Fractionation and Long-Term Laboratory Incubation to Measure Soil Organic Matter Dynamics

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Soil organic matter (SOM) in agricultural soils comprises a significant part of the global terrestrial C pool. It has often been characterized by utilizing a combination of chemical dispersion of the soil followed by physical separation. We fractionated soil samples under continuous corn (*Zea mays* L.) rotations at four long-term sites in the Corn Belt to determine the concentration of C and N associated with soil fractions (light fraction [LF], particulate organic matter [POM], silt size, clay size, and Bradford reactive soil protein [BRSP]) and to identify the change in C concentration and $\delta^{13}C$ signal of each fraction using laboratory incubations. Light fractions comprised 3 to 5% of the soil organic carbon (SOC), with no significant difference between conventional tillage (CT) and no-till (NT) treatments. The POM fraction accounted for 5 to 11% of the SOC in the soils with >30% clay and 17 to 23% for the soils with <20% clay. The clay-size fraction contained the highest proportion of SOC. Measurement of ^{13}C during long-term incubation showed that the average mean residence time (MRT) of corn-derived C in the LF was 3.5 yr, whereas the POM fractions ranged from 6 to 12 yr. The ^{13}C changes during incubation show that both fractions consist of a mixture of active and resistant materials, with movement between fractions. The BRSP has long MRTs except in the NT Hoytville soil. Measurement of the dyna mics of these fractions provides a basis for C models to test the impacts of land use and management on C sequestration.

Abbreviations: BRSP, Bradford reactive soil protein; CT, conventional tillage; KBS, Kellogg Biological Station; LF, light fraction; MRT, mean residence time; NT, no-till; OM, organic matter; POM, particulate organic matter; SOC, soil organic carbon; SOM, soil organic matter.

Soil organic matter (SOM) in agricultural soils comprises a significant part of the global terrestrial C and N pools. Agriculture has caused significant reductions in SOM levels in most soils, contributing to increases in atmospheric CO_2 levels. Reductions in residue returns to agricultural fields have resulted in poor soil physical conditions, greater risk of soil erosion, poor water retention, and less cycling of nutrients through organic forms. Altered management practices (e.g., reduced tillage, decreased bare fallow, increased residue input, or conversion to native vegetation) can potentially mitigate the loss of SOM and increase soil C and N levels (Paustian et al., 1997; Flach et al., 1997).

Soil organic matter is composed of a continuum of materials ranging in age from days for plant residues and root exu-

dates to >1000 yr for the resistant humics (Campbell et al., 1967a, 1967b). Soil ages vary with parent material, landscape position, vegetation, and changes in SOM fractions due to agricultural management. Atmospheric CO_2 contains both a long-lived radioactive (^{14}C) and a stable (^{13}C) isotope. These are incorporated into plants by photosynthesis and into soils by microbial transformations and humification with little change in the isotopic signal (Boutton, 1996). The majority of soils within the Corn Belt of North America were formed in glacial deposits (till, outwash, or loess overlying glacial till) originating from the Late Wisconsinan glacial drift (ca. 14,500 yr BP). These soils developed under forest in the eastern Corn Belt and prairie vegetation in the west. The switch from C_3 forest vegetation to C_4 corn is reflected in the change in the $\delta^{13}C$ content of the soil (Balesdent et al., 1988; Gregorich et al., 1997; Collins et al., 2000). This provides a useful signal for ^{13}C studies in soils representing a range of soil types, parent materials, and climates.

Collins et al. (1999) calculated the turnover of C_3– and C_4–C residues remaining, in long-term experimental sites, with known C inputs and management history.

Extended laboratory incubations and curve fitting were used to determine the turnover kinetics (k_a, k_s) and pool sizes (C_a, C_s) of the active and slow C fractions and determine the relative contribution of varying crop residue inputs (C_3, C_4) to soil organic C (SOC) pools (Collins et al., 2000). They

reported that during 800 d of laboratory incubation, the quantity of SOC mineralized ranged from 10.3 to 15.4% of the total C. It was similar in both conventional tillage (CT) and no-till (NT) treatments for Wooster and Hoytville, OH, soils but was significantly different for soils at the Kellogg Biological Station (KBS). The SOC loss determined by direct measurement of total SOC after 800 d of incubation indicated a loss ranging from 14 to 21% in NT and 13 to 19% in CT. The SOC loss, determined using direct SOC measurement, was higher for NT than for CT treatment sites. The higher SOC loss from NT than CT treatments could be attributed to the initial higher SOC content. A relatively higher SOC loss was observed with direct total soil C determination than with the CO_2 evolution measurement. More of the SOC was lost during the first 440 d (8–16%) than between 440 and 800 d (2–9%) during incubation of these soils.

The SOC is not lost equally from all fractions of the SOM during incubation. This can cause shifts in the relative abundance of C in the different size and density fractions (Haile-Mariam et al., 2000). Numerous studies have fractionated SOM by utilizing a combination of chemical dispersion of the soil followed by physical separation. These fractions include a light fraction (LF) consisting of material with a density of <1.7 g cm^{-3}, a particulate organic matter fraction (POM) that is associated with sand-size particles and partially protected fractions within aggregates, and the SOM associated with silt- and clay-size particles (Stevenson and Elliott, 1989; Janzen et al., 1992; Beare et al., 1994; Six et al., 1998). Analysis of these fractions using ^{13}C nuclear magnetic resonance spectroscopy indicated that they are chemically different (Sohi et al., 2005). Decomposability of SOC associated with textural fractions decreases in the order of sand, clay, whole soil, and silt in some soils (Christensen, 1987). In others, the coarse clay fraction is the oldest (Campbell et al., 1967a, 1967b). Gregorich et al. (1995), studying eastern Canadian soils, indicated that mineralization of the LF-C (<1.7 g cm^{-3}) was more rapid (mean residence time [MRT] ~8 yr) than that associated with any other particle-size fraction. The organic C of the LF and sand-sized (POM) fractions are mineralized faster than that of the silt- and clay-size fractions (Tiessen and Stewart, 1983; Dalal and Mayer, 1986a, 1986b; Gregorich et al., 1995).

