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# Soil organic matter distribution and microaggregate characteristics as affected by agricultural management and earthworm activity

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## Summary

Stable microaggregates can physically protect occluded soil organic matter (SOM) against decomposition. We studied the effects of agricultural management on the amount and characteristics of microaggregates and on SOM distribution in a marine loam soil in the Netherlands. Three long-term farming systems were compared: a permanent pasture, a conventional-arable system and an organic-arable system. Whole soil samples were separated into microaggregates (53–250  $\mu\text{m}$ ), 20–53  $\mu\text{m}$  and < 20  $\mu\text{m}$  organo-mineral fractions, sand and particulate organic matter, after complete disruption of macroaggregates. Equal amounts of microaggregates were isolated, irrespective of management. However, microaggregates from the pasture contained a larger fraction of total soil organic C and were more stable than microaggregates from the two arable fields, suggesting greater SOM stabilization in microaggregates under pasture. Moreover, differences in the relative contribution of coarse silt (> 20  $\mu\text{m}$ ) versus fine mineral particles in the microaggregates of the different management systems demonstrate that different types of microaggregates were isolated. These results, in combination with micromorphological study of thin sections, indicate that the great earthworm activity under permanent pasture is an important factor explaining the presence of very stable microaggregates that are relatively enriched in organic C and fine mineral particles. Despite a distinctly greater total SOM content and earthworm activity in the organic- versus the conventional-arable system, differences in microaggregate characteristics between both arable systems were small. The formation of stable and strongly organic C-enriched microaggregates seems much less effective under arable conditions than under pasture. This might be related to differences in earthworm species' composition, SOM characteristics and/or mechanical disturbance between pasture and arable land.

## Introduction

Conventional agriculture has caused large losses of soil organic matter (SOM) from cultivated land worldwide. This has detrimental effects on soil structure and soil fertility and has contributed to global warming by further increasing the atmospheric concentration of CO<sub>2</sub> (Lal & Kimble, 1997). Within certain climatic conditions and a given soil type, the factors controlling SOM concentrations are determined largely by land use and management. The SOM content of agricultural soils can therefore be increased through the adoption of alternative management systems that increase the amount of

organic inputs and/or slow down SOM turnover. To define such management systems a better understanding is needed of the effects of management on SOM dynamics and the interactions with soil structure.

Tillage activities and the amount and quality of manure and organic residues returned to the soil affect the activity of soil organisms, soil structural characteristics and the chemical and physical stabilization of SOM (Hendrix *et al.*, 1986; Oades, 1988). Organic matter and mineral particles bind together to form aggregates of different size and stability depending on the SOM content and the type of binding agent (Tisdall & Oades, 1982). According to the conceptual model of aggregate hierarchy of Tisdall & Oades (1982) microaggregates (< 250  $\mu\text{m}$ ) and organic residues are bound into stable macroaggregates

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(> 250  $\mu\text{m}$ ) by a network of roots and hyphae. Such stable macroaggregates are known to slow down the rate of decomposition of the occluded SOM (Elliott, 1986; Gupta & Germida, 1988; Six *et al.*, 1998). However, the roots and hyphae that bind microaggregates into macroaggregates are 'temporary binding agents' that do not persist unless they are replaced continuously (Tisdall & Oades, 1982; Oades, 1984). Many studies have shown that cultivation leads to a decline in water-stable macroaggregates, resulting in a rapid loss of SOM that binds microaggregates into macroaggregates (Tisdall & Oades, 1982; Elliott, 1986). By comparison, native grasslands or no-tillage systems lead to increased macroaggregate stability, and greater sizes and mean residence times of mineralizable SOM pools (Gupta & Germida, 1988; Six *et al.*, 1998).

Oades (1984) suggested that macroaggregates do not just bind existing microaggregates together, but that new microaggregates are formed preferentially within the stable macroaggregates. Mucilage produced during decomposition of organic fragments inside the macroaggregate interacts with clay, which begins to encrust the organic fragment, eventually to an extent where the degradation of the organic material is retarded (Oades, 1984). Over time, the binding agents in macroaggregates degrade, resulting in a loss of macroaggregate stability and the release of stable microaggregates, which become the building blocks of the next cycle of macroaggregate formation (Six *et al.*, 2000). Microaggregates exhibit greater stability than macroaggregates and better protect SOM against microbial decay (Tisdall & Oades, 1982; Skjemstad *et al.*, 1990). Six *et al.* (2000) and Denef *et al.* (2001) reported that the formation of these microaggregates within macroaggregates is negatively related to the rate of macroaggregate turnover and therefore strongly affected by management factors such as tillage and residue management. Therefore, the degree of stable microaggregation, rather than stable macroaggregation, might play a more important and direct role in the relation between SOM sequestration and management of agricultural soils.

In addition, earthworms can play an important role in the incorporation of organic residues into the soil matrix (Parnell *et al.*, 1990) and the formation of relatively stable macroaggregates that tend to be enriched in SOM compared with uningested soil (Scullion & Malik, 2000). The ingestion and digestion of soil and litter by earthworms is also known to induce the formation of new microaggregates. Shipitalo & Protz (1989) and Barois *et al.* (1993) reported how such microaggregates are formed: organic particles are fragmented and pre-existing aggregates are dispersed in the earthworm gut; dispersed clay is then brought into intimate association with mucilage-coated, decomposing organic fragments and rearranged into newly formed microaggregates that are excreted in casts. Earthworm activity depends strongly on management practices such as tillage, manure inputs and pesticide use (Mackay & Kladvik, 1985; Berry & Karlen, 1993; Whalen

*et al.*, 1998). Therefore, differences in earthworm activity are expected to further enhance management effects on microaggregate formation and associated SOM stabilization.

We aimed to study the effects of agricultural management on microaggregation and SOM distribution in a marine loam soil in the Netherlands. Three long-term farming systems were compared: a permanent pasture, a conventional-arable farming system and an organic-arable system. For the soil series studied, an increase in total SOM content, water-stable macroaggregation and earthworm activity in the order conventional-arable land < organic-arable land < permanent pasture has been found in earlier studies (Jongmans *et al.*, 2001; Pulleman *et al.*, 2003). We therefore hypothesized that the soil under permanent pasture contains more microaggregates than under conventional-arable farming, whereas organic-arable farming holds an intermediate position. Moreover, it was expected that these microaggregates protect occluded SOM against decomposition.

To test these hypotheses, we isolated microaggregates (53–250  $\mu\text{m}$ ) from soil samples of the three long-term farming systems located in a single soil series. The organic C distribution across particulate organic matter (POM), microaggregates and smaller organo-mineral fractions were studied, as well as microaggregate characteristics.

## Materials and methods

### Site conditions

We selected one soil series developed in marine loam deposits in the southwestern part of the Netherlands, and three fields with different long-term (> 70 years) management histories: a permanent pasture (PP), a conventional-arable field (CA) and an organic-arable field (OA). PP is a conventional grassland that is grazed by cows and supplied with mineral fertilizers and animal manure. This grassland system represents a relatively undisturbed system in terms of soil tillage and organic inputs. CA represents the most common farming system in the region. Soil fertility is maintained with mineral fertilizers (on average 130 kg N and 64 kg P ha<sup>-1</sup> year<sup>-1</sup>) and the use of animal manure is negligible. The soil is ploughed in the autumn to a depth of 25–30 cm. The rotation consists of arable crops (3 years), mainly cereals, potatoes and sugar beet, in rotation with grass (2–3 years). The OA system has similar tillage activities and crop rotation, but animal manure is applied instead of synthetic fertilizers and no biocides are used. At the time of sampling both arable fields had been under grass for 2 years. The soil series is classified as a Calcaric Fluvisol (FAO–UNESCO, 1997) and has developed in polders that were reclaimed from the sea in the 14th–16th century. Soil particle-size distribution, carbonate contents, pH and organic C contents for the three fields are given in Table 1.

In a previous study of the same management systems, the volume % of worm-worked soil in thin sections of undisturbed

**Table 1** Soil particle-size distribution ( $n=4$ ), total soil organic C contents ( $n=4$ ) and carbonate content and pH (composite samples) of the permanent pasture (PP), organic-arable field (OA) and conventional-arable field (CA). Standard errors are given in parentheses. Values that are significantly different between the management systems or depths are marked with different letters or an asterisk, respectively ( $P < 0.05$ )

Depth /cm	Field	Particle-size distribution					Organic C	Carbonates	pH(H <sub>2</sub> O)
		Coarse sand 250–2000 $\mu\text{m}$	Fine sand 53–250 $\mu\text{m}$	Coarse silt 20–53 $\mu\text{m}$	Fine silt 2–20 $\mu\text{m}$	Clay < 2 $\mu\text{m}$			
0–10	PP	15 (2) <sup>a</sup>	349 (14) <sup>a</sup>	269 (7) <sup>a</sup>	148 (3) <sup>a</sup>	219 (9) <sup>b</sup>	38.9 (0.5) <sup>a*</sup>	60	7.8
	OA	16 (1) <sup>a</sup>	281 (7) <sup>a</sup>	284 (5) <sup>a</sup>	153 (3) <sup>a</sup>	267 (3) <sup>a</sup>	14.5 (0.4) <sup>b</sup>	91	8.2
	CA	2 (0) <sup>b</sup>	375 (14) <sup>a</sup>	272 (5) <sup>a</sup>	130 (6) <sup>b</sup>	220 (9) <sup>b</sup>	9.4 (0.5) <sup>c</sup>	95	8.4
10–20	PP	11 (1) <sup>b</sup>	339 (11) <sup>a</sup>	277 (8) <sup>a</sup>	144 (5) <sup>ab</sup>	228 (10) <sup>b</sup>	15.0 (0.5) <sup>a</sup>	83	8.1
	OA	16 (1) <sup>a</sup>	280 (6) <sup>b</sup>	280 (4) <sup>a</sup>	157 (3) <sup>a</sup>	267 (3) <sup>a</sup>	13.5 (0.6) <sup>a</sup>	96	8.3
	CA	2 (0) <sup>c</sup>	378 (13) <sup>a</sup>	269 (3) <sup>a</sup>	131 (6) <sup>b</sup>	221 (9) <sup>b</sup>	8.5 (0.4) <sup>b</sup>	95	8.5

soil (0–20 cm depth) was found to increase from 8% under CA to 28% under OA and 52% under PP (Pulleman *et al.*, 2003). Regarding the species' composition of the earthworm populations, in all three management systems epigeic and endogeic species (*Lumbricus rubellus*, *Aporrectodea caliginosa* and *A. rosea*) were present. However, anecic (*L. terrestris* and *A. longa*) were present only in the soil under permanent pasture (J. Marinissen, personal communication).

