Pergamon

PII: S0038-0717(95)00159-X

Soil Biol. Biochem. Vol. 28, No. 4/5, pp. 665–676, 1996

0038-0717/96 $15.00 + 0.00

SOIL AGGREGATE FORMATION AND THE ACCRUAL OF PARTICULATE AND MINERAL-ASSOCIATED ORGANIC MATTER

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(Accepted 16 October 1995)

Summary—The degradation of soil aggregates appears to be a primary mechanism in the loss of organic matter caused by long-term cultivation, but little information exists on how the formation and stabilization of macroaggregates control the process of C aggradation when disturbance is reduced or eliminated. A chronosequence of restored tallgrass prairie was used to investigate the relationships between the formation of stable macroaggregates (> 212 μm dia) and the accrual of particulate and mineral-associated organic matter. Changes in the percentage of macroaggregates and in the accumulation of whole-soil organic C across the chronosequence were both described with a simple exponential model. The rate constant (k) for change in aggregation was more than 35 times the k for total organic C accumulation. Thus, the time required to reach 99% of equilibrium was 10.5 y for macroaggregates and 384 y for whole-soil organic C, providing evidence for the existence of a phased relationship between macroaggregate formation and C accrual. The input rate for whole-soil organic C to a 10-cm depth was estimated at 1.16 g kg^{-1} y^{-1} or 0.133 kg m^{-2} y^{-1} (assuming an average bulk density of 1150 kg m^{-3} for previously cultivated soils in the chronosequence). An increase in macroaggregate-associated C-to-N ratios with time since cultivation suggested that the accumulating organic matter was not "highly processed", but less than 20% of the accrued C occurred in the form of particulate organic matter (density $\leq$ 1.85 g cm^{-3}). Rather, most of the accumulated C occurred in the mineral-associated fraction of macroaggregates, suggesting that inputs of organic debris were rendered relatively rapidly into particles or colloids that are associated with mineral matter and thus are physically protected, slowing decomposition and promoting the development of stable microaggregates within macroaggregates.

INTRODUCTION

The mechanisms involved in the binding of soil particles into stable aggregates vary with a variety of factors related to soil parent material, climate, vegetation, and management practices (e.g. see reviews by Martin *et al.*, 1955; Harris *et al.*, 1966; Kay, 1990). In soils where organic matter is the major binding agent, the formation of aggregates appears to be hierarchical in that primary particles and clay microstructures are bound into microaggregates (up to 250 μm dia), which, in turn, are bound into larger macroaggregates (up to several millimeters in dia) (Tisdall and Oades, 1982; Oades and Waters, 1991). The hierarchical packing of variously sized units also creates a hierarchy of pore sizes and contact points (Dexter, 1988). Hence, a variety of binding mechanisms may function more or less simultaneously at different spatial scales to stabilize a single large macroaggregate (Tisdall and Oades, 1982).

Tisdall and Oades (1982) suggested that plant roots and fungal hyphae bind microaggregates into macroaggregates. However, a number of researchers have proposed that the growth of roots and hyphae serves to physically form macroaggregates and that microaggregates are then formed and stabilized within these macroaggregates as a result of a number of processes, including decomposition of organic debris; deposition of microbial by-products; reorientation of clay platelets; and physicochemical reactions between polyvalent cations, organic molecules, and clays (e.g., Allison, 1968; Oades, 1984; Elliott and Coleman, 1988; Tiessen and Stewart, 1988; Oades and Waters, 1991).

Tisdall and Oades (1980) found greater concentrations of organic C in macroaggregates than in microaggregates and suggested that the presence of decomposing roots and hyphae within macroaggregates not only increased C concentrations but also contributed to their stabilization. Elliott (1986) suggested that macroaggregates have elevated C concentrations because of the organic matter binding microaggregates into macroaggregates and that this organic matter is "qualitatively more labile and less highly processed" than the organics stabilizing microaggregates. Further, he proposed that inter-microaggregate binding agents are the primary source of organic matter lost when native sod soils are cultivated. More recently, Cambardella and Elliott (1992; 1993, 1994) suggested that most of this labile

pool within macroaggregates is either light-fraction particulate organic matter (POM) or relatively low-density, mineral-associated organic matter, probably of microbial origin.

Elliott and Coleman (1988) hypothesized that a hysteretic effect separates the rates of organic matter degradation and aggradation and that different types of macroaggregate binding mechanisms will appear to be important depending on the process being observed. They point out that intermicroaggregate organic matter seems most important when native grassland soils are cultivated because large amounts of it are exposed and mineralized, causing relatively rapid disintegration of macroaggregates into microaggregates. In contrast, when soils subjected to long-term cultivation are converted to reduced-tillage systems or returned to perennial vegetation (particularly grasses), roots and hyphae appear important because they probably serve as initial binding agents. Elliott and Coleman suggest that with time, however, accumulation of POM and deposition of microbial by-products between microaggregates become important mechanisms in further stabilizing macroaggregates and result in the accrual of soil organic matter.

Although the loss of macroaggregate-associated organic matter with cultivation has been studied in some detail (e.g., Dormaar, 1983; Elliott, 1986; Gupta and Germida, 1988; Cambardella and Elliott, 1992, 1993, 1994), little information exists on how macroaggregate formation and stabilization influence the process of C aggradation. The rapid formation and stabilization of macroaggregates observed in a chronosequence of restored tallgrass prairie (Jastrow, 1987) provides an excellent opportunity to investigate the accrual of macroaggregate-associated organic matter. Hence, the prairie chronosequence was used to determine whether the redevelopment of macroaggregate structure results in the concomitant accrual of soil organic matter in restored prairie soils and, if so, to ascertain the quality and form of organic matter accumulated within macroaggregates.

