DIVISION S-3—SOIL BIOLOGY & BIOCHEMISTRY

Carbon Dynamics of Aggregate-Associated Organic Matter Estimated by Carbon-13 Natural Abundance

J. D. Jastrow,* T. W. Boutton, and R. M. Miller

ABSTRACT

A major factor controlling soil organic matter dynamics is believed to be the differing degrees of protection from decomposition afforded by the spatially hierarchical organization of soil aggregate structure. Changes in the natural ^{13}C content and in the concentration of soil organic C resulting from the growth of C3 pasture grasses (low $\delta^{13}C_{PDB}$) on former C4 cropland (high $\delta^{13}C_{PDB}$) were used to investigate the turnover and inputs of organic C in water-stable aggregates of different sizes. After removal of *free and released* particulate organic matter (POM) in aggregate size separates (POM with a density ≤ 1.85 g cm^{-3} that was either exterior to aggregates in situ or released from unstable aggregates by slaking), organic C concentrations were greater in macroaggregates (>212 μm) than in microaggregates (53–212 μm). The turnover time ($1/k$) for C4-derived C was 412 yr for microaggregates, compared with an average turnover of 140 yr for macroaggregates, indicating that old C associated with microaggregates may be both biochemically recalcitrant and physically protected. Net input rates of C3-derived C increased with aggregate size (0.73–1.13 g kg^{-1} yr^{-1}), supporting the concept of an aggregate hierarchy created by the binding of microaggregates into increasingly larger macroaggregates. The net input rate for microaggregates, however, was equal to the rates for small macroaggregates, suggesting that the formation and degradation of microaggregates may be more dynamic than is predicted by their stability in cultivated soils or by the observed turnover times for old C.

THE CONCEPT of an aggregate hierarchy in soils for which organic matter is the major binding agent (Tisdall and Oades, 1982; Oades and Waters, 1991) has recently stimulated mechanistic studies of aggregate formation, stabilization, and degradation (e.g., Elliott, 1986; Gupta and Germida, 1988; Gregorich et al., 1988, 1989; Miller and Jastrow, 1990; Cambardella and Elliott, 1993, 1994). In this hierarchical view, primary particles are cemented together to form microaggregates (up to 250 μm in diameter), which are bound together into macroaggregates. Increasingly larger macroaggregates (up to several millimeters in diameter) may, in turn, be formed by the binding together of small macroaggegates. The binding mechanisms involved at each hierarchical level are determined largely by a similar hierarchy of pore sizes and contact points, resulting from the spatial packing of different-sized aggregates (Dexter, 1988; Oades, 1993).

Organic binding agents have been classified into three broad categories on the basis of their temporal persistence (Tisdall and Oades, 1982): (i) transient (mainly polysaccharides), (ii) temporary (roots, fungal hyphae, bacterial cells, and algae), and (iii) persistent (aromatic humic materials associated with polyvalent metal cations and polymers strongly sorbed to clays). Microaggregates are believed to be stabilized mainly by persistent agents and perhaps some transient materials, whereas macroaggregates are bound together by transient and temporary agents (Tisdall and Oades, 1982).

These concepts lead to a number of hypotheses regarding the formation of soil aggregates and the subsequent accrual and turnover of soil organic matter. First, macroaggregates will contain more organic C than microaggregates because the former are composed of microaggregates plus the organic materials that bind microaggregates together (Tisdall and Oades, 1982). Second, the relative stabilities of micro- and macroaggregates are a function of several factors, including (i) the strength of the physicochemical attractions between the organic and mineral components; (ii) the lability of the binding agents; and (iii) the size and location of organic binding agents within the hierarchical packing, which influence their accessibility to attack by various soil organisms (Tisdall and Oades, 1982; Oades, 1984; Elliott and Coleman, 1988; Kay, 1990; Oades and Waters, 1991). Consequently, the turnover rates for organic matter serving as binding agents for micro- and macroaggregates are expected to differ, with slower turnovers occurring for microaggregate-associated organic matter (Elliott and Coleman, 1988). Hence, in soils with a stable microaggregate structure, most C accrual and intermediate-term (~ 50 yr) turnover are presumed to be a function of the protection provided by the formation and stabilization of macroaggregates (Elliott and Coleman, 1988).

