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Long-Term Manure Impacts on Soil Aggregates and Aggregate-Associated Carbon and Nitrogen

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Long-term studies document that soil properties influenced by management practices occur slowly. The objectives of this study were to evaluate 70 yr of manure (M) and commercial fertilizer (F) additions and moldboard plowing on soil organic C (SOC), soil total N (STN), water-stable aggregates (WSA), and aggregate-associated C and N. The Knorr–Holden plots have been in furrow irrigated continuous corn (*Zea mays* L.) since 1912 on a Tripp sandy loam (coarse-silty, mixed, superactive, mesic Aridic Haplustoll). Soil samples were collected from the 0- to 5-, 5- to 10-, 10- to 15-, and 15- to 30-cm depths in 2011. Soils were fractionated by wet sieving into four aggregate-size classes (>1000, 500–1000, 250–500, and 53–250 μm). Continuous M amendment increased the SOC in the 0- to 30-cm depth approximately 1.7-fold compared with the F treatment. The combination of F + M further increased SOC in the 0- to 15-cm depth by approximately 36% for the M treatment receiving 90 kg N ha^{-1} of F (90 + M) and by 16% for the M treatment receiving 180 kg N ha^{-1} of F (180 + M) compared with the 15- to 30-cm depth. Macroaggregates increased with M and F + M when compared with F with the corresponding increase in microaggregate quantities associated with the F and no-N treatment. In the 0- to 30-cm depth, microaggregates were approximately 1.8 to 4.9 times greater than the macroaggregates. Aggregate-associated C masses were greater in microaggregates than in macroaggregates, which reflects greater amounts of microaggregates present in the soil. A significant, positive correlation was observed between SOC and aggregate-associated C. Overall, the addition of manure-based amendments, with or without F, increased SOC and enhanced aggregate stability.

Abbreviations: F, fertilizer; M, manure; SOC, soil organic carbon; SOM, soil organic matter; STN, soil total N; WSA, water-stable aggregates; 0 + M, M treatment with no F added; 90 + M, M treatment receiving 90 kg N ha^{-1} of F; and 180 + M, M treatment receiving 180 kg N ha^{-1} of F.

The Knorr–Holden plot was initiated in 1910 in the North Platte Valley of western Nebraska during construction of the first large-scale irrigation projects in the state. The Knorr–Holden continuous corn plot was developed in response to a need to determine the best methods of farming for irrigated lands in the early 1900s. Today, the Knorr–Holden research plot is the oldest long-term irrigated corn plot in North America. The Knorr–Holden continuous corn plot was officially listed in the National Register of Historic Places in June of 1992 (Hergert and Nielsen, 2012) when it was determined to be a historic property worthy of preservation. Long-term studies are unique because they al-

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low the examination of long-term changes in soil properties that can result from crop management practices over time (Brown, 1991; Peterson et al., 2012). Long-term studies help improve the quantitative knowledge available on soil management, soil quality, and sustainability that may not be possible to quantify in short-term studies (Peterson et al., 2012). Long-term studies were established to answer a specific question when they were initiated. Over time, these studies have gained importance by allowing the determination of long-term management practices on soil quality and productivity (Brown, 1991; Peterson et al., 2012; Mikha et al., 2013).

Soil aggregation is an important property related to soil quality, soil structure (Six et al., 2000; Nimmo and Perkins, 2002; Lado et al., 2004), soil sustainability (Lado et al., 2004; Bronick and Lal, 2005), and soil organic matter (SOM) conservation (Webb et al., 2012). Previous research documented that improving SOM has a positive influence on increasing soil aggregation, aggregate stability, and soil C conservation (Six et al., 2000; Mikha and Rice 2004; Mikha et al., 2010). In addition, improving aggregate stability has the potential to increase resistance to erosion, especially in reference to wind erosion (Blanco-Canqui et al., 2009; Blanco-Moure et al., 2012). Soil organic matter is considered the primary binding agent responsible for improving aggregate stability in microaggregates (<250 μm) and macroaggregates (>250 μm) (Tisdall and Oades, 1982; Angers, 1998, Wright and Upadhyyaya, 1998). In addition, SOM can be physically protected by being encapsulated within soil aggregates (Tisdall and Oades, 1982; Golchin et al., 1994) or by adsorption to the clay minerals (Hassink et al., 1993).

Management practices, such as tillage and adding organic amendments to the soil can have an influence on soil aggregation, aggregate stability (Blanco-Canqui et al., 2009; Blanco-Moure et al., 2012), and aggregate-associated C and N (Mikha and Rice, 2004; Zibilske and Bradford, 2007; Mikha et al., 2013). Previous studies documented that tillage practices enhance SOM decomposition (Six et al., 2002; Blanco-Moure et al., 2012) and reduce the "life cycle" of soil macroaggregates (Six et al., 2000). Thus, tillage prevents the formation and stabilization of macroaggregates, reduces the physical protection of SOM within macroaggregates, and promotes soil C losses (Six et al., 2000; Six et al., 2002; Zibilske and Bradford, 2007; Blanco-Moure et al., 2012). Adding an organic amendment, such as manure, is one of the management practices that can improve SOM and, consequently, soil nutrient status and physical properties. Soil amended with manure was found to increase the formation and stabilization of soil macroaggregates, improve macroaggregate-associated C and N (Aoyama et al., 1999a, 1999b; Mikha and Rice, 2004; Wortmann and Shapiro, 2008), reduce soil crust formation, and decrease water runoff (Pagliai et al., 2004). Aoyama et al. (1999b) also reported that the accumulation of macroaggregate-protected C and N was a result of manure addition as a nutrient source.

For the last few decades, research on Knorr–Holden study plots focused on grain yields, soil N content, and soil erosion (Anderson and Peterson, 1973; Eghball et al., 1995; Gilley et al., 1999). However, the effects that 10 decades of annual moldboard plowing and disking, combined with the additions of manure in subsequent years, have on soil quality have not been extensively documented. The specific objectives of this study were to evaluate SOC and STN, aggregate-size distribution, and aggregate-associated C and N after 100 yr of tillage practice and 70 yr of manure additions. The hypothesis for this study is that the addition of manure will increase total SOC and aggregate-associated C and, thereby, increase aggregate stability, even under long-term tillage.

MATERIALS AND METHODS

Site Description and Field Treatments

The Knorr–Holden continuous corn plot site is located approximately 8 km north and 2 km west of Scottsbluff, NE at 41.56° N lat and 103.42° W long. The research plot is located in a semiarid climate region at an elevation of 1240 m that has a total annual precipitation of 401 mm. The 30-yr average frost free period (occurrence of 0°C) is May 7th to September 29th (National Climatic Data Center, 2015). The soil is Tripp, a very-fine, sandy loam, with approximately 500, 320, and 180 g kg^{-1} sand, silt, and clay, respectively (Blanco-Canqui et al., 2015).

