

Long-term soil quality effects of soil and crop management in organic and conventional arable cropping systems

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ARTICLE INFO

Handling Editor: Ingrid Kögel-Knabner

Keywords:

Soil health
Soil structural stability
Soil organic C
Earthworms

ABSTRACT

Improving or maintaining soil health is crucial to support human needs, with the concept of soil quality connecting soil functions and sustainability concerns. In 2019, we assessed soil chemical, physical and biological properties in a long-term crop rotation experiment initiated in 1997 at Foulum, Denmark, with the aim of determining the long-term soil quality effects of the use of cover crops, animal manure, different crop sequences (with or without a legume-based ley) and organic vs conventional management. The concentration of soil organic carbon has been relatively stable across all treatments for 14 years prior to this investigation; in 2019, we found high aggregate stability, porosity, air permeability and pore organization in all treatments. Bulk density, air permeability and pore organization were affected to some extent by soil and crop management, with bulk density being the lowest in the organic treatment without cover crops, which had the most frequent harrowing. Earthworm density was the greatest in the organic system with grass-clover, especially following the ley year, thanks to a combination of high quality plant input and reduced soil disturbance. From a system perspective, none of the treatments investigated represented extremes, and all maintained good soil quality in the long-term. This indicates that long-term management should take into account the combination of different factors affecting soil quality.

1. Introduction

The importance of healthy soils for sustainable development has gained increasing attention during the last decade ([Safeguarding our soils, 2017](#)). Soils provide essential services that include food production, nutrient cycling, water filtration and carbon (C) storage ([Batjes, 1996](#)). While undisturbed soils can maintain their characteristics over time, cultivation alters this ability, challenging the long-term provision of services that support human needs. In addition, soil cultivation practices that induce the release of stored soil C have played an important role in anthropogenic greenhouse gas emissions in the last century ([Amundson et al., 2015](#)). Therefore, it is crucial to identify and implement management strategies to restore and safeguard soil health. In this study, we focus on how sustainable agricultural production can affect soil functions.

Amongst others, soil functions are related to several physical properties, such as bulk density, wet stability of aggregates and porosity. Wet

stability of aggregates reflects the ability of soil to resist disintegration induced by external stresses, such as soil cultivation, water or wind. A low wet stability of aggregates may thus impair the potential for crop establishment and early growth by increasing the risk of soil cementing and hard and non-friable aggregates ([Schjønning et al., 2012](#)), soil erosion ([Le Bissonnais, 2016](#)) as well as the risk of transport of fine particles carrying pollutants to the water environment ([Nørgaard et al., 2013](#)). [Pulido Moncada et al. \(2015\)](#) indicated that if the percentage of water stable aggregates (WSA, 1–2 mm air-dry aggregates) was above 70 then soil is very stable, across different soil types. The pore-size distribution of a given soil is crucial for water and nutrient availability, microbial activity, percolation and hence soil organic matter turnover and availability of water and nutrients essential for plant growth ([Kravchenko and Guber, 2017](#); [Rabot et al., 2018](#)). Total soil porosity can be divided in > 30 µm and < 30 µm pore size classes, which mainly is defined by soil structure and soil texture, respectively ([Dexter et al., 2008a](#)). A low volume of > 30 µm pores may reduce soil gas exchange

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<https://doi.org/10.1016/j.geoderma.2021.115383>

Received 29 March 2021; Received in revised form 28 July 2021; Accepted 4 August 2021

Available online 16 August 2021

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and affect root growth negatively, while a low volume of $< 30 \mu\text{m}$ pores relates to a decrease in the capacity to store plant-available water. Lipiec and Hatano (2003) identified an air-filled porosity of $0.10 \text{ m}^3 \text{ m}^{-3}$ as a critical limit for soil aeration. In addition to the pore-size distribution, the degree of pore continuity or pore organization is an important parameter for soil aeration as well as infiltration of water and thus crop growth (Schjøning et al., 2007).

Several soil physical and biological properties, e.g., bulk density, aggregate stability and soil microbial biomass are related to the content and turnover of soil organic matter (SOM). Organic matter and clay are intimately linked by a range of physical, chemical and biological processes, playing a crucial role in the formation of soil aggregates, affecting stability at different scales (Totsche et al., 2018). Thus, it is vital to include both clay and organic matter when identifying critical thresholds for soil functioning. In particular, the content of soil organic C (SOC) interacting with clay is of critical importance in determining soil physical behavior. Dexter et al. (2008b) identified a critical threshold in soils, where clay/SOC ratio values below 10 had higher soil structural stability and were less impacted by management practices (Jensen et al., 2017; Jensen et al., 2019). Among soil biological properties, earthworm abundance can be used as an indicator of soil quality; earthworms play an important role in the transformation of litter and in the formation of soil aggregates, and respond to several agricultural practices (Pulleman et al., 2012).

In cultivated systems, factors such as crop rotation, cover crops, fertilizer applications and tillage events have been found to influence soil functions by affecting both physical and biological properties (Riley et al., 2008; Munkholm et al., 2013). Riley et al. (2008) found adding leys into the crop rotation improved soil structure, increased aggregate stability and reduced bulk density. In addition, the inclusion of at least one year of ley provides favorable conditions for proliferation of earthworms, especially when ley cuts are mulched, resulting in greater cast production that contributes to formation of SOM and availability of nitrogen (N) (Froseth et al., 2014). In a similar way, the inclusion of legume-based cover crops was shown to have a positive effect on soil structure (Munkholm et al., 2013), to increase SOC concentration, soil microbial biomass and mycorrhiza colonization and to reduce bulk density (Daryanto et al., 2018).