Several studies have demonstrated greater concentrations of organic C in macroaggregates than in microaggregates (Tisdall and Oades, 1980; Dormaar, 1983; Elliott, 1986; Gupta and Germida, 1988; Cambardella and Elliott, 1993) and greater mineralization of intramacroaggregate organic matter compared with mineralization of organic matter associated with microaggregates (Elliott, 1986; Gupta and Germida, 1988; Beare et al., 1994a). In contrast, others have reported higher concentrations of organic C (Beare et al., 1994b) or greater mineralizable C

J.D. Jastrow and R.M. Miller, Environmental Res. Div., Argonne Natl. Lab., Argonne, IL 60439; and T.W. Boutton, Dep. of Rangeland Ecology and Management, Texas A&M Univ., College Station, TX 77843-2126.
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Abbreviations: POM, particulate organic matter; PDB, Pee Dee belemnite reference standard.

(Seech and Beauchamp, 1988) in microaggregates than in macroaggregates.

Quantitative information on the relative turnover rates of organic matter in micro- and macroaggregates, however, is sparse. Buyanovsky et al. (1994) followed the short-term (4 yr) decomposition of a pulse of ^{14}C-labeled soybean [*Glycine max* (L.) Merr.] residues in micro- and macroaggregates. Initial turnover times for this residue in cultivated soil were faster in macroaggregates (1–3 yr) than in microaggregates (7 yr). After the first 0.5 yr, however, residue degradation slowed in smaller macroaggregates (turnover time ≈ 10 yr) but remained constant in microaggregates.

Recent studies have demonstrated the usefulness of ^{13}C natural abundance for estimating longer term turnover and C dynamics in soils where the photosynthetic pathway of the original vegetation has changed (Balesdent et al., 1987, 1988; Martin et al., 1990; Skjemstad et al., 1990). Plants with the C3 pathway discriminate against $^{13}CO_2$ during photosynthesis, causing the $^{13}C/^{12}C$ ratios of their phytomass to be depleted in ^{13}C relative to those of C4 plants (Smith and Epstein, 1971). The isotopic composition of soil organic C reflects the plant material from which it is derived, with relatively minor isotopic fractionation as it undergoes decomposition (Dzurec et al., 1985; Balesdent et al., 1987, 1988; Martin et al., 1990). Hence, the introduction of vegetation with a different photosynthetic pathway provides an in situ label enabling quantification of both the loss rate of the original organic matter and the net input rate from the new source.

To date, most studies (e.g., Balesdent et al., 1987, 1988; Martin et al., 1990; Bonde et al., 1992) have used this technique to examine C dynamics on whole soils and/or particle-size fractions. Skjemstad et al. (1990), however, compared turnover times for micro- and macroaggregates from an Australian soil developed under subtropical rain forest. Although microaggregates in surface soils had slower turnover times (75 yr) than macroaggregates (60 yr), the two types of aggregates were not remarkably different.

Soil organic C in mollisols developed under tallgrass prairie in North America have a predominantly C4 signature that has often been enhanced by the long-term cultivation of corn (*Zea mays* L.). These soils also have a relatively stable, well-developed microaggregate structure due, in part, to their formation under productive grasses. Conversion of these temperate mollisols from C4 cropland to C3 pasture grasses provides an excellent opportunity to utilize the ^{13}C natural abundance method to test hypotheses based on the concept of aggregate hierarchy. Specifically, we tested the hypotheses that (i) macroaggregates contain higher amounts of organic C than microaggregates, (ii) microaggregate-associated organic C turns over at a slower rate than macroaggregate-associated organic C, and (iii) more C is accumulated in macroaggregates than microaggregates when long-term cropland is converted to perennial vegetation.

MATERIALS AND METHODS

Study Sites and Sample Collection

The study sites were a corn field and an ungrazed pasture dominated by C3 Eurasian grasses. Both sites were located within the Fermilab National Environmental Research Park, Batavia, IL, 48 km west of Chicago on Mundelein silt loam (fine-silty, mixed, mesic Aquic Argiudoll), which developed under tallgrass prairie dominated by C4 grasses. The area was first cultivated during the mid-1800s and has been cropped with corn for most of that period. Both sites have 0 to 2% slopes and are underlaid by drainage tiles. The ungrazed pasture was planted with the C3 grasses, smooth brome (*Bromus inermis* Leyss.) and Kentucky bluegrass (*Poa pratensis* L.), in the fall of 1971 and also supported a large population of the volunteer C3 grass, quackgrass [*Agropyron repens* (L.) P. Beauv.]. When samples were collected in late June 1989, the pasture was beginning its 18th growing season. During the same period, the corn field was continuously in corn.

