



## Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use

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### Abstract

The type of land use and soil cultivation are important factors controlling organic carbon storage in soils and they may also change the relative importance of different mechanisms of soil organic matter stabilization. Our objectives were: i) to quantify the soil organic carbon (SOC) and nitrogen (N) storage in silty soils under wheat, maize, grassland and spruce, ii) to determine the SOC and N storage in water-stable aggregates of different size (<53  $\mu\text{m}$ , 53–250  $\mu\text{m}$ , 250–1000  $\mu\text{m}$ , 1000–2000  $\mu\text{m}$ , >2000  $\mu\text{m}$ ) and in density fractions (Mineral-associated soil organic matter >2  $\text{g cm}^{-3}$  (Mineral-SOM), free particulate organic matter <1.6  $\text{g cm}^{-3}$  (free POM), light occluded particulate organic matter <1.6  $\text{g cm}^{-3}$  (occluded POM<sub><1.6</sub>) and dense occluded particulate organic matter 1.6 to 2.0  $\text{g cm}^{-3}$  (occluded POM<sub>1.6–2.0</sub>)) and iii) to analyse the stability and turnover of these SOC fractions in the maize soil on the basis of the  $\delta^{13}\text{C}$  values. Total SOC stocks down to a depth of 60 cm and including the humus layer were larger at the spruce site (10.3  $\text{kg C m}^{-2}$ ) as compared with the grassland, wheat and maize (7 to 8  $\text{kg C m}^{-2}$ ). However, SOC stocks in the mineral soil were smaller in the forest soil than in the agricultural soils. In the arable soils, the aggregate fractions 53–250  $\mu\text{m}$  and 250–1000  $\mu\text{m}$  were the most abundant size fractions, whereas aggregates >1000  $\mu\text{m}$  were most abundant in the grassland and forest soil. The SOC concentration and the C/N ratio were greater for macroaggregates (>250  $\mu\text{m}$ ) than microaggregates (<250  $\mu\text{m}$ ) in the field and grassland soils. At the maize site the percentage of maize-derived C was smallest in the fraction <53  $\mu\text{m}$  with 24% and steadily increased with increasing aggregate size to 47% in the fraction >1000  $\mu\text{m}$ .

The major part (86–91%) of the SOC was associated with the heavy mineral fraction at the grassland, maize and wheat site. In the A horizon of the spruce stand, the particulate organic matter accounted for 52% of the total SOC content. The C/N ratios of density fractions decreased in the order free POM<sub><1.6</sub>>occluded POM>Mineral-SOM for all soils and depths.

The mean age of organic carbon in the water-stable aggregates in the Ap horizon of the maize site increased with decreasing aggregate size from 35 yr (>1000  $\mu\text{m}$ ) to 86 yr (<53  $\mu\text{m}$ ). For the density fractions the order was free POM (22 yr)<dense occluded POM<sub>1.6–2.0</sub> (49 yr)<Mineral-SOM (63 yr)<light occluded POM<sub><1.6</sub> (83 yr). The results showed that

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the type of land use affected the distribution pattern of litter carbon to functionally different SOM pools and that increasing SOC concentrations were closely associated with the formation of macroaggregates.

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## 1. Introduction

The type of land use is an important factor controlling organic matter storage in soils since it affects the amount and quality of litter input, the litter decomposition rates and the processes of organic matter stabilization in soils (Römken et al., 1999; Six et al., 1999, 2002a; Shepherd et al., 2001). Several mechanisms have been proposed to contribute to SOM stabilization in soils (Sollins et al., 1996): soil organic matter (SOM) can be (1) biochemically stabilized through the formation of recalcitrant SOM compounds, (2) protected by intimate association with silt and clay particles and (3) physically stabilized through aggregate formation. Land use and soil cultivation can change the total amount of SOM stabilized but they may also change the relative importance of the SOM protecting processes. These changes have to be known in order to understand, assess and predict land use effects on the storage and stability of SOM.

Physical fractionation techniques such as particle size fractionation, aggregate fractionation and density fractionation emphasize the role of soil minerals in SOM stabilization and turnover and they are considered chemically less destructive than chemical fractionation procedures, and their results are anticipated to relate more directly to the structure and function of SOM in situ (Christensen, 1992). These techniques have been applied to determine the association of SOM with primary particles and to quantify the amount of particulate organic matter between and within soil aggregates (Beare et al., 1994; Six et al., 1998; Aoyama et al., 1999; Puget et al., 2000). Macroaggregation was found to be sensitive to changes in land use and cultivation practice, whereas microaggregation was less sensitive (Franzuebbers and Arshad, 1997; Puget et al., 2000) and the dynamics of aggregate formation seems to be closely linked with SOM storage in soils (Golchin

et al., 1997). According to the hierarchical model proposed by Tisdall and Oades (1982), stable microaggregates (<250  $\mu\text{m}$ ) are bound together to form stable macroaggregates (>250  $\mu\text{m}$ ) with organic compounds of different origin as intermicroaggregate binding agents (Elliott, 1986; Golchin et al., 1994b). Tisdall and Oades (1982) and Hassink (1997) proposed the concept that organic matter addition to soils results first in the formation of SOM associations with clay and silt particles and with microaggregates (<250  $\mu\text{m}$ ) and that macroaggregate (>250  $\mu\text{m}$ ) formation starts if the SOM binding capacity of the clay and silt fraction is saturated.

Only a few studies exist where stable isotopes were used to trace the turnover of C in different aggregate fractions (Aita et al., 1997; Angers et al., 1997; Monreal et al., 1997; Puget et al., 2000; Denef et al., 2001; Frey et al., 2003). A field experiment where  $^{13}\text{C}^{15}\text{N}$ -labelled wheat straw was incorporated into a silty soil showed a fast association of litter C and N with macroaggregates (Aita et al., 1997; Angers et al., 1997), but the enrichment of  $^{13}\text{C}$  of the macroaggregates decreased at later stages of the experiment. At day 574, the 50–250  $\mu\text{m}$  fraction accounted for approximately 50% of the residual  $^{13}\text{C}$ , indicating that stable aggregates appear to be involved in the storage and stabilization of C from residues in silty soils (Angers et al., 1997). Monreal et al. (1997) reported for a long-term experiment with continuous maize cultivation on a Gleysol that the mean turnover time of soil organic carbon was 14 yr in macroaggregates, 61 yr in microaggregates and 275 yr in the fraction <50  $\mu\text{m}$ . However, distribution of recently incorporated litter carbon is not homogeneous in soil aggregates. Puget et al. (2000) found that maize residue C was enriched in particular in the particulate organic matter located in macroaggregates.

Summarising the findings above, there is still considerable uncertainty about the effects of different

land use and soil management on organic carbon storage in soil physical SOM fractions and how these fractions differ in soil organic carbon (SOC) stability. We applied a combined soil aggregate and density fractionation procedure to silty soils with different land use (forest, grassland, wheat, maize) and determined the  $^{13}\text{C}/^{12}\text{C}$  isotopic ratio of SOC fractions in order to analyse SOC storage and stability.

The objectives of our study were: i) to quantify total SOC and total N storage in loess-derived soils with different land use, ii) to determine the SOC and N storage in water stable aggregates of different size and in density fractions and iii) to analyse the stability of these SOC fractions in the maize soil on the basis of the  $\delta^{13}\text{C}$  values.

## 2. Materials and methods

### 2.1. Study sites

Samples were taken from the long-term experiment of the 'Höhere Landbauschule' at Rotthalmünster, which is located in the 'tertiäres Hügelland' of the lower Rottal (N48°21'47", E13°11'46"). The field trial is located 360 m above sea level. The mean annual precipitation is 886 mm and the mean annual temperature is 8.7 °C. The soil type was a stagnic Luvisol derived from loess. Soil texture was a silty loam at all experimental plots with a nearly identical grain size distribution (mean  $\pm$  standard deviation) in the topsoils:  $10.6 \pm 0.9\%$  sand,  $73.1 \pm 1.7\%$  silt and  $16.4 \pm 1.2\%$  clay. The predominant minerals of the clay fraction in the A horizon were illite > kaolinite > vermiculite (M. Kleber, personal communication).

Soil samples were taken from the following sites: (i) A continuous wheat plot (*Triticum aestivum* L.) established in 1969 on former grassland; in most years *Sinapis alba* L. was used as an intercrop. Since 1998, conservation tillage with grubbing instead of ploughing was conducted. (ii) A continuous maize plot (*Zea mays* L.), where only grains were harvested, established in 1979. Previous vegetation on the maize plot was grassland until 1970, subsequently spring wheat and winter wheat were planted. (iii) A grassland established in 1961 and additionally, (iv) a nearby spruce stand (*Picea abies* L.) with an age of about 80 yr.

