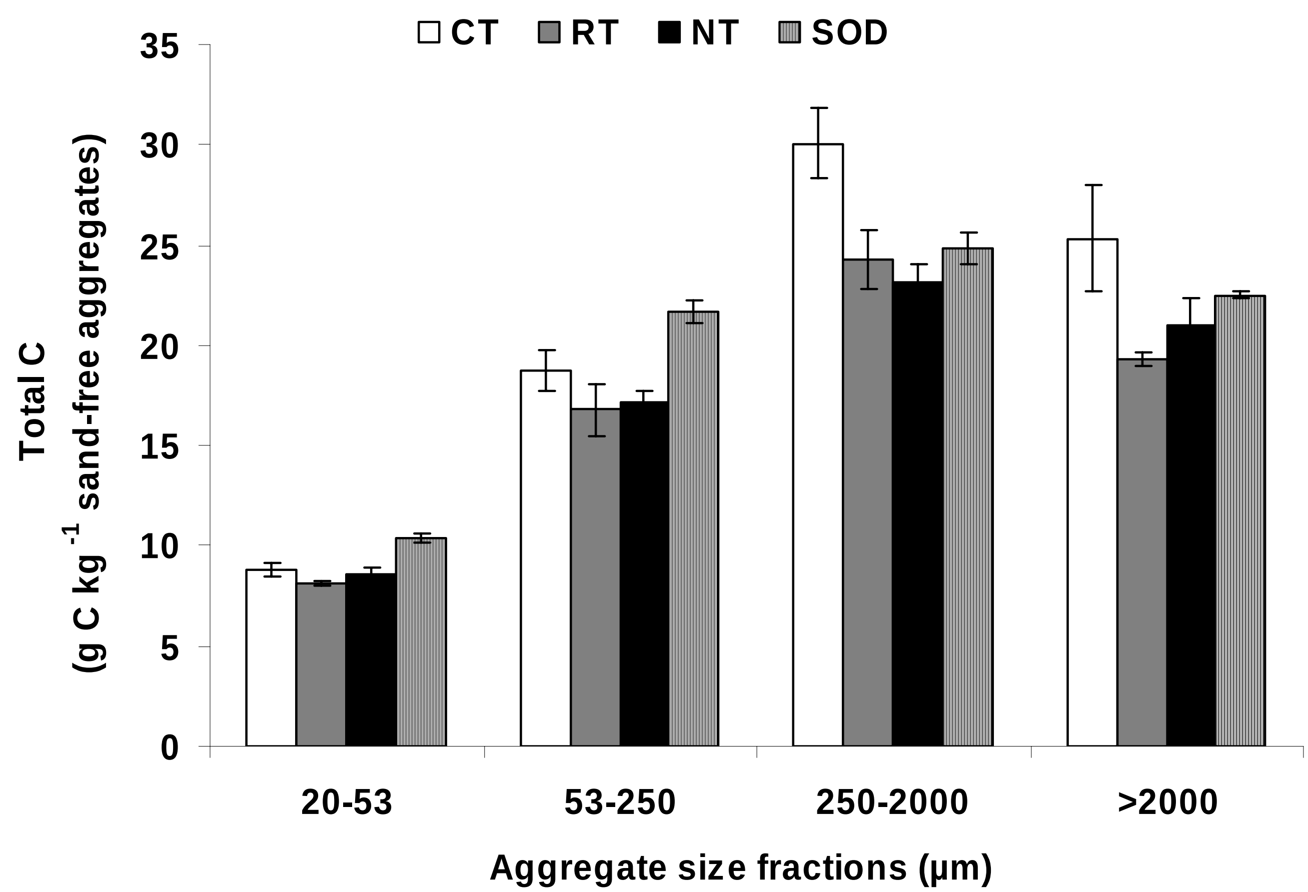

Figure 3-5. Total C normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment. Error bars represent the standard error of the mean (n=4).

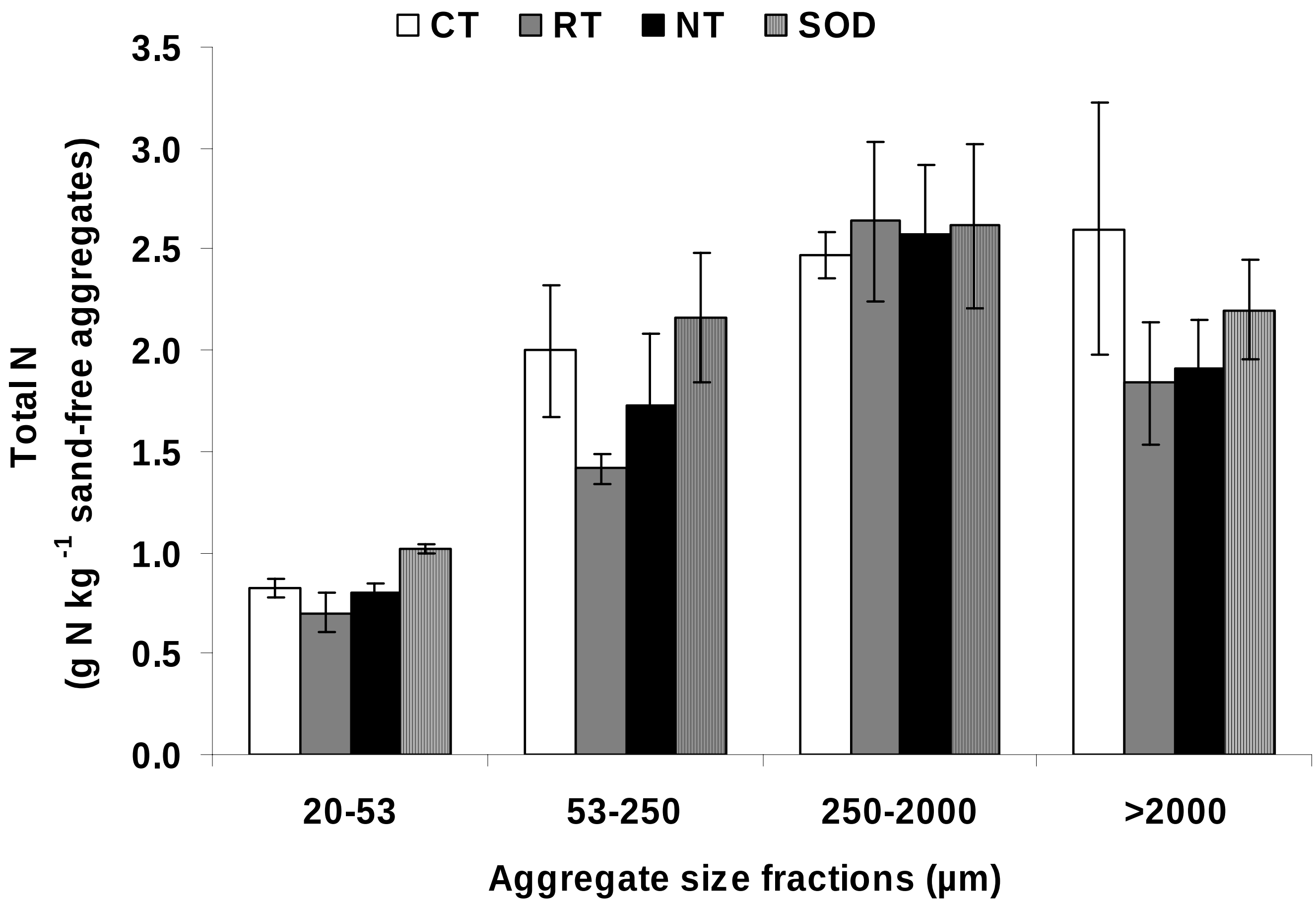

Figure 3-6. Total N normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment. Error bars represent the standard error of the mean (n=4).

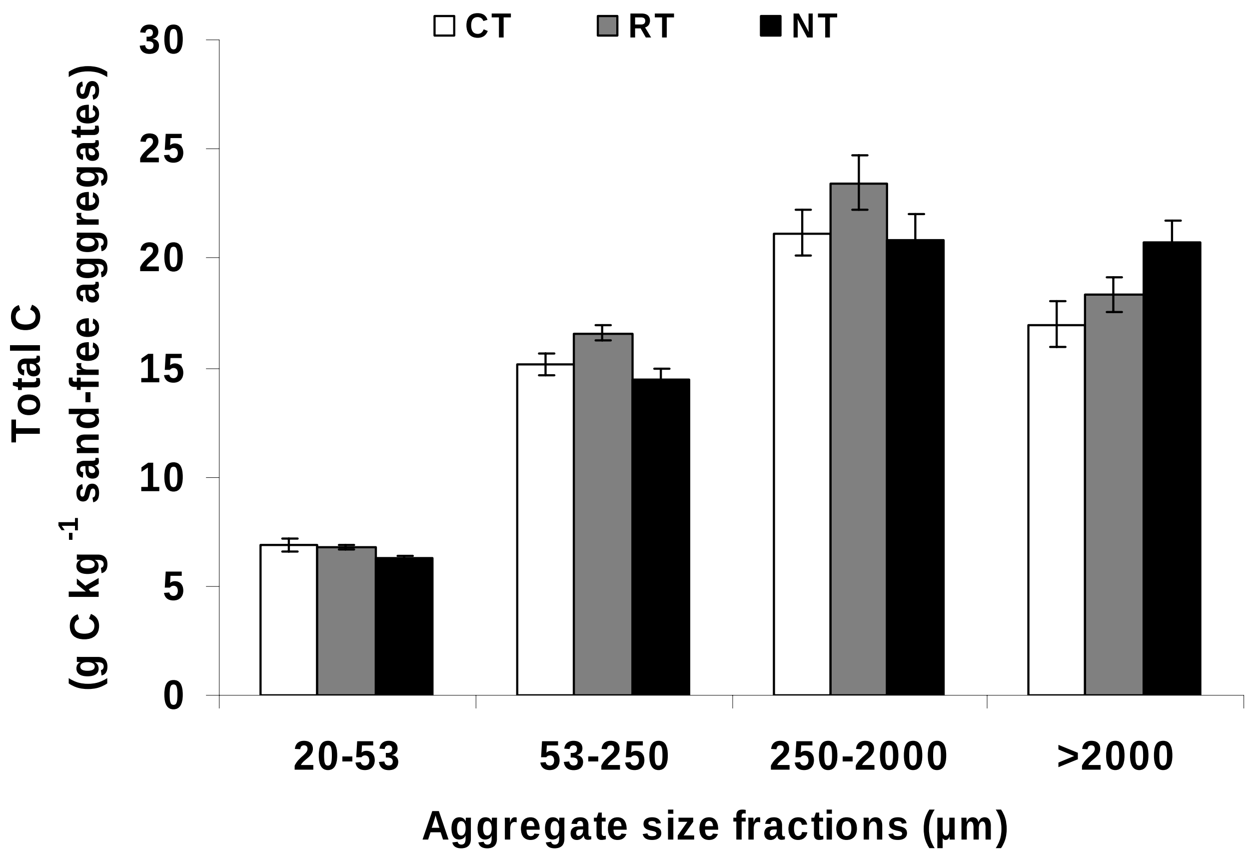

Figure 3-7. Total C normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) for Parsons experiment. Error bars represent the standard error of the mean (n=4).

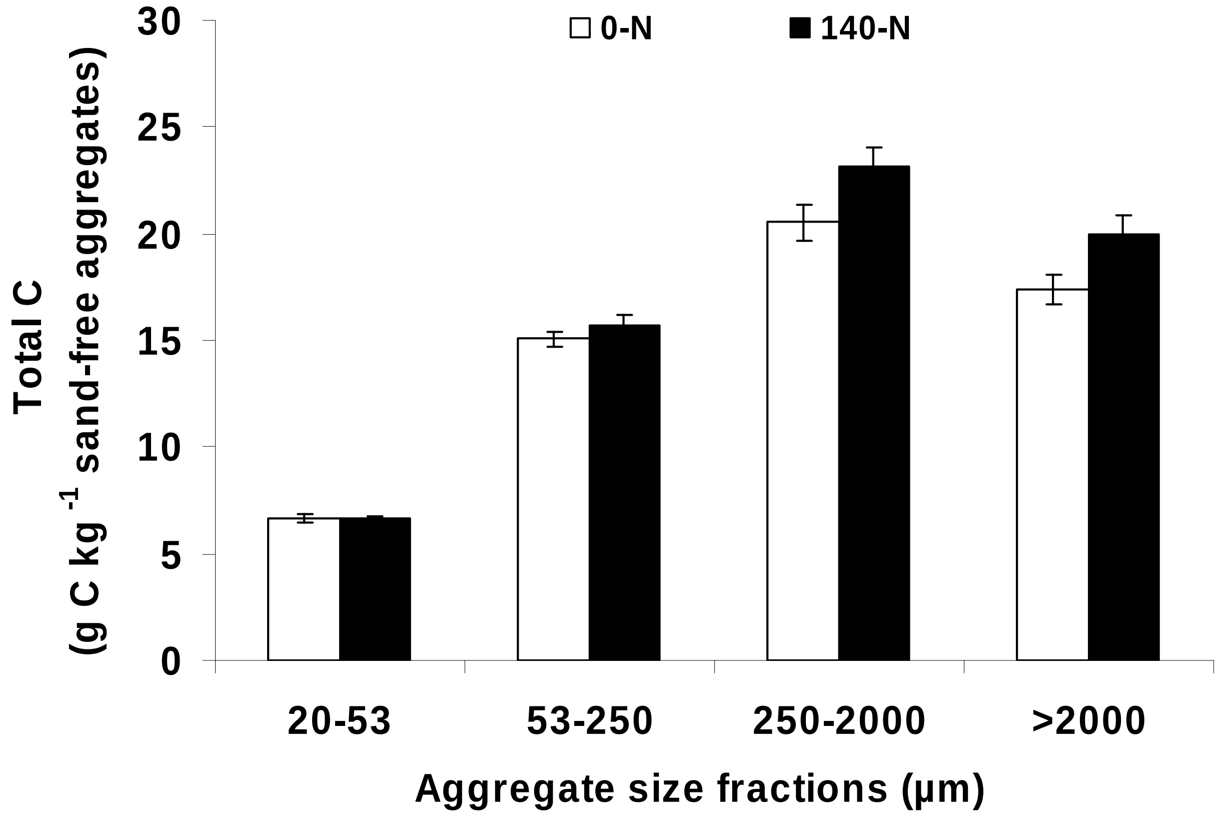

Figure 3-8. Total C normalized to sand-free basis in each water stable aggregates affected by N application, 0 and 140 kg N ha^{-1}, 0-N and 140-N, respectively, for Parsons experiment. Error bars represent the standard error of the mean (n=4).

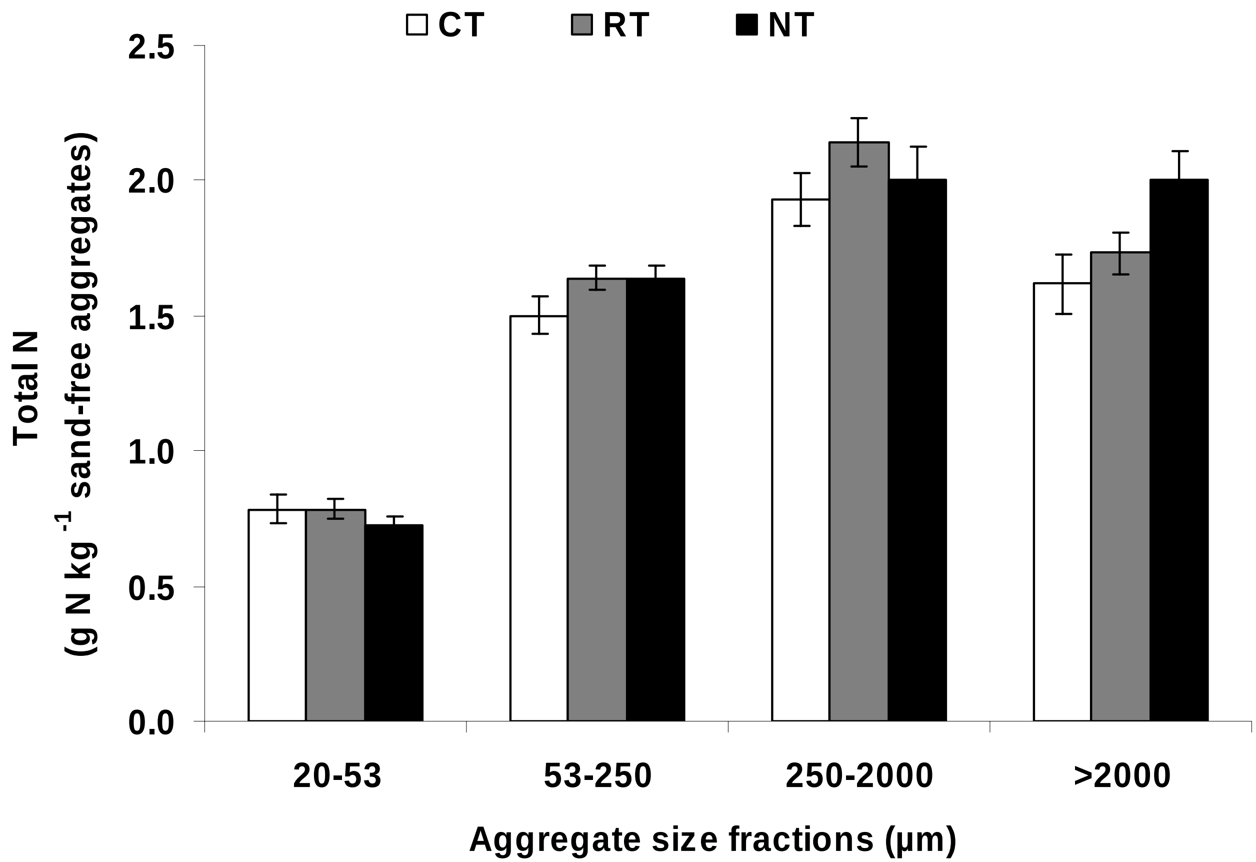

Figure 3-9. Total N normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) for Parsons experiment. Error bars represent the standard error of the mean (n=4).

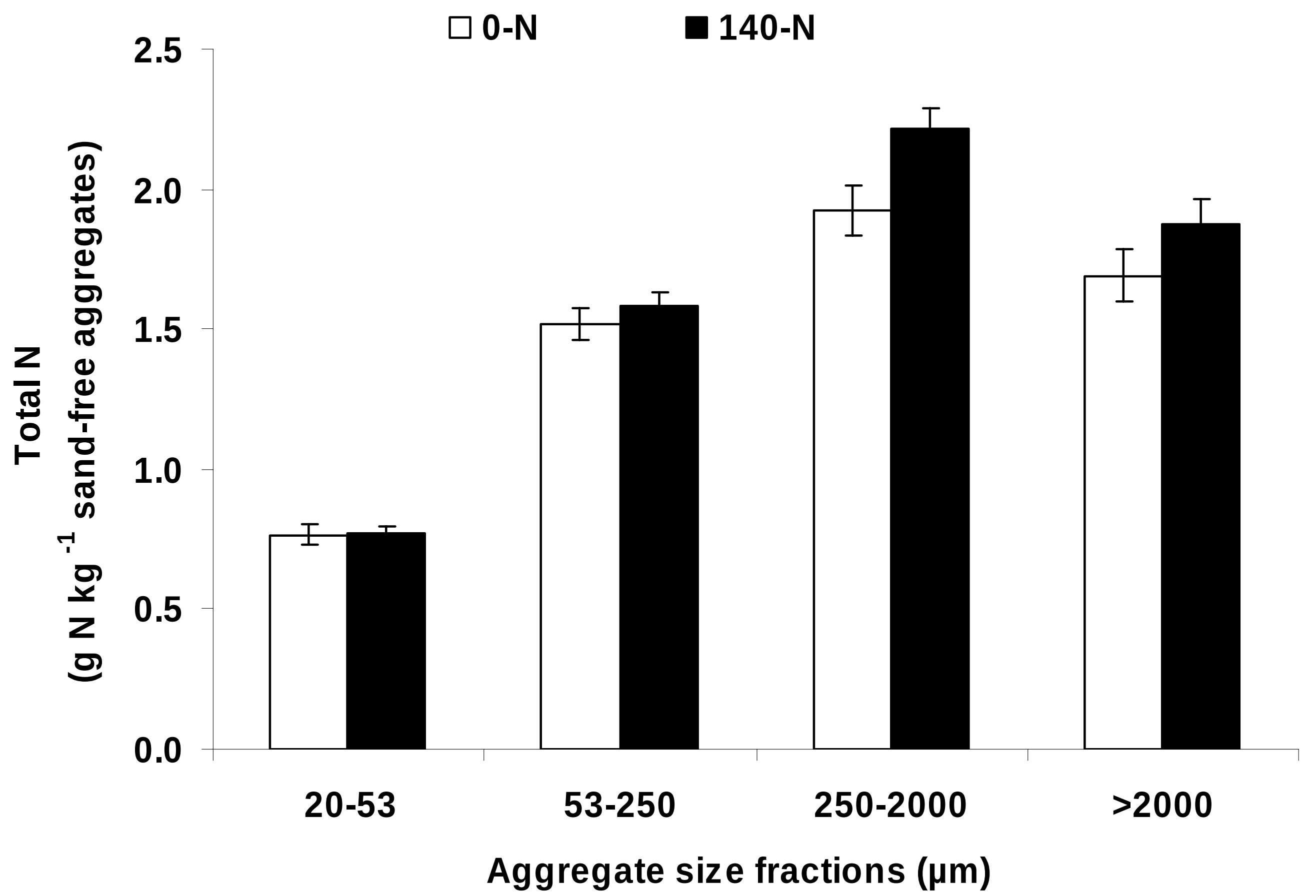

Figure 3-10. Total N normalized to sand-free basis in each water stable aggregates affected by N application, 0 and 140 kg N ha^{-1}, 0-N and 140-N, respectively, for Parsons experiment. Error bars represent the standard error of the mean (n=4).

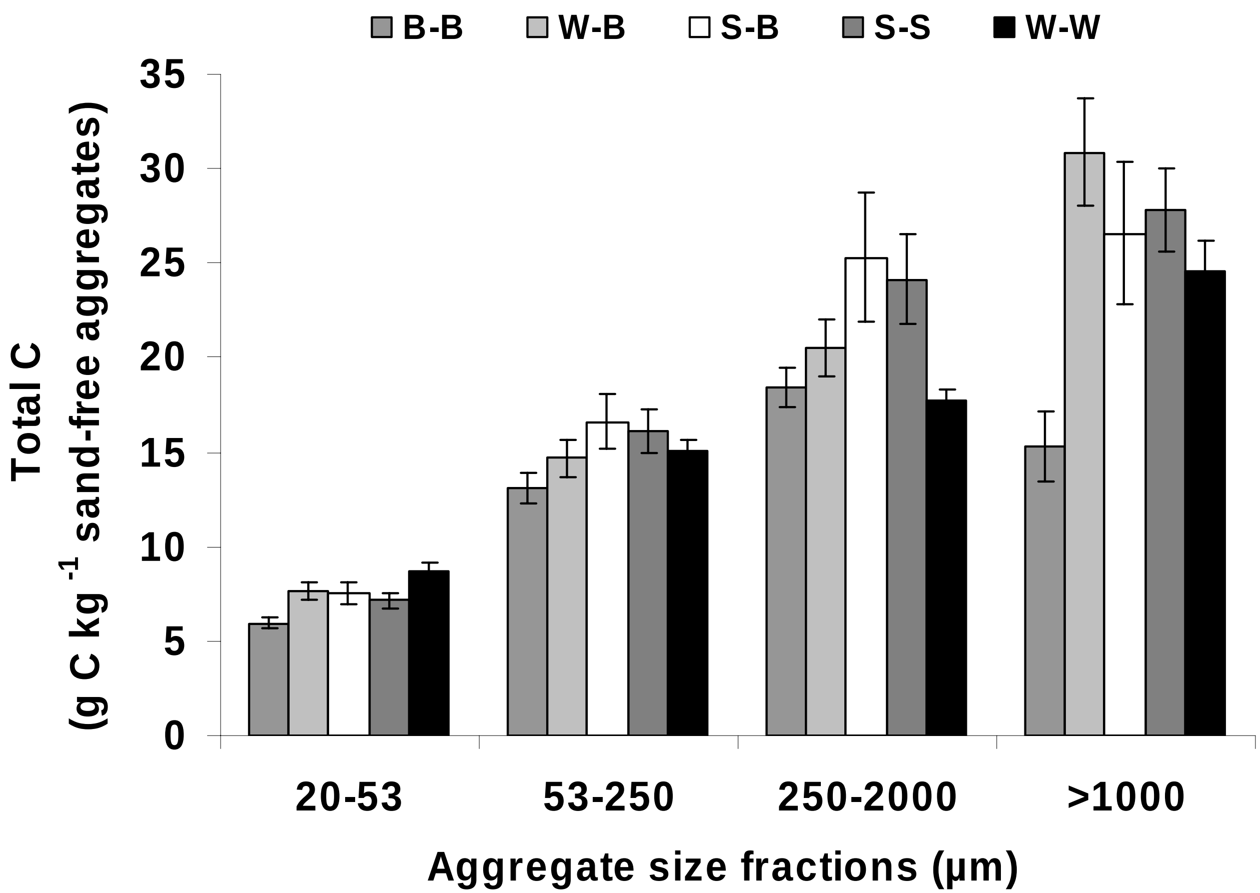

Figure 3-11. Total C normalized to sand-free basis in each water stable aggregates affected by crop rotation in Manhattan experiment. Continuous wheat (W-W), wheat-soybean (W-B), continuous sorghum (S-S), sorghum-soybean (S-B), continuous soybean (B-B) rotation. Error bars represent the standard error of the mean (n=4).

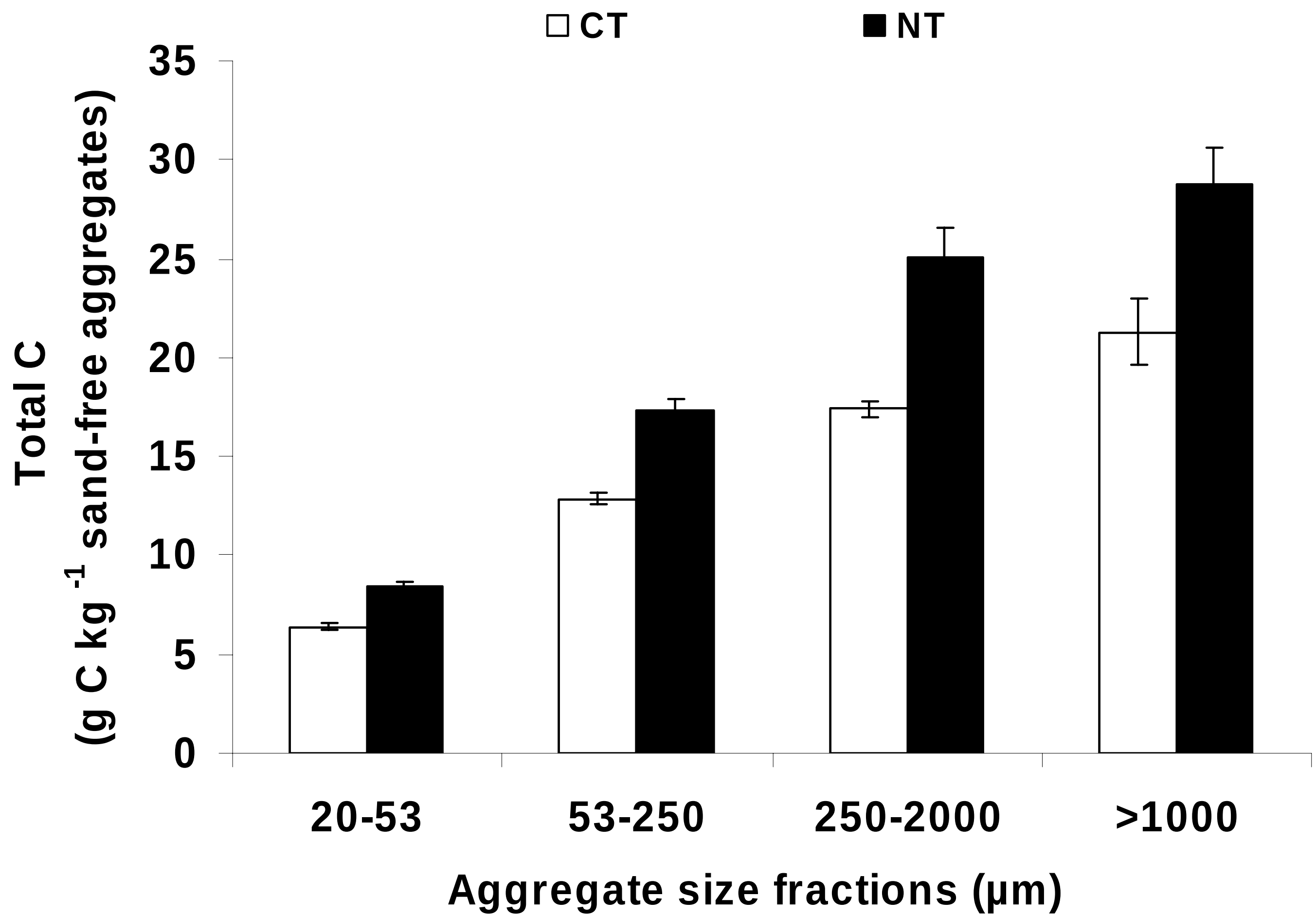

Figure 3-12. Total C normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), and no-tillage (NT) for Manhattan experiment. Error bars represent the standard error of the mean (n=4).

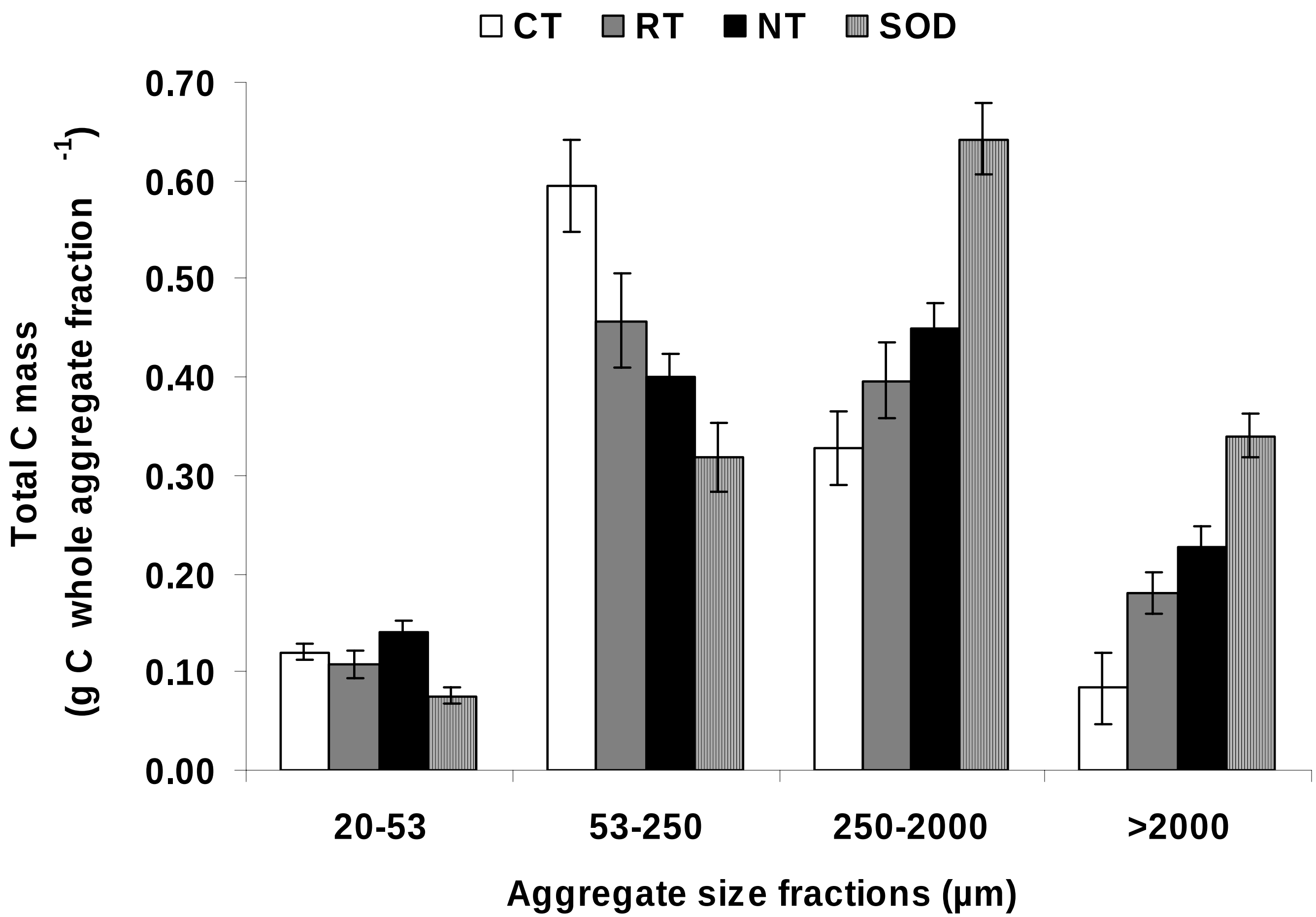

Figure 3-13. Total C mass in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment. Error bars represent the standard error of the mean (n=4).

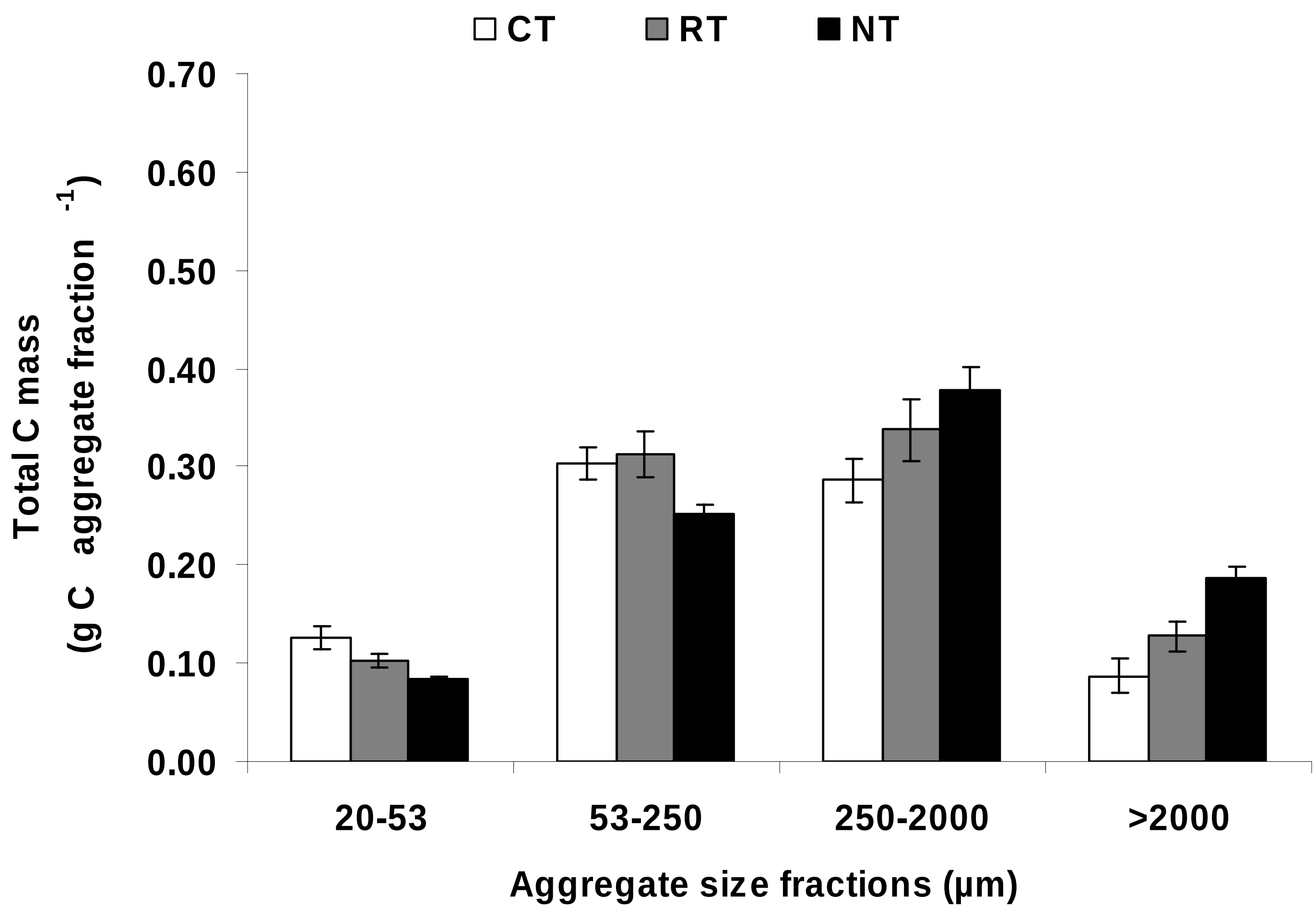

Figure 3-14. Total C mass in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) for Parsons experiment. Error bars represent the standard error of the mean (n=4).

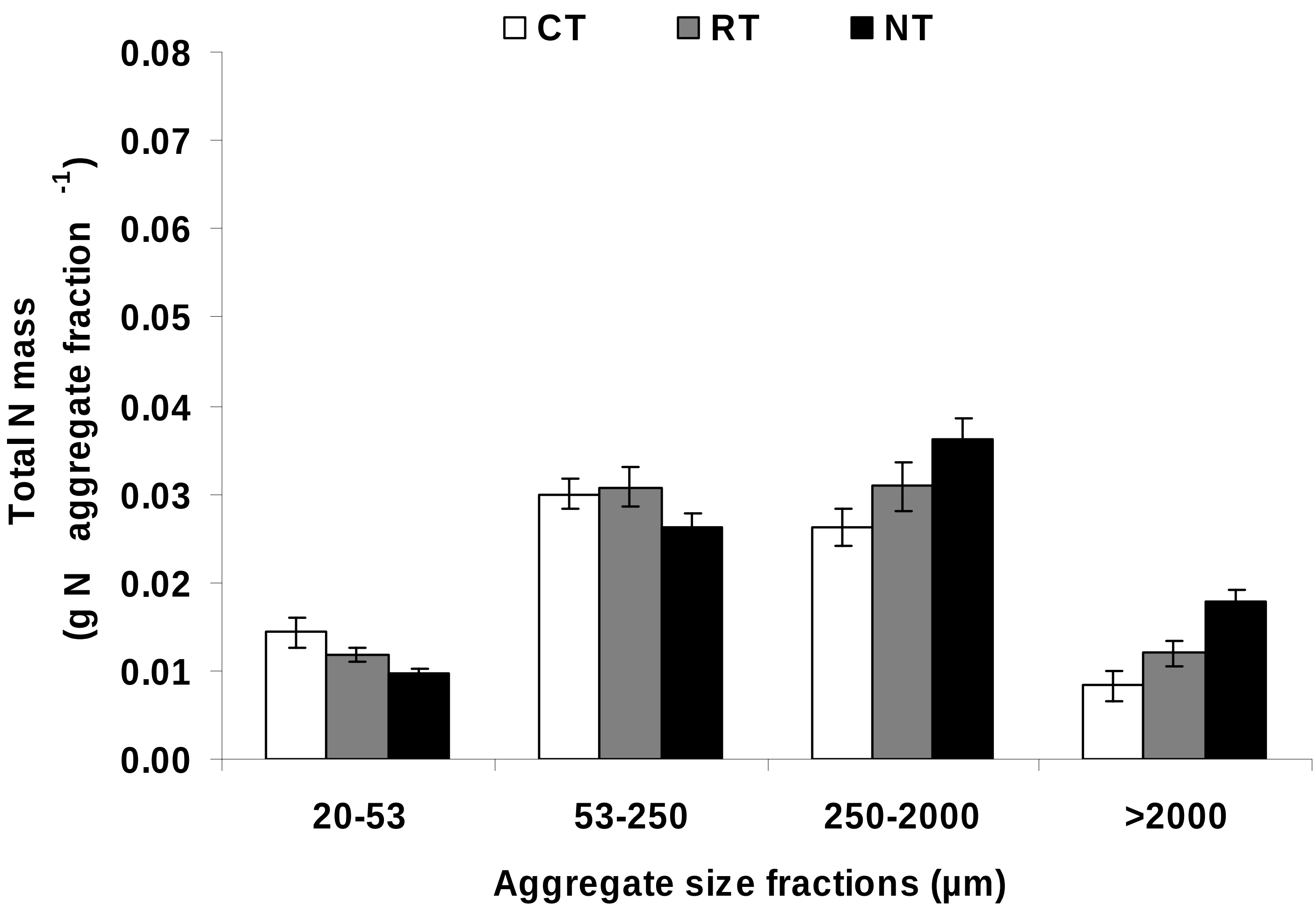

Figure 3-15. Total N mass in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) for Parsons experiment. Error bars represent the standard error of the mean (n=4).

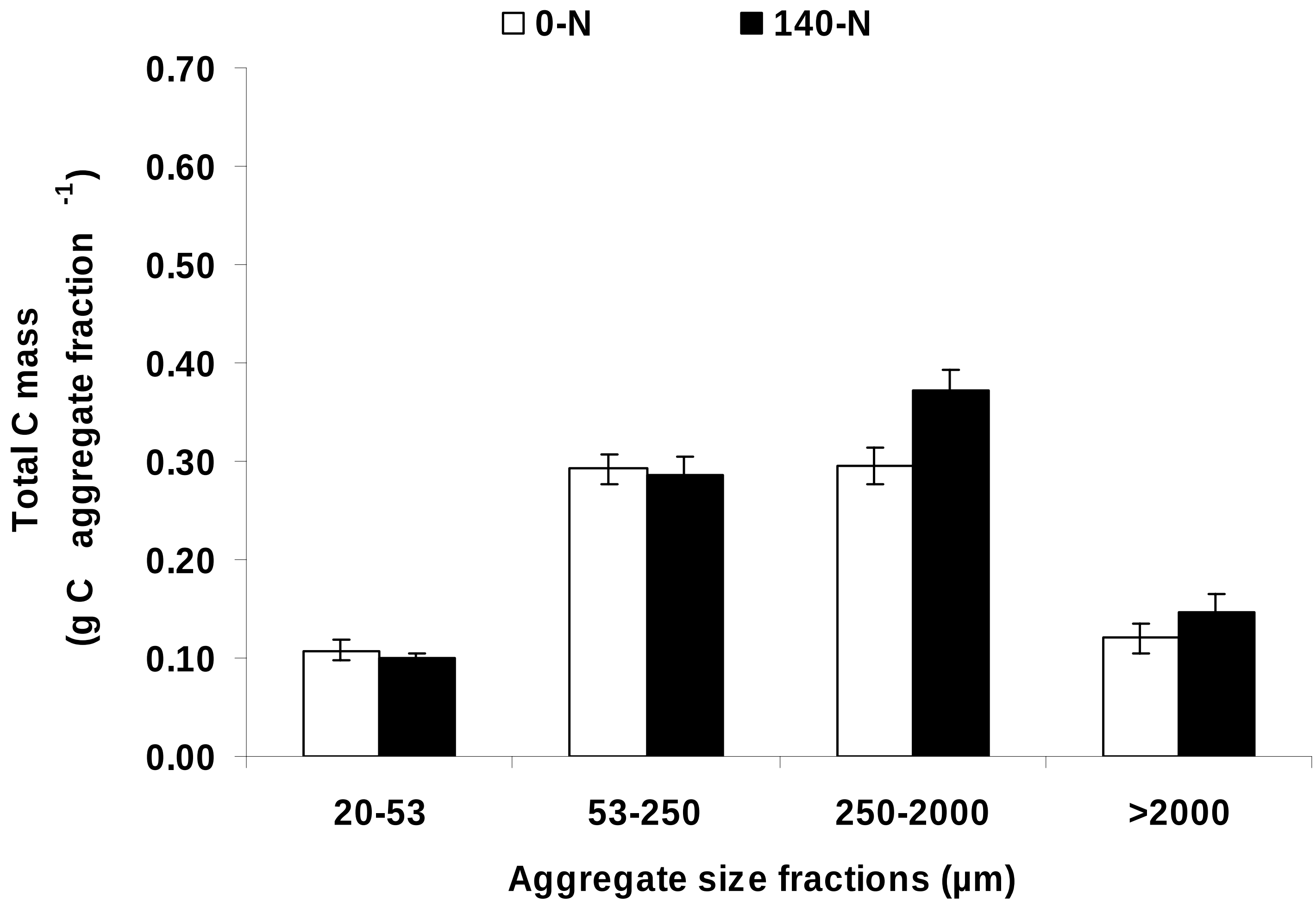

Figure 3-16. Total C mass in each water stable aggregates affected by N application, 0 and 140 kg N ha^{-1}, 0-N and 140-N, respectively for Parsons experiment. Error bars represent the standard error of the mean (n=4).

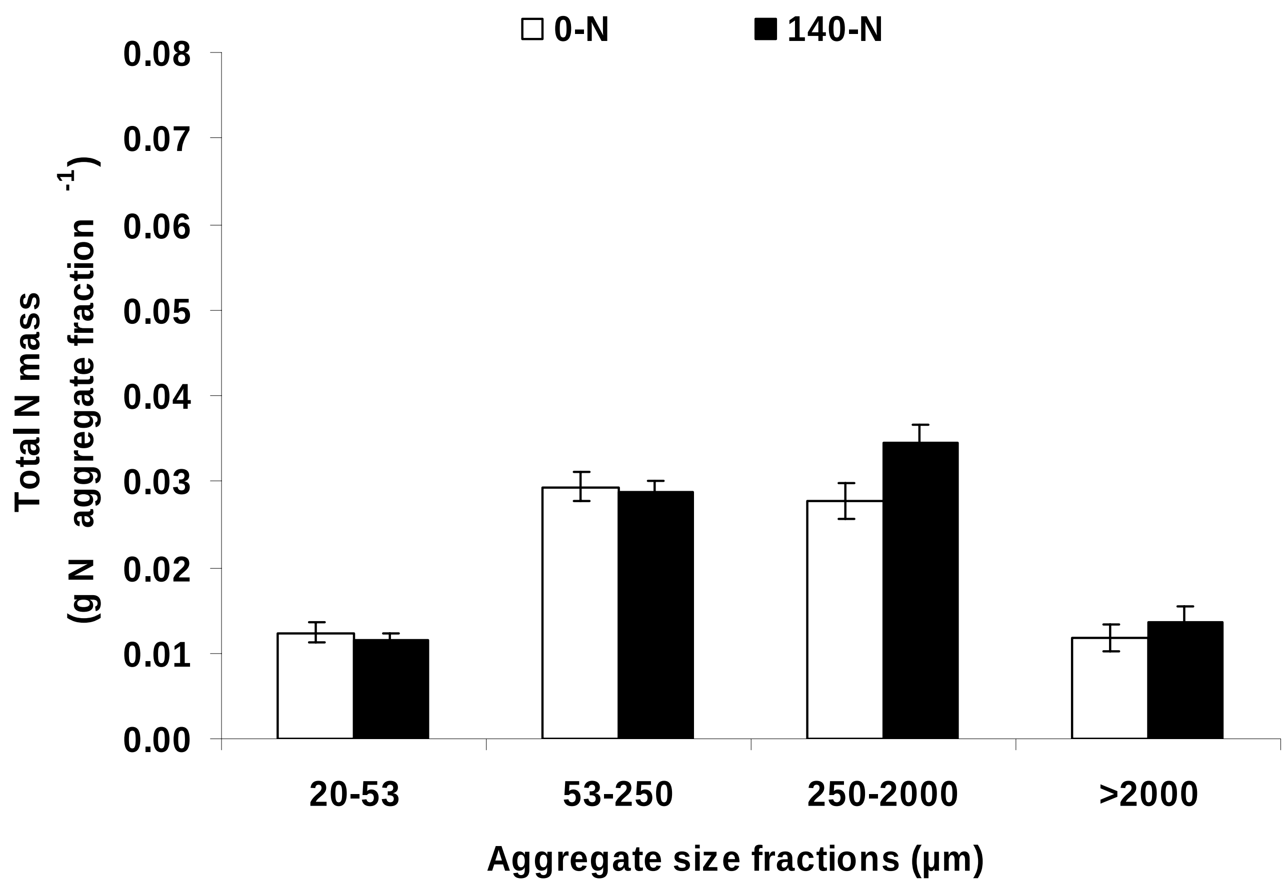

Figure 3-17. Total N mass in each water stable aggregates affected by N application, 0 and 140 kg N ha^{-1}, 0-N and 140-N, respectively for Parsons experiment. Error bars represent the standard error of the mean (n=4).