The formation of SOM is largely dependent on the quality and amount of organic material inputs. Thus, adequate fertilization is crucial to promote plant production and the resulting return of C (and N) in residues to the soil, as well as the stabilization of C in soils by increasing the availability of nutrients for microbial processes (Kirkby et al., 2014). Since fertilization with animal manure adds organic matter to the soil, it generally leads to greater SOC as well as lower soil bulk density and greater content of soil microbial biomass C compared to mineral fertilizers (Edmeades, 2003; Schjøning et al., 2007).

Changes in SOC as a consequence of different management practices require time, thus long-term crop rotation experiments are valuable tools to assess effects that would not be detectable in the short term (Autret et al., 2016). In a previous study, based on the long-term crop rotation experiment in Foulum, the temporal variation in SOC was investigated (Hu et al., 2018). We continue and build upon this long-term crop rotation experiment by investigating additional soil quality aspects, including chemical, physical and biological properties. The overall goal of this study is to assess the long-term soil quality effects of the use of cover crops, animal manure, different crop sequences (with or without a legume-based ley) and organic vs conventional management. We hypothesized that soil physical properties will be affected only to a limited extent by different management strategies, due to the low clay and high SOC content in the study soil (clay/SOC = 4) at the start of the experiment. In addition, we hypothesized that earthworms will benefit from the inclusion of legume-based ley and cover crops, and that differences in nutrient management (e.g., mineral fertilization, use of animal manure) will induce differences in the content of available P, K and Mg in soil, arising from variations in crop and nutrient management.

2. Material and methods

2.1. Study site

Soil samples and earthworm counts were acquired in 2019, at the end of the fifth cycle of the long-term crop rotation experiment initiated in 1997 at Foulum, Denmark ($56^\circ 30' \text{ N}$, $9^\circ 34' \text{ E}$). The study site is characterized by a temperate oceanic climate (Cfb in the Köppen classification), with an average annual temperature of 8.7° C and average cumulative precipitation of 749 mm per year, in the period from 2015 to 2018 (fifth cycle of the experiment). According to the Danish 30-year climate normal (1981–2010), the average annual temperature is 8.3° C and the average cumulative annual precipitation is 746 mm across Denmark (Cappelen, 2019). The soil is classified as a Mollic Luvisol according to FAO WRB, and it is a sandy loam with 90 g clay ($< 2 \mu\text{m}$) kg^{-1} soil, 130 g silt ($2\text{--}20 \mu\text{m}$) kg^{-1} soil and 780 g sand ($> 20 \mu\text{m}$) kg^{-1} soil, and an initial average content of SOC of 23 g kg^{-1} soil in the top 25 cm (Djurhuus and Olesen, 2000). Thus, the clay/SOC ratio at the start of the experiment was approximately 4.

2.2. Experimental design and management

The experiment started in 1997 and, as described by Djurhuus and Olesen (2000) had a factorial randomized block design with two blocks, each divided into two sub-blocks (Fig. 1). In its current setup, the experiment includes two organic cropping systems and one conventional cropping system with 4-year crop rotations. All crops in all systems were represented every year in the experiment. During the fifth cycle of the experiment (2015–2018), the crop sequence included spring barley (*Hordeum vulgare* L.), spring wheat (*Triticum aestivum* L.) and spring oats (*Avena sativa* L.) as cereal crops. The crop sequence differed in the two organic systems for the inclusion of either grass-clover as a green manure ley (OGM) or faba bean (*Vicia faba* L.) as grain legume (OGL). In OGM, grass-clover was a mixture of perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) and it was established by undersowing it in spring barley. The conventional system (CGL) had the same crop sequence as OGL (Table 1).

The plots were 12 m wide and 18 m long, and were each divided into four subplots ($3 \times 18 \text{ m}$) of which the two central ones have been used as harvest plots and the two side ones for additional sampling since the start of the long-term experiment (Fig. 1). In the organic systems, treatment factors were use of a legume-based cover crop mixture (CC) and animal manure (M), combined in three treatments for each OGL and OGM: +M/+CC, +M/–CC/ and – M/+CC. Plots that did not receive animal manure were amended with Patentkali®, a potash fertilizer used in organic farming, containing 30% K_2O , 10% MgO and 43% SO_3 in water-soluble forms. In the conventional system, all plots received surface applied mineral fertilizer (F, NPK 21–3–10) and the use of non-legume cover crops distinguished the two + F/+CC and + F/–CC treatments. In total, this led to eight treatments, which combined with all the crops in rotation (four) being represented every year gave 32 plots in each block. The current combination of cropping systems and treatments was introduced in 2005, as explained in detail by Hu et al. (2018). Rates of N applied to the crops in 2018 are reported in Table 1, and follow Danish national standards (Landbrugsstyrelsen, 2020).

The legume-based cover crop mixture consisted of perennial ryegrass, chicory (*Chicorium intybus* L.), white clover and red clover, and it was undersown in May in the inter-row space of organic main crops. The row spacing was 12 cm in conventional systems and 24 cm in organic systems. In the conventional system, the cover crop was either perennial ryegrass undersown in May or a mixture of fodder radish (*Raphanus sativus* L.) and winter rye (*Secale cereale* L.) sown after harvest of the main crop. All cover crop types were left in the field during winter and were terminated by harrowing in the following spring. Prior to sowing of the next main crop, cover crop biomass was incorporated into the soil by ploughing to a depth of 20 cm.

