

DIVISION S-7—FOREST & RANGE SOILS

Measuring and Understanding Carbon Storage in Afforested Soils by Physical Fractionation

J. Six,* P. Callewaert, S. Lenders, S. De Gryze, S. J. Morris, E. G. Gregorich, E. A. Paul,
and K. Paustian

ABSTRACT

Forested ecosystems have been identified as potential C sinks. However, the accuracy of measurement and understanding of the underlying mechanisms for soil organic C (SOC) storage in forested ecosystems needs to be improved. The objective of this study was to use aggregate and soil organic matter (SOM) fractionation techniques to identify SOC pools that preferentially stabilize SOC in the long term and elucidate SOC sequestration mechanisms in forested soils. At two sites (Wildlife area, Ohio and Kemptville, Ontario) representing two different soils (Hapludalf and Hapludoll), we sampled soils under agriculture, afforestation, and forest and separated them into aggregates. Different size classes of intra-aggregate particulate organic matter (iPOM) fractions were isolated by density flotation, dispersion, and sieving. At both sites, aggregation and whole SOC content were greater in the forested than in the agricultural ecosystems. The greater aggregation in forested ecosystems resulted in greater iPOM C concentrations, especially the iPOM C fractions associated with microaggregates (53–250 μm) and microaggregates occluded within macroaggregates (mM) (250–2000 μm). The sum of C in these fractions (microaggregate protected C) was 468 ± 29 , 696 ± 171 , 673 ± 70 g C m^{-2} in the agricultural, afforested, and forested soils at Kemptville, respectively. The difference in the microaggregate protected C between the agricultural and the afforested soils accounted, on average, for 20% of the difference in whole SOC stocks between the soils. We conclude, SOC is stabilized for a relatively longer term within microaggregates formed in afforested and forest systems. Therefore, we suggest a new fractionation scheme to isolate this microaggregate associated SOC for assessing the impact of land use, land management, and climate change on C storage.

IN RECENT YEARS, increasing amounts of CO_2 in the atmosphere and the suggested emission limitations based on a C credit trading system in the Kyoto protocol have led to an increased interest in the dynamics of organic C in soils (Intergovernmental Panel on Climate Change, 1997). Soil-vegetation systems may play an important role in the reduction or increase of atmospheric CO_2 concentrations. These systems can act as a source

or sink of atmospheric CO_2 depending on the rate of SOC formation and decomposition (Van Breemen and Feijtel, 1990). It is therefore important to understand and quantify the dynamics of SOC and its link with the current or previous vegetation type. Physical soil properties such as soil structure or aggregation need to be evaluated because they mediate many biological and chemical soil processes and hence decomposition and formation of SOC.

Carbon stored in forest ecosystems represents a substantial part of the global C budget. It is estimated that forest standing biomass constitutes about 82 to 86% of all aboveground C (Richter et al., 1999), and forest soils about 70 to 73% of all SOC (Birdsey et al., 1993). Following cultivation of previously forested lands, SOC can be rapidly lost as a result of enhanced SOM decomposition. Ellert and Gregorich (1996) estimated that 30 to 35% of SOC originally present in the A and B horizons of native temperate forest soils was lost after cultivation for 30 yr or more. Forest plantations, on the other hand, may sequester SOC in soil, especially in cases of establishment on cultivated lands where soil C has been depleted (Johnson, 1992). Several researchers have studied SOC sequestration by afforestation of agricultural land (Huntington, 1995; Ellert and Gregorich, 1996; Bashkin and Binkley, 1998; Post and Kwon, 2000). For example, SOC increased by 0.8 to 4.0 $\text{Mg ha}^{-1} \text{yr}^{-1}$ during secondary forest succession on land that had been cultivated for 100 to 300 yr in Puerto Rico (Lugo and Sanchez, 1986). Bouwman and Leemans (1995) reported that reforestation stores 50 Mg ha^{-1} of SOC in 30 yr, giving a tentative estimate of global SOC accumulation rate in tropical tree plantations of 0.07 Pg yr^{-1} .

Spatial and temporal variability in forest soils reduces the accuracy of SOC stock measurements. In addition, the detection of changes in SOC stocks with management, climate, or land use change is difficult because of the small magnitude of such changes relative to the large forest SOC stocks. Physical fractionation techniques can augment the detection limits for SOC storage by isolating SOC pools more sensitive to changes in management, climate, or land use. Physical fractionation techniques can also elucidate soil processes and mechanisms involved in the storage of SOC. It has been established

J. Six, Dep. of Agronomy and Range Science, One Shields Avenue, Univ. of California, Davis, CA 95616; E.A. Paul and K. Paustian, Natural Resource Ecology Lab., Colorado State Univ., Fort Collins, CO 80523; P. Callewaert, S. Lenders and S. Degryze, Dep. of Land Management, Faculty of Agricultural and Applied Biological Sciences, K.U. Leuven, Kasteelpark Arenberg 20, 3001 Heverlee, Belgium; S.J. Morris, Dep. of Biology, Bradley Univ., 1501 West Bradley Avenue, Peoria, IL 61625; E.G. Gregorich, Eastern Cereal & Oilseed Research Center, Agriculture Canada, Central Experimental Farm, Ottawa, ON, Canada K1A 0C6. Received 7 Jan. 2002. *Corresponding author (jwsix@ucdavis.edu).