The plots were established in 1910 by plowing native soil near Mitchell, NE that was formed under short-grass prairie (Hastings 1936). The area then was planted to oat *(Avena sativa* L.) in 1911. In 1912, a nonreplicated rotation study including corn, sugar beet (*Beta vulgaris* L.), potato (*Solanum tuberosum* L.), and alfalfa (*Medicago sativa* L.) was initiated. In 1942, the continuous corn part of the plots was split into two beef cattle (*Bos taurus*) manure treatment levels (0 and 27 Mg $ha^{-1}yr^{-1}$). In 1953, a second replication was added. The same year, the two replications of the manure treatments were split into subplots to include six inorganic fertilizer treatments (0, 45, 90, 135, and 180 kg N ha^{-1}, and 135 kg N ha^{-1} + 20 kg P ha^{-1}). The experiment is a split-plot design in which the two manure treatments are main plots and the six N fertilizer treatments are subplots replicated twice. The main plots are 33 m wide and 12.5 m long while the subplots are 5.5 m wide by 12.5 m long. Row-width has varied from 90-cm spacing in the early years to 76-cm row spacing starting in the 1970s and 56-cm row spacing since 2004.

Site descriptions, rotations, and management practices from 1910 to 1938 have been previously reported in detail by Scofield and Holden (1927), Hastings (1936), Nuckols (1937), and Hastings et al. (1938). Ammonium nitrate (34-0-0) was used as the inorganic N source but urea (46-0-0) has been used since 2005. The field site has been consistently managed according to the indicated treatments since 1953. All plots are furrow-irrigated with surface water from the Bureau of Reclamation reservoirs that are part of the North Platte project. A more detailed description of this site and the history of the long-term (100 yr) irrigated corn research plots were reported by Anderson and Peterson (1973), Eghball et al. (1995), and more recently Hergert and Nielsen (2012). Since 1990, corn stalks have been shredded in early April and disked to a 10- to 13-cm depth. Manure and mineral fertilizer are then applied, and the area is plowed to a 20- to 25-cm depth. After moldboard plowing, the study site is leveled and packed with a Brillion roller packer (Brillion Iron Work Inc., Brillion, WI) before planting. Before the widespread use of herbicides, the area was cultivated one or two times for weed control before furrow formation.

Soil samples were collected in March 2011, which is approximately 100 yr after the research plots were established. Treatments sampled from this study site were from F treatment at 0, 90, and 180 kg N ha^{-1} and from F + M treatments at 0 + M, 90 + M, and 180 + M. The F treatment of 0 kg N ha^{-1} in no manure plots represents the control treatment (no-N added). The 0 + M treatment in manure plots represents M treatment with no F added. Composite soil samples were collected using a 2.5-cm-diam. probe from the 0- to 5-, 5- to 10-, 10- to 15-, and 15- to 30-cm depths of each N treatment using a Giddings soil hydraulic probe (Giddings Machine Co., Windsor, CO). Six cores were taken from each plot at 0 to 15 cm, divided into three 5-cm increments, and the 5-cm increments were composited. Two soil cores were taken from each plot at the 15- to 30-cm depth and composited. During field sampling, the composited soil samples were placed in sterile polypropylene bags, kept in coolers, and then stored at 4°C until processing. The field-moist soil samples were manually passed through a 6-mm sieve to remove stones and coarse organic matter, to homogenize the soil samples, and to define the initial dimensions for aggregate analysis. The sieved soil samples were air-dried before aggregate-size distribution and SOC evaluation.

Aggregate-Size Distributions

The modified apparatus reported by Mikha et al. (2005) was used to measure WSA. The apparatus used consisted of three (>1000, 500–1000, 250–500 μm) sieves (12.7 cm diam.) stacked on top of one another, which allowed for complete recovery of different aggregate fractions from individual samples. The air-dried sieved soil samples from each N treatment were fractionated into macroaggregate (>1000, 500–1000, 250–500 μm) and microaggregate (53–250 μm) size classes as defined by Tisdall and Oades (1982). A detailed description of this procedure for sand-free WSA-size distribution was reported by Mikha et al. (2005). However, aggregate-size distribution was reported as sand-free WSA on an oven-dry basis (105°C).

Soil and Aggregate-Associated Organic Carbon and Total Nitrogen

After 100 yr of management, the soil pH at the 0- to 30-cm depth averaged 6.5, indicating the absence of soil carbonates at this soil depth. Therefore, all C present within the 0- to 30-cm depth, whether in bulk soil or associated with soil aggregates, was considered to be organic C. The SOC was evaluated by direct combustion (950°C) using a Leco CHN-2000 (Leco Co., St Joseph, MI). Air-dried soils were ground to a fine powder using a roller mill and about 0.2 g of ground soil was used for SOC and STN analysis.

Throughout the manuscript, aggregate-associated organic C and aggregate-associated total N will be presented as aggregate-associated C and N. Aggregate-associated C and N contents were determined by direct combustion (950°C) using a Thermo Scientific, Flash 2000 C and N analyzer (CE Elantech, Inc., Lakewood, NJ). A small sample size (especially from macroaggregates >1000 μm) was generated from 100 g of soil segregated into different aggregates sizes classes. The Thermo Scientific C and N analyzer was used to evaluate aggregate-associated C and N content because the analyzer requires a small sample size of aggregates. The subsamples of whole aggregates were ground to a fine powder using mortar and pestle, and about 18 to 20 mg of ground soil was used for SOC and STN evaluation. Aggregate-associate C and N were calculated as grams C and N per kilogram of sand-free WSA in each aggregate-size class. The mass of aggregate-associated C and N was then calculated as the amount contained in the total mass of aggregates per kilogram of soil. The percent proportion of the organic C associated with different aggregate-size fractions relative to the SOC was determined as the ratio between aggregate-associated C and total SOC within the whole soil and is as follows:

$$\text{Aggregate-associated C\%} = \left[\frac{\text{Aggregate-associated C}\left(\text{g C kg}^{-1}\text{soil}\right)}{\text{SOC}\left(\text{g C kg}^{-1}\right)}\right]100 \quad [1]$$

Statistical Analyses

Whole soil total SOC and STN were analyzed using a randomized complete block split-plot design, with M as the whole plot factor and commercial F as the subplot factor. Aggregate-size class was considered an independent variable and analyzed as a sub-subplot factor in a split-split plot design. The PROC Mixed (SAS version 9.3, SAS Institute Inc., 2010) was used for analysis of variance and mean separation differences. The *F*-test was used to explain multiple comparisons of means using treatment differences. All results were considered significantly different at $p < 0.05$ unless noted otherwise. Simple linear regression and correlation analyses between SOC and aggregate-associated C were generated across soil depths for each individual N source.

RESULTS AND DISCUSSIONS

Soil Organic Carbon and Soil Total Nitrogen

Whole SOC and STN at all depths studied were significantly ($P < 0.0001$) influenced by N treatments (Table 1). At any individual depth studied, SOC and STN were greater with the treatment receiving inorganic F + M compared with the treatment that received F alone. Furthermore, SOC and STN were increased the most with the treatment receiving M along with inorganic F at the rate of 180 kg N ha^{-1}. These data agree with the previous research (Tirol-Padre et al., 2007; Kaur et al., 2008; Brar et al., 2013) that reported an increase in SOC when farm yard manure was applied in conjunction with inorganic F (NPK) compared with the addition of inorganic F alone. At any depth studied, no significant differences in SOC and STN were observed with M treatments receiving no F addition (0 + M) when

Table 1. Soil organic C (SOC) and soil total N (STN) as influenced by long-term N treatments (M, manure; F, inorganic fertilizer; and no N added [control]) at different depth increments.

