

# Soil organic carbon pool changes following land-use conversions

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## Abstract

Carbon (C) can be sequestered in the mineral soil after the conversion of intensively cropped agricultural fields to more extensive land uses such as afforested and natural succession ecosystems. Three land-use treatments from the long-term ecological research site at Kellogg biological station in Michigan were compared with a nearby deciduous forest. Treatments included a conventionally tilled cropland, a former cropland afforested with poplar for 10 years and an old field (10 years) succession. We used soil aggregate and soil organic matter fractionation techniques to isolate C pools that (1) have a high potential for C storage and (2) accumulate C at a fast rate during afforestation or succession. These fractions could serve as sensitive indicators for the total change in C content due to land-use changes. At the mineral soil surface (0–7 cm), afforestation significantly increased soil aggregation to levels similar to native forest. However, surface soil (0–7 cm) C did not follow this trend: soil C of the native forest site (22.9 t C ha<sup>-1</sup>) was still significantly greater than the afforested (12.6 t C ha<sup>-1</sup>) and succession (15.4 t C ha<sup>-1</sup>) treatments. However, when the 0–50 cm soil layer was considered, no differences in total soil C were observed between the cropland and the poplar afforested system, while the successional system increased total soil C (0–50 cm) at a rate of 0.786 t C ha<sup>-1</sup> yr<sup>-1</sup>. Afforested soils sequestered C mainly in the fine intra-aggregate particulate organic matter (POM) (53–250 µm), whereas the successional soils sequestered C preferentially in the mineral-associated organic matter and fine intra-aggregate POM C pools.

## Nomenclature

- POM = particulate organic matter, i.e. organic matter of size >53 µm
- intra-POM = POM material contained within an aggregate
- fine intra-POM = intra-POM of size 53–250 µm
- coarse intra-POM = intra-POM of size 250–2000 µm
- total light POM = POM material having a density <1.85 g cm<sup>-3</sup>
- total heavy POM = POM material having a density >1.85 g cm<sup>-3</sup>
- free light POM = POM not contained in a soil aggregate having a density <1.85 g cm<sup>-3</sup>
- mineral-associated OM = carbon associated with the silt and clay fraction (<53 µm)
- MWD = mean weight diameter, the estimated median size of all soil aggregates
- OM = organic matter
- SOM = soil organic matter
- macroaggregate = aggregate of size >250 µm
- large macroaggregate = aggregate of size >2000 µm
- small macroaggregate = aggregate of size 250–2000 µm
- microaggregate = aggregate of size 53–250 µm
- silt and clay = soil fraction of size <53 µm.

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## Introduction

During the past two centuries, land-use practices such as deforestation and tillage have resulted in a net loss of soil carbon (C) to the atmosphere. Recent concerns about rising carbon dioxide (CO<sub>2</sub>) concentrations in the atmosphere have focused attention on the possibility of sequestering C back into the soil system. This may be achieved by means of afforestation and other land-use conversions. It has been noted that these C sinks represent only a partial solution given that the capacity of soils to store carbon is finite (Hassink, 1996; Six *et al.*, 2002b) and that increases in soil organic matter (SOM) can be reversed if proper management is not maintained (Paustian *et al.*, 2000). Nevertheless, soil C sequestration could be important in providing a mechanism to temporarily buffer rising atmospheric CO<sub>2</sub> concentrations.

Carbon stored in forest ecosystems represents a substantial part of the global C stock. Worldwide, forests contain ~70% of all plant C and ~20% of all soil C. Furthermore, the average C content of forest soils is relatively large: 120 t C ha<sup>-1</sup> vs. a mean value of all ecosystem soils of 79 t C ha<sup>-1</sup> (Amthor, 1998). The C content of forest soils, however, varies greatly with climatic and soil properties. Post *et al.* (1982) reported that forests in cooler climates often contain nearly twice as much soil C as warm temperate forests.

Many studies show large losses of soil C after forest clearing. For example, Ellert & Gregorich (1996) estimated that 30–35% of C originally present in the A and B horizons of native temperate forest soils was lost after 30 or more years of cultivation. On the other hand, these large losses of soil C after forest clearing suggest that forest regrowth could lead to C sequestration. Therefore, agriculture to forest conversions after 1950 were hypothesized to be a 'missing sink' in the global C cycle (Johnson & Sharpe, 1983). Estimates of this sink ranged from 0.6 to 2.3 Gt C yr<sup>-1</sup> during the 1980s and from 2 to 4 Gt C yr<sup>-1</sup> during the 1990s (Schimel *et al.*, 2001). However, Post & Kwon (2000) estimated that only 5–10% of the inferred terrestrial sink could be explained by soil C accumulations during forest growth. Most likely, the missing sink is a combination of smaller sinks, such as C uptake by forests, and also by peatlands or wetlands combined with a higher ocean uptake than previously estimated (Schindler, 1999). This debate illustrates that significant uncertainties remain regarding the role of forests in the global C

cycle and the environmental response to rising concentrations of atmospheric CO<sub>2</sub> (Schimel *et al.*, 2001).

Recent studies about the potential and rate of C sequestration in forests were reviewed by Rollinger *et al.* (1997) and Post & Kwon (2000). The latter authors found an average rate of soil C accumulation after afforestation of 0.338 t C ha<sup>-1</sup> yr<sup>-1</sup> although values vary greatly among studies. Jenkinson (1971) found an increase of 0.596 t C ha<sup>-1</sup> yr<sup>-1</sup> after conversion of an old field to an oak forest while Robertson & Vitousek (1981) reported a value of only 0.094 t C ha<sup>-1</sup> yr<sup>-1</sup> for the same vegetation conversion type. The source of this variation is only partly understood.

In general, mechanistic C sequestration studies can be divided into two groups: (1) studies that correlate total SOM accumulation with ecosystem driving variables (e.g. soil texture, soil structure, annual rainfall, temperature) and (2) studies that compare organic matter (OM) fractions among treatments. Fractionation of OM implies the separation of the total OM into different parts that are thought to be functionally homogeneous with respect to physicochemical properties and turnover rate. The separation can be carried out by physical or chemical means: e.g. sieving, flotation, dispersion. The difference in C content between fractions of an agricultural field and the corresponding native vegetation can yield information about the mechanisms of C sequestration (Six *et al.*, 2002a; Del Galdo *et al.*, 2003). Furthermore, fractions that are more sensitive to land-use changes than the total C can serve as an early indicator of changes (Six *et al.*, 2002a).

We followed the second approach and used two different fractionation schemes: (1) a complex fractionation combining sieving, dispersion and flotation steps and (2) a particulate organic matter (POM) fractionation with subsequent flotation (at 1.85 g cm<sup>-3</sup>) to separate light and heavy POM. The objectives of this study were to (1) investigate potential C sequestration and the rate at which C accumulates in the mineral soil of afforested and successional plots, and (2) isolate fractions that have a high potential for C storage and that accumulate C at a fast rate.

