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CONTRIBUTIONS OF ROOTS AND ORGANIC MATTER TO SOIL AGGREGATE STABILIZATION

By

Djail Santos

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ABSTRACT

CONTRIBUTIONS OF ROOTS AND ORGANIC MATTER TO SOIL AGGREGATE STABILIZATION

By

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Soil aggregates and associated porosities control many of the below ground biological, chemical and physical processes and strongly influence plant root activities. This study examines three specific objectives which address the inter-relationships between soil aggregates and the retention and spatial distributions of carbon (C) contents within individual aggregates. First, a field study was sampled to assess crop rotation and conventional- (CT) and no-tillage (NT) modifications of total C contents and the concomitant incorporation of ^{13}C into soil macroaggregates. Accumulations of C were greatest in aggregates sampled at the 0-5 cm depth. Analysis of ^{13}C-natural abundance indicated that 65% of the C in the outer layers of CT aggregates (-26.5‰) originated from alfalfa roots ($\delta^{13}C$ of -27.9‰), while 25% of the NT aggregate C, of the outer layer, originated from the alfalfa. Aggregate samples taken at 5-15 cm depth had less negative $\delta^{13}C$ values which were similar to the bulk soil. $\delta^{13}C$ values in the inner regions of soil aggregates were similar at both depths. The second field study was designed to investigate the distribution of original or native C_3- and more recent or contemporary C_3- and C_4-derived C in aggregates having different sized-fractions. The proportion of more recent crop-derived C was 53 to 76% higher in NT soil aggregates at the 0-5 cm depth. Depositions of crop-derived C in CT soils ranged from 22 to 45% and appeared to not be affected by soil depth. Aggregate stabilities were lower in CT and NT soils than in never-tilled grassland soils. Never-tilled soils contained proportionally greater quantities of water-stable aggregates among fractions > 0.5 mm, whereas CT and NT soils contained more stable aggregates having fractions < 0.5 mm. On average for both soil depths, the mean weight diameter of aggregates (MWD), < 6.5 mm, was 62% lower in CT soil than in the never-tilled grassland soil. However, following 11 years of NT, the MWD of NT soil aggregates, were improved by 40% over those from CT. Thirdly, a greenhouse study was designed to investigate amounts and distributions of recently released root C in soil aggregates by ryegrass plants under water stressed and non-stressed conditions. Water availability during a six week growth period significantly changed ryegrass root parameters to depths of 10 cm. Higher C contents were detected in aggregates associated with non-stressed plants. New inputs of C_3-C into whole aggregates, at 0-5 cm depth, increased by as much as 62% when ryegrass was subjected to drought stress and 25% in the non-stressed treatment. Aggregates associated with non-stressed ryegrass roots, at the 0-5 cm depth, developed higher MWDs than aggregates from unplanted treatments. Soil aggregates associated with non-stressed ryegrass roots also had greater MWDs than aggregates from ryegrass roots under water stress. The soil abrasion techniques for peeling concentric layers from aggregates, described in these studies, appear to be useful for quantifying the sequestration of recently released root C by soil aggregates and provide a novel approach for further elucidating soil processes associated with the formation, stability, and function of soil aggregates.

To Silvanda and Lucas -

for your love and the joy

you bring me

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INTRODUCTION

Carbon (C) and nitrogen (N) compounds that originate from soil organic matter (SOM) are essential to the functioning of terrestrial ecosystems. Distribution of C and N compounds within the soil are dependent on soil chemical, physical and biological properties, root distribution and water infiltration (Stewart, 1987). Cycling of C and N compounds from plant litter, root turnover, microbial decomposition and mineralization occur within heterogeneous microbial habitats. Soils contain an interstitial mosaic of microsites within a matrix of solid components and pore spaces where sequestration and transformation of added chemicals and SOM take place (Bamforth, 1985).

Aggregation results when primary soil particles and microaggregates combine intimately into secondary units of varying sizes and relative stability, cohesive forces and cementing agents. The structural stability of soil aggregates and the pores between them influence and sometimes control important soil processes such as plant emergence, water and solute infiltration, soil aeration and drainage, erosion, plant root and microbiological activities and crop productivity (Tisdall, 1996). Different mechanisms, operating for different size classes of aggregates, were proposed by Tisdall and Oades (1982) and Oades (1984). Macroaggregates greater than 250 μm in diameter, and specially those greater than 2 mm in diameter, appear to be held together largely by fine roots and fungal hyphae. The stability of microaggregates with diameter of 20-250 μm is usually greater than that of aggregates with larger diameters. Binding agents for this size class appear to be rather persistent. The stability of microaggregates in the diameter range of 2-20 μm depends upon the formation of cation bridges. Units smaller than 2 μm in diameter consist of clay particles held together by inorganic and organic matter and electrostatic bonds (Oades, 1984; 1993).

Plant roots strongly affect aggregate stabilization. During plant growth, a portion of root and soil derived C is constantly stabilized in soil through the production of new microbial biomass. Different plant species and cultivars may significantly influence soil microbial activity and soil structure formation through variation in supply of available root-derived C (Juma, 1994) and by a branched network formed by roots, which may physically enmesh soil microaggregates (Tisdall and Oades, 1982). As soil aggregates are being formed, the newly added organic C, solutes and particulate matter move through the larger soil pores and deposit mainly on or near the surfaces of the aggregates. Successive depositions result in the development of coating materials somewhat richer in those components compared with concentric inner fractions (Ghadiri and Rose, 1991). The conventional sieving methods used to separate different particles sizes within aggregates have provided some information about the effect of soil structure on turnover of soil organic matter. It may, however, redistribute the soil organic matter (Elliott and Cambardella, 1991) giving results that sometimes are difficult to interpret. Knowledge of relationships between C gradients within soil aggregates and soil microbial biomass activities is important to determine the mechanisms involved in soil aggregate development and stability and the best approaches to effectively manage the soil for sustaining intensive agricultural production systems.

In addition to rhizodeposition, large amounts of organic material are returned to the soil after annual crops are harvested and residues are ploughed back (Haynes and Francis, 1993). Such incorporation of organic residues may contribute to soil aggregate formation indirectly by providing a substrate for microorganisms which, in turn, will produce polysaccharide binding agents (Lynch and Bragg, 1985). The degree of soil disturbance associated with the incorporation of these residues should influence the spatial habitat for biological activity through effects on soil bulk density, pore continuity and size distribution, and aggregation (Doran, 1987). Yet, limited information exists in the literature on the effect of soil management, e.g., minimum-tillage or no-tillage, on the nutrient contents of soil aggregates and their associated organic C (Cambardella and Elliott, 1993; Beare et al., 1994).

Aggregation impacts the kinetics of microbial decomposition of organic residues and influences the cycling of C in soils. Kilbertus (1980) found that only 6% of the inner volume of soil aggregates was colonized by microorganisms. Upon soil structure disruption by cultivation, the organic matter previously less or none accessible to microbial metabolism, e.g., within pores $< 0.5\ \mu m$ (Emerson et al., 1986) and within aggregates (Robert and Chenu, 1995), becomes potentially more accessible for microbial activity. However, Edwards and Bremner (1967) hypothesized that organic material inside aggregates $< 250\ \mu m$ is physically inaccessible to microorganisms. This agrees with the observation that microaggregates are not disintegrated with tillage (Tisdall and Oades, 1980; Elliott, 1986). These microhabitats provide protection against predation because the pores are water-saturated most of the time and subjected to few hydric variations (Robert and Chenu, 1995). Although survival of microorganisms may occur, the lack of available O_2 becomes limiting, inducing anaerobic conditions that decreases their activity and, by consequence, accessibility of substrates (Robert and Chenu, 1995). Carbon dioxide production and microbial biomass C was found to be greatest in microaggregates (< 250 μm) under aerobic conditions and biomass declined with increasing aggregate diameter (Seech and Beauchamp, 1988). Extensive predation may occur in frequently unsaturated large pores, e.g., in sandy soils or on the surfaces of aggregates, with drastic water potential fluctuations. These pores are essentially aerobic, and more substrates could be accessible to microbes due to water flow (Robert and Chenu, 1995).

Conventional tillage practices break up root systems, disrupt soil macroaggregates, expose more organic matter to microbial attack and decreases the fungal biomass. The reduction in macroaggregation may provide a short-term increase in soil fertility through the release of nutrients from the labile organic matter that binds micro- and macroaggregates (Elliott, 1986). Prolonged cultivation, will, therefore, result in a rapid decline in the number of macroaggregates and in labile organic matter (Waters and Oades, 1991) since the incorporation of plant residues results in a more homogeneous distribution of substrate for the microbes (Coleman and Elliott, 1986). Organic matter may be protected from microbial metabolism to some extent by adsorption to clay minerals (Oades, 1984) and the formation of microaggregates (Edwards and Bremner, 1967; Gregorich et al., 1989) by isolation in micropores (Adu and Oades, 1978) and by physical protection within stable macroaggregates (Elliott, 1986).

A relatively recent tool used to study C dynamics in soils is the analysis of the stable isotope ^{13}C. This technique, also known as the natural abundance of ^{13}C or $\delta^{13}C$, is based on the fact that plants using C_3 photosynthesis (Calvin-cycle) incorporate less ^{13}C than plants with a C_4 (Hatch-Slack) photosynthetic cycle and, therefore, differ in their discrimination between ^{12}C and ^{13}C isotopes (Smith and Epstein, 1971). These $\delta^{13}C$ differences are transferred to SOM since the $\delta^{13}C$ values in C_3 plants are around -27‰, while in C_4 plants they are about -14‰ (Balesdent et al., 1987). This natural tracer of *in situ* produced organic C has proved to be useful to study C sequestration into different soil fractions and aggregate sizes (Vitorello et al., 1989; Martin et al., 1990; Angers and Giroux, 1996). The $\delta^{13}C$ technique has been used mainly in long-term studies.

The dynamics of C derived from plants with different metabolisms on soil aggregate stability still need to be tested. Contributions of corn root-derived C to SOM in bulk soil samples with C_3 vegetation history vary with soil management and may not be detectable at the end of the growing season (Balesdent and Balabane, 1992). After five years of continuous corn on a previous tobacco-cultivated soil Ryan and Aravena (1994) estimated that 55% of the soil microbial biomass under conventional till management was derived from C_4-C, while under no-till management the estimated value was 30%. As organic C appears to be relatively uniform across aggregate size fractions, the level of water-stable macroaggregation should provide an estimation of the amount of organic C stored and physically protected in the soil, especially the labile fractions (Angers and Carter, 1996). Organic matter contained in the macroaggregates was shown to be younger than that in the microaggregates (Skjemstad et al., 1990). They found, using the $\delta^{13}C$

technique, that the microaggregates of an Australian soil contained older C than the macroaggregates. In an other experiment, larger C contents in stable macroaggregates in a soil with previous C_3 vegetation, were attributed to the young C_4-derived organic C (Puget et al., 1995). Carbon-13 natural abundance, however, has not been fully applied to the study of the spatial and temporal characteristics of C dynamics in the development and stabilization of soil aggregates which, in turn, may help to elucidate some of these questions on a short-time basis.

Information on the form and persistence of the aggregating constituents and the extent over which they are operative is vital for understanding the reasons for the rapid deterioration of the structure of some soils when continuous row cropping is introduced (Baldock et al., 1987). This information will help design sustainable agricultural systems that can decrease or reverse the trends in soil degradation associated with current cropping practices. This study is an attempt to better understand the relationship between soil aggregates and the retention and spatial distribution of C and N contents within them. The first objective of this study was to assess the influence of soil and plant managements can exert on recent C deposition by contemporary root systems and its contribution to C gradients within individual soil aggregates. A second objective was to investigate the distribution of original C_3-derived and contemporary C_3 and C_4-derived C in water-stable aggregates under different tillage management systems and its interactions with aggregate stability. It was intended to verify whether disturbance (e.g., conventional- *vs.* no-tillage) influence the size distribution of water-stable aggregates and their C contents and compositions, compared to undisturbed soils. Finally, the effects of contemporary cropping of a C_3 plant species on the amount, composition, and distribution of recently released root C in soil aggregates under conditions of water-stress and non-stress was investigated. Crop-water-soil interactions were investigated in terms of their influences on the incorporation of C of recent deposition into soil aggregates and on aggregate physical properties.

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Uniform Separation of Concentric Surface Layers from Soil Aggregates

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Uniform Separation of Concentric Surface Layers from Soil Aggregates

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ABSTRACT

It is well accepted that soil organic matter improves soil structure; however, current methods limit our knowledge of the many physical–chemical mechanisms that control the temporal processes of soil aggregation. A soil aggregate erosion (SAE) apparatus was designed to uniformly remove concentric surface layers of soil aggregates in an enclosed erosion chamber. The SAE was used to test for the presence of concentration gradients within aggregates of different soil types. The SAE method generally improved the separation of ion concentrations between the external and internal layers. Concentrations of C, Ca, and Mg were 8 to 19, 20 to 21, and 29 to 33% lower, respectively, at the external layers of aggregates for Haplaquoll and Hapludalf soils. Removal of surface layers had no effect on the tensile strength of soil aggregates. Therefore, we concluded that the SAE apparatus improves the separation of ion gradients within natural soil aggregates. Identification of the mineralogical, physical, chemical, and biological properties of this improved separation of concentric layers from soil aggregates should lead to further advancements in our knowledge of the functional mechanisms associated with the development and stability of soil aggregates.

SOIL AGGREGATE DEVELOPMENT has been reported to result from the cementing of smaller aggregates into larger aggregates containing various soil components (Dexter et al., 1988). This assembly activity is influenced by soil water, ions, minerals, organic matter, plant roots, and microorganisms, which utilize numerous stabilizing mechanisms (Lynch and Bragg, 1985). The structural stability of soil aggregates and the pores between them affect the infiltration, movement, and storage of water; aeration; erosion; plant root and microbiological activities; and crop productivity (Tisdall, 1996). During the formation of aggregates, many components of the soil are redistributed within the structural unit, developing gradients within individual soil aggregates. Shrinking and swelling processes result not only in the well-known accumulations of fine textural separates but also in variations in chemical and physical properties within each aggregate (Horn, 1990).

Horn et al. (1994) demonstrated external surface layers of aggregates contained more microorganisms than internal regions. These layers also varied in their chemical and physical properties. Consequently, different functions associated with these layered regions within soil aggregates resulted from specific physical and chemical gradients. Additionally, soil aggregation processes depend upon the number of wetting and drying events as well as on the intensity and frequency of shrinking and swelling. Increased numbers of wetting and drying events cause more soil particles to be rearranged within each aggregate, resulting in fewer changes in the hydraulic properties and lower states of free entropy associated with soil aggregation (Horn et al., 1994).

Since plant roots are known to grow preferentially in weak aggregates or in voids between aggregates (McSweeney and Naussen, 1984; Dexter, 1986), certain gradients may develop in the presence of roots. In addition to root growth, preferential flow of soil solutions, gaseous exchange, microbial colonization, and solute sequestration at aggregate surfaces enhance conditions that further develop biological, chemical, and physical gradients from the external layers to the internal regions of soil aggregates (Horn et al., 1994). Methods used to obtain separated layers of soil aggregates include manual peeling (Qureshi and Jenkins, 1987; Horn, 1987; Ghadiri and Rose, 1991; Priesack and Kisser-Priesack, 1993), dry sieving (Ghadiri and Rose, 1991), combined freezing and wet sieving (Kayser et al., 1994; Wilcke et al., 1996), electron probe microanalyses (Qureshi and Jenkins, 1987), electron microscopy (Foster, 1994), ultraviolet photo-oxidation (Ladd et al., 1993), and selective staining of specific cells within resin-impregnated soil sections (White et al., 1994). Manual peeling of soil aggregates is a time-consuming and tedious process and does not provide an opportunity for processing large quantities of samples and is subject to individual errors. Although electron microscopy has provided visual evidence of many aggregate components (Foster, 1994), sample preparation renders them unavailable for further analyses. Recent attempts to investigate the interiors of natural soil aggregates using laser confocal microscopy, by our laboratory, have not been conclusive (H. Taubner, J. Whallon, and A. Smucker, 1994, unpublished data). Therefore, new methods were tested for eroding surface layers from soil aggregates, providing samples for further analyses. A new method needed to be developed that could uniformly produce separates from different regions within soil aggregates. Quantification of these separate components could enhance our understanding of several cementing mechanisms associated with the dynamic soil aggregation responses of different soil types to specific tillage and cropping rotations. Concentric separation of aggregate layers will also contribute to our knowledge of the fate and efficacy of surface-applied materials such as crop residues, pesticide granules and sprays, weed seeds, or fertilizer granules that are controlled by the breakdown and reformation of soil aggregates. A soil aggregate erosion (SAE) device could also be used to study the mechanisms of aggregate life expectancies, e.g., length of time between the formation and breakdown of soil aggregates, as described by Staricka et al. (1992).

The objectives of this study were (i) to improve a mechanized SAE device and compare its quantitative

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efficiency with manual peeling and (ii) verify physical and chemical concentration gradients within soil aggregates from different soil types.

MATERIALS AND METHODS

Equipment Description and Operation

The SAE apparatus was developed by modifying a seed cleaning machine (Agriculex, Inc., Guelph, Ontario, Canada), using the principles proven by an earlier aggregate particle erosion system reported by Schroeder and Smucker (1988). The SAE apparatus (Fig. 1) includes a squirrel-cage fan and chamber (Fig. 1B) and electric motor (Fig. 1A). A wind-control cover (Fig. 1C) is attached at the air inlet, to manually control air flow rates. A deflecting air foil (Fig. 1D) is located at the top of the fan chamber, placing it directly below the base of the removable sample erosion chamber (Fig. 1E). The air foil (Fig. 1D) causes vertical flowing wind to be deflected into a revolving cyclone pattern inside the removable sample erosion chamber (Fig. 1E). Aggregates are retained in the sample erosion chamber by fine 1.0-mm (16 mesh) nylon screens glued onto the base of the chamber and confined in the sample erosion chamber by a similar screen attached to the lower end of the vertical wind duct, directly above the removable sample erosion chamber. The interior of the removable sample erosion chamber is lined with abrasive stainless steel grating materials, assembled from commercially available kitchen grating utensils, and slides into the removable sample erosion chamber and is retained stationary by friction during operation and could be removed from the erosion chamber for cleaning.

Uniform soil aggregates, ranging in widths from 3 to 15 mm, are placed into the sample erosion chamber (Fig. 1E). An upward cyclone pattern of air flow, controlled by the manually adjusted wind-control cover (Fig. 1C), suspends and rotates each soil aggregate. Weak soil aggregates are identified at the beginning of each erosive run by subjecting natural aggregates to the SAE apparatus for 30 s, causing some to split. Split aggregates are discarded, and many of the tips or acute angles of the unbroken aggregates are abraded during this abrasive pre-run treatment. Following a complete cleaning of the SAE, by an air nozzle, selected unbroken and uniform aggregates are comprehensively eroded by exposure to the cyclone air-flow pattern for the time periods required to remove layers of soil materials as concentric shells from the aggregates. Uniform layers of soil material are eroded from each soil aggregate as the air-suspended and rotating aggregates make contact with abrasive surfaces along the walls of the sample erosion chamber (Fig. 1E). Removal of each surface layer by the SAE apparatus results in uniform and time-dependent smaller volumes of the remaining internal portions of each aggregate.

Air containing eroded soil particles is transported to the dust collector (Fig. 1F). The dust collector reduces the speed of air flow, resulting in the deposition of soil particles into the sample collector (Fig. 1G) located at the base of the dust collector. Air exits from the dust collector through a fine 0.20-mm (130 mesh) screen via the air vent (Fig. 1H) at the base of the exhaust port (Fig. 1I). At the end of each run, the dust collector is gently tapped to remove soil particles that adhere to the fine screen at the air exit or on the walls of the dust collector. The entire SAE apparatus is further cleaned between samples by directing high-speed jets of compressed air throughout the chamber.

Run times required to remove soil materials associated with each region of the aggregates depend upon soil type and soil water content and are determined by weighing soil materials that accumulate in the sample collector. The desired amount of soil to be removed from each regional layer is controlled by the duration of run time as presented by the summation curve in Fig. 2. Experience has demonstrated that the external surface layers of air-dried soil aggregates erode more rapidly, producing a linear relationship between run time and summation percentage for each soil type. Following the removal of external layers of aggregates, the next region or zone of the aggregate, which we refer to as the transitional layer, is removed more slowly, producing a nonlinear relationship between time and the accumulation of eroded soil material. This phenomenon appears to be the result of a combination of smaller surface areas of progressively eroded aggregates and more durable layers within the internal regions of aggregates. The internal region of soil aggregates erodes even more slowly, producing a parabolic relationship between run time and summation curve (Fig. 2). More than two concentric layers can be removed from the surfaces of soil aggregates, as depicted

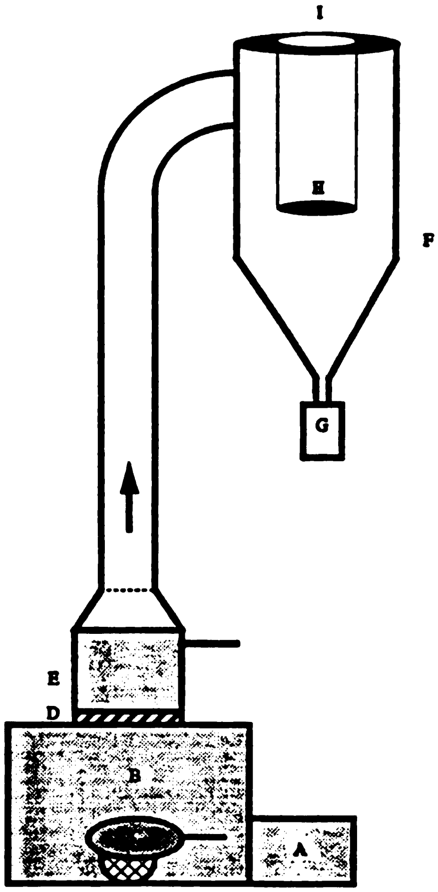

Fig. 1. The pneumatic soil aggregate erosion (SAE) apparatus. Aggregates are placed in the sample chamber (E). An upward cyclone pattern of air flow is established by passing an air stream, generated by the motor (A), fan (B), and air-flow controller (C) through the air foil (D). Eroded soil components are carried by the air stream to the dust collector (F) and accumulate in the sample bottle (G). The fine-mesh screen (H) filters soil particles from the exhaust air (I). Distinct layers can be removed from soil aggregates by stopping the SAE, changing the sample bottle (G), and repeating the surface-abrasion process.

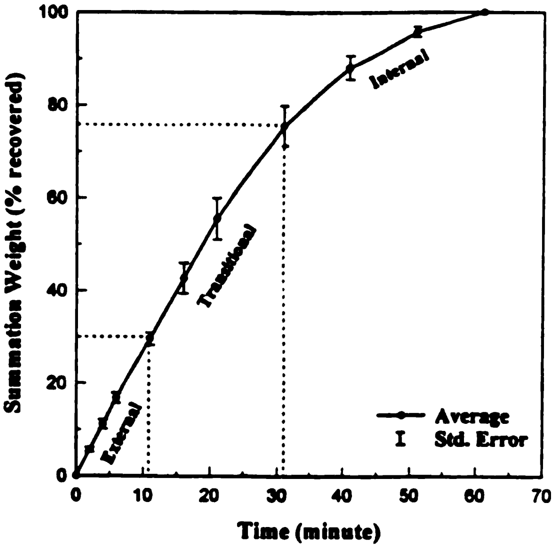

Fig. 2. Summation percentage curve, by weight, of a 60-min abrasion process for removing soil particles from the external layer (first linear portion of graph, 0–11 min.), transitional layer (second linear portion of graph, 11–32 min.), and internal region (parabolic portion of graph, 32–60 min.) of soil aggregates from the Ap horizon of a Conover loam (n = 3; 10 aggregates each).

by the nine data points on the summation graph of Fig. 2. The thickness of each layer can be estimated by the integral equation describing the relationship between mass of clay and the thickness of abraded zones as presented by Kay and Dexter (1990). Their equation can be rearranged as

$$D_e = x\rho Cf \qquad [1]$$

where D_e is the amount (Mg m^{-2}) of eroded material extracted from each concentric layer, x (m), ρ is the bulk density (Mg m^{-3}) of the aggregate layer, C is the total clay content (Mg Mg^{-1}) of the eroded layer from the aggregate, and f is the fraction of the total clay content from the eroded layer during SAE run times from t_1 to t_2.

Soil Sample Preparation and Erosion Protocol

Field soil samples from the Ap horizons of a Kalamazoo loam (fine-loamy, mixed, mesic Typic Hapludalf) and a Colwood loam (fine-loamy, mixed, mesic Typic Haplaquoll) were extracted in larger volumes and transported to the laboratory in foam packing materials. Large soil blocks were gently broken into smaller aggregates (widths <20 mm) and air dried. Chemical compositions of manually peeled internal and external layers of soil aggregates were compared with those mechanically eroded by the SAE apparatus. Ten aggregates, 3 to 5 mm in diameter, were selected and manually or mechanically separated by the SAE method into external layers and internal regions; transitional layers were not analyzed. Aggregates were separated manually by peeling the same quantity of soil (using a sharp knife and gloved hands), by weight, from each aggregate as was eroded by the SAE method of separation. Composite samples were extracted and combined until at least 5 g of soil materials were accumulated for chemical analyses. Exchangeable Ca, Mg, K, and Na were extracted by shaking to equilibrium in 1:10 soil to solution of 1 M NH_4OAc or NH_4Cl. Ion concentrations were assessed by atomic absorption spectrophotometry, as described by Thomas (1982). Organic C was determined by dry combustion, as described by Nelson and Sommers (1982). Where necessary, additional soil aggregates were eroded from concentrically smaller soil aggregates by the SAE method, until similar quantities of soil mass were accumulated for each layer and region, thus minimizing analytical errors due to different sample sizes.

In a second experiment, tensile strengths of air-dried whole or natural aggregates and machine-eroded aggregates containing only the internal region of each soil aggregate, i.e., the transitional layer and internal zone, were measured by the crushing test of Dexter and Kroesbergen (1985) to determine whether removal of surface layers would modify aggregate stability. Samples from the B horizons of the following four soils were used: a Conover loam (fine-loamy, mixed, mesic Udollic Ochraqualf), located at the MSU Agronomy Farm, East Lansing, MI; a Parabraunerde soil (Typic Hapludalf), located in Siggen, Germany; a Podsol soil (Haplorthod), located in Oberwarmensteinach, Germany; and a Podsolige Braunerde (Spodic Dystrochrept), located in Wülfersreuth, Germany. Replicated soil samples for these measurements were collected in cylinders (12 by 12 cm) at close proximity to minimize horizontal heterogeneity. Following the removal of compacted soil adjacent to the cylinder walls, soil volumes were gently separated into natural aggregates of several size classes. Twenty natural soil aggregates (13–15 mm diam.) and twenty aggregates (8–10 mm diam.) whose external layers had been eroded by the SAE apparatus were measured for aggregate strength by individually crushing each between two flat parallel plates. A constantly increasing vertical force was applied to each aggregate by the top plate, which was connected to a computer-controlled stepper motor. The bottom flat plate was the platform of an electronic digital balance, which was connected to a computer. The computer continuously recorded and graphed the applied forces on a monitor. The graphic peak associated with the first major decline in force, applied to the aggregate, was identified by solving for the first derivative of force vs. time. Resistance strength, in kPa, for each aggregate (y), was calculated by the following equation:

$$y = \frac{0.576F}{d^2} \qquad [2]$$

where F is the crushing force required to fracture the aggregate. F is determined by

$$F = bg \qquad [3]$$

where b is the measured balance reading (in kilograms) and g is the acceleration force due to gravity (9.807 m s^{-2}). The parameter d is the effective spherical diameter of each aggregate and can be calculated as $d = (6m/\pi\rho)^{1/3}$, where m is the mass (in kilograms) of the individual aggregate and ρ is the mean aggregate bulk density. Bulk densities of the aggregates were determined by the paraffin-sealing and water displacement methods (Blake and Hartge, 1986).

RESULTS AND DISCUSSION

Soil layers can be uniformly abraded from individual or groups of aggregates by eroding from 1 to 15 aggregates in the SAE apparatus (Fig. 1) for different periods of time. Although the times for removing concentric layers from soil aggregates are different for each soil type, external layers are removed most easily, producing a linear portion (e.g., 2.7% per 60 s) of the erosion summation curve (Fig. 2). Consequently, external layers

Table 1. Organic C and exchangeable C and Mg concentrations by two methods for separating the external and internal layers of soil aggregates from two soils in Michigan.

Soil	Zone	Organic C Manual	Organic C SAE†	Ca Manual	Ca SAE	Mg Manual	Mg SAE
		g kg^{-1}		cmol$_c$ kg^{-1}			
Typic Haplaquoll (Colwood)	External	24.6	21.9	12.00	10.35	2.19	1.70
	Internal	22.0	23.8	11.72	12.48	2.15	2.28
LSD (0.05)		1.8		1.30		0.34	
Typic Hapludalf (Kalamazoo)	External	17.9	17.2	4.27	4.22	0.47	0.45
	Internal	18.3	18.9	4.34	5.08	0.49	0.58
LSD (0.05)		2.2		0.39		0.06	

† SAE = soil aggregate erosion.

of aggregates from Conover soils were designated as the soil removed during the first linear portion (0–11 min) of the erosion summation curve (Fig. 2). The second linear region of this erosion summation graph (11–32 min.) is designated to be the transitional layer between the external layer and the internal volume of aggregates associated with a Conover loam soil (Fig. 2). External and transitional layers of soil eroded from aggregates by the SAE method and the remaining internal volumes or regions of aggregates (indicated by the nonlinear region in the summation curve) were collected separately and analyzed. In this way, the mass of each concentric layer for a given soil was nearly uniform, regardless of the aggregate number and size. It is acknowledged that the uniformity of abraded soil collected at the end of each run time is a function of original aggregate homogeneity. The relatively small error bars associated with aggregate erodibility in Fig. 2 suggest a uniform sampling of soil aggregates. Therefore, the SAE method appears to overcome much of the heterogeneity among aggregates peeled manually.

Chemical gradients between the external layer and internal regions of soil aggregates from two different soil types in Michigan can be identified by the SAE method of separation (Table 1). Higher C contents in the manually separated external layer of the Typic Haplaquoll soil may have resulted from the uneven cutting or mixing, or both, of samples from the two layers during manual removal, as C concentrations in both the external and internal layers were significantly higher and lower than the same layers separated by the SAE. However, average C concentrations for both the external and internal zones were not significantly different, 23.3 vs. 22.9 g kg^{-1}, for both methods of separation. Manual and SAE methods of layer removal demonstrated very similar C concentrations in both zones within the aggregates of the Typic Hapludalf soil type (Table 1). These C deposits, which appear to remain at or near the surfaces of formed aggregates, result in somewhat richer coatings of organic C, compared with inner fractions (Ghadiri and Rose, 1991; Puget et al., 1995). Therefore, differences in the C concentrations for these two soils, at the external and internal regions of aggregates, may have resulted from plant cultures for each site at the time of sampling.

The SAE appeared to be a better method for separating aggregate layers, as concentrations of both Ca and Mg were significantly greater for internal regions of aggregates for both soil types separated by this method (Table 1). Greater differences in the concentration gradients for Ca and Mg, which exist between external and internal regions of aggregates, separated by the SAE apparatus, suggest that this method improves the separation of aggregate regions containing contrasting chemical gradients. More accurate extraction by the mechanical SAE procedure may be due to less cross contamination, a problem often associated with manual methods of aggregate separation. Discrepancies between the two methods may have been caused by differential losses of the fine particles associated with the two methods of separating aggregate layers. Sample losses by the SAE method were ≈10% by weight. Most of these losses resulted from either adhesion of fine particles to the vertical plastic walls of the SAE by static electricity or via the air exhaust. An improved model of the SAE, made of metal and recycled air, is presently being designed to overcome these losses.

Concentrations of Ca, Mg, and K within aggregates of a Udollic Ochraqualf loam were significantly higher in the internal layers, when separated by the SAE method. In contrast, gradients of K were higher for external layers of the Typic Hapludalf soil (Table 2).

Table 2. Exchangeable cation concentrations in the external and transitional layers and the internal zones of aggregates from one soil type from Michigan and one from Germany, separated by the soil aggregate erosion (SAE) method (n = 10).