Within each site, 10 sample stations consisting of a 0.25-m^2 circular quadrat were located by using a stratified random design. After removing the aboveground standing crop from inside each quadrat, a soil core (48-mm diameter) was taken to a depth of 10 cm from the center of each quadrat. Each core was transferred intact to a polyethylene bag and frozen until analysis. Three of the 10 cores from each site were randomly selected for use in this study.

Physical Separations and Carbon Analyses

Size fractions of water-stable aggregates were collected by slaking of air-dry soil followed by wet sieving. The method was modified from Kemper and Chepil (1965). After thawing overnight in a refrigerator, each core was cut into three sections of approximately equal lengths. Each section was gently broken apart along its natural breaking points to pass a 9.5-mm sieve. Roots entering larger aggregates were cut off at the surface, and larger roots, rhizomes, and pieces of organic debris were removed. After air drying for 3 to 5 d, subsamples were taken from each section, pooled, and oven dried at 105°C for moisture corrections. For samples from the pasture, each section (50–70 g) was distributed evenly across the top sieve in a stack of sieves with hole widths of 4750, 2000, 1000, 500, and 212 μm, immersed for 10 min at atmospheric pressure in a column of deionized water, and oscillated for 10 min (30 up-down cycles min^{-1}). After wet sieving, the water columns were drained through a 53-μm sieve to collect microaggregates. The same procedure was used for samples from the corn field, except that each core section was divided in half and sieved separately to prevent clogging of the smaller sieves. Aggregates were dried at 70°C, pooled by size class for each core, and weighed. Subsamples from each size fraction were dried at 105°C for moisture corrections.

Subsamples of the size fractions and of whole soil (before wet sieving) were ground in a mortar and pestle to pass a 250-μm sieve. Any roots or debris >1 mm in length were removed. Total C was determined by dry combustion at 800°C in a boat sampler attached to a Dohrmann DC-180 Infrared C Analyzer (Tekmar-Dohrmann, Cincinnati, OH). Because carbonates were not present, total C was equivalent to organic C.

Substantial amounts of free and released POM were present in most size fractions. This material was exterior to aggregates and consisted of a mixture of two functionally different pools (i.e., free in situ and released by slaking). To evaluate aggregate-associated C, we removed free and released POM from each size fraction by density flotation (except the >4750-μm

fraction because it could be readily sampled by separating individual intact aggregates from any contaminating POM).

After drying overnight at 105°C, 1.25-g subsamples of unground aggregate size separates in all size classes (except >4750 μm) 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 J kg^{-1} to remove air entrapped in aggregate pore spaces and 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 with deionized water, backwashed into pyrex pans, and dried at 70°C. The soil was removed from the pans, dried at 105°C, weighed, ground to pass a 250-μm sieve, and analyzed for aggregate-associated C by using the Dohrmann C Analyzer.

Some soluble organic C accumulated in the sodium polytungstate; therefore, fresh heavy liquid was used for each flotation to prevent potential cross-contamination of samples to be used in stable isotope analysis. Because sodium polytungstate could not be removed completely from the soil, aggregate-associated C was expressed as the total amount of C in the >1.85 g cm^{-3} fraction per gram of original sample before removal of free and released POM (sensu Cambardella and Elliott, 1993).

Stable Isotope Analysis and Calculations

Natural abundance stable isotope ratios were measured for total and aggregate-associated C in each aggregate size fraction (i.e., before and after removal of free and released POM). Ground soil (<250 μm in diameter) was combusted with CuO in sealed, evacuated quartz tubes at 900°C according to the procedures outlined by Boutton (1991). The purified CO_2 was analyzed with a VG 903 dual inlet, triple collector isotope ratio mass spectrometer (VG Isogas Ltd., Middlewich, UK). Results were expressed in standard $\delta^{13}C_{PDB}$ notation and reported relative to the international PDB standard (Boutton, 1991). The precision (±1 SD) for multiple analyses of a single sample was 0.2‰.