## Materials and methods

### Site description

Michigan State University's W.K. Kellogg biological station is located in southwest Michigan (42°24'N,

85°24'W) at an elevation of 288 m. Annual rainfall averages 890 mm yr<sup>-1</sup> (half as snow and half as rain). Potential evapotranspiration exceeds precipitation for approximately 4 months. Mean annual temperature is 9.7 °C. The soil is a Kalamazoo loam: fine-loamy, mixed, semiactive, mesic Typic Hapludalf. A soil horizon description is given in Table 1.

In April and May 1999, we sampled the native deciduous forest and three treatments from the ongoing long-term ecological research experiment (LTER). This is a randomized complete block design with six replicate blocks and seven treatments. Before establishment of the LTER experiment in 1989, the site has been in agriculture for at least 100 years. Sampled treatments were: conventionally tilled cropland rotation (soybean, *Glycine max* L.; wheat, *Triticum aestivum* L. and corn, *Zea mays* L.), a cropland afforested with a fast growing Poplar, *Populus* clone, a natural succession managed by occasional burning or felling of woody biomass, and a native oak-hickory deciduous forest (*Quercus* sp.–*Carya* sp.). Before establishment of treatments, all plots were under the same corn–soybean rotation, except the sixth replicate plot, which was under alfalfa, *Medicago sativa* L. Mean aboveground primary production for the treatments were: 9.24 ± 1.41 Mt ha<sup>-1</sup> yr<sup>-1</sup> for the agricultural, 10.17 ± 4.00 Mt ha<sup>-1</sup> yr<sup>-1</sup> for the afforested and 4.24 ± 0.37 Mt ha<sup>-1</sup> yr<sup>-1</sup> for the successional treatment (Robertson *et al.*, 2000). Sampling of the mineral soil was done in three layers (0–7, 7–25 and 25–50 cm). The complete experimental design is explained in <http://lter.kbs.msu.edu/experimentalDesign.html>. Further biological, physical and agronomical background information can be found at <http://lter.kbs.msu.edu/Data>.

Six subsamples were taken from the mineral soil with a 6.7 cm diameter core from each replicate plot. Samples were taken at three points along the plot diagonal. At each point, two samples were taken approximately 60 cm apart. In the native forest, three trees were randomly selected and samples were taken at the base of the tree and 60 cm away from the tree. A subsample of each core was dried at the standard temperature of

110 °C to determine gravimetric water content (Jarrell *et al.*, 1999). Bulk density was determined for each sample by dividing soil core volume by dry soil weight. All samples were passed through an 8 mm sieve by gently crumbling the moist soil. The six samples were composited per plot and depth. Subsamples of ~150 g were put into paper bags, allowed to dry at room temperature before samples were taken for analysis of total C and N and fractionations.

#### Aggregate separation

The complete fractionation sequence is illustrated in Fig. 1. Aggregates were separated by wet sieving as described by Elliott (1986). Prior to sieving, the soil (100 g) was submerged for 5 min in deionized water. Four aggregate fractions were obtained: >2000 µm (large macroaggregates), 250–2000 µm (small macroaggregates), 53–250 µm (microaggregates) and <53 µm (silt and clay fraction). All fractions were oven dried overnight (60 °C) and stored in closed glass jars at room temperature. Fraction weights were corrected for different sand contents before comparison. The mean weight diameter (MWD), which is an approximation of the median size of the aggregates, was calculated as:

$$\text{MWD} = \frac{2000 + 8000}{2} [A] + \frac{2000 + 250}{2} [B] + \frac{250 + 53}{2} [C] + \frac{53}{2} [D],$$

where MWD is the mean weight diameter in µm, [A], [B], [C] and [D] are weight percentages of the aggregate fractions and the silt and clay particles, respectively.

#### Fractionation scheme A: separation of free light POM and intra-aggregate POM

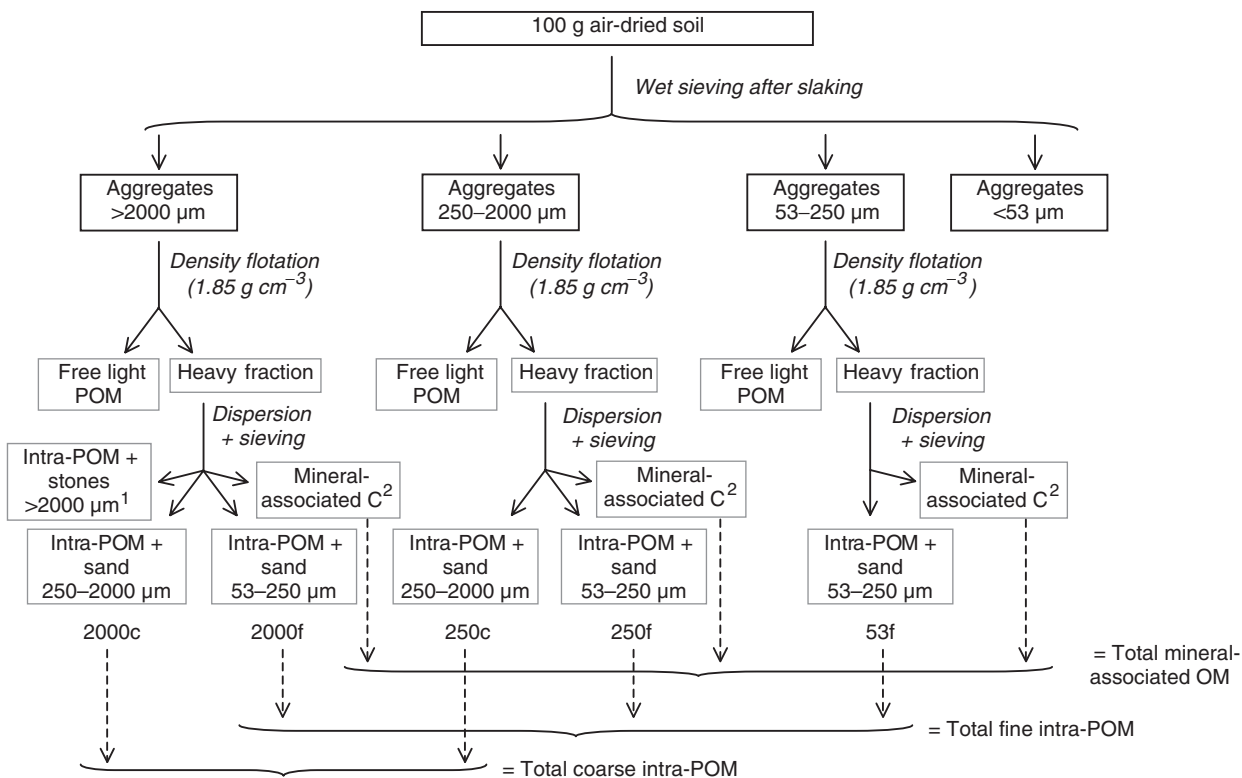
Aggregates from the 0–7 and 7–25 cm depths were analyzed for free light POM and intra-aggregate POM (intra-POM) using the method from Six *et al.* (1998). Soil taken from the 25–50 cm soil layer had too little

