MANAGEMENT EFFECTS ON LABILE ORGANIC CARBON POOLS IN A TEXAS COTTON-CROPPING SYSTEM

A Thesis

by

SCOTT MICHAEL KOLODZIEJ

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Approved as to style and content by:

David A. Zuberer
(Co-Chair of Committee)

Frank M. Hons
(Chair of Committee)

Thomas W. Boutton
(Member)

Mark A. Hussey
(Head of Department)

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It is well documented that increases in soil organic matter (SOM) improve soil physical properties and increase the overall fertility and sustainability of the soil. Research in SOM storage has recently amplified following the proposal that agricultural soils may provide a significant carbon (C) sink that may aid in the mitigation of increasing atmospheric carbon dioxide. Observed differences in lint yield and nitrogen response from a cotton performance study at the Texas A&M University Experimental Farm near College Station, TX prompted us to examine the effects of tillage and rotation on soil organic C (SOC), soil microbial biomass C (SMBC), 38-day cumulative C mineralization (38-day CMIN), hot-water extractable organic C (hot-WEOC), carbohydrate C, and total glomalin. The treatments examined included conventional-till continuous cotton (CT), reduced-till continuous cotton (RT), and conventional-till cotton after corn rotation (CC) treatments. In pre-plant soil samples, SOC, SMBC, and 38-day CMIN in the top 5 cm were 33, 58, and 79 % greater in RT and 29, 32, and 36 % greater in CC vs. CT. Comparable differences were observed for hot-WEOC and carbohydrate C. Little seasonal variation was observed for labile-C pools throughout the growing season, suggesting minimal C input from cotton roots. Water-stable aggregation was not significantly affected by management, and did not correlate with
labile-C pools or total glomalin. Labile-C pools were generally more responsive to management vs. SOC and were strongly correlated with one another. Carbohydrate C of hot-water extracts exhibited the strongest relationships with SMBC and 38-day CMIN, even though it comprised only 3 and 5 % of these pools, respectively. Our data suggest that increasing SOC in Texas cotton-cropping systems through conservation management is possible. Long-term data are still needed to fully address SOC storage potentials in Texas, but increases in labile-C pools resulting from conservation management are attainable and have the potential to positively impact soil fertility.
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INTRODUCTION

Managing soils to provide an environment conducive to organic matter storage has gained new impetus as recent research has suggested that the proper management of agricultural lands may be an effective tool in mitigating atmospheric carbon dioxide. The earth’s soils contain the largest terrestrial stock of organic carbon and the soil organic carbon (SOC) pool is sensitive and responsive to ecosystem performance and soil and crop management practices (Carter, 1996). Thus, with proper management, soils have the potential to act as a significant carbon (C) sink (Paustian et al., 1998a). Utilizing agricultural soils to sequester C is deemed as a “win-win” situation because management practices that increase soil organic matter (SOM) generally increase the fertility of the soil and involve reduced labor and input costs (Paustian et al., 1998b). Increases in SOM also increase soil structural stability, water-holding capacity, and help maintain the long-term productivity of the soil (Lal et al., 1997). A better understanding of SOC storage potentials for various soil types and how management may be utilized to maximize SOC storage in these soils is imperative to creating successful C sequestration programs. Substantial work has been done with respect to understanding SOC storage and dynamics under various crop-management systems, but few studies have been done in assessing SOC storage and dynamics under cotton management in warm climates.

An ongoing study of the effects of tillage and rotation on cotton performance at the Texas A&M University Experimental Farm near College Station, TX has shown significant increases in lint yield from conventional-till (CT) cotton/corn rotation and reduced-till (RT) continuous cotton treatments versus CT continuous cotton. The CT cotton/corn rotation and RT continuous cotton treatments have also exhibited decreased

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response to added nitrogen (N) when compared to the CT continuous cotton treatment (Hons et al., 2004). Efforts to conclusively explain these yield and N-response differences through assessment of soil physical and chemical properties have failed, suggesting that soil biological processes may be responsible for the observed management-induced changes. The observed differences in yield and N response also suggest that organic matter storage and dynamics have likely been affected by tillage and rotation in these cotton systems. A better understanding of such systems could provide valuable insight into SOC dynamics and microbial activity under varying cotton management systems.
REVIEW OF LITERATURE

Even though the results of individual mitigation measures seem modest, improved management has the potential to significantly increase C storage in soils over time. Soils have the potential to globally store 20-30 Pg (Pg = 10^{15} grams) of C in the next 50-100 years at approximate rates of 0.4-0.9 Pg C yr^{-1} (Paustian et al., 1998b). This potential equals approximately 6-10% of current C emissions from fossil fuel combustion (Paustian et al., 1998a). These estimates take into account the improved management of existing agricultural lands, the restoration of degraded lands, permanent conversion of surplus agricultural lands, and the restoration of wetlands. From a management perspective, the widespread adoption of conservation tillage may lead to global C sequestration of 1480-4900 Tg (Tg = 10^{12} grams) of C (Lal et al., 1997). Sperow et al. (2003) used the United Nations’ Intergovernmental Panel on Climate Change’s (IPCC) SOC inventory method in combination with the Natural Resource Inventory to gauge the potential for C storage in U.S. soils. They reported that U.S. soils have the potential to increase C sequestration by an additional 60-70 Tg yr^{-1} above the current rate of 17 Tg yr^{-1}, and that the adoption of no-till management on all annually cropped land in the country could increase C sequestration by 47 Tg C yr^{-1}.

Soil Organic Matter Composition and Characterization

Soil organic matter is a fundamental but dynamic component of soils that influences the many chemical, physical, and biological properties that regulate soil productivity (Carter, 1996). SOM is a mixture of carbon-rich organic materials derived from plant and animal residues that have been degraded by soil microorganisms to varying degrees. These materials may react with one another to form organic complexes and may also be stabilized by soil minerals (Cheng and Molina, 1995).
Organic C ordinarily comprises about 48 to 58% of SOM mass, and for this reason SOM estimates are usually derived from measurements of total SOC (Collins et al., 1997).

It has long been accepted that SOM contributes to soil fertility, and that the primary contribution is through the capacity to supply plant available nutrients, especially N. Generally, about 95% or more of the N and S and between 20 and 75% of the P in surface soils are components of the SOM (Duxbury et al., 1989). The average C:N:P:S ratio for SOM is 140:10:1.3:1.3 but this ratio is variable for each soil type (Stevenson, 1986).

SOM is heterogeneous with respect to biological degradation (Duxbury et al. 1989) and can be characterized by various pools or fractions with varying decomposition rates and turnover times ranging from days to 1000’s of years. Labile SOM fractions (e.g. the microbial biomass and carbohydrates) turn over more rapidly, while recalcitrant fractions (e.g. humic and fulvic acids) have much larger turnover times (Theng et al., 1989). Current models generally group SOM into a number of discrete pools based upon decomposition rates (Duxbury et al., 1989). In a survey of 20 SOC degradation models, Molina et al. (1994) showed that SOC could be viewed as a continuum of pools with a wide range of decay rates characterizing SOC into a series of labile to recalcitrant fractions. The method of partitioning SOM into discrete pools has some mathematical advantages, but is limited because the system is likely to be continuously variable (Duxbury et al., 1989). However, these models could provide valuable information on the nature and magnitude of pools that represent bio-reactive SOM fractions (Cheng and Molina, 1995).

Stevenson (1986) characterized SOM into three interrelated pools including plant and animal residues, the microbial biomass, and the resistant humus fraction. The
author stated that the humus fraction may have residence times ranging from 250 to 1000’s of years. SOM can more simply be divided into two fundamental fractions, one labile and the other stable or resistant (Theng et al., 1989; Körschens, 1997). The labile fraction generally consists of plant roots and non-decomposed plant residues, the soil microfauna and macrofauna, light fraction (LF) or macro-organic matter, water-soluble organic matter, and other non-humic organic material. The stable or resistant fraction generally consists of humic substances that have an intrinsic resistance to microbial oxidation. This resistance is related to the high molecular weight and chemical complexity of these substances as well as their interactions with clay minerals and metal cations, and their protection by aggregates (Theng et al., 1989). In temperate soils, up to 50% of SOM may be included in this resistant fraction, which may have a residence time of 1000 or more years, and is not generally considered significant with respect to C and nutrient cycling (Jenkinson and Rayner, 1977).

**Soil Organic Carbon Storage and Dynamics**

Amounts of total SOC in soil vary considerably. These amounts may be 1% or less in coarse-textured or tropical soils, up to 3.5% in prairie grassland soils, and up to 10% in poorly drained soils (Stevenson, 1986). In simplified terms, the levels of SOC in terrestrial systems are determined by the difference between organic matter inputs and outputs (Paustian et al., 1997; Gregorich et al., 1998). Inputs in agronomic soils generally consist of crop residues (including roots) and organic soil amendments such as manures and composts. The predominant output is the mineralization of organic material by heterotrophic soil microorganisms that generate CO₂ as the major product (Paustian et al., 1997). Leaching of dissolved organic carbon (DOC) may also function as an output, but these losses are relatively minor (Gregorich et al., 1998).
The capacity for SOC storage is governed by many factors including climate, soil type, landscape position, vegetation inputs, and soil management (Carter, 1996). Though SOC storage is primarily controlled by the quantity and quality of organic inputs and mineralization by soil microorganisms, climate sets gross restraints for SOC turnover (Anderson and Flanagan, 1989). Climate is the most important single factor in determining vegetation composition, primary productivity, and the intensity of microbial decomposition at any given location (Stevenson, 1986). Jenny (1930), described climate as the most important of the soil forming factors with respect to influence on soil total N.

The influence of climate on SOC storage involves the relationship between mean annual precipitation and temperature. Generally, wet and cool climates exhibit reduced decomposition and promote organic matter accumulation, while warmer climates favor accelerated decomposition of organic matter (Jenny, 1941; Carter, 1996). However, this general rule of thumb does not always apply. In a comparison of agricultural sites, Sanchez et al. (1982) found no effect of climate on average SOM within given soil orders. Also, SOC levels across the central U.S. reflect, to some extent, climatic patterns, but there is significant local variability associated with varying topography, drainage, and parent material (Paustian et al., 1997).

Climate sets overall constraints on soil organic matter by acting as a proximal control over primary productivity and decomposition, but the influence of climate on SOC storage is usually modified and sometimes overridden by soil physiochemical characteristics and landscape. Edaphic conditions, such as soil porosity, texture, structure, clay mineralogy, moisture, and pH can significantly influence SOC storage
Protection of Soil Organic Matter

The physical and chemical protection of organic matter by various mechanisms exerts a major influence on soil SOC storage and dynamics. Soil organic matter may be protected from decomposition through three basic mechanisms: (1) through associations with silt and clay particles, (2) through physical protection by aggregation, and (3) by biochemical stabilization in the form of recalcitrant humic compounds. If not protected by one of the above mechanisms, SOM is considered to be oxidizable by soil microorganisms (Six et al. 2002).

Silt and clay particles function to protect SOM through the formation of organo-mineral complexes. Thus, soils with higher clay contents typically are higher in SOM than coarser-textured soils (Stevenson, 1986). Hassink (1995) observed lower C and N mineralization per unit of microbial biomass in finer-textured soils when compared to coarser-textured soils. He attributed this observation to enhanced protection of organic matter in finer-textured soils. Hassink (1997) observed a positive relationship between the silt/clay fraction and silt/clay associated C. The author reported that C and N associated with the silt/clay fraction were better protected against decomposition than C and N associated with other soil fractions. He also defined a soil’s capacity to protect C based upon the observed silt/clay associations and found that this capacity, in the soils studied, was finite. Puget et al. (1999) observed enrichment of microbial carbohydrates in the silt/clay fraction obtained from stable aggregates in both conventional-till (CT) and no-till (NT) plots, supporting that carbohydrates bind to soil minerals, and that aggregate formation is partially mediated by microbial polysaccharides.
Biochemical protection of SOC is a result of the formation of complex organic (humic) substances possessing an intrinsic resistance to microbial decomposition. The resistance of these humic substances is related to their chemical structure and complexity (Theng et al., 1989). The dominant theory of humus formation involves numerous complex biochemical reactions in a multi-step process that can be simplified into: (1) the decomposition of plant materials into simple C compounds, (2) the assimilation and repeated cycling of C through the microbial biomass, and (3) the concurrent polymerization of microbially synthesized polyphenols and alteration of plant-derived lignin to form high-molecular-weight polymers (Collins et al., 1997). The complexity of the humic material results from the chemical composition of the original plant material and the actions of various decomposition and condensation reactions that increase the complexity of the compounds (Six et al., 2002).

**Soil Aggregate Formation**

Aggregate protection of organic matter also plays an important role in SOC storage. Aggregate formation is dependent upon many factors including soil texture, clay mineralogy, composition of exchangeable ions, and organic matter content (Kay, 1997). Tisdall and Oades (1982) proposed a hierarchical mechanism of aggregate formation where organic matter binds to clays through various bond types. These organo-clay complexes then bind to one another to form microaggregates that, in turn, bind to each other to form macroaggregates. Tisdall and Oades (1982) classified the organic binding agents as transient, temporary, and persistent based upon their chemical structures and residence times in the soil.

Oades (1984) and Elliott and Coleman (1988) discussed an alternate view of aggregate formation where microaggregates form within macroaggregates through
association with the particulate organic matter (POM) occluded in macroaggregates. Microaggregates formed in this manner are thought to be relatively stable (Beare et al. 1994a). In reality, both suggested mechanisms of aggregate formation may occur simultaneously in soil systems (Jastrow and Miller, 1997).

The macroaggregates referred to in the latter discussed mechanism for aggregation are thought to be a result of interactions by plant roots and fungal hyphae. Several researchers have suggested relationships between aggregation and plant roots, and between aggregation and fungal hyphae (Elliott and Coleman, 1988; Oades and Waters, 1991; and Jastrow et al. 1997). Roots and hyphae are thought to form a framework of aggregation through the binding action of the mucilages and exudates on their exteriors (Jastrow et al., 1997). Recently, Wright and Upadhyaya (1996) discovered glomalin, a ubiquitous and persistent glycoprotein that is excreted through the hyphae of arbuscular mycorrhizal fungi. After surveying a group of diverse soils, Wright and Upadhyaya (1998) discovered that glomalin was relatively abundant in soils and showed a strong positive correlation with water-stable aggregation. A particular fraction of glomalin termed immunoreactive, easily extractable glomalin (IREEG) exhibited the strongest relationship with aggregation in their study.