MATERIALS AND METHODS

Study sites

Seven sites were sampled, including four plots in a chronosequence of tallgrass prairie restorations, a virgin prairie remnant, an ungrazed pasture dominated by cool-season Eurasian grasses, and a rowcrop field. Six of the sites were located within the National Environmental Research Park at Fermi National Accelerator Laboratory (Fermilab), Batavia, Illinois, 48 km west of Chicago. The prairie remnant, which consisted of a 0.5-ha plot within the 48-ha West Chicago Prairie, was located 6 km north of the Fermilab Research Park. The rowcrop field (adjacent to the accelerator ring) had been variously cultivated for over 100 y according to the practices of the time. The field has been in continuous corn (*Zea mays* L.) using conventional tillage practices since at least 1969 (when Fermilab was established). The prairie restorations (inside the accelerator ring) had been similarly cultivated for row crops, but in 1969–1970 the area was taken out of cultivation and allowed to revert to an old-field condition until each plot was plowed, disked, and planted with prairie species. The four sampled restorations (planted in spring 1975, autumn 1977, spring 1981 and spring 1984) encompassed the range of plot ages within the chronosequence. At the time of sampling (17–27 June 1985), the plots had completed 10, 7, 4, and 1 growing seasons (gs), respectively. The ungrazed pasture (adjacent to the cornfield) also was planted on similarly cultivated soils in the fall of 1971 and had completed 13 gs.

All sampled sites were located entirely on Mundelein silt loam (fine-silty, mixed, mesic Aquic Argiudoll), except the youngest (1 gs) prairie restoration, which was on Drummer silty clay loam (fine–silty, mixed, mesic Typic Haplaquoll), and the oldest (10 gs) restoration, which was mostly on Wauconda silt loam (fine–silty, mixed, mesic Udollic Ochraqualf) with some small areas of Drummer. All three soils are closely related as part of a drainage association. More detailed descriptions of the sites, prairie restoration methods, burn histories, and plant species composition were reported previously (Jastrow, 1987).

Chronosequence study methods

Within each of the sites, 10 sample stations consisting of a 0.5-m^2 circular quadrat were located by using a stratified random design. The aboveground standing crop inside each quadrat was clipped to within 2 cm of the surface and removed. After clipping, a soil core (4.8-cm dia) was taken to a depth of 10 cm from each quadrat and transferred intact to a polyethylene bag for aggregate analysis. Bulk density (to 10 cm) was determined by the core method (Blake and Hartge, 1986). Three additional 10-cm cores were pooled to determine total soil organic C for each quadrat. All soil samples were frozen until analysis.

The pooled soil cores were broken apart, and most roots and large organic debris were removed. After thorough mixing and air drying, the soil was passed through a 2-mm sieve. Whole soil organic C was measured colorimetrically on Walkley–Black dichromate digests (Nelson and Sommers, 1982). No carbonates were present in the samples.

Water-stable macroaggregates were collected by slaking of air-dried soil followed by wet sieving. A modification of the method of Kemper and Chepil (1965) was used as detailed by Jastrow (1987). After overnight thawing in a refrigerator, each core was gently broken apart along its natural breaking points to pass a 9.5-mm sieve, and large roots and organic debris were removed. The samples were air dried,

immersed in deionized water at atmospheric pressure for 10 min, and wet sieved for 10 min through a nest of sieves with hole widths of 4750, 2000, 1000, and 212 μm on a sieving machine at a rate of 30 up–down cycles min^{-1}.

Subsamples of aggregate size separates were ground in a mortar and pestle to pass a 250-μm sieve. During grinding, any roots and plant debris longer than about 1 mm were removed with forceps. Organic C was determined as above. Total Kjeldahl N was measured by wet oxidation on a block digestor and analyzed with a Technicon TRAACS 800 autoanalyzer (Nelson and Sommers, 1980).

Fractionation of organic matter

To further investigate the form of organic matter in macroaggregates from different stages in the chronosequence, a second study was conducted using samples from the cornfield, two prairie restorations (4 and 10 gs), and the prairie remnant. Macroaggregates in three size classes (212–1000, 1000–2000, and 2000–4750 μm) from three randomly selected quadrats in each site were fractionated into mineral-associated C and POM-associated C by using methods modified from Elliott *et al.* (1991) and Cambardella and Elliott (1992, 1993).

All C concentrations were determined by dry combustion at 800°C in a boat sampler attached to a Dohrmann DC-180 Infrared C Analyzer. Dry combustion values for total C were equivalent to organic C because no carbonates were present in the sampled soils. Because of the relatively small sample sizes used, all samples were dried overnight at 100°C prior to C analysis (to reduce variability due to moisture), and the C concentrations determined for each sample were the average of at least three replicate analyses with the C analyzer. For the quadrats used in this study, the total organic C concentrations of each macroaggregate size separate and of the whole soil were reanalyzed with the Dohrmann C Analyzer on subsamples ground to pass a 250-μm sieve.

After overnight drying at 100°C, 1.25-g subsamples of intact water-stable macroaggregates in each size class (including the "free and released" POM that was either exterior to aggregates *in situ* or released from unstable aggregates by slaking) were weighed into 50-ml conical centrifuge tubes and suspended in 15 ml of sodium polytungstate adjusted to a density of 1.85 g cm^{-3}. The suspended soil was evacuated for 5 min at $-$ 86 kPa to remove air trapped in aggregate pore spaces and then centrifuged at 900 *g* for 10 min. Free and released POM was aspirated from the top of the heavy liquid. The remaining soil was washed twice with deionized water, backwashed into pyrex pans, and dried at 70°C. Samples were then removed from the pans, dried at 100°C, weighed, ground to pass a 250-μm sieve, and analyzed for macroaggregate-associated C.