Because C3 plants were grown on soils that previously supported C4 vegetation, the $\delta^{13}C_{PDB}$ signature of the pasture soils at the time of sampling reflected the combined inputs of organic matter from both C4 and C3 vegetation. Thus, the $\delta^{13}C_{PDB}$ of each isolated fraction from the pasture soil was partitioned as

$$\delta_S \approx f\,\delta_O + (1 - f)\delta_I$$

where δ_S is the $\delta^{13}C_{PDB}$ of a given fraction isolated from pasture soil, δ_O is the $\delta^{13}C_{PDB}$ of that fraction before the switch from C4 to C3 vegetation, δ_I is the $\delta^{13}C_{PDB}$ of organic inputs from C3 grasses, f is the proportion of fraction organic C derived from C4 vegetation, and $(1 - f)$ is the proportion derived from C3 grasses (Wolf et al., 1994). The value of δ_I was estimated by the average $\delta^{13}C_{PDB}$ value measured for roots from the pasture (-26.3‰). Values for δ_O were estimated by the $\delta^{13}C_{PDB}$ values of isolated fractions from the corn field. This assumption was possible because both sites were cultivated for >100 yr before the pasture was planted and, thus, were probably at equilibrium for C inputs and loss at the time of planting (Lee et al., 1993).

Because particles that passed through the 53-μm sieve were not collected, their C content was estimated from the other size fractions by mass balance. The calculations (and necessary assumptions) are documented in footnotes to the tables. Estimates of C associated with particles <53 μm were required to compute C turnover and inputs for the whole soil.

RESULTS AND DISCUSSION

Carbon Concentrations and Aggregate Size Distributions

Total organic C in the whole soil increased from 27.4 g kg^{-1} in the corn field to 41.5 g kg^{-1} in the 17-yr pasture. The C concentrations of each size fraction from each site (Table 1) were significantly reduced (paired *t* tests; $P \leq 0.10$) by the removal of contaminating free and released POM. The concentrations of aggregate-associated organic C in all but two pasture size fractions (212–500 μm and >4750 μm) were significantly higher (two-tailed *t* tests; $P \leq 0.10$) than in corresponding fractions from the corn field. For both sites, microaggregates had lower C contents that macroaggregate size fractions, as reported by others (e.g., Tisdall and Oades, 1980; Dormaar, 1983; Elliott, 1986). Furthermore, in the pasture, the concentration of aggregate-associated organic C steadily increased as aggregate size increased. These trends were consistent with the hypothesis that microaggregates are bound together by various organic agents to form macroaggregates (Tisdall and Oades, 1982).

The quantity of macroaggregates resistant to slaking increased substantially under long-term pasture grasses (Table 2). In the corn field, most of the soil slaked into microaggregates (53–212 μm) or macroaggregates in the 212- to 500-μm size fraction, but 75% of the pasture soil remained in stable macroaggregates >1000 μm in diameter. The amounts of C associated with each aggregate size fraction (grams per kilogram whole soil) were highly correlated with the amounts of soil in each size fraction ($r = 0.94$ for total C and 0.93 for aggregate-associated C; $P \leq 0.0001$). Thus, for the pasture soil as a whole, more C was present in larger macroaggregates (Table 2) primarily because of the increase in the amount of water-stable macroaggregates under perennial grasses.

Stable Isotope Ratios

The $\delta^{13}C_{PDB}$ values for all aggregate size classes from the corn field (Fig. 1) were greater than -18‰, indicating that they were enriched in ^{13}C relative to native prairie soils in the area (avg. ≈ -19‰; R.M. Miller and J.D. Jastrow, 1987, unpublished data). Balesdent et al. (1988) reported a comparable $\delta^{13}C_{PDB}$ of -18.6‰ for virgin prairie soil in Missouri, where vegetative

Table 1. Mean total C concentrations (standard error in parentheses; $n = 3$) in aggregate size fractions of soils from the corn field and pasture before and after removal of free and released particulate organic matter (FRPOM).

	Corn field		Pasture	
Size fraction	+ FRPOM	− FRPOM	+ FRPOM	− FRPOM
μm	g kg^{-1} fraction			
53–212	24.8 (4.3)	22.1 (3.8)	39.2 (4.1)	33.7 (2.2)
212–500	29.4 (5.1)	24.8 (3.9)	42.3 (3.8)	34.6 (3.6)
500–1000	32.4 (4.4)	24.8 (3.4)	41.1 (3.8)	35.6 (3.0)
1000–2000	31.6 (4.5)	25.0 (2.4)	41.3 (3.7)	36.3 (2.3)
2000–4750	29.9 (4.5)	23.6 (3.5)	38.8 (3.0)	36.9 (2.9)
>4750	−†	27.7 (6.0)	–	41.2 (4.5)

† Analyzed samples from the >4750-μm size fraction had no FRPOM because individual aggregates were subsampled for analysis.