#### Sampling and analyses

We collected soil material from each field in April 2000 by randomly taking four replicate undisturbed samples (25 cm  $\times$  20 cm  $\times$  10 cm) from 0–10 and 10–20 cm depth. The samples were transferred to the laboratory, living grass roots removed and the soil gently broken along natural planes of weakness into natural aggregates (diameter < 25 mm). The aggregates were then air-dried at room temperature for 7 days and thoroughly mixed. Representative subsamples were crushed with a pestle and mortar to pass a 2-mm sieve. Organic particles > 2 mm were discarded.

Total carbonate contents and pH(H<sub>2</sub>O) were measured in composite soil samples of four replicates according to Buurman *et al.* (1996). For each replicate, soil particle-size analyses were performed by the pipette method (Buurman *et al.*, 1996). Finely ground subsamples were analysed for total C and N content with a LECO CHN-1000 analyzer (Leco Corp., St Joseph, MI). Inorganic C was determined with a pressure transducer from the difference in voltage due to released CO<sub>2</sub> after reaction of 1 g of ground soil material with 2 ml of 6 M HCl + 3% FeCl<sub>2</sub> in a sealed vial with a volume of 20 ml (Sherrod *et al.*, 2002). The organic C (OC) content was calculated from the difference between total and inorganic C concentrations.

#### Microaggregate separations

Microaggregates (53–250  $\mu\text{m}$ ) were isolated from whole soil samples with a device developed by Six *et al.* (2000), which

completely breaks up macroaggregates with minimal disruption of microaggregates. A 90-g soil sample was divided into six subsamples of 15 g, each of which was slaked in deionized water for 10 minutes to disrupt large macroaggregates and clods. The samples were then transferred to a 250- $\mu\text{m}$  mesh screen on top of the device and shaken with 50 glass beads (diameter 4 mm) until all macroaggregates had been broken up. The microaggregates released were immediately flushed through the 250- $\mu\text{m}$  sieve, and deposited onto a 53- $\mu\text{m}$  sieve by a continuous flow of water through the device. Sand and coarse POM retained on the 250- $\mu\text{m}$  mesh screen were washed off. The sieve holding the material 53–250  $\mu\text{m}$  was moved 50 times up and down in the suspension containing the < 53  $\mu\text{m}$  fraction. Then the remaining suspension holding the < 53  $\mu\text{m}$  fraction was sieved similarly over a 20- $\mu\text{m}$  mesh. Particles < 20  $\mu\text{m}$  were discarded. All fractions were oven-dried (60°C) and weighed. The amount of microaggregates in the 53–250  $\mu\text{m}$  size fraction was calculated by subtracting the amount of sand in the 53–250  $\mu\text{m}$  size fraction, as derived from the particle-size analysis. Representative subsamples of the separated fractions were ball-mill ground and OC and total N were determined as described above for total soil samples. The amount and OC and N content of the < 20  $\mu\text{m}$  fractions (including soluble fractions) were calculated by difference. Tests on our fractionation procedure have revealed that C released as dissolved organic carbon (DOC) during the physical fractionation is not more than 1–2% of the total C (J. Six, unpublished data). Therefore DOC losses during fractionation were considered negligible.

#### Free fine POM and intra-microaggregate POM and coarse silt

Free fine POM (53–250  $\mu\text{m}$ ) retained on the 53- $\mu\text{m}$  sieve together with fine sand and microaggregates was isolated by density flotation of 10 g of the material in sodium polytungstate solution ( $\rho = 1.85 \text{ g cm}^{-3}$ ) and subsequent centrifugation

(1250 g) at 20°C for 60 minutes (Six *et al.*, 1998). The floating organic particles were aspirated, collected on a 20- $\mu\text{m}$  nylon filter and transferred to a Whatman GF/A glass microfibre filter paper (1.6  $\mu\text{m}$ , diameter 47 mm) of known weight. The filter paper holding the free fine POM was dried at 60°C, ground and OC and total N were measured as described above. The amount of free fine POM and OC and N contents were calculated after correction for the mass of the filter paper.

The remaining material was dispersed by shaking in 15 ml of  $5\text{ g l}^{-1}$  Na-hexametaphosphate solution overnight (18 hours). Intra-microaggregate POM > 53  $\mu\text{m}$  and fine sand were collected by sieving the suspension over a 53- $\mu\text{m}$  sieve. The material that passed this sieve was then sieved over a 20- $\mu\text{m}$  sieve to collect intra-microaggregate POM 20–53  $\mu\text{m}$  with the coarse silt. Both size fractions were oven-dried at 60°C, weighed and ground, and analysed for OC and total N as described above.

We double-checked the intra-microaggregate POM + sand or coarse silt fractions under a microscope to see if all organo-mineral material had been broken up. This was the case for all fractions except for those of the 0–10 cm depth layer of PP. Microaggregates from the upper 10 cm of PP were only partly dispersed in Na-hexametaphosphate solution, even after the concentration was increased to  $50\text{ g l}^{-1}$ . Therefore, we tested two additional dispersion techniques: (i) application of ultrasonic energy for 30 minutes (corresponding to an applied energy of  $310\text{ J ml}^{-1}$  of suspension), which was found to be the minimum energy required to break up all the microaggregates from the 0–10 cm layer of PP, and (ii) dispersion in 75 ml of a 1:1 mixture of 0.1 M NaOH and 0.1 M Na-pyrophosphate solutions (NaOH–Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>). After the headspace had been rinsed with N<sub>2</sub>, the bottles containing the soil and NaOH + Na-pyrophosphate suspension were closed and shaken overnight for 18 hours. Occluded POM fractions and coarse silt were separated and quantified as described above for samples treated with Na-hexametaphosphate solution.

### Microscopy

To enable microscopic analysis of microaggregates, subsamples of the 53–250  $\mu\text{m}$  size fractions were transferred to a small cardboard box that was divided into 2 cm × 2 cm × 2 cm compartments. Thin sections were prepared according to the method of Fitzpatrick (1970) and studied under plain light and cross-polarized light with a petrographic microscope. For scanning electron microscope (SEM) analysis, intact, oven-dried microaggregates were mounted on aluminium stubs and sputter-coated with Au before examination using secondary- and backscatter-electron detection. SEM analysis of the internal architecture of microaggregates and the occluded SOM was performed on small thin sections that were polished with 60 nm polishing cloth, and coated with C. The thin sections were examined using backscatter detection so that features composed of elements with a small average

atomic number, such as organic matter, are recognized by a dark-grey colour. Mineral particles have a large average atomic number and are recognized as solid white to light-grey grains.

### Microaggregate stability and coarse silt contents

The stability of the microaggregates against water dispersion was determined by adding 150 ml demineralized water to 15 g of the total 53–250  $\mu\text{m}$  size fraction in a 250-ml polyethylene bottle. The suspension was shaken overnight (18 hours) on an end-over-end shaker and subsequently sieved over a 53- $\mu\text{m}$  sieve. The material retained on the sieve was dried at 60°C and weighed. Ten grams of this material was dispersed with a 1:1 mixture of 0.1 M NaOH and 0.1 M Na-pyrophosphate (NaOH–Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>) solutions to determine fine sand and coarse silt contents as described above for total microaggregate fractions. The amount of stable microaggregates was calculated after correction for the amount of sand in the 53–250  $\mu\text{m}$  size fraction.

