Tillage and Manure Effects on Soil and Aggregate-Associated Carbon and Nitrogen

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ABSTRACT

In agricultural systems, maintenance of soil organic matter (SOM) has long been recognized as a strategy to reduce soil degradation. No-tillage and manure amendments are management practices that can increase SOM content and improve soil aggregation. We investigated the effects of 10-yr of different tillage systems and N sources on soil aggregate-size distribution and aggregate-associated C and N. The study was a split-plot design replicated four times. The main plot treatment was tillage (no-tillage, NT; conventional tillage, CT) and the subplot treatment was N source (manure, M; NH_4NO_3 fertilizer, F). The experiment was established in 1990 on a moderately well-drained Kennebec silt loam (Fine-silty, mixed, superactive mesic Cumulic Hapludoll) with continuous corn *(Zea mays* L.). In 1999, soil samples were collected (0- to 5-cm depth) from the field treatments and separated into four aggregate-size classes (>2000, 250–2000, 53–250, and 20–53 μm) by wet sieving. Labile C and N content of all aggregate-size fractions were measured using 28-d laboratory incubations of intact and crushed aggregates. No-tillage and M treatments significantly increased total C and N and the formation of macroaggregates. Conventional tillage in comparison with NT significantly reduced macroaggregates with a significant redistribution of aggregates into microaggregates. Aggregate protected labile C and N were significantly greater for macroaggregates, (>2000 and 250–2000 μm) than microaggregates (53–250 and 20–53 μm) and greater for M than F indicating physical protection of labile C within macroaggregates. No-tillage and M a lone each significantly increased soil aggregation and aggregate-associated C and N; however, NT and M together further improved soil aggregation and aggregate-protected C and N.

Soil structure is an important property that mediates many soil physical and biological processes and controls SOM decomposition (van Veen and Kuikman, 1990). Soil aggregates are the basic unit of soil structure and are composed of primary particles and binding agents (Edwards and Bremner, 1967; Tisdall and Oades, 1982; Haynes et al., 1991). Organic matter is considered a major binding agent that stabilizes soil aggregates (Tisdall and Oades, 1982; Haynes et al., 1991). Aggregate stability depends on the bonding mechanisms of clay and organic matter, such as chemical bonding by organic compounds and physical binding of particles by fungal hyphae and plant roots (Miller and Jastrow, 1990; Angers, 1998). Soil organic matter can be physically protected from microbial decomposition through sorption to clay minerals (Hassink et al., 1993) and encapsulation within soil aggregates (Tisdall and Oades, 1982; Golchin et al., 1994). Golchin et al. (1994) divided SOM based on difference in position within the soil matrix and accessibility to soil organisms into (i) free particulate organic matter (POM) and (ii) occluded POM (POM within aggregates). Mineralization studies of C and N in intact versus crushed aggregates (protected) indicated that aggregate-protected C and N pools were more labile than unprotected pools since the protected pools were less accessible to microbial decomposition (Elliott, 1986; Cambardella and Elliott, 1993; Beare et al., 1994b).

In agricultural systems, the amount and turnover of SOM can be altered by different management practices (Paustian et al., 1997). Cultivation affects soil structure by destructing soil aggregates that results in loss of SOM (Tisdall and Oades, 1982; Elliott, 1986; Angers et al., 1992). In general, incorporating plant residues in soil can affect the soil microclimate and increase plant residue contact with soil. This will increase residue decomposition and organic matter transformation (Beare et al., 1992; Cambardella and Elliott, 1993; Paustian et al., 1997). Tillage enhances decomposition of SOM by mixing of plant residues into the soil, increasing aeration, and enhancing dry-wet and freeze-thaw cycles (Beare et al., 1994b; Paustian et al., 1997). Tillage also disrupts soil aggregates and expose physically protected organic material (Blevins and Frye, 1993; Beare et al., 1994b; Paustian et al., 1997). In contrast, NT reduces soil mixing and soil disturbance, which allows SOM accumulation (Blevins and Frye, 1993). Many studies have shown that NT improves soil aggregation and aggregate stability (Beare et al., 1994b; Six et al., 1999). Fungal growth (Frey et al., 1999) and mycorrhizal fungi (O'Halloran et al., 1986), which are promoted by NT contribute to the formation and stabilization of macroaggregates (Tisdall and Oades, 1982; Beare and Bruce, 1993). Six et al. (2000a) observed a substantial increase in the mass of macroaggregates with NT and a decrease in microaggregates compared with CT.

Manure amendment is a management practice that can improve the nutrient status of the soil and increase soil organic C (SOC) levels (Rochette and Gregorich, 1998). Aoyama et al. (1999a) observed an increase in SOM with addition of manure and consequently the formation of slaking-resistant macroaggregates (250–1000 μm diam). Aoyama et al. (1999b) concluded that manure application contributed to the accumulation of macroaggregate-protected C and N.

Water-stable aggregates (WSA) >250 μm in diam.