Table 2. Mean size distribution of aggregates (standard error in parentheses; $n = 3$) and total amounts of organic C associated with each aggregate size fraction in corn field and pasture soils.

Size fraction	Distribution of soil in size fractions	Total fraction-associated C	
		+ FRPOM†	− FRPOM
μm	%	g kg^{-1} whole soil	
	Corn field		
<53	8.9 (0.7)	2.6‡	2.6§
53–212	48.6 (2.5)	12.0	10.7
212–500	16.7 (1.5)	4.9	4.1
500–1000	7.9 (1.0)	2.6	2.0
1000–2000	4.5 (0.4)	1.4	1.1
2000–4750	5.7 (0.2)	1.7	1.3
>4750	7.8 (0.7)	2.2	2.2
Whole soil	100.0	27.4	24.1¶
	Pasture		
<53	2.2 (0.2)	2.2‡	2.2§
53–212	4.7 (0.3)	1.8	1.6
212–500	6.8 (0.1)	2.9	2.4
500–1000	11.2 (0.4)	4.6	4.0
1000–2000	23.4 (1.0)	9.6	8.5
2000–4750	39.3 (0.8)	15.2	14.5
>4750	12.4 (1.6)	5.1	5.1
Whole soil	100.0	41.5	38.2¶

† FRPOM = free and released particulate organic matter.
‡ Calculated from amount in whole soil minus the sum of the other size fractions.
§ Assumed to equal the value for + FRPOM because POM separated in this study was ≥ 53 μm in diameter.
¶ Calculated as the sum of all size fractions.

composition was ≈ 75% C4 grasses and 25% C3 species. Thus, the higher values for the corn field reflect the inputs of 100% C4 vegetation under continuous corn. As expected, pasture aggregates had significantly lower $\delta^{13}C_{PDB}$ values (two-tailed t tests; $P \leq 0.01$) relative to both the corn field and virgin prairie after 17 yr of C3 inputs.

For both sites, aggregate-associated C was consistently enriched in ^{13}C compared with the total C in each size fraction (Fig. 1), although the differences generally were not statistically significant (paired t tests; $P > 0.10$). Thus, free and released POM may be slightly depleted in ^{13}C relative to aggregate-associated organic matter. In the pasture, some depletion was expected because of inputs from C3 vegetation. In the corn field, however, it probably indicates that much of the readily decomposable components of the POM have been lost, leaving behind components depleted in ^{13}C, such as lignin (Benner et al., 1987). In native grassland and cultivated soils from western Nebraska, POM had an average lignin content of 47% and a lignocellulose index (Melillo et al., 1989) of 0.7, indicating that it was in the latter stages of decay and characterized by very low rates of decomposition (Cambardella and Elliott, 1992).

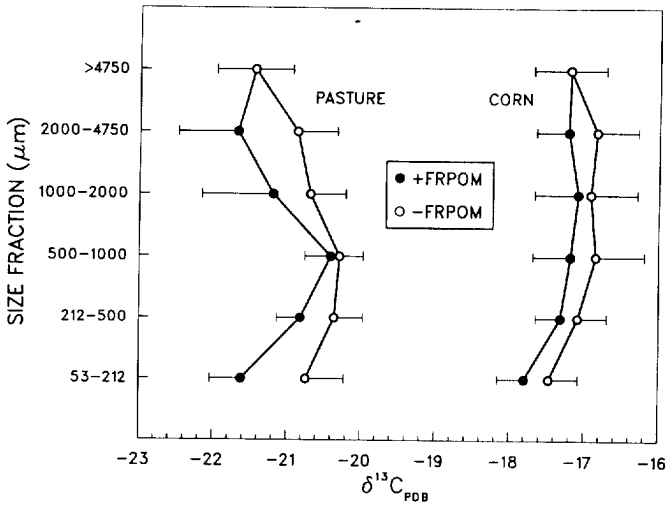

Fig. 1. Mean $\delta^{13}C_{PDB}$ values (error bars indicate standard error; $n = 3$) for aggregate size fractions of soils from the corn field and pasture before and after removal of free and released particulate organic matter (FRPOM). Analyzed samples from the >4750-μm size fraction had no FRPOM because individual aggregates were subsampled for analysis.