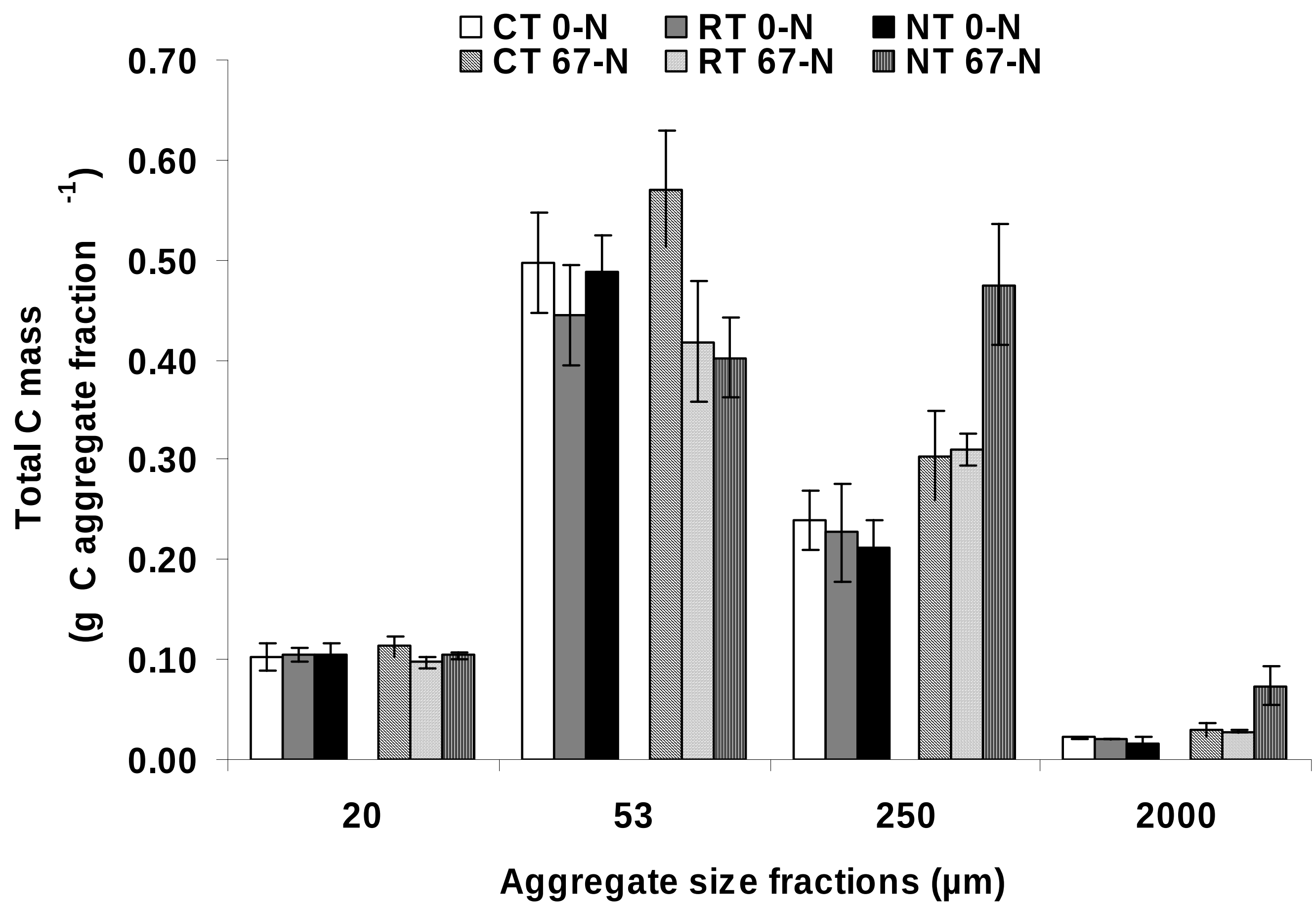

Figure 3-18. Total C mass in each water stable aggregates under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} in Hays experiment. Error bars represent the standard error of the mean (n=4).

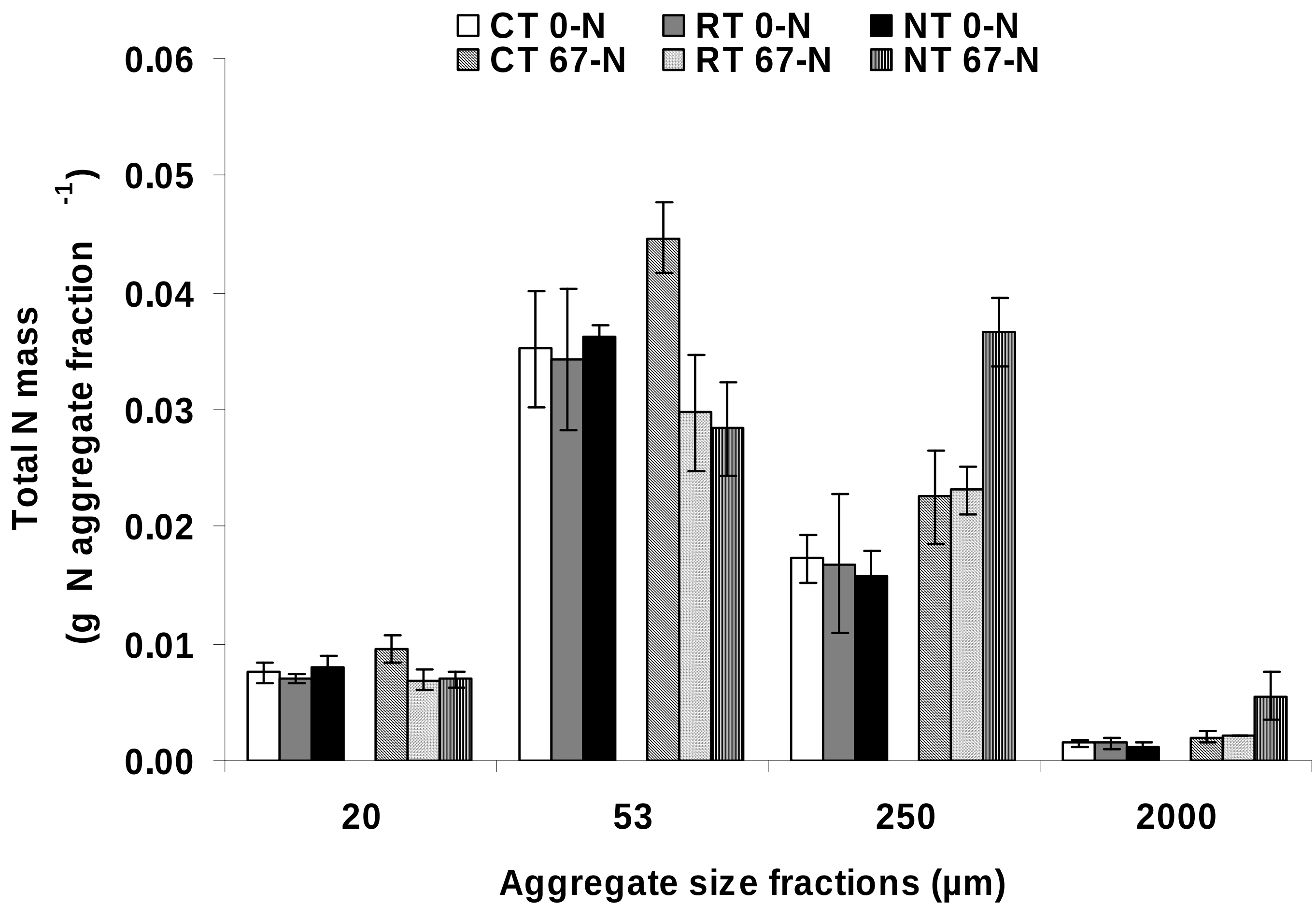

Figure 3-19. Total N mass in each water stable aggregates under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} in Hays experiment . Error bars represent the standard error of the mean (n=4).

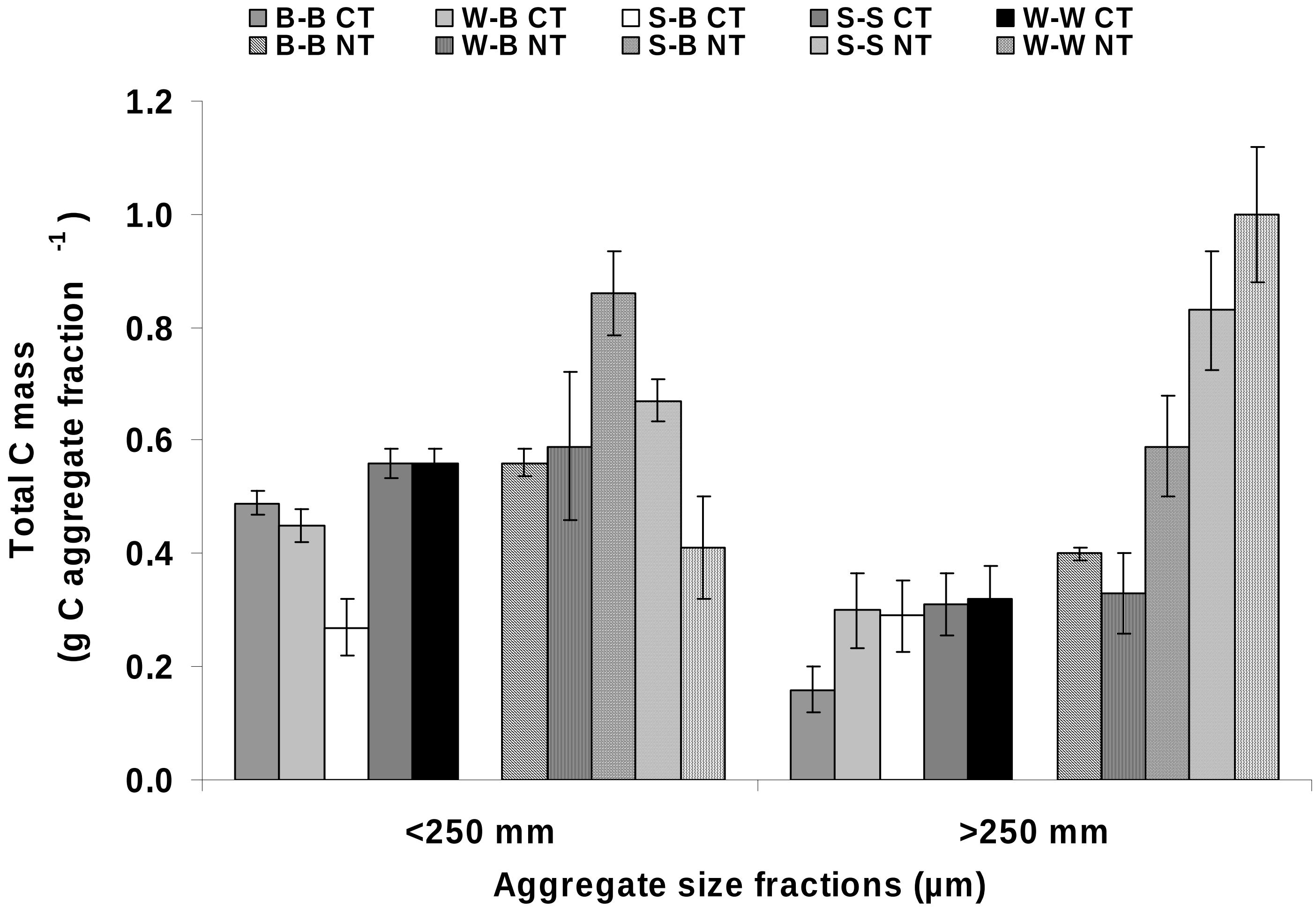

Figure 3-20. Total C mass in each water stable aggregates under conventional till (CT), reduced till (RT), no-tillage (NT) in five crop rotations: continuous wheat (W-W), wheat-soybean (W-B), continuous sorghum (S-S), sorghum-soybean (S-B), and continuous soybean (B-B) for Manhattan experiment. Error bars represent the standard error of the mean (n=4).

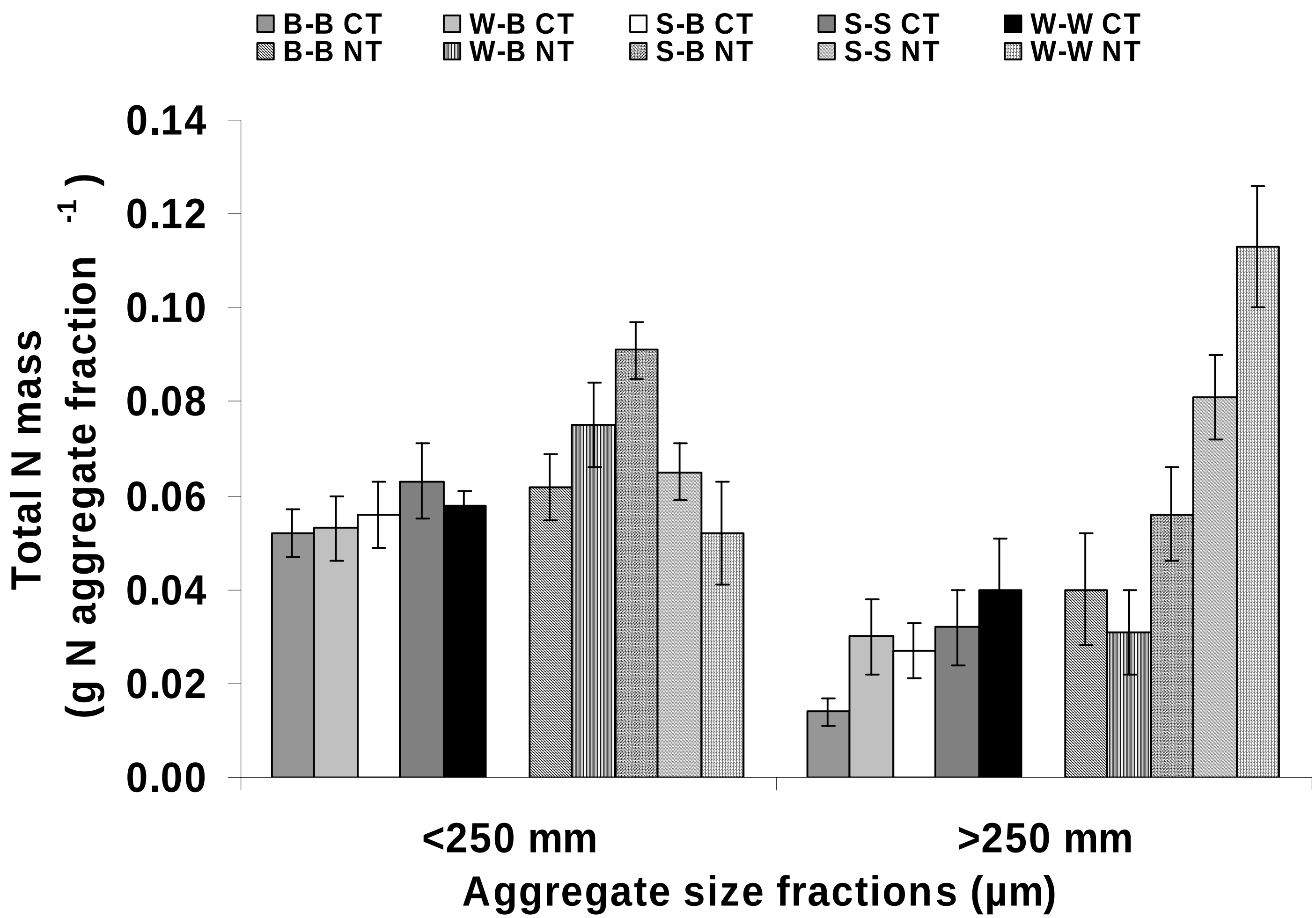

Figure 3-21. Total C mass in each water stable aggregates under conventional till (CT), reduced till (RT), no-tillage (NT) in five crop rotations: continuous wheat (W-W), wheat-soybean (W-B), continuous sorghum (S-S), sorghum-soybean (S-B), and continuous soybean (B-B) for Manhattan experiment. Error bars represent the standard error of the mean (n=4).

CHAPTER 4 - SOIL ORGANIC CARBON AND NITROGEN POOLS IN AGRICULTURAL MANAGEMENT SYSTEMS

ABSTRACT

Soil organic matter (SOM) is important for sustaining soil quality. Management practices such as crop rotation, tillage, and fertilization can influence soil biological activities through their effects on the quantity, structure, and distribution of soil organic carbon (SOC). The objective of our study was to determine the influence of different long-term management practices (tillage, N fertilization, and crop rotations) in different locations in Kansas on SOC and N pools. Three long-term experiments were sampled. All the sites evaluated (Tribune, Manhattan, and Hays) included three tillage systems: conventional tillage (CT), reduced tillage (RT) and no-tillage (NT). Nitrogen fertilization was evaluated at Hays (0, and 67 kg N ha^{-1}). Crop rotations were continuous wheat (W-W) and wheat-soybean (W-B) at Manhattan. Soil samples were taken at 0-5 and 5-15 cm depth and analyzed for total, mineralizable, and microbial biomass C and N (MBC and MBN, respectively). The impact of management practices on MBC and MBN were reflected at the soil surface (0-5 cm). At Hays, MBC was greater in the 0-N than in the 67-N treatments. No-tillage presented the lowest MBN concentration in the control treatment. Cultivation reduced MBC, MBN, and mineralizable C and N compared with native prairie sod at Tribune site. At Manhattan, NT in the W-B rotation had the greatest MBC and N compared with CT and RT within the same rotation, and with all the tillage on the W-W rotation. At Hays, mineralizable C and N were greater in the NT systems with N application at 0-5 cm. At Tribune, mineralizable C and N were similar between NT and RT but greater than CT. At Manhattan no differences were found in mineralizable N, but mineralizable C was greater under W-B RT and W-W CT than W-W NT and W-W RT. The C_0:N_0 ratio was lower under NT and RT than under CT. The mineralizable pools, C_0 and N_0 represented on average 25, 23, and 32% of the total C and 17,11, and 11% of the TN for Hays, Tribune, and Manhattan, respectively.

Keys words: microbial biomass, potentially mineralizable C and N, tillage, fertilization, crop rotation.

INTRODUCTION

The atmospheric greenhouse gases (GHG) which include CO_2, N_2O, and CH_4 have increased over the last 150 years creating concern of anthropogenic-driven global climate change. Climate change affects agriculture through impacts on water resources nutrient cycles, and pest cycles (CAST, 2004). Agriculture can be a source of GHG through changes in land use, tillage, crop residue removal and burning, and livestock management. However, agriculture can also be a sink for CO_2 through improvement of soil and crop management practices such as reduced tillage intensity, a reduction in bare fallow period, intensive crop rotations, use of winter cover crops, and water management (Lal et al., 1998; Paustian et al., 2000; West and Post, 2002; Lal, 2004; Post et al., 2004).

Soil organic carbon (SOC) is a central element of soil quality, plant productivity, biodiversity, and sustainability (Lal et al., 1997; Rice, 2002). Soil organic C also is central to soil structure (Tisdall and Oades, 1982; Karlen et al., 1994; Chenu et al., 2000; McVay et al., 2006).

Because of the importance of soil organic C, research efforts have been directed to the understanding of the complexity and formation of stabilized soil C. Several studies report that different fractions of SOC could better reflect changes due to management than total SOC (Cambardella and Elliot, 1992; Wander et al., 1994). Often SOC is partitioned into three or more compartments or pools. Paul and Clark (1996) partitioned SOC into active, slow, and recalcitrant pools. These pools are often used for modeling, including CENTURY (Parton et al., 1987; Falloon and Smith, 2002) and RothC (Coleman and Jenkinson, 1996; Coleman et al., 1997; Falloon and Smith, 2002). The active pool is comprised of microbial biomass and labile organic compounds that have a rapid turnover time (less than 1 yr), and represents less than 5% of SOC. The slow pool, representing up to 20-40% of the total organic C, consists of plant and microbial byproducts and some resistant C with a turnover time of decades. Finally, the recalcitrant pool makes up to 60-70% of the total organic C and is material that is difficult to degrade and contains humic and fulvic acids (Paul and Clark, 1996). Carbon in the recalcitrant pool has a turnover time of hundreds to thousands of years.

Microbial biomass participates in different processes including nutrient transformations, which are essential for plant nutrient availability (Rice et al., 1996). Microbial biomass often is considered a sensitive indicator of changes induced by tillage systems, residue incorporation, N fertilizer management, and crop rotations (Powlson et al., 1987; Rice et al., 1996; McCarty and Meisinger, 1997). Potentially mineralizable C and N may be a good indicator for assessing soil quality as they are sensitive to changes in management practices (Franzluebbers et al., 1994, 1995; Turco et al., 1994; Omay et al., 1997; Needelman et al., 1999).

Changes in management can affect the partitioning of C and N into these different pools, which can then impact soil function. This research is important for evaluating the combined effect of management practices in agricultural systems. Therefore, the objective of this study was to determine the effect of soil management including the interactions of tillage, N fertilization and crop rotation on soil C and N pools.

MATERIALS AND METHODS

Site description

The Hays experiment is located in central Kansas (38º 51'N, 99º 20'W). This experiment was initiated in 1965 on a Harney silt loam soil (fine, smectitic, mesic Typic Argiustoll). The 30-yr average annual precipitation was 533 mm with an annual mean temperature of 11.9 ºC. The crop rotation was wheat-grain sorghum-fallow, with three tillage systems: conventional tillage (CT), reduced tillage (RT), and no-tillage (NT). Conventional tillage consisted of using tools such as disk, one-way plow, and mulch treader. Reduced tillage included V-blade, sweeps or rod weeder. No-tillage consisted of planting directly into the residue. From the beginning of the experiment to 1975, N rates were 0 and 45 kg N ha^{-1}. Since 1975 four N rates were evaluated, 0 (0-N), 22 (22-N), 45 (45-N), and 67 (67-N) kg N ha^{-1} yr^{-1}. Nitrogen fertilizer was applied as ammonium nitrate in the previous fall for June sorghum planting and in August prior to September wheat planting. The experimental design was split-plot with four replications, with tillage as the main plot and N as sub-plot

The Tribune experiment, initiated in 1988, was located in western Kansas (38º 30'N, 101º 41'W). The 30-yr average annual precipitation is 422 mm, with an annual mean temperature of 11.3 ºC. The soil was classified as Richfield silt loam (fine-smectitc, mesic Aridic Argiustolls). The crop rotation was wheat-grain sorghum-fallow, with three tillage systems: CT, RT, and NT. Conventional tillage consisted of three or four operations per year with a sweep plow between crop harvest and planting the next crop. The RT system used a combination of tillage (primarily sweep plow) and herbicides for weed control during fallow. The number of tillage operations with RT was approximately 50% of CT. No-tillage consisted of planting directly into the residue. Nitrogen fertilizer as urea ammonium nitrate (UAN) was broadcast applied at 67 kg N ha^{-1} yr^{-1} to 112 kg N ha^{-1} yr^{-1}, depending on the year or crop. Native sod was included as part of the experimental design, which represented the natural vegetation types of C_3 and C_4 grass, with the dominant species being buffalograss (*Buchloe dactyloides*). The treatments were arranged in a randomized complete block design with four replications.

The Manhattan experiment, initiated in 1974, was located on the Kansas State University Agronomy Farm, Manhattan (Riley County; 39º 07'N, 96º 37'W). Soils were Muir silt loam (fine-silty, mixed, mesic Cumulic Haplustoll) and Reading silt loam (fine, mixed, mesic Typic Argiudoll). The 30-yr average annual precipitation was 813 mm, which was mainly concentrated in the spring-summer period, with an annual mean temperature of 11.3 ºC. Crop rotation and tillage systems were evaluated in this experiment. The experimental design was split-plot with four replications, with rotation as the main plot and tillage as sub-plot. The three crops, soybean (B) *(Glycine max (L.) Merrill)*, grain sorghum (S) *(Sorghum bicolor (L.) Moench)*, and winter wheat (W) *(Triticum aestivum L.),* were combined in five rotations: continuous sorghum (S-S), sorghum-soybean (S-B), continuous soybean (B-B), wheat-soybean (W-B), and continuous soybean (B-B). The three tillage treatments were CT, RT, and NT systems. Conventional tillage included chisel, disk, and field cultivator. Reduced tillage included disk and field cultivator. No-tillage consisted of planting directly into the residue and chemical weed control. A blend of urea and diammonium phosphate fertilizer providing 112 kg N ha^{-1} and 11.3 kg P ha^{-1} was broadcast applied prior to the last tillage operation before planting of each crop and year.

Soil Sampling

Soil samples were taken from each plot at 0 to 5 and 5 to 15 cm depths. A sterile polypropylene bags (3.78 L) were filled with soil samples collected randomly from each plot using a 2-cm diam. Oakfield soil-probe (Forestry Suppliers, Inc., Jackson, MS). For Hays, samples were collected March 2003 (before planting) on the sorghum rotation phase of the experiment. For Tribune, samples were collected April 2004 on the sorghum rotation phase and native prairie. For Manhattan, samples were taken May 2004 before wheat harvesting. All soil samples were passed through a 4-mm sieve, roots removed, and the soil stored at 4ºC until further analyzed as described in the following sections.

Soil Microbial Biomass

Soil microbial biomass was determined using the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976). Soil (25 g) was added to duplicate 125 mL Erlenmeyer flasks. The gravimetric soil water content was adjusted to 0.25 g H_2O g soil^{-1} and pre-incubated for 5 d at 25ºC. After pre-incubation, one flask was fumigated with chloroform in a vacuum dessicator. After 18 to 24 h, the dessicator was evacuated to remove residual chloroform and then the flasks were placed in 950 mL mason jars and incubated for 10 days at 25 ºC. After 10 days, headspace CO_2-C concentration was determined by gas chromatograph (Shimadzu Gas Chromatograph-8A, Kyoto, Japan) equipped with a thermal conductivity detector (TCD) and a 2-m Porapak column. The column temperature was 70ºC and the carrier gas was He at a flow rate of 14 mL min^{-1}. Soil inorganic N was determined by adding 100 mL 1 *M* KCl to the flask, shaken for 45 min at 300 RPM on an orbital shaker followed by filtration through Whatman No. 2 filter paper (Fisher Scientific, Fair Lawn, NJ). The soil extracts were analyzed for NH_4^+-N and NO_3^--N on an Alpkem Autoanalyzer (Alpkem Corp., Bulletin A303-S021 and A303-S170). Microbial biomass C (MBC) and N (MBN) were calculated as suggested by Voroney and Paul (1984):

$$\text{Microbial biomass C} = \frac{(C_f - C_{unf})}{0.41}$$

$$\text{Microbial biomass N} = \frac{(N_f - N_{unf})}{k_N}$$

where C_f = CO_2-C evolved from the fumigated samples

C_{unf} = CO_2-C evolved from the unfumigated samples

N_f = NH_4^+ + NO_3^- from fumigated samples

N_{unf} = NH_4^+ + NO_3^- from unfumigated samples

$K_N = (-0.041\ C_f/N_f) + 0.39$

Mineralizable C and N

Mineralizable C and N were determined by laboratory incubation following the method proposed by Cabrera and Kissel (1988) as modified by Garcia (1992). Soil samples collected from the 0 to 5 and 5 to 15 cm were sieved through a 4-mm sieve, packed into PVC cores (5 cm diam. by 10 cm height) to a bulk density of 1.0 Mg m^{-3}. Cores were placed in a 960 mL mason jars equipped with a rubber septum on the lid and incubated at 35 ºC. Water was added to the jar to maintain soil water content through the incubation.

For mineralizable N, soil samples were leached with 300 mL of 0.01 M $CaCl_2$ during the first 100 days, and 150 mL afterwards due to slow infiltration. Leaching was done every week for a month, biweekly for another month and monthly thereafter. The NH_4^+-N and NO_3^--N concentrations in the leachate were determined as described earlier. After leaching, an N-free nutrient solution as described by Garcia (1992) (50 mL) was added to each core and a vacuum of –0.033 MPa applied for 6 h to adjust to constant water content. Between leaching events, the cores were placed in 950-mL Mason jars and incubated at 35ºC.

For mineralizable C, CO_2-C evolved from the cores was determined with 0.5 mL gas samples taken from the headspace of the mason jars and injected to a gas chromatograph (Shimadzu Gas Chromatograph-8A, Kyoto, Japan) equipped with a thermal conductivity detector (TCD) and a 2-m Porapak column. The column temperature was 70ºC and the carrier gas was He at a flow rate of 14 mL min^{-1}.

Carbon and N mineralization were described by first order kinetics. The Marquardt option NLIN, a nonlinear curve fitting procedure model (SAS Institute Inc., 2002) was used to fit a one-pool model (Stanford and Smith, 1972) to determine cumulative C and N mineralization with time. The model is:

$$C_m \text{ or } N_m = C_0 \text{ or } N_0 \, [1 - \exp(-kt)]$$

where C_m = mineralized C in μg CO_2-C g^{-1} soil

N_m = mineralized N in μg N g^{-1} soil

C_0 = potentially mineralizable C in μg CO_2-C g^{-1} soil

N_0 = potentially mineralizable N in μg N g^{-1} soil

k = rate constant of mineralization in day^{-1}

t = time in days

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.

RESULTS

Soil Microbial Biomass

At Hays, there was a significant Nitrogen x Depth interaction for MBC; with greater MBC with the 0-N treatment at 0-5 cm (Table 4.1, Fig. 4.1). Microbial biomass N was significantly affected by the interaction between Tillage x Nitrogen x Depth (Table 4.2). At 0-5 cm, CT and NT without N (0-N treatment) had higher levels of MBN compared with the other treatments. At the deeper depths, no differences were found among treatments, except NT 0-N that had higher MBC at 15-30 cm (Table 4.2, Fig. 4.2).

At Tribune, MBC was significantly affected by tillage and depth (Table 4.3). The native prairie had significantly greater MBC than the cropped systems. Conventional tillage had greater MBC than RT but similar to NT, which did not differ from RT. There was higher MBC at 5-15 cm than at 0-5 and 15-30 cm (Table 4.3). Microbial biomass N had a significant Tillage x Depth interaction (Table 4.4), where no differences in MBN was detected among tillage systems at 0-5 and 5-15 cm. At 15-30 cm NT and CT had higher or similar MBN than RT (Table 4.4). Microbial biomass N was higher in the native prairie at all depths.

At Manhattan, there was a significant 3-way interaction (Rotation x Tillage x Depth) for MBC and MBN (Table 4.5 and 4.6). At 0-5 cm, MBC was significantly greater under the W-B NT treatment than all the other treatments (Table 4.5). No differences among tillage systems were found at deeper depths, but on average the W-B rotation had higher MBC than the W-W rotation. The highest values of MBN occurred in the W-B under NT and RT at 0-5 and 15-30 cm.

Carbon and N Mineralization

At Hays, C_0 and N_0 were significantly greater with NT and N fertilizer at 0-5 cm (Table 4.7) with no difference among treatments at 5-15 cm (Table 4.8). Cumulative C and N mineralized curves are presented in the appendix (Fig. B.1 to B.8) At 0-5 cm, K_c was similar among tillage systems with N fertilizer, but was higher under NT and CT than RT without N (Table 4.7). Fertilizer resulted in significantly higher K_n (Table 4.7), indicating a faster N mineralization rate

At Tribune, C_0 and N_0 were significantly greater, 40% and 44%, respectively, in the native prairie than the tillage systems at 0-5 cm (Table 4.9). Cumulative C and N mineralized curves are presented in the appendix (Fig. B.9 to B.16). Among tillage, C_0 and N_0 were similar in NT and RT but significantly greater than CT. At 5-15 cm, C_0 was similar between prairie and NT but significantly greater than RT and CT (Table 4.10). This indicates that the loss of mineralizable C was confined to the surface 5 cm and thus preserved mineralizable C deeper in the soil profile. For N_0, no differences were found among tillage but were significantly lower than native prairie. The prairie K_c was greater than the tillage systems. Conversely, K_n was significantly greater under CT than RT, NT and prairie (Table 4.9). No differences were found on the K_n values at 5-15 cm; however, K_c was significantly lower under NT than the other treatments.

At Manhattan, C_0 and N_0 were significantly affected by the Rotation x Tillage interaction at 0-5 cm (Table 4.11). Cumulative C and N mineralized curves are presented in the appendix (Fig. B.17 to B.24). Mineralizable C was similar among tillage in the W-B rotation, but was lower under RT or NT in the W-W rotation. At 5-15 cm, CT and RT in the W-W and CT in the W-B rotation had greater C_0 values (Table 4.12). At 0-5 cm K_c was greater under RT and NT in the W-W and W-B rotation, respectively (Table 4.11) with no differences at 5-15 cm. No differences were found among treatments for N_0 at 0-5 cm (Table 4.11). Potentially mineralized N and K_n were affected by tillage, in which CT and RT had significantly greater N_0 ($P<0.10$) than NT, but CT had significantly greater k_n ($P<0.10$) than NT and RT (Table 4.12).

Relationships among C_0, N_0, microbial biomass, Total C and Total N

At Hays, the MBC:C_0 ratio was significantly higher in CT and RT than in NT. Across tillage, MBC:C_0 was significantly higher under 0-N compared to 67-N treatment. The MBN:N_0 ratio was similar among tillage at the 0-N rate, but lower under NT than CT and RT at the 67-N rate. At 5-15 cm, the C_0:N_0 ratio was greater under CT than NT; and the MBN:N_0 ratio under 0-N rate was greater than 67-N rate (Table 4.14). The microbial pool represented 2-3% of the Total C and 1-6% of the Total N (Fig. 4.3, 4.4). The control treatments (0-N) had a greater percentage of microbial C and N than the fertilized treatments (67-N) (Fig. 4.3, 4.4). The mineralizable pool represented 20% to 12% of the Total C and 16% to 8% of the Total N, at 0-5 and 5-15 cm, respectively. The proportion of mineralizable C and N to the total was significantly greater in NT with N fertilizer at 0-5 cm (Table 4.13; Fig. 4.3, 4.4). Conversely, the recalcitrant pool was significant lower for the NT 67-N than the other treatments. The recalcitrant pool represented 66% and 84% of the Total C and 78% and 88% of the Total N at 0-5 and 5-15 cm respectively.

At Tribune, cultivation affected the relationships between pools (Table 4.15 and 4.16). At 0-5 cm, the C_0:N_0 ratio was significantly higher under CT than NT systems. The MBC:C_0 ratio was similar under CT and native prairie but significantly greater than RT and NT. The MBN:N_0 ratio was greater under native prairie than the cropped systems. The microbial pool represented < 2 % of the total C, and <5% of total N (Figure 4.5, 4.6). The proportion of microbial C pool of the total was similar under NT and RT, but lower than CT and native prairie at 0-5 cm. However, the proportion of microbial N pool of the total was significantly greater under native prairie than the tillage systems at both depths. The mineralizable pool accounted for up to 27% % of the total C; and 10% of the total N. A significant lower proportion of mineralizable C was found for CT at 5-15 cm. The native prairie had the greatest proportion of mineralizable N compared with cropped systems. The recalcitrant pool represented 80 % of the total C, and 86% of the total N. The proportion of recalcitrant C was similar among treatments at 0-5 cm, but greater under CT at 5-15 cm. A significantly lower proportion of recalcitrant N was found in the native prairie than the tillage systems at 0-5 and 5-15 cm.

For Manhattan, the C_0:N_0 ratio was significantly greater under CT than RT and NT systems at 0-5 cm, and greater in the W-W than W-B rotation at 5-15 cm (Table 4.17 and 4.18). At 0-5 cm, the MBC:C_0 ratio was greater under W-B than W-W in the CT, similar among rotations in the RT, and significantly lower under W-W than W-B in the NT systems. The MBN:N_0 ratio was similar among tillage systems in the W-W rotation, but greater under NT in the W-B rotation. The microbial pool accounted for <3 % of the total C and <2.5 % of the total N. The proportion of microbial C and N was significantly greater in the W-S than in W-W rotation, except for microbial N at 5-15 in which no differences were detected (Fig. 4.7, 4.8). The mineralizable pool represented around 30% of the Total C and 10 % of the total N. At 0-5 cm, CT had a greater proportion of mineralizable C than RT and NT systems. The proportion of mineralizable N was greater under W-S than under W-W rotation at 0-5 cm. The recalcitrant pool accounted for 68% of the total C, and 88% of the total N. Conversely, NT systems had a greater proportion of recalcitrant C than RT and CT, and also the proportion of recalcitrant N was greater in the W-W rotation.

DISCUSSION

Differences in soil microbial biomass in response to management could be related to fluctuations in microbial activity due to crop type, sampling time, temperature, moisture and microclimatic conditions (Carter and Rennie, 1982; Doran 1987; Franzluebbers et al., 1994; McCarty et al., 1995; Deng et al. 2000). There was a significant response to N application, in which MBC and MBN were reduced with the application of N fertilizer. Microbial biomass in unfertilized systems has been reported to be higher than with added fertilizer. (Bierderbeck et al., 1984,1994; Omay et al.,

1997) which may be due a greater proportion of dormant cells and retention of N by microbes to degrade higher C/N ratio residue in unfertilized systems (Bierderbeck et al., 1984, 1994; Omay et al., 1997).

Comparing cropping systems, wheat-soybean rotation had greater MBC and N than wheat monoculture, with greater differences under NT. Similar to our results, Franzluebbers et al. (1994, 1995) reported greater MBC with increasing cropping intensity, and the differences were greater under NT than CT systems. Greater soil microbial biomass under reduced tillage may be a result of the accumulation of crop residues near the soil surface and greater soil water content, aggregation and C content compared with CT (Doran, 1987; Balota et al., 2003).

Fertilized NT systems increase SOC and total N and also increase the mineralizable C pool. A similar tendency occurred for soil organic N; however, the changes were not as pronounced as for C. It appears the gain in soil organic C in NT is reflected in the mineralizable pool. This would be expected as plant C is decomposed, the C flows through the microbial biomass into the mineralizable pool (Paul and Clark, 1996). Eventually the C in the mineralizable pool would be expected to be transformed into the recalcitrant pool. This would be desirable as these pools are more stable when considering soil C sequestration. NT also increased in the mineralizable N pool, which is the source for plant available N during the growing season (Mikha et al., 2006; Omay et al., 1997). Several authors reported greater mineralizable N under NT systems (Carter and Rennie, 1982; Liang et al., 2004; Doran, 1980). Greater levels of mineralizable N with no-tillage reflect either greater immobilization, less mineralization, or both, and also lower losses by erosion as compared with plowed soils (Doran, 1987; Franzluebbers et al., 1995; Doran et al., 1998; Needelman et al., 1999).

The introduction of cropping systems in native prairie regardless of tillage systems decreased SOC and total N, as well as microbial biomass and mineralizable C and N. Tillage exposes SOC to microbial activity due to the disruption of soil aggregates (Beare et al., 1994b; Paustian et al., 1997). However, a reduction in tillage intensity, such as in NT or RT systems, reduced the loss of soil C and N through less disturbance and physical protection of the C and N.

Crop rotations affected soil C, where continuous wheat had greater SOC than wheat-soybean rotation. The rotation effect could be related to the residue quality as wheat residues would have a higher C/N ratio and lower turnover rates compared with sorghum and soybean residues (Wright and Hons, 2005a). The effect of crop residue quality also was reflected in the different fractions. Continuous wheat had lower pools of microbial biomass C and mineralizable C but greater recalcitrant C under NT, which could explain the increase in C storage observed under this rotation. The greater C in the recalcitrant fraction indicates that under 29 yr of NT C had been located in the most stables fractions which is important for the long-term storage. However, tillage eliminated the crop effect on C dynamics, as mineralizable C and N and SOC were similar across rotations with CT. The increase in SOC under NT in the continuous wheat could also be related to the effect that crop residue has on aggregation. Wright and Hons (2004, 2005a,b) reported greater soil aggregation with wheat than sorghum and soybean, which they attributed to differences in the amount and quality of the residues.

Residues from cropping sequences including sorghum or soybean are more readily decomposed than wheat residue (Wright and Hons, 2004).

The mineralizable pools, C_0 and N_0, represented on average 23 to 32% of the total C and 11 to 17% of the TN across the three sites. These results are within the range of 5 to 18% reported for potentially mineralizable N (Bonde et al., 1988; Cabrera and Kissel, 1988; Omay et al., 1997) and 20- 29% for potentially mineralizable C (Omay et al., 1997, Rice and Garcia, 1994). The greater proportion of C_0 of the SOC in Manhattan could indicate a faster C turnover compared with the other sites, and could be related with the greater amount of precipitation at this site.

The quantification of these pools is important to understand nutrient dynamics that could lead to mineralization-immobilization of nutrient in short-term, and significant long-term storage of nutrients, and also to the identification of the management practices that would favor the sequestration of C and N, and then the long-term sustainability of the agroecosystems. Overall, our results indicate that no-till systems tend to increase the amount of mineralizable C and recalcitrant pools which would favor a greater C and N stabilization.

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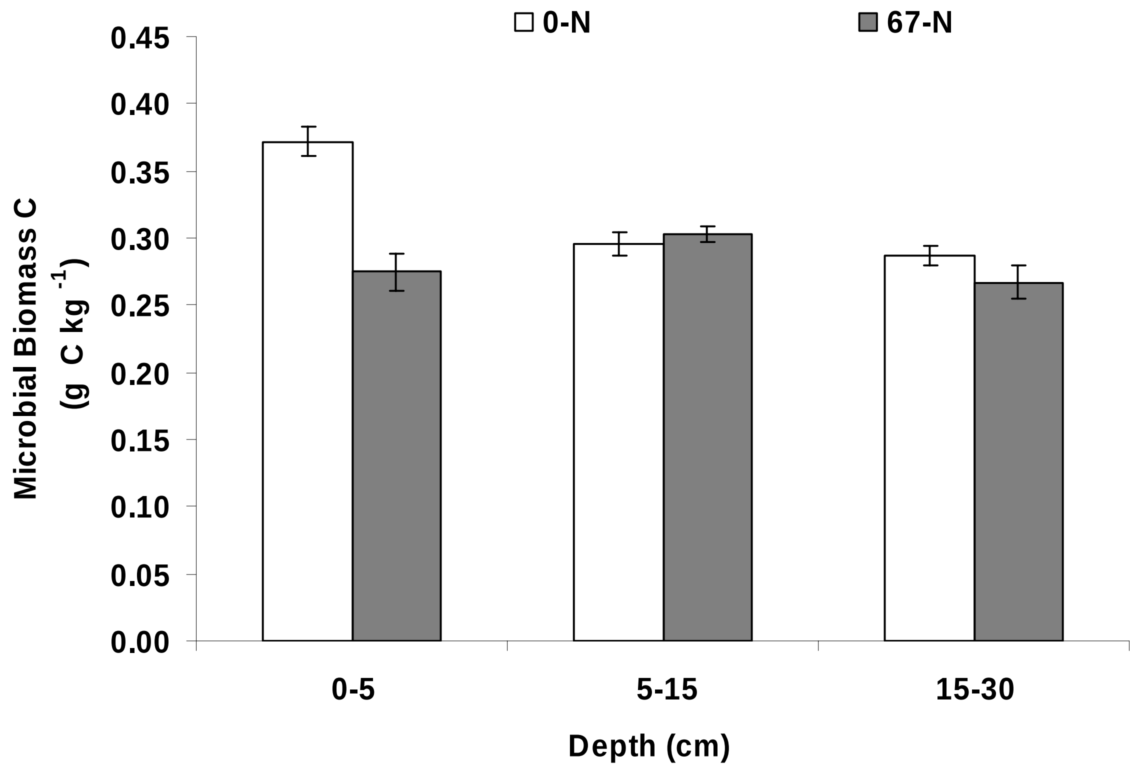

Figure 4-1. Microbial biomass C affected by N application at 0-5, 5-15, and 15-30 cm soil depths in Hays experiment. Error bars represent the standard error of the mean (n=4).

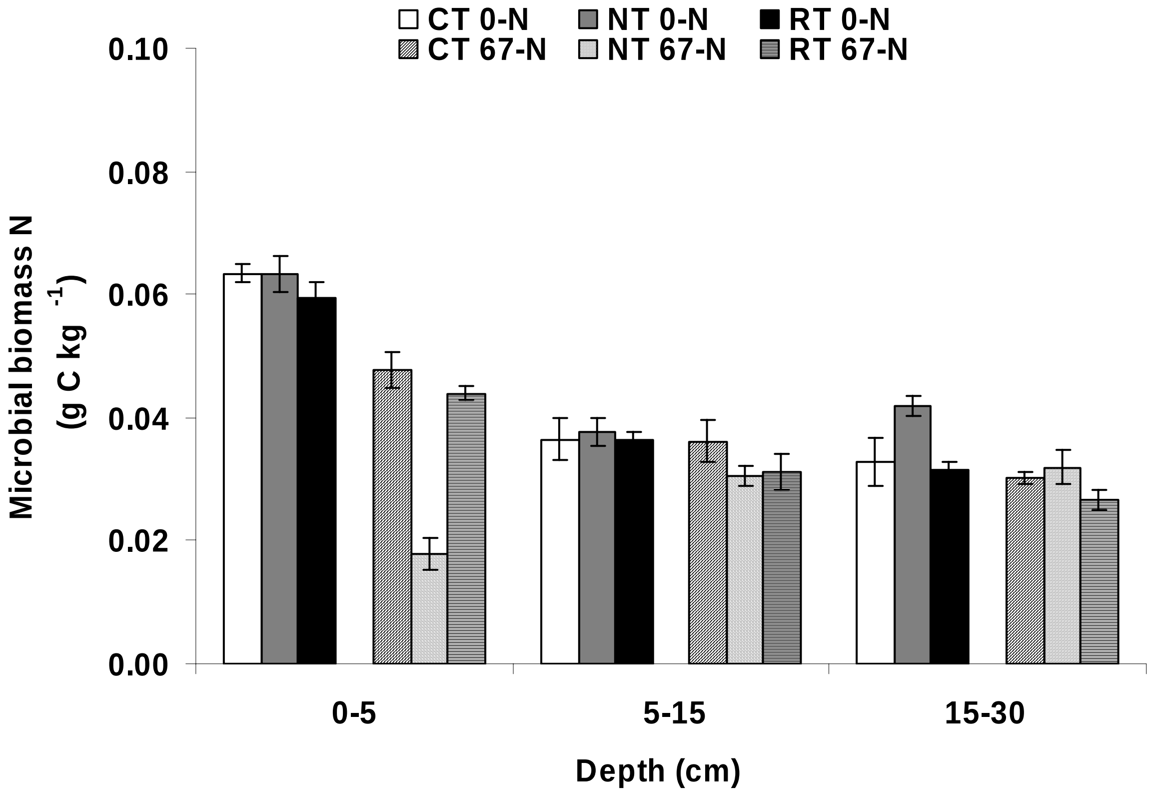

Figure 4-2. Microbial biomass N under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} at 0-5, 5-15, and 15-30 soil depth in Hays experiment. Error bars represent the standard error of the mean (n=4).