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Abbreviations: CT, conventional tillage; F, NH_4NO_3 fertilizer; M, manure; NT, no-tillage; POM, particulate organic matter; SOC, soil organic C; SOM, soil organic matter; WSA, water-stable aggregate.

rapidly increases with changes in management practices (Six et al., 1999, 2000b; Lupwayi et al., 2001). Water-stable aggregates respond to different management practices such as tillage and manure application. Water-stable aggregates have been associated with various labile SOM fractions such as POM (Cambardella and Elliott, 1993; Jastrow and Miller, 1997), labile carbohydrates (Haynes and Francis, 1993), fungal biomass (Beare et al., 1992), and hydrophobic components (Capriel et al., 1990). Elliott (1986) reported more labile and readily mineralized SOM was associated with macroaggregates than microaggregates and was the primary source of nutrients lost during cultivation. A wet-sieving procedure using either air-dry or field-moist soil that is wetted rapidly or slowly (Yoder, 1936; Kemper and Rosenau, 1986) commonly determines WSAs. For more than 40 yr, the effect of cultivation on soil aggregate disruption and SOM losses has been studied intensively (Low, 1954; Tisdall and Oades, 1982; Elliott, 1986; Cambardella and Elliott, 1993; Six et al., 2000a). Recently, a few studies have been conducted to determine the effect of manure application on aggregate-size distribution and aggregate-associated organic matter (Aoyama et al., 1999a, 1999b). However, it is unknown if the effects of reduced tillage and manure addition on the distribution of C and N among aggregates are additive. The objectives of this study were to determine aggregate-size distribution and aggregate-associated C and N after 10 yr of NT and M application.

MATERIALS AND METHODS

Site Description

Soil was sampled from a long-term tillage N source study in continuous corn established in 1990 at the North Agronomy Farm located at Kansas State University, Manhattan, KS. The soil was a moderately well-drained Kennebec silt loam. Selected physical and chemical properties of the soil are presented in Table 1. Treatments were arranged in a completely randomized split plot design with four replications. Tillage treatments included NT and chisel-disk (CT; fall chisel plow and spring offset disk). Applying 321 g L^{-1} of atrazine (2-chloro-4-ethylamine-6-isopropylamino-S-triazine) and 400 g L^{-1} of metolachlor [2-chloro-6′-ethyl-N-(2-methoxy-1-methylethyl) acet-o-toluidide] (Bicep 6L, Ciba-Geigy) controlled weeds at the rate of 4.76 L ha^{-1} within one month of corn emergence. Subplots were based on N sources, including cattle manure (M) or F, both at 168 kg N ha^{-1} yr^{-1}. Manure application was made with assumption that 100% of M inorganic N and 30% of M organic N will be available during each growing season.

Soil samples were taken at 0- to 5-cm depth on 29 Oct. 1999, approximately 3 wk after corn harvest. Sterile polypropylene bags (3.78 L) were filled with soil samples collected randomly from each plot using a 2-cm diam. Oakfield soil probe (Forestry Suppliers, Inc., Jackson, MS). Samples were stored at field water content at 4°C. All samples were presieved (6-mm diam.) before wet sieving to remove stones and coarse organic matter and to define the initial dimensions of the aggregates for analysis.

Aggregate-Size Distributions

Aggregate size was treated as an independent variable, where it was considered as a sub-subplot in the ANOVA. Water-stable aggregates were separated using an instrument similar in principle to the Yoder wet-sieving apparatus (Yoder, 1936). The apparatus was modified and designed to handle stacked sieves (12.7 cm diam.) and to allow for complete recovery of all particle fractions from individual samples. Four aggregate-size classes were collected from each treatment (n = 4), >2000-, 250- to 2000-, 53- to 250-, and 20- to 53-μm diam. Macroaggregates were defined as >2000- and 250- to 2000-μm size fractions; microaggregates were defined as 53- to 250- and 20- to 53-μm size fractions. Sieves with mesh opening ≥250-μm diam. were contained in the oscillation cylinders. The amount of soil used was ≤0.4 g of air-dried soil cm^{-2} of sieve area. Soils were air dried for 24 h and evenly distributed over the nested sieve surfaces (>2000- and 250- to 2000-μm diam.). The nest was set at the highest point when the oscillation cylinders were filled with distilled water to the point where the bottom sieve (250-μm diam.) was completely covered with water without reaching the top screen (2000-μm diam.). Four 50-g subsamples of air-dried soil were placed on the top sieve of each nest. To slake the air-dried soil, 1 L of distilled water was rapidly added to each cylinder until the soil sample and top screen was covered with water. The soils were submerged in water for 10 min before the start of the wet-sieving action. The apparatus specifications of oscillation time (10 min), stroke length (4 cm), and frequency 30 cycle min^{-1} were held constant.

Following wet sieving, soil plus water remaining in the oscillation cylinder was poured onto the finer sieves (53 and 20 μm in diam.). Each sieve was shaken horizontally for 1 min to allow water and particle fractions smaller than the sieve size to pass through. Material remaining on each sieve was backwashed into a round aluminum pan (11-cm top diam., volume of 200 mL) and dried at 50°C for 24 h. The dried aggregate from each size class was weighed and stored in crush-resistant containers at room temperature. Floating organic matter (density <1 g cm^{-3}) was removed from the >2000-μm aggregate-size class since it was mostly plant debris. However, organic matter from other aggregate-size classes was considered organic matter associated with the size class and not removed. Aggregates <20-μm diam. were discarded and soil recovery calculated. Subsamples (0.2–2.0 g) of WSA from each size class were dried at 105°C for 24 h to allow correction for dry weight.

