

Effect of manure and fertilizer application on the distribution of organic carbon in different soil fractions in long-term field experiments

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Abstract

We studied soil organic carbon (SOC) pools using physical fractionation in two long-term arable field experiments in Hungary (Martonvásár and Keszthely), which started in 1950 and 1963, respectively (clay loam and loam soil). Replicate experimental plots of three different manure and fertilizer treatments and one unfertilized control object were sampled in March 2004 at both sites. Samples from all fields were separated into five size and density fractions. Fertilization had a distinct influence on both the OC amount present in two free particulate organic matter (POM) fractions (+19–230% compared to the control treatment) and their relative proportion on the whole-soil OC. This increase in OC was equal or smaller for the POM occluded in microaggregates (53–250 μm) (+16–97% compared to the control treatment), and much smaller for the amount of OC present in the silt + clay sized fraction (+–2 to 30% compared to the control treatment). The considered SOC fractions were associated with different conceptual SOC pools. Differences in the relative effect of management on the amount of OC present in the fractions consequently resulted in shifts of the relative proportion of the OC present in these conceptual OC pools. The measurability of these changes at the time scale of decades shows that it may be possible to use experimental data such as the data obtained from this study for the development and calibration of SOC models with conceptual OC pools which coincide with physical counterparts.

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1. Introduction

Soil organic matter (SOM) is a heterogeneous, dynamic substance that varies in C and N content, molecular structure, decomposition rate and turnover time (Oades, 1988). In most current SOM models, SOM is considered to be composed of several discrete pools which differ in their intrinsic decomposition rate and in the factors controlling the decomposition rates. While such simulation models can be successfully validated using measurements of total SOC and isotopic ratios of total C, the individual pools are generally only loosely associated with measurable quantities (Six et al., 2002b). However, if measured SOM fractions can be shown to be unique and non-composite, they can be used as model pools and give the opportunity of detailed model initialization and evaluation (Smith et al., 2002). Six et al. (2002b) proposed such a model in which SOM is sep-

arated into conceptual pools where OM is retained by different SOM protection mechanisms: physically protected SOC, recalcitrant chemically and biochemically protected SOC and lastly, labile unprotected SOC.

A closer linkage between such theoretical and measurable pools of SOM can be made by explicitly defining model pools to coincide with measurable quantities or by devising more functional laboratory fractionation procedures or both (Six et al., 2002b). Indeed, the whole concept behind physical fractionation of soil emphasizes the role of soil minerals and aggregation in the processes of SOM stabilization and turnover (Christensen, 2001). Six et al. (2000) suggested a physical fractionation procedure, which allows isolation and direct measurement of the SOC contained within the different pools of their conceptual model.

Several studies have elucidated the relationship between aggregate and associated SOM dynamics (Elliott, 1986; Jastrow, 1996; Six et al., 1998, 2000). Tisdall and Oades (1982) presented an aggregate hierarchy theory for aggregate stabilization in temperate soils which has been widely accepted and refined (Oades et al., 1984; Golchin et al., 1994; Angers et al., 1997).

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This theory has been corroborated by many others, as summarized in detail by Six et al. (2004). The current hypothesis of the aggregate hierarchy concept is that free primary particles are bound together into microaggregates (50–250 μm) by persistent binding agents (e.g. humified OM). These stable microaggregates are bound together into macroaggregates. Microaggregate stability is higher and less dependent on agricultural management than macroaggregate stability. Since long it has been an aspiration to integrate these mechanisms of the physical protection of SOM within aggregates into a SOM model (Elliott et al., 1996). The physical fractionation procedure and the associated conceptual SOM model, which Six et al. (2002b) proposed, specifically takes physical protection of OM into account since it differentiates POM inside aggregates from POM outside of these aggregates.

Soil management induced changes on the equilibrium between input of primary OM and decomposition, e.g. by additional input of OM from plants or manure on one hand, or by enhanced aeration due to tillage on the other, lead to measurable changes in OC contents of organic–mineral fractions (Schulten and Leinweber, 2000). Many studies have reported relative increases in OC content of particle-size fractions of manured fields compared to soils which received only mineral fertilizer (Christensen, 1988, 1992; Angers and N'Dayegamiye, 1991; Gerzabeck et al., 2001). None of these studies, however, used fractionation techniques which specifically aim at isolating fractions that can be related to conceptual SOM model pools. In contrast, the objective of this study was specifically to assess the extent to which OM management affects, and thus is measurable by, the distribution of SOC in such conceptual pools. Soil samples of the plough layer from different OM-treatments from two long-term Hungarian field experiments were fractionated into size and density fractions using a methodology similar to the one proposed by Six et al. (2000). We examined the effects of mineral fertilizer and animal manure, as compared with unfertilized soil on the OC distribution in these SOC fractions. Thereby, a distribution of SOC into conceptual pools with differing SOM stabilization mechanisms is obtained.