Soil	Zone	Ca	Mg	K	Na
		cmol$_c$ kg^{-1}			
Udollic Ochraqualf (Conover)	External	1.00	0.14	0.05	0.08
	Transitional	1.02	0.15	0.05	0.10
	Internal	1.49	0.21	0.07	0.08
LSD (0.05)		0.25	0.05	0.02	NS†
Typic Hapludalf (Parabraunerde)	External	7.09	3.26	0.44	0.21
	Transitional	7.20	3.27	0.46	0.21
	Internal	7.00	3.00	0.35	0.22
LSD (0.05)		NS	0.25	0.04	NS

† NS = not significant.

Table 3. Tensile strengths of natural and soil aggregate erosion (SAE)-processed soil aggregates for one soil type from Michigan and three from Germany ($n = 20$).

	Aggregates	
Soil (series)	**Natural**	**SAE**
	kPa	
Udollic Ochraqualf (Conover)	**88 (32)†**	**88 (38)**
Typic Hapludalf (Parabraunerde)	**609 (331)**	**597 (231)**
Haplorthod (Podsol)	**144 (80)**	**148 (50)**
Spodic Dystrochrept (Podsolige Braunerde)	**85 (46)**	**105 (56)**

† Numbers in parentheses are the standard deviation of the means.

No significant ion concentration gradients could be detected between external and internal layers of soil aggregates for the Haplorthod and Spodic Dystrochrept soils from Germany (Table 2). Sodium concentration gradients were both low and not significant for any of the soils tested. Variations in the degree of aggregation and differences in the textural separates identified in aggregate layers have been reported to cause higher concentrations of exchangeable cations in the internal regions of soil aggregates (Horn, 1987).

Tensile or resistance strengths of soil aggregates from four different natural and SAE-abraded soil types of this study indicate that aggregates from the Typic Hapludalf were substantially stronger than the other soil aggregates (Table 3). Tensile strengths of the air-dry soil aggregates in this study were highly variable and no differences were observed between noneroded and SAE treatments of the soil aggregates (Table 3). Therefore, it appears that when artificial soil drying rates of aggregates exceed in situ drying rates for corresponding sampling depths, then the analyses of soil aggregates by any method could provide unrealistic information. Intensive drying pretreatment and other artificial processing of soil aggregates may lead to little or no measurable differences in natural ion gradients.

CONCLUSIONS

The SAE apparatus provides a new approach for removing concentric layers of particles from soil aggregates of different soil types. The SAE can be used to determine ion concentration gradients for natural conditions or specific treatments. Extraction of the surface layers of aggregates can also provide a novel approach for preparing the internal surfaces of aggregates for further analyses by microscopy and other techniques without need for embedding soil volumes. Quantification of the chemical gradients and associated mechanisms that control soil aggregate stabilization is essential to improve management of soil structure and contribute significantly to our understanding of agricultural and environmental soil physics.

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CHAPTER 2

DISTRIBUTION OF ALFALFA ROOT-DERIVED ORGANIC CARBON IN SOIL AGGREGATES IN TILLED AND NON-TILLED ROTATIONS

INTRODUCTION

Soil structure directly influences most physical, chemical and biological processes. Rates of certain soil processes have been shown to be spatially distributed within aggregates (Horn, 1990; Santos et al., 1997). These heterogeneously spaced accumulations may influence root development and the availability of resources to plants. Interaggregate voids, resulting from successive wetting and drying cycles or from the active plant root growth, constitute the dynamic interface between plant roots, the soil matrix, and associated soil solution. These voids are the focus of pedogenic activity and tend to be planar and relatively permanent (Qureshi and Jenkins, 1987).

The composition of external surfaces of aggregates is generally different from that of the bulk soil. Illuviation of clay, organic matter modifications, preferential leaching of acidic solutions, and preferential weathering of aggregate surfaces are some of the processes taking place at the surfaces of soil aggregates (Kayser et al., 1994; Smucker et al., 1996; Wilcke and Kaupenjohann, 1997; Santos et al., 1997). Lower concentrations of organic C and total N in the center of the aggregates have been suggested to result from prolonged anaerobic conditions in the inner zones (Dexter, 1988). Micro-environment variations within and between aggregates influence SOM dynamics since the water content may be greater within aggregates but oxygen is often less than between aggregates

(Sextone et al., 1985). These important but here-to-fore neglected processes need to be considered when designing sustainable soil management practices. Aggregate scale gradients are not taken into account with bulk soil sampling, resulting in improper assessment of plant root environments. Implications for plant growth and development are important as roots will preferentially grow in the macropores separating aggregates (Heil and Buntley, 1965; McSweeney and Jansen, 1984; Logsdon and Allmaras, 1991; Passioura, 1991). Therefore, the root environment will be confined to aggregate surfaces rather than the bulk soil (Qureshi and Jenkins, 1987) and the zones of influence exerted by roots and root hairs are reduced. Under these conditions, the interiors of soil aggregates are not accessible to the roots, contributing minimal quantities of nutrients and soil water (Logsdon and Allmaras, 1991; Passioura, 1991; Amato and Ritchie, 1995). Passioura (1991) pointed out that, in such circumstances, the time needed for water and nutrients to flow to the roots may be such a large proportion of the life of a crop that they will not be used during the growing season. Thus, certain gradients within aggregates may develop. These gradients can be determined by sampling and analyzing individual or groups of aggregates. Management practices that result in higher soil compaction are expected to affect soil-plant interactions at the rhizosphere level and enhance the expression of these gradients. As the density of each structural unit or ped increases, more roots are confined to ped surfaces and unable to extract water from the centers of compacted units (Smucker and Allmaras, 1993).

Much research effort has been made to understand aggregate formation and stability because of its influence on soil quality properties and agricultural productivity.

Methods used to assess the distribution of soil organic matter (SOM) relative to aggregate structure, usually combine the impact of dry or wet sieving and abrasion of large masses of soil. The sampling of surface layers of aggregates has the potential to provide information on the mechanisms associated with the dynamic soil aggregates responses of different soil types to specific tillage and cropping rotations. Santos et al. (1997) reviewed the methods used to obtain separated layers of soil aggregates and presented a soil aggregate erosion (SAE) apparatus that provides quantitative removal of successive surface layers of soil aggregates by mechanical abrasion. The method is of particular interest to study the relationship between organic matter content and stable macroaggregates composition. The extent of surfaces exposed is expected to be related not only to the amount of mechanical energy applied to the sample but also to differences in properties of the surfaces exposed at different time intervals.

The resistance to abrasion of air-dry soil aggregates will be mainly related to clay mineralogy and content, and nature and amount of cementing agents. There is evidence that clay content and some chemical properties are different when surfaces and interiors of aggregates are compared (Horn, 1990; Smucker et al., 1996; Santos et al., 1997). Thus, the amount of soil material abraded from the surfaces associated with the weakest failure zones (i.e., the original exterior of aggregates) will be different from that abraded from surfaces associated with stronger failure zones (i.e., the interior of original aggregates) which are exposed only when greater energy is applied (Kay and Dexter, 1990). Changes in management practices may influence both exposed aggregate surfaces and its erodibility. Conventional cultivation accelerates decomposition of roots, fungal hyphae, which stabilize aggregates larger than 0.25 mm (Oades, 1984).

Few studies have been conducted to determine the influence of root systems on individual aggregates. A relatively new approach, based on the difference in $^{13}C/^{12}C$ ratio ($\delta^{13}C$) between plants with different photosynthetic pathways has been used to partitioning soil organic carbon (SOC) according to its origins (Balesdent et al., 1987; Balesdent and Balabane, 1996). Plants using the C_4 photosynthetic pathway have higher $\delta^{13}C$ (mean values of -12‰) than C_3 plants (-28‰). Delta ^{13}C of SOM is assumed to correspond closely to the ^{13}C content of the plant material from which it is derived. This technique was used in the present study to determine the storage of SOM and turnover of plant residue C in different spatial compartments of soil macroaggregates.

The objectives of this study were: i) to utilize an improved Soil Aggregate Erosion (SAE) apparatus to mechanically remove external surface layers from groups of soil aggregates, and ii) to investigate the effect of crop rotations and tillage practices on SOM content and isotopic composition ($\delta^{13}C$) across soil aggregates.

MATERIALS AND METHODS

Study Site

The soil samples were collected at the Agroecosystem Interactions experimental site that is part of the Long Term Ecological Research (LTER) project at the W.K. Kellogg Biological Station, located in Hickory Corners, 15 miles northeast of Kalamazoo, in southwestern Michigan (42°24'N; 85°24'W). Mean annual temperature is 9°C and precipitation averages 920 mm yr^{-1} (URL:http://lter/kbs.msu.edu/description.html). The experimental site contains Kalamazoo (fine-loamy, mixed, mesic) and Oshtemo (coarse-loamy, mixed, mesic) soil series (Typic Hapludalfs). The soils have a loam-textured surface horizon, a sandy loam lower argillic (Bt) horizon, and a stratified sand and loamy sand textured parent material (Whiteside, 1982). These soils, developed on glacial till, are well-drained with moderate permeability in the upper part and highly permeable in the lower part of the profile. Original vegetation in the region included oak-hickory (*Quercus-Carya*) forests with minor areas of elm-ash (*Ulmus-Fraxinus*) and beech-maple (*Fagus-Acer*) (Küchler, 1964). Selected properties of the soils in the experimental plots are shown in Appendix A1.

The experiment was initiated in the spring of 1986 in a moldboard plowed area under corn/soybean (*Zea mays* L./*Glycine max* (L.) Merr.) rotation for more than 50 years. Experimental plots (40 x 27 m) were installed in a randomized complete block with four replications (Appendix A2). Treatments were conventional-tillage (CT) and no-tillage (NT). Nitrogen fertilizer was broadcast shortly after planting at the rate of 168 kg N ha^{-1}

yr^{-1}. An additional treatment consisting of never-tilled plots under successional grass vegetation (NeTSG) established in order to compare long-term soil structural changes, was also evaluated. The inclusion of these plots in the study serves as a comparative value, as the four replicated plots are not randomized within the main experiment. The never-tilled successional plots were first cleared of timber in the late 50's and have been mowed annually since, which maintained a largely herbaceous community (URL:http://lter/kbs.msu.edu/description.html).

The CT and NT treatments were continuously cropped with the following crops since 1986: corn ('86-88), soybeans ('89), corn ('90-94), and alfalfa (*Medicago sativa* L.) (September '94 - May 96). Alfalfa hay was harvested three times during the 1995 growing season (on 12 June, 22 July, and 1 September) with all alfalfa biomass removed from the plots. Alfalfa plants were spray-killed on 2 May '96. Conventional tillage plots were moldboard plowed in early spring of each year followed by one or two passes of a disk and/or field cultivator. Direct drilling was used in NT plots since the spring of 1985. All plots have received glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine] at the rate of 7 L ha^{-1} in early spring of each year and were treated with pre- and post-emergence herbicides.

Soil sampling and preparation

Four soil samples were collected per plot in May 1996 from the 0-5 and 5-15 cm layers using a flat shovel. Maximum sampling depth of 15 cm was chosen to avoid large variations in bulk density of soil aggregates when approaching the plow pan. Previous measurements in these experimental plots indicated bulk densities of 1.25, 1.45 and 1.19 Mg m^{-3} at the 0-7.6 cm depth, and of 1.28, 1.44 and 1.46 Mg m^{-3} at 7.6-15 cm depth, for CT, NT and NeTSG treatments (Reinert, 1990).

Subsamples were collected in rows free of wheel traffic. Subsamples were pooled together, manually separated along lines of least resistance, air-dried and gently sieved through a 20-mm screen, by hand, to remove stones and plant residues. Dry soil materials were further gently sieved by hand to obtain aggregates ranging from 12.5 to 16 mm across. This size range was chosen because distinction between intact and broken aggregates could be difficult with smaller sizes. Aggregates were selected by hand to avoid inclusion of broken aggregates in the samples, carefully packed on foam containers and kept at room temperature. Aggregates within this size were not available in the 5-cm surface soil of NeTSG plots.

Soil aggregate fractionation

Aggregates, 12.5-16 mm in diameter, were mechanically separated into outer and transitional layers, and the inner region using a modified version of the SAE apparatus described by Santos et al. (1997) that was manufactured by Agriculex, Inc. (Guelph, Ontario, Canada). Briefly, six aggregates from each replicated plot and from each depth were placed into the sample erosion chamber (Fig. 2.1). Samples were first processed for 15 seconds for the aggregates from the NeTSG plots, or 1 minute for the aggregates from CT and NT treatments. Any broken aggregates were removed from the erosion chamber and not included in the final sample. The aggregates were mechanically eroded by removing concentric layers of soil from each aggregate as they rotated against an abrasive stainless steel screen along the walls of the erosion chamber. Removal of surface layers resulted in smaller spherical aggregates with a diameter depending on abrasion time. Eroded samples included primary soil particles and microaggregates of two concentric layers, collected in separate sample bottles, which were placed at the bottom of the funnel (Fig. 2.1). The inner regions of the aggregates remained in the aggregate chamber.

To evaluate the efficacy of the SAE method, the calibration procedure of Amelung and Zech (1996) was used. For calibration, 15 aggregates from each replicated plot and from each depth, were individually peeled for periods ranging from 0.2 to 25 min. Aggregates that abraded completely during the assigned peeling time, were not considered in the calibration procedure. The mass percentage of each aggregate layer was expressed as a linear function of the total aggregate weight, W_i, and the peeling time, t, according to the following equation:

$$\text{\% Aggregate Surface Fraction (ASF)} = \mathrm{a} + \mathrm{b}t + \mathrm{c}W_i \qquad (1)$$

where a, b, and c are regression coefficients (Amelung and Zech, 1996).

The eroded layer thickness (mm) of the outer and transitional regions of the aggregate were inferred from their respective mass percentages. Aggregates were assumed to have a spherical shape. The mean bulk densities of aggregates from each treatment (Table 2.1), were measured using two distinct groups of aggregates, before and following removal of the outer layer. These values were considered for the outer and transitional layers, respectively. The procedure allowed calculation of the mass percentage of the outer and transitional layers which had been eroded for at least 2 minutes for the NeTSG plots, and at least 15 minutes for CT and NT plots, [Eq. 2]. Preliminary abrasion tests indicated lower abrasion resistance for aggregates of the NeTSG plots. The following multiple regression equation was used to estimate the thickness of the eroded layer:

$$\%\ \mathrm{ASF}_{layer} = 100 - W_i^{-1}\, 4\pi/3\ (\, r_{\mathrm{agg}} - layer\ x)^3\ 100\ \rho_{agg} \qquad (2)$$

where r_{agg} and ρ_{agg} are the radius (mm) and density (g 1000 mm^{-3} = g cm^{-3} x 10^{-3}) of the aggregate. Layer x refers to the thickness (mm) of the layer to be removed. By inserting

$$r_{\mathrm{agg}} = [W_i\, \rho_{agg}{}^{-1}\ (0.75\pi^{-1})]^{1/3} \qquad (3)$$

and Eq. 2 into Eq. 1, the following equation is obtained

$$t_{layer\,x} = \mathrm{b}^{-1}\ [100 - W_i^{-1}\, 4\pi/3\ (W_i\, \rho_{agg}{}^{-1}\ 0.75\pi^{-1})^{1/3}\ -\ layer\ x)^3\ 100\ \rho_{agg} - a\ - cW_i] \quad (4)$$

where $t_{layer\,x}$ is the time needed to remove a layer of thickness x from the surface of the aggregates. Equation 4 can also be expressed in terms of layer x:

$$Layer\ x = (W_i\, \rho_{agg}{}^{-1}\ 0.75\pi^{-1})^{1/3}\ +\ \{[(t\, b + a + c\ W_i - 100)^{1/3}]\ /\ (400\ \pi\ \rho_{agg})\} \qquad (5)$$

The effect of aggregate size on the mass percentage of aggregate layers was minimized by using an aggregate size fraction as narrow as possible (12.5-16 mm dia.).

Aggregate bulk density

Bulk densities of aggregates before and after peeling were determined by the clod method (Blake and Hartge, 1986). Each aggregate was suspended by a thread, weighed, and dipped in saran solution until the sample became water proof. The saran-coated aggregates were then weighed once in the air and once in water. Aggregate bulk density (ρ_{agg}) was calculated using the equation:

$$\rho_{agg} = (\rho_w \mathrm{W}_{\mathrm{ods}}) / [\mathrm{W}_{\mathrm{sa}} - \mathrm{W}_{\mathrm{spw}} + \mathrm{W}_{\mathrm{pa}} - (\mathrm{W}_{\mathrm{pa}} \rho_w / \rho_s)] \qquad (6)$$

where ρ_w = density of water, g cm^{-3}; $\mathrm{W}_{\mathrm{ods}}$ = oven-dry mass of aggregate, g; W_{sa} = net mass of aggregate in the air, g; $\mathrm{W}_{\mathrm{spw}}$ = net mass of the sample and saran in the water, g; W_{pa} = mass of saran coating in the air, g; and ρ_s = density of the saran, g cm^{-3}.

Tensile stength of soil aggregates.

Tensile strength of 10-15 aggregates was measured by crushing the aggregates between parallel plates using a pressure gauge (Dexter and Kroesbergen, 1985). Aggregates were oven-dried at 105 °C for 24 h, removed from the oven and allowed to cool in a desiccator, thus preventing atmospheric water absorption. Aggregates were removed one at a time immediately before being crushed. The tensile strength (Y) was calculated for each aggregate using the equation:

$$Y = 0.576\ F / D^2 \tag{7}$$

where D is the aggregate effective spherical diameter and 0.576 is a proportionality constant relating maximum compressive stress to the tensile strength at failure (Dexter and Kroesbergen, 1985). F is the force (N) required to fracture the aggregate and is determined by $F = b\ g$, where b is the reading (in kilograms, kg) and g is gravity (9.807 m s^{-2}). The effective spherical diameter of each aggregate was calculated as $D = (6m/\pi\rho)^{1/3}$ where m is the mass (kg) of the individual aggregate and ρ is the mean aggregate bulk density (g cm^{-3}) of a specific treatment and depth, determined by the methods outlined above.

Total carbon and nitrogen, and carbon isotope ratios

Replicated soil samples from the 3 aggregate regions were ground to < 250 μm using a mortar and pestle and analyzed for total C and total N by the dry combustion technique using a CHN analyzer (Carlo Erba, Italy). Delta $(\delta)^{13}$C was determined by combustion on a Isotope Ratio Mass Spectrometer Model 2020 (Europa Scientific, UK). The δ^{13}C value was calculated from the measured $^{13}C/^{12}C$ ratios (R) of the sample and standard gases as:

$$\delta^{13}C\ (‰) = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3 \tag{8}$$

and expressed relative to the international standard PDB. Soil samples taken in 1994, before alfalfa replaced corn, were used as the reference. The proportion of C derived from alfalfa residues for the period of September 94 to May 96 (x) was calculated as:

$$x = (\delta_{as} - \delta_{cs})/(\delta_{a} - \delta_{cs}) \tag{9}$$

where $\delta_{as} = \delta^{13}C$ value of sample (isolated layer or whole soil from alfalfa planted soil), $\delta_{cs} = \delta^{13}C$ value of the corn-planted soil of the corresponding sample, and δ_{a} = measured $\delta^{13}C$ value of alfalfa residues (-27.9‰).

Statistical analyses

Statistical analyses were performed with the Statistical Analysis System (SAS Institute, 1996). Where appropriate, one-way analyzes of variance (ANOVA-GLM) were run, either comparing bulk samples, outer and transitional layers, and inner regions of aggregates with each tillage, or across tillage within these sample and aggregate components. Fisher's LSD mean separation test was used to determine significant differences among means at 0.05 probability level. A *t*-test and a *F* test were used to compare the regression coefficients of the calibration equations.

RESULTS AND DISCUSSION

Evaluation of the peeling method

Thicknesses of the eroded layers ranged from 1.35 to 2.59 mm for the outer layer and from 1.30 to 3.81 mm for the transitional layers of aggregates from the field replicated treatments (Table 2.1). Thicker outer and transitional layers abraded for the same time period, were associated with soils under NT management. Particle losses ranged from 2.2 to 6.6% by weight. Higher losses were associated with the fractionation of the transitional layer of NT soil aggregates sampled at 0-5 cm depth. The losses were primarily a result of soil particles adherence to internal surfaces of the aggregate chamber and collector funnel of the SAE apparatus, despite a thorough brushing and cleaning between runs for recovery of particles. Nevertheless, the observed losses were much lower than those observed (≈ 10% by weight) by an earlier model of the SAE (Santos et al., 1997). Using a fractionation method based on freezing and sieving, Wilcke and Kaupenjohann (1998) observed mass percentage variations of 10 to 90% for outer layers of aggregates ranging 2-20 mm in diameter.

For a same abrasion time, the amount of soil removed in the transitional layer (Table 2.1) was generally lower than in the outer layer but not always directly associated with higher aggregate bulk density values (Table 2.2). Results might be improved by peeling a smaller number of aggregates or even individual aggregates (data not reported). The disadvantage of peeling individual aggregates is that several subsamples might be required to obtain enough soil for analysis.

Peeling times for removal of similar quantities of soil particles from the surfaces of aggregates ranged from two (NeTSG plots) to 30 minutes (NT treatment at 0.05-0.15m depth) (Table 2.1). Lower aggregate erodibility results in larger amounts of aggregates being abraded during a specified peeling time. Soil aggregates from NeTSG plots showed very low abrasion resistance probably due to a large grass root distribution around and through them. Aggregate surface fractions corresponding to about one-third of their total mass were isolated as outer (31.5%) and transitional (34%) layers after peeling times as short as 2 and 3 minutes (Table 2.1).

Regression coefficients of bivariate regressions of ASF yields *vs.* peeling time (t) and aggregate weight (Wi) are shown in Table 2.3. The regression coefficients b and c indicate the relative contribution of its variables, t and Wi, respectively, to the coefficient of determination, R^2. The regression coefficients are highly significant ($p < 0.01$) for peeling time but not for aggregate weight (Table 2.3). The only exception was for the CT treatment in the top soil layer. The high significance observed for the regression coefficients seems to indicate that the narrow range of aggregate diameter used (12.5-16 mm) was efficient in reducing the variation in mass percentage of ASF. A final conclusion on that is precluded since no other aggregate sizes were tested. Values of R^2 for CT and NT treatments ranged from 0.80 to 0.88 and were significantly different from zero ($p < 0.05$). The lowest R^2 value (0.60), was observed for the NeTSG treatment (Table 2.3). This poor relationship was due to the fact that the aggregates from the NeTSG plots had a very low resistance to abrasion. Since many of these weakly stable aggregates broke down during the assigned peeling time, they were not included in the calculations.

Aggregate bulk density

Whole air-dry aggregates had average bulk densities varying from 1.62 (NeTSG) to 1.79 (CT) Mg m^{-3} while in the inner region the values ranged from 1.51 (NeTSG) to 1.80 (CT) Mg m^{-3} (Table 2.2). Aggregate bulk densities were not significantly different between tillage systems in the topsoil layer. Bulk densities of whole aggregates increased significantly with soil depth for CT, probably resulting from decreased SOM contents. Cultivation of NeTSG plots substantially increased bulk densities of aggregates, especially at depths of 5-15 cm. The lower bulk density of NT soil aggregates at 5-15 cm depth could have been the result of partial incorporation of crop residues into the aggregates and the undisturbed organic debris of previous crops' root systems as suggested by Dao (1993). Aggregate bulk density has been considered to either directly or inversely influence root growth and development. Lower bulk densities of aggregates (15-25 mm dia.) under a grass sod in Texas were attributed to a larger amount of fine roots in the surface layers (Zobeck et al., 1995). Conversely, Voorhees et al. (1971) observed that, as aggregate density decreased, root growth not only increased but roots also became relatively more effective in proliferating and exploring the soil aggregates. Our aggregate bulk densities were 11 to 40% higher than values obtained previously by the core method for these soils (Reinert, 1990). Greater bulk densities of aggregates than from soil cores are the result of greater interaggregate porosities associated with larger soil volumes (Blake and Hartge, 1986).

Tensile strength of soil aggregates

Large differences in tensile strength of aggregates were observed between treatments at each sampling depth (Table 2.2). Tensile strengths were 50 and 40% greater on aggregates from conventional tilled soils at 0-5 and 5-15 cm depths (Table 2.2). In the present study, the physical reinforcement of aggregates by alfalfa root system may have potentially contributed to the increasing strengths of the aggregates during the previous 20 months time period. Increased tensile strengths in CT compared to NT system seems consistent not only with the increase in aggregate bulk density of whole aggregates but also with a potential shorter duration of wet periods in moldboard plowed soils (Kay et al., 1994). The surface layer of moldboard-plowed soils (CT treatment) is expected to experience larger amplitudes in drying events during wetting/drying cycles (Gregorich et al., 1993). Moreover, the closeness of the association between roots and the soil matrix and the intensity of localized soil water extraction by roots will enhance drying-wetting cycles leading to changes in aggregate strength and stability (Van Noordwijk et al., 1993; Materechera et al., 1994).

Total carbon and nitrogen

Within tillage systems, significant differences in total C concentration between aggregate layers within CT and NT tillage systems were only observed for the 0-5 surface soil (Fig. 2.2). In the 0-5 cm aggregates, C concentrations decreased from 22.6 to 10.8 g kg^{-1} from the outer layer to the inner region for CT samples and from 29.9 to 9.7 g kg^{-1} for NT samples (Fig. 2.2). For the 5-15 cm aggregates, C contents decreased from 12.8

(outer) to 9.8 g kg^{-1} (inner) for CT, and from 12.7 to 10.4 g kg^{-1} for NT treatments. Carbon gradients from inner regions to outer layers of soil aggregates ranged from 14.8 to 21.4 g kg^{-1} for NeTSG samples (Fig. 2.2). At the 0-5 cm depth, aggregates from NeTSG plots displayed greatest C concentrations in the transitional layer (Fig. 2.3). Absence of significant differences in total C between CT and NT treatments for the bulk soil (Fig. 2.2), is consistent with previous observations conducted on these plots (Daroub, 1994). Several other studies at various locations, have indicated that management effects on soil C are generally confined to the near surface of soil under NT compared to CT (Blevins et al., 1983; Havlin et al., 1990; Mahboubi et al., 1993; Ismail et al., 1994; Beare et al., 1994). Comparing 20 years of CT and NT continuous corn, Ismail et al. (1994) reported increments of soil organic C ranging from 74% and 3% for 0-5 and 5-15 cm depths, respectively. The main reason for soil C gradients with depth seems to be that plant residues are not mechanically incorporated into the plow layer therefore accumulating at the soil surface. Moreover, tillage increases the rate of organic matter decomposition and C and N mineralization (Blevins et al., 1977).

Soil C concentration in the outer layers of aggregates increased in comparison to that of the inner regions of aggregates by as much as 200% for NT and 100% for CT aggregates collected in the top 5 cm layer (Fig. 2.2). Despite the significant enrichment of C in the outer layer of aggregates of CT and NT soils at the depth of 0-5 cm, a considerable reduction in the amount of C was observed in these treatments, as compared to the NeTSG plots (Fig. 2.3a). No C concentration gradients were observed in aggregates from NeTSG plots, which contrasts with the results reported by Amelung and

Zech (1996) and with the idea that better oxygen supply in aggregate surface layers facilitates SOM turnover by aerobic microorganisms (Sextone et al., 1985). A possible explanation resides in the key role played by grass root systems in NeTSG soils, as they favored distribution and mixing of large quantities of organic materials into the soil (Oades, 1984). This homogeneous distribution of organic materials across aggregates appears to not limit the activity of microorganisms regardless of their location within the NeTSG aggregates, resulting in minimal gradients within aggregates.

The transitional layers and inner regions of CT and NT aggregates showed similar levels of C ($\approx$10 g C kg^{-1} soil) at the 5-15 cm depth (Fig. 2.2) but much lower than the levels for the inner regions of the NeTSG aggregates ($\approx$18 g C kg^{-1} soil) (Fig. 2.3a). These C losses from tilled aggregates can be attributed to the disruption of macroaggregate structures by cultivation, exposing SOM formerly protected within the aggregate structure (Tisdall and Oades, 1982; Beare et al., 1994). As a result, the quantity of organic binding agents, such as carbohydrates, can be expected to decline in cultivated soils with loss of structural stability (Tisdall and Oades, 1982).

The higher C contents in the outer and transitional layers compared to the inner regions of aggregates from CT and NT results partly from recent additions of organic materials from alfalfa roots and root exudates, as indicated by the more negative δ^{13}C ratios (Fig. 2.4). The newly added organic matter seems to have been deposited mainly on or near the surfaces of the aggregates causing the outer layer to be significantly richer in organic C and N contents compared to the inner regions. Fine root residues of annual crops, such as corn, tend to be a high C:N ratio at harvest time since the plants generally remobilize N from vegetative storage tissues to the seeds (Van Noordwijk and Brouwer, 1997). By contrast, perennial crops may have a greater root turnover during the growing season and higher N contents at harvest time. Considering that over 70% of the alfalfa root biomass is concentrated in the upper 20 cm of the soil profile (Jodari-Karimi et al., 1983), the high incorporation of alfalfa-derived C into the surfaces of aggregates observed for the relatively short time period of 20 months, corroborates the observation that the amount of organic material released by roots is related to the total root mass (Shamoot et al., 1968; Tisdall and Oades, 1982). In structured soils, plant roots tend to use available voids which they modify partly or completely by pressure during growth so that contact between roots and soil matrix is increased. Provided that there is a root anchorage immediately behind the root tips (Voorhees et al., 1971), fine roots and root hairs would extend through and around the large aggregates leading to enmeshment of the soil matrix and improved aggregate stability (Tisdall and Oades, 1982).

Wilcke et al. (1996), suggested that preferential sorption of dissolved forms of C from overlying organic layers into the aggregate surfaces could be one of the explanations for increments of soil organic C of 38% to 61% for aggregates collected from the upper 23 cm of a Podzol soil. In the present study, however, contributions from dissolved organic materials resulting from the decomposition of alfalfa shoots were not expected since all material was removed from the plots upon cutting. Despite showing higher total C levels, most of the C in the outer layers of aggregates from NT treatment comes from the previous corn crop, as indicated by the less negative $\delta^{13}C$ ratio values compared to CT (Fig. 2.4). Accordingly, previous corn root measurements indicated that the proportions of roots in Ap horizon were greater in NT than CT plots, by 52% in 1991 and 61% in 1992 (Huang, 1995). Higher bulk densities and lower macroporosities, suggesting a reduction of interaggregate pore space, were also observed in NT plots in November 1989 (Reinert, 1990). Greater root proportions in NT plots can be partly explained by the accumulation or clustering of roots in the more compacted soil layers along the cracks or planes of weakness between aggregates (McSweeney and Jansen, 1984; Vepraskas and Wagger, 1990; Staricka et al., 1992; Smucker and Allmaras, 1993). By occupying these large pores and growing around the larger aggregates, plant roots would greatly enhance C incorporation in outer (29.9 g kg^{-1}) and transitional (22.7 g kg^{-1}) layers of NT aggregates, compared to the bulk soil sample (13.3 g kg^{-1}) (Fig. 2.2). In contrast to NT system, soil loosening caused by moldboard plowing in CT plots, probably facilitated the incorporation of alfalfa-derived C within aggregates. The observed two-fold increase in total C contents of outer and transitional layers of CT aggregates, as compared to the inner zone, suggests that the inner cores of the aggregates at some point in time were aggregates of smaller diameter before plowing took place. Due to their greater tensile strength, small aggregates are more resistant to breakdown than large aggregates (Hadas, 1987). Following tillage, active incorporation of organic matter would enhance enmeshment and deposition of fresh organic materials, roots, and microbial biomass on the surfaces of the initial aggregate (Angers and Chenu, 1998). On a long run, these mechanisms would be further enhanced through root activity and rhizodeposition, associated with faunal activity, wetting and drying, and potential breakage and reformation of the aggregates.

In general the effect of tillage on total N at both depths (Fig. 2.5), was similar to that of C (Fig. 2.2.). In the surface 5 cm there was significantly more total N in NT, while below 5 cm no significant changes were observed (Fig. 2.5). These data suggest that N availability was comparable in NT and CT treatments. Since there were no large differences in N inputs between tillage treatments, the lower N content in the outer layer of CT aggregates indicates greater net N mineralization in the cultivated soil. Cambardella and Elliott (1994) reported changes in residue decomposition due to alteration in soil structure by increased tillage intensity. They observed that no-tillage soils had more labile N associated with macroaggregates than more intensely tilled soils.