At the wheat and maize fields, depth of tillage was 30 cm and the straw was returned to the field after harvest. The mean N-fertilization was 171 kg N ha<sup>-1</sup> yr<sup>-1</sup> for wheat and 180 kg N ha<sup>-1</sup> yr<sup>-1</sup> for maize (Schnellhammer and Sirch, 2001). Liming was done every few years, the last time in 2001. The grassland was cut four times a year and the nitrogen fertilization rate was 160 kg N ha<sup>-1</sup> yr<sup>-1</sup>.

The mean amount of straw C (above ground) incorporated into the soil was 0.17 kg m<sup>-2</sup> yr<sup>-1</sup> for wheat and 0.46 kg m<sup>-2</sup> yr<sup>-1</sup> for maize. Assuming that the ratio (root biomass)/(aboveground yield) was 0.2 (Balesdent and Balabane, 1992), the total annual input of maize litter C was 0.63 kg C m<sup>-2</sup> (rhizodeposition was not taken into account).

### 2.2. Soil and plant sampling

Undisturbed (250 cm<sup>3</sup>) and mixed soil samples (four replicates each) were collected from different depths (Table 1) at all sites in September 2002. Additionally, the humus layer of the forest soil was sampled by taking the complete humus layer from small plots of 0.0625 m<sup>2</sup> (25 cm  $\times$  25 cm). Plant residues were collected on the maize field (roots and aboveground biomass), on the wheat field (straw), on the grassland (roots and aboveground biomass) and at the spruce stand (needles, cones and twigs).

### 2.3. Fractionation of water stable aggregates

Following the method described by Six et al. (1998) and Puget et al. (2000), 100 g soil (dried at 40 °C) was immersed in distilled water for 10 minutes to allow slaking. Then, aggregates were separated by moving a 2000  $\mu\text{m}$  sieve up and down by about 3 cm with 50 repetitions in 10 min. The aggregates >2000  $\mu\text{m}$  were collected and the same sieving procedure was repeated for the <2000  $\mu\text{m}$  fraction with the 1000  $\mu\text{m}$  sieve. Then, the fractions 250–1000  $\mu\text{m}$  and 53–250  $\mu\text{m}$  were obtained by sieving. The supernatant water of all aggregate fractions was collected and combined. Fine particles in the supernatant were precipitated with 0.5 M AlCl<sub>3</sub> and the precipitate was combined with the fraction <53  $\mu\text{m}$ . All aggregate size classes were air dried at 25 °C. C concentrations in the sandfree

Table 1

Bulk density (BD), pH ( $10^{-2}$  M  $\text{CaCl}_2$ ), soil organic carbon (SOC), C/N ratio and  $\delta^{13}\text{C}$  values measured in different depths of the soil (sieved  $<2$  mm) at the experimental sites with continuous wheat, continuous maize, grass and spruce (forest)

Plot	Depth [cm]	BD	pH [g $\text{cm}^{-3}$ ]	SOC [g $\text{m}^{-2}$ ]	C/N	$\delta^{13}\text{C}$ [‰ $_{\text{PDB}}$ ]
Wheat	0–30	1.45	6.5	5186 (326)	9.4	–26.6 (0.1)
	30–45	1.58	6.6	1073 (39)	7.5	–25.6 (0.1)
	45–60	1.48	6.6	600 (11)	6.8	–25.1 (0.1)
Maize	0–30	1.38	6.9	5353 (170)	9.6	–21.3 (0.3)
	30–45	1.53	6.5	1549 (46)	8.7	–23.4 (0.1)
	45–60	1.52	6.6	930 (29)	7.4	–23.7 (0.3)
Grass	0–10	1.23	5.9	3025 (73)	10.3	–28.0 (0.1)
	10–20	1.47	5.6	1700 (189)	9.1	–26.7 (0.2)
	20–30	1.49	5.6	1281 (22)	8.7	–26.3 (0.0)
	30–45	1.47	5.7	1217 (36)	7.8	–25.9 (0.1)
	45–60	1.43	5.5	732 (44)	7.2	–25.7 (0.1)
Forest	L (+12 to +8)	0.11	3.4	2111 (379)	25.4	–26.0 (0.2)
	Of (+8 to +3)	0.05	3.0	1170 (287)	26.0	–25.8 (0.1)
	Oh (+3 to +0)	0.09	2.9	827 (252)	25.2	–26.0 (0.0)
	0–7	0.91	3.2	2569 (34)	21.9	–25.7 (0.0)
	7–25	1.21	3.6	2073 (33)	19.1	–25.2 (0.1)
	25–40	1.49	3.7	732 (7)	nd <sup>a</sup>	–24.9 (0.1)
	40–60	1.59	4.0	829 (7)	nd <sup>a</sup>	–24.5 (0.2)

Means and S.E. ( $n=4$ ).

<sup>a</sup> Denotes “not determined”.

aggregates (sandfree  $C_{\text{fraction}}$ ) were calculated as (Six et al., 1998):

sandfree  $C_{\text{fraction}}$

$$= C_{\text{fraction}} / (1 - (\text{sand proportion}_{\text{fraction}})) \quad (1)$$

where  $C_{\text{fraction}}$  was the C [%] in the respective aggregate and sand proportion<sub>fraction</sub> was the relative proportion of sand in the aggregate fraction.

Mean weight diameters (MWD) were determined as (Shepherd et al., 2001):

$$\text{MWD} = \sum (\text{Weight \% sample on sieve} \times \text{mean inter-sieve size}/100), \quad (2)$$

where the upper and lower limits were 10000 and 0  $\mu\text{m}$ , respectively.

#### 2.4. Density fractionation of soil

We used the procedure summarized in Fig. 1 to obtain density fractions of soil organic matter (Golchin et al., 1994a). Ten grams of air dried soil ( $<2$  mm) was placed in a centrifugation tube and 40 ml of sodium polytungstate solution (Sometu, Berlin, Germany) of a

density of  $1.6 \text{ g cm}^{-3}$  was added. The tube was gently turned upside down by hand about five times. After sedimentation for 30 min, the solution was centrifuged at  $5100 \times g$  for 1 h. The supernatant with floating particles was filtered ( $0.45 \mu\text{m}$ ) using vacuum and washed with distilled water to gain the free particulate organic matter  $<1.6 \text{ g cm}^{-3}$  (fPOM<sub><1.6</sub>). Then, the pellet was dispersed with 40 ml of sodium polytungstate solution ( $1.6 \text{ g cm}^{-3}$ ) using a test tube shaker and 10 glass beads with a diameter of 5 mm were added and the solution was shaken for 16 h with a frequency of 60 movements per minute to crack the soil aggregates (Balesdent et al., 1991). After disaggregation, the soil suspension was centrifuged for 1 h at  $5100 \times g$ . The supernatant with floating particles (light occluded particulate organic matter with a density  $<1.6 \text{ g cm}^{-3}$ , oPOM<sub><1.6</sub>) was filtered ( $0.45 \mu\text{m}$ ) using vacuum and washed with distilled water. Again, the pellet was dispersed as described above but using sodium polytungstate solution of a density of  $2.0 \text{ g cm}^{-3}$ . The supernatant with floating particles (dense occluded particulate organic matter with a density of  $1.6\text{--}2.0 \text{ g cm}^{-3}$ , oPOM<sub>1.6–2.0</sub>) was filtered under negative pressure and washed. To remove the salt, the pellet containing the mineral fraction ( $>2.0 \text{ g cm}^{-3}$ ,

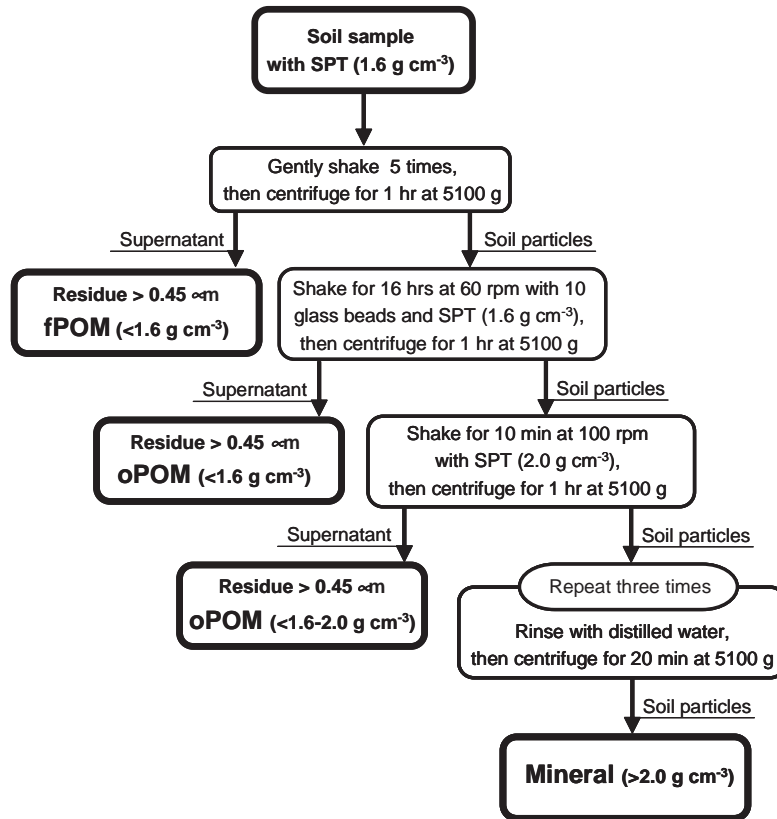


Fig. 1. Density fractionation scheme, using sodium polytungstate solution (SPT) as heavy liquid. Modified after Balesdent et al. (1991) and Golchin et al. (1994a).

