0038-075X/04/16906-000–000
Soil Science
Copyright © 2004 by Lippincott Williams & Wilkins, Inc.
June 2004
Vol. 169, No. 6
Printed in U.S.A.

BIOCHEMICALLY PROTECTED SOIL ORGANIC CARBON AT THE NORTH APPALACHIAN EXPERIMENTAL WATERSHED

Z. X. Tan[1], R. Lal[1], R. C. Izaurralde[2], and W. M. Post[3]

Land use and soil management affect the balance between labile and stable organic matter fractions in surface soils. This study was conducted to quantify the contribution of nonhydrolyzable carbon (NHC) to total soil organic carbon (SOC) and to identify the role of biochemical protection mechanisms in SOC sequestration by comparing the NHC fraction associated with aggregates from 0 to 5-, 5 to 10-, and 10 to 20-cm depths under forest, meadow, no-till (NT) and conventional tillage (CT) treatments. The NHC contribution to SOC declined from 53% under forest to 37% under CT and 39% under NT, implying that the conversion from forest to cultivation led mainly to a reduction in the NHC. Aggregate-associated NHC concentration increased with aggregate size (except for CT treatment). Conversion from CT to NT enriched NHC in all aggregate fractions, but even more so in the >250-μm fraction, underscoring the importance of macroaggregate fractions in encapsulating and thus protecting SOC from microbial processes. The formation of macroaggregates is coupled with the depletion of microaggregates, which can be quantitatively described by functions (for each aggregate class) expressed in terms of the aggregate mass and the proportion NHC/SOC. Both the NHC fraction and the nonhydrolyzable C:N ratio increased with the increase in SOC concentration in soils under meadow and forest, suggesting a large potential for SOC sequestration through biochemical protection mechanisms. (Soil Science 2004;169:000–000)

Key words: Soil organic matter, nonhydrolyzable fraction, soil aggregate, Alfisols, no-till, conventional tillage.

SOIL can be either a source or sink of CO_2, depending on the management-induced CO_2 fluxes between soil and the atmosphere. Soil acts as a carbon (C) sink through three principal mechanisms that stabilize soil organic carbon (SOC): physical protection through aggregation to reduce accessibility of organics to microbes and enzymes; chemical protection by forming organo-mineral complexes; and biochemical protection through chemical recalcitrance of organic molecules (Sollins et al., 1996; Jastrow and Miller, 1998). The biochemically protected SOC pool is referred to as the *passive* or *recalcitrant* pool (Parton et al., 1987) in soil organic matter (SOM) models, a pool similar in magnitude to the nonhydrolyzable C (NHC) that remains in a soil sample after acid hydrolysis with 6 *M* HCl (Leavitt et al., 1996; Paul et al., 1995, 1997, 2001; Stevenson, 1994). Acid hydrolysis is an appropriate technique for quantifying such a pool (Paul et al., 1995, 1999, 2001; Six et al., 2002). Because of its high resistance to decomposition (Paul et al., 2001), the NHC fraction represents almost all stable SOC components and comprises a large proportion of total SOC pool. Nonhydrolyzable C accounts for 33 to 65% of the SOC pool in agricultural soils in the Midwestern U.S. (Paul et al., 2001) and 20 to 50% of the total SOC pool in topsoils in most soils of the temperate zone (Jenkinson and Rayner, 1977).

[1]Carbon Management and Sequestration Center, The Ohio State University, Columbus OH. Dr. Tan is corresponding author. 210 Kottman Hall, 2021 Coffey Rd., Columbus, OH 43210. E-mail: tan.129@osu.edu

[2]Joint Global Change Research Institute, College Park, MD.

[3] Oak Ridge National Laboratory, Oak Ridge, TN.

Received Nov.7, 2003; accepted March 10, 2004.

DOI: 10.1097/01.ss.0000131227.51226.68

SS-06/04-148440-Tan 5/7/04 4:11 PM Page 2

The nature of the stable SOM and SOC concentrations may be related to soil management and ecosystem properties such as land use, tillage, and soil texture (Anderson and Paul 1984; Becker-Heidmann and Scharpenseel 1992). Whitbread (1994) and Körschens (1996) suggested that soil management affects the relative distribution of SOC pool between the labile and stable fractions. Metherell et al. (1993) also emphasized the importance of soil texture. Management practices such as no-till (NT) or reduced till (RT) promote the maintenance and accumulation of SOC and improvement of physical, chemical, and biological soil characteristics (Kern and Johnson, 1993; Burke et al., 1995; Paustian et al., 1997; Hendrix et al., 1998). The increased macroaggregate quantity under NT plays an important role in reducing losses of labile SOM, whereas tillage practices disrupt aggregates and lead to enhanced aggregate turnover and decomposition of SOM (Six et al., 1998).

Studies on the mass distribution of SOC among aggregate classes (Cambardella and Elliott, 1993; Six et al., 1998, 1999, 2000b) suggest that an increase in soil aggregation through adoption of best management practices usually leads to SOC sequestration. However, the dynamics governing the distribution of sequestered SOC between the labile and stable C fractions, as well as the specific contribution of each fraction to soil aggregation, remain to be documented.

The biochemical protection mechanism is also important for SOC sequestration, but there is little quantitative information about its contribution to the total SOC pool or within different aggregate size fractions. Furthermore, land use and management practices affect the balance between labile and stable SOM in surface soils strongly. It is hypothesized that the magnitude of NHC in topsoil layers is differentiated by tillage practice and land use and may be used to evaluate the potential of altering land use and tillage practice for SOC sequestration. Therefore, this study was conducted to quantify the magnitude of NHC and its contribution to total SOC and to identify the role of biochemical protection mechanisms in SOC sequestration.