SOC	Soil depth (cm) 0–5	5–10	10–15	0–15	15–30
Treatments			SOC (g C kg^{-1} soil)		
0†	6.39 e‡	6.15 d	6.17 d	6.24 e F§	5.93 e F
90	8.43 d	8.13 c	7.95 c	8.17 d D	7.50 d E
180	9.07 c	8.67 c	7.93 c	8.57 c D	8.39 c D
0+M¶	17.29 b	15.25 b	14.52 b	15.69 b B	11.51 b C
90+M	17.05 b	15.52 b	14.81 b	15.79 b B	11.63 b C
180+M	19.75 a	17.32 a	15.93 a	17.67 a A	15.26 a B
			PR > F		
Treatment	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
				PR > F	
Treatment (Trt)				< 0.0001	
Depth (D)				< 0.0001	
Trt × D				< 0.0001	
STN					
Treatments			STN (g N kg^{-1} soil)		
0	0.60 e	0.60 d	0.60 d	0.60 e F	0.57 e F
90	0.73 d	0.73 c	0.72 c	0.72 d DE	0.70 d E
180	0.86 c	0.79 c	0.71 c	0.79 c D	0.80 c D
0+M	1.64 b	1.47 b	1.35 b	1.49 b B	1.13 b C
90+M	1.64 b	1.49 b	1.44 b	1.52 b B	1.12 b C
180+M	1.89 a	1.66 a	1.58 a	1.71 a A	1.52 a B
			PR > *F*#		
Treatment	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
				PR > F	
Treatment (Trt)				< 0.0001	
Depth (D)				< 0.0001	
Trt × D				< 0.0001	

† Represents F treatment at different rates (0, 90, and 180 kg N ha^{-1}).

‡ Lowercase letters represent significant differences among the treatments within each depth for SOC or STN (ANOVA); $P < 0.05$.

§ Means with different uppercase letters represent significant differences of the treatment × depth interaction among the treatments at 0- to 15- and 15- to 30-cm depths for SOC or STN (ANOVA); $P < 0.05$.

¶ Represents M added approximately at 27 Mg ha^{-1} in addition to *F* at the rates of 0, 90, and 180 kg N ha^{-1}.

The *F*-test was used to explain multiple comparisons of means using treatment differences.

compared to the 90 + M treatment. There were no differences in SOC and STN observed among the 0- to 5-, 5- to 10-, and 10- to 15-cm soil depths.

Soil organic C was significantly ($P < 0.0001$) influenced by N treatment, depth studied, and the two-way interaction (N treatment × depth). After 100 yr, vertical stratification of SOC and STN was observed (Table 1). Summed across the 0- to 5-, 5- to 10-, and 10- to 15-cm depths, SOC associated with F + M treatments in the 0- to 15-cm depth increment was approximately 36% greater than in the 15- to 30-cm depth increment for both 0 + M and 90 + M and was 16% greater for 180 + M than the SOC in the 15- to 30-cm depth increment. There were no differences in SOC associated with F between the 0- to 15- and 15- to 30-cm depth increments except that SOC with 90 kg N ha^{-1} of F was approximately 9% greater at 0 to 15 cm compared with 15 to 30 cm. Similar trends were observed with STN, which was approximately 31, 36, and 12.5% greater in the 0- to 15-cm depth increment than in the 15- to 30-cm depth increment for 0 + M, 90 + M, and 180 + M, respectively (Table 1). Observations from this study agree with previously reported research that found significantly greater SOC at the surface 0 to 15 cm compared with 15- to 30-cm depth (Clark et al., 1998; Kaur et al., 2008; Brar et al., 2013). Greater amounts of SOC observed in the surface 15 cm could be related to plant and root biomass accumulation through the duration of the study compared with the 15- to 30-cm depth. Although disking and moldboard plowing are the dominant tillage practices at this site, the moldboard plowing operation did not exceed a 25-cm depth. Therefore, a substantial amount of SOC remained in the surface 15 cm of the soil, especially with M amended treatments. Similar results were previously reported (Kaur et al., 2008; Brar et al., 2013) where the variation in SOC was observed with depth and it was attributed to plant root, plant biomass, and root exudates. At the same study site, Eghball et al. (1996) observed an increase in SOC by an average of 97% in the 0- to 15-cm depth increment than the 15- to 30-cm depth increment with 0 + M for the 1993 sampling date. From 1993 to 2011, SOC in the 0 + M treatment increased by approximately 25 and 80% in the 0- to 15-c and 15- to 30-cm depth, respectively. The SOC associated with the control treatment increased by approximately 5% in the 0- to 15-cm depth and by approximately 48% in the 15- to 30-cm depth after 18 yr of management. Greater changes in SOC with time associated with M application were most probably related to organic amendments added compared with control treatment. Mikha et al. (2014) also observed an increase in SOC at the surface 0 to 15 cm due to continuous M addition.

Soil Aggregation

Aggregate-size distributions (normalized to the sand-free basis) were influenced ($P < 0.0001$) by N treatment at all depths studied (Fig. 1; Table 2). The addition of M significantly increased macroaggregates (>1000, 500–1000, and 250–500 μm) compared with F alone or control treatments. The combination of different rates of F + M further increased macroaggregates compared with M alone (Fig. 1). Increasing macroaggregates associated with the combination of F + M corresponded with a decrease in microaggregates compared with F alone or control treatment. For any N treatment and depth, most of the soil aggregates were associated with microaggregate (53–250 μm) ranging from 60 to 91% of the total sand-free aggregates, followed by macroaggregates in the 250- to 500-μm size classes ranging from 8 to 28% of the total sand-free aggregates. These data agree with previous research (Aoyama et al., 1999a; Mikha and Rice, 2004; Jiao et al., 2006; Yu et al., 2012) that reported an increase in macroaggregate amounts associated with the addition of M or the combination of F + M, with the corresponding shift in