**Aggregate Protection of Soil Organic Matter**

Aggregate protection of SOM is thought to be a function of the compartmentalization of SOM that renders it inaccessible to microorganisms, and of the reduced diffusion of oxygen into aggregates creating a poor environment for oxidation (Six et al., 2002). The increase in C mineralization observed from crushed aggregates versus intact aggregates (Elliott, 1986; Gupta and Germida, 1988) supports this. Elliott (1986) observed that macroaggregates on average contained more SOM than
microaggregates, and that SOM associated with macroaggregates was generally more labile than SOM associated with microaggregates. He suggested that macroaggregate-protected organic matter is a main source of nutrients lost when soils are cultivated. Beare et al. (1994b) found that the disruption of macroaggregates from a NT soil resulted in increased SOM mineralization when compared to those from a CT soil. Aggregate-protected SOM also accounted for a greater proportion of mineralizable C and N in NT than in CT. The authors suggested that macroaggregate protection is an important mechanism of SOM protection under NT.

On the other hand, recent studies have suggested that microaggregates also play a large and possibly more significant role in the protection of SOM (Six et al., 2002). Gregorich et al. (1989) observed significantly higher C mineralization from soils when higher disruptive energies where used to disrupt microaggregates as opposed to just macroaggregates and Angers et al. (1997) reported that wheat-derived C was predominantly stored and stabilized in free microaggregates. Six et al. (1999) introduced a mechanism that stresses the importance of microaggregate protection of C with respect to aggregate turnover. The authors concluded that in CT systems, macroaggregates are disrupted more frequently than in NT systems, thus rendering enclosed, C-rich microaggregates susceptible to more frequent microbial oxidation.

Researchers have observed reductions in stable aggregates when native systems are cultivated (Elliott, 1986; Angers et al., 1992). Reduced aggregation has also been documented in CT versus NT systems (Bruce et al., 1990; Carter, 1992; Beare et al., 1994a). Fundamentally, tillage reduces aggregation by physical disruption of aggregate structures. This disruption releases previously protected and relatively labile organic matter, resulting in SOM depletion. Since various fractions of SOM
significantly contribute to aggregation, this SOM depletion may also lead to further aggregate destabilization (Jastrow and Miller, 1997).

**Management Effects on Soil Organic Carbon**

Conversion of land to agriculture renders a fraction of protected SOM susceptible to mineralization, disrupts the internal cycling of nutrients, and increases the potential for nutrient and C loss from the system (Duxbury et al., 1989). Researchers have documented significant SOC losses following the cultivation of native soils (Mann, 1986; Bowman et al., 1990; Davidson and Ackerman, 1993). In a study evaluating C sequestration in Conservation Reserve Program (CRP) lands, Gebhart et al. (1994) discovered that SOC in cropland and CRP land was significantly lower than in native pastures, and that CRP lands contained significantly more SOC than croplands. It has been accepted that cultivation typically leads to decreased stocks of SOM, but there are various conservation practices that can be utilized to increase SOC storage in agricultural soils. These include the use of reduced tillage, crop rotations, organic C amendments, and cover crops. Such practices have been documented to increase SOC storage and improve soil quality (Karlen and Cambardella, 1996).

Tillage generally affects the soil C balance process in two basic ways. First, tillage physically disturbs the soil and results in the disruption of aggregates. This leads to the release of previously protected organic C that becomes available to the soil microflora. Second, tillage controls the incorporation and distribution of plant residues into the soil (Paustian et al., 1997). Several researchers have documented increases in SOC content in NT systems vs. CT systems under various cropping schemes, but others have published contradictory results.
Doran (1980) reported significantly higher SOC levels in the top 7.5 cm of NT soils vs. CT soils in long-term experimental plots at seven locations. After examining microbial populations and the relative abundance of microorganisms with varying physiological types, the author suggested that the biochemical environment in NT soils is less oxidative when compared to CT soils. Havlin et al. (1990) observed higher SOC levels in NT vs. CT soils in a long-term study involving continuous corn, continuous sorghum, continuous soybean, and sorghum-soybean and corn-soybean rotations sampled at various increments down to a 30-cm depth. Carter (1992) observed 10-17% increases in SOC in NT and reduced-till (RT) plots vs. CT after only 3-5 years of management. The author reported that NT and RT plots contained more water-stable aggregates than CT plots, and that SOC was more enriched in macroaggregates than in the whole soil. Beare et al. (1994a) also reported concomitant increases in SOC content and water-stable aggregation in NT vs. CT plots cropped to sorghum and winter rye for 13 years. Hu et al. (1995) reported significantly greater SOC levels in NT vs. CT after 12 years in a sorghum-rye rotation experiment.

Bruce et al. (1990) reported significantly greater aggregate stability in NT and chisel-plowed soils vs. CT after 8 years of management, but failed to observe measurable increases in SOC storage under NT and chisel-plow management when compared to CT. Carter and Rennie (1982) also failed to observe significant increases in SOC in NT vs. CT plots sampled after 2, 4, 12, and 16 years of cropping with spring wheat. The authors did observe significantly higher soil microbial biomass C and N in the surface of NT systems when compared with CT, but management-induced effects were not sufficient to significantly affect the total SOC pool.
In an examination of data collected in eleven long-term studies that focused on the effects of NT vs. CT on SOC storage, Paustian et al. (1997) reported that SOC (0-30 cm) was 5-20 % higher under NT when compared to CT. Increases in SOC were attributed to reduced litter decomposition rates and less disturbance to soil structure associated with NT when compared to CT. The authors also stated that SOC in the studies appeared to increase rapidly following conversion to NT, but that rates of SOC increase slowed thereafter, supporting a trend of stabilization. This observation coincides with that of Dick et al. (1991), in which the authors reported the most rapid changes in SOC under NT occurred during the first 10 years of a 25-year experiment.

The fundamental influence of crop rotations on SOC storage is related to the amount of crop residues returned to the soil system. Utilizing crops that increase residues returned to the soil in a given rotation has the potential to increase SOC. Typically, C₄ feed grains produce the highest amounts of residues with respect to row crops (Paustian et al., 1997). Several studies have documented changes in SOC proportional to the amount of residues returned to the soil by various rotations. Campbell and Zentner (1993) found that rotation significantly affected SOC and organic N levels in the 0 to 15-cm depth. Changes in SOC were directly related to the amount of residues produced by different rotations. Zielke and Christenson (1986) found that changes in SOC were correlated to the amount of residue returned by six different rotations that included corn, oats, alfalfa, navy bean, and sugar beet. The authors also reported that SOC generally increased with the increasing frequency of corn in the rotation. Havlin et al. (1990) also observed a direct relationship between rotation effects on SOC levels and the amount of residues returned to the soil. The authors reported that continuous corn and sorghum resulted in greater residue return and SOC than
corn/soybean and sorghum/soybean rotations. Continuous soybean resulted in the lowest residue return and lowest SOC levels.

**Soil Organic Carbon in Cotton Agroecosystems**

The effects of management practices on SOC in cotton agroecosystems are not as well documented. Mitchell and Entry (1998) observed SOC levels of 0.42 % in soils cropped to continuous cotton with no cover crop from long-term plots in Alabama’s “Old Rotation” experiment. The authors observed that planting of winter legumes and utilization of rotations involving corn, small grain, and soybean resulted in SOC levels reaching 1.21 %. Potter et al. (1998) observed greater SOC levels in NT and minimal-till cotton/corn rotations in a 15 year study. After assessing the potential for C sequestration in Texas soils by examining management effects on SOC levels at 3 long-term experimental sites, the authors found that C sequestration decreased with increasing mean annual temperature. The authors also suggested that climatic conditions in Texas may limit C storage. Salinas-Garcia et al. (1997) and Zibilske et al. (2002) observed 64% and 58% increases in SOC, respectively, in the surface (0-5 cm) of NT managed cotton/corn rotations when compared to CT rotations. Zibilske et al. (2002) also reported low amounts of readily oxidizable carbon in both NT and CT treatments, suggesting that a significant amount of SOC in the system was of a relatively recalcitrant nature.

**Soil Microbial Biomass**

Soil microbial biomass (SMB) is considered an active fraction of SOM that plays a fundamental role in regulating soil fertility (Smith and Paul, 1990). SMB is an important source and sink of various soil nutrients and it drives nutrient cycles through its role as a transforming agent (Schnürer et al., 1985; Duxbury et al., 1989). The
growth and functioning of SMB is usually limited by C (Smith and Paul, 1990), and SMB is generally in a resting state with periodic flushes of activity and growth (Follett, 1997). A supply of available organic C is needed for SMB turnover and the mineralization of organically bound nutrients (Theng et al., 1989). Since SMB depends on degradable C for growth and maintenance, the size and activity of the SMB pool are usually correlated with SOM (Schnürer et al., 1985; Collins et al., 1997).

The magnitude and activity of the SMB is also related to other factors, such as climate and soil texture. In a study examining data from 12 long-term agricultural experimental fields in differing climates, Insam et al. (1989) reported that SMBC per unit SOC correlated well with several climatic variables. Integrative climatic variables accounted for up to 68% of the variation in SMBC per unit SOC. The authors stated that the remaining variation was likely due to differences in clay content, pH, and management practices. Insam (1990) also observed relationships between macroclimatic variables and SMB, and reported that basal respiration was higher in soils from warmer climates when compared to soils from cooler climates. Franzluebbers et al. (2001) reported that higher mean annual temperature resulted in greater SMBC, basal respiration, and potential N mineralization. The authors concluded that although thermic regions were not capable of maintaining SOC levels as high as more frigid regions, active organic fractions per unit SOC were up to 2-3 times greater in thermic regions when compared with frigid regions.

In examining the effects of soil texture on SMB, Hassink (1994) reported that values of SMB per unit SOC were higher in fine-textured soils when compared with coarse-textured soils. The author also stated that SMB activity was lower in the clay soils that were studied. Similar results were observed by Franzluebbers et al. (1996a)
who reported that SMBC per unit SOC increased with increasing clay content. The authors also pointed out that mineralizable C and N and basal respiration per unit SMBC decreased with increasing clay content, and that soil texture significantly influenced C and N turnover in the eight Texas soils studied. A protective effect of clay content was also observed by Wang et al. (2003). The authors observed that C mineralization in laboratory incubations was initially correlated with indexes of available C substrate and did not correlate well with clay content. However, after 7 days of incubation, a significant protective effect of clay content on C mineralization was exhibited.

The soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) pools generally comprise 2 to 5 % of SOC and 1 to 5 % of soil total N, and the estimated size of the SMBN pool suggests that the microbial biomass is large enough to significantly impact plant-available soil N (Smith and Paul, 1990). Laboratory incubation studies have supported this contention. In a 12-week incubation study, Carter and Rennie (1982) found that SMBN accounted for 40 to 60 % of potentially mineralizable N. Bonde et al. (1988) observed that N mineralization was related to the decrease in SMB over the course of a 40-week incubation period. The authors reported that SMBN accounted for 55 to 89 % of N mineralized in the incubation. In a long-term field study comparing the effects of various tillage practices, Follett and Schimel (1989) found that SMBN comprised a significant proportion of the active-phase soil nitrogen pool determined using $^{15}$N.

Management-induced changes in SOM are slow and difficult to determine in the short-term due to the relatively large background size of the SOM pool (Bonde et al., 1988). The SMB is more sensitive and responsive to changes in management and data
support that SMB may be used as an early indicator of SOM changes resulting from various management practices. Powlson and Brookes (1987) examined the effects of 18 years of annual straw incorporation at two sites on SOM and the SMB and found that while straw incorporation only increased SOC and total N by approximately 5 and 10 % respectively, SMBC and SMBN increased between 37 and 50 %. Saffigna et al. (1989) also reported that SMB provided a more sensitive indicator of management-induced change. They observed that SOC was 7 % greater in NT vs. CT soils averaged across differing residue management treatments in a 6-year study. The corresponding increase in SMBC ranged from 14 to 21 %. A similar effect was observed by Carter and Rennie (1982) who failed to see significant differences in SOC storage, but observed significantly greater SMBC and SMBN in NT vs. CT systems cropped with spring wheat.

Management Effects on Soil Microbial Biomass

Numerous studies document the effects of management practices on SMB, and practices that lead to increased C substrate also generally result in increases in SMB and its activity. Gupta and Germida (1988) reported a reduction in SMB, its activity, and enzyme activity following cultivation of native prairie, and stated that this reduction was most pronounced in macroaggregates. In a study where native sod was converted to wheat and managed with NT, stubble mulch, or CT for 16 years, Follett and Schimel (1989) observed that SMB was reduced to 57, 52, and 36% of that of the native sod for NT, stubble mulch, and CT, respectively. Microbial activity measured by C mineralization was significantly greater in NT and stubble mulch treatments vs. CT, and C mineralization was greatest in native sod.

Several other studies have compared the effects of NT vs. CT on SMB. Doran (1980) observed greater microbial numbers and phosphatase and dehydrogenase
activity in the 0 to 7.5-cm depth of NT vs. CT. These effects were related to SOC storage and were reversed at a greater depth. The author suggested that the distribution of residues, controlled by tillage, affected microbial numbers by providing C and N substrates and influencing water content. Lynch and Panting (1980) reported that SMB determined by chloroform fumigation-incubation (CFI) was significantly greater in NT vs. CT in soils cropped to wheat. Carter (1992) documented increased SMBC under NT vs. CT systems and reported an enrichment of SMBC in macroaggregates of NT vs. CT. Franzluebbers and Arshad (1997) also reported greater SMBC and C mineralization in NT vs. CT macroaggregates.

In a study that examined the effects of NT vs. CT on continuous sorghum and a sorghum-wheat/soybean rotation, Franzluebbers et al. (1995a) found that SMBC averaged 65 % greater in NT vs. CT at 0-5 cm and 22 % greater in NT vs. CT at 5-12.5 cm. Microbial biomass activity measured by potential C mineralization followed similar trends. The SMBC pool and C mineralization also averaged 18 % greater in the rotation when compared to continuous sorghum. Collins et al. (1992) also observed a rotation effect on SMB. The authors reported significantly higher SOC, SMBC, and SMBN in annual cropping vs. wheat/fallow rotations. Rotation influences on the SMB were related to residue management and resulting increases in available soil C. Angers et al. (1993) observed greater SMBC in NT and chisel-plow barley and 2-year barley/red clover rotations when compared to CT. The authors also reported an enrichment of SOC as SMBC in the rotation vs. continuous barley. The largest differences in SMBC in the study (approximately 600 mg kg⁻¹ C in NT vs. 300 mg kg⁻¹ C in CT) resulted from NT plots under rotation, signifying a tillage/rotation interaction. Sorghum residue retention resulted in increases of 12 and 23 % in SMBC and SMBN in a study by Saffigna et al.
The authors also reported that residue retention increased C mineralization by 45% in a 30-day incubation. In the same study, NT vs. CT resulted in 14-21% greater SMBC in the top 10 cm, and the effect of NT on SMBC was greatest (31% vs. CT) when NT was combined with residue retention.