**Table 1** Mineral soil horizon description of Kalamazoo soil

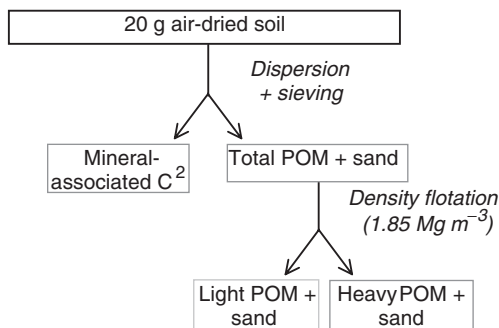
Horizon	Depth (cm)	Texture	CEC (cmol <sub>c</sub> kg <sup>-1</sup> )	Total C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	pH
Ap	0–30	Loam	8.4	12.85	1.31	5.5
E	30–41	Loam	11.5	3.25	0.53	5.7
Bt1	41–69	Silty clay loam	15.3	2.25	0.42	5.3
2Bt2	69–88	Silty loam	4.1	0.67	0.42	5.2
2E/Bt	88–152	Silt	2.3	0.20	0.18	5.6

Adapted from the Kellogg Biological Station data catalog (Crum & Collins, 1992). No error data were available. CEC, Cation Exchange Capacity.

Fractionation scheme A



Fractionation scheme B



<sup>1</sup> This material was not further analyzed, as organic material >2000  $\mu\text{m}$  is not considered POM.

<sup>2</sup> This material was not fractionated but rather calculated by difference between total C content and C content of subfractions.

Fig. 1 Graphical representation of the two fractionation schemes used.

intra-POM material to measure. A 5 g subsample was suspended in 45 ml of  $1.85 \text{ g cm}^{-3}$  sodium polytungstate. After centrifugation, the floating material (i.e. the free light POM) was aspirated. The heavy fraction was dispersed in 0.5% hexametaphosphate, and sieved through 2000, 250 and 53  $\mu\text{m}$  sieves, depending on the aggregate size being analyzed. The material remaining on the sieves was dried and weighed.

*Fractionation scheme B: separation of total heavy and light POM and texture analysis*

Total POM was separated by dispersing a 30 g subsample (see previous section) and sieving this through a 53  $\mu\text{m}$  sieve. From this material, the total light POM ( $< 1.85 \text{ g cm}^{-3}$ ) was separated by flotation as described in the previous section. The silt and clay that

passed through the sieve were transferred to sedimentation cylinders and used to measure clay content using the hydrometer method. The amount of silt was calculated by difference.

#### *Carbon and nitrogen analysis*

Total C and nitrogen (N) concentrations in aggregate fractions and intra-POM were measured with a LECO CHN-1000 analyzer (Leco Corp., St Joseph, MI, USA). When not enough material was available, samples were measured on an ANCA 20–20 GSL analyzer (PDZ Europe Ltd, Cheshire, UK), which requires less material for analysis. No carbonates were present. Total C and N concentrations of all fractions were normalized on a sand-free basis (Six *et al.*, 1998). Weights of intra-POM + sand were used to sand-correct aggregate C and intra-POM C contents (see fractionation scheme A in Fig. 1) and weights of total heavy POM + sand and total light POM + sand were used to correct C and N contents of total heavy POM, total light POM and total POM (see fractionation scheme B).

#### *Carbon accumulation rates*

The rate of C accumulation for all treatments was calculated by dividing the difference in soil C between each treatment (afforested and successional) and the control treatment (agricultural) by the duration of the experiment (Huntington, 1995; Van Laer *et al.*, 1995). Two assumptions were made: (1) at the beginning of the experiment there was no difference in soil C between the afforested, successional and control treatments (the agricultural field) and (2) the C content of the agricultural field did not significantly change between 1989 and 1999 (Post & Kwon, 2000). Assumption (1) is valid since C values (0–15 cm) from the baseline sampling at the start of the experiment did not differ significantly:  $9.04 \pm 0.84$ ,  $8.64 \pm 0.9$  and  $9.69 \pm 0.9$  g C kg<sup>-1</sup> for the agricultural, afforested and successional treatments, respectively (Kellogg biological station's long-term ecological research data catalog, Robertson & Paul, 1996). Assumption (2) is harder to test since the baseline data were sampled at different depths than in this experiment (0–15 cm instead of 0–7 and 7–25 cm). We estimated values for the 7–15 cm layer using second-order interpolation. Total C concentrations for the 0–15 cm layer could then be calculated by taking weighed averages for the 0–7 and 7–15 cm layers. Since the estimate for C in the 0–15 cm layer ( $9.08 \pm 0.63$ ) was almost identical to the value in 1989 ( $9.04 \pm 0.84$ ), assumption (2) also seems valid. Standard deviation for C accumulation rates was calculated by considering the accumulation rate as a contrast of two

means from independent groups (Sahai & Ageel, 2000). Group independency follows from the fact that the agricultural and afforested systems are on different plots. Significance was tested using the procedure outlined in Sahai & Ageel (2000).

#### *Statistical analysis*

The data were analyzed as a complete randomized design using the SAS statistical package for analysis of variance ANOVA with the MIXED procedure (SAS/STAT, SAS Institute, 2001). Treatment, size class and depth were considered as fixed effects whereas replicate was a random effect. Differences between means were tested with the Tukey procedure for multiple comparisons (DIFF option of the LSMEANS statement) and with Fisher's LSD test.

## **Results**

#### *Whole-soil characteristics*

Differences in total organic C of the mineral soil among ecosystems increased in the order agricultural < afforested < successional  $\ll$  native forest systems for the 0–7 and 7–25 cm layers (Table 2). No significant differences were observed at a depth of 25–50 cm. The native forest had less clay and lower bulk density compared with the systems at the main site. Whole-soil organic C at depth 0–25 cm (Table 3) followed the same trends as described for depth 0–7 cm. At depth 0–50 cm, the agricultural, afforested and successional systems did not differ, while the native forest was significantly greater. Organic C accumulation rates were significant for both the afforested and the successional systems at the 0–7 cm depth. At depths 0–25 and 0–50 cm, only the successional system had a significant increase in C (Table 3).

#### *MWD and aggregate size distribution*

The MWD was strongly influenced by cultivation (Fig. 2). At 0–7 and 7–25 cm depths, the agricultural field had a significantly smaller MWD compared with the other systems. The non-cultivated systems were very similar at all depths. Differences in MWD were mainly due to differences in the large macroaggregate size class (>2000  $\mu$ m) (Fig. 3). The cultivated system had a larger proportion of soil in the microaggregate size class (53–250  $\mu$ m).