After separation of the free and released POM, 1-g subsamples of the remaining macroaggregate-associated soil were dried at 100°C, soaked overnight in 30 mL of 5 g l^{-1} sodium hexametaphosphate, sonicated with a LabLine Ultratip Labsonic System at 120 W for 45 s (rated energy supply = 180 J ml^{-1} but actual probe output energy to the soil suspension was somewhat lower; see Gregorich *et al.* (1988)), and sieved (< 53 μm). The sand and intramacroaggregate POM retained on the sieve were rinsed several times, blotted from below to absorb excess water, and washed into a 50-ml centrifuge tube with sodium polytungstate (1.85 *g* cm^{-3}) to a volume of 15 ml. After centrifugation at 900 *g* for 10 min, intramacroaggregate POM was aspirated from the top of the heavy liquid, washed with 250 ml of deionized water on a 20-μm nylon filter, transferred into an aluminum weighing pan, dried at 100°C, and analyzed for C content. The remaining sand was also washed, dried, and weighed. Checks of this procedure indicated that no more than 10% and usually < 5% of the POM freed from aggregates by the sonication energies employed in this study were lost through the 53-μm sieve.

All C concentrations were corrected to a sand-free (silt plus clay) basis to facilitate comparisons among size separates (Elliott *et al.*, 1991). Because some sodium polytungstate remained in the soil and intramacroaggregate POM, all C contents were expressed on the basis of the original sample prior to separation of free and released POM (Cambardella and Elliott, 1993). Mineral-associated C was calculated as the difference between macroaggregate-associated C and intramacroaggregate POM C. Similarly, the difference between total C in the size separate and macroaggregate-associated C was used to estimate free and released POM C.

The contribution of each organic matter fraction to total organic C in the whole soil was estimated by summing the products of (1) the C concentrations for each organic matter fraction in each size separate and (2) the amount of soil in that size class. Because it was impossible to take unbiased 1.25-g subsamples of the > 4750-μm size separate for organic matter fractionation, the amount of soil in the > 4750-μm size separate was pooled with that of the 2000- to 4750-μm size separate for the purpose of these calculations. To facilitate comparisons across plots (particularly with the virgin prairie), the data were converted to a volume basis by using data on bulk density for each quadrat. Although the soil that passed through the 212-μm sieve during the wet sieving was not collected and analyzed for C content, the total amount of C in this size class was estimated by subtracting the amount of C contained in the other size classes from the total amount of C in the whole soil.

Statistical analyses and model description

Two-way analysis of variance (ANOVA) was used to determine differences between plots and size separates. Fisher's protected least significant difference was used for mean separations when ANOVA results were significant.

Changes in C and N over time were modelled by using the Marquardt method in the NLIN procedure (SAS Institute Inc., 1985). A simple exponential model for net accumulation of organic C and N was employed (Jenny, 1980; Dalal and Mayer, 1986a; Harden *et al.*, 1992). The model assumes a zero-order input and a first-order rate of loss

$$dC/dt = I - kC, \quad (1)$$

where C is the soil C or N content at a given time (t), I is the rate of input, and k is the first-order rate constant for loss. The solution to this equation is

$$C = I/k \times (1 - ye^{-kt}) = C_e(1 - ye^{-kt}), \quad (2)$$

where $y = 1 - (k/I)C_o = 1 - (C_o/C_e)$, C_o is the initial C or N content ($t = 0$), and C_e is the equilibrium C or N inventory for the undisturbed soil.

The C and N contents of soils in the virgin prairie remnant were assumed to provide a good estimate of equilibium inventories and were assigned an age of 800 y for modelling purposes (roughly one-tenth of the time prairies have occurred in Illinois). Organic C and N inventories at the time of planting to prairie ($t = 0$) were not available. However, values for the cornfield are probably underestimates, because some organic matter likely accumulated during the old-field period after rowcrop agriculture ceased and before the plots were planted to prairie. Similarly, values for the youngest restored prairie are probably overestimates, because this plot was located on Drummer soil, which has characteristically higher organic C contents under arable conditions than the Mundelein and Wauconda soils on which the other plots were located. Thus, the best estimate of C_o was assumed to be the average of values for the cornfield and the youngest restored prairie.

The same model was used to describe changes in aggregation across the chronosequence on the basis of the theoretical model of Kay *et al.* (1988) and the empirical model of Perfect *et al.* (1990). Kay *et al.* (1988) proposed that (1) changes in relative aggregate stability compared to a reference state are a function of changes in the level of stabilizing materials relative to their amount in the reference state and (2) the changes in stabilizing materials are a function of time. Perfect *et al.* (1990) found that the empirical regression equation that best described their data was of the form

$$S/S_{max} = 1 - Me^{bt}, \quad (3)$$

where S/S_{max} is relative aggregate stability, M is a constant that is a function of water content at the time of sampling, and b is the rate constant. By relating Equation 3 to the theoretical model of Kay *et al.* (1988), Perfect and Kay (1990) demonstrated that b is equivalent to $-k$ and can be considered the rate constant for the accumulation of active stabilizing materials. Thus, Equation 3 is equivalent to Equation 2 if (1) both sides are multiplied by S_{max}, (2) aggregate stability at an uncultivated equilibrium is assumed to be equivalent to S_{max}, and (3) M is consistent for all soils of interest. As with C and N inventories, the percentage of macroaggregates found in the cornfield probably underestimates conditions at the time of planting to prairie. Thus, the best estimate of the percentage of macroaggregates at $t = 0$ also was assumed to be the average of the values for the cornfield and youngest restored prairie.