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Abbreviations: fPOM, fine particulate organic matter; iPOM, intra-aggregate particulate organic matter; LF, light fraction; mM, microaggregates within macroaggregates; imMPOM, intra-microaggregate particulate organic matter; MWD, mean weight diameter; POM, particulate organic matter; SOC, soil organic C; SOM, soil organic matter.

that aggregation increases in less disturbed systems and that organic materials within soil aggregates (especially microaggregates) have lower decomposition rates than those located outside of aggregates (Oades, 1984; Elliott and Coleman, 1988; Six et al., 2000). Therefore, the objective of our study was to use physical fractionation techniques to identify SOC pools that are preferentially stabilized in the long term under forest vegetation and elucidate SOC sequestration mechanisms in forest soils.

MATERIALS AND METHODS

Sites and Sampling

Soils were sampled from six different sites in the Eastern Forest Region of North America: Wildlife Area (Wild), Chem-lawn, and Maumee in Ohio; Kellogg Biological Station in Michigan; Kemptville and Renfrew in Ontario, Canada. In this paper we focus on data collected from the Wildlife area site (Wild; 40°24' N lat. and 83°01' E long.) and the Kemptville site (45°01' N lat. and 75°38' E long.). Both sites had treatments under agriculture, afforestation, and native forest. Corn (*Zea mays* L.) was the dominant crop in the agriculture treatments at both sites. The vegetation of the afforested system was a successional mixture of maple (*Acer* L.), elm (*Ulmus* L.), and walnut (*Juglans* spp.) at Wild and a planted red pine (*Pinus resinosa*) at Kemptville. The vegetation of the forest was mixed deciduous in Wild; the species were hickory (*Carya* spp.), walnut, oak (*Quercus* spp.), and cherry (*Prunus* spp.). In Kemptville it was a mixed deciduous-conifer forest consisting of poplar (*Populus* spp.), pine (*Pinus* spp.), birch (*Betula* spp.), and cedar (*Thuja* spp.). In Wild, the mean annual

temperature is 10.8°C and mean annual precipitation is 934 mm. Mean annual temperature is 8°C and annual precipitation is 729 mm in rainfall and 186 mm in snow at Kemptville.

Soil samples from the A horizon were taken by the core method at Wild and from pits at Kemptville. At Wild, six cores (diam. = 6.6 cm) were taken in each replicate treatment plot. In the agricultural systems the six cores were taken along a transect with 3 m between two cores. In the afforested and forested systems, three trees were randomly chosen and two sets of cores were taken at each tree. Each set consisted of three cores taken at 0.5, 1, and 1.5 m from the base of the tree. At Kemptville, three randomly chosen 0.5 m² pits were dug in each treatment plot. Soil types are mesic Typic Hapludalf (silty loam) at Wild and Melanic Brunisol (Canadian taxonomy) or Hapludoll (sandy loam) at Kemptville.

Aggregate Separation

The aggregate separation was done according to Elliott (1986) by wet sieving the soil through a series of three sieves (2000, 250, and 53 μ m) to obtain four aggregate-size classes (Fig. 1). Prior to wet sieving, all field moist soil samples were passed through an 8-mm sieve and air-dried. A subsample of 100 g of air-dried soil was submersed in deionized water on top of a 2000- μ m sieve for 5 min. prior to sieving. This process leads to slaking, which is the breaking of unstable aggregates because of air and pressure buildup inside the aggregates upon submersion in water. The sieving was done manually by moving the sieve up and down 3 cm, 50 times in 2 min. Organic material floating on the water in the 2000- μ m sieve was removed after the 2-min cycle, because it is by definition not considered SOM. The fraction that remained on the 2000- μ m