MATERIALS AND METHODS

Site and Soils

The North Appalachian Experimental Watershed (NAEW) research station was established in the late 1930s and is located about 16 km northeast of Coshocton, Ohio (40°24′N; 81°48′W). The altitude ranges from 244 to 381 m, mean annual temperature is 10.5 °C, and mean annual precipitation is 1000 mm. Soil samples for this study were taken from the northwestern part of the NAEW. Three predominant soil series are involved in this study: Berks (loamy-skeletal, mixed, mesic, Typic Dystrochrepts), Coshocton (fine-loamy, mixed, mesic Aquultic Hapludalfs), and Rayne (fine-loamy, mixed, mesic, Typic Hapludalfs) (Kelley et al., 1975). These soils are dominated by silt (50 to 65%), with clay-size particles making up only 13 to 19% of the whole. Land use and soil management treatments have been practiced on small natural watersheds (0.5 to 1 ha) since 1938. Five distinctly managed watersheds were selected: (i) conventional-moldboard plow (20 cm depth) in continuous corn (*Zea mays* L.) (CTc) since 1984, where a 4-year rotation of corn/wheat (*Triticum aestivum*)/meadow/meadow had been practiced from 1938 to 1970; (ii) no-till continuous corn (NTc) since 1970; (3) no-till corn-soybean (*Glycine max* [L.] Merr.) (NTcs) in rotation with ryegrass (*Lolium perenne* L.) as a cover crop practiced since 1984; (iv) meadow of orchard grass (*Dactylis glomerata* L.) for hay that was converted from no-till corn in 1988; and (v) secondary forest of white oak (*Querus alba* L.) and red oak (*Querus rubra* L.), mixed with yellow poplar (*Liriodendron tuliperifera*).

Soil Sampling and Treatment

Soil samples were obtained during November 2001 (after corn harvest for the cultivated watersheds) from positions excluding the summit slope of the watershed to minimize the confounding effect of soil erosion. Disturbed soil samples were taken using a soil probe (2.5 cm diameter) to three depths: 0 to 5, 5 to 10, and 10 to 20 cm. Three sets of about 20 soil cores (distributed across each watershed area as evenly as possible) were pooled together to constitute three pseudo field replicates. All soil samples were gently broken apart by hand and passed through a 6.75-mm sieve while still at field moisture content and then air dried. Soil moisture contents of subsamples were determined by drying at 105 °C for 24 h. Soil pH (1:2.5 soil: DI-water ratio) ranged from 7.1 in agricultural soils to 4.6 in forest soil, without any indication of carbonate presence. A subsample of about 20 g from each treatment was set aside for C and N determination, and another subsample was set aside for nonhydrolyzable C analysis.

SS-06/04-148440-Tan 5/7/04 4:11 PM Page 3

Water Stable Aggregate Fractionation

A 30-g sample of air-dried bulk soil (passing a 6.7-mm sieve) was placed in the top sieve of a set of three nested sieves (2000, 250, and 53 μm) (Elliott, 1986). Samples were wetted gently with DI-water for 30 min. in order to allow slaking (Kemper and Roseneau, 1986). The nested sieves were then oscillated gently (5-cm amplitude of 25 strokes min^{-1}) within a column of water for 30 min. Free floating particulate organic matter (POM) was removed during the wet sieving and not included in the current analysis. Aggregate fractions were then recovered and dried at 60 °C.

Acid Hydrolysis

For acid hydrolysis, the bulk soil samples and fractions greater than 250 μm were ground using the 8000 SPEX CertiPrep Mixer/Mill at a moderate speed (Spex CertiPrep Inc., Metuchen, NJ) and passed through a 250-μm sieve, by which all gravels greater than 250 μm were removed.

Approximately 1.0 g of 60 °C dried bulk soil or aggregate fraction sample (passing a 250-μm sieve) was weighed and placed into a 50-mL digestion flask; 25 mL of 6 *N* HCl (Paul et al., 2001) were then added to this flask and shaken thoroughly by hand. A digestion block was used to heat samples to about 100 °C for 18 h. The residue was transferred to a 50-mL graduated centrifugation tube with DI-water to 40 mL and centrifuged at 1250 g for 30 min. The supernatant was discarded. The HCl was then removed from the residue by washing twice with 40 mL DI-water and centrifuging; the supernatant was discarded each time. Finally, the residue was dried at 60 °C overnight and weighed immediately to compute the mass loss.

C and N Determination and Data Analysis

For C and N determination, all unhydrolyzed samples were ground first as those to be used for acid hydrolysis. Determinations of C and N of all samples were performed on a CN analyzer (Vario Max CN Analyzer, Elementar Americas, Inc., Germany).

Land use and management practices (i.e., treatments) were considered a main effect for same sampling depth, with the replications treated as a random effect. A two-way ANOVA with depth and treatment as the main effects was performed to calculate the Fisher LSD (Least Significant Difference) that was used to separate the means between treatments and between sampling depths. A general linear model (GLM) was used to assess the effects of both treatment and depth on C and N concentrations of bulk soils, aggregates, and nonhydrolyzable fraction (NHF). Significance was reported at $P < 0.05$ from each treatment and depth separately.

RESULTS AND DISCUSSION

The Nonhydrolyzable Carbon Concentration in Bulk Soils

The NHC concentration in bulk soil ranged from 2.7 to 26.1 g kg^{-1} with a mean value of 7.9 g kg^{-1}, varying largely among treatments (Table 1). For all sampling depths, the highest NHC concentration of 16.7 g kg^{-1} was observed under forest and the lowest concentration under CTc. Differences in NHC concentrations were significant ($P < 0.05$) among treatments except for that between NTc and Meadow. Overall, soils under NTc and NTcs contained, respectively, 78% and 47% more NHC than soil under CT. NHC concentrations averaged across treatments decreased with depth from 12.8 g C kg^{-1} in the top depth interval to 4.9 g C kg^{-1} in the third depth interval. As shown in Table 1, the NHC concentration in the top depth interval was highest under forest, followed by meadow, NTc, NTcs, and CTc. Differences among treatment means were significant except for those between NTc and meadow. Following the general trend, the NHC concentration of each treatment also decreased with depth, except for CTc treatment which showed little variation with depth due to the homogenization effect of plowing.