RESULTS

The changes in both the percentage of macroaggregates > 212 μm and the accumulation of whole-soil organic C across the chronosequence fit the exponential model (Fig. 1). Data for the ungrazed pasture and cornfield were not included in model calculations for aggregate stability, because (1) the pasture vegetation is different from that of the restoration chronosequence and may not be following the same trajectory in macroaggregate formation and stabilization (Jastrow, 1987) and (2) the percentage of macroaggregates in the cornfield was not the best estimate of the value for C_o (see model description). The rate constant for change in aggregation was more than 35 times that for total organic C accumulation. Consequently, the time ($t_{1/2}$) required to reach a point midway between conditions at $t = 0$ (time of planting to prairie) and equilibrium (i.e. $t_{1/2} = -\ln[0.5]/k$; see Perfect *et al.* (1990)) was 1.6 y for macroaggregates and 58 y for whole-soil organic C. Similarly, the time required to reach 99% of equilibrium was 10.5 y for macroaggregates and 384 y for whole-soil organic C. The input rate for whole-soil organic C [$C_e(k)$] was estimated at 1.16 g kg^{-1} y^{-1}.

Organic C and total Kjeldahl N in the four macroaggregate size separates did not vary significantly [Fig. 2(A and B), Table 1]. Furthermore, the absence of significant interactions in the ANOVA indicated that the lack of significant differences among size separates was consistent across all plots. When data for all macroaggregate size separates were pooled by plot, the changes in C and N [Fig. 2(A and B)] could again be described by the exponential model (Table 2). However, the value of k for organic C in macroaggregates was less than k for whole-soil organic C (Fig. 1). The rate constant for total Kjeldahl N in macroaggregates was about 70% of k for organic C, resulting in a $t_{1/2}$ of 147 y for N, compared to 102 y for C. Furthermore, the annual rate of C input was 18 times the input rate for N.

Although the C-to-N ratio did not vary signifi-

Table 1. ANOVA results for organic C, total Kjeldahl N, and C-to-N ratio in macroaggregate size separates from the cornfield, restored prairie, pasture, and virgin prairie

Variable	Source of variation	*df*	*F*	*P*	R^2
Organic C	Age	6	227.52	0.0001	0.85
	Size	3	2.05	0.1073	
	Age × size	18	0.31	0.9973	
	Error	252			
Total Kjeldahl N	Age	6	301.50	0.0001	0.88
	Size	3	2.05	0.1075	
	Age × size	18	0.17	1.0000	
	Error	252			
C-to-N ratio	Age	6	17.48	0.0001	0.33
	Size	3	0.36	0.7843	
	Age × size	18	1.05	0.4059	
	Error	252			

cantly across the four macroaggregate size separates (Table 1), it did increase with time since the last cultivation (Fig. 2C). The C-to-N ratios of all size separates in the virgin prairie, however, were significantly lower than those in any of the other plots.

Just as total organic C did not vary among macroaggregate size classes, the mineral-associated, intramacroaggregate POM, and free and released POM C fractions within each size separate also exhibited no significant differences among size classes. Because ANOVA interactions were not significant, the data for all size classes were pooled by plot (Table 3). After the contaminating free and released POM was removed, the concentration of sand-free, macroaggregate-associated C tended to increase with time since cultivation, but this trend was not significant. After 10 gs in restored prairie, macroaggregate-associated C concentrations were still only 45% of that in virgin prairie. Intramacroaggregate POM C in the virgin prairie was more than twice that found in the other plots. However, intramacroaggregate POM C was greater in the cornfield than in the 10-gs restored prairie. As a percentage of macroaggregate-associated C, intramacroaggregate POM C was relatively low and fairly consistent in all plots, ranging from 3.7% in the 10-gs restored prairie to 6.0% in the cornfield. Consequently, the concentrations of sand-free, mineral-associated organic C in macroaggregates also tended to increase with time since cultivation, from 36% of virgin prairie concentrations in the cornfield to 46% in the 10-gs prairie.

The total amount of intramacroaggregate POM C for a given volume of soil is dependent on both the amount of soil present as macroaggregates and the concentration of POM C g^{-1} macroaggregates. Although concentrations varied somewhat within plots of different ages, variations in the total amount of intramacroaggregate POM C appeared to be a function of the amount of macroaggregates in the soil, with the cultivated soils conforming to one function and virgin prairie soils to another (Fig. 3), mostly because of differences in bulk density. Similar functions were found to describe the amounts of mineral-associated, macroaggregate-associated, and total organic C present in size separates (Table 4).

Hence, the amounts of both POM C and mineral-associated C in macroaggregates increased with time since cultivation (Table 5). However, most of the C accumulated in restored prairie soils was in the mineral-associated fraction of macroaggregates. Although the amount of macroaggregates in the 10-gs restored prairie increased by a factor of 2.18 over the amount of macroaggregates in the cornfield, the amount of mineral-associated C in those macroaggregates increased by a factor of 2.83. In contrast, the amount of intramacroaggregate POM C increased by a factor of only 1.79. The proportion of POM C found inside macroaggregates was relatively constant in the cornfield and restored prairie (17–21%) and somewhat greater in the virgin prairie (29%).

DISCUSSION

Aggregate formation and organic matter accumulation

The higher rate constant for changes in macroaggregate percentage compared to that for the accumulation of whole-soil and macroaggregate-associated organic C (Fig. 1, Table 2) indicates, as suggested by Elliott and Coleman (1988), that macroaggregate formation occurs first. This phased relationship between macroaggregate formation and

Table 2. Changes in macroaggregate-associated organic C and total Kjeldahl N with time since last cultivation on the basis of the exponential model (Equation 2) ($n = 70$)

Variable	y^c	C_e (g kg^{-1})	k (y^{-1})	I^a (g kg^{-1} y^{-1})	$t_{1/2}{}^b$ (y)	r^2
Organic C	0.59	92.9	0.0068	0.63	102	0.97
Total Kjeldahl N	0.61	7.51	0.0047	0.035	147	0.97

[a] $I = C_e(k)$.
[b] $t_{1/2} = -\ln(0.5)/k$.
[c] $y = 1 - C_o/C_e$, where C_o was assumed to be 38.2 g C kg^{-1} and 3.01 g N kg^{-1}; see text.

