

# **Biotic and abiotic binding and bonding mechanisms in soils with long-term differences in management**

*Susanne Elmholt, Kasia Debosz, Lars J. Munkholm and Per Schjønning*

Danish Institute of Agricultural Sciences, Department of Crop Physiology and Soil Science, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, DENMARK

E-mail: [susanne.elmholt@agrsci.dk](mailto:susanne.elmholt@agrsci.dk)

## **Summary**

During the last decades Denmark has experienced a growing interest in low-input farming systems like organic farming. These systems rely on a high soil fertility to maintain nutrient availability and plant health. Soil aggregation contributes to this fertility, because it is crucial to soil porosity, aeration and infiltration of water. This paper reports a study of two pairs of differently managed, neighboring fields. The aim was to elucidate long-term effects of the different farming systems on physical and biological variables with influence on bonding and binding mechanisms of soil aggregation. Each pair consists of an organically grown dairy farm soil, based on a forage crop rotation system, including grass (Org-FCS(G)) and a conventionally managed soil. One of the conventional farms has a forage crop rotation with annual cash crops and no grass (Conv-FCS(NG)) and one has been grown continuously with small grain cereals and rape (Conv-CCS). Our results indicate that the Org-FCS(G) soils stimulate biotic soil aggregating agents as measured by extracellular polysaccharides (EPS) and hyphal length measurements, respectively. Generally, the Conv-CCS soil, which relies exclusively on synthetic fertilisers and cereal production, offered poor conditions for the biotic binding and bonding agents. Nevertheless this soil contained a large amount of stable macro-aggregates. This is explained by the physical results, which indicated that the strong macro-aggregation was due to clay dispersion and cementation processes rather than to biotic processes.

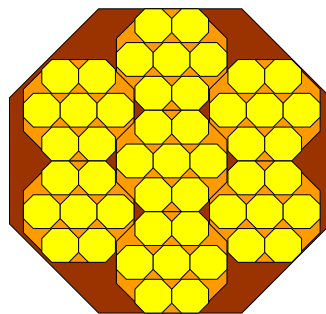
**Keywords:** soil structure, farming systems, stability, hyphal lengths, polysaccharides, microbial biomass, dispersible clay




## **Introduction**

Denmark experiences a growing interest in low-input management systems like organic farming. These systems cannot be manipulated by agrochemicals. Nutrient availability and plant health rely on a high fertility of the soil and a proper physical environment for plants and microorganisms. The Danish Research Center for Organic Farming (DARCOF) was established in 1996 with the aim to co-ordinate Danish research and development for organic farming. A number of research projects were initiated with the intention to facilitate a conversion from conventional to organic farming, while encouraging a sustainable development of the economic, ecological and social aspects of agriculture. Among these projects is a study on 'Soil fertility and soil tillage as influenced by organic farming practice and

soil tillage'. One purpose of this project was to elucidate long-term effects of distinctly different farming systems on the interactions between physical and biological properties, which play a role in soil aggregation. Soil aggregation is crucial for ensuring a desirable soil structure for plant growth (i.e. good aeration, infiltration of water, soil root contact and a low resistance against root penetration).

The hierarchical model of soil aggregation, presented by Hadas (1987) and Dexter (1988), assumes that a range of different mechanisms will combine primary particles (clay, silt, sand) and organic matter into floccules, micro-aggregates (63-250 $\mu\text{m}$ ) and gradually larger macro-aggregates (>250 $\mu\text{m}$ ) (Figure 1). There are typically  $10^{3\pm 1}$  particles of a given hierarchical order in a single particle of the next higher order (Dexter, 1988).



**Figure 1.** Conceptual model of soil aggregation, illustrating micro-aggregates , small macro-aggregates  and large macro-aggregates  (based on Dexter, 1988).

According to theories on soil aggregating mechanisms (Tisdall & Oades, 1982; Degens, 1997), these can be divided into three groups: 1) The 'persistent bonding agents', a range of humic compounds, which are associated with metal ions. This group is not addressed in the present paper as it is considered less susceptible to soil management than the other groups. 2) The 'transient bonding agents', which 'glue' (bond) together primary soil particles into micro-aggregates. They consist primarily of extracellular polysaccharides (EPS) and are produced by bacteria, fungi and plants. 3) The 'temporary binding agents', which enmesh (bind) primary particles and micro-aggregates to larger aggregates and are assumed to play their main role in macro-aggregation. They consist of fungal hyphae and plant roots.