### Calculations

**Sand corrections.** The OC contents of total soil samples (t) and the different fractions (f), expressed on a sand-free basis, were calculated as follows:

$$\text{OC}_{(t)} (\text{mg g}^{-1} \text{ sand-free soil}) = \frac{\text{OC}_{(t)} (\text{g kg}^{-1} \text{ soil}) \times 1000}{1000 - \text{Sand}_{(t)} (\text{g kg}^{-1} \text{ soil})}; \quad (1)$$

$$\begin{aligned} &\text{OC}_{(f)} (\text{mg g}^{-1} \text{ sand-free size fraction}) \\ &= \frac{\text{OC}_{(f)} (\text{mg g}^{-1} \text{ size fraction}) \times \text{Fraction}_{(f)} (\text{g kg}^{-1} \text{ soil})}{\text{Fraction}_{(f)} (\text{g kg}^{-1} \text{ soil}) - \text{Sand}_{(f)} (\text{g kg}^{-1} \text{ soil})}. \end{aligned} \quad (2)$$

**Relative OC distribution.** For the different management systems and depths, the contribution of each fraction (f) to total soil OC was calculated as:

$$\begin{aligned} &\text{OC}_{(f)} (\text{mg g}^{-1} \text{ total soil OC}) \\ &= \frac{\text{OC}_{(f)} (\text{mg g}^{-1} \text{ fraction}) \times \text{Fraction}_{(f)} (\text{g kg}^{-1} \text{ soil})}{\text{Total soil OC} (\text{g kg}^{-1} \text{ soil})}. \end{aligned} \quad (3)$$

**OC enrichment factor.** As a measure of the relative accumulation of OC in each fraction, the OC enrichment relative to CA was calculated for both depth layers of the three management systems (s). The amount of OC in each fraction (f) relative to the average amount of OC in the corresponding fraction of CA was divided by the amount of total soil OC relative to the average amount of total soil OC in CA:

$$\text{OC enrichment factor}_{(s,f)} = \frac{\text{OC}_{(s,f)} (\text{mg g}^{-1} \text{ sand-free size fraction}) / \langle \text{OC}_{(CA,f)} (\text{mg g}^{-1} \text{ sand-free size fraction}) \rangle}{\text{Total soil OC}(s) (\text{g kg}^{-1} \text{ sand-free soil}) / \langle \text{Total soil OC}(CA) (\text{g kg}^{-1} \text{ sand-free soil}) \rangle} \quad (4)$$

where  $\langle \dots \rangle$  indicates the average of the four replicate samples.

### Statistics

To test whether average values of four replicates were significantly different between the management systems, depths or treatments, the data were analysed using the SPSS statistical package for analysis of variance (ANOVA; SPSS, 1997). We used one-way analysis of variance with management being the main factor in the model and depth and treatment as secondary factors. Separation of mean values was tested using a post hoc multiple comparisons' test (Tukey's honestly significant difference; SPSS, 1997) assuming equal variances and with a significance level of  $P < 0.05$ .

## Results

### Microaggregate separations

The mass distribution across the various size fractions after microaggregate separation showed only very small differences between the management systems and depth layers (Table 2). Microaggregates contributed as much as the fine sand to the total soil mass in all three management systems (351–382 g kg<sup>-1</sup>). No significant variations in the amount of microaggregates occurred with management or depth. The size fractions 20–53  $\mu\text{m}$  and  $< 20 \mu\text{m}$  amounted to 162–241 and 73–102 g kg<sup>-1</sup> soil, respectively.

### OC contents of the fractions

In all management systems and both depth layers, except for the 0–10 cm layer of PP, the OC content of the fractions decreased in the order: free fine POM 53–250  $\mu\text{m}$  > fraction  $< 20 \mu\text{m}$  > microaggregates 53–250  $\mu\text{m}$  > fraction 20–53  $\mu\text{m}$  (Table 3). In the upper layer of PP, however, more OC was present in the microaggregates than in the fraction  $< 20 \mu\text{m}$ . The OC concentration in free POM  $> 250 \mu\text{m}$  was not calculated, because its contribution to the total mass of the  $> 250 \mu\text{m}$  size fraction was too small for accurate determination. Considerably less OC was present in microaggregates from the 10–20 cm layer of PP and both layers of the arable soils, especially of CA. Also, at 10–20 cm depth, the OC content of the microaggregates was greater in PP than in CA, whereas microaggregates of OA had an intermediate OC content.

### Relative OC distribution

In all three management systems, when expressed on a total soil basis, microaggregates accounted for the main part of total soil OC (Figure 1a,b). In the upper 10 cm of PP, however, a significantly greater fraction of total soil OC was present in microaggregates than in OA and CA (716 versus 505–532 mg g<sup>-1</sup>, respectively) and a smaller fraction of total soil OC was represented by the size fraction  $< 20 \mu\text{m}$  (98 mg g<sup>-1</sup> in PP versus 262 mg g<sup>-1</sup> in OA and CA). At 10–20 cm depth, no significant differences were found between the management types in the contribution of microaggregates (530–624 mg g<sup>-1</sup>), or other organo-mineral fractions, to total soil OC. The contribution of coarse POM to total soil OC was significantly greater in OA (42–46 mg g<sup>-1</sup>) than in the other two systems at both depths. The relative amount of OC present in coarse POM decreased with depth in PP (29 to 10 mg g<sup>-1</sup>) and CA (32 to 24 mg g<sup>-1</sup>). The 10–20 cm layer of PP contained relatively less OC in

**Table 2** Relative mass distribution of size fractions after microaggregate separation for the permanent pasture (PP), organic-arable field (OA) and conventional-arable field (CA). Standard errors are given in parentheses. Values that are significantly different between the management systems or depths are marked with different letters or an asterisk, respectively ( $P < 0.05$ )

Depth /cm	Field	Size fraction				Microaggregates‡ 53–250 $\mu\text{m}$
		$> 250 \mu\text{m}$	53–250 $\mu\text{m}$	20–53 $\mu\text{m}$	$< 20 \mu\text{m}$	
0–10	PP	15 (1) <sup>a*</sup>	714 (14) <sup>a</sup>	193 (5) <sup>b*</sup>	78 (11) <sup>a</sup>	365 (21) <sup>a</sup>
	OA	16 (0) <sup>a</sup>	655 (10) <sup>b</sup>	239 (10) <sup>a</sup>	90 (3) <sup>a*</sup>	373 (12) <sup>a</sup>
	CA	6 (0) <sup>b</sup>	758 (18) <sup>a</sup>	162 (14) <sup>b</sup>	74 (4) <sup>a</sup>	382 (11) <sup>a</sup>
10–20	PP	10 (1) <sup>b</sup>	690 (12) <sup>b</sup>	227 (7) <sup>a</sup>	73 (4) <sup>b</sup>	351 (3.6) <sup>a</sup>
	OA	17 (1) <sup>a</sup>	640 (11) <sup>c</sup>	241 (7) <sup>a</sup>	102 (3) <sup>a</sup>	360 (12) <sup>a</sup>
	CA	5 (0) <sup>c</sup>	739 (15) <sup>a</sup>	177 (18) <sup>b</sup>	78 (2) <sup>b</sup>	361 (12) <sup>a</sup>

‡Total 53–250  $\mu\text{m}$  size fraction – fine sand 53–250  $\mu\text{m}$  (derived from particle-size analysis).

**Table 3** Organic C (OC) contents of the different fractions, in the permanent pasture (PP), organic-arable field (OA) and conventional-arable field (CA). Standard errors are given in parentheses. Values that are significantly different between the management systems or depths are marked with different letters or an asterisk, respectively ( $P < 0.05$ )

Depth /cm	Field	OC content			
		Free fine POM 53–250 $\mu\text{m}$	Microaggregates‡ 53–250 $\mu\text{m}$	Coarse silt 20–53 $\mu\text{m}$	Fine silt and clay < 20 $\mu\text{m}$
		/mg g <sup>-1</sup> fraction			
0–10	PP	231.2 (5.2) <sup>b</sup>	76.7 (5.3) <sup>a*</sup>	25.3 (1.6) <sup>a*</sup>	53.1 (21.1) <sup>a</sup>
	OA	320.6 (14.4) <sup>a</sup>	19.6 (0.4) <sup>b*</sup>	10.4 (0.5) <sup>b</sup>	42.7 (5.7) <sup>a</sup>
	CA	364.8 (21.8) <sup>a*</sup>	13.0 (0.3) <sup>a</sup>	9.5 (0.6) <sup>b*</sup>	34.0 (6.3) <sup>a</sup>
10–20	PP	344.9 (18.3) <sup>a</sup>	26.7 (0.9) <sup>b</sup>	10.5 (0.9) <sup>a</sup>	42.2 (12.8) <sup>a</sup>
	OA	290.7 (25.5) <sup>a</sup>	21.7 (1.8) <sup>c</sup>	11.3 (0.2) <sup>a</sup>	22.9 (5.9) <sup>a</sup>
	CA	273.7 (34.7) <sup>a</sup>	12.4 (0.1) <sup>c</sup>	6.8 (0.3) <sup>b</sup>	30.6 (10.7) <sup>a</sup>

‡Total 53–250  $\mu\text{m}$  size fraction – fine sand 53–250  $\mu\text{m}$  (derived from texture analysis).

free fine POM (10 mg g<sup>-1</sup>) than the upper 10 cm (24 mg g<sup>-1</sup>) and than both arable soils (16–21 mg g<sup>-1</sup>).