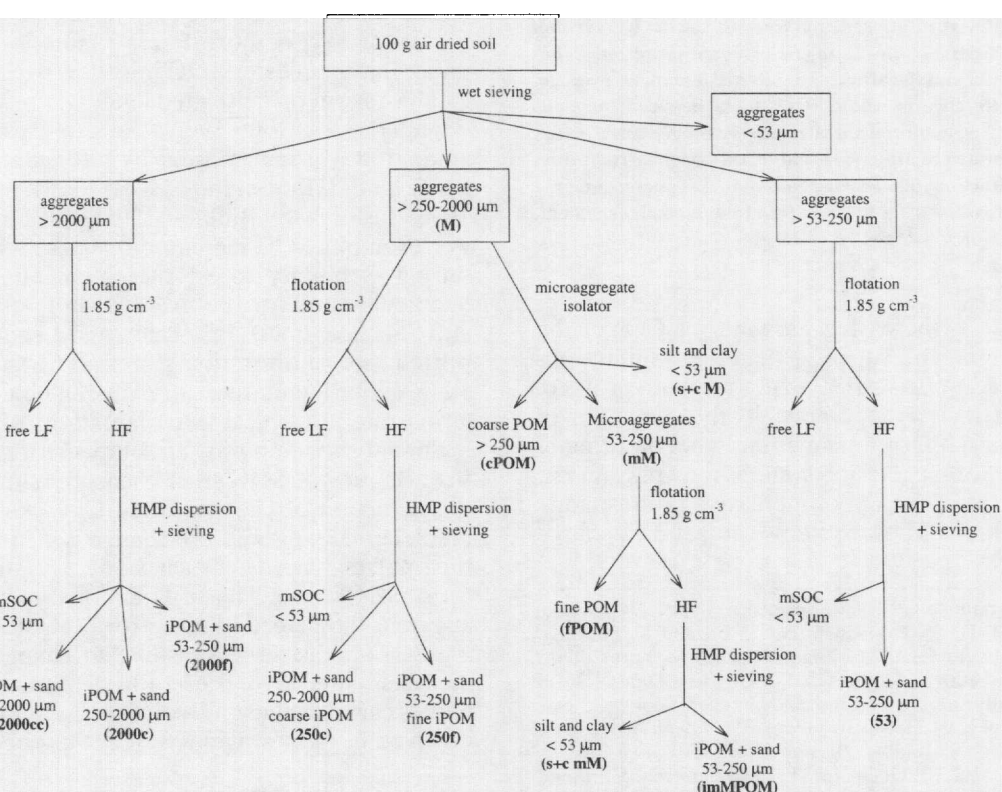


Fig. 1. Fractionation scheme to isolate aggregate and aggregate-associated organic matter fractions. cc = very coarse, c = coarse, f = fine, HF = heavy fraction, HMP = hexametaphosphate, i = intra-aggregate, LF = light fraction, mSOC = mineral associated soil organic C, mM = microaggregates within macroaggregates, M = small macroaggregates, POM = particulate organic matter, s + c = silt and clay (Adapted from Six et al., 1998 and 2000).

sieve was collected in an aluminum pan and oven dried. Water plus soil that went through the 2000- μm -sieve was poured onto the next sieve and sieving was repeated. All fractions were gently back-washed into an aluminum pan and dried overnight (50°C). The next day, the dry rocks in the largest fraction (>2000 μm) were removed and all fractions were weighed. During sieving, both aggregates and sand particles of the same size as the aggregates were retained on the sieves. Sand contents of all aggregate fractions were determined and aggregate weight percentages were corrected, to make comparisons between soils with different sand contents.

$$\frac{\text{aggregate weight \%} - \text{total fraction weight \%}}{\sum \text{ sand corrected weights}} \quad [1]$$

The mean weight diameter (MWD) was calculated according to van Bavel (1949). The MWD equals the sum of products of the mean diameter, \bar{x}_i , of each size fraction and the proportional weight, w_i , of the corresponding size fraction. The mean diameter for the largest fraction was 5000 μm .

$$\text{MWD} = \sum_{i=1}^n \bar{x}_i w_i \quad [2]$$

Free Light Fraction and Intra-Aggregate Particulate Organic Matter

The method for separation of the free light fraction (LF) (i.e., particulate organic matter [POM] outside of the aggregates) and iPOM is adopted from Six et al. (1998) (Fig. 1). Briefly, the free LF associated with the different aggregate-size classes was isolated by density flotation in 1.85 g cm⁻³ sodium polytungstate. After isolation of the LF, the aggregates were dispersed in 0.5% sodium hexametaphosphate by shaking on a reciprocal shaker for 18 h. The dispersed fraction was then passed through 2000-, 250-, or 53- μm sieves depending on the original aggregate-size class. Sodium polytungstate was recycled according to Six et al. (1999a) to avoid cross contamination of C among fractions.

Isolation of Microaggregates out of Macroaggregates

The method for isolation of coarse POM (>250 μm), fine POM (53–250 μm) (fPOM), intra-microaggregate POM (imPOM), and silt + clay fractions held within macroaggregates was adopted from Six et al. (2000). A device (Fig. 2) was used that allowed complete break up of macroaggregates while minimizing the break down of the released microaggregates. Ten grams of macroaggregates were immersed in deionized water on top of a 250- μm mesh screen and shaken with 50 glass beads (diam. = 4 mm). A continuous and steady water flow through the device flushed all released microaggregates immediately onto a 53- μm sieve, thus avoiding further disruption by the beads. After complete breakup of the macroaggregates, only sand and coarse POM remained on the 250- μm mesh screen (Fig. 1). The fraction collected on the 53- μm sieve was sieved (as described above) to separate the water-stable microaggregates from the silt + clay particles. The fine POM, imPOM, and the silt + clay fraction from the microaggregates were isolated by density flotation and dispersion as described above (Fig. 1).