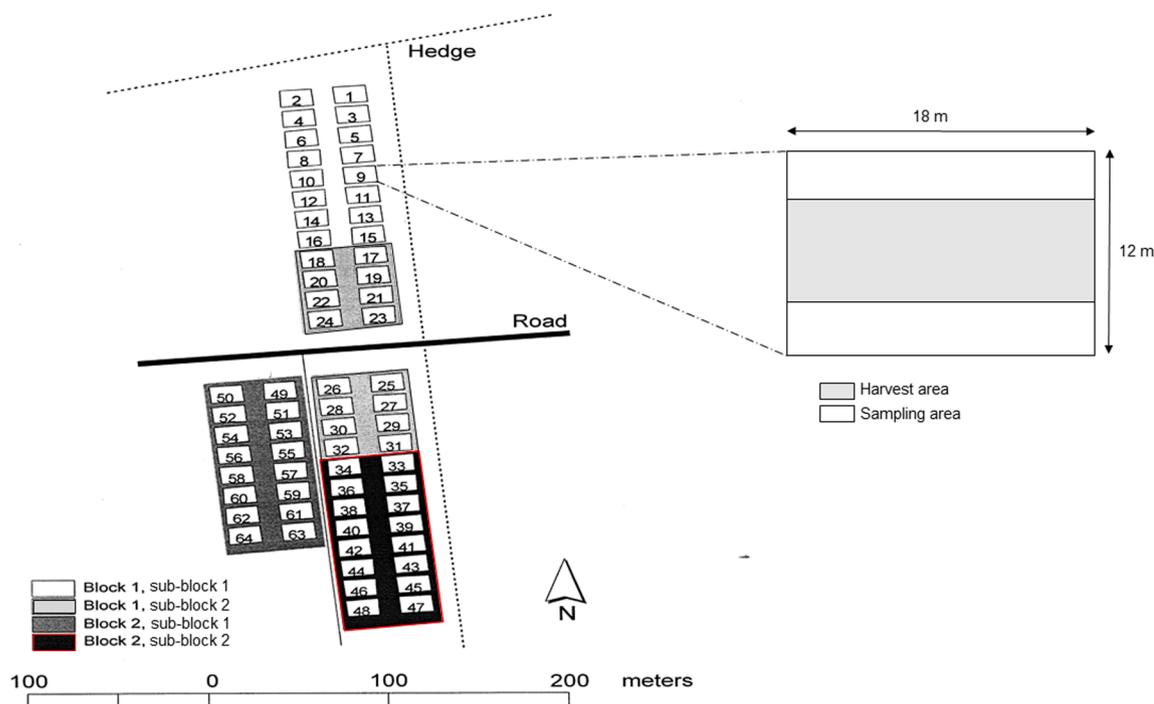


Fig. 1. Field map representing the layout of plots (white rectangles, 1–64) in blocks and sub-blocks (Djurhuus and Olesen, 2000). On the right, plot size and structure.

Table 1

Crop sequences and application rates of nutrients in animal manure (OGM and OGL) and mineral fertilizer (CGL). In OGM and OGL, -M treatments received K and Mg in the rates reported in brackets.

Cropping system	Crop sequence	Total N	P	K	Mg
OGM	Oat ^{CC}	75	13	62 (50)	10 (12)
	Spring barley:Grass clover	85	15	70 (50)	11 (12)
	Grass clover	–	–	149 (75)	36 (18)
	Spring wheat ^{CC}	105	18	86 (50)	14 (12)
OGL	Oat ^{CC}	75	13	62 (50)	10 (12)
	Spring barley ^{CC}	85	15	70 (50)	11 (12)
	Faba bean ^{CC}	–	–	75 (75)	18 (18)
	Spring wheat ^{CC}	105	18	86 (50)	14 (12)
CGL	Oat ^{CC}	85	17	71	6
	Spring barley ^{CC}	120	15	56	6
	Faba bean ^{CC}	–	20	104	6
	Spring wheat ^{CC}	135	17	63	7

OGM = Organic with Green Manure; OGL = Organic with Grain Legume; CGL = Conventional with Grain Legume; ^{CC} = position of cover crops in + CC treatments.

After harvest, cereal straw was removed from all the plots, while management of grass-clover cuttings (four in 2018) varied between + M and -M treatments, as explained in detail by Brozyna et al. (2013). Briefly, grass-clover residues were left on the field (mulched) in -M treatments, in order to increase N availability in the absence of external N inputs. Conversely, grass-clover cuttings were removed from + M

plots, to simulate a scenario where the residues were used for biogas production and the digested material was redistributed as a fertilizer for the main crops. The residue management practices had varied in the prior rotation cycles, where a larger part of the cereal straw was returned (Hu et al., 2018).

Field operations in the 2018–2019 cropping season included harrowing and ploughing in spring in all the plots, except the ones with grass-clover. Weed control in organic systems was performed by inter-row hoeing in spring, followed by inter-row hoeing in the cover crops after harvest of the main crops, while organic plots without cover crops were harrowed twice after harvest of grain crops, in early September and October. Weeds, pests and diseases in conventional systems were controlled with pesticides according to recommended practices.

