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A re-evaluation of the enriched labile soil organic matter fraction

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Summary

Identifying 'functional' pools of soil organic matter and understanding their response to tillage remains elusive. We have studied the effect of tillage on the enriched labile fraction, thought to derive from microbes and having an intermediate turnover time. Four soils, each under three regimes, long-term arable use without tillage (NT), long-term arable under conventional tillage (CT), and native vegetation (NV), were separated into four aggregate size classes. Particle size fractions of macro- (250–2000 μm) and microaggregates (53–250 μm) were isolated by sonication and sieving. Subsequently, densiometric and chemical analyses were made on fine-silt-sized (2–20 μm) particles to isolate and identify the enriched labile fraction. Across soils, the amounts of C and N in the particle size fractions were highly variable and were strongly influenced by mineralogy, specifically by the contents of Fe and Al oxides. This evidence indicates that the fractionation procedure cannot be standardized across soils. In one soil, C associated with fine-silt-sized particles derived from macroaggregates was 567 g C m^{-2} under NV, 541 g C m^{-2} under NT, and 135 g C m^{-2} under CT, whereas C associated with fine-silt-sized particles derived from microaggregates was 552, 1018, 1302 g C m^{-2} in NV, NT and CT, respectively. These and other data indicate that carbon associated with fine-silt-sized particles is not significantly affected by tillage. Its location is simply shifted from macroaggregates to microaggregates with increasing tillage intensity. Natural abundance ^{13}C analyses indicated that the enriched labile fraction was the oldest fraction isolated from both macro- and microaggregates. We conclude that the enriched labile fraction is a 'passive' pool of soil organic matter in the soil and is not derived from microbes nor sensitive to cultivation.

Introduction

Cultivation generally decreases the total organic matter in the soil, but the behaviour of different constituents is less well understood. Physical fractionation of soil has been successful in identifying fractions of soil organic matter (SOM) that are more sensitive to cultivation than total organic C or N (Cambardella & Elliott, 1992; Janzen *et al*., 1992). Most of these physically defined fractions are predominantly derived from plants and have a turnover time of 10–50 years (i.e. intermediate). For example, particulate organic matter and light fraction are two fractions derived from plants and have been shown to be sensitive to cultivation and management practices (Cambardella & Elliott, 1992; Janzen *et al*., 1992). However, physical fractionation has been less successful in isolating fractions derived from microbes, i.e. dead microbes and products from microbial metabolism with an intermediate turnover time, that are strongly affected by management practices and cultivation.

Chemical extractions have shown that microbial-derived SOM is sensitive to management practices and cultivation. For example, Arshad *et al*. (1990) found that there was more microbial-derived acid-hydrolysable carbohydrates, amino acids and amino sugars in soil under no-tillage than in that under conventional tillage. Beare *et al*. (1997) and Ball *et al*. (1996) also reported more microbial-derived carbohydrates under no-tillage than conventional tillage.

However, chemical extractions, by themselves, provide no information on the relation between the organic matter extracted and its location within the soil matrix. Moreover, extractions of specific microbial compounds (e.g. carbohydrates and amino sugars) typically isolate only a small fraction (< 10%) of the soil organic matter (Christensen, 1992). A clear influence of soil structure on the dynamics of SOM has been shown (Elliott, 1986; Beare *et al*., 1994; Jastrow, 1996; Six *et al*., 1998, 1999a, 2000b). Physical fractionation analyses have the potential to elucidate relations between the dynamics

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of SOM and changes in soil structure. Aggregation and aggregate dynamics slow turnover of SOM by physically protecting it. Microbial products bind soil into aggregates and they thereby become physically protected within the aggregates. Consequently, the dynamics of the microbial-derived SOM should be closely linked to that of the aggregates. Therefore, if we are to understand the behaviour of microbial-derived SOM within aggregate structure and its loss on cultivation when structure disintegrates we must be able to isolate the microbial SOM.

Tisdall & Oades (1982) suggested that microbial-derived organic materials bind microaggregates into macroaggregates. Cambardella & Elliott (1994) thought that the loss of stable macroaggregates caused by intensive tillage was accompanied by a substantial loss of these microbial-derived materials. They designed a method to measure this fraction, employing gentle sonication to break apart macroaggregates into their constituent parts. They reasoned that the particles most enriched in these microbial-derived materials could then be separated according to their relative density. In an agricultural soil that had formerly been under grass, they identified a fraction rich in organic C and N that declined significantly with increasing intensity of tillage, and they termed it the enriched labile fraction (ELF). They suggested that this material, along with the particulate organic matter fraction, represented SOM having an intermediate turnover time that was most susceptible to loss on cultivation. We therefore set out to test the method for its generality across a range of soils and determine the age of the ELF.