Wright and Upadhyaya (1996) described a hydrophobic, citric-soluble organic component of SOC that reacted with the general Bradford protein reagent. The name *glomalin* was used to describe this material that has been found to contain high concentrations of Fe and promote aggregate stability. Glomalin has been found to be co-extracted with other SOC components such as humic acid (Nichols and Wright, 2005; Schindler et al., 2007; Rosier et al., 2006). In this study, we refer to glomalin as Bradford reactive soil protein (BRSP). This BRSP was found to be highly correlated to total soil C and comprise from 3.8 to 7.8% of the soil C depending on land use type and soil depth (Rillig et al., 2003). Incubation has shown the BRSP content to be reduced by 25% during a 150-d incubation (Steinberg and Rillig, 2003) and by 50% in different soils during a 453-d incubation (Rillig et al., 2003)

This study, utilizing the ability of soil enzymes produced by the soil biota during extended laboratory incubation to fractionate the different pools, determined the changes of C and N content in SOM fractions. The availability of soils from 440 and 800 d of incubation from the study by Collins et al. (2000) made it possible to determine the distribution of SOC fractions as the level of whole SOC changed through time. We analyzed soil samples from CT and NT continuous corn rotations at three long-term sites in the Corn Belt and one N fertilizer experiment in the Corn Belt to: (i) determine the concentration of C associated with five soil fractions (LF, POM, silt, and clay, as well as citric acid soluble C that reacts with the Bradford protein assay (BRSP); (ii) identify the change in C concentration and $\delta^{13}C$ signal of each fraction during extended laboratory incubations; and (iii) calculate the MRT of those fractions that showed enough changes to produce different ^{13}C contents during incubation.

MATERIALS AND METHODS

Soils from four long-term agricultural experiment sites in the Corn Belt region of the United States were sampled in 1992 and 1993 (Table 1). The Lamberton, MN, and Wooster and Hoytville, OH, sites had been in continuous corn for at least 30 yr. Except for a soybean [*Glycine max* (L.) Merr.] planting in 1989, the KBS site was under continuous corn cultivation from 1986 until the soils were sampled. The history and description of the sites as well as methods of sampling were described in Collins et al. (1999, 2000). Briefly, long-term rotations (8–33 yr) of conventional tillage (moldboard plow) and conservation tillage (no-till) cropped continuously to corn were used in this study. Six cores (5.4 cm) to a depth of 1 m were collected using a truck-mounted hydraulic soil probe and composited for each replicate. Before compositing, each soil core was divided into four depth increments: 0 to 20, 20 to 25, 25 to 50, and 50 to 100 cm. Soil samples were sieved moist to pass a 2-mm screen and recognizable plant fragments were removed. Only soil from the 0- to 20-cm depth increment was analyzed in this study.

Total soil C content was determined with an NCS elemental analyzer (Carlo Erba NA1500 series II, Milan, Italy) and $\delta^{13}C$ was determined on a Europa mass spectrometer Model 2020 (PDZ Europa, Norwich, UK). Working standards for ^{13}C were sugarbeet (*Beta vulgaris* L. ssp. *vulgaris*) sucrose (–25.68‰ V-PDB) and sugarcane (*Saccharum officinarum* L.) sucrose (–10.45‰ V-PDB). The working standards were calibrated against international standards NBS-22 (–29.74‰ V-PDB) and ANU sucrose (IAEA-C-6,

Table 1. Geographical location, soil series, classification, parent material, particle size, and mean annual temperature by site.

Site	Geographic location	Soil series and texture	Classification	Parent material	Fraction (<2 mm)			MAT†
					Sand	Silt	Clay	
					——%——			°C
Wooster, OH	40°48′ N, 82°00′ W	Wooster silt loam	Typic Fragiudalfs	glacial till	22	59	19	9.1
Hoytville, OH	41°00′ N, 84°00′ W	Hoytville silty clay loam	Mollic Ochraqualfs	lacustrine	19	31	50	9.5
Kellogg Biological Stn., MI	42°18′ N, 85°30′ W	Kalamazoo loam	Typic Hapludalfs	glacial outwash	43	38	19	9.0
Lamberton, MN	44°14′ N, 95°18′ W	Normania loam	Typic Haplustolls	glacial till	33	36	31	6.2

† Mean annual air temperature.

–10.43‰ V-PDB). Isotopic ratios are expressed as $\delta^{13}C$ values, $\delta^{13}C$ (‰) = $(R_{sample}/R_{reference} - 1)1000$, where $R = {}^{13}C/{}^{12}C$ (Balesdent and Balabane, 1992).

The LF of the 0- to 20-cm depth was determined by adding 10 g of soil to a 100-mL beaker to which 40 mL of NaI solution (specific gravity ~1.70 g cm^{-3}) was added (Janzen et al., 1992). The floating material was ground (<150 μm) and analyzed for total C and $\delta^{13}C$. After the LF was removed, soil samples were washed three times with 50 mL of distilled water to remove the NaI, then dispersed in 30 mL of 5 g L^{-1} Na hexametaphosphate by shaking for 15 h on a reciprocal shaker (Cambardella and Elliott, 1992). Following dispersion, samples were passed through a 53-μm sieve and rinsed with water to remove silt and clay. The POM plus sand retained on the sieve was dried at 60°C overnight. The slurry passing through the sieve was fractionated to silt and clay by sedimentation and decantation. The sand plus POM, silt, and clay were ground to pass a 250-μm screen and analyzed for total C and $\delta^{13}C$.

Bradford reactive soil protein, also identified as glomalin, was extracted with 50 mmol L^{-1} citrate, pH 8.0, at 121°C for 1 h (Wright and Upadhyaya, 1996). Samples were centrifuged and the supernatant decanted and saved. The procedure was repeated until the supernatant was straw colored (usually three extractions or more). Supernatants from the sequential extraction were combined and frozen, and 1 mL subsequently used for the protein assay.

Fractionations of unincubated soils were compared with soil samples following the extended laboratory incubations (440 and 800 d) of Collins et al. (2000). Briefly, the extended laboratory incubations were conducted on duplicate 25-g, moist-sieved samples from the 0- to 20-cm depth increment of each field replicate, adjusted to 60% of water holding capacity and incubated in 160-mL bottles in the dark at 25°C.

Headspace CO_2 was measured using a Beckman Model 865 infrared gas analyzer (Beckman Instruments, Fullerton, CA) initially at 10-d intervals, then at approximately 21-d intervals after 100 d of incubation. Following CO_2 analysis, each sample was returned to ambient CO_2 by degassing with compressed air. After the long-term laboratory incubation, each soil sample was air dried and stored in air-tight containers until fractionated in the current study. We analyzed the proportion of C derived from corn residues for each SOC fractions using the equation: C derived from corn (%) = $(\delta - \delta_f)/(\delta_{corn} - \delta_f)100$, where δ = $\delta^{13}C$ values of the SOC fraction or whole soil from corn-cultivated soil, δ_f = $\delta^{13}C$ value of the native forest soil, and δ_{corn} = $\delta 12.0$‰. The $\delta^{13}C$ of corn stover was determined for triplicate samples collected across all sites. We estimated the turnover rates of the LF and POM by curve fitting the 0-, 440-, and 800-d LF and POM concentrations to a first-order decay model $C_t = C_0 exp(-kt)$ using nonlinear regression in SAS (SAS Institute, Cary, NC), where C_t is total C in the fraction at time t, C_0 is the initial C content, and MRT is the reciprocal (k^{-1}) of the decomposition rate constant, k.