Carbon to N ratios are usually higher for NT than CT because younger organic matter (with higher C:N ratio) accumulates near the soil surface under NT (Frye and Blevins, 1997). The mixing of young and old organic matter by plowing would diminish much of the effect of age of SOM on C:N ratio under CT. Carbon to N ratios were similar between tillage systems (Fig. 2.6). A significantly higher C:N ratio for CT was found in the outer layer of aggregates in the surface 5 cm. The C:N ratio in the outer and intermediate layers of CT and NT aggregates were consistently wider than in corresponding bulk soil and inner regions sampled at 0-5 cm depth (Fig. 2.6). The C:N ratio of alfalfa and corn plant materials is about 14 and 58, respectively (Ajwa and Tabatabai, 1994). The high C:N ratios suggests that the accumulating SOC in the external layers of aggregates is not highly processed and probably includes relatively large quantities of particulate organic matter. High C:N ratios can be attributed to incompletely humified organic material and suggests that active binding agents are root hairs and fungi

(Buyanovsky et al., 1994). A different type of organic matter, highly processed and more stable than the one associated with more external layers, would be located inside the cultivated aggregates from cultivated plots (C:N ratios ≈10). The interior of the aggregates is protected from microbial attack by structural organization of the primary particles into aggregates and by the chemical structure of organic matter as it bonds with mineral particles (Buyanovisky et al., 1994). For the 5-15 cm sampling depth, the C:N ratios of CT and NT aggregates did not differ from those of NeTSG plots (Fig. 2.3c).

Soil organic matter associated with the outer and transitional layers may have been processed by soil organisms to a lesser extent than that of the inner regions and bulk soil samples. Thus, the flow of microbial by-products may depend on the location within the aggregate (Monreal and Kodama, 1997). The hypothesis that oxidative decay of SOM is enhanced at the aggregate surfaces and inhibited in inner regions (Amelung and Zech, 1996), does not explain the lower SOC values in the inner regions of cropped soil aggregates in the present study.

Carbon isotope ratios

The average $\delta^{13}C$ of soil under successional grass vegetation across aggregate fractions and bulk soil, at the 0-15 cm depth, was -25.7‰ (Fig. 2.3d), confirming that the inputs of C have been from C_3 vegetation. Mean values (± standard deviation, $n = 4$) of $\delta^{13}C$ of plant shoot and root samples from successional grass vegetation were -27.7‰ (± 0.41‰) and -27.6‰ (± 0.31‰), respectively. There was a slight enrichment in ^{13}C of the soil C compared to the native vegetation. Similar soil ^{13}C enrichments have been reported to result from the discrimination in C isotopes that occur during microbial breakdown (Balesdent et al., 1987), or from the presence of some C_4-type organic matter. The presence of some C_4 weed species in NeTSG may be related to this enrichment.

Surface (0-20 cm) bulk soil samples, taken in 1994, before alfalfa was planted, indicated that, at that time, cropping practices had increased the $\delta^{13}C$ from -25.7‰ (NeTSG) to -22.7‰ (± 1.0‰, $n = 2$) under CT, and to -23.5‰ (± 0.52‰, $n = 2$) under NT. Until 1985, these soils had been primarily under C_4 (corn) and C_3 (wheat/soybeans) rotation for over 80 years. The mixture of these previous crop residues was calculated to have an average $\delta^{13}C$ of -17.1‰ (Collins et al., 1997). Since the difference in $\delta^{13}C$ content between tillage systems is probably not significant, an average $\delta^{13}C$ value of -23.1‰ for CT and NT was calculated. Thus, after 9 years, with estimated productions of 5 Mg C ha^{-1} yr^{-1} for corn residues (6 years) and 3 Mg C ha^{-1} yr^{-1} for other crop residues, the inputs of total soil C by crops planted before alfalfa were estimated to be as much as 30% (Paul et al., 1998).

Surface soil samples taken in 1986 across the entire experimental area, before the experiment was set up, showed an average soil organic C of 7.9 g C kg^{-1} ($n = 98$; Reinert, 1990). At that time, reference soil OC levels (estimated from the 5-15 cm NeTSG treatment, Fig. 2.3a), had decreased by 63%. By the spring of 1989, OC contents for CT and NT were 7.2 and 8.6 g C kg^{-1}, respectively ($n = 12$; Reinert, 1990). These values represented 34 and 40% of the reference OC levels. The samples taken in 1994 contained 8.9 and 8.6 g C kg^{-1} for CT and NT, respectively, indicating no substantial increases in C levels over the 5-year period.

The introduction of alfalfa in the continuous corn cropping system allowed us to analyze the distribution of the new C_3-C in the soil. Though not stratified by depth, $\delta^{13}C$ data from samples collected in 1994 in CT (-22.7‰ ± 1.0‰, *n* = 2), and NT (-23.5‰ ± 0.52‰, *n* = 2) plots were used as baselines to estimate inputs of alfalfa-derived SOC in the bulk soil samples taken in 1996. $\delta^{13}C$ values (± standard deviation, *n* = 2) for outer, intermediate, and inner regions of these soil aggregates were, -23.5‰, -24.3‰, and -23.7‰ for CT and -23.1‰, -23.8‰, and -23.4‰ for NT, respectively. After 20 months of alfalfa cropping, $\delta^{13}C$ in CT bulk soil samples were similar in the top 5 cm (-22.7‰ *vs.* -22.8‰) and at the 5-15 cm depth (-22.7‰ *vs.* -23.5‰). Alfalfa-derived C was not detected in the bulk samples under NT treatment. Changes in $\delta^{13}C$ are not generally detected in such a short time span. However, when the isotopic composition was measured in the concentrically layered fractions separated from individual macroaggregates, a consistent pattern of deposition of alfalfa-derived C was observed between aggregate layers. The $\delta^{13}C$ signature decreased (became more negative) in the outer and transitional layers of aggregates (Fig. 2.4). On average, for aggregates sampled in the 0-5 cm soil depth of CT and NT plots, 67 and 43% of the C associated with outer and transitional layers of macroaggregates, respectively, were derived from alfalfa, whereas no new C was found in the inner regions of the aggregates (Fig. 2.7). Gradients of $\delta^{13}C$ with soil depth in CT treatments may have occurred due to the absence of moldboard plowing for nearly 2 years, during alfalfa growth. The accumulation of alfalfa root-derived C was surprisingly high. Perennial legume forages have been associated with increases in aggregate stability and have the potential to favor soil C storage on a long-term basis. A short-term increase in aggregate stability after only two years of alfalfa cropping did not reflect significant increases in SOC of bulk soil samples, in neither our studies (Fig. 2.2) nor those reported by Angers (1992). Studying the incorporation of corn-root C into soils, Balesdent and Balabane (1996), observed a large accumulation of below ground C, probably resulting from a high below ground production and a slow biodegradation of root-derived material. In this study, the very active fine root production by alfalfa, along with a rapid root decomposition rate (Rasse and Smucker, 1998a, b) potentially explains the high proportion of alfalfa-derived C on aggregate surfaces.

A large proportion of the recently deposited organic material is accumulated predominantly in macroaggregates (Angers and Giroux, 1996; Puget et al., 1995) supposedly being responsible for increased aggregate stability of these fractions. This organic matter is highly labile, easily available to heterotrophic microbes, and consequently subject to potential decomposition upon macroaggregate disruption (Golchin et al., 1997; Angers and Carter, 1996). Thus, increased macroaggregate stability is a transient process and its maintenance strongly depends upon continuous additions of C sources to the soil matrix. The incorporation of young alfalfa-derived C in outer and transitional aggregate layers in our study seems to confirm the observation of Besnard et al. (1996) that, with cultivation, a partial breakdown and reformation of soil macroaggregates may occur. In the present case, the outer and transitional layers could have been developed over the 20-month period of alfalfa on pre-existing smaller macroaggregates, which later became inner regions of larger macroaggregates. The initial steps of this process involve rhizosphere activity, which enhances microaggregation by extensive root exudation, and cell debris and mucigel production (Domaar and Foster, 1991; Foster, 1994). Residues of mucigel products and root hair are mainly on the surfaces of already formed aggregates and are rapidly colonized by associated microbial populations. The microflora and its by-products are strongly adhesive to mineral particles (Angers and Chenu, 1998). This process would lead to the formation of 20-250μm microaggregates (Golchin et al., 1997) which would then be surrounded and enveloped by fungal hyphae, root residues, illuvial clays, etc., into concentric layers which form larger macroaggregates of several millimeters in diameter.

The high degree of fragmentation of the particles removed by the fractionation technique prevented a further fractionation of each layer and region of the aggregates. Techniques to discriminate for the contributions of plant- and microbial-derived C among different sized fractions, within each layer, will provide additional information about the origins of organic matter in macroaggregates and provide additional mechanisms associated with their formation, stabilization, and degradation. Successive measurements of ^{13}C values in compartments of aggregates of several sizes across crop rotations and tillage practices will assist in the determination of aggregate life expectancies and possible microniches of SOM and associated microbial activities.

CONCLUSIONS

Quantities of C continuously deposited onto soil aggregates can be considered one of the major factors relating crop and soil management systems to soil structure. The results of this experiment indicated greater concentrations of C located in the outer or surface layers of soil aggregates. Accumulation patterns of recently deposited C in aggregates were shown to be highly tillage-specific with differences being reflected on the form and amount of carbon entering the soil and its spatial distribution in the soil aggregates. $\delta^{13}C$ analyzes of CT and NT soils demonstrated that more C from a contemporary alfalfa crop was deposited on surface of aggregates from CT than from NT. Interior regions of aggregates from NT soils remained less negative than soils from CT treatments. Accumulations of C_3-C were greater for aggregates sampled near the soil surface. The differences in SOC between layers may be related to differences in vertical distribution of C resulting from the two different tillage systems. No-tillage management appeared to be more effective at accumulating or slowing the decomposition of newly incorporated C and N in soil aggregates. The results indicate that, on a short-term basis, the below-ground plant system has an important role in the site-specific deposition of SOC and the dynamics of structure. The novel small scale sampling design used in these studies provided opportunities for quantifying accumulations of organic matter in soil aggregates and its dynamic interactions within soil structural units, as affected by crop rotations.

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Table 2.1. Peeling time, layer thickness, aggregate surface fraction (ASF, as mass % of the whole aggregates), and losses associated with the peeling procedure of outer and transitional layers of soil aggregates (12.5-16 mm dia.) from conventional tillage (CT), no-tillage (NT), and never-tilled successional grass (NeTSG) treatments at 0-5 and 5-15 cm depths. Means ± standard error; n =4.

Treatment	Outer layer[†]				Transitional layer[†]				Overall
	Time	Thickness	ASF	Loss	Time	Thickness	ASF	Loss	Losses
	min	mm	---------- % ---------		min	mm	---------- % ----------		-------- % -------
					0 - 5 cm depth				
CT	15	1.58±0.10	37.0±6.4	2.6±0.6	15	1.45±0.25	19.9±1.8	2.9±0.3	5.5±0.4
NT	15	2.05±0.12	29.8±3.4	2.1±1.5	15	1.87±0.16	31.1±5.6	4.5±1.5	6.6±1.5
					5 - 15 cm depth				
CT	15	1.35±0.05	33.0±2.6	1.3±0.1	15	1.30±0.12	21.6±1.0	1.2±0.1	2.5±0.2
NT	30	2.59±0.81	36.8±7.8	0.7±0.4	15	2.09±0.60	26.2±3.6	1.5±0.1	2.3±0.3
NeTSG	2	1.50±0.15	31.5±5.3	1.4±0.4	3	3.81±0.78	34.0±3.8	2.9±1.5	4.3±1.9

[†] Estimates of the thickness of outer and transitional layers were based on average bulk density values of whole aggregates and inner regions of aggregates, respectively (Table 2.2).

Table 2.2. Bulk densities and tensile strengths of air-dry soil aggregates (12.5-16 mm dia.) from conventional tillage (CT), no-tillage (NT), and never-tilled successional grass (NeTSG) treatments at 0-5 and 5-15 cm depths. Means ± standard error.

Treatment	Bulk density				Tensile strength	
	Whole aggregate		Inner region		Whole aggregates	
	n	Mg m^{-3}	*n*	Mg m^{-3}	*n*	kPa
			0 - 5 cm depth			
CT	17	1.69 (0.02)	15	1.80 (0.02)	39	160 (11.1)
NT	16	1.67 (0.02)	13	1.78 (0.02)	49	107 (6.8)
			5 - 15 cm depth			
CT	16	1.79 (0.02)	15	1.63 (0.02)	45	177 (9.8)
NT	16	1.70 (0.01)	15	1.69 (0.01)	48	126 (7.2)
NeTSG	16	1.62 (0.02)	7	1.51 (0.05)	35	111 (8.5)

Table 2.3. Comparison of regression coefficients (a, b, and c) of the calibration equations of the form (ASF = a + b*t* + c*Wi*) for aggregate surface fraction (ASF), and the variables time (*t*) and aggregate initial weight (*Wi*) for soil aggregates from conventional tillage (CT), no-tillage (NT), and never-tilled successional grass (NeTSG) treatments at 0-5 and 5-15 cm depths.

Treatment	a	b	c	$R^{2\dagger}$	ASF_{max} (%)	*n*
			<u>0 - 5 cm depth</u>			
CT	13.68	2.78^{**}	-2.05^{**}	$0.81^{\ddagger}$	77.05	15
NT	16.92	3.25^{***}	-1.94	$0.88^{\ddagger}$	88.80	13
			<u>5 - 15 cm depth</u>			
CT	12.88	2.47^{***}	-1.87	$0.88^{\ddagger}$	81.13	15
NT	3.77	1.83^{***}	0.69	$0.80^{\ddagger}$	67.98	14
NeTSG	37.17	19.85	-9.09	0.60	98.90	7

**, *** Significant (single-tail *t*-test) at the $p < 0.05$ and $p < 0.01$ levels, respectively, for evaluating the uselfulness of the variables *t* and *Wi* to predict ASF.

† Coefficient of determination.

‡ Significantly different from zero ($p < 0.05$, *F* test).

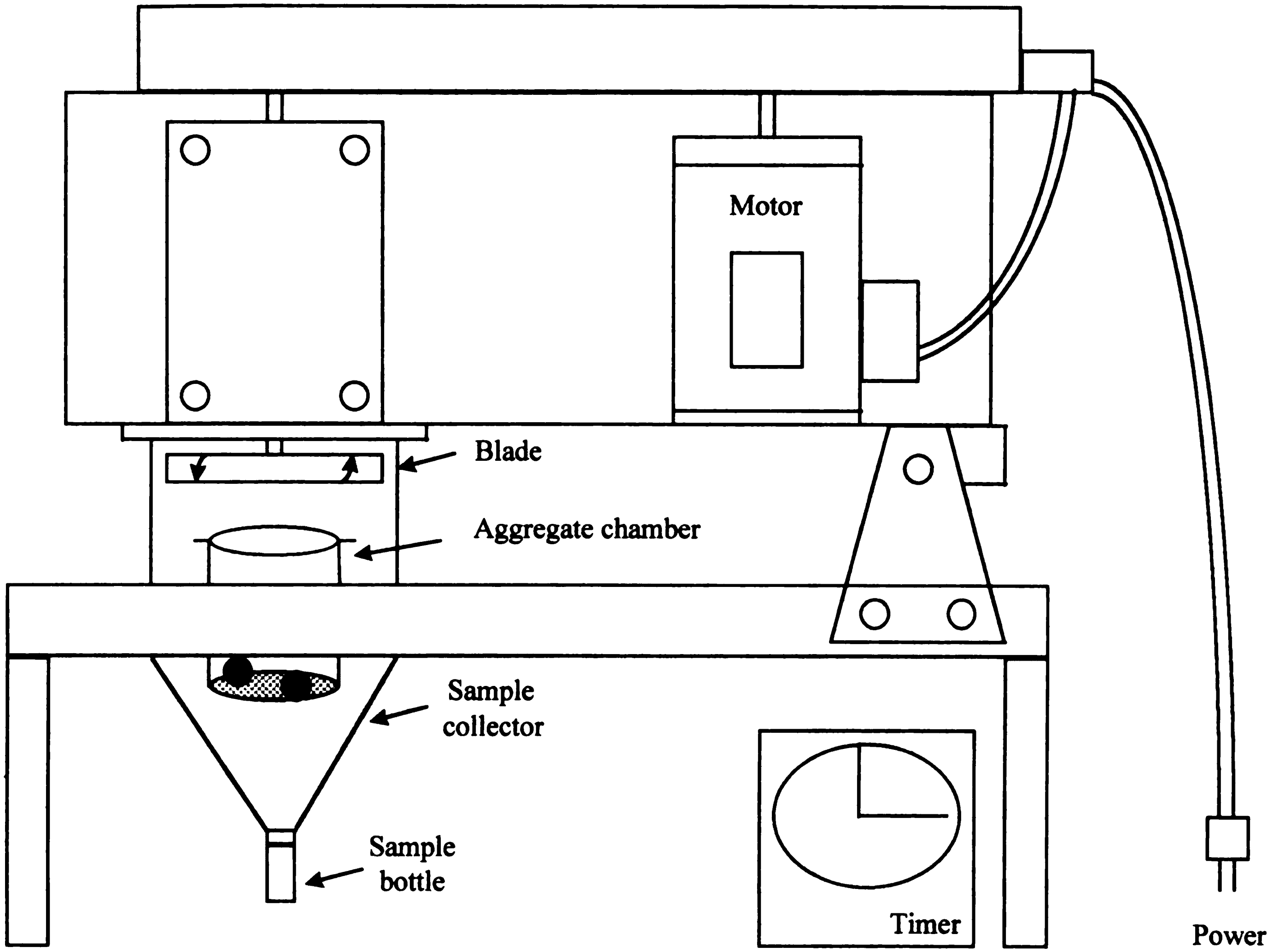

Figure 2.1. Diagramatic representation of the improved soil aggregate erosion (SAE) apparatus. Aggregates are placed in the removable aggregate chamber. A downward cyclone pattern of air flow is established by a blade connected to a motor and positioned on the top of the chamber. Eroded soil particles are deposited in the sample collector and accumulate in the sample bottle. As in the previous SAE model (Santos et al., 1997), distinct layers can be separated from soil aggregates by stopping the SAE, changing the sample bottle, and repeating the process.

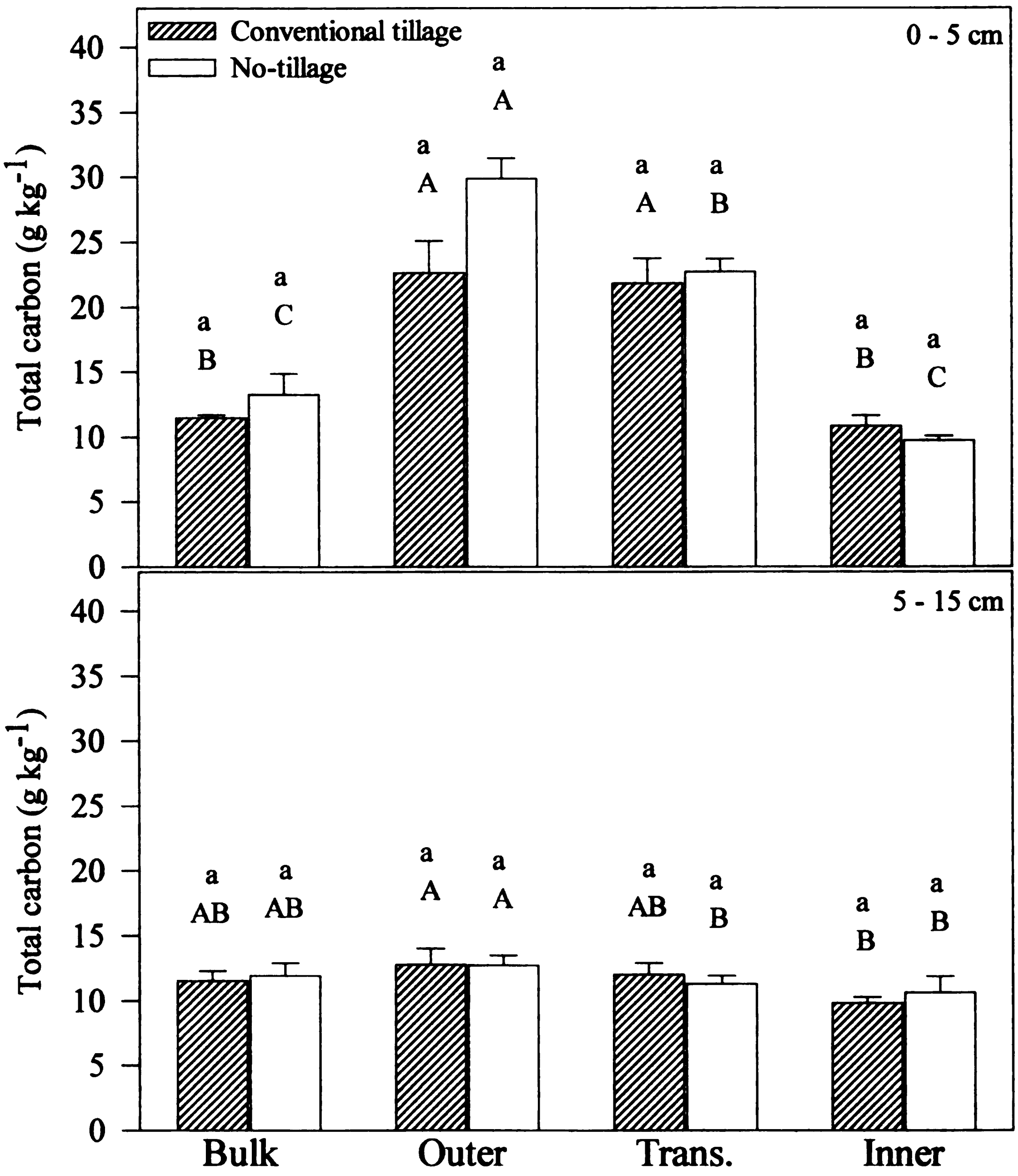

Figure 2.2. Total carbon contents of bulk soil samples, outer and transitional layers, and inner regions of soil aggregates from conventional tillage (CT) and no-tillage (NT), at 0-5 and 5-15 cm depths. Values followed by the same uppercase letter within a tillage treatment and between soil and aggregate samples, and by the same lowercase letter within soil and aggregate samples and between tillage treatments are not significantly different at $p > 0.05$ according to Fisher's LSD mean separation test.

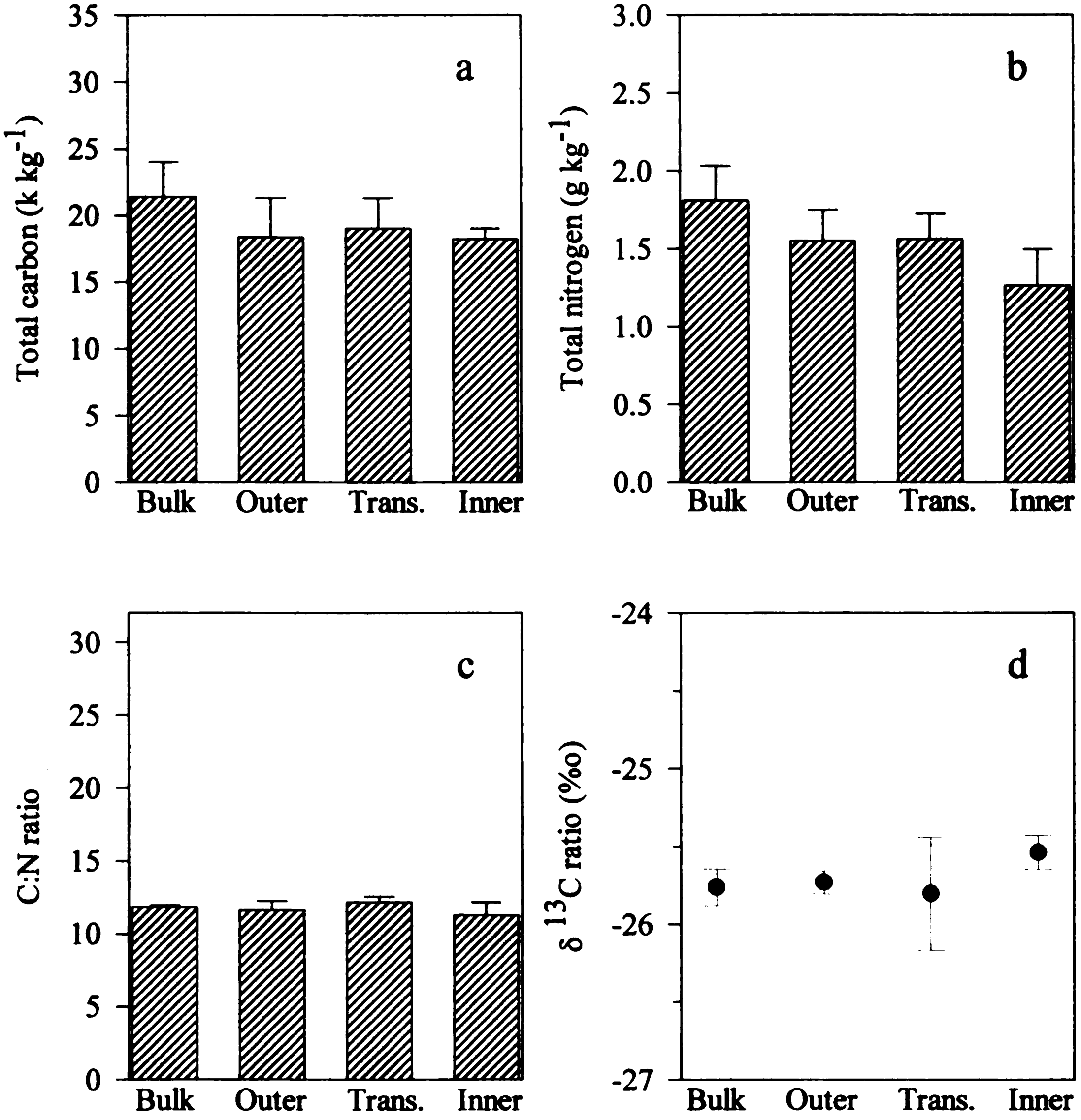

Figure 2.3. Total carbon (a), total nitrogen (b), C:N ratio (c), and δ^{13}C ratio (d) of bulk soil samples, outer and transitional layers, and inner regions of soil aggregates from the never-tilled successional grass (NeTSG) treatment, at 5-15 cm depth. Means ± standard error; $n = 4$.

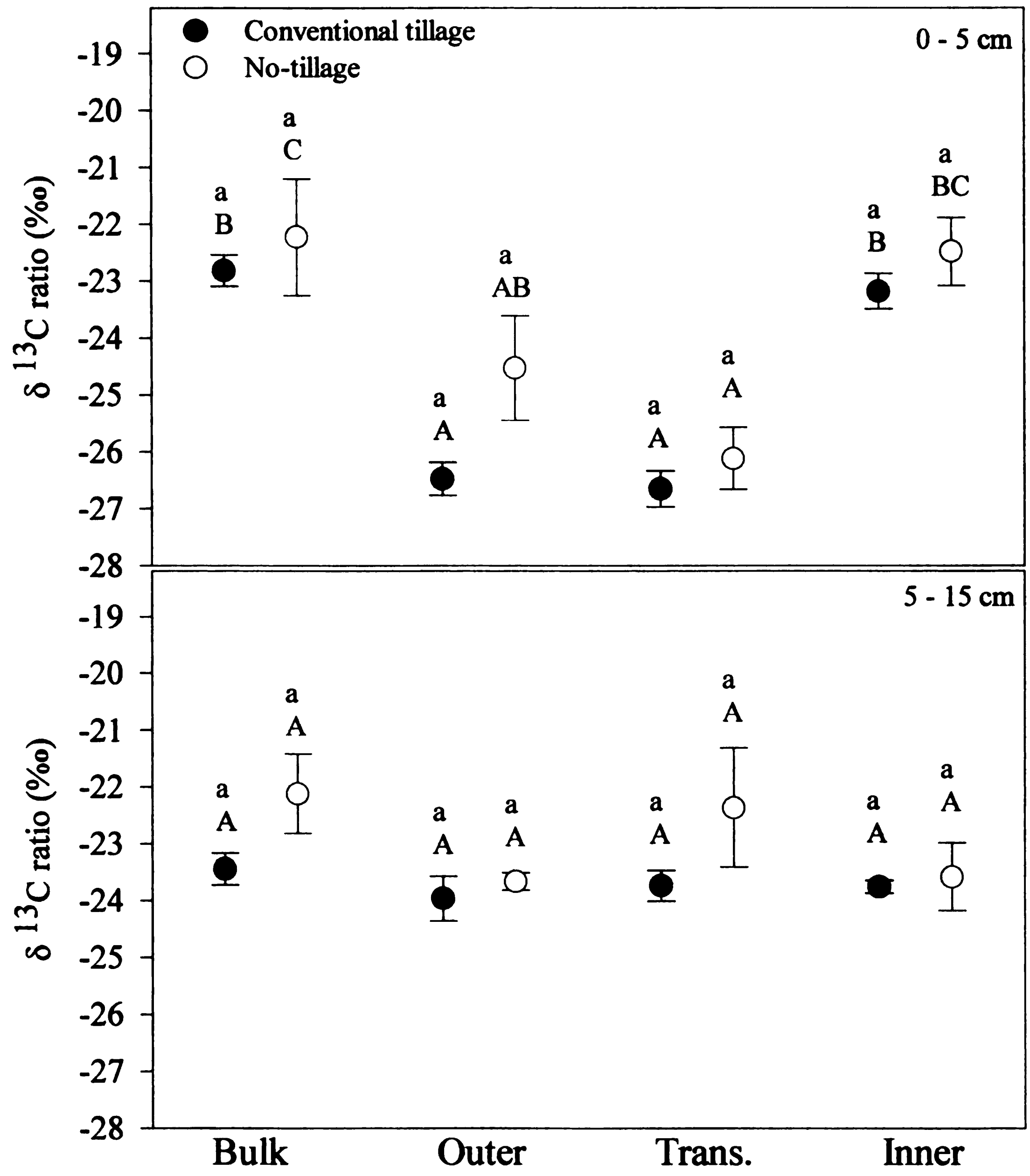

Figure 2.4. δ^{13}C ratios of bulk soil samples, outer and transitional layers, and inner regions of soil aggregates from conventional tillage (CT) and no-tillage (NT), at 0-5 and 5-15 cm depths. Values followed by the same uppercase letter within a tillage treatment and between soil and aggregate samples, and by the same lowercase letter within soil and aggregate samples and between tillage treatments are not significantly different at $p > 0.05$ according to Fisher's LSD mean separation test.

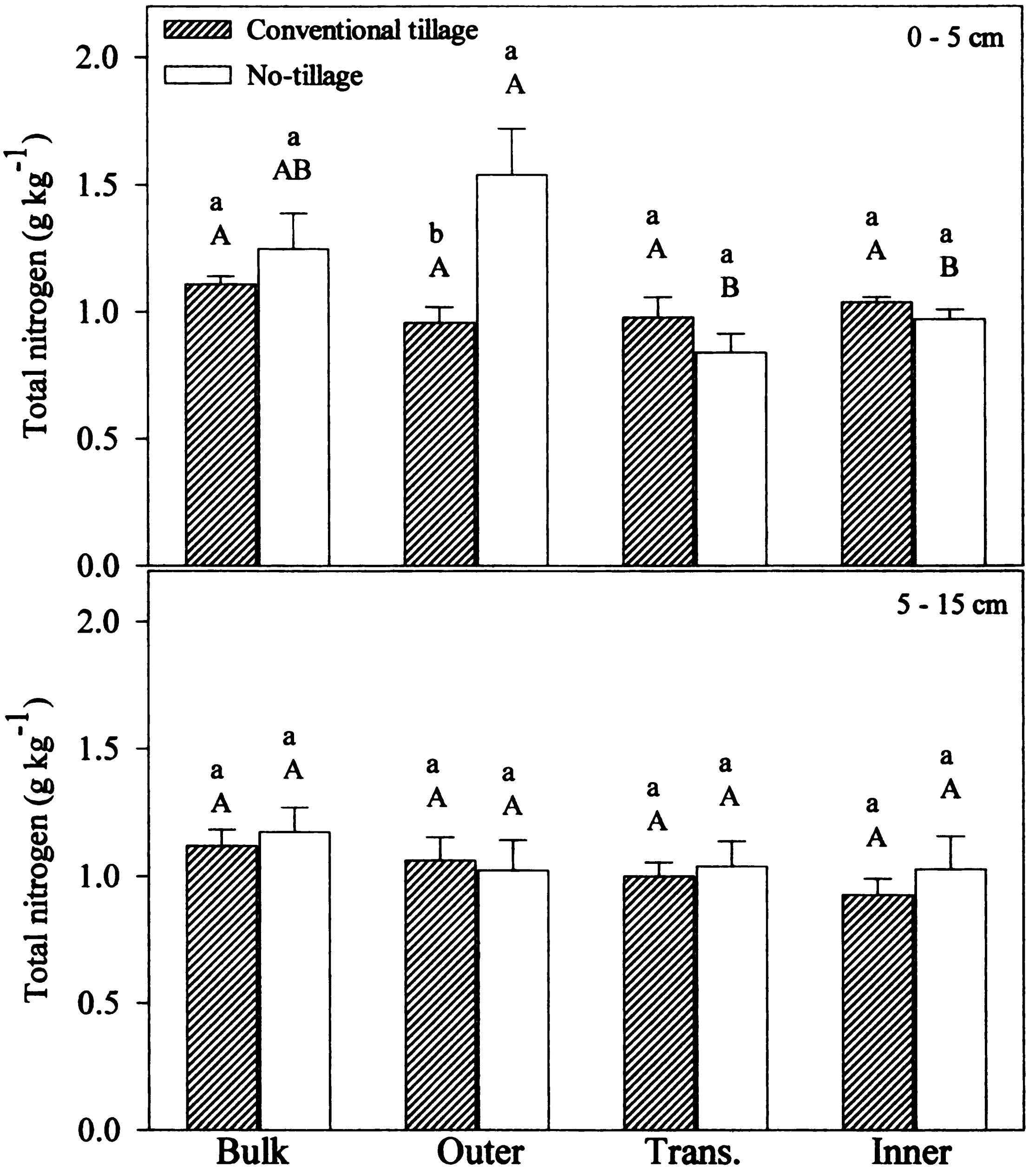

Figure 2.5. Total nitrogen of bulk soil samples, outer and transitional layers, and inner regions of soil aggregates from conventional tillage (CT) and no-tillage (NT), at 0-5 and 5-15 cm depths. Values followed by the same uppercase letter within a tillage treatment and between soil and aggregate samples, and by the same lowercase letter within soil and aggregate samples and between tillage treatments are not significantly different at $p > 0.05$ according to Fisher's LSD mean separation test.