In order to elucidate the effect of soil management on the interaction between soil physical and biological variables it is important to use a multidisciplinary approach, integrating these different disciplines of soil science. Therefore both physical and biological variables that indicate bonding and binding (groups 2 and 3, cf. above) mechanisms were assessed. In this paper we present some of our results on the physical condition of the soils (aggregate stability, soil porosity), on indicators of biotic binding (hyphal lengths) and bonding (EPS) as well as abiotic bonding mechanisms (clay dispersion/cementation). Results on microbial biomass are presented to indicate the living conditions for soil microorganisms.

## Materials and Methods

Four differently managed arable soils, Soil Pair I and Soil Pair II, were used. The soils are located on the island of Sealand and a few relevant chemical and physical characteristics of the soils are shown in Table 1. A more detailed description of physical, chemical and management characteristics can be found in Schjøning et al. (*submitted*).

**Table 1.** Selected chemical and physical characteristics of the four soils. For further characteristics and details on methodology and analyses of variation, cf. Schjøning et al., in press. ‘Forage Crop System’ (FCS) or ‘Continuous Cereal System’ (CCS). Crop rotation with Grass (G) or with No Grass (NG).

	Soil Pair I		Soil Pair II	
	FCS(G)	FCS(NG)	FCS(G)	CCS
Soil type	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Clay (< 2µm), %	20	21	17	19
Organic matter, %	3.9	3.5	3.5	2.4
pH (CaCl <sub>2</sub> )	6.7	7.1	6.2	6.1
Bulk density, g cm <sup>-3</sup>	1.35	1.35	1.36	1.49

### Soil Pair I (SP I)

In SP I, one dairy farm soil had been grown organically (Org) since 1951 with high amounts of animal manure (estimated yearly 233 kg N/ha from slurry, composted farmyard manure and grazing) and a diversified crop rotation, based on grass/clover leys and cereals (forage crop system with grass, FCS(G)). The Org-FCS(G) soil had an estimated yearly input of incorporated dry matter of 5.6 t/ha. This soil was referenced by a conventional dairy farm having a forage crop rotation with no grass (Conv-FCS(NG)). The crop rotation of the Conv-FCS(NG) soil consisted of annual cash crops only, including beets for sugar production. The dairy farm characteristics of this soil were primarily reflected in a rather high amount of animal manure application (estimated yearly 261 kg N/ha from slurry). The Conv-FCS(NG) soil had an estimated yearly input of incorporated dry matter of 4 t/ha.

### Soil Pair II (SP II)

In SP II, the dairy farm soil had been grown organically since 1958 and was managed nearly identically to the Org-FCS(G) soil of SP I (same owner). This soil has a forage crop rotation too but lacked grazing and had only one year of grass/clover ley in the rotation. The Org-FCS(G) soil had fairly low, estimated yearly inputs of animal manure (128 kg N/ha from slurry and composted farmyard manure) and high inputs of incorporated dry matter (4.5 t/ha). It was referenced by a conventionally managed soil (Conv). This soil had not received animal manure for a minimum of twenty years and had been grown continuously with small grain cereals and rape (continuous cereal system, CCS), generally without mulching of plant residues (estimated yearly input of dry matter 0.8 t/ha). Despite this, the input of nitrogen to the Conv-CCS soil was higher than to the Org-FCS soil (estimated yearly input from synthetic fertilisers 159 kg N/ha).

### **Soil sampling and analysis**

The time and strategy of soil sampling aimed to avoid/minimise effects of temporal variations in the studied variables caused by short-term effects of e.g. tillage, fertilisation, crop rotation, rhizodeposition and drying/wetting of the soil. The four fields were grown to winter wheat at the time of sampling, *Triticum aestivum* L. ssp. *spelta* in the organically managed SP I-FCS and SP II-FCS fields and *Triticum aestivum* L. ssp. *vulgare* in the conventionally managed SP I-FCS(NG) and SP II-CCS fields. For each soil nine sampling points were identified as the intersection of a 10 x 10m grid, from which undisturbed soil blocks were collected at 6-13 cm depth. This depth was chosen to avoid soil, which had been directly disturbed by seedbed preparation and sowing operations. The soil blocks (~ 650 cm<sup>3</sup>) were retrieved by carefully hammering a metal shovel sideways into a soil 'wall' and cutting the block from the bulk soil by pressing down a metal plate. The soil blocks from each of the nine sampling points were placed in plastic boxes with dimensions corresponding exactly to nine block units and covered with a plastic lid. A slight air exchange to the surrounding atmosphere secured oxic conditions in the boxes. Undisturbed soil cores (100 cm<sup>3</sup>) were collected in metal cylinders forced into the soil by means of a hammer. After removal of the soil-filled cylinder from the bulk soil, the end surfaces were trimmed with a knife and mounted with plastic caps to protect the soil from mechanical disturbance and evaporation. Two replicate samples were collected at each of the nine sampling points in each field. The soil (both blocks and cores) was stored at 2°C until analysis for soil porosity, WAS, clay dispersibility and microbial biomass. The soil for measurements of EPS and hyphal lengths was air-dried immediately after sampling and sieved into sizes 4-8mm, 0.50-1mm and 0.063-0.25mm. All results are expressed on a dry weight basis (gravimetric determination of water content by drying the soil for 24 hours at 105°C).