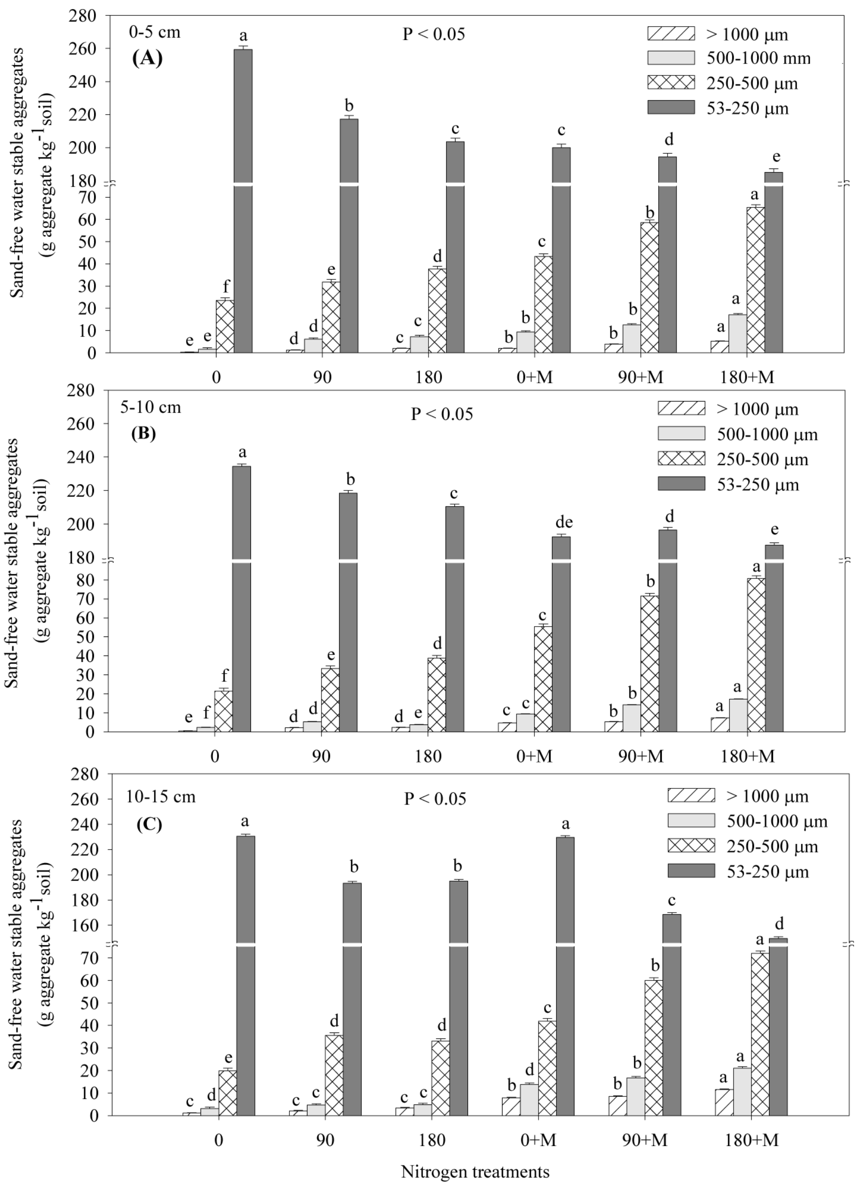

Fig. 1. Sand-free water stable aggregates (grams aggregates per kilogram soil) at (a) 0- to 5-cm, (b) 5- to 10-cm, and (c) 10- to 15-cm depths as affected by different inorganic fertilizer (F) rates (0, 90, and 180 kg N ha^{-1}) and the combination of manure (M) at a rate of 27 Mg ha^{-1} with F at 0, 90, and 180 kg N ha^{-1} (0 + M, 90 + M, and 180 + M, respectively). Lowercase letters represent significant differences ($P \leq 0.05$) among N treatments within the same aggregate-size classes. The error bars represent standard errors of the mean.

Table 2. Sand-free water stable aggregate-size distribution (grams C aggregate per kilogram soil; normalized to a sand-free basis) as influenced by long-term N treatments (M, manure; F, inorganic fertilizer; and no N added [control]) at 0- to 15- and 15- to 30-cm depths.

Treatments	Sand-free water stable aggregate-size distribution			
	g aggregates kg^{-1} soil			
0- to 15-cm depth	>1000 µm	500–1000 µm	250–500 µm	53–250 µm
0†	0.56 a‡	2.35 f	21.61 h	238.13 ab
90	1.80 a	5.33 e	33.59 ef	209.65 c
180	2.78 a	5.25 e	36.49 de	202.96 c
0 + M§	4.81 a	10.84 d	46.86 c	207.30 c
90 + M	6.01 a	14.46 b	63.38 b	186.49 d
180 + M	8.00 a	18.01 a	72.70 a	173.93 e
15- to 30-cm depth				
0	1.52 a	5.33 e	22.53 h	240.70 a
90	2.92 a	9.00 d	30.19 g	196.82 d
180	3.31 a	9.48 d	31.24 fg	201.62 c
0 + M	5.52 a	5.73 e	39.60 d	229.02 b
90 + M	7.08 a	11.22 c	49.06 c	168.58 e
180 + M	9.34 a	15.32 b	60.11 b	153.39 f
	PR > *F*¶			
Treatment (Trt)	<0.0001	<0.0001	<0.0001	<0.0001
0 (mean)	1.04 E#	3.84 E	22.07 E	239.41 A
90 (mean)	2.36 D	7.27 D	31.89 D	203.24 C
180 (mean)	2.89 D	7.37 D	33.86 D	202.29 C
0 + M (mean)	5.16 C	8.23 C	43.23 C	218.16 B
90 + M (mean)	6.54 B	12.84 B	56.22 B	177.54 D
180 + M (mean)	8.67 A	16.86 A	66.41 A	163.66 E
Depth (D)	0.0021	0.7224	<0.0001	0.0275
0–15 cm (mean)	3.94 B	9.44 A	45.77 A	303.08 A
15–30 cm (mean)	4.95 A	9.38 A	38.79 B	198.36 B
Trt × D	0.9404	<0.0001	0.0039	0.0022

† Represents F treatment at different rates (0, 90, and 180 kg N ha^{-1}).

‡ Lowercase letters represent significant differences of the treatment ´ depth interaction among the treatments within each aggregate-size fractions (ANOVA); $P < 0.05$.

§ Represents M added approximately at 27 Mg ha^{-1} in addition to F at the rates of 0, 90, and 180 kg N ha^{-1}.

¶ The *F*-test was used to explain multiple comparisons of means using treatment differences.

Means with different uppercase letters between treatments or depths within each aggregate-size fraction are significantly different (ANOVA); $P < 0.05$.

microaggregate amounts associated with the addition F alone and control treatments.

In both of the 0- to 15- and 15- to 30-cm depths (Table 2), the amounts of soil macroaggregates (>1000, 500–1000, and 250–500 μm) were greater with the combination of F + M treatments compared with F alone and control treatments. Microaggregates (53–250 μm) were substantially lower with the combination of F + M treatments compared with other N treatments. Overall, microaggregates had approximately 1.8 to 4.9-fold greater quantities compared with the combination of all macroaggregates associated with each N treatment combination. As the SOC increased (Table 1) with the addition of M alone or in combination with F, there was an increase in soil macroaggregates compared with F alone or control treatments. Previous research reported that microbial activity can be enhanced with organic additions (Puget et al., 1995) that promote soil particles binding into macroaggregates (Six et al., 1999). Mikha and Rice (2004) also observed increased macroaggregates with long-term M additions compared with F, where the shift to a higher proportion of microaggregates with F compared with M treatment. Averaged across N treatments (Table 2), macroaggregates >1000 μm had smaller quantities at the 0- to 15-cm depth than the 15- to 30-cm depth. There were no differences in 500- to1000-μm macroaggregates observed between the 0- to 15- and 15- to 30-cm depths. However, 250- to 500-μm macroaggregates and 53- to 250-μm microaggregates were significantly higher in the 0- to 15-cm depth than the 15- to 30-cm depth (Table 2). Continuous moldboard plowing and disking for the last 100 yr might contribute to destruction of macroaggregates, especially those >1000 μm, into smaller aggregates-size classes in the surface layer compared with deeper depths, where tillage has less effect. Previous research observed a greater susceptibility of macroaggregates to destruction and higher turnover rates with tillage practices compared with less disturbed soil (Six et al., 2000; Mikha and Rice, 2004; Mikha et al., 2013). The data generated from this study indicates that aggregate-size distributions at different depths were influenced by long-term organic amendments and continuous tillage.