2.3. Crop yield

Since the beginning of the long-term crop rotation experiment, data on yield and other crop productivity measures have been collected (Shah et al., 2017; De Notaris et al., 2018; Pullens et al., 2021). Grain yield of cereal and grain legumes were determined by harvesting two sub-plots of each plot using a plot combine harvester. Dry matter in grains was determined by near-infrared spectroscopy (InfratecTM 1241 Grain Analyzer, Foss A/S). Samples of total aboveground biomass were taken in two 0.5 m² sample areas in each plot 1–2 weeks prior to crop harvest in the cereals and grain legumes. Samples of total aboveground biomass in the grass-clover ley were taken in two 0.5 m² sample areas in each plot at each cut. Samples of aboveground biomass of cover crop and weeds were taken around 1 November in two 0.5 m² plots in each plot.

2.4. Soil sampling

Soil for physical and chemical analyses was sampled in March 2019, before the field operations for the new season were performed. For physical analyses, undisturbed soil cores (100 cm³) were extracted from the 6–10 cm soil layer, and a minimally-disturbed soil cube (650 cm³) was sampled from the 6–13 cm layer. Three soil cores and one cube were

extracted from each plot and stored at 2 °C until analyses. The three soil cores were used for measuring water retention and air permeability at –100 hPa matric potential as well as bulk density; for each plot, we used an average of the three. The soil cube was used for measuring wet stability of aggregates. Soil from the cubes was carefully fragmented by hand and left to air-dry. For chemical analyses, 16 soil cores (2 cm diameter) were sampled from each plot from the 0–25 cm soil layer. The composite sample consisting of 16 soil cores per plot was dried at 40 °C, mixed and sieved < 2 mm prior to chemical analyses.

2.5. Physical analyses

Wet stability of aggregates was determined at plot level by means of a wet sieving apparatus (Eijkelkamp Agrisearch Equipment, the Netherlands) using soil from the cubes and as described by Kemper and Rosenau (1986). Briefly, 4 g of 1–2 mm air-dry aggregates were transferred to a sieve with 250 µm openings and rewetted with a vaporizer. Subsequently, the sieve was moved up and down in artificial rainwater (0.012 mM CaCl₂, 0.150 mM MgCl₂, and 0.121 mM NaCl; pH 7.82; EC $2.24 \times 10^{-3} \text{ S m}^{-1}$) for 3 min (34 cycles min⁻¹; stroke length 13 mm). Wet stability of aggregates was calculated as the fraction of water stable aggregates (WSA) remaining on the sieve and corrected for mineral particles > 250 µm (sand particles).

Undisturbed soil cores were placed on top of a tension table and saturated with water from beneath after which soil water retention was determined at –100 hPa matric potential (Dane and Hopmans, 2002). The soil cores were oven-dried and bulk density calculated. Soil porosity was estimated from bulk density and a particle density of 2.65 g cm⁻³. A particle density of 2.65 g cm⁻³ is typically being used for arable mineral soils and corresponds to the mean particle density found in a study, including soils distributed throughout Denmark (Schjøning et al., 2017). Next, the volume of pores with tube-equivalent diameter > 30 µm (air-filled at –100 hPa) reflecting structural porosity was calculated as the difference between porosity and the water retained at –100 hPa. The volume of pores < 30 µm reflecting textural porosity corresponds to the water retained at –100 hPa. Air permeability was measured using the cores adjusted to –100 hPa matric potential according to Schjøning and Koppelgaard (2017). Pore organization was calculated from air permeability and air-filled porosity.

2.6. Chemical analyses

Total C and N content was determined on 1 g dried sieved soil by dry combustion using a Vario Max Cube (Elementar Analysensysteme GmbH, Hanau, Germany). Olsen extractable P (Olsen et al., 1954) was measured by extracting 1 g dried soil with 20 ml of 0.5 M NaHCO₃ (pH 8.5) for 30 min at 20 °C according to Banderis et al. (1976). The concentration of P in the clear extract was measured colorimetrically using the molybdate blue method for water samples (ISO, 2004) after appropriate dilutions and adjustments of pH. Soil pH was determined with a glass-electrode in a 1:2.5 w/v suspension of dried sieved soil and 0.01 M CaCl₂ 1 h after mixing the soil and the solution.

Exchangeable potassium (K) and magnesium (Mg) were measured in the filtrate after shaking 10 g dried sieved soil in 100 ml 0.5 M Ammoniumacetate (CH₃COONH₄) solution for 30 min. The concentration of K in the extract was determined by continuous flow analysis with Atomic Emission Spectroscopy for detection and the concentration of Mg was determined by Atomic Absorption Spectroscopy.

2.7. Earthworm density

In August 2019, earthworms were counted in two 0.25 × 0.25 m² micro-plots within each plot using a mustard solution, which works as an expellant and allows to extract earthworms from a 10–15 cm depth (Valckx et al., 2011). Briefly, half of a solution obtained by mixing 2 L of water and 20 g of ground yellow mustard was slowly poured in each

micro-plot. Earthworms coming to the surface were collected for 5 min, after which the remaining solution was poured and earthworms were collected for 5 additional minutes.

2.8. Statistical analyses

Statistical analyses and data exploration, including visual investigation with multi-panel dot plots and boxplots (Zuur et al., 2010), were performed using R (R Core Team, 2016). The effects of treatment factors on physical, chemical and biological soil properties measured in 2019 and crop yield were tested by analysis of variance (ANOVA), after organizing the full data set into relevant subsets. In particular, the effect of crop sequence was tested by comparing OGL and OGM (rotation), the effect of organic vs conventional was tested by comparing OGL + M and CGL (system), the effect of cover crops was tested on a subset excluding –M treatments and the effect of manure (only relevant for OGL and OGM) was tested on a subset excluding –CC treatments. This minimized the possible confounding effects derived by an incomplete factorial design.