Soil porosity: The core samples in the metal cylinders were weighed before saturating them on sandboxes with capillary water from beneath. The cores were drained to -100hPa and oven-dried (105°C, 24 hs). The weight of each sample was recorded at each matric potential before and after drying. Results are given as the percentage of soil pores in relation to the soil volume, either as total porosity or macro-porosity (pores > 30µm).

Wet aggregate stability (WAS): Core subsamples of approx. 45 g from each of the nine blocks of each soil were gently passed through an 8 mm sieve and taken to a 250 µm sieve installed in a wet-sieving apparatus (Yoder, 1936). Thirty seconds of initial capillary wetting were followed by 2 min of vertical movement of the sieve (stroke length 32mm, 38 strokes/min). Stable aggregates remaining on the sieve (macro-aggregates) were transferred quantitatively to a beaker, water was evaporated at 80°C and the soil further dried at 105°C. Finally the soil was dispersed by end over end shaking (24 hours with a 0.002 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> solution) and poured through the 250µm sieve. Primary particles >250µm were weighed following drying at 105°C. Results are given as gram wet stable aggregates per gram soil.

Clay dispersibility: Subsamples of 1.5 g from each soil block of a given soil, drawn from the 8 mm sieved samples described in the section on WAS, were weighed into cylindrical centrifuge bottles and applied with 50 ml of 0.002 M  $\text{Na}_4\text{P}_2\text{O}_7$  solution. The bottles were shaken end over end (33 rpm) for 24 hours. Clay dispersed from the soil samples was determined from the turbidity of a clay-holding suspension, siphoned off the shaking bottles after a specified time period (Pojasok & Kay; 1990; Watts et al., 1996), in this case 24hs. Correction was made for primary particles >2 mm in the given soil. Results are given as dispersed clay in relation to the clay fraction of the sample.

Hyphal length: Subsamples from each soil block of a given soil were taken representatively from the air-dried, fractionated samples of aggregates 4-8mm, which were sieved (<2 mm) before subsampling. Hyphal lengths were essentially determined according to procedures by West (1988): Subsamples were dispersed in 0.0033 M sodium hexametaphosphate and the suspension was sieved (38  $\mu\text{m}$ ) to remove clay and silt. The remaining material was blended, diluted, stained for 2 hours with calcofluor white, filtered (Nuclepore 110659, black polycarbonate, diameter 25mm, mesh width 0.8 $\mu\text{m}$ ) and examined at a magnification of 200 x with an epifluorescent microscope, using the gridline intersect method (Olson, 1950). Results are given as meter of hyphae per gram soil.