2. Methods

2.1. Site description and soils

In both field experiments several mineral fertilizer and farmyard manure treatments were established at both experimental

sites (Table 1). The crop rotations at Martonvásár (wheat–wheat–maize–maize) and at Keszthely (wheat–wheat–sugar beet–maize–maize) were comparable. From the Martonvásár field experiment, four contrasting treatments were included in this study. 1° A control receiving no manure or NPK fertilizer (M1); 2° application of 40 tonnes FYM every 4 years (M2); 3° application of 40 tonnes FYM plus an equivalent amount of NPK in mineral fertilizer every 4 years (M3); 4° application of 80 tonnes FYM every 4 years (M4). Similar treatments were selected from the Keszthely field experiment to be able to compare results with the Martonvásár site as much as possible. 1° A control receiving no manure or NPK fertilizer (K1); 2° application of 35 tonnes FYM every 5 years (K2); 3° application of 35 tonnes FYM plus an equivalent amount of NPK in mineral fertilizer every 5 years (K3); 4° application of 104 tonnes FYM every 5 years (K4).

Table 2 summarizes the total amount of N, P and K from both mineral and organic fertilizers applied calculated on a yearly basis, the average crop yield of maize and winter wheat and the estimated average yearly OC input from crop residues and farmyard manure into the soil. The crop residue OC input was calculated based on crop yields by assuming a constant proportion of root and above ground residues to the harvested part of the maize and wheat. No yield data were available of the sugar beets, therefore an estimate of the OC input from root residues from this crop was calculated using average yield figures for Hungary (Anonymous, 2004). The OC and nutrient input from the FYM was calculated using a constant OC content of 105 kg OC tonnes⁻¹ fresh manure and NPK contents of 6.2 kg N tonnes⁻¹ fresh manure, 1.8 kg P tonnes⁻¹ fresh manure and 5.8 kg K tonnes⁻¹ fresh manure based on literature data by Anonymous (2004) which reflect current Hungarian agricultural practice.

Samples from the top soil (0–20 cm) were taken in March 2004 from two replicate experimental plots for each treatment using an auger bore. First, the field moist soil was gently broken apart by hand and was passed through an 8 mm sieve to break down large macro-aggregates. The soil was then dried at 50 °C.

2.2. Physical fractionation of soil organic matter

Sub samples from every replicate of the different treatments were separately used for the physical fractionation. The fractionation procedure was carried out in triplicate, yielding a total of six repetitions per treatment. To avoid possible slaking of microaggregates during wet sieving, all soil samples were pre-wetted according the method used by Gale et al. (2000): a 10 g sub sam-

Table 1
Summary of the long-term fertilization experiments in Keszthely and Martonvásár (Hungary)

Site	Latitude/longitude (°)	Mean annual temperature (°C)	Mean annual rainfall (mm)	Soil texture (USDA)	World Reference Base ^a —soil group and subunit	Soil pH _{KCl} (–)
Martonvásár	47.32N 19.00E	10.3	453	Clay loam	Calcic Chernozem	5.9
Keszthely	46.46N 17.00E	10.0	623	Loam	Eutric Cambisol	6.7

^a FAO (1998).

Table 2
Description of the different treatments of the long-term fertilization experiments in Keszthely and Martonvásár (Hungary)

Treatment	Total fertilization			OC input ^a (tonnes OC ha ⁻¹ year ⁻¹)		Grain Yield (tonnes DM ha ⁻¹) ^c	
	N (kg N ha ⁻¹ year ⁻¹)	P (kg P ha ⁻¹ year ⁻¹)	K (kg K ha ⁻¹ year ⁻¹)	FYM	Plant ^a	Maize	Wheat
Martonvásár							
M1: Control treatment	0	0	0	0	0.6	4.9	2.4
M2: 40 tonnes FYM ha ⁻¹ /4 years	62	18	58	1.1	0.8	6.4	3.0
M3: 40 tonnes FYM ha ⁻¹ + NPK/4 years	124	36	116	1.1	0.9	6.6	3.6
M4: 80 tonnes FYM/4 years	124	36	116	2.1	1.0	7.1	4.6
Keszthely							
K1: Control treatment	0	0	0	0	0.4	2.5	2.9
K2: 35 tonnes FYM ha ⁻¹ /5 years	43	13	41	0.7	0.5	3.3	3.2
K3: 35 tonnes FYM ha ⁻¹ + NPK/5 years	87	26	82	0.7	0.7	5.6	4.5
K4: 104 tonnes FYM/5 years ^b	129	37	121	2.2	0.6	3.8	4.2

^a Estimated from crop yields.

^b Farmyard manure was applied in two separate portions.

^c DM, dry matter.

ple was weighed on a 20 µm nylon filter on top of a glass-fiber filter (Whatman GF/A) in a Petri-shell. The soil samples were wetted by slowly adding water to the edges of the glass-fiber filter and by allowing it to be absorbed by the soil. The samples were left to equilibrate overnight in a refrigerator.