Materials and methods

Sampling

In November 1995, soils from four long-term agricultural field experiments were sampled. The sites were Sidney, Nebraska (41°14′N, 103°00′W), Wooster, Ohio (40°48′N, 82°00′W), W. K. Kellogg Biological Station (KBS), Michigan (42°24′N, 85°24′W), and Lexington, Kentucky (38°07′N, 84°29′W). All sites had uncultivated 'native' vegetation (NV), no-tillage (NT), and conventional tillage (CT) treatments. The soil at Lexington has a mixed clay mineralogy dominated by vermiculite and kaolinite; the soil at other sites is dominated by 2:1 layer clay types (Table 1). In addition, the concentration of amorphous and poorly crystalline Fe and Al oxides is 2–16 times greater in the Lexington soil than in the Sidney, Wooster

Table 1 General characteristics of the agricultural experiment field sites

	Sidney, NE	Wooster, OH	KBS, MI	Lexington, KY
Soil classification	Pachic Haplustoll	Typic Fragiudalf	Typic Hapludalf	Typic Paleudalf
Soil series	Duroc	Wooster	Kalamazoo and Oshtemo	Maury
Texture	Loam	Silt loam	Sandy loam	Silty clay loam
Mineralogy[a]	Illite, chlorite	Chlorite, illite	Chlorite, illite	Vermiculite, kaolinite, illite
MAT /°C[b]	8.5	9.1	9.2	13.1
MAP /mm[c]	440	905	920	1127
Crop rotation	Winter wheat–fallow	Continuous maize	Maize–soybean–winter wheat	Continuous maize (84 kg N)
Prior vegetation	Short grass prairie	Grass meadow	Grassland	Bluegrass pasture
Year of establishment	1969	1962	1986	1971
Organic C (0–20 cm)[d] /g C m^{-2}				
Native vegetation[e]	4090 a	4008	2944	5036
No-tillage	3428 ab	3806 a	2422 a	3742 a
Conventional tillage	2907 b	3380 b	2209 a	3125 b
Organic N (0–20 cm) /g N m^{-2}				
Native vegetation	396 a	300	241	789
No-tillage	366 a	346 a	218 a	441 a
Conventional tillage	312 a	294 b	204 a	348 b

[a]Dominant clay minerals in order of dominance. Data adopted from Six *et al.* (2000a).
[b]MAT, mean annual air temperature.
[c]MAP, mean annual precipitation.
[d]Data adopted from Six *et al.* (1999a).
[e]Values followed by a different lowercase letter are significantly different ($P < 0.05$). The native vegetation treatment could not be included within the statistical analyses at Wooster, KBS and Lexington because this treatment was not included within the experimental design of the agricultural experiment fields.

and KBS soils (Six *et al*., 2000a). For further details of site characteristics see Table 1.

When samples were taken, the litter layer was removed, and the soil cores (5.5 cm diameter) were divided into two depths: 0–5 cm and 5–20 cm. Eight cores per treatment replicate were taken. Once in the laboratory, the field-moist soil was gently broken apart to pass an 8-mm sieve, composited, air-dried and stored at room temperature.

Physical fractionation

The method used for isolation of the enriched labile fraction was modified from Cambardella & Elliott (1994). Briefly, a 100-g subsample of air-dried soil was wet-sieved through a series of three sieves to obtain four aggregate fractions: (i) $>2000\,\mu$m (large macroaggregates), (ii) 250–2000 μm (small macroaggregates), (iii) 53–250 μm (microaggregates), and (iv) $<53\,\mu$m (silt-plus clay-sized particles). Immersion of the air-dried soil in water causes slaking, i.e. the disruption of unstable aggregates caused by an increase in the internal air pressure during rapid wetting. The aggregates were oven-dried (at 50°C), weighed and stored in Wheaton jars at room temperature.

Macro- and microaggregates (10 g of each) were broken into their constituents by sonication (Ultrasonics Heat Systems Model W-375) in 60 ml water with little energy, i.e. 6.8 J s^{-1} and a total energy input of 1225 J (North, 1976). This energy was determined to be the minimum to break macroaggregates into non-associated organic matter, sand- and silt-sized microaggregates and primary particles (Cambardella & Elliott, 1994). Four size fractions were isolated by sieving the sonicated macroaggregates: (i) 250–2000 μm, (ii) 53–250 μm, (iii) 20–53 μm, and (iv) $<20\,\mu$m. The fourth size fraction was divided into two fractions (2–20 μm and $<2\,\mu$m) by centrifuging (100 ***g*** for 9 min). The four smallest size fractions were also obtained from sonicated microaggregates. Because rather little energy was used for sonication, these size fractions were not primary particles, except for the $>250\,\mu$m and 53–250 μm fractions, which consist of sand and particulate organic matter.

The density separation of the 2–20 mm sized fraction (fine-silt-sized particles) is the part of the methodology which was modified the most and is therefore described in more detail. In contrast to determining the ELF by calculating differences between successive fractions as done by Cambardella & Elliott (1994), we isolated ELF with a sequential density flotation method (devised by W. J. Gale, personal communication). In the final density separation three fractions were obtained: (i) a light fraction (<2.00 g cm^{-3}), (ii) an intermediate fraction ($2.00 - x$ g cm^{-3}), and (iii) a heavy fraction ($>x$ g cm^{-3}). The density limit, x, for the intermediate density fraction was chosen as the point where the cumulative concentration of carbon declined as density increased, as specified by Cambardella & Elliott (1994). For the Sidney soil they determined 2.22 g cm^{-3} as the upper density limit.