Under NT, the soil from 0 to 5 cm depth contained significantly more NHC under NTc than under NTcs, suggesting that corn residue might have higher resistance to microbial decomposition than that of soybean. This could be one reason why the mean SOC pool in surface soil under continuous corn was greater than that under corn-soybean in the present study and in others (Hao et al., 2002; Paustian et al., 1997).

The correlation analysis conducted on the data in Table 1 indicated that the NHC concentration was positively correlated with total SOC concentration regardless of the land use, management, and sampling depth ($r = 0.97$, $P < 0.001$). On average, the NHC fraction accounted for 42% of the total SOC, ranging from 45% in the 0 to 5-cm depth to 40% in the 10 to 20-cm depth, indicating a decrease in the size of the NH fraction with depth, as reported by Stevenson (1994) and Paul et al. (2001). Grouped by treatment, the highest NHC proportion (% of total SOC) was ob-

SS-06/04-148440-Tan 5/7/04 4:11 PM Page 4

TABLE 1

Total and nonhydrolyzable C concentrations in bulk soils

Treatment	Depth cm	Bulk density		Soil organic matter (SOM)				NHF†				NHC/SOC %
		Mg m^{-3}	St dev	g C kg^{-1}	St dev	C:N	St dev	g C kg^{-1}	St dev	C:N	St dev	
CTc	0–5	1.49	0.08	9.4	0.2	10.3	0.2	3.4	0.1	16.6	1.4	36.2
NTc		1.37	0.09	28.3	0.5	12.0	0.5	11.3	0.6	21.7	1.3	40.1
NTcs		1.42	0.08	19.1	0.6	10.5	0.6	7.9	0.3	23.7	1.3	41.2
Meadow		1.32	0.10	26.6	0.4	10.7	0.4	12.0	0.6	24.6	1.8	45.3
Forest		1.05	0.11	45.8	2.1	13.8	2.1	26.1	1.3	45.2	0.5	57.1
CTc	5–10	1.69	0.09	9.4	0.4	9.6	0.4	3.5	0.2	16.4	0.2	36.8
NTc		1.64	0.11	11.9	0.7	9.6	0.7	4.8	0.1	17.2	0.6	40.1
NTcs		1.57	0.10	11.2	0.0	9.6	0.0	4.3	0.0	21.0	1.5	38.6
Meadow		1.58	0.10	13.6	0.6	9.8	0.6	5.3	0.2	21.5	1.4	39.3
Forest		1.26	0.18	25.6	1.3	11.8	1.3	13.0	1.0	42.3	0.6	50.6
CTc	10-20	1.74	0.11	10.3	0.1	10.0	0.1	4.0	0.3	17.4	1.1	39.0
NTc		1.74	0.05	7.5	0.5	8.9	0.5	2.7	0.2	14.7	1.5	35.3
NTcs		1.59	0.12	9.7	0.3	9.3	0.3	3.8	0.2	18.8	2.4	38.8
Meadow		1.63	0.09	7.9	0.1	8.6	0.1	3.0	0.1	16.1	2.8	37.3
Forest		1.55	0.17	21.8	1.6	11.0	1.6	11.1	0.9	42.7	0.9	50.9
LSD (0.05)		0.14		1.5		0.7		1.0		2.6		20
CTc	All depths	1.64	0.13	9.7	0.5	10.0	0.3	3.6	0.3	17.0	0.5	37.0
NTc		1.58	0.19	16.4	10.9	10.2	1.6	6.5	4.5	17.9	3.6	38.3
NTcs		1.53	0.09	13.3	5.0	9.9	0.6	5.3	2.2	21.1	2.5	39.5
Meadow		1.51	0.16	16.0	9.6	9.7	1.0	6.8	4.7	20.8	4.3	40.8
Forest		1.29	0.25	31.1	12.9	12.2	1.5	16.7	8.2	43.4	1.6	52.9
LSD (0.05)		0.10		0.8		0.4		0.6		1.5		1.2

†Non-hydrolyzable fraction and non-hydrolyzable C (NHC) concentration is on the basis of NHF mass.

served under forest (53%), whereas the least was measured under CTc (37%), but there were no substantial differences in NHC proportion among CTc, NTc, and NTcs treatments. More importantly, however, the proportion of NHC increased with total SOC ($r = 0.88$, $P < 0.001$) (Fig. 1), being in agreement with results for the soils of the Midwestern U.S. (Paul et al., 2001). These data imply that the conversion from forest to cultivation results mainly in a loss of the NHF of SOM.

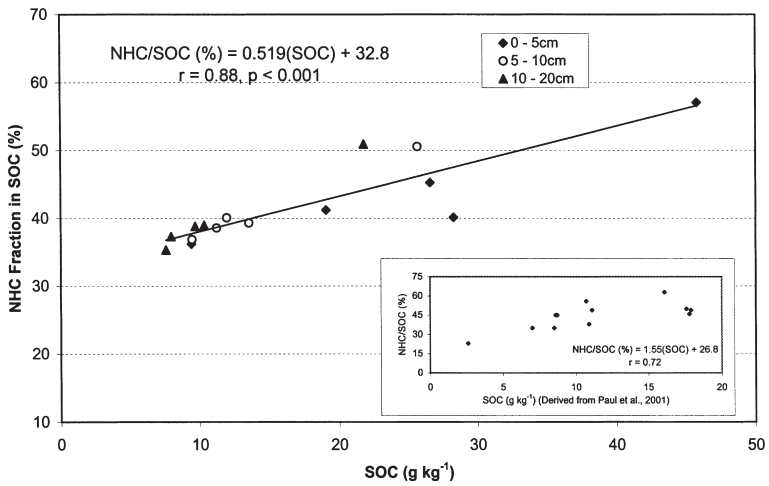

Fig. 1. Relationship between nonhydrolyzable carbon (NHC) proportion and total soil organic carbon (SOC).

