Acid hydrolysis of easily dispersed and microaggregate-derived silt- and clay-sized fractions to isolate resistant soil organic matter

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Summary

The current paradigm in soil organic matter (SOM) dynamics is that the proportion of biologically resistant SOM will increase when total SOM decreases. Recently, several studies have focused on identifying functional pools of resistant SOM consistent with expected behaviours. Our objective was to combine physical and chemical approaches to isolate and quantify biologically resistant SOM by applying acid hydrolysis treatments to physically isolated silt- and clay-sized soil fractions. Microaggegrate-derived and easily dispersed silt- and clay-sized fractions were isolated from surface soil samples collected from six long-term agricultural experiment sites across North America. These fractions were hydrolysed to quantify the non-hydrolysable fraction, which was hypothesized to represent a functional pool of resistant SOM. Organic C and total N concentrations in the four isolated fractions decreased in the order: native > no-till > conventional-till at all sites. Concentrations of non-hydrolysable C (NHC) and N (NHN) were strongly correlated with initial concentrations, and C hydrolysability was found to be invariant with management treatment. Organic C was less hydrolysable than N, and overall, resistance to acid hydrolysis was greater in the silt-sized fractions compared with the clay-sized fractions. The acid hydrolysis results are inconsistent with the current behaviour of increasing recalcitrance with decreasing SOM content: while %NHN was greater in cultivated soils compared with their native analogues, %NHC did not increase with decreasing total organic C concentrations. The analyses revealed an interaction between biochemical and physical protection mechanisms that acts to preserve SOM in fine mineral fractions, but the inconsistency of the pool size with expected behaviour remains to be fully explained.

Introduction

A current trend in studies of soil organic matter (SOM) dynamics is to develop methods to isolate and quantify functional pools of bioreactive vs. resistant soil organic C (Xu *et al.*, 1997), which is expected to lead to a better understanding of soil C dynamics and better models of these. The goal of any physical or chemical treatment used to isolate biologically resistant SOM is to quantify residual organic materials that are only slowly degraded or metabolized by the soil microbial biomass and its enzymes. A resistant SOM pool could thus be defined as one that is lost slowly after the cultivation of soil under native vegetation, and increases proportionally as total organic C decreases.

A large number of studies have demonstrated that SOM is not distributed evenly across all particle size fractions (e.g.

Anderson *et al.*, 1981; Kiem *et al.*, 2002; Jolivet *et al.*, 2003). The fine silt and coarse clay fractions contained not only the most C, but also the oldest (Anderson & Paul, 1984; Scharpenseel & Becker-Heidmann, 1992; Six *et al.*, 2000). Finer soil particle-size fractions protect SOM by virtue of their reactive surfaces. In addition, silt-sized microaggregates often resist dispersion by even the most rigorous methods, and thus may contain well-protected and therefore resistant SOM. Anderson & Paul (1984) found that the fine clay fraction was younger than coarse clay, and more recent studies have found that silt- and clay-sized fractions can consist of both labile and resistant fractions (Balabane & Balesdent, 1995; Balesdent *et al.*, 1998). The results of these latter studies indicate that physical means alone will not succeed in isolating resistant SOM.

A common chemical treatment to isolate a pool of resistant SOM is acid hydrolysis, originally applied to soil samples to fractionate soil N (Bremner, 1949). Refluxing soil samples in 6 M hydrochloric acid (HCl) removes chemically labile

materials such as proteins, nucleic acids and polysaccharides, leaving behind more resistant compounds such as aromatic, humified components and wax-derived long-chain aliphatics (Martel & Paul, 1974a; Schnitzer & Preston, 1983; Paul *et al.*, 2000). Studies have found that the non-hydrolysable fraction from a wide range of samples represents 35–65% of the total soil C, and is generally 1300–1800 years older than total soil C (Leavitt *et al.*, 1996; Paul *et al.*, 1997b; Paul *et al.*, 2001). While not the oldest material within soil organic matter because of its composite nature, the acid hydrolysis method has been broadly adopted in studies of SOM dynamics, and coupled with ^{14}C-dating is widely used to estimate the size and turn-over rate of the stable SOM pool in models (Leavitt *et al.*, 1996; Falloon *et al.*, 1998; Paul *et al.*, 2000).

The objective of the current study was to combine physical and chemical approaches to isolate and quantify resistant SOM. We applied the acid hydrolysis technique to silt- and clay-sized fractions physically isolated from a range of soils. While acid hydrolysis may isolate a fraction of SOM that is generally older, it is confounded by the fact that a significant proportion of recently deposited plant materials, primarily lignin, may resist hydrolysis (Follett *et al.*, 1997). Pre-treatments such as density fractionations or handpicking of visible plant residues have been adopted to remove particulate organic matter from samples prior to hydrolysis, with the aim of improving the results. We expected that acid hydrolysis of only fine soil fractions (< 53 μm) would minimize the impact of particulate organic matter and focus the analysis on mineral-associated SOM.

Materials and methods

Site descriptions and sampling

Soil samples were collected as part of a larger project from several long-term agricultural field experiments (Table 1). Site,

soil, crop and management characteristics are described in detail in Paul *et al.* (1997a), except for the Breton experiment, where we sampled a long-term tillage-residue-fertilization experiment (Nyborg *et al.*, 1995; Plante *et al.*, 2006) and not the Classical plots. At each site, soil cores were collected manually or by coring truck and separated into 0–5 and 5–20 cm subsamples in the field. Each field experiment replicate from no-till and conventional treatments was sampled, as well as pseudo-replicates from adjacent native forests or grasslands. Three to 10 cores were collected for each replicate (depending on the site) and were returned to the laboratory field-moist for bulk density analysis. The individual core samples for each replicate were combined, then passed through an 8-mm aperture sieve by gently breaking apart the soil by hand. Samples were then air-dried, sieved to 2 mm, and stored at room temperature.

Soil particle-size analysis

Soil particle-size analyses were performed using a modified version of the standard hydrometer method without removal of carbonates or SOM (Gee & Bauder, 1986). Briefly, 30 g of air-dry, 2-mm sieved soil were shaken for 16 hours in a 250-ml Nalgene bottle with 100 ml of 5 mg l^{-1} sodium hexametaphosphate and 10 glass beads (diameter 6 mm). Soil clay contents were determined using hydrometer readings taken at 1.5 and 24 hours and appropriate interpolation calculations. Sand contents were determined by pouring and washing the suspension over a 53-μm aperture sieve after the sedimentation was completed. Materials retained on the sieves were oven dried at 60°C and weighed. Soil silt contents were determined by difference.

Physical fractionation

Whole-soil samples were partially dispersed using the micro-aggregate isolation method of Six *et al.* (2002). Fifty grams of

Table 1 Soil, climate and treatment characterization for long-term field experiments sampled in the current study