The assumptions of normality and homoscedasticity were checked with the Shapiro-Wilk test and visual examination of the residuals against fitted values. Logarithmic (ln) transformation was performed on air permeability and pore organization to yield normality. Boxplots and the boxplot.stats()\$out function were used to identify outliers; we identified one for air permeability and two for pore organization, which were removed. For earthworm density, we excluded measurements from OGL plots cropped with oat in 2019, since exceptional tillage was performed in August to manage weeds.

In its original layout, the experimental field was arranged into two blocks each divided into two sub-blocks, to account for varying initial conditions in SOM (C and N content) in the 0–25 cm soil layer (Djurhuus and Olesen, 2000). In each block, all the treatments with fertilization (+M or +F) and +CC were placed in the same sub-block, while the other treatments were placed in the other sub-block; treatments were randomized within each sub-block. Prior to testing the effects of treatments on soil properties, we tested the effect of block and sub-block, which were significant for soil chemical parameters and were thus included in the subsequent analyses. In line with what already observed at the beginning of the experiment (Djurhuus and Olesen, 2000), soil physical properties were not significantly affected by sub-block. For the relevant subsets, we tested the interaction effect between cropping system and cover crop, manure and preceding crop and, when significant, a post hoc comparison was performed using the Tukey HSD test.

As an example of the models used, the effect of rotation (OGL vs CGL) on SOC was tested as:

$$dt1 \leftarrow dt[dt\$Rotation != "OGM",]$$

$$\text{summary}(m1 \leftarrow \text{aov}(SOC \sim \text{Rotation} + \text{Block} \cdot \text{SubBlock}, \text{data} = dt1))$$

Changes in SOC occur over several years, thus the effect of long-term management can be assessed by comparing a specific treatment to a reference at a given time (e.g., use of cover crops compared to no cover crops). However, due to the initial variability in SOC content at our field site (Djurhuus and Olesen, 2000), we investigated the change in SOC content in time, i.e. in 2004, 2008, 2012, 2016 and 2019, for each treatment to get a better understanding of the differences observed in 2019.

Possible correlations among different soil properties were assessed with Pearson correlation coefficients, using the cor.test function of the R Stats package. For all statistical tests we used $\alpha = 0.05$.

3. Results

3.1. Effects of management on crop productivity

Based on the yield data collected throughout the fifth cycle of the crop rotation (2015–2018), the average dry matter yield of cereal grain crops followed the order CGL > OGM > OGL ($p < 0.001$), with 5.8, 4.8

and 4.4 Mg ha⁻¹ year⁻¹, respectively (for better comparison, in the organic systems this includes + M treatments only). Within OGL, manure application and use of cover crops increased the average cereal grain yield by 0.8 and 0.4 Mg ha⁻¹ year⁻¹, respectively, while in OGM only by 0.4 and 0.05 Mg ha⁻¹ year⁻¹, respectively. In the latter system, the grass-clover produced an average dry matter of 16.5 Mg ha⁻¹ year⁻¹ aboveground biomass (sum of three to four cuts per year), which was mulched in the -M treatment and removed from + M. Across the full rotation, aboveground cover crop biomass, sampled every year in early November, ranged from an average of 1.1 Mg ha⁻¹ year⁻¹ in CGL to 1.5 Mg ha⁻¹ year⁻¹ in OGM -M, with no significant differences among treatments.

3.2. Effects of management on soil chemical properties

At the end of the fifth crop rotation cycle, in spring 2019, the content of SOC was on average 22 g kg⁻¹ soil, with no statistically significant difference between cropping systems and in relation to manure application, but with a positive effect of cover crop ($p < 0.05$) (Fig. 2). Overall, the lowest average SOC was measured in CGL without cover crops (20 g kg⁻¹ soil) and the greatest in OGM with cover crops (24 g kg⁻¹ soil) (Table 2).

When looking at the development of SOC since November 2004, the different treatments followed similar trends, with a general decline in SOC until 2016 (Fig. 3). The only significant variation in SOC content from 2004 until spring 2019 was observed for OGL + M + CC, where SOC increased by 2 g kg⁻¹ soil, going from 19 to 21 g kg⁻¹ soil ($p < 0.05$). In 2019, soil total N followed a similar trend as SOC (Table 2). Thus, soil C/N ratio was similar across treatments, with an average of 12.

Soil pH was on average 5.6, being higher in OGL than in both OGM and CGL ($p < 0.001$), but no significant effect of other treatment factors was observed (Tables 2 and 3). Available P was higher in CGL than in OGL ($p < 0.001$). Treatments without cover crops had a higher available P than the ones + CC ($p < 0.05$) and, in the organic systems, -M treatments had a significantly lower P than + M ($p < 0.001$). Available K was generally higher in OGL than in OGM ($p < 0.001$), and -M treatments had a higher K content than + M treatments ($p < 0.01$). Available Mg was higher in OGL than in CGL ($p < 0.001$), and -M treatments had higher Mg than + M ($p < 0.05$).

Table 2

Soil properties for the different treatments. Data reported are mean values, and numbers in brackets are standard errors ($n = 8$).