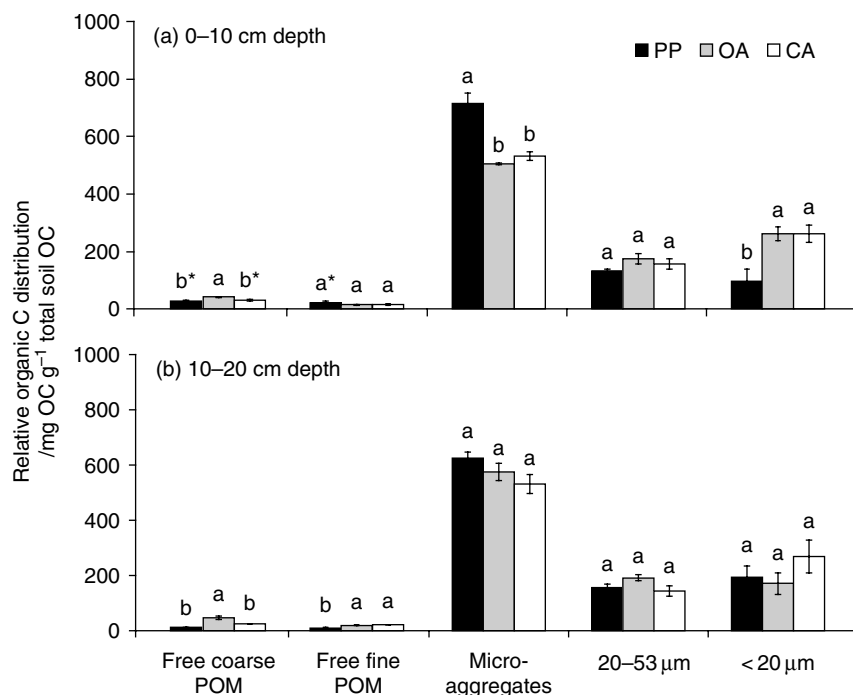
#### OC enrichment factor

Microaggregates in PP were significantly enriched in OC relative to CA by factors of 1.28 and 1.45 at 10–20 and 0–10 cm depth, respectively (Table 4). Microaggregates from OA were also somewhat enriched in OC, although not significantly. The 20–53  $\mu\text{m}$  and < 20  $\mu\text{m}$  fractions of PP and OA were relatively depleted in OC, except for the 20–53  $\mu\text{m}$  fraction of OA at 10–20 cm depth. However, the OC depletion was significant only for PP at 0–10 cm depth, with factors of 0.66 and 0.38, respect-

ively. In PP at 10–20 cm depth, the free fine POM was significantly depleted in OC relative to OA and CA, and relative to the upper layer of PP, as indicated by an enrichment factor of 0.50.

#### Intra-microaggregate POM-OC

The amount of intra-microaggregate POM-OC could not be determined for the microaggregates from the 0–10 cm layer of PP. These microaggregates were extremely stable and were only partly dispersed using 5 g l<sup>-1</sup>, or even 50 g l<sup>-1</sup>, Na-hexametaphosphate solution. Both alternative dispersion techniques (i.e. sonication for 30 minutes, and dispersion in NaOH + Na-pyrophosphate solution) could break up all the



**Figure 1** Relative organic C (OC) distribution across different size fractions (free coarse POM, 20–53  $\mu\text{m}$  and < 20  $\mu\text{m}$ ) and size-density fractions (free fine POM and microaggregates) of the permanent pasture (PP), organic-arable field (OA) and conventional-arable field (CA) at (a) 0–10 cm depth and (b) 10–20 cm depth. Bars indicate standard errors. Values that are significantly different between the management systems or with depth are marked with different letters or an asterisk, respectively ( $P < 0.05$ ).

**Table 4** Organic C (OC) enrichment factors of the different size fractions in the permanent pasture (PP) and organic-arable field (OA), relative to the conventional-arable field (CA). Standard errors are given in parentheses. Values that are significantly different between the management systems or depths are marked with different letters or an asterisk, respectively ( $P < 0.05$ )

Depth /cm	Field	OC enrichment factor			
		Free fine POM 53–250 $\mu\text{m}$	Microaggregates 53–250 $\mu\text{m}$	Coarse silt 20–53 $\mu\text{m}$	Fine silt and clay < 20 $\mu\text{m}$
0–10	PP	1.60 (0.33) <sup>a*</sup>	1.45 (0.10) <sup>a</sup>	0.66 (0.06) <sup>b</sup>	0.38 (0.15) <sup>b</sup>
	OA	1.13 (0.17) <sup>a</sup>	1.10 (0.04) <sup>b</sup>	0.80 (0.05) <sup>ab*</sup>	0.92 (0.10) <sup>a</sup>
	CA	1.00 (0.07) <sup>a</sup>	1.00 (0.03) <sup>b</sup>	1.01 (0.08) <sup>a</sup>	0.98 (0.14) <sup>a</sup>
10–20	PP	0.50 (0.03) <sup>b</sup>	1.28 (0.07) <sup>a</sup>	0.91 (0.10) <sup>b</sup>	0.81 (0.23) <sup>a</sup>
	OA	1.06 (0.09) <sup>a</sup>	1.25 (0.08) <sup>ab</sup>	1.18 (0.03) <sup>a</sup>	0.53 (0.13) <sup>a</sup>
	CA	1.00 (0.05) <sup>a</sup>	1.01 (0.04) <sup>b</sup>	1.00 (0.04) <sup>ab</sup>	0.98 (0.30) <sup>a</sup>

microaggregates, including those from the 0–10 cm layer of the pasture soil. Those treatments, however, reduced the amount of intra-microaggregate POM-OC recovered by 7–33% (sonication) and 48–75% (NaOH + Na-pyrophosphate) relative to the amount of POM-OC that was recovered after dispersion in Na-hexametaphosphate solution. Therefore, we have presented only the amounts of intra-microaggregate POM-OC as determined after dispersion in Na-hexametaphosphate solution for OA and CA and the 10–20 cm depth layer of PP. The total amount of intra-microaggregate POM-OC ranged from 379 to 411 mg g<sup>-1</sup> microaggregate OC, of which the main part was > 53  $\mu\text{m}$  (Table 5). No significant differences were found between management types and with depth. The amounts of intra-microaggregate POM-OC of the size fractions > 53  $\mu\text{m}$  and 20–53  $\mu\text{m}$  correspond to 3.8–6.9 and 0.9–2.0 mg g<sup>-1</sup> microaggregate, or 149–174 and 38–53 mg g<sup>-1</sup> total soil OC, respectively (not shown). The ratio between free fine POM and occluded fine POM was calculated as a measure of the degree of incorporation of POM-OC into

microaggregates. This ratio was considerably larger in the pasture soil (10–20 cm depth) than in both arable soils (Table 5).

#### C:N ratios

The C:N ratios of total SOM ranged from 8.5 to 9.2, without significant differences between management systems or depths (Table 6). The C:N ratios of the different fractions, however, did show significant variations between management types and depths. The C:N of coarse POM ranged from 14.6 to 17.2, except for coarse POM in the 0–10 cm layer of PP, which had a significantly smaller C:N of 11.9. In all cases, the C:N ratios of the fractions decreased in the order coarse POM > free fine POM > microaggregates  $\approx$  fraction 20–53  $\mu\text{m}$ . The C:N ratios of the size fraction < 20  $\mu\text{m}$  are not presented since the N contents in this fraction were too small to calculate an accurate ratio. The decrease in C:N ratio from coarse POM to the fraction 20–53  $\mu\text{m}$  was least pronounced in PP at 0–10 cm

**Table 5** Contribution of the intra-microaggregate POM fractions to total microaggregate OC, and the ratio between occluded POM-OC and free fine POM-OC in the permanent pasture (PP), organic-arable field (OA) and conventional-arable field (CA). Standard errors are given in parentheses. Values that are significantly different between the management systems or depths are marked with different letters or an asterisk, respectively ( $P < 0.05$ )

Depth /cm	Field	Intra-microaggregate POM-OC		Intra-microaggregate POM-OC:Free fine POM-OC
		> 53 $\mu\text{m}$ /mg g <sup>-1</sup> microaggregate OC	20–53 $\mu\text{m}$	
0–10	PP	ND <sup>‡</sup>	ND <sup>‡</sup>	ND <sup>‡</sup>
	OA	317 (15) <sup>a</sup>	82 (9) <sup>a</sup>	13.4 (1.2) <sup>a</sup>
	CA	304 (37) <sup>a</sup>	102 (10) <sup>a</sup>	13.9 (0.9) <sup>a*</sup>
10–20	PP	240 (15) <sup>a</sup>	75 (3) <sup>a</sup>	20.4 (0.3) <sup>a</sup>
	OA	318 (30) <sup>a</sup>	93 (8) <sup>a</sup>	12.8 (1.2) <sup>b</sup>
	CA	307 (52) <sup>a</sup>	72 (7) <sup>a</sup>	9.7 (0.8) <sup>b</sup>

<sup>‡</sup>Not determined because microaggregates of PP resisted dispersion.



**Table 6** C:N ratios of the different fractions in the permanent pasture (PP), organic-arable field (OA) and conventional-arable field (CA). Standard errors are given in parentheses. Values that are significantly different between the management systems or depths are marked with different letters or an asterisk, respectively ( $P < 0.05$ )

Depth /cm	Field	Total soil	C:N ratio			
			Free POM		Microaggregates	
			250–2000 $\mu\text{m}$	53–250 $\mu\text{m}$	53–250 $\mu\text{m}$	20–53 $\mu\text{m}$
0–10	PP	8.8 (0.1) <sup>a</sup>	11.9 (0.3) <sup>b</sup>	10.2 (0.1) <sup>a</sup>	8.2 (0.1) <sup>a</sup>	7.7 (0.2) <sup>a*</sup>
	OA	9.2 (0.3) <sup>a</sup>	17.1 (0.9) <sup>a</sup>	10.8 (0.8) <sup>a</sup>	6.3 (0.2) <sup>b</sup>	6.2 (0.4) <sup>a</sup>
	CA	8.8 (0.4) <sup>a</sup>	16.7 (1.1) <sup>a</sup>	9.3 (0.5) <sup>a</sup>	5.7 (0.2) <sup>b</sup>	6.6 (0.5) <sup>a*</sup>
10–20	PP	9.0 (0.4) <sup>a</sup>	14.6 (2.3) <sup>a</sup>	10.3 (0.2) <sup>a</sup>	7.3 (0.4) <sup>a</sup>	6.0 (0.2) <sup>b</sup>
	OA	8.6 (0.3) <sup>a</sup>	17.2 (1.7) <sup>a</sup>	11.3 (0.6) <sup>a</sup>	6.8 (0.2) <sup>a</sup>	6.9 (0.0) <sup>a</sup>
	CA	8.5 (0.1) <sup>a</sup>	15.9 (0.5) <sup>a</sup>	11.5 (0.6) <sup>a*</sup>	5.4 (0.2) <sup>b</sup>	4.5 (0.3) <sup>c</sup>

depth. Consequently, microaggregates in the top 10 cm of PP had a significantly greater C:N ratio than those in OA and CA. At 10–20 cm depth microaggregates of both PP and OA had a greater C:N ratio than microaggregates from CA.