Aggregate-Associated Carbon and Nitrogen

Aggregate-associated C (grams C per kilogram sand-free aggregates) at 0- to 5-, 5- to 10-, and 10- to 15-cm depths (Fig. 2) was significantly influenced by N treatment, depth, and the two-way interaction (N treatments × depths). At the surface 0- to 5-cm depth, substantial amounts of C were associated with macroaggregates (>1000, 250–500, and 53–250 μm) when different rates of F were combined with M (0 + M, 90 + M, and 180 + M) compared with F or control treatments (Fig. 2a). These results agree with previous research that documented an increase in aggregate-associated C with organic amendment additions (Mikha and Rice, 2004; Jiao et al., 2006; Yu et al., 2012). Greater amounts of C were associated with macroaggregates (500–1000 μm) when F rates were 90 and 180 kg N ha^{-1} compared with the control and combination of F + M treatments (Fig. 2a). Similar patterns for aggregate-associated C were observed at the 5- to 10- and 10- to 15-cm depth (Fig. 2b and 2c). The differences in aggregate stability and C conservation associated with F treatment between macroaggregates >1000 μm and those 500 to 1000 μm may contribute to greater SOC accumulation within macroaggregates 500 to 1000 μm compared to macroaggregates >1000 μm, especially with F treatment. These results agree with previous research that found that tillage can reduce macroaggregate stability, promote macroaggregate turnover, and reduced soil C conservation (Six et al., 2000; Blanco-Moure et al., 2012; Mikha et al., 2013). However, M additions can improve macroaggregate stability and increase aggregate-associated C (Aoyama et al., 1999a; Mikha and Rice, 2004; Wortmann and Shapiro, 2008; Yu et al., 2012) in tilled systems.

The amount of soil C associated with microaggregates (53–250 μm) was approximately two times greater with the M and combination of F + M treatments compared with F alone and control treatments (Fig. 2), indicating that the addition of M alone or in combination with F improves soil C conservation associated with microaggregate-size classes. A similar pattern was observed with aggregate-associated N. These results agree with Mikha and Rice (2004) who observed a greater amount of microaggregate-associated C and N with M compared to F treatments. Yu et al. (2012) reported an increase in microaggregate-associated C with combination of F and organic amendment compared with F alone and control treatments. Averaged across N treatments, aggregate-associated C was greater at the 0- to 15-cm depth than the 15- to 30-cm depth (Table 3) except for 250- to 500-μm aggregates. Across aggregate-size classes, aggregate-associated C was 64% greater at the 0- to 15-cm depth compared with the 15- to 30-cm depth. A similar pattern was observed with aggregate-associated N. This study indicates that M addition influenced soil C and N distributions and C and N conservation associated with different aggregate-size classes and that the effects are depth dependent.

Carbon and Nitrogen Mass in Relation to Total Soil Aggregates

The mass of C and N associated with the whole mass of individual aggregate-size classes recovered per kilogram soil, normalized to sand-free WSA, at 0- to 5-, 5- to 10-, and 10- to 15-cm depths (Fig. 3) was significantly influenced by N treatment, depth, and the two-way interactions (N treatments × depths). However, depth has no effect on the mass of C and N for 250- to 500-μm aggregates. Evaluating aggregate-associated C and N on the aggregate mass recovered from a fixed soil weight provides the actual representation of soil C and N masses as it exists in the fixed weight of this soil. Aggregate-associated C and N (Fig. 3) depend on the mass of aggregates associated with each N treatment and depth. At any depth studied (Fig. 3), aggregate-associated C was greater with 53- to 250-μm microaggregates than with any other aggregate-size class. Averaged across N treatment, 53- to 250-μm microaggregate-associated C represents approximately 60% for the 0- to 5-cm depth, 59% for the 5- to 10-cm

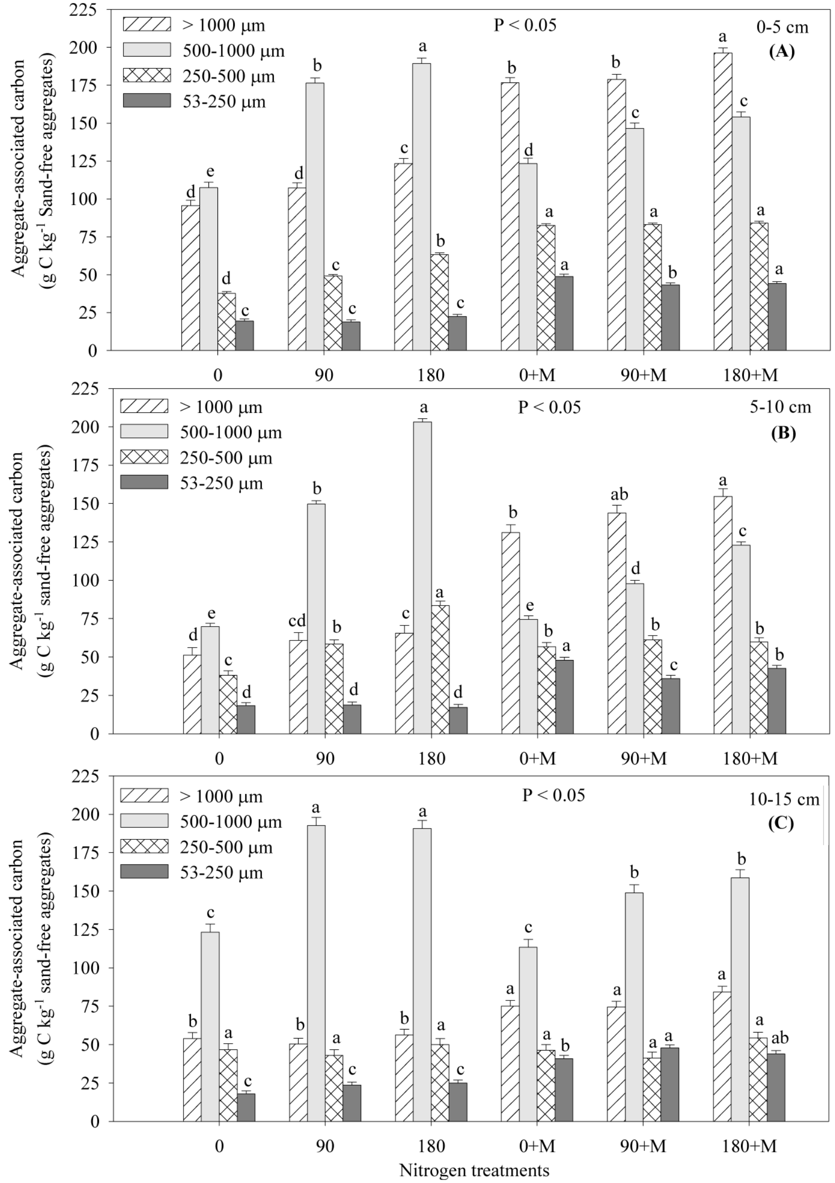

Fig. 2. Aggregate-associated C (grams C per kilogram; normalized to sand-free water stable aggregates) at (a) 0- to 5-cm, (b) 5- to 10-cm, and (c) 10- to 15-cm depths as affected by different inorganic fertilizer (F) rates (0, 90, and 180 kg N ha^{-1}) and the combination of manure (M) at a rate of 27 Mg ha^{-1} with F at 0, 90, and 180 kg N ha^{-1} (0 + M, 90 + M, and 180 + M, respectively). Lowercase letters represent significant differences ($P \leq 0.05$) among N treatments within the same aggregate-size classes. The error bars represent standard errors of the mean.