The wet sieving method used was based on the procedure described by Six et al. (2000) (Fig. 1). A sieving setup was used that allows complete break up of macroaggregates into microaggregates (53–250 µm) while minimizing disruption of the released microaggregates. The 10 g soil sample was placed on a 250 µm sieve and was gently shaken with 4 mm glass beads on top of a reciprocal shaker. A constant water flow through the

sieving column consisting of a lid, the 250 µm sieve and a pan with water outlet (Eijkelcamp Agrisearch Equipment) flushed the <250 µm fraction directly onto a 53 µm sieve, thus avoiding further disruption of the microaggregates by the beads. The water + soil fraction <53 µm passing through the 53 µm sieve was collected in buckets. The coarse free particulate organic matter (coarse fPOM) and sand which had been retained on the 250 µm sieve was collected in a pre-weighed 400 ml measuring beaker, dried at 105 °C and weighed after cooling down in a desiccator. The same procedure was followed for micro-aggregates, fine free POM (53–250 µm) (fine fPOM) and fine sand, which had been retained on the 53 µm sieve. The water in the buck-

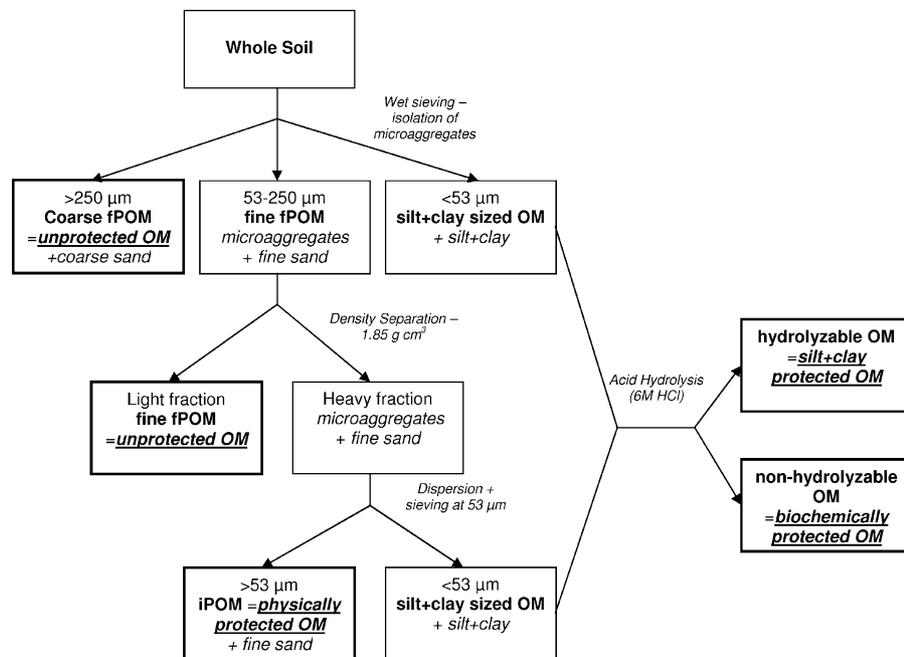


Fig. 1. Schematic of the applied physical fractionation procedure for the isolation of coarse free particulate organic matter (coarse fPOM), fine free POM (fine fPOM), intra-microaggregate POM (iPOM) and silt and clay sized OM, with indication of the four isolated conceptual SOM pools according to the model of Six et al. (2002b) (unprotected OM, physically protected OM, silt and clay protected OM and biochemically protected OM).

ets was aspirated after sedimentation of the silt and clay and the remaining water + soil was collected in measuring beakers, dried and weighed. All fractions were stored in glass vials.

The fine fPOM between the microaggregates was isolated from the fine sand, the intra-microaggregate POM (iPOM) and the clay + silt fraction within the microaggregates by density flotation with 1.85 g cm^{-3} sodium polytungstate (SPT) (Sometu Europe, Falkenriedt 4, Berlin, Germany) following a procedure similar to the one used by Six et al. (1998). Five grams of the dried 53–250 μm fraction was weighed and pre-wetted as described above. Each sample was rinsed from the nylon filter into an 80 ml nalgene centrifuge tube using 50 ml SPT (the SPT was adjusted to 1.87 g cm^{-3} as the water inside the aggregates would lower the density to 1.85 g cm^{-3} , as described by Gale et al. (2000)). The samples were centrifuged at $1250 \times g$ for 60 min at 20°C . The floating material, i.e. the fine fPOM, was aspirated and filtered on a pre-weighed 20 μm nylon filter. The material on the filter was rinsed with water to remove remains of SPT, and the filters were dried at 50°C . The pellet was rinsed into a 1000 ml measuring beaker and was left to sediment overnight after which the water + dissolved remains of SPT were aspirated. This procedure was repeated to ensure complete removal of the SPT. Afterwards the remaining water and soil were transferred to a pre-weighed 250 ml beaker, dried at 105°C and weighed.

A sub sample of 4 g of the isolated microaggregates was dispersed in 25 ml sodium hexametaphosphate (5 g l^{-1}) inside a 250 ml Erlenmeyer by shaking 18 h on a reciprocal shaker. The dispersed samples were passed through a 53 μm sieve and were rinsed thoroughly with water. The material retained on the sieve (fine sand and iPOM) was backwashed into a pre-weighed measuring beaker and was dried at 105°C and weighed.

2.3. Chemical fractionation of the silt + clay (<53 μm) associated OM

The <53 μm fraction contains OM which is stabilized both as a consequence of its complex chemical composition and by adsorption to silt and clay particles. Several studies have found that the non-hydrolyzable OM fraction in temperate soils includes very old C (for a review see Six et al., 2002b). A simple hydrolysis technique with 6 M HCl, based on a technique used by Leavitt et al. (1996), was used to differentiate older and passive OC from the more labile OC associated with the <53 μm soil fraction.