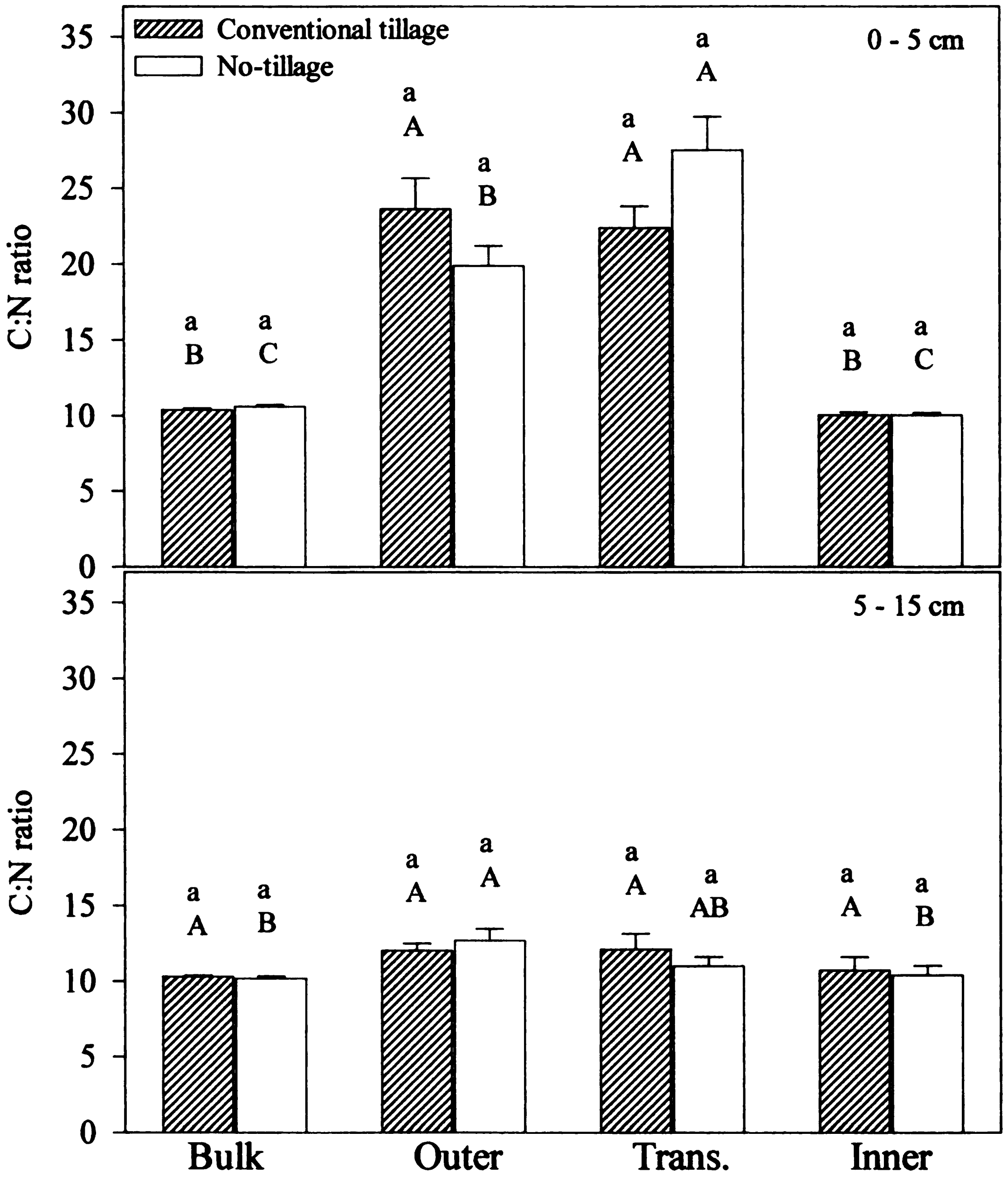

Figure 2.6. C:N ratios of bulk soil samples, outer and transitional layers, and inner regions of soil aggregates from conventional tillage (CT) and no-tillage (NT), at 0-5 and 5-15 cm depths. Values followed by the same uppercase letter within a tillage treatment and between soil and aggregate samples, and by the same lowercase letter within soil and aggregate samples and between tillage treatments are not significantly different at $p > 0.05$ according to Fisher's LSD mean separation test.

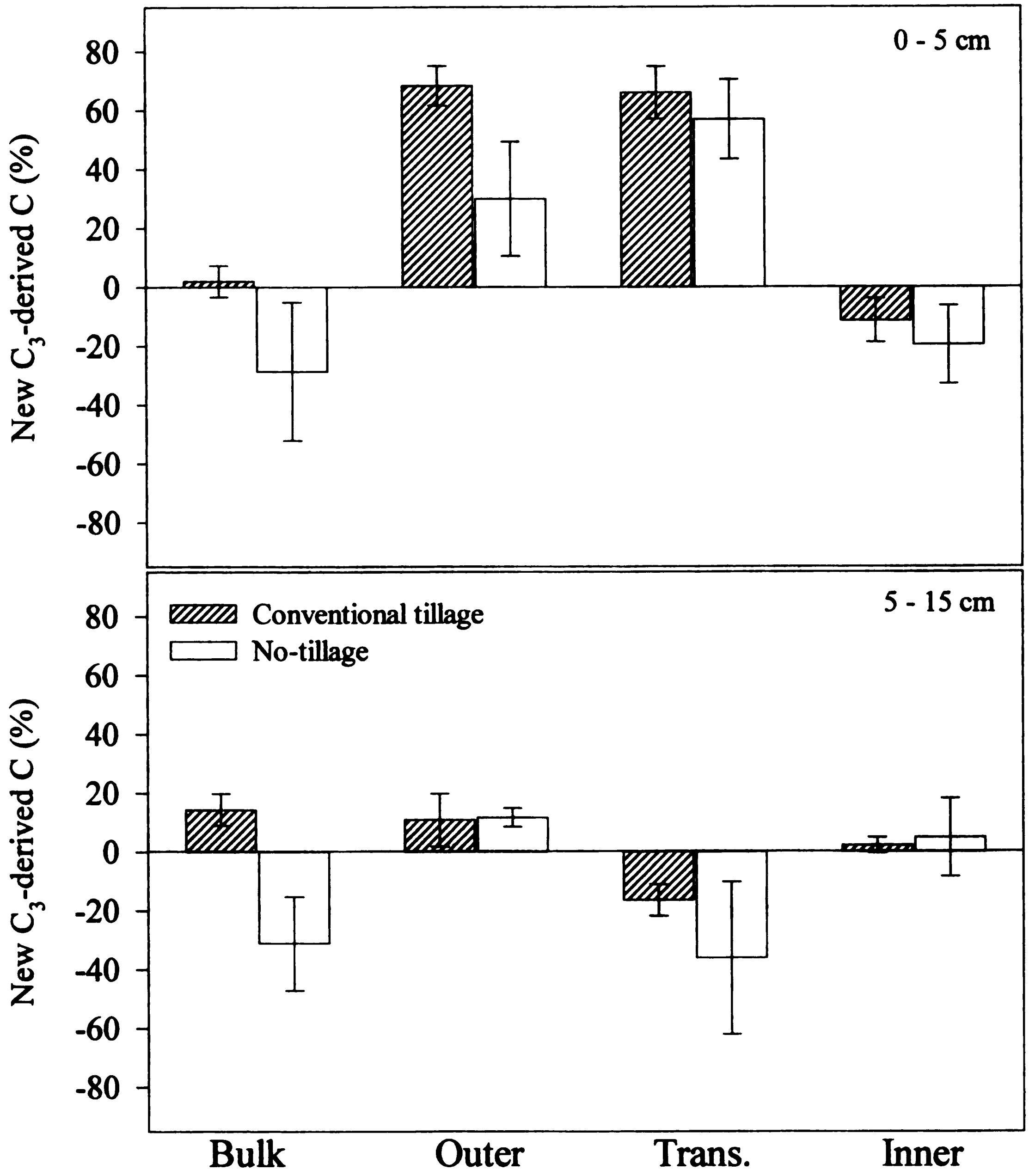

Figure 2.7. Percentage of new C_3-derived carbon in bulk soil samples, outer and transitional layers, and inner regions of soil aggregates from conventional tillage (CT) and no-tillage (NT), at 0-5 and 5-15 cm depths.

CHAPTER 3

MODIFICATIONS OF DISTRIBUTION AND COMPOSITION OF ORGANIC MATTER IN WATER-STABLE AGGREGATES OF UNDISTURBED AND CULTIVATED AGROECOSYSTEMS

INTRODUCTION

Organic matter content in soils is the result of a balance between rates of its loss through biological oxidation and its storage after decomposition of plant shoot and root materials incorporated into the soil. Accumulation and preservation of soil organic matter (SOM) is generally affected by management practices that result in larger amounts of biomass such as crop choice, fertilization levels, and climate conditions that enhance residue decomposition and SOM transformations (Beare et al., 1994; Reicosky et al., 1995). Disturbed or cultivated soils generally contain greater proportions of SOM in the finer aggregates and particle fractions indicating that macroaggregates are more susceptible to disruptive forces imposed upon the soil during cultivation (Angers et al., 1993; Cambardella and Elliott, 1993). Organic C of more recent deposition has been suggested to be the temporary binding agents between microaggregates and the major SOM pool depleted upon cultivation of native grassland soils, resulting in 25-50% reduction in macroaggregation (Elliott, 1986; Gupta and Germida, 1988). Reduced- or no-tillage management systems have been shown to improve soil aggregation (Carter, 1992; Beare et al., 1994) and reverse the decline in SOM as a result of slower biomass decomposition and mineralization (Reicosky et al., 1995; Lal and Kimble, 1997).

Restoration or maintenance of optimum levels of soil productivity requires a better understanding of how soil management affects the stabilization and storage of SOM, soil biology and aggregation processes. In recent years, the potential detrimental effects of excessive tillage operations on soil properties and agricultural sustainability as they influence environmental quality have become a concern. No-tillage, reduced tillage, and other conservation tillage practices have been adopted as alternatives to the conventional intensive plowing practices. Benefits of conservation tillage practices include maximization of surface residue retention and enhancement of OM levels near the soil surface, resulting in reduced evaporative losses, increased rainfall infiltration, reduced water runoff and water erosion hazard (Blevins et al., 1998). Conservation tillage affects C cycling by increasing soil organic carbon content and its distribution within the soil profile, among soil aggregate fractions, and the rate of SOM turnover by soil biota (Blevins et al., 1998). In the long run, conservation tillage practices also promote reduction in energy, labor, and production costs (Blevins and Frye, 1993). It has been stated that no-tillage management may be the single most promising technology that can be applied to agroecosystems that combines economic viability through high crop productivity and increased C storage in soil, due to lower CO_2 emission to the atmosphere (Dick and Durkalski, 1998).

Several studies have been conducted to evaluate the effects of cropping and conservation tillage management on physical properties. Aggregate stability and size distribution show significant short-term variations with cropping and tillage practices even during a growing season. Soil aggregation is commonly measured with a wet-sieving procedure (Kemper and Rosenau, 1986), which provides information on the potential disruption and fractionation of aggregates under the combined impact of water and abrasion. This technique is often used for evaluating the effects of conservation tillage practices on soil quality because of its relationship to resistance of soil to erosion. Physical fractionation according to particle size has been used to study the distribution, composition and stabilization and turnover of SOM as related to its location in the soil aggregates (Elliott and Cambardella, 1991; Puget et al., 1995; Angers and Giroux, 1996).

Isotopic ^{13}C fractionation has been successfully used to investigating the fate of recent inputs of OM to soil and estimate SOM dynamics associated with whole soils and aggregate size fractions (Balesdent et al., 1990; Puget et al., 1995; Angers and Giroux, 1996; Jastrow et al., 1996). Physiological differences in enrichments of ^{13}C by C_3 and C_4 plants allows one to distinguish recently-incorporated SOM by the latest crop from the older SOM of previous vegetation provided that different plants are cultivated in a sequence (Balesdent and Mariotti, 1996). The $^{13}C/^{12}C$ ratio of SOM corresponds closely to that of the original vegetation from which it was derived. Based on these C isotope ratios, organic matter can be either C_4-derived (high, or less negative $\delta^{13}C$, e.g. -12‰) or C_3-derived (low, or more negative, $\delta^{13}C$ e.g. -28‰) (Boutton, 1996). Relatively little information is available on the combined effect of tillage practices and cropping variations on the organic matter distribution and fractionation of soil aggregates. In this study, the contribution of contemporary C_3- and C_4-derived tilled crop C in stable aggregates of a loam soil originally under C_3 vegetation was investigated. The amount and fate of recently deposited C in soil aggregates of an undisturbed grass soil converted to no-till C_4 cropland and its effects on aggregate stability were also assessed.

MATERIALS AND METHODS

Study site

Soil samples were collected from the Agroecosystems Interactions Site established in 1986 at the W.K. Kellogg Biological Station (KBS), located in southwest Michigan (42°24' N, 85°24' W). The soils in the experimental site are well drained [Typic Hapludalfs either Kalamazoo (fine-loamy) or Oshtemo series (coarse-loamy)], mixed, mesic developed on glacial outwash (Whiteside, 1982). Mean annual temperature is 9° C and precipitation averages 920 mm year^{-1} (URL:http://lter.kbs.msu.edu/description.html). The area was first cultivated during the last decades of the last century and have been cropped with maize for most of that period (Robertson et al., 1993). Selected soil properties of the surface horizons are shown in Appendix A1.

The experimental design is a randomized complete block with four treatments and four replicates. The treatments are: two tillage (conventional and no-tillage) by two nitrogen fertilization levels (0 and 168 kg N ha^{-1} yr^{-1}). The no-tillage areas have not been tilled since the spring of 1985. The treatments were applied to large plots of 40 by 27 m (Appendix A2). Two additional treatments were established on a directly adjacent site which had not been tilled after clearing of the native oak-hickory (*Quercus-Carya* spp.) forest in the late 1950's. The present successional vegetation in these plots is dominated by herbaceous perennial dicots and grasses, including *Trifolium pratense*, *Rosa* spp., *Medicago lupilina*, *Achillea millefolium*, *Bromus* spp. and *Poa* spp. (Robertson et al., 1993). The additional treatments were established in a randomized complete block design with four replicated plots of 15 by 40 m with never-tilled successional grass (NeTSG) and never-tilled successional with maize (NeTSM) treatments. Neither treatment in the never-tilled area received any type of fertilizer. In the NeTSG treatment vegetation is clipped once each year (late fall) with no removal of residues. The CT treatment involved moldboard plowing to a depth of 20 cm each spring, followed by secondary tillage with a disc cultivator as needed. For both CT and NT, annual field operations included pre- and post-emergence herbicide treatment, and post-emergence N fertilization.

Sample collection

Soil samples were obtained in the spring of 1997 from the N-fertilized CT and NT treatments, and from the NeTSG and NeTSM treatments. Four subsamples were taken with a flat shovel in each of the four replicated plots per treatment, from the 0-5 and 5-15 cm layers. Samples were pooled per plot, gently broken apart along their planes of weaknesses, air-dried and gently manually sieved through a 6.3-mm sieve. Subsamples of air-dry soil were weighed and oven dried at 105 °C for moisture determinations.

Aggregate size distribution and stability

The size distribution of aggregates was measured on air-dry soil samples by wet sieving (Kemper and Rosenau, 1986) using an apparatus with four stacks of sieves to collect six aggregate size classes from each sample: 4-6.3, 2-4, 1-2, 0.5-1, 0.25-0.5, and 0.106-0.25 mm, plus microaggregates and colloidal material < 0.106 mm. One-hundred grams of soil were spread across the surface of the 4-mm sieve and directly immersed in a water bath of distilled water (Angers and Giroux, 1996). Each sieve stack was immersed in separate containers and the water level was adjusted so that the aggregates on the upper sieve were just submerged at the highest point of oscillation. The oscillation rate was 33 cycles per minute, the amplitude of the sieving was 3.5 cm, and the sieving duration of each replication was 20 min.

After wet sieving, the individual water baths were held stationary and sedimentation was allowed to occur for a period of 48 hours to collect the < 0.106 mm fraction. Flocculation of suspended particles was aided by pouring 20 ml of $CaCl_2$ 0.5 M in each container. Floating organic debris and/or pieces of crop residues were excluded from the analysis. The soil fractions were washed into beakers, oven-dried at 60°C for 24 hours, and weighed. Samples on each sieve were split. About one-half of the soil in each fraction was shaken and dispersed in a solution of Na-hexametaphosphate (5 % w/v) for 20 minutes. They were then passed through a 53-μm sieve to collect sand-size mineral grains, oven-dried and weighed. The distribution of sands accounts for much of the differences in the composition of aggregate size fractions reported in the literature (Elliott et al., 1991; Cambardella and Elliott, 1993; Beare et al., 1994). The reason is because some of the sand grains are retained on the finer sieves and included in these size fractions of aggregates during the sieving process. Thus, the mass of each size fraction was calculated on a sand-free (> 53 μm) basis to normalize the expression of C distribution in the size fractions. The mean-weight diameter (MWD) is an index of aggregate stability and was calculated according to the summation equation:

$$\mathrm{MWD} = \sum_{i=1}^{n} x_i w_i \tag{1}$$

where x_i is the mean diameter of the aggregate size fraction and w_i is the proportion of the total sample weight present in the size fraction (Kemper and Rosenau, 1986). The remaining half of the wet-sieved soil in each fraction was ground in a ball mill (< 250 μm) before analysis.

Total carbon and stable isotope analyses

Ground samples were used for the determination of total C and ^{13}C contents. Samples were then carefully weighed and packed into tin capsules, and wrapped into pellets so that total soil C and δ^{13}C could be determined by combustion on a Isotope Ratio Mass Spectrometer Model 2020 (Europa Scientific, UK). The δ^{13}C is calculated from the measured ^{13}C/^{12}C ratios (R) of the sample and standard gases and expressed relative to the international standard PDB (as δ^{13}C, ‰). The proportion of C derived from crops (x) was calculated by the modified mixing equation (Balesdent and Mariotti, 1996):

$$x = (\delta_{cs} - \delta_{rs}) / (\delta_c - \delta_{rs}) \tag{2}$$

where δ_{cs} = δ^{13}C value of sample (isolated fraction from cropped soil), δ_{rs} = δ^{13}C value of the reference soil of the corresponding fraction, and δ_c = measured δ^{13}C value of crop residues. The never-tilled successional grass plots (NeTSG) were used as the reference to calculate the proportion of C derived from crops (both C_4 and C_3 plants), in each size fraction, in CT and NT treatments, and the proportion of maize-derived C in the NeTSM treatment. For CT and NT soils, the proportion of C derived from crops was calculated assuming a mixture residue return of two parts maize to one part non-maize crops (mostly soybeans) over the 100-year period these plots had been under cultivation, before the experiment began. Maize residues return more biomass to the soil than soybean, and soybean residues decompose faster because of their lower C:N ratio. The residue mixture was calculated to have an average $\delta^{13}C$ of -17.1‰ (Collins et al., 1997).

RESULTS AND DISCUSSION

Aggregate size distribution and stability

Long-term cultivation resulted in substantial changes in aggregate size distribution and a reduction in aggregate stability. There were more stable macroaggregates ranging from 1 to > 4 mm in the never-tilled successional grass (NeTSG) (Fig. 3.1) than in the cultivated (CT and NT) soils (Fig. 3.2). High levels of SOM and the continual presence of roots in grassland soils promote the conditions necessary for microbial and invertebrate reworking of soil material which develop aggregates with enhanced water stability. Under these conditions, there would be little or no exposure of the organic material to decomposition due to absence of disruption by tillage (Cambardella and Elliott, 1993). A favorable balance of physical forces, such as enmeshment by pressure exerted by growing roots (Tisdall and Oades, 1982; Douglas and Goss, 1982) and localized drying caused by root water extraction (Kay, 1990), has been associated with enhanced water-stable aggregation of undisturbed soils. The effect of roots in physically enmeshing fine particles of soil into macroaggregates is persisting and usually goes beyond the root death (Tisdall and Oades, 1982).

When the never-tilled soil was cropped with a no-tillage maize (NeTSM) the percentage of aggregates > 4 mm in the top 5 cm was higher than in the reference plot under grass (Fig. 3.1). Reductions in aggregate stability upon cultivation of native soils are more pronounced in soil macroaggregates whereas the stability of microaggregates usually remains unchanged (Tisdall and Oades, 1984; Oades, 1984). The increase in the percentage of aggregates > 4 mm under NeTSM may indicate an additive effect of maize roots on macroaggregate stabilization as roots are thought to play an important role on aggregation by supplying large quantities of organic material to soils. However, the C concentration at the depth of 0-5 cm was up to 17% lower in this fraction (> 4 mm) after 12 years of slot maize cropping (Fig. 3.3a).

In recently tilled sod soils changes in aggregate stability often occur before significant declines in total SOM can be detected (Angers et al., 1992; Haynes and Swift, 1990). In the present study, the slot planted maize showed reductions in C levels but relatively small changes in aggregate stability after 11 years. Based on their findings during the first four years after the commencement of the present field trial, Reinert et al. (1990) predicted that cropping the never-tilled soil to maize, even when under no-tillage management, should decrease its stability on a long-term basis. They concluded that the soils under long-term cultivation were much more sensitive to cyclic variations in weather fluctuations, i.e., rainfall patterns and associated soil water contents, than undisturbed soils. Cyclic variations in aggregate stability were more dramatic in the CT treatment because of the tillage disturbance, whereas there was a considerable similarity between the cycles in NT and NeTSG soils (Reinert et al., 1990). Annual cycles in aggregate stability (especially > 4 mm size) followed a trend towards structural stability degradation from fall to early spring and a restoration action starting early spring and reaching the maximum in the late summer (Reinert et al., 1990). The cyclical variations observed appeared to be associated with soil temperature and moisture conditions favorable for biological activity. In view of these data collected earlier in these plots, it can not be ruled out that the higher percentage of aggregates > 4 mm in the NeTSM, as indicated by an increased MWD value (Table 3.1), is little more than the result of temporal variations in aggregation than influenced by maize root activity.

Overall, there was a greater percentage of water stable aggregates soil in the small macroaggregates and in the microaggregate size classes (< 0.5 mm) in the cultivated soils. Lower percentages of soil in the < 0.106 mm size-class were associated with less disturbance by tillage with CT showing the highest and NeTSG the lowest values. While tillage intensity is an important factor in aggregate disruption, the relatively lower residue cover (visual observation), leading to intensive drying/wetting and freezing/thawing, may have contributed to the destruction of aggregates into smaller particles. It seems that for these soils the limit of macro and microaggregation would be somewhere between 0.5 and 0.25 mm. This is because above and below this size the trends in size distribution among the treatments are in the opposite direction to intensity of cultivation. In general, the undisturbed (NeTSG and NeTSM) soils had a proportionally higher percentage of soil aggregates in the fractions > 0.5 mm (Fig. 3.1), whereas CT and NT management resulted in more soil in the fractions < 0.5 mm (Fig. 3.2). Thus, macroaggregates of undisturbed soils were more stable than of cultivated soils (CT and NT). As discussed by Elliott (1986), the large shift in fractions distribution greater and smaller than 0.3 mm, and the small amount of material smaller than < 0.1 mm even under disruption conditions by slaking would support the model of soil structure proposed by (Tisdall and Oades, 1982) in which macroaggregates are formed by an assemblage of highly stable microaggregates. The effect of slaking is to disrupt the soil due to internal forces and results in the production of particles of a more fundamental nature compared to a less aggressive pre-treatment of samples (Elliott, 1986). It has been suggested that upon wetting, large air-dried soil aggregates (>1 mm) are susceptible to disruption by slaking breaking down into aggregates of 0.25 to 0.50 mm in diameter (Angers, 1992). A greater shift in the aggregate size distribution of a sample towards the smaller size classes upon disruption by rapid wetting indicates its lower aggregate stability (Kemper and Rosenau, 1986).

The quantity of macroaggregates resistant to slaking was substantially higher under the undisturbed long-term grass treatments as it can be seen from the small proportion of microaggregates at both depths (Fig. 3.1). Comparing native pasture soils to adjacent soils continuously cropped for > 60 years, Skidmore et al. (1975) concluded that the structure of the sod soil was much more stable than of the tilled soil, which was corroborated by a large number of roots and cavities in aggregates of sod soils. In the present study, the effect of NT on macroaggregation (> 0.25 mm), as compared to CT, was clear at both depths (Fig. 3.2). Greater macroaggregation under NT than under CT was only offset by a decrease in the 0.25-0.5 mm class at the 0-5 cm depth and similar values for > 4 mm aggregates at the 5-15 cm depth. The experimental area had been under conventional tillage for over 100 years before the CT and NT experimental plots were established. Thus, as opposed to several studies in which CT and NT plots are started out of undisturbed soils, the aggregate stability data reported here indicate the resilience capacity of this soil to restore macroaggregation under NT management, over a relatively short time span. In general, a decrease in aggregate size and stability (i.e., reduction in stable macroaggregates) with increasing intensity of cultivation is expected and fairly well

documented in the literature (Oades, 1984; Blevins and Frye, 1993; Beare et al., 1994; Franzluebbers and Arshad, 1996; Bajracharya et al., 1997; Monreal and Kodama, 1997).

Aggregate stability of the soils was also evaluated based on their MWD which is an aggregation index that takes into account the different size particles which breakdown and are separated during wet sieving. The MWD exhibited a pattern similar to macroaggregation except for the 0-5 cm depth, where the value for the NeTSM soil was higher than for NeTSG (Table 3.1). No-tillage had the greatest impact on MWD at the depth of 0-5 cm with an increase up to 53% compared with CT and smaller impact at the depth of 5-15 cm (mean increase of 27% compared with CT). The highest values, however, were associated with the undisturbed treatments. The mean weight diameter decreased on cultivation from 2.02 mm in the NeTSG to 0.75 mm (CT) and 1.16 mm (NT) after over 100 years of cropping. This indicates a decrease in the MWD by as much as 63% under CT management. Mean weight diameter of NT soils was 55% higher than that of CT, indicating the beneficial effects of NT practices after 11 years. Studying a fine sandy loam, Carter (1992) observed that the MWD under no-tillage was 43-53% greater than under conventional tillage at the depth of 0-5 cm after a 3-5 year period. According to the classification of Le Bissonnais (1996) the MWD values for NeTSG, NeTSM and NT correspond to very stable soils (MWD > 2.0 mm) and those for CT to soils with low stability (MWD < 0.8 mm).

Total carbon contents in aggregate size fractions

Total C concentrations in aggregate size classes were strongly influenced by the different proportions of sand in each size class, which agrees with previous reports (Beare et al., 1994; Elliott, 1986; Puget et al., 1995). Therefore, to describe interaggregate differences in organic C concentrations, as well as the effects of the treatments and soil depth, all total C values were normalized on a sand-free basis. Cultivation influenced the relative distribution of C in different aggregate size classes. For all aggregate size-classes and depths, C concentrations were always higher for the never-tilled treatments (NeTSG and NeTSM) (Fig. 3.3) than for the cultivated soils (CT and NT) (Fig. 3.4) and higher in the 0-5 cm layer. Higher organic matter levels in undisturbed, steady-state soils are expected because little vegetation is removed, wetting and drying are slow, and oxidation is at minimum due to the absence of periodical soil mixing (Dormaar and Carefoot, 1996). In addition, root and crown tissues production is much greater for native grasses than for cultivated crops, making up a higher proportion of net primary productivity (Sala et al., 1988).

When total C distribution in the different size fractions of NeTSG (Fig. 3.3) and CT (Fig. 3.4) soils are analyzed in conjunction with the distribution of water-stable aggregates (Fig. 3.1 and Fig. 3.2), there seems to be an agreement with the observation that cultivation results in a smaller proportion of soil containing 'high-nutrient macroaggregates' and larger proportion of soil containing 'low-nutrient microaggregates' (Elliott, 1986). Gupta and Germida (1988) and Cambardella and Elliott (1993) also reported that C concentrations were higher in aggregates of native sod than in those of long-term cultivated grasslands. Organic matter losses are rapid and usually exponential, with rapid declines during the first decades, followed by smaller annual losses, finally approaching a new equilibrium characteristic of the particular system considered. For example, the A horizons of prairies have lost 35 to 50%, based on concentration, or 15 to 30%, based on mass, of their OM since they were broken 100 years ago (Dormaar and Carefoot, 1996).

Carbon concentrations were generally equal to one another in the five larger aggregate size classes (> 0.25 mm) and were always higher than in the smaller size class (< 0.106 mm) (Fig. 3.3 and Fig. 3.4). Never-tilled grass (NeTSG) soil had higher C contents in all fractions compared with the never-tilled grass under maize (NeTSM). Absolute differences in C between size classes were relatively smaller in NeTSM compared to NeTSG (Fig. 3.3), and in CT compared to NT (Fig. 3.4), at the depth of 0-5 cm. In the deeper layer, the absolute differences were smaller in NeTSG.

There was a considerable decrease in the C concentrations of NeTSG due to maize cropping (NeTSM) after 11 years, although the decreasing effects of maize in C were not always coupled with reduction in macroaggregate structure, as discussed earlier. Changes in C contents in the several size fractions ranged from 10 to 18 g C kg^{-1} soil fraction at the depth of 0-5 cm and from 8 to 15 g C kg^{-1} at the depth of 5-15 cm (Fig. 3.3). An average across all water-stable aggregate fractions indicated that C contents under maize decreased by 1.2 g kg^{-1} yr^{-1} (0-5 cm) and 0.9 g kg^{-1} yr^{-1} (5-15 cm). It is important to mention that in the NeTSM treatment maize has been planted after only a mowing late in the previous fall, without any fertilization. The biomass production has been very low and the residues returned to soil (mainly from roots) probably contribute too little to the pool of organic material. Because the maize is slot planted, one possible explanation for decreased SOM under NeTSM is the disruption of soil aggregates and increased aeration by growing maize roots. Even if small, this disturbance still may have provided oxygen supply and accessibility of new easily oxidizable material, resulting in further substantial biological oxidation of the residual C in the system (Reicosky et al., 1995).