SS-06/04-148440-Tan 5/7/04 4:11 PM Page 5

The C:N ratios in the NHF were strikingly different from that in whole SOM (24.4 vs. 10.4, derived from Table 1) and were also differentiated by treatment and sampling depth (Table 1). The variation of the C:N ratios among land uses agreed with that of SOC concentrations but was relatively less influenced by management practices. For example, the lack of significant differences in C:N ratios of the NHF between CTc and NTc may be attributed to their similar cropping history with C_4 corn. NTcs and meadow had similar C:N ratios in the NHF because of their comparable cropping background of C_3-C_4 crop rotation. The data provided by $d^{13}C$ analysis on the same samples as those used in this study (Puget et al., unpublished data) indicate that the amount of corn-derived SOC in the top 20 cm depth was 3.1 kg m^{-2} under NTc and only 1.6 kg m^{-2} under NTcs. However, the nonhydrolyzable C:N ratios under both NTcs and meadow were substantially greater (but much less than in forest) than those under both CTc and NTc. These data suggest that the SOC derived from corn residues (C_4) have a lower nonhydrolyzable C:N ratio than that from soybean or C_3 crop. In addition, the nonhydrolyzable C:N ratio decreased significantly with sampling depth except for CTc, which is in agreement with the results documented by Collins et al. (2000). Furthermore, the nonhydrolyzable C:N ratio was positively a function of the NHC concentration, as indicated by the data in Table 1 (C:N ratio = 5.42 Ln(NHC), $r = 0.87$, $P < 0.01$), suggesting that the accumulation of the NHC be the result primarily of the C components with enhanced C:N ratios.

NHC Mass Distribution among Water Stable Aggregates

Only the data from the NT_C treatment were used for comparison with CT_C and forest treatments because both NT_C and CT_C had the same cropping system and organic matter origin. Data in Table 2 indicate that the soil under CT was composed mainly of microaggregates whereas the soils under NT and forest were dominated by macroaggregate fractions, especially by aggregates of the 250 to 2000-μm fraction.

The data in Table 2 show that the NHC concentration generally increased with aggregate size but decreased with sampling depth, except for those under CTc where the NHC fraction was evenly distributed among sampling depths, and the highest NHC concentration occurred in aggregates 250 to 2000 μm in size. The NHC concentrations averaged across these three treatments for aggregates <53, 53 to 250, 250 to 2000, and >2000 μm were 5.8, 6.3, 8.5, and 9.8 g C kg^{-1} fractions, respectively. A big difference was observed between aggregates greater than 250 μm

TABLE 2

Characteristic comparison of water stable aggregates in top the 20 cm depth

Treatment	Aggregate		SOM				NHF†			
	Size	Mass	g C kg^{-1}		C:N		g C kg^{-1}		C:N	
	μm	%	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
CTc	<53	27.2	9.3	0.2	8.0	0.6	3.1	0.6	14.8	2.8
	53–250	35.7	8.3	0.4	8.0	0.4	2.7	0.6	14.9	2.9
	250–2000	28.2	12.2	1.0	9.4	0.2	4.2	0.7	17.7	3.5
	>2000	9.0	10.9	0.4	8.8	1.0	3.1	0.5	15.1	3.1
NTc	<53	14.6	10.8	7.4	8.2	4.8	3.9	3.3	13.5	6.5
	53–250	32.1	11.7	8.2	8.8	3.7	4.1	3.3	14.3	6.3
	250–2000	39.9	17.7	11.2	11.9	6.4	6.9	5.5	17.1	5.9
	>2000	13.3	19.1	10.3	11.7	4.7	7.2	5.0	17.2	5.6
Forest	<53	16.4	20.3	10.3	12.3	3.1	10.5	7.8	33.2	15.1
	53–250	26.3	24.0	13.4	13.3	3.4	12.2	10.1	32.4	14.8
	250–2000	44.0	28.3	13.3	15.5	4.5	14.5	10.7	35.6	12.4
	>2000	13.4	35.2	14.4	16.9	5.3	19.3	12.0	39.7	9.5
All treatments & depths	<53	19.4	13.5	8.2	9.5	3.6	5.8	5.5	20.5	12.7
	53-250	31.3	14.6	10.6	10.0	3.6	6.3	6.9	20.5	12.1
	250–2000	37.4	19.4	11.2	12.3	4.7	8.5	7.6	23.5	11.6
	>2000	11.9	21.8	13.9	13.4	5.4	9.8	9.8	24.0	13.1
LSD	(0.05)	15.1	10.7		7.3		12.3		11.9	

†Non-hydrolyzable fraction and non-hydrolyzable C (NHC) concentration is on the basis of NHF mass.

SS-06/04-148440-Tan 5/7/04 4:11 PM Page 6

and those less than 250 μm, except for the 10 to 20-cm depth interval. The NHC concentration under forest was 1 to 3 times higher than that under NTc for all aggregate fractions and sampling depths. However, no significant differences in aggregate-associated NHC concentration were observed between NT and CT for 5 to 10-cm and 10 to 20-cm depths. The NHC concentration under CT was the lowest and the least variable among all aggregate classes and was especially lower in aggregates greater than 2000 μm than in aggregates 250 to 2000 μm for all sampling depths, suggesting that the SOM responsible for formation aggregates less than 2000 μm under CT treatment is dominated by hydrolyzable components. This may be attributed to soil disturbance and mixing by plowing caused by: (i) the periodic breakdown of soil structure towards smaller size fractions under CT, resulting in a permanently young age for the newly formed macroaggregates and their binding organic matter, and (ii) the breakdown of soil structure, which leads to the exposure of the SOC trapped inside all macroaggregates greater than 250 μm, enhances its oxidation, and shortens the life span of affected C components (Puget et al., 1999; Six et al., 2000a; Plante and McGill, 2002).