To determine this variable upper density limit, x, for the other soils, a gradient was made with increasing densities (2.10 g cm^{-3}–2.7 g cm^{-3}) for the fine-silt-sized fraction derived from the macroaggregates from the 0–5 cm soil layer of the NT plots. In a first step, 5 ml of 2 g cm^{-3} density sodium polytungstate (SPT) was added to 0.75 g of the size fraction in a 50-ml centrifuge tube. The sample was gently mixed and another 5 ml of 2 g cm^{-3} density SPT was added to rinse off soil particles adhering to the sides of the tube. The samples were then centrifuged for 10 min at 850 ***g*** and the supernatant plus floating particles were poured off into a second centrifuge tube. This density flotation step was repeated with 2 g cm^{-3} SPT and the supernatant was added to the same tube. This tube then contained the light fraction (<2 g cm^{-3}) while the remaining pellet contained material having a density of >2 g cm^{-3}. This remaining pellet was then suspended in SPT with a heavier density (2.05–2.7 g cm^{-3} with sequential step increases of 0.05–0.10 g cm^{-3} across different centrifuge tubes). The supernatant was then poured off into a third centrifuge tube, and the density flotation was repeated. Consequently, the first tube contained the heaviest fraction, the second tube the lightest fraction and the third tube the intermediate density fraction. For each tube containing a given density fraction, distilled water was added, and the tube was shaken to break the pellet formed. The tubes were centrifuged for 10 min at 850 ***g***. The clear supernatant was aspirated with a Pasteur pipette attached to a vacuum flask. This washing procedure was repeated but with a solution of 0.25 M $CaCl_2$ plus 0.25 M $MgCl_2$ to avoid excessive dispersion of the material. The procedure was repeated with distilled water for a final rinse. The samples were washed into preweighed aluminium pans and dried at 50°C. The SPT used for the density separation was cleaned and recycled as described by Six *et al*. (1999b) to avoid cross-contamination of C and N between samples, which would compromise natural abundance ^{13}C analyses.

Density gradients were also determined on fine-silt-sized particles derived from macroaggregates from the 0–5 cm layer in NT after oxidation of SOM with H_2O_2. This was done to determine the influence of the mineral composition on the shape of the density gradient curve. A 10-g subsample was taken from the respective macroaggregate fractions and sieved through a 2-mm sieve. The 2-mm sieved soil was treated with 30% H_2O_2 at 60–70°C until there was no reaction upon addition of H_2O_2, then rinsed and used for the density gradient analyses. This procedure resulted in a $\pm$ 80% reduction of C associated with the fine-silt-sized particles.

Carbon and nitrogen, and isotope analyses

Carbon concentrations were measured using a LECO CHN-1000 analyser (Leco Corp., St. Joseph, MI) or a Carlo Erba NA

1500 CN analyser (Carlo Erba, Milan, Italy) depending on the sample size. Differences in sand content between field treatments and size fractions, and the fact that sand particles do not bind with organic matter, necessitates a correction for sand content when comparing the C and N content of size fractions (Elliott *et al*., 1991). This sand correction is important for comparisons between treatments at the Sidney site because CT has a coarser texture compared with NV and NT in the 0–5 cm layer. Concentration of C as proportions of sand-free soil fractions, denoted $[C]_{sf}$, is

$$[C]_{sf} = \frac{[C]}{1 - [S]}, \quad (1)$$

where [C] is the carbon content of the fraction and [S] the proportion of sand in the fraction. Carbon-isotope ratios of SOM fractions were determined using a Carlo Erba NA 1500 CN analyser coupled to a Micromass VG isochrom-EA mass spectrometer (Micromass UK Ltd, Manchester) (Continuous flow measurement). Isotope ratios were expressed as $\delta^{13}C$ values:

$$\delta^{13}C = \frac{[^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{reference}]}{{}^{13}C/^{12}C_{reference}} \times 1000. \quad (2)$$

The reference used is the international Pee Dee Belemnite (PDB) standard.

Statistical analyses

The data were analysed as a complete randomized block design. All treatments were included in the statistical analysis for Sidney, but for the other sites the NV was not included in the analysis because it was not replicated. Within depth, tillage treatment was the main factor in the model, with fraction size class and replicate as secondary factors (see Table 2).

Results and discussion

The general decrease in total organic C and N and aggregation in the order NV > NT > CT, for the soils in this study (Six *et al*., 2000b), suggests a relation between the loss of organic C and N and the loss of structural stability with increasing intensity of cultivation. Earlier work at the Sidney site by Elliott (1986) suggested that the loss of SOM on cultivation was a result of a loss of macroaggregates rich in C and an increase in microaggregates depleted in C. This relation between the loss of structural stability and SOM with increasing cultivation was confirmed for several other soils in which there is a hierarchy of aggregates (Six *et al*., 2000b).

Carbon distribution among macroaggregate constituents

An analysis of variance for Sidney (Table 2) indicates that there are significant effects of tillage and depth on the concentrations of C and N in the constituents of macroaggregates. The concentrations were generally less at 5–20 cm depth than in the surface layer. There were significant interactions between depth and treatment: the differences in concentration were larger between depths in the NV and NT than in the CT.