Sand-free WSA was measured using a subsample of intact aggregates (2–5 g) and combined with fivefold volume

Table 1. Selected soil physical and chemical properties at the 0- to 5-cm depth of no-tillage (NT) and conventional tillage (CT) systems in the Kennebec silt loam soil.

Year	Total C		Total N		Bulk density		Particle analysis		
	NT	CT	NT	CT	NT	CT	Sand	Silt	Clay
	Mg ha^{-1}				Mg m^{-3}		%		
1990	9.0	8.7	0.68	0.68	1.18	1.18	9	69	22
1999	16.4 a†	12.4 b	2.1 a	1.7 b	1.5	1.4			

† Means with different letters between tillage systems within C and N measurement are significantly different (ANOVA); $P < 0.05$.

(10–25 mL) of 5 g L^{-1} sodium hexametaphosphate, left overnight and shaken on an orbital shaker at 350 revolutions per minute for 4 h. The dispersed organic matter and sand was collected on a 53-μm mesh sieve, washed with deionized water, and dried at 105°C for 24 h, and the aggregate weights were recorded for estimating the sand-free correction.

Aggregate-Associated Carbon and Nitrogen

Total C and N contents of aggregates were determined by direct combustion using a Carlo Erba C/N Analyzer (Carlo Erba Instruments, Milan, Italy). Subsamples of whole aggregates were ground to a fine powder using mortar and pestle. Calculations for total C and N in different aggregate-size fractions were adjusted to oven-dry weight for sand-free WSA.

To determine the protected labile SOM for aggregates from each size class, a subsample was crushed using mortar and pestle to pass through a 20-μm sieve. Subsamples (2–5 g) from each size class (intact and crushed) were added to 160-mL serum bottles and incubated for 28 d. Deionized water was added to adjust the aggregates to a water content corresponding to a potential of −0.033 MPa. Water retention (−0.033 MPa) for individual aggregate-size classes (data not shown) was determined following the method outlined by Klute (1986). The initial total weight (serum bottle + soil + deionized water) was recorded. The samples were incubated at 25°C after being sealed with a rubber stopper and aluminum seals. The CO_2 evolved was measured weekly by taking a 0.5-mL gas sample of the headspace. The concentration of the CO_2–C was measured on a Shimadzu Gas Chromatograph-8A (Shimadzu Inc., Kyoto, Japan). The gas chromatograph was equipped with a thermal conductivity detector (TCD) and a 2-m Porapak column (Shimadzu Scientific Instruments, Columbia, MD). The column temperature was 70°C and the carrier gas was He at a flow rate of 14 mL min^{-1}. After the headspace gas was sampled, the serum bottles were opened for about 10 min to allow equilibration with the atmosphere. Before the serum bottles were sealed, the soil water content was adjusted to the initial weight by adding deionized water. Aggregate inorganic N was determined at the end of incubation by adding 25 mL of 1 *M* KCl to the serum bottles and shaking for 1 h at 300 revolutions per minute on an orbital shaker. Supernatant was filtered through (2W) Whatman filter paper No. 2 (Fisher Scientific, Fair Lawn, NJ) and stored at 4°C until analyzed for NH_4^+–N and NO_3^-–N on an Alpkem Autoanalyzer (Alpkem Corp., Clackamas, OR, Bulletins A303-S021 and A303-S170). Aggregate-associated C and N were presented per gram or kilogram of sand-free WSAs. However, to determine the total mass of C and N associated with whole mass of individual aggregate-size class recovered from 100 g soil, aggregate C and N were presented as mass of C and N per whole mass of sand-free WSAs.

Statistical Analyses

Whole soil total C and N were analyzed using a split-plot randomized complete block design, with tillage as the whole plot factor, and N source as the subplot factor. However, aggregate-size class was considered as an independent variable and analyzed as a sub-subplot factor in a split-split plot design. The ANOVA F-test was used for treatment factor main effects and interactions. The F-protected *t* test was used on pairwise comparisons to follow up any significant findings. Proc Mixed was used for analysis of variance and mean separation differences (SAS Institute Inc., 1999). All results were considered significantly different at $P < 0.05$ unless noted otherwise.

Table 2. Total C and N (whole soil) as affected by no-tillage (NT), conventional tillage (CT), manure (M), and fertilizer (F) management practices.

Treatments	Total C	Total N
	Mg ha^{-1} soil	
NT-M	18.7	2.3 a‡
CT-M	13.0	1.7 b
NT-F	14.2	1.9 b
CT-F	12.0	1.7 b
	PR > F	PR > F
Tillage (T)	0.01	0.009
NT (mean)	16.5 A†	2.0 A
CT (mean)	12.4 B	1.7 B
N source (NS)	0.01	0.02
M (mean)	14.9 A	1.9 A
F (mean)	12.9 B	1.7 B
T × NS	0.07	0.02

† Means with different lowercase letters between management practices within total N are significantly different (ANOVA); $P < 0.05$.
‡ Means with different uppercase letters between management practices within total N or total C are significantly different (ANOVA); $P < 0.05$.

RESULTS

Whole soil total C was significantly affected by tillage and N source with no significant interaction (Table 2). No-tillage significantly increased C, as did the addition of manure. However, with fertilizer, neither total C nor N was significantly affected by tillage. These results suggest that added organic matter, either through plant residues or manure, was conserved to a greater extent with NT.