Carbon and Nitrogen Analyses

Aggregate and iPOM C and N concentrations were measured on a LECO CHN-1000 analyzer (Leco Corp., St. Joseph,

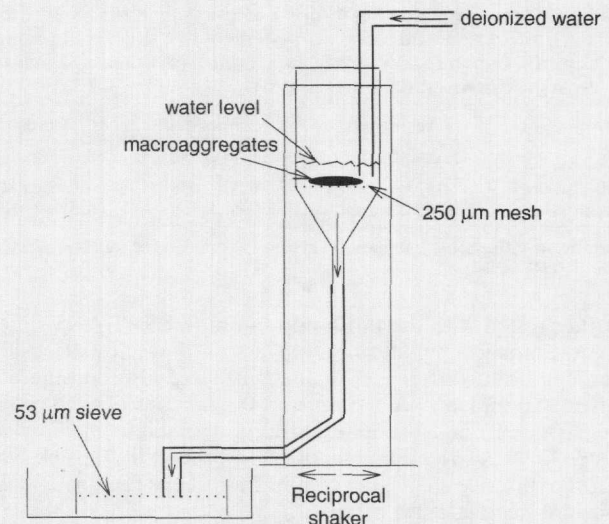


Fig. 2. Schematic presentation of the microaggregate isolator.

MI). Because of smaller sample sizes, LF C was measured on an ANCA 20-20 GSL (PDZ Europe Ltd., Cheshire, UK), which requires less C for analysis. We preferred to use the LECO analyzer if sufficient material was available, to minimize the subsampling error.

Because carbonates were present in our samples, total and C fraction values had to be corrected for inorganic C. In a closed glass vial, 2 mL of acid (6 M HCl + 3% FeCl₂) was added to a soil subsample of 0.2 to 1 g, according to the amount of C present in the sample (Wagner et al., 1998). After 2 h, pressure inside the vial was measured using a pressure transducer. A series of standards and blanks were used to calculate the carbonate content.

Since sand contents differed among aggregate-size classes, SOC concentrations were corrected to minimize confounding effects of sand dilution on C contents (Six et al., 1998).

$$\text{iPOM C (g kg}^{-1} \text{ sand free aggregate)} = \frac{\text{iPOM C (g kg}^{-1} \text{ aggregate)}}{1 - \text{sand content of aggregate}} \quad [3]$$

Differences in sampling depth and bulk density made it necessary to correct for different soil masses. Ellert and Bettany (1995) found that the actual value selected as equivalent mass was less important than the need for using the same standard or reference soil mass for comparisons of organic matter and nutrient storage. The mass of the lightest soil was designated as the equivalent mass of soil. Equivalent SOC and N masses were calculated using the equation:

$$M_c = [\text{Conc}_c \times D_b \times \text{depth} - \text{Conc}_c (M_{\text{soil}} - M_{\text{soil, equiv}})] \times 10 \quad [4]$$

where M_c equals Equivalent C or N mass (g m⁻²); Conc_c equals Organic C or N concentration (g kg⁻¹); D_b equals bulk density (g cm⁻³); Depth equals depth of horizon (cm); M_{soil} equals soil mass (g cm⁻²); $M_{\text{soil, equiv}}$ equals equivalent soil mass (g cm⁻²).

Statistical Analysis

The data were analyzed using the SAS statistical package for analysis of variance ANOVA with the MIXED procedure of the SAS system (SAS/STAT, SAS institute, Cary, NC). Treatment and size class were considered as fixed effects and replicate as a random effect. Differences between means were

Table 1. Total organic C (g C m^{-2}) of the A horizon in agricultural, afforested, and forest ecosystems at two sites (Kemptville, Ontario, Canada and Wildlife area, Ohio). (average \pm standard error), (Morris et al., unpublished).

Site	Agriculture	Afforested	Forest
	g C m^{-2}		
Wildlife area	$1823 \pm 29^*$	$2491 \pm 458^{**}$	5065 ± 410
Kemptville	$2624 \pm 163^*$	$3996 \pm 569^{**}$	3578 ± 505

Significant differences between ecosystems are indicated as $^{**}(P < 0.05)$ and $^*(P < 0.10)$.

further tested with the DIFF option of the LSMEANS statement. Statistical significance was assessed at the 0.05 level, but significant differences at the 0.10 were also considered. Given experimental constraints on the number of field replicates ($n = 3$) and the inherent large variability in forested systems, we also considered the 0.10 probability level in an effort to balance chances of committing a Type II error against those of making a Type I error.