The C:N ratio of the NHF was related highly to aggregate-associated NHC concentration, and it increased with an increase in NHC concentration of respective aggregates but differed among treatments (Fig. 2). This ratio was less than 20 and had the least variation under CT treatment and the highest under forest, where the C:N ratio increased with an increase in aggregate-associated NHC concentration following a logarithm function model ($r = 0.98$, $P < 0.001$). The C:N ratio

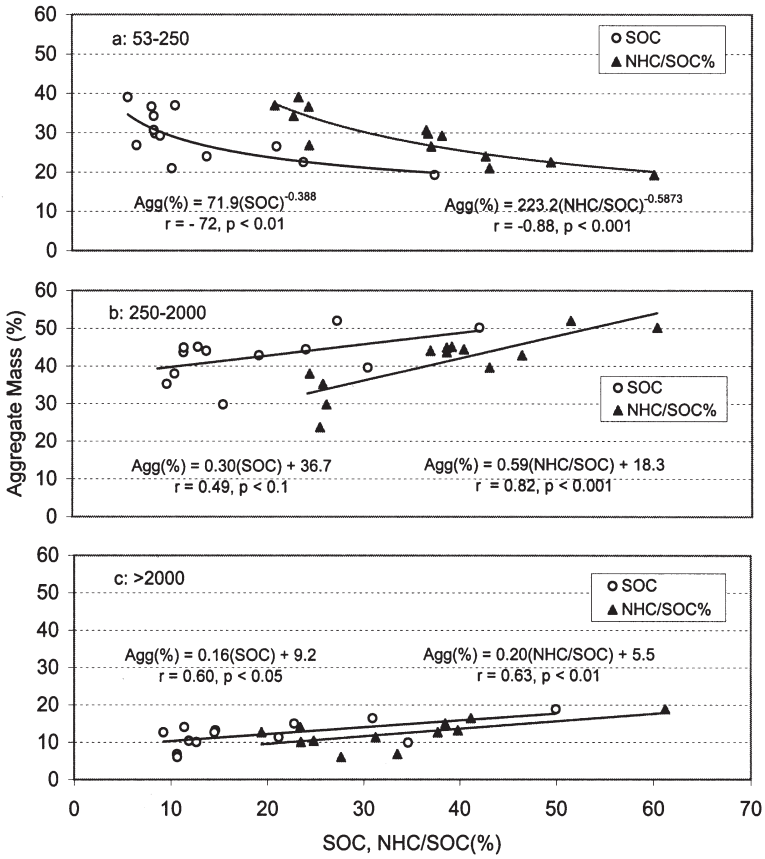

Fig. 2. Relationships between aggregate mass proportion and either SOC or NHC concentrations for each aggregate size class: (a) 53–250 μm; (b) 250–2000 μm; (c) >2000 μm.

(Note: Data from 0 to 5 cm depth under CT were not included because plowing artificially destroys macroaggregates and leads to a predominant proportion of the 53 to 250 μm aggregates).

SS-06/04-148440-Tan 5/7/04 4:11 PM Page 7

in the NHF generally increased with increase in aggregate size (except for aggregates > 2000 μm).

Aggregation as Related to the Nonhydrolyzable Fraction

Aggregates greater than 2000 μm and 250 to 2000 μm enriched SOC by 36% and 12%, respectively, compared with bulk soils (Table 1 vs Table 2). These results are in accord with the hierarchy theory of soil structure that suggests that the surplus amount of SOC acts as a binding agent between microaggregates to form macroaggregates (Angers and Giroux, 1996; Oades and Waters, 1991; Puget et al., 1995; Six et al., 2000a). In general, the added SOM responsible for soil aggregation is less humified and likely corresponds to particulate organic matter (POM) (Gale et al., 2000; Jastrow, 1996; Puget et al., 2000). According to these investigations and the currently accepted SOM model, the extra proportion of SOM occluded in macroaggregates versus that in microaggregates is more transient, fresher, and younger, and is not likely associated with NHC. This is, however, not necessarily true for the formation of aggregates 250 to 2000 μm. Because both aggregates greater than 2000 μm and those 250 to 2000 μm were also enriched with more NHC by 27% and 13%, respectively, compared with bulk soils, but aggregates 250 to 2000 μm contained much less hydrolyzable SOM (by 9% C in mass) than aggregates greater than 2000 μm. Furthermore, the mass proportion of aggregates 250 to 2000 μm in size significantly increased with an increase in the proportion NHC/SOC (in %) ($r = 0.82$, $P < 0.001$, Fig. 3 (b)), whereas the mass proportion of aggregates 53 to 250 μm decreased with an increase in either the NHC concentration or the proportion NHC/SOC, following a negative power model ($r = -0.88$, $P < 0.001$). Probably, more microaggregates 53 to 250 μm will be bound by NHC components to form aggregates 250 to 2000 μm if there are more NHC components available in soils that are dominated by 2:1 clay minerals, with neither oxides nor carbonates, such as the soils used in the present study.