The carbon in macroaggregate constituents responded differently to tillage intensity, according to size (Figure 1). Particles >250 μm did not show a consistent trend across management treatments, whereas the 53–250 μm and 20–53 μm fractions generally decreased in the order NV > NT > CT. Fine-silt-sized particles (2–20 μm) did not differ in C content across management treatments. This suggests that SOM associated with the fine-silt-sized particles was the least affected by tillage.

In Sidney, the fine-silt-sized particles contained more C than all other macroaggregate constituents (Figure 1). This agrees with the data of Cambardella & Elliott (1994) for the same site. Other researchers have found the silt fraction to be the

Table 2 Summary of analysis of variance of carbon and nitrogen distribution among constituents of macroaggregates obtained by slaking at Sidney

	Carbon			Nitrogen		
Source:	d.f.	Mean square	*F*-value	d.f.	Mean square	*F*-value
Size	3	339.7	336.6*	3	5.2	273.2*
Treatment	2	58.8	58.2*	2	0.7	36.4*
Depth	1	65.3	64.7*	1	0.8	41.75*
Treatment × size	6	31.7	31.4*	6	0.3	14.6*
Depth × size	3	14.0	13.9*	3	0.1	7.85*
Depth × treatment	2	29.5	29.3*	2	0.2	11.2*
Depth × treatment × size	6	11.7	11.6*	6	0.1	6.4*

*Significant at the 0.0001 probability level.

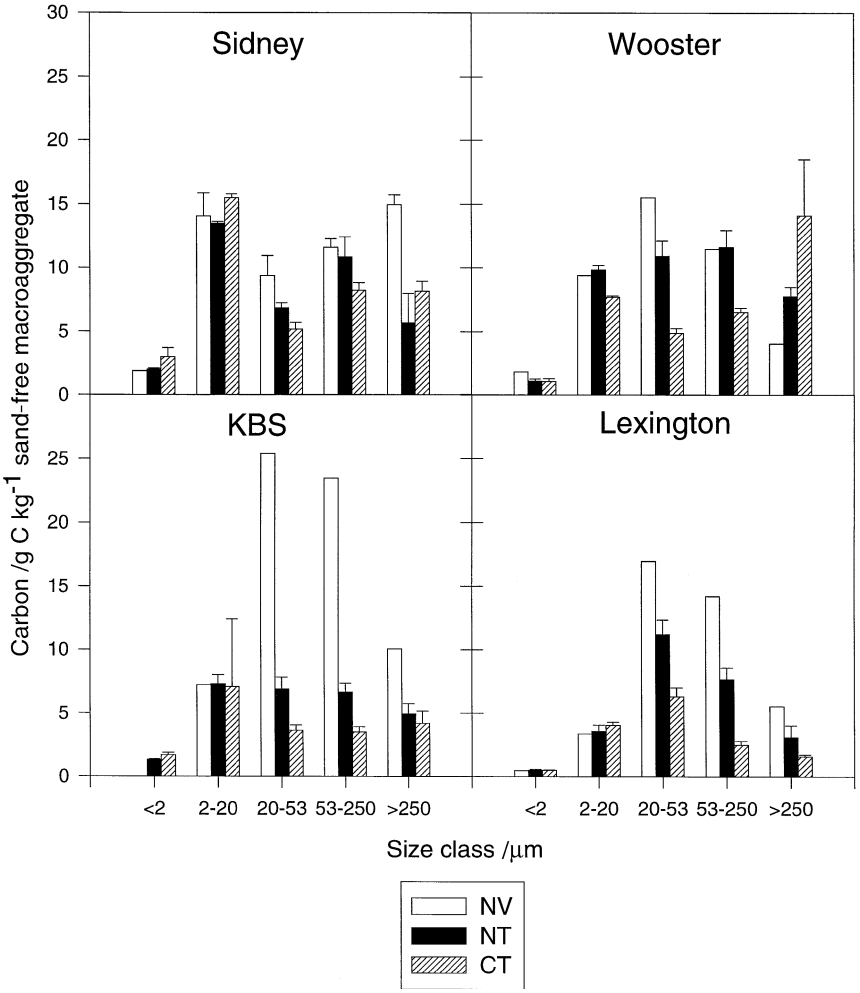

Figure 1 Carbon distribution (g C kg^{-1} sand-free macroaggregate) among constituents of macroaggregates obtained by slaking in the surface layer (0–5 cm) of four long-term agricultural experiment sites with three management treatments. NV, native vegetation; NT, no-tillage; CT, conventional tillage. Bars are standard errors.

most rich in C of all primary particle size fractions (Turchenek & Oades, 1978; Tiessen & Stewart, 1983; Oades, 1990). However, this richness of the fine-silt-sized particles of macroaggregates was not observed at our other three sites. In Wooster and KBS, the richest organo–mineral fraction (i.e. fractions $<53\,\mu$m) differed with management treatment, i.e. the fine-silt-sized particles were the richest in CT, fine- and coarse-silt-sized particles were equally enriched in NT, and the coarse-silt-sized particles were the richest in NV. In Lexington, the largest concentration of C was in the coarse-silt-sized particles (20–53 μm). The distribution of N was similar to that of C (data not shown).