Aggregate-size distribution was significantly ($P < 0.0001$ and $P < 0.01$) influenced by the interaction with tillage and N source, respectively (Table 3). Percentage of soil recovery from wet sieving was about 90% from soil used. Aggregates in the 53- to 250-μm class comprised the greatest proportion of the whole soil, followed by aggregates in the 20- to 53- and 250- to 2000-μm classes. Significantly greater amounts of macroaggregates (>2000 and 250–2000 μm) were present in NT compared with CT (Table 3), with a corresponding shift in the proportion of microaggregates (53–250 and 20–53 μm) in CT.

Table 3. Distribution of sand-free water-stable aggregates as affected by no-tillage (NT), conventional tillage (CT), manure (M), and fertilizer (F) management practices.

	Sand-free water-stable aggregates			
Treatments	20–53 μm	53–250 μm	250–2000 μm	>2000 μm
	g 100 g^{-1} soil			
NT-M	19.8	18.9	24.6	16.5
CT-M	25.5	32.5	19.4	6.5
NT-F	23.2	23.5	24.6	10.2
CT-F	28.3	30.9	19.2	3.1
		PR > F		
Tillage (T)		0.77		
N source (NS)		0.8		
T × NS		0.7		
Aggregate (Agg)		0.0001		
T × Agg		0.0001		
NT (mean)	21.5 b†	21.2 b	24.6 a	13.2 a
CT (mean)	25.9 a	31.7 a	19.3 b	4.8 b
NS × Agg		0.01		
M (mean)	21.7 b	25.7 b	22.0 a	11.5 a
F (mean)	25.7 a	27.2 a	21.9 a	6.6 b
T × NS × Agg		0.3		

† Means with different lowercase letters between tillage practices or N sources within each row are significantly different (ANOVA); $P < 0.05$.

Manure significantly increased aggregates >2000 μm (Table 3).

Total C and N associated with sand-free WSA were significantly affected by tillage ($P < 0.005$), N source ($P < 0.002$), aggregate-size fractions ($P < 0.001$), and the two-way interactions (Tillage × Aggregate; $P < 0.0004$) and N source × Aggregate; $P < 0.01$). The three-way interaction was not significant. Aggregate total C and N were significantly greater with NT than CT, except for the 20- to 53-μm aggregates (Fig. 1A,C). The same pattern was observed across tillage treatment, where aggregate total C and N were significantly greater with M than F (Fig. 1B,D). In general, aggregate total C and N were significantly greater ($P < 0.001$) with macroaggregates than microaggregate averaged across tillage and N source ($LSD_{[0.05]} = 1.8$ and 0.18 for total C and N, respectively).

Aggregate associated labile C was significantly affected by tillage ($P < 0.009$), N source ($P < 0.001$), and aggregate-size fractions ($P < 0.001$). Aggregate labile C (Fig. 2) was significantly greater with NT than CT and with M than F, except for the 20- to 53-μm aggregates where tillage and N source had no significant effect on labile C and N (Fig. 2A). Aggregate labile N was significantly affected by the interaction between aggregate-size classes and N source with tillage (three way interaction; $P < 0.0001$). Aggregate labile N was significantly greater for aggregates >2000 with NT than CT and at aggregates >2000-μm and 53- to 250-μm diam. with M than F (Fig. 2C,D). In general, aggregate labile C and N were significantly greater with macroaggregates than microaggregates.

The masses of total C and N were significantly associated with aggregate 250- to 2000-μm NT and M, while total C and N were significantly associated with aggregates 53- to 250-, and 250- to 2000-μm diam. for CT and F, respectively (Fig. 3). Labile C was significantly associated with NT and M in the 250- to 2000-μm aggregate (Fig. 4A,B) compared with CT and F, respectively. Aggregate labile N was significantly associated with macroaggregates (>2000- and 250- to 2000-μm diam.) in NT and with aggregates 53- to 250-μm diam. for CT (Fig. 4C). Nitrogen was greater with M than F treatment in aggregate-size classes 53 to 250 and >2000 (Fig. 4D), while labile C was greater in all aggregate-size classes except for those of 20 to 53 μm (Fig. 4B). In general, NT and M significantly increased retention of C and N.

Crushing the aggregates increased the release of labile C, compared with intact aggregates, in the >2000-μm aggregates in the first 7 d of incubation by 367 and 285 (both about 1.6-fold) and 180 (1.2-fold) μg C g^{-1} aggregate for NT-M, NT-F, and CT-M, respectively. Labile C mineralized (data not shown) was significantly greater with crushed compared with intact aggregates for the >2000- and 250- to 2000-μm size classes. After 28 d of incubation, significantly greater labile N was measured with crushed aggregates >2000 μm by 16 (1.1-fold), 12, and 13 (both about 1.2-fold) μg N g^{-1} aggregate for NT-M, NT-F, and CT-M, respectively. This indicates labile C and N of the aggregates were physi-