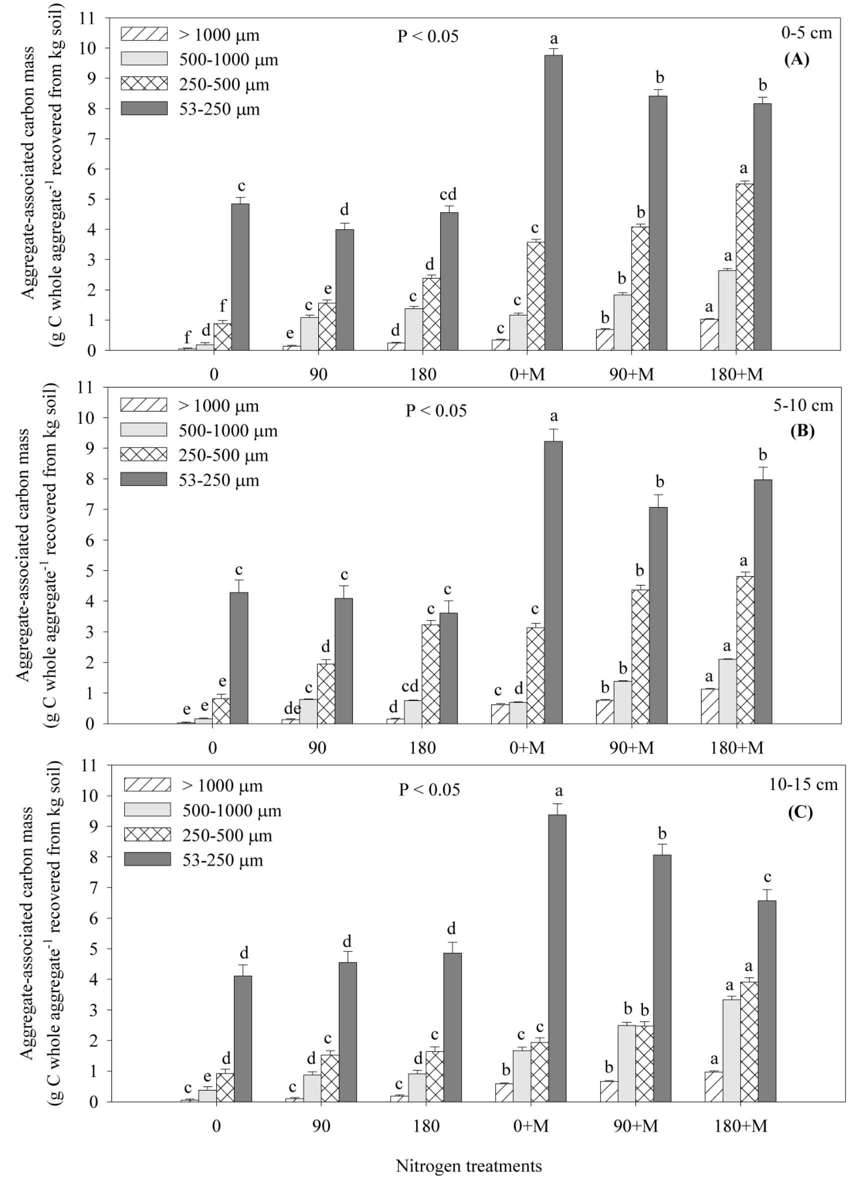

Fig. 3. Aggregate-associated C mass (grams C per whole aggregates recovered from 1 kg of soil; normalized to sand-free water stable aggregates) at (a) 0- to 5-cm, (b) 5- to 10-cm, and (c) 10- to 15-cm depths as affected by different inorganic fertilizer (F) rates (0, 90, and 180 kg N ha^{-1}) and the combination of manure (M) at a rate of 27 Mg ha^{-1} with F at 0, 90, and 180 kg N ha^{-1} (0 + M, 90 + M, and 180 + M, respectively). Lowercase letters represent significant differences ($P \leq 0.05$) among N treatments within the same aggregate-size classes. The error bars represent standard errors of the mean.

Table 3. Aggregate-associated total C (grams C per kilogram water-stable aggregate; normalized to sand-free basis) in water-stable aggregates at different size fractions as influenced by long-term N treatments (M, manure; F, inorganic fertilizer; and no N added [control]) at 0- to 15- and 15- to 30-cm depths.

Treatments	Aggregate-associated C			
	g C kg^{-1} sand-free aggregate			
0- to 15-cm depth	>1000 µm	500–1000 µm	250–500 µm	53–250 µm
0†	66.91 de‡	100.15 f	40.80 e	18.52 de
90	72.79 cd	172.95 b	50.09 d	20.23 de
180	81.69 c	194.39 a	65.64 b	21.49 de
0 + M§	127.85 b	103.77 de	61.79 bc	45.88 a
90 + M	132.34 b	131.08 d	61.78 bc	42.36 a
180 + M	145.01 a	145.16 c	66.03 b	43.54 a
15- to 30-cm depth				
0	37.89 g	59.06 f	32.94 f	14.89 e
90	44.60 fg	51.33 f	57.88 c	21.67 d
180	51.80 f	52.48 f	64.07 bc	20.72 de
0 + M	61.75 e	53.06 f	51.86 d	35.77 b
90 + M	62.64 e	60.55 f	93.42 a	29.11 c
180 + M	61.91 e	55.03 f	97.72 a	45.05 a
	PR > *F*¶			
Treatment (Trt)	<0.0001	<0.0001	<0.0001	<0.0001
0 (mean)	52.40 C#	79.61 E	36.87 E	16.71 C
90 (mean)	58.69 C	112.14 B	54.00 D	20.95 C
180 (mean)	66.75 B	123.43 A	64.85 C	21.10 C
0 + M (mean)	94.40 B	78.51 E	56.83 D	40.83 A
90 + M (mean)	97.49 A	95.82 D	75.36 B	35.74 B
180 + M (mean)	103.46 A	105.09 C	81.88 A	45.00 A
Depth (D)	<0.0001	<0.0001	0.0011	0.0070
0–15 cm (mean)	104.61 A	140.35 A	57.69 B	32.00 A
15–30 cm (mean)	53.43 B	56.95 B	66.32 A	27.86 B
Trt × D	0.0002	<0.0001	0.0005	0.0241

† Represents F treatment at different rates (0, 90, and 180 kg N ha^{-1}).