Data concerning the effects of tillage and rotation on SMBC in cotton agro-ecosystems are less abundant. Entry et al. (1996) reported SMBC values of 20 mg kg\(^{-1}\) in long-term continuous cotton with no N application in Alabama’s “Old Rotation” experiment as determined by chloroform fumigation-extraction (CFE). SMBC in a 2-year cotton/corn rotation with a winter legume was more than twice that of the continuous system (55 mg kg\(^{-1}\)). Balota et al. (2003) observed average SMBC values of 372 mg kg\(^{-1}\) and 145 mg kg\(^{-1}\) under NT and CT cotton/wheat rotations, respectively, in southern Brazil. They concluded that NT systems resulted in higher SMB and suggested that nutrient cycling may be enhanced in NT systems due to the larger SMB pool. Feng et al. (2003) reported 60, 140, and 75% greater SMBC (determined by CFI) in the surface layer of NT vs. CT continuous cotton in February, May, and October, respectively. The authors also observed a significant influence of tillage on microbial communities as determined by analysis of phospholipid ester-linked fatty acid profiles.

**Temporal Variability of Soil Microbial Biomass**

Although the SMB pool is usually limited by organic C inputs from plant litter, it is still susceptible to influences of seasonal moisture and temperature (Follett, 1997). However, clarifying the relative magnitude of contributions of different environmental factors to SMB temporal variability can prove to be difficult. Collins et al. (1992) observed greater SMB in the early spring in a climate dominated by cool and wet winters and warm and dry summers. Deluca and Keeney (1994) recorded peak SMBC
values in late February after thaw. The authors related the peak in SMB to soil moisture and a possible post-thaw increase in microbial activity due to the disruption of microbial tissues. Angers et al. (1993) observed peak SMB in mid-May followed by a decrease in SMB during the summer and a subsequent increase in the fall. The authors also reported that SMB was loosely correlated with soil water content. Other studies have reported that soil moisture had the greatest influence on temporal variability of the SMB (McGill et al., 1986; van Gestel et al., 1992).

In a study by Lynch and Panting (1980) SMB levels increased during the growth of wheat and returned to lower and relatively constant levels after harvest. The authors related this increase in SMB to inputs by crop roots and rising temperatures associated with the growing season. Franzluebbers et al. (1994) and Franzluebbers et al. (1995b) also observed an influence of crop input on seasonal fluctuations of SMB in continuous wheat and wheat rotations involving soybean and sorghum under NT and CT. The authors concluded that seasonal inputs from crop roots and residues resulted in seasonal effects on SMB and potential mineralization of C and N. The authors also concluded that N dynamics may be dependent upon short-term seasonal inputs associated with cropping.

Other studies have failed to detect significant influences of crops on the temporal variability of the SMB. Ritz and Robinson (1988) observed no relationship between SMB and crop growth throughout the growing season of spring barley, and Joergensen et al. (1994) observed only minor SMB fluctuations under a wheat system. The authors also failed to find significant variations in SMB throughout the 1-year study that was marked by significant differences in weather conditions.
In a publication examining data from 58 previous studies, Wardle (1998) found no difference in temporal variability among forest, grassland, and arable ecosystems, or between NT and CT arable systems. When assessing the entire data set, the author found that a 3-component model including pH, soil C, and latitude best predicted temporal variability of the SMB. Variations concerning latitude were related to increasing latitudes experiencing higher inter-seasonal variations in temperature.

**Water-soluble Organic Carbon**

Water-soluble organic matter (WSOM) in soils plays an important role in the nutrient cycling of C, N, and P (Kalbitz et al., 2000), and appears to be an immediate source of available organic C for soil microorganisms, making its replenishment key in mediating SMB turnover (McGill et al. 1986). Major sources of WSOM include surface and subsurface litter (including roots), rhizosphere exudation, and soil humus. Other sources may include organic fertilizers, lysed microbial biomass, and faunal excreta (Zsolnay, 1996). Studies in both the field and laboratory have shown that litter and humus are the most important sources of WSOM in soils (Kalbitz et al., 2000).

Fluxes of WSOM are primarily controlled by the physical processes of diffusion and convection, which are regulated by the proportion of immobile SOM to WSOM and the gradient of the soil’s water content, respectively. Numerous biochemical reactions in the soil also regulate fluxes in WSOM (Zsolnay, 1996). The observed concentration or flux of WSOM in soil is the net result of processes releasing WSOM (e.g. leaching from litter and desorption from solid-phase organic matter), and processes removing WSOM (e.g. adsorption, decomposition, and leaching) (Kalbitz et al., 2000). In general, the production and consumption of WSOM depend primarily on microbial activities and the equilibrium with solid-phase organic matter (Chantigny, 2003).
Using $^{13}$C compositions of SOM, SMB, and WSOC, Gregorich et al. (2000) reported that a clear distinction existed between WSOM and SMB. The authors found that WSOM was closely related to SOM, and stated that SOM dominated WSOC composition even though the WSOM pool was considered to be very active. This suggests that the SOM pool, which is generally much larger than the WSOM pool, exists in equilibrium with the pool of WSOM. Seto and Yanagiya (1983) studied relationships between WSOM and respiration in 65 soils from both agricultural and forested sites. They observed that WSOM was only sufficient to support small amounts of respiration, but that WSOM was replaced rapidly. The authors reported no measurable decreases in WSOM for a 6-week period following an initial loss after the first week. A better understanding of the rate at which WSOM is generated from SOM and made available to SMB could improve insight into SMB activity and turnover with respect to WSOM substrate (Jandl and Sollins, 1997).

Water-extractable organic matter (WEOM) is WSOM obtained by extraction of a given mass of soil with an aqueous solution, and is usually quantified as water-extractable organic carbon (WEOC) (Zsolnay, 1996). Total extraction of soluble C is unlikely, due to the equilibrium of sorption and desorption, and a portion of soluble C always remains in soil samples following extraction (Tao and Lin, 2000). Extractions carried out with hot water hydrolyze part of the SOM and solubilize a portion of the microbial biomass, thus creating WSOM that does not exist \textit{in situ}. However, hot-water extractions may be helpful in assessment of potentially available organic material and in indicating changes in soil fertility associated with management (Leinweber et al., 1995; Zsolnay, 1996). In general, amounts of WEOC extracted will vary with extraction conditions, thus WEOC is considered to be operationally defined (Zsolnay, 1996).
Results from several published studies concerning the availability of WEOM imply that this material consists of different fractions that vary in biodegradability, and that WEOM composition can vary with soil type and land-use. Burford and Bremner (1975) observed a high correlation ($r^2=0.96$) between WSOC and mineralizable C, and characterized WSOM as highly susceptible to decomposition. In support that WSOM is readily available to the soil microorganisms, Xu and Juma (1993) reported that the majority of $^{14}$C respired during a 10-day incubation came from water-soluble organic $^{14}$C.

Other authors have published results that imply WEOM contains a fraction that is recalcitrant or less susceptible to degradation. Cook and Allan (1992) noted that C mineralization was related to WSOM in the early stages of an incubation study, but that this relationship became less apparent in the latter stages of incubation. In a study involving WSOC from forest floor, soil solution, and stream water samples, Qualls and Haines (1992) observed that only 14-33% of WSOC was decomposed during 134 days of laboratory incubation. Comparable data were reported by Boissier and Fontvieille (1993) who found that only 3.8-39.9% of WEOC from 2 different soils was available to microorganisms in laboratory incubations. Similar results were also reported by Nelson et al. (1994) who documented WEOC availabilities ranging from 3 -22% throughout the profile of an agricultural soil and Kalbitz et al. (2003) who reported that labile WEOC comprised 14-25% of total WEOC extracted from agricultural soils.

Several studies have reported that WEOC consists of two distinguishable pools with respect to degradability. Zsolnay and Steindl (1991) observed that, independent of depth, WEOC was composed of two distinct fractions, one labile and the other recalcitrant. Jandl and Sollins (1997) reached a similar conclusion and also reported
that the high-molecular-weight acid fraction of WEOC was less degradable than the neutral fraction. Kalbitz et al. (2003) assayed the biodegradability of WEOC from 13 samples including maize straw, forest floor, and agricultural soil through laboratory incubations and found that WEOC decomposition fit a double exponential decay model dividing WEOC into rapidly and slowly decomposable pools. Gregorich et al. (2003) also found that both hot-WEOC and cold-WEOC from soils cropped to maize fit similar models, further supporting the characterization of WEOC into two distinguishable pools based upon degradability. The authors also noted that the proportion of degradable WEOC was affected by extraction temperature, and that hot-WEOC contained a larger proportion of degradable C than cold-WEOC. The authors also suggested that WEOM contained a significant amount of labile, N-rich compounds that related to decomposability of WEOM in their study.

**Management Effects on Water-extractable Organic Carbon**

Management may influence WEOC storage and dynamics by controlling the quantity and quality of organic matter inputs, and by affecting the microbial degradation of organic matter (Kalbitz et al., 2000). In the long-term, WEOC contents tend to be proportional to SOM content (Chantigny, 2003), thus management practices that influence SOM will likely effect WEOC. In reviewing land-use influences on WEOC, Chantigny (2003) reported that WEOC is generally highest in forest soils followed by grassland soils and then arable soils, respectively. However, Boyer and Groffman (1996) documented unexpectedly greater concentrations of WEOC in the surface soils of agricultural plots when compared to forest soils. The authors attributed this difference largely to the increased soluble humic acid fraction observed in agricultural soils.
Data describing the effects of management practices on WEOC are not as abundant as those describing management effects on SOC and SMB. Leinweber et al. (1995) observed that long-term fertilization with NPK and farmyard manure resulted in significantly greater hot-WEOC vs. control plots. Gregorich et al. (2003) also observed that manure inputs increased hot-WEOC and cold-WEOC when compared to control plots. The authors also reported that the portion of biodegradable hot-WEOC was greater in manured vs. control plots, and that water-extractable C and N were strongly related to whole soil C and N.

Gregorich et al. (2003) also examined the effects of continuous maize vs. maize/soybean rotation on WEOC, and found that the rotation had little effect on hot-WEOC and cold-WEOC content vs. continuous sorghum. The authors also found that rotation did not affect the proportion of WEOC degraded over a 42-day incubation. Campbell et al. (1999a) observed higher WEOC in continuous wheat when compared to wheat/fallow systems, and found similar effects for light fraction and mineralizable C and N. They found that wheat/fallow reduced WEOC by 22 % compared to continuous wheat after 29 years of management. The authors stated that the effects were related to historically higher crop residue inputs and reduced tillage intensity associated with continuous wheat. Similar results were observed by Campbell et al. (1999b) in wheat rotations involving legumes and alfalfa.

With respect to tillage, Leinweber et al. (2001) reported that tillage intensity altered the composition of water-soluble fulvic acids. In comparisons of NT vs. CT on WEOC in soils from Illinois, Kentucky, Nebraska, and Minnesota, Linn and Doran (1984) generally found that NT increased WEOC vs. CT in the top 7.5 cm. In most cases, a
reverse effect was observed in the 7.5-15 cm depth. The authors reported that, overall, the differences in WEOC resulting from tillage were not significant.

Ghani et al. (2003) described hot-WEOC as a sensitive and consistent indicator of management-induced change at several sites, with WEOC levels following the pattern native soil > sheep/beef pasture > dairy pasture > cropped soil. The authors reported that hot-WEOC levels also responded to grazing intensity and N and P fertilization in pastures. Good relationships between hot-WEOC and other labile organic matter fractions were observed.

**Soil Carbohydrates**

Soils carbohydrates generally comprise 5-25 % of the total SOM, and are considered readily degradable components of the SOM that act as major energy sources for soil microorganisms (Cheshire, 1985). They are also involved in the formation of soil structure (Cheshire, 1979) and have been strongly correlated with aggregation (Chaney and Swift, 1984; Haynes et al., 1991; Ball et al., 1996).

The carbohydrate fraction of the SOM is composed of a complex mixture of mono and polysaccharides (Folsom et al., 1974), and soil carbohydrates also exist in the form of various humic-polysaccharide complexes (Cheshire, et al. 1992). Carbohydrates in soils include those of both plant and microbial origin (Cheshire, 1979), and much of the soil carbohydrate is considered to consist of polysaccharides synthesized by soil microorganisms (Folsom et al., 1974).

Soil carbohydrate composition can provide some insight into carbohydrate origin. Oades (1984) suggested that galactose + mannose to arabinose + xylose ratios of < 0.5 and > 2.0 were characteristic of plant-derived carbohydrates and microbiially-derived carbohydrates, respectively. Hu et al. (1995) reported that significantly higher mannose
to xylose ratios were observed in microaggregates vs. macroaggregates, and suggested that organic matter was more highly processed in microaggregates when compared to macroaggregates.

Most studies have involved acid-hydrolyzable carbohydrates (AHC) as opposed to the water-soluble carbohydrates (WSC). Generally, WSC comprise only a small proportion of AHC, but both fractions respond similarly to tillage (Angers et al. 1993). The composition of WSC most likely includes free sugars and microbial polysaccharides, while AHC include these components in addition to more complex polysaccharides of plant and microbial origin (Cheshire, 1979). Hot-water extractable carbohydrates have exhibited strong relationships with aggregate stability, and Haynes and Francis (1993) suggested that the importance of this soluble fraction to aggregation was related to the high content of microbially-derived sugars in the fraction. Ball et al. (1996) later reported that monosaccharide ratios of hot-water extractable carbohydrate indicated a contribution of microbial polysaccharides to this fraction.

**Management Effects on Soil Carbohydrates**

Researchers have found that soil carbohydrate content is responsive to management, and that effects on carbohydrates are generally related to effects on SOC and other labile SOC pools. Dalal and Henry (1988) found a greater proportion of carbohydrates in light fraction organic matter as opposed to SOM, and found that light fraction decline upon cultivation resulted in a substantial reduction of carbohydrates. In the study, carbohydrate response to cultivation was similar to that of SOM. Ball et al. (1996) observed concomitant stratification of both SOM and carbohydrates in NT vs. CT soils, and reported that hot-water extractable carbohydrate content was best correlated with aggregate stability compared to other SOM pools.
Hu et al. (1995) found that carbohydrates were significantly greater in the top 5 cm of NT vs. CT plots in a long-term experiment involving a sorghum/rye rotation. The authors reported that carbohydrate content reflected changes in SOC. Hu et al. (1997) observed that carbohydrate content was generally greater in forest soils when compared to agricultural soils, but that the proportion of SOC consisting of carbohydrate was greater in agricultural soils vs. forest soils. The authors also observed substantially higher carbohydrate contents (752 μg g⁻¹ vs. 364 μg g⁻¹) in long-term NT vs. CT plots cropped to sorghum or soybean with a winter rye cover crop. Such increases have not always been observed. In a long-term tillage experiment cropped to continuous barley for 10 years, Arshad et al. (1990) observed that carbohydrate content was only 10% greater in NT vs. CT treatments.