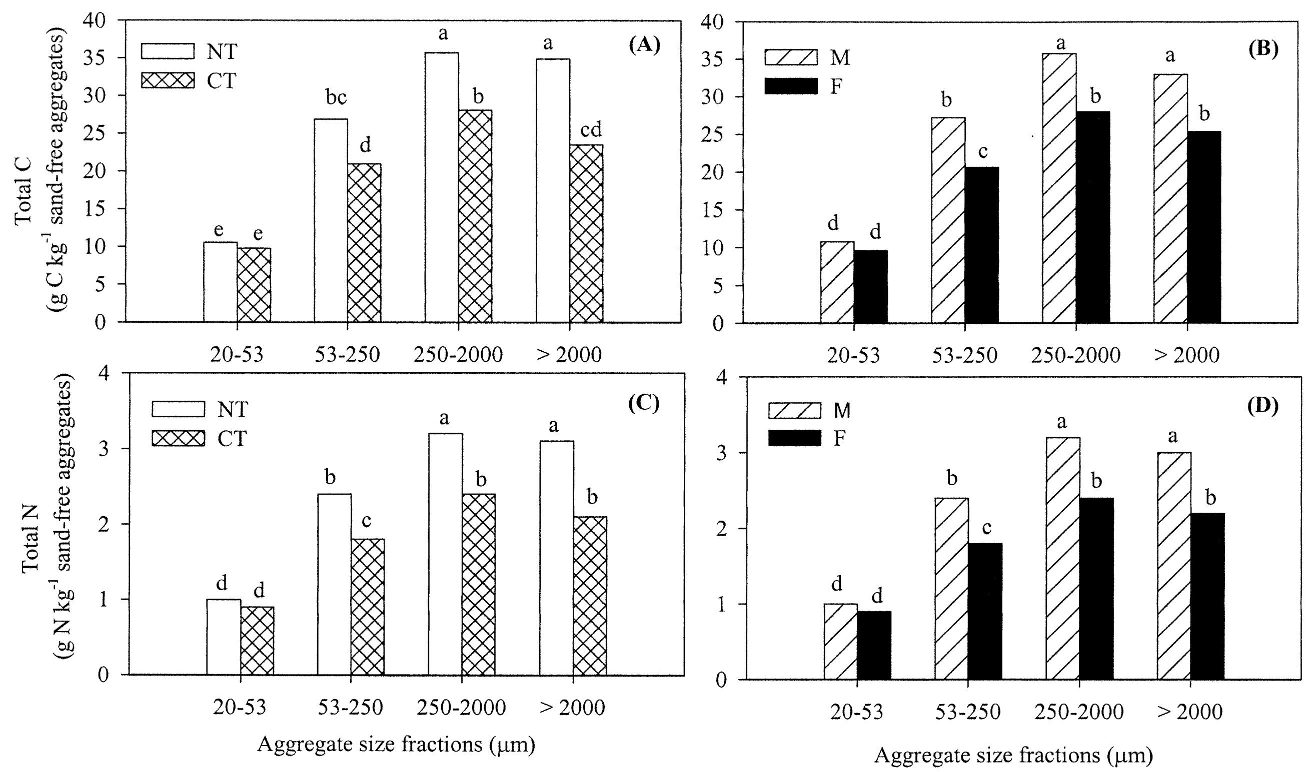

Fig. 1. Total C and N (g kg^{-1}; normalized to sand-free basis) in water-stable aggregates (n = 4). A and C represent aggregate total C and N for no-tillage (NT) and conventional tillage (CT) averaged across manure (M) and fertilizer (F), lowercase letters indicate significant differences between aggregate-size fraction and tillage; $P < 0.05$; B and D represent aggregate total C and N for manure and fertilizer averaged across tillage, lowercase letters indicate significant differences between aggregate-size fraction and N source; $P < 0.05$.