In the corn field, macroaggregates appeared to be slightly enriched in ^{13}C compared with microaggregates (Fig. 1), suggesting that recent inputs of C4-derived organic matter were greater in macroaggregates, particularly for those >500 μm in diameter. Trends for the pasture were not as straightforward, even after the removal of free and released POM. Inputs of C_3-derived material were apparently greater in microaggregates and the larger macroaggregates than in smaller macroaggregates (212–500 and 500–1000 μm size fractions). One explanation may be that microbial activity is relatively greater in these size fractions. Microbial biomass and the residual substrate remaining in soil after microbial degradation are usually enriched in ^{13}C relative to the substrate's initial composition (Mary et al., 1992). Also, more microbial biomass, especially fungal biomass, was found in similarly sized small macroaggregates than in microaggregates or larger macroaggregates for both native and cultivated mollisols in western Canada (Gupta and Germida, 1988).

Turnover and Net Inputs of Organic Carbon

The relative proportions of C4-derived C in all size fractions (±free and released POM) from the pasture were >0.5 and ranged as high as 0.65 (Table 3). In general, the proportions of C4-C were higher for aggregate-associated C than for total C in each size fraction. However, the total concentrations of C4-C were higher than aggregate-associated concentrations in all size fractions <2000 μm in diameter. This suggests that a substantial portion of the free and released POM was of C4 origin, particularly in the 212- to 500-μm (46%) and 500- to 1000-μm (71%) fractions. Much of this material may have been located inside larger but unstable aggregates and released when these aggregates were disrupted by slaking. After 20 yr of cultivation on grassland soils in Nebraska, significant amounts of POM derived from native grasses remained in the soil, especially under no-till management (Cambardella and Elliott, 1992).

Although the rate of loss of C4-C does not strictly follow first-order kinetics, Skjemstad et al. (1990) used the assumption of exponential decay as a means for comparing relative decay rates and turnover times in micro- and macroaggregates. First-order rate constants (k) were estimated as

$$k = -\ln(C4_P/C4_O)/t$$

Table 3. Average proportions and concentrations of C4-derived organic C in aggregate size fractions and C4-C allocation among size fractions in whole soil for pasture as determined by ^{13}C natural abundance.

Size fraction	Proportion of C4-C in size fraction		C4-C in size fraction		Fraction-associated C4-C in whole soil	
	+ FRPOM†	− FRPOM	+ FRPOM	− FRPOM	+ FRPOM	− FRPOM
μm			g kg^{-1} fraction		g kg^{-1} whole soil	
<53	−‡	−	−	−	1.6§	1.6§
53–212	0.551	0.630	21.6	21.2	1.0	1.0
212–500	0.610	0.645	25.8	22.3	1.8	1.5
500–1000	0.647	0.636	26.6	22.7	3.0	2.5
1000–2000	0.554	0.597	22.9	21.7	5.3	5.1
2000–4750	0.509	0.574	19.7	21.2	7.7	8.3
>4750	0.533	0.533	22.0	22.0	2.7	2.7
Whole soil	0.559¶	0.597¶	23.2#	22.8#	23.2††	22.8††

† FRPOM = free and released particulate organic matter.
‡ dash = not determined.
§ Value for <53-μm size fraction estimated from total fraction-associated C for this size fraction (Table 2) times an assumed value of 0.75 for the proportion of C4-C in this size fraction [based on data for similarly sized particles in Balesdent et al. (1988)]. Computation of this value was necessary to estimate the total amount of C4-C in whole soil by summation of data for all size fractions.
¶ Value for whole soil calculated as the total amount of C4-C per kilogram of whole soil divided by the total amount of organic C per kilogram of whole soil in the pasture (Table 2).
# Value for whole soil equivalent to the value obtained by summation of fraction-associated C4-C in whole soil.
†† Calculated as the sum of all size fractions.

where $C4_P$ is the concentration of C4-C remaining in each size fraction from the pasture (Table 3), $C4_O$ is the concentration of C4-C in each size fraction before the switch to pasture grasses as represented by data for the corn field (Table 1), and t is the length of time in pasture (17 yr).