The profile presented in Fig. 3 may actually describe the dynamics of different aggregates in response to SOM dynamics. It quantitatively confirms the hierarchy theory of soil structure and interprets both physically and biochemically protected mechanisms involved in SOC sequestration. Fig. 3 indicates that a soil whose SOC concentration is lower than 10 g kg^{-1} is usually dominated by microaggregates with few macroaggregates. With an increase in SOC concentration or the proportion NHC/SOC in this soil, the formation of aggregates 250 to 2000 μm takes place by entrapping free microaggregates (note that C contained in free microaggregates has a high resistance (Jastrow et al., 1996; Six et al., 2000a)), resulting in a decrease in the amount of microaggregates. Meanwhile, macroaggregates > 2000 μm form from newly formed aggregates of 250 to 2000 μm. Assuming that the aggregation follows the mass balance principle, the loss of microaggregate quantity should be equivalent to the gain of all macroaggregates. Therefore, the depletion rate of microaggregates is greater than

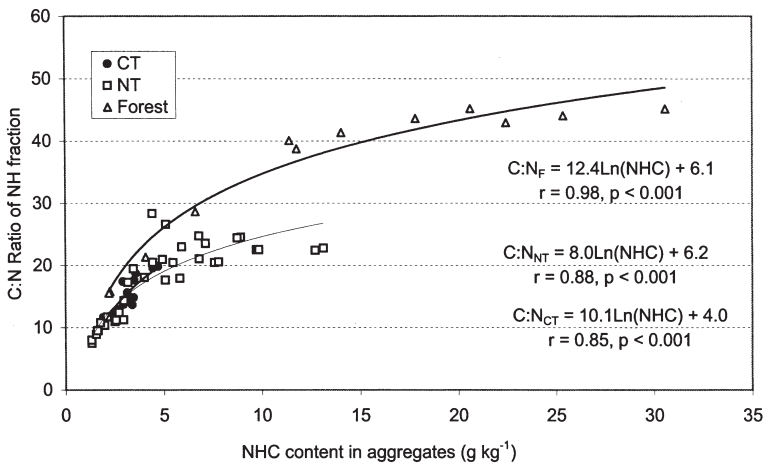

Fig. 3. Relationships between C/N ratio of nonhydrolyzable fraction and aggregate-associated NHC content in aggregates (NT includes NTc, NTcs, and meadow treatments).

SS-06/04-148440-Tan 5/7/04 4:11 PM Page 8

the formation rate of each macroaggregate fraction. Figure 3 also shows that the formation rate of aggregates 250 to 2000 μm in size depends first on the availability of SOM and then on the availability of microaggregates 53 to 250 μm. Similarly, the formation rate of macroaggregates greater than 2000 μm relies first on the availability of SOM and then on that of aggregates of 250 to 2000 μm. This may be the reason why the aggregation rate is smaller for aggregates greater than 2000 μm than for aggregates 250 to 2000 μm, as shown in Fig. 3 (b and c) and why the model describing the depletion of microaggregate with an increase in either SOC or the proportion NHC/SOC differs from that describing the formation of macroaggregates.

The contribution of aggregate-associated NHC to total NHC in bulk soil presented in Fig. 4 is basically similar to the distribution pattern of aggregate masses. Of the total NHC contained in bulk soils, about 50% was associated with aggregates 250 to 2000 μm in both 0 to 5-cm and 5 to 10-cm depths, except for 0 to 5-cm depth under CT in which only 23% was contributed by aggregates 250 to 2000 μm and about 70% by aggregates less than 250 μm. The contribution of aggregates greater than 2000 μm varied widely with treatments and sampling depths: substantially more in meadow and forest but less in other treatments and the least under CT treatment. These results suggest that soil aggregation is coupled with the accumulation of NHC and that NHC acts as a major binding agent for the formation of aggregates 250 to 2000 μm. This, however, seems to contradict the currently accepted model, which suggests that macroaggregates are bound by transient, fresh, and young OM (Kay, 1998; Miller and Jastrow, 1990; Oades and Waters. 1991; Tisdall and Oades, 1979), not materials likely to be associated with the NHC. The question remains: are all fresh and young OM hydrolyzable? And, if so, what is the source of the

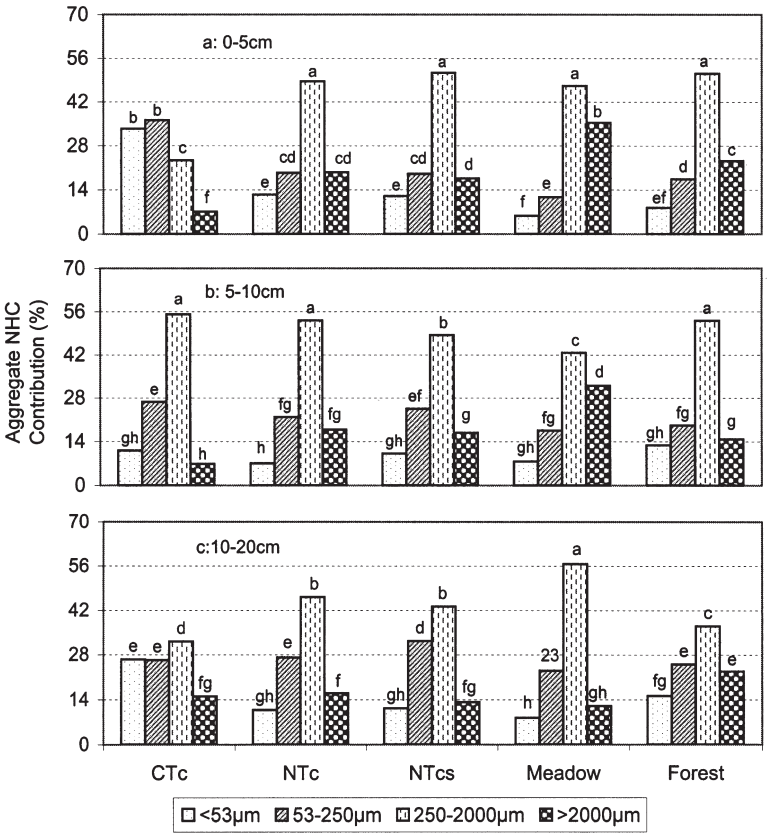

Fig. 4. Mass contribution of aggregate-associated nonhydrolyzable carbon (NHC) to the total NHC in bulk soils (as 100%) (same letter along the top of individual aggregate class bars for same depth means no significant difference at $P < 0.05$).