These differences in C and N distribution across different soils is probably due to a difference in stability of silt-sized particles (both 2–20 μm and 20–53 μm size classes) rather than to differences in SOM dynamics between soils. The macroaggregates were sonicated with the same low energy level to isolate the constituents. Consequently, differences in stability of the silt-sized particles ($<53\,\mu$m) within macroaggregates would lead to a difference in C and N distribution among the silt-sized particles obtained after sonication. That is, a more stable soil will contain more C and N in the coarse-silt-sized particles than in the fine-silt-sized and clay-sized ($<2\,\mu$m) particles. We found the concentration of citrate–ascorbate and citrate–dithionite extractable Fe and Al differing in the order Sidney < KBS < Wooster < Lexington (Six *et al*., 2000a). Since Fe and Al oxides can lead to stable silt-sized particles (Turchenek & Oades, 1978; Cassel & Lal, 1992) we predict that the stability of the silt-sized particles would follow the same order as the Fe and Al oxide concentration and consequently the distribution of C and N concentration in the particles too. Indeed, the relative amounts of C and N increased with particle size ($<2\,\mu$m < 2–20 μm < 20–53 μm) in the order Sidney < KBS < Wooster < Lexington (Figure 1), suggesting that an increase in concentrations of Fe and Al oxide leads to a stability of larger organo–mineral particles and more C and N in these particles.

The differences in distribution of C and N caused by differences in physical and chemical properties between soils suggest that the energy used to disrupt the macroaggregates should not be held constant to obtain fractions with similar dynamics across soils. The soil's stability is determined by the

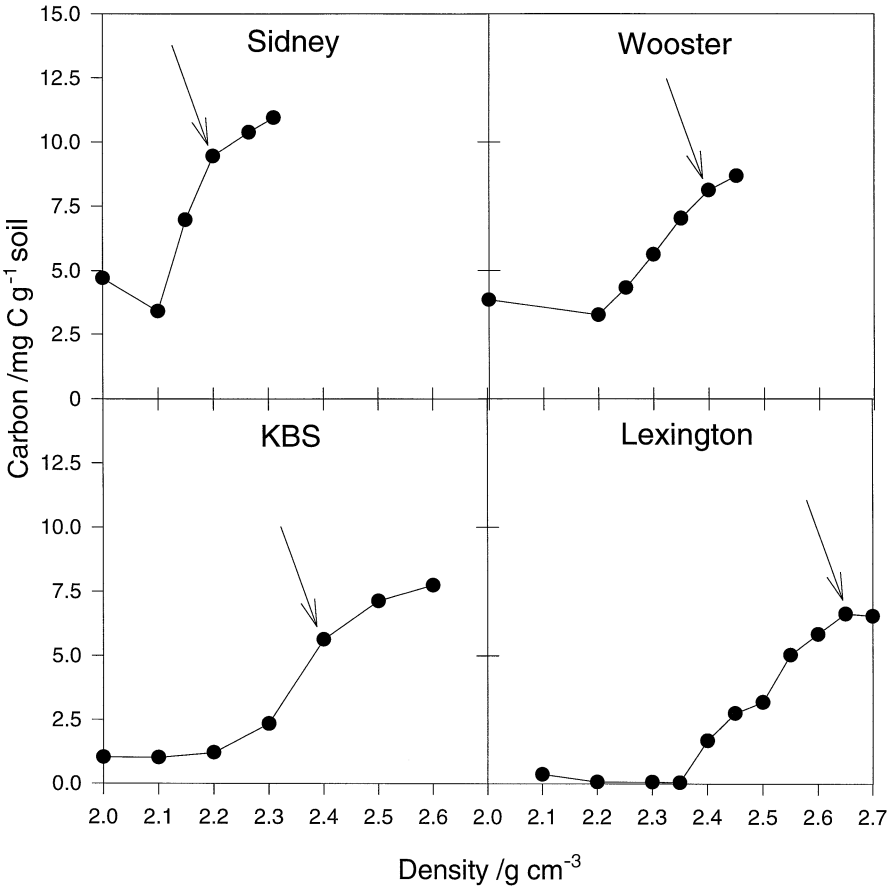

Figure 2 Amount of carbon associated with increasing density fractions from macroaggregate-derived fine-silt-sized particles from four surface soils under long-term no-tillage management (mg C g^{-1} soil). Arrows indicate the density limit for the soils.

amount of macroaggregates surviving a certain disruptive force, but once the macroaggregates are isolated, the stability of soil components is no longer of interest. To isolate the desired macroaggregate constituents the energy to disrupt macroaggregates should be varied. However, one must not create artefacts by using too much energy which would cause organic matter to detach from minerals and be diverted into other particle size fractions (Christensen, 1992; Cambardella & Elliott, 1994). In addition, it is not obvious what criteria should determine the amount of energy to be used to isolate the constituents. One attempt was made by Raine & So (1993), who proposed a method in which they could determine the dispersive energy consumed for dispersion and found that this consumed dispersive energy was a characteristic of each soil and probably depends on the bonding mechanisms within the aggregates. Consequently, this method could be used to separate the constituents from aggregates. However, the disadvantage of the method is that temperature must be measured with an accuracy of 0.01°C to calculate energy consumption.