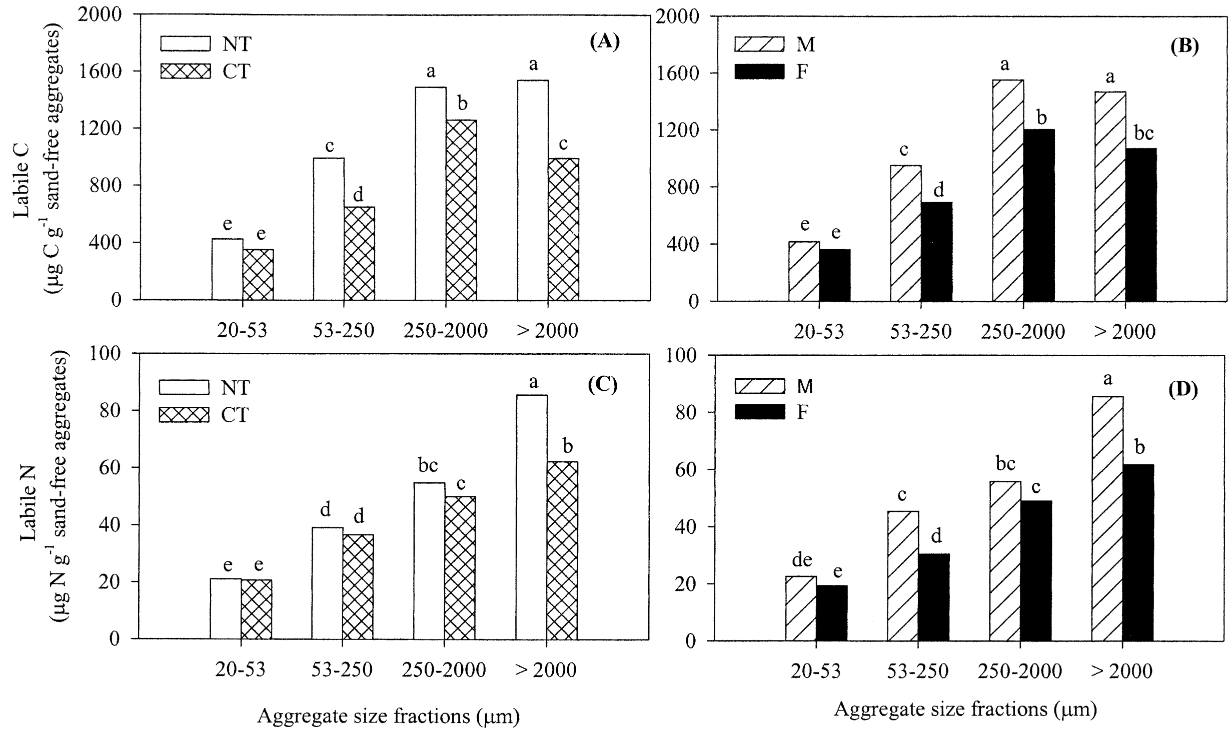

Fig. 2. Labile C and N after 28 d of incubation ($\mu g\ g^{-1}$; normalized to sand-free basis) in water-stable aggregates (n = 4). A and C represent aggregate labile C and N for no-tillage (NT) and conventional tillage (CT) and averaged across manure (M) and fertilizer (F), lowercase letters indicate significant differences between aggregate-size fraction and tillage; $P < 0.05$; B and D represent aggregate labile C and N for manure and fertilizer averaged across tillage, lowercase letters indicate significant differences between aggregate-size fraction and N source; $P < 0.05$.

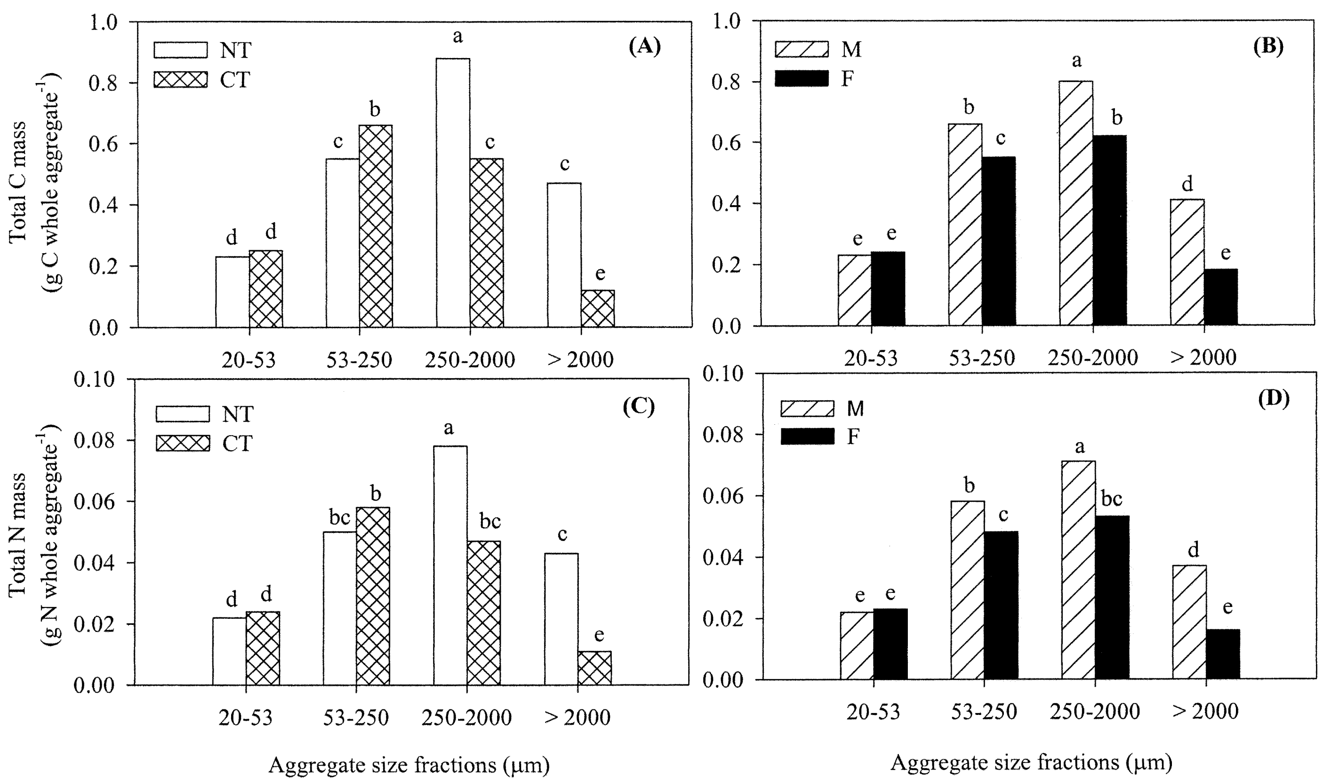

Fig. 3. Total C and N masses (g whole aggregate^{-1}; normalized to sand-free basis) in water-stable aggregates (n = 4). (A and C) represent aggregate total C and N for no-tillage (NT) and conventional tillage (CT) averaged across manure (M) and fertilizer (F), lowercase letters indicate significant differences between aggregate size fraction and tillage; $P < 0.05$; B and D represent aggregate total C and N for manure and fertilizer averaged across tillage, lowercase letters indicate significant differences between aggregate-size fraction and N source; $P < 0.05$.