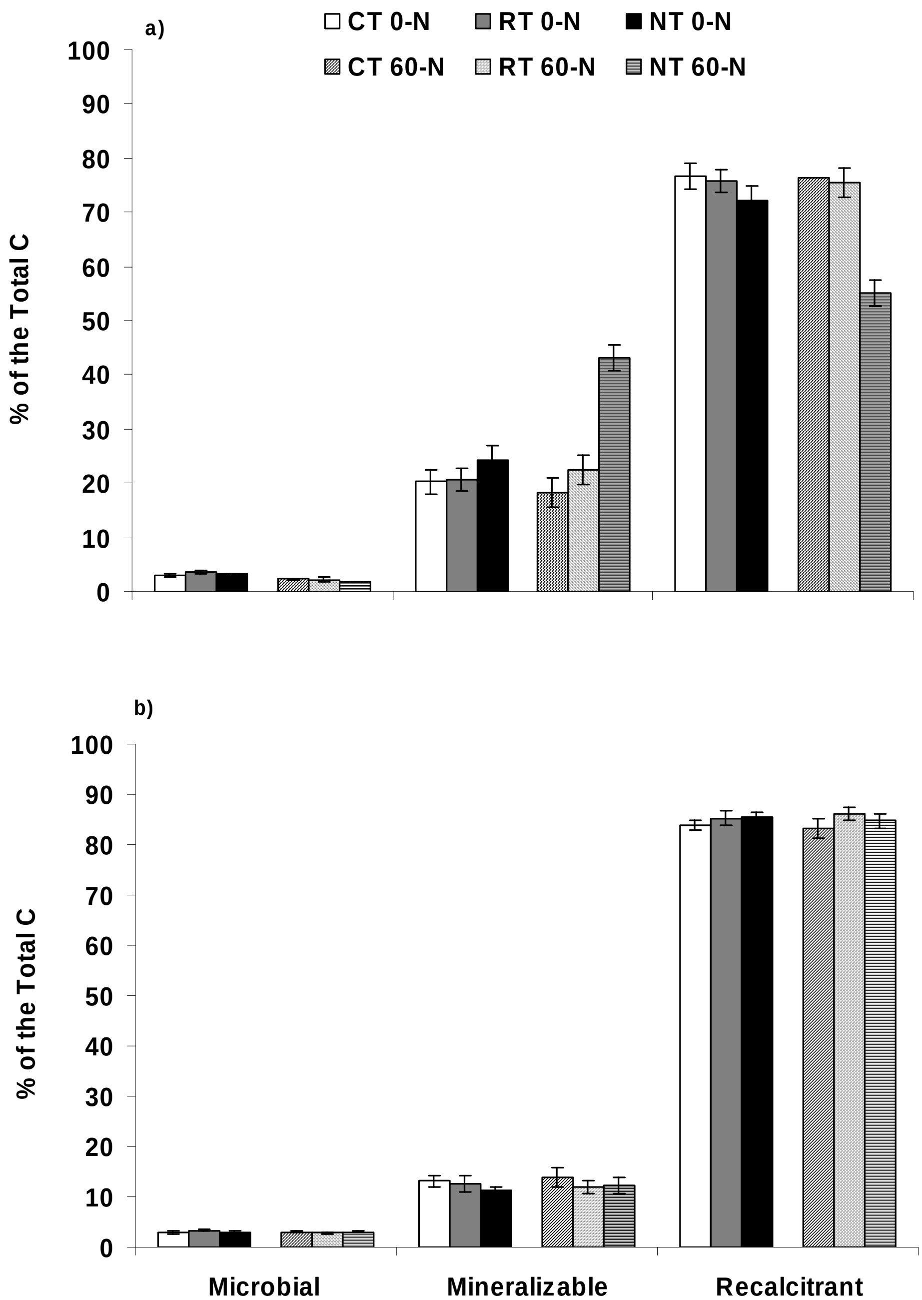

Figure 4-3. Distribution of the soil organic C pools under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} at 0-5 (a) and 5-15 cm (b) in Hays experiment Error bars represent the standard error of the mean (n=4).

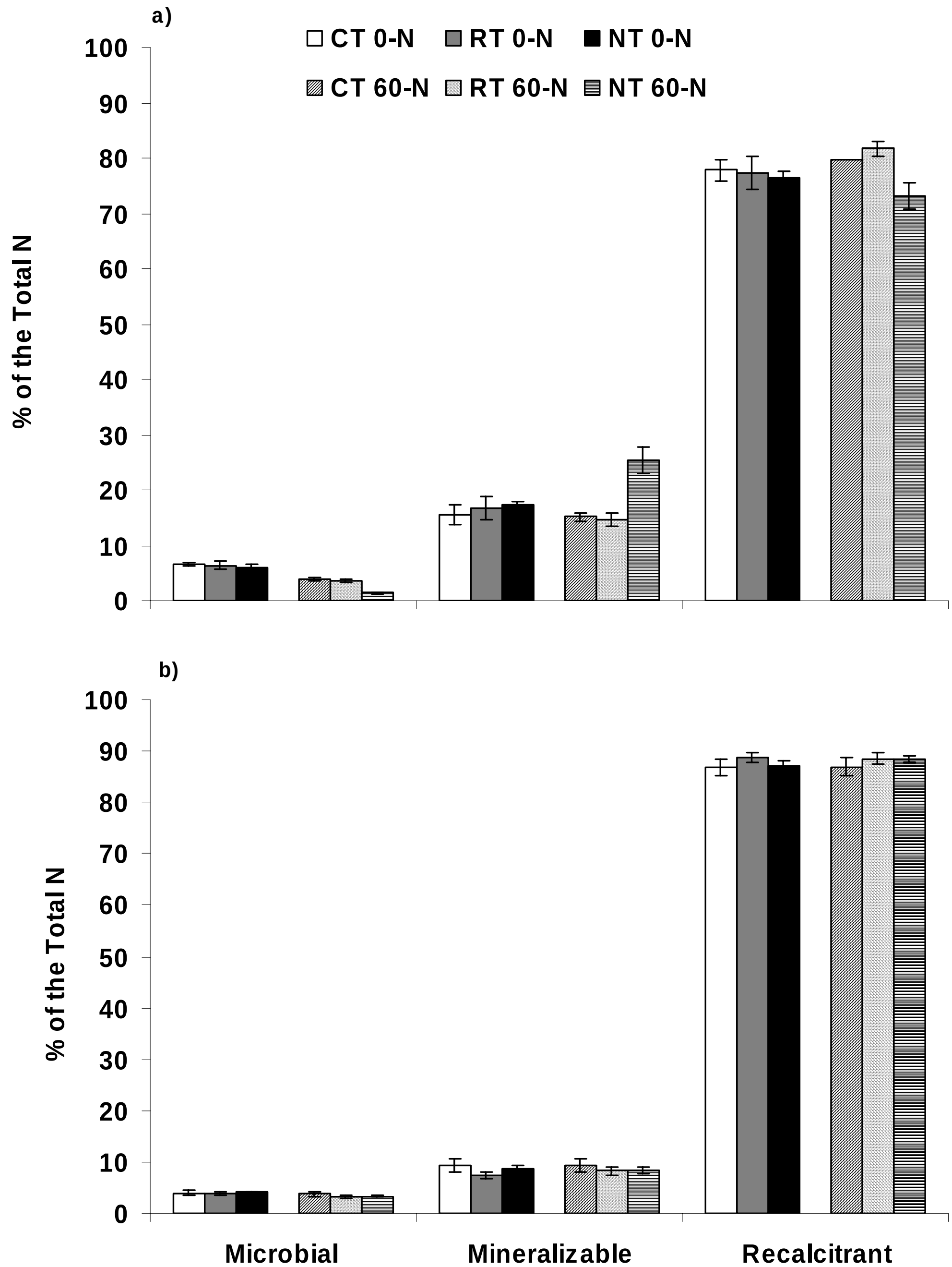

Figure 4-4. Distribution of the soil organic N pools under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} at 0-5 (a) and 5-15 cm (b) in Hays experiment. Error bars represent the standard error of the mean (n=4).

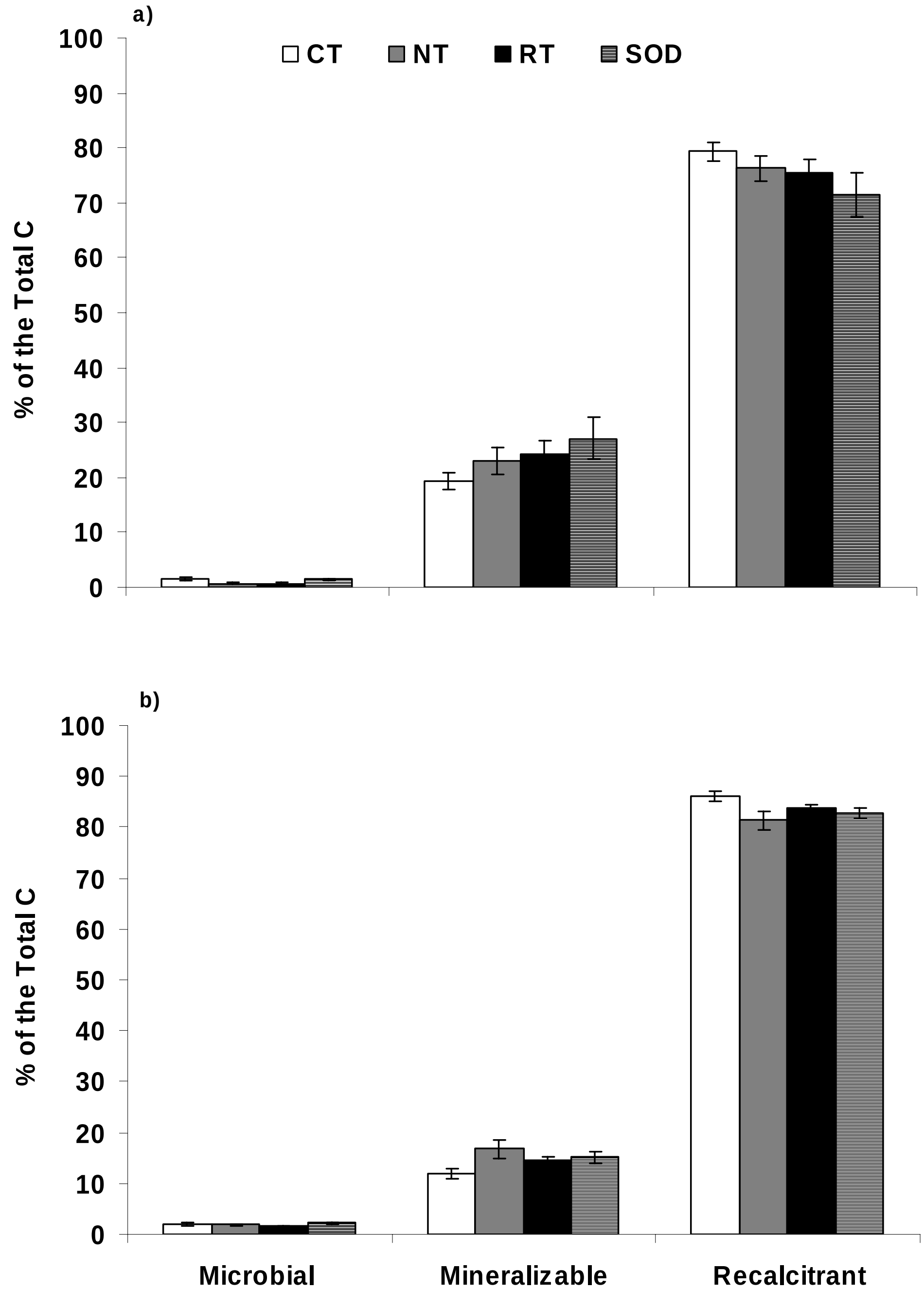

Figure 4-5. Distribution of the soil organic C pools under conventional till (CT), reduced till (RT), no-tillage (NT) and native prairie sod (SOD) at 0-5 (a) and 5-15 cm (b) in Tribune experiment. Error bars represent the standard error of the mean (n=4).

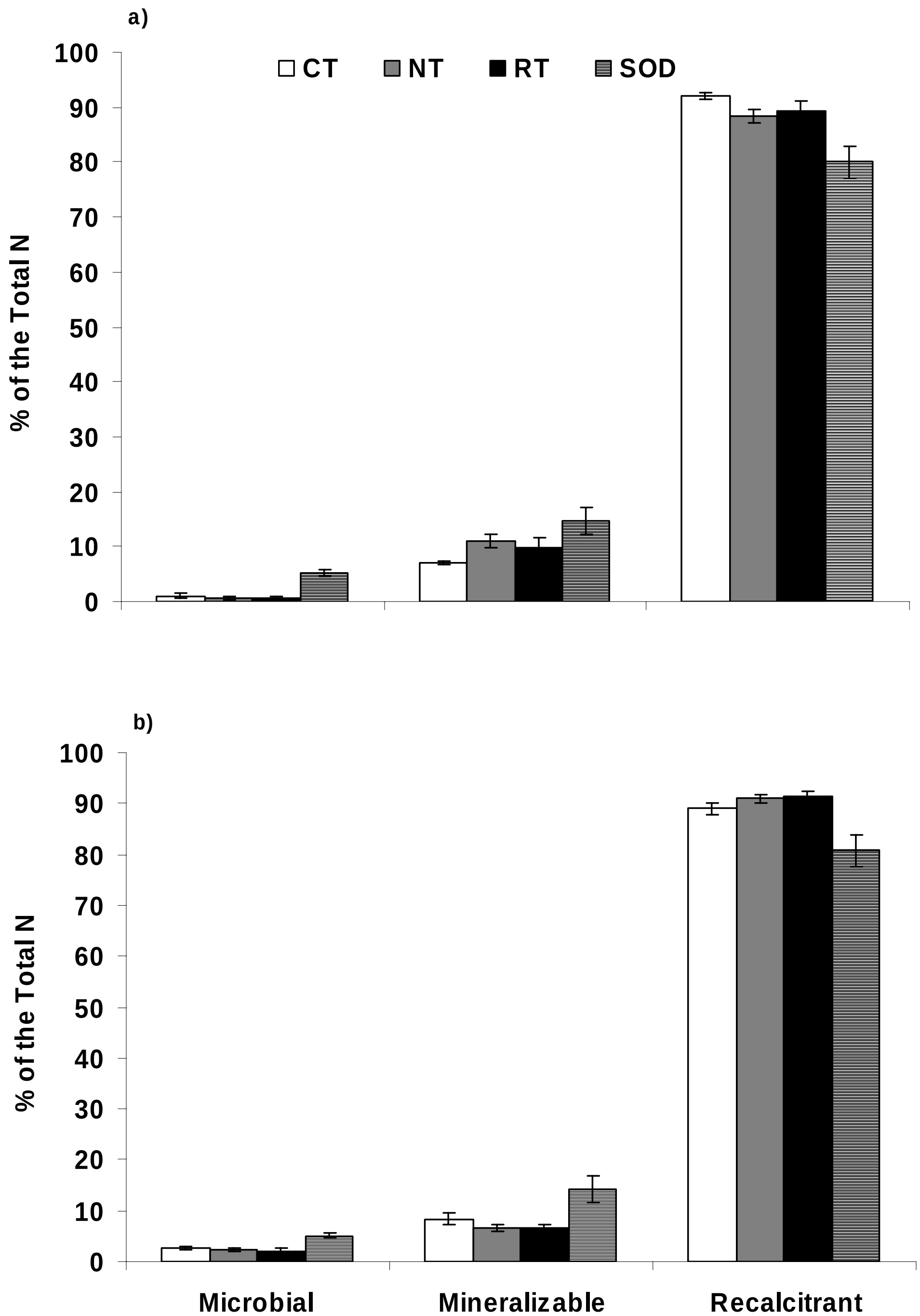

Figure 4-6. Distribution of the soil organic N pools under conventional till (CT), reduced till (RT), no-tillage (NT) and native prairie sod (SOD) at 0-5 (a) and 5-15 cm (b) in Tribune experiment. Error bars represent the standard error of the mean (n=4).

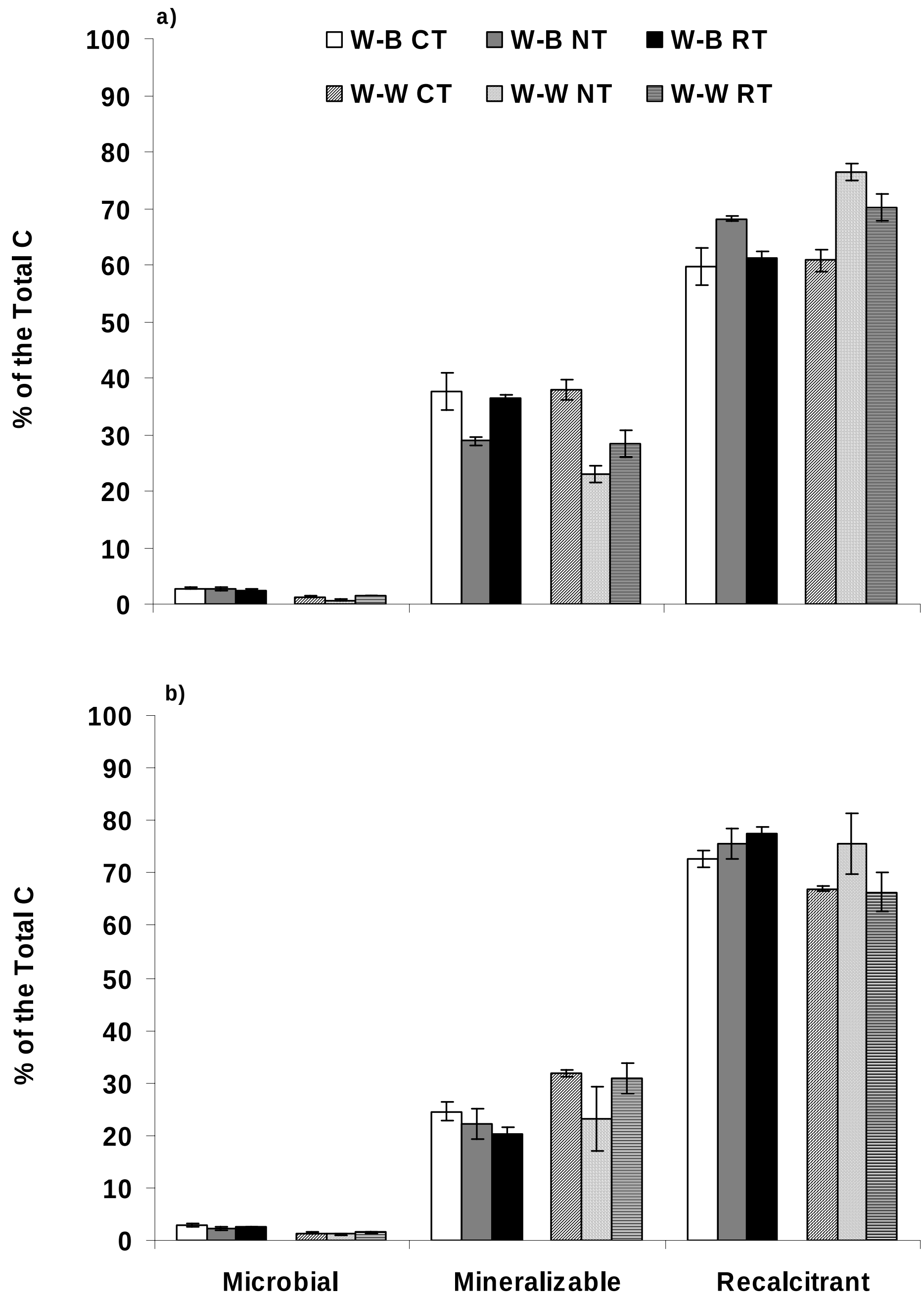

Figure 4-7. Distribution of the soil organic C pools under conventional till (CT), reduced till (RT), no-tillage (NT) in two crop rotations wheat-soybean (W-B) and wheat-wheat (W-W) at 0-5 (a) and 5-15 cm (b) in Manhattan experiment. Error bars represent the standard error of the mean (n=4).

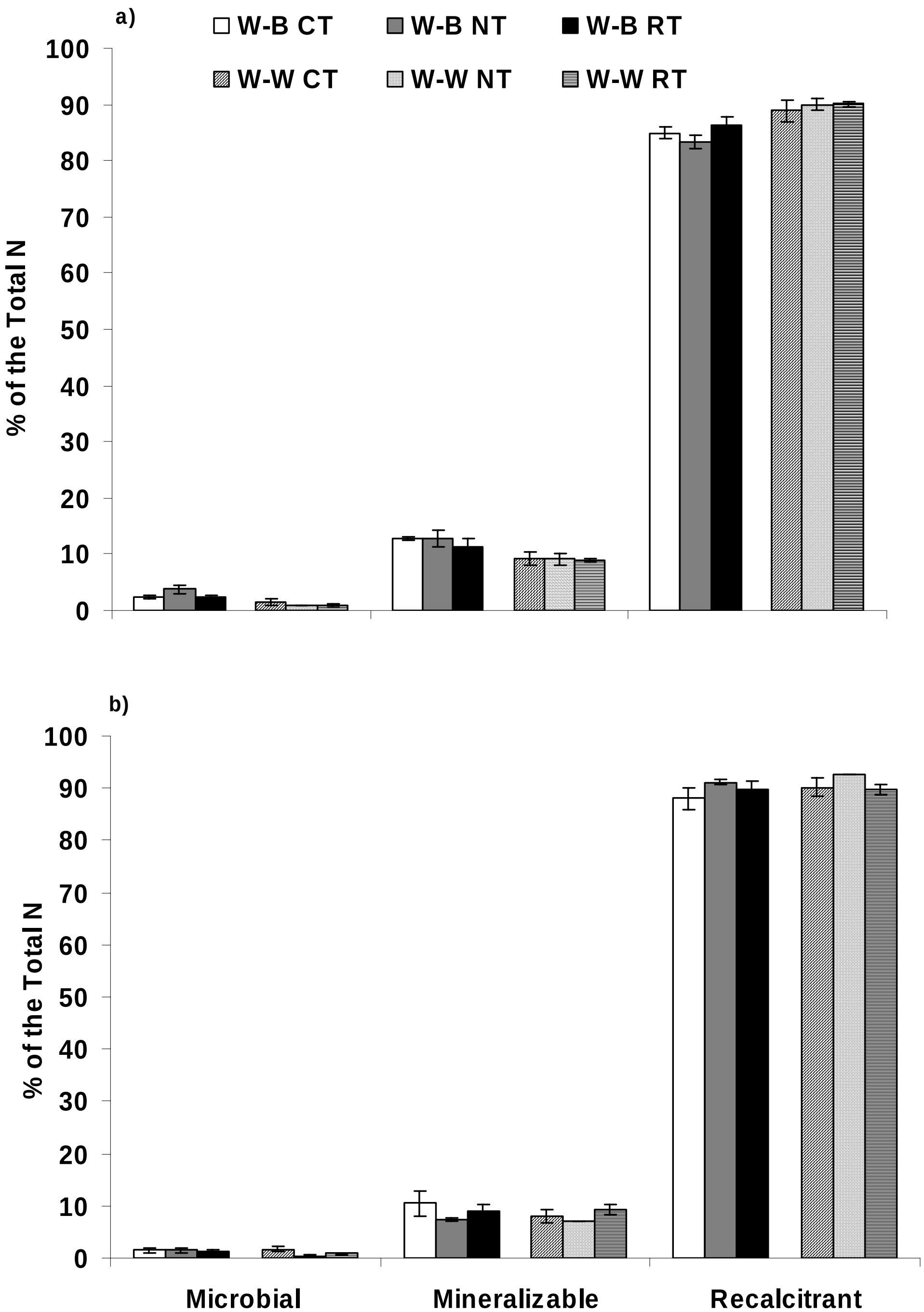

Figure 4-8. Distribution of the soil organic N pools under conventional till (CT), reduced till (RT), no-tillage (NT) in two crop rotations wheat-soybean (W-B) and wheat-wheat (W-W) at 0-5 (a) and 5-15 cm (b) in Manhattan experiment. Error bars represent the standard error of the mean (n=4).

Table 4-1. Soil microbial biomass carbon (C) under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1} for Hays experiment.

	Soil microbial biomass C		
	0-5 cm	**5-15 cm**	**15-30 cm**
	……………………g C kg^{-1}……………..		
CT 0-N	0.354	0.300	0.277
RT 0-N	0.385	0.324	0.307
NT 0-N	0.377	0.265	0.277
CT 67-N	0.290	0.323	0.299
RT 67-N	0.272	0.301	0.260
NT 67-N	0.265	0.287	0.247
	…………………………*P* values……………………..		
Tillage (T)		0.1643	
Nitrogen (N)		0.0020	
T x N		0.0704	
Depth (D)		0.0002	
T x D		0.6687	
N x D		0.0001	
0-N (mean)	0.372 **a**†	0.296 **bc**	0.287 **bc**
67-N (mean)	0.276 **bc**	0.303 **b**	0.267 **c**
T x N x D		0.7445	

† Different letters represent significant differences between N rates and depth.

Table 4-2. Soil microbial biomass nitrogen (N) under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1} for Hays experiment.

	Soil microbial biomass N		
	0-5 cm	**5-15 cm**	**15-30 cm**
	……………………g N kg^{-1}……………..		
CT 0-N	0.064 **a**†	0.037 **def**	0.033 **efgh**
RT 0-N	0.059 **b**	0.036 **defg**	0.031 **efgh**
NT 0-N	0.063 **a**	0.038 **cde**	0.042 **bcd**
CT 67-N	0.047 **b**	0.036 **defg**	0.030 **gh**
RT 67-N	0.044 **bc**	0.031 **efgh**	0.027 **h**
NT 67-N	0.018 **i**	0.030 **fgh**	0.032 **efgh**
	…………………………*P* values………………………		
Tillage (T)		0.2341	
Nitrogen (N)		0.0001	
T x N		0.0019	
Depth (D)		0.0001	
T x D		0.0001	
N x D		0.0001	
T x N x D		0.0002	

† Different letters represent significant differences among N rates, tillage and depth.

Table 4-3. Soil microbial biomass carbon (C) under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) and native prairie sod (SOD) for Tribune experiment.

	Soil microbial biomass C		
	0-5 cm	**5-15 cm**	**15-30 cm**
	……………………g C kg $^{-1}$……………..		
CT	0.218	0.278	0.186
RT	0.099	0.192	0.165
NT	0.116	0.252	0.193
SOD	0.331	0.318	0.290
	…………………………*P* values………………………		
Tillage (T)		0.0021	
CT (mean)		0.227 **b**†	
RT (mean)		0.152 **c**	
NT (mean)		0.187 **bc**	
SOD (mean)		0.313 **a**	
Depth (D)		0.0296	
	0.191 **ab**††	0.260 **a**	0.208 **b**
T x D		0.3320	

† Different letters represent significant differences among tillage.
†† Different letters represent significant differences among depth

Table 4-4. Soil microbial biomass nitrogen (N) under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) and native prairie sod (SOD) for Tribune experiment.

	Soil microbial biomass N		
	0-5 cm	**5-15 cm**	**15-30 cm**
	……………………g N kg $^{-1}$……………..		
CT	0.017 **ef**†	0.040 **c**	0.023 **def**
RT	0.012 **f**	0.030 **cde**	0.017 **f**
NT	0.013 **f**	0.035 **cd**	0.036 **cd**
SOD	0.103 **a**	0.068 **b**	0.044 **c**
	………………………….*P* values………………………		
Tillage (T)		0.0001	
Depth (D)		0.0007	
T x D		0.0001	

† Different letters represent significant differences among tillage and depth

Table 4-5. Soil microbial biomass carbon (C) under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) in continuous wheat (W-W) and wheat-soybean (W-B) rotation for Manhattan experiment.

	Soil microbial biomass C		
	0-5 cm	**5-15 cm**	**15-30 cm**
	……………………g C kg $^{-1}$……………..		
W- B CT	0.347 **b**†	0.337 **b**	0.284 **bc**
W- B RT	0.358 **b**	0.304 **b**	0.312 **b**
W- B NT	0.450 **a**	0.283 **bcd**	0.312 **b**
W- W CT	0.178 **e**	0.168 **e**	0.174 **e**
W- W RT	0.207 **cde**	0.193 **ed**	0.184 **e**
W- W NT	0.129 **e**	0.151 **e**	0.143 **e**
	…………………………*P* values………………………		
Rotation (R)		0.0039	
Tillage (T)		0.7697	
R x T		0.2018	
Depth (D)		0.0029	
R x D		0.0046	
T x D		0.3326	
R x T x D		0.0254	

†Different letters represent significant differences among rotation, tillage and depth

Table 4-6. Soil microbial biomass nitrogen (N) under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) in continuous wheat (W-W) and wheat-soybean (W-B) rotation for Manhattan experiment.

	Soil microbial biomass N		
	0-5 cm	**5-15 cm**	**15-30 cm**
	……………………g N kg $^{-1}$……………..		
W- B CT	0.030 **bcd**†	0.018 **bcde**	0.028 **bcd**
W- B RT	0.033 **bc**	0.016 **bcde**	0.077 **a**
W- B NT	0.061 **a**	0.019 **bcde**	0.039 **b**
W- W CT	0.020 **bcde**	0.022 **bcde**	0.025 **bcde**
W- W RT	0.015 **cde**	0.012 **de**	0.015 **cde**
W- W NT	0.016 **cde**	0.005 **e**	0.023 **bcde**
	…………………………*P* values………………………		
Rotation (R)		0.0101	
Tillage (T)		0.5069	
R x T		0.0150	
Depth (D)		0.0001	
R x D		0.0197	
T x D		0.0165	
R x T x D		0.0081	

†Different letters represent significant differences among rotation, tillage and depth

Table 4-7. Parameters of the one-pool model for C and N mineralization under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1}, for Hays experiment at 0-5 cm.

	SOC	**Total N**	**C_0**	**k_c**	**N_0**	**k_n**
	g C kg^{-1}	g N kg^{-1}	g C kg^{-1}	day^{-1}	g N kg^{-1}	day^{-1}
CT 0-N	11.6	0.96	2.31 **b**†	0.0055 **ab**	0.148 **b**	0.0050
RT 0-N	10.9	0.95	2.25 **b**	0.0050 **b**	0.164 **b**	0.0045
NT 0-N	11.7	1.06	2.82 **b**	0.0070 **a**	0.177 **b**	0.0050
CT 67-N	13.5	1.19	2.46 **b**	0.0060 **ab**	0.179 **b**	0.0063
RT 67-N	13.05	1.23	2.91 **b**	0.0058 **ab**	0.178 **b**	0.0057
NT 67-N	14.9	1.34	6.32 **a**	0.0043 **b**	0.336 **a**	0.0055
		…………………………	…*P* values…	……………………		
Tillage (T)	0.2163	0.2213	0.0002	0.7068	0.0039	0.1683
Nitrogen (N)	0.0001	0.0001	0.0004	0.2605	0.0003	0.0034
0-N (mean)	11.4 **b**	0.99 **b**				0.0046 **b**
67-N (mean)	13.8 **a**	1.25 **a**				0.0056 **a**
T x N	0.3023	0.7115	0.0041	0.0082	0.0027	0.5569

† Different letter in the same column means differences by tillage systems and N fertilization.

Table 4-8. Parameters of the one-pool model for C and N mineralization under conventional tillage (CT), reduced tillage (RT), and no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1}, for Hays experiment at 5-15 cm.

	SOC	**Total N**	**C_0**	**k_c**	**N_0**	**k_n**
	g C kg^{-1}	g N kg^{-1}	g C kg^{-1}	day^{-1}	g N kg^{-1}	day^{-1}
CT 0-N	10.6	0.95	1.41	0.0028	0.086	0.0027
RT 0-N	9.6	0.97	1.23	0.0030	0.071	0.0028
NT 0-N	9.30	0.92	1.04	0.0033	0.078	0.0026
CT 67-N	10.83	0.99	1.52	0.0030	0.090	0.0025
RT 67-N	10.1	0.98	1.19	0.0030	0.080	0.0027
NT 67-N	9.6	0.93	1.19	0.0033	0.077	0.0029
	…………………………*P* values……………………					
Tillage (T)	0.1509	0.6210	0.1090	0.4686	0.1356	0.8417
Nitrogen (N)	0.3301	0.4726	0.5894	0.7292	0.4271	0.9327
T x N	0.9702	0.8959	0.8372	0.8839	0.7637	0.7789

Table 4-9. Parameters of the one-pool model for C and N mineralization under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment at 0-5 cm.

	SOC	**Total N**	**C_0**	**k_c**	**N_0**	**k_n**
	g C kg^{-1}	g N kg^{-1}	g C kg^{-1}	day^{-1}	g N kg^{-1}	day^{-1}
CT	15.7 **c**†	1.71 **c**	2.99 **c**	0.0087 **b**	0.117 **c**	0.020 **a**
RT	16.8 **bc**	1.83 **b**	3.95 **b**	0.0070 **b**	0.172 **b**	0.012 **b**
NT	17.2 **b**	1.83 **b**	3.86 **b**	0.0085 **b**	0.202 **b**	0.012 **b**
SOD	22.8 **a**	2.04 **a**	5.97 **a**	0.0112 **a**	0.291 **a**	0.009 **b**
		…………………………*P* values………………………				
Tillage	0.0001	0.0142	0.0001	0.0616	0.0004	0.0206

†Different letter in the same column means differences by tillage systems.

Table 4-10. Parameters of the one-pool model for C and N mineralization under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment at 5-15 cm.

	SOC	**Total N**	**C_0**	**k_c**	**N_0**	**k_n**
	g C kg^{-1}	g N kg^{-1}	g C kg^{-1}	day^{-1}	g N kg^{-1}	day^{-1}
CT	13.1	1.49	1.60 **b**†	0.0049 **a**	0.129 **b**	0.0038
RT	12.8	1.47	1.77 **b**	0.0045 **a**	0.091 **b**	0.0048
NT	12.9	1.49	2.18 **a**	0.0028 **b**	0.099 **b**	0.0037
SOD	14.3	1.32	2.20 **a**	0.0049 **a**	0.191 **a**	0.0032
	………………………….*P* values………………………					
Tillage	0.2766	0.5743	0.0530	0.0092	0.0809	0.5258

†Different letter in the same column means differences by tillage systems

Table 4-11. Parameters of the one-pool model for C and N mineralization under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) in continuous wheat (W-W) and wheat-soybean (W-B) rotation for Manhattan experiment at 0-5 cm.

	SOC	**Total N**	**C_0**	**k_c**	**N_0**	**k_n**
	g C kg^{-1}	g N kg^{-1}	g C kg^{-1}	day^{-1}	g N kg^{-1}	day^{-1}
W- B CT	12.7†	1.29	4.73 **ab**	0.0038 **bc**	0.170	0.0073
W- B RT	14.4	1.41	5.37 **a**	0.0037 **bc**	0.163	0.0100
W- B NT	16.8	1.60	4.93 **ab**	0.0050 **a**	0.208	0.0088
W- W CT	13.6	1.37	5.18 **a**	0.0033 **c**	0.128	0.0135
W- W RT	12.3	1.19	4.08 **b**	0.0050 **a**	0.138	0.0218
W- W NT	18.2	1.76	4.18 **b**	0.0045 **ab**	0.160	0.0075
		…………………………	*P* values	………………………		
Rotation (R)	0.0799	0.1025	0.0885	0.7227	0.1054	0.2187
Tillage (T)	0.0001	0.0001	0.3444	0.0117	0.1581	0.1257
CT (mean)	13.1 **c**	1.33 **c**				
RT (mean)	14.5 **b**	1.46 **b**				
NT (mean)	17.5 **a**	1.68 **a**				
R x T	0.2431	0.4818	0.0191	0.0371	0.8244	0.2262

†Different letter in the same column means differences by tillage systems and N fertilization.

Table 4-12. Parameters of the one-pool model for C and N mineralization under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) in continuous wheat (W-W) and wheat-soybean (W-B) rotation for Manhattan experiment at 5-15 cm.

	SOC	**Total N**	**C_0**	**k_c**	**N_0**	**k_n**
	g C kg^{-1}	g N kg^{-1}	g C kg^{-1}	day^{-1}	g N kg^{-1}	day^{-1}
W- B CT	11.9	1.20	2.98 **ab**†	0.0025	0.127	0.0100
W- B RT	12.3	1.23	2.48 **b**	0.0020	0.110	0.0035
W- B NT	12.3	1.23	2.70 **b**	0.0023	0.090	0.0043
W- W CT	10.6	1.25	4.03 **a**	0.0027	0.098	0.0093
W- W RT	12.8	1.29	3.98 **a**	0.0033	0.120	0.0085
W- W NT	12.3	1.19	2.73 **b**	0.0023	0.085	0.0065
		…………………………	*P* values	……………………		
Rotation (R)	0.2346	0.6892	0.1220	0.1891	0.4948	0.2106
Tillage (T)	0.9168	0.3917	0.0378	0.7444	0.0960	0.0610
CT (mean)					0.112 **a**	0.0096 **a**
RT (mean)					0.115 **a**	0.0060 **b**
NT (mean)					0.088 **b**	0.0054 **b**
R x T	0.8586	0.2209	0.0363	0.3909	0.3483	0.2718

†Different letter in the same column means differences by tillage systems and N fertilization.

Table 4-13. Relationships between C_0, N_0, total C, total N, microbial biomass C, and microbial biomass N under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1} for Hays experiment at 0-5 cm.

	C_0/N_0	C_0/Total C	MBC/C_0	N_0/Total N	MBN/N_0
CT 0-N	15.68	0.203 **b**†	0.157	0.156 **b**	0.436 **a**
RT 0-N	14.26	0.206 **b**	0.177	0.167 **b**	0.365 **a**
NT 0-N	15.79	0.244 **b**	0.139	0.174 **b**	0.358 **ab**
CT 67-N	13.74	0.183 **b**	0.117	0.152 **b**	0.271 **ab**
RT 67-N	17.47	0.125 **b**	0.334	0.073 **b**	0.535 **b**
NT 67-N	19.14	0.430 **a**	0.043	0.254 **a**	0.054 **c**
Tillage (T)	0.1640	0.0002	0.0007	0.0057	0.0024
CT (mean)			0.102 **a**		
RT (mean)			0.105 **a**		
NT (mean)			0.054 **b**		
Nitrogen (N)	0.3570	0.0094	0.0003	0.1623	0.0001
0-N (mean)			0.123 **a**		
67-N (mean)			0.051 **b**		
T x N	0.2156	0.0049	0.4521	0.0260	0.0176

† Different letter in the same column means differences by tillage systems and N fertilization

Table 4-14. Relationships between C_0, N_0, total C, total N, microbial biomass C, and microbial biomass N under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1} for Hays experiment at 5-15 cm.

	C_0/N_0	C_0/Total C	MBC/C_0	N_0/Total N	MBN/N_0
CT 0-N	17.04	0.131	0.230	0.092	0.427
RT 0-N	17.47	0.125	0.334	0.073	0.535
NT 0-N	13.57	0.112	0.257	0.086	0.485
CT 67-N	17.40	0.138	0.234	0.093	0.405
RT 67-N	16.15	0.224	0.099	0.147	0.250
NT 67-N	15.15	0.122	0.270	0.084	0.396
	……………………………*P* values………………………				
Tillage (T)	0.0529	0.2388	0.2516	0.6424	0.3633
CT (mean)	28.6 **a**				
RT (mean)	25.3 **ab**				
NT (mean)	22.1 **b**				
Nitrogen (N)	0.8825	0.7027	0.6165	0.6959	0.0073
0-N (mean)					0.482 **a**
67-N (mean)					0.398 **b**
T x N	0.2701	0.7194	0.7681	0.7080	0.2359

Table 4-15. Relationships between C_0, N_0, total C, total N, microbial biomass C, and microbial biomass N under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) and native prairie sod for Tribune experiment at 0-5 cm.

	C_0/N_0	**C_0/Total C**	**MBC/C_0**	**N_0/Total N**	**MBN/N_0**
CT	25.66 **a**†	0.193	0.072 **a**	0.069 **b**	0.139 **b**
RT	23.6 **ab**	0.241	0.029 **b**	0.099 **b**	0.071 **b**
NT	19.19 **b**	0.230	0.032 **b**	0.110 **ab**	0.066 **b**
SOD	20.92 **ab**	0.271	0.056 **ab**	0.148 **a**	0.366 **a**
	………………………….*P* values………………………				
Tillage (T)	0.0712	0.2284	0.0403	0.0160	0.0013

†Different letter in the same column means differences by tillage systems

Table 4-16. Relationships between C_0, N_0, total C, total N, microbial biomass C, and microbial biomass N under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) and native prairie sod for Tribune experiment at 5-15 cm.

	C_0/N_0	C_0/Total C	MBC/C_0	N_0/Total N	MBN/N_0
CT	13.27	0.120 **b**†	0.174	0.084 **b**	0.328
RT	20.69	0.146 **ab**	0.111	0.065 **b**	0.327
NT	21.99	0.167 **a**	0.118	0.067 **b**	0.358
SOD	13.31	0.151 **ab**	0.146	0.142 **a**	0.386
		…………………………*P* values………………………			
Tillage (T)	0.1024	0.0603	0.1709	0.0014	0.8270

†Different letter in the same column means differences by tillage systems

Table 4-17. Relationships between C_0, N_0, total C, total N, microbial biomass C, and microbial biomass N under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) in continuous wheat (W-W) and wheat-soybean (W-B) rotation for Manhattan experiment at 0-5 cm.

	C_0/N_0	**C_0/Total C**	**MBC/C_0**	**N_0/Total N**	**MBN/N_0**
W- B CT	31.65	0.375	0.074 **b**†	0.128	0.023 **b**
W- B RT	29.76	0.364	0.066 **bc**	0.114	0.023 **b**
W- B NT	26.73	0.289	0.103 **a**	0.129	0.038 **a**
W- W CT	42.54	0.379	0.035 **de**	0.092	0.014 **bc**
W- W RT	30.38	0.284	0.051**cd**	0.892	0.010 **c**
W- W NT	27.33	0.229	0.031 **e**	0.091	0.009 **c**
	………………………….*P* values………………………				
Rotation (R)	0.2195	0.0554	0.0001	0.0049	0.0043
W-B (mean)		0.352 **a**		0.119 **a**	
W-W (mean)		0.309 **b**		0.087 **b**	
Tillage (T)	0.0487	0.0002	0.1310	0.7250	0.2378
CT (mean)	38.65 **a**	0.387 **a**			
RT (mean)	28.5 **b**	0.335 **b**			
NT (mean)	31.6 **b**	0.269 **c**			
R x T	0.3552	0.1359	0.0017	0.8377	0.0628

†Different letter in the same column means differences by tillage systems and rotation

Table 4-18. Relationships between C_0, N_0, total C, total N, microbial biomass C, and microbial biomass N under in continuous wheat (W-W) and wheat-soybean (W-B) rotation for Manhattan experiment at 5-15 cm.

	C_0/N_0	C_0/Total C	MBC/C_0	N_0/Total N	MBN/N_0
W- B CT	26.71	0.246	0.118	0.104	0.015
W- B RT	23.56	0.202	0.125	0.089	0.013
W- B NT	30.11	0.221	0.112	0.074	0.016
W- W CT	41.13	0.318	0.041	0.080	0.018
W- W RT	33.89	0.310	0.049	0.093	0.009
W- W NT	33.84	0.231	0.074	0.070	0.004
	………………………… *P* values ………………………				
Rotation (R)	0.0545	0.0370	0.0020	0.4280	0.6823
W-B (mean)	26.8 **b**	0.217 **b**	0.118 **a**		
W-W (mean)	36.3 **a**	0.299 **a**	0.054 **b**		
Tillage (T)	0.6356	0.2011	0.8086	0.1680	0.3120
R x T	0.6433	0.2609	0.5618	0.4849	0.2755

CHAPTER 5 - SOIL ORGANIC MATTER AND MICROBIAL ECOLOGY OF MOLLISOLS, VERTISOLS, AND OXISOLS: COMPARISONS OF NATIVE AND AGROECOSYSTEMS

ABSTRACT

C sequestration is a viable short-term option for mitigating increased atmospheric CO_2. In agriculture soils, some of the strategies are the adoption of best management practices such as no-tillage, cover crops, and improved crop rotations. Cultivation decreases soil organic carbon (SOC) and aggregate stability. The objective of our study was to determine the influence of different long-term tillage practices on SOC and total N, soil aggregation and aggregate-associated C and N in three soils an Oxisol (Brazil), Vertisol (Argentina), and Mollisol (Kansas,USA). Tillage systems were conventional tillage (CT), reduced tillage (RT) and no-tillage (NT) and a native vegetation treatment. Soil samples were taken at 0-5 cm, 0-15 and 15-30 cm. Water-stable aggregates (WSA) were separated using a wet sieving method. Total C and N contents were determined by dry combustion. For all three soils, SOC was significantly greater in NT than CT for 0-5 cm. In the Oxisol NT had greater amounts of large macroaggregates (>2000 μm) than CT; this was accompanied by a corresponding decrease in the aggregates <2000 μm. In the Vertisol and Mollisol, no significant differences were detected among tillage, but NT tended to have greater amounts of large macroaggregates than CT. Carbon and N concentrations in the aggregates differed according to the soil types in response to management. In general, C and N in the native vegetation of the Oxisol were similar to, or greater than, NT and greater than CT. However, in the Vertisol, NT had greater C and N concentrations in the macroaggregates than did soil from the native site. In the Mollisol, the native site had greater C and N concentrations in the aggregates compared to either tillage systems. Cultivation of native vegetation sites reduced the mass of macroaggregates and the associated C and N concentration; however NT tended to be more similar to the native grassland sites. Microbial communities were also affected by management being greater the levels under native grassland and NT systems. The tendency of had greater abundance of fungi PLFA and AM fungi biomarkers under NT than CT together with the better aggregation could contribute to explain the increase in SOC under no-tillage systems.

Keys words: aggregates, tillage, grassland

INTRODUCTION

Soil C sequestration is considered a viable near-term option for mitigating increased atmospheric CO_2 (Post et al., 2004; Caldeira et al., 2004). In agriculture, some of the strategies to increase soil C include the adoption of best management practices such as no-tillage, cover crops, and improved crop rotation (Lal et al., 1998; Paustian et al., 2000; West and Post, 2002; Lal, 2004; Post et al., 2004).

Cultivation decreases soil organic carbon (SOC) and reduces soil aggregate stabiltiy (Tisdall and Oades, 1980). Tillage can affect SOC dynamics through changes in soil environment (temperature, moisture) thus affecting microbial activity; and also through the disruption of soil structure (Balesdent et al., 2000). The bare soil surface is exposed to continuous wet-dry and freeze-thaw cycles (Beare et al., 1994; Paustian et al., 1997) thus making the aggregates more susceptible to disruption. Also, changes in soil climate promote increases in the decomposition rates (Cambardella and Elliott, 1993), and affect the microbial community composition (Beare et al., 1993).

Carbon stabilization in temperate and tropical soils is mediated by soil biota, soil structure and their interactions, and also by agricultural management (Six et al., 2002). Some of the factors that greatly differ among tropical and temperate regions are climate, parent material, and vegetation. Tropical soils generally have low activity clays (1:1 clays) which are characterized by low specific surface and cation exchange capacity (CEC). The climate is generally characterized by high temperatures and high precipitation relative to temperate systems. As a result of the soil and climate, microbial activity is high, and consequently Oxisols have a lower capacity to stabilize C. Conversely, temperate soils have a predominance of high activity clays (2:1 clays), with high specific surface area and CEC; therefore, a greater capacity to stabilize C (Six et al. 2002).

Soil organic matter and biological processes play a primary role in the aggregation of temperate soils dominated by 2:1 clay mineralogy. However, in weathered soils, it has been suggested that soil organic matter and biological processes play a secondary role in the binding of aggregates (Six et al., 1999; Denef et al., 2002; Denef and Six, 2004). The primary factor in these soils is mineral-mineral bonding due to electrostatic interactions between oxides and 1:1 clay minerals.

Management practices can alter the composition and function of microbial communities thus affecting soil C dynamics. Bacteria and fungi are the most abundant microorganisms in soils, and play a key role in organic matter decomposition (Six et al., 2006). The proportion of microbial biomass composed of fungi can increase with less disturbance such as NT (Beare, 1997; Frey et al., 1999; Watson and Rice, 2004). The degree of disturbance, soil moisture, and residue placement are factors controlling the proportions of bacterial and fungal biomass in NT and CT systems (Six et al., 2006; White and Rice, 2007). Different techniques have been used to assess variations in microbial communities and composition. Guggenberger et al. (1999) reported that the ratio of the fungal-derived amino sugar glucosamine to the bacterial-derived muramic acid was significantly higher under NT than under CT, indicating greater accumulation of fungal cell wall residues in NT soils. Frey et al. (1999) used direct counts to report a greater proportion of fungal biomass in NT than CT. Phospholipid fatty acid (PLFA) analysis has been applied to detect responses of soil microbial communities to land use changes or ecosystems disturbance (Hedrick et al., 2000; Fang et al., 2001; Harris, 2003). Specific PLFA markers can identify groups of organisms in soils (Zak et al., 1994; Zelles, 1997; Zogg et al., 1997; Mckinley et al., 2005).

The introduction of conservation tillage as a strategy to reduce soil erosion, improve soil structure, reduce soil C loss, and promotion of sustainable agriculture has been gaining importance in different parts of the world. However, changes in management practices can influence the dynamics of C in soils affecting the quantity and quality of SOM, soil aggregation, and microbial populations to different degrees according to soil type and climate. Thus, the objective of our study was to evaluate the effect of agroecosystems and native vegetation on SOC, aggregation, and microbial community structure using a PLFA technique in three different soil orders: Oxisols, Vertisols, and Mollisols.