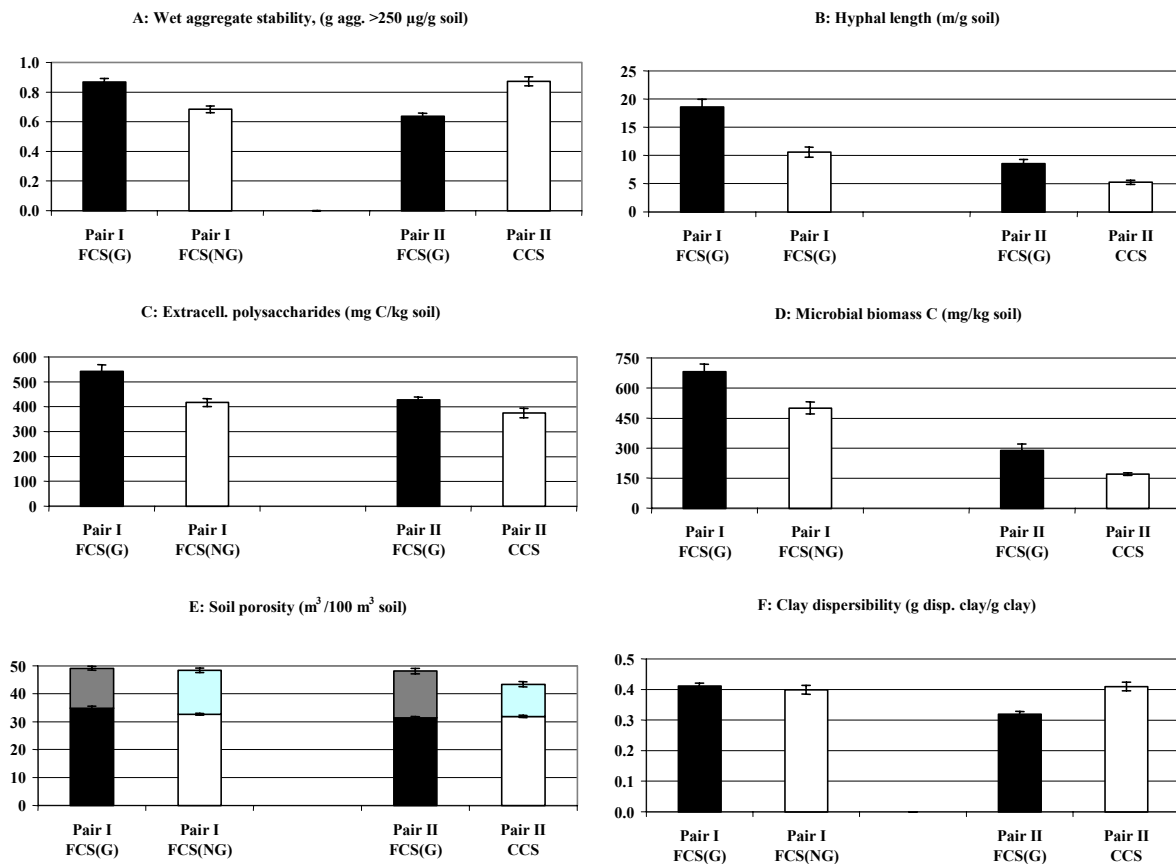
Extracellular polysaccharides (EPS): An easy extractable carbohydrate fraction was extracted from air-dry aggregates of 4-8mm according to the method described by Ball et al. (1996). The following modifications were used: The air-dried aggregates were shaken with hot water (80° C) using 1: 6 soil extractant ratio (wt/vol.) for 16 h. Before extraction, highly soluble substances and floating plant material were removed by shaking the soil with distilled cold water (20°C). The carbohydrate content of the hot water extract after centrifugation (5800 x g, 10 min) was analysed using a reaction with thymol in strongly acid solution. The results are given as mg EPS-C per kg soil.

Microbial biomass: Microbial biomass C was determined in field-moist soil by the fumigation-extraction method (Vance et al., 1987). Three core samples from each of the nine soil blocks were sieved (2 mm) and mixed thoroughly. Soluble C and chloroform labile C (C solubilized by  $\text{CHCl}_3$  during an 18 h fumigation period) were extracted with 0.5 M  $\text{K}_2\text{SO}_4$  in a soil:solution ratio of 1:4 (wt:vol). Organic C in all 0.5 M  $\text{K}_2\text{SO}_4$  extracts was determined by an automated UV persulphate oxidation procedure using a Dorhmann DC-180 Carbon Analyzer (Wu et al., 1990). Biomass C was calculated as soluble C in fumigated minus soluble C in non-fumigated soil using 0.45 as the  $k_c$ -factor (Kaiser et al., 1992). The results are given as mg biomass C per kg soil.

## **Results and Discussion**

Figure 2 presents a range of physical and biological results from the four soils. Figure 2A shows the mechanical stability of the soil, WAS, expressed as the amount of wet-stable macro-

aggregates (g) per g soil. For Soil Pair I, the macro-aggregate stability is higher in the ORG-FCS(G) soil than in the CONV-FCS(NG) soil.