RESULTS AND DISCUSSION
Soil Organic Carbon Fractions in Conventional Tillage and No-Till Soils

The distribution of SOC in the LF, POM, and silt- and clay-size fractions varied among sites and was dependent on the SOC content and soil particle size distribution. Light fractions separated using a density of 1.7 g cm^{-3} at time zero before incubation had SOC contents ranging from 0.5 to 0.8 g C kg^{-1} and accounted for 3 to 5% of the SOC with no significant difference between CT and NT treatments (Table 2). Skjemstad et al. (1986) reported that the LF in a cultivated soil accounted for 1% of the total SOC and Tiessen and Stewart (1983) found

Table 2. Organic C distribution among light fraction (LF), particulate organic matter (POM), silt, and clay soil fractions, soluble C, and the whole soil during long-term incubation of the Wooster and Hoytville, OH, and Kellogg Biological Station (KBS), MI, soils by absolute content (Abs.) and proportion (Prop.).

Soil fraction	Conventional tillage						No-till					
	Wooster, OH		Hoytville, OH		KBS, MI		Wooster, OH		Hoytville, OH		KBS, MI	
	Abs.	Prop.	Abs.	Prop.	Abs.	Prop.	Abs.	Prop.	Abs.	Prop.	Abs.	Prop.
	g C kg^{-1}	%	g C kg^{-1}	%	g C kg^{-1}	%	g C kg^{-1}	%	g C kg^{-1}	%	g C kg^{-1}	%
						Initial						
LF	0.6 (0.1)†	5.4	0.7 (0.2)	3.5	0.5 (0.1)	4.5	0.6 (0.1)	3.6	0.8 (0.2)	3.0	0.6 (0.1)	4.6
POM + sand	2.2 (0.1)	20.6	2.1 (0.8)	11.0	2.2 (0.3)	20.4	3.6 (0.2)	22.9	2.2 (0.2)	8.4	2.0 (0.3)	17.4
Silt	3.0 (0.1)	28.5	4.4 (0.8)	23.1	1.9 (0.2)	17.5	4.3 (0.4)	27.4	4.7 (0.5)	18.4	2.0 (0.1)	17.6
Clay	3.8 (0.1)	35.7	10.1(4.6)	52.9	5.3 (0.1)	48.5	5.6 (0.8)	36.3	15.5 (0.2)	60.6	5.8 (0.3)	50.6
Soluble C‡	1.0§	10.0	1.8§	9.5	1.0§	9.3	1.5§	9.5	2.4§	9.6	1.1§	9.7
Whole soil	10.6 (0.9)		19.0 (0.5)		10.9 (0.3)		15.6 (1.0)		25.6 (0.3)		11.5 (0.2)	
						440-d incubation						
LF	0.3 (0.02)	2.9	0.4 (0.02)	2.3	0.3 (0.02)	3.3	0.3 (0.03)	2.3	0.5 (0.02)	2.0	0.3 (0.02)	2.8
POM + sand	2.3 (0.1)	23.9	2.3 (0.7)	12.9	2.3 (0.3)	24.1	3.3 (0.1)	23.9	2.4 (0.1)	10.3	2.0 (0.3)	20.5
Silt	2.5 (0.2)	26.2	4.3 (0.8)	24.4	1.4 (0.3)	15.1	3.4 (0.4)	25.0	5.0 (0.7)	22.1	1.7 (0.4)	18.1
Clay	3.5 (0.3)	37.2	8.6 (2.0)	49.4	5.2 (0.1)	54.1	5.2 (0.7)	38.3	14.6 (2.2)	64.3	5.5 (0.4)	57.8
Soluble C	0.8§	9.7	1.8§	11.0	0.3§	3.3	1.3§	10.5	0.4§	1.4	0.5§	0.9
Whole soil	9.5 (0.3)		17.4 (0.3)		9.6 (0.2)		13.6 (0.3)		22.8 (0.6)		9.6 (0.5)	
						800-d incubation						
LF	0.2 (0.03)	2.1	0.2 (0.01)	1.0	0.2 (0.02)	2.9	0.2 (0.05)	1.8	0.3 (0.02)	1.2	0.2 (0.01)	2.0
POM + sand	2.0 (0.1)	22.1	2.3 (0.1)	13.8	2.2 (0.2)	25.0	3.3 (0.3)	25.7	2.3 (0.1)	11.4	1.8 (0.1)	19.5
Silt	2.6 (0.8)	29.0	4.3 (0.2)	25.8	1.6 (0.3)	18.4	3.8 (0.7)	29.9	5.2 (0.8)	25.5	1.9 (0.6)	21.1
Clay	3.4 (0.7)	38.1	8.4 (1.4)	50.8	4.2 (0.6)	47.4	4.3 (0.9)	33.6	10.8 (1.1)	52.8	4.4 (0.8)	48.0
Soluble C	0.8§	8.8	1.4§	8.7	0.6§	6.3	1.1§	9.0	1.9§	9.2	0.9§	9.4
Whole soil	9.0 (0.3)		16.6 (0.2)		8.8 (0.6)		12.6 (0.5)		20.4 (0.3)		9.1 (0.6)	

† Values in parentheses are standard errors of the mean at $P = 0.05$.

‡ Soluble C defined as the C unaccounted for following the fractionation process.

§ Estimated by subtraction.

Table 3. Organic C distribution, δ^{13}C, and C derived from C$_4$ residue inputs among light fraction (LF), particulate organic matter (POM), silt, and clay soil fractions, soluble C, and the whole soil during long-term incubation of the Lamberton, MN, soil. Native soil δ^{13} C = −19.3 (0.4) ‰.