MATERIALS AND METHODS

Site description

Characteristics of the experimental sites are summarized in Table 5.1. The Oxisol was located at the Center of Experimentation and Research FUNDACEP in Cruz Alta (RS), Brazil (28º 36' S, 53º 40'W). This experiment was initiated in 1985 on a clay Rhodic Hapludox and referred to in the text as Oxisol. The average annual precipitation was 1727 mm without a dry season and an annual mean temperature of 19.2 ºC. The crop rotation was: black oat (*Avena strigosa* Schreber) - soybean (*Glycine max* (L) Merrill) - black oat + vetch (*Vicia sativa* (L.) *Walp.)- maize (Zea mays L.) - radish oil (Raphanus sativus L.)*- wheat *(Triticum* aestivum L.) - soybean, under conventional tillage (CT) and no-tillage (NT) systems. Conventional tillage consisted of using tandem disk and disk plow. No-tillage consisted of planting directly into the residue. 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). The experimental design was a randomized block with three replications. A native vegetation site (Native site) was included in the experiment, which represented the natural vegetation of the area with species such as *Andropogon lateralis, Paspalum notatum, Conyza bonariensis, Eryngium horridum, Desmodium incanum, Cyperus spp.,* and *Digitaria spp.*

The Vertisol was located at the Experimental Station INTA Parana, Entre Rios (31º 50’ 07” S, 60º 32’ 19” W). The averaged annual precipitation was 995 mm with an annual mean temperature was 18.5 ºC. The soil was classified as an argic chromic Peludert (very fine, montmorillonitic slighly akaline, thermic Peludert) and referred to in the text as Vertisol. The crop rotation, initiated in 1997, was wheat/soybean-maize, with two tillage systems: reduced tillage (RT) and no-tillage. Reduced tillage consisted of two or three operations with disk or chisel plow. No-tillage consisted of planting directly into the residue. A native site (Native site) was included as a treatment. The natural vegetation of the area was a savanna with xenomorphic species (*Prosopis spp*. and *Acacia spp*.) and grasses such as *Bromus spp*., *Setaria spp*., and *Stipa spp*. The treatments were arranged in a randomized complete block design with four replications.

The Mollisol was located at the North Agronomy Farm located at Kansas State University, Manhattan, Kansas (Riley County; 39º 13’ 12” N, 96º 36’ 0” W). The soil was a moderated well-drained Kennebec silt loam (fine-silty, mixed, superactive mesic Cumulic Hapludoll) and referred in the text as Mollisol. The 30-yr average annual precipitation was 800 mm, which was mainly concentrated in the spring-summer period, with an annual mean temperature of 11.4 ºC. The experiment, initiated in 1990, was a split-plot with four replications, with tillage as the main plot and N as the sub-plot. For this study, samples were taken only in the CT and NT plots receiving 168 kg N ha^{-1} as ammonium nitrate. Conventional tillage consisted of fall chisel plow and spring offset disk. No tillage consisted of planting directly into the residue. Similar to the other experiments, a native site was included for comparison; the natural vegetation was a tallgrass prairie dominated by warm-season grasses: big bluesterm (*Andropogon* gerardii Vit.), indiangrass (*Sorgastrum nutans* (L.), and switchgrass (*Panicum virgatum* Michx.).

Soil Sampling

Soil samples were taken from each plot at 0-5, 0-15, and 15-30 cm depth. A sterile polypropylene bags (3.78 L) were filled with soil samples collected randomly from each plot using a 2-cm diam. Oakfield soil-probe (Forestry Suppliers, Inc., Jackson, MS) or shovel. Samples were collected August 2005 for the Oxisol, November 2005 for Mollisol, and March 2006 for the Vertisol. Soil samples were passed through 8-mm sieve, roots removed, and stored at 4ºC until use.

Aggregate-Size Distribution

Water-stable aggregates (WSA) were separated using a wet-sieve method described by Yoder (1936) with modifications by Mikha and Rice (2004). Soil was air-dried and 50 g placed on the top of the sieve of each nest. To slake the air-dried soil, 1 L of distilled water was rapidly added until soil was covered with water. Soils were submerged in water for 10 min followed by 10 min of wet sieving. Four aggregate size classes were collected from each treatment >2000, 250-2000, 53-250, and 53-20 μm diam. Water stable aggregates were dried and a subsample was used to determine sand content of each fraction (Mikha and Rice, 2004). Large macroaggregates were defined as >2000 μm, small macroaggregates 250-2000 μm, microaggregates 250-53 μm, and silt plus clay by 20-53 μm size fraction.

Sand-free WSA was measured using a subsample of intact aggregates (2-5g) and combined with fivefold volume (10-25 mL) of 5 g L^{-1} sodium hexametaphosphate, left overnight and shaken on an orbital shaker at 350 RPM for 4h. The dispersed organic matter and sand was collected on a 53 μm mesh sieve, washed with deionized water, and dried at 105ºC for 24 h, and the aggregate weights were recorded for estimating the sand-free correction.

Total C and N

Soil samples were dried and ground to a fine powder using a mortar and pestle. Total C and N contents were determined by dry combustion using a C/N Elemental Analyzer (Flash EA 1112 Series ThermoFinnigan Italia S.p.A., MI, Italy). Calculations for total C and N in different aggregate-size fraction were adjusted for sand-free water stable aggregates.

Microbial Community Structure: Phospholipid Fatty Acid Analysis.

Phospholipids and neutral lipid fatty acids (PLFA and NLFA) analyses were determined following a modification of the Bligh and Dyer (1959) method (White and Ringelberg, 1998). Lipids were extracted with a single phase chloroform:methanol:phosphate buffer solution (Blight and Dyer, 1959) for 2 h from 5 g of freeze-dried soil. Total lipid extracts were separate into neutral lipids, polar lipids and glycolipids using preconditioned silica gel disposable extraction columns (J.T. Baker, Phillipsburg, NJ, USA). Neutral and polar lipids were subject to alkaline methanolysis to cleave the fatty acids from the glycerol molecule and replace it with methyl groups, creating fatty acid methyl esters. Samples were analyzed by gas chromatography (HP 6890, Agilent Incorporated, Palo Alto, CA, USA). A 25-m Ultra-2 (J &W Scientific, Agilent Technologies, Palo Alto, CA, USA) column was used and He was the carrier gas at 1 mL min^{-1}. The initial temperature was 80ºC for 1 min followed by an increase of 20ºC min^{-1} until 155ºC, and a second increase at 5ºC min^{-1} until 27.ºC. Peaks were identify using retention times of fatty acid standards and by comparing spectra from a library (Wiley 138K mass spectral database). Samples peak were quantified based on comparison of the abundance with an internal standard nonadecanoic acid methyl ester (19:0) in terms of nmol g^{-1} dry soil or mol %.

Fatty acids were designated ***a:b***, where a is the total number of carbons and ***b*** are the number of double bonds. An ***ω*** refers to the position of the double bond from the aliphatic end of the fatty acid. The prefixes ***a*** and ***i*** refer to ***anteiso*** and ***iso*** branching, the suffixes ***c*** and ***t*** indicate ***cis*** and ***trans*** conformations. Methyl groups were indicated by ***aMe***, where ***a*** indicates the position of the methyl group.

Fatty acids were grouped into Gram positive bacteria (i15:0, a15:0, 10Me16:0, i17:0, and a17:0), Gram negative bacteria (18:1ω7c and cyclic 19:0), actinomycetes (10Me18:0 and 10Me17:0), and fungi (18:2ω6,9c and 18:1ω9c) (McKinley et al., 2005).

Statistical Analysis

Analysis of variance was performed using SAS PROC MIXED (SAS Institute, 2002) to assess differences between treatments. Because the native site treatment was not included in the experimental design two different analyses were performed: 1) comparison between tillage treatments, NT or RT versus CT, at each site, and 2) comparison between tillage treatments and the native site treatment at each site. Results were considered statistically significant at $P < 0.05$ unless noted otherwise. Means were compared using LSD values.

RESULTS

Total C and N

For all three soils, SOC was significantly ($P<0.01$) greater in NT than CT for 0-5 cm (Table 5.2). For the Oxisol, SOC also was significantly greater in NT than CT for 0-15 cm ($P<0.05$); however SOC at 0-15 cm was not different between tillage systems for the Vertisol and the Mollisol. There were no differences between tillage systems for SOC at 15-30 cm for all three soils (Table 5.2).

The native grassland for all three soils had greater SOC concentrations at 0-5 cm ($P<0.05$), which were significant for the Oxisol and the Mollisol. At 0-15 and 15-30 cm the native grassland had also significantly greater SOC than both cropped treatments in the Mollisol and Vertisol.

For the Mollisol and Vertisol, total N was significantly ($P<0.01$) greater in NT than CT for 0-5 cm (Table 5.2). There were no differences between tillage systems for total N at 0-15 and 15-30 cm ($P>0.05$), except in the Oxisol where CT had greater total N than NT ($P<0.01$). The native grassland had greater total N concentrations compared to tillage treatments at all depths in Mollisol ($P<0.05$).

Sand-free water stable aggregates

The level of aggregation was much greater for the Oxisol than the other two soils (Fig. 5.1). For the Oxisol at 0-5 cm, sand-free WSA was significantly affected by the interaction of tillage x aggregate size ($P<0.05$) (Fig. 5.1a). No-tillage had significantly greater amounts of large macroaggregates than CT at the expense of smaller aggregates. No differences were observed for the aggregates associated with the silt plus clay size. The native site had similar amounts of large macroaggregates as NT but was significantly greater than CT (Fig. 5.1a). The differences in aggregates between tillage systems at 0-5 cm were not apparent at 0-15 and 15-30 cm (Fig. 5.1b, c) indicating the response was confined to the surface 5 cm of the no-till soil. The native site had greater amounts of macroaggregates (>2000 μm) than the cropped systems except for 0-5 cm where NT had significantly greater amounts of macroaggregates than the native site. The native site had significantly lower amounts of small macroaggregates and microaggregates at 0-15 cm (Fig. 5.1b). At 15-30 cm, the Native site had similar amounts of large macroaggregates as the CT but less than NT (Fig. 5.1c).

For the Vertisol there were generally no differences (P>0.05) between tillage systems at all depths (Fig. 5.2). The native site had greater amount of macroaggregates at all depths (Fig. 5.2).

For the Mollisol, no significant differences ($P>0.05$) in aggregates were detected between tillage systems at all depths (Fig. 5.3). The native site had significantly greater amounts of macroaggregates ($P<0.05$) than the cropped treatments (Fig. 5.3).

Concentrations of C and N in each aggregate fraction

The Oxisol had similar C concentrations between tillage systems in the macroaggregate fraction at 0-5 cm; however, NT had significantly greater (P<0.05) C concentrations than CT in the microaggregate fraction, which might indicate a loss of microaggregate-associated C as a result of intensive tillage (Fig. 5.4a). At 0-15 and 15-30 cm, no significant differences were observed between tillage systems for C concentrations (Fig. 5.4 b, c). The C concentrations of the aggregates from the native site were greater or similar to NT but significantly greater than CT indicating that tillage had accelerated the loss of C in all aggregates while no-till may preserve aggregate-associated C. At 0-5 cm, total N concentration was significantly greater under NT than CT in all aggregate size fractions (Fig. 5.5). The native site had significantly greater N concentrations in the microaggregates than CT and NT at all depths (Fig. 5.5). At 0-15 and 15-30 cm, no differences between tillage were observed; however at 0-15 cm the native site had significantly greater (*P*<0.05) concentrations of N than the cropped systems for all aggregate size fractions, except in the < 53 um fraction (Fig. 5.5b). Total C and N mass are presented in the appendix (Fig. C.1,C.2).

For the Vertisol, at 0-5 cm, total C and N concentration was significantly greater (P<0.05) under NT than CT in the macroaggregate fraction, with no differences in the microaggregate fraction (Fig. 5.6a, Fig. 5.7a). The total C and N concentration in the native site was significantly lower than both tillage systems in the large macroaggregates. At 0-15 cm, NT had significantly greater (*P*<0.05) C and N concentration in large macroaggregates (Fig. 5.6b, Fig. 5.7b). Conversely, CT at 15-30 cm had significantly greater (P<0.05) C and N concentration than NT (Fig. 5.6c, Fig. 5.7c). Total C and N mass are presented in the appendix (Fig. C.3,C.4).

Total C and N concentrations for the Mollisol were significantly greater (P<0.05) under NT than CT for the small macroaggregates at 0-5 cm, with no differences between cropped treatments in the microaggregates (Fig. 5.8a, Fig. 5.9a). At 0-15 cm no differences were observed in C and N concentrations between CT and NT (Fig. 5.8b, Fig. 5.9b). At 15-30 cm, CT had greater C and N concentrations in the large macroaggregates than NT, but no differences were observed in the other size classes (Fig. 5.8c, Fig. 5.9c). The native site had greater C and N than the cropped treatments in the small macroaggregate and microaggregate fraction at 0-5 cm and 0-15 cm (Fig. 5.8a,b; Fig. 5.9a,b). The concentrations of C and N in the native site was greater than both cropped treatments for all aggregate size classes at 15-30 cm, except in the < 53 um fraction (Fig. 5.8c, Fig. 5.9c). Total C and N mass are presented in the appendix (Fig. C.5,C.6).

Microbial Community Structure

Oxisol

Microbial biomass estimated as total PLFA was significantly greater ($P<0.10$) under NT than CT (Fig. 5.10a). No-tillage had 60% more microbial biomass than CT. The native site was similar to NT but had greater microbial biomass than CT ($P<0.05$).

The abundance of the PLFA fungal markers responded significantly to the tillage treatments ($P<0.05$) (Fig. 5.11a). No-tillage systems had a greater abundance of fungi than CT. The native site had similar fungal abundance as NT but significantly greater ($P<0.05$) than CT. Actinomycete indicators estimated with PLFA were similar among treatments ($P>0.05$) (Fig. 5.11a). The abundance of Gram-positive and Gram-negative bacteria was significantly greater ($P<0.10$) under NT than CT (Fig. 5.11a). Similar values were observed between NT and the native site, but they were significantly greater ($P<0.05$) than CT (Fig. 5.11a). The relative abundance of each PLFA indicator is presented on Figure 5.11b. No differences were detected between tillage systems in the relative abundance, except for Gram-negative bacteria that showed greater abundance in NT than CT systems. In the native site, Gram-negative were more abundant than the cropped systems.

The native site had significantly greater ($P<0.05$) abundance of the NLFA fungal marker than the cropped systems. Besides, NT had significantly greater abundance of fungal NLFA biomarker than CT ($P<0.10$) (Fig. 5.12a). Similar abundance of the NLFA AMF marker was observed between the NT and native site but was significantly greater ($P<0.05$) than CT (Fig. 5.12a). The relative abundance of the NLFA fungal indicator reflects significant differences between tillage systems and the native site ($P<0.05$) (Fig. 5.12b).

Vertisol

Microbial biomass estimated by total PLFA was similar among treatments ($P>0.05$) (Fig. 5.10b). The abundance of PLFA indicators was similar between tillage treatments ($P>0.05$), and the native prairie and tillage systems ($P>0.05$), except for Gram-negative bacteria, which were significantly greater under NT than CT (Fig. 5.13a).

The relative abundance of each PLFA indicator is presented on Figure 5.13b. No differences were detected between tillage systems in relative abundance, except for Gram-positive bacteria that showed greater abundance for the native site than CT systems ($P<0.05$).

No-tillage had significantly greater ($P<0.10$) abundance of the NLFA fungal indicator than CT (Fig. 5.14a). The native site had significantly greater ($P<0.05$) abundance of the AM than the cropped systems which may be due the reliance of the vegetation for P uptake by AM symbiosis. Total fungi, including the AM fungi, was similar under NT and the native site, but significantly differed from CT (Fig. 5.14a).

The relative abundance of NLFA AM fungal indicator was significantly greater under native site than CT and NT ($P<0.10$) (Fig. 5.14b).

Mollisol

Microbial biomass estimated as total PLFA was significantly greater ($P<0.10$) under NT than CT (Fig. 5.10c). No-tillage had 44% more microbial biomass than CT treatments. When the native site was compared with tillage systems, the native site had significant greater biomass ($P<0.05$) than NT and CT.

The abundance of fungi, actinomycetes, Gram-positive, Gram-negative responded significantly to the tillage treatments ($P<0.10$) (Fig. 5.15a). No-tillage systems had greater abundance than CT. The native site had significant greater abundance of actinomycetes, Gram-positive and Gram-negative than CT and NT ($P<0.05$). The relative abundance of each PLFA indicator is presented in Figure 5.15b. No differences were detected between tillage systems in the relative abundance. Differences with the native site were only observed in the actinomycetes and Gram-negative indicator, in which the prairie had greater relative abundance than CT and NT systems.

The native site had significantly greater ($P<0.05$) abundance of AMF than the cropped systems (Fig. 5.16a). The relative abundance of NLFA fungal indicator reflects significant differences between tillage and native site ($P<0.05$), but no differences ($P>0.05$) between CT and NT (Fig. 5.16b). The native site had greater relative abundance of AMF, but lower relative abundance of fungi than CT and NT treatments ($P<0.05$) (Fig. 5.16b).

DISCUSSION

Despite the differences in soil and climate among sites, our results reflect that the no-tillage effect was concentrated in the first 0-5 cm soil depth, where SOC and total N were greater under NT than under CT. Similar results have been reported in the literature (Six et al.,1999; West and Post, 2002; Deen and Kataki, 2003; Fabrizzi et al., 2003; Mikha and Rice, 2004; McVay et al., 2006; Wright and Hons, 2004, 2005a,b; Amado et al., 2006). In the Oxisol, however, NT increased SOC content to a depth of 15 cm. The impact of NT on the depth of SOC increases may be a function of the soil, climate, cropping systems and time. Positive gains with no-tillage management have been reported to a depth of 30 cm (Cambardella and Elliot, 1994; Six et al., 1999; Fabrizzi et al., 2003); however, other studies have report no increase in SOC under NT systems (Angers et al., 1997; Franzluebbers et al., 1999; Needelman et al., 1999; Puget and Lal, 2005; Sainju et al., 2006).

Cultivation decreased SOC and total N concentration. However NT in the Vertisol had similar C and total N concentrations as the native site, which may be a function of prior history. The Vertisol was the youngest of the three experiments, which may have limited the time for differences to develop. There were significant differences in C concentration at 0-15 and 15-30 cm between tilled and native for the Oxisol and the Mollisol. The lack of differences in the Vertisol may be due to the self-mixing of the shrink-swell clays that minimize stratification that would develop under NT. Leinweber et al. (1999) and Schulten and Leinweber (2000) mentioned that the drying and wetting, resulting in the swell and shrink dynamics and lead to pedoturbation and mixing of mineral-associated organic matter within the soil profile.

Several authors have reported an increase in the proportion of macroaggregates under NT systems (Beare et al., 1994a; Mikha and Rice, 2004; Wright and Hons, 2005a). Differences in soil aggregation induced by tillage systems in our study were more pronounced in the Oxisol at 0-5 cm. NT had greater proportion of large macroaggregates than CT. In the Vertisol and Mollisol, the amount of large macroaggregates under CT was small or even not detectable than the smaller macroaggregates and microaggregates, which may be a response to frequency of disruption and lack of stability under CT. Some reasons for the greater response of the Oxisol than temperate soils to change in management could be related to the direct and indirect impact of tillage and soils dominated by 1:1 clay and lower CEC that have faster turnover and less stabilized C (Six et al., 2004). Tillage may expose aggregates to more frequent wet-dry cycles; increase SOC decomposition; and change microbial communities, especially reducing fungal growth and proliferation that contribute to macroaggregate formation (Six et al., 1998).

Cultivation of native ecosystems reduced the mass of macroaggregates. Similar to our results, Elliott (1986) reported more stables macroaggregates in native sod than in cultivated soils; and this can be related to the importance of roots, fungi and bacteria on aggregation by enmeshing and binding of soil particles (Bronick and Lal, 2005). Tillage decreases the length of the roots and breaks up the hyphal networks resulting in decreased aggregation under long-term cultivation (Tisdall and Oades, 1980). The influence of microbial activity or products on aggregation is related to different scales of influence of fungi vs. bacteria, soil texture and mineralogy (Six et al., 2004). Denef and Six (2004) reported a significant correlation between aggregation and microbial biomass in the Mollisol, but no effect of the microbial biomass on the Oxisols. Our results were similar to those reported (Fig. 4.17b). According to the aggregate hierarchy theory and the pore exclusion principle, fungal mycelium is important to the formation of macroaggregates, whereas the production of mucilages from bacteria and fungi promotes the formation of microaggregates (Six et al., 2004).

The lack of differences in C and N content across aggregate–size classes in the Oxisol (Fig.4.4) indicate that these soils do not follow the aggregate hierarchy proposed by Tisdall and Oades (1982). In these tropical soils, 1:1 clays and Al and Fe oxides are the principal agents of aggregation, and SOM play a secondary role as binding agent (Six et al., 2000a,b, 2002; Denef et al., 2002; Zoratelli et al. 2005). Conversely, the Mollisol and Vertisol showed an increase of C and N concentration with increasing aggregate-size (Fig. 4.6 and 4.8) supporting the aggregate hierarchy (Six et al., 2000b). The Oxisol had a decrease in macroaggregates with an increase in microaggregates with cultivation, but there was no C depletion at any aggregate size fraction which indicated that for these soils there is not a direct link between loss of aggregates and C loss (Six et al., 2000b; Zoratelli et al., 2005). However, in Mollisols, cultivation results in the loss of C-rich macroaggregates and an increase of C depleted microaggregates (Six et al., 2000b) as reflected in our results.

The greater amount of macroaggregates in the Oxisol than in the Mollisol and the Vertisol can be related to the formation of bridges between primary and secondary particles through the formation of a coat of oxides on the clay surface (Norrish, 1983; Muggler et al., 1999, cited by Six et al., 2004). The binding of oxides to minerals will reduce the CEC of kaolinite and increases positive charge, promoting aggregation through electrostatic binding (Dixon, 1989, cited by Six et al., 2004). The importance of these physical mechanisms on the aggregate formation in Oxisols is also shown by the poor relationship between SOC content and microbial biomass and amount of macroaggregates in these soils (Fig. 4.17). However, in the Mollisol there was a strong correlation between SOC or microbial biomass and the amount of macroaggregates indicating the importance of biological agents on aggregate formation (Fig. 4.17).

Total PLFA biomass was greater under NT than CT in the Oxisol and Mollisol, but was similar among treatments in the Vertisol. The lack of differences in the Vertisol could be related to the lower intensity of the tillage and time under treatments. The native site had greater or similar total PLFA biomass than NT but greater than CT in these evaluated experiments.

In general, NT had greater abundance of fungal and AMF than CT and similar abundance compared to the native site. The differences between management on the fungal NLFA biomarker followed the same pattern and were more pronounced than PLFAs, except in the Vertisol. For the Gram positive and Gram-negative bacteria, the native site had similar or greater abundance than NT, but both were greater than CT systems. When the biomarkers were expressed as a proportion of the total biomass, changes in management practices did not affect the relative abundance.

Several studies have reported a greater proportion of the microbial biomass composed by fungi in NT than CT (Beare, 1997; Frey et al., 1999; Watson and Rice, 2004). The tendency to have greater fungal than bacterial biomass under NT than CT could be related with three major factors: 1) disturbance, less disturbance favors fungal growth and activity due to enhanced establishment and maintenance of extensive hyphal networks; 2) soil moisture, fungi can maintain activity in the dry surface litter environment in NT systems, and 3) residue placement, because fungi can bridge the soil-residue interface and utilize the spatially separated C resources by translocating N into the C-rich surface residues (Frey et al., 1999; Six et al., 2006). Thus, the shifts in microbial communities due to management practices can have important implications on the soil C dynamics and soil aggregation. Thus, soil environment favoring fungi growth can be an alternative to sequester C in soils, such as no-tillage systems.

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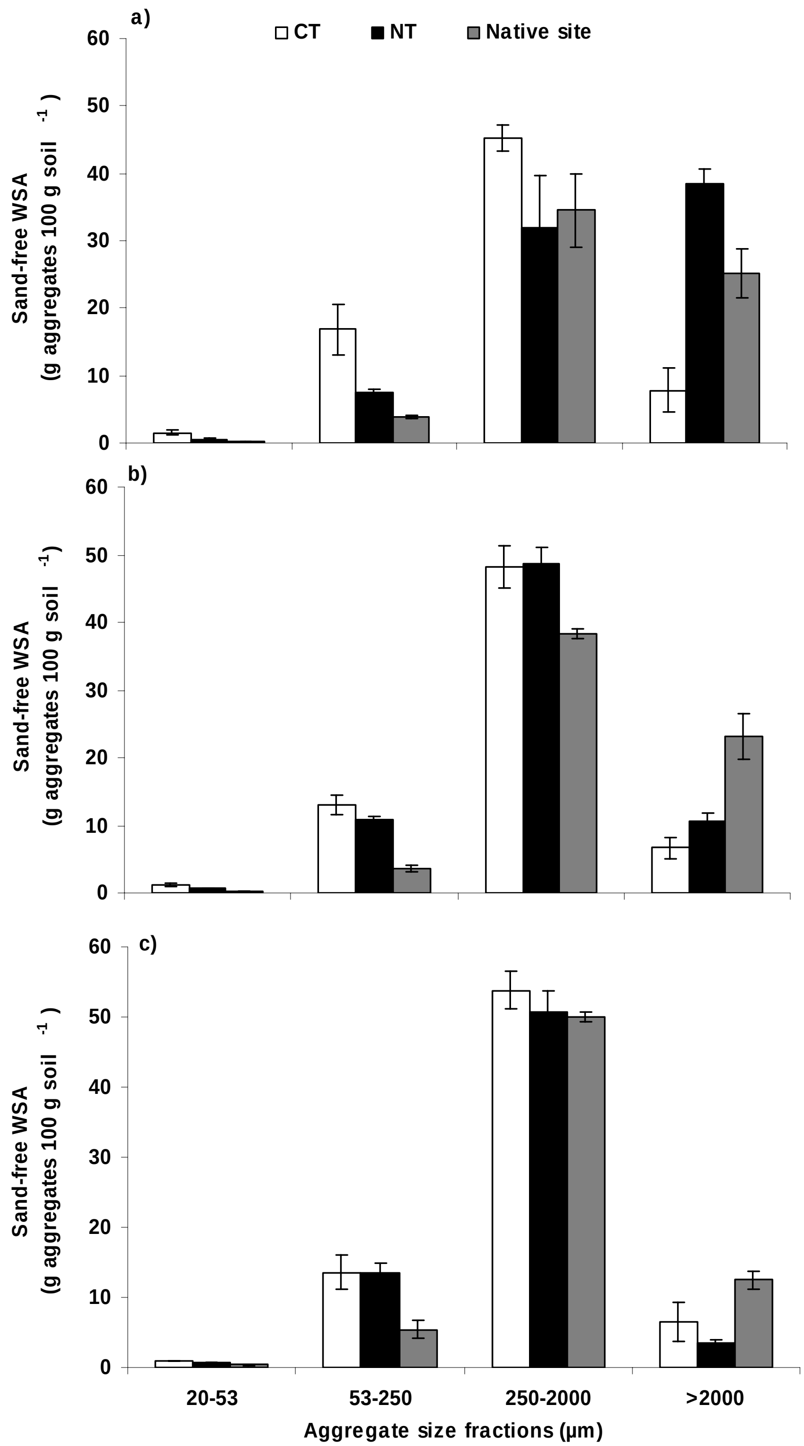

Figure 5-1. Distribution of sand-free water stable aggregates (WSA) under conventional tillage (CT), no-tillage (NT) and native vegetation at 0-5 cm (a), 0-15 (b), and 15-30 (c) for the Oxisol. Error bars represent the standard error of the mean.

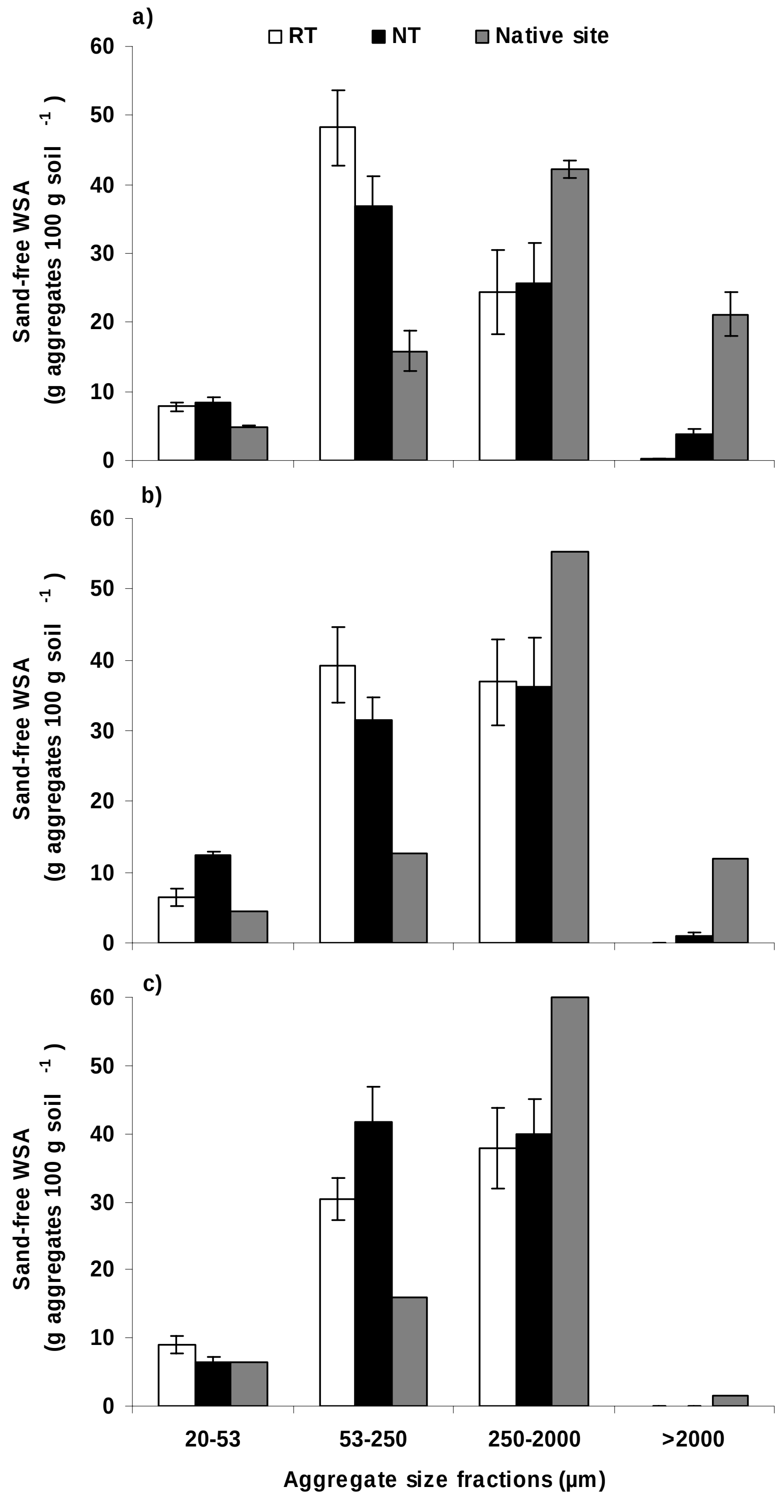

Figure 5-2. Distribution of sand-free water stable aggregates (WSA) under reduced tillage (RT), no-tillage (NT) and native vegetation at 0-5 cm (a), 0-15 (b), and 15-30 (c) for the Vertisol. Error bars represent the standard error of the mean.

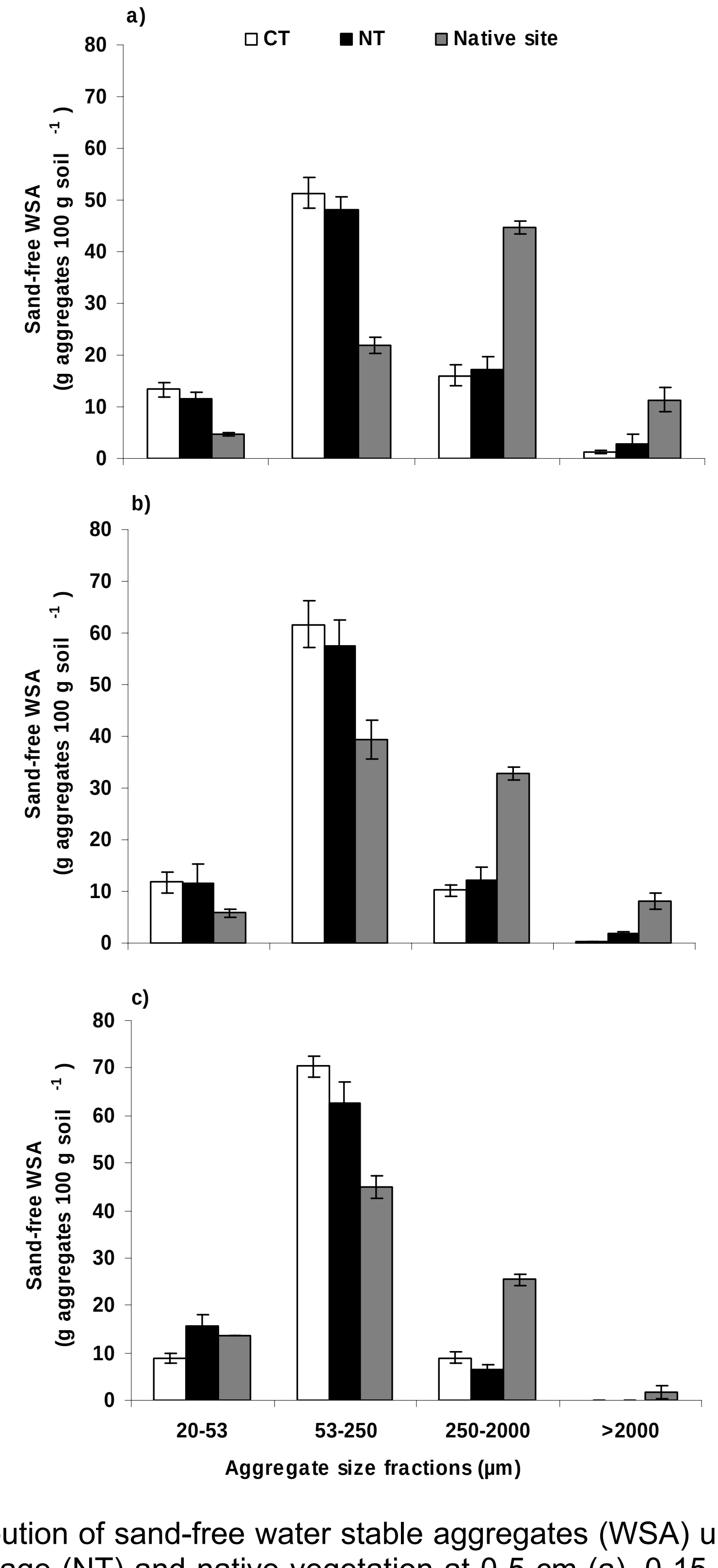

Figure 5-3. Distribution of sand-free water stable aggregates (WSA) under conventional tillage (CT), no-tillage (NT) and native vegetation at 0-5 cm (a), 0-15 (b), and 15-30 (c) for the Mollisol. Error bars represent the standard error of the mean.

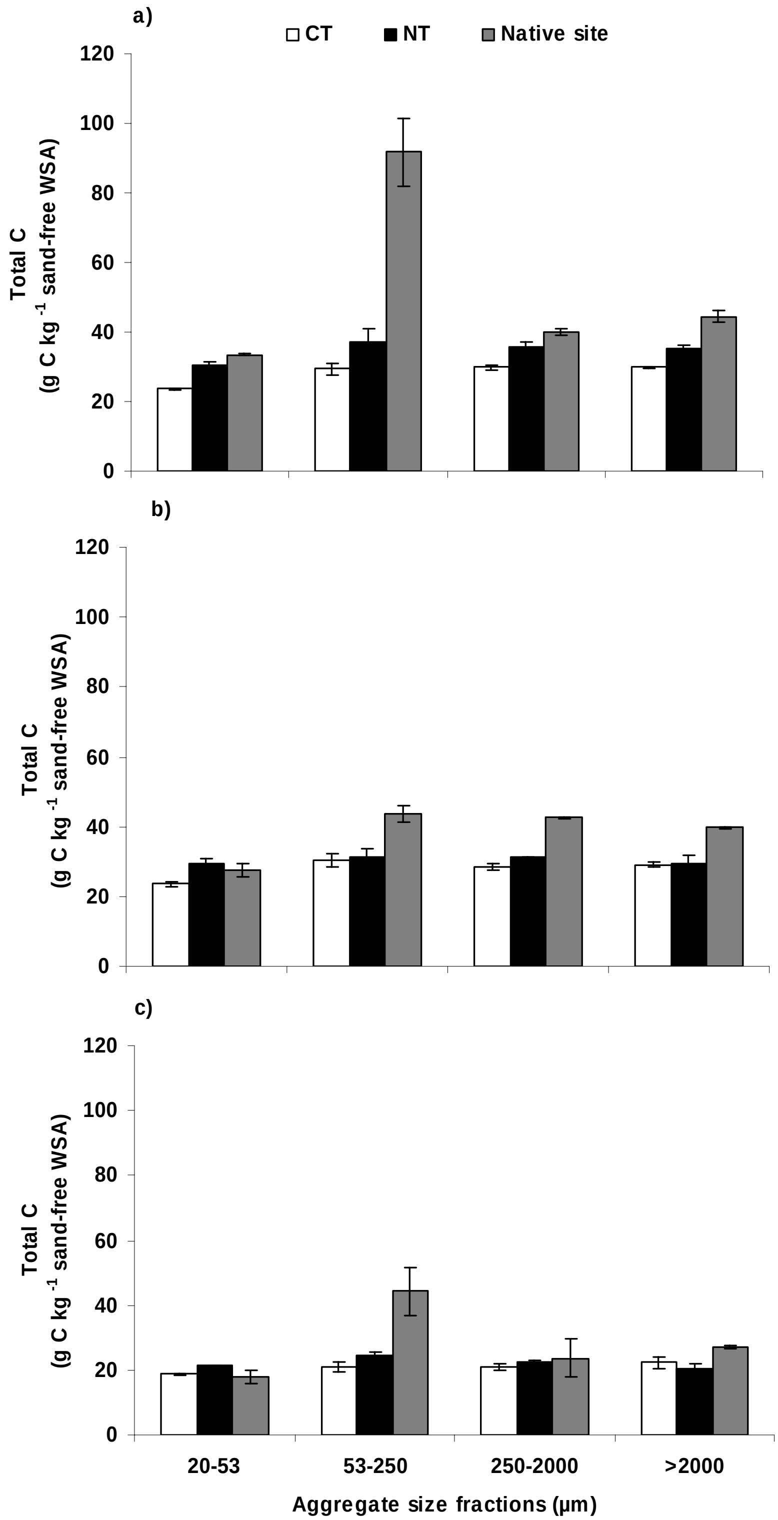

Figure 5-4. Total C in the sand-free water stable aggregates (WSA) under conventional tillage (CT), no-tillage (NT) and native vegetation at 0-5 cm (a), 0-15 (b), and 15-30 (c) for the Oxisol. Error bars represent the standard error of the mean.

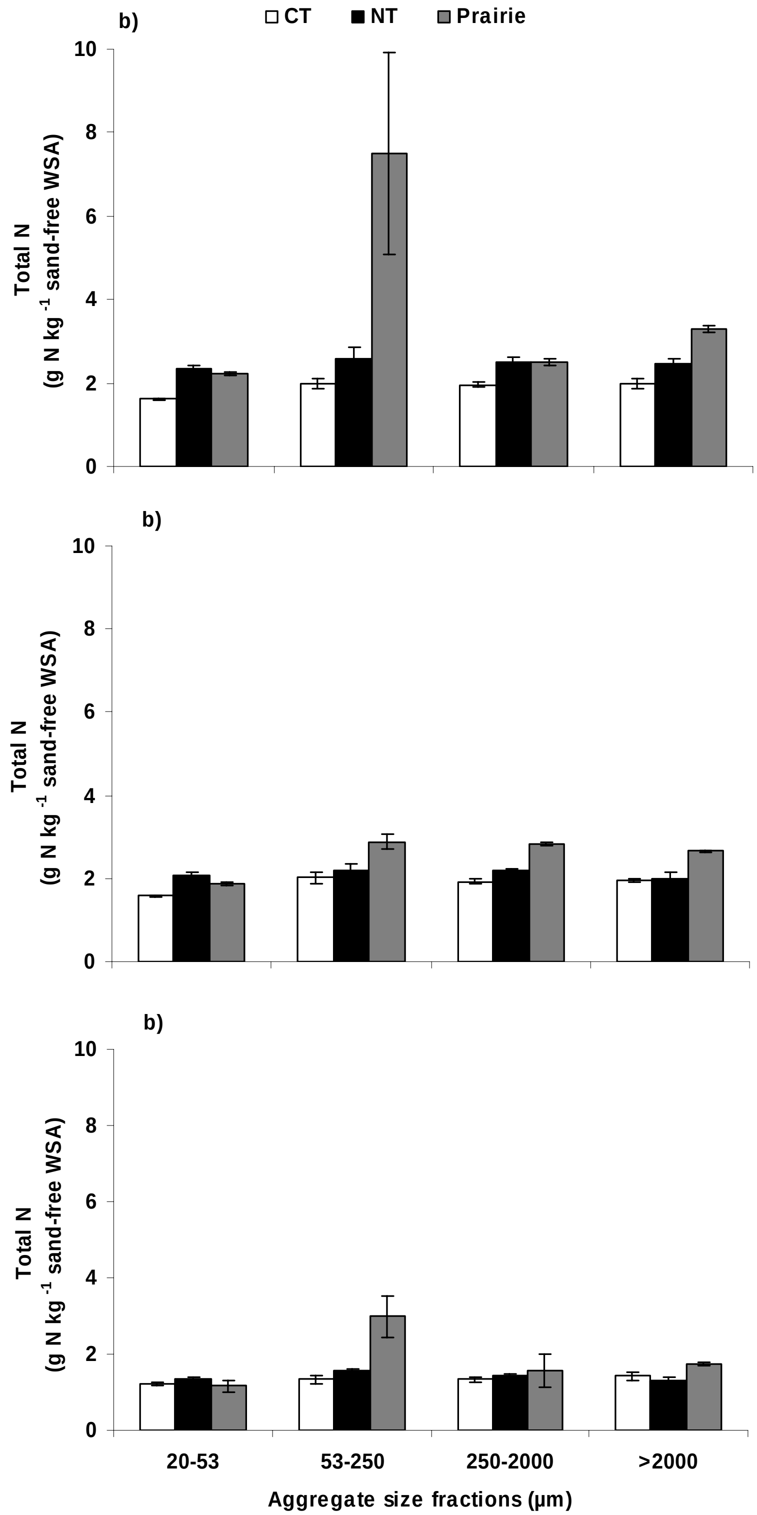

Figure 5-5. Total N in the sand-free water stable aggregates (WSA) under conventional tillage (CT), no-tillage (NT) and native vegetation at 0-5 cm (a), 0-15 (b), and 15-30 (c) for the Oxisol. Error bars represent the standard error of the mean.

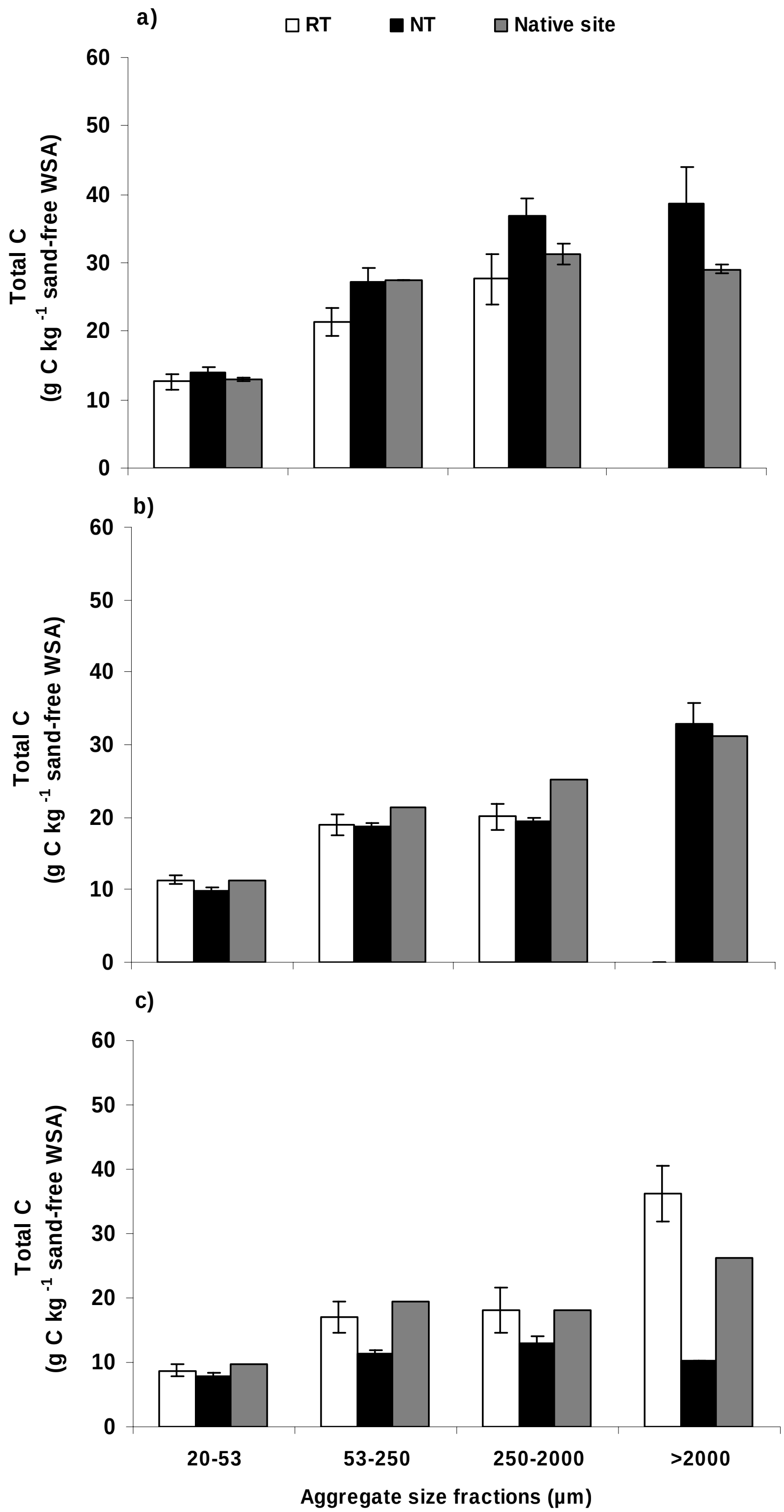

Figure 5-6. Total C in the sand-free water stable aggregates (WSA) under reduced tillage (RT), no-tillage (NT) and native vegetation at 0-5 cm (a), 0-15 (b), and 15-30 (c) for the Vertisol. Error bars represent the standard error of the mean.