Since the amount of material left of the <53 μm fraction was insufficient, additional soil samples were used for the hydrolysis. Whole-soil samples (20 g) were dispersed by shaking in distilled water for 18 h with 10 glass beads inside a 400 ml glass beaker in accordance to Carter et al. (2003). The dispersed sample was passed through a 53 μm sieve and rinsed with water and collected in 600 ml measuring beakers and was dried at 105°C . Prior to hydrolysis, CaCO_3 present in the <53 μm samples was removed by shaking the soil inside a glass Erlenmeyer with 50 ml 0.1 M HCl on a reciprocal shaker for 16 h. The sample + 0.1 M HCl solution were then separated by filtration on a Whatman 2 filter using a Büchner funnel. The soil material retained on the filter was collected and dried in the oven at 105°C . Five g

of this CaCO_3 -free <53 μm soil fraction was rinsed with 25 ml 6 M HCl into a 500 ml round-bottom flask, and the mixture was gently boiled for 16 h under reflux using Liebig coolers which were attached on top of the flasks. Afterwards plenty of distilled water was added to the flasks and the remaining material was again separated by filtration and dried as described above. The fraction of non-hydrolyzable OC is the ratio of the OC content of the <53 μm sample after hydrolysis to the OC before hydrolysis.

2.4. Carbon analysis

Sub samples (200 mg) of the separated iPOM fraction + fine sand and the silt + clay (<53 μm) sieving fraction was analyzed for both OC and inorganic carbon (IC) content with a Shimadzu TOC analyzer, because all soils contained CaCO_3 (Martonvásár: 1.1–1.3%, Keszthely: 0.5–0.6%). Because of the very low quantities of fine iPOM obtained after the density fractionation (5–30 mg), the fine fPOM obtained from the three fractionation replicates per field replicate of each treatment were bulked for the C analysis. The coarse and fine fPOM fractions were assumed to contain no CaCO_3 and were analyzed with an Elementar Variomax CNS-analyzer which allows for faster measurement but measures total C. The non-hydrolyzable material obtained from the chemical fractionation was also analyzed for total C using the CNS-analyzer, as it contains no carbonate (acid digestion).

3. Results

3.1. SOC content of the whole soil

Table 3 gives the soil texture for all treatments and the CaCO_3 -C content of the bulk soil (0–20 cm). There was very little variability in these soil properties among treatments of the same field experiment. However, the soil texture of the K4 treatment at Keszthely was slightly heavier than for the other treatments at that site, which may influence the results of the physical fractionation. CaCO_3 -C contents were low, but

Table 3
Soil texture, CaCO_3 -C and SOC content and comparison with the bulk soil OC balance by summing the different OC fractions from the physical fractionation

Treatment	Soil texture			CaCO_3 -C C (%)	SOC measured OC (%)	SOC sum fractions OC (%)
	Sand (%)	Silt (%)	Clay (%)			
Martonvásár						
M1	30.5	35.7	33.8	0.14	1.65	1.56
M2	31.0	35.7	33.4	0.14	1.75	1.57
M3	31.1	35.2	33.8	0.14	2.12	1.95
M4	31.0	36.0	33.1	0.15	2.17	2.00
Keszthely						
K1	44.1	33.9	22.1	0.08	0.92	0.87
K2	44.4	34.1	21.6	0.10	1.04	0.99
K3	46.1	32.6	21.4	0.09	1.00	0.98
K4	43.4	32.8	23.8	0.08	1.24	1.22

not negligible for physical fractionation, particularly for Martonvásár. The Calcic Chernozem soil at Martonvásár had a much higher SOC content than the Eutric Cambisol at Keszthely for all treatments, although both experiments were comparable in management and crop rotation. OC content of the bulk soil samples increased with both mineral fertilizer and manure applications as compared to the control for both sites. However, there was no further increase in SOC between the K2 and K3 treatments at Keszthely. The mean difference between the measured whole-soil OC content and the calculated whole-soil OC content after the physical fractionation amounted 7.9 and 3.5% on an average for Martonvásár and Keszthely, respectively. This difference was, however, only significant for the M4 treatment.

3.2. Soil DM distribution in the isolated soil fractions

Table 4 summarizes the distribution of the soil dry matter (DM) over the various isolated soil fractions. The >250 μm fraction (sand+coarse fPOM) and the fine fPOM fraction accounted for 2.4–4.2 and 0.1–0.4% of the soil DM for Martonvásár and 2.2–2.5 and 0.02–0.04% for Keszthely, respectively. The third sand-sized soil fraction, containing the iPOM, which was obtained after density separation and dispersion of the 53–250 μm sieving fraction, amounted to 25.8–28.0 and 37.9–42.0% of the whole-soil DM for Martonvásár and Keszthely, respectively. The sum of DM percentages of these three fractions was roughly equal to the percentage of sand (recalculated on a whole-soil basis by taking into account the %OM and %CaCO₃).

For Martonvásár, there were notable differences among treatments for all fractions considered. There was no clear effect however, of treatment on the DM proportion of any of the isolated soil fractions of the Keszthely site, except for the proportion of the fine fPOM fraction but no consistent trend with manure and fertilizer treatment was observed. For the coarse fPOM+sand fraction and the fine fPOM fraction there was a clear increase of the relative amount of DM in the order: control < mineral fertilizer < mineral fertilizer + FYM < FYM \times 2. For the

other fractions no consistent trend with treatment could be found.