Soil fraction	Unfertilized				Fertilized (160 kg N ha^{-1})			
	C distribution		δ^{13}C	C derived from C$_4$ inputs	C distribution		δ^{13}C	C derived from C$_4$ input
	Content	Proportion			Content	Proportion		
	g C kg^{-1}	%	‰	%	g C kg^{-1}	%	‰	%
				Initial				
LF	0.5 (0.1)†	2.5	−12.9 (0.2)	87.7 (3.1)	0.7 (0.1)	3.5	−12.7 (0.4)	90.3 (7.9)
POM + sand	1.1 (0.1)	5.3	−15.1 (0.4)	59.1 (1.3)	1.2 (0.1)	5.8	−14.6 (0.2)	65.9 (3.7)
Silt	3.4 (0.3)	16.5	−16.4 (0.2)	41.9 (1.3)	3.1 (0.4)	15.1	−15.8 (0.2)	49.2 (4.4)
Clay	13.5 (1.0)	66.1	−16.7 (0.2)	37.4 (2.0)	13.9 (1.2)	68.4	−16.3 (0.2)	42.6 (2.2)
Soluble C‡	1.9§	9.6			1.5§	7.2		
Whole soil	20.4 (0.4)		−16.4 (0.1)	39.3 (0.9)	20.4 (0.5)		−16.4 (0.3)	41.5 (5.5)
				440-d incubation				
LF	0.2 (0.02)	1.1	−14.6 (0.5)	64.5 (6.5)	0.3 (0.1)	1.4	−14.2 (0.3)	69.7 (4.0)
POM + sand	1.4 (0.1)	7.4	−16.8 (0.2)	34.7 (3.3)	1.5 (0.1)	8.3	−16.6 (0.3)	37.0 (3.5)
Silt	3.3 (0.2)	17.8	−18.3 (0.5)	13.3 (1.5)	3.2 (0.5)	17.8	−17.7 (0.2)	22.2 (1.5)
Clay	12.4 (1.5)	66.1	−16.7 (0.6)	35.6 (0.6)	12.6 (1.4)	66.8	−16.6 (0.2)	36.9 (2.8)
Soluble C	1.4§	7.7			0.4§	2.7		
Whole soil	18.7 (0.6)		−16.9 (0.2)	32.8 (2.4)	18.0 (0.4)		-16.6 (0.3)	37.1 (4.4)
				800-d incubation				
LF	0.2 (0.01)	0.8	−15.6 (0.6)	51.4 (5.2)	0.2 (0.08)	0.8	−16.3 (0.4)	40.6 (4.9)
POM + sand	1.3 (0.2)	7.7	−17.6 (0.4)	24.0 (3.6)	1.6 (0.05)	9.3	−17.9 (0.2)	22.2 (3.0)
Silt	3.4 (0.2)	19.8	−18.8 (0.2)	7.6 (1.4)	3.5 (0.2)	19.8	−18.4 (0.1)	12.2 (1.4)
Clay	10.8 (1.2)	62.3	−17.9 (0.2)	19.7 (1.2)	10.8 (0.6)	61.2	−17.7 (0.4)	22.1 (5.1)
Soluble C	1.7§	9.4			1.5§	9.0		
Whole soil	17.4 (0.4)		−17.0 (0.1)	31.6 (2.1)	17.6 (0.1)		−16.8 (0.5)	34.3 (4.6)

† Values in parentheses are standard errors of the mean at P = 0.05.

‡ Soluble C defined as the C unaccounted for following the fractionation process.

§ Estimated by subtraction.

that floatable organic C (which is comparable to the POM fraction of our study) accounted for 8 to 14% of the SOC in cultivated soil. The POM fraction accounted for 5 to 11% of the SOC in the fine-textured (>31% clay) soils at Lamberton, MN (Table 3), and Hoytville, OH (Table 2), compared with a range of 17 to 23% of the SOC in the KBS, MI, and Wooster, OH, soils that contained <20% clay (Table 2). There was no significant difference in the concentrations of LF and the POM fractions between the two tillage treatments. The highest proportion of the SOC content was found in the clay-size fraction ranging from 36% (3.8 g C kg^{-1}) of the SOC in the CT treatment of the Wooster soil (Table 2) to 68.4% (13.9 g C kg^{-1}) in the fertilized Lamberton soil (Table 3). The NT treatments among all sites showed a higher C enrichment in the clay fraction than in CT. In contrast, silt fractions were more enriched in C in the CT than NT soils before incubation. Silt-associated organic C accounted for 16 to 28% of the SOC.

The proportion of C contained in the LF decreased an average of 65% among all treatments in the samples incubated for 800 d. The majority of the loss in LF-C occurred during the first 400 d of incubation. There was no significant difference in the loss of LF-C among CT and NT treatments for any of the incubated soils (Table 2). The loss of C from LF was attributed to mineralization of the labile fractions that contain partly decomposed plant residues, as well as the turnover of microbial biomass (Anderson et al., 1981; Tiessen and Stewart, 1983; Janzen et al., 1992; Gregorich et al., 1995). The C concentration and distribution (percentage of SOC) in the POM, silt-associated, and clay fractions did not change significantly during 800 d of incubation. The decomposition of the LF might have contributed to a transfer of C to the POM and silt fractions, as suggested by Tiessen and Stewart (1983). The fractionation procedure used in this study resulted in losses of soluble organic C and some of the mineral fractions, with a maximum estimated loss of 10%.

The Carbon and Nitrogen Composition of the Fractions

The LF and POM, being closely related to plant residues and associated microbial decomposition products, had the expected wide C/N ratios (Greenland and Ford,1964; Dalal and Mayer, 1986a; Christensen, 1992). It decreased from ~21 in the LF to around 8 in the clay fraction of Hoytville (Table 4). The C/N ratio of the POM fraction (~14.5) was intermediate between the LF and heavy fractions. That of the silt was similar to the whole soil. The clay fraction had the narrowest C/N ratio (6.3–10.2). Christensen and Sorensen (1985) incubated soils for 5 yr with ^{15}N–NH$_4$ and ^{14}C-hemicellulose and found that the C/N ratio of labeled, organic C associated with clay was lower and that associated with silt was higher than that of the whole soils. The Hoytville soil had the highest clay content (50%) and this clay had the narrowest C/N ratio (6.3–6.7).

There are two possible explanations for the narrow C/N ratio of the clay. Fixed NH$_4$, the NH$_4^+$ ion fixed within the lattice of clay minerals, has been found to occur in clays of the U.S. Midwest. Others (Smith and Young, 1975; Stevenson and Dhariwal, 1959) have reported that clays similar to those in the soils of this study had a fixed NH$_4$ content of 2 cmol kg^{-1} clay. Correcting the C/N ratios of the clay for fixed NH$_4$ would result in ratios of C/organic N of 9.5 for the clay from Wooster, 7.4 for Hoytville, 9.3 for KBS, and 10.1 for Lamberton. The corrected values are similar to the C/N ratios of the total soil C.

Table 4. Carbon/nitrogen ratio of light fraction (LF), particulate organic matter (POM), silt, and clay soil fractions and the whole soil initially and after 440 or 800 d of laboratory incubation.