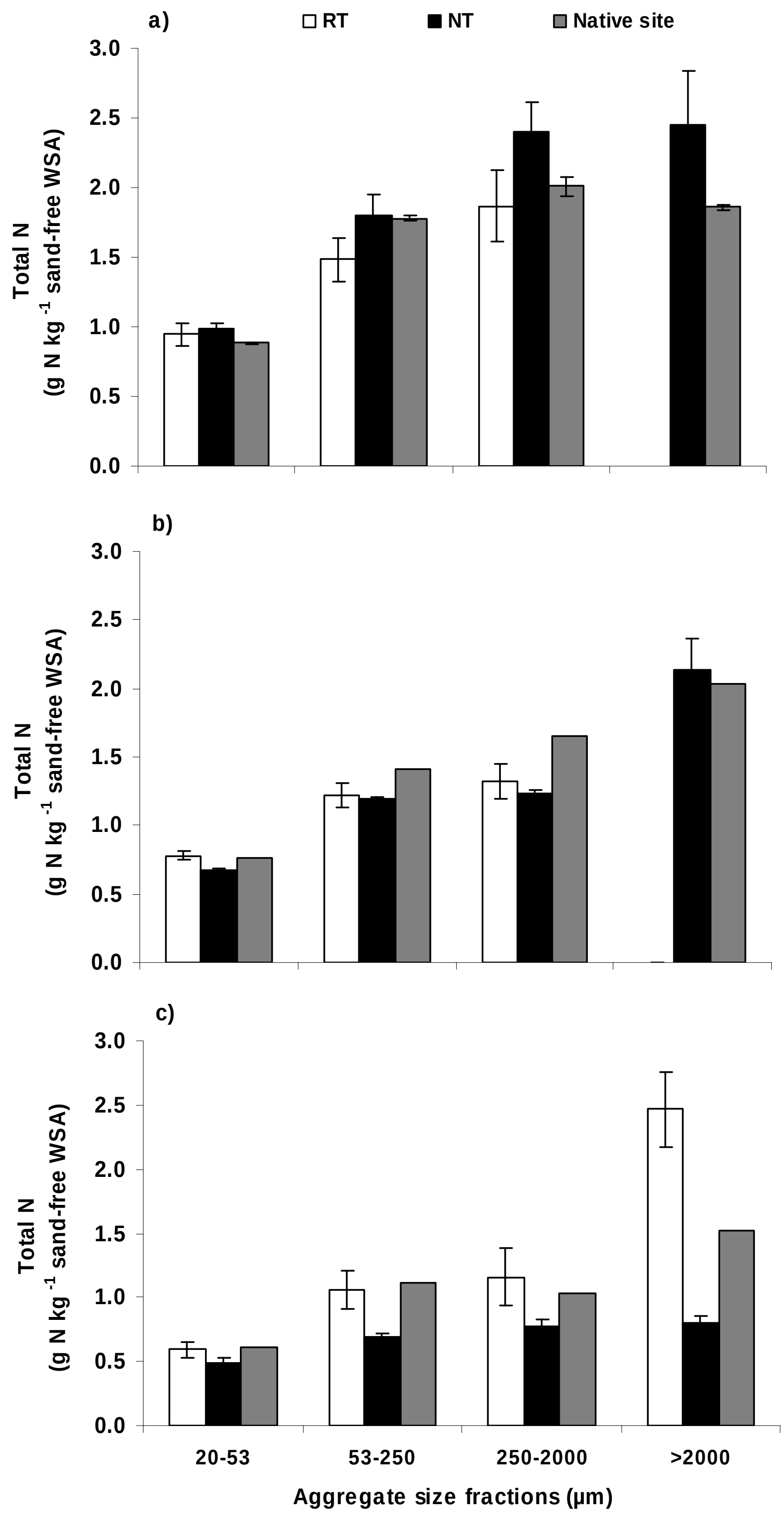

Figure 5-7. Total N in the sand-free water stable aggregates (WSA) under reduced tillage (RT), no-tillage (NT) and native vegetation at 0-5 cm (a), 0-15 (b), and 15-30 (c) for the Vertisol. Error bars represent the standard error of the mean.

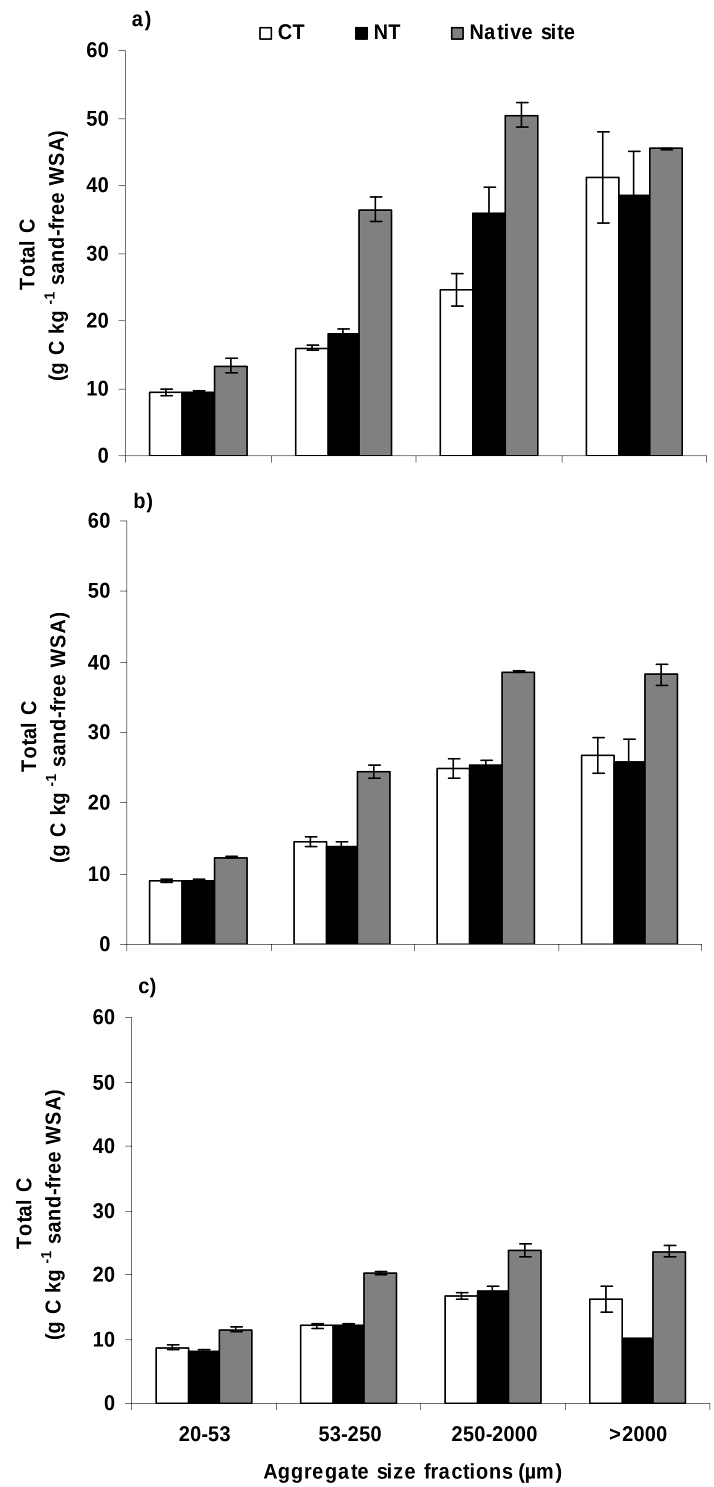

Figure 5-8. Total C in the sand-free water stable aggregates (WSA) under conventional tillage (CT), no-tillage (NT) and native vegetation at 0-5 cm (a), 0-15 (b), and 15-30 (c) for the Mollisol. Error bars represent the standard error of the mean.

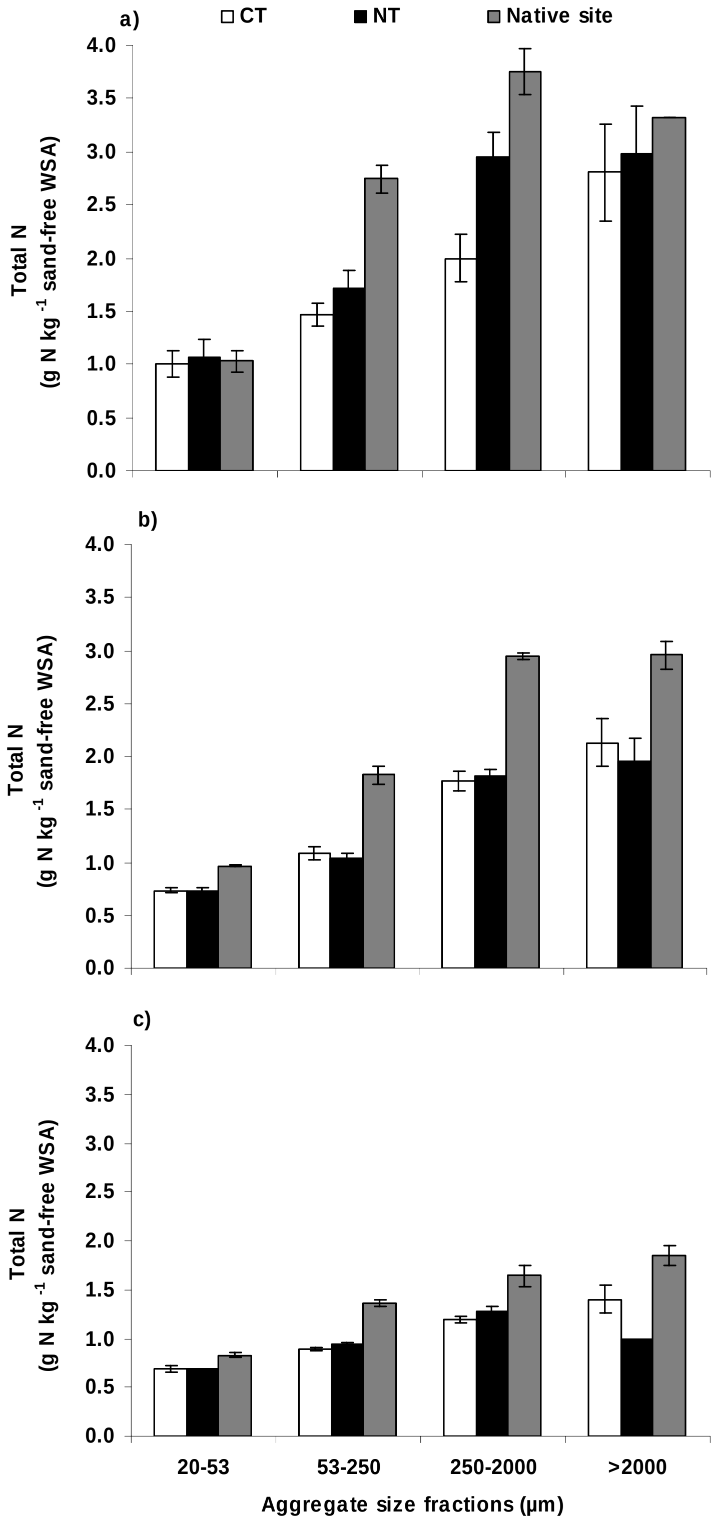

Figure 5-9. Total N in the sand-free water stable aggregates (WSA) under conventional tillage (CT), no-tillage (NT) and native vegetation at 0-5 cm (a), 0-15 (b), and 15-30 (c) for the Mollisol. Error bars represent the standard error of the mean.

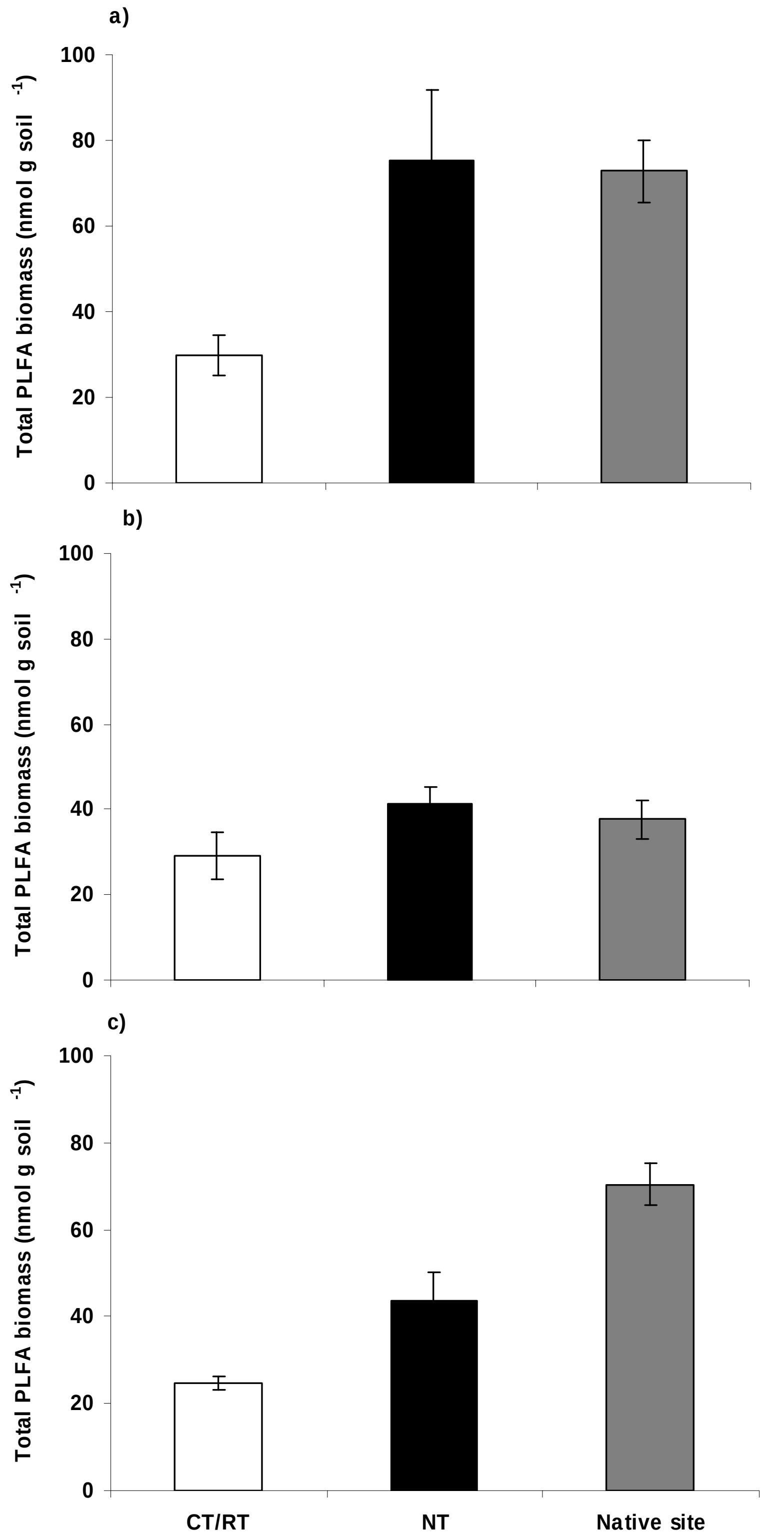

Figure 5-10. Total phospholipid fatty acid (PLFA) biomass under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) and native vegetation at 0-5 cm, for the Oxisol (a), Vertisol (b) and Mollisol (c). Error bars represent the standard error of the mean.

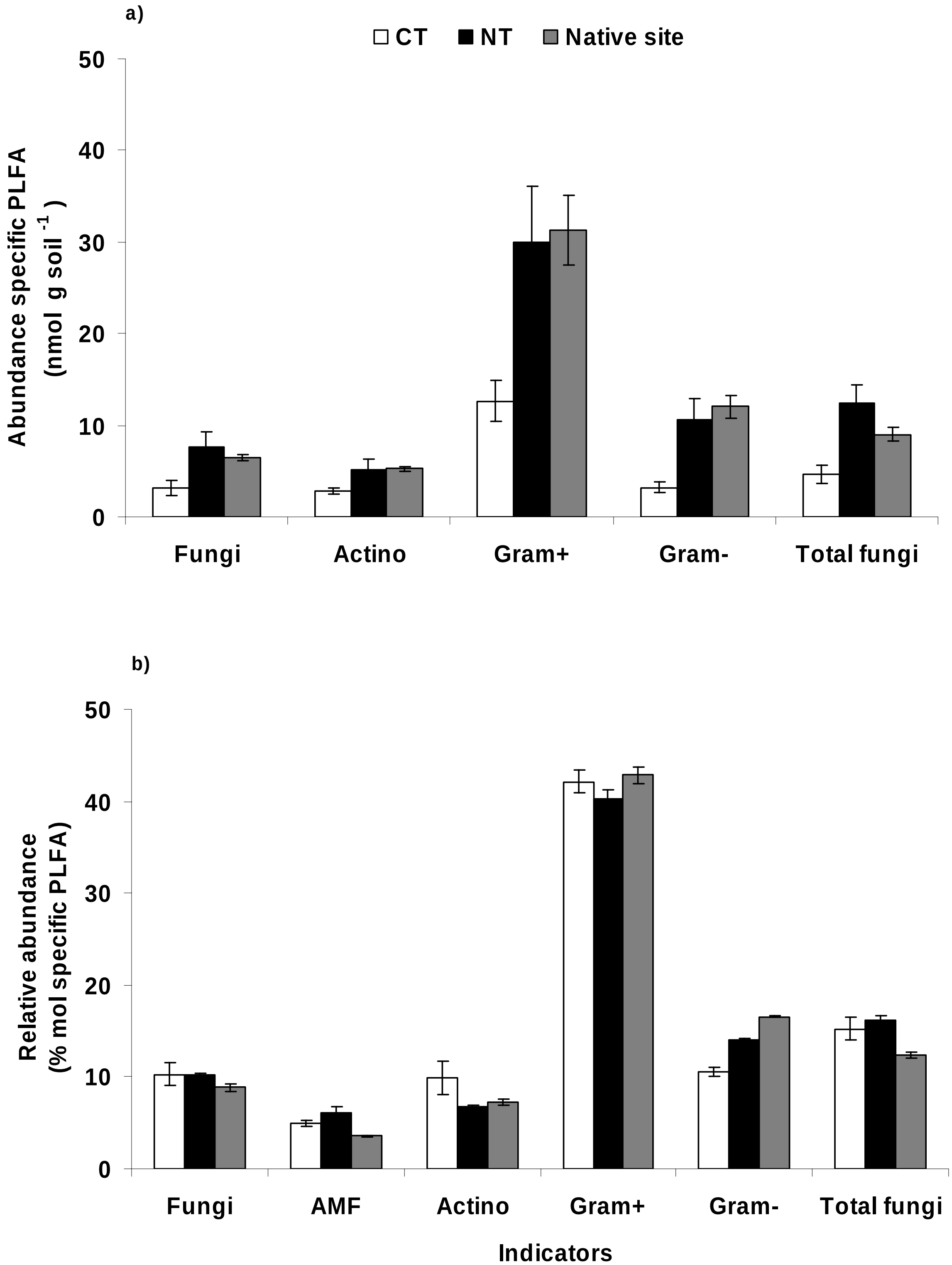

Figure 5-11. Abundance of specific phospholipid fatty acid (PLFA) (a) and relative abundance (b) under conventional tillage (CT), no-tillage (NT) and native vegetation at 0-5 cm, for the Oxisol. Error bars represent the standard error of the mean.

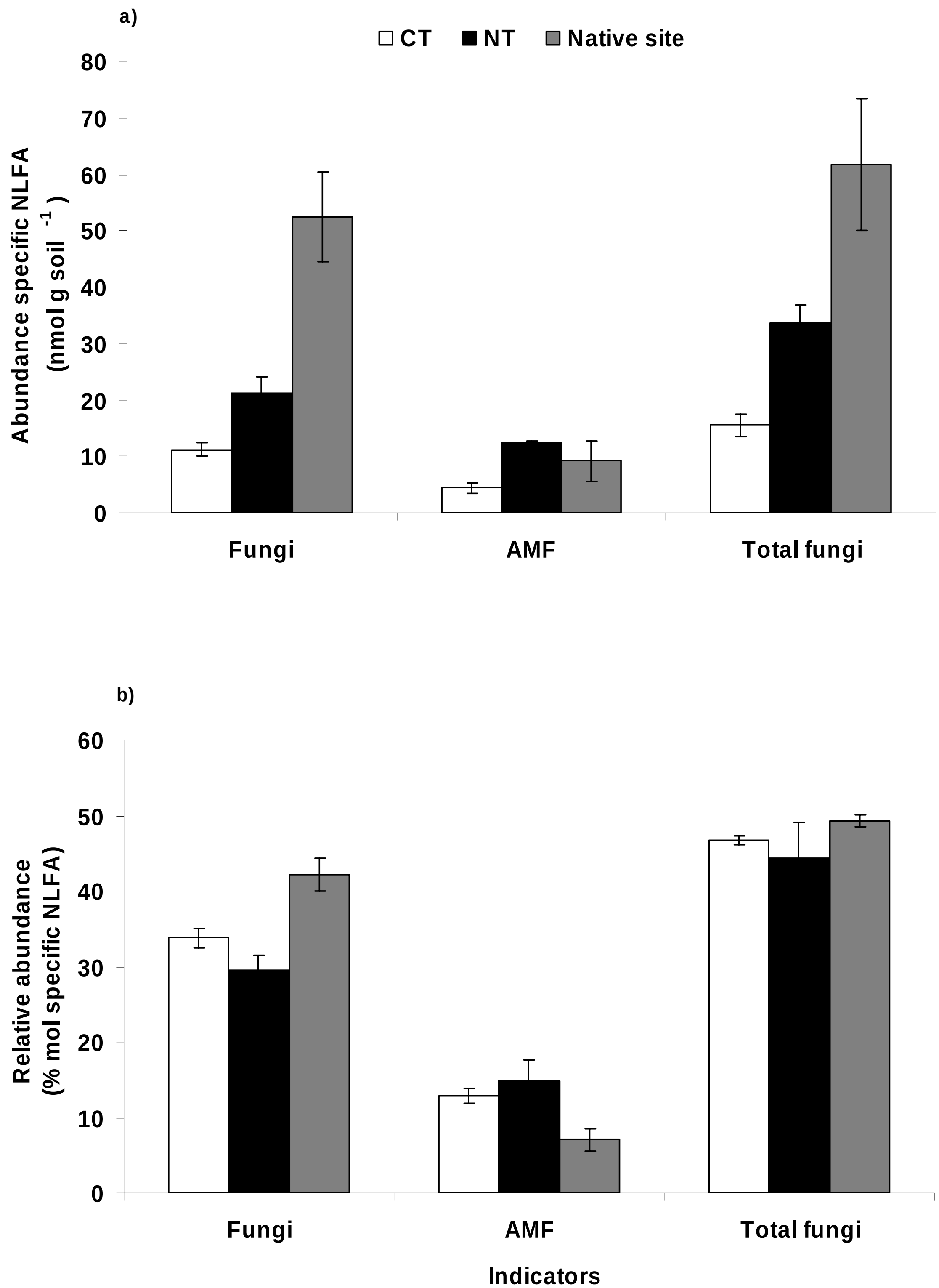

Figure 5-12. Abundance of specific neutralipid fatty acid (NLFA) (a) and relative abundance (b) under conventional tillage (CT), no-tillage (NT) and native vegetation at 0-5 cm, for the Oxisol. Error bars represent the standard error of the mean.

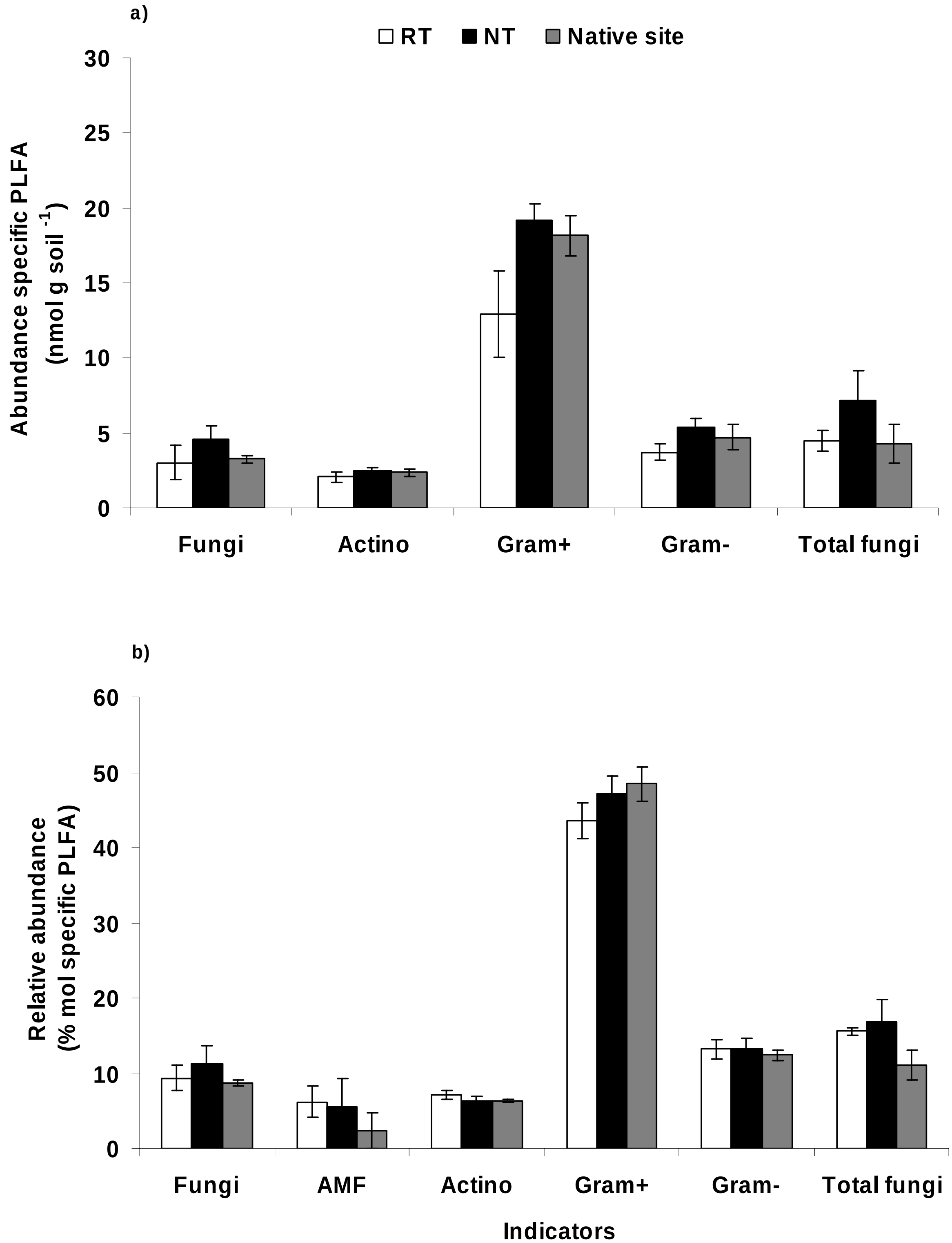

Figure 5-13. Abundance of specific phospholipid fatty acid (PLFA) (a) and relative abundance (b) under reduced tillage (RT), no-tillage (NT) and native vegetation at 0-5 cm, for the Vertisol. Error bars represent the standard error of the mean.

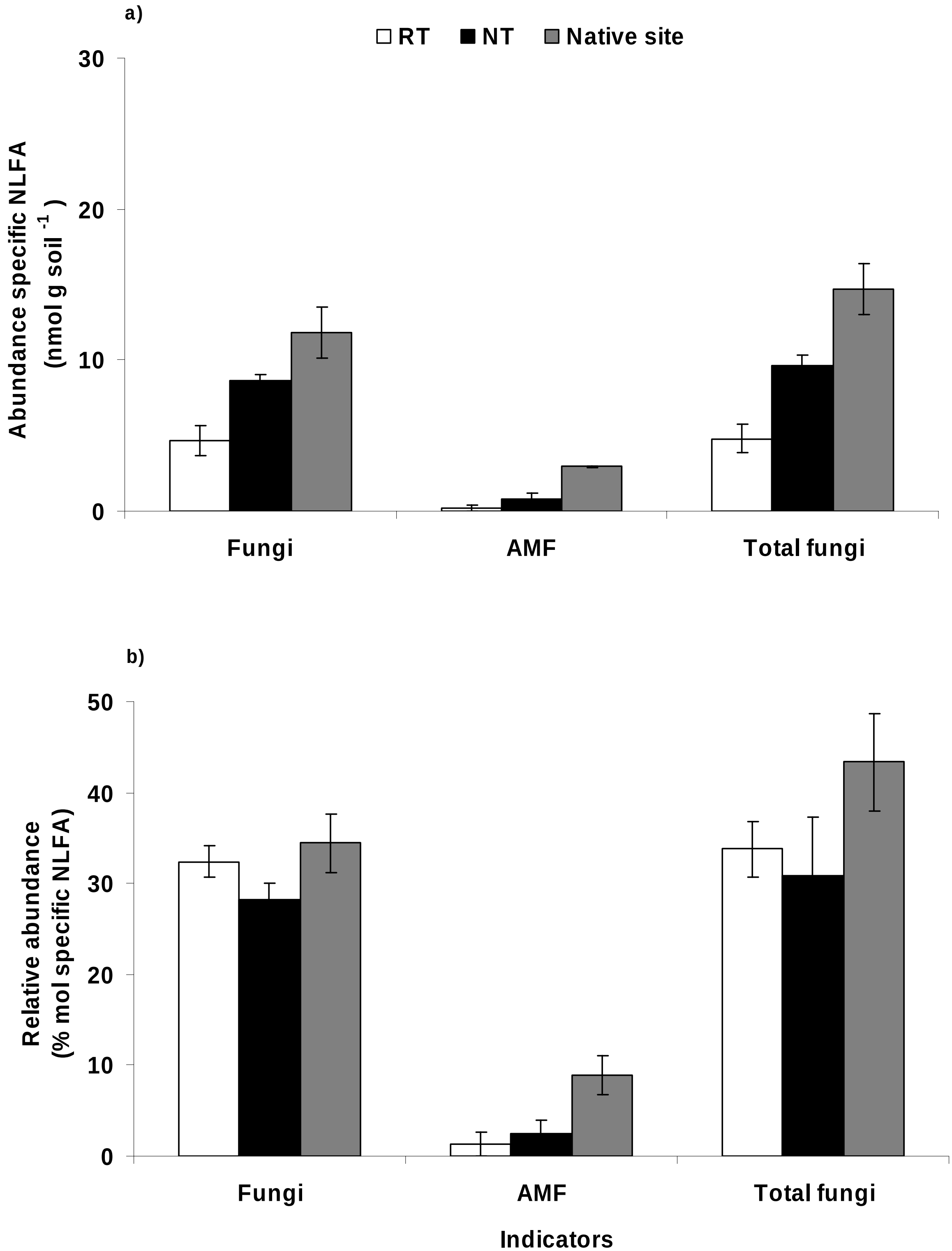

Figure 5-14. Abundance of specific neutralipid fatty acid (NLFA) (a) and relative abundance (b) under reduced tillage (RT), no-tillage (NT) and native vegetation at 0-5 cm, for the Vertisol. Error bars represent the standard error of the mean.

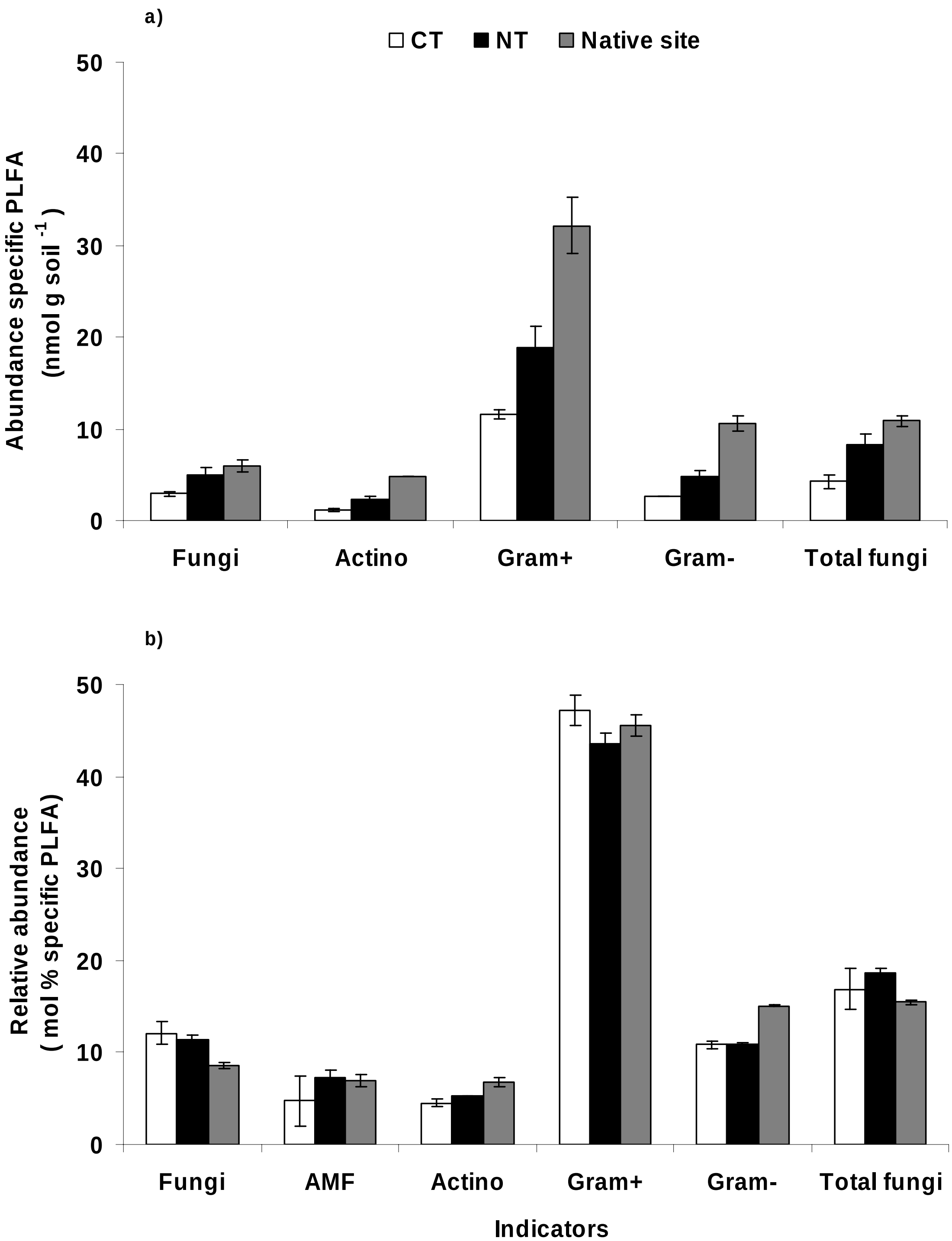

Figure 5-15. Abundance of specific phospholipid fatty acid (PLFA) (a) and relative abundance (b) under conventional tillage (CT), no-tillage (NT) and native vegetation at 0-5 cm, for the Mollisol. Error bars represent the standard error of the mean.

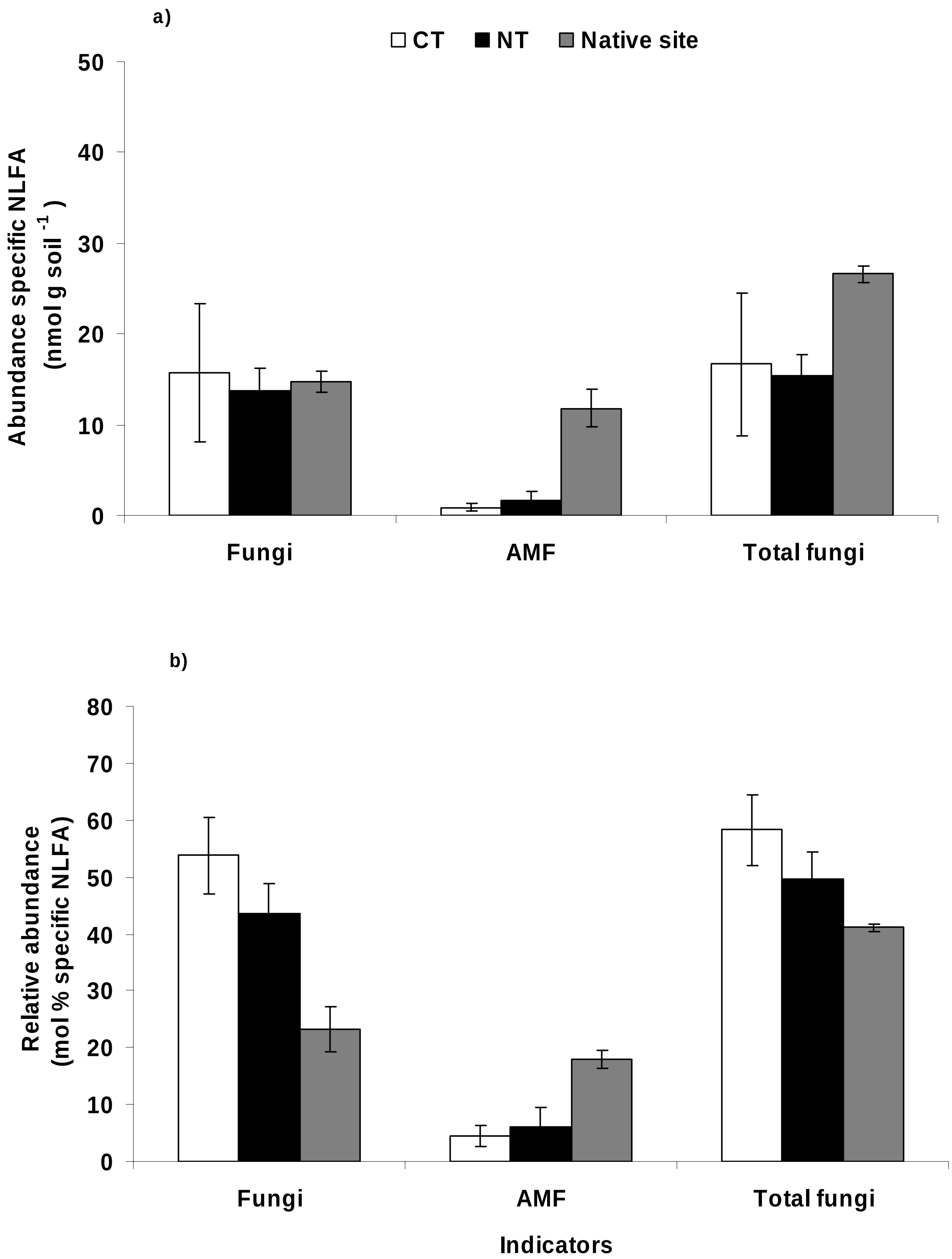

Figure 5-16. Abundance of specific neutralipid fatty acid (NLFA) (a) and relative abundance (b) under conventional tillage (CT), no-tillage (NT) and native vegetation at 0-5 cm, for the Mollisol. Error bars represent the standard error of the mean

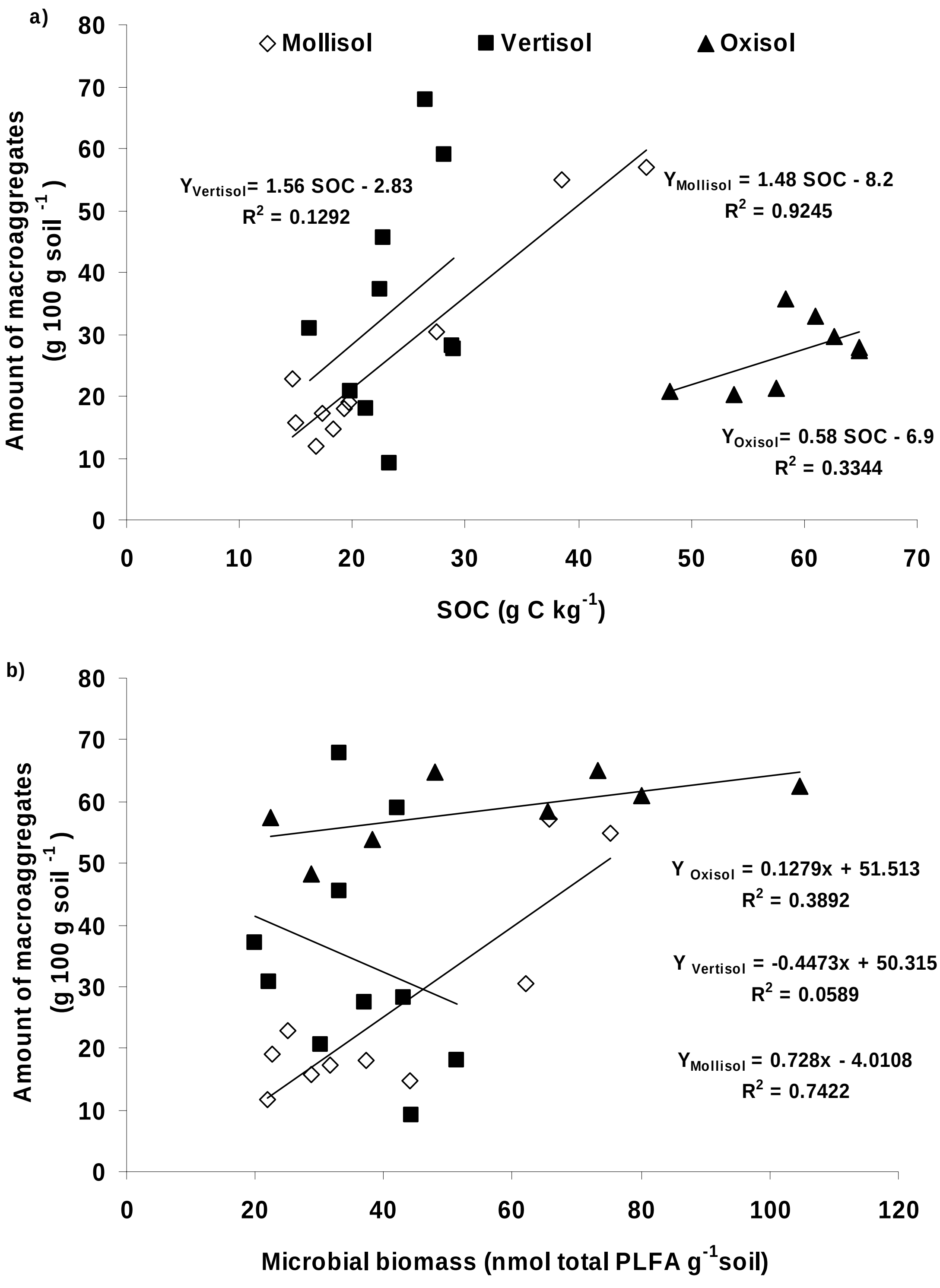

Figure 5-17. Relationship between the soil organic content (SOC) and the amount of macroaggregates (a) and microbial biomass estimated through the PLFA technique and the amount of macroaggregates (b) (> 250 μm) for the Oxisol, Vertisol, and Mollisol.

Table 5-1. Soil characteristics of the three sites evaluated in Brazil, Argentina, and Kansas (USA) at 0-5 cm.

Site	Soil type		pH	Bray-P	Ca^{2+}	K^{+}	Mg^{2+}	Na^{+}	CEC	Sand	Silt	Clay
				………………………… mg kg^{-1} ………………					cmol $_{(+)}$ kg^{-1}	…………… % …………		
Brazil	Oxisol	NT	5.1	26.8	1004	261	261	4.3	17.1	25	24	51
		CT	5.1	18.4	681	146	189	3.2	16.2	25	23	52
		Native site	5.3	3.7	826	185	336	4.8	20.3	28	28	44
Argentina	Vertisol	NT	7.4	35.8	6290	994	389	11.6	43.3	8	49	43
		CT	7.5	29.3	7011	804	374	24.3	41.2	8	49	44
		Native site	6.3	36.4	4340	527	544	49.7	39.2	6	52	41
Kansas	Mollisol	NT	5.8	55.0	2137	318	265	10.2	18.4	12	68	20
		CT	6.2	54.9	2260	371	297	14.5	17.1	10	70	20
		Native site	5.7	65.0	2472	659	412	19.6	24.7	9	59	32

Table 5-2. Soil organic carbon (C) and total nitrogen (Total N) for cropped (conventional tillage (CT), no-tillage (NT)) and native vegetation for the Oxisol, Vertisol, and Mollisol.

	Soil organic C			Total N		
	0-5 cm	0-15 cm	15-30 cm	0-5 cm	0-15 cm	15-30 cm
	……………………g C kg^{-1}…………….			……………………g N kg^{-1}…………….		
OXISOLS						
CT	20.8 **Bb**	20.2 **Bb**	15.6 **Ba**	2.10	2.43 **Ba**	1.41
NT	28.3 **Ba**	22.0 **Ba**	15.9 **Ba**	2.84	1.72 **Bb**	1.58
Native site	34.2 **A**	27.9 **A**	19.9 **A**	2.87	3.00 **A**	1.62
P values[1]	0.0086	0.0111	0.5940	0.1823	0.0076	0.6838
P values[2]	0.0023	0.0004	0.0092	0.2949	0.0006	0.8262
VERTISOLS						
CT	20.4 **Bb**	18.4	12.8	1.43 **Bb**	1.31	0.93
NT	25.5 **Aa**	17.2	14.7	1.84 **Aa**	1.17	0.96
Native site	27.3 **A**	18.7	12.2	1.88 **A**	1.30	0.79
P values[1]	0.0068	0.2118	0.6421	0.0057	0.1396	0.5777
P values[2]	0.0620	0.1848	0.9124	0.0697	0.1739	0.3248
MOLLISOLS						
CT	16.5 **Bb**	14.8 **Ba**	12.8 **Ba**	1.50 **Bb**	1.24 **Ba**	1.00**Ba**
NT	20.6 **Ba**	15.4 **Ba**	12.4 **Ba**	1.98 **Ba**	1.30 **Ba**	0.98 **Ba**
Native site	42.3 **A**	31.7**A**	19.5 **A**	3.74 **A**	2.85 **A**	1.71 **A**
P values[1]	0.0472	0.2643	0.4084	0.0108	0.2757	0.5931
P values[2]	0.0006	0.0001	0.0002	0.0002	0.0001	0.0001

[1] Indicates comparisons between CT and NT. [2] Indicates comparisons between CT and NT with Native site. Lowercase letters indicate differences between tillage. Uppercase letters indicates differences between tillage and the native site.

GENERAL SUMMARY

Agricultural activities can be a source and a sink for greenhouse gases (GHGs). Concern about climate change due to the increasing concentrations of GHGs in the atmosphere has created attention on mitigation strategies to reduce GHGs. Soil C sequestration appears to be a viable short-term option to mitigate the increase of CO_2 in the atmosphere, because it is relatively low cost and can be rapidly deployed across large areas.

Our results indicate that combining management practices was positive for increased soil C sequestration. Practices that reduced disturbance, such as no-tillage (NT), combined with rotations that contribute to increase amounts of residue, showed the greatest rates of C sequestration. Fallow in the rotation reduced C sequestration even with no-tillage management, for which a small rate or no change was observed.

Evaluation of the changes in SOC due to land use, agricultural practices, and climate is necessary for regional estimates of C sequestration, and for policy makers who develop policies to reduce greenhouse gases (GHG) emissions. The Intergovernmental Panel on Climate Change (IPCC) has developed guidelines to determine the National Greenhouse Gas Inventory through the estimation of emissions and sinks of GHG. They have developed coefficients to estimate soil C stocks by different agricultural land-use and management practices. The IPCC has established a default value of 1.1 for SOC in NT relative to conventional tillage (CT). Our results indicated a factor of 1.14 for a change from CT to NT, and 1.08 from CT to RT for 0-30 cm. The value for reduced tillage is important to establish for those farmers that either cannot or are unwilling to adopt no-tillage. These results can be used to validate models that will extrapolate C sequestration rates for the state of Kansas for various management practices and systems.

One of the proposed mechanisms for increasing soil C is the development of macroaggregates in soil. Development of macroaggregates also has additional benefits of improving soil structure thereby improving the quality and physical environment of the soil. Nitrogen application and crop rotations that included wheat or sorghum improved aggregation however the impact was enhanced when combined with no-till. In addition to the increased level of aggregation, the C and N associated with the macroaggregates increased, supporting the idea of physical protection of C and N within the soil. Thus, for these soils, buildup and maintenance of macroaggregates seems to be one of the primary mechanisms for C retention in these temperate agroecosystems.

Soil organic C and N often are divided into different pools of different turnover times and stability. The most common division is into three pools, including microbial biomass, mineralizable, and recalcitrant C. Soil microbial biomass made up a small fraction of the total C (0.7-3%) and N (0.7-6%) pools and was more variable in response to different management practices. It appears the gain in soil organic C in NT is reflected in the mineralizable pool, which can be expected as C flows through the microbial biomass into the mineralizable pool and would be expected to be transformed into the recalcitrant pool.