3.3. OC distribution in the isolated SOM size and density fractions

The relative distribution of the whole-soil OC over the different fractions (expressed as g C g⁻¹ soil C) is shown in Fig. 2. The relative OC contribution for all fractions differed significantly between fertilizer treatments for Martonvásár. Particularly, treatments M3 and M4 had higher proportions of both free POM fractions (coarse fPOM and fine fPOM). The proportion of OC in fine fPOM and iPOM fractions increased consistently with fertilizer and manure treatment. As a consequence of the larger proportions of both free and occluded POM fractions to whole-soil OC, the silt+clay associated OC was significantly smaller in the M3 and M4 treatments than in the other treatments. For Keszthely, the fertilizer treatments had a clear influence on the relative OC contribution of all fractions except for the coarse fPOM fraction. Only very little OC was present in the fine fPOM fraction (0.01–0.03 g C g⁻¹ soil C). The relative amount of iPOM was lower in the K1 (control) treatment than in the other treatments and its relative amount of silt+clay associated OC was notably higher.

3.4. Hydrolysis of the <53 μm soil fraction

Table 5 gives the OC content and the corresponding amount of OC expressed on a whole-soil basis of the <53 μm fraction prior to hydrolysis and the measured proportion of non-hydrolyzable OC in this fraction. Both the percentage of OC and the total amount of OC present in this fraction increased with fertilizer and manure additions. The non-hydrolyzable OM proportion of the silt+clay fraction varied among treatments but a clear relation with management could not be deduced. This fraction was some 15% larger for Martonvásár than for Keszthely. The total amount of non-hydrolyzable OC present in the <53 μm fraction expressed on a whole-soil basis is presented in Fig. 3. An increasing trend with augment-

Table 4

Amount of soil dry matter present in the size and density fractions (g 100 g⁻¹ soil) obtained by the physical fractionation (average \pm S.D.)

Treatment	Coarse sand + coarse POM (>250 μm)	Microaggregates + fine free POM + fine sand (53–250 μm)			Silt + clay (<53 μm)
		Light fraction (fine POM)	Heavy fraction		
			>53 (μm)	<53 (μm)	
Martonvásár					
M1	2.4 \pm 1.5	0.13 \pm 0.01	26.7 \pm 0.8	35.4 \pm 2.7	35.6 \pm 3.7
M2	2.5 \pm 1.3	0.17 \pm 0.01	26.0 \pm 1.9	35.0 \pm 1.4	36.3 \pm 4.1
M3	3.2 \pm 0.6	0.26 \pm 0.02	28.0 \pm 0.8	32.3 \pm 2.4	36.3 \pm 3.1
M4	4.2 \pm 2.4	0.39 \pm 0.09	25.8 \pm 0.7	34.6 \pm 4.0	35.0 \pm 5.1
Keszthely					
K1	2.5 \pm 1.0	0.04 \pm 0.00	39.0 \pm 2.0	35.5 \pm 2.7	22.9 \pm 2.0
K2	2.2 \pm 0.6	0.05 \pm 0.00	40.1 \pm 3.4	35.2 \pm 4.4	22.4 \pm 1.5
K3	2.5 \pm 1.5	0.03 \pm 0.00	42.0 \pm 1.7	33.9 \pm 1.5	21.6 \pm 1.8
K4	2.5 \pm 0.3	0.02 \pm 0.00	37.9 \pm 1.9	36.8 \pm 2.7	22.8 \pm 3.6

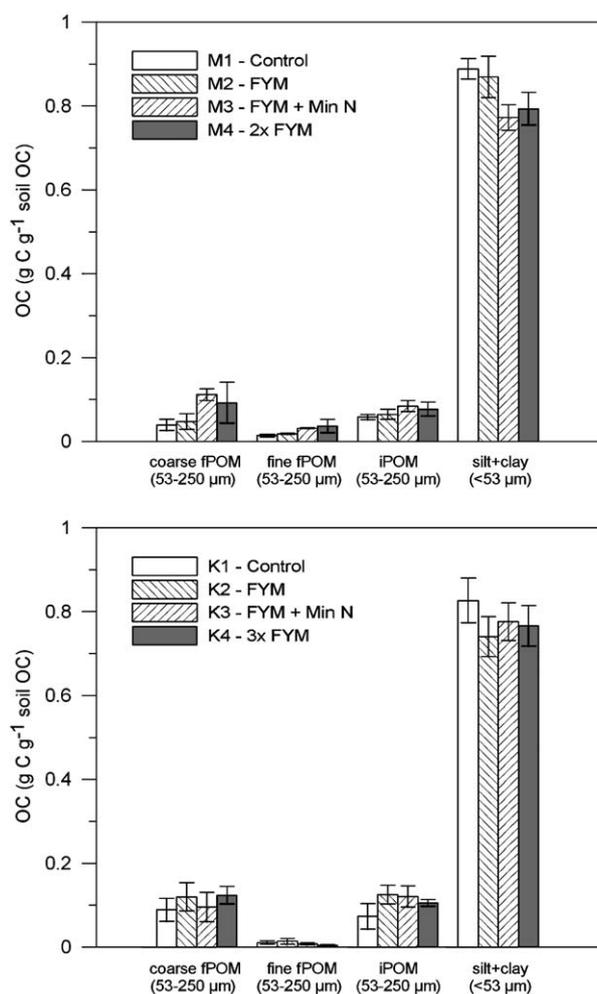


Fig. 2. Relative distribution of the total OC over the coarse fPOM (>250 μm), the fine fPOM (53–250 μm), the iPOM (53–250 μm) and the silt + clay associated (<53 μm) SOM fractions for the different treatments at the Martonvásár (above) and Keszthely (below) sites. Columns represent average values of all repetitions; Y-error bars represent the corresponding standard deviation.