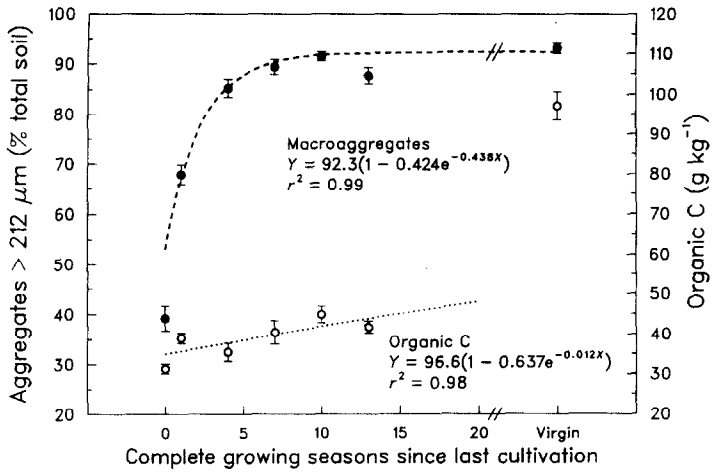

Fig. 1. Changes in percentage of macroaggregates and accumulation of whole-soil organic C with time since cultivation. Error bars indicate standard errors (n = 10).

C accrual also explains why macroaggregate development in our restored prairie system is more strongly related to the growth of roots and mycorrhizal fungal hyphae than to changes in organic C, total hydrolysable carbohydrates, or water-soluble C (Miller and Jastrow, 1990; J. D. Jastrow, unpub. data). Angers (1992) also reported that increases in soil organic C lagged behind more rapid increases in stable aggregate formation under alfalfa (*Medicago sativa* L.). However, because of the relatively short duration of the study (5 y), this conclusion was based on differences in the shapes of the curves used to describe changes in aggregation and organic C rather than on direct comparisons of rate constants.

The rate constant for macroaggregate formation was of similar magnitude to but higher than those reported by Kay (1990) for three other grassland or forage systems. The rapidity of macroaggregate formation in restored tallgrass prairie compared to other systems has been noted before (Jastrow, 1987) and is likely due to a combination of factors, including (1) the rapid, extensive proliferation of roots and mycorrhizal hyphae (Cook *et al.*, 1988; Miller and Jastrow, 1992a), (2) the types of plant species involved (Jastrow, 1987; Miller and Jastrow, 1990), (3) the presence of favorable quantities of clays and polyvalent cations (Emerson *et al.*, 1986; Muneer and Oades, 1989), (4) a microaggregate structure that is relatively resilient to the effects of cultivation (Jastrow *et al.*, in press; J. D. Jastrow, unpublished data), and (5) favorable climatic conditions.

The input rates (I) calculated for whole-soil and macroaggregate-associated organic C (0.133 and 0.072 kg m^{-2} y^{-1}, respectively, assuming a 10-cm depth and using the average bulk density of 1150 kg m^{-3} for previously cultivated soils in the chronosequence) were considerably higher than C input rates reported by Harden *et al.* (1992), except for Alaskan spodosols (0.055 kg m^{-2} y^{-1}). Their data, however, were based on long-term chronosequences (tens of thousands of years) of soils developed on freshly exposed mineral substrates such as glacial deposits, except for the Alaskan chronosequence, which encompassed only 1000 years. Furthermore, the input rates calculated on an areal (m^2) basis for the Fermilab site are probably underestimates because of the decrease in bulk density that occurs with C aggradation over time and because they only include the surface 10 cm.

Nevertheless, in comparison to estimates of decomposition rates and turnover of belowground biomass in tallgrass prairie, the calculated input rates for whole-soil C and macroaggregate-associated organic C were not unreasonable. From sequential harvest data for a 10-gs restored prairie at the Fermilab site, peak standing crops (10-cm depth) were 9.0 mg cm^{-3} for roots and 15.6 mg cm^{-3} for total belowground phytomass, with estimated annual turnovers of 0.35 and 0.38, respectively (D. R. Reinhardt, R. M. Miller and J. D. Jastrow, unpublished data). By assuming a first-order annual decomposition rate of -0.66 and a C content of 44% for tallgrass prairie roots (Seastedt *et al.*, 1992), the annual belowground input of C to a 10-cm depth (assuming that all decomposition is lost as respiration) can be estimated at 0.072 kg m^{-2} for roots and 0.135 kg m^{-2} for total belowground phytomass. These values are remarkably close to those calculated from the model. Furthermore, the model input rate for whole soil is similar to the rate measured for pasture at Fermilab by using stable C isotopes (0.122 kg m^{-2} y^{-1}, assuming a 10-cm depth and an average pasture bulk density of 1130 kg m^{-3}) (J. D. Jastrow *et al.*, in press).

Similar C input rates to soil organic matter (0.115 kg m^{-2} y^{-1}) were reported for tallgrass prairie in Missouri; however these rates were modeled for a 50-cm profile (Buyanovsky *et al.*, 1987). Thus, input rates to the top 10 cm were somewhat lower than the inputs to Fermilab soils, even though 90% of