Density fractions

There were differences not only in the amounts of C and N across the constituents of macroaggregates but also in the characteristics of the soils in the density flotation step of the ELF fractionation. The upper density limit, which defines the density window for the ELF fractionation, differed substantially among soils (Figure 2). The upper density limits were 2.22 for Sidney, 2.40 for Wooster and KBS, and 2.65 g cm^{-3} for Lexington (Figure 2). In addition, the original fixed lower density of 2.00 g cm^{-3} could be changed to 2.20 g cm^{-3} for Wooster and KBS and to 2.35 g cm^{-3} for Lexington because there was little additional C isolated between 2.00 g cm^{-3} and these densities (Figure 2). This would lead to density windows of 2.00–2.22 g cm^{-3} for Sidney, 2.20–2.40 g cm^{-3} for Wooster, 2.20–2.40 g cm^{-3} for KBS, and 2.35–2.65 g cm^{-3} for Lexington. We proposed the hypothesis that the density window varied mainly as a function of mineral densities and that the difference in the amount of SOM relative to minerals was of secondary importance. To test it we determined the density gradient on peroxide-treated fine-silt-sized particles (Figure 3). For each soil, the density gradient with and without SOM showed similar trends (Figure 3), but with the mineral-only curve shifted to the right due to the smaller density of the organic matter than that of the minerals. Thus the mineral composition of each soil largely determined the shape of the curve and consequently the density window. Oades (1990) also indicated that the type of clay influenced the critical density to isolate organic fractions. He found that illite and kaolinite

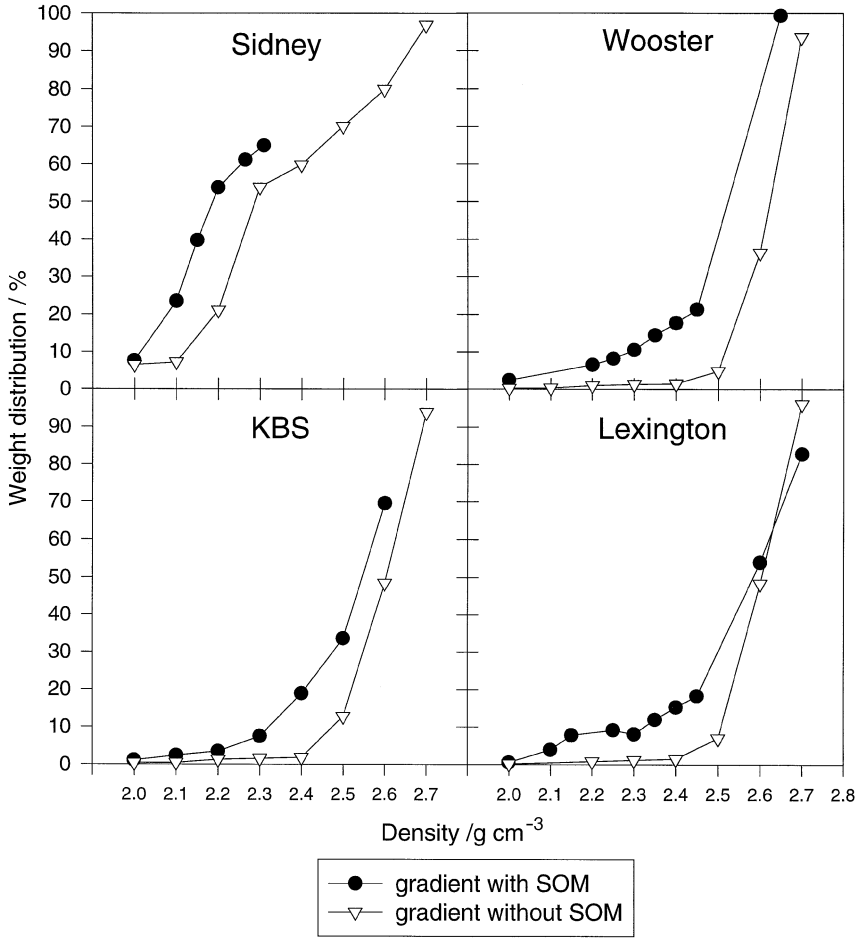

Figure 3 Density gradient for macroaggregate-derived fine-silt-sized particles from four surface soils under long-term no-tillage management with and without SOM associated.

separated out at higher densities than interstratified minerals and smectites. Thus, in contrast to the idea of Cambardella & Elliott (1994) that an organic fraction that is more labile (ELF) could be isolated by density fractionation, we found that the density curve was determined by the mineralogy and not by the characteristics of the associated organic matter.

Carbon and nitrogen dynamics of SOM fractions in Sidney soil

On a whole soil basis (0–20 cm depth and corrected for differences in bulk density), fine-silt-sized particles contained the most C and N of all organo–mineral constituents derived from macroaggregates and microaggregates (Table 3). However, the changes in C and N of fine-silt-sized particles caused by tillage were similar to those observed in the clay and coarse silt fractions (Table 3). The contents of carbon and nitrogen of fractions derived from macroaggregates differed in the order NV = NT > CT, whereas organo–mineral associated C and N from the microaggregates increased in the order NV < NT < CT (Table 3). The same was observed for all density fractions of the fine-silt-sized particles (Table 3). The percentage loss of N from macroaggregate-derived organo–mineral particles upon bringing the NV under NT and CT management were similar (Table 4). This suggests that the N associated with the different organo–mineral particles was similarly affected by cultivation intensity and does not greatly differ in lability. Furthermore, the same was observed for the three density fractions of the fine-silt-sized particles (Table 5), suggesting that the density fractions do not differ in lability.