Treatment			SOC	STN	pH	P	K	Mg
			g kg ⁻¹ soil			mg kg ⁻¹ soil		
OGM	+M	+CC	24	1.91	5.55	3.4	73	62
			(1)	(0.06)	(0.05)	(0.1)	(4)	(4)
	-CC		21	1.72	5.45	3.6	81	63
			(1)	(0.07)	(0.04)	(0.2)	(5)	(4)
	-M	+CC	21	1.78	5.58	2.8	122	64
			(1)	(0.10)	(0.04)	(0.1)	(7)	(5)
OGL	+M	+CC	21	1.72	5.65	3.4	114	59
			(1)	(0.08)	(0.04)	(0.1)	(6)	(3)
	-CC		23	1.81	5.76	3.8	138	64
			(1)	(0.05)	(0.02)	(0.2)	(5)	(2)
	-M	+CC	23	1.78	5.62	2.7	100	74
			(1)	(0.05)	(0.04)	(0.1)	(7)	(3)
CGL	+F	+CC	22	1.75	5.51	3.9	113	46
			(1)	(0.05)	(0.04)	(0.1)	(3)	(2)
	-CC		20	1.61	5.39	4.3	125	41
			(1)	(0.10)	(0.02)	(0.1)	(7)	(1)

OGM = Organic with Green Manure; OGL = Organic with Grain Legume; CGL = Conventional with Grain Legume; M = Manure; F = mineral Fertilizer; CC = Cover Crop; SOC = Soil Organic Carbon; STN = Soil Total Nitrogen.

3.3. Effects of management on soil physical properties

Water stable aggregates ranged from a minimum value of 80.5 to a maximum of 90.8 %. Notably, there was no significant difference in aggregate stability between organic and conventional systems (OGL vs CGL), different crop sequences (OGL vs OGM) and in organic treatments with and without animal manure (Fig. 4). Use of cover crops increased aggregate stability, with an average of 87.1 % WSA in fertilized treatments with cover crops and 85.7 % without cover crops ($p = 0.06$).

Bulk density was significantly greater in CGL compared to OGL, with average values of 1.33 and 1.25 g cm⁻³ ($p < 0.01$), respectively. This difference was mainly related to the bulk density in OGL - CC being lower compared to CGL-CC (Table 4). Use of cover crops increased the fraction of soil volume represented by pores < 30 μm ($p < 0.01$), which was on average 0.28 and 0.26 m³ m⁻³ in fertilized (+F and +M) + CC treatments and -CC, respectively. However, in the organic systems the

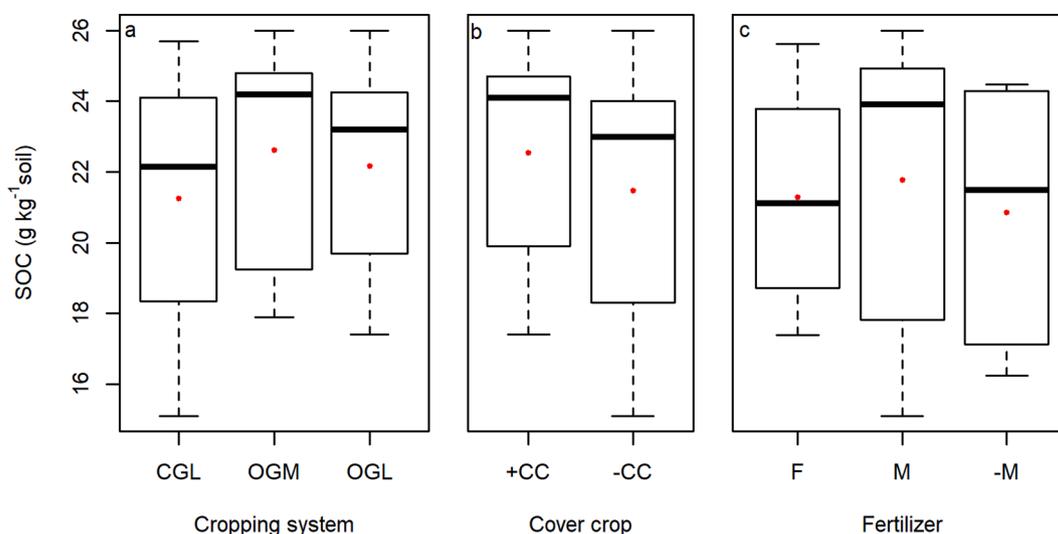


Fig. 2. Soil organic carbon (SOC, g kg⁻¹ soil) for (a) the different cropping systems, (b) with cover crop or without and (c) fertilizer types. For balanced comparisons, only treatments + M were included for OGM and OGL in (a) and (b), and only treatments + CC were included in (c). Red dots indicate mean values. Lines within the boxes represent median values, box boundaries include the 25th and 75th percentiles, and the whiskers extend from the box boundary to the largest and smallest values no further than 1.5 times the inter-quartile range. OGM = Organic with Green Manure; OGL = Organic with Grain Legume; CGL = Conventional with Grain Legume; CC = Cover Crop; M = Manure; F = mineral Fertilizer.