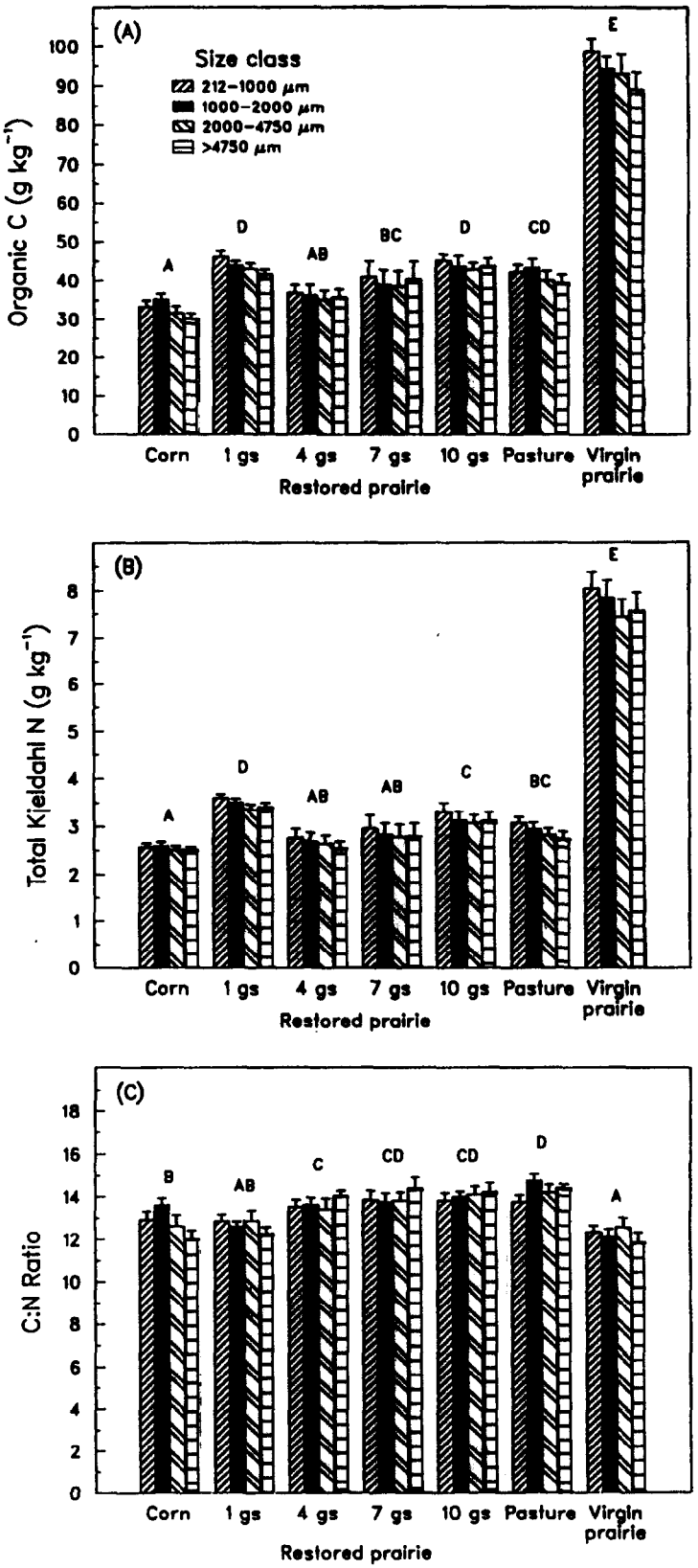

Fig. 2. Changes in (A) organic C, (B) total Kjeldahl N, and (C) C-to-N ratio of macroaggregate size separates with time since cultivation. Error bars indicate standard errors ($n = 10$). Sites indicated by the same letter have mean values for all macroaggregates > 212 μm dia ($n = 40$) that are not significantly different at $P \leq 0.05$ on the basis of Fisher's protected least significant difference (requires a significant F in the ANOVA).

Table 3. Changes with time in the mean concentrations (g kg^{-1}; with standard errors in parentheses) of sand-free organic C in different organic matter fractions of macroaggregate size separates (n = 9)

Organic matter fraction	Corn	Restored prairie		Virgin prairie
		4 gs[a]	10 gs	
Mineral-associated C	27.57A[b]	29.08A	34.58A	75.80B
	(0.77)	(2.22)	(2.36)	(4.22)
Intramacroaggregate POM-C	1.76B	1.37AB	1.32A	3.99C
	(0.13)	(0.14)	(0.10)	(0.24)
Macroaggregate-associated C	29.33A	30.45A	35.89A	79.79B
	(0.75)	(2.33)	(2.32)	(4.44)
Free and released POM-C in size separate	8.72BC	6.10A	7.23AB	11.25C
	(0.94)	(0.60)	(0.72)	(2.03)
Total C in size separate	38.05A	36.55A	43.12A	91.04B
	(1.15)	(2.66)	(2.75)	(5.23)

[a]gs = complete growing seasons since last cultivation.
[b]Means within each row followed by the same letter are not significantly different at $P \leq 0.10$ on the basis of Fisher's protected least significant difference (requires a significant F in the ANOVA).

belowground production at the Missouri site occurred in the A1 horizon (top 25 cm) and 60% of that was found in the top 5 cm. The reasons for the differences between these two prairie sites are probably related to lower primary production at the Missouri site and climate effects on decomposition rates.

The rate constant (k) and, consequently, the input rate (I) were both lower for organic C in macroaggregates than for whole-soil C, because the ratio of the concentration of C in macroaggregates to that in whole soil was slightly > 1 in the cornfield and the two youngest restored prairies but a little < 1 in all plots over 4 gs old. Thus, the value for k was lower for macroaggregates than for whole soil, because of a flatter slope to a lower equilibrium value. This phenomenon can be explained by the proportion of soil remaining as stable macroaggregates after slaking. There can be little difference between the C concentration of macroaggregates and that of the whole soil if most of the soil is tied up in macroaggregates that survive slaking and wet sieving (as in the older restored prairie, pasture, and virgin prairie). Only when a significant portion of the soil is not stabilized can macroaggregates be C-enriched relative to the whole soil or to microaggregates not incorporated into macroaggregates, as reported by others (e.g., Tisdall and Oades, 1980; Dormaar, 1983; Elliott, 1986; Gupta and Germida, 1988; Carter, 1992). This is particularly true for systems, like this one, that exhibit no significant differences in the C concentrations of different macroaggregate size classes. The lower C concentrations in macroaggregates compared to whole soil in the older plots probably resulted from the removal of more fine roots and organic debris via flotation during the course of wet sieving than could be accomplished in the preparation of whole soils for analysis.