Mineral-SOM<sub>>2.0</sub>) was washed three times with distilled water. Finally, the sample was centrifuged and the supernatant was discarded. All fractions were dried at 40 °C, the POM fractions were ground with a mortar.

### 2.5. General soil analysis and isotopic analysis

Soil pH was determined in a 10<sup>-2</sup> M CaCl<sub>2</sub> solution (soil:solution ratio 1:2.5). Soil bulk density was determined gravimetrically from undisturbed soil cores. Bulk soil (<2 mm), water-stable aggregates and the fraction 'Mineral-SOM<sub>>2.0</sub>' were ball-milled, samples from the topsoil of wheat, maize and grassland were acidified with 10% HCl. SOC and N<sub>t</sub> contents of the samples were determined by an automated C and N analyzer (Heraeus Elementar Vario EL, Hanau, Germany).

<sup>13</sup>C/<sup>12</sup>C isotope ratios of all samples and the C and N contents of the POM fractions (fPOM<sub><1.6</sub>, oPOM<sub><1.6</sub>,

oPOM<sub>1.6-2.0</sub>) were measured by an isotope ratio mass spectrometer (IRMS, Finnigan MAT, DELTA<sup>plus</sup>, Bremen, Germany) connected to an elemental analyzer (NA 2500, Fisons, Milan, Italy).

<sup>13</sup>C/<sup>12</sup>C isotope ratios were expressed as δ<sup>13</sup>C values:

$$\delta^{13}\text{C}(\text{‰}) = [(\delta_{\text{sam}}/\delta_{\text{std}}) - 1] \times 10^3, \quad (3)$$

where  $\delta_{\text{sam}}$  = <sup>13</sup>C/<sup>12</sup>C ratio of the sample, and  $\delta_{\text{std}}$  = <sup>13</sup>C/<sup>12</sup>C ratio of the reference standard (PDB).

The proportion of C derived from maize in a sample was calculated according to Balesdent and Mariotti (1996):

$$f = (\delta_{\text{sam}} - \delta_{\text{ref}})/(\delta_{\text{maize}} - \delta_{\text{wheat}}), \quad (4)$$

where  $f$  stands for the relative proportion of maize-derived C in the sample,  $\delta_{\text{sam}}$  for the measured δ<sup>13</sup>C of the sample,  $\delta_{\text{ref}}$  for the δ<sup>13</sup>C of the corresponding sample from the wheat field as C<sub>3</sub> reference soil, and

$\delta_{\text{maize}}$  and  $\delta_{\text{wheat}}$  for the  $\delta^{13}\text{C}$  values of the crop residues of maize and wheat.  $\delta^{13}\text{C}$  values (mean  $\pm$  standard deviation) were  $-12.7 \pm 0.2\text{‰}_{\text{PDB}}$  for maize and  $-26.8 \pm 0.1\text{‰}_{\text{PDB}}$  for wheat, resulting in a difference of  $14.1\text{‰}_{\text{PDB}}$ . The standard error S.E. of maize-derived proportions of  $n$  samples was calculated from the standard deviations of  $\delta_{\text{ref}}$  ( $s_{\delta_{\text{ref}}}$ ) and  $\delta_{\text{sam}}$  ( $s_{\delta_{\text{sam}}}$ ) assuming that the contributions of the uncertainties in  $\delta_{\text{maize}}$  and  $\delta_{\text{wheat}}$  were negligible:

$$\text{S.E.} = \left( (s_{\delta_{\text{ref}}} / (\delta_{\text{maize}} - \delta_{\text{wheat}}))^2 + (s_{\delta_{\text{sam}}} / (\delta_{\text{maize}} - \delta_{\text{wheat}}))^2 \right)^{0.5} / n^{0.5} \quad (5)$$

### 2.6. Calculation of the apparent turnover time or mean age of organic carbon

The apparent turnover time  $T$  of soil organic carbon fractions of the maize site was calculated as:

$$T = 1/k = -(t - t_0) / \ln(C_t / C_{t_0}) \quad (6)$$

where  $k$  is the rate constant of the first-order decay equation,  $t$  gives the time of sampling (year),  $t_0$  the time of vegetation change (year),  $C_t$  the proportion of remaining carbon labelled from  $\text{C}_3$ -derived C in the soil at the time of sampling [%] and  $C_{t_0}$  the percentage of carbon labelled from  $\text{C}_3$  plants at  $t_0$  [%]. For our continuous maize cropping plot  $t$  was 2002,  $t_0$  was 1979,  $C_{t_0}$  was 100%, and  $C_t$  was calculated as  $100\% - (f \cdot 100\%)$ .

The calculated apparent turnover times of organic carbon in aggregate fractions and in the occluded particulate organic matter (oPOM) do not describe C turnover within aggregates since the dynamics of organic matter in aggregate fractions is strongly interrelated with the life-time of aggregates and carbon may have resided in other fractions before entering aggregates. We therefore use the term “mean age” instead of “apparent turnover time” for organic carbon of soil aggregate fractions.

## 3. Results

### 3.1. Soil properties of the study sites

Soil organic carbon storage in the upper mineral soil (0–30 cm) decreased in the order grassland ( $\sim 6.0$

$\text{kg C m}^{-2}$ ) > maize  $\approx$  wheat ( $\sim 5.3 \text{ kg C m}^{-2}$ ) > forest ( $\sim 4.9 \text{ kg C m}^{-2}$ ) (Table 1). In the subsoil (30–60 cm), organic carbon stocks were smaller in the forest soil than in the agricultural soils. Total SOC stocks down to a depth of 60 cm and including the humus layer were larger at the spruce site ( $\sim 10.3 \text{ kg C m}^{-2}$ ) as compared with the grassland and fields ( $\sim 7$  to  $8 \text{ kg C m}^{-2}$ ). The corresponding nitrogen stocks were approximately  $0.5 \text{ kg N m}^{-2}$  in the forest soil and  $0.8 \text{ kg N m}^{-2}$  in the agricultural soils.

The C/N ratios decreased with soil depth at all sites and they were significantly greater in forest soil (26 in the humus layer to  $\sim 20$  in the subsoil) than in the agricultural soils ( $\sim 10$  to 7) (Table 1). No difference in pH occurred among the cropped soils (pH 6.5 to 6.9), pH was lower in the grassland soil (5.5 to 5.9) and lowest in the acidified forest soil (pH 3 to 4).

### 3.2. $\delta^{13}\text{C}$ values of plant litter and soil organic carbon

The litter  $\delta^{13}\text{C}$  values (mean  $\pm$  standard error) were lowest for the grassland with  $-28.9 \pm 0.2\text{‰}_{\text{PDB}}$  for the above ground biomass and  $-29.2 \pm 0.1\text{‰}_{\text{PDB}}$  for the root biomass. Spruce litter showed widely differing  $\delta^{13}\text{C}$  values: needles ( $-28.3 \pm 0.1\text{‰}_{\text{PDB}}$ ), twigs ( $-26.6 \pm 0.5\text{‰}_{\text{PDB}}$ ) and cones ( $-25.9 \pm 0.1\text{‰}_{\text{PDB}}$ ). Wheat residues had a mean  $\delta^{13}\text{C}$  value of  $-26.8 \pm 0.1\text{‰}_{\text{PDB}}$  and maize showed the typical  $^{13}\text{C}$  enrichment of  $\text{C}_4$  plants with  $-12.7 \pm 0.1\text{‰}_{\text{PDB}}$  in the above ground litter and  $-12.8 \pm 0.1\text{‰}_{\text{PDB}}$  in the root biomass.