The rate constant for loss of aggregate-associated C4-C was much less for microaggregates than for macroaggregates (Table 4). Consequently, the turnover time ($1/k$) of aggregate-associated C4-C in microaggregates was 412 yr compared with an average of 157 yr for macroaggregates 212 to 4750 μm in diameter and 74 yr for aggregates >4750 μm in diameter. These turnover times for a temperate Mollisol were much slower than those reported by Skjemstad et al. (1990) for both micro- (75 yr) and macroaggregates (60 yr) from Australian soils supporting long-term pasture grasses after clearing of native subtropical rain forest. Since they also used heavy liquids to remove POM and charcoal confounding the $\delta^{13}C_{PDB}$ signatures of their slaked aggregate fractions, the faster turnover times probably were related largely to higher temperatures and precipitation. However, site differences in edaphic factors such as clay mineralogy and base status also could affect the turnover of persistent organo-mineral complexes (Emerson et al., 1986).

The turnover time reported for the whole soil (Table 4) was also much slower than the 83-yr turnover time calculated from changes in the total organic C contents of soils from a chronosequence of restored prairie, also located at the Fermilab site (Jastrow, 1996). However, the C analyses of chronosequence soils included free and released POM, which precludes direct comparison to the rates in Table 4. Calculations including free and released POM for the pasture soil yielded a whole-soil turnover time (102 yr) similar to that obtained from the chronosequence.

The measured turnover times for micro- and macroaggregates (Table 4) provide substantial evidence to support the concept of aggregate hierarchy (Oades and Waters, 1991) and the proposed differences in the persistence of organic materials involved in the binding of micro- vs. macroaggregates (Tisdall and Oades, 1982; Oades, 1984). However, if macroaggregates are composed of microaggregates, then calculated turnover times for macroaggregates are actually a function of the turnover of microaggregate-associated C and of the organic materials binding microaggregates into macroaggregates. Consequently, the macroaggregate turnover times in Table 4 overestimate the turnover times for transient and temporary organic materials (Tisdall and Oades, 1982) binding microaggregates together into macroaggregates. These binding agents (by themselves) probably have turnover times more like the 1- to 10-yr estimates of Buyanovsky et al. (1994) for ^{14}C-labeled soybean residues in macroaggregates.

The net input rate for whole soil from the pasture was similar to that obtained for whole soil in the Fermilab chronosequence study (1.16 g kg^{-1} yr^{-1}; Jastrow, 1996) especially if free and released POM was included (1.08 g kg^{-1} yr^{-1}). Furthermore, net input rates of C3-C to water-stable aggregates increased with aggregate size, particularly for macroaggregates >1000 μm in diameter (Table 4). This finding supports the hypothesis that larger aggregates are bound together initially by the growth of roots and mycorrhizal hyphae [sensu the *sticky string*

Table 4. Average rate constant (k) for loss and turnover time ($1/k$) of C4-C and average net input rate of C3-C in aggregate size fractions of pasture soil as determined by ^{13}C natural abundance after removal of free and released particulate organic matter.

Size fraction	k	$1/k$	Net input rate†
μm	yr^{-1}	yr	g kg^{-1} fraction yr^{-1}
53–212	0.0024	412	0.73
212–500	0.0062	162	0.72
500–1000	0.0054	186	0.76
1000–2000	0.0083	120	0.86
2000–4750	0.0063	159	0.92
>4750	0.0136	74	1.13
Whole soil	0.0032	314	0.91

† The concentration of C3-C in each size fraction divided by 17 yr in pasture.

bag concept of Oades and Waters (1991)]. As these roots and hyphae senesce and begin comminution and decomposition, they probably will be incorporated into the intraaggregate POM of larger aggregates first.

The net input rate for microaggregates, however, was equivalent to rates for small macroaggregates, which is inconsistent with the concept of extremely stable microaggregates that, once formed, are turned over very slowly (Tisdall and Oades, 1980, 1982). One possible explanation is that most of the C3-C associated with microaggregates is located on aggregate surfaces. In soils with very stable macroaggregates, such as those in the pasture, the few microaggregates released by slaking were probably bound into macroaggregates in situ but were not stabilized sufficiently to survive the slaking process. Hence, they could be coated with intermicroaggregate binding agents of relatively new origin. This scenario is consistent with observations by Cambardella and Elliott (1994) of a labile fraction within macroaggregates, which they hypothesized is of microbial origin and functions as an intermicroaggregate binding agent. A similar pool of recent labile material could contribute to macroaggregate stability at the Fermilab site and might adhere in significant quantities to the surfaces of microaggregates released from slaked macroaggregates.