Soil fraction	C/N ratio					
	Conventional tillage			No-till		
	Initial	440 d	800 d	Initial	440 d	800 d
Wooster, OH						
LF	22.2 a†	21.8 a	21.9 a	20.9 a	20.4 a	20.1 a
POM + sand	14.4 b	14.3 b	14.2 b	15.7 b	14.8 b	14.5 b
Silt	10.1 c	8.5 d	8.1 d	10.3 c	9.6 c	9.1 cd
Clay	8.2 d	7.8 d	7.6 d	8.2 d	7.5 d	7.4 d
Whole soil	10.0 c	9.3 c	8.5 d	11.2 e	10.4 c	8.2 d
Hoytville, OH						
LF	19.6 c	19.5 c	19.4 c	19.4 b	19.3 b	19.2 c
POM + sand	15.0 de	14.6 de	14.4 d	14.9 d	14.3 de	14.2 ef
Silt	10.3 g	9.3 h	9.0 fg	10.1 f	9.8 hi	9.5 gh
Clay	6.5 j	6.4 l	6.3 k	6.7 h	6.4 k	6.4 l
Whole soil	9.8 gh	9.0 hi	8.0 ij	10.2 f	9.4 hi	8.2 j
Kellogg Biological Stn., MI						
LF	21.0 b	20.1 bc	20.4 b	21.2 a	20.6 a	20.6 a
POM + sand	15.2 d	15.0 d	14.0 d	15.2 cd	14.99 c	14.5 ef
Silt	10.0 gh	9.4 gh	8.8 gh	10.5 ef	9.6 hi	8.9 i
Clay	8.5 i	7.8 jk	7.7 j	7.8 g	7.8 j	7.8 k
Whole soil	10.4 g	10.7 f	8.8 fgh	10.3 f	9.8 gh	9.0 i
		Unfertilized			160 N kg ha^{-1}	
Lamberton, MN						
LF	21.0 b	20.6 b	20.5 b	20.7 a	20.3 a	16.7 d
POM + sand	14.9 de	14.5 de	14.1 d	14.7 d	14.2 e	14.1 f
Silt	11.4 f	10.4 f	10.3 e	10.5 ef	10.4 f	9.7 g
Clay	9.4 h	9.3 h	9.2 fg	10.2 f	9.3 i	9.3 hi
Whole soil	10.4 g	10.0 fg	9.5 f	11.0 e	10.3 fg	9.5 gh

†Comparisons were made within a site, among fractions, and between treatments. Means with the same letter are not significantly different at $p = 0.05$ level.

The C content of the clay ranged from 20 g C kg^{-1} clay in the Wooster and Hoytville soils to 28 g C kg^{-1} clay in KBS and 43 C kg^{-1} clay in the Lamberton soil. Buyanovsky et al. (1994) suggested that the C associated with the clay fraction may be highly processed and stable compared with organic matter (OM) associated with silt and sand fractions. Anderson and Paul (1984) found the relative ^{14}C content of silt, coarse clay, and fine clay to depend on soil type. In some soils, coarse clay had higher MRTs than silt. In all cases, the fine clays had low MRTs and many showed the influence of recent bomb C. Sorensen (1972) showed the preferential absorption of amino acids to clay, and recent literature (Sollins et al., 2006) has suggested that clays build up amino-rich compounds in an onion-like structure of amino acids layered onto the clay. This could, in part, cause the narrow C/N ratios. The correction for fixed NH$_4$ changes the C/N ratio of the clay to nearly that of the silts, suggesting that this may be the more important mechanism of the two.

Carbon-13 Values of Soil Organic Carbon Fractions before and after Incubation

On average, across all sites, δ^{13}C values of the native soils differed by 3‰ from the adjacent cultivated whole soil, enabling us to identify changes in the composition of C$_3$ (forest and prairie) and C$_4$ (corn) C with time of incubation and calculate the mean residence time of C within different organic fractions in the whole soil. The δ^{13}C values of the LF ranged from –12.7‰ at Lamberton, MN (Table 3), to –18.2‰ at KBS, MI, before incubation (Table 5). The more negative δ^{13}C value for the KBS LF was probably due to the shorter period (~8 yr) this site was under continuous corn

compared with >30 yr for the other three sites. The δ^{13}C values for the LF of the Wooster, Hoytville (Table 5), and Lamberton (Table 3) sites were –14.9, –14.1, and –12.7‰, respectively, indicating that it consists mainly of corn (C$_4$–C)-derived residues. The δ^{13}C values of the POM, silt, and clay fractions of all sites show a consistent decline in enrichment of C$_4$–C. The δ^{13}C contents of the whole soils were similar to the values of the silt fractions among all sites. Collins et al. (2000) reported that the δ^{13}C values of the native soils were –26.1‰ at KBS, –25.5‰ at Wooster, –24.8‰ at Hoytville, and –19.5‰ at Lamberton.

After 800 d of incubation, δ^{13}C values of the LF became less enriched in C$_4$–C than that of the POM, silt, and clay organic fractions. The δ^{13}C of the LF decreased 6‰ in the Wooster soil and 3 to 5‰ at the other sites (Table 5). Although initial δ^{13}C values of the LF for the Wooster and Hoytville soils (Table 5) were similar, the greater decrease in Wooster (6‰) than Hoytville (4‰) soils reflects a higher turnover rate of C$_4$–C at Wooster (Table 6). The rate of turnover decreased in the order Wooster > Hoytville > KBS > Lamberton based on changes in the δ^{13}C values of the LF with incubation. As with the LF, the POM in the Wooster and Hoytville soils had greater depletion (~4.5‰) than the KBS or Lamberton (~2.5‰) soils. Since POM is commonly associated with aggregates, it is better protected from decomposition than the LF, resulting in less depletion. The silt and clay fractions showed less depletion, ranging from 2.6‰ to almost no change with incubation. The silt-size organic fraction was most depleted at Lamberton (2.6‰) and the least depletion was observed at Hoytville (0.8‰). The clay-size organic fraction depletion ranged from 1.91‰ at Wooster to 0.46‰ at KBS. The clay-size organic

fraction of the Hoytville soils showed an average of 0.1‰ enrichment with 800 d of incubation.

The type of tillage was not a factor in affecting the loss of C_4–C during incubation. Light fraction and POM had turnover times averaging 4 and 9 yr, respectively, on a field-equivalent basis. The POM fraction after 800 d of incubation had ^{13}C values similar to that of the whole soil. The silt fraction was more depleted than the clay fraction after 440 d of incubation in the coarser textured sites (KBS and Lamberton). During the incubations of these soils, Collins et al. (2000) showed that >50% of the CO_2 evolved during the first 100 d was derived from corn residue and, even after >800 d of incubation, the $\delta^{13}C$ of the CO_2 from the Hoytville soil was enriched by 2‰.