Table 5

OC content and total amount of OC present in the <53 μm fraction and the percentage non-hydrolyzable OM of the <53 μm fraction (average \pm standard deviation)

Treatment	OC content of <53 μm fraction (% OC)	OC in <53 μm fraction (g C kg^{-1} soil)	Non-hydrolyzable OM in 6M HCl (%)
Martonvásár			
M1	1.96 ± 0.09	13.9 ± 1.1	80.3 ± 5.3
M2	1.91 ± 0.18	13.6 ± 0.9	88.6 ± 13.5
M3	2.23 ± 0.06	15.5 ± 1.0	78.3 ± 12.1
M4	2.28 ± 0.09	15.7 ± 0.5	81.6 ± 7.3
Keszthely			
K1	1.23 ± 0.12	7.2 ± 0.9	63.8 ± 9.8
K2	1.27 ± 0.05	7.3 ± 0.7	68.5 ± 5.5
K3	1.37 ± 0.14	7.6 ± 0.7	75.4 ± 6.8
K4	1.57 ± 0.07	9.4 ± 0.6	70.8 ± 4.4

ing fertilizer and manure applications can be found for both sites.

4. Discussion

4.1. Whole-soil OC, texture, CaCO_3 and DM distribution

Significant changes in SOC due to land management practices can only be observed after long time periods (Gerzabeck et al., 2001). N-fertilization increases SOC and N by increasing plant biomass production (Gregorich et al., 1996). Long-term applications of animal manure increase SOM in two ways: by adding OM contained in the manure and by increased OM in crop residues due to higher crop yields in soils receiving manure (Whalen and Chang, 2002). The differences in SOC content between different fertilizer and manure treatments were detectable and considerable after several decades in both field experiments studied here (Table 3). The increase of the SOC contents of the treatments relative to the control objects were consistent with the estimated extra OC input from manure and plant residues (Table 2). As other possible factors such as soil

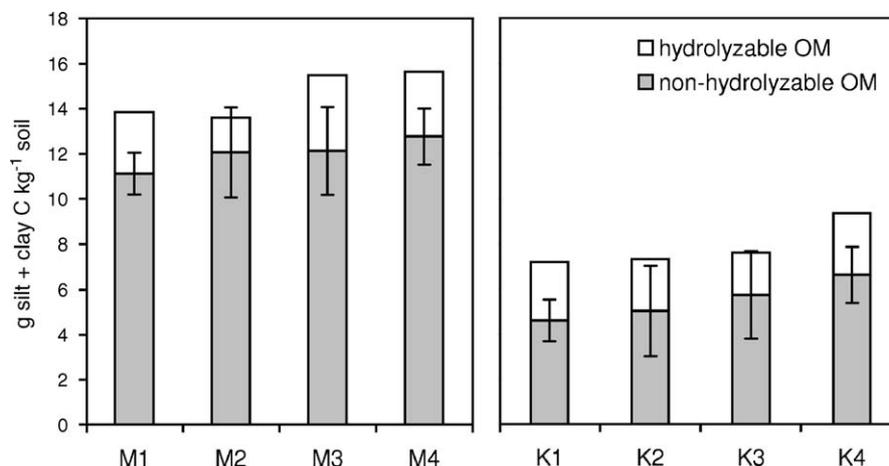


Fig. 3. Non-hydrolyzable and hydrolyzable OC in the silt + clay (<53 μm) sized soil fraction in the different fertilizer and OM treatments of the Martonvásár (left) and Keszthely (right) field experiments. Columns represent average values of all repetitions; Y-error bars represent the corresponding standard deviation.

texture and pH (data not shown) were equal among treatments, it can be concluded that management was the sole factor influencing the SOC levels. The fact that SOC levels were higher at Martonvásár compared to Keszthely, in spite of the fact that both experiments received very similar OC inputs, can be explained by the finer texture of the Martonvásár soils and possibly as well by the fact that the Calcic Chernozem soil in Martonvásár by nature is rich in OM in the form of stable humus.

It is generally accepted that calcium is a critical element for stabilization of SOM and aggregates (Six et al., 2004). Because calcium exerts its influence at the scale of the organo-mineral complexation, its stabilization effect is mostly observed at the microaggregate level. As there were no differences in soil CaCO₃ content between treatments no effect on micro-aggregation should be expected. Organic matter application both from manure (Aoyama et al., 1999; Gerzabeck et al., 2001) and plant residues (Sainju et al., 2003) also promotes aggregate formation. Manure contains polysaccharides and aliphatic and aromatic compounds that can bind to soil particles and create organo-mineral complexes important for flocculating aggregates <0.2 μm (Tisdall and Oades, 1982). In addition, manure is a source of energy and nutrients for soil micro-organisms and plant roots that produce extracellular polysaccharides known to flocculate soil mineral particles into aggregates. In spite of this, no increase in the amount of soil present in the micro-aggregate + fine sand fraction (53–250 μm) was found in the FYM treatments. This fraction constitutes 61% of the soil DM in Martonvásár and constitutes a considerably larger part of 75% for Keszthely. As the %sand was about 13% higher in Keszthely, this larger DM proportion of the micro-aggregate + fine sand fraction of these soils is very likely due to their larger proportion of sand.