The similar dynamics of the different density fractions (Table 5) and the original size separates (Table 4) indicate that the density separation does not result in the isolation of a more labile fraction, as postulated by Cambardella & Elliott (1994). The density separation did yield a richer intermediate density fraction but was similar to the light and heavy fractions in regard to its lability. Ladd & Amato (1980) found on incubating soil with glucose and KNO_3-^{15}N for 160 days that the bioavailability of ^{15}N was similar for the light (< 2.06 g cm^{-3}) and heavy (> 2.06 g cm^{-3}) subfractions of silt particles. They suggested, however, that the use of a 2.00 g cm^{-3} density limit would result in a different biological availability of the subfractions. This is contradicted by our data, and consequently we agree with Christensen (1992) that densiometric analyses of silt-sized organo–mineral complexes does not lead

Table 3 Carbon and nitrogen contents in g m^{-2} (standard errors in parentheses) in sonicated and density fractions of macroaggregates (M) and microaggregates (m) under native grassland (NV), no-tillage (NT) and conventional tillage (CT) (Sidney, 0–20 cm). The entries in bold are for the fraction of most concern in the text

	Sonicated fractions											
	Carbon /g C m^{-2}						Nitrogen /g N m^{-2}					
	NV		NT		CT		NV		NT		CT	
Size class /μm	M	m	M	m	M	m	M	m	M	m	M	m
250–2000	296 (17)		98 (28)		51 (6)		27 (2)		8 (1)		7 (1)	
53–250	358 (51)	424 (16)	312 (31)	308 (15)	80 (6)	334 (23)	35 (5)	44 (2)	28 (2)	37 (5)	8 (1)	34 (3)
20–53	355 (26)	339 (26)	294 (13)	454 (7)	51 (3)	442 (34)	41 (3)	42 (5)	32 (3)	48 (4)	6 (0)	55 (7)
2–20	**567 (53)**	**552 (11)**	**541 (28)**	**1018 (46)**	**135 (20)**	**1302 (36)**	**67 (9)**	**70 (2)**	**63 (6)**	**128 (6)**	**17 (3)**	**152 (5)**
<2	107 (6)	87 (2)	90 (11)	154 (22)	29 (5)	191 (10)	14 (1)	12 (1)	11 (2)	20 (3)	4 (1)	24 (1)
	Density fractions for 2–20 μm size fraction											
	Carbon /g C m^{-2}						Nitrogen /g N m^{-2}					
	NV		NT		CT		NV		NT		CT	
Density /g cm^{-3}	M	m	M	m	M	m	M	m	M	m	M	m
<2.00	131 (10)	128 (6)	122 (10)	246 (17)	37 (6)	335 (8)	11 (1)	10 (2)	10 (1)	19 (2)	3 (1)	27 (2)
2.00–2.22	**310 (30)**	**320 (6)**	**317 (14)**	**590 (25)**	**78 (13)**	**750 (31)**	**36 (4)**	**35 (1)**	**37 (1)**	**72 (3)**	**9 (1)**	**87 (6)**
>2.22	64 (7)	62 (1)	63 (3)	99 (3)	13 (3)	108 (4)	10 (1)	9 (1)	10 (0)	15 (1)	2 (0)	17 (2)

Table 4 Per cent loss of nitrogen within the sonicated fractions of macroaggregates (M) and macroaggregates + microaggregates (M + m) with increasing cultivation intensity (Sidney, 0–20 cm)

	((NV – NT)/NV) × 100		((NT – CT)/NT) × 100	
	M	M + m	M	M + m
Size class /μm	/% loss			
250–2000	69	69	11	11
53–250	19	17	71	36
20–53	22	3	83	25
2–20	**7**	**–26**[a]	**73**	**11**
<2	22	–18	68	–4

[a]A negative value indicates a gain of N in the fraction with increasing cultivation.

Table 5 Per cent loss of nitrogen within the density fractions of fine silt (2–20 μm) derived from macroaggregates with increasing cultivation intensity (Sidney, 0–20 cm)

Density /g cm^{-3}	((NV – NT)/NV) × 100	((NT – CT)/NT) × 100
	/% loss	
<2.00	12	72
2.00–2.22	**–2**[a]	**75**
>2.22	5	82

[a]A negative value indicates a gain of N in the fraction with increasing cultivation.

to distinct and homogeneous pools of organic matter with different turnover times.

The C and N contents of the fine-silt-sized particles from macro- plus microaggregates in NT and CT were equal to or exceeded that in NV (Table 3), and this suggests that this fraction is stable under tillage. The main effect of tillage was to redistribute this material from macroaggregates to microaggregates. Calculations of the loss of C and N of this material support this redistribution, i.e. there is only a small loss of C and N associated with the fine-silt-sized particles when macro- and microaggregates are added together (Table 4). Therefore, we suggest that the fine-silt-sized particles are occluded within microaggregates, within macroaggregates, rather than existing between microaggregates within macroaggregates, as suggested by Cambardella & Elliott (1994).