Site	Location	Mean annual air temperature/°C	Mean annual precipitation/mm	Soil classification by US Soil Taxonomy	Soil textural class	Native vegetation type
AB	Breton, Alberta, Canada (53°07′ N, 114°28′ W)	2.1	547	Typic Cryoboralf	Silty clay loam	Forest
CO	Akron, Colorado, USA (40°09′ N, 103°09′ W)	9.2	420	Aridic Paleustoll	Silt loam	Grassland
GA	Watkinsville, Georgia, USA (33°54′ N, 83°24′ W)	17	1252	Typic Kanhapludult	Clay	Grassland
KY	Lexington, Kentucky, USA (38°07′ N, 84°29′ W)	13	1140	Typic Paleudalf	Silt loam	Grassland
OH	Hoytville, Ohio, USA (41°13′ N, 84°45′ W)	9.5	845	Mollic Ochraqualf	Silty clay loam	Forest
SC	Swift Current, Saskatchewan, Canada (50°17′ N, 107°48′ W)	3.5	358	Aridic Haploboroll	Silt loam	Grassland

air-dried whole-soil were slaked in deionised water for 30 minutes and then partially dispersed in the isolator chamber by shaking (120 rev minutes^{-1}) with 50 glass beads (diameter 6 mm) until all macroaggregates were disrupted. The < 250 μm materials were continuously flushed out of the shaker onto a 53-μm aperture sieve by a water flow. The materials retained on the 53-μm sieve were wet sieved for 2 minutes at approximately 25 cycles per minute, and the finer materials were gently washed off to isolate the microaggregate fraction (53–250 μm). The easily dispersed silt-sized and clay-sized fractions were isolated from the suspension passing the 53-μm sieve by centrifugation. The suspension was spun at 127 *g* for 7 minutes to separate the silt-sized fraction. The supernatant was subsequently separated, flocculated with 0.25 M CaCl$_2$-MgCl$_2$ and spun at 1730 *g* for 15 minutes to separate the clay-sized fraction. The isolated microaggregate fraction was subjected to a density separation procedure to isolate free and intra-aggregate particulate organic matter (POM) by means of 1.85-g cm^{-3} sodium polytungstate solution, then dispersed by shaking overnight with 12 glass beads (diameter 4 mm) in deionised water. The microaggregate-derived silt- and clay-sized fractions were separated by centrifugation as described for the easily dispersed fractions. All fractions were oven dried at 60°C and weighed.

Acid hydrolysis

The easily dispersed and microaggregate-derived silt- and clay-sized fractions were subjected to acid hydrolysis with a modified version of the method described by Paul *et al.* (1997b). Briefly, 0.3–0.5 g of sample was refluxed at 95°C for 16 hours in 25 ml of 6 M HCl. If insufficient material was recovered during the physical fractionation, individual replicates were combined. After refluxing, the suspension was filtered and washed with deionised water over a glass-fibre filter. The residue was oven-dried at 60°C, and weighed. The hydrolysability of samples is expressed as the percentage of non-hydrolysable C or N (%NHC or %NHN), and was calculated using the following equation, which accounts for mass loss of the sample during hydrolysis and incomplete recovery during filtration:

$$\%NHC, \%NHN = \frac{\left(\frac{g\,C, N}{kg\,sample}\right)_{after} \times \frac{mass_{after}}{mass_{after}}}{\left(\frac{g\,C, N}{kg\,sample}\right)_{before}},$$

where the terms represent the sample organic C or total N concentrations and masses before and after acid hydrolysis.

Organic carbon and total nitrogen analyses

Total C and N analyses were performed on the isolated silt- and clay-sized fractions before and after acid hydrolysis using a CHN autoanalyser (LECO CHN-1000, Leco Corp.,

St Joseph, MI, USA). Results of weak acid additions for detecting the presence of soil carbonates indicated that carbonates were not present (data not reported), and thus total C concentrations were equated to organic C concentrations.

Statistical analyses

All results are reported as mean ± standard error, unless otherwise noted. Linear relationships between total organic C and non-hydrolysable C, and total N and non-hydrolysable N concentrations in the silt- and clay-sized fractions were determined by Model II regression, also called major axis analysis (Webster, 1997; Legendre & Legendre, 1998), with total organic C or total N as the *x*-axis variable. Estimates for the slope and intercept of the major axis, along with their 95% confidence intervals, were determined using the Model II regression software package (Legendre, 2001). Comparisons of sample hydrolysability (%NHC or %NHN), between sites, depths, treatments and fractions were performed using analysis of variance by PROC MIXED with tests of covariance in SAS v9.1 (SAS Institute, Cary, NC, USA) where sites and replicates were treated as random variables.

Results

Recovery of silt- and clay-sized fractions was significantly different between the fractionation procedures compared with soil particle-size analyses (Table 2). During fractionation, more silt-sized material and less clay-sized material was recovered compared with soil particle-size analysis.

Organic C and total N concentrations in the four fractions isolated from the 0–5 and 5–20 cm depth increments decreased significantly in the order: native > no-till > conventional-till (Tables 3 and 4, Figures 1 and 2). Cultivation of soil resulted generally in a two- to threefold decrease in organic C and total N concentrations, with the largest decrease observed at Breton, AB. The C/N ratios of each fraction were smaller in tilled versus native soils, but showed no differences between no-till and conventional-till (Figure 3). Overall, organic C and total N concentrations were greater ($P < 0.001$ for each of organic C and total N) in clay-sized fractions compared with silt-sized fractions. In addition, microaggregate-derived silt- and clay-sized fractions contained more total N than did easily dispersed fractions ($P = 0.003$ for silt and 0.027 for clay), while only the silt-sized fraction showed a difference in organic C concentrations ($P = 0.005$ for silt and 0.810 for clay). In all sites, depths and treatments combined, the C/N ratio of the isolated fractions decreased in the order: μagg-silt ≥ d-silt > d-clay > μagg-clay.

Concentrations of NHC and NHN in each of the isolated fractions were strongly correlated with total organic C and total N, respectively, across all sites, depths and treatments (Tables 3 and 4). The slopes of the major axes differed significantly ($P < 0.001$) between the four fractions for both

Table 2 Total soil silt- and clay-sized contents of the 0–5 cm depth increments determined by soil particle-size analysis compared with the sum of the easily dispersed (d-) and microaggregate-derived (μagg-) fraction masses recovered by physical fractionation using the Six *et al.* (2002) procedure (mean ± standard error)

Site[1]	Treatment[2]	Silt/%		Clay/%	
		Particle-size	d-Silt + μagg-Silt	Particle-size	d-Clay + μagg-Clay
AB	NF	39.7 ± 2.6	35.5 ± 2.8	23.1 ± 0.7	6.5 ± 1.0
	NT-S-N	40.3 ± 2.2	55.9 ± 1.8	31.0 ± 2.3	10.5 ± 0.7
	CT-S-N	39.5 ± 1.1	55.7 ± 1.7	30.5 ± 1.4	11.9 ± 0.7
	NT-NS-NN	37.5 ± 1.7	52.7 ± 1.5	31.6 ± 2.0	9.9 ± 0.8
	CT-NS-NN	36.3 ± 2.2	54.4 ± 3.0	30.4 ± 3.2	10.7 ± 1.2
CO	NG	40.0 ± 1.5	48.6 ± 2.9	27.0 ± 1.2	11.3 ± 0.7
	NT-WCSF	40.4 ± 2.5	52.1 ± 2.0	27.1 ± 1.4	10.0 ± 0.7
	NT-WF	41.7 ± 3.2	51.9 ± 2.5	26.9 ± 0.08	9.5 ± 0.7
	CT-WF	40.3 ± 6.5	56.4 ± 2.0	29.7 ± 2.2	9.7 ± 0.7
GA	NG	12.3 ± 0.7	14.3 ± 0.8	16.4 ± 0.5	3.8 ± 0.7
	NT	9.6 ± 0.5	18.0 ± 1.0	19.9 ± 0.6	6.6 ± 0.8
	CT	13.6 ± 0.6	23.5 ± 1.2	21.2 ± 0.5	8.1 ± 0.9
KY	NG	68.4 ± 2.0	82.3 ± 1.0	26.0 ± 1.6	9.3 ± 1.0
	NT	67.3 ± 0.7	82.1 ± 2.4	26.9 ± 0.5	9.6 ± 0.4
	CT	68.5 ± 0.4	82.0 ± 2.2	27.2 ± 0.3	11.1 ± 0.7
OH	NF	44.6 ± 1.1	61.5 ± 1.1	38.3 ± 0.9	14.5 ± 1.3
	NT-C**OH**	38.5 ± 0.5	61.2 ± 1.8	41.0 ± 0.8	14.4 ± 0.9
	NT-**C**S	37.2 ± 0.2	59.5 ± 1.2	41.3 ± 0.02	15.0 ± 0.8
	PT-C**OH**	36.4 ± 0.04	63.6 ± 1.5	45.1 ± 0.1	15.5 ± 0.5
	PT-**C**S	36.1 ± 2.3	66.8 ± 0.8	46.5 ± 1.8	13.4 ± 1.3
SC	NG	43.3 ± 3.9	48.7 ± 6.1	25.7 ± 1.8	8.7 ± 1.2
	NT	53.7 ± 2.5	59.6 ± 3.6	24.3 ± 0.5	9.1 ± 1.0
	CT	44.7 ± 3.5	57.3 ± 2.6	30.4 ± 2.1	11.1 ± 1.0

[1]AB, Breton, Alberta; CO, Akron, Colorado; GA, Watkinsville, Georgia; KY, Lexington, Kentucky; OH, Hoytville, Ohio; SC, Swift Current, Saskatchewan.