4.2. SOC distribution in isolated soil fractions

At the Martonvásár and Keszthely sites the OC in the coarse fPOM and the fine fPOM combined made up 5–14 and 10–13% of the total OC. Köbl and Kögel-Knabner (2004) also found the proportion of these fractions to vary between 2.5 and 13.4% of the bulk soil OC for sandy to loamy soils. According to Christensen (2001) the uncomplexed fraction usually makes up less than 10% of the OM in the tilled layer of long cultivated arable soils. Results for both sites correspond fairly with these results. The light fraction (LF) and POM, especially coarse POM (>250 μm) are mainly derived from plant material, which has been subject to partial microbial decomposition (Six et al., 2001). Both the LF and POM have often been suggested to be good indicators for labile organic matter (Bremer et al., 1994; Balesdent, 1996; Carter et al., 2003). For example, using ¹³C natural abundance techniques, Balesdent (1996) concluded that POM has a short mean residence time compared to other soil fractions, indicating its high lability. Six et al. (2002b) suggested that LF and POM, not occluded within micro-aggregates, which correspond to the fine fPOM and coarse fPOM in our case, constitute a conceptual “unprotected” SOM pool in which both fractions are considered to be similar.

At Martonvásár, the clear increasing trend of the proportion of the amount of OC present in the coarse fPOM and the fine fPOM with manure and fertilizer treatment confirm them to be good indicators of management. There was no further significant increase from the M3 to the M4 treatments. There are indeed indications that this unprotected C pool can become saturated, as in various field studies no further increase in LF-C and/or POM-C with increasing OM input could be observed (e.g. Carter et al., 2003; Six et al., 2002b), whereas at lower OM input a distinct relationship between carbon inputs and LF-C or POM-C is observed. In a study by Carter et al. (2003) on a sandy loam soil in Ottawa the proportions of C in the POM and C in the LF on the total SOC were higher in manure amended plots (50 tonnes ha⁻¹ per rotation) than in unamended plots, but no further increase was observed between a plot receiving 50 tonnes ha⁻¹ and a plot receiving 100 tonnes ha⁻¹. For Keszthely, there was no clear increasing trend with management of C in both coarse and fine fPOM. Both K2 and K4 treatments tended to have a larger proportion of C in coarse fPOM than the control K1 object. This seems to confirm the distinct response of these labile fractions to management.

Köbl and Kögel-Knabner (2004) reported that the OC of occluded POM constituted 5.0–9.8% of the whole-soil OC for loam to silty clay loam soils. For the Martonvásár and Keszthely soils, the physically protected OC of the iPOM fraction held a similar proportion of the whole-soil SOC (5.7–8.4 and 7.3–12.0%, respectively). Aoyama et al. (1999) concluded from a field experiment lasting 18 years, that there was no effect of NPK fertilization on the amount of C physically protected within aggregates, whereas cattle manure application (20 tonnes ha⁻¹ year⁻¹) did increase the proportion of protected pools of C in small macro-aggregates (250–1000 μm). The proportion of the iPOM fraction on whole-soil OC increased for all treatments compared to the control object for both experiments. There were not any further large differences between manure and fertilizer treatments, except for the M4 (2× farmyard manure) treatment, which held a larger proportion of the whole-soil SOC. From these results it can be concluded that several decades of manure application did result in a larger proportion of physically protected SOC. Further increases of the OM input had only limited additional effects compared to the M2 and K2 treatments in which a single dose of FYM manure was given per rotation. This increase in the OC proportion of the micro-aggregate + fine sand (53–250 μm) soil fraction occurred while there were no appreciable differences between treatments in the DM proportion of this fraction. These results are in contrast with results reported for example by Elliott (1986) who stated that the distribution of OC in aggregate fractions is primarily controlled by the amount of soil present in that fraction. Del Galdo et al. (2003), however, obtained similar results to ours as they reported that the amount of C present in the 53–250 μm soil fraction was 38% lower in a cultivated grassland soil compared to a native grassland, while the DM proportion of this soil fraction did not differ between both. Furthermore, Six et al. (2002a) concluded that C sequestration after afforestation occurred through a C stabilization within microaggregates while this increase did not appear to be pro-

moted by a stimulation of microaggregate or macroaggregate formation.