The C:N ratios of intra-microaggregate POM  $> 53 \mu\text{m}$  ranged from 13.3 to 16.4 and increased in the direction PP (10–20 cm)  $<$  OA (0–10 cm)  $<$  OA (10–20 cm) (Table 7). For both depth layers of CA the C:N ratios of intra-microaggregate POM 53–250  $\mu\text{m}$  are not presented. Again, the N contents were too small to enable calculation of an analytically sound C:N ratio. Intra-microaggregate POM 20–53  $\mu\text{m}$  had a smaller C:N ratio than intra-microaggregate POM  $> 53 \mu\text{m}$ , with values between 7 and 10 and without significant differences between management systems or with depth.

**Table 7** C:N ratios of intra-microaggregate POM fractions in the permanent pasture (PP), organic-arable field (OA) and conventional-arable field (CA). Standard errors are given in parentheses. Values that are significantly different between the management systems or depths are marked with different letters or an asterisk, respectively ( $P < 0.05$ )

Depth /cm	Field	C:N ratio of intra-microaggregate POM	
		$> 53 \mu\text{m}$	20–53 $\mu\text{m}$
0–10	PP	ND‡	ND‡
	OA	14.3 (0.2) <sup>b</sup>	7.2 (0.4) <sup>a</sup>
	CA	ND§	7.7 (0.7) <sup>a</sup>
10–20	PP	13.3 (0.4) <sup>c</sup>	9.9 (0.7) <sup>a</sup>
	OA	16.4 (0.4) <sup>b*</sup>	9.2 (0.9) <sup>a</sup>
	CA	ND§	8.6 (1.0) <sup>a</sup>

‡Not determined because microaggregates of PP resisted dispersion.

§Not determined because the N content was too small to calculate an analytically sound C:N ratio.

### Microscopy

SEM images of intact microaggregates from PP (0–10 cm) at different magnifications are presented in Figure 2(a–c). Most microaggregates had an irregular surface, but spherical aggregates with a smooth surface, identified as faecal pellets, were also present (Figure 2a). The same general picture was obtained for microaggregates from the arable fields, although fewer faecal pellets were observed (pictures not shown).

Thin sections of the 53–250  $\mu\text{m}$  size fraction studied with a light microscope showed that microaggregates from the upper 10 cm of PP were more intensely stained with organic pigment than microaggregates in the arable soils, and to a lesser extent than microaggregates from the 10–20 cm layer of PP. Under all three management types we observed POM fragments inside microaggregates, but much more under pasture than under both arable systems (pictures not shown). That organic matter was most abundant in microaggregates from the top 10 cm of PP was also visible when comparing the SEM images of the thin sections (Figure 3a,c,f). Microaggregates of PP often consisted of one or several cores of organic matter enclosed by a layer of clay (Figure 3g,h). Such features were also observed in OA and CA, but much less frequently (Figure 3d,e). The organic matter inside the microaggregates was mostly difficult to identify, although some remains of either plant or faunal cell walls were observed at higher magnifications (Figure 3e,i,j). The thin sections further revealed that microaggregates from the arable fields contain more coarse skeleton grains ( $> 20 \mu\text{m}$ ) than microaggregates from PP, especially when compared with the upper 10 cm. The 53–250  $\mu\text{m}$  fraction of CA, and to a lesser extent of OA, contained fragments of crusts that indicate the occurrence of slaking during oven-drying of the fraction (Figure 3a).

### Microaggregate stability and coarse silt contents

At 0–10 cm depth, the fraction of total microaggregates that survived water dispersion decreased significantly in the order

PP > OA > CA (741, 363 and 450 g kg<sup>-1</sup>, respectively; Figure 4a). On a total soil basis, these values correspond to 274, 136 and 86 g kg<sup>-1</sup> (Figure 4b). Microaggregate stability decreased significantly with depth in PP to 450 g kg<sup>-1</sup> of total microaggregates, which was still greater than for OA (320 g kg<sup>-1</sup>) and CA (200 g kg<sup>-1</sup>), although the difference between PP and OA was not significant at this depth. The amounts of stable microaggregates at 10–20 cm depth correspond to 158, 116 and 73 g kg<sup>-1</sup> total soil for PP, OA and CA, respectively.

Measurements of coarse silt (20–53 µm) contents provided quantitative evidence of our micromorphological observation that more coarse skeleton grains are present in microaggregates under arable management than under PP. Although coarse silt contents of total soil were almost similar (269–284 g kg<sup>-1</sup>) for all management systems and depth layers (Table 1), coarse silt contents of total microaggregates increased in the order PP 0–10 cm < PP 10–20 cm ≈ OA < CA (Table 8). The same trend was found for coarse silt contents of the microaggregates that were stable against water dispersion. Moreover, in all management systems stable microaggregates contained less coarse silt than total microaggregates, but the difference between total and stable aggregates was significant only for both layers of OA.

## Discussion

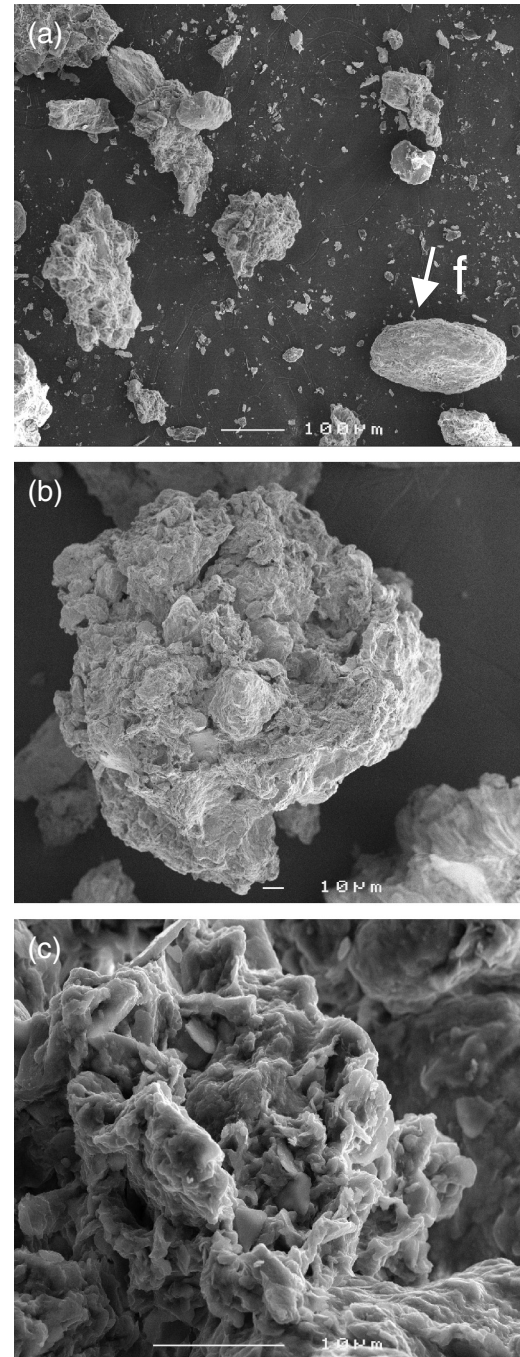
### Total amounts of microaggregates and organic C and N distribution

Similar total amounts of microaggregates were isolated from soil samples of the three management systems (on average 37% of total soil mass). Significant differences were found, however, in OC distribution across the different fractions, as well as in microaggregate morphology and stability.

The greater relative OC enrichment of microaggregates from the 0–10 cm layer of PP compared with the arable systems by up to 1.45 times, accompanied by relative OC depletion in the < 53 µm organo-mineral fractions, demonstrate that relatively more SOM has accumulated in microaggregates under pasture than in arable land. Moreover, these microaggregates had a greater C:N ratio than the microaggregates of the arable soils, despite a smaller C:N ratio of free coarse POM in PP, which suggests that the SOM occluded in microaggregates of PP is less decomposed. A similar pattern was found at 10–20 cm depth, although the differences were less pronounced than at 0–10 cm and significant only for PP versus CA. Especially at this depth, the observed differences are illustrative of the relatively strong accumulation of SOM in microaggregates of PP: ploughing of the soil under arable land causes a considerable input of organic residues below the rooting zone. This has resulted in a comparatively greater amount of OC present as free POM at 10–20 cm depth than in the same layer of PP. Nevertheless, also at this depth, microaggregates from PP were

relatively enriched in OC by a factor 1.28 compared with microaggregates from CA.

A major part of the OC in microaggregates consisted of very fine mineral-associated organic matter. Although



**Figure 2** SEM images (normal detection) of the 53–250 µm size fraction separated from 0–10 cm depth of the permanent pasture soil (PP), showing intact microaggregates at different magnifications. (a) Overview; f, faecal pellet. (b) Microaggregate at greater magnification. (c) Detail of (b).