Some authors have reported soil carbohydrate response to management in the short-term. Angers et al. (1993) reported a relatively short-term effect of tillage on soil carbohydrates, observing that WSC and AHC were on average 40% greater in NT vs. CT continuous barley and 2-year barley/red clover rotations after 4 years of management. Carbohydrate levels were also slightly higher in the rotation compared to continuous barley after 4 years. The authors also reported that soil carbohydrate per unit SOM was greater in NT vs. CT. Angers and Mehuys (1989) observed significantly greater soil carbohydrates after 2 years of cropping to barley and alfalfa when compared to fallow or corn. Effects of management were not significant with respect to SOC and total N in their study, suggesting that soil carbohydrates were more sensitive to management-induced change. In contrast, no significant short-term (2 years) effects of NT vs. CT management on soil carbohydrates were observed by Hu et al. (1997).
RESEARCH OBJECTIVES

This research project was intended to provide insight into the effects of management on SOC storage and dynamics in a cotton-cropping system located in south-central Texas. Emphasis was placed on labile SOM pools that have the potential to act as nutrient source/sinks, and play significant roles in C and nutrient cycling.
MATERIALS AND METHODS

The experimental plots were located in the Brazos River floodplain in south-central Texas (30°32'N, 96°26'W). Annual temperature and precipitation in the area average 20 ºC and 978 mm, respectively. The soil is a Weswood silty clay loam (fine, mixed, superactive, thermic, Fluventic Ustochrept) with an average of 350 and 520 g kg\(^{-1}\) of clay and silt, respectively. The soil is calcareous (~140 g CaCO\(_3\) kg\(^{-1}\)) and has an average surface pH of 8.2.

The plots were arranged in an incomplete factorial within a randomized complete block design. The three management systems included conventional-till continuous cotton (CT), reduced-till continuous cotton (RT), and a conventional-till cotton/corn rotation (CC). The plots did not include a reduced-till cotton/corn rotation treatment. The tillage treatments were in place for 10 years prior to sampling, and the rotation with corn had been in place for 6 years. The type of reduced tillage practiced was ridge-till, in which tillage is used to re-establish seed beds prior to planting. Ridge-till plots were also minimally tilled to help maintain water furrows during the growing season. The study was partially irrigated. Various N rates were superimposed upon these management systems and each distinctive treatment (including N rate) contained four field replicates. Sets of replicate plots with N rates of 0 and 90 kg N ha\(^{-1}\) were sampled for this study. Replicate size was 4, 1-m wide rows that were ~13 m in length.

Soil Sampling and Preparation

Thirty-five soil cores (2.54 cm dia.) were taken from each replicate plot at four different times during the spring and summer of 2003. These four sampling periods consisted of a pre-plant sampling and three later samplings that coincided with different growth stages of cotton. These growth stages included pinhead-square, peak bloom,
and full maturity. Sampling at these stages was performed based upon the growth of cotton in the CC treatment, which was the most advanced at each stage. Soil cores were separated into 0-5 cm and 5-15 cm depth increments and additional 0-5 cm cores were taken to obtain equal amounts of soil for both depths. All soil samples were air-dried and gently crushed to pass through a 5-mm sieve in order to prepare samples for analysis of water-stable aggregation. Soils not examined for water-stable aggregates were then ground to pass a 2-mm sieve for all other analyses. All samples were stored at room temperature. Field replicates (n=4) were used as laboratory replicates in the analysis of all parameters.

**Water-stable Aggregation**

Structural stability was measured as degree of water-stable aggregation. Water-stable aggregates were determined through a wet sieving procedure modified from Elliott (1986). Subsamples (100 g) from the 0-5 cm depth section were capillary-wetted on Whatman #1 filter paper for 3 hrs and then washed through a succession of sieves. Material remaining on top of each sieve was then backwashed into pre-weighed containers, which were dried at 55 ºC to determine the weight of soil in each aggregate size class.

Size classes collected included large macroaggregates (>2000-µm fraction), small macroaggregates (2000- to 250-µm), microaggregates (250- to 53-µm), and the silt-clay fraction (< 53-µm). The amount of soil in the silt-clay fraction was determined by difference. Water-stable aggregates were not determined for soils from the 5-15 cm depth due to physical complications experienced when attempting to pass the soils through the 5-mm sieve, which would have significantly biased the analysis.
**Total Glomalin**

Total glomalin was extracted by autoclaving 1-g soil samples with 8 mL of 50 mM Na-citrate (pH 8.0) in 40-mL centrifuge tubes at 121 °C for 1 hour. Extracts were then centrifuged at 5,000 xg for 20 minutes to pellet the soil, and supernatants were decanted. Total glomalin of the extracts was approximated by the Bradford protein assay using a bovine serum albumin standard (Wright and Upadhyaya, 1996).

**Soil Organic Carbon**

Soil samples (40 g) were prepared for total SOC analysis by removing visible organic residues and grinding the soils to pass a 200-mesh sieve. SOC was determined by dry combustion on 200-mg subsamples using an Elementar multi-element analyzer. Combustion was performed at 650 °C to allow SOC determination while preventing the combustion of inorganic carbonates.

**Carbon Mineralization**

Carbon mineralization (CO$_2$ evolution) was determined using a laboratory incubation method. Soil samples (100 g) were placed in 550-mL gas-tight jars and wetted to 25 % moisture. Alkali traps (10 mLs of 1N NaOH) were placed inside the jars, which were sealed and incubated at 25 °C. Alkali traps were removed and titrated with 1N HCL to determine CO$_2$-C evolved (Anderson, 1982) after 3, 10, 24, and 38 days of incubation.

**Soil Microbial Biomass Carbon**

Soil microbial biomass carbon (SMBC) was determined using the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976). Soil samples (35 g) were wetted to 25 % moisture, placed in gas-tight jars, and pre-incubated for 10 days at 25 °C to allow for equilibration of the SMB (Franzleubbers et al., 1996b). SMBC was
quantified from the amount of CO$_2$-C evolved over a ten-day period using an efficiency factor of 0.41 (Voroney and Paul, 1984). Data reported are without subtraction of a control. SMBC data without subtraction of a control were less variable than data where a control was subtracted. SMBC without subtraction of a control also correlated better with other labile-C parameters when compared to SMBC with subtraction of a control.

**Hot-water Extractable Organic Carbon**

Hot-water extracts were obtained by placing 40 g of soil and 160 mL of deionized water in 250-mL Nalgene® bottles and briefly agitating them by hand. The solutions were then heated at 80 ºC for 16 hours in a water bath and subsequently shaken on a wrist-action shaker at high agitation for 30 minutes. Extracts were then centrifuged at 5,000 x g for 20 minutes and filtered through pre-washed 0.45-µm cellulose-acetate membranes. Extracts were frozen until analysis for soluble total organic C with an OI Analytical Model 1010 TOC analyzer using a potassium hydrogen phthalate standard (OI Corp., College Station, TX).

**Carbohydrate Carbon**

Carbohydrate content of hot-water extracts was determined with the bicinchoninic acid (BCA) assay using glucose standards (Joergensen et al., 1996). Test-tubes containing 2 mL of hot-water extract and 2 mL of BCA reagent were heated in a water bath at 60 ºC for 2 hours. Absorbance was read at 592 nm.

**Nitrogen Mineralization**

Soil subsamples were obtained from soils subjected to the 38-day laboratory incubation at 25 ºC, and respective soils not subjected to the incubation. Subsamples (7 g) were shaken in 28 mL of 2N KCL for 1 hour, filtered, and analyzed for NH$_4$-N and NO$_3$-N on a Technicon Autoanalyzer. Potential N mineralization was calculated by
subtracting time-zero extractable NO$_3^-$-N and NH$_4^+$-N (non-incubated soils) from extractable NO$_5^-$-N and NH$_4^+$-N from incubated soils.

**Statistical Analysis**

One-way ANOVA was used to indicate significant differences between treatments and the Student-Newman-Keuls multiple comparison procedure was used to separate means. Data not meeting normality or equal variance assumptions of ANOVA were log transformed for statistical analyses. In few cases, outlier replicates with exceptionally high or low values were dropped from data analyses. These instances are noted in the thesis by n=3. Kruskal-Wallis rank-based ANOVA was used in examination of the data for water-stable aggregation. Linear regression was used to evaluate the correlation of parameters. All statistical analyses were performed using Sigma Stat, Version 3.0 (SPSS Inc., 2003).
RESULTS AND DISCUSSION

Water-stable Aggregation

Overall, reduced tillage and rotation with corn did not significantly affect water-stable aggregation in the top 5 cm of the cotton plots studied. Water-stable, small macroaggregates dominated over other aggregate-size fractions across all treatments, constituting roughly 50% of the samples used in the analysis (Fig. 1). Similar distributions of soil within different aggregate-size classes were observed for all treatments. The only statistically significant difference in aggregation was observed in the CC plots receiving 90 kg N ha⁻¹, which contained significantly more large macroaggregates and significantly fewer microaggregates than all other treatments.

The significant increase in large macroaggregates observed in the CC plots receiving N application was not observed in the CC plots not receiving N, and the CC plots receiving N did not exhibit significantly greater concentrations of SOC, SMBC, or mineralizable C than any of the other treatments in the study. Therefore, we could not conclude that additional organic matter inputs due to rotation with corn, or enhanced microbial activity under corn were likely responsible for enhancing macroaggregation in our study.

One could speculate that the enhanced macroaggregation observed in the CC plots receiving N resulted from the root system of corn, which is generally denser than that of cotton, especially in the surface. Researchers have suggested important relationships between plant roots and aggregation (Oades and Waters, 1991; Jastrow et al., 1997), and a more dense and fibrous rooting system could provide the potential for greater development of macroaggregates. However, we did not see significantly enhanced macroaggregation in CC plots not receiving N.
Fig. 1. Water-stable aggregates in the 0-5 cm depth of soil samples taken pre-plant from cotton plots at the Texas A&M University Experimental Farm. CC, CT, and RT indicate conventional-till cotton/corn rotation, conventional-till continuous cotton and reduced-till continuous cotton, respectively. 0N and 90N indicate kg added N ha\(^{-1}\). (*) Indicates significant difference (P<0.05) for given aggregate size class.
We failed to observe enhanced aggregation in RT plots. Our original hypothesis was that reduced physical disruption by tillage would lead to more water-stable aggregates in RT vs. CT. Studies have documented increased aggregation in NT vs. CT agricultural systems, (Bruce et al., 1990; Carter, 1992; Beare et al., 1994a); however, we failed to see any notable differences between RT and CT. The type of reduced tillage used in our study was ridge-till, which incorporated tillage in the re-establishment of seed beds prior to planting, and in the maintenance of furrows throughout the growing season. These tillage steps likely played a role in the physical disruption of aggregates in the RT system, and it is possible that enhanced aggregation may have been observed within a strictly no-till system.

Researchers have proposed that aggregate formation is largely a function of biological processes and SOM content. Aggregation has been positively correlated with SOM (Chaney and Swift, 1984), microbial biomass (Chan and Heenan, 1999), hot-water extractable carbohydrates (Haynes and Swift, 1990), glomalin (Wright and Upadhyaya, 1998), root length (Oades and Waters, 1991; Jastrow et al., 1997), and length of fungal hyphae (Oades and Waters, 1991; Jastrow et al., 1997). In our study, no correlations were observed between aggregation within any size fraction and SOC, labile SOC pools, or total glomalin. This was largely due to the lack of differences in aggregation between treatments in our plots.

**Total Glomalin**

Total glomalin ranged from approximately 2300 to 2800 mg kg\(^{-1}\) soil, and was well correlated with SOC (Fig. 2). Glomalin is a recently discovered glycoprotein excreted by the hyphae of arbuscular mycorrhizal fungi that is considered to be ubiquitous and persistent in soils (Wright and Upadhyaya, 1996). The protein has also
Fig. 2. Total glomalin in soil samples taken pre-plant from cotton plots (A). CC, CT, and RT indicate conventional-till cotton/corn rotation, conventional-till continuous cotton and reduced-till continuous cotton, respectively. 0N and 90N indicate kg added N ha\(^{-1}\). Lower-case letters indicate significant differences (P<0.05) between tillage/rotation treatment for columns representing N and depth. Upper-case letters indicate significant differences within tillage/rotation treatment. Relationship between SOC and total glomalin at pre-plant (P<0.001) (B).
been linked to aggregation, and has correlated well with aggregate stability across various soil types (Wright and Upadhyaya, 1998); however, some studies have reported poor correlations between aggregation and glomalin (Franzluebbers et al., 2000a; Bird et al., 2002; Rillig et al., 2003). We did not observe a positive correlation between water-stable aggregation and total glomalin due to the lack of differences in aggregation in our study.

Though we were unable to link glomalin with aggregation, we did observe that glomalin comprised a significant proportion of SOC in the cotton-cropped soils studied. It is estimated that glomalin contains approximately 30 % C by weight (Bird et al., 2002). Based on this estimate, total glomalin C accounted for roughly 7 to 9 % of SOC in our study. Total glomalin also showed responses similar to SOC concerning the effects of tillage and rotation, and was well correlated with SOC (P<0.001, r²=0.87).

Total glomalin in the top 5 cm of RT and CC was 16 and 10 % greater vs. CT (Fig. 2). Possible explanations for the slight increases include reduced disruption of hyphal networks by RT and the greater root biomass near the surface associated with corn in CC. Tillage and rotation could also indirectly affect glomalin content through improving plant growth and increasing below-ground biomass. If conservation management improves soil fertility and leads to increases in yield, one would expect that greater root biomass would support more mycorrhizal fungi and lead to greater glomalin levels. Studies have reported that tillage (Wright et al., 1999) and rotation (Wright and Anderson, 2000) affect glomalin concentrations. However, there is a current need for more data concerning management effects on glomalin in agroecosystems.
Differences in glomalin between tillage/rotation treatments were less than those observed for SOC, suggesting that glomalin was less responsive to management when compared to bulk SOC. In general, the bulk SOC pool reflects management effects slowly due to its large relative magnitude (Bonde et al., 1988), and labile-C pools are considered better indicators of management-induced change. We also found that the ratio of glomalin C to SOC was higher for CT than for CC and RT (8.64 vs. 7.56 and 7.61, respectively). This indicates that glomalin responded less to tillage and rotation relative to SOC even though it only constituted roughly 7 to 8.5 % of SOC. In general, our data support the suggestion that glomalin does represent a relatively stable pool of organic matter (Wright and Upadhyaya, 1996; Lutgen et al., 2003).

**Soil Organic Carbon**

Soil organic carbon (SOC) ranged from 8.2 to 11.4 g C kg\(^{-1}\) soil in our study and was significantly affected by tillage and rotation (Fig. 3). SOC in the 0-5 cm depth of RT and CC was 33% and 29% higher when compared to CT. When averaged across both depths (0-15 cm), SOC concentrations in both RT and CC were approximately 26% greater when compared to CT. Tillage and rotation affect SOC storage through altering residue distribution and physical disruption of the soil, and through controlling the amount of residues returned to the soil system, respectively (Paustian et al. 1997). Results from our study suggested that altered residue distribution and less frequent physical disruption associated with RT and additional organic matter inputs associated with CC were sufficient enough to affect SOC storage in these cotton-cropping systems.