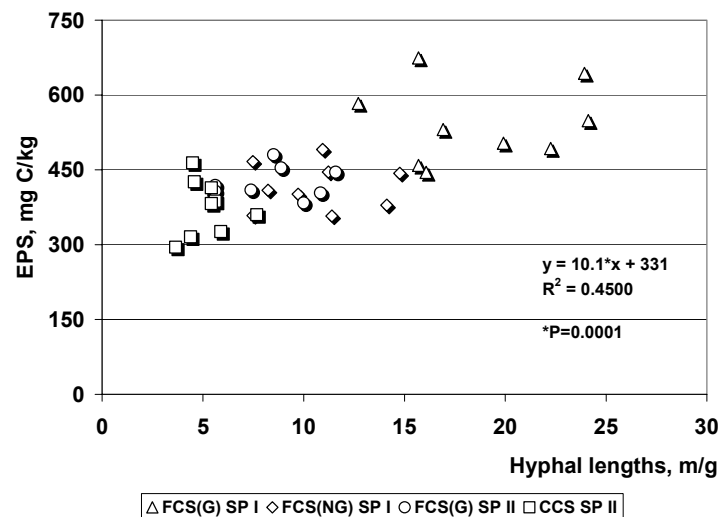


**Figure 2.** Physical and biological characterisation of the two Soil Pairs (I and II). Pair I, FCS(G): Organically managed, forage crop rotation with grass. Pair I, FCS(NG): Conventionally managed, forage crop rotation without grass. Pair II, FCS(G): Organically managed, forage crop rotation with grass. Pair II, CCS: Conventionally managed, cereal crop system. All results are shown as mean values for the samples from the nine grid points, the bars giving the standard error of the mean. In 2E the bottom stacks show the volume of pores <30 µm and the top stacks show the macro-porosity (pores > 30µm).

In Soil Pair II, however, WAS is higher in the CONV-CCS soil than in the ORG-FCS(G) soil. This indicates that bonding and especially binding agents - which are supposed to play the larger role in macro-aggregation - are more abundant in the ORG-FCS(G) soil of Pair I and in the CONV-CCS soil of Pair II.

Figure 2B shows that the fungal hyphae, which are supposed to be one of the major agents of macro-aggregation, are about twice as abundant in the two organically managed soils (both being FCS soils with grass in the rotation) as compared with the conventionally managed soils. The lowest values were found in the CCS soil. It should be noted, however, that Schjønning et al. (*submitted*) report results on the soil ergosterol content, which indicate a higher fungal biomass in the SP I-FCS(NG) soil than in the SP I-FCS(G) soil. For the SP II soils both the hyphal length

measurements and the soil ergosterol contents show higher results for the FCS soil than for the CCS soil. The results on hyphal measurements reported here are in accordance with Tisdall & Oades (1982). They put forward the hypothesis that an increase in the frequency of grass will increase the percentage of organic carbon in the soil and - what is interesting in this context - that this will primarily affect the temporary binding agents, *i.e.* the roots and hyphae. Both organically managed soil have a crop rotation with grass leys and they have higher percentages of organic matter than their conventional reference soils (Table 1).



**Figure 3.** Correlation between hyphal lengths and EPS, expressed as a linear regression for all samples.

According to Tisdall & Oades (1982), the bonding agents will be less susceptible to changes in crop rotation and organic matter. Figure 2C shows the results for the EPS, which are considered important bonding agents. The pattern is the same as for the hyphae although the differences between the organically and the conventionally managed soils are less pronounced. At grid point level a significant correlation was found between hyphal lengths and EPS (Figure 3), which is also in accordance with the theory of Tisdall & Oades (1982).

Figure 2D shows the microbial biomass C, which is regarded an important indicator of soil quality (Doran & Parkin, 1994). Like the results on biotic binding and bonding agents (Figure 2B and C), these results also indicate that the conditions for microbial life are poor in the CCS soil as compared with the other three soils. This may be related to poorer soil porosity in the CCS system, especially regarding macro-pores  $>30 \mu\text{m}$  (top stacks in Figure 2E). At sample level our results show a significant correlation between hyphal lengths or EPS, respectively, on the one hand and total soil porosity on the other (results not shown).

All the biological results as well as the pore characteristics indicate the lowest soil quality in the CCS soil. Bonding as well as binding agents appear to have poor conditions, indicating that biotic soil aggregation is poor in this soil. Nevertheless the WAS results showed a high