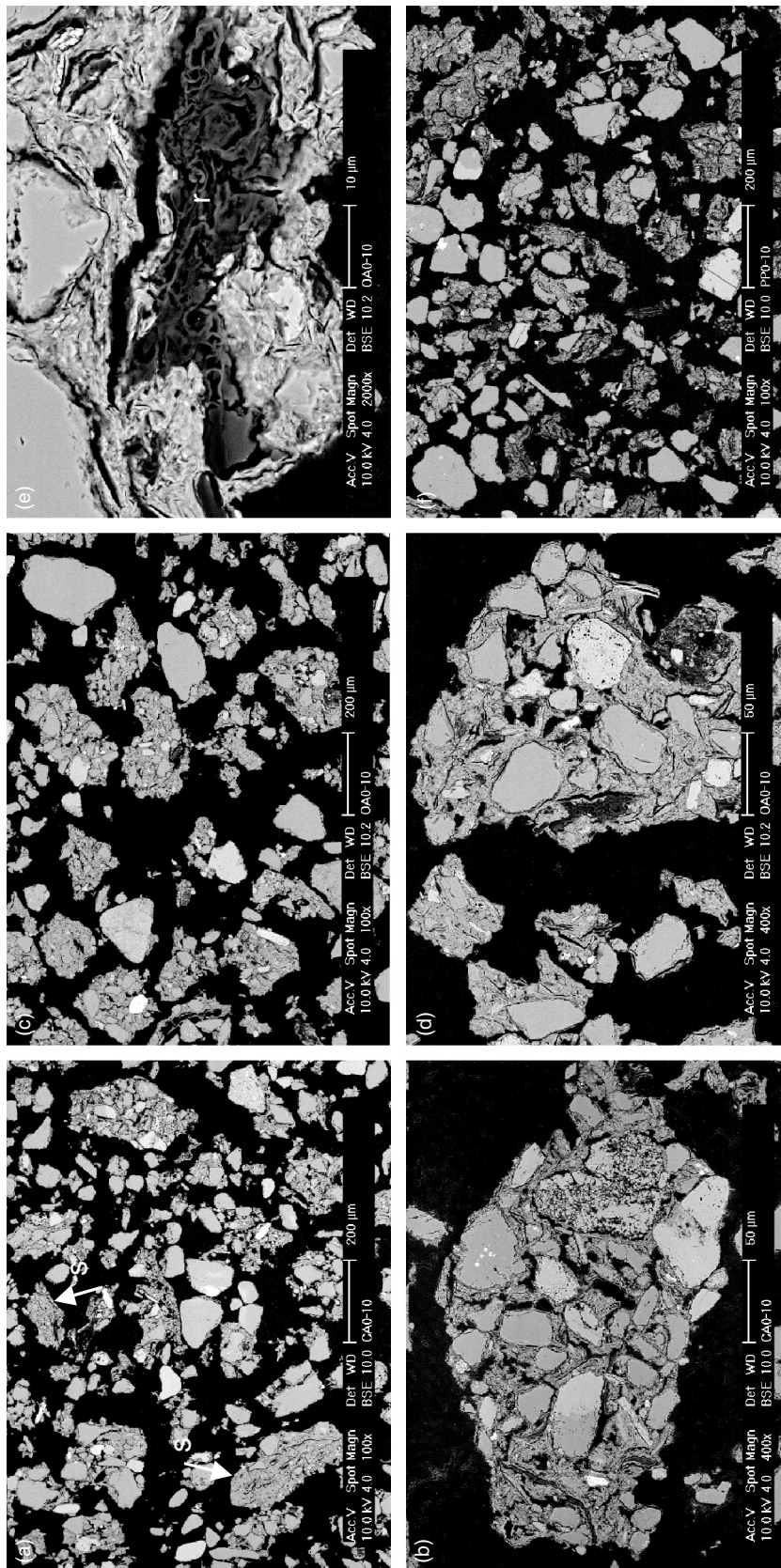
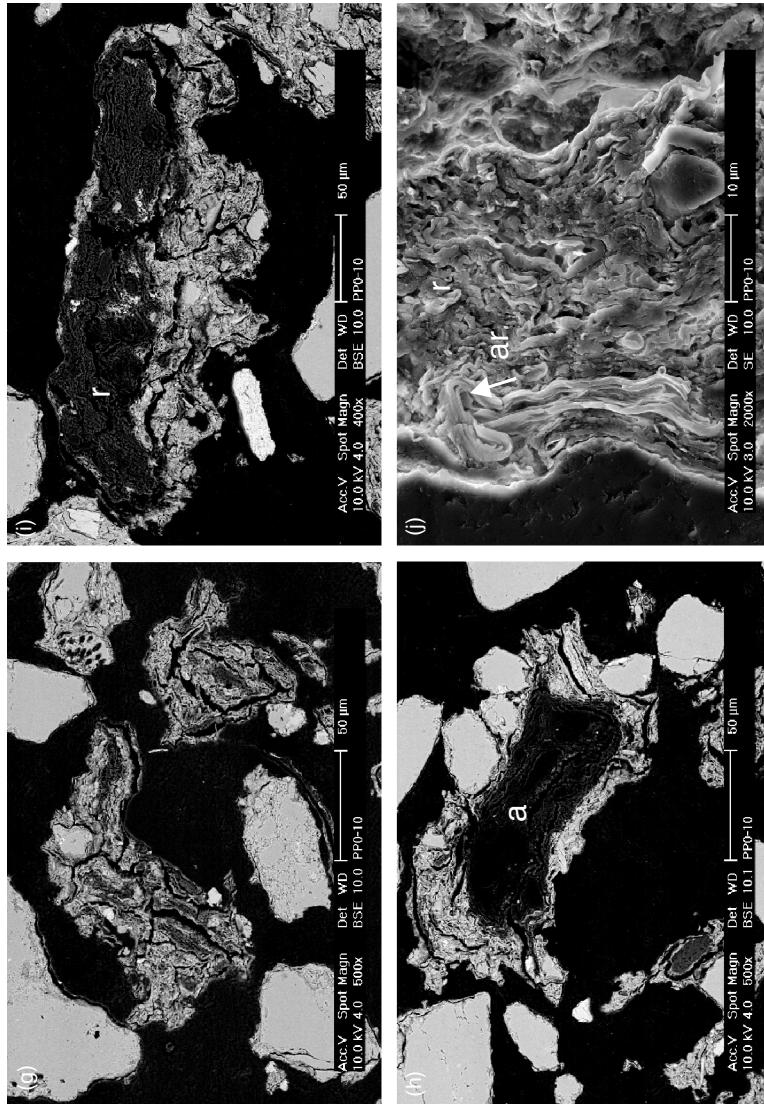
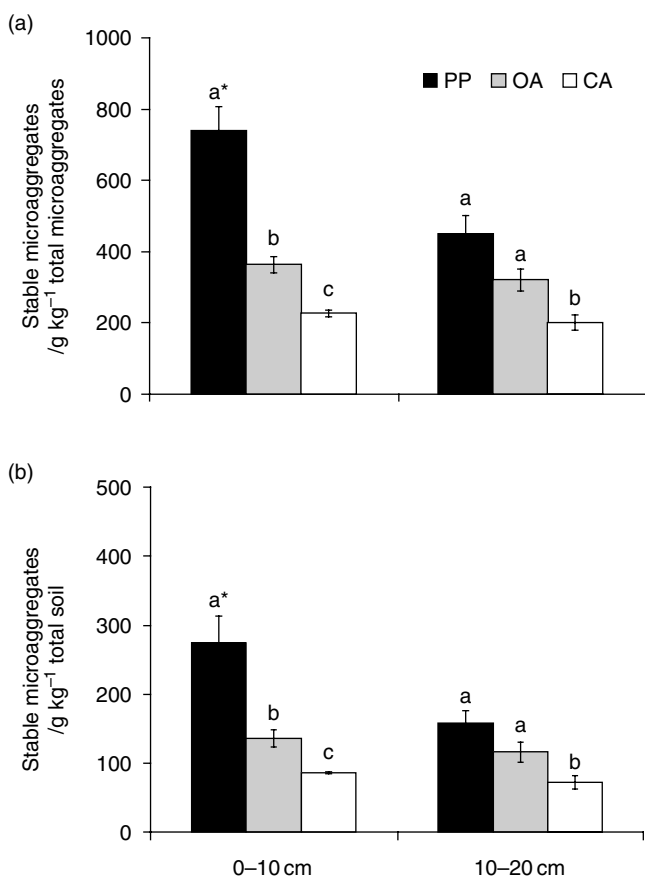


Figure 3 Continued



**Figure 3** SEM images of thin sections from the 0–10 cm depth layers of the conventional-arable field (CA) (a,b), the organic-arable field (OA) (c–e) and the permanent pasture (PP) (f–j). (a) Overview of the 53–250  $\mu\text{m}$  size fraction separated from CA (backscatter detection). (b) Detail of (a), showing a microaggregate from CA (backscatter detection). (c) Overview of the 53–250  $\mu\text{m}$  size fraction separated from OA (backscatter detection). (d) Detail of (c), showing a microaggregate from OA (backscatter detection). (e) Detail of (d), showing organic matter occluded in a microaggregate of OA (backscatter detection). (f) Overview of the 53–250  $\mu\text{m}$  size fraction separated from PP (backscatter detection). (g) Detail of (f) (backscatter detection). (h) Detail of (g), showing a microaggregate consisting of a core of organic matter surrounded by clay (backscatter detection). (i) Detail of (h), showing a microaggregate, probably a faecal pellet, consisting of organic matter, clay and fine silt (backscatter detection). (j) Detail of (i), showing organic matter (normal detection). r, organic matter showing some visible cell structures; a, remnants of plant cell structures (membranes) filled with amorphous organic material; ar, exoskeleton of soil fauna (arthropod).