No-tillage as a strategy to reduce soil erosion, improve soil structure, reduce soil C loss and to promote sustainable agriculture has gained importance around the world. No-tillage affects soil aggregation and the composition and function of microbial communities thus affecting soil C dynamics. The responses in the soil to no-tillage may vary according to the genetics of the soil and the climate. We examined the impact of tillage systems and native grass on SOC and total N, and the microbial ecology of three genetic soils; an Oxisol in Brazil, a Vertisol in Argentina, and a Mollisol in USA. Introduction of cropping systems generally decreased aggregation and SOC and total N. However, the reduction in C and N and aggregation compared with soil under native grass was less with NT systems than with CT systems. Our results showed the importance of the physical mechanisms on the aggregate formation in the Oxisol, while in the Mollisol the strong correlation between SOC or microbial biomass and the amount of macroaggregates suggested that SOM plays a key role in the aggregate formation. Cultivation decreased the amount of macroaggregates with an increase in microaggregates, but there was no C depletion at any aggregate size fraction in the Oxisol indicating that there was not a direct link between loss of aggregates and C loss; however, in the Mollisol, cultivation resulted in the loss of C-rich macroaggregates and an increase in C-depleted microaggregates.

Greater microbial biomass was observed under native grassland and NT systems. Fungi and AM fungi were more abundant in NT than CT, with NT tending to be more similar to the native grass. This change in microbial communities to increased abundance of fungi in less disturbed systems could explain the greater levels of aggregation and the resulting levels of soil C storage, particularly in the Mollisol. In any case less soil disturbance (NT or native) increased aggregation and SOC and total N across all three soils. Shifts in microbial communities due to management practices can have important implications on the soil C dynamics and soil aggregation. Thus, soil environments favoring fungal growth can contribute to C sequestration in soils, such as in no-tillage systems.

Overall, the adoption of management practices that increase productivity while enhancing C sequestration in soil and preserving the environment would be important to maintain the sustainability of agricultural systems.

Future research is needed to evaluate the effects of the shift in microbial communities structure due to soil disturbance and its implications in the incorporation, turnover and stabilization of C in agricultural soils under different climatic and soil conditions. Integration of data that characterize soil aggregation and microbial community composition into ecosystems models will improve knowledge of the global impact of management practices on soil C sequestration.

Appendix A - Chapter 3

Table A-1. Distribution of sand-free water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment.

	Sand-free water stable aggregates			
	20-53 μm†	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………g 100 g^{-1} soil……………………			
CT	13.6 **g**	42.6 **a**	13.9 **g**	3.3 **j**
RT	13.3 **g**	33.9 **b**	19.7 **de**	10.8 **gi**
NT	16.4 **ge**	28.9 **c**	22.9 **d**	12.4 **g**
SOD	7.3 **i**	19.6 **e**	31.9 **bc**	17.9 **e**
	…………………………*P* values………………………			
Tillage (T)	0.5448			
Size (S)	<.0001			
T x S	<.0001			

† Different letter means differences by tillage systems and aggregate size fraction.

Table A-2. Distribution of sand-free water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 140 (140-N) kg N ha^{-1} for Parsons experiment.

	Sand-free water stable aggregates			
	20-53 μm	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………. g 100 g^{-1} soil …………………….			
CT 0-N	20.3	29.1	15.8	5.25
RT 0-N	14.7	27.7	17.8	9.2
NT 0-N	13.2	25.0	22.9	11.3
CT 140-N	16.1	28.2	19.6	7.2
RT 140-N	15.5	26.0	19.9	8.48
NT 140-N	13.2	24.0	23.4	11.2
	…………………………*P* values……………………			
Tillage (T)	0.7498			
Nitrogen (N)	0.9467			
T x N	0.9829			
Size (S)	<.0001			
T x S	<.0001			
CT (mean)	18.2 **cg**†	28.6 **a**	17.7 **cg**	6.2 **h**
RT (mean)	15.1 **dg**	26.8 **af**	18.9 **c**	8.8 **eh**
NT (mean)	13.2 **d**	24.5 **bf**	23.2 **b**	11.2 **de**
N x S	0.2477			
T x N x S	0.6065			

† Different letter means differences by tillage systems and aggregate size fraction.

Table A-3. Distribution of sand-free water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1} for Hays experiment.

	Sand-free water stable aggregates			
	20-53 μm†	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………. g 100 g^{-1} soil …………………….			
CT 0-N	15.01 **f**	49.48 **ab**	17.15 **ef**	1.35 **g**
RT 0-N	16.06 **f**	50.31 **a**	16.19 **f**	1.29 **g**
NT 0-N	16.82 **f**	50.51 **a**	15.59 **f**	0.91 **g**
CT 67-N	14.59 **f**	49.81 **ab**	17.34 **ef**	1.53 **g**
RT 67-N	14.87 **f**	44.23 **b**	22.98 **ed**	1.85 **g**
NT 67-N	13.93 **f**	36.95 **c**	28.82 **d**	4.03 **g**
	…………………………*P* values………………………			
Tillage (T)	0.9802			
Nitrogen (N)	0.9802			
T x N	0.9989			
Size (S)	0.0001			
T x S	0.0387			
N x S	0.0001			
T x N x S	0.0036			

† Different letter means differences by tillage systems, nitrogen application and aggregate size fraction.

Table A-4. Distribution of sand-free water stable aggregates under conventional tillage (CT), and no-tillage (NT) in different rotations, continuous wheat (W-W), wheat-soybean (W-B), continuous sorghum (S-S), sorghum-soybean (S-B) and continuous soybean (B-B) for Manhattan experiment.

ROTATION	TILLAGE	Sand-free water stable aggregates			
		20-53 μm†	**53-250 μm**	**250-1000 μm**	**>1000 μm**
		…………………… g 100 g^{-1} soil ……………………			
W-W	**CT**	15.17 **hijklm**	38.18 **a**	18.41 **ghijk**	2.37 **q**
S-S	**CT**	29.76 **abcde**	32.38 **abcd**	11.88 **jklmnop**	4.83 **opq**
S-B	**CT**	28.91 **bcdef**	34.06 **abc**	13.88 **ijklmn**	3.71 **pq**
W-B	**CT**	16.53 **ghijkl**	32.71 **abcd**	17.78 **ghijkl**	1.34 **pq**
B-B	**CT**	34.30 **ab**	32.79 **abcd**	10.02 **klmnopq**	2.01 **q**
W-W	**NT**	12.22 **jklmnop**	20.39 **fghij**	36.24 **ab**	16.81 **ghijkl**
S-S	**NT**	20.44 **fghij**	31.06 **abdce**	21.60 **efghi**	9.07 **lmnopq**
S-B	**NT**	23.89 **defgh**	36.02 **ab**	15.17 **hijklm**	6.00 **mnopq**
W-B	**NT**	17.19 **ghijkl**	34.64 **ab**	13.62 **ijklmno**	1.68 **q**
B-B	**NT**	25.36 **cdefg**	32.08 **abcd**	16.11 **ghijkl**	5.99 **nopq**
		………………………*P* values………………………			
Rotation (R)		0.2756			
Tillage (T)		0.4870			
T x R		0.8654			
Size (S)		0.0001			
R x S		0.0001			
T x S		0.0001			
R x T x S		0.0020			

† Different letter means differences by tillage systems, crop rotation and aggregate size fraction.

Table A-5. Distribution of sand-free water stable aggregates (WSA), total C mass, and total N for macroaggregates (>250 μm) and microaggregates (<250 μm) fractions under conventional tillage (CT), and no-tillage (NT) in different rotations, continuous wheat (W-W), wheat-soybean (W-B), continuous sorghum (S-S), sorghum-soybean (S-B) and continuous soybean (B-B) for Manhattan experiment.

ROTATION	**TILLAGE**	**WSA**		**Total C mass**		**Total N mass**	
		>250 μm†	**<250 μm**	**>250 μm**	**<250 μm**	**>250 μm**	**<250 μm**
		g 100 g^{-1} soil		g C kg^{-1} sand-free aggregates		g N kg^{-1} sand-free aggregates	
W-W	**CT**	20.8 **ef**	53.34 **bc**	0.32 **ef**	0.56 **cd**	0.040 **efg**	0.058 **ed**
S-S	**CT**	16.7 **f**	62.1 **ab**	0.31 **ef**	0.56 **cd**	0.032 **fgh**	0.063 **cd**
S-B	**CT**	17.6 **f**	63.0 **ab**	0.29 **ef**	0.27 **cd**	0.027 **gh**	0.056 **ed**
W-B	**CT**	18.8 **f**	49.2 **c**	0.30 **ef**	0.45 **ed**	0.030 **fhg**	0.053 **def**
B-B	**CT**	12.0 **f**	67.1 **a**	0.16 **f**	0.49 **cde**	0.014 **h**	0.052 **def**
W-W	**NT**	53.1 **bc**	32.6 **d**	1.00 **a**	0.41 **ed**	0.113 **a**	0.052 **ef**
S-S	**NT**	30.7 **ed**	51.5 **bc**	0.83 **ab**	0.67 **bc**	0.081 **bc**	0.065 **cd**
S-B	**NT**	21.2 **def**	59.9 **abc**	0.59 **cd**	0.86 **ab**	0.056 **ed**	0.091 **ab**
W-B	**NT**	15.3 **f**	51.8 **bc**	0.33 **ef**	0.59 **cd**	0.031 **fhg**	0.075 **bcd**
B-B	**NT**	22.10 **def**	57.4 **abc**	0.40 **ed**	0.56 **cd**	0.040 **efg**	0.619 **cde**

† Different letter in the same column means differences by tillage systems and crop rotation

Table A-6. Total carbon normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment.

	Total C			
	20-53 μm†	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………g C kg^{-1} sand-free aggregates……………………			
CT	8.8 **h**	18.7 **dg**	30.0 **a**	25.3 **b**
RT	8.1 **h**	16.8 **d**	24.3 **bf**	19.3 **eg**
NT	8.6 **h**	17.2 **dg**	22.9 **bce**	21.0 **e**
SOD	10.4 **h**	21.7 **ce**	24.9 **b**	22.5 **cf**
	…………………………*P* values………………………			
Tillage (T)	0.0015			
Size (S)	<.0001			
T X S	0.0020			

† Different letter in the same column means differences by tillage systems in each aggregate size fraction.

Table A-7.Total nitrogen normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment.

	Total N			
	20-53 μm	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………g N kg^{-1} sand-free aggregates……………………			
CT	0.8	2.0	2.5	2.6
RT	0.7	1.4	2.6	1.8
NT	0.8	1.7	2.6	1.9
SOD	1.0	2.2	2.6	2.2
	…………………………*P* values………………………			
Tillage (T)	0.1905			
Size (S)	<.0001			
Size (mean)	0.84	1.83	2.57	2.14
T X S	0.7060			

Table A-8.Total carbon normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 140 (140-N) kg N ha^{-1} for Parsons experiment.

	Total C			
	20-53 μm	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………. g C kg^{-1} sand-free aggregates …………………….			
CT 0-N	7.1	15.5	21.2	16.1
RT 0-N	6.6	15.8	20.5	16.7
NT 0-N	6.3	13.9	19.9	19.2
CT 140-N	6.7	14.8	21.1	17.9
RT 140-N	7.0	17.4	26.4	20.0
NT 140-N	6.3	14.9	21.8	22.2
	…………………………*P* values………………………			
Tillage (T)	0.4314			
Nitrogen (N)	0.0054			
T x N	0.0769			
Size (S)	<.0001			
T x S	<.0001			
CT (mean)	6.9 **e**†	15.1 **d**	21.1 **b**	17.0 **cf**
RT (mean)	6.8 **e**	16.6 **df**	23.5 **a**	18.4 **c**
NT (mean)	6.3 **e**	14.4 **d**	20.9 **b**	20.7 **b**
N x S	0.0046			
0-N (mean)	6.7 **e**‡	15.1 **d**	20.5 **b**	17.4 **c**
140-N (mean)	6.7 **e**	15.7 **d**	23.1 **a**	20. 0 **b**
T x N x S	0.2631			

† Different letter means differences by tillage systems and aggregate size fraction
‡ Different letter means differences by N application and aggregate size fraction

Table A-9. Total nitrogen normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 140 (140-N) kg N ha^{-1} for Parsons experiment

	Total N			
	20-53 μm	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	…………………… g N kg^{-1} sand-free aggregates ……………………			
CT 0-N	0.8	1.5	1.9	1.5
RT 0-N	0.8	1.6	1.9	1.6
NT 0-N	0.7	1.4	1.9	1.9
CT 140-N	0.8	1.5	1.9	1.7
RT 140-N	0.8	1.7	2.3	1.8
NT 140-N	0.7	1.6	2.1	2.1
	…………………………*P* values………………………			
Tillage (T)	0.3412			
Nitrogen (N)	0.0247			
T x N	0.2841			
Size (S)	<.0001			
T x S	<.0001			
CT (mean)	0.8 **d**†	1.5 **c**	1.93 **a**	1.6 **bc**
RT (mean)	0.8 **d**	1.6 **bc**	2.1 **a**	1.7 **b**
NT (mean)	0.7 **d**	1.5 **c**	2.0 **a**	2.0 **a**
N x S	0.0292			
0-N (mean)	0.8 **e**‡	1.5 **d**	1.9 **b**	1.7 **c**
140-N (mean)	0.8 **e**	1.6 **dc**	2.1 **a**	1.9 **b**
T x N x S	0.6228			

† Different letter means differences by tillage systems and aggregate size fraction
‡ Different letter means differences by N application and aggregate size fraction

Table A-10. Total carbon normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1} for Hays experiment.

	Total C			
	20-53 μm	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………. g C kg^{-1} sand-free aggregates …………………….			
CT 0-N	6.79	11.61	15.94	19.78
RT 0-N	6.66	10.07	15.57	18.56
NT 0-N	6.19	10.95	15.59	29.30
CT 67-N	7.69	13.16	19.98	22.27
RT 67-N	6.52	10.60	15.66	19.60
NT 67-N	7.45	12.45	20.25	22.44
	…………………………*P* values………………………			
Tillage (T)	0.0639			
Nitrogen (N)	0.2330			
T x N	0.4515			
Size (S)	0.0001			
Size (mean)	6.88 **d**	11.47 **c**	17.10 **b**	21.94 **a**
T x S	0.1416			
N x S	0.2919			
T x N x S	0.2474			

Table A-11. Total nitrogen normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1} for Hays experiment

	Total N			
	20-53 μm	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	…………………… g N kg^{-1} sand-free aggregates ……………………			
CT 0-N	0.519	0.821	1.150	1.350
RT 0-N	0.454	0.781	1.114	1.407
NT 0-N	0.467	0.019	1.163	1.972
CT 67-N	0.654	0.181	0.395	0.118
RT 67-N	0.467	0.750	1.178	1.467
NT 67-N	0.507	0.875	1.451	1.651
	…………………………*P* values………………………			
Tillage (T)	0.0906			
Nitrogen (N)	0.1028			
T x N	0.2035			
Size (S)	0.0001			
Size (mean)	0.511 **d**	0.847 **c**	1.256 **b**	1.574 **a**
T x S	0.1400			
N x S	0.3868			
T x N x S	0.5716			

Table A-12.Total carbon normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), and no-tillage (NT) in different rotations, continuous wheat (W-W), wheat-soybean (W-B), continuous sorghum (S-S), sorghum-soybean (S-B) and continuous soybean (B-B) for Manhattan experiment.

		Total C			
		…………………………*P* values………………………			
Rotation (R)		0.0009			
Tillage (T)		0.0001			
T x R		0.0440			
Size (S)		0.0001			
R x S		0.0001			
T x S		0.0001			
R x T x S		0.3372			
		20-53 μm	**53-250 μm**	**250-1000 μm**	**>1000 μm**
	Rotation	…………………… g C kg^{-1} sand-free aggregates ……………………			
R x S	**W-W**	8.64 **h**†	15.03 **gf**	17.72 **def**	24.55 **c**
	W-B	7.66 **h**	14.67 **gf**	20.52 **d**	30.86 **a**
	S-S	7.18 **h**	16.15 **efg**	24.13 **c**	27.81 **ab**
	S-B	7.55 **h**	16.61 **ef**	25.28 **bc**	26.59 **bc**
	B-B	5.96 **h**	13.10 **g**	18.44 **ed**	15.31 **gf**
	Tillage				
T x S	**CT**	6.37 **f**‡	12.85 **e**	17.40 **d**	21.26 **c**
	NT	8.42 **f**	17.37 **d**	25.05 **b**	28.79 **a**

†Different letter means differences by crop rotation and aggregate size fraction
‡ Different letter means differences by tillage systems and aggregate size fraction

Table A-13.Total nitrogen normalized to sand-free basis in each water stable aggregates under conventional tillage (CT), and no-tillage (NT) in different rotations, continuous wheat (W-W), wheat-soybean (W-B), continuous sorghum (S-S), sorghum-soybean (S-B) and continuous soybean (B-B) for Manhattan experiment.

ROTATION	TILLAGE	Total N mass			
		20-53 μm[1]	53-250 μm	250-1000 μm	>1000 μm
		…………………… g N kg^{-1} sand-free aggregates ……………………			
W-W	**CT**	0.81	1.43	2.04	2.24
S-S	**CT**	0.61	1.63	1.92	2.23
S-B	**CT**	0.66	1.20	1.75	1.61
W-B	**CT**	0.69	1.66	2.41	4.58
B-B	**CT**	0.52	1.30	1.50	1.16
W-W	**NT**	1.16	2.11	2.16	2.88
S-S	**NT**	0.79	1.81	3.04	2.82
S-B	**NT**	1.05	2.09	3.16	2.84
W-B	**NT**	1.81	2.08	3.10	0.81
B-B	**NT**	0.63	1.78	2.16	1.67
		………………………….*P* values……………………			
Rotation (R)		0.1671			
Tillage (T)		0.0021			
T x R		0.5607			
Size (S)		0.0004			
Size (mean)		1.15 **c**	1.67 **bc**	2.14 **ab**	2.29 **a**
R x S		0.3161			
T x S		0.8715			
R x T x S		0.4249			

Table A-14.Total carbon mass in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment.

	Total C mass			
	20-53 μm†	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………g C aggregate fraction^{-1} ……………………			
CT	0.12 **f**	0.59 **a**	0.33 **d**	0.08 **f**
RT	0.11 **f**	0.46 **b**	0.40 **bd**	0.18 **e**
NT	0.14 **f**	0.40 **bd**	0.45 **b**	0.23 **e**
SOD	0.08 **f**	0.32 **d**	0.64 **a**	0.34 **d**
	…………………………*P* values………………………			
Tillage (T)	0.0541			
Size (S)	<.0001			
T X S	<.0001			

†Different letter means differences by tillage systems in each aggregate size fraction.

Table A-15.Total nitrogen mass in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT), and native prairie sod (SOD) for Tribune experiment.

	Total N mass			
	20-53 μm†	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………. g N aggregate fraction^{-1} …………………….			
CT	0.0113 **d**	0.0633 **a**	0.0283 **cf**	0.007 **d**
RT	0.0095 **d**	0.0388 **bc**	0.0433 **bc**	0.0058 **df**
NT	0.0130 d	0.0400 **bc**	0.0505 **ab**	0.0210 **cde**
SOD	0.0075 **d**	0.0318 **bcf**	0.0670 **a**	0.0333 **bce**
	…………………………*P* values………………………			
Tillage (T)	0.2178			
Size (S)	<.0001			
T X S	<.0001			

†Different letter means differences by tillage systems and aggregate size fraction.

Table A-16.Total carbon mass in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 140 (140-N) kg N ha^{-1} for Parsons experiment

	Total C mass			
	20-53 μm	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	…………………… g C aggregate $fraction^{-1}$ ……………………			
CT 0-N	0.14	0.32	0.25	0.07
RT 0-N	0.10	0.31	0.28	0.12
NT 0-N	0.08	0.25	0.36	0.17
CT 140-N	0.11	0.29	0.32	0.11
RT 140-N	0.11	0.31	0.39	0.13
NT 140-N	0.08	0.25	0.40	0.20
	…………………………*P* values………………………			
Tillage (T)		0.2409		
Nitrogen (N)		0.0477		
T x N		0.6214		
Size (S)		<.0001		
T x S		<.0001		
CT (mean)	0.13 **f**†	0.30 **bc**	0.29 **b**	0.09 **f**
RT (mean)	0.10 **f**	0.31 **bc**	0.34 **ac**	0.13 **f**
NT (mean)	0.08 **f**	0.25 **d**	0.38 **a**	0.19 **e**
N x S		0.0091		
0-N (mean)	0.11 **c**‡	0.29 **b**	0.29 **b**	0.12 **d**
140-N (mean)	0.10 **c**	0.29 **b**	0.37 **a**	0.14 **d**
T x N x S		0.7812		

†Different letter means differences by tillage systems and aggregate size fraction
‡ Different letter means differences by N application and aggregate size fraction

Table A-17.Total nitrogen mass in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 140 (140-N) kg N ha^{-1} for Parsons experiment.

	Total N mass			
	20-53 μm[1]	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	…………………… g N aggregate $fraction^{-1}$ ……………………			
CT 0-N	0.0164	0.0313	0.0228	0.006
RT 0-N	0.0113	0.0309	0.0267	0.0117
NT 0-N	0.0095	0.0260	0.0339	0.0172
CT 140-N	0.0124	0.0287	0.0296	0.0102
RT 140-N	0.0123	0.0307	0.0351	0.0123
NT 140-N	0.0098	0.0266	0.0385	0.0186
	…………………………*P* values………………………			
Tillage (T)		0.1573		
Nitrogen (N)		0.0966		
T x N		0.8075		
Size (S)		<.0001		
T x S		<.0001		
CT (mean)	0.014 **e**†	0.030 **b**	0.026 **b**	0.01 **e**
RT (mean)	0.012 **de**	0.031 **bf**	0.031 **f**	0.012 **e**
NT (mean)	0.01 **de**	0.026 **bf**	0.036 **a**	0.018 **c**
N x S		0.0192		
0-N (mean)	0.012 **d**‡	0.029 **b**	0.027 **b**	0.012 **d**
140-N (mean)	0.012 **d**	0.029 **b**	0.034 **a**	0.014 **d**
T x N x S		0.8327		

†Different letter means differences by tillage systems and aggregate size fraction
‡ Different letter means differences by N application and aggregate size fraction

Table A-18.Total carbon mass in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1} for Hays experiment

	Total C mass			
	20-53† μm	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………. g C aggregate $fraction^{-1}$ …………………….			
CT 0-N	0.102 **gh**	0.498 **ab**	0.240 **ef**	0.022 **h**
RT 0-N	0.104 **gh**	0.445 **bc**	0.227 **ef**	0.020 **h**
NT 0-N	0.105 **gh**	0.489 **a**	0.213 **f**	0.017 **h**
CT 67-N	0.113 **g**	0.571 **a**	0.304 **e**	0.029 **gh**
RT 67-N	0.097 **gh**	0.418 **bc**	0.311 **ed**	0.028 **gh**
NT 67-N	0.104 **gh**	0.402 **cd**	0.475 **abc**	0.074 **gh**
	…………………………*P* values………………………			
Tillage (T)	0.2184			
Nitrogen (N)	0.0222			
T x N	0.4493			
Size (S)	0.0001			
T x S	0.0398			
N x S	0.0010			
T x N x S	0.0292			

†Different letter means differences by tillage systems, N application and aggregate size fraction

Table A-19.Total nitrogen mass in each water stable aggregates under conventional tillage (CT), reduced tillage (RT), no-tillage (NT) with two nitrogen rates 0 (0-N) and 67 (67-N) kg N ha^{-1} for Hays experiment.

	Total N mass			
	20-53 μm†	**53-250 μm**	**250-2000 μm**	**>2000 μm**
	……………………. g N aggregate $fraction^{-1}$ …………………….			
CT 0-N	0.0075 **gh**	0.0353 **bc**	0.0170 **e**	0.0013 **h**
RT 0-N	0.0067 **gh**	0.0343 **bc**	0.0168 **ef**	0.0015 **h**
NT 0-N	0.0080 **gh**	0.0363 **b**	0.0160 **ef**	0.0013 **h**
CT 67-N	0.0095 **fg**	0.0448 **a**	0.0225 **ed**	0.0018 **h**
RT 67-N	0.0067 **gh**	0.0298 **bcd**	0.0233 **ed**	0.0020 **h**
NT 67-N	0.0070 **gh**	0.0288 **cd**	0.0365 **b**	0.0055 **gh**
	…………………………*P* values………………………			
Tillage (T)	0.2395			
Nitrogen (N)	0.0184			
T x N	0.3180			
Size (S)	0.0001			
T x S	0.0173			
N x S	0.0009			
T x N x S	0.0047			

†Different letter means differences by tillage systems, N application and aggregate size fraction

Table A-20. Total carbon mass In each water stable aggregates under conventional tillage (CT), and no-tillage (NT) in different rotations, continuous wheat (W-W), wheat-soybean (W-B), continuous sorghum (S-S), sorghum-soybean (S-B) and continuous soybean (B-B) for Manhattan experiment.

ROTATION	TILLAGE	Total C mass			
		20-53 μm†	**53-250 μm**	**250-1000 μm**	**>1000 μm**
		……………………. g C aggregate fraction^{-1}			…………………….
W-W	**CT**	0.111 **nopqr**	0.447 **cde**	0.272 **hijklm**	0.046 **qr**
S-S	**CT**	0.187 **jklmnop**	0.371 **efgh**	0.204 **jklmno**	0.102 **opqr**
S-B	**CT**	0.177 **klmnop**	0.389 **dfgh**	0.232 **ijklmn**	0.064 **pqr**
W-B	**CT**	0.107 **nopqr**	0.341 **efghi**	0.272 **hijklm**	0.041 **qr**
B-B	**CT**	0.185 **jklmnop**	0.304 **fghij**	0.147 **mnopqr**	0.019 **r**
W-W	**NT**	0.123 **nopqr**	0.287 **ghijk**	0.624 **ab**	0.381 **defgh**
S-S	**NT**	0.165 **klmnopq**	0.504 **cbd**	0.568 **abc**	0.265 **hijklm**
S-B	**NT**	0.214 **jklmno**	0.642 **a**	0.413 **def**	0.178 **klmnop**
W-B	**NT**	0.152 **lmnopqr**	0.580 **abc**	0.284 **hijkl**	0.049 **qr**
B-B	**NT**	0.162 **klmnopq**	0.401 **defg**	0.290 **fghijk**	0.114 **nopqr**
		………………………….*P* values………………………			
Rotation (R)		0.0042			
Tillage (T)		0.0001			
T x R		0.2641			
Size (S)		0.0001			
R x S		0.0001			
T x S		0.0001			
R x T x S		0.0001			

†Different letter means differences by tillage systems, crop rotation and aggregate size fraction

Table A-21. Total nitrogen mass in each water stable aggregates under conventional tillage (CT), and no-tillage (NT) in different rotations, continuous wheat (W-W), wheat-soybean (W-B), continuous sorghum (S-S), sorghum-soybean (S-B) and continuous soybean (B-B) for Manhattan experiment.

ROTATION	TILLAGE	Total N mass			
		20-53 μm†	**53-250 μm**	**250-1000 μm**	**>1000 μm**
		……………………. g N aggregate fraction^{-1} …………………….			
W-W	**CT**	0.013 **jklmn**	0.045 **bc**	0.035 **cdefg**	0.005 **nm**
S-S	**CT**	0.018 **ijklmn**	0.045 **bc**	0.022 **ghijkl**	0.001 **lmn**
S-B	**CT**	0.019 **hijklm**	0.036 **cdefg**	0.022 **fghijkl**	0.005 **nm**
W-B	**CT**	0.012 **klmn**	0.042 **cd**	0.028 **defghij**	0.003 **nm**
B-B	**CT**	0.018 **ijklmn**	0.034 **cdefgh**	0.013 **ijklmn**	0.002 **n**
W-W	**NT**	0.014 **ijklmn**	0.037 **cdef**	0.071 **a**	0.043 **cd**
S-S	**NT**	0.016 **ijklmn**	0.049 **bc**	0.058 **ab**	0.023 **fghijkl**
S-B	**NT**	0.025 **fghijkl**	0.066 **a**	0.041 **cde**	0.015 **ijklmn**
W-B	**NT**	0.013 **ijklmn**	0.063 **ab**	0.026 **efghijk**	0.005 **nm**
B-B	**NT**	0.0156 **ijklmn**	0.046 **bc**	0.029 **defghi**	0.011 **klmn**
		…………………………*P* values………………………			
Rotation (R)		0.0119			
Tillage (T)		0.0001			
T x R		0.2400			
Size (S)		0.0001			
R x S		0.0001			
T x S		0.0011			
R x T x S		0.0007			

†Different letter means differences by tillage systems, crop rotation and aggregate size fraction

Appendix B - Chapter 4

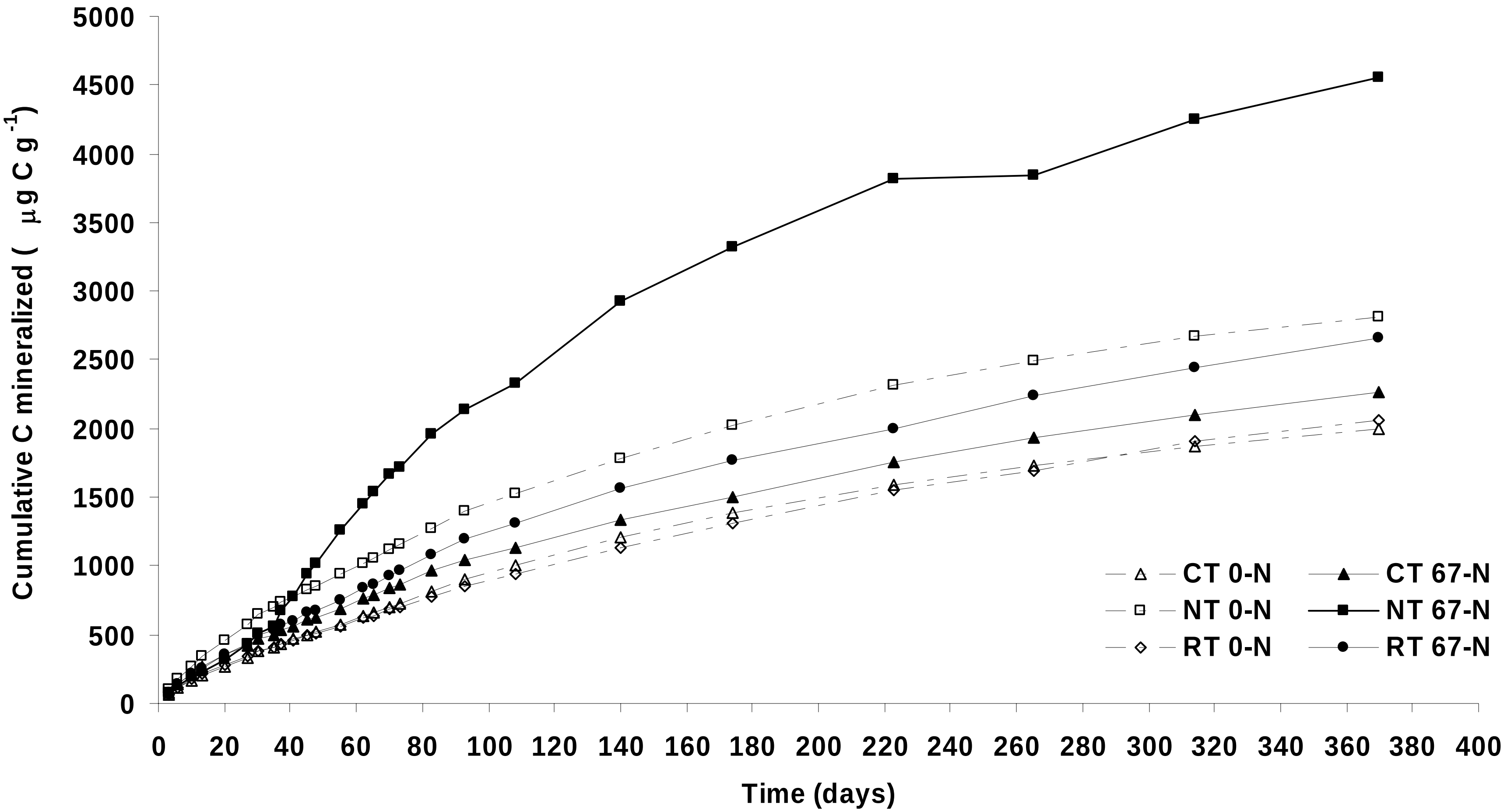

Figure B-1. Cumulative C mineralized during 370 days of incubation under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} at 0-5 cm in Hays experiment.

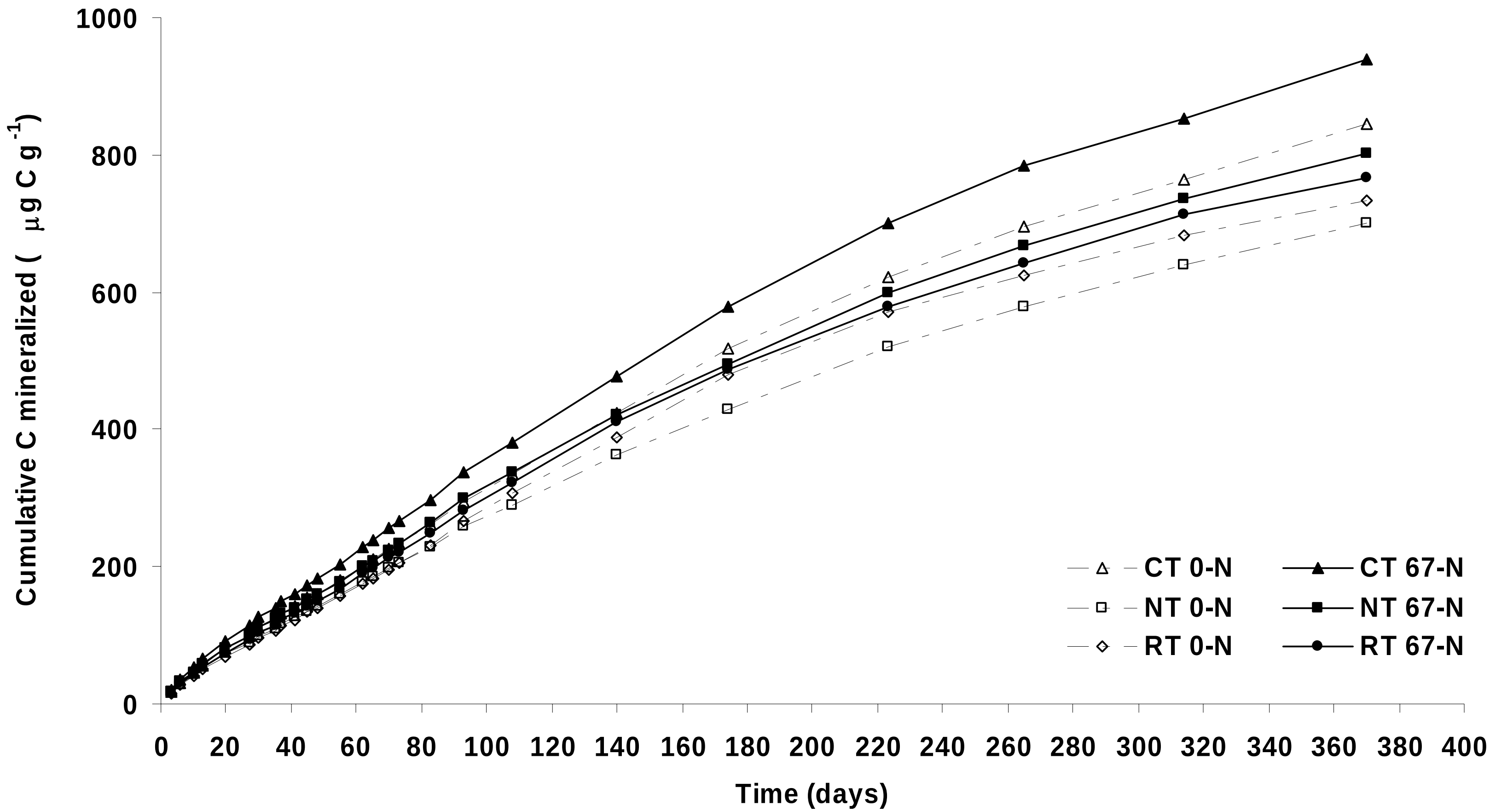

Figure B-2. Cumulative C mineralized during 370 days of incubation under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} at 5-15 cm in Hays experiment.

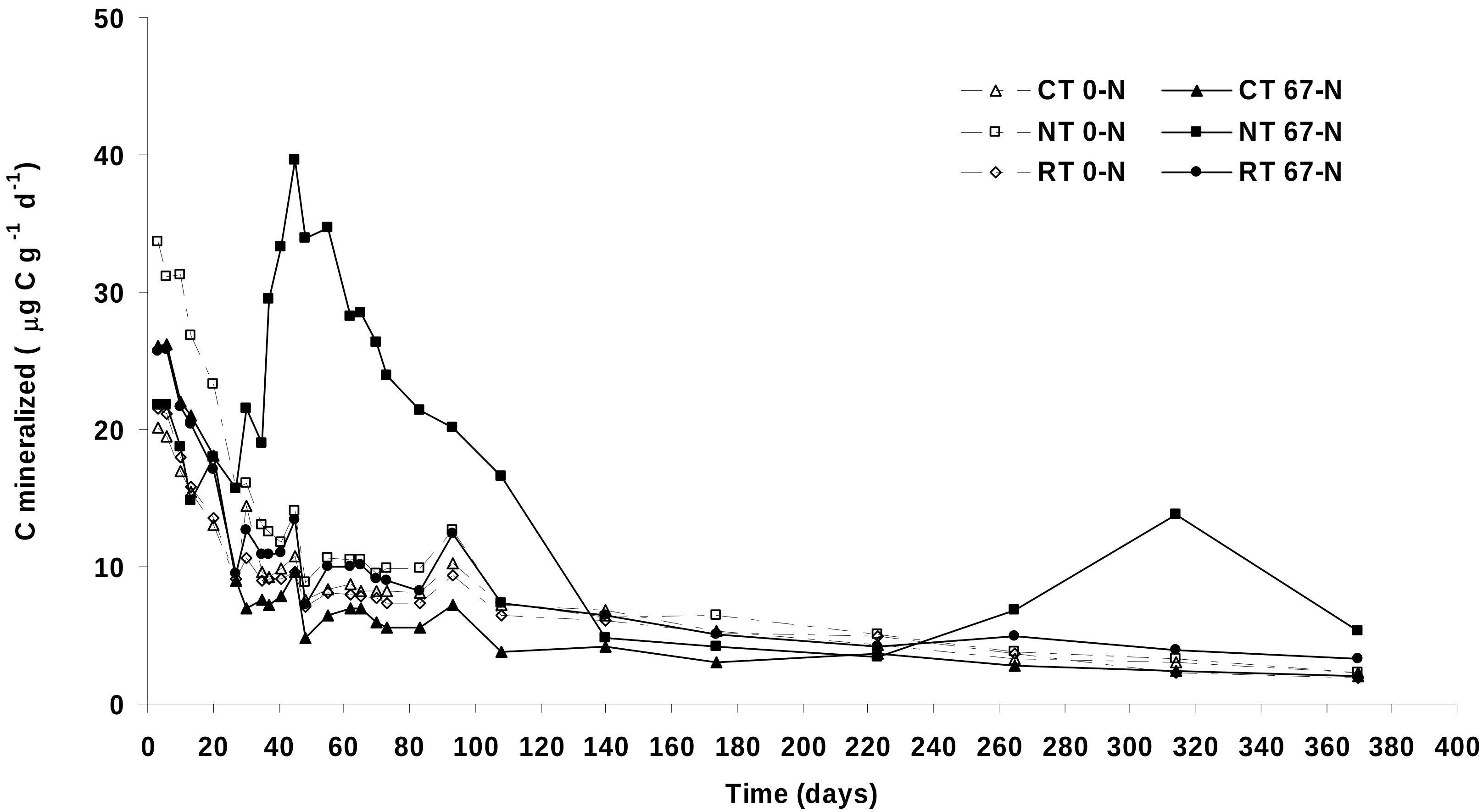

Figure B-3. Carbon mineralization rate during 370 days of incubation under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} at 0-5 cm in Hays experiment.

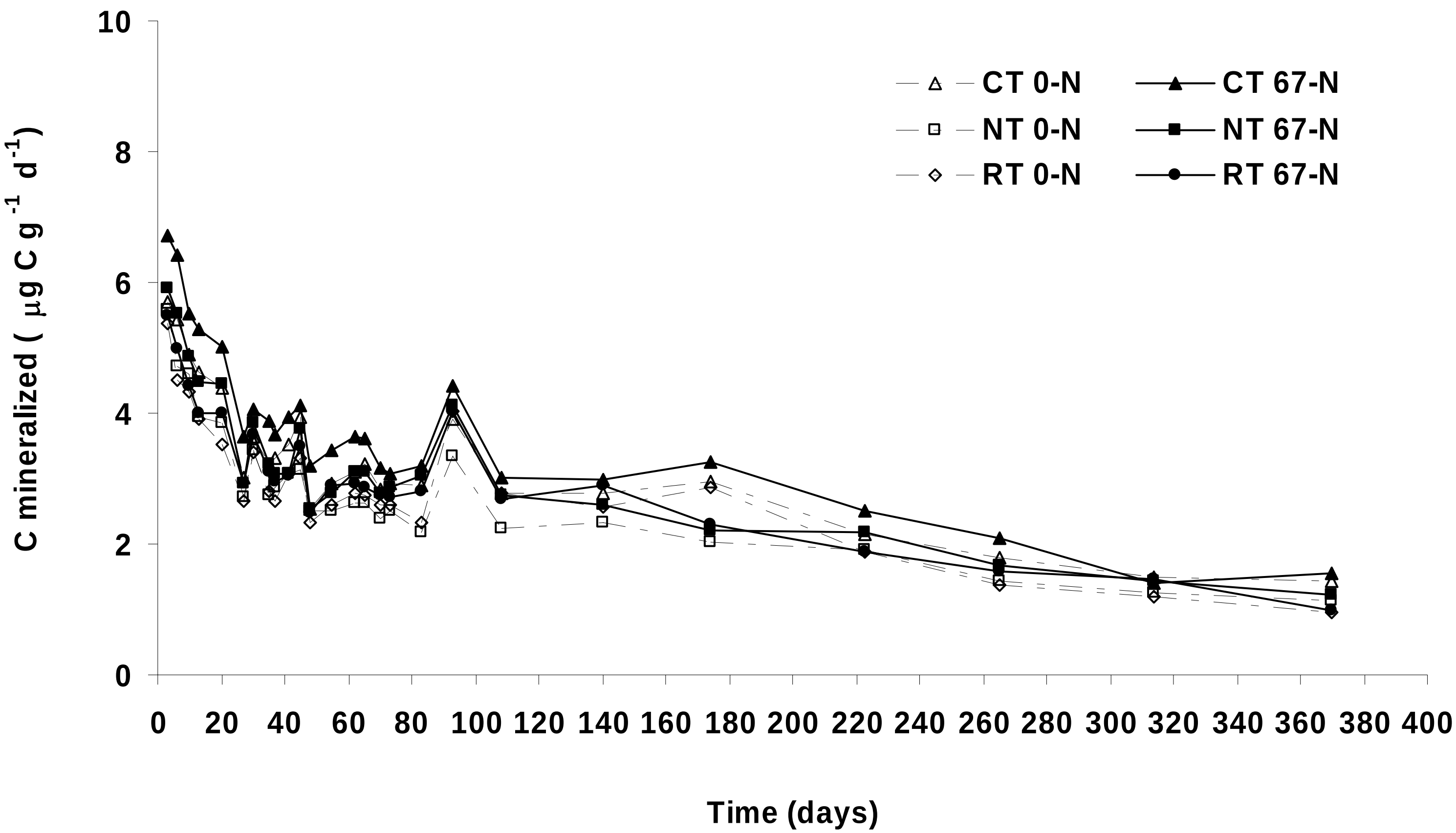

Figure B-4. Carbon mineralization rate during 370 days of incubation under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1}, at 5-15 cm in Hays experiment.

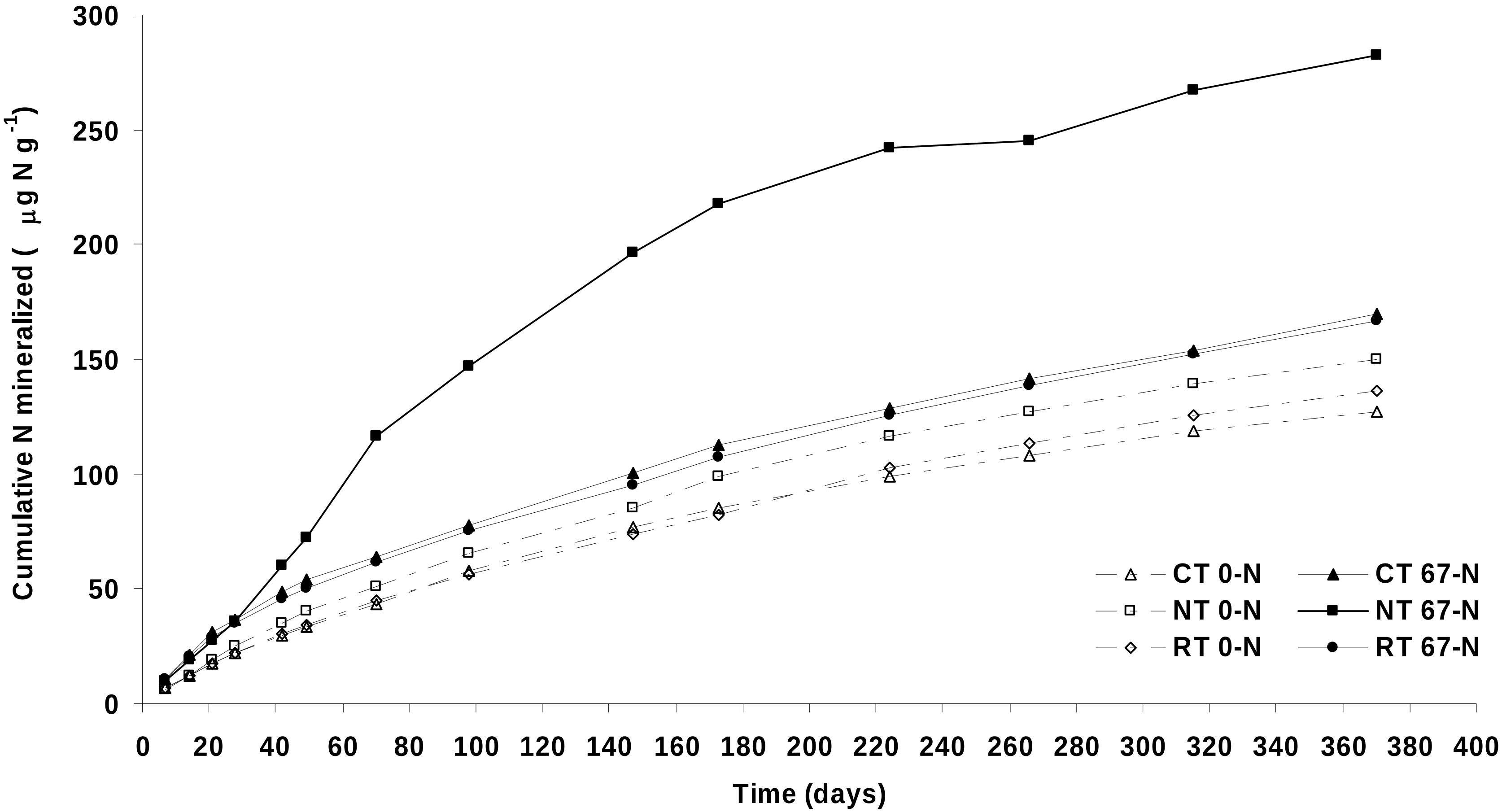

Figure B-5. Cumulative N mineralized during 370 days of incubation under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} at 0-5 cm in Hays experiment.