Quality and form of accumulated organic matter

The increase in macroaggregate-associated C-to-N ratios with time since cultivation (Fig. 2C) suggests that the accumulating organic matter was "less highly processed", as proposed by Elliott (1986). Light-fraction materials usually consist of relatively recent detritus and consequently have elevated C-to-N ratios compared to the mineral (heavy) fraction (Spycher *et al.*, 1983; Sollins *et al.*, 1984). Although undisturbed soils at equilibrium for C accrual often contain more light-fraction POM than recently

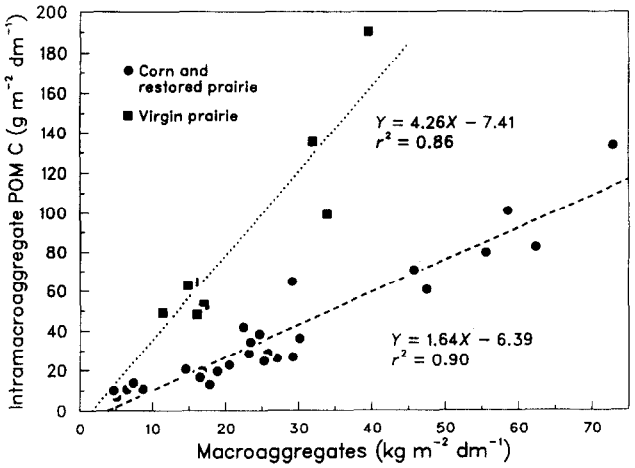

Fig. 3. Relationships between the amount of soil contained in macroaggregates and intramacroaggregate POM C on the basis of soil volume for corn and restored prairie (n = 27) and virgin prairie (n = 9).

Table 4. Relationships between the amount of macroaggregates (kg m^{-2} dm^{-1}) and the amounts (g m^{-2} dm^{-1}) of mineral-associated C, macroaggregate-associated C, and total organic C in macroaggregate size separates for corn and restored prairie ($n = 27$) and virgin prairie ($n = 9$)

Organic matter fraction	Corn and restored prairie		Virgin prairie	
	Model	r^2	Model	r^2
Mineral-associated C	$Y = 27.87X + 21.86$	0.87	$Y = 73.22X + 4.17$	0.87
Macroaggregate-associated C	$Y = 29.51X + 15.47$	0.88	$Y = 77.48X - 3.24$	0.87
Total C	$Y = 33.73X + 65.71$	0.87	$Y = 80.49X + 131.57$	0.87

cultivated soils (Whitehead *et al.*, 1975; Cambardella and Elliott, 1992), the C-to-N ratio in macroaggregates from the virgin prairie was the lowest of all soils studied. However, this pattern may not be unusual; Whitehead *et al.* (1975) reported lower C-to-N ratios in the light-fraction POM extracted from long-term pasture (several hundred years) than in material from arable soils or short-term pasture (c. 20 y) and suggested that the POM in long-term pasture had been subjected to greater microbial attack.

The concentrations of intramacroaggregate plus free and released POM C associated with macroaggregates from the Fermilab site (Table 3) were not predicted by the C-to-N ratios of the macroaggregate size separates before extraction of POM. In particular, the concentrations of POM C associated with macroaggregate size separates from the restored prairie were less than those for cornfield macroaggregates. Consequently, the observed pattern of macroaggregate C-to-N ratios may be more reflective of changes in the quality of mineral-associated organic matter at the Fermilab site. Mineral-associated organic C accounted for 72.5% of the total C concentration in macroaggregate size separates from the cornfield, about 80% in the restored prairie plots, and 83.3% in the virgin prairie (Table 3).

Thus, significant amounts of recent organic inputs apparently were associated with the mineral fraction of the soil. This association may occur if POM in this system is broken down relatively quickly by the activities of soil fauna and microbes into pieces < 53 μm that become physically associated with mineral matter (probably encrusted with clays). Because of the relatively low sonication energies used in this study, many smaller microaggregates (< 53 μm dia) with cores of POM that are strongly associated with soil minerals probably were not completely disrupted (Golchin *et al.*, 1994). Any mineral-associated POM remaining after sonication would be denser than the heavy liquid used to extract it (Golchin *et al.*, 1994). If true, this explanation coupled with the rapid formation of macroaggregates (Fig. 1) supports the hypothesized formation of microaggregates within macroaggregates as clay particles become reoriented around and encrust bits of organic debris (e.g. Oades, 1984; Elliott and Coleman, 1988; Oades and Waters, 1991). Indeed, related work using stable C isotopes on Fermilab soils demonstrated significant incorporation of recent organic C into both micro- and macroaggregates (Jastrow *et al.*, in press).

In addition, some of the accumulating mineral-associated organic matter in our soils may be similar to the enriched labile fraction isolated from macroaggregates of grassland soils in the Great Plains (Cambardella and Elliott, 1994). This fraction is believed to include significant quantities of fungal cell wall residues and to contribute to the binding and stabilization of microaggregates into macroaggregates. The potential existence of a significant pool of this type is supported by the relatively large amounts of mycorrhizal fungal hyphae found in this system and their correspondingly large contributions to aggregate formation (Miller and Jastrow, 1990; Miller *et al.*, 1995). A large pool of this type could also explain why the macroaggregates in this system are more stable than those from many other soils.