RESULTS

Wildlife Area Site

At Wild, total SOC of the A horizon of each ecosystem increased in the order agricultural < afforested < forested (Table 1). There was a 37% increase in SOC going from the agricultural system to the afforested system. However, SOC content in the forest was almost double the SOC content in the afforested system. Even with the large difference in SOC content between afforested and forested systems, the difference in aggregate distribution was minimal between these two ecosystems.

Wild (A-horizon)

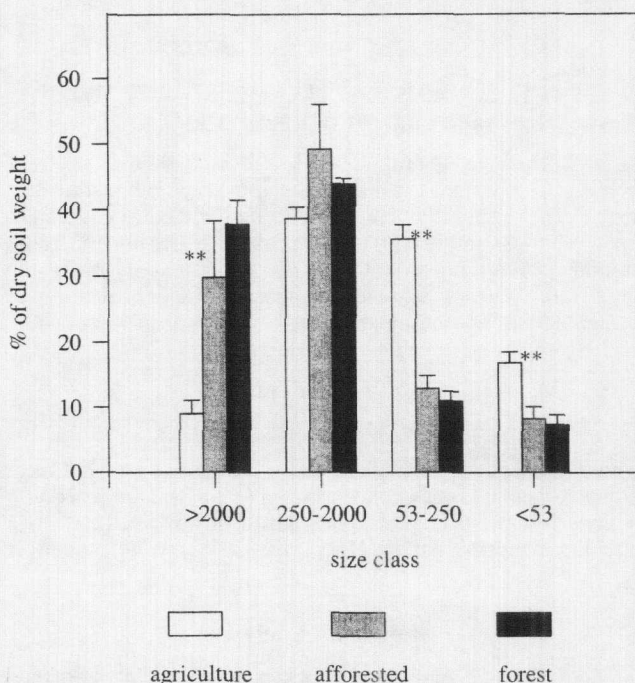


Fig. 3. Aggregate-size distribution in the A horizon of agriculture, afforested, and forest ecosystems at the Wildlife area, Ohio. Bars represent mean \pm standard error. Significant differences between ecosystems are indicated as $^{**}(P < 0.05)$.

On the other hand, a great difference in percentage of large macroaggregates ($>2000 \mu\text{m}$) was observed between the agricultural and the afforested systems (Fig. 3). The percentage of large macroaggregates increased from 10% in the agricultural system to 30 and 40% in the afforested and forest systems, respectively. In all ecosystems, the aggregate distribution was dominated by small macroaggregates ($250\text{--}2000 \mu\text{m}$). Relative amounts of this aggregate fraction were comparable among treatments.

The iPOM C concentration (g kg^{-1} sand-free aggregate) within the large macroaggregates were not significantly different among the ecosystems (Fig. 4). Within the small macroaggregates, the coarse iPOM (250c) was significantly higher in the forest compared with the agricultural and afforested agricultural systems. Surprisingly, the fine iPOM (250f) was the lowest in the afforested treatment. However, the greatest difference between ecosystems was observed in the microaggregate iPOM fraction (53). The microaggregate iPOM C was seven times greater in the forest than in the afforested system. When calculated on an equivalent soil mass basis, the total C amounts of the microaggregate iPOM were 67 ± 7 , 44 ± 12 , and $218 \pm 27 \text{ g C m}^{-2}$ in the agricultural, the afforested, and the forest systems, respectively. In all systems, C levels (on a total soil basis) of the fine iPOM (250f) were the greatest of all the iPOM fractions, followed by the microaggregate POM. Both fractions together accounted for 242 ± 28 , 117 ± 17 , and $456 \pm 33 \text{ g C m}^{-2}$ in the agricultural, afforested, and forest systems, respectively.

Wild (A-horizon)