#### *Aggregate carbon*

Aggregate C content of the mineral soil generally decreased in the following order: macroaggregate

**Table 2** Mineral soil organic carbon, texture (mean  $\pm$  standard error) and bulk densities at three soil depths (0–7, 7–25 and 25–50 cm) of an agricultural, poplar afforested, successional and native forest system

Depth (cm)	Treatment	Organic C (g C kg <sup>-1</sup> soil)	Clay (%)	Silt (%)	Sand (%)	Bulk density (g cm <sup>-3</sup> )
0–7	Agricultural	10.0 C a	26 $\pm$ 6	37 $\pm$ 3	37 $\pm$ 4	1.4 A
	Afforested	14.0 BC a	24 $\pm$ 5	38 $\pm$ 2	38 $\pm$ 4	1.3 A
	Successional	16.0 B a	24 $\pm$ 5	39 $\pm$ 2	37 $\pm$ 4	1.4 A
	Native forest	29.3 A a	9	24	67	1.1 B
7–25	Agricultural	8.5 BC a	26 $\pm$ 7	39 $\pm$ 6	35 $\pm$ 5	1.5 A
	Afforested	7.9 C b	26 $\pm$ 6	37 $\pm$ 4	37 $\pm$ 2	1.4 A
	Successional	9.9 AB b	25 $\pm$ 6	40 $\pm$ 5	34 $\pm$ 5	1.4 A
	Native forest	11.1 A b	21 $\pm$ 9	25 $\pm$ 17	54 $\pm$ 26	1.3 B
25–50	Agricultural	3.7 A b	26 $\pm$ 6	37 $\pm$ 5	37 $\pm$ 3	1.6 A
	Afforested	3.4 A c	28 $\pm$ 2	36 $\pm$ 3	36 $\pm$ 1	1.6 A
	Successional	3.6 A c	25 $\pm$ 5	38 $\pm$ 3	37 $\pm$ 4	1.6 A
	Native forest	3.5 A b	18 $\pm$ 8	25 $\pm$ 12	57 $\pm$ 19	1.6 A

Within a depth, treatments followed by a different uppercase letter differ significantly among treatments ( $P < 0.05$ ). Within a treatment, depths followed by a different lowercase letter differ significantly among depths ( $P < 0.05$ ).

**Table 3** Mineral soil organic carbon and organic carbon (OC) accumulation rate at three soil depths (0–7, 0–25, 0–50 cm) of an agricultural, poplar afforested, successional and native forest system

Depth (cm)	Treatment	OC (t C ha <sup>-1</sup> )	OC accumulation rate (t C ha <sup>-1</sup> yr <sup>-1</sup> )
0–7	Agricultural	9.4 D	NR
	Afforested	12.6 C	0.318*
	Successional	15.4 B	0.596*
	Native forest	22.9 A	NR
0–25	Agricultural	31.9 C	NR
	Afforested	32.3 C	0.035
	Successional	40.0 B	0.813*
	Native forest	48.6 A	NR
0–50	Agricultural	46.7 B	NR
	Afforested	45.7 B	-0.107
	Successional	54.6 AB	0.786*
	Native forest	62.4 A	NR

NR, not relevant. Within a depth, treatments followed by a different uppercase letter differ significantly among treatments ( $P < 0.05$ ). Accumulation rates followed by an asterisk (\*) are significant ( $P < 0.05$ ).

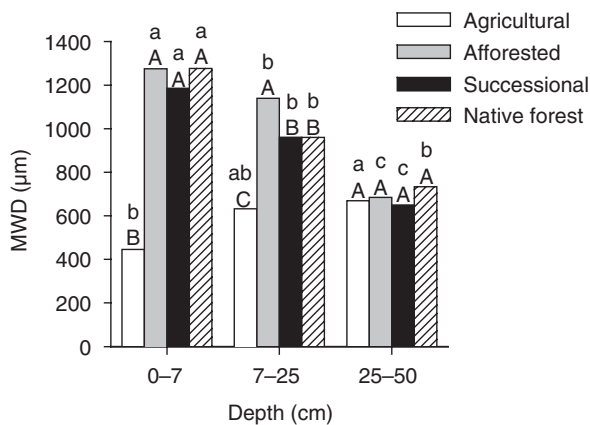
(>250  $\mu$ m) associated C > microaggregate (53–250  $\mu$ m) associated C > C associated with silt and clay particles (<53  $\mu$ m) (Fig. 4). Within a size class, aggregate C concentrations of the agricultural, afforested and successional systems were generally similar while aggregate C in the native forest was significantly larger. Among systems, differences generally decreased with increasing depth. The largest differences in C among systems were found in the large macroaggregate fraction.

#### Carbon content of intra-POM and free light POM (fractionation scheme A)

The mineral associated OM fraction accounts for most of the total C content and was largest in the native

forest system (Fig. 5). The amount of C in the free light (<1.85 g cm<sup>-3</sup>) POM increased in the following order: agricultural < afforested  $\approx$  successional < native forest systems. Total coarse (>250  $\mu$ m) intra-POM C and total fine (53–250  $\mu$ m) intra-POM C of the native forest were significantly greater than in the other systems. No differences existed in total coarse intra-POM C among the agricultural, afforested and successional systems whereas the total fine intra-POM of the agricultural system was significantly less than in the afforested and successional systems. There was about half as much C in the free light, total fine and total coarse POM fractions at a depth of 7–25 than at 0–7 cm.

Figure 6 shows the C concentrations in the separate intra-POM fractions of the separate aggregate size classes. For the afforested, native forest and successional



**Fig. 2** Mean weight diameter (MWD) of slaked soils at three mineral soil depths (0–7, 7–25 and 25–50 cm) of an agricultural, poplar afforested, successional and native forest system. Within a treatment, bars with a different lowercase letter differ significantly among depths ( $P < 0.05$ ). Within a depth, bars with a different uppercase letter differ significantly among treatments ( $P < 0.05$ ).

systems, the greatest C concentration was observed in the intra-POM of the microaggregates (53–250 µm) (53f in Figs 1 and 6). Fine intra-POM C concentration (2000f, 250f and 53 in Figs 1 and 6) was largest in the native forest and larger in the afforested than the agricultural field. Coarse intra-POM C concentration (2000c and 250c) was also largest in the native forest, but there were no differences among agricultural, afforested and successional systems. No significant differences existed among the intra-POM C concentrations of the agricultural, afforested and successional systems at a depth of 7–25 cm.

For both the afforested and successional systems, there was significant C accumulation in all POM fractions and particularly in the fine intra-POM fraction (Table 4). Furthermore, the afforested system had a very significant increase in the coarse intra-POM fraction. The succession had a very significant ( $P < 0.01$ ) increase in the mineral-associated OM. At a depth of 7–25 cm, C accumulation rates were not significant.