SOC was significantly greater in RT plots when compared to CT plots, except in the 5-15 cm depth when 90 kg N ha\(^{-1}\) were applied. SOC in CC plots was also not significantly greater than SOC in CT plots at the 5-15 cm depth when N was applied, or
Fig. 3. Soil organic carbon (SOC) in soil samples taken pre-plant from cotton plots. CC, CT, and RT indicate conventional-till cotton/corn rotation, conventional-till continuous cotton and reduced-till continuous cotton, respectively. 0N and 90N indicate kg added N ha\(^{-1}\). Lower-case letters indicate significant differences (P<0.05) between tillage/rotation treatment for columns representing N and depth. Upper-case letters indicate significant differences within tillage/rotation treatment.
at the 0-5 cm depth when N was applied (Fig. 3). These results suggest that N application may have indirectly increased SOC in CT relative to CC and RT systems. Previous data from this tillage/rotation experiment have shown that CT plots have exhibited a greater N response relative to RT and CC plots (Hons et al., 2004). However, N application had no significant effect on SOC storage when examined within any of the tillage/rotation treatments.

We did not observe a significant effect of depth on SOC storage within any of the tillage/rotation treatments. The RT plots did show the greatest accumulation of SOC in the surface, containing 16 % more SOC in the 0-5 vs. the 5-15 cm depth, but this difference was not statistically significant. Many studies reporting increased SOC under NT or RT systems have observed stratification of SOC in the soil surface as a result of the surface accumulation of crop residues in these systems (Havlin et al., 1990; Salinas-Garcia et al., 1997; Zibilske et al., 2002) We failed to observe significant stratification of SOC under RT, suggesting that ridge-tillage practices likely redistributed crop residues within the top 15 cm of the soil and reduced organic matter stratification.

The increases in SOC that we observed in the top 5 cm of soil were relatively similar to those of other studies in Texas. Salinas-Garcia et al. (1997) and Zibilske et al. (2002) observed 64 % and 58 % greater SOC in the top 5 cm of NT cotton/corn rotations vs. CT rotations in studies near Corpus Christi and Weslaco, respectively. These differences involved strict no-till systems as opposed to the ridge-till system in our study, and they also involved the combination of NT and rotation with corn. Zibilske et al. (2002) also employed ridge-tillage, which resulted in differences more comparable to our study. They observed 44 % greater SOC in ridge-till vs. CT in the top 4 cm. Our study, which showed 33 % greater SOC in the top 5 cm of RT vs. CT, did not include a
treatment that combined RT and rotation with corn. It is possible that the combined effects of RT and rotation with corn might have resulted in additive increases on SOC storage in the cotton plots studied.

Climate sets the gross restraints for SOC turnover (Anderson and Flanagan, 1989) and warmer climates generally favor accelerated decomposition of organic matter (Jenny, 1941; Carter, 1996). In a study involving 3 long-term cropping systems in Texas, Potter et al. (1998) observed that C sequestration decreased with increasing mean annual temperature. The authors suggested that C sequestration in Texas was possible, but that warm climatic conditions would limit storage potentials. In our study, when averaged across the top 15 cm of the soil, both RT and CC treatments increased SOC by approximately 26% compared to CT after ten and six years of management, respectively. These increases in SOC storage are respectable considering results from other C sequestration studies. For example, Paustian et al. (1997) reported SOC increases of 5-20 % from NT vs. CT systems (0-30 cm) in an examination of data from 11 long-term studies, many of which were from much cooler climates. Still, comprehensive long-term data evaluating management effects on SOC storage in cotton-cropped systems are lacking.

One limitation of our study concerning evaluation of C sequestration is that samples were only taken from 0-15 cm due to experimental emphasis on labile C pools. When evaluating C storage increases, it is necessary to include the top 30 cm of soil (or depth to plow-pan) to rule out the possibility that increases were only due to NT or RT systems redistributing residues near the surface (Paustian et al. 1997). It is possible that the effects of ridge-till on increased SOC may have been less when assessed throughout the top 30 cm in our study. In a similar system, Salinas-Garcia et al. (1997)
observed significantly greater SOC in the 12.5 – 20 cm depth of CT vs. NT cotton/corn rotations. In our study, SOC in the top 15 cm of both CC and RT was 26 % greater than in CT. Assuming that residue distribution by RT has resulted in less SOC below 15 cm compared to CC and CT, CC plots may have been more effective than RT in sequestering C relative to CT.

When assessing the potential for C sequestration, it is important to understand those factors involved in determining C storage limits. It has been accepted that the warm Texas climate will play an important role in limiting C sequestration potentials. Additionally, high clay content of many soils in Texas may play an important role in determining storage capacities. Organic matter associations with clay and silt particles are considered an important mechanism of SOM protection (Six et al. 2002), and silt-clay content has been related to silt-clay protected C (Hassink, 1997). Although studies have found higher soil microbial biomass (SMB) per unit SOC in finer-textured soils (Hassink, 1994; Franzluebbers et al. 1996a), observations of lower C mineralization per unit SMB with increasing clay content suggest a protective effect of clay on SOM (Hassink, 1994; Hassink, 1995; Franzluebbers et al., 1996a; Wang et al., 2003).

Soil Microbial Biomass Carbon

Soil microbial biomass carbon (SMBC) ranged from approximately 270 to 550 mg kg⁻¹ as examined across all treatments, including N application and depth, in soil samples collected pre-plant (Fig. 4). RT and CC plots contained significantly more SMBC than CT plots at both 0-5 cm and 5-15 cm depths with the exception of CC plots at 5-15 cm when 90 kg N ha⁻¹ were applied. The greatest influence of management was observed in the surface 5 cm where SMBC was 58 % and 32 % greater under RT and CC management when compared to CT.
Fig. 4. Soil microbial biomass carbon (SMBC) at various growth stages of cotton. CC, CT, and RT indicate conventional-till cotton/corn rotation, conventional-till continuous cotton and reduced-till continuous cotton, respectively. 0N and 90N indicate kg added N ha\(^{-1}\). Lower-case letters indicate significant differences (P<0.05) between tillage/rotation treatment for columns representing N and depth. Upper-case letters indicate significant differences within tillage/rotation treatment. RT 0N 0-5 cm at pinhead-square (n=3).
These results suggest that altered residue distribution by RT and additional organic matter inputs from corn in CC provided the soil microflora with more substrate as compared to CT. The soil microbial biomass (SMB) relies on C substrates for growth and maintenance; therefore the size and activity of the SMB pool are generally related to organic matter content (Schnürer et al., 1985; Collins et al., 1997). In accordance, we observed that management resulting in increased SOC also increased SMBC. Furthermore, SOC was strongly correlated (P<0.001) with both SMBC and 38-day cumulative carbon mineralization (CMIN) as measured at pre-plant (Fig. 5).

The 58 % increase in SMBC that we observed in the top 5 cm of RT vs. CT was relatively similar to the 65 % increase that Franzleubbers et al. (1995a) observed in NT vs. CT sorghum and sorghum-wheat/soybean rotations from a similar soil. Additionally, they reported 18 % greater SMBC in rotation vs. monoculture and related this effect to increased organic-C inputs and a shorter fallow period. We also observed an effect of rotation with CC plots containing 32 % greater SMBC in the top 5 cm vs. CT. We attribute this increase to additional organic-C inputs provided by corn in the rotation when compared to cotton monoculture. Typically, C₄ feed grains such as corn and sorghum produce the highest residue levels for row crops (Paustian et al., 1997), and it is likely that additional residue production from corn increased both SOC and SMBC in CC vs. CT. Our study did not include a treatment combining ridge-tillage and rotation with corn, and it is likely that increased organic-C inputs from corn and reduced decomposition associated with RT would have had an additive effect on SMBC.

Effects of management on SMBC in our study were generally consistent with those involving other cotton-cropping systems. Balota et al. (2003) observed increased
Fig. 5. Relationships of 38-day cumulative C mineralization (38-day CMIN) and soil microbial biomass C (SMBC) measured in pre-plant soil samples with soil organic C (SOC). Relationships significant at P<0.001.
SMBC in NT vs. CT cotton/wheat rotations (372 mg kg\(^{-1}\) and 145 mg kg\(^{-1}\), respectively) in Brazil. The relative difference between tillage treatments was greater when compared to our study, but their study involved rotation with wheat and a strict no-till system. These management practices likely resulted in less soil disturbance and greater C inputs vs. the ridge-till continuous cotton system in our study. Acosta-Martínez et al. (2004) reported that SMBC was higher in the top 5 cm of wheat-fallow-rye-cotton rotation (237 mg kg\(^{-1}\)) vs. continuous cotton (124 mg kg\(^{-1}\)) when sampled under rye and cotton.

SMBC measured at pinhead-square, peak bloom, and full maturity sampling periods was not different than SMBC at pre-plant and showed essentially the same effects from tillage and rotation. In general, there was little seasonal variation in SMBC throughout the growing season of cotton. Seasonal variability of SMBC is discussed further in the *Seasonal Variation of Labile Organic Carbon Pools* section.

At a given depth, plots receiving 90 kg N ha\(^{-1}\) had slightly greater SMBC than plots not receiving N. This trend suggested that N application may have indirectly affected SMBC through enhancing crop growth and subsequent plant biomass that was ultimately returned to the soil. Similar trends were observable in data for other labile-C pools; however, these trends were not significant enough to draw any concrete conclusions.

Depth or N application had no significant impact on SMBC in pre-plant soil samples within any of the tillage/rotation treatments with the exception that RT at 0-5 cm contained significantly more SMBC where N was applied vs. no N application. It is possible that the addition of fertilizer N may have lowered C:N ratios in the surface of RT soils, which may have led to increased decomposition of organic residues and
subsequent increases in SMB in these plots. However, the magnitude of this observed
difference is partly a result of the data distribution of the RT plots (0-5 cm) receiving N.
The distribution was skewed by one of the field replicates that contained unusually high
SMBC with respect to the rest of the replicates. Therefore, we could not make any
conclusions regarding the effect of N application in this situation.

Stratification of the SMB was observed in RT plots, which contained 31 % more
SMBC in the 0-5 cm depth vs. the 5-15 cm depth at pre-plant. Researchers have
observed increased microbial abundance in the surface of RT and NT managed soils
(Doran, 1980; Carter and Rennie, 1982; Carter, 1992). This stratification is usually
related to the distribution of more organic matter near the surface of RT and NT soils.

The observed stratification of SMB in our RT plots was relatively modest, but it
was greater than the stratification of SOC in these plots (31 % vs. 16 %, respectively).
Carter and Rennie (1982) observed similar results with SMBC showing considerably
greater differences between NT and CT when compared to SOC in soils cropped to
spring wheat. Changes in SOC can be hard to measure against background SOC, and
SMB has proven to be a more sensitive indicator of management-induced change
(Powlson and Brookes, 1987; Saffigna et al., 1989). SMBC reflected management
practices more sensitively when compared to SOC concerning stratification under RT
management in our study. SMBC was also a more sensitive indicator of management-
induced change vs. SOC with respect to the increases in these parameters in both RT
and CC vs. CT after 10 years of tillage management and 6 years of rotation with corn.

SMBC as a percentage of SOC ranged from approximately 3 to 5 % in our
study. Smith and Paul (1990) reported that SMBC may constitute approximately 2 to 5
% of SOC. Though organic matter stocks are generally lower in areas experiencing
warmer climate (Jenny, 1941), it has been reported that active organic matter fractions, such as the microbial biomass and mineralizable C, can constitute relatively larger proportions of SOM in warmer regions (Franzluebbers et al., 2001). Potential SOC storage in our cotton cropping systems may be limited by warm climatic conditions, but increases in SMBC are apparently not limited. In a long-term study from subtropical Brazil, Balota et al. (2003) reported SMBC (chloroform fumigation-extraction) values of 372 mg kg⁻¹ and 145 mg kg⁻¹ under NT and CT cotton/wheat rotations, respectively. Significant management-induced changes in SMBC were still attainable despite the warm climate, and the authors suggested that increased SMB resulting from NT would enhance nutrient cycling in the systems studied. Though the potential for C sequestration in Texas may be limited by the warm climate, increases in SMB resulting from NT or RT positively impact soil fertility and should be considered when deciding on management practices.

The microbial biomass pool significantly impacts nutrient cycling by acting as both a source and sink of nutrients and through its role as a transforming agent (Schnürer et al., 1985; Duxbury et al. 1989). SMB estimates suggest that the pool is large enough to impact plant-available N (Smith and Paul, 1990) and laboratory studies have suggested that SMBN comprises a significant proportion of mineralizable N (Carter and Rennie, 1982; Follett and Schimel, 1989). It is possible that enhanced SMB resulting from conservation management in warm climates could positively contribute to nutrient cycling, especially concerning N. We were unable to directly address contributions of SMB to potentially mineralizable N in our study because SMBN was not measured.
**Carbon Mineralization**

Thirty-eight day cumulative C mineralization (38-day CMIN) ranged from 162 mg kg\(^{-1}\) to 365 mg kg\(^{-1}\) in pre-plant soil samples. For samples taken pre-plant, this parameter provided the most sensitive indicator of change resulting from tillage and rotation when compared to SOC and other measured labile-C pools. As with SMBC, relative differences between treatments were greatest near the surface. In the 0-5 cm depth, 38-day CMIN in RT and CC was 79 % and 36 % greater than 38-day CMIN in CT at pre-plant (Fig. 6). In the 5-15 cm depth, these differences were 20 % and 29 %, respectively. 38-day CMIN in RT and CC was significantly greater than 38-day CMIN in CT at both depths suggesting that RT and CC management increased the amount of mineralizable organic matter in top 15 cm of the soil.

Little variation in 38-day CMIN was observed throughout the growing season of cotton, and CMIN measured at pinhead-square, peak bloom, and full maturity was relatively similar to that measured at pre-plant for all treatments. A trend involving a slight increase in 38-day CMIN throughout the growing season was observed for CT. This may be attributable to inputs of mineralizable organic matter by cotton roots. However, root-input effects would be expected in all treatments, and we failed to observe this trend in RT and CC. When considering variation, the increase of 38-day CMIN throughout the growing season in CT did not support any solid conclusions. Further discussion of seasonal variation in CMIN can be found in the section entitled *Seasonal Variation of Labile Organic Carbon Pools*.