The  $\delta^{13}\text{C}$  values of the soil organic carbon in the A horizons reflected the differences in the  $^{13}\text{C}$  abundance of the litter. The most negative  $\delta^{13}\text{C}$  value occurred in the surface soil (0–10 cm) of the grassland ( $-28.0\text{‰}_{\text{PDB}}$ ; Table 1). The  $^{13}\text{C}$  abundance in the SOC of the wheat and forest soil was similar with  $\delta^{13}\text{C}$  values of approximately  $-26.0\text{‰}_{\text{PDB}}$  in the A horizon and  $-25.0\text{‰}_{\text{PDB}}$  in the subsoil. Continuous maize cropping for 24 yr resulted in a significant  $^{13}\text{C}$  enrichment in SOC (Table 1). The  $\text{C}_4$ -C in total SOC decreased with depth from 35.7% in the Ap-horizon to 15.4% in 30–45 cm to 10.2% in 45–60 cm (Table 2). The total amount of maize-derived C in the soil profile was  $2.24 \text{ kg C m}^{-2}$ . Assuming an annual C input of  $0.63 \text{ kg C m}^{-2}$  (Section 2.1), the mean portion of maize litter C stabilized in soil was approximately 15%. The apparent turnover time of SOC increased

Table 2

Maize-derived C in total SOC (bulk soil) and in SOC fractions (means and S.E.), and mean apparent SOC turnover times with lower and upper limit, determined from the standard error of the  $k$  value of Eq. (6) ( $n=4$ )

SOC fraction		Maize-derived C [%]	Turnover times [yr]
Bulk soil <2 mm	0–30 cm depth	35.7 (2.1)	54 (+4/–4)
	30–45 cm depth	15.4 (0.8)	144 (+9/–8)
	45–60 cm depth	10.2 (1.9)	223 (+55/–37)
Water-stable aggregates	>2000 $\mu\text{m}$	46.7 (nd) <sup>a</sup>	38 (nd) <sup>a</sup>
	1000–2000 $\mu\text{m}$	49.5 (2.8)	35 (+2/–3)
	250–1000 $\mu\text{m}$	38.3 (1.2)	50 (+2/–2)
	53–250 $\mu\text{m}$	33.1 (1.6)	60 (+3/–4)
	<53 $\mu\text{m}$	24.4 (0.8)	86 (+3/–4)
Density fractions	fPOM	64.8 (5.2)	22 (+4/–3)
	oPOM <sub>&lt;1.6</sub>	25.0 (2.3)	83 (+10/–8)
	oPOM <sub>1.6–2.0</sub>	38.3 (2.7)	49 (+5/–4)
	Mineral	31.4 (1.4)	63 (+4/–3)

<sup>a</sup> Value determined from composite sample.

with soil depth. Based on the  $\delta^{13}\text{C}$  values, Eq. (4) and by assuming a first-order decay, we calculated a mean SOC turnover time of 54 yr (Ap horizon), 144 yr (30–45 cm) and 223 yr (45–60 cm) (Table 2).

### 3.3. Water-stable aggregates

#### 3.3.1. Distribution of water-stable aggregates

The distribution of aggregate size classes in the A horizons was influenced by the type of land use and by land cultivation: The most frequent aggregate fractions were the microaggregates (53–250  $\mu\text{m}$ ) for maize, the smaller macroaggregates (250–1000  $\mu\text{m}$ ) for wheat and the megaaggregates (>2000  $\mu\text{m}$ ) for the grassland and forest soils (Table 3). The mean weight diameter in the A horizon (mean  $\pm$  standard error) calculated according to Eq. (2) increased in the order wheat (757  $\pm$  115  $\mu\text{m}$ ) < maize (934  $\pm$  227  $\mu\text{m}$ )  $\ll$  grassland (2734  $\pm$  309  $\mu\text{m}$ ) < forest (2859  $\pm$  119  $\mu\text{m}$ ). However, differences between wheat and maize and between forest and grassland were not significant. Soil aggregation was much less pronounced in the subsoil. The mean weight diameters of the subsoils of grassland (618  $\pm$  68  $\mu\text{m}$ ) and forest (984  $\pm$  111  $\mu\text{m}$ ) were not significantly different from each other.

#### 3.3.2. C and N storage and C/N in water-stable aggregates

Within a horizon, SOC concentration was always smallest in the fraction <53  $\mu\text{m}$  (Table 3). At the agricultural sites (wheat, maize, grassland), the SOC

concentration increased in the order: (<53  $\mu\text{m}$ ) < microaggregates < macroaggregates. This order was also found in the forest and grassland subsoils. SOC concentrations increased in all aggregate fractions with increasing total SOC content, thus C concentrations in aggregate fractions from the A horizon of the forest were always higher than in its counterparts from the grassland and the grassland aggregates had always greater SOC concentrations than the corresponding aggregate classes of the fields (Table 3). The relationship between the total C stored in macroaggregates (>250  $\mu\text{m}$ ) of 1 kg soil and the total SOC in 1 kg soil had a greater slope and a greater correlation coefficient ( $r=0.99$ ) than the relationship between the total C stored in microaggregates (<250  $\mu\text{m}$ ) and the total SOC storage ( $r=0.72$ ). Fig. 2 shows the contribution of C and N in different aggregate fractions to the total SOC and N stocks in different horizons of the experimental sites. For wheat and maize, the major part of the total SOC was stored in the aggregate fraction 250–1000  $\mu\text{m}$ . By contrast, the aggregates >2 mm were the most important fraction for C storage in the A horizon of the grassland and the forest soil. In the subsoil of these sites the aggregates 250–1000  $\mu\text{m}$  stored the most C. The correction for the sand content of the size fractions as explained in Section 2.3 slightly increased the C concentration of all aggregate classes >53  $\mu\text{m}$  (Table 3) but it did not change the relative contribution of the different aggregate classes to SOC storage. The relative distribution of soil nitrogen among the aggregate fractions was similar as the C

Table 3

Total dry matter yield [%], C concentration [%], C/N and  $\delta^{13}\text{C}$  in water-stable aggregates from different depths of the soil of the continuous wheat field, continuous maize field, grassland and spruce stand (forest)

Plot	Aggregate size [ $\mu\text{m}$ ]	Yield [%]	C [%] in total aggregates	C [%] in sand-free aggregates	C/N	$\delta^{13}\text{C}$ [‰ <sub>PDB</sub> ]
Wheat, 0–30 cm	>2000	5.6 (1.6)	3.51 (0.05) <sup>a</sup>	4.92 (nd) <sup>a</sup>	16.2	–27.1 (0.2)
	1000–2000	7.7 (2.7)	1.68 (0.13) <sup>b</sup>	2.26 (nd) <sup>b</sup>	9.7	–26.6 (0.0)
	250–1000	41.3 (3.4)	1.41 (0.03) <sup>b,c</sup>	1.63 (0.03) <sup>c</sup>	8.6	–26.6 (0.0)
	53–250	26.8 (4.7)	1.27 (0.02) <sup>c</sup>	1.54 (0.04) <sup>c</sup>	8.4	–26.6 (0.1)
	<53	17.2 (2.5)	0.97 (0.07) <sup>d</sup>	0.97 (0.07) <sup>d</sup>	6.0	–26.1 (0.1)
Maize, 0–30 cm	>2000	10.9 (4.7)	1.49 (nd)	2.18 (nd)	10.0	–20.4 (nd)
	1000–2000	3.4 (0.8)	1.50 (0.26) <sup>a</sup>	2.48 (nd)	10.4	–20.0 (0.4)
	250–1000	27.7 (3.0)	1.49 (0.05) <sup>a</sup>	1.72 (0.06) <sup>b</sup>	9.1	–21.2 (0.2)
	53–250	35.0 (2.8)	1.23 (0.04) <sup>a,b</sup>	1.51 (0.04) <sup>b</sup>	8.8	–21.9 (0.2)
	<53	20.1 (0.5)	0.87 (0.03) <sup>b</sup>	0.87 (0.03) <sup>c</sup>	7.5	–23.1 (0.1)
Grass, 0–10 cm	>2000	38.3 (6.0)	2.67 (0.13) <sup>a</sup>	3.00 (0.14) <sup>a</sup>	10.2	–28.2 (0.0)
	1000–2000	16.4 (0.9)	2.62 (0.06) <sup>a</sup>	3.08 (0.07) <sup>a</sup>	10.9	–28.0 (0.0)
	250–1000	26.7 (1.3)	2.69 (0.05) <sup>a</sup>	3.08 (0.06) <sup>a</sup>	10.1	–27.9 (0.0)
	53–250	15.3 (2.2)	2.43 (0.02) <sup>a</sup>	3.07 (0.05) <sup>a</sup>	9.0	–27.9 (0.0)
	<53	8.5 (2.6)	1.14 (0.05) <sup>b</sup>	1.14 (0.05) <sup>b</sup>	7.3	–27.4 (0.0)
Grass, 10–30 cm	>2000	3.9 (nd)	1.45 (nd)	2.02 (nd)	10.2	–26.9 (nd)
	1000–2000	6.5 (1.4)	1.20 (nd)	1.58 (nd)	10.4	–26.7 (nd)
	250–1000	36.6 (4.7)	1.00 (0.06) <sup>b</sup>	1.13 (0.06) <sup>b</sup>	9.6	–26.4 (0.1)
	53–250	31.7 (3.5)	0.84 (0.06) <sup>b</sup>	1.03 (0.08) <sup>c</sup>	9.2	–26.4 (0.1)
	<53	21.4 (3.2)	0.57 (0.07) <sup>c</sup>	0.57 (0.07) <sup>d</sup>	8.6	–26.0 (0.2)
Forest, 0–7 cm	>2000	41.7 (2.4)	5.16 (0.17) <sup>a</sup>	6.28 (0.21) <sup>a</sup>	21.2	–25.5 (0.1)
	1000–2000	13.5 (0.9)	4.73 (0.61) <sup>a</sup>	6.17 (0.78) <sup>a</sup>	20.8	–25.5 (0.0)
	250–1000	19.9 (1.0)	4.32 (0.16) <sup>a</sup>	5.14 (0.16) <sup>a</sup>	19.6	–25.7 (0.0)
	53–250	16.8 (0.9)	4.97 (0.21) <sup>a</sup>	5.90 (0.27) <sup>a</sup>	19.6	–25.8 (0.0)
	<53	7.6 (1.0)	2.24 (0.07) <sup>a</sup>	2.24 (0.07) <sup>b</sup>	15.4	–25.9 (0.0)
Forest, 7–25 cm	>2000	10.9 (2.2)	1.30 (0.05) <sup>a</sup>	1.82 (0.07) <sup>b</sup>	nd	nd (nd)
	1000–2000	10.0 (0.2)	1.27 (0.02) <sup>a</sup>	2.34 (nd)	25.2	–25.4 (0.1)
	250–1000	20.9 (1.0)	0.99 (0.04) <sup>b</sup>	1.24 (0.06)	20.9	–25.3 (0.0)
	53–250	22.0 (1.2)	0.77 (0.03) <sup>c</sup>	0.98 (0.04) <sup>d</sup>	18.0	–25.3 (0.0)
	<53	35.8 (2.0)	0.37 (0.02) <sup>d</sup>	0.37 (0.02) <sup>e</sup>	13.3	–25.2 (0.2)