[2]NF, native forest; NG, native or long-term grassland; NT, no-till; CT, conventional till; S, straw returned after harvest; NS, straw removed after harves; N, urea-N fertilized; NN, no urea-N fertilizer; WCSF, wheat-corn-soybean-fallow rotation; WF, wheat-fallow rotation; COH, corn-oats-hay rotation; CS, corn-soybean rotation. Bold letters represent the crop within the rotation at time of sampling.

organic C and total N (Table 5). It should also be noted that the slopes of the major axes were generally greater than the mean percentage non-hydrolysable C (e.g. slope for easily dispersed silt = 0.625, mean %NHC = 52.9%), and were generally less than the percentage non-hydrolysable N (e.g. slope = 0.222, mean %NHN = 38.3%).

In each fraction, with all sites, depths and treatments combined, organic C was less hydrolysable than N, such that %NHC was significantly greater than %NHN ($P < 0.001$ for each fraction, using a paired *t*-test analysis; Figure 4). The result was that C/N ratios were significantly greater after acid hydrolysis compared with before hydrolysis (data not shown).

Carbon hydrolysability within each of the isolated fractions was not affected by site, depth or treatment. When combined over all of these experimental variables, however, %NHC was significantly different between the fractions ($P < 0.001$): 52.9 ± 1.8% in d-silt, 47.5 ± 1.0% in d-clay, 57.8 ± 0.6% in μagg-silt and 44.3 ± 0.3% in μagg-clay (Figures 5 and 6).

Overall, resistance to acid hydrolysis was greater ($P < 0.001$) in the silt-sized fractions (55.8 ± 0.7%) compared with the clay-sized fractions (46.3 ± 0.5%). In addition, hydrolysability of C within the silt- and clay-sized fractions differed depending on their source, e.g. whether they were easily dispersed or microaggregate-derived. The easily dispersed silt-sized fraction C was less resistant to acid hydrolysis than microaggregate-derived silt C (53.9 ± 1.1% vs. 57.8 ± 0.7%, $P < 0.001$) but conversely, easily dispersed clays were more resistant than microaggregate-derived clays (47.9 ± 0.8% vs. 44.4 ± 0.6%, $P = 0.002$).

Unlike C hydrolysability, %NHN was not statistically different between fractions ($P = 0.198$) when combined over all experimental variables: 38.3 ± 1.2% in d-silt, 34.7 ± 1.6% in d-clay, 33.3 ± 1.8% in μagg-silt and 31.7 ± 0.4% in μagg-clay (Figures 7 and 8). Hydrolysability of N within each of the isolated fractions was not affected by site or depth; however, statistically significant differences

Table 3a Total organic C and non-hydrolysable C concentrations in fractions isolated from the 0–5 cm depth increments (mean ± standard error)

Site[1]	Treatment[2]	Organic C/g 100 g^{-1} fraction				Non-hydrolysable C/g 100 g^{-1} fraction			
		d-Silt	d-Clay	µagg-Silt	µagg-Clay	d-Silt	d-Clay	µagg-Silt	µagg-Clay
AB	NF	3.30 ± 0.22	6.73 ± 0.26	5.28 ± 0.34	9.47 ± 0.48	2.07 ± 0.18	3.97 ± 0.28	3.54 ± 0.17	5.28 ± 0.16
	NT-S-N	1.87 ± 0.11	3.21 ± 0.14	2.15 ± 0.25	2.92 ± 0.19	1.19 ± 0.07	1.76 ± 0.09	1.47 ± 0.18	1.40 ± 0.14
	CT-S-N	1.72 ± 0.07	2.98 ± 0.13	1.91 ± 0.13	2.46 ± 0.11	1.10 ± 0.06	1.69 ± 0.10	1.26 ± 0.09	1.05 ± 0.08
	NT-NS-NN	1.40 ± 0.02	2.31 ± 0.07	1.31 ± 0.06	2.24 ± 0.11	0.88 ± 0.03	1.62 ± 0.28	0.91 ± 0.04	1.03 ± 0.05
	CT-NS-NN	1.42 ± 0.06	2.27 ± 0.08	1.18 ± 0.03	2.19 ± 0.10	1.00 ± 0.05	1.29 ± 0.08	0.84 ± 0.02	1.09 ± 0.09
CO	NG	1.17 ± 0.09	3.01 ± 0.21	1.60 ± 0.15	3.11 ± 0.08	0.49 ± 0.07	1.58 ± 0.15	0.79 ± 0.08	1.39 ± 0.04
	NT-WCSF	0.69 ± 0.03	1.63 ± 0.08	0.91 ± 0.07	1.36 ± 0.11	0.39 ± 0.03	0.89 ± 0.05	0.53 ± 0.02	0.64 ± 0.06
	NT-WF	0.74 ± 0.09	1.71 ± 0.14	0.94 ± 0.03	1.44 ± 0.19	0.40 ± 0.04	0.93 ± 0.09	0.57 ± 0.03	0.71 ± 0.06
	CT-WF	0.76 ± 0.04	1.76 ± 0.07	0.92 ± 0.05	1.53 ± 0.13	0.42 ± 0.04	0.97 ± 0.08	0.54 ± 0.04	0.75 ± 0.09
GA	NG	3.45 ± 0.19	5.81 ± 0.15	4.97 ± 0.32	6.72 ± 0.28	1.89 ± 0.18	2.63 ± 0.16	2.57 ± 0.09	3.53 ± 0.73
	NT	2.10 ± 0.12	2.62 ± 0.22	3.03 ± 0.17	3.14 ± 0.32	1.00 ± 0.08	0.91 ± 0.08	1.67 ± 0.06	1.17 ± 0.13
	CT	1.54 ± 0.07	1.94 ± 0.11	1.78 ± 0.07	2.06 ± 0.12	0.73 ± 0.08	0.62 ± 0.07	0.86 ± 0.08	0.69 ± 0.05
KY	NG	1.73 ± 0.12	3.25 ± 0.25	2.29 ± 0.21	2.73 ± 0.17	0.62 ± 0.06	1.12 ± 0.10	1.05 ± 0.13	1.04 ± 0.07
	NT	1.58 ± 0.07	2.86 ± 0.10	1.89 ± 0.08	2.35 ± 0.07	0.58 ± 0.03	1.02 ± 0.04	0.97 ± 0.08	0.94 ± 0.05
	CT	0.94 ± 0.04	1.99 ± 0.07	1.00 ± 0.07	1.93 ± 0.35	0.33 ± 0.02	0.67 ± 0.03	0.43 ± 0.04	0.84 ± 0.20
OH	NF	6.69 ± 0.33	6.61 ± 0.20	7.56 ± 0.17	5.69 ± 0.37	4.25 ± 0.34	3.78 ± 0.18	5.10 ± 0.17	3.05 ± 0.28
	NT-COH	3.21 ± 0.17	3.21 ± 0.15	3.84 ± 0.22	3.16 ± 0.14	1.58 ± 0.09	1.44 ± 0.10	2.14 ± 0.14	1.62 ± 0.07
	NT-CS	2.43 ± 0.15	2.83 ± 0.03	4.41 ± 1.32	2.76 ± 0.03	1.28 ± 0.09	1.31 ± 0.02	2.40 ± 0.65	1.47 ± 0.04
	PT-COH	1.76 ± 0.05	1.92 ± 0.14	2.21 ± 0.06	1.88 ± 0.07	0.95 ± 0.01	0.94 ± 0.01	1.50 ± 0.03	0.98 ± 0.06
	PT-CS	1.70 ± 0.02	1.90 ± 0.08	2.00 ± 0.12	1.82 ± 0.03	0.89 ± 0.01	0.83 ± 0.01	1.28 ± 0.03	0.95 ± 0.03
SC	NG	2.10 ± 0.21	4.66 ± 0.44	3.18 ± 0.37	4.04 ± 0.52	1.06 ± 0.12	2.36 ± 0.29	1.77 ± 0.20	1.47 ± 0.03
	NT	1.54 ± 0.09	3.97 ± 0.17	2.08 ± 0.09	3.38 ± 0.20	0.78 ± 0.04	2.11 ± 0.09	1.20 ± 0.06	1.53 ± 0.06
	CT	1.44 ± 0.11	3.57 ± 0.24	1.81 ± 0.23	3.37 ± 0.13	0.73 ± 0.07	1.87 ± 0.15	1.04 ± 0.13	1.56 ± 0.05