#### Carbon content of total, light and heavy POM (fractionation scheme B)

On average, total POM (see fractionation scheme B in Fig. 1) accounted for ~28% of all C in the mineral surface soil (0–7 cm) and ~17% at the 7–25 cm depth (Fig. 5). The native forest had more total heavy POM ( $> 1.85 \text{ g cm}^{-3}$ ) at the surface (0–7 cm) than the other systems. No significant differences in total heavy POM among systems were observed at depth 7–25 cm. At the surface (0–7 cm), the agricultural system contained the

least total light POM ( $< 1.85 \text{ g cm}^{-3}$ ). At 7–25 cm, the native forest contained the most total light POM.

Figure 5 compares the two fractionation schemes. Ideally, total amounts of C (bar heights) and total amounts of POM should be the same. Because of the many steps of fractionation scheme A in comparison with fractionation scheme B and often the limited number of aggregates available, total POM in fractionation scheme A is generally smaller than total POM in fractionation scheme B and mineral associated OM in fractionation scheme A was greater than in fractionation scheme B (except for the native forest).

#### Carbon to nitrogen ratios

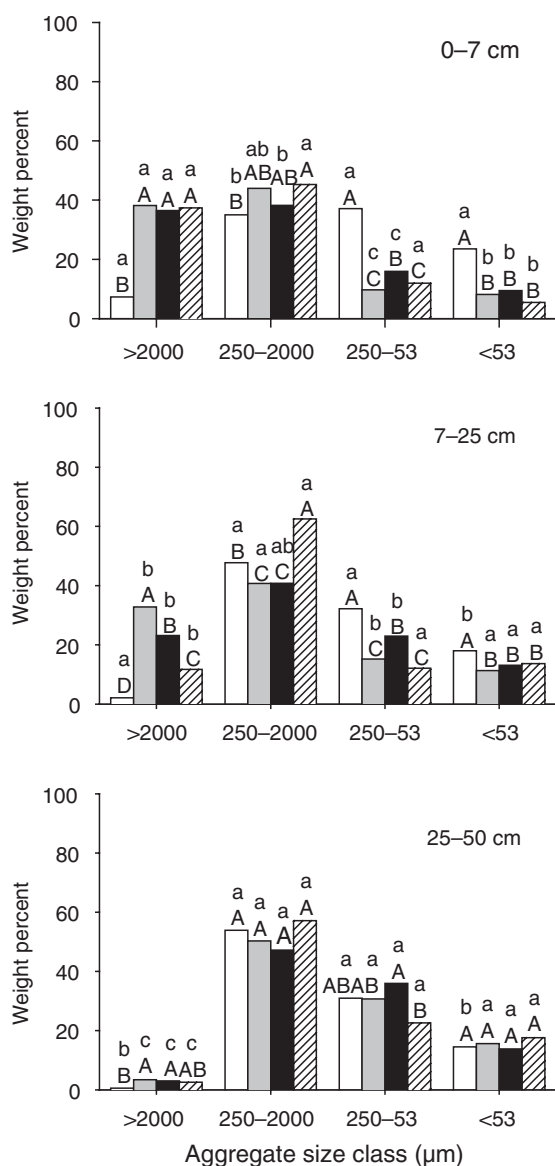
For almost all fractions, the native forest had the largest C-to-N ratios and the agricultural system had the smallest (Table 5). At the 7–25 cm depth, only the C–N ratio of aggregate fractions and light POM ( $< 1.85 \text{ g cm}^{-3}$ ) differed among systems. At both soil depths, total soil C–N ratios decreased significantly in the order: native forest  $>$  successional  $\approx$  afforested  $>$  agricultural systems. While no clear differences in C–N ratios of intra-POM size classes were observed, the total light POM fraction had significantly greater C–N ratios compared with the total heavy POM fraction.

## Discussion

#### Carbon sequestration: rate and extent

The experimental treatments represent possible land-use changes from conventional agriculture that can potentially lead to C sequestration and a mitigation of greenhouse gas emissions. The natural succession treatment is representative of land abandonment and is similar to conservation set-asides such as in the Conservation Reserve Program. It has a typical perennial grass-dominated vegetation that established quickly. The poplar treatment is managed as a short-rotation biomass crop that could be used for energy production and/or as an industrial feedstock.

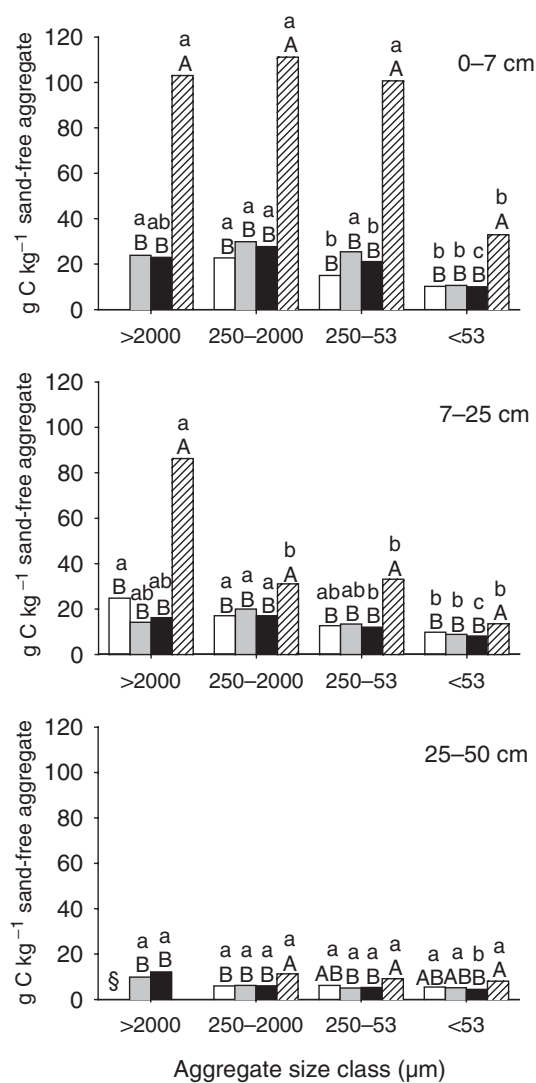
The difference in C content of the mineral soil between the native forest and the agricultural system serves as an indicator for the overall C sequestration potential (Ellert & Gregorich, 1996). Our data suggest that there is considerable potential for C sequestration in the surface layer (0–7 cm) of these soils. Cultivation reduced surface soil (0–7 cm) C by 60% compared with forest soils (Table 3). This is in agreement with other studies. Johnson & Pflieger (1992) found soil C losses of 30–60% (0–7 cm) and Ellert & Gregorich (1996) found a 34% loss of C. However, sequestration potential decreases sharply with increasing depth. At a depth



**Fig. 3** Weight percentages of slaking resistant aggregates at three mineral soil depths (0–7, 7–25 and 25–50 cm) of an agricultural, poplar afforested, successional and native forest system. Within a depth and treatment, bars with a different lowercase letter differ significantly among size classes ( $P < 0.05$ ). Within a depth and size class, bars indicated by a different uppercase letter differ significantly among treatments ( $P < 0.05$ ).

of 7–25 cm, inferred cultivation losses were only 10% and at a depth of 25–50 cm C concentrations between native forest and the agricultural system were not significantly different (Table 2).