Corn-Derived Carbon and Turnover Time

After ~30 yr of cropping, the proportion of C derived from corn was 22, 38, and 42% in the whole soil for the Wooster, Hoytville (Table 5), and Lamberton (Table 3) sites, respectively. The KBS site, with a shorter period of continuous corn cropping, showed the least incorporation of corn residue (21% for CT and 22% for NT) into the SOM (Table 5). Hoytville, although cropped for 30 yr, showed a similar low incorporation of C_4–C in the whole soil (Table 5). This may have resulted from the high clay content and poorly drained characteristics of this site. Our findings are similar to the 25 to 35% estimate of Gregorich et al. (1997) after 25 yr and the 34% estimate by Balesdent et al. (1988) after 36 yr of continuous corn cropping. The proportion of corn-derived C in the LF ranged from 56 to 84% under CT and 60 to 85% in the NT treatments. The LF, which is relatively undecomposed and has a C/N ratio of ~21 (Table 4), is biologically very active. Except for the KBS site, <20% of the C_3–derived organic C has remained in the LF. The KBS site has been continuously cropped to corn for 8 yr, before which the rotation was a corn–soybean–wheat (*Triticum aestivum* L.) rotation.

In the POM fraction, corn-derived C ranged from 38 to 64% under CT and 46 to 70% in NT soils (Table 5). As Goldchin et al. (1995) and Gregorich et al. (1997) pointed out, aggregation is a continuous process and thus the composition of the organic pool depends on the cropping history. They suggested that the physically protected LF, and in this case the POM, is a relatively older stabilized pool of C compared with the unprotected LF. In both fractions, slightly more corn-derived C is found under NT than CT due to slower decomposition of residue at the surface of the NT treatments. Soil C derived from corn residue in the silt averaged 19, 20, 36, and 46%, and 23, 11, 30, and 40% in the clay size fractions,

Table 5. The δ^{13} C distribution and C derived from C_4 inputs of light fraction (LF), particulate organic matter (POM), silt, and clay soil fractions and the whole soil during long-term incubation of the Wooster and Hoytville, OH, and Kellogg Biological Station (KBS), MI, soils.

Soil fraction	Conventional tillage						No-till					
	Wooster, OH		Hoytville, OH		KBS, MI		Wooster, OH		Hoytville, OH		KBS, MI	
	δ^{13}C	C from C$_4$ inputs	δ^{13}C	C from C$_4$ inputs	δ^{13}C	C from C$_4$ inputs	δ^{13}C	C from C$_4$ inputs	δ^{13}C	C from C$_4$ inputs	δ^{13}C	C from C$_4$ inputs
	‰	%	‰	%	‰	%	‰	%	‰	%	‰	%
					Initial							
LF	−14.9 (0.2)†	78.0 (1.8)	−14.1 (0.3)	83.6 (3.1)	−18.2 (0.5)	55.7 (5.0)	−14.2 (0.2)	84.1 (1.0)	−13.9 (0.3)	84.9 (3.3)	−17.5 (0.3)	60.9 (3.1)
POM + sand	−16.9 (0.1)	64.0 (0.8)	−17.8 (0.5)	55.7 (5.8)	−20.7 (0.3)	38.1 (3.7)	−16.4 (0.1)	67.3 (0.4)	−17.2 (0.5)	60.2 (4.1)	−19.1 (0.5)	46.0 (5.8)
Silt	−20.7 (0.9)	35.7 (2.7)	−22.4 (0.1)	20.0 (0.9)	−23.5 (0.5)	18.4 (3.0)	−20.6 (0.4)	36.4 (4.4)	−22.4 (0.3)	19.9 (2.9)	−23.3 (0.5)	19.7 (6.5)
Clay	−21.1 (0.5)	32.6 (1.3)	−23.5 (0.1)	11.2 (1.0)	−23.2 (0.5)	21.2 (2.4)	−21.6 (0.6)	29.0 (6.0)	−23.6 (0.1)	10.7 (1.1)	−22.5 (0.4)	25.2 (1.7)
Whole soil	−20.3 (0.1)	38.3 (0.4)	−22.1 (0.1)	22.4 (0.3)	−23.1 (0.6)	21.3 (6.2)	−20.6 (0.5)	36.2 (3.8)	−22.6 (0.7)	18.6 (5.6)	−21.9 (0.4)	29.3 (4.5)
					440-d incubation							
LF	−20.7 (0.2)	35.3 (1.6)	−17.3 (0.8)	58.4 (6.2)	−22.2 (0.8)	28.0 (8.6)	−19.4 (0.6)	45.1 (4.6)	−17.2 (0.6)	59.7 (4.4)	−19.5 (0.6)	47.0 (6.3)
POM + sand	−21.8 (0.5)	27.3 (3.9)	−20.3 (0.3)	35.5 (2.4)	−22.6 (0.1)	24.8 (0.8)	−20.7 (0.3)	35.3 (2.3)	−20.0 (0.5)	37.7 (3.8)	−22.5 (0.3)	25.9 (3.1)
Silt	−21.9 (0.3)	26.5 (2.5)	−22.8 (0.2)	15.6 (1.6)	−24.4 (0.2)	11.9 (1.7)	−20.9 (0.5)	34.2 (3.8)	−23.5 (0.4)	10.4 (2.8)	−24.1 (0.1)	14.2 (1.6)
Clay	−22.1 (0.3)	25.5 (1.9)	−23.3 (0.1)	12.1 (0.4)	−23.1 (0.1)	21.1 (0.5)	−21.9 (0.3)	26.4 (2.3)	−23.2 (0.3)	12.3 (0.2)	−22.7 (0.1)	23.7 0.5)
Whole soil	−20.9 (0.7)	34.0 (5.3)	−22.5 (0.7)	18.1 (5.3)	−23.4 (0.9)	19.5 (5.0)	−20.8 (0.4)	34.9 (2.6)	−22.7 (0.1)	16.4 (0.9)	−22.8 (0.5)	23.4 (5.3)
					800-d incubation							
LF	−21.2 (0.7)	32.0 (5.5)	−18.2 (0.8)	51.9 (6.1)	−22.2 (0.1)	27.7 (0.2)	−20.9(0.1	34.4 (1.1)	−17.9 (0.2)	53.6 (1.2)	−22.0 (0.1)	28.8 (0.8)
POM + sand	−21.8 (0.1)	27.3 0.7)	−22.2 (0.3)	20.7 (2.2)	−22.9 (0.5)	22.7 (5.1)	−21.0 (0.4)	33.7 (2.7)	−21.5 (0.2)	25.8 (1.7)	−22.8 (0.1)	23.4 (1.1)
Silt	−21.8 (0.1)	27.2 (0.9)	−23.2 (0.1)	12.4 (0.7)	−24.4 (0.3)	11.9 (3.2)	−21.5 (0.3)	30.0 (2.5)	−23.7 (0.1)	8.3 (0.5)	−24.6 (0.3)	10.5 (3.1)
Clay	−22.7 (0.3)	21.0 (1.9)	−23.5 (0.3)	10.6 (0.8)	−23.6 (0.1)	19.8 (1.0)	−23.5 (0.4)	14.9 (3.1)	−23.5 (0.1)	10.3 (0.4)	−23.9 (0.1)	15.8 (0.7)
Whole soil	−21.4 (0.9)	30.5 (6.3)	−22.5 (0.1)	17.7 (0.3)	−23.2 (0.9)	20.5 (5.1)	−21.0 (1.2)	33.5 (6.0)	−22.8 (0.3)	15.5 (2.0)	−23.8 (0.1)	16.1 (0.7)