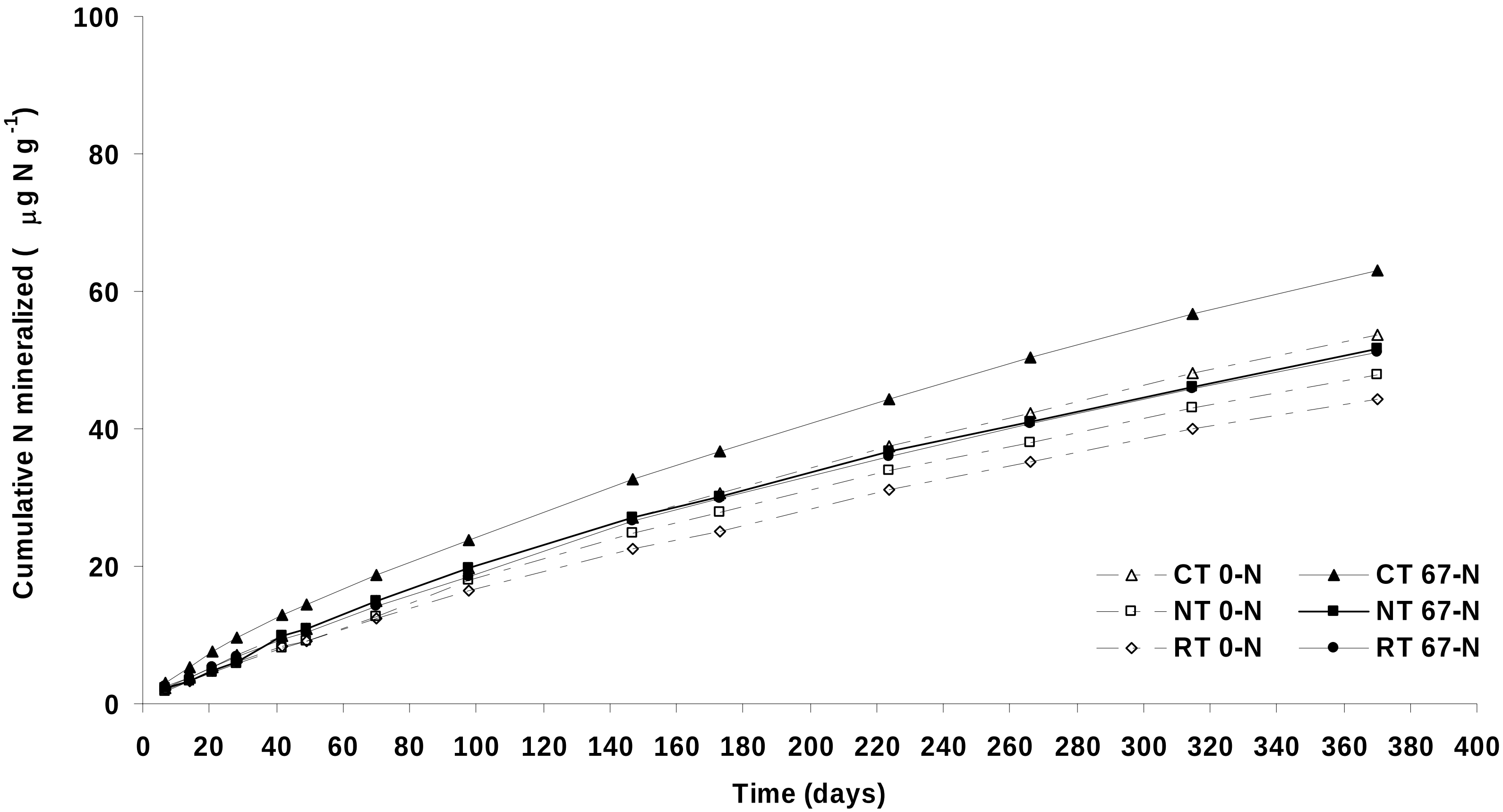

Figure B-6. Cumulative N mineralized during 370 days of incubation under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} at 5-15 cm in Hays experiment.

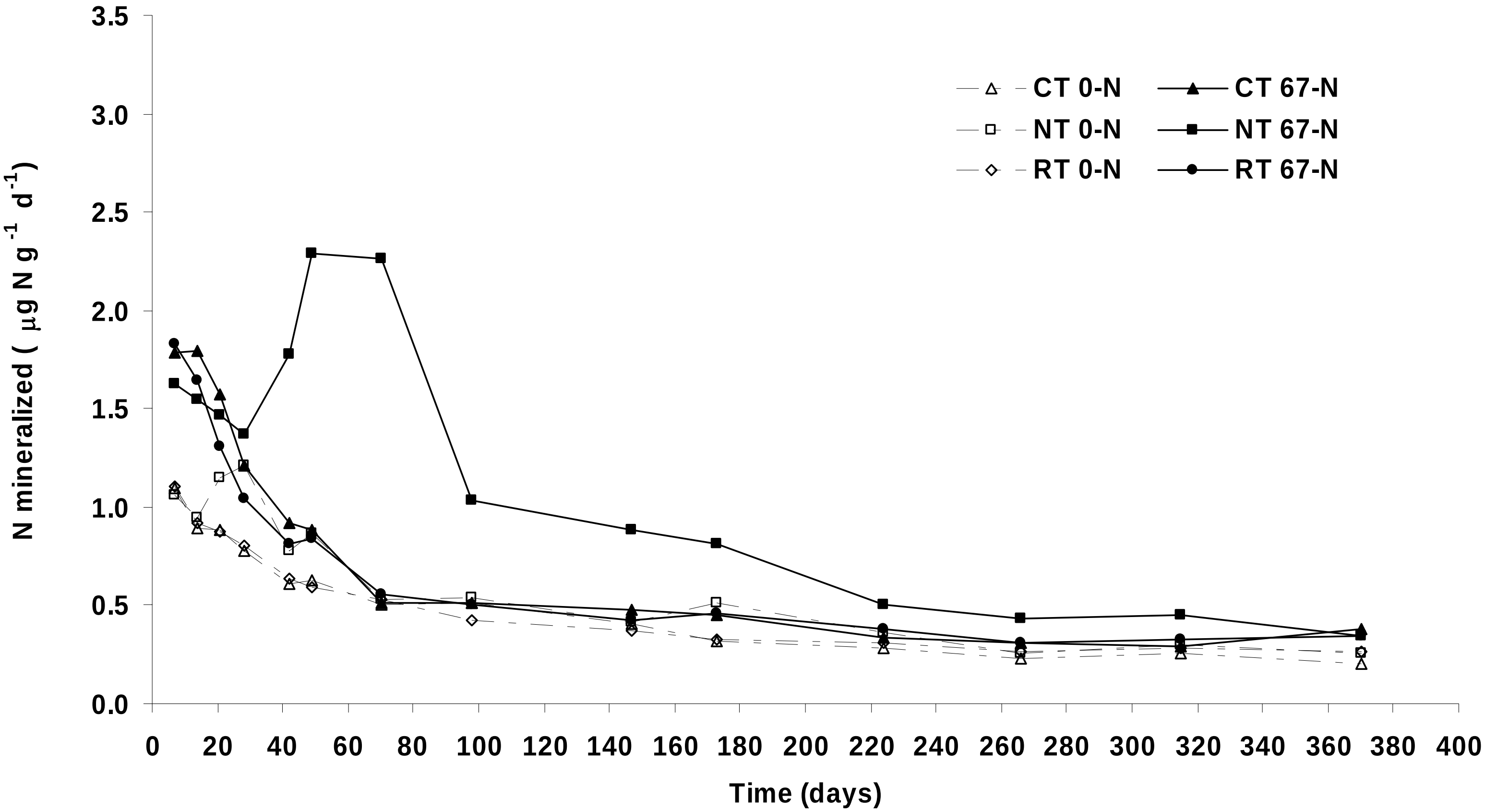

Figure B-7. Nitrogen mineralization rate during 370 days of incubation under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1} at 0-5 cm in Hays experiment.

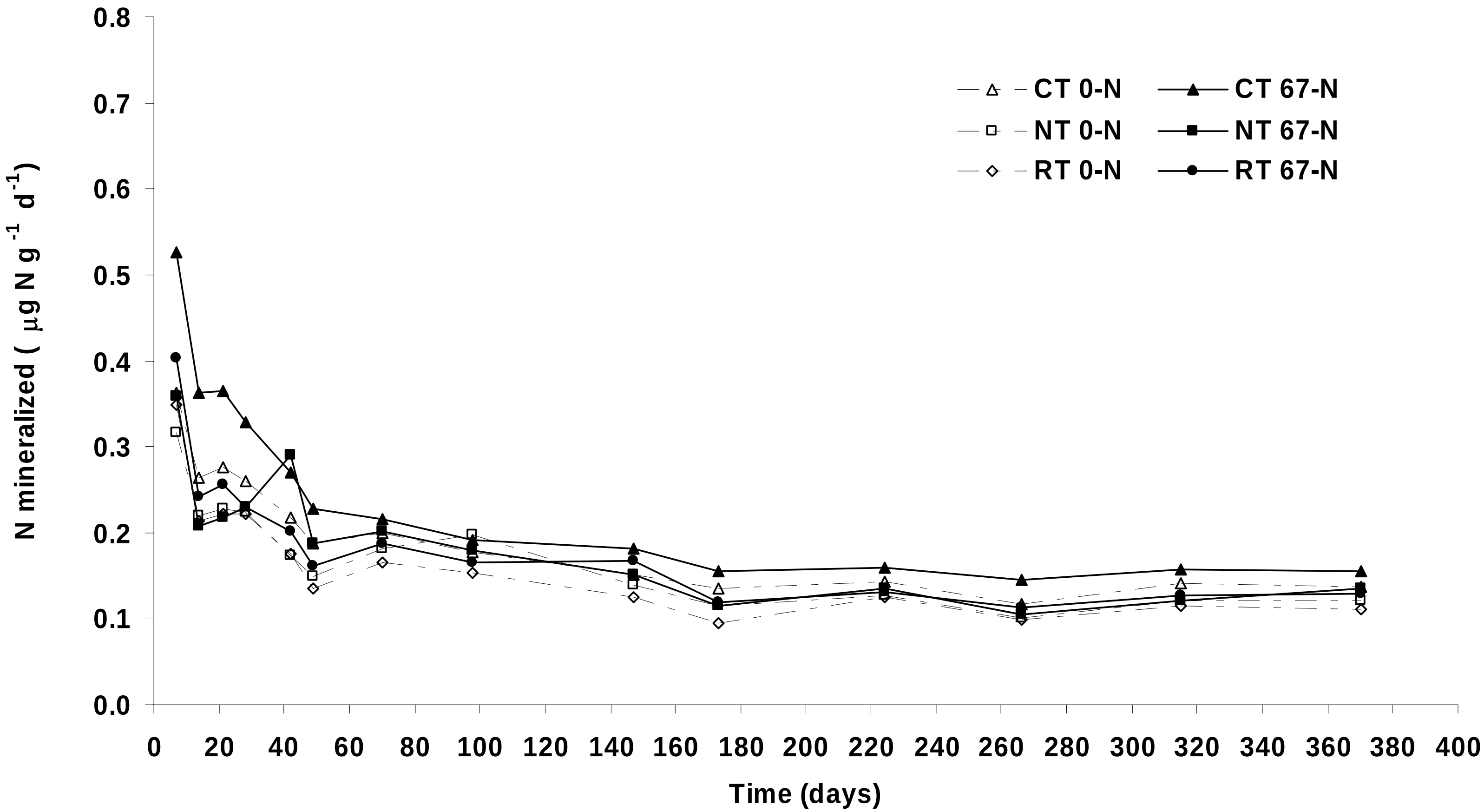

Figure B-8. Nitrogen mineralization rate during 370 days of incubation under conventional till (CT), reduced till (RT), and no-tillage (NT) with two N rates 0 (0-N) and 67 (67-N) kg N ha^{-1}, at 5-15 cm in Hays experiment.

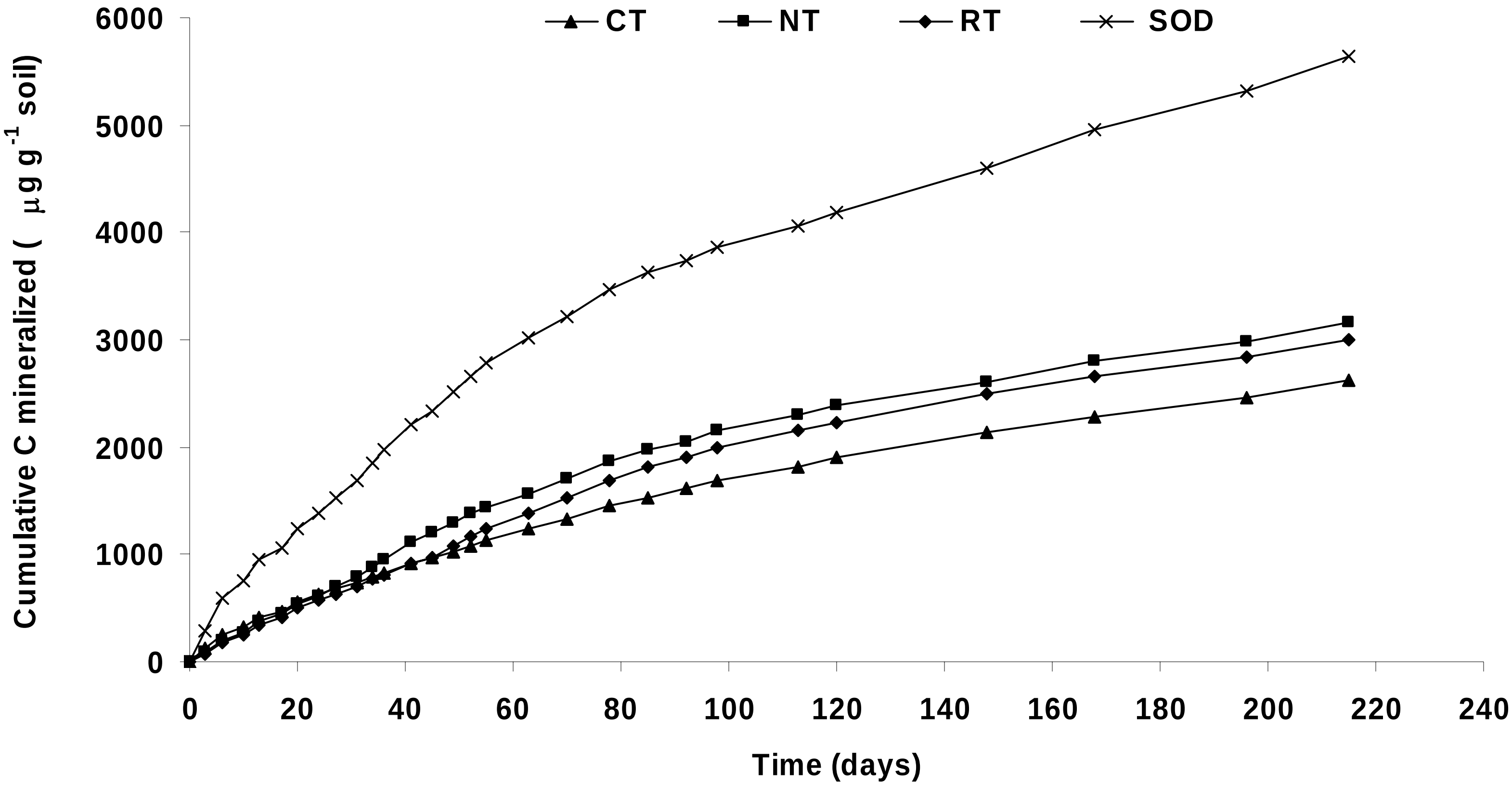

Figure B-9. Cumulative C mineralized during 215 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT), and native prairie sod (SOD) at 0-5 cm in Tribune experiment.

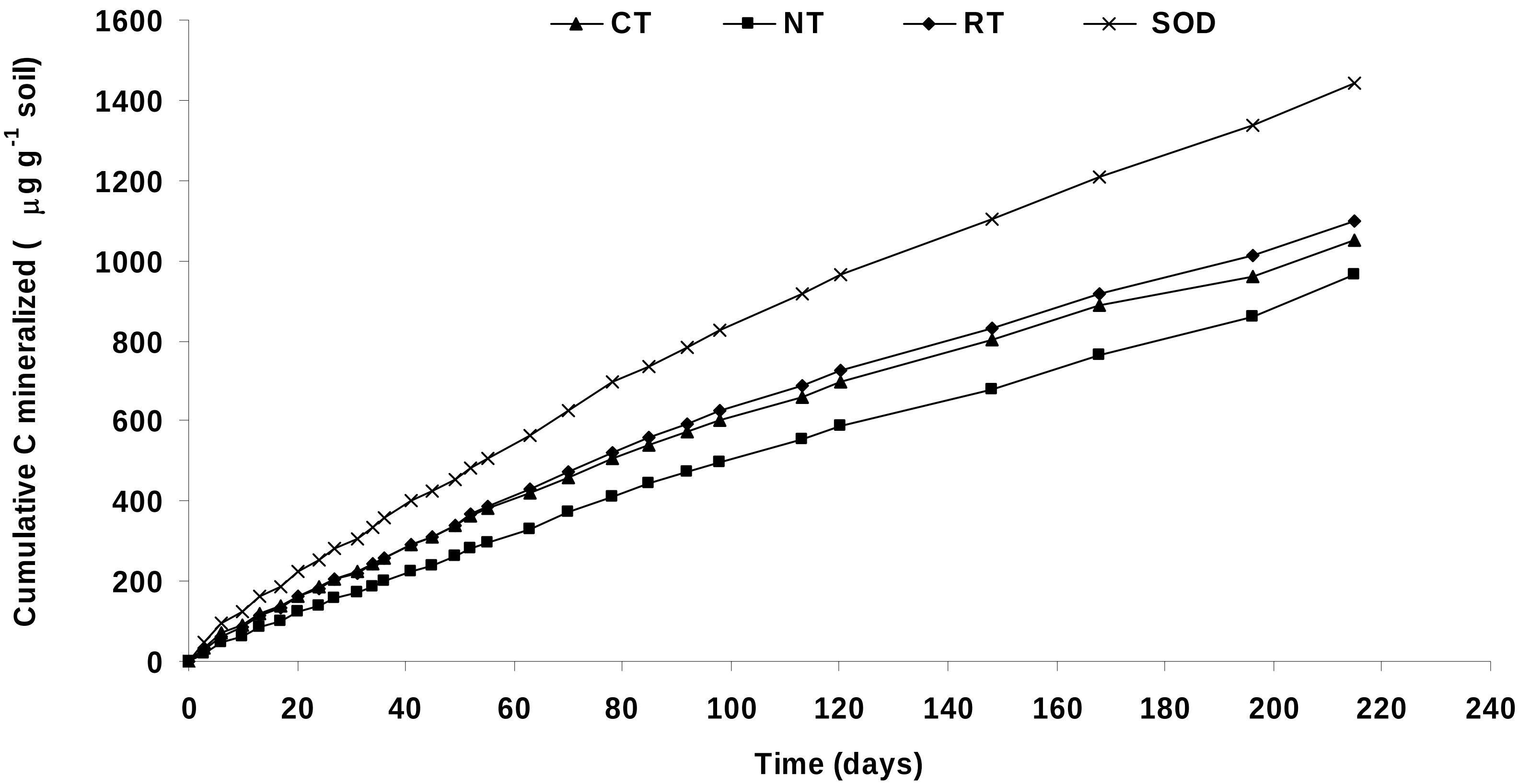

Figure B-10. Cumulative C mineralized during 215 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT), and native prairie sod (SOD) at 5-15 cm in Tribune experiment.

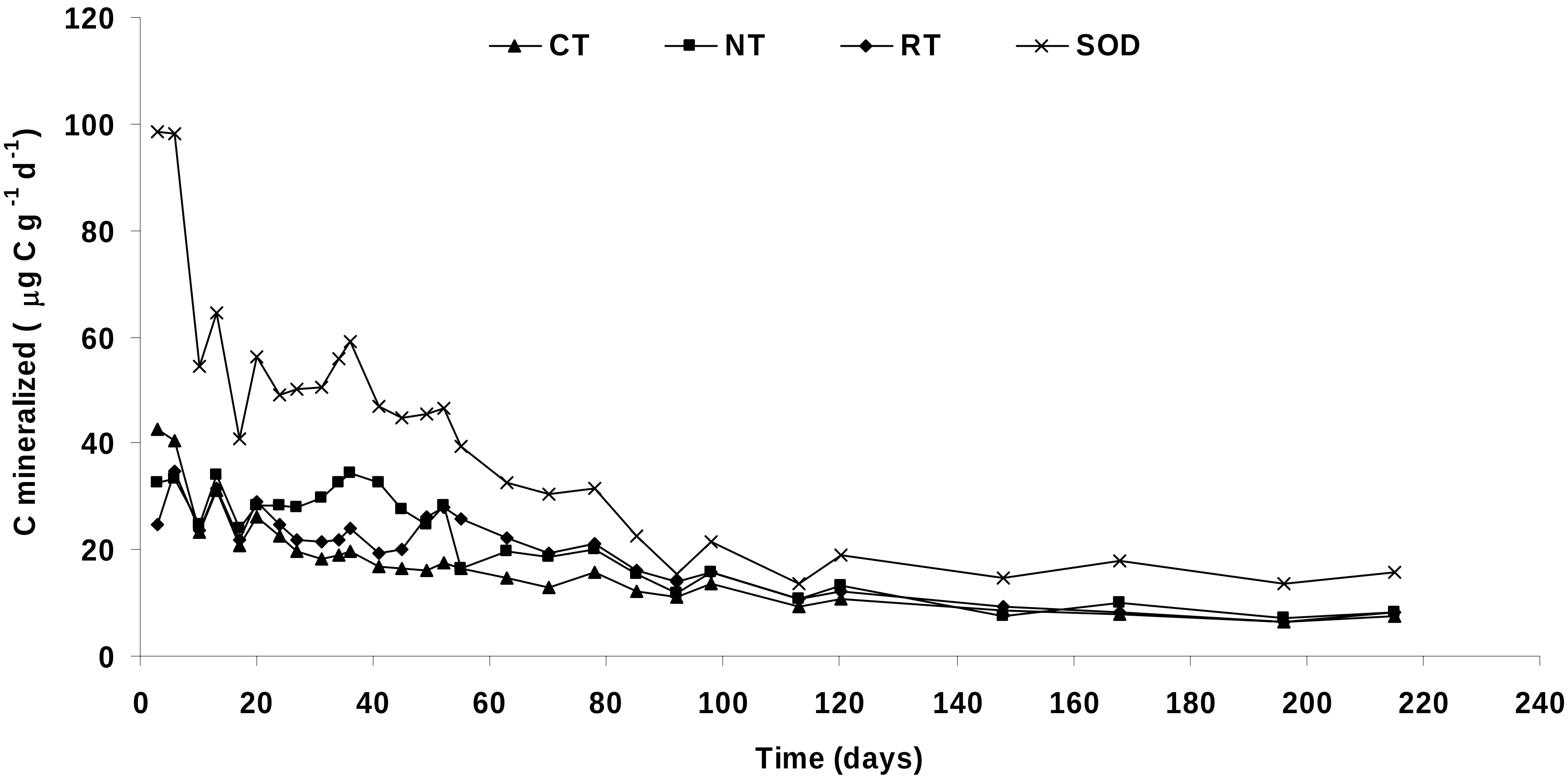

Figure B-11. Carbon mineralization rate during 215 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT), and native prairie sod (SOD) at 0-5 cm in Tribune experiment.

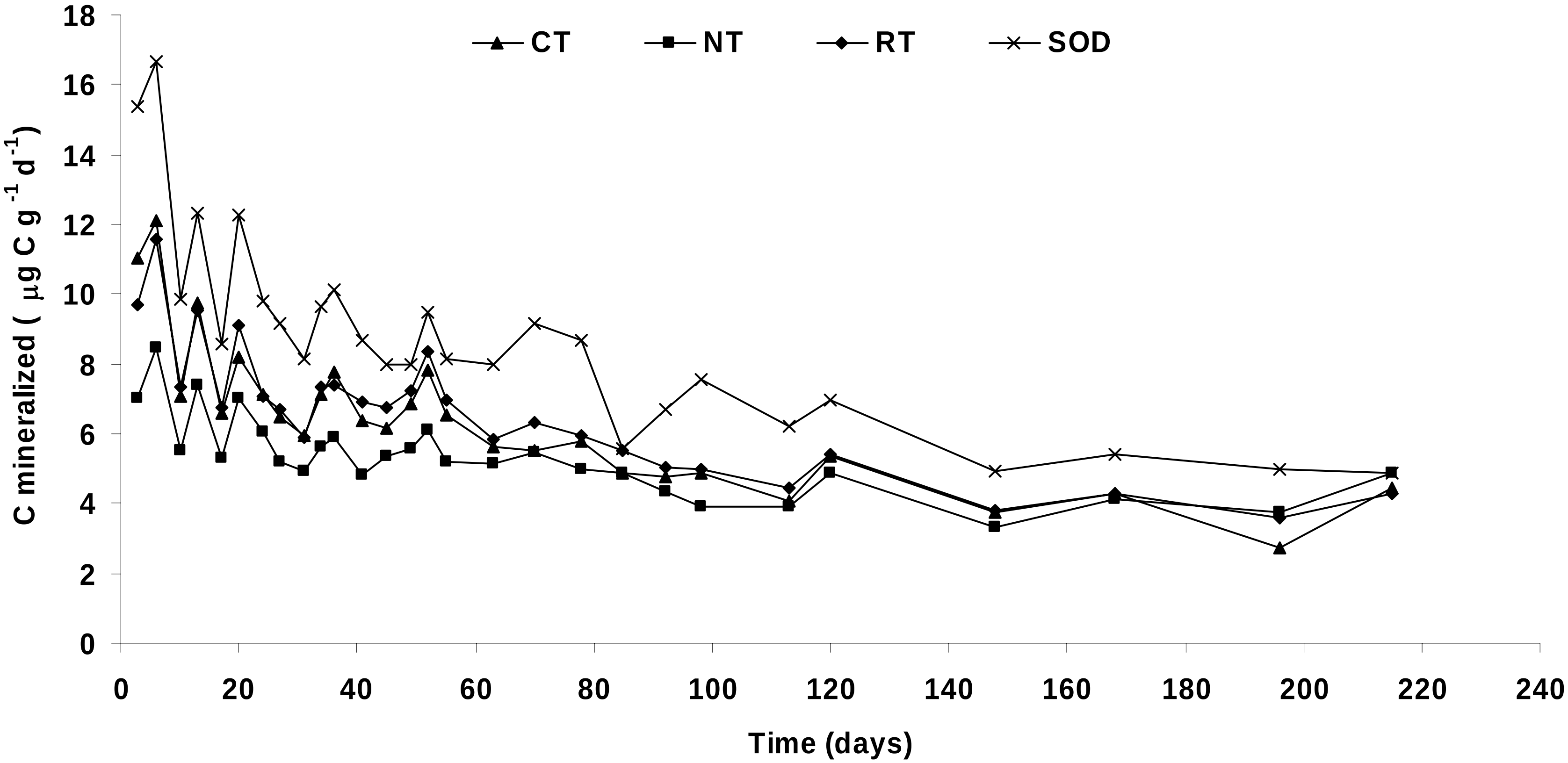

Figure B-12. Carbon mineralization rate during 215 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT), and native prairie sod (SOD) at 5-15 cm in Tribune experiment.

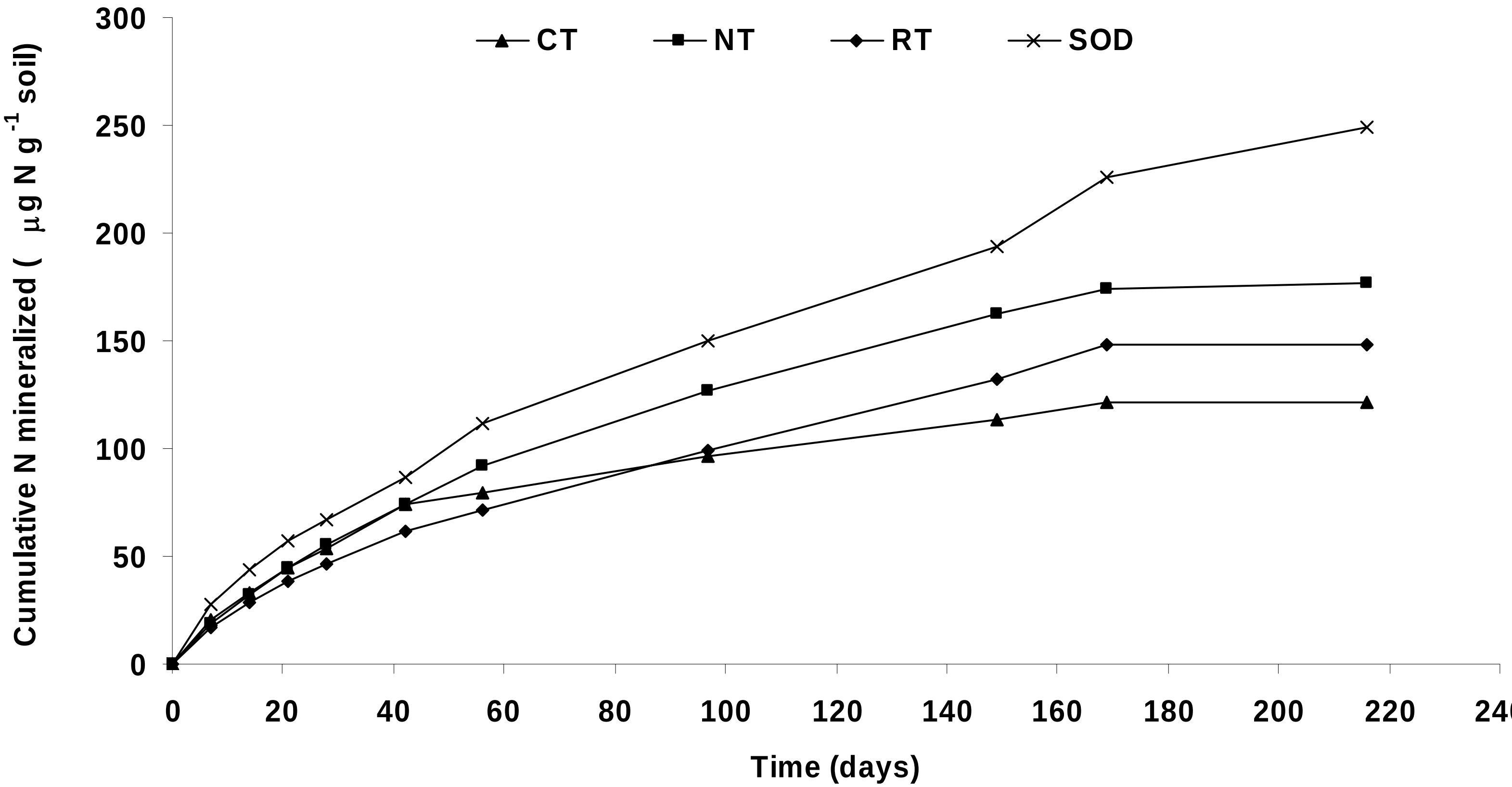

Figure B-13. Cumulative N mineralized during 215 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT), and native prairie sod (SOD) at 0-5 cm in Tribune experiment.

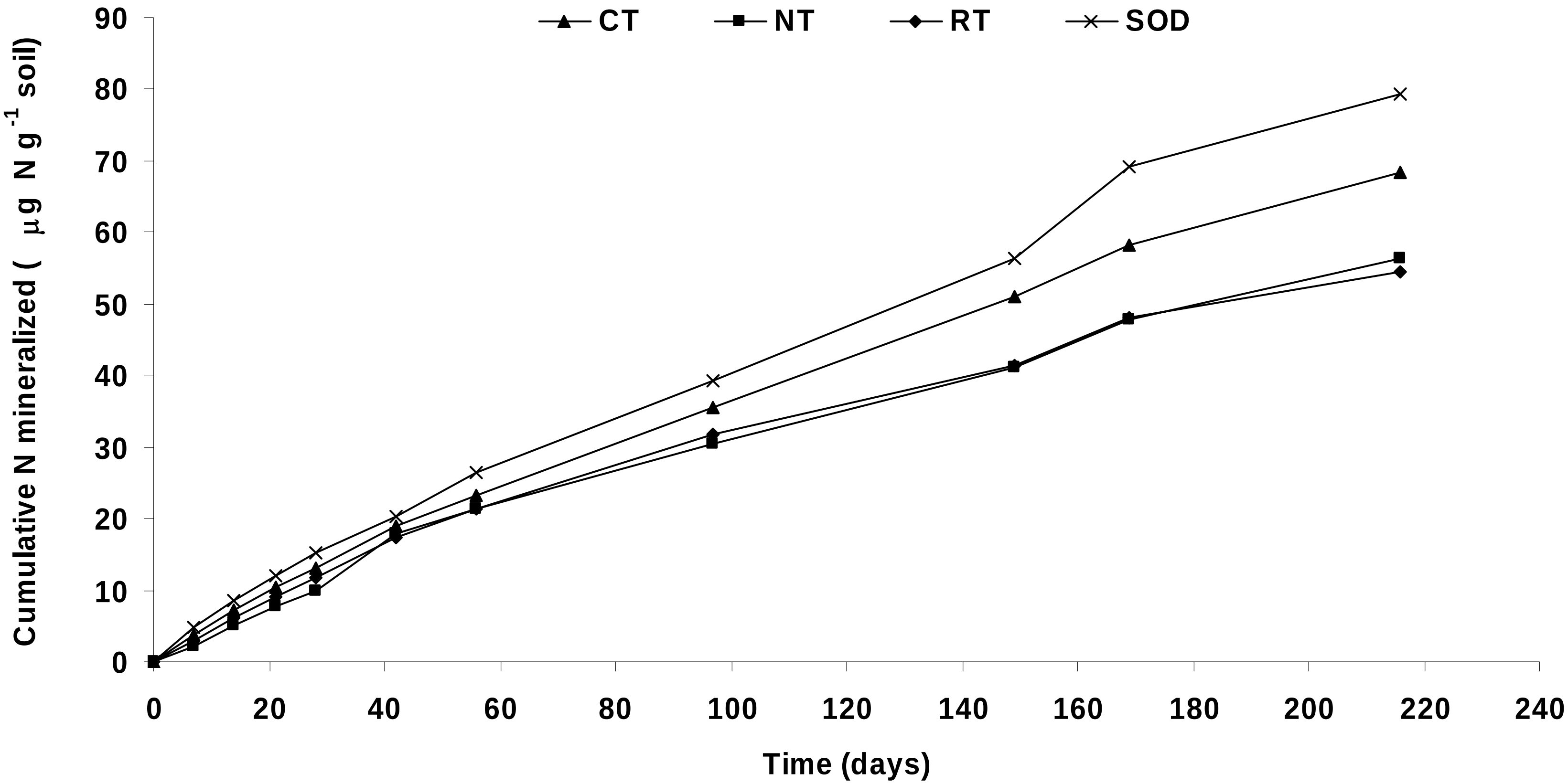

Figure B-14. Cumulative N mineralized during 215 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT), and native prairie sod (SOD) at 5-15 cm in Tribune experiment.

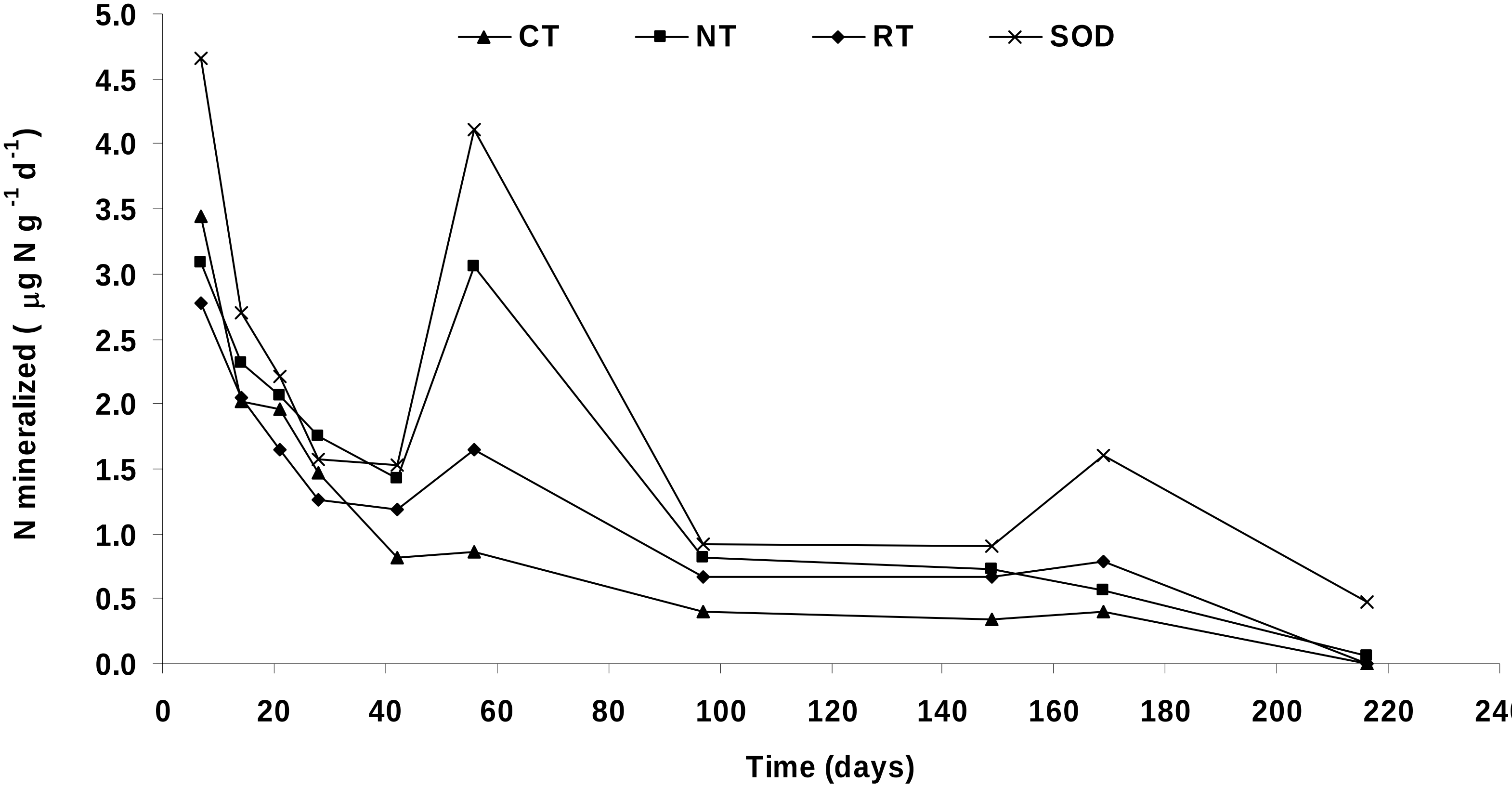

Figure B-15. Nitrogen mineralization rate during 215 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT), and native prairie sod (SOD) at 0-5 cm in Tribune experiment.

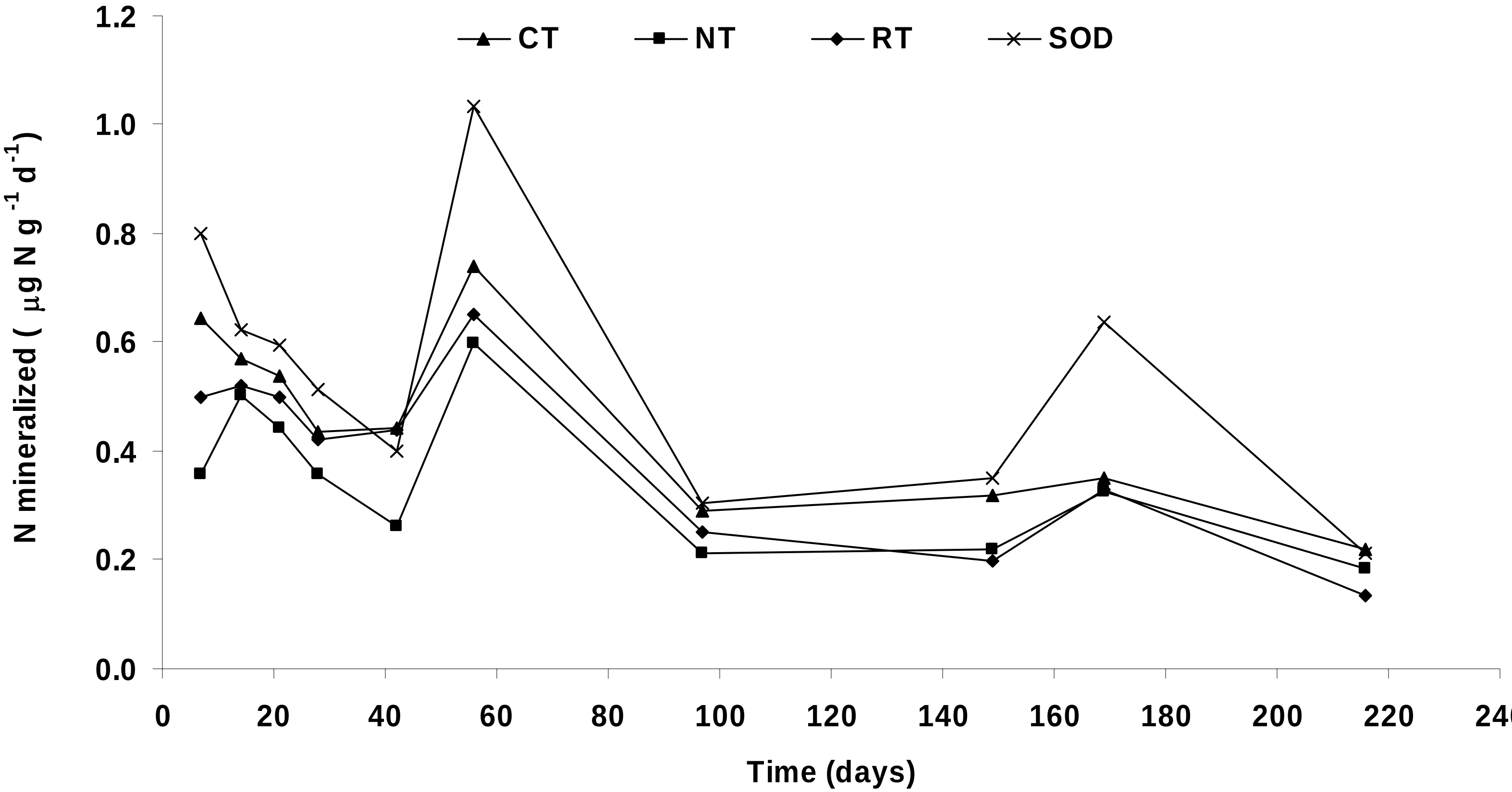

Figure B-16. Nitrogen mineralization rate during 215 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT), and native prairie sod (SOD) at 5-15 cm in Tribune experiment.

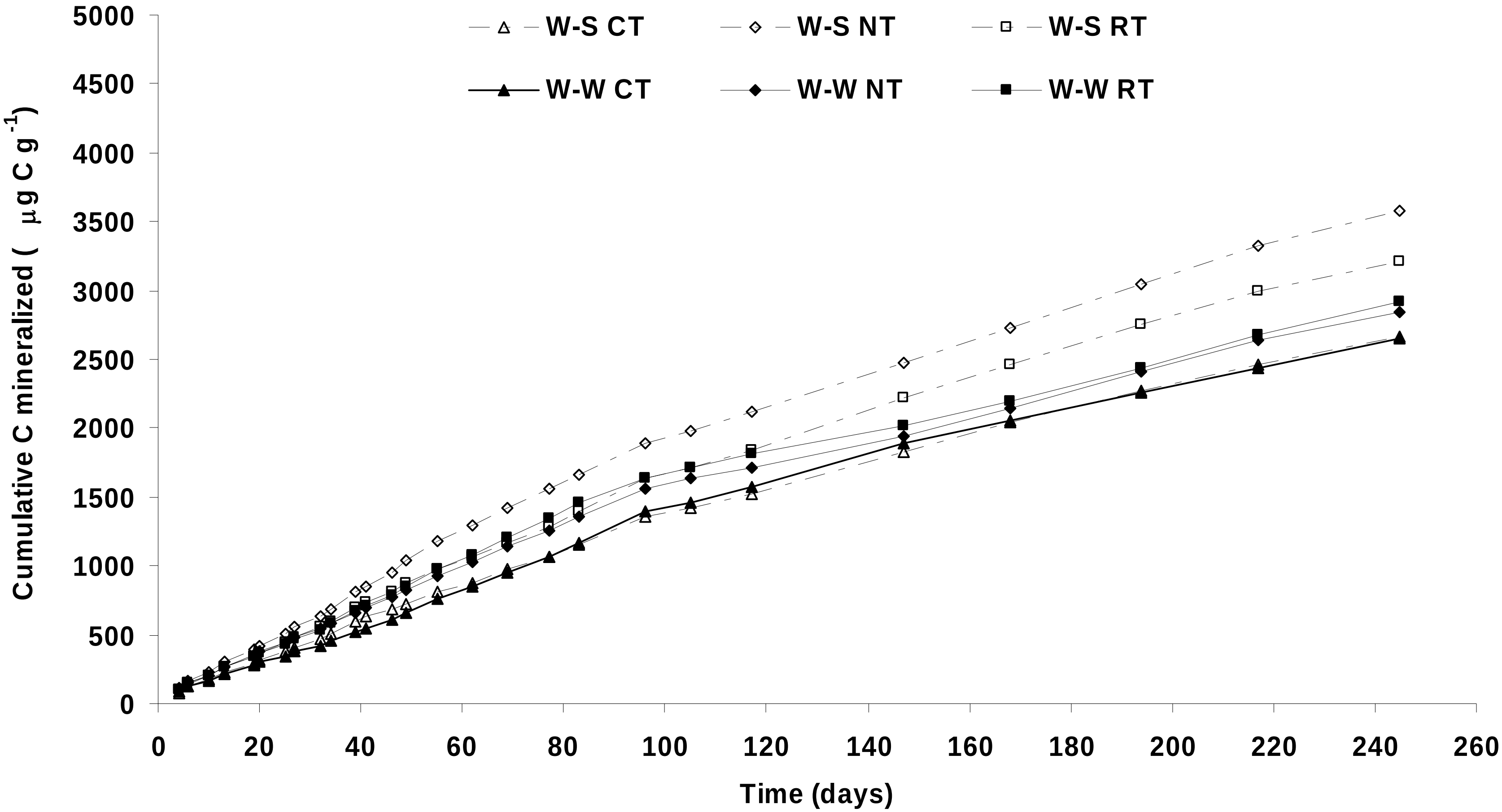

Figure B-17. Cumulative C mineralized during 245 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT) for wheat-soybean (W-B) and wheat-wheat (W-W) rotation at 0-5 cm in Manhattan experiment.

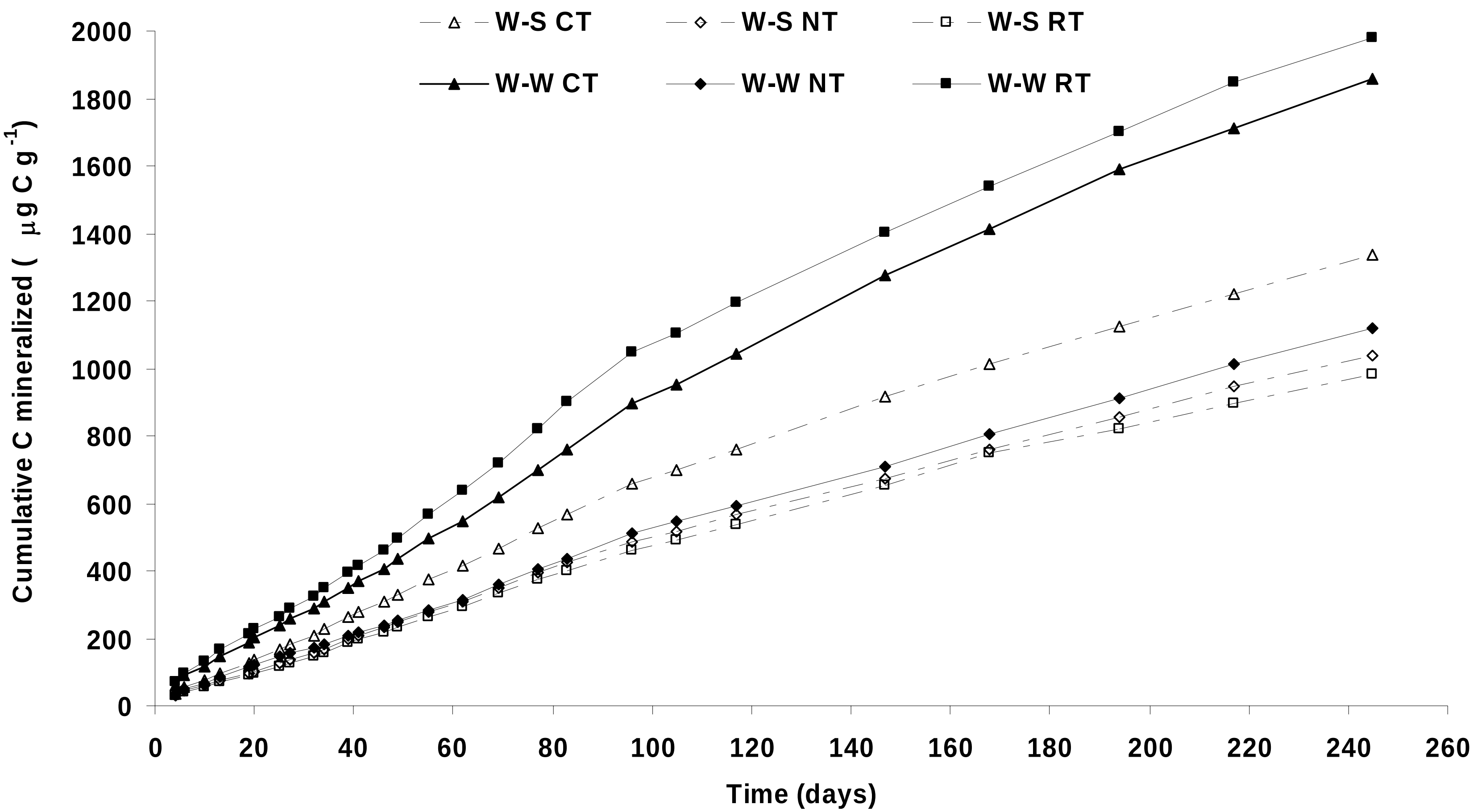

Figure B-18. Cumulative C mineralized during 245 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT) for wheat-soybean (W-B) and wheat-wheat (W-W) rotation at 5-15 cm in Manhattan experiment.