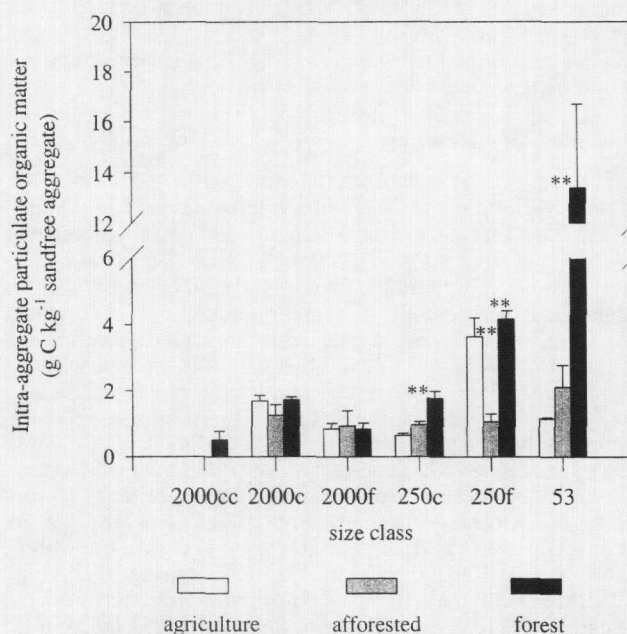


Fig. 4. Intra-aggregate particulate organic matter C contents in the A horizon of agriculture, afforested, and forest ecosystems at the Wildlife area, Ohio. cc = very coarse ($>2000 \text{ mm}$), c = coarse ($250\text{--}2000 \text{ mm}$) f = fine ($53\text{--}250 \text{ mm}$). Bars represent mean \pm standard error. Significant differences between ecosystems are indicated as $^{**}(P < 0.05)$.

Kemptville Site

In contrast to the great difference in SOC contents of the A horizon between afforested and the forest ecosystems at Wild, Kemptville showed less difference in the SOC content of the afforested system and the forest (Table 1). However, the SOC content of the afforested system was 52% higher than the SOC content of the agricultural system. The relative proportion of large macroaggregates was not different among any of the ecosystems but the relative amount of small macroaggregates increased from 30% in the agricultural ecosystem to approximately 50% in the afforested system and forest (data not shown).

A detailed analyses of the SOC fractions within macroaggregates revealed that the imMPOM had the greatest SOC concentration of all fractions in the afforested and the forest systems (Fig. 5). In addition, the greatest difference between ecosystems is observed in the imMPOM and to a lesser extent in the silt + clay fraction not associated with the microaggregates. The other fractions did not differ significantly between the ecosystems. On a soil mass equivalent basis, total C amounts of the imMPOM were 177 ± 3 , 508 ± 104 , and 499 ± 85 g C m⁻² in the agricultural, afforested, and forest systems. The sum of imMPOM and the microaggregate POM (53) constitutes the total POM fraction stabilized within microaggregates. This sum was 468 ± 29 , 696 ± 171 , 673 ± 70 g C m⁻² in the agriculture, afforested, and

forest ecosystems, respectively and accounted for 18, 17, and 19% of the whole SOC content in the respective ecosystems.

DISCUSSION

Concern about the effects of increasing atmospheric CO₂ on climate change has focused considerable debate on uncertainties in the global C budget. Estimates of sources and sinks for CO₂ have consistently provided evidence for an imbalance indicating the existence of a substantial terrestrial sink located in the northern temperate region of the Northern Hemisphere (Tans et al., 1990; Detwiler and Hall, 1988). The identity, magnitude, and understanding of the processes associated with this terrestrial C sink are crucial to predicting environmental response to continued atmospheric loading of CO₂.

Our data suggest a large potential for SOC sequestration in an afforested ecosystem under pine and a smaller potential under the mixed deciduous afforested ecosystem (Table 1). Based on the SOC content (g C m⁻²), reforestation with pine leads to an average annual C sequestration rate of 47 ± 25 g C m⁻² yr⁻¹ in the A horizon over 29 yr. The C sequestration rate over 50 yr was only 13 ± 10 g C m⁻² yr⁻¹ at the Wild site. These sequestration rates are of a similar magnitude as the average C sequestration rate of 33.8 g C m⁻² yr⁻¹ reported by Post and Kwon (2000) and of 30 g C m⁻² yr⁻¹ by Schlesinger (1990) for afforested systems. If we assume that the SOC contents in the here studied forest systems can be used as a measure of the C sequestration potential, then the afforested system at the Wild site is, even after 50 yr, quite far from reaching this potential. In contrast, the afforested system at Kemptville did reach (and even surpassed) its potential after only 29 yr. Our results indicate that the rate and period of C sequestration differ to a great extent between afforested ecosystems and great errors are associated with these estimates.

In afforested and forested ecosystems at both sites, aggregation and intra-aggregate C were significantly higher than in the agricultural systems (Fig. 3–5). The SOC distribution among the intra-aggregate fractions indicates the importance of microaggregates as the primary site of long-term C sequestration in soils, since the highest amount of POM C was stabilized within the microaggregates of the less disturbed systems (afforested and forest). Previous studies have demonstrated that stable microaggregates can stabilize and sequester C in the long term (Skjemstad et al., 1990; Jastrow et al., 1996; Six et al., 1999b; Balesdent et al., 2000). At the Wild site, the high enrichment of microaggregate POM C (53) in the forest ecosystem illustrated this high stabilization of SOC by microaggregates. In addition, the fine iPOM (250f), a fraction stabilized by mM (Six et al., 2000), was the most important fraction on a whole soil basis and was stabilized under the forest. Because of these findings, we decided to isolate the microaggregates within macroaggregates and the fractions associated with these microaggregates at the Kemptville site (Fig. 5). The small concentration of fine POM and the enrich-