Angers and N'Dayegamiye (1991) reported increases in the silt + clay associated OC after 10 years of solid cattle manure application. In a study by Gerzabeck et al. (2001), silt (2–63 μm) and clay (0.1–2 μm) size OC fractions exhibited clear responses to 42 years of different OM treatments. Christensen (1988) also measured increases in silt and clay sized fractions in soil after 61 years of fertilizer and manure application. In the present study, the increases in both the %OC and the amount of OC present in the <53 μm fraction with increasing manure application resulted in additional OC in the mineral associated OC pool for both the Martonvásár and Keszthely sites. However, these increases were, relatively speaking, much lower than the increases in the whole-soil OC, which supports the hypothesis of preferential storage of OC in the POM fractions at the time scale of decades. Evidently, this then resulted in a lower proportion of the silt and clay associated OC, on the total OC. This implies that in arable soils which receive high OM inputs, relatively less OM is stabilized either by association with the silt + clay mineral fraction or by the inherent biochemical recalcitrance of the OM. The amount of C in the non-hydrolyzable OM of the <53 μm soil fraction followed a similar increasing trend with management. This result is opposed to the reasoning that the non-hydrolyzable silt + clay associated OM is a very stable OM pool which is hypothesized to be unaffected by management except at very long time-scales. Although several authors have separated such an OM fraction using hydrolysis techniques (for a review see Six et al., 2002b), we argue that the hydrolysis technique is not adequate to yield such a distinct OM pool which contains very old stabilized OM. Balesdent (1996) did not find any great differences in dynamics between the non-hydrolyzable and hydrolyzable OC fraction and therefore also questioned the relationship between biodegradability and hydrolyzability. More powerful analytical tools, such as analytical pyrolysis combined with mass spectrometry or ^{13}C NMR spectroscopy for example, may be needed to characterize the silt + clay associated OM fraction.

Considering the conceptual model of Six et al. (2002b) which defines an “unprotected” SOC pool to be constituted by the C in the coarse fPOM and the fine fPOM, there was clearly a large influence of management on the amount of “unprotected” OC. This model also defines a “physically” protected SOC pool as the C in the iPOM, which was affected by management to a lesser extent than the “unprotected pool” at the Martonvásár site and to an equal extent at the Keszthely site. Increases in the silt + clay size fraction associated OC, which is hypothesized to constitute a “(bio-) chemically protected pool”, with manure and fertilizer treatment was, relatively speaking, much smaller than for the other two pools. Table 6 gives the amount of OC present in these conceptual pools and the relative difference between the control treatment and the manure and fertilizer treatments. The difference in the responsiveness of these conceptual pools resulted in a shift in the proportion of the whole-soil OC towards relatively more labile OC with increasing OM input.

The measured shifts in the distribution of SOM over distinct fractions are likely to be reflected in changes in the general chemical composition of the SOM. Several studies (Golchin et

Table 6

Total amount of OC present ($\text{g C } 100 \text{ g}^{-1} \text{ DS}$) in the conceptual pools of the model of Six et al. (2002a,b) and the relative differences (%) with the control treatment (in brackets)

Treatment	Coarse fPOM + fine fPOM (unprotected)	iPOM (physically protected)	OC in <53 μm soil fraction ((bio-) chemically protected)
Martonvásár			
M1	0.083	0.090	1.384
M2	0.103 (23.8)	0.101 (12.3)	1.360 (–1.7)
M3	0.252 (202.8)	0.151 (67.4)	1.550 (12.0)
M4	0.277 (232.0)	0.163 (80.7)	1.565 (13.1)
Keszthely			
K1	0.087	0.064	0.719
K2	0.134 (53.0)	0.124 (92.9)	0.733 (1.9)
K3	0.104 (19.4)	0.118 (83.9)	0.761 (5.8)
K4	0.158 (80.1)	0.128 (99.6)	0.935 (30.1)

al., 1994; Köbl and Kögel-Knabner, 2004) found the occluded POM to contain more alkyl C and less *O*-alkyl C than the free POM or LF. These data suggest that during the transformation of free to occluded POM, there is a selective decomposition of easily decomposable carbohydrates and a preservation of recalcitrant long-chained C. If so, the relative larger increase in fPOM compared to iPOM with increasing OM application over the M1 to M4 treatments is expected to be accompanied by an enrichment of *O*-alkyl C.

Using pyrolysis field ionization mass spectroscopy, Schulten and Leinweber (1996) found soils receiving farmyard manure applications to be enriched in lipids and lignin-dimers compared to unfertilized soil. In line with these results, the increased supply of lignin-dimers in the straw in the farmyard manure may particularly have yielded an enrichment of lignin in the M4 and K4 treatments and to a lesser extent in the other FYM treatments. On the other hand, Schulten and Leinweber (1996) found unfertilized soil to be richer in N-containing compounds, particularly so in the clay sized fraction, and they attributed this to the reduced import of fresh OM having high C:N-ratio's as opposed to a continuous input of N-containing metabolites. The reduced import of plant OM (Table 2) in the control treatments (M1 and K1) on top of the larger proportion of the silt + clay sized OM fraction may well have caused an enrichment of N-containing compounds in M1 and K1.

5. Conclusions

Manure and fertilizer application can increase the amount of OC present in free POM, occluded POM and mineral associated OM at the time scale of several decades. Differences in the relative increases of the OC present in different soil fractions lead to a shift in the relative distribution of the bulk soil OC in these fractions. Based on fractionation results of two long-term arable field experiments, it can be concluded from this study that at the time scale of decades manure and fertilizer application result in relatively more labile OC present in the soil compared to an unfertilized control treatment. A consequence of the measurability of these changes at this time scale is that it may be possible to

use such experimental data for the development and calibration of SOC models with conceptual OC pools which correspond to existing physical fractions. The theoretical model suggested by Six et al. (2002b), used in this study, may qualify as such a model. However, chemical fractionation of the silt + clay associated OC by a simple hydrolysis technique seems to be unable to separate an inert SOC pool, which is virtually unaffected by management, and alternative techniques will be needed here.

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