Our results are consistent with those of other studies that have measured effects of management on CMIN. Follett and Schimel (1989) and Franzluebbers et al. (1995a) both observed greater CMIN in NT vs. CT, and both studies showed concomitant...
Fig. 6. 38-day cumulative C mineralization (38-day CMIN) at various growth stages of cotton. CC, CT, and RT indicate conventional-till cotton/corn rotation, conventional-till continuous cotton and reduced-till continuous cotton, respectively. 0N and 90N indicate kg added N ha$^{-1}$. Lower-case letters indicate significant differences (P<0.05) between tillage/rotation treatment for columns representing N and depth. Upper-case letters indicate significant differences within tillage/rotation treatment.
increases in SMBC. Franzluebbers et al. (1995a) also observed 18% greater CMIN in sorghum-wheat/soybean rotations vs. continuous sorghum, citing that additional organic-C inputs from rotation resulted in the increase. Saffigna et al. (1989) observed greater SMBC and activity as measured by CMIN in soils where sorghum residues were retained vs. those where they were removed. Overall, our data suggested that tillage and rotation practices that increased SOC, by either reducing decomposition or providing additional C inputs, or both, generally led to increases in SMB and potentially mineralizable C.

The effects of depth were well illustrated for 38-day CMIN. In RT managed soils, 38-day CMIN was significantly greater (69%) in the 0-5 cm depth vs. the 5-15 cm depth (Fig. 6). This result suggests stratification of organic matter in RT plots. This stratification was insignificant as represented by SOC measurements, thus 38-day CMIN was more sensitive in assessing the effects of stratification by RT. The stratification of mineralizable organic matter in these soils is likely due to the distribution of more crop residues near the surface of RT soils in these systems. As anticipated, no significant depth effect was seen for CC and CT due to the greater redistribution of crop residues by conventional tillage when compared to ridge-till.

With the exception of soils in the 5-15 cm depth under CT management, the application of N had no significant effect on 38-day CMIN within any of the tillage/rotation treatments. Nitrogen application resulted in significantly greater 38-day CMIN at this depth in CT-managed soils. This effect may be a result of the higher N response exhibited by CT-managed cotton. If a greater yield response to N application resulted in increased crop residues, one would expect that the incorporation of these residues by tillage would lead to greater C mineralization in these soils. This N effect
was not as pronounced in the 0-5 cm depth of CT-managed soils. In all tillage/rotation treatments, at a given depth, soils receiving 90 kg N ha\(^{-1}\) generally had slightly greater 38-day CMIN than soils not receiving N. This trend was observed for all labile-C pools studied, but was generally not significant for any of the pools.

Assessments of CMIN also included 3-day CMIN and basal soil respiration (BSR), which was calculated as the rate of CMIN measured between 24 and 38 days of incubation. BSR varied little across the growing season of cotton and was significantly greater in the top 5 cm of RT vs. CC and CT when averaged across seasonal sampling periods (Fig. 7). BSR in the top 5 cm of RT was 73 and 47 % higher than in CT and CC, respectively. In most cases, BSR was not significantly higher in CC vs. CT. In the 0-5 cm depth, BSR in CC was only 18 % greater than in CT. Smaller differences in BSR between CC and CT were observed when compared to differences between these treatments concerning SMBC and 38-day CMIN. The effect of depth was greatest for RT, for which BSR was 88 % greater in the 0-5 cm depth vs. the 5-15 cm depth. This difference was greater than the effect of depth as shown for 38-day CMIN.

These data suggest that greater organic residue distribution near the surface of RT played an important role in providing soil microorganisms with a steady source of organic C in RT. It is likely that light fraction or macro-organic matter near the surface of RT provided an intermediately labile source of C for the soil microflora, which led to greater C mineralization later in the incubation. Higher mineralization rates in RT near the end of the incubation (BSR) were a factor in enhancing the differences between RT and CC/CT with regards to 38-day CMIN.

We observed a strong correlation (P<0.001, \(r^2=0.81\)) between SMBC and its activity as measured by BSR (Fig. 8), but the relationship between BSR and SMBC was
Fig. 7. Basal soil respiration in soils from cotton plots averaged across all seasonal sampling periods. CC, CT, and RT indicate conventional-till cotton/corn rotation, conventional-till continuous cotton and reduced-till continuous cotton, respectively. 0N and 90N indicate kg added N ha\(^{-1}\). Lower-case letters indicate significant differences (P<0.05) between tillage/rotation treatment for columns representing N and depth. Upper-case letters indicate significant differences within tillage/rotation treatment.
not as strong as relationships between SMBC and other labile-C pools. The size of the SMB pool and SMB activity are not always clearly related. Discrepancies between SMB magnitude and activity result from varying proportions of inactive vs. active SMB and soil environmental conditions (Smith and Paul, 1990). In a study assessing relationships between microbial biomass, soil respiration, and substrate, Wang et al. (2003) concluded that respiration under favorable conditions was principally determined by substrate supply rather than SMB pool size.

We did observe the characteristic flush in CMIN following the rewetting of air-dried soil. Our data showed a strong relationship (P<0.001, r²=0.90) between 3-day CMIN following rewetting of dried soils and SMBC (Fig. 8). It has been shown that the re-wetting of dried soil creates a flush of C and N mineralization, and short-term measurements of CMIN following rewetting have strongly correlated with SMBC and NMIN (Franzluebbers et al., 1996b; Franzluebbers et al., 2000b; Haney et al., 2001). Drying kills a portion of the microbial biomass (Sorensen, 1974), which is lysed upon rewetting (Kieft et al., 1987). Surviving organisms then utilize metabolites released from lysed cells (Jenkinson, 1966). The flush in mineralization observed after rewetting is likely a result of the contributions of SMB and other labile organic matter pools (Haney et al., 2001), though chemical and physical disruptions associated with rewetting dried soils may also increase organic matter mineralization (van Gestel et al., 1991).

Researchers have suggested that short-term CMIN following rewetting could provide a rapid and easy method of estimating SMB and potential N mineralization (Franzluebbers et al., 1996b; Haney et al., 2001). Our data do support that the CMIN flush following rewetting relates well to SMBC as measured by chloroform fumigation-incubation. Our soils were air-dried, and it is possible that oven-drying soils at low
Fig. 8. Relationships of basal soil respiration and 3-day cumulative C mineralization (3-day CMIN) with soil microbial biomass C (SMBC). Data are averaged across seasonal sampling periods. Relationships significant at P<0.001.
temperatures may have enhanced this relationship. Drying soils to a greater extent would have resulted in the desiccation of more microbial biomass, which would have been lysed upon rewetting and made available as a growth-substrate for the surviving biomass.

**Nitrogen Mineralization**

We hypothesized that enhanced organic matter mineralization observed in RT and CC managed plots would lead to greater mineralization of organic N to plant-available forms when compared to CT. The increases in CMIN and SMBC that were observed in RT and CC vs. CT suggested that plant-available N may have been positively affected by these types of management. We hypothesized that differences in labile SOM pools would affect N cycling, and that differences in N mineralization (NMIN) would coincide with the yield differences observed on these plots by Hons et al. (2004). Through a number of years, yield data showed that CC and RT consistently outperformed CT, and that CC usually resulted in the highest yields.

NMIN, as measured from a 38-day incubation, ranged from 9.5 to 17.2 mg kg\(^{-1}\) at pre-plant and from 11.1 to 22.4 mg kg\(^{-1}\) at full maturity (Fig. 9). We did observe greater NMIN in RT vs. CT, but we failed to see enhanced NMIN in CC vs. CT. NMIN measured at both pre-plant and full maturity was relatively similar concerning CC vs. CT. At pre-plant, RT exhibited 66 and 53 % greater NMIN when compared to CC and CT at the 0-5 cm depth. At full maturity these differences were 72 and 67 %, respectively. Roughly the same general differences between tillage/rotation treatments were observed at full maturity, though data at full maturity were less variable when compared to pre-plant. Labile organic C pools in treatments followed the consistent pattern RT > CC > CT, and CC usually contained significantly more labile-C when
Fig. 9. Soil N mineralization measured from 38-day incubations of soil samples collected at pre-plant and full maturity. CC, CT, and RT indicate conventional-till cotton/corn rotation, conventional-till continuous cotton and reduced-till continuous cotton, respectively. 0N and 90N indicate kg added N ha\(^{-1}\). Lower-case letters indicate significant differences (P<0.05) between tillage/rotation treatment for columns representing N and depth. Upper-case letters indicate significant differences within tillage/rotation treatment. RT 90N 0-5 cm at full maturity (n=3).
compared to CT. This trend was not seen for NMIN, which was not significantly greater in CC compared to CT. This suggests that while enhanced labile organic matter fractions contributed to greater NMIN in RT, enhancement of these fractions did not result in improved N economy in CC.

Nitrogen application and depth had relatively little effect on NMIN. NMIN was greater in the top 5 cm of RT compared to the 5-15 cm depth, but this stratification was not as enhanced as it was for labile-C pools or BSR. This effect suggested that while greater SOM results in more SMB in the surface of RT plots, the decomposition of residues and light-fraction organic matter in the surface of RT may partially affect NMIN through immobilization processes.

We observed relatively poor relationships between CMIN and NMIN in our study (Fig. 10). The lack of a relationship between CMIN and NMIN in CC largely contributed to the poor overall relationships between the parameters. Relationships between CMIN and NMIN were stronger for RT, suggesting that increased labile-SOM resulting from RT enhanced the potentially mineralizable N pool. Nitrogen transformations in soil are largely dependent upon transformations of C (McGill et al., 1975), and enhanced CMIN may be linked to enhanced NMIN (Smith, 1994). Franzleubbers et al. (1996b) observed strong relationships between CMIN and NMIN in soils from several geographic regions, and short-term CMIN has been reported as a good predictor of 24-day potential NMIN (Haney et al., 2001). However, N turnover is dependent upon complex mineralization and immobilization processes which vary with changes in the quality and quantity of the SOM and SMBC (Jansson and Persson, 1982).

The absence of enhanced NMIN and strong relationships between NMIN and CMIN in CC may be due to the immobilization of N as a result of changes in the quality
Fig. 10. Relationships between C mineralization (CMIN) and N mineralization (NMIN) from 38-day incubations of soils collected at pre-plant (P=0.068) and full maturity (P=0.002).

**Pre-plant**

$r^2=0.30$

$y = 0.02x + 7.0$

**Full Maturity**

$r^2=0.69$

$y = 0.08x - 3.8$
or composition of SOM and/or SMB under rotation vs. continuous cotton. NMIN is dependent upon microbial metabolism (activity), and the C/N ratios of SMB and SOM (Smith, 1994). Possible alteration of these ratios under CC management may have been responsible for the observed results.

We also observed that NMIN and CMIN correlated more poorly at pre-plant \( (r^2=0.30) \) than at full maturity \( (r^2=0.69) \) (Fig. 10). We have no explanation for this observation. The differences in variability could be partially attributed to spatial variation in the field or laboratory variation. Bonde and Rosswall (1987) observed that potential NMIN decreased throughout the growing season as a result of organic matter reduction. If one assumed a depletion in organic matter throughout the growing season, initial differences in SOM quantity/quality could be reduced by various decomposition processes. This could lead to more consistent relationships in the data. This assumption does not include the effects of below-ground-C inputs during the growing season.

It is known that C-inputs are deposited in the form of plant-root-exudates during the growing season, and that these inputs can increase microbial activity in the rhizosphere (Whipps, 1990) and possibly result in N immobilization, although these relationships are still not well understood (Smith, 1994). Franzluebbers et al. (1994) observed increases in SMBC and CMIN from planting to flowering of wheat with the highest SMBC and CMIN levels occurring at flowering. They suggested that short-term C inputs by crops during the growing season affected NMIN dynamics. We failed to observe meaningful differences in NMIN between pre-plant and full maturity, although NMIN was slightly greater at full maturity. We also failed to observe biologically significant differences in SMBC and CMIN throughout the growing season, suggesting
that below-ground-C inputs from cotton were not sufficient to affect microbial biomass or activity. Thus, one would not expect these inputs to be large enough to affect N dynamics either.

**Hot-water Extractable Organic Carbon**

Hot-water extractable carbon (hot-WEOC) ranged from approximately 261 to 445 mg kg\(^{-1}\) at pre-plant in all treatments including N and depth (Fig. 11). This range was comparable to values observed for 38-day CMIN and SMBC although hot-WEOC was slightly less than SMBC and slightly higher than 38-day CMIN. Relative differences between tillage/rotation treatments were also similar to those observed for SMBC and 38-day CMIN with hot-WEOC being significantly greater in RT and CC vs. CT. Hot-WEOC was 53 and 29 % greater in the top 5 cm of RT and CC when compared to CT at pre-plant. These differences in the 5-15 cm depth were 33 and 30 %, respectively. Differences between treatments were similar across all seasonal sampling periods, but a reduction in hot-WEOC was observable from pre-plant to full maturity. This reduction was more apparent for CC and CT vs. RT. This observation is further discussed in the *Seasonal Variation of Labile Organic Carbon Pools* section.

Little effect of depth or N was observed for hot-WEOC across all seasonal sampling periods. The effect of depth in RT was not as pronounced for hot-WEOC as it was for SMBC and CMIN, and it was generally not significant with the exception of the pinhead-square sampling. At pre-plant, hot-WEOC was only 19 % greater in the top 5 cm vs. the 5-15 cm depth in RT. This stratification in RT was more comparable to the stratification observed for SOC (16 %) than that observed for SMBC (31 %) or 38-day CMIN (69 %).
Fig. 11. Hot-water extractable organic C (hot-WEOC) at various growth stages of cotton. CC, CT, and RT indicate conventional-till cotton/corn rotation, conventional-till continuous cotton and reduced-till continuous cotton, respectively. 0N and 90N indicate kg added N ha\(^{-1}\). Lower-case letters indicate significant differences (P<0.05) between tillage/rotation treatment for columns representing N and depth. Upper-case letters indicate significant differences within tillage/rotation treatment.
WEOC is derived from several sources including surface and sub-surface litter, rhizosphere exudates, soil humus, and lysed microbial biomass (Zsolnay, 1996). However, litter and humus are considered to be the most important sources of WEOC in soils (Kalbitz et al., 2000). Hot-water extractions hydrolyze part of soil organic matter (SOM) and solubilize a portion of the microbial biomass (Zsolnay, 1996); thus, they may be successful in indicating labile organic matter or management-induced change (Leinweber et al., 1995; Zsolnay, 1996). It is likely that the WEOC we obtained through hot-water extraction was derived from both microbial sources and various SOM fractions (i.e. the light fraction, free and aggregate-associated particulate organic matter, and the recalcitrant humic fraction). Therefore, increases in SOC, mineralizable C, and SMB resulting from RT and CC likely contributed to the observed increases in hot-WEOC.

Rewetting of air-dried soils prior to hot-water extraction probably resulted in a higher microbial contribution due to enhanced desiccation and lysing of microbial cells. Strong correlations (P<0.001) between hot-WEOC and SMBC ($r^2=0.90$) and hot-WEOC and 38-day CMIN ($r^2=0.84$) were observed. These relationships indicate that hot-WEOC represents a relatively labile pool of organic matter. Though some studies have considered WEOC to be labile in nature (Burford and Bremner, 1975; Xu and Juma, 1993), others have suggested that recalcitrant organic matter comprises of a significant proportion of WEOC (Qualls and Haines, 1992; Boissier and Fontvieille, 1993; Nelson et al., 1994).