In order to balance the oxidation of the original SOM and maintain a new level closer to the original level, large additions of SOM from decomposing residues should be added to the soil. In an experiment in Indiana, Barber (1979) concluded that in cropping practices with no incorporation of residue over a period of years following a previous practice where the residue was returned to the soil, the SOM level would decrease until it comes to an equilibrium with the new practice. He observed that after 11 years of continuous maize with no residue return, the SOM level decreased to by 10 percent of its original level. A higher amount of residues returned to the soil under NeTSM would also offset the disturbance effects on physical properties such as the degree of aggregation. Reid et al. (1982) reported that detrimental effects of maize cropping on soil aggregates were directly related to maize root mucilages, although their explanation has been contested (Pojasok and Kay, 1990). During a 28-day growth study of maize, Helal and Sauerbeck (1989) observed a short-term positive effect on aggregation, which they attributed to enhanced root exudation and microbial biomass. However, 60 days after harvest, they verified that OM levels and aggregate stability were lower in the maize planted soil than in the control. Despite the fact that for most crops, root decay contributes to the total amount of OM in the soil (Goss, 1987), the beneficial effects are confined to the topsoil and rapidly disappear when additional organic surfaces are exposed to microbial attack in root-disrupted soil aggregates (Helal and Sauerbeck, 1989). Root released materials are more readily available to microbes than are residues from even the previous crop and there is less breakdown of these materials in the presence of living roots than in fallowed soils (Reid and Goss, 1982). Organic matter content in soil aggregates seems to be dictated by the ability of structural units to pack small-size organic fragments; it is largely influenced by the activity of soil microorganisms and plant roots in undisturbed soils and by tillage intensity in cultivated soils (Monreal and Kodama, 1997). These authors verified that aggregates from a forest soil had greater amounts of plant debris, SOM, larger diameter particles, and higher water stability than those from a cultivated site.

The lower C contents of CT and NT soils (Fig. 3.4), compared to the NeTSG plots (Fig. 3.3), is a result of their intensive cultivation since the end of the last century. The average reduction in C with cultivation, across all the measured aggregate size fractions in the 0-5 cm layer of CT soil, was in the order of 33 g kg^{-1}. For the 5-15 cm layer, C reduction averaged 16 g kg^{-1}. This illustrates a natural decline in C content of cultivated soils, particularly when small amounts of crop residue are returned to the soil. Nearly all the differences in aggregate C contents between CT and NT treatments were found in the top 5 cm of soil (Fig. 3.4), with an average net increase of 6.6 g kg^{-1} in NT. Coleman et al. (1994) attributed similar effects to the lower abundance and turnover of macroaggregates in the top soil surface of CT as opposed to NT soils.

The lower C contents in the top 0-5 layer of our CT treatment reflects a redistribution effect of organic matter by plowing. Beare et al. (1994) reported that aggregates of CT had a lower capacity to protect SOM than aggregates of NT in the surface 5 cm of a sandy clay soil. Frequent disruption of aggregates are expected to reduce the quantity of macroaggregate-protected SOM and increase the quantity of unprotected SOM in proportion to the total aggregate SOM (Coleman et al., 1994). Reducing tillage intensity and increasing surface residue cover inhibit loss of organic matter from soil. Twenty years of bare-fallow, stubble-mulch, and no-till management on a grassland soil of Nebraska reduced the surface total C by 28, 18, and 12% of the original level (Cambardella and Elliott, 1992). Plant residues decompose more slowly on the soil surface than when they are incorporated into the soil, mainly as a result of changes in soil temperature and moisture, and availability and location of substrate. At the 5-15 cm depth, except for the > 4 mm aggregate size, there were no significant effects of tillage on organic C contents (Fig. 3.4b). The higher values of C in macroaggregates > 4 mm under CT probably resulted from the redistribution or deep burial of C-enriched surface aggregates by tillage, as indicated by similar concentrations of C at both depths in CT. The reduction in C with depth was greater in NT and would probably be even greater if a more stratified sampling of the top 5 cm had been performed. From whole soil samples taken in the spring of 1996, it was observed that after 10 years of CT and NT management at this experimental site, C concentrations in NT (13.2 g kg^{-1}) were somewhat greater than in CT (11.5 g kg^{-1}) (Santos and Smucker, 1998). While repeated cultivation of soils enhances SOM decomposition and changes the composition of the residual organic matter, the effects of crop rotations and reduced tillage on SOM usually become significant only after several years of their adoption. Several studies have shown greater amounts of C in soils that have been under reduced or no-tillage for long periods compared with soils under moldboard plow tillage (Havlin et al., 1990; Beare et al., 1994; Franzluebbers and Arshad, 1996). Blevins et al. (1983) reported 37 and 12% more organic C (at 0-5 cm depth) under NT and CT maize, respectively, than in an unfertilized soil.

Measurements of living fractions of SOM such as soil microbial biomass C (SMBC), have indicated early changes in SOM for relatively short periods before these can be detected by chemical analysis (Powlson et al., 1987). Although SMBC evaluations were out of the scope of the present study, measurements taken twice during 1996 (Sissoko and Smucker, 1998), indicated that the higher the intensity of cultivation the lower the SMBC values will be. For samples taken in November, SMBC values at 0-20 cm were consistently higher in NeTSG (310 ± 16 μg C g^{-1} soil; where $n = 3$; ± standard deviation) and NT (210 ± 12 μg C g^{-1} soil), than in CT (175 ± 22 μg C g^{-1} soil). Soil microbial biomass measured in May in CT and NT averaged 32% greater than in November, whereas seasonal variation in NeTSG was only 13% (Sissoko and Smucker, 1998). The decrease in SMBC in November may have been the result of a long drought period during the 1996 growing season. Nevertheless, the observed decrease in SMBC with CT compared to NT is similar to that reported by Salinas-Garcia et al. (1997). When the NeTSG soil is compared, the results are similar to that reported by Follett and Schimel (1989) who found that SMBC decreased in the order of sod soil > NT > CT.

δ^{13}C values in aggregate size fractions

The δ^{13}C of the whole soil under the present successional vegetation, cleared from forest in the 1950's, shows a mean value of -25.7 ‰ (Paul et al., 1998), indicating that the organic matter in this soil was derived mainly from C_3 vegetation. This δ^{13}C value is relatively similar to that in the surface layer of the native vegetation of deciduous oak-hickory forest (-26.1‰) (Paul et al., 1998). The δ^{13}C values (± standard deviation, $n = 4$) of random grass plant samples were -27.6 ‰ (± 0.41‰) and -27.6 (± 0.31‰) for shoots and roots, respectively. This indicates a slight δ^{13}C enrichment (1.9 ‰) of the SOM. Since plant residue decomposition in soils is a microbial-mediated process, a discrimination against the heavier ^{13}C could have resulted in an increase in the δ^{13}C value of the soil (Balesdent and Mariotti, 1996). Also, separate constituents of plant residues are isotopically distinct from whole plant tissue and decompose at different rates. More rapid decomposition of cellulose and hemicellulose (1-2 ‰ enrichment in ^{13}C) than lignin (2-6 ‰ depletion in ^{13}C) fractions could deplete ^{13}C enrichment of recently added plant material as it is incorporated into the SOM pool (Boutton, 1996; Huggins et al., 1998). The presence of some C_4 plants in the pool of grass species in the successional vegetation could be another explanation. Several other causes for this enrichment are discussed in detail by Boutton (1996).

The δ^{13}C values of whole soil samples of CT and NT, taken at the depth of 0-5 cm, were -22.8 ± 0.27 ‰ (means ± standard error, $n = 4$) and -22.2 ± 1.03 ‰, respectively. For the 5-15 cm samples, the values were -23.6 ± 0.28‰ for CT and -22.1 ± 0.70‰ for NT (Santos and Smucker, 1998). Small differences in δ^{13}C were observed between the several size fractions of the NeTSG soil (Table 3.5), without exceeding 0.8‰. For all aggregate fractions at both depths of CT and NT treatments, the $\delta^{13}C$ values were greater than -25.7‰ (Fig. 3.6), indicating an enrichment in ^{13}C relative to the successional vegetation (NeTSG). Changes in the original $\delta^{13}C$ signature of the successional vegetation soil in NeTSM and NT treatments were greater in the surface soil than in the 5-15 cm soil layer (Fig. 3.5 and 3.6, respectively). In the CT treatment, mixing of soil and burial of residues by tillage produced a more uniform $\delta^{13}C$ signature across the 0-15 cm depth. In samples from 0-5 cm layer of the NeTSM treatment and from both depths of the CT treatment, macroaggregates appeared to be slightly enriched in ^{13}C. This seems to indicate that SOM is more decomposed in smaller than in larger aggregates. However, increase in $\delta^{13}C$ (less negative) with increase in aggregate size, found by Anger and Giroux (1996), was observed for the NT treatments only in the 5-15 cm layer. For the 0-5 cm layer of NT, inputs of C_4-derived material were apparently greater in microaggregates and smaller macroaggregates (<0.5 mm). If microbial activity is relatively greater in these size fractions (Jastrow et al., 1996), microbial biomass and the residual substrate remaining in the soil after microbial degradation would be enriched in ^{13}C relative to the initial composition of the substrate (Jastrow et al., 1996). However, the high variation in the $\delta^{13}C$ values for NT at this depth (Table 3.6), probably resulted from undecomposed or partly decomposed maize residues, in addition to potential disruption by slaking during the sieving step, prevents any final conclusion. The results indicate a slight trend for increasing $\delta^{13}C$ values in the direction of macroaggregates, similar to those reported by Puget et al. (1995) for a silty soil under NT and superficial tillage.

Assuming that the organic matter in the NeTSG treatment had reached a steady state by the time the soils were first cultivated, its total C content represents the initial carbon (C_3-derived) in the soils (Fig. 3.3). These values were used as a baseline to calculate the changes in the C_3-C content as a result of cultivation for over 100 years, as well as the inputs of C from the crops during this period. The relative contents (Fig. 3.7) and proportions (Fig. 3.8) of recently deposited C in the CT and NT treatments were almost always higher in macroaggregates (> 0.25 mm). The opposite trend of higher percentage of "new" C (from crops) in aggregates < 0.5 mm in the NT treatment at 0-5 cm seems to be related to redistribution of fresh residues to smaller fractions during wet sieving, as indicated by the high standard errors of the $\delta^{13}C$ values (Fig. 3.6).

The proportions of C_4-derived C in the NeTSM treatment after 11 years of maize cropping ranged from 9.5 to 14% and from 1 to 3%, for the 0-5 and 5-15 cm depths, respectively (Fig. 3.8). The proportion of crop-derived C was much higher in NT soil at the depth of 0-5 cm depth, ranging from 53 to 76% and representing almost twice the proportion in the 5-15 cm depth (Fig. 3.8). For CT soils, these proportions were not greatly affected by depth and ranged from 28 to 35% at 0-5 cm and from 22 to 45% at the 5-15 cm depth. The similarity in the proportions in CT indicates the influence of the topsoil mixing by annual plowing. Since the organic matter responsible for stabilizing microaggregates is considered to turn over much more slowly than that of macroaggregates (Tisdall and Oades, 1982), the higher proportion of new C in the microaggregates of the NT soil seems to be inconsistent. Jastrow et al. (1996) detailed several possible explanations suggesting that not all C associated with microaggregates may be biochemically recalcitrant. Some of their hypotheses are summarized here: i) most of the new-C associated with microaggregates might come from the surfaces of larger aggregates. In soils with highly stable aggregates, such as those in NT, the microaggregates released by disruption upon slaking could be previously bound into macroaggregates *in situ* but were not stabilized enough to resist the disruption; ii) a recent pool of labile material could be associated in significant quantities to the surfaces of microaggregates released from slaked macroaggregates; and iii) incrustation of organic debris and clay particles could produce slaking-stable microaggregates in which the core newly incorporated debris are physically protected from rapid decomposition. The present findings and the results reported by Jastrow et al. (1996) suggest that microaggregates are probably more dynamic than suggested by the current models of soil aggregation.

CONCLUSIONS

The results of this study show important differences in water-stable aggregate distributions between NT and CT soils, and in a recently cultivated soil. These differences may explain their contrasting retentions of SOM. In comparison to the permanent grass treatment, 11 years of non-tilled and non-fertilized maize cropping caused a decline in C levels but the effects were not clear on aggregate stability. In the long-term cultivated soils (CT and NT), macroaggregation was greatly reduced with a concomitant increase in the proportion of small macroaggregates and microaggregates. The introduction of no-tillage greatly increased C levels in the top 5-cm soil, with trends in improvements in stability of macroaggregates at both depths after 11 years. The development and/or stabilization of new and old macroaggregates in NT management may indicate that the resilience capacity of this soil is an important mechanism for storage and protection of SOM that is otherwise lost under CT. The extent to which aggregation and SOM contents change as a result of cropping and tillage practices are often difficult to determine based on other reports since it depends on several site-specific factors, such as soil properties, climate, type of crop, and management and fertilization practices.

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Table 3.1. Mean weight diameter (MWD) under conventional tillage (CT), no-tillage (NT), never-tilled successional grass (NeTSG), and never-tilled successional with maize (NeTSM) at two sampling depths. (Means ± standard error; $n = 4$).

Depth	CT	NT	NeTSG	NeTSM
cm	------------------------------- MWD (mm) ----------------------------			
0 - 5	0.75 ± 0.07	1.16 ± 0.05	2.02 ± 0.09	2.12 ± 0.08
5 - 15	0.75 ± 0.09	0.94 ± 0.10	1.91 ± 0.12	1.75 ± 0.11

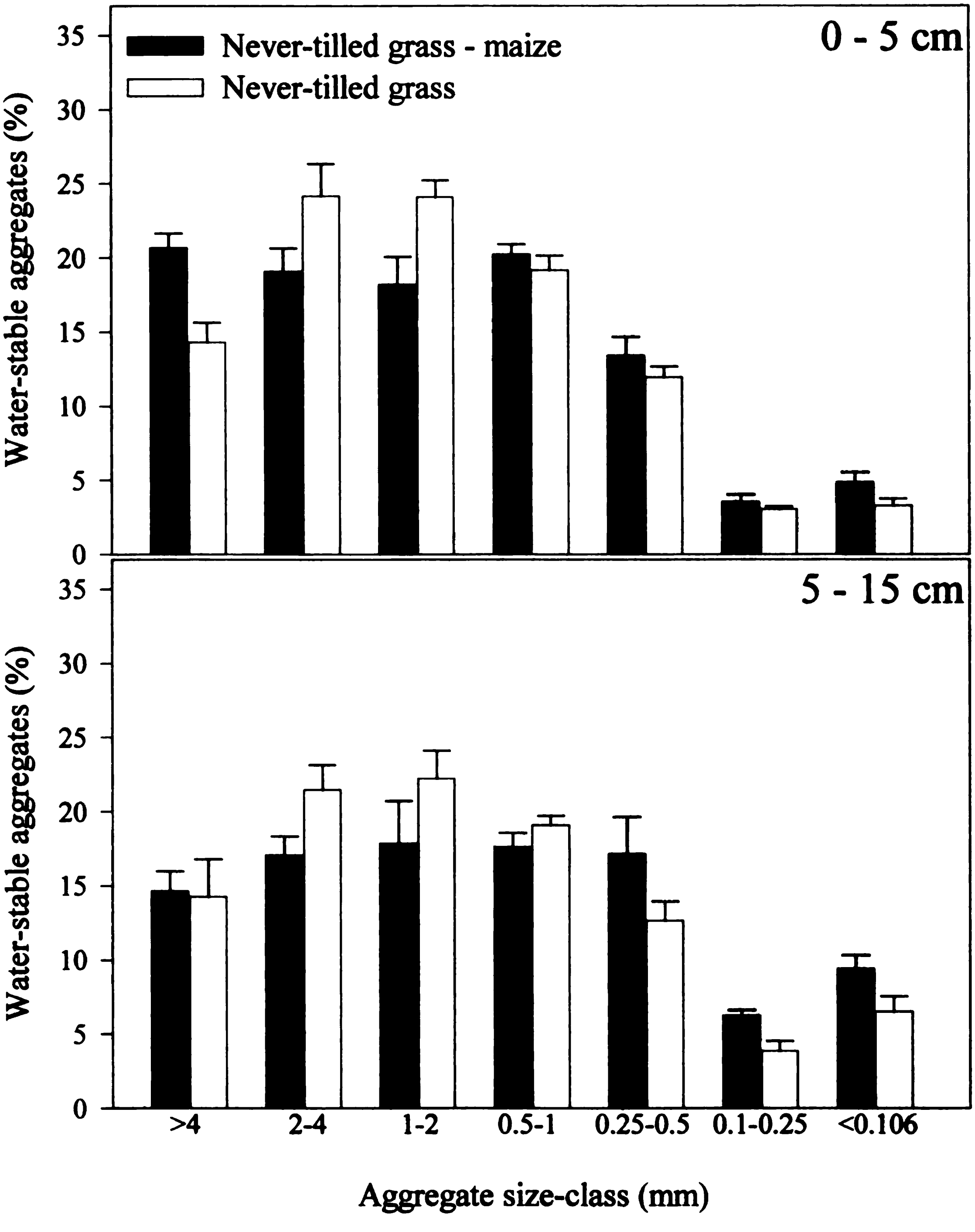

Figure 3.1. Size distribution of intact water-stable aggregate fractions under never-tilled successional grass (NeTSG), and never-tilled successional with maize (NeTSM) at 0-5 and 5-15 cm depths. Means ± standard error, $n = 4$.

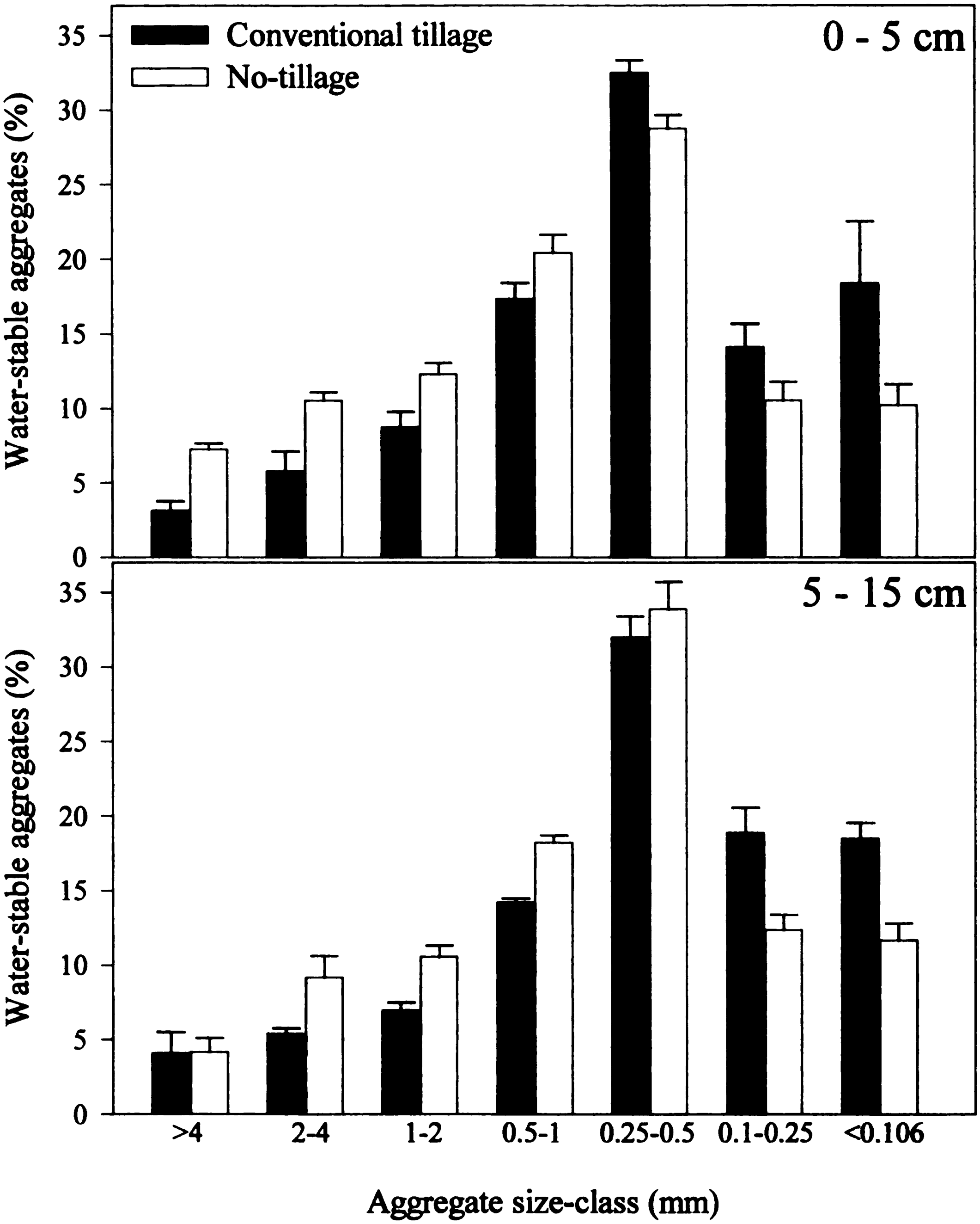

Figure 3.2. Size distribution of intact water-stable aggregate fractions under conventional tillage (CT), and no-tillage (NT) at 0-5 and 5-15 cm depths. Means ± standard error, *n* = 4.

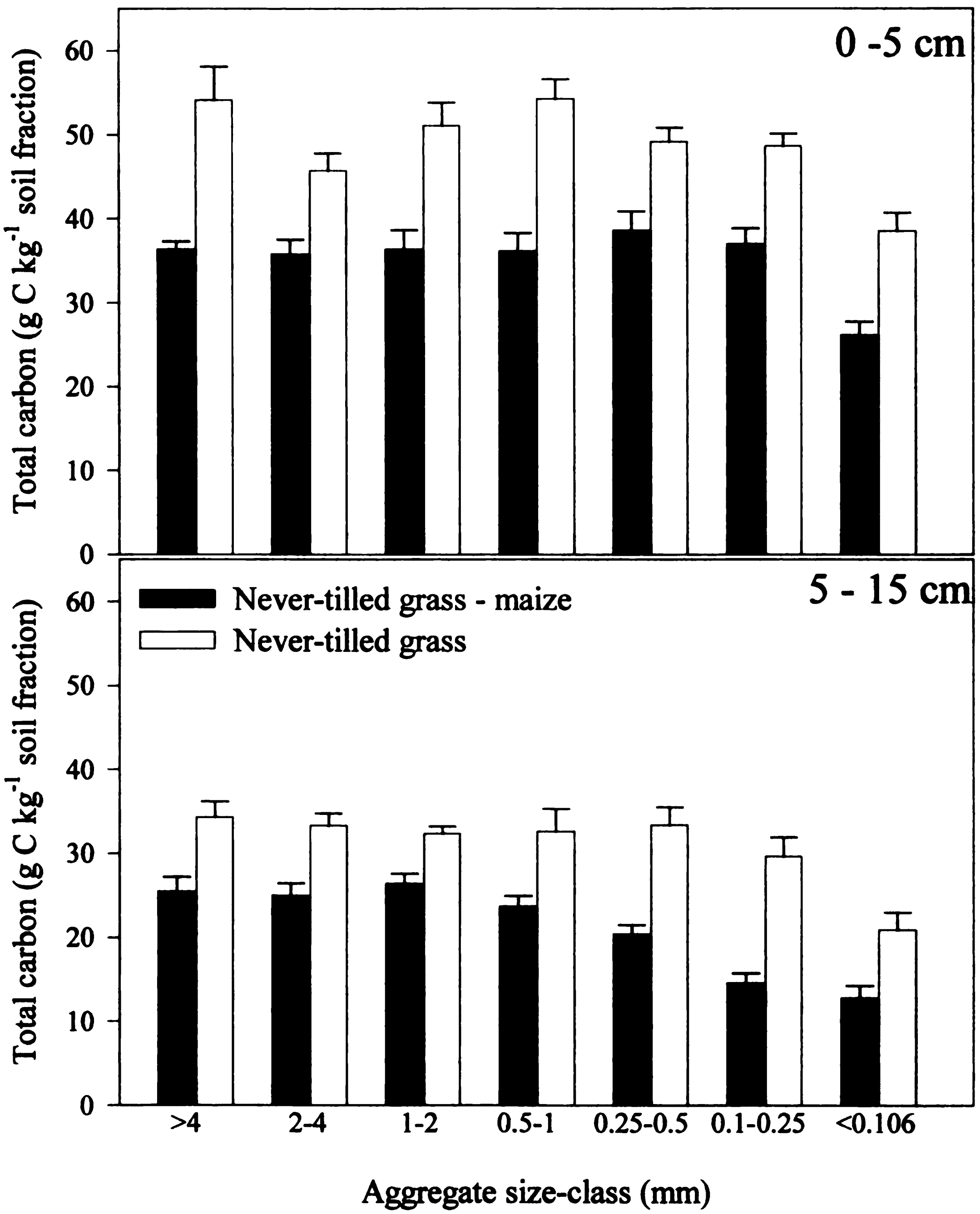

Figure 3.3. Total carbon contents (normalized to a sand-free basis) of water-stable aggregate fractions under never-tilled successional grass (NeTSG), and never-tilled successional with maize (NeTSM) at 0-5 and 5-15 cm depths. Means ± standard error, *n* = 4.

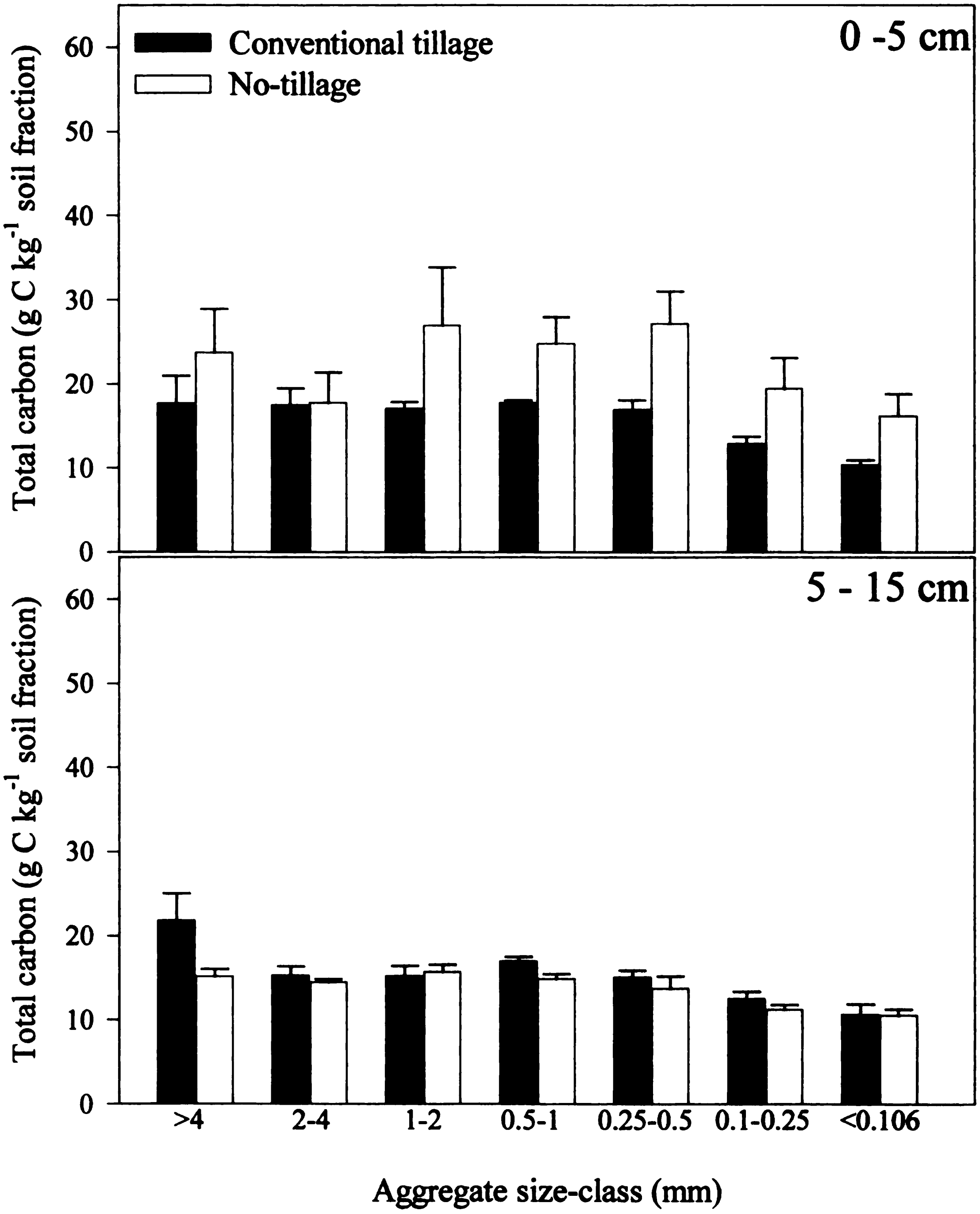

Figure 3.4. Total carbon contents (normalized to a sand-free basis) of water-stable aggregate fractions under conventional tillage (CT), and no-tillage (NT) at 0-5 and 5-15 cm depths. Means ± standard error, *n* = 4.

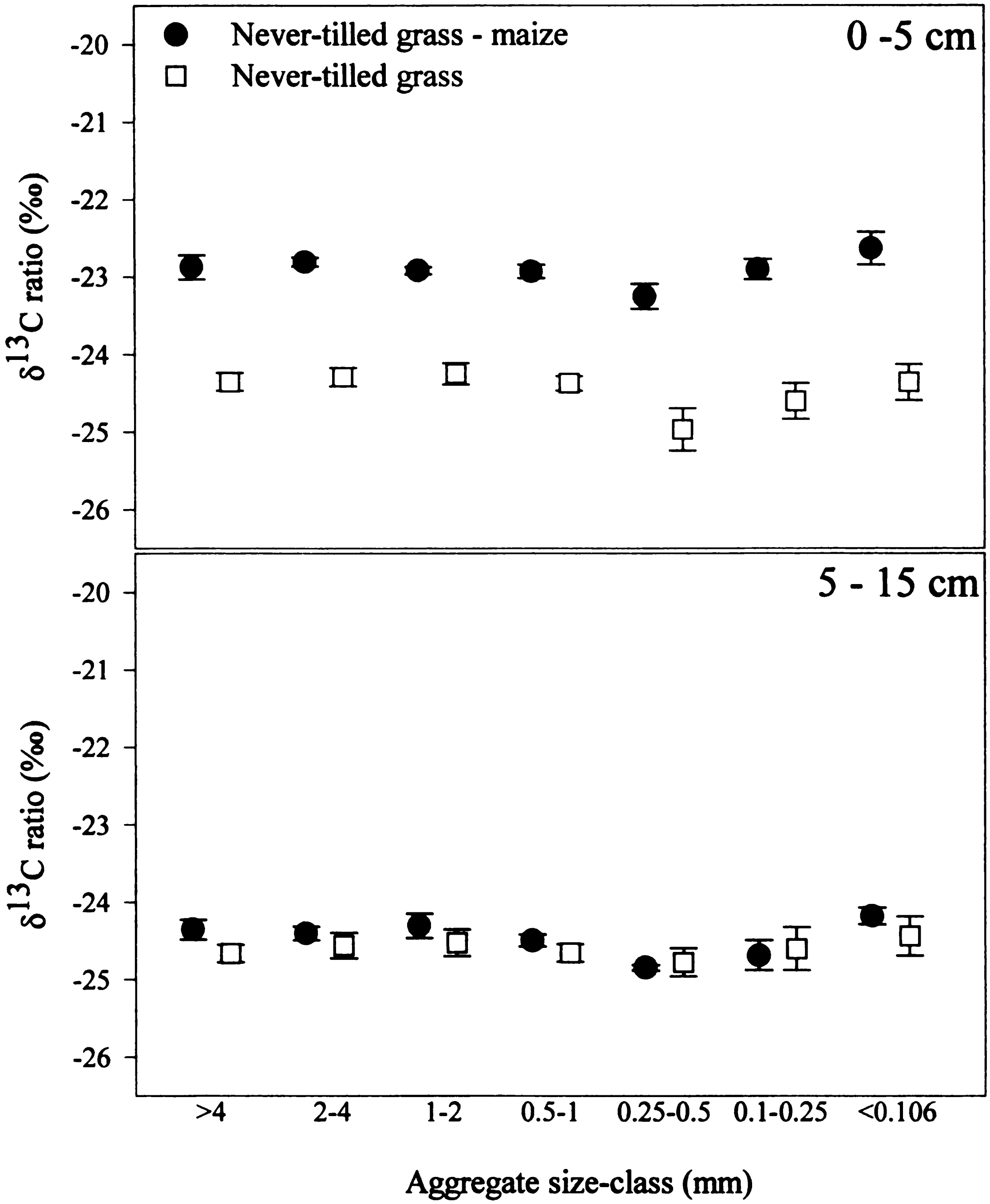

Figure 3.5. $\delta^{13}C$ ratios in water-stable aggregate size fractions under never-tilled successional grass (NeTSG), and never-tilled successional with maize (NeTSM) at 0-5 and 5-15 cm depths. Means ± standard error, $n = 4$.