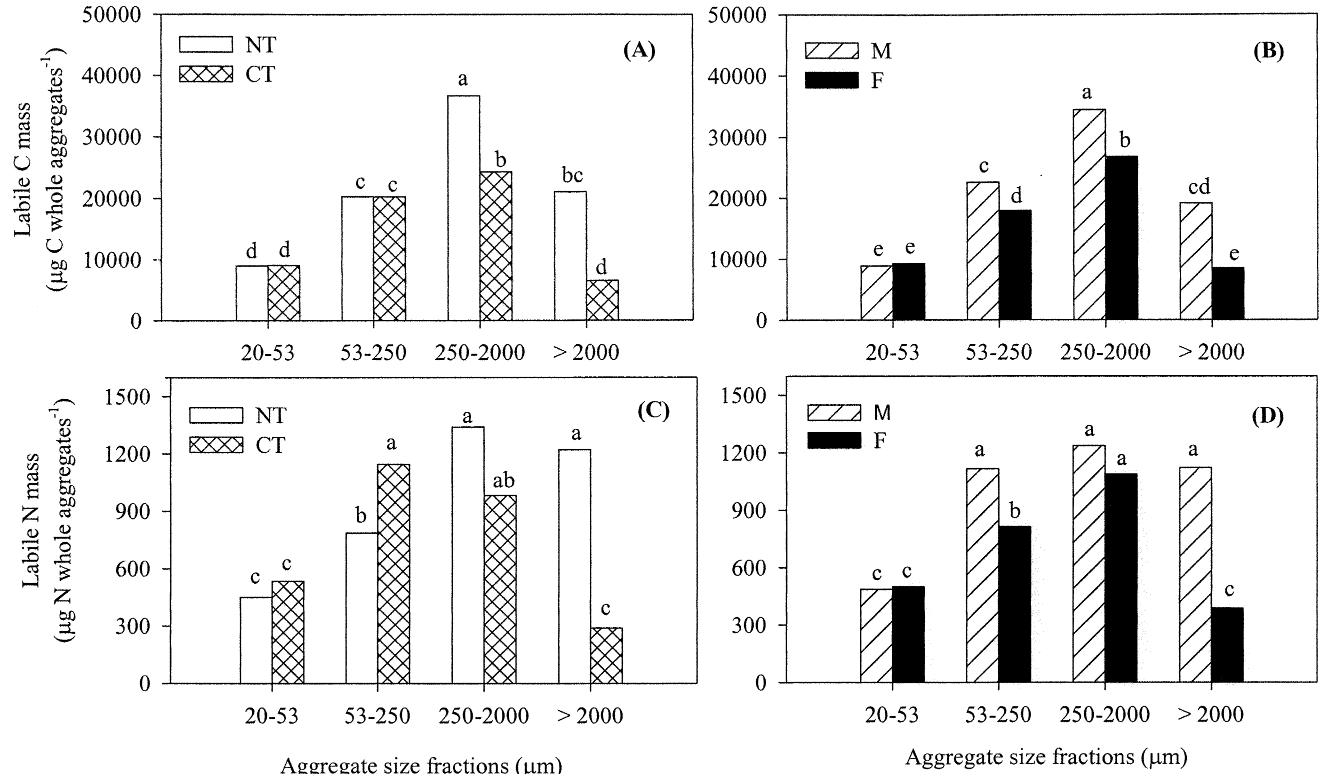

Fig. 4. Labile C and N mass after 28 d of incubation (µg whole aggregate^{-1}; normalized to sand-free basis) in water-stable aggregates (n = 4). A and C represent aggregate labile C and N for no-tillage (NT) and conventional tillage (CT) averaged across manure (M) and fertilizer (F), lowercase letters indicate significant differences between aggregate size fraction and tillage; $P < 0.05$; B and D represent aggregate labile C and N for manure and fertilizer averaged across tillage, lowercase letters indicate significant differences between aggregate size fraction and N source; $P < 0.05$.

cally protected, especially with NT and M management practices and mostly in aggregates >2000 µm.

DISCUSSION

Ten years of NT significantly increased soil total C and N. Differences in total C and N between tillage treatments were the result of less soil disturbance. A control on conservation of C in soil includes microbial activity, as well as physical and chemical protection of SOM (Rice and Angle, 2004). Throughout the 10 yr of this study, physical protection had the greatest influence on conservation of soil C and N. Substrate quality and clay content were similar between treatments in this study with the major difference being soil disturbance. Soil water content, soil temperature, and corn biomass production did not significantly differ among treatments over the 10 yr (data not shown). Other research has also shown that soil disturbance (i.e., tillage) disrupts soil aggregates (Six et al., 2000a), and increases respiration (loss of C as CO_2) as a result of the release of protected SOM (Six et al., 2000a).