‡ Lowercase letters represent significant differences of the treatment ´ depth interaction among the treatments within each aggregate-size fractions (ANOVA); $P < 0.05$.

§ Represents M added approximately at 27 Mg ha^{-1} in addition to F at the rates of 0, 90, and 180 kg N ha^{-1}.

¶ The *F*-test was used to explain multiple comparisons of means using treatment differences.

Means with different uppercase letters between treatments or depths within each aggregate-size fraction are significantly different (ANOVA); $P < 0.05$.

depth, and 63% for the 10- to 15-cm depth of total aggregate-associated C. The combination of macroaggregate-associated C (>1000, 500–1000, and 250–500 µm) represents 40, 41, and 37% for 0- to 5-, 5- to 10-, and 10- to 15-cm depths, respectively. The greater amount of microaggregate-associated C was a consequence of the substantial amount of microaggregates observed with any treatment at any depth studied (Fig. 1). Results presented in Fig. 3 are in contrary to those data presented in Fig. 2, because microaggregates amounts averaged between 4 and 4.8 times greater mass than the macroaggregates at all depths studied. Although greater concentrations of C were associated with macroaggregates (Fig. 2), the low amount of macroaggregates present per kg of soil (Fig. 1) caused the apparent differences in

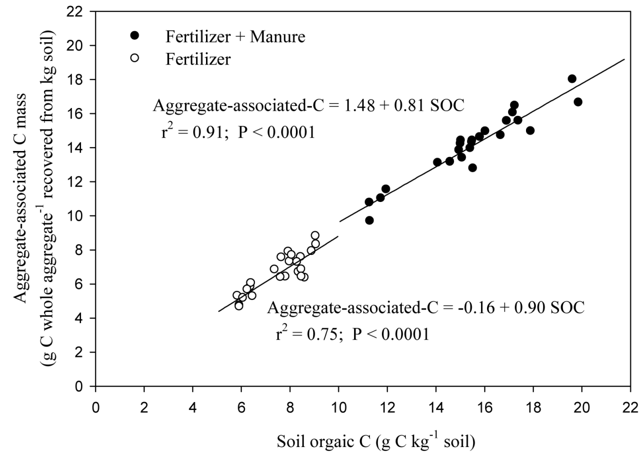

Fig. 4. Relationship between soil organic C (grams C per kilogram soil) and aggregate-associated C mass (grams C per whole aggregates recovered from 1 kg of soil; normalized to sand-free water stable aggregates) influenced by inorganic fertilizer (F) and the combination of F + manure (M) at the 0- to 30-cm depth.

results between results presented in Fig. 2 and 3. A similar pattern was observed with aggregate-associated N (data not shown). Data generated from this study agrees with Mikha and Rice (2004) and Zibilske and Bradford (2007), who observed greater amounts of aggregate C correlated with a greater mass of soil aggregate-size classes when aggregate C was presented as relative to aggregate mass.

Averaged across depths, the combination of F + M increased aggregate-associated C compared with other treatments (Table 4). The 0- to 5-cm depth contained a substantial amount of aggregate-associated C compared with the 15- to 30-cm depth. Microaggregate-associated C, however, was approximately 1.5 times greater than the combination of macroaggregate-associated C. The greater amount of microaggregate-associated C was a consequence of greater amounts of microaggregates compared with macroaggregates at this study site. A greater amount of microaggregates than macroaggregates could be due to continuous breakdown of macroaggregates during tillage operation. These results indicate that SOC and STN have a potential to be lost from this site, specifically through wind erosion, due to their greater association with microaggregates rather than macroaggregates. This conclusion is based on previous research that soil wind erodibility can be influenced by aggregate stability and their size distribution (Merrill et al., 1999; Blanco-Canqui et al., 2009). Soils are more susceptible to erosion and SOC losses as microaggregates increase relative to macroaggregates (Jiao et al., 2006; Zhang et al., 2007; Blanco-Moure et al., 2012).

Relation between Aggregate-Associated Carbon and Total Soil Organic Carbon

The aggregate-associated C (grams C per whole aggregate recovered per kilogram soil; normalized to sand-free WSA) relative to the SOC at the 0- to 15-cm and 15- to 30- cm depths was influenced by N treatments, depth, and treatment × depth interaction for all aggregate-sizes except for 53 to 250 µm (Table 5). The SOC associated with 250- to 500-µm macroaggregates was approximately 1.3 to 2 times greater in the 0- to 15-cm depth and 1.9 to 4.1 times greater at the 15- to 30- cm depth compared with the combination of the >1000- and 500- to 1000-µm aggregate-size classes. These results indicate that the majority of SOC is present in the 250- to 500-µm macroaggregates-size class compared with other macroaggregate-size classes evaluated. These results agree with those presented by Mikha and Rice (2004), who observed greater C enrichment with 250- to 2000-µm macroaggregates. The SOC associated with 53- to 250-µm microaggregates ranged between 42 and 71% at both depths (Table 5). This is mainly due to a higher mass of microaggregate present in soil compared with other aggregate-size classes (Table 2). Similarly, Zibilske and Bradford (2007) observed greater amounts of C associated with aggregate-size classes that predominate in the volume of soil.

Table 4. Aggregate-associated total C (grams C per whole water-stable aggregate recovered from 1 kg of soil; normalized to sand-free basis) at different size fractions as influenced by long-term N treatments (M, manure; F, inorganic fertilizer; and no N added [control]) at 0- to 15- and 15- to 30-cm depths.

Treatments	Aggregate-associated C			
	g C whole sand-free aggregate^{-1}			
	>1000 µm	500–1000 µm	250–500 µm	53–250 µm
0- to 15-cm depth				
0†	0.03 i‡	0.25 h	0.88 g	4.42 de
90	0.12 gh	0.91 e	1.68 f	4.21 de
180	0.19 f	1.01 d	2.42 de	4.34 de
0 + M§	0.52 c	1.14 c	2.88 d	9.46 a
90 + M	0.70 b	1.90 b	3.64 c	7.85 c
180 + M	1.04 a	2.69 a	4.74 b	7.57 c
15- to 30-cm depth				
0	0.06 hi	0.32 h	0.74 g	3.58 e
90	0.13 fg	0.47 g	1.75 f	4.26 de
180	0.18 fg	0.50 g	2.00 ef	4.18 de
0 + M	0.34 e	0.31 h	2.06 ef	8.18 b
90 + M	0.44 d	0.68 f	4.58 b	4.91 d
180 + M	0.58 c	0.99 e	5.88 a	6.91 c
	PR > F¶			
Treatment (Trt)	<0.0001	<0.0001	<0.0001	0.0001
0 (mean)	0.05 E#	0.28 D	0.81 E	4.00 C
90 (mean)	0.13 D	0.69 C	1.71 D	4.24 C
180 (mean)	0.18 D	0.76 C	2.21 C	4.26 C
0 + M (mean)	0.43 C	0.72 C	2.47 C	8.82 A
90 + M (mean)	0.57 B	1.29 B	4.11 B	6.38 B
180 + M (mean)	0.81 A	1.84 A	5.31 A	7.24 B
Depth (D)	<0.0001	<0.0001	0.3447	0.0021
0–15 cm (mean)	0.43 A	1.31 A	2.75 A	6.31 A
15–30 cm (mean)	0.29 B	0.54 B	2.84 A	5.34 B
Trt × D	<0.0001	<0.0001	0.0048	0.03

† Represents F treatment at different rates (0, 90, and 180 kg N ha^{-1}).
‡ Lowercase letters represent significant differences of the treatment × depth interaction among the treatments within each aggregate-size fractions (ANOVA); $P < 0.05$.
§ Represents M added approximately at 27 Mg ha^{-1} in addition to F at the rates of 0, 90, and 180 kg N ha^{-1}.
¶ The *F*-test was used to explain multiple comparisons of means using treatment differences.
\# Means with different uppercase letters between treatments or depths within each aggregate-size fraction are significantly different (ANOVA); $P < 0.05$.