Means and S.E. ( $n=4$ ). Within columns, values followed by the same letter (a–d) are not significantly different ( $p<0.05$ ) in between one soil horizon. nd—denotes “not determined”.

distribution, however, total N concentration was greater in the grassland than in the forest soil because of the smaller C/N ratio of the soil organic matter.

For all soils, C/N ratios were smallest in the fraction <53  $\mu\text{m}$  and greatest in the aggregates >1000  $\mu\text{m}$ . The C/N ratio of the microaggregates (53–250  $\mu\text{m}$ ) and the small macroaggregates (250–1000  $\mu\text{m}$ ) took an intermediate position (Table 3).

### 3.3.3. $\delta^{13}\text{C}$ values and maize-derived C in water-stable aggregates

The  $\delta^{13}\text{C}$  values of the SOC in aggregate fractions tended to decrease with aggregate size in the wheat and grassland soil (Table 3). For the maize site,

changes in  $\delta^{13}\text{C}$  showed the contribution of young, maize-derived C to the SOC stocks in different aggregate classes. The percentage of maize-derived C was smallest in the fraction <53  $\mu\text{m}$  with 24.4% and increased with increasing aggregate size to 33% in the fraction 53–250  $\mu\text{m}$ , 38% in the fraction 250–1000  $\mu\text{m}$ , and about 48% in the fractions >1000  $\mu\text{m}$ . The corresponding mean age of SOC in the aggregate fractions ranged between 35 and 86 yr (Table 2). However, these SOC turnover times have to be assessed carefully, since the  $\delta^{13}\text{C}$  values may not exclusively be interpreted as a result of SOC turnover in aggregates, because aggregate turnover also affects SOC storage in the aggregates. Nevertheless, these



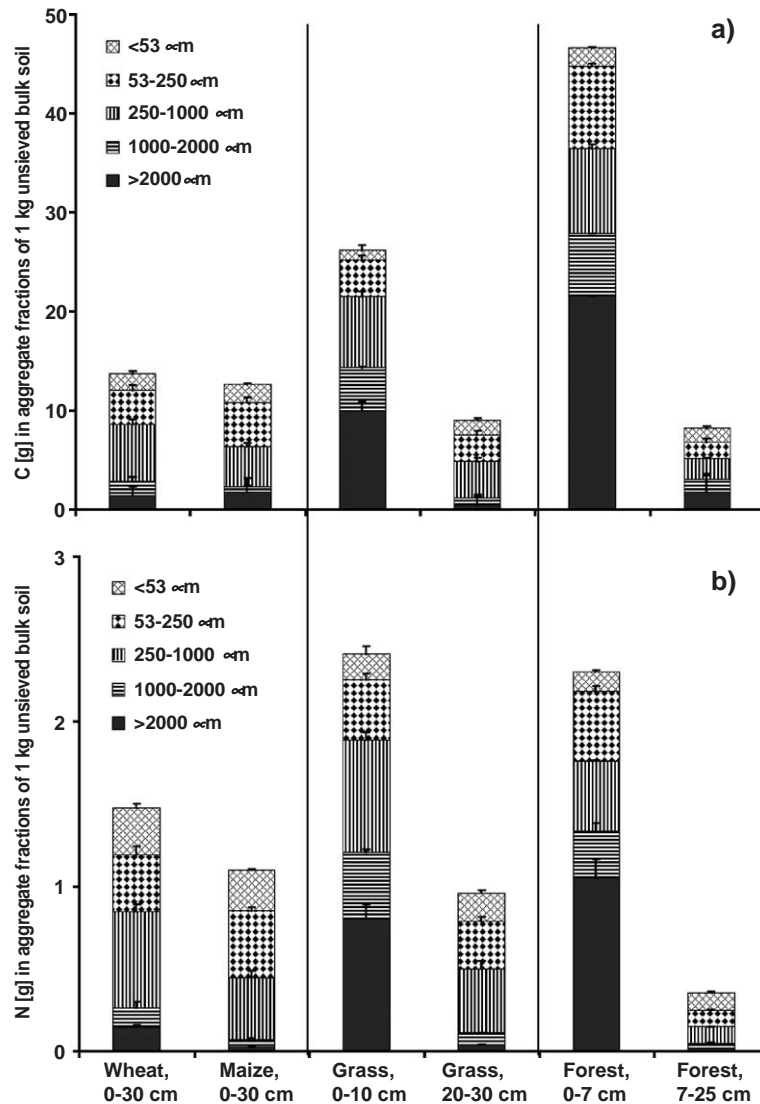


Fig. 2. a) Distribution of organic carbon in 1 kg soil among water-stable aggregates in different depths of the soils of the experimental sites. Means and S.E. ( $n=4$ ) and b) distribution of total nitrogen in 1 kg soil among water-stable aggregates in different depths of the soils of the experimental sites. Means and S.E. ( $n=4$ ).

results show that young, less decomposed maize-derived organic carbon is enriched preferentially in larger aggregates.