Manure application significantly increased soil total C and N compared with fertilizer addition. The increase in soil C and N were due in part to increased organic residue input with manure addition. According to Rochette and Gregorich (1998), about half of added manure C was retained in the soil at the end of the season. Similarly, Aoyama et al. (1999a) reported that additional accumulation of SOM with M was mainly from M application itself and not from changes in plant biomass.

Additions of manure significantly increased the proportion of aggregates >2000 µm (Table 3). The increased in macroaggregates could be attributed to the input of additional fresh organic residue and available C to the soil resulting in enhanced microbial activity and thus binding of aggregates. Aoyama et al. (1999a) also observed an increase in POM and mineral-associated organic matter in aggregate fractions with manure application. They explained that some of the mineral-associated organic matter derived from the decomposition of POM stimulates formation and stabilization of macroaggregates. Organic residues can be a catalyst for microbial activity (Puget et al., 1995) and induce binding of soil particles into macroaggregates (Six et al., 1999). Aggregation is promoted by increased hyphal mass from fungi and polysaccharide production (Haynes and Francis, 1993).

Tillage significantly reduced macroaggregates (>2000- and 250- to 2000-µm diam.) with a concomitant increase in microaggregates (53- to 250- and 20- to 53-µm diam.). The reduction in macroaggregates with CT could be mainly due to physical disruption of macroaggregates and reduced aggregate stability. Tillage increases the effect of drying and rewetting, which increase macroaggregates susceptibility to disruption. According to Six et al. (2000a), tillage increases the decomposition rate and turnover of macroaggregates, where macroaggregate turnover was about two times greater in CT than in NT. Beare et al. (1994b) observed that macroaggregates from CT were much less stable than those from NT, reflecting their greater susceptibility to dispersion. Similarly, Elliott (1986) and Six et al. (2000b) observed aggregates of cultivated soil were more susceptible to slaking.

In the current study, the same amount of manure N and fertilizer N were added to NT and CT. Although there was no significant interaction ($P < 0.05$) between tillage and N source, NT tended to conserve the added C and N to a greater extent. Both manure and NT enhance microbial activity by providing a nutrient source (with manure) and reducing water loss. Rochette and Gregorich (1998) reported manure addition significantly increased microbial biomass C and enhanced CO_2 flux by factor of 2.6, compared with fertilizer. Enhanced microbial activity would increase the production of microbial polysaccharides that act as binding agents between soil aggregates (Tisdall and Oades, 1982).

Results of our study showed a significant increase in aggregate total C and N due to NT practices and manure application. The effect of NT and manure on total C and N were more pronounced in macroaggregates than in microaggregates, indicating a greater sensitivity of macroaggregates to this management practices. Six et al. (2000b) reported that increased cultivation intensity decreased C-rich macroaggregates and increased C-depleted microaggregates, leading to a loss of C that binds microaggregates into macroaggregates. Other studies have similarly reported proportionally greater reduction of macroaggregate C and N, as well as water stability, as a result of tillage (Cambardella and Elliott, 1993; Puget et al., 1995).

A significantly greater proportion of labile C and N were associated with macroaggregates than microaggregates. This indicates that macroaggregates contributes more to nutrient cycling than microaggregates. Similarly, Elliott (1986) and Beare et al. (1994a) reported macroaggregates have higher C and N mineralization potentials than microaggregates. Puget et al. (1995) suggested the greater C content in macroaggregates could be due to lower decomposable SOM associated with these aggregates, and also the direct contribution of SOM to the stability of macroaggregates resulting in only C-rich macroaggregates being able to withstand slaking. Manure addition and NT not only increased labile C in macroaggregates, but also significantly increased labile C in aggregates at 53 to 250 μm in diameter (Fig. 2A). Increasing labile C in microaggregate (53–250 μm) indicated that NT and M addition improved SOM conservation and reduced nutrient lost. These results are similar to that of Angers et al. (1997) who showed that the amount of residual new-labeled ^{13}C and ^{15}N recovered in microaggregates increased with time. Similarly, Six et al. (2000a) reported the proportion of fine intra-aggregate POM C (iPOM-C) held in microaggregates within macroaggregates were younger and much greater in NT than CT (90 vs. 58%, respectively).

The location of SOM within aggregates influences their decomposition (Besnard et al., 1996). Grinding or crushing increases mineralization of SOM (Elliott, 1986; Beare et al., 1994a; Aoyama et al., 1999b). The effect of aggregate crushing in our study increased labile C for the first 7 d and N for the first 28 d of incubation from NT macroaggregates. The lack of crushing effect on microaggregates was probably because more recalcitrant SOM was associated with microaggregates (Puget et al., 1995; Six et al., 2000b). Manure application increased protected labile C and N with NT and CT, whereas fertilizer increased protected labile C and N with NT only. Aoyama et al. (1999b) observed a three-fold increase protected C and a four-fold increase in protected N following manure application in aggregates 250 to 1000 μm in diameter.

In summary, NT significantly increased soil total C and N, WSAs, and labile C and N associated with macroaggregates compared with CT. Conventional tillage likely enhanced disruption of soil aggregates resulting in loss of SOM. Manure significantly increased total C and N (compared with F) through improved formation of WSAs and increased aggregate-associated C and N. In general, the combination of NT and M significantly improved soil aggregation and aggregate-associated C and N compared with CT and F.

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