[1]AB, Breton, Alberta; CO, Akron, Colorado; GA, Watkinsville, Georgia; KY, Lexington, Kentucky; OH, Hoytville, Ohio; SC, Swift Current, Saskatchewan.
[2]NF, native forest; NG, native or long-term grassland; NT, no-till; CT, conventional till; S, straw returned after harvest; NS, straw removed after harvest; N, urea-N fertilized; NN, no urea-N fertilizer; WCSF, wheat-corn-soybean-fallow rotation; WF, wheat-fallow rotation; COH, corn-oats-hay rotation; CS, corn-soybean rotation. Bold letters represent the crop within the rotation at time of sampling.

were found between treatments in each fraction ($P = 0.001$ for d-silt, 0.002 for d-clay, 0.037 for µagg-silt and 0.020 for µagg-clay). In each of the isolated fractions, %NHN increased in the order native < no-till = conventional-till, except in the d-silt fraction where no-till and conventional-till were essentially equal. Scatter in N hydrolysability was greater at small initial N concentrations and tapered as N concentrations increased. This pattern was also apparent in the C hydrolysability data (Figures 5 and 6), but was not as pronounced.

Discussion

The current paradigm of SOM dynamics, supported by a predominantly biochemical model of organic matter decomposition (e.g. Gregorich *et al.*, 1996), suggests that as total SOM decreases, the proportion of resistant SOM will increase. For instance, Kiem *et al.* (2000) found greater decreases of *O/ N*-alkyl C species than aromatic C species in C-depleted soils compared with fertilized soils, which they described as

consistent with the degree of biodegradability of these structures. The CENTURY model (Parton *et al.*, 1987), a reasonable barometer for our current conceptual understanding of SOM dynamics in mineral soils, predicts an increase in the proportion of 'passive C' from 40.8% of total soil C to 77.0% after the conversion from native grassland to long-term conventionally tilled wheat-fallow production at Akron, CO (data not shown). While our N hydrolysis data did show a trend of increasing %NHN with increasing cultivation intensity, our data on C hydrolysis appear to partly contradict the paradigm because we did not see any increase in %NHC upon conversion to agriculture.

These observations of C hydrolysability are, however, supported by previous observations reported in the literature. Martel & Paul (1974a) observed a significant decrease in SOM content and a significant increase in radiocarbon age after 60 years of cultivation of virgin grassland, but they did not observe an appreciable change in the proportion of non-hydrolysable materials. This led the authors to conclude that biochemical recalcitrance played only a minor role in

Table 3b Total organic C and non-hydrolysable C concentrations in fractions isolated from the 5–20 cm depth increments (mean ± standard error)

Site[1]	Treatment[2]	Organic C/g 100 g⁻¹ fraction				Non-hydrolysable C/g 100 g⁻¹ fraction			
		d-Silt	d-Clay	µagg-Silt	µagg-Clay	d-Silt	d-Clay	µagg-Silt	µagg-Clay
AB	NF	0.92 ± 0.13	1.96 ± 0.22	1.54 ± 0.21	2.78 ± 0.46	0.46 ± 0.06	0.91 ± 0.10	0.87 ± 0.12	0.94 ± 0.14
	NT-S-N	1.16 ± 0.11	1.80 ± 0.21	1.28 ± 0.25	1.63 ± 0.27	0.74 ± 0.09	0.99 ± 0.15	0.86 ± 0.17	0.71 ± 0.13
	CT-S-N	1.16 ± 0.16	1.85 ± 0.32	1.15 ± 0.17	1.79 ± 0.39	0.76 ± 0.10	1.11 ± 0.19	0.75 ± 0.12	0.87 ± 0.24
	NT-NS-NN	0.78 ± 0.04	1.19 ± 0.07	0.79 ± 0.06	0.88 ± 0.07	0.42 ± 0.03	0.57 ± 0.06	0.45 ± 0.01	0.31 ± 0.06
	CT-NS-NN	0.89 ± 0.05	1.33 ± 0.09	0.78 ± 0.10	1.03 ± 0.08	0.54 ± 0.05	0.68 ± 0.05	0.51 ± 0.09	0.45 ± 0.06
CO	NG	1.02 ± 0.05	2.29 ± 0.15	1.21 ± 0.10	2.04 ± 0.11	0.46 ± 0.06	1.07 ± 0.11	0.84 ± 0.11	0.93 ± 0.08
	NT-WCSF	0.66 ± 0.01	1.45 ± 0.12	0.81 ± 0.01	1.23 ± 0.11	0.41 ± 0.02	0.74 ± 0.11	0.53 ± 0.03	0.58 ± 0.08
	NT-WF	0.73 ± 0.05	1.53 ± 0.16	0.78 ± 0.07	1.39 ± 0.20	0.33 ± 0.03	0.79 ± 0.16	0.54 ± 0.07	0.68 ± 0.14
	CT-WF	0.73 ± 0.04	1.64 ± 0.15	0.89 ± 0.01	1.41 ± 0.15	0.47 ± 0.02	0.84 ± 0.13	0.54 ± 0.03	0.63 ± 0.10
GA	NG	1.33 ± 0.11	2.27 ± 0.20	1.79 ± 0.12	2.73 ± 0.23	0.63 ± 0.06	0.95 ± 0.10	0.97 ± 0.11	1.02 ± 0.17
	NT	1.12 ± 0.06	1.59 ± 0.17	1.55 ± 0.10	2.00 ± 0.39	0.55 ± 0.06	0.58 ± 0.08	0.73 ± 0.07	0.64 ± 0.16
	CT	1.27 ± 0.07	1.51 ± 0.16	1.58 ± 0.15	1.83 ± 0.24	0.55 ± 0.07	0.50 ± 0.10	0.76 ± 0.11	0.60 ± 0.10
KY	NG	1.12 ± 0.05	2.33 ± 0.10	1.20 ± 0.06	2.29 ± 0.11	0.37 ± 0.02	0.76 ± 0.03	0.66 ± 0.08	0.75 ± 0.13
	NT	1.09 ± 0.03	2.27 ± 0.12	1.31 ± 0.07	2.63 ± 0.13	0.41 ± 0.02	0.74 ± 0.05	0.57 ± 0.03	N/D
	CT	0.96 ± 0.03	2.02 ± 0.06	1.00 ± 0.05	2.11 ± 0.20	0.34 ± 0.01	0.69 ± 0.05	0.54 ± 0.06	0.69 ± 0.06
OH	NF	4.20 ± 0.31	3.85 ± 0.18	4.73 ± 0.26	3.56 ± 0.32	2.40 ± 0.24	1.92 ± 0.12	2.79 ± 0.20	1.85 ± 0.18
	NT-**COH**	2.09 ± 0.06	2.18 ± 0.06	2.72 ± 0.12	2.25 ± 0.06	1.20 ± 0.03	1.12 ± 0.05	1.53 ± 0.09	1.22 ± 0.19
	NT-**CS**	1.85 ± 0.06	2.08 ± 0.07	2.44 ± 0.02	2.16 ± 0.03	1.18 ± 0.03	1.02 ± 0.02	1.48 ± 0.03	1.12 ± 0.01
	PT-**COH**	1.77 ± 0.04	1.81 ± 0.09	2.23 ± 0.09	1.83 ± 0.10	1.00 ± 0.01	0.89 ± 0.03	1.37 ± 0.03	1.02 ± 0.08
	PT-**CS**	1.65 ± 0.03	1.76 ± 0.03	2.08 ± 0.10	1.81 ± 0.10	1.02 ± 0.03	0.94 ± 0.02	1.29 ± 0.08	0.95 ± 0.05
SC	NG	2.30 ± 0.16	2.90 ± 0.20	4.07 ± 0.55	3.64 ± 0.53	0.89 ± 0.07	1.73 ± 0.40	1.24 ± 0.16	1.69 ± 0.36
	NT	1.45 ± 0.24	1.98 ± 0.45	3.14 ± 0.40	2.97 ± 0.31	1.29 ± 0.22	1.93 ± 0.25	1.11 ± 0.28	1.16 ± 0.18
	CT	1.38 ± 0.06	1.70 ± 0.18	2.73 ± 0.16	2.79 ± 0.15	1.23 ± 0.09	1.68 ± 0.03	0.87 ± 0.08	1.25 ± 0.21