### 3.4. Density fractions

#### 3.4.1. C storage and C/N in density fractions

The mean C recovery of the density fractionation procedure was 97% of the total SOC. In all soils, the

major part of the SOC was associated with the heavy mineral fraction (Fig. 3). The C concentration of the mineral fraction ranged between about  $11 \text{ g kg}^{-1}$  in the arable soils and about  $21 \text{ g kg}^{-1}$  in the Ah horizon of the grassland and forest soil. The particulate organic matter (POM) contributed approximately 13% (fields), 14% (grassland) and 52% (forest) to the total SOC stored in the A horizon (Table 4). The POM fraction was divided in the free POM located between

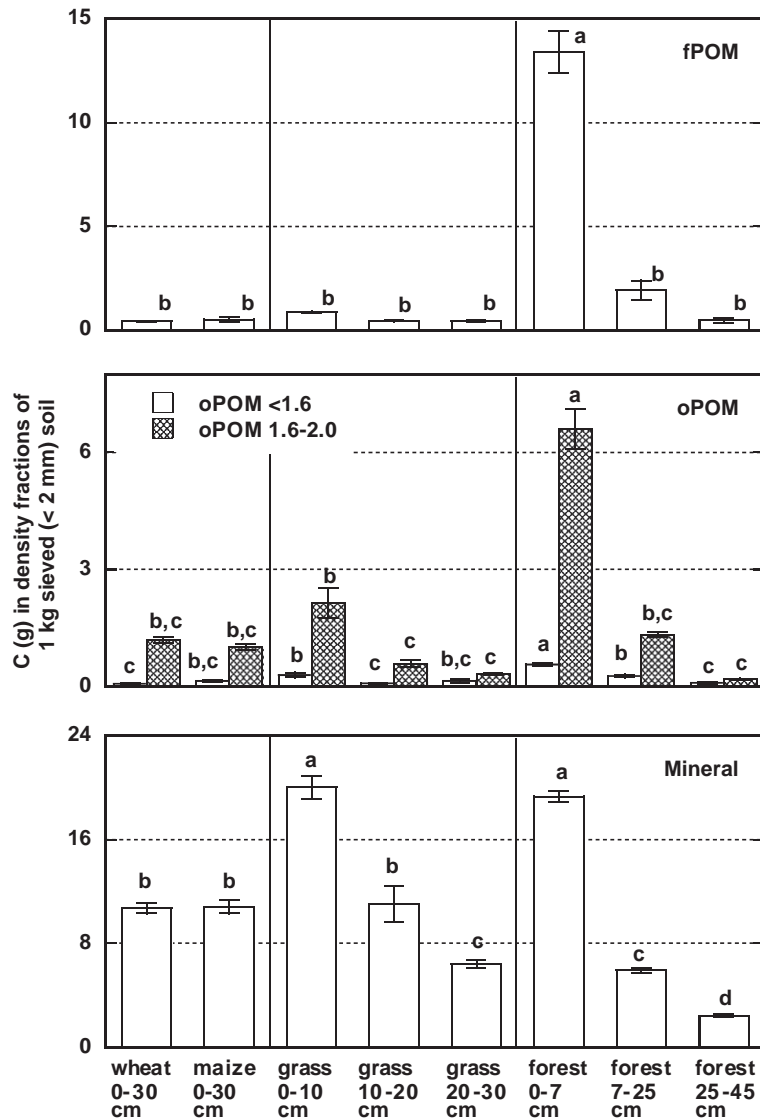


Fig. 3. Distribution of organic carbon in 1 kg soil among density fractions in different depths of the soils of the experimental sites. Carbon stocks in one type of density fraction followed by the same letter (a–d) were not significantly different ( $p < 0.05$ ).

aggregates (fPOM) and the POM occluded in aggregates (oPOM). The latter fraction was divided in oPOM  $<1.6\text{ g cm}^{-3}$  and oPOM 1.6 to  $2.0\text{ g cm}^{-3}$ . For the wheat, maize, and grassland soil, of the POM fractions, oPOM<sub>1.6–2.0</sub> was most important (4.4–9.6% of total C), followed by the fPOM<sub><1.6</sub> (3.3–5.9% of total C) whereas the oPOM<sub><1.6</sub> (0.5–1.8% of total C) was only of minor importance for C storage (Table 4). In the forest soil, fPOM<sub><1.6</sub> (14.6–33.6% of total C) stored the major part of the POM fractions, followed by

oPOM<sub>1.6–2.0</sub> (5.7–16.6% of total C) and oPOM<sub><1.6</sub> (1.4–2.8% of total C).

The C/N ratios of density fractions showed the same decreasing trend for all soils and depths in the order fPOM<sub><1.6</sub> > oPOM > Mineral-SOM (Table 4).

### 3.4.2. $\delta^{13}\text{C}$ values and maize-derived C in density fractions

There were little or no differences in the  $\delta^{13}\text{C}$  values of the POM fractions obtained from the same

Table 4

Organic carbon yield [% of total SOC], C/N and  $\delta^{13}\text{C}$  in density fractions from different depths of the soil of the continuous wheat field, continuous maize field, grassland and spruce stand (forest)

Plot	Density fraction	C yield [% of SOC]	C/N of the fraction	$\delta^{13}\text{C}$ [% $_{\text{EPDB}}$ ]
Wheat, 0–30 cm	fPOM	3.3	15.0	–26.5 (0.3)
	oPOM $_{<1.6}$	0.5	17.3	–27.4 (0.1)
	oPOM $_{1.6-2.0}$	9.6	12.0	–27.4 (0.0)
	Mineral	86.6	7.3	–26.5 (0.1)
Maize, 0–30 cm	fPOM	4.1	19.0	–17.3 (0.7)
	oPOM $_{<1.6}$	1.0	19.0	–23.9 (0.3)
	oPOM $_{1.6-2.0}$	8.1	13.9	–22.0 (0.4)
	Mineral	86.8	7.5	–22.1 (0.2)
Grass, 0–10 cm	fPOM	3.7	15.9	–28.9 (0.1)
	oPOM $_{<1.6}$	1.2	16.2	–28.6 (0.1)
	oPOM $_{1.6-2.0}$	9.1	12.1	–28.7 (0.1)
	Mineral	85.9	6.7	–28.0 (0.1)
Grass, 10–20 cm	fPOM	3.6	22.2	–27.3 (0.4)
	oPOM $_{<1.6}$	0.6	18.4	–27.2 (0.3)
	oPOM $_{1.6-2.0}$	4.8	13.5	–27.3 (0.1)
	Mineral	91.0	7.5	–26.9 (0.2)
Grass, 20–30 cm	fPOM	5.9	24.7	–27.3 (0.2)
	oPOM $_{<1.6}$	1.8	nd <sup>a</sup>	–26.8 (0.4)
	oPOM $_{1.6-2.0}$	4.4	nd <sup>a</sup>	–26.8 (0.3)
	Mineral	87.9	6.6	–26.3 (0.1)
Forest, 0–7 cm	fPOM	33.6	19.8	–25.1 (0.1)
	oPOM $_{<1.6}$	1.4	21.4	–26.1 (0.1)
	oPOM $_{1.6-2.0}$	16.6	19.1	–25.9 (0.1)
	Mineral	48.4	13.6	–26.1 (0.1)
Forest, 7–25 cm	fPOM	20.4	24.5	–25.2 (0.1)
	oPOM $_{<1.6}$	2.8	21.3	–25.9 (0.3)
	oPOM $_{1.6-2.0}$	14.0	26.5	–26.1 (0.1)
	Mineral	62.8	10.3	–25.4 (0.1)
Forest, 25–40 cm	fPOM	14.6	25.9	–25.5 (0.1)
	oPOM $_{<1.6}$	2.5	nd <sup>a</sup>	–26.4 (0.1)
	oPOM $_{1.6-2.0}$	5.7	21.9	–26.2 (0.1)
	Mineral	77.2	6.3	–25.0 (0.2)

Means and S.E. in brackets ( $n=4$ ).

<sup>a</sup> Denotes “not determined”.

horizon except for the maize soil (Table 4). The maize-derived percentages (Table 2) were greatest in the C of fPOM $_{<1.6}$  with 65% and lowest in oPOM $_{<1.6}$  with 25%. The fraction oPOM $_{1.6-2.0}$  showed an intermediate value with 38% which was not significantly different from 31% in the C of the mineral fraction. The  $^{13}\text{C}$  abundance in the SOC of density fractions of the maize soil indicated distinct differences in the stability of these SOC pools. The mean age of the C increased in the order fPOM $_{<1.6}$  (22 yr) < oPOM $_{1.6-2.0}$  (49 yr) < Mineral-SOM (63 yr) < oPOM $_{<1.6}$  (83 yr).

## 4. Discussion

### 4.1. Carbon and nitrogen stocks

Our results show that despite an increasing SOC concentration in the A horizons in the order arable soils < grassland soil < forest soil, total SOC stocks stored in the mineral soil (0–60 cm) were smallest in the spruce stand and there was no difference in the SOC stocks of the fields and the grassland. The difference in total nitrogen stocks in the mineral soil was even more pronounced. The agricultural soils

stored about twice as much N as the mineral soil of the spruce stand. The reduced C and N stocks in the mineral soil of the forest site were a result of soil acidification and the associated accumulation of litter in the humus layer. This accumulation is driven mainly by reduced decomposition rates, the lack of earthworms, and the withdraw of roots from the mineral soil into the humus layer (Jentschke et al., 2001; Goldbold et al., 2003). If the humus layer is included in the calculation of SOC and soil N stocks, our results agree with the conclusion that cultivation of forest soils results in decreased SOC stocks (Houghton, 1995).