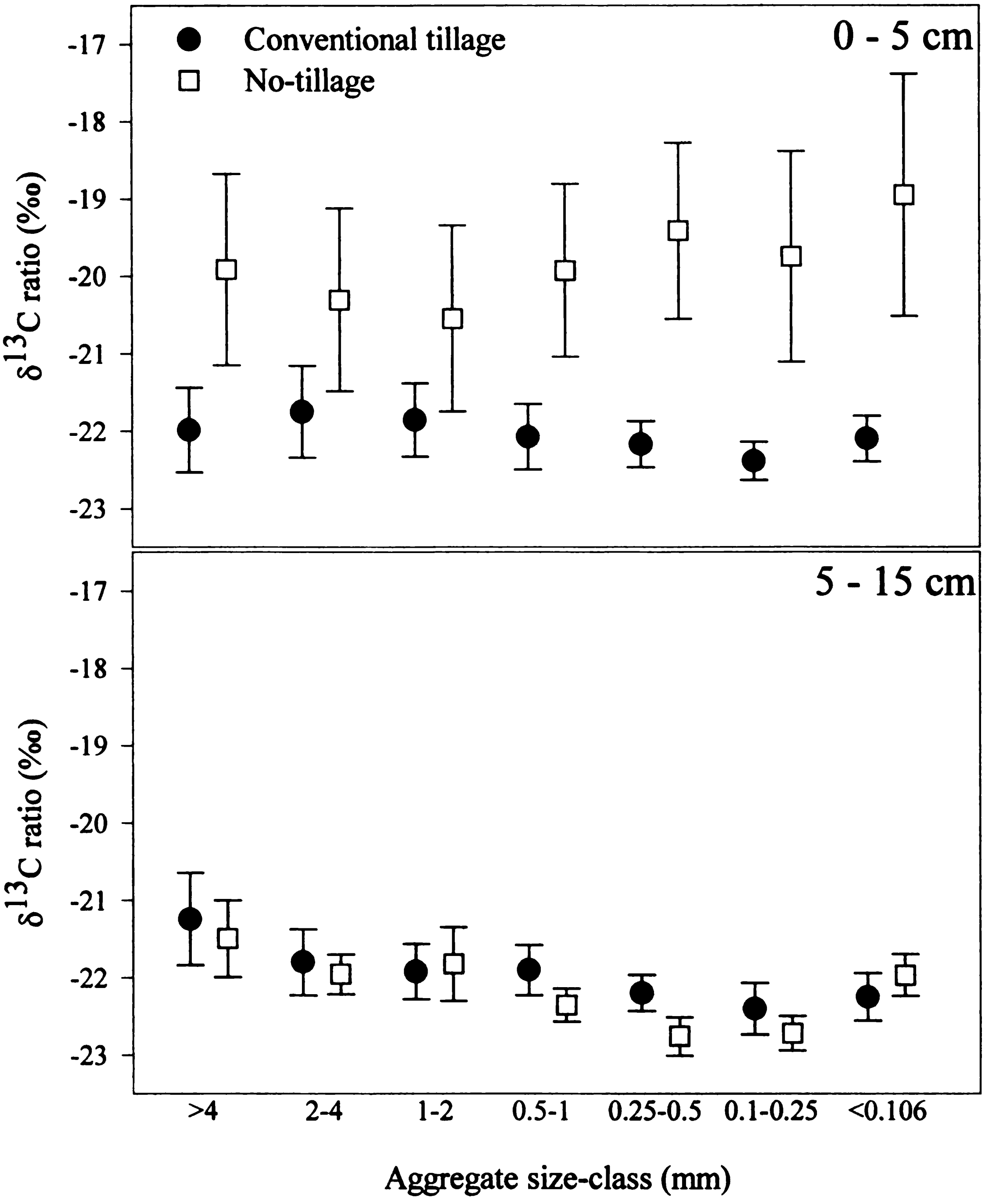

Figure 3.6. δ^{13}C ratios in water-stable aggregate size fractions under conventional tillage (CT), and no-tillage (NT) at 0-5 and 5-15 cm depths. Means ± standard error, *n* = 4.

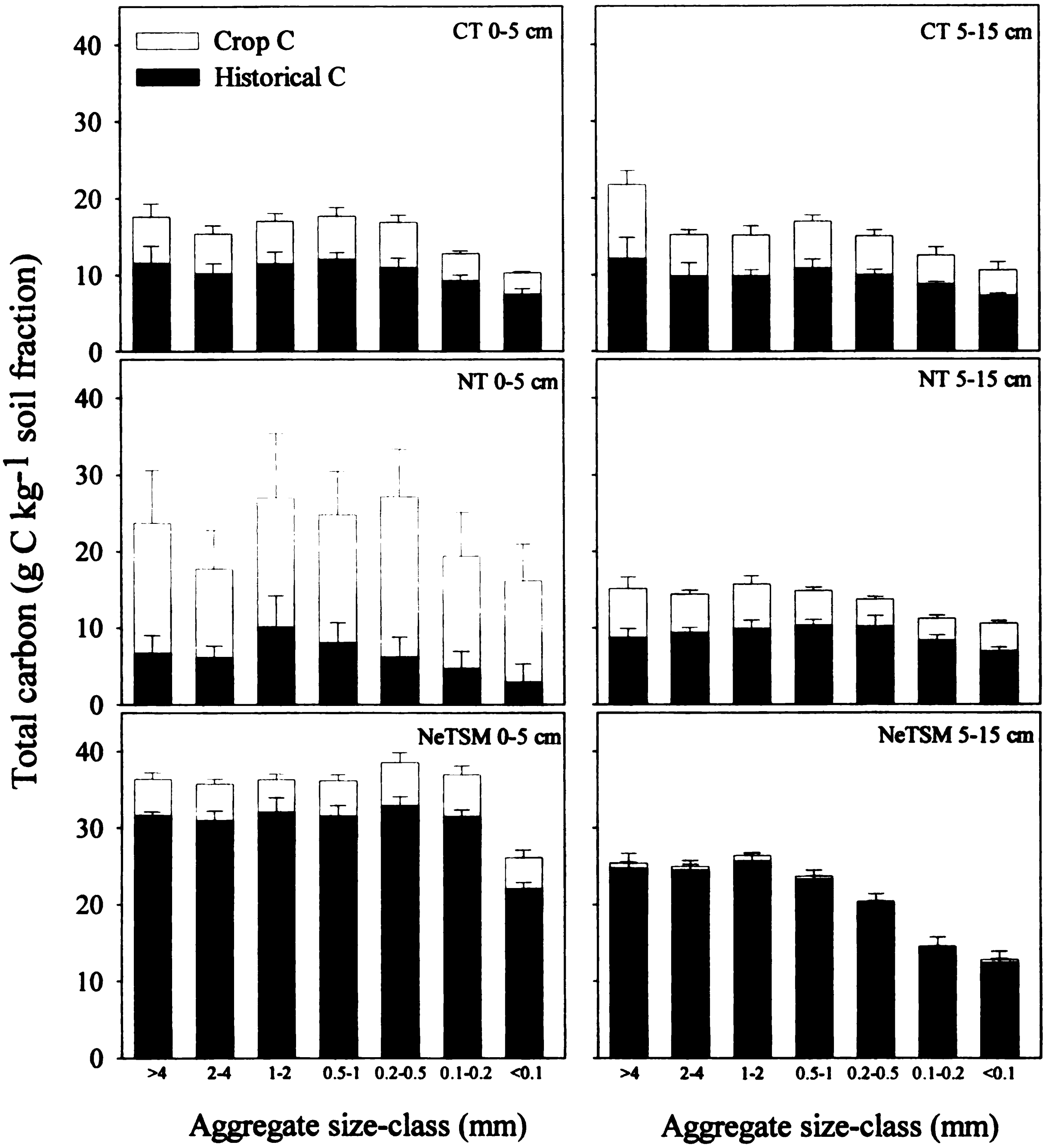

Figure 3.7. Contents of total C from historical and crop sources (normalized to a sand-free basis) in water-stable aggregate fractions under conventional tillage (CT), no-tillage (NT), and never-tilled successional with maize (NeTSM) at 0-5 and 5-15 cm depths. Means ± standard error, *n* = 4.

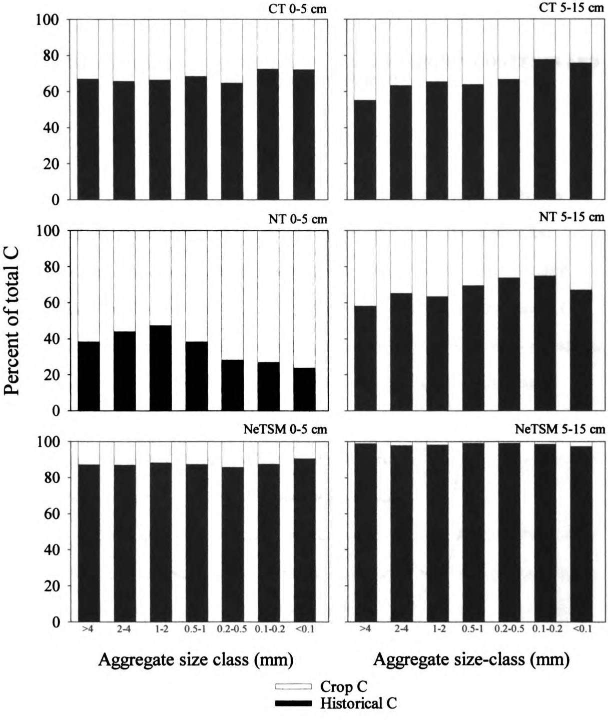

Figure 3.8. Percent of the total carbon derived from historical and crop sources in water-stable aggregate fractions under conventional tillage (CT), no-tillage (NT), and never-tilled successional with maize (NeTSM) at 0-5 and 5-15 cm depths. Means ± standard error, $n = 4$.

CHAPTER 4

PLANT ROOT EFFECTS ON SOIL AGGREGATE CARBON CONTENTS AND PHYSICAL PROPERTIES

INTRODUCTION

The primary source of C inputs to soils is through photosynthetic fixation by plants. The C that is translocated to the roots is present in soils as living roots and rhizomes, root exudates, mucilages, sloughed root cells and tissues, and as microbial tissues and metabolites (Qian et al., 1997; Balesdent and Balabane, 1992). Organic C released from growing roots may stabilize aggregates directly, or indirectly by providing a source of energy for microorganisms in the rhizosphere which may in turn produce stabilizing materials (Tisdall and Oades, 1979). Soil aggregation is thought to play an important role in the C dynamics of cultivated soils since it affects, and is affected by, the microbial-regulated C transformations and cycling in soils.

Soil aggregate surfaces reflect soil structural processes since they provide much of the contact area between roots and rhizosphere soil. As soil aggregates are being formed and root systems elongate around or through them, newly added root C, solutes and particulate organic matter move through the large soil pores and accumulate on or near the surfaces of aggregates. However, in aggregated soils, the role of structure in soil organic matter (SOM) dynamics has received little attention (Amelung and Zech, 1996), despite evidence that physical protection of SOM within aggregates is important in controlling its decomposition (Elliot, 1986; Gupta and Germida, 1988; Oades, 1988; Van

Veen and Kuikman, 1990; Gregorich et al., 1997). Yet, most of the information available on the amounts of C derived from roots is based on analysis of bulk soil samples through sieving techniques. One of the limitations of the sieving method is the redistribution of SOM among aggregate fractions that have been produced by the method itself. As a result, wet sieving data can be difficult to interpret (Elliott and Cambardella, 1991). The distribution of organic compounds and minerals within aggregates can be assessed with greater reliability by sequential removal and analysis of surface and inner fractions of aggregates (Ghadiri and Rose, 1991; Kayser et al., 1994; Santos et al., 1997). In this way, surface accumulations of recently deposited C on soil aggregates by contemporary root systems can be estimated (Santos and Smucker, 1998).

Recent studies have shown the usefulness of the $\delta^{13}C$ technique for estimating soil C dynamics in a situation where the original vegetation has been replaced by plants with a different photosynthetic pathway (Balesdent et al., 1987; Skjemstad et al., 1990). The natural abundance of ^{13}C is higher in C_4 compared to C_3 species. Plants with the C_3 pathway (mean $\delta^{13}C$ of -28‰), discriminate against $^{13}CO_2$ during photosynthesis, causing the $^{13}C/^{12}C$ ratios of their phytomass to be depleted in ^{13}C relative to those of C_4 plants (mean $\delta^{13}C$ of -12‰) (Smith and Epstein, 1971). By growing C_4 plants on soils previously only exposed to C_3 plants, this difference in isotope ratio may be utilized to study C partitioning in plant-soil systems. This technique can be not sensitive enough for detecting changes in C composition of disturbed bulk soil samples during and/or after a single growing season (Balesdent and Balabane, 1992). Plant C allocation to the soil is known to be influenced by physiological factors such as the stage of plant development (Keith et al.,

1986) and water availability (Boutton, 1996). To date, most studies using the natural ^{13}C abundance technique (e.g., Balesdent et al., 1987; Martin et al., 1990; Angers and Giroux, 1996) have dealt with C dynamics on whole disturbed soils and/or aggregate fractions sieved out of bulk samples. Its use in successive external surfaces of soil aggregates could estimate, on a short-term basis, the quantity and spatial location of root-derived organic C recently deposited on or near soil aggregates. Differences among the sequestration rates are important because the dynamics of C derived from C_4 are different than from C_3 plants resulting in contrasting C concentrations in a range of soil particle fractions (Huggins et al., 1995). In this case, the finer textured fractions accumulated more total C under C_4 (corn) than under C_3 (soybean) crops.

Objectives of the present study were to assess the effects of a C_3 crop on the quantity, composition and distribution of recently released root C to soil aggregates from a fallow soil dominated by C_3 grasses for the past 40 years. Using a short-term plant growth experiment, C originating from both a C_3 vegetation site and from contemporary ryegrass plants were traced and the plant root effects on some soil physical and chemical properties were investigated.

MATERIALS AND METHODS

Soil

The experimental soil was collected from the A horizon (0-20 cm) of an uncultivated site, under C_3-grass vegetation for at least 40 years, at the Kellogg Biological Station, near Hickory Corners, MI. Bulk samples of the soil, a Kalamazoo loam (fine loamy, mixed, mesic, Typic Hapludalf), were sieved to remove root materials and pebbles, and obtain aggregates in the size range of 3-7 mm in diameter. Selected properties of the soil aggregates are shown in Table 4.1.

Soil columns (construction and packing)

Polyvinyl chloride (PVC) columns, 20 cm diameter and 34 cm length, were split in half horizontally and reassembled with duct tape (Fig. 4.1). The bottom end of each column was covered with stainless steel wire screen (1 mm opening size) and eight layers of cheese-cloth secured with duct tape. Columns were placed on a 1-cm layer of steam-sterilized (220°C for 24 hours) pea gravel contained in a 30-cm wide aluminum foil plate with a drainage hole in the center. Two pairs of holes, 3 mm in diameter and 5 cm apart, were drilled in one side of the columns, 10 and 26 cm from the top. Two pairs of 19-cm long stainless steel waveguides, connected to a coaxial cable and embedded in resin at one end, were inserted through the holes in the PVC soil columns to monitor volumetric water content in each column by the Time Domain Reflectometry (TDR) technique (Topp et al., 1980).

Eight kg of air dry white silica sand was packed in layers of 2 kg at the bottom half of each column. The top half of each column was then filled with 30 alternating layers of previously weighed amounts of aggregates (94 g layer^{-1}) and sand (390 g layer^{-1}), respectively, so that the surface was about 2 cm from the top of the column. The columns were topped with 0.5 cm sand to minimize evaporation. The use of a mixture of sand and aggregates as growth media took into consideration that: (i) penetration resistance of sand has been reported to vary little with water content across a wide range of water contents (Cook et al., 1997); (ii) during a preliminary trial, a desirable quantity of preferential root growth occurred around soil aggregates; and (iii) plant roots can be easily washed free from the sand to ensure the entire root system is removed. Water characteristic curves of the growth medium (silica sand plus aggregates) and of the silica sand were determined on a ceramic pressure plate apparatus (Klute, 1986) and are reported in Fig. 4.2.

Planting

Columns were saturated with a half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) at a rate of 10 L per column and allowed to drain for 4 days. They were then saturated with distilled water and allowed to drain for approximately 1 day when the water content of the soil column was considered to be at 'container capacity'. On July 5 1997, ryegrass seeds (*Lolium perenne* L. 'Dimension'), with an average weight of 0.18 g per 100 seeds, were planted at a rate to provide about 4 plants per cm^2 of the column surface area. The experiment was set up and maintained in a greenhouse at 24 °C ± 2 day/night ambient temperature. Natural daylight was supplemented by high-pressure sodium vapor lights at 36,000 ft candles (387.5 lx), placed at a height of 1.5 m above the top of the columns, for a 16-h photoperiod. For the first two days after planting the top of the columns were covered with aluminum foil to reduce evaporative losses. On July 15, a copper sulphate ($CuSO_4.5H_2O$) solution (5% w/v) was sprayed on the surface sand to eliminate and prevent algae development.

Water regimes

One week after planting, columns were irrigated four times daily with a half-strength Hoagland's nutrient solution using 3 drip emitters per column, equally spaced, with a delivery rate of 10 mL min^{-1}. An automated irrigation system was set up to deliver the appropriate solution amounts to the treatments at 10:00, 14:00, 18:00, and 22:00 h. The influence of drought stress on allocation of root C to soil aggregates was studied by dividing the columns into two random groups, after initial wetting. On July 25, water stress was randomly imposed on one group of columns at the one-tiller stage by applying one-fourth (1 minute irrigation time) of the amount of water in the adequately watered columns (4 minutes irrigation time). Then , the number of drip emitters in the unplanted control treatment was changed to two per column. Irrigation was applied at 5:30, 9:30, 13:30, and 17:30 h. After July 31, the irrigation was exclusively distilled water in an attempt to reduce mineral toxicity symptoms in corn plants. On August 8, the number of emitters in the unplanted control treatment was reduced to one per column.

The amount of water applied was monitored in each column by the TDR technique. The two pairs of waveguides from each column were connected to a series of multiplexers by 2-m length 50-Ω coaxial cables which were then connected to a Metallic TDR cable tester, model Tektronix 1502C (Tektronix, Inc., Redmond, OR 97756). The cable tester was connected to an IBM-compatible PC (Fig. 4.1). The waveforms obtained on the cable tester were analyzed and converted to volumetric water content using a software written in BASIC language (J. Ferguson, unpublished) which utilizes the linear calibration equation of Lidieu et al. (1986).

Plant, soil and root sampling

The total growth period was for 6 weeks following planting. At harvest, plant shoots were cut at the soil surface and oven dried (65 °C) for determining dry matter and $\delta^{13}C$. The columns were then left without irrigation for a few days so that the soil columns would not be too wet when disassembled. Upon sampling, the soil was moist but not too wet so that aggregates were not excessively sheared during the procedure. A wooden piston apparatus was used to push the mass of sand plus aggregates out of each PVC column. The cylindrical segments were then cut longitudinally into 1/4 sections. Two 1/4 sections of the growth media in the top halves of the columns were kept for root analysis and the other two combined for soil aggregate analysis. In order to collect these samples, the top halves of the columns were further divided into three 5-cm layers to evaluate the influence of root density on C allocation to aggregates. The two 1/4 sections designated for soil analyzes were air-dried. The sand was gently separated from the soil aggregates and associated roots by brief manual sieving through 2-mm (15 cm wide) sieve. Large roots and associated soil aggregates were picked out by hand. Care was taken to separate those aggregates enmeshed by roots and discarding those with roots growing through them and those free of roots. Root samples were taken for determination of their $\delta^{13}C$ ratios.

The other two 1/4 sections of the growth media designated for root analyzes, plus the roots in the bottom halves of the columns, were bagged and stored at 4°C and later washed from sand by the hydropneumatic elutriation method (Smucker et al., 1982). Roots present in the bottom halves of each column were collected to investigate the potential effects of varying water content on root growth in pure sand. Washed roots were stored in whirlpack plastic bags containing about 200 mL 15% (v/v) methanol solution and preserved at 4°C. Root samples were dyed with Malachite green oxalate by injecting 5 mL of 1% (w/v) dye solution into the plastic storage bag (Smucker, 1990). Stained roots were rinsed with distilled water on an ultra fine (25 μm) nylon screen and uniformly distributed on a clear glass tray where the roots were covered with a thin water film for image video recording. The glass tray was illuminated from below by a translucent light table (to provide contrast between the roots and the background) and video recorded by a computer controlled robotic camera on VHS video tapes (Smucker, 1990). To avoid overlapping of roots, samples with large amounts of roots were split into 2 or more trays for the video recording step and then recombined for dry weight measurements. Roots were then dried at 60°C for 48 hours and their weights recorded. Video recorded root images (100 per tray samples) were processed by a Sun Ultra-based computer algorithm in the Root Image Processing Laboratory (RIPL), at Michigan State University (URL:http://www.rootdig.css.msu.edu) (Pietola and Smucker, 1998). The version of the program for washed roots (WR-RIPL version 3) was used for measurements of root length by classifying roots into 5 classes, based on their diameter, using an image resolution of 276 pixels per 1 cm by the image processing system. Average root width classes were Class 1 (0.2 mm), Class 2 (0.5 mm), Class 3 (0.9 mm), Class 4 (1.4 mm), and Class 5 (2.1 mm). Root length was measured for each class and a summation of roots in all classes was also obtained. All data reported are for roots without debris (Dowdy et al., 1998). Root surface area (RSA) was calculated as a sum of all image profile areas in all width classes, assuming a uniform cylindrical root shape, with the formula $A = 2 \pi r L$, where r is root radius and L total root length. Root volumes (RV) were calculated as $V = \pi r^2 L$.

Subsamples of intact aggregates of each class were analyzed for physical and chemical properties. Water-stability of air-dry aggregates was assessed with the wet sieving technique (Kemper and Rosenau, 1986). The tensile strength of dried aggregates was measured by the crushing test (Dexter and Kroesbergen, 1985). Soil aggregates were first air-dried and then oven dried at 105 °C for 24 hours and cooled in a desiccator before being crushed. Each aggregate was measured for its strength by a vertical force applied between two flat parallel plates (Fig. 4.3). A constantly increasing vertical force was applied to each aggregate by the top plate, which was connected to a computer-controlled stepper-motor. The bottom flat plate was the platform of a digital balance, whose output was recorded by a computer. The computer continuously recorded and graphed the applied forces on a monitor. The graphic peak associated with the first major decline in force, applied to the aggregate, was identified by solving for the first derivative of applied force *vs*. time using a program written in BASIC (John Ferguson, unpublished). The tensile strength of each aggregate was calculated by the equation

$$Y = 0.576 \; (F / D^2) \qquad (1)$$

where D is the aggregate effective spherical diameter and 0.576 is a proportionality constant relating maximum compressive stress to tensile strength at failure (Dexter and Kroesbergen, 1985). F is the force (N) required to fracture an aggregate and was calculated by

$$F = b\, g \qquad (2)$$

where b is the measured balance reading (in kilograms, kg) and g is the acceleration force due to gravity (9.807 m s^{-2}). The mean sieve diameter, d, of all the aggregates to be crushed (40 aggregates per treatment) was determined from the average of the sieve sizes ($d = (0.003 + 0.007)/2) = 0.005$ m). The effective spherical diameter (D) of each aggregate was then determined according to their individual masses, m, in kilograms. The adjustment was done on the assumption that all aggregates within each treatment had the same density, ρ (Dexter and Kroesbergen, 1985). If m_o is the mean mass of a batch of aggregates, then

$$\rho = (6\, m_o \;/\; \pi\, d^3 \,) = (\, 6\, m / \pi\, D^3 \,) \qquad (3)$$

which results in

$$D = d\,(m/m_o)^{1/3} \qquad (4)$$

Groups of soil aggregates of each class were mechanically separated into external and internal fractions using a modified version of the soil aggregate erosion (SAE) method described by Santos et al. (1997). In the present study, soil aggregates were abraded using the stainless steel (SS) mesoSAE chambers, 2.5 cm in diameter, developed by the Soil Biophysics Laboratory, at Michigan State University. A detailed abrasion protocol is given in Appendix A3. Briefly, each of 8-10 aggregates was placed in individual SS mesoSAE chambers and rotated at 300 rpm in a reciprocal shaker, having a rotational diameter of 2.5 cm. The shaker was stopped when 33% (± 2%) of the original aggregate mass was abraded (outer layer) and passed through the 356 μm screen inside the chamber. Soil material from each aggregate passing through the screen was then thoroughly mixed and stored in glass vials while the sand particles (> 356 μm) remaining on the screen were discarded. Another run was conducted so that a transitional layer of aggregates, representing about 33% of the remaining aggregate mass, could be collected. The percentages of the mass remaining at the time the outer and transitional layers were collected was considered to be the inner region of the aggregate (Appendix A3). Replicated soil samples from each concentric layer and whole aggregates were ground using a mortar and pestle. Total C was determined by dry combustion in a CHN analyzer (Carlo Erba, Italy) and $\delta^{13}C$ was determined by mass spectrometry (Europa Scientific, UK). The $\delta^{13}C$ value was expressed relative to the international standard PDB (as $\delta^{13}C$, ‰). The fraction of organic C derived from ryegrass plants was calculated using the modified form of the mixing equation (Balesdent and Mariotti, 1996):

$$New\ C_3\text{-}C\ (\%) = (\delta_s - \delta_{gs})/(\delta_{rye} - \delta_{gs}) \times 100 \qquad (5)$$

where δ_s is $\delta^{13}C$ value for an aggregate layer or whole aggregate cultivated with ryegrass, δ_{gs} is the $\delta^{13}C$ value for the corresponding aggregate layer or whole aggregate from the grass soil (unplanted control treatment), and δ_{rye} is the $\delta^{13}C$ value of ryegrass root residues.

Experimental design

A factorial experiment (3 x 2) was arranged in a split plot design with 8 replications per treatment (32 columns total). The main treatments were ryegrass, maize (data not reported), and an unplanted control, split into two water treatments (water-stressed and non-stressed control).

RESULTS AND DISCUSSION

Volumetric water content

The watering treatments produced differences in the water contents of the planted columns. Beyond 17 days after planting, soil water contents of the stressed treatments were always lower than that of the non-stressed treatment (Fig. 4.4). Top portions of the non-stressed columns of the unplanted control, had an average soil water content in the range of 0.17 cm^3 cm^{-3} while in the ryegrass columns it varied from 0.10 to 0.16 cm^3 cm^{-3}. Soil water contents in both top and bottom portions of the ryegrass columns of both stressed and non-stressed treatments dropped sharply, particularly in the last two weeks of the experimental period, indicating a very profuse growth of the ryegrass root system. Twenty-two days after planting, all treatments were watered to container capacity in a flow test to check for drainage limitations in any of the columns, as evidenced by the rise in water content seen on the graphs (Fig. 4.4). No limitations in drainage were observed.

Root and shoot dry weight

Differences in water content affected the accumulation of dry matter in ryegrass plants. Shoot and root dry weight, and root:shoot ratio decreased significantly with imposition of water stress (Table 4.2). These results differ from an earlier report concerning ryegrass's response to drought occurring under greenhouse environments. Jupp and Newman (1987) reported no significant differences in shoot dry weight and root:shoot ratio between *L. perenne* plants under severe and long-term water stressed and non-stressed treatments. However, they reported significant increases in lateral root initiation and elongation under prolonged low water potential, with total length of lateral roots increasing between three to five times compared to non-stressed plants. Also, the increase in root:shoot ratio of ryegrass plants with a decrease in water availability in loam soils, observed by Davidson (1969), was not verified in the present study using 75% sand.

Root parameters

Water deficit significantly reduced ryegrass total root length to the depth of 10 cm (Table 4.3). The distribution of the total RL across the several root width classes indicated an even higher proportion of roots in the Classes 1 and 2 (< 0.5 mm dia.). In the top of the columns, the percent of roots < 0.5 mm was 99% of the total RL for both stressed and non-stressed plants. In the lower column, the proportion of roots in the Classes 1 and 2 was even higher, but similar for both water treatments, approaching nearly 100% of the total RL (Table 4.4). From these results it is clear that fine roots (< 0.2 mm), make the largest contribution to the total root length. Low water contents had a deleterious effect on root dry weights at the depths of 0-5 and 5-10 cm, possibly because many of these roots had grown before drought conditions were initiated and may have died.

Ryegrass root surface area (RSA), volume (RV), length density (RLD) and dry weight density (RDWD) parameters were significantly lower in the stressed treatment at the 0-5 and 5-10 cm depth (Table 4.5). Root length density, an index of water uptake capacity, was comparable to values of 6.9 and 2.4 cm cm^{-3}, reported by Haynes and Francis (1993) for field-cultivated perennial ryegrass at the depths of 0-10 and 10-20 cm.

However, RLD values were much lower than values of 27.6 and 23.6 cm cm^{-3} for a sandy loam and a silty loam soil, reported by Reid and Goss (1981) for 42-d old ryegrass plants grown in a greenhouse. Although the present study did not include a pure soil aggregate bed for comparisons, these variations could be a result of the less favorable conditions for root growth in the sand plus soil aggregates mixture used in the present experiment.

Root LD in the bottom portion of the columns was 9 and 15 times lower than in the top portions, for water-stressed and non-stressed treatments, respectively. Similarly, RDWD values were 10 and 20 times lower in the bottom columns, respectively. Reduction in root parameters in the top 10 cm of the column was a direct effect of reduced root growth of plants under water deficit conditions. Higher RLD and RDWD for non -stressed plants were expected due to the ryegrass characteristic fine root networks and large branching and root length densities. The lack of significant differences between water treatments for the 10-15 and 17-34 cm depths, indicates that plant roots responded to decreasing soil water contents by greater growth in the wetter region of deeper soil. In this regard, severe water stress would cause damage to the root system while moderate stress would enhance its growth (Eghball and Maranville, 1993; Smucker et al., 1991). From Fig. 4.4 it can be seen that the imposed stress may have been too severe towards the end of the experimental period, especially in the top 10 cm, causing the rapid death of root hairs and cortical cells (Jupp and Newman, 1987). As there is some indication that roots of perennial ryegrass are relatively short-lived with a large proportion of roots living for less than 14 days at 27 °C (Forbes et al., 1997), recovery following an imposed stress would have a great effect on root turnover and regrowth.

Carbon isotope variation in plant tissues

Differences in $\delta^{13}C$ of both shoot and root of ryegrass were observed in plants grown under water stressed conditions. Significantly higher (less negative) $\delta^{13}C$ were observed in plants subjected to water stress (Table 4.6). The mean $\delta^{13}C$ value across water treatments for ryegrass was -29.8‰ for shoots, and -26.3‰ and -28.1‰ for stressed and non-stressed roots, respectively, across the three sampling depths. Differences in soil water availability and soil fertility, have a greater impact on stomatal conductance and photosynthetic capacity, respectively, of C_3 compared to C_4 plants (Balesdent and Mariotti, 1996; Boutton, 1996). Plants under adequate water levels would have open stomata to maximize the rate of CO_2 diffusion into the leaves. This results in a relatively high ratio of intercellular CO_2 concentration in the leaves (p_i) to the partial pressure of atmospheric CO_2 (p_a) and a smaller $\delta^{13}C$ (or larger Δ value, as expressed in the plant ecophysiological literature) (Farquhar et al., 1989; Boutton, 1996). The p_i/p_a ratio indicates the rate at which CO_2 diffuses into the leaves as opposed to the rate at which it is utilized by photosynthesis. Conversely, plants under conditions of water stress would decrease stomatal aperture to prevent water losses. Closure of stomata would also limit CO_2 diffusion into the leaves, resulting in low p_i/p_a and larger $\delta^{13}C$ (or smaller Δ values) (Boutton, 1996).

Soil C and ^{13}C ratio

Total C contents (0-5 cm depth) were higher for whole aggregates, outer layer and inner regions of soil aggregates cultivated with ryegrass under well-watered conditions compared to the unplanted soil (Fig. 4.5). For the water-stressed treatment, differences were found only when whole aggregates were analyzed indicating that the reduced root dry weights and lengths of plants under water stress explored lesser volumes of soil. For the 5-10 cm depth, C contents of whole aggregates and their outer layers, under non-stressed ryegrass differed significantly from the unplanted soil. Carbon contents in soil aggregates under plants with adequate water supply was always higher than for those under water deficit conditions in the top 5 cm (Fig. 4.5). Differences in C contents between water treatment and aggregates of the unplanted soil were generally not distinct for any of the depths. However, there was a trend of increased C content from 0-5 to 5-10 cm depth for whole aggregates and their outer layers. This could be an indication of partial leaching of the original C from the aggregates in the top 0-5 cm.