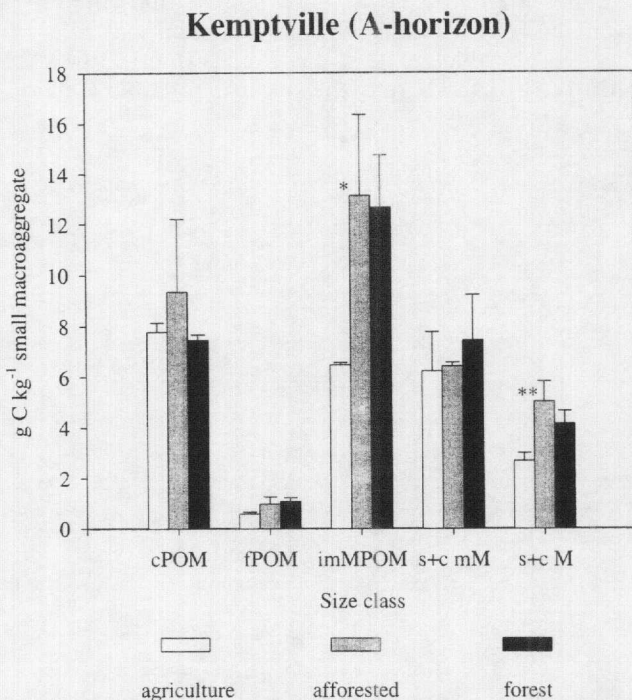


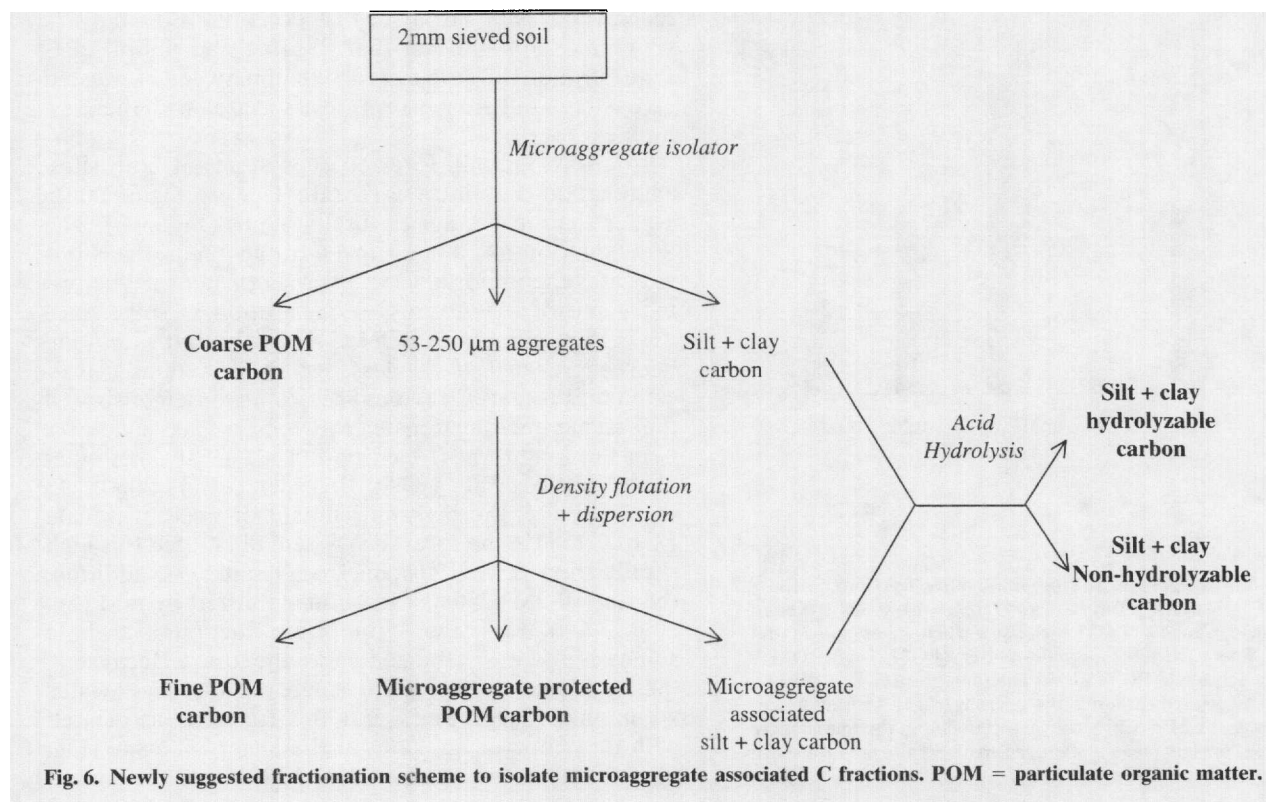
Fig. 5. Intra-macroaggregate soil organic matter fractions in the A horizon of agriculture, afforested, and forest ecosystems at Kemptville, Ontario, Canada. cPOM = coarse particulate organic matter, fPOM = fine particulate organic matter (POM), imMPOM = intra-microaggregate POM within macroaggregates, s + c mM = silt + clay associated carbon in the microaggregates within macroaggregates, s + c M = silt + clay associated C in the macroaggregates. Bars represent mean \pm standard error. Significant differences between ecosystems are indicated as ** ($P < 0.05$) and * ($P < 0.10$).