#### 4.2. $\delta^{13}\text{C}$ values of soil organic carbon and plant litter

The measured  $^{13}\text{C}$  abundance in the litter of the  $\text{C}_3$  plants depicted the natural variability of the isotopic composition of different plant species and plant organs. Gebauer and Schulze (1991) reported a similar variation of  $\delta^{13}\text{C}$  values of spruce litter from different sites.

The  $\delta^{13}\text{C}$  values of the SOC of the wheat, grassland and forest site resembled those of the plant material. The  $\delta^{13}\text{C}$  values of SOC were higher in the A horizons than in the subsoils and higher in the humus layer than in the Ah of the forest soil. This has been found in many studies and is mainly due to the  $^{13}\text{C}$  discrimination occurring during the microbial degradation of organic carbon (Balesdent and Mariotti, 1996; Bol et al., 1999; Bird et al., 2002; Novak et al., 2003).

The accumulation of maize-derived C in the soil profile (Table 2) agreed well with observations from other sites. In a clayey soil, Gregorich et al. (1996) found that after 32 yr of maize cultivation 22–30% of soil C was maize-derived under NPK treatment as compared to 15–20% under an unfertilized field. Collins et al. (1999) found that of eight soils in the maize belt of the US with a texture range from silty clay to loam that had continuously grown maize for 8 to 35 yr, the  $\text{C}_4$ -derived C in the surface soils (0–20 cm) ranged from 23% to 60% and from 9% to 32% in the subsoils (25–50 cm). Wanniarachchi et al. (1999) measured 10% of maize-derived C in the SOC of a sandy loam in Ontario cropped with maize for 6 yr under conventional tillage.

We found that 85% of the total maize-derived SOC were located in the Ap horizon and that 15% were stored in the subsoil. Assuming that the major part of the root biomass was located in the Ap horizon (~80%, Balesdent and Balabane, 1992), about 95% of the total maize residues (aboveground and roots) were incorporated into the Ap horizon after harvest and only 5% were located in the subsoil. Several processes may have contributed to the relative difference between the C input and the C stabilization in the Ap horizon and the subsoil: The most important were probably i) an additional transport of maize C into the subsoil by earthworms and by DOC leaching and ii) a smaller decomposition and greater stabilization rate of maize-derived organic carbon in the subsoil as compared to the A horizon.

Our data indicate that on a long-term basis only about 15% of the total maize C input during the recent 24 yr was stored as SOC. This result is slightly lower than the mean of 19.6% proportion of maize root and shoot residue C retained as SOM, calculated from literature data that was given by Bolinder et al. (1999).

Our data show an increasing SOC stability with soil depth (Table 2) with a mean apparent SOC turnover time of 54 yr in the Ap horizon and of more than 200 yr in the subsoil. Similarly, Trumbore (1993) found that the age of the SOC was increasing with depth. Balesdent et al. (1990) observed only small amounts of maize-derived C in the subsoil compared to the surface soil after 17 yr of continuous maize cropping.

#### 4.3. Water-stable aggregates

##### 4.3.1. Distribution of water-stable aggregates and C storage in water-stable aggregates

Our results showed that the MWDs were significantly greater in soils without tillage (grassland and forest) than in the soils with tillage (maize, wheat). Comparable to our results, using a similar method, Wander and Bollero (1999) found significantly higher MWD in non-disturbed soils (1.18 mm) as compared to no-tillage (0.67 mm) or conventional tillage (0.58 mm). In soils of New Zealand, Shepherd et al. (2001) found that the MWD of water-stable aggregates declined by 64–71% from pasture after 4 yr of cropping.

The distribution of aggregate sizes showed that macroaggregates, in particular the fraction  $>1000 \mu\text{m}$ , were destroyed by tillage. Similar to our results, Six et al. (2000) found that at three out of four sites, the most abundant aggregate fraction was  $53\text{--}250 \mu\text{m}$  under conventional and  $250\text{--}1000 \mu\text{m}$  under no tillage. From the loamy soil under continuous maize, 32% and 29% of the total dry weight were located in the fractions  $<50 \mu\text{m}$  and  $50\text{--}250 \mu\text{m}$ . Additionally, our results agree with the observation of Besnard et al. (1996) who found that macroaggregates were the most important aggregate fraction (52.4%) in a forest soil and that 7 yr after its conversion to maize, 28.6% of the total soil were located in the fraction  $>200 \mu\text{m}$ , 18.0% in the fraction  $50\text{--}200 \mu\text{m}$  and 49.5% in the fraction  $<50 \mu\text{m}$ .

We found increasing SOC concentrations with increasing aggregate size in all agricultural soils and in the subsoils of the forest and the grassland. These results were in line with the concept of aggregate hierarchy according to which microaggregates are bound together into macroaggregates by transient binding agents (i.e., microbial- and plant-derived polysaccharides) and temporary binding agents (roots and fungal hyphae) (Tisdall and Oades, 1982; Six et al., 2000). The consequences of this aggregate hierarchy are an increase in C concentration with increasing aggregate-size class because larger aggregate-size classes are composed of smaller aggregate-size classes plus organic binding agents (Elliott, 1986). In all soils, the lowest SOC concentration was found in the aggregate fraction  $<53 \mu\text{m}$ . This fraction contained no sand particles but it might be that the low SOC concentration in this fraction was influenced by the accumulation of free silt particles with low or no binding capacity of SOC (e.g. small quartz grains).

We found that sand particles were included in soil aggregates and that this sand fraction can contain organic carbon. This is contradictory to Six et al. (1998) who observed that sand particles were not included in aggregates and therefore they proposed the calculation of sand-free aggregates. Our finding may be a result of the low sand content of the soil (10.6% sand) that consisted mainly of fine sand.

Tisdall and Oades (1982) and Hassink (1997) proposed the concept that formation of macroaggregates ( $>250 \mu\text{m}$ ) starts after saturation of the SOM

binding capacity of silt and clay particles. This concept did not hold for our study sites since formation of aggregates  $>250 \mu\text{m}$  occurred even in the subsoils where the C concentration of the fraction  $<53 \mu\text{m}$  was very low (0.37 to 0.57% C) and far away from the C saturation of this fraction (Table 3). Our data indicate that the formation of macroaggregates increased with increasing C saturation of the silt and clay fraction.

We found that for the same aggregate size, SOC concentration increased with the total SOC content of the soil. The closer correlation of the SOC stored in macroaggregates to the SOC in the bulk soil as compared to the correlation between the SOC in microaggregates to the total SOC suggests that increased SOC storage was closer associated with macroaggregation than microaggregation. The results also proved that the amount of C stored in microaggregates was less influenced by cultivation than C in macroaggregates and that C was mainly lost from macroaggregates if land use change from forests to grasslands or further to ploughed soils took place. The increasing C/N ratios with increasing aggregate size suggest that SOC in macroaggregates is younger and more labile than SOC in microaggregates.

#### 4.3.2. $\delta^{13}\text{C}$ values and maize-derived C in water-stable aggregates

Increasing  $^{13}\text{C}$  enrichment of SOC with decreasing aggregate size indicated different portions of young plant residues in the aggregates. The  $\delta^{13}\text{C}$  values measured in the macroaggregate SOC were close to that of the plant litter. Only the strongly acid, biologically less active forest soil did not fit with this general trend. Similar to the results of our study, Angers and Giroux (1996) found that  $^{13}\text{C}$  contents were increasing with decreasing aggregate size under a meadow. They found  $\delta^{13}\text{C}$  values of  $-26.8\text{‰}_{\text{PDB}}$  in the fraction  $<50 \mu\text{m}$  and  $-27.5\text{‰}_{\text{PDB}}$  in the fraction  $>2 \text{ mm}$ . The different sources of SOC in aggregate fractions became clearer from the maize soil where  $\delta^{13}\text{C}$  values showed the portion of young maize-derived C in the aggregates. The increasing percentage of maize-derived C in SOC with increasing aggregate size reflected the greater mean age of SOC in microaggregates and in the silt and clay fraction. SOC contained in macroaggregates was younger and more labile than SOC in microaggre-

gates. Our results agree with the findings of [Angers and Giroux \(1996\)](#) from a 15 yr maize monoculture. They found for water-stable aggregates a decreasing percentage of maize-derived C with decreasing aggregate size: 20.5% (>2000  $\mu\text{m}$ ), 18.2% (1000–2000  $\mu\text{m}$ ), 11.6% (500–1000  $\mu\text{m}$ ), 8.2% (250–500  $\mu\text{m}$ ), 3.4% (50–250  $\mu\text{m}$ ) and 0.3% (<50  $\mu\text{m}$ ) were maize-derived.