The higher C contents in soil aggregates cultivated with ryegrass and located in the top layers is likely to be a result of additions of organic materials to the soil from roots and root exudates. The higher proportion of ryegrass roots at 0-5 than at 5-10 cm (Table 4.5) corroborate this inference. Materechera et al. (1992) reported 27 to 30% increases of C in 2-4 mm aggregates after the soil was cropped to a different ryegrass species (***Lolium rigidum***) for 4-5 months. They attributed this increase to be the result of the high root mass and length density. The addition of C through senescence of root segments and root hairs growing around and within aggregates may have occurred in the present study. The more negative $\delta^{13}C$ values seem to confirm these effects in the inner regions of aggregates especially for plants under non-stressed conditions at 0-5 cm depth (Fig. 4.6). The changes in $\delta^{13}C$ even in the inner regions of aggregates are in agreement with our concept of aggregate invasions by root hairs of ryegrass and associated fungi which invade the interior of larger aggregates. Trends in $\delta^{13}C$ values in aggregates from the ryegrass columns under water stress were not as clear. These effects were evident due to the high root length density and root mass of ryegrass, consistent with the observation of Tisdall and Oades (1979, 1982) and Haynes and Francis (1993). These researchers reported that the amount of organic material released by roots is related to their length and mass. Greater root growth means greater possibilities for C production by root exudates as well as greater physical entanglement of particles by roots (Monroe and Kladivko, 1987; Reid and Goss, 1981). Thus the ability of crop roots to provide OC to soil structural units is an important contribution for long-term soil stability.

The cultivation of soil aggregates originally with an average initial $\delta^{13}C$ of -24.3‰ with ryegrass ($\delta^{13}C$ of roots ranging from -28.1 to -26.3‰) provided another opportunity for determining new C_3-C contributions to the soil system, using Eq. 5. After 6 weeks of ryegrass growth, $\delta^{13}C$ values for the whole aggregates became more negative by ~1‰ at both sampling depths with similar values for water-stressed and non-stressed treatments. This decrease strongly suggests that much of the C present in surfaces of aggregates originates from the current ryegrass plants. $\delta^{13}C$ values of outer layers and inner regions of aggregates decreased to a greater extent under non-stressed than in stressed conditions, with values similar to those in the whole aggregates (Fig. 4.6). In the 5-10 cm depth, $\delta^{13}C$ values were similar for outer layer and inner regions, regardless of irrigation treatment. At both depths, the greatest variations in $\delta^{13}C$ values were associated with aggregates from water stressed treatments, as values ranged from 1 to nearly 2 ‰ units. This seems to be a result of the high variability in the isotopic composition of plants under environmental stress (Balesdent and Mariotti, 1996) and/or an indication that the root-aggregate contact were more erratic under water stress conditions.

New C_3-C in whole aggregates was 62% (4.39 g kg^{-1}) for stressed ryegrass and 25% (2.07 g kg^{-1}) for the non-stressed treatment in soil aggregates located in the 0-5 cm region (Fig. 4.7). External layers and inner aggregate regions had 20% (0.88 g kg^{-1}) and 8% (0.39 g kg^{-1}) of new C_3-C, respectively. The sum of C_3-C contents in external layers and internal regions of aggregates was less than the values observed for whole aggregates because the transitional region (representing 1/3 of the aggregate mass) was not reported. This newly sequestered C that became part of the soil organic matter pool in the aggregates may have included some root debris that had been located within the aggregates. Except for these debris, all other visible root material, specifically very fine roots growing on the aggregate surfaces, had been carefully removed with the aid of a magnifier lens, prior to the aggregate peeling procedure.

Soil aggregates from the unplanted control columns showed a slight variation in $\delta^{13}C$ values with an increase of about on-half ‰ unit. Since the unplanted control samples were used as background to obtain the proportion of new C_3-C (Fig. 4.7) these variations were reflected in the estimated inputs. Further peeling of the original aggregates used in the columns could provide some insights into these variations. In addition, variations in the calculated proportions between stressed and non-stressed treatments for similar initial and final $\delta^{13}C$ values (e.g. outer layer of aggregates) were due to the different $\delta^{13}C$ assigned to roots when using Eq. 5. On average for all depths, $\delta^{13}C$ values of roots of water stressed plant were 1 to 2 ‰ units less negative than those of non-stressed plants (Table 4.6), leading to differences of 37% in the calculated new C_3-C inputs between water treatment (Fig. 4.7). This indicates the importance of the characterization of a specific root $\delta^{13}C$ to correct for differences in C inputs by plants under physiological stresses.

Mean weight diameter and aggregate tensile strength

Plants with greater root mass and root length density are usually expected to increase soil aggregate stability (Reid and Goss, 1980, 1981; Haynes and Francis, 1993). However, a significant effect of ryegrass on MWD was observed only for the 0-5 cm depth of non-stressed plants compared to stress treatment (Fig. 4.8). This effect is expected to persist for several months because roots decompose slowly (Tisdall and Oades, 1980). The lower MWD values in the ryegrass soil under water stress conditions may result from the fact that the aggregates used in this study were under meadow for at least 40 years, with relatively low soil C contents (Table 4.1). Partly because of their low C contents, these aggregates are not strongly bound together and drying causes acute development of incipient fracture faults, while rewetting, resulting in greater slaking (Haynes and Swift, 1990; Haynes and Beare, 1997). More frequent wetting and drying events seemed to influence in the MWD of aggregates in the unplanted control treatment with an indication of higher values towards the surface layer (Fig. 4.8). The apparent no change in MWD with depth for non-stressed conditions corroborate this observation. Fewer roots of ryegrass at the depth of 10-15 cm resulted in lower MWD values, compared to the unchanged MWD in the control treatment.

Factors such as the intensity and frequency of wetting and drying, the previous management of the soil, the proportion of clay, and continued wetting and drying cycles decrease the percentage of water-stable aggregates and tensile strength of stabilized aggregates by tilth mellowing (Utomo and Dexter, 1981). The high RLD and RDWD of ryegrass probably resulted in very fine roots penetrating the aggregates which, in turn, increased the chance of aggregate breakage. Although tensile strength measurements were conducted on visibly intact aggregates, the values for aggregates under ryegrass at the top 5 cm were significantly lower than at 5-10 cm (Fig. 4.9). For the non-stressed ryegrass treatment, tensile strength values were similar in the top 10 cm while at 5-15 cm showed the lowest value compared to the unplanted control.

CONCLUSIONS

The experiment showed important influences of water availability on growth of plant roots and its effects on properties of soil aggregates. There were significant differences in shoot and root dry weights of ryegrass plants in response to water stress. The majority of total RL was comprised by roots < 0.5 mm diameter indicating the importance of fine root contribution to the total RL. Water availability significantly changed ryegrass rooting patterns and root activity to the depth of 10 cm. In general, RSA, RLD, and RDWD of ryegrass decreased with water stress. After 6 weeks of ryegrass growth, C contents were higher in the whole aggregates and their outer and inner regions under well-watered conditions. For samples subjected to water stress higher C contents were observed only when the whole aggregates were analyzed. Regardless of water treatment, $\delta^{13}C$ values for whole aggregates became ~1‰ more negative in relation to the initial $\delta^{13}C$ value of -24.3‰ for this soil. The aggregate abrasion technique described was useful in quantifying the amount of recently released root C into soil aggregates. Inputs of new C_3-C in whole aggregates were estimated to be 62% (4.39 g kg^{-1}) and 25% (2.07 g kg^{-1}) of the total C in stressed and non-stressed ryegrass treatments at the 0-5 cm depth. For external layers and inner regions of aggregates associated with stressed plants new C_3-C accounted for 20% (0.88 g kg^{-1}) and 8% (0.39 g kg^{-1}) of the total C. Significant influences of water and plant treatments on soil aggregate stability and tensile strength were not evident from this experiment, probably as a result of the relatively short time.

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Table 4.1. Selected properties of aggregates (3-7 mm dia.) of the Kalamazoo loam soil used in the greenhouse experiment. Kellogg Biological Station, Hickory Corners, MI. 1996.

Property	Status
pH (water)	6.5
CEC	6.3 cmol kg^{-1}
Total C	6.3 g kg^{-1}
Total N	1.6 g kg^{-1}
$\delta^{13}C$ signature	-23.4 ‰
P	0.12 g kg^{-1}
K	0.18 g kg^{-1}
Ca	0.91 g kg^{-1}
Mg	0.15 g kg^{-1}
Zn	4 mg kg^{-1}
Mn	24 mg kg^{-1}

Table 4.2. Dry weights of leaves, stems, shoots, and roots and shoot/root ratio of ryegrass plants grown in a sand (75%) and soil aggregate (25%) matrix for 6 weeks under water stressed and non-stressed conditions (Means ± standard error, *n* = 8).

	Leaf	Stem	Shoot	Root[†]	Total R/S ratio
	----------------------------------	---------- g	----------	----------------------------------	
			Ryegrass		
Stress	10.29 ± 0.95	4.15 ± 0.41	14.44 ± 1.27	4.63 ± 0.61	0.32 ± 0.03
Non-stress	14.41 ± 1.44	5.53 ± 0.59	19.94 ± 1.47	7.23 ± 0.64	0.37 ± 0.03

[†] Root data are the sum of all the roots in the two 1/4 sections of the top portions (0-15 cm) of each column, multiplied by two, plus the roots in the bottom (17-34 cm) portion of each column.

Table 4.3. Lengths of five different root width classes of ryegrass plants grown in a sand (75%) and soil aggregate (25%) matrix for 6 weeks under water stressed and non-stressed conditions. (Means ± standard error; $n = 8$).

	Root width classes					Total
	Class 1 (0.2 mm)	Class 2 (0.5 mm)	Class 3 (0.9 mm)	Class 4 (1.4 mm)	Class 5 (2.1 mm)	
	Root length (m)					
			0 - 5 cm depth			
Stress	57.4 ± 8.5	6.2 ± 0.9	1.5 ± 0.5	0.17 ± 0.07	0.009 ± 0.005	65.3 ± 8.9
Nonstress	95.7 ± 13.0	13.0 ± 2.3	1.6 ± 0.4	0.25 ± 0.09	0.028 ± 0.013	110.6 ± 14.3
			5 - 10 cm depth			
Stress	21.8 ± 4.6	1.0 ± 0.3	0.04 ± 0.02	0.01 ± 0.01	0.001 ± 0.001	22.9 ± 4.8
Nonstress	70.3 ± 6.6	6.4 ± 0.8	0.11 ± 0.02	0.01 ± 0.01	0.004 ± 0.003	76.8 ± 7.2
			10 - 15 cm depth			
Stress	28.4 ± 7.2	2.2 ± 0.8	0.15 ± 0.12	0.02 ± 0.02	0.002 ± 0.002	30.8 ± 7.8
Nonstress	43.5 ± 10.7	4.5 ± 1.2	0.15 ± 0.05	0.01 ± 0.06	0.001 ± 0.001	48.2 ± 11.8
			17 - 34 cm depth			
Stress	24.7 ± 6.9	4.4 ± 1.3	0.10 ± 0.02	0.01 ± 0.01	0.00	29.2 ± 8.1
Nonstress	29.2 ± 6.5	5.4 ± 1.4	0.05 ± 0.01	0.00	0.00	34.7 ± 7.9

Table 4.4. Percentage of roots in individual root width classes of ryegrass plants grown in a sand (75%) and soil aggregate (25%) matrix for 6 weeks under water stressed and non-stressed conditions. (Means ± standard error; *n* = 8).

	Root width classes				
	Class 1	Class 2	Class 3	Class 4	Class 5
	(0.2 mm)	(0.5 mm)	(0.9 mm)	(1.4 mm)	(2.1 mm)
			%		
			<u>0 - 5 cm depth</u>		
Stress	87.9 ± 2.3	9.4 ± 1.4	2.4 ± 0.8	0.3 ± 0.1	0.01 ± 0.01
Nonstress	85.4 ± 2.3	12.4 ± 1.7	1.8 ± 0.5	0.3 ± 0.1	0.04 ± 0.02
			<u>5 - 10 cm depth</u>		
Stress	94.6 ± 1.5	4.4 ± 0.9	0.7 ± 0.6	0.2 ± 0.2	0.03 ± 0.03
Nonstress	91.5 ± 0.6	8.3 ± 0.6	0.2	0.0	0.01
			<u>10 - 15 cm depth</u>		
Stress	91.5 ± 1.5	8.0 ± 1.4	0.4 ± 0.2	0.0	0.02 ± 0.02
Nonstress	90.4 ± 1.2	9.2 ± 1.0	0.4 ± 0.2	0.0	0.00
			<u>17 - 34 cm depth</u>		
Stress	85.2 ± 1.3	14.3 ± 1.2	0.4 ± 0.3	0.1 ± 0.1	0.02 ± 0.02
Nonstress	85.6 ± 1.5	14.2 ± 1.5	0.1	0.0	0.00

Table 4.5. Total root dry weight (RDW), length (RL), surface area (RSA), volume (RV), length density (RLD), and dry weight density (RDWD), for all root width classes of ryegrass plants grown in a sand (75%) and soil aggregate (25%) matrix for 6 weeks under water stressed and non-stressed conditions. (Means ± standard error; *n* = 8).

	RDW (g)	RL (m)	RSA (cm^2)	RV (cm^3)	RLD ($cm\ cm^{-3}$)	RDWD ($\mu g\ cm^{-3}$)
			<u>0 - 5 cm depth</u>			
Stress	1.61 ± 0.20	65.20 ± 8.93	506.93 ± 63.44	4.24 ± 0.63	8.30 ± 1.14	1541.53 ± 400.03
Nonstress	2.35 ± 0.29	110.65 ±14.33	865.07 ± 108.15	7.09 ± 0.98	14.09 ± 1.82	2989.07 ± 374.37
			<u>5 - 10 cm depth</u>			
Stress	0.48 ± 0.05	22.84 ± 4.83	154.08 ± 32.88	0.92 ± 0.20	2.91 ± 0.62	529.87 ± 92.45
Nonstress	0.73 ± 0.06	76.85 ± 7.21	546.58 ± 51.51	3.57 ± 0.34	9.79 ± 0.92	931.44 ± 77.14
			<u>10 - 15 cm depth</u>			
Stress	0.49 ± 0.15	30.79 ± 7.83	218.87 ± 57.03	1.47 ± 0.43	3.92 ± 1.00	621.24 ± 186.78
Nonstress	0.36 ± 0.05	48.14 ± 11.84	348.61 ± 85.56	2.36 ± 0.58	6.13 ± 1.51	451.95 ± 62.11
			<u>17 - 34 cm depth</u>			
Stress	0.40 ± 0.15	29.14 ± 11.52	226.52 ± 89.33	1.70 ± 0.66	0.55 ± 0.15	75.03 ± 28.03
Nonstress	0.37 ± 0.06	34.64 ± 11.11	269.26 ± 88.93	2.00 ± 0.69	0.65 ± 0.15	68.41 ± 11.21

Table 4.6. $\delta^{13}C$ signatures of shoot (n = 8) and root (n = 4) parts of ryegrass plants grown in a sand (75%) and soil aggregate (25%) matrix for 6 weeks under stressed and non-stressed water conditions. (Means ± standard error).

	Stressed	Non-stressed
	‰	
	Shoot	
	-29.6 ± 0.20	-30.1 ± 0.23
	Root	
Depth		
0-5 cm	-25.8 ± 1.40	-27.9 ± 0.66
5-10 cm	-26.0 ± 1.72	-28.1 ± 0.72
10-15 cm	-27.3 ± 0.71	-28.3 ± 0.79
Mean (n=12)	-26.3 ± 0.73	-28.1 ± 0.38

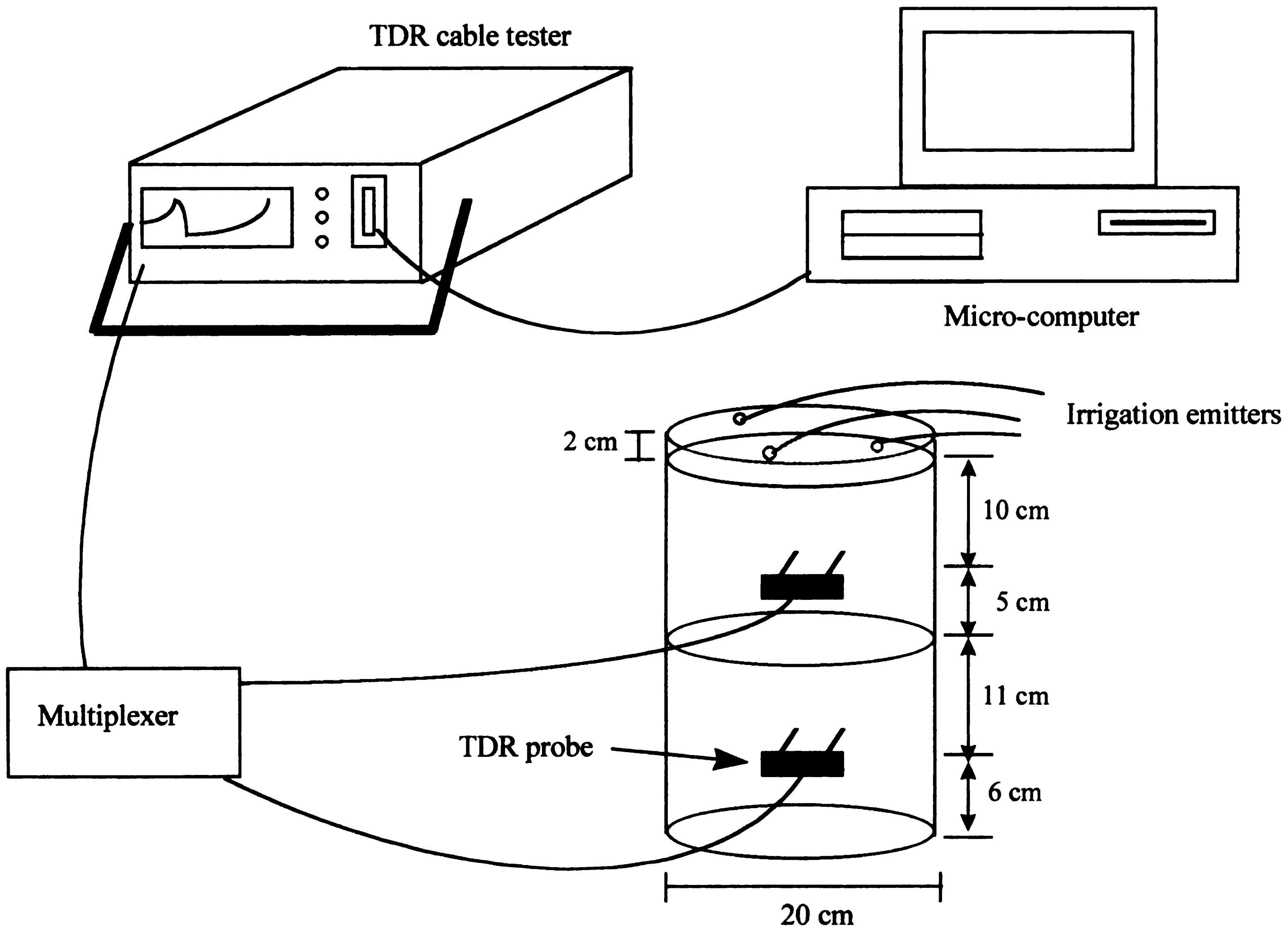


Figure 4.1. Simplified representation of the system used for drip irrigation and measurements of water contents at the depths of 10 and 26 cm in the PVC columns using an automated and multiplexed time-domain reflectometry (TDR) equipment.

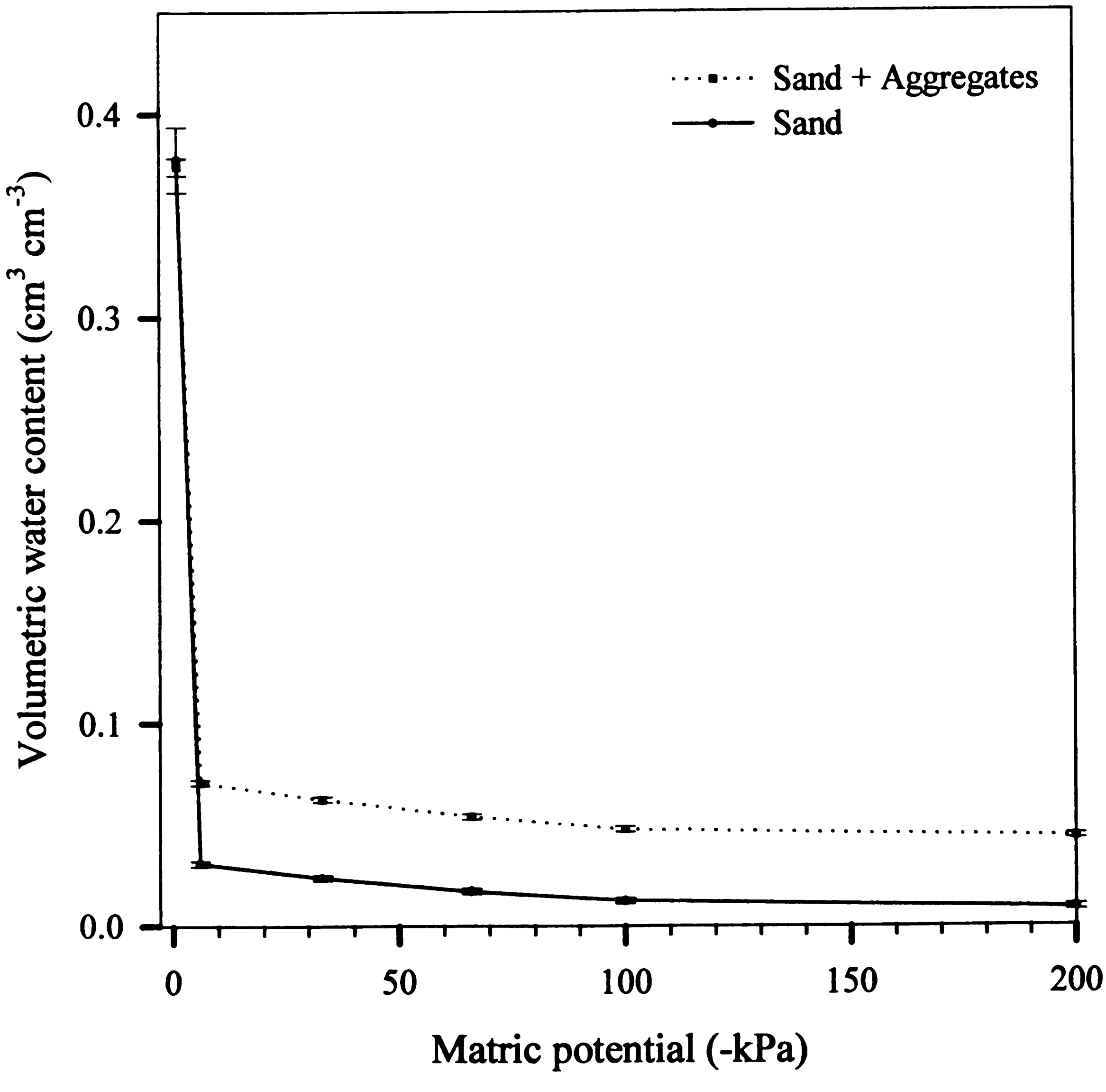


Figure 4.2. Water characteristic curves for the growth medium (silica sand and soil aggregates) and pure silica sand used in the greenhouse experiment.

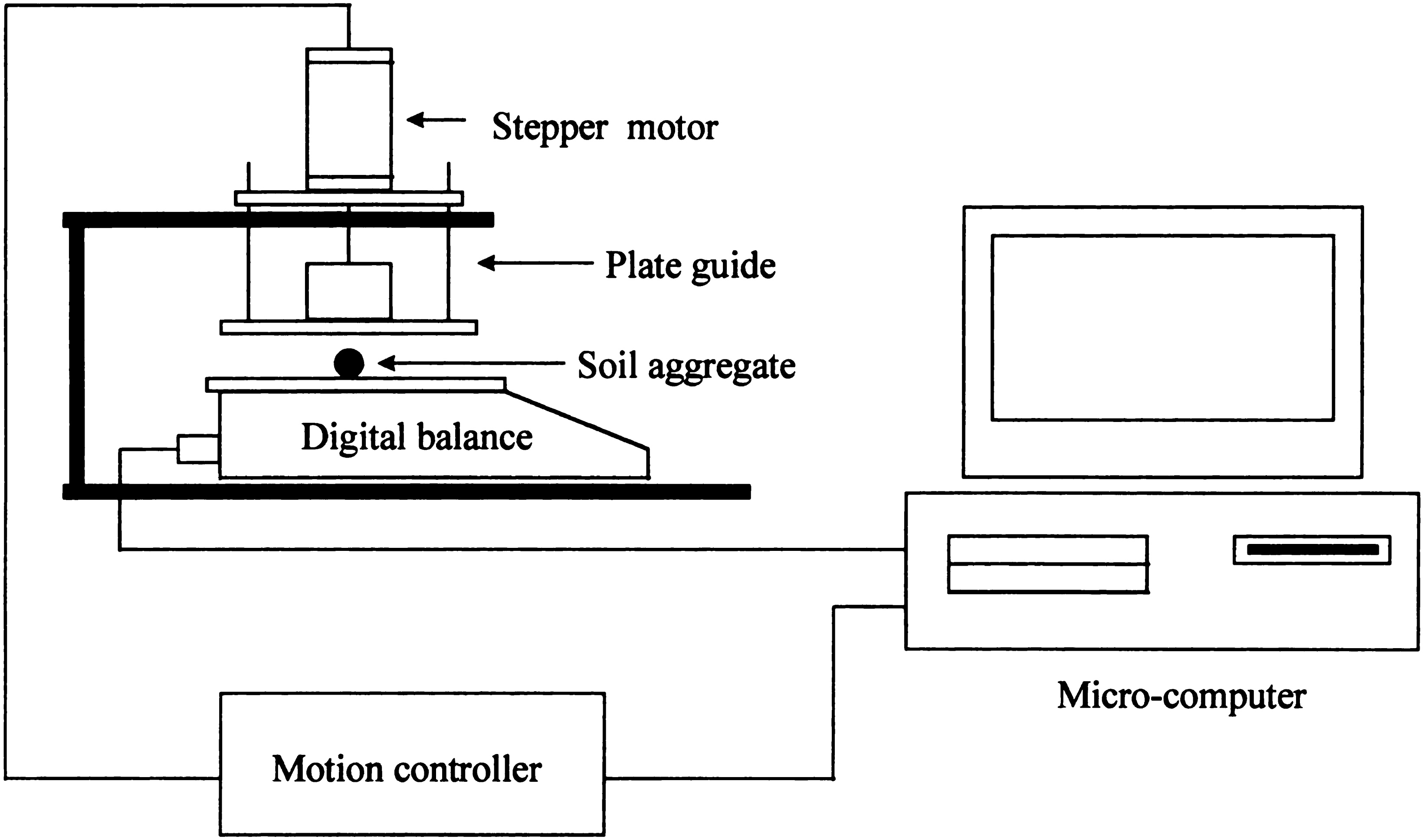


Figure 4.3. Diagrammatic representation of the apparatus used for automated measurements of tensile strength of individual soil aggregates.

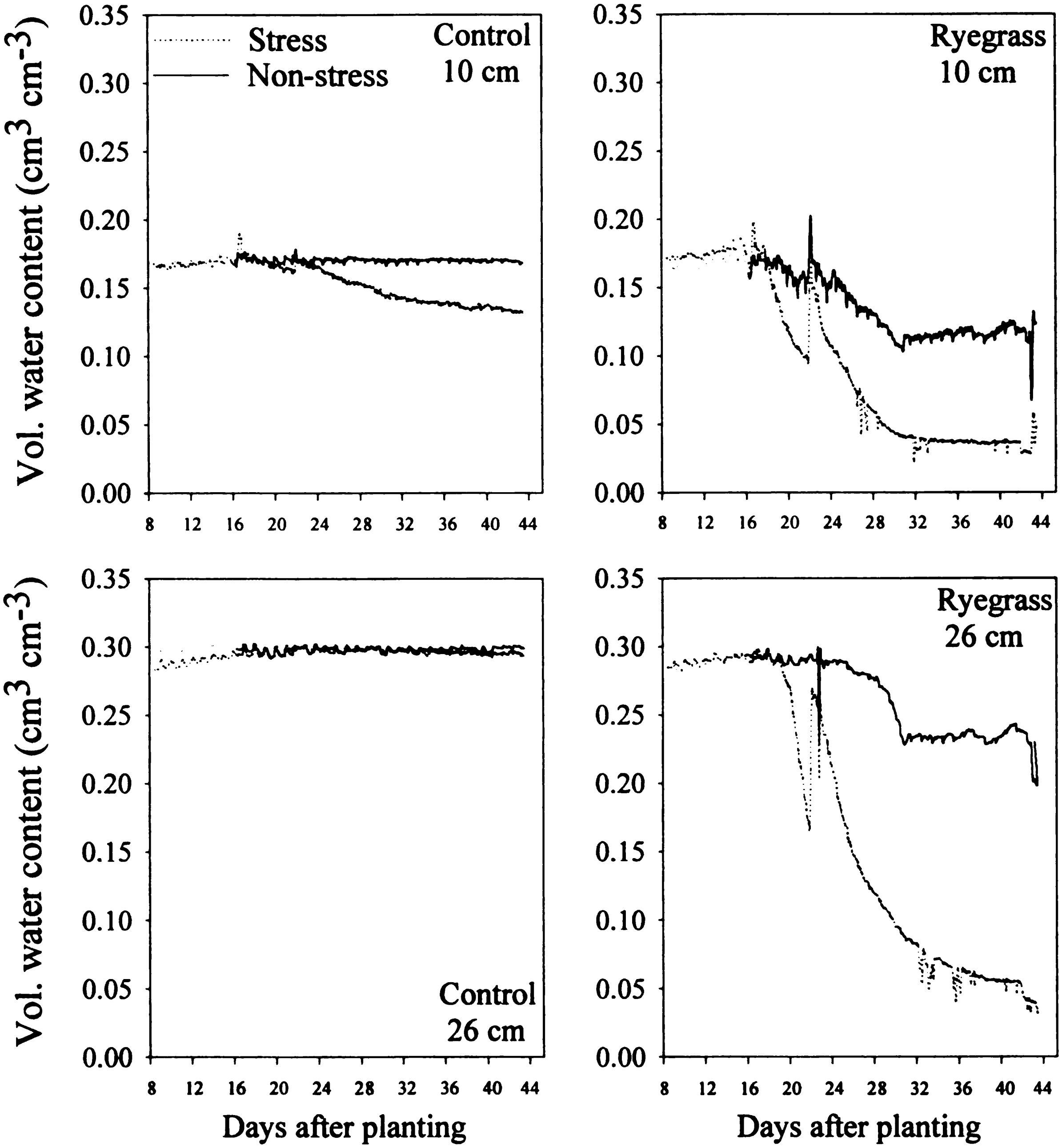


Figure 4.4. Volumetric water contents in the control and ryegrass treatments, as a function of water level applied (stressed and non-stressed plants), and position of the TDR probes (10 and 26 cm depths). Mean values, *n* = 8.