SS-06/04-148440-Tan 5/7/04 4:11 PM Page 9

NHC? The data presented indicate that neither all binding OM of macroaggregates is hydrolyzable nor is all such SOM fresh and young. It is possible for some free NHC materials or charcoal particles either to participate in (as a binding agent) the formation of macroaggregates or to be bound along with other microaggregates to form macroaggregates. The physical protection under minimally disturbed soils makes the young C components occluded within aggregates become older with time. Moreover, that the amount of NHC in surface soil was larger (except for CT treatment) than that in deeper soil also implies that not all OM accumulated in upper soil layers must be hydrolyzable. Golchin et al. (1994) proposed that during decomposition of the organic materials associated with soil aggregates, more labile portions of those organic materials are consumed, and the more resistant plant structure materials are concentrated as occluded particles within the aggregates. Compton and Boone (2000; 2002) suggested that the light fraction of SOM has a similar recalcitrance to that of the heavy fraction of SOM. Therefore, it is important to examine the recalcitrance of the SOM occluded within macroaggregates but not that within microaggregates.

SUMMARY

Influences of land use and soil management practices on the amount and nature of SOC entrapped in aggregates can be explained to a great extent by the NH fraction. Conversion from forest to cultivation has led mainly to a reduction in NHC. The contribution of the NHC fraction increased with total SOC concentration, increasing aggregate-associated NHC with aggregate size, especially under forest where there were substantial differences among all aggregate fractions in the 0 to 5-cm depth. This finding indicates the important role of the NHC fraction in soil aggregation and aggregate stabilization. The conversion from CT to NT led to an enrichment of NHC in all aggregate fractions with the largest increase occurred in aggregates 250 to 2000 μm, suggesting that the capacity of preserving SOC depends on the quantity of aggregates greater than 250 μm and the NH fraction of SOM. The formation of macroaggregates is coupled with the depletion of microaggregates, which can be quantitatively described by a series of functions expressed in terms of the aggregate mass and the proportion NHC/SOC for each aggregate fraction. Because a much higher NHC concentration and C:N ratio in the NHF were observed in association with aggregates greater than 2000 μm in meadow and forest soils, there may be a large potential for SOC sequestration through biochemical protection mechanisms. The NHF is, to a greater extent, responsible for the long-term SOC sequestration.

REFERENCES

Anderson, D. W., and E. A. Paul. 1984. Organomineral complexes and their study by radiocarbon dating. Soil Sci Soc Am J. 48:298–301.

Angers, D. A., and M. Giroux. 1996. Recently-deposited organic matter in soil water-stable aggregates. Soil Sci. Soc. Am. J. 60:1547–1551.

Becker-Heidmann, P., and H. W. Scharpenseel. 1992. Studies of soil organic matter dynamics using natural carbon isotopes. Sci. Total Environ. 117:305–312.

Burke, I. C., E. T. Elliott, and C. V. Cole. 1995. Influence of macroclimate, landscape position and management on soil organic matter in agroecosystems. Ecol. Appl. 5:124–131.

Cambardella, C. A., and E. T. Elliott. 1993. Carbon and nitrogen mineralization in aggregates from cultivated and native grassland soils. Soil Sci. Soc. Am. J. 57:1071–1076.

Collins, H. P., E. T. Elliott, K. Paustian, L. G. Bundy, W. A. Dick, D. R. Huggins, A. J. M. Smucker, and E. A. Paul. 2000. Soil carbon pools and fluxes in long-term Corn Belt agroecosystems. Soil Biol. Biochem. 32:157–168.

Compton, J. E., and R. D. Boone. 2000. Long-term impacts of agriculture on soil carbon and nitrogen in New England forests. Ecology 81:2314–2330.

Compton, J. E., and R. D. Boone. 2002. Soil nitrogen transformations and the role of light fraction organic matter in forest soils. Soil Biol. Biochem. 34:933–943.

Elliott, E. T. 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50:518–524.

Gale, W. J., C. A. Cambardella, and T. B. Bailey. 2000. Root-derived carbon and the formation and stabilization of aggregates. Soil Sci. Soc. Am. J. 64:201–207.

Golchin, A., J. M. Oades, J. O. Skjemstad, and P. Clarke. 1994. Soil structure and carbon cycling. Aust. J. Soil Res. 32:1043–1068.

Hao, Y., R. Lal, L. B. Owens, R. C. Izaurralde, W. M. Post, and D. L. Hothem. 2002. Effect of cropland management and slope position on soil organic carbon pool at the Appalachian Experimental Watersheds. Soil Tillage Res. 68:133–142.

Hendrix, P. F., A. J. Franzluebbers, and D. V. McCracken. 1998. Management effects on C accumulation and loss in soils of the southern Appalachian Piedmont of Georgia. Soil Tillage Res. 47:245–251.

Jastrow, J. D. 1996. Soil aggregate formation and the ac-

SS-06/04-148440-Tan 5/7/04 4:11 PM Page 10

crual of particulate and mineral-associated organic matter. Soil Biol. Biochem. 28:665–676.

Jastrow, J. D., T. W. Boutton, and R. M. Miller. 1996. Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. Soil Sci. Soc. Am. J. 60:801–807.

Jastrow J. D., and R. M. Miller. 1998. Soil aggregate stabilization and carbon sequestration: Feedbacks through organomineral associations. *In* Soil Processes and the Carbon Cycle. R. Lal, J. Kimble, R. Follett, and B.A. Stewart (eds.). CRC Press, Boca Raton, FL, pp. 207–223.

Jenkinson, D. S., and J. H. Rayner. 1977. The turnover of soil organic matter in some of the Rothamsted classic experiments. Soil Sci. 123:298–305.