Differences in mineral soil C between the land-use change treatments and the agricultural system after 10 years of establishment serve as an indicator for C sequestration rates. Rates of C accumulation varied



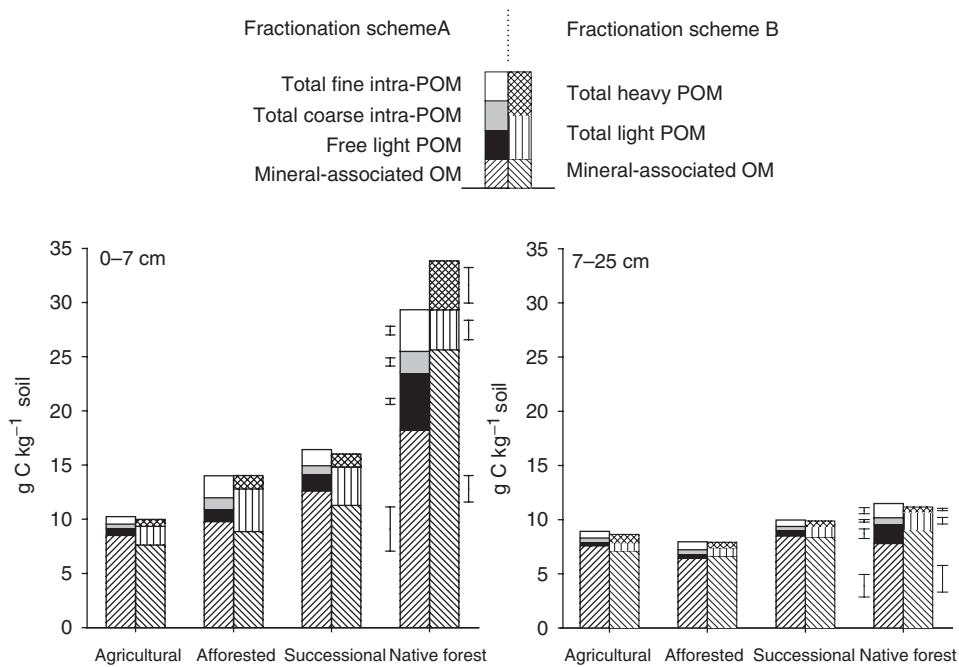
§ Not enough material was available

**Fig. 4** Total sand-corrected aggregate carbon at three mineral soil depths (0–7, 7–25 and 25–50 cm) of an agricultural, poplar afforested, successional and native forest system. Within a depth and treatment, bars with a different lowercase letter differ significantly among size classes ( $P < 0.05$ ). Within a depth and size class, bars indicated by a different uppercase letter differ significantly among treatments ( $P < 0.05$ ).

considerably between afforested and successional systems and among depths (Table 3). At the surface (0–7 cm), cessation of cultivation and conversion to perennial vegetation increased total soil C in both treatments. Rates of C accumulation in the soil surface (0–7 cm) were comparable with the average accumulation rate of 0.30–0.50 t C ha<sup>-1</sup> yr<sup>-1</sup> reported for afforested systems by Post & Kwon (2000) and Six *et al.* (2002a).

Some authors have suggested that forest soils could sequester C in the subsoil (Huntington, 1995; Post &





**Fig. 5** Total carbon content divided into C fractions at two mineral soil depths (0–7 and 7–25 cm) of an agricultural, poplar afforested, successional and native forest system. See Fig. 1 for explanation of the fractions. Within a depth, treatment and fractionation scheme, error bars represent the Fisher's LSD ( $P < 0.05$ ) among fractions.

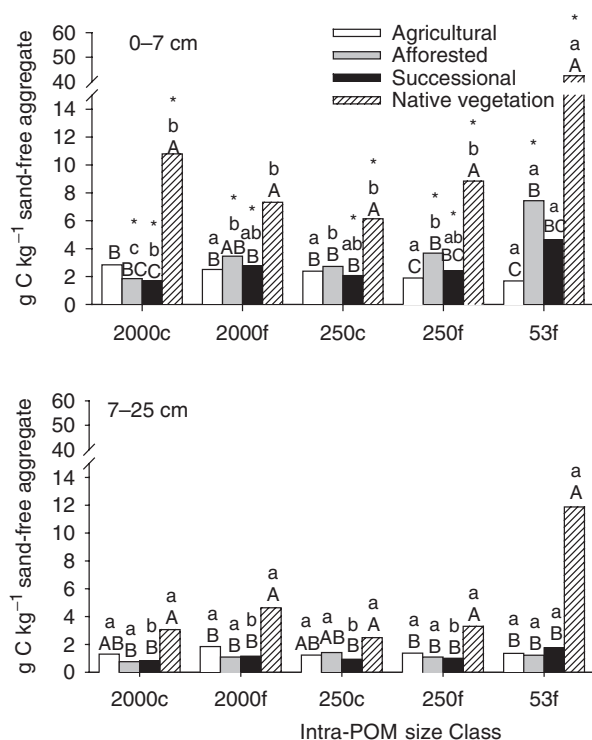
**Table 4** Carbon accumulation rates for four fractions at two mineral soil depths (0–7, 7–25 cm) (means  $\pm$  standard deviation) of a poplar afforested and a successional system

Treatment	Depth (cm)	C accumulation rate ( $\text{t C ha}^{-1} \text{ yr}^{-1}$ )			
		Free light POM	Total fine intra-POM	Total coarse intra-POM	Mineral-associated OM
Afforested	0–7	$0.046 \pm 0.019^*$	$0.117 \pm 0.020^{**}$	$0.054 \pm 0.014^{**}$	$0.087 \pm 0.105$
	7–25	$-0.008 \pm 0.015$	$0.022 \pm 0.040$	$0.005 \pm 0.018$	$-0.412 \pm 0.215$
	0–25	$0.038 \pm 0.027$	$0.139 \pm 0.053^*$	$0.059 \pm 0.028$	$-0.325 \pm 0.298$
Successional	0–7	$0.063 \pm 0.022^*$	$0.075 \pm 0.026^{**}$	$0.031 \pm 0.011^*$	$0.415 \pm 0.091$
	7–25	$0.035 \pm 0.017$	$-0.013 \pm 0.022$	$-0.008 \pm 0.015$	$0.096 \pm 0.178$
	0–25	$0.098 \pm 0.023^{**}$	$0.062 \pm 0.046$	$0.0023 \pm 0.024$	$0.511 \pm 0.186^*$

Free light particulate organic matter (POM) is material  $< 1.85 \text{ g cm}^{-3}$ , total fine intra-POM = total fine intra-aggregate POM (53–250  $\mu\text{m}$ ), total coarse intra-POM = total coarse intra-aggregate POM (250–2000  $\mu\text{m}$ ) and mineral associated OM is material associated with the silt and clay fraction ( $< 53 \mu\text{m}$ ). Accumulation rates significantly different from 0 are marked with \* for  $P < 0.05$  and \*\* for  $P < 0.01$ .