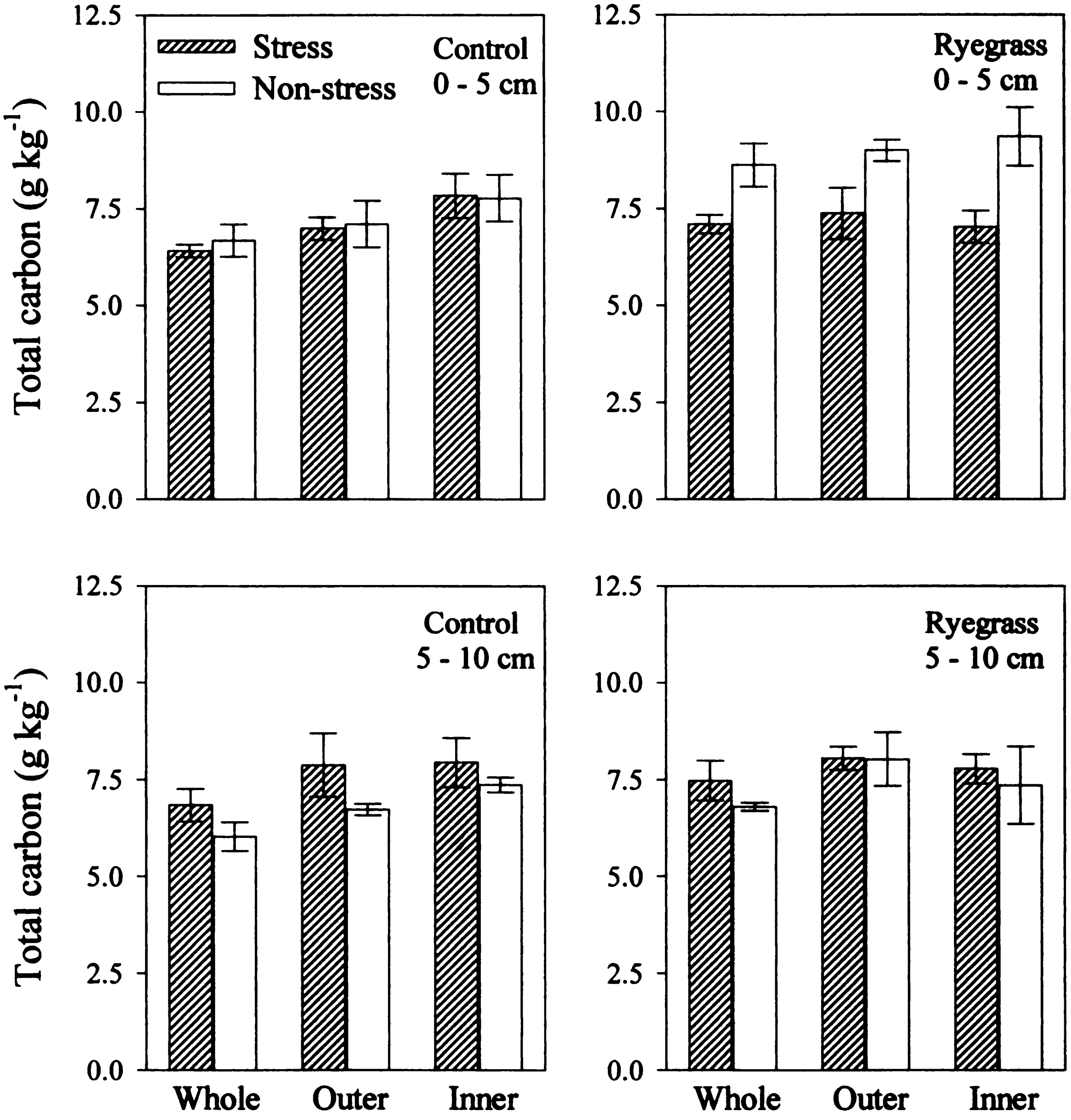


Figure 4.5. Total carbon contents in whole soil aggregates, outer layers, and inner regions of soil aggregates from the control and ryegrass treatments in a sand (75%) and soil aggregate (25%) matrix after 6 weeks under water stressed and non-stressed conditions. Means ± standard error, *n* = 4.

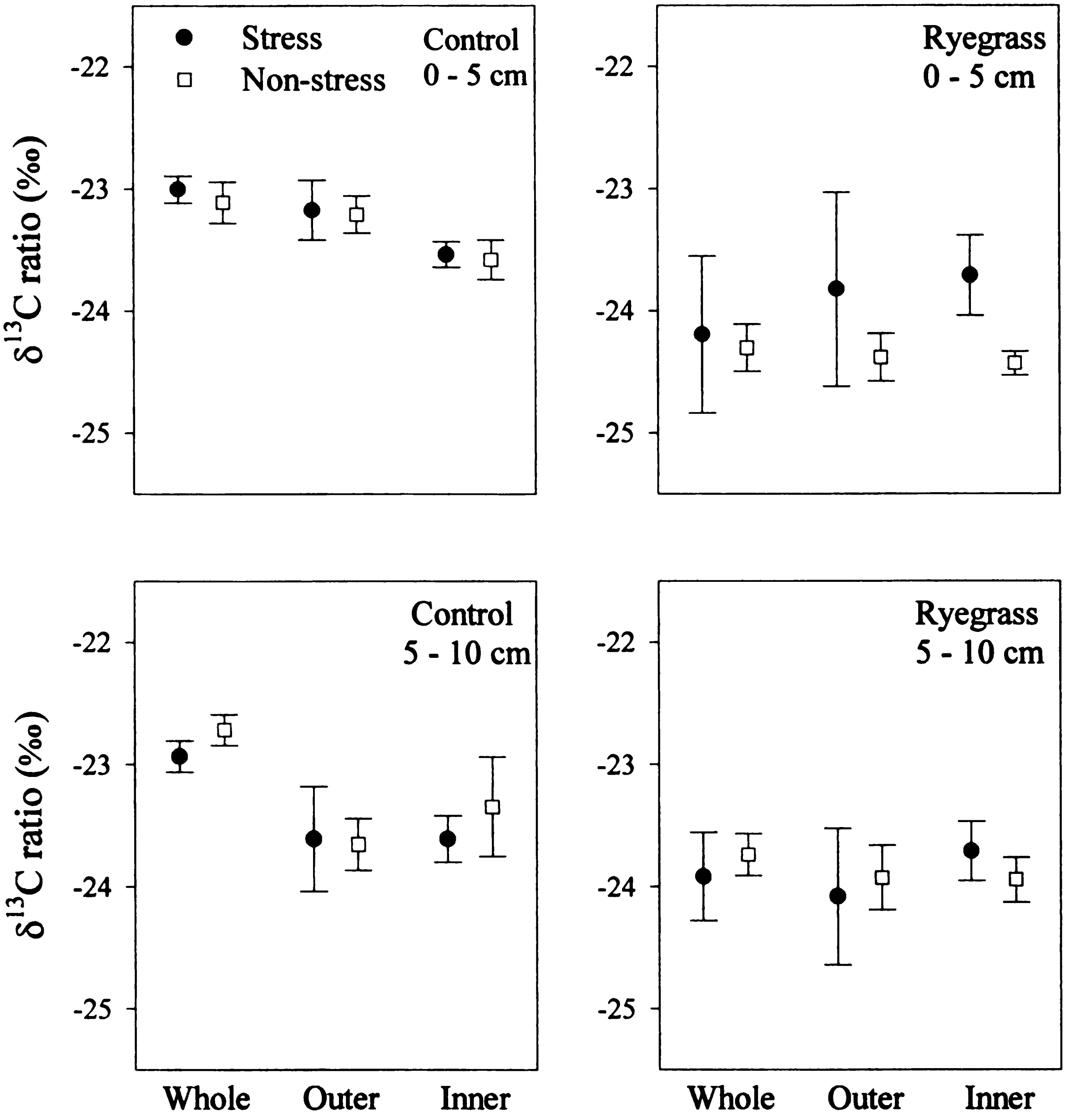


Figure 4.6. $\delta^{13}C$ ratios in whole soil aggregates, outer layers, and inner regions of soil aggregates from the control and ryegrass treatments in a sand (75%) and soil aggregate (25%) matrix after 6 weeks under water stressed and non-stressed conditions. Means ± standard error, *n* = 4.

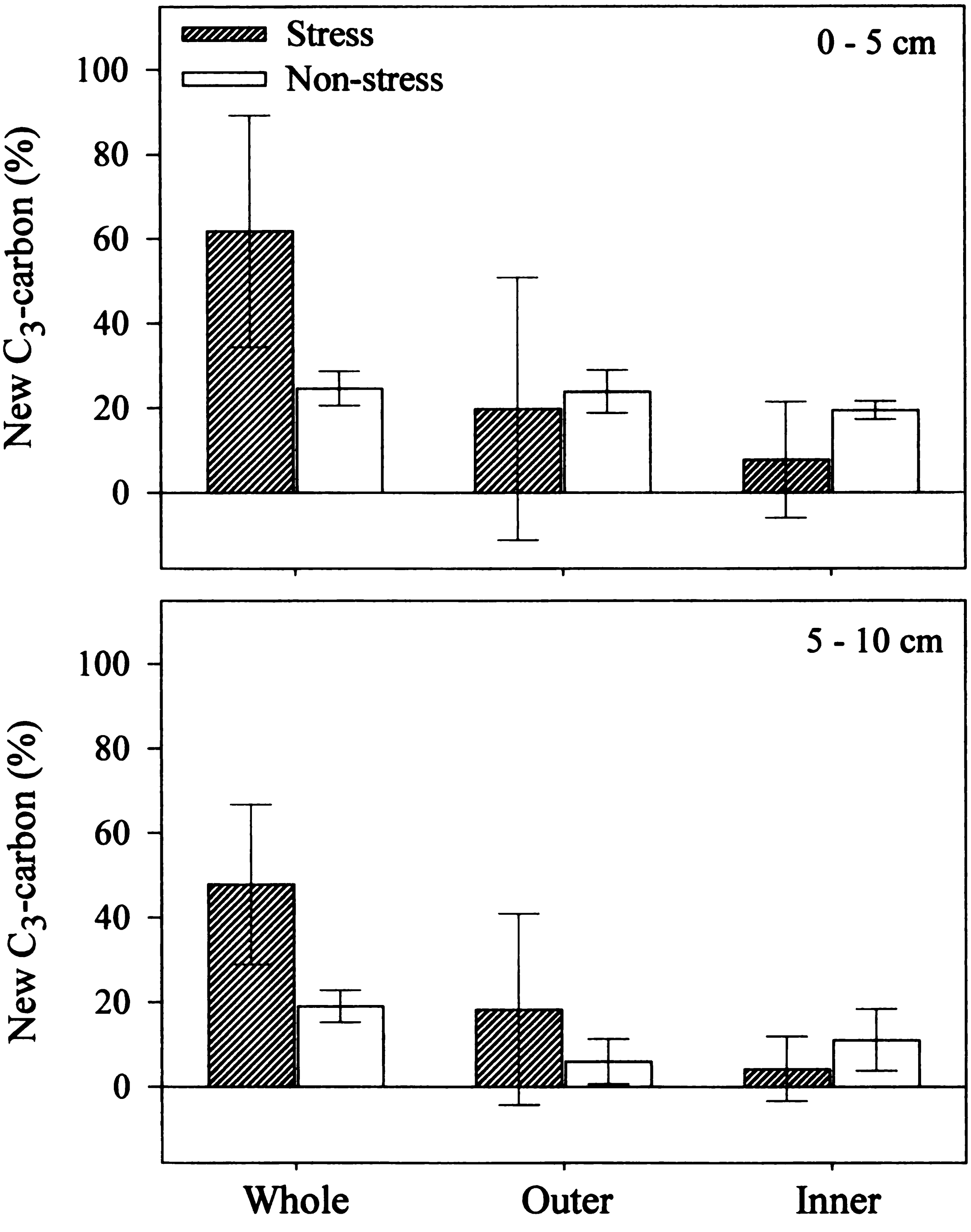


Figure 4.7. Percentage of new C_3-carbon in whole soil aggregates, outer layers, and inner regions of soil aggregates the control and ryegrass treatments in a sand (75%) and soil aggregate (25%) matrix after 6 weeks under water stressed and non-stressed conditions. Means ± standard error, *n* = 4.

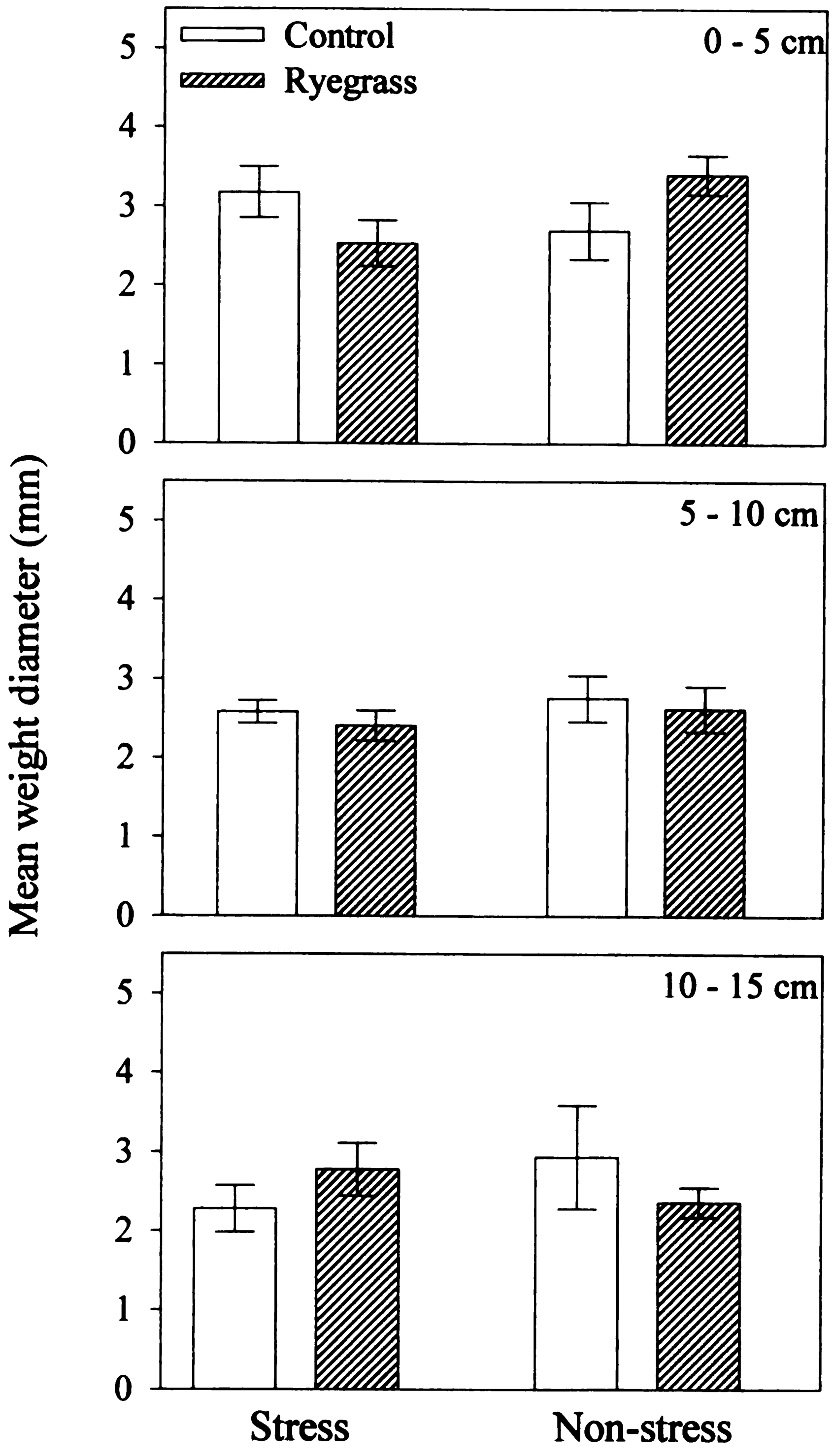


Figure 4.8. Mean weight diameter (MWD) of soil aggregate samples from the control and ryegrass treatments in a sand (75%) and soil aggregate (25%) matrix after 6 weeks under water stressed and non-stressed conditions. Means ± standard error, *n* = 4.

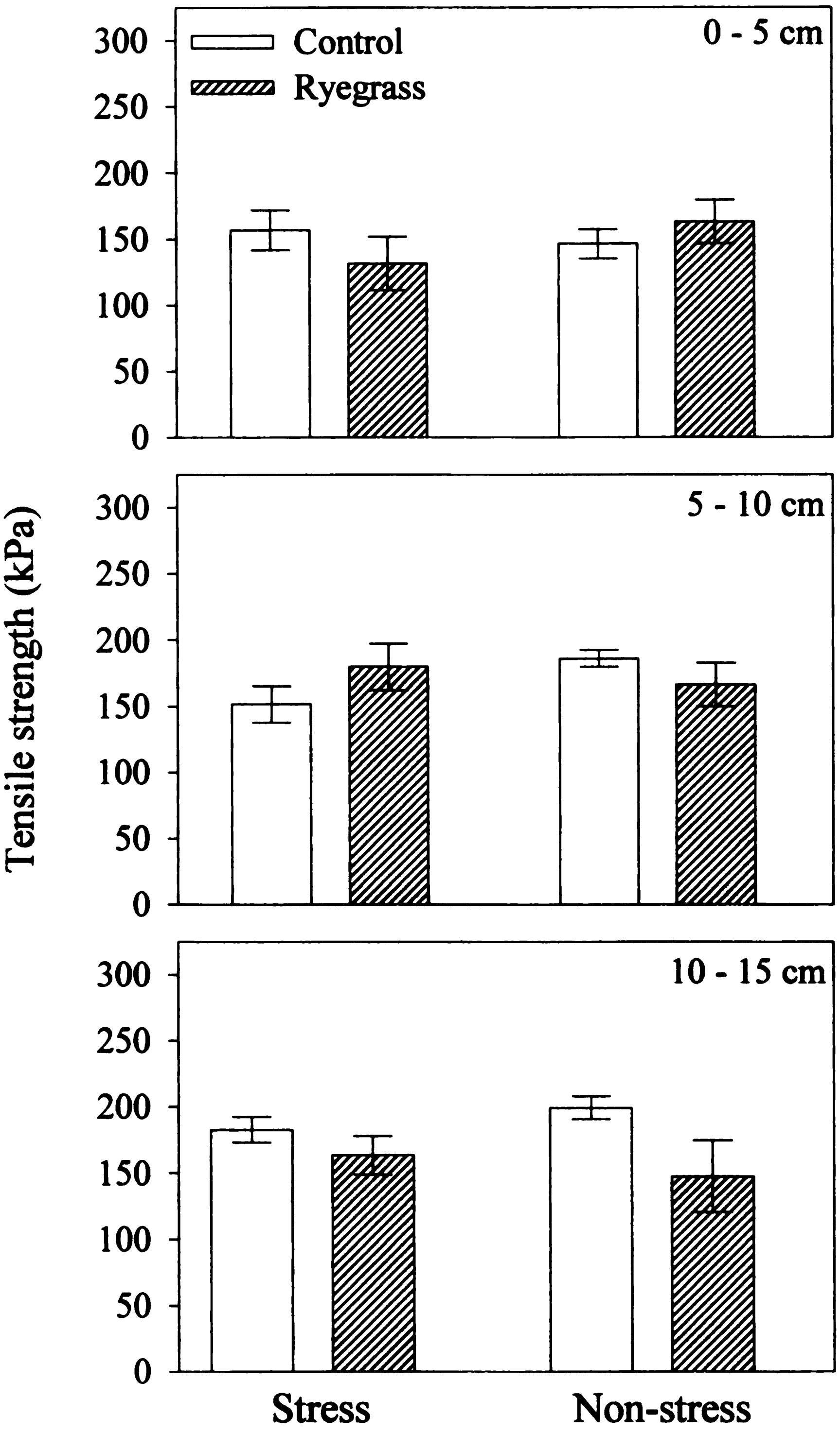


Figure 4.9. Tensile strength of of soil aggregate samples from the control and ryegrass treatments in a sand (75%) and soil aggregates (25%) matrix after 6 weeks under water stressed and non-stressed conditions. Means ± standard error, $n = 4$.

GENERAL CONCLUSIONS

The improved version of the Soil Aggregate Erosion (SAE) apparatus, designed and built for this study based on an earlier version (Chapter 1), was a useful tool for peeling soil aggregates. This novel approach provided opportunities for quantifying the accumulation of C and ions within specific regions of soil aggregates. Aggregate abrasion measurements by the SAE apparatus appear to also provide a method for determining dry soil aggregate stability.

Deposition of plant root C to soil aggregates was evaluated by locating recent additions of C_3-C within their concentric SAE-peeled layers using the $\delta^{13}C$ technique. The results demonstrated greater concentrations of C accumulated on external layers than in the interior regions of aggregates sampled from 0-5 cm depths. Approximately 70% of root-deposited C accumulated on external layers of conventional tillage (CT) aggregates during 20 months of alfalfa (C_3-C) growth. Though total C inputs were higher in no-tillage (NT), deposition of C_3-C on external layers was lower than in aggregates from CT soils.

No-tillage treatments caused significant increases in the stability of aggregates > 0.5 mm. Following 11 years of NT, aggregate C concentrations increased with improvements in macroaggregate stability at depths of 0-5 and 5-15 cm. Eleven years of NT maize without N fertilization caused a reduction in aggregate C contents with little modification of aggregate stability. In soils under long-term cultivation (CT and NT), macroaggregation was greatly reduced with a concomitant increase in the proportion of microaggregates. NT showed clear trends in the sequestration of C during the development of new macroaggregates and/or stabilization of old macroaggregates. Therefore NT of this soil might be an important approach for increasing the storage and protection of SOM which may be lost during CT.

Drought stresses changed ryegrass rooting patterns and activity as indicated by decreases in RDW, RL, RSA, RV, RLD, and RDWD to depths of 10 cm. After 6 weeks of ryegrass growth in a sand (75%) and soil aggregate (25%) matrix, C contents were higher in whole soil aggregates, in both their outer and inner regions, when ryegrass was irrigated. Under drought stress conditions, higher soil C contents were observed only when whole aggregates were analyzed. Inputs of new C in whole aggregates were estimated to be 62% in drought-stressed and 25% in non-stressed ryegrass treatments at the 0-5 cm depth. New C accounted for 20 and 8% of the total C in the external layers and inner regions of aggregates. Several constraints and difficulties are associated with studies of plant drought stress under controlled conditions: i) Regulating water-stress regimes in sand for some crops may be a limiting factor when using sand growth media instead of soil; ii) To simulate field processes drought stress in the greenhouse should develop gradually; and iii) Soil volumes occupied by root systems and associated water availability in the rooting zone are confined to the column *vs*. fractions of the root system in field-grown plants.

Several new questions were raised by this study which deserve further investigation: i) NT soils appeared to accumulate more C from the previous maize crop and/or slow the decomposition of the newly deposited C from alfalfa; ii) Developments in aggregate abrasion techniques and soil sampling provide a new processing approach for peeling aggregates of several sizes for further confirmation of C deposition patterns within soil structural units; iii) Wet sieving methods have some limitations as redistribution of SOM among the sieved aggregate fractions may be produced by the method itself; iv) optimization of the test conditions, e.g., pretreatment of soil aggregates and duration of sieving are required before wet sieving can be universally applied to C studies for all soil types; and v) Additional research using different soils and crop species, accompanied by wetting/drying cycles and sequential soil sampling would provide further insights into the factors affecting root C deposition into aggregates and the effects of soil C on the chemical and physical processes of soil aggregate stability.

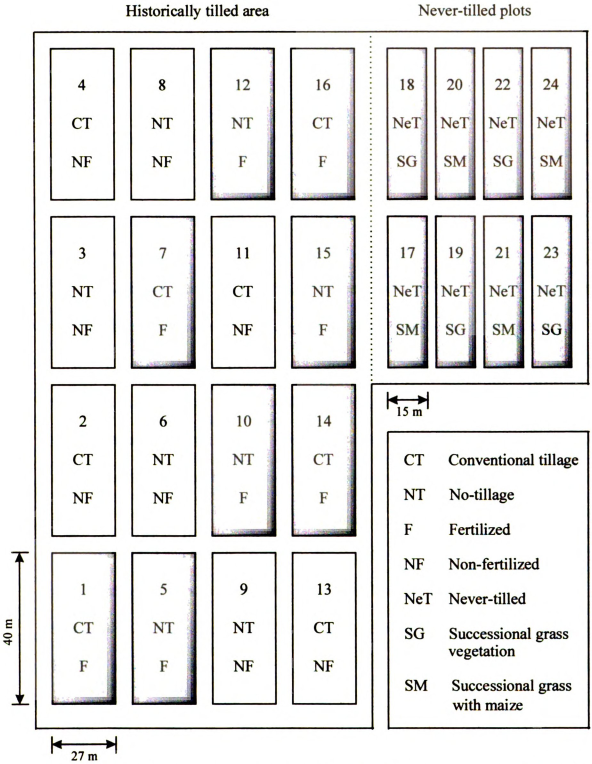
APPENDICES

Appendix A1. Selected characteristics of bulk surface soil samples of conventional tillage (CT), no-tillage (NT), and never-tilled successional grassland (NeTSG) of the Agroecosystems Interations site at W.K. Kellogg Biological Station, Hickory Corners, MI.

Treatment	Particle size Sand	 Silt	 Clay	Org. C	N	WSA	pH†	CEC†
	g kg^{-1}					%		cmolc kg^{-1}
CT	421	431	147	12.4	1.1	78.5	6.3	9.0
NT	422	443	135	11.3	1.0	87.6	5.5	10.0
NeTSG	391	468	141	18.2	1.6	90.3	5.3	8.9

WSA = water-stable aggregates (> 250 μm).

† From Daroub (1994).



Appendix A2. Plot layout and sampled plots (shaded) of the Agroecosystems Interactions site at W.K. Kellogg Biological Station. Hickory Corners, MI.

Appendix A3. Steps for the progressive and quantitative erosion of concentric layers from soil aggregates by the mesoSAE chambers. (Smucker, A.J.M., J. Wagester, and D. Santos, unpublished).

Pre-cleaning

1. Place 10-20 grams of coarse sand particles, which can be retained on the screen of the SAE chambers to pre-clean them. Place the chambers into the bases, cap the chambers with plastic bottle tops or rubber stoppers, enclose in foam supports, and place them onto a rotational shaker. Run for 30 minutes at 300 rpm.

   Note: In order to secure the mesoSAE chambers onto the shaker it may be necessary to place them into beakers. Try placing 100mL glass beakers into the clamps on the rotational shaker. Then cut pieces of foam about 25 cm long and 5 cm wide. Back the foam with pieces of duct tape. Place the mesoSAE chambers in the middle of the foam with the taped side to the outside. Wrap the foam around the mesoSAE chamber and then place them into the 100mL beakers which are secured to the clamps on the rotational shaker.
2. Discard the sand and thoroughly brush the knurled walls of the SAE chambers with a wire brush or toothbrush. Clean the screen of the SAE chambers by turning them upside down and brushing the screen with a steel wire brush. First, use a stiff painting brush to remove any material from the base and then compressed air to blow the SAE chambers and the bases clean. Inspect for trapped material and remove it.
3. Separately weigh the clean bases and the clean SAE chambers to the nearest 0.1 mg. Record the weights in a permanent file.

Preliminary Erosion Data

4. Select a minimum of 25 uniform air-dry soil aggregates from the same sample. Aggregates 4-5 mm in diameter are recommended, however, aggregates from 0.2 mm to 18 mm can be abraded in the mesoSAE chambers. Weigh each aggregate to the nearest 0.1 mg and record the weight. Gently place it onto the screen of the SAE chamber. Weigh the SAE chamber with the aggregate inside of it and weigh the base. Record the weights. The weight of the SAE chamber with the aggregate inside of it and the weight of the base will be the weights for time 0. This will expedite the collection of preliminary soil erosion data. Repeat this procedure for each of the 25 selected aggregates.
5. Cap the 25 mesoSAE chambers and rotate them at 300 rpm for separate runs of 1, 2, 3, 4, 5, 6, 9, 12, 24, 48, and 60 minutes. Note: Each time includes the sum of the previous times, i.e., a time of 12 minutes is 9 of the minutes already run with three additional minutes of rotation. If one was to calculate the amount of minutes of actual rotation it would be the following: 1, 1, 1, 1, 1, 1, 3, 3, 12, 24, and 12 minutes. After each run, weigh the SAE chamber with the aggregate and the particles inside of it, and weigh the base with the eroded materials in it. For maximum accuracy, the sum of the SAE chamber with the aggregate inside of it and the base at time 0 should always equal the sum of the SAE chamber with the aggregate in it and the base with the eroded particles in it for all timed runs.
6. Once all of the timed runs have been completed, examine the contents of each SAE chamber. If the center of the aggregate remains, separately weigh the aggregate center,

the contents of the SAE chamber, and the contents of the base. To weigh an aggregate or an aggregate center, extract the aggregate or aggregate center and place it onto a piece of tared weighing paper that is on a balance. Record the weight to the nearest 0.1 mg. Remove the weighing paper with the aggregate or aggregate center on it. Re-tare the balance with a new sheet of weighing paper. Use this weighing paper for the contents of the SAE chamber. Firmly slam the SAE chamber (upside down) onto the weighing paper, which should be placed on a hard, smooth surface. While inverted on the paper, brush the screen clean with a steel wire brush so that the dislodged particles are added to the SAE chamber sample. Weigh the sample. Re-tare the balance with a new piece of weighing paper. Slam the base (upside down) onto the tarred weighing paper to remove its contents. Brush out any remaining material from the base into the sample using a stiff painting brush. Weigh the sample.

7. If an aggregate center remains, place it into the mesoSAE chamber and rotate it for an additional 60 minutes to obtain a 120 minute run. Separately weigh the aggregate center (if it remains), the contents of the SAE chamber, and the contents of the base. Repeat the weighing procedure outlined in step 6. The sum of the contents of the SAE chamber, the contents of the base, and the remaining aggregate center (if it remains) should equal the weight of the aggregate center at time 60. If the aggregate center remains after 120 minutes, rotate it in the mesoSAE chamber for an additional 60 minutes to create a 180 minute run. Repeat the weighing procedure used for the 120 minute run.

8. For each run of 1, 2, 3, 4, 5, 6, 9, 12, 24, 48, 60, 120, and 180 calculate an erosion weight per minute (mg per minute). Determine how long each run should be to extract the external layer, the transitional layer, and the internal layer. Each layer is 1/3 of the weight of the aggregate at time 0. For the external layer, calculate the amount of time it took to erode 1/3 of the weight of the aggregate at time 0. For the transitional layer calculate the time to erode 2/3 of the weight of the aggregates at time 0. The internal layer would be the aggregate center remaining after the transitional layer has been removed by abrasion. It should be 1/3 of the aggregate weight at time 0.
9. An erosion weight per minute can also be determined by using inflection points, which are points where the aggregate begins to erode more slowly (i.e., the external [outer] layer, transitional layer, and internal layer [region] described by the two inflection points in Fig. 2 (Santos et al., Soil Science Society of America Journal, 61:720-724, 1997). On Kalamazoo loam soils these two points are between 2 and 6 minutes and 12 and 24 minutes. The soil erosion rate declines from milligrams to 0.1 milligrams per minute between 2 and 6 minutes and then from 0.1 milligrams to 0.001 milligrams per minute between 12 and 24 minutes. Once the erosion times for separating soil aggregates into three distinct components (i.e. layers) have been established, rotation of the mesoSAE chambers only needs to be stopped at these previously established times.

Sample Aggregate Erosion

10. Select 12 closely uniform aggregates from each sample (use more aggregates if more soil is needed for the analyses of each layer). Weigh each aggregate on a piece of tared

weighing paper and then place it into the bottom of the SAE chamber. Place the SAE chamber into the base and cap the top. Rotate the mesoSAE chambers on the shaker at 300 rpm for the amount of time to erode 1/3 of the weight of each aggregate. Note: Layers are eroded at different rates for each aggregate.

11. Weigh the aggregate to determine how much has been eroded by abrasion. Weigh the sand-grain sized contents of the SAE chamber and store it in a labeled glass scintillation vial if desired. Weigh the contents of the base and store them in a glass scintillation vial. The contents of the base are the external layer. Clean both the base and the SAE chamber as done in step 2. Place the remaining aggregate into the SAE chamber, put the SAE chamber into the base, cap the top, and rotate for the amount of time to erode half of the weight of the remaining aggregate (e.g., the second 1/3 of the weight at time 0). This would be the time that was calculated to erode 2/3 of the aggregate at time 0. Therefore, at the end of the run the remaining aggregate should be 1/3 of the weight of the aggregate at time 0. Note: If any aggregates become broken during the abrasion of either the external or transitional layers, discard the sample and begin the abrasion process again with new aggregates. Figure 1 shows the location of the external, transitional, and internal layers of a soil aggregate.
12. Weigh the aggregate to determine how much has been eroded by abrasion. Weigh the contents of the SAE chamber and store them in a labeled glass scintillation vial if desired. Weigh the contents of the base and store them in a labeled glass scintillation vial. The contents of the base are the transitional layer. Clean both the base and the SAE chamber as done in step 2. Place the remaining aggregate, which is the internal

layer, into the SAE chamber, place the SAE chamber into the base and cap the top. Rotate it until the internal layer has been completely eroded so that all of the fine particles have passed through the screen into the base.

Note: The abrasion of the internal layer (region) may take several hours or even days of rotation.

13. Weigh the contents of the SAE chamber and store it in a labeled glass scintillation vial if desired. Weigh the contents of the base and store it in a labeled glass scintillation vial. The contents of the base are the internal layer. Clean both the base and the SAE chamber as outlined in step 2.
14. Select new aggregates and repeat the procedure as outlined in steps 10 – 13. Note: When using different soil types or samples that have received very different treatments, repeat the procedure as outline in steps 4-13. This will provide greater accuracy in the collection of aggregate layers.
15. Process each layer or region of these peeled soil aggregates according to the experimental protocol.