Kay, B. D. 1998. Soil Structure and Organic Carbon: A Review. *In* Soil Processes and the Carbon Cycle. R. Lal, J. M. Kimble, R.F. Follett, and B.A. Stewart (eds.). CRC Press, Boca Raton, FL, pp. 169–197.

Kelley, G. E., W. M. Edwards, L. L. Harrold, and J. L. McGuinness. 1975. Soils of the North Appalachian Experimental Watershed. Miscellaneous Publication No. 1296. ARS and SCS-USDA, Washington DC.

Kemper, W. D., and R. Roseneau. 1986. Aggregate stability and size distribution. *In* Methods of Soil Analysis, Part I. Physical and Mineralogical Methods. A.L. Page (ed.). SSSA, Madison, WI, pp. 425–442.

Kern, J. S., and M. G. Johnson. 1993. Conservation tillage impacts on national soil and atmospheric carbon levels. Soil Sci. Soc. Am. J. 57:200–210.

Körschens, M. 1996. Long-term datasets from Germany and Eastern Europe. *In* Evaluation of Soil Organic Matter Models Using Existing, Long-Term Datasets. NATO ASI series No. 1, Vol. 38. D.S. Powlson, P. Smith, and J.U. Smith (eds.). Springer, Berlin, pp. 69–80.

Leavitt, S. W., R. F. Follett, and E. A. Paul. 1996. Estimation of slow- and fast-cycling soil organic carbon pools from 6N HCl hydrolysis. Radiocarbon 38:231–239.

Metherell, A. K., L. A. Harding, C. V. Cole, and W. J. Parton. 1993. CENTURY soil organic matter model environment. Technical documentation, agroecosystem version 4.0. Great Plains System Research Unit Technical Report No. 4. USDA-ARS, Fort Collins, CO.

Miller, R. M., and J. D. Jastrow. 1990. Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biol. Biochem. 22:579–584.

Oades, J. M., and A. G. Waters. 1991. Aggregate hierarchy in soils. Aust. J. Soil Res. 29:815–828.

Parton, W. J., Schimel, D. S., Cole, C. V., and D. Ojima. 1987. Analysis of factors controlling soil organic levels of grasslands in the Great Plains. Soil Sci Soc Am J. 58:530–536.

Paul, E. A., H. P. Collins, S. W. Leavitt. 2001. Dynamics of resistant soil carbon of Midwestern agricultural soils measured by naturally occurring ^{14}C abundance. Geoderma 104:239–256.

Paul, E. A., R. F. Follett, S. W. Leavitt, A. Halvorson, G. A. Peterson, and D. J. Lyon. 1997. Radiocarbon dating for determination of soil organic matter pool sizes and dynamics. Soil Sci. Soc. Am. J. 61: 1058–1067.

Paul, E. A., W. R. Horwath, D. Harris, R. Follett, S. W. Leavitt, B. A. Kimball, and K. Pregitzer. 1995. Establishing the pool sizes and fluxes in CO2 emissions from soil organic matter turnover. *In* Soils and Global Change. R. Lal, J. Kimble, E. Levine, and B.A. Stewart (eds.). CRC Press, Boca Raton, FL, pp. 297–304

Paul, E. A., D. Harris, H. P. Collins, U. Schulthess, and G. P. Robertson. 1999. Evolution of CO2 and soil carbon dynamics in biologically managed, row-crop agroecosystems. Appl. Soil Ecol. 11:53–65.

Paustian K., H. P. Collins, and E. A. Paul. 1997. Management controls in soil carbon. *In* Soil Organic Matter in Temperate Agroecosystems; Long-Term Experiments in North America. E. A. Paul et al. (eds.). CRC Press, Boca Raton, FL, pp 15–49

Plante, A. F., and W. B. McGill. 2002. Intraseasonal soil macroaggregate dynamics in two contrasting field soils using labeled tracer spheres. Soil Sci. Soc. Am. J. 66:1285–1295.

Puget, P., D. A. Angers, and C. Chenu. 1999. Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. Soil Biol. Biochem. 31:55–63.

Puget, P., J. Balesdent, and C. Chenu. 2000. Dynamics of soil organic matter associated with particle-size fractions of water-stable aggregates. Eur. J. Soil Sci. 51:595–605.

Puget, P., C. Chenu, and J. Balesdent. 1995. Total and young organic-matter distribution in aggregates of silty cultivated soils. Eur. J. Soil Sci. 46:449–459.

Six, J., R. T. Conant, E. A. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241:155–176.

Six, J., E. T. Elliott, K. Paustian, and J. W. Doran. 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Sci. Soc Am J. 62:1367–1377.

Six, J., E. T. Elliott, and K. Paustian. 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Sci. Soc. Am. J. 63:1350–1358.

Six, J., E. T. Elliot, and K. Paustian. 2000a. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32: 2099–2103.

Six, J., K. Paustian, E. T. Elliott, and C. Combrick. 2000b. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Sci. Soc. Am. J. 64:681–689.

Sollins, P., P. Homann, and B. A. Caldwell. 1996. Stabilization and destabilization of soil organic matter: Mechanisms and controls. Geoderma 74:65–105.

SS-06/04-148440-Tan 5/7/04 4:11 PM Page 11

Stevenson, F. J. 1994. Humus Chemistry: Genesis, Composition and Reactions, 2nd Ed. John Wiley, New York.

Tisdall, J. M., and J. M. Oades. 1979. Stabilization of soil aggregate by the root systems of rye grass. Aust. J. Soil Res. 17:429–441.

Whitbread, A. M. 1994. Soil organic matter: Its fractionation and role in soil structure. *In* Soil Organic Matter Management for Sustainable Agriculture, ACIAR workshop, Ubon, Thailand, August 24–26, 1994. R.D.B. Lefroy, G.J. Blair, and E.T. Craswell (eds.). ACIAR, Canberra, Australia, 124–131