[1]AB, Breton, Alberta; CO, Akron, Colorado; GA, Watkinsville, Georgia; KY, Lexington, Kentucky; OH, Hoytville, Ohio; SC, Swift Current, Saskatchewan.

[2]NF, native forest; NG, native or long-term grassland; NT, no-till; CT, conventional till; S, straw returned after harvest; NS, straw removed after harvest; N, urea-N fertilized; NN, no urea-N fertilizer; WCSF, wheat-corn-soybean-fallow rotation; WF, wheat-fallow rotation; COH, corn-oats-hay rotation; CS, corn-soybean rotation. Bold letters represent the crop within the rotation at time of sampling.

protecting the soil C, while physical protection mechanisms played a more dominant role. Plante *et al.* (2004) observed similar results when treating isolated clay fractions with hydrogen peroxide. Clays were isolated from two sets of soils. The first set consisted of soils from a cultivation chronosequence: native forest soil and the same soil converted to maize cultivation for 7 and 35 years. The second set consisted of a different soil under long-term cereal production and under long-term bare fallow. The proportion of peroxide-resistant C did not differ between all soils and represented approximately 12% of initial clay-associated organic C. One possible explanation for these observations is that chemical fractionations do not properly capture the range of behaviours of a modelable recalcitrant pool of C. A strictly biochemical model of organic matter dynamics is incapable of predicting the observed constancy of the non-hydrolysable pool, as well as other observations of the retention of seemingly labile C species such as carbohydrates in C-depleted soils (e.g. Kiem *et al.*, 2000).

Acid hydrolysis is most frequently performed on whole-soil samples after handpicking plant debris or density separation by flotation. However, the hydrolysis of fractions < 53 µm ensures that the contribution of recent plant lignin is minimized and that the analysis is being performed on mineral-associated organic material. Few studies have reported acid hydrolysis of isolated physical fractions, and most reports concern the hydrolysability of N (e.g. Anderson *et al.*, 1981; Leinweber & Schulten, 1998). Leinweber & Schulten (1998) measured non-hydrolysable N in particle size fractions isolated from soils from several long-term fertilization experiments. The values ranged from 9 to 31% of total N and varied inconsistently with variations in particle size and management practices. Previously, Anderson *et al.* (1981) found that N hydrolysability increased with decreasing particle size, leading them to suggest that SOM associated with the finer fractions was relatively labile but protected from decomposition by surface adsorption or occlusion in microaggregates. The absence of differences in N hydrolysability between fractions observed

Table 4a Total N and non-hydrolysable N concentrations in fractions isolated from the 0–5 cm depth increments (mean ± standard error)

Site[1]	Treatment[2]	Total N/g 100 g^{-1} fraction				Non-hydrolysable N/g 100 g^{-1} fraction			
		d-Silt	d-Clay	μagg-Silt	μagg-Clay	d-Silt	d-Clay	μagg-Silt	μagg-Clay
AB	NF	0.27 ± 0.02	0.58 ± 0.02	0.39 ± 0.03	0.92 ± 0.05	0.09 ± 0.01	0.17 ± 0.01	0.14 ± 0.01	0.25 ± 0.03
	NT-S-N	0.22 ± 0.01	0.41 ± 0.01	0.22 ± 0.02	0.42 ± 0.02	0.08 ± 0.01	0.14 ± 0.01	0.09 ± 0.01	0.15 ± 0.02
	CT-S-N	0.19 ± 0.01	0.36 ± 0.02	0.19 ± 0.01	0.36 ± 0.01	0.08 ± 0.01	0.14 ± 0.01	0.06 ± 0.01	0.09 ± 0.01
	NT-NS-NN	0.17 ± 0.01	0.30 ± 0.01	0.14 ± 0.01	0.32 ± 0.02	0.07 ± 0.01	0.13 ± 0.01	0.07 ± 0.01	0.13 ± 0.01
	CT-NS-NN	0.15 ± 0.01	0.28 ± 0.01	0.12 ± 0.01	0.30 ± 0.01	0.07 ± 0.01	0.13 ± 0.01	0.07 ± 0.01	0.13 ± 0.01
CO	NG	0.12 ± 0.01	0.35 ± 0.03	0.20 ± 0.02	0.43 ± 0.01	0.05 ± 0.01	0.12 ± 0.01	0.05 ± 0.01	0.10 ± 0.01
	NT-WCSF	0.07 ± 0.01	0.21 ± 0.01	0.14 ± 0.01	0.24 ± 0.01	0.05 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.08 ± 0.01
	NT-WF	0.09 ± 0.01	0.23 ± 0.02	0.14 ± 0.01	0.24 ± 0.02	0.05 ± 0.01	0.10 ± 0.01	0.05 ± 0.01	0.08 ± 0.01
	CT-WF	0.09 ± 0.01	0.21 ± 0.01	0.12 ± 0.01	0.26 ± 0.02	0.04 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.08 ± 0.01
GA	NG	0.35 ± 0.02	0.63 ± 0.02	0.52 ± 0.03	0.77 ± 0.04	0.08 ± 0.01	0.12 ± 0.01	0.11 ± 0.02	0.14 ± 0.01
	NT	0.20 ± 0.01	0.31 ± 0.03	0.33 ± 0.01	0.39 ± 0.04	0.06 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
	CT	0.17 ± 0.01	0.26 ± 0.01	0.19 ± 0.01	0.29 ± 0.02	0.05 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
KY	NG	0.21 ± 0.01	0.42 ± 0.03	0.27 ± 0.02	0.41 ± 0.03	0.04 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.10 ± 0.01
	NT	0.18 ± 0.01	0.36 ± 0.01	0.21 ± 0.01	0.33 ± 0.02	0.05 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.11 ± 0.01
	CT	0.12 ± 0.01	0.27 ± 0.01	0.12 ± 0.01	0.30 ± 0.04	0.03 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.12 ± 0.01
OH	NF	0.68 ± 0.03	0.77 ± 0.03	0.83 ± 0.02	0.73 ± 0.03	0.18 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.01
	NT-C**OH**	0.34 ± 0.02	0.43 ± 0.01	0.45 ± 0.02	0.48 ± 0.02	0.10 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
	NT-**C**S	0.29 ± 0.02	0.41 ± 0.01	0.48 ± 0.12	0.43 ± 0.03	0.10 ± 0.01	0.13 ± 0.01	0.11 ± 0.02	0.15 ± 0.02
	PT-C**OH**	0.20 ± 0.01	0.29 ± 0.02	0.26 ± 0.01	0.32 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.12 ± 0.01
	PT-**C**S	0.20 ± 0.01	0.28 ± 0.01	0.25 ± 0.01	0.32 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.11 ± 0.01
SC	NG	0.22 ± 0.02	0.52 ± 0.05	0.33 ± 0.03	0.48 ± 0.07	0.06 ± 0.01	0.13 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
	NT	0.16 ± 0.01	0.45 ± 0.02	0.22 ± 0.01	0.43 ± 0.01	0.04 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.13 ± 0.01
	CT	0.16 ± 0.01	0.41 ± 0.02	0.21 ± 0.03	0.44 ± 0.01	0.05 ± 0.01	0.13 ± 0.01	0.07 ± 0.01	0.14 ± 0.01