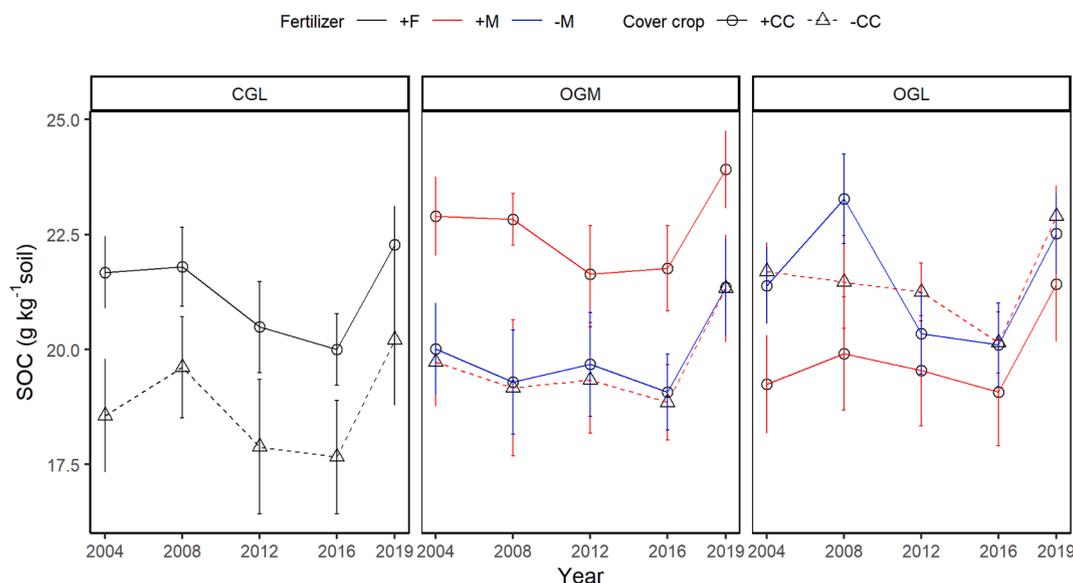


Fig. 3. Evolution of soil organic carbon (SOC, g kg⁻¹ soil) at treatment level. Data reported are mean values, and error bars represent standard errors (n = 8). OGM = Organic with Green Manure; OGL = Organic with Grain Legume; CGL = Conventional with Grain Legume; F = mineral Fertilizer; M = Manure; CC = Cover Crop.

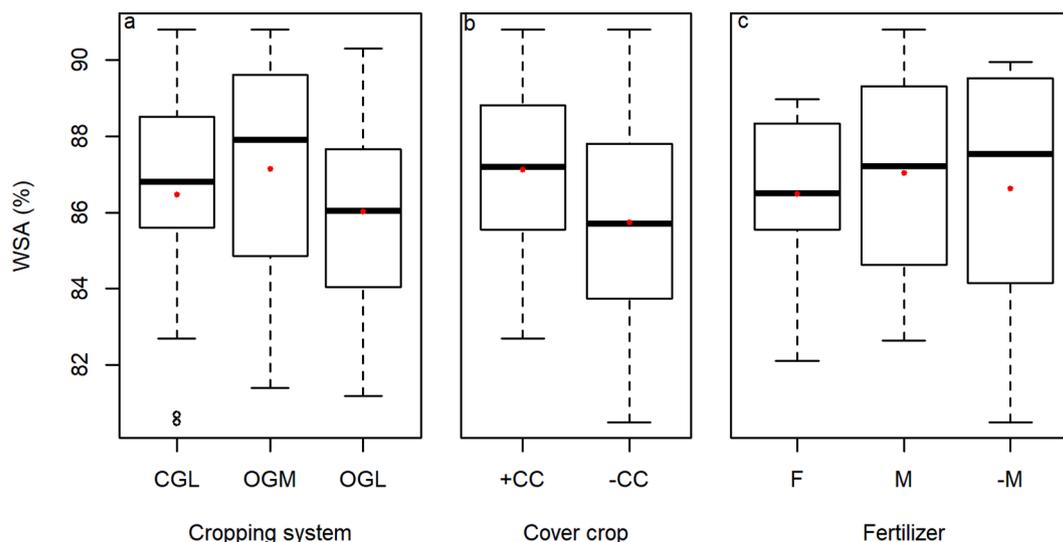


Fig. 4. Water stable aggregates (WSA, %) for (a) the different cropping systems, (b) with cover crop or without and (c) fertilizer types. For balanced comparisons, only treatments + M were included for OGM and OGL in (a) and (b), and only treatments + CC were included in (c). Red dots indicate mean values. Lines within the boxes represent median values, box boundaries include the 25th and 75th percentiles, and the whiskers extend from the box boundary to the largest and smallest values no further than 1.5 times the inter-quartile range. OGM = Organic with Green Manure; OGL = Organic with Grain Legume; CGL = Conventional with Grain Legume; M = Manure; F = mineral Fertilizer; CC = Cover Crop; WSA = Water Stable Aggregates.

opposite effect was observed for $> 30 \mu\text{m}$ pores, which were reduced by the use of cover crops ($p < 0.05$). In addition, OGL - CC had a significantly greater fraction of soil volume represented by pores $> 30 \mu\text{m}$ than CGL - CC ($p < 0.001$) (Table 4).

Overall, OGL was the system with the greatest air permeability and pore continuity, with average values of 43 and 168 μm^2 , respectively, at a cropping system level. Average air permeability and pore continuity in CGL were 26 and 108 μm^2 , respectively, and 26 and 100 μm^2 in OGM. The lowest average values were found for the + M + CC treatment in OGM. Differently than for the other physical parameters, both air permeability and pore continuity were significantly affected by the crop grown in the previous year ($p < 0.05$) (Table 5), and were the greatest following spring wheat.

3.4. Effects of management on earthworm density

Earthworm density recorded in August 2019 was overall greater in OGM compared with OGL ($p < 0.01$), and use of cover crops had a general positive effect ($p < 0.05$) (Fig. 5). The greatest density of earthworms was recorded in plots with grass-clover in 2019, with an average of 258 earthworms m^{-2} , which was significantly greater than following other main crops ($p < 0.001$).