†Values in parentheses are standard errors of the mean at P = 0.05.

Table 6. Mean residence time (MRT) of C_4–C for the light fraction (LF), particulate organic matter (POM), silt, and clay fractions of cultivated soils under conventional tillage (CT) and no-till (NT).

Site and treatment	Light fraction		Particulate organic matter		Silt		Clay	
	Lab MRT	Field MRT†	Lab MRT	Field MRT	Lab MRT	Field MRT	Lab MRT	Field MRT
	d	yr	d	yr	d	yr	d	yr
Wooster, OH								
CT	321 (33)‡	2.6 (0.3)	676 (110)	5.6 (0.9)	1927 (199)	15.9 (1.6)	1451 (138)	12.0 (1.1)
NT	386 (29)	3.2 (0.2)	800 (159)	6.6 (1.3)	2524 (180)	20.8 (1.5)	860 (92)	7.1 (0.5)
Hoytville, OH								
CT	459 (36)	3.7 (0.3)	971 (91)	7.8 (0.7)	1597 (26)	12.8 (0.5)	3342 (153)	26.8 (1.2)
NT	495 (6)	4.0 (1.0)	1068 (32)	8.6 (0.3)	1034 (34)	8.3 (0.3)	2003 (16)	16.1 (0.2)
Kellogg Biological Stn., MI								
CT	467 (69)	3.9 (0.6)	1368 (136)	11.4 (1.1)	1314 (75)	10.9 (1.0)	1985 (150)	16.5 (0.5)
NT	459 (12)	3.8 (0.1)	1377 (134)	11.4 (1.1)	1174 (130)	9.7 (1.1)	1078 (98)	9.0 (1.0)
Lamberton, MN								
0 N	379 (27)	3.8 (0.3)	1267 (118)	12.8 (1.2)	469 (20)	4.7 (1.0)	910 (17)	9.2 (0.2)
160 kg N	337 (11)	3.4 (0.1)	1111 (74)	11.2 (0.8)	628 (10)	6.3 (0.4)	881 (51)	8.9 (0.5)

†Mean residence times converted to field rates using a Q_{10} of 2 [$2^{(25-t)/10}$], where t = mean annual temperature.

‡Standard error of the mean in parentheses.

for the KBS, Hoytville, Wooster (Table 5), and Lamberton sites (Table 3), respectively.

The silt- and clay-size organic fractions had much less corn-derived C than LF or POM, suggesting that finer size particles with greater surface area provide physical protection of the older C_3–C. Higher concentrations of organic C (50–70% of total soil C) and relatively more stable organic C was found in the clay-size fractions as indicated by the isotopic composition. Thus, after >30 yr of corn cropping, the silt- and clay-size organic fractions are dominated by older C_3–C (Table 7). The silt- and clay-size organic C is more stable than the LF and POM and contributes to long-term soil fertility. Clay and OM content appear to have a greater correlation in most soils due to physical protection of the OM by mineral particles and resistance to biochemical reaction in the soil.

The change in the percentage of SOC derived from corn and incorporated into LF, POM, silt, clay, and whole soils after 440 and 800 d of laboratory incubation is shown in Tables 3 and 5. The greatest loss of C_4–derived C was observed for the LF (31–59%) and POM (40–66%) fractions. After 800 d of

Table 7. Field mean residence times (MRT)†‡ of C_3–C for the light fraction (LF), particulate organic matter (POM), silt, and clay fractions of cultivated soils under conventional tillage (CT) and no-till (NT). Assumes C at time of study initiation was all C_3–C and that the concentration of C_3–C did not increase due to cropping of continuous corn.

Site and treatment	LF MRT	POM MRT	Silt MRT	Clay MRT
		yr		
Wooster, OH				
CT	20.5	30.3	70.2	78.6
NT	16.7	27.7	68.5	90.5
Hoytville, OH				
CT	17.2	38.1	139	261.0
NT	16.4	33.7	140	273.9
Kellogg Biological Stn., MI				
CT	19.7	33.4	47.1	40.0
NT	17.0	26.0	43.5	31.8
Lamberton, MN				
0 N	15.8	36.9	60.8	70.5
160 kg N	14.1	30.7	48.7	59.5

† Mean residence times converted to field rates using a Q^{10} of 2 [$2^{(25-t)/10}$], where t = mean annual temperature.

incubation, almost half of the original C_4–derived C had been mineralized, which suggests that these fractions are responsible for the short- and medium-term fertility of the soil.

The clay-sized C fraction showed the least loss of C_4-derived C, followed by the silt-size fraction. The loss of clay-size C_4–derived C was greater than the silt-size fraction in Wooster soils. This supports previous studies (Martel and Paul, 1974; Dalal and Mayer, 1986a, 1986b) that the clay-fraction OM is more labile than the silt-associated organic C. In all the other sites, the silt-size organic C fraction showed relatively faster turnover of corn-derived organic C and this observation (silt-associated OM is more labile than clay) is not common in temperate soils (Christensen, 1987). Balesdent et al. (1987) observed that the organic C associated with the clay fraction was very heterogeneous with regard to degradability. Anderson and Paul (1984) found the SOC of coarse clays to have a high MRT while the fine clays were much younger. This study did not fractionate the clay into two sizes.