Our data generally support the concept that hot-WEOC represents an available pool of organic-C, but we did not conduct a biodegradability assay to properly characterize the extracts. These types of assays have generally shown that WEOC
consists of both labile and recalcitrant fractions (Zsolnay and Steindl, 1991; Gregorich et al., 2003; Kalbitz et al., 2003). This is probably the case concerning the hot-WEOC obtained from our cotton-cropped plots. Although hot-WEOC was successful in reflecting management effects, there were instances where hot-WEOC was a less sensitive indicator vs. SMBC and CMIN. This was most evident with respect to the stratification in RT discussed above. Also, hot-WEOC did not correlate with SMBC and CMIN as well as hot-water extractable carbohydrate C. These results suggest some involvement of more recalcitrant fractions included in hot-WEOC.

Studies concerning the effects of management on WEOC in agroecosystems are not as abundant as those involving effects on SMBC. Generally, WEOC tends to be proportional to SOM content (Chantigny, 2003) and management practices leading to greater SOM would be expected to increase WEOC. Campbell et al. (1999a) and Campbell et al. (1999b) observed positive effects of rotation on WEOC in wheat systems and related increases in WEOC to greater organic inputs. In contrast, Gregorich et al. (2003) observed little effect of maize/soybean rotation vs. continuous maize on hot- or cold-WEOC. Linn and Doran (1984) observed that no-till increased WEOC in the top 7.5 cm vs. conventional-till in soils from Illinois, Kentucky, Nebraska, and Minnesota, but these increases were not, overall, considered to be significant. We observed that CC and RT resulted in significantly greater hot-WEOC vs. CT, and similar results were observed for SOC, SMBC, and CMIN. This supports the notion that there are consistent relationships between these pools and suggests that these pools respond similarly to management practices that increase SOC storage by reducing C outputs or increasing C inputs.
Ghani et al. (2003) stressed the importance of hot-WEOC as a sensitive indicator of management-induced change. The authors found that hot-WEOC sensitively reflected land-use and grazing intensities. They also observed strong relationships between hot-WEOC and fractions of labile organic matter. We observed greater relative differences between treatments for hot-WEOC when compared to SOC, suggesting that hot-WEOC more sensitively reflected changes in management. We also found that hot-WEOC correlated well with other labile-C fractions. Relative differences between treatments for hot-WEOC were greater when compared to SOC, and were similar to those observed for other measured labile-C pools.

**Hot-water Extractable Carbohydrate Carbon**

The effects of management on the carbohydrate C content of hot-water extracts followed patterns similar to those for hot-WEOC, SMBC, and 38-day CMIN. RT and CC generally contained significantly more carbohydrate C when compared to CT, and carbohydrate C was highest in the top 5 cm of RT soils. Carbohydrate C, including both N and depth treatments, ranged from approximately 9 to 15 mg kg⁻¹ in pre-plant soil samples (Fig 12). The carbohydrate C pool was much smaller than SMBC, 38-day CMIN, and hot-WEOC, all of which measured roughly in the 160 to 550 mg kg⁻¹ range. However, carbohydrate C responded similarly to the other measured labile-C pools concerning management, and was well correlated with these pools.

Carbohydrate C in the top 5 cm was 43 and 24 % greater in RT and CC vs. CT at pre-plant. These differences in the 5-15 cm depth were 24 and 33 %, respectively. Similar relative differences between treatments for carbohydrate C were observed at pinhead-square, peak bloom, and full maturity. We observed a weak trend for reduction in carbohydrate C throughout the growing season. This trend was also seen for some
Fig. 12. Carbohydrate C of hot-water extracts at various growth stages of cotton. CC, CT, and RT indicate conventional-till cotton/corn rotation, conventional-till continuous cotton and reduced-till continuous cotton, respectively. 0N and 90N indicate kg added N ha\(^{-1}\). Lower-case letters indicate significant differences (P<0.05) between tillage/rotation treatment for columns representing N and depth. Upper-case letters indicate significant differences within tillage/rotation treatment. RT 90N 0-5 cm at pinhead-square (n=3).
of the other labile-C pools, but these trends were not strong enough, in light of sample variability, to draw any definite conclusions about seasonal dynamics.

Nitrogen application and depth had little overall effect on carbohydrate C within tillage/rotation treatment. The effect of depth was greatest in RT plots where carbohydrate C in the top 5 cm was 28 % greater than carbohydrate C in the 5-15 cm depth at pre-plant. Similar differences were seen throughout the season with the stratification in RT being most pronounced at pinhead-square where carbohydrate C was 68 % greater in the 0-5 vs. the 5-15 cm depths.

A slight trend was also observed where plots receiving 90 kg N ha\(^{-1}\) contained slightly more carbohydrate C than plots not receiving N. This trend could be attributed to the indirect effects of N on SOC, whereby enhancement of crop growth resulting from N application leads to greater organic residue returns to the soil. This trend was observable for data from all labile-C pools studied, but was generally not significant.

Other studies have reported increases in carbohydrate C resulting from conservation tillage (Arshad et al. 1990; Angers et al. 1993; Hu et al., 1995; Hu et al., 1997) and rotation (Angers and Meyhus, 1989; Angers et al., 1993). In many cases, increased carbohydrate C was found with concomitant increases in SOC or labile-C pools. In contrast to this study, most of these studies involved analyses of acid-hydrolyzable carbohydrates (AHC) and not water-soluble or -extractable carbohydrates (WSC). While WSC most likely consist of free sugars and microbial polysaccharides, AHC likely consist of these components in addition to more complex polysaccharides of plant and microbial origin that are hydrolyzed by concentrated acid (Cheshire, 1979).

Generally, WSC comprise only a small fraction of AHC. Puget et al. (1999) observed that hot-water extractable carbohydrates accounted for 0.78 to 2.47 % of
SOC, while AHC accounted for roughly 16 to 25 % of SOC in the clay + silt and particulate organic matter fractions of a Typic Hapludalf. Angers et al. (1993) reported that WSC only comprised 5 to 7 % of AHC, but both fractions responded similarly to tillage and rotation. Hot-water extractable carbohydrate C only accounted for a very small proportion of SOC (~0.12 %) in the present study. However, carbohydrate C showed significant responses to management, and responses were similar to those of SOC, SMBC, CMIN, and hot-WEOC.

We used the bicinchoninic acid (BCA) assay to determine the carbohydrate C content of our hot-water extracts. This method was utilized by Joergensen et al. (1996) to measure total carbohydrates in 0.5 M K$_2$SO$_4$ extracts. The authors observed that carbohydrate C ranged from 1.5 to 15.7 mg kg$^{-1}$ in a collection of 58 arable soils. The range of values that we observed (9 to 15 mg kg$^{-1}$ in pre-plant samples) were consistent with this range.

Carbohydrate determination using the BCA reagent is based primarily upon the reduction of Cu$^{2+}$ to Cu$^{+}$ by reducing sugars (Mopper and Gindler, 1973). Then BCA, a sensitive and stable reagent for Cu$^{+}$, binds (2:1) with Cu$^{+}$ under alkaline conditions resulting in the development of a purple color that is monitored at an absorbance maximum of 592 nm (Mikkelsen and Cortón, 2004).

We refer to our results in terms of carbohydrate C because glucose standards were used in the BCA procedure. However, in hot-water extracts, it is likely that a mixture of various carbohydrates and proteins (amino acids) released upon exposure of air-dried soil to hot water are capable of reducing Cu$^{2+}$ to Cu$^{+}$, and driving the reaction with the BCA reagent. BCA can be used to effectively quantify both total proteins and carbohydrates that are capable of reducing Cu$^{2+}$, but other substances including
acidifying agents, and common membrane lipids and phospholipids may interact with the reagent (Mikkelsen and Corton, 2004). Joergensen et al. (1996) reported that interference by proteins was negligible due to their low concentrations in 0.5 M K$_2$SO$_4$ extracts.

**Relationships Between Labile Organic Carbon Pools**

We observed strong relationships between the labile organic C pools measured in the study. Management practices that increased SOC generally led to increased SMBC, CMIN, hot-WEOC, and carbohydrate C. Though some labile organic C pools responded more sensitively to tillage or rotation practices than others, all responded in a similar fashion. Overall, 38-day CMIN exhibited the greatest relative differences between treatments and was the best indicator of management-induced change in pre-plant samples. Relative differences between tillage/rotation treatments were generally similar for all measured labile-C pools in pinhead-square, peak bloom, and full maturity samples. In soil samples from peak bloom and full maturity, hot-WEOC showed greater relative differences between RT vs. CC and CT. We partially attribute these increased differences to the reduction of hot-WEOC in CC and CT throughout the growing season.

A strong correlation was observed between SMBC and 38-day CMIN (P<0.001, $r^2=0.93$) as averaged across all four seasonal sampling periods (Fig. 13). The observed correlation is probably due to the close relationship of the pools. CMIN is essentially a measure of the activity of the SMB. The microbial biomass is limited by available C, and in its presence, the biomass oxidizes C substrates to meet growth requirements and produces CO$_2$ (Smith and Paul, 1990). This relationship can be complicated by factors that influence the energy requirement of the SMB or substrate availability including the presence of inactive vs. active SMB (Smith and Paul, 1990), the effects of
Fig. 13. Relationship between 38-day cumulative C mineralization (38-day CMIN) and soil microbial biomass C (SMBC) as averaged across all seasonal sampling periods. Relationship significant at \( P<0.001 \).

\[
\text{SMBC mg kg}^{-1} \\
200 250 300 350 400 450 500 \\
\]
\[
\text{38-day CMIN mg kg}^{-1} \\
160 180 200 220 240 260 280 300 320 340 \\
\]

\( r^2=0.93 \)

\( y = 0.7x + 0.1 \)
disturbance (Insam and Domsch, 1988), and the protective effect of clay (Wang et al., 2003).

The measurement of 38-day CMIN also included the CMIN flush that followed the rewetting of air-dried soils. This flush was measured by 3-day CMIN in our study, and correlated well with SMBC ($r^2=0.90$). The flush of CMIN following rewetting has been correlated to SMBC (Franzluebbers et al., 1996b; Franzluebbers et al., 2000b), and is likely a result of the utilization of metabolites released from lysed cells by surviving organisms (Jenkinson, 1966). It is likely that readily available SOC or non-biomass SOC made available by drying/rewetting also contributed to 3-day CMIN. The flush of mineralization after 3 days accounted for 27% of 38-day CMIN as averaged across all treatments at pre-plant. Data support that SMB turnover contributes significantly to 3-day CMIN, and the addition of this component into 38-day CMIN likely contributed to the strength of correlation between the two parameters.

Both hot-WEOC and carbohydrate C were strongly correlated ($P<0.001$) with SMBC and 38-day CMIN (Fig. 14). Hot-water extractions were also carried out on air-dried soils, so it is likely that microbial cells were lysed from both osmotic shock (Kieft et al., 1987) and by hot-water (Nelson et al. 1994; Zsolnay, 1996). High temperatures also hydrolyze organic materials and dissociate organics from inorganic colloids (Nelson et al., 1994). Thus, the combination of rewetting dried soils and subsequently subjecting them to hot-water extraction should attain a significant proportion of the microbial biomass in addition to a significant fraction of labile SOM. However, not all hot-WEOC is considered labile. Gregorich et al. (2003) observed that both cold- and hot-water extracts consisted of rapidly decomposable (29-36% of WEOC) and recalcitrant WEOC pools.
Fig. 14. Relationships of hot-water extractable organic C (hot-WEOC) and carbohydrate C with soil microbial biomass C (SMBC) and 38-day cumulative C mineralization (38-day CMIN) as averaged across all seasonal sampling periods. All relationships significant at $P<0.001$. 
Other researchers have observed strong relationships between hot-WEOC and SMBC (Sparling et al., 1998; Ghani et al., 2003) and between hot-WEOC and 3-day CMIN (Fischer, 1993). The observed relationships between hot-WEOC and SMBC likely result from a combination of two cases: 1) hot-water extracts contain significant amounts of microbial material, and 2) hot-water extracts contain labile SOM that supports microbial growth.

Hot-water extractable carbohydrate C correlated strongly with SMBC and 38-day CMIN (Fig. 14), though it only accounted for a small proportion of these pools. It is interesting that these relationships were the strongest observed throughout the entire study because carbohydrate C only comprised 3 and 5 % of SMBC and 38-day CMIN, respectively. As discussed earlier, the BCA reagent used in determining carbohydrate content likely reacted with a mix of polysaccharides, amino acids, and other compounds capable of reducing Cu$^{2+}$. The lysis of cells by hot-water would release many of these types of compounds into solution, in addition to those compounds extracted from other SOM fractions and dissociated from soils colloids.

Our results suggested that use of this reagent in conjunction with hot-water extraction identified a labile pool of C. The strong relationships with SMBC and 38-day CMIN support the likelihood that the BCA reagent apparently focused on more labile-C pools in contrast to hot-WEOC measurements. It is known that WEOC includes a recalcitrant component (Qualls and Haines, 1992; Boissier and Fontvieille, 1993; Nelson et al., 1994), which would generally not be considered part of the SMB or degradable by the SMB. Thus, using a method that discriminates labile C from recalcitrant C in hot-water extracts would enhance relationships with the SMB and other labile-C pools. As with hot-WEOC, the relationships observed (carbohydrate C vs.
SMBC and 38-day CMIN) likely resulted from the extraction and measurement of both microbial material, and readily available C that is used for microbial metabolism. Though researchers have suggested a significant microbial contribution to hot-water extracts (Leinweber et al., 1995; Ball et al., 1996), the material is likely chemically heterogeneous due to extraction of both microbial materials and non-microbial organic matter (Sparling et al., 1998).

We were unable to formally assess the effectiveness of using carbohydrate C as an index of labile SOC because our experiment only included soil from one series and texture (Weswood silty clay loam) with an SOC content ranging from roughly 8 to 11 g C kg⁻¹. However, the strong relationships that we observed suggest that there is a potential for usage of carbohydrate C as an index of labile SOC. The use of hot-water extractable carbohydrate C (determined by BCA) as an index for labile-C, or possibly SMB, needs to be further evaluated on a diverse group of soils including broad ranges of SOC, SMB, texture, and pH.