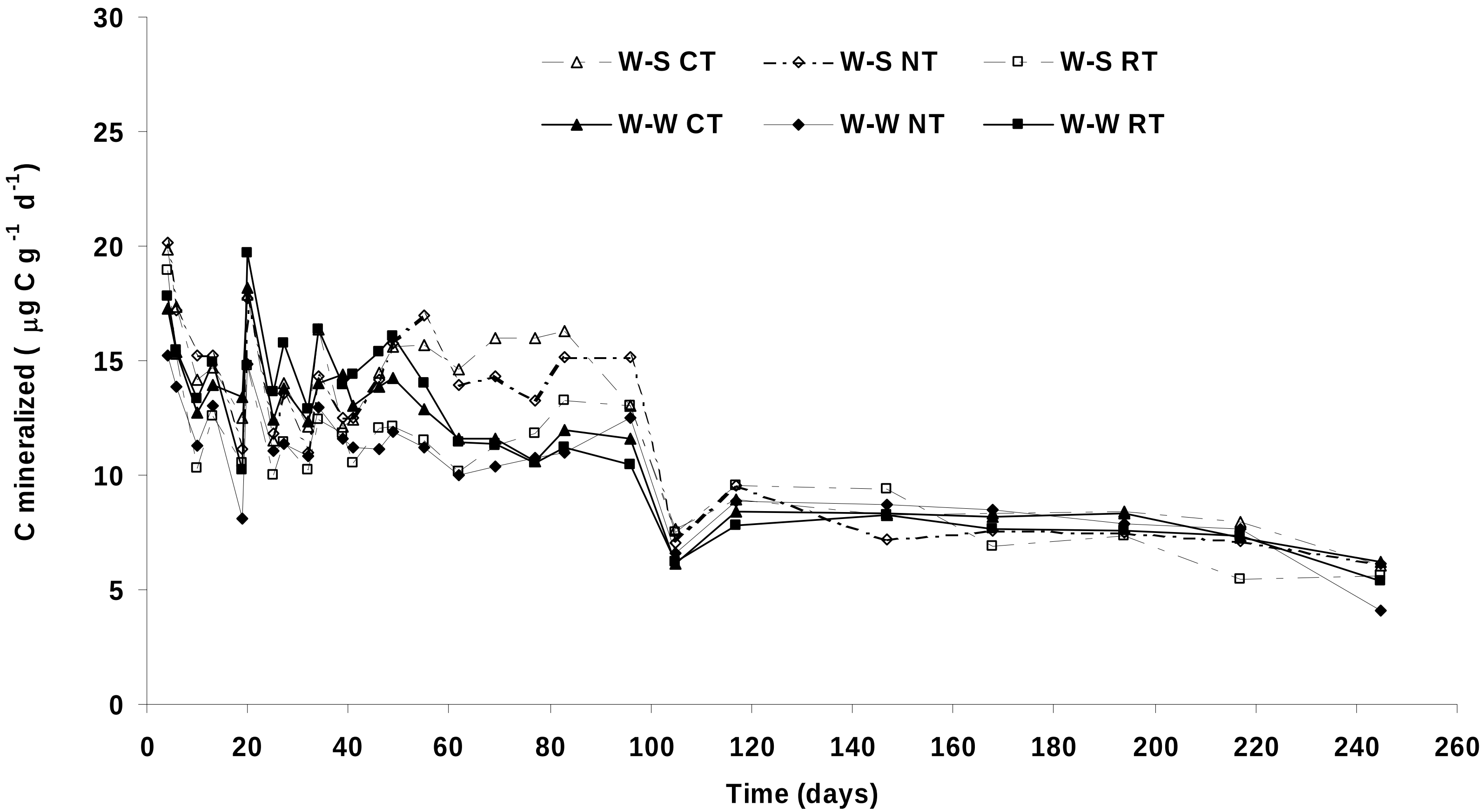

Figure B-19. Carbon mineralization rate during 245 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT) for wheat-soybean (W-B) and wheat-wheat (W-W) rotation at 0-5 cm in Manhattan experiment.

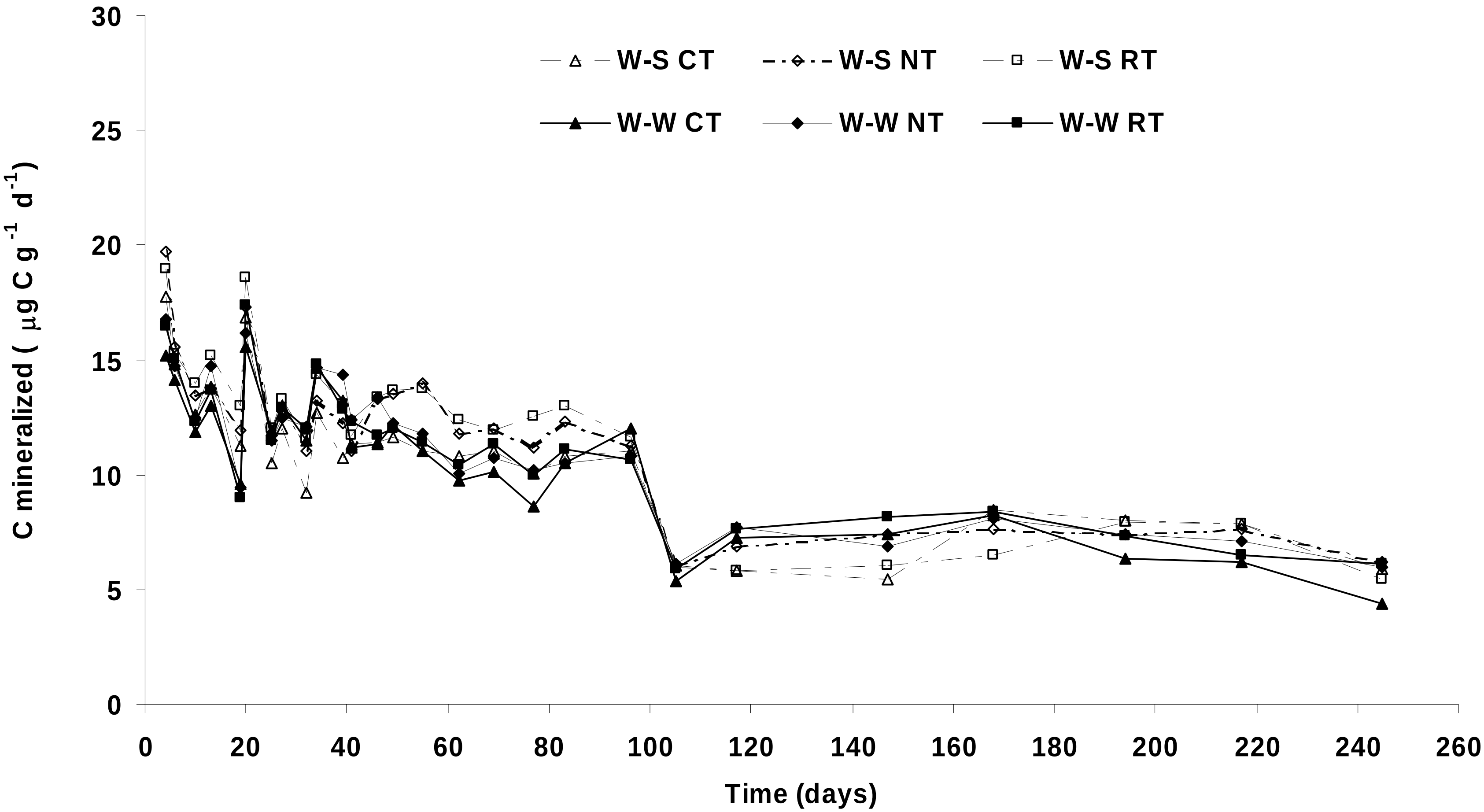

Figure B-20. Carbon mineralization rate during 245 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT) for wheat-soybean (W-B) and wheat-wheat (W-W) rotation at 5-15 cm in Manhattan experiment.

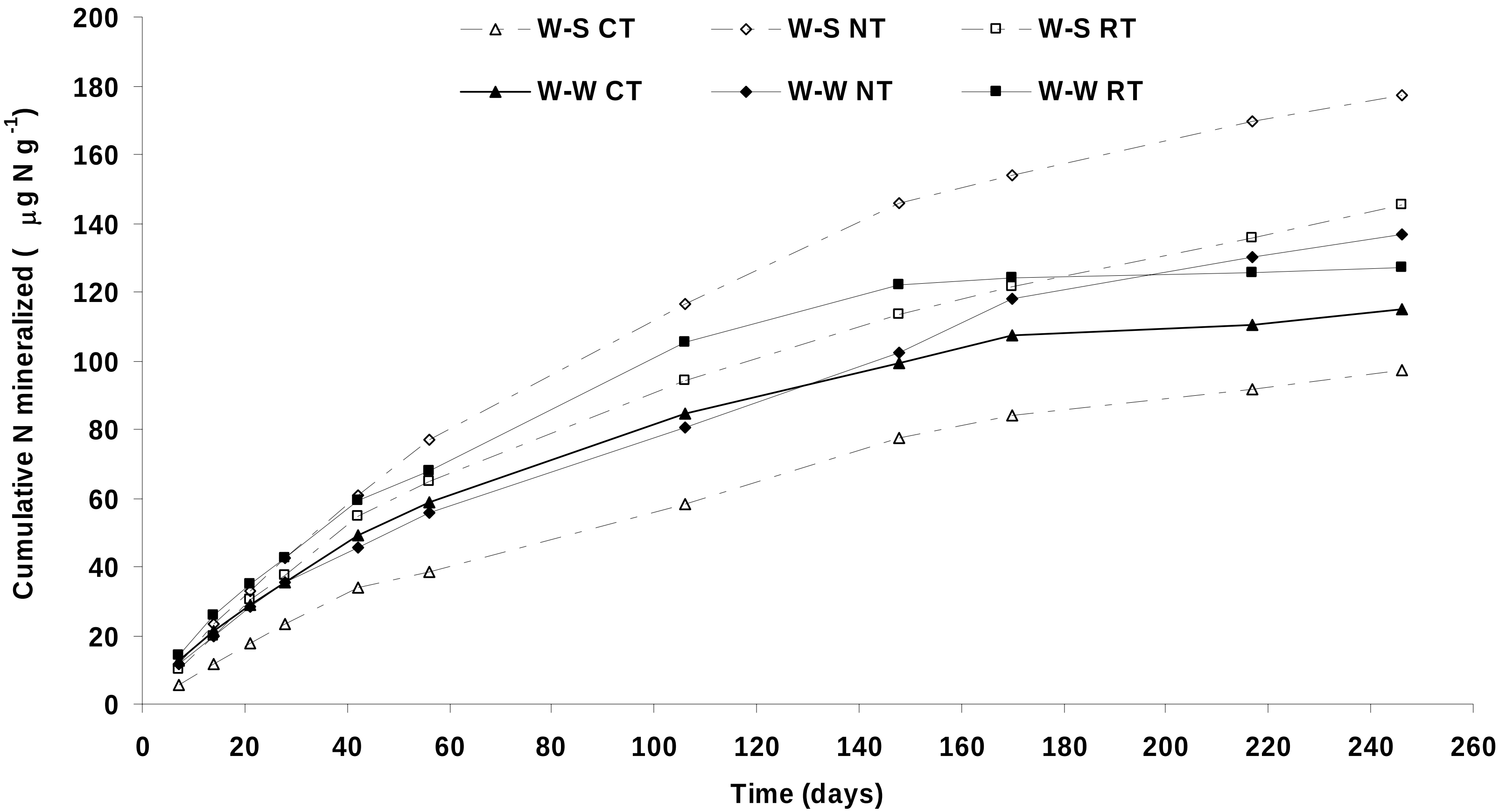

Figure B-21. Cumulative N mineralized during 245 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT) for wheat-soybean (W-B) and wheat-wheat (W-W) rotation at 0-5 cm in Manhattan experiment.

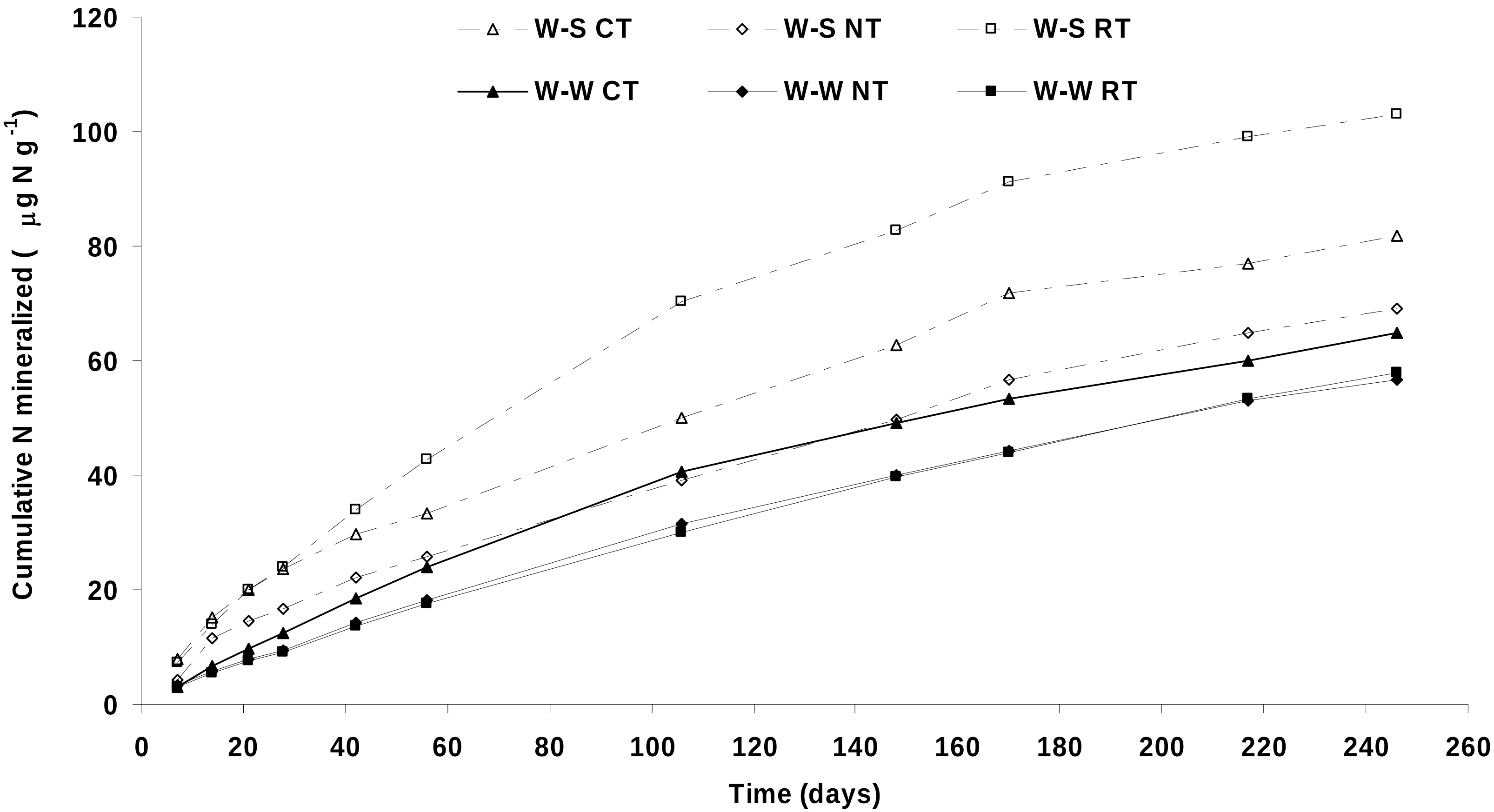

Figure B-22. Cumulative N mineralized during 245 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT) for wheat-soybean (W-B) and wheat-wheat (W-W) rotation at 5-15 cm in Manhattan experiment.

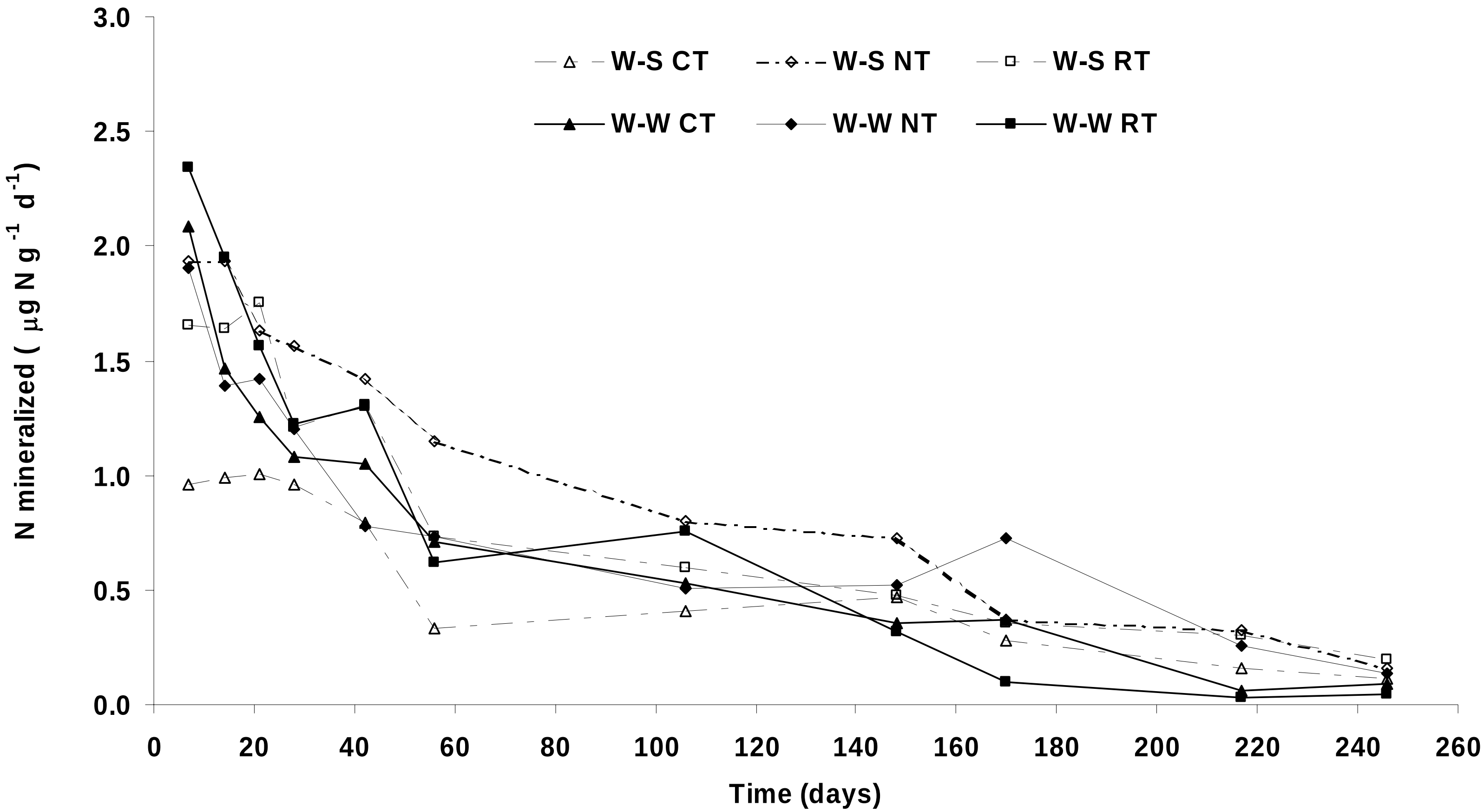

Figure B-23. Nitrogen mineralization rate during 245 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT) for wheat-soybean (W-B) and wheat-wheat (W-W) rotation at 0-5 cm in Manhattan experiment.

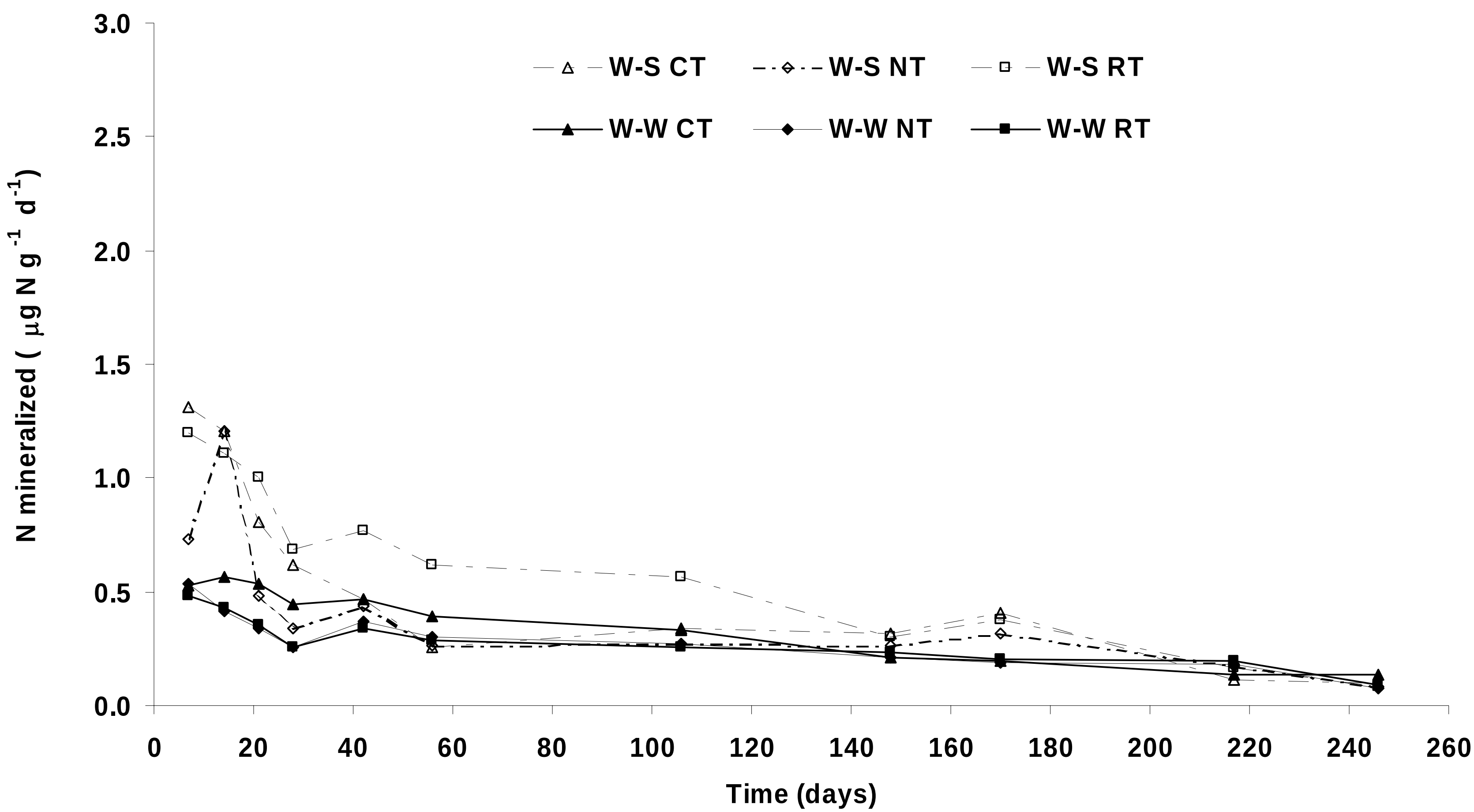

Figure B-24. Nitrogen mineralization rate during 245 days of incubation under conventional till (CT), reduced till (RT), no-tillage (NT) for wheat-soybean (W-B) and wheat-wheat (W-W) rotation at 5-15 cm in Manhattan experiment.

Appendix C - Chapter 5

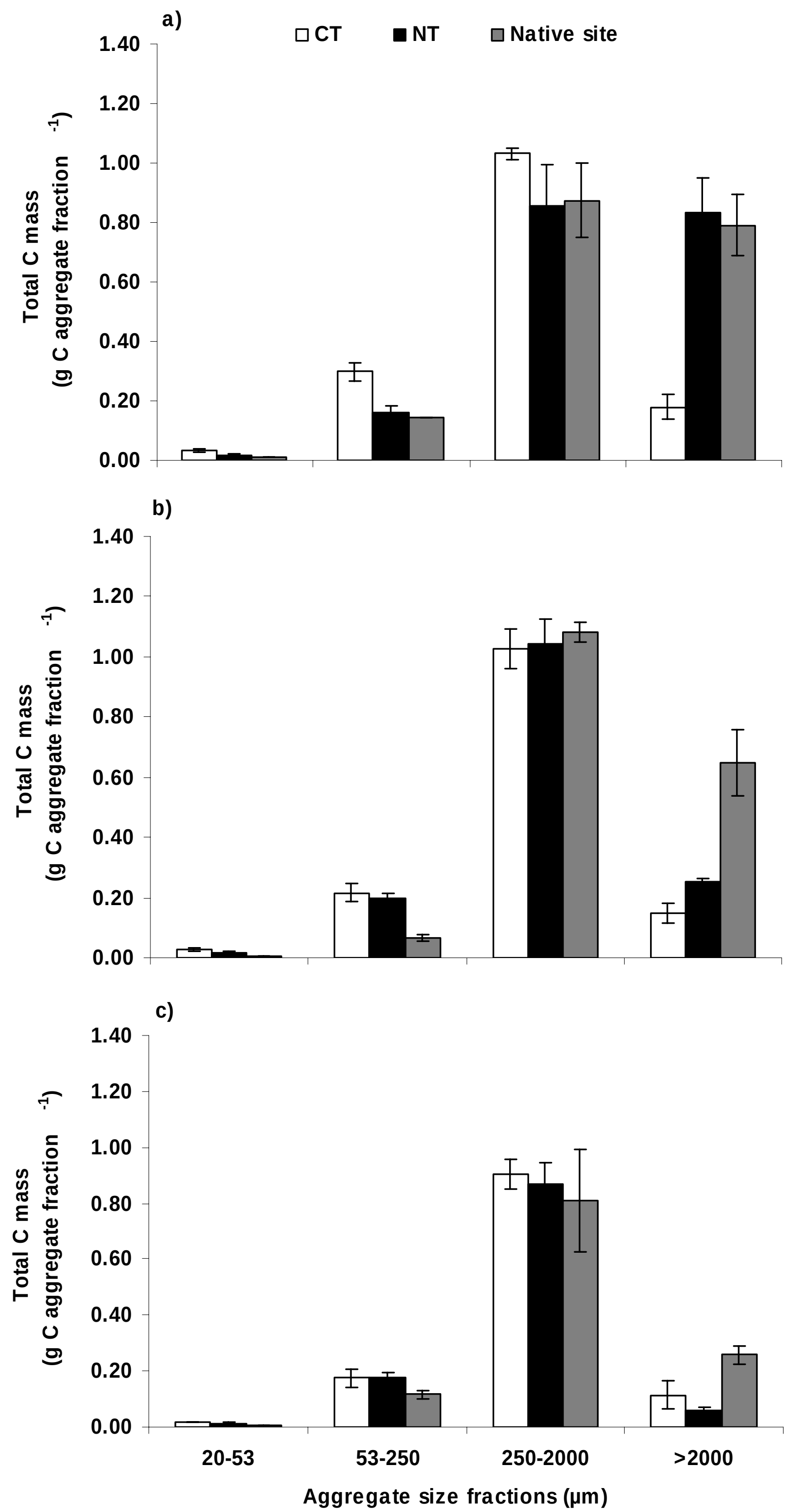

Figure C-1. Total C mass under conventional tillage (CT), no-tillage (NT) and native site at 0-5 cm (a), 0-15 (b), and 15-30 cm (c) for the Oxisol site.

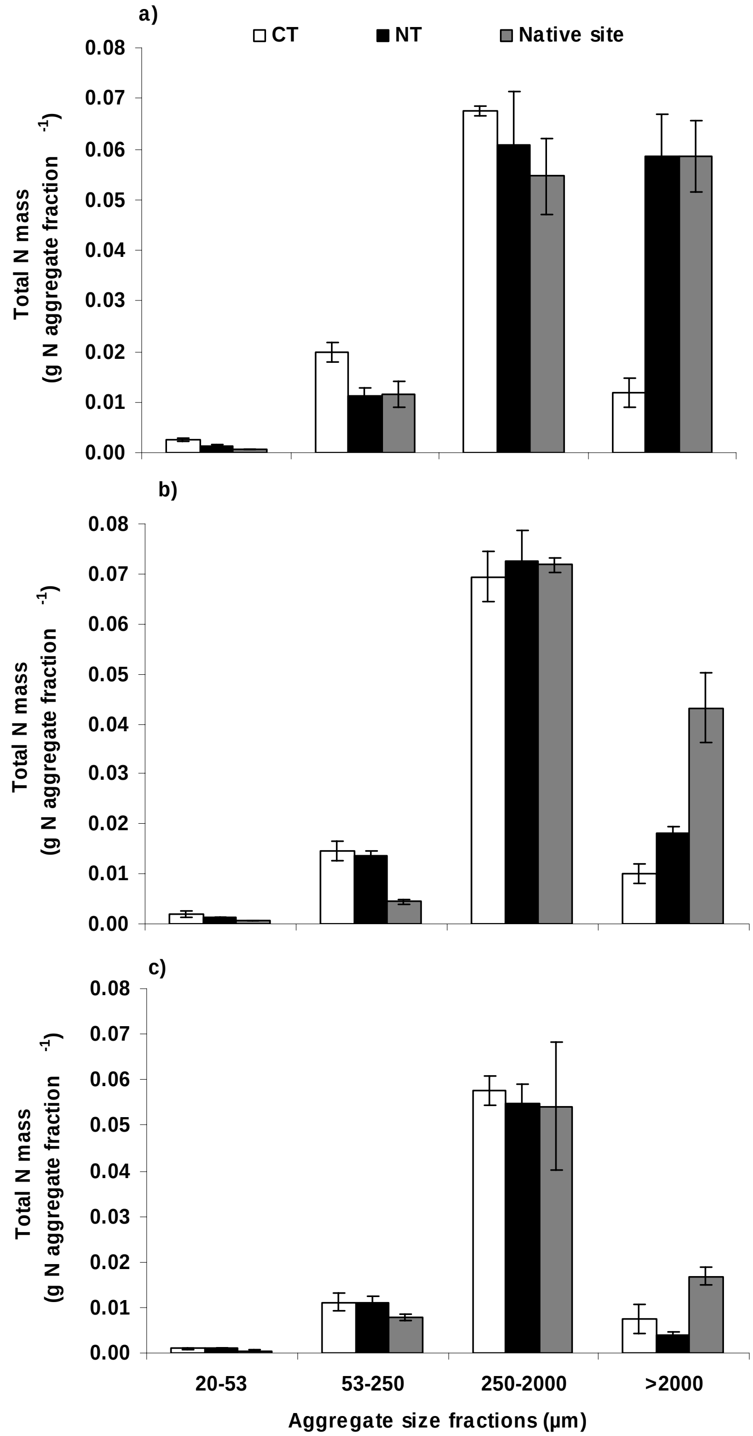

Figure C-2. Total N mass under conventional tillage (CT), no-tillage (NT) and native site at 0-5 cm (a), 0-15 (b), and 15-30 cm (c) for the Oxisol site.

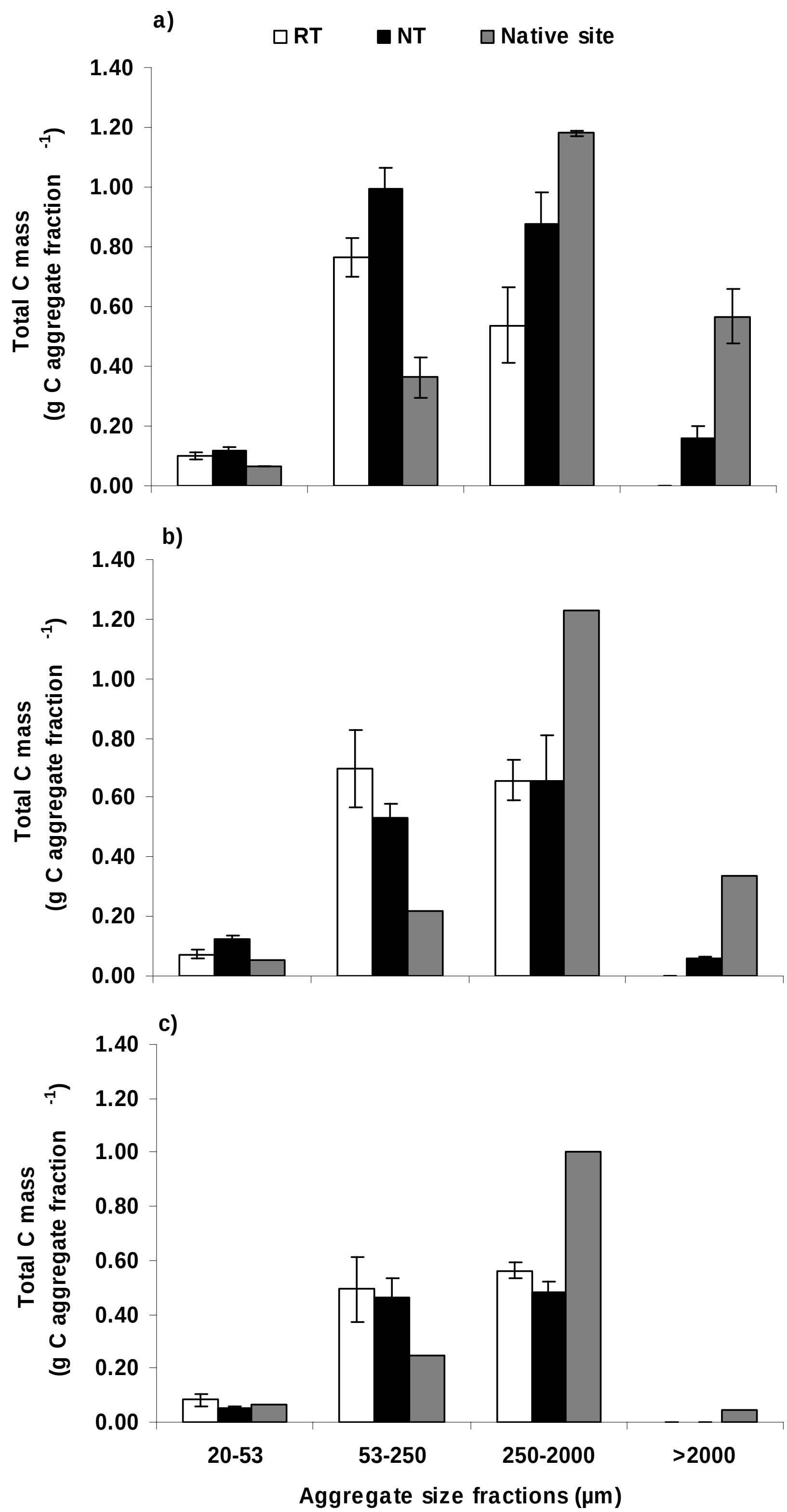

Figure C-3. Total C mass under reduced tillage (RT), no-tillage (NT) and native site at 0-5 cm (a), 0-15 (b), and 15-30 cm (c) for the Vertisol site.

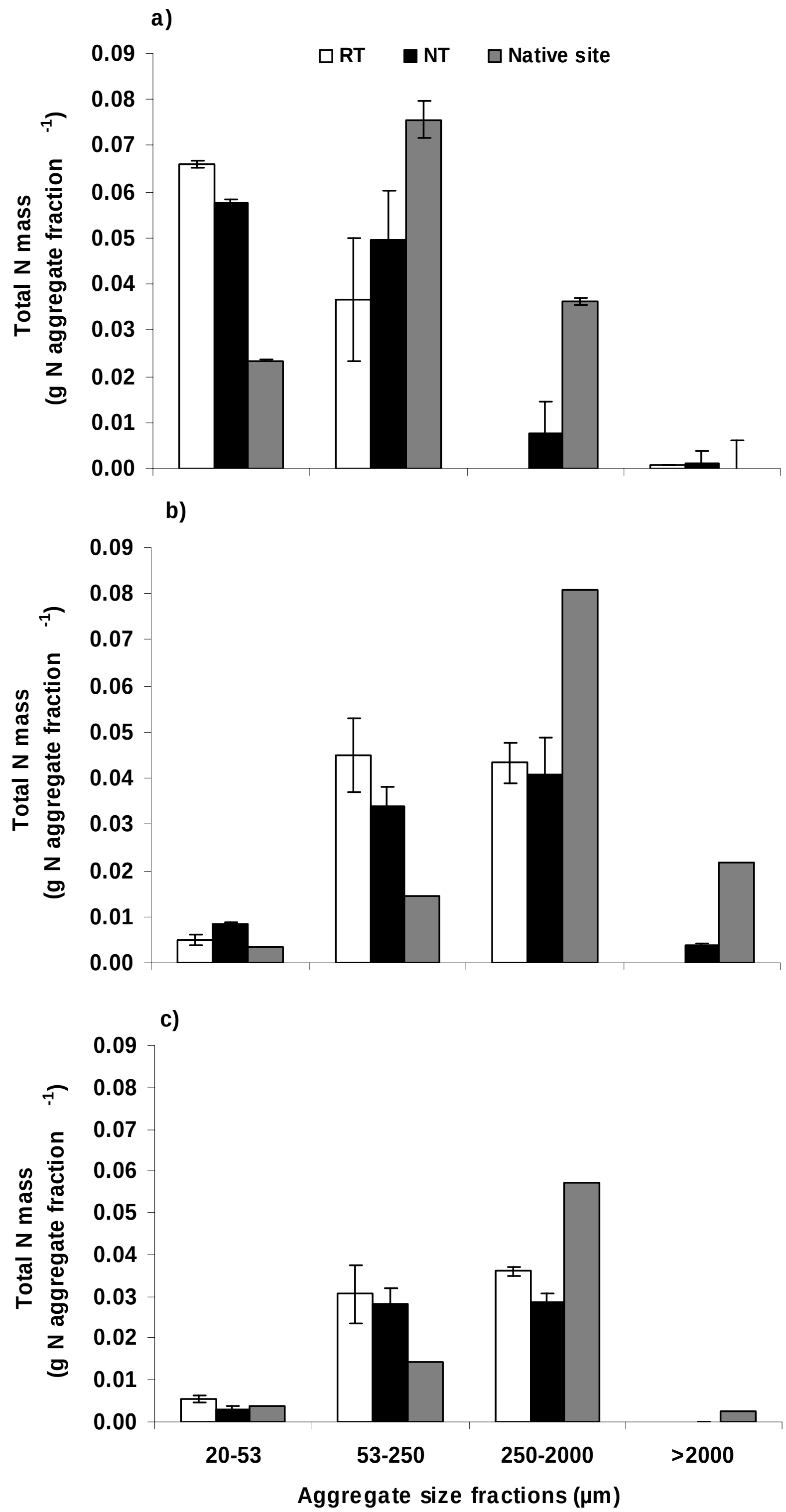

Figure C-4. Total N mass under reduced tillage (RT), no-tillage (NT) and native site at 0-5 cm (a), 0-15 (b), and 15-30 cm (c) for the Vertisol site.

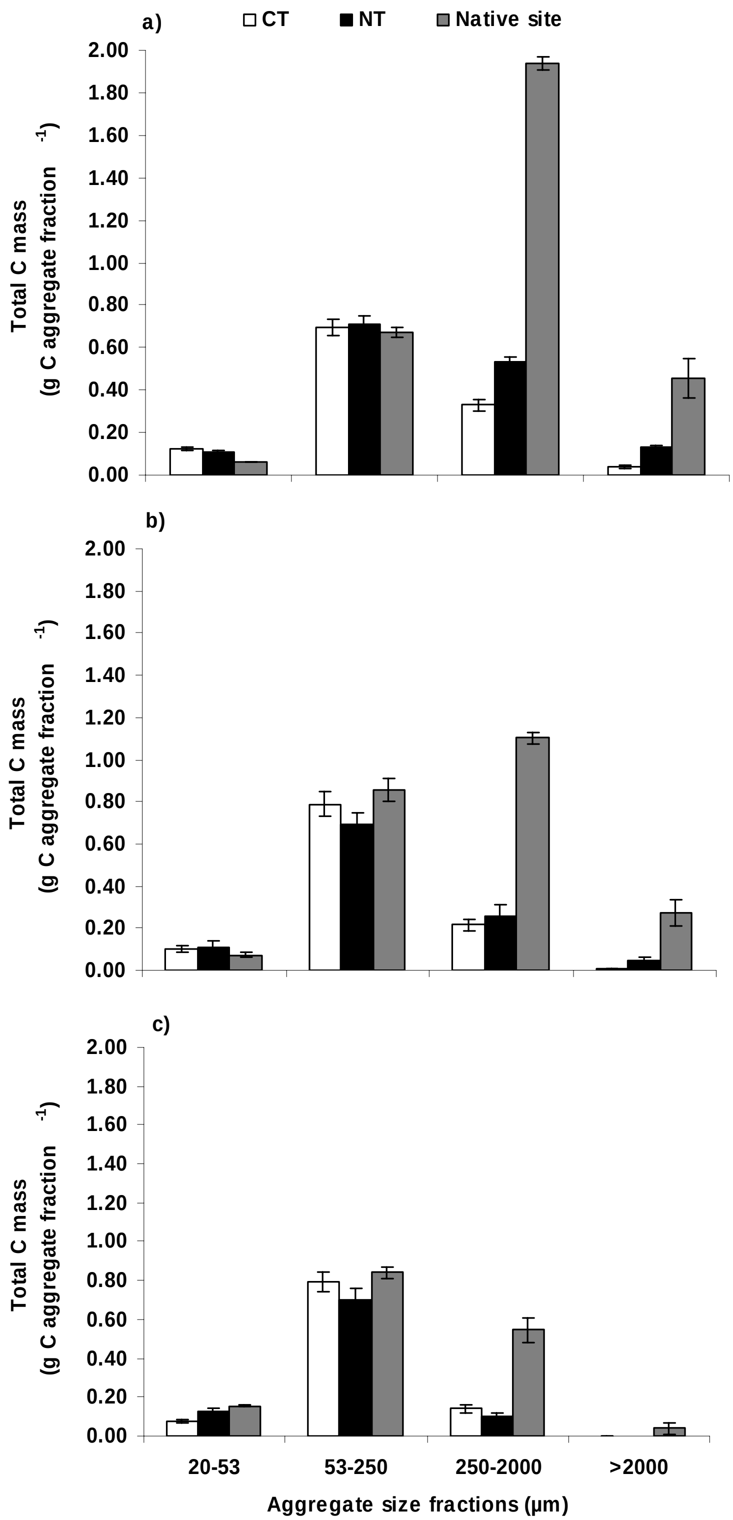

Figure C-5. Total C mass under conventional tillage (CT), no-tillage (NT) and native site at 0-5 cm (a), 0-15 (b), and 15-30 cm (c) for the Mollisol site.

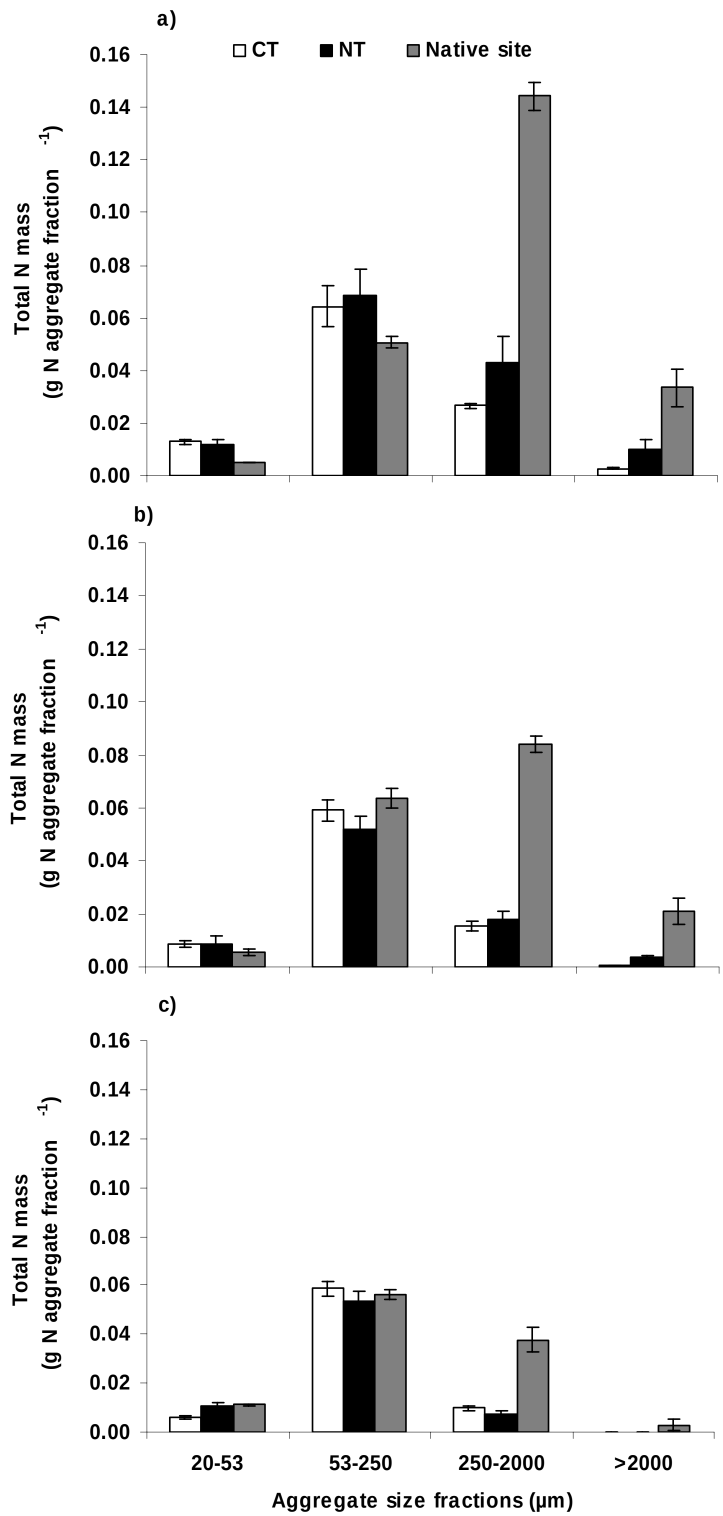

Figure C-6. Total N mass under conventional tillage (CT), no-tillage (NT) and native site at 0-5 cm (a), 0-15 (b), and 15-30 cm (c) for the Mollisol site.