ment of imMPOM corroborated the finding of Six et al. (2000) that most fine iPOM (250f) is accumulated in microaggregates within macroaggregates. The enrichment of imMPOM under the afforested system and the forest also reinforces the importance of the microaggregates in the stabilization of SOC and their functioning within the soil as a C sink. The stabilization of imMPOM in less disturbed systems such as forest is due to a slower macroaggregate turnover in these systems compared with a more disturbed (agricultural) system (Six et al., 1998, 1999b, 2000). In less disturbed systems, macroaggregate turnover is slower, which leads to the formation of stable microaggregates (within macroaggregates) that stabilize and protect SOC in the long term (Six et al., 2000).

Since the microaggregate POM (53) and the imMPOM both seem to be stabilized in less disturbed ecosystems and they are related to the same functional aggregate fraction, we calculated the sum of these two fractions for the Kemptville site and designated it as the microaggregate protected POM fraction. The microaggregate protected POM accounted on average for 18% of the whole SOC stock and was significantly influenced by ecosystem type. The microaggregate protected POM in the agricultural ecosystem was 228 g C m^{-2} lower than the forest ecosystem, and 205 g C m^{-2} lower than the agricultural ecosystem, which accounts for 17 and 22% of the difference in whole SOC stocks, respectively. These percentages are very similar to the average 20% contribution of the fine iPOM C (250f) differences to the lower whole SOC stocks in conventional tillage compared with no-tillage systems (Six et al., 1999b). In conclusion, the microaggregate protected SOC forms

an SOC pool that is sensitive to ecosystem changes, accounts for a substantial amount of the whole SOC stocks, explains a considerable proportion of the difference in whole SOC stocks between ecosystems, and is stabilized in the long term. Therefore, the microaggregate protected SOC in a SOC pool that can help address questions concerning the impact of climate change, land use, and land management on SOC storage.

To isolate the microaggregate protected POM C pool and other functional SOC pools, such as the nonhydrolyzable SOC (Paul et al., 2001), we developed a new and less complex fractionation scheme (Fig. 6). In a first step, coarse POM, microaggregates, and silt + clay SOC are isolated from 2-mm air-dried sieved soil. These fractions can be isolated with the microaggregate isolator described in the MATERIALS AND METHODS section. In a second step, fine POM that was collected together with the microaggregates on the sieve is isolated by density flotation (Six et al., 1998). Subsequently, microaggregates are dispersed to isolate microaggregate-protected POM versus silt and clay associated C. The silt and clay associated C fractions from Step 1 and 2 are then hydrolyzed to differentiate the silt + clay hydrolyzable C versus nonhydrolyzable C. The hydrolysis is suggested in an attempt to separate a passive from labile and predominantly microbial-derived C pool associated with the silt and clay particles (Paul et al., 2001). We found in some preliminary tests of this fractionation scheme that the microaggregate-protected SOC pool is strongly influenced by land-use changes and land management (e.g., no-tillage versus conventional tillage), the hydrolyzable fraction has a consistently low C/N ratio of 6 to 8 whereas the nonhydrolyzable fraction



had a higher C/N ratio (Six et al., unpublished data, 2002). The C/N ratio of 6 to 8 of the hydrolyzable fraction is consistent with a microbial-derived SOC pool.

CONCLUSIONS

Conversion of cultivated land to forest resulted in increased aggregation and a greater SOC stock in the A horizon at both sites, indicating afforestation on these sites created a soil C sink. However, the magnitude of the C sinks, the rate of C sequestration, and the period of C sequestration differs substantially between afforested ecosystems. Nevertheless, the microaggregates (both the free microaggregates and the microaggregates occluded within the macroaggregates) and their capacity to protect POM C in the longer term were crucial for the SOC sequestration in the forested systems at both sites. On average, 18% of the whole soil C was microaggregate-protected POM and it accounted for 20% of the difference in whole SOC between ecosystems. Consequently, the microaggregates protect an SOC pool in the longer term that forms a substantial proportion of the whole SOC stock and accumulates under forested ecosystems. We also propose a new and simpler fractionation scheme that enables the isolation of this fraction on a more routine basis.

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