Kwon, 2000). The deep rooting system of trees and the mixing of soil horizons due to the uprooting of trees from events such as windthrow (Schaezel, 1986) would supply a large amount of OM deep in the soil. Additionally, decomposition is believed to occur slower at depth (Cole *et al.*, 1977). However, we found no indications that C accumulated at depth as C content in the afforested and successional systems were similar to those of the agricultural system (7–25 and 25–50 cm). Many studies have reported no change or a decrease in subsurface soil C during the first years after afforesting.

Richter *et al.* (1999) found a decrease in soil C (35–60 cm) 40 years after afforesting on a sandy loam soil. In addition, using  $^{14}\text{C}$  techniques, these authors found evidence that the original crop roots decomposed quickly during the first stage of forest regrowth. Similarly, Binkley & Resh (1999) found no net change of total subsoil C after conversion of sugarcane, *Saccharum officinarum* L. to forest whereas  $^{13}\text{C}$  measurements showed a fast replacement of the sugarcane C with forest-derived C. When comparing 15 forest sites, Ellert & Gregorich (1996) found that only four sites had



**Fig. 6** Intra-aggregate POM (intra-POM) C at two mineral soil depths (0–7 and 7–25 cm) of an agricultural, poplar afforested, successional and native forest system. 2000c = coarse intra-POM (>250  $\mu\text{m}$ ) in the large macroaggregate fraction (>2000  $\mu\text{m}$ ), 2000f = fine intra-POM (53–250  $\mu\text{m}$ ) in the large macroaggregate fraction, 250c = coarse intra-POM in the small macroaggregate fraction (250–2000  $\mu\text{m}$ ), 250f = fine intra-POM in the small macroaggregate fraction (250–2000  $\mu\text{m}$ ), 53f = fine intra-POM (53–250  $\mu\text{m}$ ) in the microaggregate fraction (53–250  $\mu\text{m}$ ). Within a depth and intra-POM fraction, bars indicated by a different uppercase letter differ significantly among treatments ( $P < 0.05$ ). Within a depth and treatment, bars indicated by a different lowercase letter differ significantly among intra-POM fraction ( $P < 0.05$ ). Within a treatment and intra-POM fraction, bars with an asterisk differ significantly between the two depths ( $P < 0.05$ ).

significantly higher C in subsurface layers compared with cultivated areas. In contrast, Schiffman & Johnson (1988) found no differences in total soil C among converted plantations, old field afforested sites, native forests and control fields. In a chronosequence study, Zak *et al.* (1990) found that rates of C first declined for 10 years and then increased continuously over a 50-year period. Thus, 10–40 years may be necessary before an increase in subsurface soil C stocks of afforested systems becomes apparent.

It remains unclear how long C will accumulate in an aggrading forest as the change in accumulation rates over time varies considerably among studies. Huntington (1995) found the highest rates of C accumulation

from 30 to 110 years after afforesting and he proposed that C increased sigmoidally over time. Similar results were obtained by Poulton *et al.* (2003). Post & Kwon (2000), on the other hand, found that about half of the relevant studies in their review showed a significant slowing in the rate of C accumulation 50–100 years after afforesting.

#### *Aggregate and aggregate associated carbon regeneration*

It is generally assumed that SOM is protected in soil aggregates (e.g. Puget *et al.*, 1995; Angers *et al.*, 1997; Six *et al.*, 1998). Therefore, the relationship between aggregate regeneration and soil C within the aggregates was investigated. At the soil surface (0–7 cm), regeneration of soil structure occurred quickly following cessation of cultivation and annual cropping (Figs 2 and 3). The afforested and successional treatments had equivalent aggregate distributions in comparison with the native vegetation levels. These results are in agreement with Jastrow (1996), who observed a similarly fast (10 years) regeneration of soil structure in a restored tallgrass prairie. In contrast to the fast regeneration of soil aggregate structure, aggregate C did not follow this fast recovery (Fig. 4). This lag between soil structure reformation and C accumulation was also observed by Jastrow (1996), Angers (1992) and Six *et al.* (2002a). Although the mechanism of C protection inside aggregates is widely acknowledged, C sequestration by occlusion into aggregates appears to be a relatively slow phenomenon.

#### *Potential amount and rate of carbon sequestration among fractions*

Two different fractionation schemes were used to find fractions that are (1) C enriched, thus forming a pool where C is sequestered and (2) a sensitive indicator for the change in total C after the possible land-use changes. For the first objective, the native vegetation data should be compared with the agricultural field, whereas the different land-use conversion systems should be compared with the agricultural field for the second objective. A complex and labor-intensive fractionation scheme (fractionation scheme A in Fig. 1) isolating small but potentially very sensitive C pools is compared with a much simpler and probably less-sensitive fractionation scheme (fractionation scheme B). Both fractionation schemes distinguish between POM and mineral-associated OM <53  $\mu\text{m}$ . Fractionation scheme A separates POM enclosed (*intra-POM*) and not enclosed (*freelight POM*) within different soil aggregate size classes. This fractionation further separates intra-POM according to size: fine intra-POM (53–

**Table 5** Carbon to nitrogen ratios of total mineral soil, aggregate size classes, intra-POM fractions and total POM fractions at two soil depths (0–7 and 7–25 cm) of an agricultural, poplar afforested, successional and native forest system

	0–7 cm				7–25 cm				
	Agricultural	Afforested	Successional	Native forest	Agricultural	Afforested	Successional	Native forest	
Total soil	10.6 C	12.6 B	12.7 B	14.5 A	9.3 C	9.4 C	9.9 B	12.1 A	
Aggregate size classes	>2000	NA*	11.6 Ba	9.8 C a	14.6 A a	10.1 B a	10.2 B a	9.8 B a	16.4 A a
	2000–250	9.5 B a	11.8 AB a	10.5 AB a	12.9 A b	8.2 C ab	10 BC a	9.4 B a	14.3 A ab
	250–53	9.2 C ab	10.9 AB a	9.7 BC a	12.4 A bc	8.5 B ab	9.3 B a	9.4 B a	13.2 A ab
Intra-POM fractions	0–53	8.3 B b	8.5 B b	7.7 B b	10.8 A c	7.1 B b	8 B b	8.1 B b	10.3 A b
	2000c	11.3 B a	13.6 AB a	12 B a	16.1 A a	9.7 A a	10.9 A a	8.9 A b	8.4 A bc
	2000f	12.2 A a	14.1 A a	12.4 A a	12.9 A a	11.8 A a	12.2 A a	11 A a	12.6 A a
	250c	8.6 A a	11.3 A a	9.8 A b	11.4 A a	8.7 A a	7.7 A a	6.1 A c	6.4 A c
Total POM fractions	250f	10.1 B a	14.5 A a	11.5 AB b	13.2 AB a	10.9 A a	12.2 A a	11 A a	9.9 A ab
	53	10.7 B a	12.5 AB a	13.3 AB a	14.1 A a	11.2 A a	11.1 A a	9.1 A b	12.3 A a
	HPOM	9 B b	9.3 B b	9 B b	12.9 A b	11 A b	9 A b	NA	NA
	LPOM	22.6 AB a	22.3 AB	21 B a	27.5 A a	22.4 B a	25.2 B a	22.1 B a	36.2 A a

NA, not enough material available. Within a depth and size class, treatments followed by a different uppercase letter differ significantly ( $P < 0.05$ ) among treatments. Within a depth and treatment, size classes followed by a different lowercase letter differ significantly ( $P < 0.05$ ) among size classes.