[1]AB, Breton, Alberta; CO, Akron, Colorado; GA, Watkinsville, Georgia; KY, Lexington, Kentucky; OH, Hoytville, Ohio; SC, Swift Current, Saskatchewan.
[2]NF, native forest; NG, native or long-term grassland; NT, no-till; CT, conventional till; S, straw returned after harvest; NS, straw removed after harvest; N, urea-N fertilized; NN, no urea-N fertilizer; WCSF, wheat-corn-soybean-fallow rotation; WF, wheat-fallow rotation; COH, corn-oats-hay rotation; CS, corn-soybean rotation. Bold letters represent the crop within the rotation at time of sampling.

in our study suggested some uniformity of the N associated with the various fractions. However, the differences in the observed C hydrolysability between the fractions pointed to different mechanisms of stabilization of the SOM associated with those fractions, particularly the potential for physical protection within microaggregates.

Comparisons of yields of clay-sized fractions from our physical fractionation procedure and the soil particle-size analyses suggest that dispersion was incomplete (Table 2). Therefore the easily dispersed and microaggregate-derived silt-sized fractions are likely to be made up of an unknown combination of silt-sized primary particles and similarly sized microaggregates made of clay particles. It is therefore possible that these non-dispersed silt-sized microaggregates physically protect SOM from acid hydrolysis, resulting in the greater %NHC observed in the silt-sized fraction compared with the clay-sized fractions (Table 3). However, physical protection mechanisms alone cannot explain differences in hydrolysability of organic C between the sources of the silt- and clay-sized fractions. Once released from macro- or microaggregates, the

microaggregate-derived and easily dispersed fractions of a given size fraction (silt or clay) should not provide any differential barrier to acid hydrolysis because they no longer have their protective structure. Therefore, any difference in hydrolysability between aggregate-derived or easily dispersed fractions might reflect a difference in the chemical compositions of the two fractions. It is possible that the physical location of the silt- and clay-sized particles (dispersed vs. microaggregate-derived) might impact the resulting chemical composition, but it is more likely that the biochemical composition itself results in the differing hydrolysability and not the physical location.

There is some evidence reported in the literature that supports the idea of differing hydrolysability of isolated size fractions due to differing biochemical composition of the associated organic matter. Several studies have reported decreasing lignin phenol concentrations with decreasing particle size (Guggenberger *et al.*, 1994; Amelung *et al.*, 1999a; Six *et al.*, 2001; Kiem & Kögel-Knabner, 2003). They also reported that the acid-to-aldehyde ratio of the vanillyl and

Table 4b Total N and non-hydrolysable N concentrations in fractions isolated from the 5–20 cm depth increments (mean ± standard error)

Site[1]	Treatment[2]	Total N/g 100 g^{-1} fraction				Non-hydrolysable N/g 100 g^{-1} fraction			
		d-Silt	d-Clay	µagg-Silt	µagg-Clay	d-Silt	d-Clay	µagg-Silt	µagg-Clay
AB	NF	0.11 ± 0.01	0.22 ± 0.02	0.14 ± 0.01	0.32 ± 0.05	0.03 ± 0.01	0.08 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
	NT-S-N	0.13 ± 0.01	0.23 ± 0.02	0.12 ± 0.02	0.22 ± 0.03	0.07 ± 0.01	0.12 ± 0.01	0.07 ± 0.01	0.10 ± 0.01
	CT-S-N	0.13 ± 0.01	0.23 ± 0.04	0.11 ± 0.01	0.25 ± 0.06	0.08 ± 0.01	0.12 ± 0.01	0.06 ± 0.01	0.11 ± 0.02
	NT-NS-NN	0.11 ± 0.01	0.16 ± 0.01	0.08 ± 0.01	0.14 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
	CT-NS-NN	0.12 ± 0.01	0.17 ± 0.01	0.07 ± 0.01	0.15 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.06 ± 0.02
CO	NG	0.12 ± 0.01	0.26 ± 0.01	0.16 ± 0.01	0.28 ± 0.01	0.05 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.09 ± 0.01
	NT-WCSF	0.12 ± 0.01	0.21 ± 0.01	0.12 ± 0.01	0.20 ± 0.02	0.05 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.07 ± 0.01
	NT-WF	0.09 ± 0.01	0.18 ± 0.02	0.12 ± 0.01	0.23 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.07 ± 0.01
	CT-WF	0.09 ± 0.01	0.19 ± 0.02	0.15 ± 0.01	0.26 ± 0.02	0.05 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.08 ± 0.01
GA	NG	0.13 ± 0.01	0.26 ± 0.02	0.18 ± 0.02	0.32 ± 0.02	0.05 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.08 ± 0.01
	NT	0.12 ± 0.01	0.21 ± 0.02	0.17 ± 0.01	0.28 ± 0.05	0.06 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.07 ± 0.01
	CT	0.14 ± 0.01	0.21 ± 0.01	0.18 ± 0.01	0.25 ± 0.03	0.06 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
KY	NG	0.15 ± 0.01	0.31 ± 0.01	0.15 ± 0.01	0.35 ± 0.02	0.04 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.11 ± 0.01
	NT	0.15 ± 0.01	0.32 ± 0.01	0.17 ± 0.01	0.40 ± 0.02	0.04 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	ND
	CT	0.13 ± 0.01	0.28 ± 0.01	0.12 ± 0.01	0.34 ± 0.03	0.04 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.12 ± 0.01
OH	NF	0.39 ± 0.02	0.41 ± 0.02	0.51 ± 0.02	0.48 ± 0.04	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
	NT-COH	0.28 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.34 ± 0.02	0.10 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
	NT-CS	0.22 ± 0.01	0.29 ± 0.01	0.31 ± 0.01	0.35 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
	PT-COH	0.23 ± 0.01	0.30 ± 0.01	0.25 ± 0.01	0.30 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.12 ± 0.01
	PT-CS	0.23 ± 0.01	0.29 ± 0.01	0.25 ± 0.01	0.30 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.10 ± 0.01
SC	NG	0.23 ± 0.02	0.42 ± 0.09	0.27 ± 0.03	0.42 ± 0.08	0.07 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.12 ± 0.01
	NT	0.17 ± 0.01	0.37 ± 0.04	0.20 ± 0.04	0.37 ± 0.05	0.07 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.11 ± 0.02
	CT	0.17 ± 0.01	0.35 ± 0.01	0.19 ± 0.02	0.35 ± 0.02	0.06 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.12 ± 0.03

[1]AB, Breton, Alberta; CO, Akron, Colorado; GA, Watkinsville, Georgia; KY, Lexington, Kentucky; OH, Hoytville, Ohio; SC, Swift Current, Saskatchewan.