**Figure 4** Amounts of total and stable microaggregates in the permanent pasture (PP), organic-arable field (OA) and conventional-arable field (CA), expressed (a) per unit of total microaggregates and (b) on a total soil basis. Bars indicate standard errors. Values that are significantly different between the management systems or with depth are marked with different letters or an asterisk, respectively ( $P < 0.05$ ).

mineral-associated OC is generally greater in magnitude, the microaggregate-associated POM fractions are more responsive to management changes. Unfortunately, the amount of intra-microaggregate POM could not be calculated for the 0–10 cm depth of PP, because the microaggregates were too stable to break them up without disrupting the occluded POM at the same time. At 10–20 cm depth, however, the ratio between intra-microaggregate POM and free fine POM in PP was approximately twice that in the arable fields. The accumulation of a larger proportion of fine POM and of total SOM with a relatively large C:N ratio in microaggregates of PP indicates a higher rate of incorporation of fresh residues into newly formed microaggregates and a slower decomposition of the microaggregate-occluded SOM under pasture than in arable land.

C:N ratios of intra-microaggregate POM 53–250  $\mu\text{m}$  were available only for PP at 10–20 cm depth and for both depths of OA. In these cases, the C:N ratio of intra-microaggregate POM decreased with decreasing particle size from 53–250  $\mu\text{m}$

to 20–53  $\mu\text{m}$ , as was expected, since decomposition generally causes a decrease in POM size as well as in C:N ratio. When compared with POM outside microaggregates, the intra-microaggregate POM of the size fraction 53–250  $\mu\text{m}$  had a C:N ratio that was intermediate between coarse and free fine POM. This might indicate that POM 53–250  $\mu\text{m}$  occluded in microaggregates was less decomposed than free fine POM in the same size fraction, possibly due to physical protection. However, this is in contrast to Golchin *et al.* (1994), who found for an old pasture that POM occluded in stable aggregates < 2 mm had a more advanced state of decomposition than free POM as indicated by <sup>13</sup>C CP/MAS NMR and electron microscopy, despite a wider C:N ratio.

#### Microaggregate characteristics

The observation that a considerable part of the microaggregates from the upper 10 cm of PP could not be dispersed in Na-hexametaphosphate solution revealed that the stability of the microaggregates was considerably affected by management. This was further confirmed by the up to 3-fold larger amounts of microaggregates that survived water dispersion in PP compared with the arable soils. In addition, the mineral particle-size composition of the microaggregates was found to depend on management. Not only were the microaggregates of PP relatively enriched in fine mineral particles compared with microaggregates of OA and especially CA, but stable microaggregates also tended to be enriched in fine mineral material when compared with total microaggregates.

These differences in microaggregate characteristics demonstrate that different types of microaggregates were present in the different management systems. Microaggregates in the arable soils were not as stable as microaggregates in the pasture soil and less enriched in OC and fine mineral particles,

**Table 8** Coarse silt (20–53  $\mu\text{m}$ ) contents of total and stable microaggregates in the permanent pasture (PP), organic-arable field (OA) and conventional-arable field (CA). Standard errors are given in parentheses. Values followed by different letters or an asterisk are significantly different between management types or depths, respectively. A '#' indicates that the difference between total and stable microaggregates is significant ( $P < 0.05$ )

Depth /cm	Field	Coarse silt content	
		Total microaggregates /g kg <sup>-1</sup> microaggregates	Stable microaggregates
0–10	PP	242 (11) <sup>c</sup>	210 (13) <sup>b</sup>
	OA	332 (2) <sup>b#</sup>	250 (11) <sup>b</sup>
	CA	397 (10) <sup>a</sup>	334 (24) <sup>a</sup>
10–20	PP	330 (12) <sup>b*</sup>	261 (4) <sup>b</sup>
	OA	328 (8) <sup>b#</sup>	223 (12) <sup>b</sup>
	CA	382 (15) <sup>a</sup>	359 (39) <sup>a</sup>

suggesting that microaggregates in the arable soils are not as effective in terms of soil structural stability and C sequestration. The smaller water stability of the microaggregates under CA than under PP corresponds well with the known susceptibility to slaking of Dutch marine loam soils under conventional-arable land (Jongmans *et al.*, 2001). The small water stability also explains why breakdown of microaggregates occurred after they were isolated from the arable soils, as was indicated by the slake crusts that had formed on top of the 53–250  $\mu\text{m}$  size fractions during oven-drying.

#### *Land-use and tillage effects on microaggregation and SOM turnover*

We hypothesized that the soil under PP would contain more microaggregates than under arable land and would better protect microaggregate-associated SOM against rapid decomposition. The greater relative OC enrichment, the relatively large C:N ratio and the increased stability of microaggregates of PP soil compared with both arable systems indeed indicate a slower turnover of microaggregates and associated organic matter under PP than in arable land. This was supported by a previous study of the same soils by Nierop *et al.* (2001). Using pyrolysis GC/MS, they showed that in PP, SOM was composed of relatively little decomposed, mainly grass-derived material, whereas both arable soils contained strongly humified plant material and microbially altered proteinaceous material. The presence of a more recalcitrant SOM pool in arable soils indicates a faster SOM turnover in arable land than under pasture (Preston *et al.*, 1987). The less humified nature of SOM in PP than in the arable soils could not be explained by a greater protection at the macroaggregate level under PP. Pulleman & Marinissen (2004) found that C mineralization rates were not significantly lower in intact than in crushed (< 250  $\mu\text{m}$ ) macroaggregates of PP. However, a relatively large increase in C mineralization in finely ground compared with crushed soil suggested a more important C protection at the microaggregate level.

Our results agree with studies by Six *et al.* (2000) and Denef *et al.* (2001), who used the same device as in our study to isolate microaggregates out of stable macroaggregates instead of total soil samples. They showed that microaggregate formation and associated C stabilization increase with decreasing intensity of macroaggregate disturbance, related to either dry-wet cycles (Denef *et al.*, 2001) or tillage intensity (Six *et al.*, 2000). Greater macroaggregate stability, absence of tillage practices and reduced susceptibility to rapid wetting and drying reduces macroaggregate turnover in no-tillage systems and especially under permanent grassland when compared with conventional-arable land. Moreover, the larger amounts of organic inputs under grassland provide more intra-macroaggregate POM that acts as the centre of microaggregate formation when decomposition and gradual

encrustation with clay and microbial exudates proceeds (Oades, 1984).

#### *The role of earthworms in microaggregate formation and SOM turnover*

The observed differences in OC distribution and microaggregate characteristics between PP and the arable soils of our study suggest an important contribution of earthworms to microaggregate formation and the incorporation of organic matter in microaggregates. Blanchart *et al.* (1993) and Jongmans *et al.* (2001) observed that worm casts often consist of faecal pellets that have a core of sand and coarse silt surrounded by a clayey 'cast cortex' in which fine mineral particles and organic matter are intimately mixed. Concentration of fine mineral particles and organic matter by the abundant earthworm population under PP could explain why microaggregates of PP, especially the stable ones, were enriched not only in organic matter but also in fine mineral particles.

Additional evidence for an important role of earthworms in the formation of stable, organic matter-enriched microaggregates follows from micromorphological work (Jongmans *et al.*, 2001; Pulleman *et al.*, 2004). When comparing thin sections of undisturbed soil samples from PP and CA, Jongmans *et al.* (2001) observed abundant worm casts in PP which contained clay-rich microaggregates that were strongly stained with organic pigment and enclosed abundant fine POM (< 100  $\mu\text{m}$ ). Such microstructures were rarely observed in CA. Pulleman *et al.* (2004) separated worm casts and physicogenic macroaggregates from soil samples of PP and CA and prepared thin sections to quantify microaggregates within the different macroaggregate types. The worm casts, especially those from PP, contained considerably more organic matter-enriched microaggregates than the physicogenic macroaggregates. When recalculated to a total soil basis, microaggregates made up 30% and 8% of total soil mass under PP and CA, respectively. These values correspond remarkably well with the amounts of stable microaggregates that were found in the present study, i.e. 27% and 9%, respectively, suggesting that the microaggregates that were identified in thin sections were the same microaggregates that survived the water-dispersion test in the present study.

The direct involvement of earthworms in the formation of stable microaggregates within their casts was also demonstrated by Bossuyt *et al.* (2004a) in a short-term laboratory study. They found that newly formed macroaggregates > 2 mm contained considerably more POM and almost four times more stable microaggregates after 12 days of incubation in the presence of the endogeic earthworm species *Aporrectodea caliginosa* than in the absence of earthworms. Moreover, the worm-made microaggregates within the large macroaggregates protected a significant proportion of total soil C

and C from newly added  $^{13}\text{C}$ -labelled residues against decomposition (Bossuyt *et al.*, 2004b).

In contrast to the concepts of microaggregate formation that involve gradual microbial decomposition (Oades, 1984; Six *et al.*, 2000), organic matter that is fractionated and incorporated into stable microaggregates by earthworms becomes directly occluded within fine mineral material, as described in a model for aggregate formation in worm casts by Shipitalo & Protz (1989). However, from our study we cannot differentiate between the effects of earthworm-induced versus microbially mediated microaggregate formation on microaggregate stability and microaggregate-associated SOM stabilization in the different management systems.