POM, particulate organic matter; HPOM, total heavy POM; LPOM, total light POM.

250  $\mu\text{m}$ ), mostly stabilized within microaggregates, vs. coarse intra-POM (250–2000  $\mu\text{m}$ ) occluded within macroaggregates. Fractionation scheme B is much simpler and divides POM in two fractions according to its density: the total light POM has a density  $< 1.85 \text{ g cm}^{-3}$  and the total heavy POM has a density  $> 1.85 \text{ g cm}^{-3}$ . Light total POM is thought to be younger and more plant derived, while total heavy POM is older and more microbially derived (Six *et al.*, 2002b).

Of all fractions, the mineral associated OM fraction contains the most C (Fig. 5). Although the native vegetation has much less silt and clay compared with the other treatments, amounts of mineral-associated C in the native forest were still similar to the afforested and successional systems. As a consequence, the relative concentration of C in the silt and clay fraction was six times larger in the native forest soils than in the soils of the other treatments. Therefore, the (high-clay) successional and afforested systems have a greater (absolute) potential for C accumulation within the mineral-associated OM fraction compared with the (low clay) native forest soil. Particulate OM makes up 17% of total C for the agricultural, 30% for the afforested, 23% for the successional and 38% for the native forest systems. These values are in agreement with literature data. Marquez *et al.* (1999) found that POM comprised 18–24% of total soil C in a riparian buffer planted with poplar after 10 years. Cromack *et al.* (1999) found that total light POM fraction could constitute as much as 40% of total C and N capital following afforestation.

When comparing the agricultural and native forest systems, C accumulation potential in the surface layer (0–7 cm layer) was largest in the mineral-associated OM, the total fine intra-POM and free light POM fractions of fractionation scheme A and mineral-associated OM and total heavy POM fractions of fractionation scheme B (see Figs 1 and 5). The most sensitive fractions in C changes were the total fine intra-POM and free light POM ( $< 1.85 \text{ g cm}^{-3}$ ) in the afforested system, and the mineral-associated OM fraction in the successional system (Fig. 1 and Table 4). The difference in location of C accumulation between the afforested and successional systems indicates that ecosystem-specific factors play a role in this process.

The free light POM fraction as an unprotected C pool seems to be consistently a sensitive indicator for ecosystem change. After the conversion of a native forest to corn, a substantial decline in free light POM C and N was observed by Besnard *et al.* (1996). Likewise, Arrouays & Pelissier (1994) found that the rapid decrease in C during the first years after clearcutting was related to a decrease in free light POM.

Fractionation schemes A and B are complementary approaches. Fractionation scheme A focuses on mechanistic fractions, but tends to underestimate total POM, due to the many experimental steps. Fractionation scheme B increases the sensitivity of C measurements in comparison with a total C analysis. It gives a strong indication of the total amount of POM C that was sequestered in forest soils.

### Mechanism of carbon accumulation

Three general mechanisms are hypothesized to be important for C sequestration in a mineral soil: (1) increased plant net primary production and hence a higher C input in the soil, (2) higher litter recalcitrance and/or less favorable decomposition conditions and (3) enhanced physical or physicochemical protection of decomposition products.

The afforested system produces about as much aboveground biomass as the agricultural system but about twice as much as the succession. Still, differences in total C between the successional and afforested systems were small. Although no data were available to rigorously compare belowground production, these data already indicate that aboveground primary production changes are not the main factor in soil C accumulation in poplar afforested systems. The C contents associated with free light POM and coarse intra-POM are considered to be mostly determined by C input levels (Six *et al.*, 1998). Therefore, the larger amount of C in the coarse intra-POM (>250  $\mu\text{m}$ ) and free light POM (<1.85  $\text{g cm}^{-3}$ ) fractions of the native forest compared with the other treatments may reflect a larger input of C in the native forest compared with the other treatments. This is also supported by the greater amount of total light POM in the native forest than in the other systems. However, the greater C-to-N ratio of the native forest fractions, especially the unprotected fractions, could also be an indication of higher litter recalcitrance.

The consistent increase of C in the microaggregate (53–250  $\mu\text{m}$ ) intra-POM fraction in both land-use change treatments compared with the agricultural system (Figs 5 and 6), corresponds with the suggestion that this fraction can serve as an early indicator of C accumulation in afforested systems (Six *et al.*, 2002a). It also corroborates the hypothesis that microaggregates physically protect particulate C (Six *et al.*, 1998, 2002a). The large C enrichment of the free microaggregate intra-POM fraction (53f in Figs 1 and 6) is in accordance with studies by Skjemstad *et al.* (1990), Balesdent *et al.* (2000), and Bossuyt *et al.* (2002) highlighting the importance of free microaggregates for C sequestration. In contrast, the coarse intra-POM fractions (2000c and 250c in Figs 1 and 6) of the land-use change treatments are much more similar to the agricultural system, signifying the relatively low degree of protection exerted by macroaggregates alone (Six *et al.*, 2002b).

### Conclusions

- Most of the carbon changes occurred in the top soil layer (0–7 cm). Whereas soil carbon (0–50 cm) did not change significantly after poplar afforesting, it

increased in the successional system at a rate of 0.8  $\text{tCha}^{-1}$ . It was hypothesized that 10–40 years may be necessary before an increase in subsurface soil carbon stocks of afforested systems becomes apparent.

- In all soils studied, most of the carbon was minerally associated. Fractionation indicated that microaggregates (53–250  $\mu\text{m}$ ) stabilize POM. The POM (>53  $\mu\text{m}$ ) fraction enclosed within microaggregates served as an early indicator for total carbon changes. The increase in POM after land-use change occurred mainly in the heavy fraction (density >1.85  $\text{g cm}^{-3}$ ).
- After poplar afforesting, the largest accumulation of carbon occurred as POM (53–250  $\mu\text{m}$ ) within soil, whereas the successional system accumulated carbon mainly as mineral-associated organic matter (<53  $\mu\text{m}$ ).
- The results indicate that agricultural lands have greater potential for C sequestration than comparison with adjacent uncultivated sites would indicate.

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