[2]NF, native forest; NG, native or long-term grassland; NT, no-till; CT, conventional till; S, straw returned after harvest; NS, straw removed after harvest; N, urea-N fertilized; NN, no urea-N fertilizer; WCSF, wheat-corn-soybean-fallow rotation; WF, wheat-fallow rotation; COH, corn-oats-hay rotation; CS, corn-soybean rotation. Bold letters represent the crop within the rotation at time of sampling.
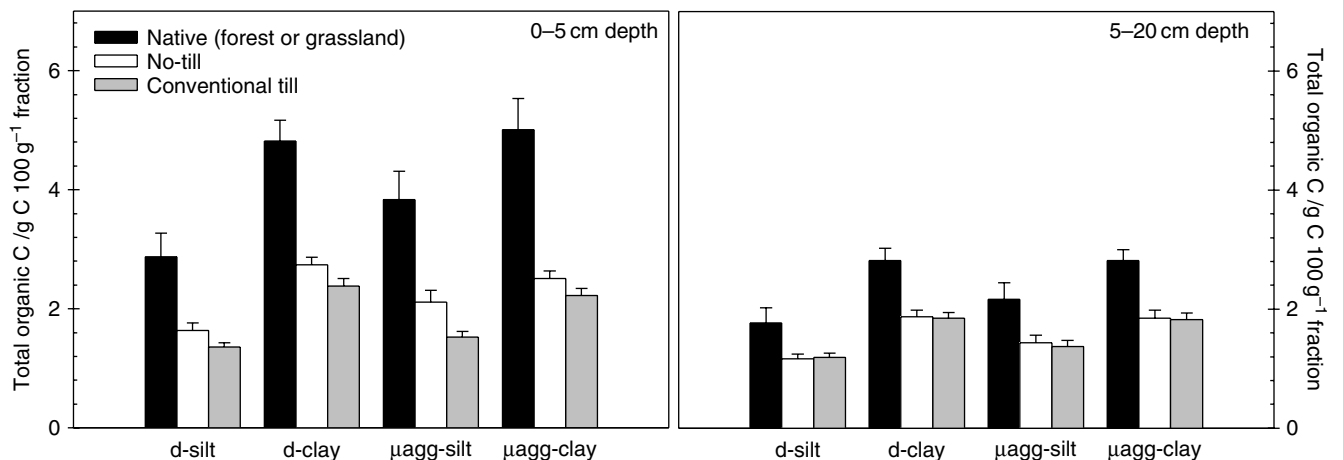

Figure 1 Total organic C concentration in each isolated fraction from the three land use types before acid hydrolysis treatment (all field sites combined, treatments combined by land use). Error bars represent standard error of the mean.
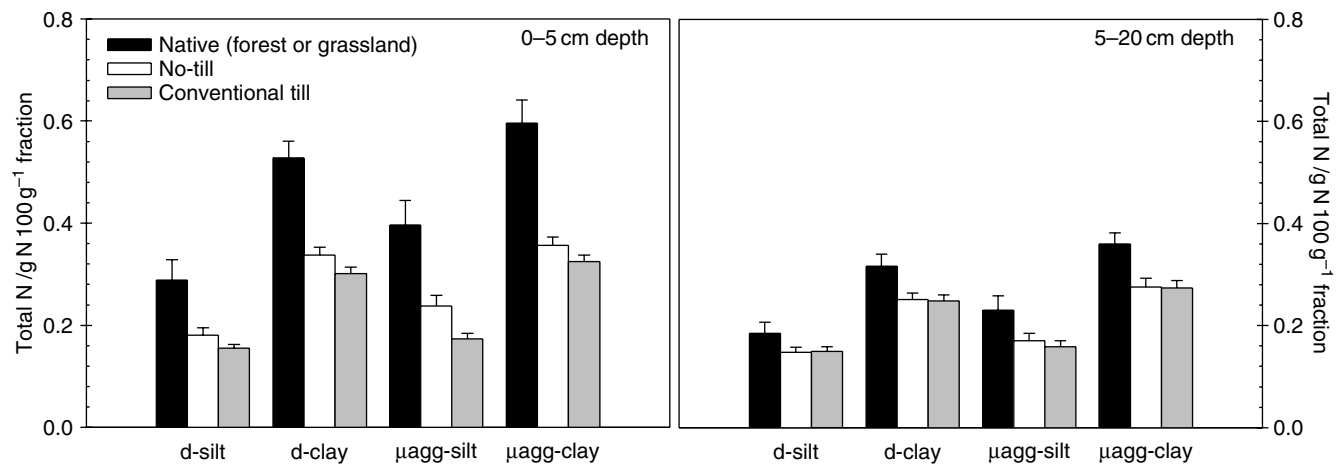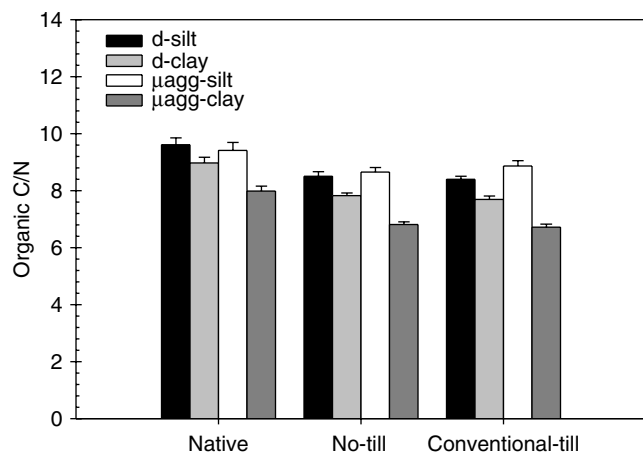
Figure 2 Total N concentration in each isolated fraction from the three land use types before acid hydrolysis treatment (all field sites combined, treatments combined by land use). Error bars represent standard error of the mean.

Figure 3 Organic C/N ratio of each isolated fraction from the three land use types before acid hydrolysis treatment (all field sites combined, treatments combined by land use). Error bars represent standard error of the mean.

syringyl units (an indicator of the degree of microbial alteration) increased with decreasing particle size. Greater carbohydrate contents have been observed in clay-sized fractions compared with silt-size fractions (Guggenberger et al., 1994; Amelung et al., 1999b; Kiem & Kögel-Knabner, 2003). The results of these biochemical analyses are consistent with the observed results of acid hydrolysability in silt- versus clay-sized fractions. Plant lignin is known to resist acid hydrolysis while amino acids, peptides and polysaccharides are easily hydrolysed (Martel & Paul, 1974a; Schnitzer & Preston, 1983; Paul et al., 2000). The greater %NHC in the silt-sized fractions therefore may be attributable to greater lignin and lesser carbohydrate concentrations, while greater hydrolysability in the clay-sized fractions may be attributable to lesser lignin and larger carbohydrate concentrations. Further biochemical analyses of these fractions before and after acid hydrolysis treatment will be necessary to confirm this.