Table 5. Percent of whole aggregate-associated C from the total soil organic C at different size fraction (>1000, 500–1000, 250–500, and 52–250 µm; normalized to sand-free basis) as influenced by long-term N treatments (M, manure; F, inorganic fertilizer; and no N added [control]) at 0- to 15- and 15- to 30-cm depths.

Treatments	Aggregate-associated C and SOC			
	%			
0- to 15-cm depth	>1000 µm	500–1000 µm	250–500 µm	53–250 µm
0†	0.53 h‡	3.86 e	14.07 ef	70.75 a
90	1.49 fg	11.18 b	20.58 d	51.86 a
180	2.24 ef	11.76 b	28.07 b	50.96 a
0 + M§	3.36 cd	7.36 c	18.19 d	60.52 a
90 + M	4.47 b	12.15 b	22.93 cd	49.79 a
180 + M	5.93 a	15.18 a	26.73 bc	42.86 a
15- to 30-cm depth				
0	0.97 gh	5.32 d	12.15 f	60.49 a
90	1.73 f	6.29 cd	23.31 cd	56.91 a
180	2.97 f	5.94 d	23.87 cd	49.85 a
0 + M	2.97 de	2.65 e	17.87 de	71.14 a
90 + M	3.79 bc	5.84 d	39.42 a	42.06 a
180 + M	3.79 bc	6.53 cd	38.51 a	45.28 a
	PR > F¶			
Treatment (Trt)	<0.0001	<0.0001	<0.0001	0.0039
0 (mean)	0.75 D#	4.59 C	13.29 E	65.62 A
90 (mean)	1.60 D	8.74 B	21.94 C	54.39 B
180 (mean)	2.65 D	8.85 B	26.00 B	50.41 BC
0 + M (mean)	3.16 C	5.01 C	18.03 D	65.83 A
90 + M (mean)	4.13 B	9.00 A	31.17 A	45.92 C
180 + M (mean)	4.86 A	11.00 A	32.62 A	44.07 C
Depth (D)	0.0138	<0.0001	0.0015	0.9329
0–15 cm (mean)	3.00 A	10.25 A	22.02 B	54.46 A
15–30 cm (mean)	2.55 B	5.43 B	25.92 A	54.29 A
Trt × D	0.0114	<0.0001	0.0011	0.1172

† Represents F treatment at different rates (0, 90, and 180 kg N ha^{-1}).
‡ Lowercase letters represent significant differences of the treatment × depth interaction among the treatments within each aggregate-size fractions (ANOVA); $P < 0.05$.
§ Represents M added approximately at 27 Mg ha^{-1} in addition to F at the rates of 0, 90, and 180 kg N ha^{-1}.
¶ The *F*-test was used to explain multiple comparisons of means using treatment differences.
\# Means with different uppercase letters between treatments or depths within each aggregate-size fraction are significantly different (ANOVA); $P < 0.05$.

Averaged across depths, a greater SOC was associated with macroaggregates when F and M were combined as N source compared with other N treatments. The lowest amount of SOC was associated with microaggregates in the F + M treatment compared with other N treatments (Table 5). Averaged across treatments, the SOC was greater at the 0- to 15-cm depth with >1000- and 500- to 1000-μm macroaggregates than the 250- to 500-μm macroaggregate. However, the percentage of SOC associated with 250- to 500-μm macroaggregates was greater than the >1000- and 500- to 1000-μm macroaggregates for the 15- to 30-cm depth compared with 0- to 15-cm depth (Table 5). These results agree with Zibilske and Bradford (2007) who observed an increase in aggregate-associated C, based on aggregate mass, at the deeper depths compared with the surface depths when tillage was performed. Zibilske and Bradford (2007) explained that high C deposition in the lower depth was probably due to increasing aggregate-associated C at the deeper depth with tillage practices. No differences in aggregate-associated C between depths were observed with 53- to 250-μm microaggregate-size classes.

The correlation between SOC and aggregate-associated C at the 0- to 30-cm depth (Fig. 4) was significantly affected by N treatments, and it was separated into two groups. The first group was represented by F + M treatment and the second group was represented by F treatments. A positive and significant linear correlation was observed between SOC and aggregate-associated C. The slope of both lines was not significantly different, suggesting that aggregate-associated C accounted for almost similar amounts of SOC (81% for F + M and 90% for F). The relationship between aggregate-associated C and SOC (r^2 = 0.91 for F + M and r^2 = 0.75 for F treatment) indicates a close relationship between the two parameters studied but with different magnitude. Overall, after 70 yr of different N treatment, the relationship between aggregate-associated C and SOC was affected by N source, F vs. F + M, and by the SOC quantities associated with different N rates (Fig. 4).

CONCLUSIONS

Seventy years of continuous manure addition substantially increased SOC and STN at all depths studied compared to the F treatment. Vertical distribution and stratification of SOC and STN within the soil, especially with F treatment, was observed due to long-term moldboard plowing. Aggregate-size distributions, at different depths, were influenced by manure additions and continuous tillage. The combination of F + M further increased macroaggregate-size classes compared with other N treatments. Microaggregates were 1.8 to 4.9 times greater than macroaggregates within the top 30-cm of soil. Interpretation of aggregate-associated C and N were influenced by the calculation approach in relation to the fixed aggregates weight (grams C or N per kilogram of aggregates) compared to fixed soil weight (grams C of N per whole aggregates recovered from 1 kg soil). Comparing aggregate-associated C as grams C per kilogram of sand-free aggregates, it appears that macroaggregate-associated C was greater compared with microaggregates. However, presenting aggregate-associated C as grams C per whole aggregate recovered from 1 kilogram of soil, normalized to sand-free WSA, microaggregate-associated C was greater compared with macroaggregates. A higher percentage of SOC was associated with microaggregates than macroaggregates due to the greater mass of microaggregates compared with macroaggregates. Results generated from this study agreed with our hypothesis that the addition of an organic amendment (M) alone or in combination of F (F + M) increased SOC, SOC content within soil aggregates, and soil aggregation, even with tillage. The quantity of SOC and its distribution among aggregate-size classes and C conservation were enhanced by adding manure. The changes in SOC and soil aggregation, compared to control treatment, occurred throughout the 100 yr of irrigation, continued moldboard plowing and disking, and 70 yr of manure additions. According to this study, the addition of M or the combination of F + M in a moldboard plow system appears to be a sustainable management practice that increases SOC and enhances soil aggregate stability.

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