The mean residence time (MRT = $1/k$) of corn-derived C in the LF and POM fractions was estimated by assuming an exponential decay of the original organic C (C_o) in each fraction with 440 and 800 d of incubation (Table 6). The estimated average MRT of corn-derived C in the LF across sites was 3.5 and 9.4 yr for the POM fraction. The POM fraction of the Wooster and Hoytville soils had a lower MRT (5.6–8.6 yr) than that of the KBS and Lamberton soils, which averaged ~12 yr. This result was expected in the field situation where the mean annual temperature in Ohio is higher than at KBS or Lamberton, indicating a faster turnover. With laboratory incubations, however, soils from cold sites (KBS and Lamberton) would be expected to turn over faster because of a greater concentration of labile OM than in the warmer soils of Ohio. Other factors such as the extent of aggregation could have played a role, but fractionation into aggregates sizes and determination of their ^{13}C signal did not show significant differences in this study (data not shown). Tillage treatment or fertilization did not significantly affect the MRT of corn-derived C in either the LF or the POM fractions.

Bradford Reactive Soil Protein

The Bradford reactive soil protein (glomalin) extracted with hot citrate comprised 1.6 to 2.5% of the SOC in the CT soils and 2.6 to 9.5% of the SOC in the NT soils at the time

Table 8. Change in Bradford reactive soil protein (BRSP) content, its C derived from C_4 residue inputs, and their mean residence time (MRT) initially and after 440 or 880 d of incubation of soils.

Site and treatment	Conventional tillage				No-till			
	C	$\delta^{13}C$	C from C_4 inputs	MRT	C	$\delta^{13}C$	C from C_4 inputs	MRT
	g C kg^{-1}	‰	%	yr	g C kg^{-1}	‰	%	yr
Wooster, OH								
Initial	0.70 (0.01)†	−19.8 (0.3)	42.2		1.23 (0.02)	−20.9 (0.3)	34.1	
440 d	0.65 (0.03)	−21.7 (0.1)	28.2		1.15 (0.01)	−21.0 (0.4)	33.3	
800 d	0.60 (0.03)	−21.5 (0.2)	29.6	42.8	0.79 (0.03)	−21.5 (0.4)	29.6	14.9
Hoytville, OH								
Initial	2.14 (0.3)	−24.0 (0.1)	7.7		5.36 (1.3)	−22.6 (1.4)	18.5	
440 d	2.03 (0.4)	−23.8 (0.4)	9.3		2.33 (0.2)	−24.6 (0.6)	13.1	
800 d	2.00 (0.5)	−24.6 (0.3)	3.1	96.5	3.00 (0.1)	−23.2 (0.2)	13.9	11.3
Kellogg Biological Stn., MI								
Initial	0.73 (0.2)	−24.5 (0.3)	17.8		0.74 (0.01)	−24.4 (0.8)	18.9	
440 d	0.77 (0.04)	−24.8 (0.1)	14.4		0.59 (0.01)	−24.9 (0.1)	13.3	
800 d	0.63 (0.1)	−24.8 (0.1)	14.4	45.1	0.57 (0.05)	−25.1 (0.1)	11.1	25.4
		Unfertilized				160 N kg ha^{-1}		
Lamberton, MN								
Initial	3.77 (0.8)	−18.2 (0.1)	17.3		4.39 (0.8)	−17.9 (0.4)	21.3	
440 d	3.60 (0.9)	−18.6 (0.3)	12.0		3.15 (0.7)	−17.3 (0.5)	29.3	
800 d	3.59 (0.6)	−18.5 (0.3)	13.3	104.5	2.82 (0.5)	−17.9 (0.5)	21.3	18.2

†Values in parentheses are stand error of the mean at $p = 0.05$. ‡MRT-Mean residence times converted to field rates using a Q_{10} of 2;($2^{(25-t)/10}$); where, t = Mean annual temperature.

of soil sampling (Table 8). Similar results were obtained from the Lamberton soils, which had a N fertilizer treatment rather than a CT–NT comparison. The proportion of BRSP derived from corn inputs, as determined by the ^{13}C contents, varied with soil type, in that the Wooster soil showed approximately one-third of the BRSP to be corn derived. The higher SOC Hoytville had <10% of the BRSP as corn derived SOC after 30 yr of continuous CT corn, as evidenced by the ^{13}C contents of about –24‰ relative to the total SOC of –22‰. This indicates a very slow turnover rate of approximately 100 yr in the field. The NT Hoytville soil had twice as much corn-derived BRSP-C and the amount of this material dropped from 9.6% of the SOC to 2% during incubation in the NT soils. The Lamberton soils had similar percentages of BRSP in both treatments, but its ^{13}C content showed more corn-derived material in the N fertilizer treatment. This was not expected, as the literature indicates that N treatment should lower the arbuscular mycorrhizal fungi in this soil. The data agree with the observations of Nichols and Wright (2005), Schindler et al. (2007), and Rosier et al. (2006) that much of the material extracted by the hot citric acid has co-extracted constituents such as humic acids and other proteinaceous material. The anomaly in the NT Hoytville soil, however, merits further investigation.

CONCLUSIONS

The analysis of soils from long-term plots before and after laboratory incubation, in which the microbial enzymes were allowed to decompose the labile soil components, provided a great deal of useful information on physically separated fractions to complement the ^{13}C (Collins et al., 1999), ^{14}C (Paul et al., 2001), and incubation data (Collins et al., 2000) of our previous studies.

There continues to be a hope that physical fractions such as the LF and POM can be used as a directly measured fraction in modeling. The plant residues remaining in soil, as represented by the LF, were increased by NT. Their stability during

incubation was not different under the two management systems nor was it site (soil) specific, showing that inherent chemical characteristics controlled turnover rates. Both the proportion of corn-derived C, as sampled in the field after continuous corn, and the ^{13}C measurements during this incubation indicated that LF and POM fractions were not homogenous. Black C or charcoal found in both the LF and POM could be one explanation; however, the corn-derived C comes from the last 30 yr of cultivation with no known history of fire and we believe that some factor other than, or in addition to, charcoal is responsible for approximately one-third of both the LF and POM that is much older than the rest.

The silt- and clay-associated C from corn was older than the LF and POM in all soils. In three soils, the clay-associated residues had the largest MRT. In one soil (Wooster), the silt-associated, corn-derived material had a longer MRT than the clay. The MRTs of the C_3–C, or non-corn-derived C, in the silt and clay, as measured during incubation, are starting to approach but are still approximately one-quarter the values of the whole soil as measured by C dating. This study further shows that SOM is a continuum of materials from very young to very old with ongoing transfers between pools. A combination of physical, chemical, and biologically defined kinetic pools can help determine its dynamics, but the hope of straightforward direct fractionations will be fulfilled only with great difficulty because of the many interactions involved.

foresight in maintaining such valuable scientific resources over the years is commendable and much appreciated.

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