**Seasonal Variability of Labile Organic Carbon Pools**

Overall, we observed little seasonal variability in the four labile-C pools monitored throughout the growing season of cotton. We failed to observe any significant seasonal variability in SMB as measured at four different growth stages (Fig. 15). Seasonal variation in SMB has been attributed to temperature (Collins et al., 1992; Angers et al., 1993), soil moisture (McGill et al., 1986; van Gestel et al., 1992), and crop inputs (Lynch and Panting, 1980; Franzluebbers et al., 1994; Franzluebbers et al., 1995b). Other studies have seen little or no seasonal variation in SMB (Ritz and Robinson, 1988; Joergensen et al., 1994)
Fig. 15. Seasonal variation of soil microbial biomass C (SMBC) (A), 38-day cumulative C mineralization (38-day CMIN) (B), hot-water extractable organic C (hot-WEOC) (C), and carbohydrate C (D) as measured at pre-plant, pinhead-square, peak bloom, and full maturity. CC, CT, and RT indicate conventional-till cotton/corn rotation, conventional-till continuous cotton and reduced-till continuous cotton, respectively. Carbohydrate C data not to scale.
It is possible that we may have observed more seasonal variability associated with temperature and moisture fluxes with more intense sampling, but our initial goal was to assess effects of C-inputs from cotton on SMB. The air-drying of soils and equilibration (10-day pre-incubation) of SMB prior to determination may have also negated minor SMB fluctuations due to variations in temperature and moisture. Thus, the predominant influence on variability in SMB would have been organic-C. Root inputs from cotton apparently were not sufficient to increase SMB, although they may have helped maintain SMB throughout the growing season. If one assumes that CMIN, starting at pre-plant, would deplete SOM throughout the growing season, one would also expect to see a reduction in SMB unless root-C inputs provided additional substrate. With respect to fluctuations due to temperature and moisture, CMIN, hot-WEOC, and carbohydrate C were all strongly correlated to SMBC, and variation of these pools should have reflected possible changes in SMBC that may have been eliminated by the 10-day pre-incubation used for determination of SMBC. We did not observe seasonal variation in these pools that would suggest seasonal effects from moisture and temperature. In general, the seasonal variation component of our study did not include enough sampling to detect these effects.

The lack of seasonal variation observed for SMBC was also observed for other labile-C pools. Slight seasonal trends existed for 38-day CMIN and carbohydrate C, but when accounting for variation, these trends were considered biologically insignificant. The greatest seasonal change occurred for hot-WEOC for which there was a slight reduction throughout the growing season. This reduction was only apparent in CC and CT, where hot-WEOC was approximately 36 % less when measured at full maturity as compared to pre-plant. This reduction suggests that root-C inputs throughout the
growing season were insignificant or not measured well by hot-WEOC. Hot-WEOC likely includes organic material from various fractions of SOM including light fraction and particulate organic matter in addition to microbial C. The reduction of these SOM fractions throughout the growing season may have been responsible for the observed reduction in hot-WEOC.
SUMMARY AND CONCLUSIONS

Ridge-tillage and rotation with corn significantly increased SOC and labile organic C in the cotton-cropping systems studied. However, these management practices had virtually no effect on water-stable aggregation in these systems. Total glomalin comprised a significant proportion of SOC (~ 7-9 %), and the two parameters were well correlated. These two parameters also showed similar responses to management, but total glomalin was less sensitive to management when compared to SOC. Labile organic C pools (SMBC, 38-day CMIN, hot-WEOC, and carbohydrate C) and SOC were similarly affected by tillage/rotation management, but labile-C pools showed greater relative differences between treatments vs. SOC. Little seasonal variation in labile-C pools was observed throughout the growing season of cotton, suggesting that root inputs by cotton were minimal.

Depth and N application had little overall effect on SOC pools. The effect of depth was greatest in RT where significant stratification of SOC pools was observed as a result of residue distribution by ridge-tillage. This stratification was most pronounced for basal respiration, suggesting that decomposing plant residues in the surface of RT contributed to an intermediately labile-C pool. Trends in SOC pools reflecting possible indirect effects of N application on organic matter storage were observed, but they were generally insignificant.

Increased labile organic matter in RT vs. CT led to enhanced potential N mineralization in RT vs. CT. This was not the case for CC where increased labile organic matter pools did not result in enhanced N mineralization, suggesting possible immobilization of N in CC. Increases in labile organic matter pools resulting from conservation management have the potential to impact plant-available N in cotton
systems, but contributions may vary as a function of complex mineralization and immobilization processes.

As expected, all labile-C pools were strongly correlated due to their close relationships. Hot-WEOC and carbohydrate C exhibited strong relationships with SMBC and 38-day CMIN and may provide easy to obtain, useful indexes of labile C. Carbohydrate C of hot-water extracts, as determined by the BCA assay, showed the strongest correlations with SMBC ($r^2=0.96$) and 38-day CMIN ($r^2=0.96$), even though it only comprised 3 and 5% of these pools, respectively. The potential for using this method as an index of labile C or SMBC needs to be evaluated across a range of soils with various physical and chemical characteristics.

Our study suggests that C sequestration is possible in cotton-cropping systems associated with the warm Texas climate. However, more long-term data are needed to comprehensively address SOC storage potentials for Texas soils under varying management. Nonetheless, increases in labile organic matter pools are attainable with conservation tillage and rotation, and these pools have the potential to enhance soil fertility and benefit producers.
REFERENCES


APPENDIX

Appendix. SOC and labile-organic-C pools at various growth stages of cotton. Values are means ± 1 standard deviation (n=4). (†) denotes n=3.

<table>
<thead>
<tr>
<th>Plot/N/Depth</th>
<th>SOC %</th>
<th>SMBC mg kg⁻¹</th>
<th>38-day CMIN mg kg⁻¹</th>
<th>hot-WEOC mg kg⁻¹</th>
<th>Carb-C mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pre-plant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC 0N 0-5 cm</td>
<td>1.13 ± 0.19</td>
<td>412 ± 30</td>
<td>264 ± 32</td>
<td>365 ± 21</td>
<td>13.1 ± 1.0</td>
</tr>
<tr>
<td>CC 0N 5-15 cm</td>
<td>1.05 ± 0.08</td>
<td>360 ± 29</td>
<td>227 ± 10</td>
<td>336 ± 29</td>
<td>11.6 ± 1.0</td>
</tr>
<tr>
<td>CC 90N 0-5 cm</td>
<td>1.01 ± 0.05</td>
<td>428 ± 32</td>
<td>272 ± 46</td>
<td>348 ± 22</td>
<td>12.7 ± 1.2</td>
</tr>
<tr>
<td>CC 90N 5-15 cm</td>
<td>0.99 ± 0.04</td>
<td>367 ± 25</td>
<td>219 ± 17</td>
<td>360 ± 44</td>
<td>13.0 ± 2.3</td>
</tr>
<tr>
<td>CT 0N 0-5 cm</td>
<td>0.82 ± 0.06</td>
<td>310 ± 23</td>
<td>189 ± 12</td>
<td>271 ± 15</td>
<td>10.2 ± 0.9</td>
</tr>
<tr>
<td>CT 0N 5-15 cm</td>
<td>0.80 ± 0.07</td>
<td>272 ± 30</td>
<td>162 ± 10</td>
<td>261 ± 14</td>
<td>8.9 ± 1.2</td>
</tr>
<tr>
<td>CT 90N 0-5 cm</td>
<td>0.85 ± 0.09</td>
<td>325 ± 39</td>
<td>204 ± 14</td>
<td>282 ± 27</td>
<td>10.5 ± 0.3</td>
</tr>
<tr>
<td>CT 90N 5-15 cm</td>
<td>0.85 ± 0.10</td>
<td>300 ± 30</td>
<td>184 ± 7</td>
<td>274 ± 28</td>
<td>9.6 ± 0.5</td>
</tr>
<tr>
<td>RT 0N 0-5 cm</td>
<td>1.09 ± 0.09</td>
<td>454 ± 48</td>
<td>337 ± 21</td>
<td>400 ± 14</td>
<td>14.3 ± 0.5</td>
</tr>
<tr>
<td>RT 0N 5-15 cm</td>
<td>0.95 ± 0.11</td>
<td>359 ± 47</td>
<td>203 ± 24</td>
<td>341 ± 50</td>
<td>11.0 ± 1.3</td>
</tr>
<tr>
<td>RT 90N 0-5 cm</td>
<td>1.14 ± 0.15</td>
<td>553 ± 87</td>
<td>366 ± 83</td>
<td>445 ± 69</td>
<td>15.3 ± 0.7†</td>
</tr>
<tr>
<td>RT 90N 5-15 cm</td>
<td>0.98 ± 0.13</td>
<td>408 ± 63</td>
<td>213 ± 10</td>
<td>368 ± 52</td>
<td>12.1 ± 1.1</td>
</tr>
<tr>
<td><strong>pinhead-square</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC 0N 0-5 cm</td>
<td>--</td>
<td>400 ± 14</td>
<td>237 ± 9</td>
<td>316 ± 29</td>
<td>11.9 ± 0.6</td>
</tr>
<tr>
<td>CC 0N 5-15 cm</td>
<td>--</td>
<td>359 ± 25</td>
<td>202 ± 8</td>
<td>285 ± 12</td>
<td>10.0 ± 0.8</td>
</tr>
<tr>
<td>CC 90N 0-5 cm</td>
<td>--</td>
<td>406 ± 41</td>
<td>243 ± 23</td>
<td>332 ± 19</td>
<td>12.2 ± 0.7</td>
</tr>
<tr>
<td>CC 90N 5-15 cm</td>
<td>--</td>
<td>349 ± 26</td>
<td>210 ± 9</td>
<td>315 ± 23</td>
<td>10.5 ± 0.6</td>
</tr>
<tr>
<td>CT 0N 0-5 cm</td>
<td>--</td>
<td>305 ± 26</td>
<td>181 ± 4</td>
<td>256 ± 8</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td>CT 0N 5-15 cm</td>
<td>--</td>
<td>267 ± 22</td>
<td>161 ± 6</td>
<td>227 ± 13</td>
<td>7.5 ± 0.7</td>
</tr>
<tr>
<td>CT 90N 0-5 cm</td>
<td>--</td>
<td>324 ± 32</td>
<td>193 ± 14</td>
<td>255 ± 17</td>
<td>9.3 ± 0.6</td>
</tr>
<tr>
<td>CT 90N 5-15 cm</td>
<td>--</td>
<td>308 ± 25</td>
<td>182 ± 10</td>
<td>255 ± 27</td>
<td>8.3 ± 0.8</td>
</tr>
<tr>
<td>RT 0N 0-5 cm</td>
<td>--</td>
<td>452 ± 8†</td>
<td>284 ± 27</td>
<td>385 ± 14</td>
<td>14.7 ± 0.6</td>
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<tr>
<td>RT 0N 5-15 cm</td>
<td>--</td>
<td>319 ± 34</td>
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<td>8.9 ± 1.0</td>
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<tr>
<td>RT 90N 0-5 cm</td>
<td>--</td>
<td>508 ± 56</td>
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<td>409 ± 27</td>
<td>16.6 ± 1.5</td>
</tr>
<tr>
<td>RT 90N 5-15 cm</td>
<td>--</td>
<td>342 ± 63</td>
<td>190 ± 21</td>
<td>282 ± 44</td>
<td>9.8 ± 1.5</td>
</tr>
<tr>
<td>Plot/N/Depth</td>
<td>SOC %</td>
<td>SMBC mg kg⁻¹</td>
<td>38-day CMIN mg kg⁻¹</td>
<td>hot-WEOC mg kg⁻¹</td>
<td>Carbohydrate C mg kg⁻¹</td>
</tr>
<tr>
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<td>-------</td>
<td>--------------</td>
<td>---------------------</td>
<td>------------------</td>
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<td><strong>peak bloom</strong></td>
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<tr>
<td>CC 0N 0-5 cm</td>
<td>--</td>
<td>347 ± 76</td>
<td>229 ± 8</td>
<td>267 ± 13</td>
<td>10.7 ± 0.8</td>
</tr>
<tr>
<td>CC 0N 5-15 cm</td>
<td>--</td>
<td>377 ± 4</td>
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<td>275 ± 14</td>
<td>10.9 ± 0.2</td>
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<tr>
<td>CC 90N 0-5 cm</td>
<td>--</td>
<td>398 ± 35</td>
<td>223 ± 11</td>
<td>271 ± 29</td>
<td>12.1 ± 0.8</td>
</tr>
<tr>
<td>CC 90N 5-15 cm</td>
<td>--</td>
<td>405 ± 34</td>
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<td>274 ± 26</td>
<td>11.3 ± 1.0</td>
</tr>
<tr>
<td>CT 0N 0-5 cm</td>
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<td>290 ± 33</td>
<td>190 ± 28</td>
<td>184 ± 13</td>
<td>8.5 ± 0.5</td>
</tr>
<tr>
<td>CT 0N 5-15 cm</td>
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<td>288 ± 9</td>
<td>195 ± 16</td>
<td>196 ± 6</td>
<td>7.9 ± 1.5</td>
</tr>
<tr>
<td>CT 90N 0-5 cm</td>
<td>--</td>
<td>324 ± 30</td>
<td>206 ± 6</td>
<td>180 ± 19</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>CT 90N 5-15 cm</td>
<td>--</td>
<td>329 ± 41</td>
<td>216 ± 15</td>
<td>201 ± 35</td>
<td>9.0 ± 1.3</td>
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<tr>
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<td>322 ± 35</td>
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<tr>
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<td>242 ± 26</td>
<td>322 ± 44</td>
<td>10.6 ± 1.8</td>
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<td>416 ± 17</td>
<td>226 ± 4</td>
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<td>369 ± 25</td>
<td>232 ± 8</td>
<td>268 ± 25</td>
<td>10.5 ± 0.8</td>
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<td>237 ± 22</td>
<td>248 ± 31</td>
<td>11.3 ± 2.0</td>
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<tr>
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<td>389 ± 26</td>
<td>226 ± 11</td>
<td>252 ± 25</td>
<td>10.2 ± 1.0</td>
</tr>
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<td>191 ± 9</td>
<td>206 ± 11</td>
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</tr>
<tr>
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<td>367 ± 36</td>
<td>230 ± 22</td>
<td>204 ± 22</td>
<td>9.7 ± 1.3</td>
</tr>
<tr>
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<td>--</td>
<td>326 ± 52</td>
<td>223 ± 7</td>
<td>197 ± 31</td>
<td>8.4 ± 0.7</td>
</tr>
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<td>--</td>
<td>446 ± 15</td>
<td>269 ± 35</td>
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<td>334 ± 15</td>
<td>200 ± 13</td>
<td>279 ± 22</td>
<td>9.2 ± 0.5</td>
</tr>
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<td>514 ± 32</td>
<td>323 ± 44</td>
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<td>14.3 ± 1.6</td>
</tr>
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<td>--</td>
<td>392 ± 56</td>
<td>221 ± 21</td>
<td>327 ± 57</td>
<td>10.2 ± 1.7</td>
</tr>
</tbody>
</table>
VITA

Scott Michael Kolodziej

Permanent Address: 3569 County Rd. 231, Falls City, TX 78113

Phone: (830) 484-3615

Email: skolodziej@excite.com

Education:  
B.S. Bioenvironmental Science, Summa Cum Laude, Texas A&M University, 5/2002  
M.S. Soil Science, Summa Cum Laude, Texas A&M University, 5/2005

Professional Experience:  
Environmental Scientist, Whitehead and Mueller, Inc.  
1/2005 - present