Alternatively, microaggregates are more dynamic than predictions based on old C turnover rates (Table 4) or on their long-term stability against physical disruption in cultivated soils (Tisdall and Oades, 1980, 1982). The production of organic acids by roots and rhizosphere organisms can disperse clays, which may then become reoriented around and bound to freshly produced mucilages or bits of organic debris as a result of localized drying or compression by roots (Oades, 1984; Emerson et al., 1986). New microaggregates produced in this way could also be very stable against slaking and would serve to physically protect the newly incorporated organic material from microbial attack.

Many microaggregates >90 μm in diameter have cores of recognizable plant debris that are physically protected from rapid decomposition by encrusting clay particles (Oades and Waters, 1991). Furthermore, readily mineralizable organic material has been isolated from within microaggregates (Gregorich et al., 1989; Cambardella and Elliott, 1994), indicating that not all C associated with microaggregates is biochemically recalcitrant with very slow turnover times, as originally suggested by Tisdall and Oades (1982).

On the basis of studies using ultrasonic dispersion, density fractionation, and spectrochemical techniques, Golchin et al. (1994) proposed that the most stable microaggregates have cores of POM that are relatively new and chemically attractive to microorganisms. Although this POM is physically protected from rapid decomposition by encrusting mineral particles, decomposition does proceed, and microbially produced mucilages and metabolites permeate the mineral coatings, thereby enhancing aggregate stability. Eventually, the more labile constituents of an organic core are consumed, production of microbial metabolites declines, the aggregate becomes less stable, and the relatively resistant remains of the POM core are no longer as strongly associated with mineral particles.

Our findings of substantial inputs of new C3-C coupled with a relatively slow turnover time for old C4-C in microaggregates support this model and suggest that microaggregate turnover may be relatively dynamic. For soils in situ, microaggregates weakened by reduced microbial activity may be more susceptible to degradation via clay dispersion and reorientation. Mineral particles freed in this manner may then be sorbed to the mucilages and metabolites on the surfaces of new (in our case, C3-derived) labile POM supporting an active microbial population. Recent studies by Buyanovsky et al. (1994) demonstrated rapid incorporation of ^{14}C-labeled soybean residues into microaggregates and estimated turnover times of ≈ 7 yr for this material. At the same time, significant quantities of C4-derived microbial metabolites and organo-mineral complexes probably remain strongly associated with mineral particles encrusting new C3-derived microaggregate cores. Because of the types of bonding involved (Oades, 1984; Emerson et al., 1986), the turnover of mineral-associated organics is likely to be much slower than the degradation of either the microaggregates themselves or their POM cores. In fact, Buyanovsky et al. (1994) observed that the organic C in silt and clay particles included both relatively labile material (turnover times of 7 to 10 yr) and more stabilized C (turnover times estimated by stable isotopes of ~ 400 yr for silt and ~ 1000 yr for clay).

CONCLUSIONS

Use of ^{13}C natural abundance as a long-term tracer following a change from C4 cropland to C3 pasture provided a powerful approach for quantifying net inputs and turnover of organic matter associated with micro- and macroaggregates and for testing the concept of aggregate hierarchy. Old (>17 yr) C in microaggregates turned over more slowly than the old C associated with macroaggregates, suggesting that microaggregate C may be both physically protected and biochemically more recalcitrant. Net inputs of new (≤ 17 yr) C increased with increasing aggregate size, confirming the existence of an aggregate hierarchy where microaggregates are bound together to form increasingly larger macroaggregates (Tisdall and Oades, 1982; Oades and Waters, 1991). Yet, net inputs of new C to microaggregates were equal to those for small macroaggregates, indicating that the formation and degradation of microaggregates may be more dynamic than has been predicted by their stability in cultivated soils (Tisdall and Oades, 1980, 1982) or by the observed turnover times for old C. Our natural abundance tracer data support the conceptual model of microaggregate turnover proposed by Golchin et al. (1994) by demonstrating substantial incorporation of new C into microaggregates. The concurrent slow turnover time for old C in microaggregates suggests that a significant portion of this older C is stabilized by intimate associations with mineral particles.

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