The calculated mean age of SOC in aggregate fractions of the maize soil (Ap horizon) varied between 35 and 86 yr. For a silty clay loam to clay loam in eastern Ontario, Canada, [Monreal et al. \(1997\)](#) found turnover times of 14 yr (macroaggregates >250  $\mu\text{m}$ ), 61 yr (microaggregates 50–250  $\mu\text{m}$ ) and 275 yr (microaggregates <50  $\mu\text{m}$ ). However, these turnover times did not characterize C turnover in aggregates since the calculated rates may be affected by the redistribution of particulate organic matter among aggregate classes through the destruction and re-formation of aggregates. [Puget et al. \(2000\)](#) assumed that stable macroaggregates exist for a few years but that microaggregates may exist for decades. However, the knowledge of soil aggregate turnover and the lifespan of different aggregates classes is still fragmentary.

#### 4.4. Density fractions

##### 4.4.1. Distribution of the C and N in density fractions

The greater SOC concentration in the A horizon of grassland as compared with the SOC concentrations in the Ap horizons was primarily due to larger amounts of SOC in the heavy mineral fraction, thus indicating that agricultural soils did not reach their maximum capacity of SOC storage in the mineral fraction. The C stocks in  $\text{fPOM}_{<1.6}$  and  $\text{oPOM}$  were also greater in the grassland soil than the field soils. However, these fractions contributed only about 15% of the difference in SOC concentration between grassland and field. The results suggest that changes in SOC contents induced by the conversion from fields to grassland or vice versa are primarily caused by changes in the mineral associated SOC pool.

The acid forest soil had much larger amounts of POM. The POM concentration in the 0–7 cm layer was about 13 times greater in the forest soil than in the field soils. The greater importance of free and occluded POM in the forest soil was probably a result of the litter

quality and the microbial activity in soil. The much lower bioavailability of spruce litter as compared with grass or crop litter and the reduced microbial activity in the acid soil had probably favoured POM accumulation in the mineral soil. Similar to our study, [Besnard et al. \(1996\)](#) found that 35 yr after the conversion from forest to a maize culture, the  $\text{fPOM}_{<1.6}$  decreased from 34.9  $\text{g kg}^{-1}$  soil in the forest soil to 7.1  $\text{g kg}^{-1}$  soil in the maize soil in the fraction >200  $\mu\text{m}$  and for the fraction 50–200  $\mu\text{m}$  the decrease was from 8.5  $\text{g kg}^{-1}$  soil to 2.5  $\text{g kg}^{-1}$  soil, respectively.

[Golchin et al. \(1997\)](#) proposed different functions for  $\text{oPOM}$  fractions with different specific weight. They assumed following their hierarchical concept described above, that  $\text{oPOM}_{<1.6}$ ,  $\text{oPOM}_{1.6-1.8}$   $\text{g cm}^{-3}$  and  $\text{oPOM}_{1.8-2.0}$   $\text{g cm}^{-3}$  derive from microaggregates and the fraction  $\text{oPOM}_{1.8-2.0}$   $\text{g cm}^{-3}$  also partly derives from macroaggregates where it acts as a binding agent between microaggregates. Therefore, as grassland (0–10 cm) and forest soil (0–7 cm) contained a higher percentage of macroaggregates, the relative contribution of the fraction  $\text{oPOM}_{1.6-2.0}$  to the total SOC was higher than at the cropped soils. Our results did not confirm this hypothesis since macroaggregation was different in grassland and field soils whereas the relative importance of the dense  $\text{oPOM}$  fraction ( $\text{oPOM}_{1.6-2.0}$ ) was similar.

The relative contribution of  $\text{fPOM}_{<1.6}$  and  $\text{oPOM}$  to SOC storage in the grassland and fields was in the same order of magnitude as it was found by [Golchin et al. \(1994a\)](#) for a grassland soil with similar clay content. [Six et al. \(2002b\)](#) found a higher C stabilization in microaggregates under forest soil than under agricultural soil, however, the differences in POM fractions caused by land use were not as pronounced as in our study.

The decreasing C/N ratios as  $\text{fPOM}_{<1.6} > \text{oPOM} > \text{Mineral-SOM}$  indicated an increasing degree of degradation and humification of the organic matter in this order. The comparison of the two  $\text{oPOM}$  fractions showed that the N concentration was higher in the dense  $\text{oPOM}_{1.6-2.0}$  than in the light  $\text{oPOM}_{<1.6}$  fraction. Our observation that the C/N ratio was higher for the light  $\text{oPOM}_{<1.6}$  fraction than the dense  $\text{oPOM}_{1.6-2.0}$  fraction is in accordance with the results of [Golchin et al. \(1994b\)](#) who found the order  $\text{oPOM}_{<1.6} > \text{OPOM}_{1.6-1.8} > \text{fPOM}_{<1.6} > \text{oPOM}_{1.6-2.0} > \text{Mineral-SOM}_{>2.0}$ .

#### 4.4.2. Distribution of $^{13}\text{C}$ and maize-derived C in density fractions

The high percentage of maize-derived C in the  $\text{fPOM}_{<1.6}$  indicated that this fraction contained considerable amounts of young crop residues. The  $\text{oPOM}$  fractions were considerably older and contained significantly less maize carbon. A similar order of the apparent turnover time of SOC in density fractions was determined by Golchin et al. (1995) under pasture, 35 and 83 yr after a forest clearing. After 35 (83) yr, the apparent turnover time of the SOC in the  $\text{fPOM}_{<1.6}$  was 19 (27) yr, for the fraction  $\text{oPOM}$  1.6–1.8  $\text{g cm}^{-3}$  it was 24 (41) yr, for  $\text{oPOM}$  1.8–2.0  $\text{g cm}^{-3}$  it was 24 (53) yr and for the fraction  $\text{oPOM}_{<1.6}$  it was 40 (49) yr. The longest apparent turnover time was observed in the mineral fraction  $>2.0 \text{ g cm}^{-3}$  with 41 (73) yr.

Using  $^{13}\text{C}$  CP/MAS NMR, Golchin et al. (1994a,b) found that the largest proportion of the organic C contained in the free light fractions of these soils was *O*-alkyl, followed by alkyl, aromatic and carbonyl C, respectively. There was more alkyl, aromatic and carbonyl C in the  $\text{oPOM}_{<1.6}$ , but less *O*-alkyl C. Thus their results proved that the  $\text{oPOM}$  was further degraded than the  $\text{fPOM}_{<1.6}$ .

Our results show that different POM pools occluded in soil aggregates ( $\text{oPOM}$ ) existed. These pools differed in specific weight and mean age. Besnard et al. (1996) found that the mean age of POM was greater inside microaggregates than inside macroaggregates and they concluded that this may reflect different turnover rates of POM fractions occluded in aggregates. Further studies are necessary to show whether this phenomenon was caused by  $\text{oPOM}$  pools differing in specific weight and how these  $\text{oPOM}$  pools contribute to the SOC content of different aggregate classes.

## 5. Conclusions

In the topsoils, the distribution of aggregate size classes was influenced by land cultivation, as tillage destroyed especially aggregates  $>1000 \mu\text{m}$ . For the tilled horizon of the maize soil, the most frequent aggregate fraction was the microaggregate fraction (53–250  $\mu\text{m}$ ). For wheat (conservation tillage since 1998) the smaller macroaggregates (250–1000  $\mu\text{m}$ ) and for the grassland and forest soils the megaa-

ggregates ( $>2000 \mu\text{m}$ ) were most important. Soil aggregation was more intensive in the surface soils than in the subsoils. Aggregate formation was associated with increased carbon storage as C contents increased with aggregate size in most cases and C contents in all aggregate fractions  $>53 \mu\text{m}$  were greater than in the silt and clay fraction ( $<53 \mu\text{m}$ ). We like to point out that our results did not prove physical protection of organic matter by aggregate formation since the mean age of SOC pools did not provide information about the mechanisms of SOC protection or stabilization. The mean age of organic carbon in the analysed SOC fractions may be also influenced by differences in the chemical structure and recalcitrance. The amount of C of 1 kg soil stored in the microaggregates was poorly correlated to the amount of SOC in the bulk soil, and hence cultivation, while the amount of SOC stored in macroaggregates was highly correlated to the total SOC concentration. The differences in the SOC concentration, C/N ratio and maize-derived C among aggregate classes were in line with the concept of aggregate hierarchy.

The greater SOC concentrations in the A horizons of the grassland and the spruce site as compared with the fields were caused by different SOC pools. In the grassland soil, the additional C was located mainly in the heavy 'Mineral-SOM' fraction, whereas in the acid forest additional C was mainly stored in the different fractions of particulate organic matter. The results indicate that SOC storage should not only be assessed in terms of total C stocks but also with respect to SOC composition and stability. More detailed information of the quantity, structure and stability of SOC pools within aggregate fractions are necessary to understand the reasons of different SOC concentrations in aggregates and the functions of these pools.

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