Table 5. Changes with time in the mean amounts (g m^{-2} dm^{-1}; with standard errors in parentheses) of sand-free organic C in macroaggregate- and nonmacroaggregate-associated organic matter fractions of the whole soil ($n = 3$)

Organic matter fraction	Corn	Restored prairie		Virgin prairie
		4 gs[a]	10 gs	
Mineral-associated macroaggregate C	1181A[b]	2548B	3348B	4692C
	(143)	(376)	(374)	(415)
Intramacroaggregate POM C	77A	131AB	138B	250C
	(21)	(15)	(18)	(29)
Macroaggregate-associated C	1258A	2679B	3485B	4942C
	(164)	(390)	(358)	(443)
Free and released POM C in macroaggregate size separates	341A	480AB	672C	597BC
	(67)	(35)	(98)	(37)
Total C in macroaggregates plus free POM	1599A	3159B	4157B	5539C
	(230)	(420)	(448)	(480)
Total C in size separates < 212 μm in diameter	1918A	837B	576B	567B
	(165)	(228)	(101)	(377)
Total C in whole soil	3517A	3996AB	4733B	6106C
	(68)	(622)	(389)	(185)

[a]gs = complete growing seasons since last cultivation.
[b]Means within each row followed by the same letter are not significantly different at $P \leq 0.10$ on the basis of Fisher's protected least significant difference (requires a significant F in the ANOVA).

Under restored tallgrass prairie, the concentration of POM C within macroaggregates (Table 3) did not increase over that found in cornfield soils, even 10 gs after cultivation ceased. However, the total amount of intramacroaggregate POM C (on the basis of whole soil volume) increased initially in restored prairie but remained constant from 4–10 gs after planting at slightly over 50% of the amounts present in virgin prairie (Table 5). This increase in intramacroaggregate POM was associated with increases in the percentage of soil tied up in macroaggregates. Although virgin soil macroaggregates had greater total amounts of intramacroaggregate POM C, this material represented a proportion of the total macroaggregate-associated C (5.1%) similar to that in the cornfield and the restored prairie (4.0–6.1%). When viewed as a proportion of total C in the whole soil, however, intramacroaggregate POM C was lowest in the cornfield (2.2%), increased slightly in the restored prairie (about 3%), and was greatest in virgin prairie (4.1%). Thus, the amount of intramacroaggregate POM C not only was related to the amount of macroaggregates, but also was a relatively constant fraction of the total organic C pool in the sampled soils.

The total of intramacroaggregate POM C plus free and released POM C in virgin prairie (Table 5) was generally 2–4 times greater than the amounts of POM C isolated from several Australian soils and a tallgrass prairie soil in Kansas (densities of 2.0 and 1.7 g cm^{-3}, respectively) (Dalal and Mayer, 1986b; Strickland and Sollins, 1987). In contrast, the amount of virgin prairie POM C was similar to the amount isolated from native sod in Nebraska (density = 1.85 g cm^{-3} as in this study), but the amounts of POM C in the cornfield and restored prairie were notably greater than the amounts observed for the Nebraska soil under various tillage treatments (Cambardella and Elliott, 1992; considering that they reported C contents to a 20-cm depth rather than the 10-cm depth used in this study).

The proportion of the whole-soil organic C found in intramacroaggregate POM C plus free and released POM C did increase with time since cultivation (ranging from 11.9% in the cornfield to 17.1% in the oldest restored prairie). However, these proportions were somewhat less than the 18–25% POM C observed in tilled Nebraska soils (Cambardella and Elliott, 1992), although some free and released POM associated with size separates < 212 μm was not recovered by the methods used in this study. From related studies (Jastrow *et al.*, in press), free and released POM probably accounts for about 11 to 15% of the total C in this size separate. Thus, total POM C was probably closer to 18 to 19% of the total amount of organic C in the cornfield and restored prairie. In contrast, intramacroaggregate POM C plus free and released POM C was only 13.9% of the total amount of organic C in the virgin prairie, whereas POM C composed 39% of the total organic C in the Nebraska native sod. Even if all of the C in the < 212 μm size fraction was POM C, POM C would account for only 23% of the total organic C in the virgin prairie. This difference between long-term undisturbed soils from these two sites was due primarily to a much greater accumulation of mineral-associated C under tallgrass prairie.

In conclusion, this study provides evidence to support the hypothesis that the formation of macroaggregates, often promoted by the growth of roots and fungal hyphae, facilitates the accrual of organic matter. Intramacroaggregate POM may be an important agent facilitating the binding of microaggregates into macroaggregates because it provides nucleating sites for the growth of fungal hyphae and for other microbial activities resulting in the deposition of extracellular polysaccharides (Tisdall and Oades, 1980; Miller and Jastrow, 1992b; Cambardella and Elliott, 1992, 1993). However, given the favorable moisture and temperature conditions of the tallgrass prairie region and parent material with adequate amounts of clay minerals and polyvalent cations, a significant proportion of organic matter inputs were found to become associated relatively rapidly with mineral matter. This process not only serves to physically protect the organic matter, facilitating its accrual, but also promotes the formation of very stable microaggregates (Oades, 1984; Emerson *et al.*, 1986; Tiessen and Stewart, 1988; Oades and Waters, 1991; Golchin *et al.*, 1994). The rate of association of organic inputs with the mineral fraction suggested by this study indicates that under favorable conditions the processes of microaggregate formation and degradation (as conceptually described by Golchin *et al.*, 1994) may be more dynamic than might be predicted by the relatively slow rate of microaggregate degradation due to cultivation (Tisdall and Oades, 1980, 1982).

Acknowledgements—I thank the National Environmental Research Park at Fermilab for allowing sampling within the park and the DuPage County Forest Preserve District for permitting sampling at the West Chicago Prairie. R. Cronn, M. Fink, and B. Fox helped with field sampling, and E. Copland, R. Cronn, T. Houchens, A. Singer, K. Spokas, and A. Tarrant provided technical assistance in the laboratory. This work was supported by the U.S. Department of Energy, Office of Energy Research, Office of Health and Environmental Research, Environmental Sciences Division, Global Change Research.

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