The observed differences in hydrolysability of C vs. N reflect differences in chemical compositions between fractions as well

Table 5 Major axis parameters for functional linear relationships between total versus non-hydrolysable organic C and total N, and mean percentage non-hydrolysable C and N (% NH) in isolated fractions (all sites, depths and treatments combined)

| Fraction		r	Slope[1]	Intercept[1]	Mean/% NH
Easily dispersed silt	C	0.949	0.625 (0.593, 0.658)	−0.125 (−0.178, −0.074)	52.9
Easily dispersed clay	N	0.830	0.222 (0.199, 0.246)	0.024 (0.019, 0.028)	38.3
	C	0.933	0.577 (0.543, 0.612)	−0.234 (−0.327, −0.144)	47.5
Microaggregate-derived silt	N	0.729	0.169 (0.144, 0.193)	0.050 (0.042, 0.058)	34.7
	C	0.974	0.636 (0.636, 0.660)	−0.108 (−0.154, −0.064)	57.8
Microaggregate-derived clay	N	0.895	0.233 (0.215, 0.252)	0.017 (0.013, 0.021)	33.7
	C	0.953	0.548 (0.519, 0.577)	−0.225 (−0.295, −0.156)	44.3
	N	0.748	0.202 (0.172, 0.232)	0.035 (0.025, 0.045)	31.7

[1] Estimate (95% confidence interval).
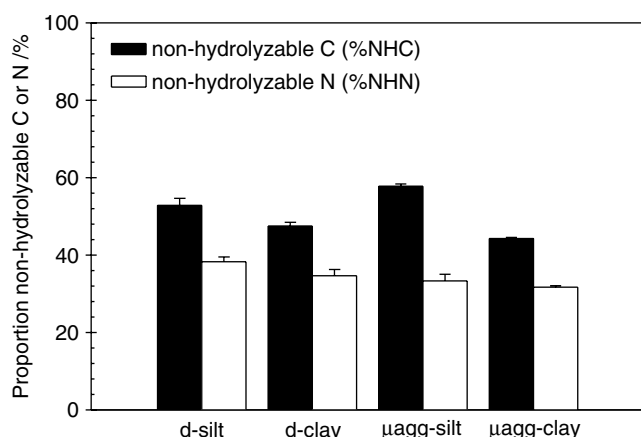
Figure 4 Proportion of non-hydrolysable C or N in each isolated fraction (all sites, depths and treatments combined). Hydrolysability of organic C is significantly different between silt- and clay-sized fractions, while N hydrolysability is not different between fractions. Error bars represent standard error of the mean.

as the differing mechanisms by which the hydrolysis acts on compounds containing these elements. Barriuso *et al.* (1987) described three steps in the degradation of organic matter by acid hydrolysis into smaller, more polar, soluble components using the following equations:

1 Protonation or electrophilic attack
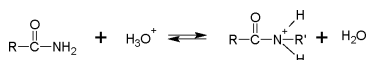
2 Hydration or nucleophilic attack

Figure 6 Proportion of non-hydrolysable C as a function of total organic C concentration in easily dispersed and microaggregate derived clay-sized fractions (all sites, depths and treatments combined).

3 Regeneration of protons
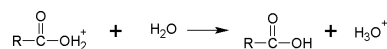
They suggested that organic compounds that are more easily protonated are more easily hydrolysable, and therefore N-containing compounds are more easily hydrolysed than C-containing compounds.

[14]C-dating of NHC provides significant evidence that it is generally older than bulk soil C. The whole-soil data of Martel & Paul (1974a, 1974b), Trumbore (1993), Leavitt *et al.* (1996) and Paul *et al.* (2001) show that, on average, the NHC of surface whole-soil samples is 1200–1400 years older than that of the total soil C. This difference is even greater in subsurface
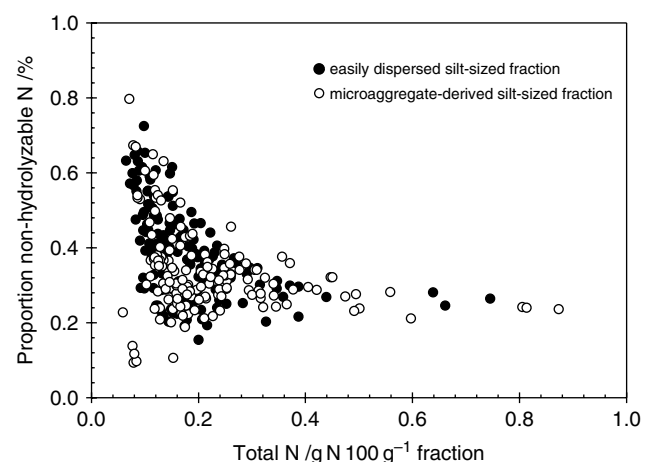

Figure 5 Proportion of non-hydrolysable C as a function of total organic C concentration in easily dispersed and microaggregate derived silt-sized fractions (all sites, depths and treatments combined).

Figure 7 Proportion of non-hydrolysable N as a function of total N concentration in easily dispersed and microaggregate derived silt-sized fractions (all sites, depths and treatments combined).
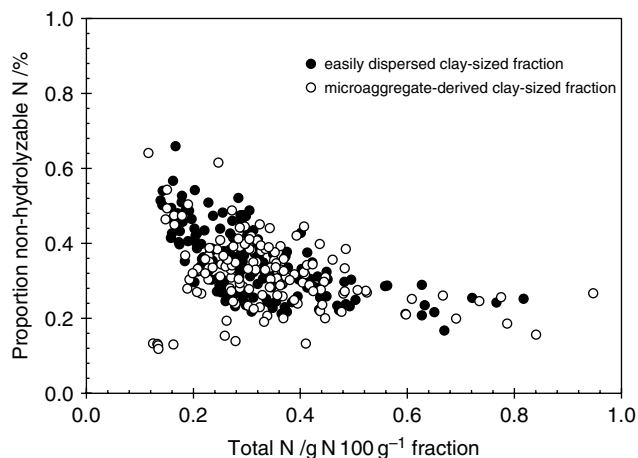
Figure 8 Proportion of non-hydrolysable N as a function of total N concentration in easily dispersed and microaggregate derived clay-sized fractions (all sites, depths and treatments combined).

samples. The greater %NHC in the silt-sized fraction is also consistent with previous observations of slower turnover of silt-associated SOM compared with clay-associated SOM. Stable C isotope analyses (^{14}C-dating and δ^{13}C) of particle size fractions also suggest that the silt- and clay-sized fractions have slower turnover times than the sand-sized fraction of SOM (Anderson & Paul, 1984; Balesdent *et al.*, 1988, 1998). Our observations suggest that the combination of physical and chemical means of isolating the silt- and clay-associated non-hydrolysable SOM may be one possible approach to parameterize the turnover time of a resistant pool of SOM, but the problems associated with determining the pool size of the resistant fraction are not properly resolved by our approach.

Conclusions

Many approaches have been proposed in the literature to produce a measurable fraction analogous to the resistant or passive modelable pool of SOM. The current understanding of SOM dynamics is that as total SOM decreases, the proportion of resistant SOM should increase. The results of acid hydrolysis treatment on silt- and clay-sized fractions in the current study partly differ from this behaviour. While %NHN was indeed greater in cultivated soils compared with their native analogues, %NHC did not increase with decreasing initial C concentrations. C hydrolysability also differed between the isolated fractions, being more resistant in silt-sized fractions than clay-sized fractions. Therefore, an interaction appears to exist between biochemical and physical protection mechanisms that act to preserve SOM in fine mineral fractions. The long mean residence times of NHC reported in the literature make this pool of C of particular scientific interest, but the inconsistency of the size of the non-hydrolysable pool with expected behaviour remains to be explained. Further characterization of

acid hydrolysis residues from native and cultivated soils may help us understand why this pool represents such a consistent proportion of total soil organic matter and its dynamics after land use changes.

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