amount of stable macro-aggregates (Figure 2A). Figure 2F shows clay dispersibility for the four soils, expressed as the amount of clay released from soil aggregates during a prolonged shaking procedure of 24 hours with  $\text{Na}_4\text{P}_2\text{O}_7$ . The biological results combined with the fact that clay is more easily dispersed from the CCS soil than from the FCS soils indicates that macro-aggregation in this soil depends on abiotic rather than biotic processes. A large dispersibility means that the clay is loosely bound to the soil aggregates and that the soil is susceptible to dispersion (slaking) and cementation during wetting and drying processes. Re-orientation and hardening of the dispersed clay minerals may eventually lead to a dense and mechanically strong soil. *In situ* studies of the soil structure confirmed this and revealed that the CCS soil had a very firm blocky structure whereas the FCS(G) soil had a more porous and crumbly structure in the studied soil layer (Munkholm, 2000). In an agronomic sense this may cause problems in preparing a proper seed bed in the CCS soil. The macro aggregates, which were retrieved by wet sieving from this soil, are therefore small, dense blocks rather than porous crumbs as discussed by Schjønning et al. (*submitted*). This seems to be the reason why the CCS soil is a poorer habitat for soil organisms than the FCS soils.

## Conclusions

Our results stress the need to integrate biological, physical and chemical methodologies to increase our understanding of soil aggregation mechanisms. The results on EPS and hyphal length measurements indicate that the FCS(G) soils stimulate biotic bonding and especially binding mechanisms. Generally, the CCS soil, the management of which is based exclusively on synthetic fertilisers and cereal production, offered poor conditions for the biotic binding and bonding agents. The strong macro-aggregation of this soil was ascribed instead to clay dispersion and cementation processes.

## References

- Ball, B.C., Cheshire, M.V., Robertson, E.A.G. & Hunter, E.A., 1996. Carbohydrate composition in relation to structural stability, compactibility and plasticity of two soils in a long-term experiment. *Soil Till. Res.* 39, 143-160.
- Degens, B.P., 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: a review. *Aust. J. Soil Res.* 35, 431-459.
- Dexter, A.R., 1988. Advances in Characterization of Soil Structure. *Soil Tillage Res.* 11, 199-238.
- Doran, J.W. & Parkin, T.B., 1994. Defining and assessing soil quality. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F. & Stewart, B.A. (eds), *Defining Soil Quality for a Sustainable Environment*. Soil Science Society of America Inc., American Society of Agronomy, Inc, Madison, Wisconsin, 3-21
- Hadas, A., 1987. Long-term tillage practice effects on soil aggregation modes and strength. *Soil Sci. Soc. Am. J.* 51, 191-197.



- Kaiser, E.A., Mueller, T., Joergensen, R.G., Insam, H. & Heinemeyer, O., 1992. Evaluation of methods to estimate the soil microbial biomass and the relationship with soil texture and organic matter. *Soil Biol. Biochem.* 24, 675-683.
- Munkholm, L.J., 2000. The spade analysis - a modification of the qualitative spade diagnosis for scientific use. DIAS report no. 28, Plant Production. Danish Institute of Agricultural Sciences, Foulum, 40 pp.
- Olson, F.C.W., 1950. Quantitative estimates of filamentous algae. *Trans. Am. Mic. Soc.* 69, 272-279.
- Pojasok, T. & Kay, B.D., 1990. Assessment of a combination of wet sieving and turbidimetry to characterize the structural stability of moist aggregates. *Can J. Soil Sci.* 70, 33-42.
- Schjønning, P., Elmholt, S., Munkholm, L.J. & Deboasz, K., (*submitted*). Soil quality aspects of humid sandy loams as influenced by different long-term management. *Agric. Ecosyst. Environ.*
- Tisdall, J.M. & Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. *J. Soil Sci.* 33, 141-163.
- Vance, E.D., Brookes, P.C. and Jenkinson, D.S., 1987. Microbial biomass measurements in forest soils: The use of chloroform fumigation-incubation method in strongly acid soils. *Soil Biol. Biochem.* 19, 697-702.
- Watts, C.W., Dexter, A.R., Dumitru, E. & Arvidsson, J., 1996. An assessment of the vulnerability of soil structure to destabilization during tillage. Part I. A laboratory test. *Soil Till. Res.* 37, 161-174.
- West, A.W., 1988. Specimen preparation, stain type, and extraction and observation procedures as factors in the estimation of soil mycelial lengths and volumes by light microscopy. *Biol. Fert. Soils* 7, 88-94.
- Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R. & Brooks, P.C., 1990. Measurement of soil microbial biomass C - an automated procedure. *Soil Biol. Biochem.* 22, 1167-1169.
- Yoder, R.E., 1936. A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. *J. Amer. Soc. Agron.* 28, 337-351.

## **Acknowledgements**

The study was funded by The Danish Research Center for Organic Farming (DARCOF). Jørgen M. Nielsen, Anette Clausen, Palle Jørgensen and Michael Koppelgaard are thanked for skilful technical assistance.