### Organic farming effects

The only significant difference in OC distribution between the two arable systems was a larger contribution of coarse free POM to total soil OC under OA at both depths, which could be explained by the addition of farmyard manure (FYM) in OA. The FYM contains straw residues and is ploughed into the soil. The addition of organic manure under OA was not only accompanied by an increase in total soil OC compared with CA but also resulted in a 3.5 times greater volume of worm-worked soil. The amount of slake-resistant water-stable macroaggregates was 1.7 times greater under OA than in CA (73% versus 42% of total soil mass, respectively) (Pulleman *et al.*, 2003). However, the small and mainly non-significant differences in microaggregate stability and OC enrichment between OA and CA indicate that there is no simple linear relationship between stable macroaggregation or earthworm activity and the presence of stable, SOM-enriched microaggregates.

The contribution of microaggregates to total soil OC and the stability and fine mineral particle content of microaggregates tended to be somewhat higher in OA than in CA, suggesting a trend of increasing OC stabilization in microaggregates under OA management. Although this trend might have been demonstrated more clearly if only stable microaggregates had been considered, differences between OA and CA were small in comparison to the differences between both arable soils and PP. Moreover, the soil under OA had a somewhat greater clay and fine silt content than the soils under CA and PP. Irrespective of possible management effects, a greater content of fine mineral particles will increase microaggregate stability, fine mineral particle content in microaggregates and SOM stabilization in microaggregates; it might thus partly explain the differences between OA and CA.

The very small effect of organic farming on microaggregate characteristics and associated C stabilization may be caused by the effect of tillage on aggregate turnover (Six *et al.*, 1998). Paucity of organic resources that stimulate the formation of microaggregates during passage through earthworms, and other biotic processes that result in the formation or stabil-

ization of microaggregates, may also play an important role. Shipitalo & Protz (1989) found that the type and extent of bonding between mineral and organic materials in worm casts depended on the amount and composition of the ingested organic debris. In addition, a possible influence of the presence of different ecological groups of earthworm species with different feeding behaviour cannot be excluded (Haynes & Fraser, 1998), since anecic species seemed to be absent in both arable fields.

### Conclusions

Although management did not affect the total amounts of microaggregates separated from whole soil samples, management did have a distinct effect on the OC enrichment, stability and morphology of the microaggregates. Microaggregates separated from the pasture soil, especially from the top 10 cm, were extremely stable and more effectively stabilized occluded organic matter than microaggregates from the arable systems. We found indications that the large and diverse earthworm population present under PP contributes to the formation of these stable, organic matter-enriched, microaggregates. Although the OC enrichment, the stability and the amount of fine mineral particles tended to be somewhat greater in microaggregates of OA than in microaggregates of CA, differences between the two arable systems were small, indicating that organic farming did not significantly affect microaggregate characteristics and OC stabilization.

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### References

- Barois, I., Villemin, G., Lavelle, P. & Toutain, F. 1993. Transformation of the soil structure through *Pontosolex corethurus* (Oligochaeta) intestinal tract. *Geoderma*, **56**, 57–66.
- Berry, E.C. & Karlen, D.L. 1993. Comparison of alternative farming systems. II. Earthworm population density and species diversity. *American Journal of Alternative Agriculture*, **8**, 21–26.
- Blanchart, E., Bruand, A. & Lavelle, P. 1993. The physical structure of casts of *Millsonia anomala* (Oligochaeta, Megascolecidae) in shrub savanna soils (Côte d'Ivoire). *Geoderma*, **56**, 119–132.

- Bossuyt, H., Six, J. & Hendrix, P.F. 2004a. Rapid incorporation of carbon from fresh residues into newly formed stable microaggregates within earthworm casts. *European Journal of Soil Science*, **55**, 393–399.
- Bossuyt, H., Six, J. & Hendrix, P.F. 2004b. Protection of soil carbon by microaggregates within earthworm casts. *Soil Biology and Biochemistry*, **37**, 251–258.
- Buurman, P., Van Lagen, B. & Velthorst, E.J. 1996. *Manual for Soil and Water Analyses*. Backhuys, Leiden.
- Denef, K., Six, J., Paustian, K. & Merckx, R. 2001. Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry–wet cycles. *Soil Biology and Biochemistry*, **33**, 2145–2153.
- Elliott, E.T. 1986. Aggregate structure and carbon, nitrogen and phosphorus in native and cultivated soils. *Soil Science Society of America Journal*, **50**, 627–633.
- FAO–UNESCO 1997. *Soil Map of the World, Volume I, Legend*. FAO, Rome.
- Fitzpatrick, E.A. 1970. A technique for the preparation of large thin sections of soils and unconsolidated material. In: *Micromorphological Techniques and Application* (eds D.A. Osmond & P. Bullock), pp. 3–13. Technical Monograph 2, Soil Survey of England and Wales, Rothamsted Experimental Station, Harpenden.
- Golchin, A., Oades, J.M., Skjemstad, J.O. & Clarke, P. 1994. Study of free and occluded particulate organic matter in soils by solid state  $^{13}\text{C}$  CP/MAS NMR spectroscopy and scanning electron microscopy. *Australian Journal of Soil Research*, **32**, 285–309.
- Gupta, V.V.S.R. & Germida, J.J. 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. *Soil Biology and Biochemistry*, **20**, 777–786.
- Haynes, R.J. & Fraser, P.M. 1998. A comparison of aggregate stability and biological activity in earthworm casts and uningested soil as affected by amendment with wheat or lucerne straw. *European Journal of Soil Science*, **49**, 629–636.
- Hendrix, P.F., Parmelee, R.W., Crossley, D.A., Jr, Coleman, D.C., Odum, E.P. & Groffman, P.M. 1986. Detritus food webs in conventional and no-tillage agroecosystems. *BioScience*, **36**, 374–380.
- Jongmans, A.G., Pulleman, M.M. & Marinissen, J.C.Y. 2001. Soil structure and earthworm activity in a marine silt loam under pasture versus arable land. *Biology and Fertility of Soils*, **33**, 279–285.
- Lal, R. & Kimble, J.M. 1997. Conservation tillage for carbon sequestration. *Nutrient Cycling in Agroecosystems*, **49**, 243–253.
- Mackay, A.D. & Kladvik, E.J. 1985. Earthworms and rate of breakdown of soybean and maize residues in soil. *Soil Biology and Biochemistry*, **17**, 851–857.
- Nierop, K.G.J., Pulleman, M.M. & Marinissen, J.C.Y. 2001. Management induced organic matter differentiation in grassland and arable soil: a study using pyrolysis techniques. *Soil Biology and Biochemistry*, **33**, 755–764.
- Oades, J.M. 1984. Soil organic matter and structural stability: mechanisms and implications for management. *Plant and Soil*, **76**, 319–337.
- Oades, J.M. 1988. The retention of organic matter in soils. *Biogeochemistry*, **5**, 35–70.
- Parmelee, R.W., Beare, M.H., Cheng, W., Hendrix, P.F., Rider, S.J., Crossley, D.A. Jr & Coleman, D.C. 1990. Earthworms and enchytraeids in conventional and no-tillage agroecosystems: a biocide approach to assess their role in organic matter breakdown. *Biology and Fertility of Soils*, **10**, 1–10.
- Preston, C.M., Shipitalo, S.E., Dudley, R.L., Fyfe, C.A., Mathur, S.P. & Levesque, M. 1987. Comparison of  $^{13}\text{C}$  CPMAS NMR and chemical techniques for measuring the degree of decomposition in virgin and cultivated peat profiles. *Canadian Journal of Soil Science*, **67**, 187–198.
- Pulleman, M.M. & Marinissen, J.C.Y. 2004. Physical protection of mineralizable C in aggregates from long-term pasture and arable soil. *Geoderma*, **120**, 273–282.
- Pulleman, M.M., Jongmans, A.G., Marinissen, J.C.Y. & Bouma, J. 2003. Effects of organic versus conventional arable farming on soil structure and organic matter dynamics in a marine loam in the Netherlands. *Soil Use and Management*, **19**, 157–165.
- Pulleman, M.M., Six, J., Uyl, A., Marinissen, J.C.Y. & Jongmans, A.G. 2004. Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. *Applied Soil Ecology*, in press.
- Scullion, J. & Malik, A. 2000. Earthworm activity affecting organic matter, aggregation and microbial activity in soils restored after opencast mining for coal. *Soil Biology and Biochemistry*, **32**, 119–126.
- Sherrod, L.A., Dunn, G., Peterson, G.A. & Kolberg, R.L. 2002. Inorganic carbon analysis by modified pressure-calimeter method. *Soil Science Society of America Journal*, **66**, 299–305.
- Shipitalo, M.J. & Protz, R. 1989. Chemistry and micromorphology of aggregation in earthworm casts. *Geoderma*, **45**, 357–374.
- Six, J., Elliott, E.T., Paustian, K. & Doran, J.W. 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. *Soil Science Society of America Journal*, **62**, 1367–1377.
- Six, J., Elliott, E.T. & Paustian, K. 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry*, **32**, 2099–2103.
- Skjemstad, J.O., Le Feuvre, R.P. & Prebble, R.E. 1990. Turnover of soil organic matter under pasture as determined by  $^{13}\text{C}$  natural abundance. *Australian Journal of Soil Research*, **28**, 267–276.
- SPSS 1997. *SPSS 7.5 for Windows*. SPSS Inc., Chicago, IL.
- Tisdall, J.M. & Oades, J.M. 1982. Organic matter and water-stable aggregates in soils. *Journal of Soil Science*, **33**, 141–163.
- Whalen, J.K., Parmelee, R.W. & Edwards, C.A. 1998. Population dynamics of earthworm communities in corn agroecosystems receiving organic or inorganic fertilizer amendments. *Biology and Fertility of Soils*, **27**, 400–407.