It is interesting that the losses of clay-sized and fine-silt-sized particles caused by tillage were less than for coarse-silt-sized particles (Table 4). This accords with the findings of Tiessen & Stewart (1983) who also observed that losses of organic materials associated with fine silt and clay were

Table 6 Delta ^{13}C-signature (standard errors in parentheses) of sonicated fractions and density fractions of micro- and macroaggregates in no-tillage (NT) and conventional tillage (CT) (Sidney, 0–5 cm)

	Sonicated fractions			
	No-tillage		Conventional tillage	
Size class /μm	Macroaggregates	Microaggregates	Macroaggregates	Microaggregates
	/‰			
250–2000	–25.69 (0.04)[a]		–23.74 (0.11)	
53–250	–23.82 (0.46)	–23.60 (0.04)	–22.40 (0.17)	–21.71 (0.05)
20–53	–21.81 (0.11)	–21.36 (0.04)	–21.49 (0.03)	–20.07 (0.10)
2–20	**–19.92 (0.09)**	**–19.65 (0.08)**	**–19.34 (0.12)**	**–18.65 (0.09)**
<2	–20.46 (0.27)	–20.15 (0.18)	–20.04 (0.49)	–18.89 (0.26)
Total aggregate	–21.70 (0.11)	–20.55 (0.06)	–21.96 (0.11)	–19.19 (0.12)
	Density fractions			
	No-tillage		Conventional tillage	
Density /g cm^{-3}	Macroaggregates	Microaggregates	Macroaggregates	Microaggregates
	/‰			
<2.00	–20.82 (0.05)	–20.76 (0.10)	–20.54 (0.17)	–20.24 (0.07)
2.00–2.22	**–19.97 (0.11)**	**–19.62 (0.08)**	**–19.52 (0.06)**	**–18.49 (0.04)**
>2.22	–20.36 (0.16)	–20.09 (0.08)	–18.88 (0.07)	–19.42 (0.10)

[a]More negative δ^{13}C values indicate younger material and a greater proportion of crop-derived C.

substantially less than those associated with coarse silt and fine clay.

The old (more positive) δ^{13}C-signature of the fine-silt-sized fraction (Table 6) confirms that the C derived from the original grassland in this fraction is largely retained on conversion to NT and CT and that this fraction is stable. Nearly all of the other size fractions contained more crop-derived organic matter, i.e. the δ^{13}C-signature is closer to that of the crop (–27.57). Balesdent *et al.* (1987) found, based on ^{13}C natural abundance analyses, that the turnover of C associated with the fine-silt fraction is slowest of all primary particle fractions. In addition, Anderson & Paul (1984) observed by ^{14}C dating that the C associated with fine-silt and coarse clay fractions were the oldest fractions. The resistance to mineralization of C associated with the fine silt is also indicated by the larger proportion of aromatic C in this fraction than in other primary particle fractions (Anderson & Paul, 1984; Oades *et al.*, 1987; Oades, 1990). Skjemstad *et al.* (1993) showed by high-energy ultraviolet photo-oxidation that organic matter is protected more in the silt fraction than in the clay fraction. They found that 36% and 23% of the organic carbon was protected in the silt and clay fractions, respectively. Christensen (1987) also found that the decomposability of organic matter decreased in the order sand > clay ⩾ whole soil > 'silt.

Several authors have indicated that the silt fraction forms a sink for microbial C and N (Ladd *et al.*, 1977; Ladd & Amato, 1980; Chotte *et al.*, 1998). Based on these authors' evidence and the stability of the C and N associated with silt, Oades (1990) concluded that microbial cell debris accumulated and was stabilized within fine-silt-sized aggregates. Cambardella & Elliott (1994) suggested that the organic N accumulated in the ELF under no-tillage regimes derived from the debris of fungal cell walls because the microbial communities in such systems are dominated by fungi (Frey *et al.*, 1999). However, our chemical characterization of ELF suggests that this fraction is neither predominantly fungal nor microbial-derived. This is not immediately surprising considering the reported aromaticity of the carbon associated with silt-sized particles, which suggests that there is a selective stabilization of residual plant-derived compounds in the silt-sized particles (Christensen, 1996).

Conclusion

We set out to isolate, from a range of soils, a pool of organic matter derived from microbes with an intermediate turnover time that is lost upon cultivation. We used the enriched labile fractionation scheme, based on concepts postulated by Cambardella & Elliott (1994). However, we found that the applicability of the method across soils is limited by differences in mineralogy. The differences in mineralogy seem to result in:

1 different macroaggregate constituents being the richest in C and N, and

2 different density windows necessary to isolate the enriched organo–mineral complexes from the fine-silt-sized particles. These observations preclude an easy standardization of the method.

Concerning the dynamics of C and N with increasing cultivation intensity, the enriched labile fractionation scheme revealed that:

1 the dynamics of C and N in constituents of macroaggregates is not very different from that of the total macroaggregates associated C and N;

2 a densiometric analysis of organo–mineral particles does not lead to an isolation of organic pools with different biological availabilities, and

3 the enriched labile fraction is not part of the intermediate pool lost on cultivation, but is the oldest of all the fractions we isolated and therefore seems to be rather a passive pool of material protected within silt-sized organo–mineral complexes.

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