3.5. Correlations between soil physical properties and organic carbon

Bulk density was negatively correlated to SOC in CGL ($p < 0.001$) and OGL ($p < 0.01$) (Fig. 6), with a greater bulk density in the conventional than in the organic system. In OGM, the greatest bulk density values were associated with grass-clover leys, especially at the lower end

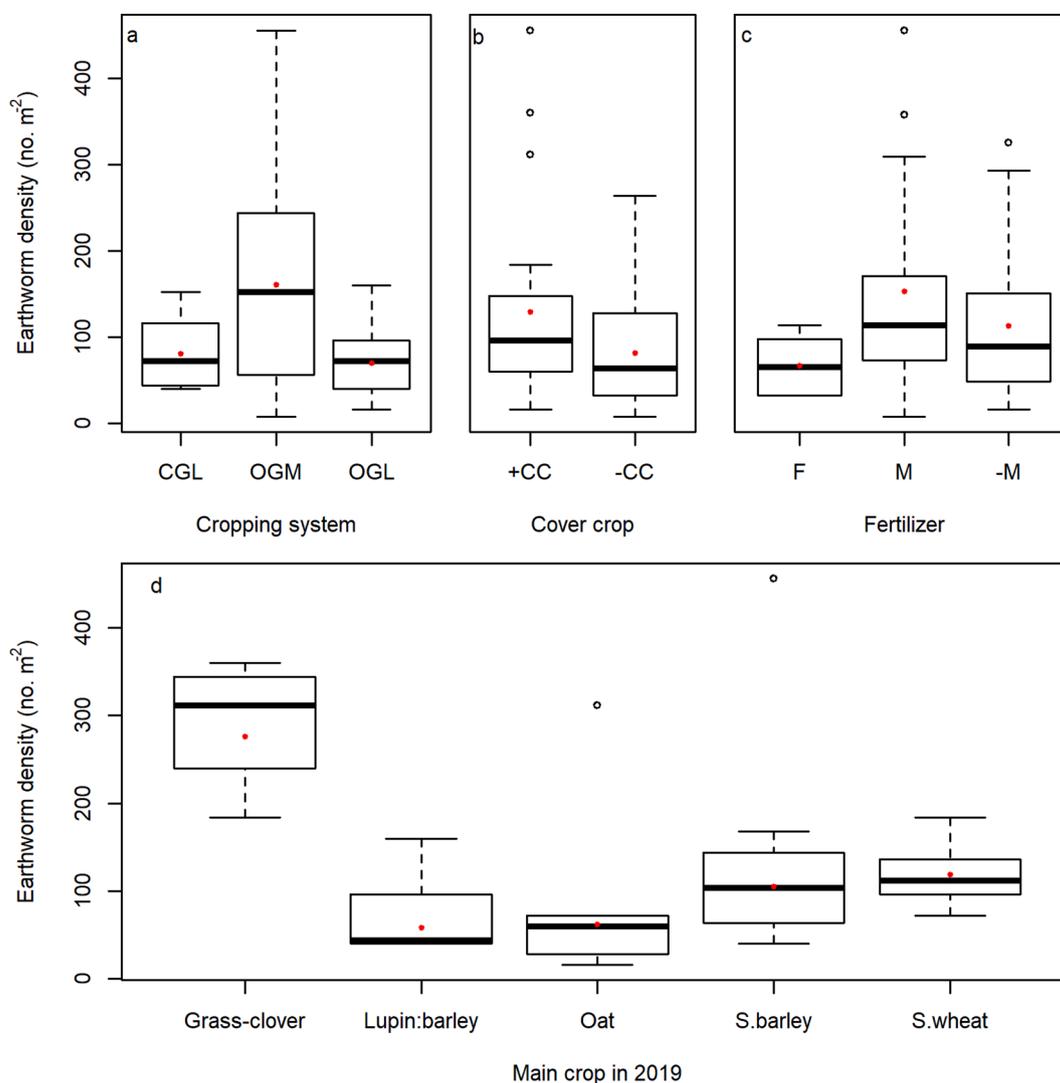


Fig. 5. Earthworm density (number m^{-2}) for (a) the different cropping systems, (b) with cover crop or without, (c) fertilizer types and (d) main crop. For balanced comparisons, only treatments + M were included for OGM and OGL in (a) and (b), and only treatments + CC were included in (c). Red dots indicate mean values. Lines within the boxes represent median values, box boundaries include the 25th and 75th percentiles, and the whiskers extend from the box boundary to the largest and smallest values no further than 1.5 times the inter-quartile range. OGM = Organic with Green Manure; OGL = Organic with Grain Legume; CGL = Conventional with Grain Legume; M = Manure; F = mineral Fertilizer; CC = Cover Crop.

of SOC concentration. Aggregate stability (WSA), air permeability and pore organization did not change as a function of SOC (Fig. 7), while porosity was correlated to SOC, but to a different extent based on cover crop treatment (Fig. 8). The use of cover crops influenced the correlation between porosity and SOC, with porosity $< 30 \mu m$ increasing more markedly with increasing SOC in + CC treatments than -CC. On the other hand, porosity $> 30 \mu m$ was not correlated with SOC in + CC treatments while there was a positive correlation in -CC treatments, even though it was not strong (Fig. 8).

4. Discussion

4.1. Effect of management on SOC

We investigated the long-term effects of using cover crops, different crop sequences and animal manure on soil quality in a field experiment initiated in 1997, where the current treatment setup started in 2005. However, the OGM and OGL treatments had been maintained since 1997. The average SOC in the top 25 cm soil layer was $23 g kg^{-1}$ soil in November 1996 (Djurhuus and Olesen, 2000), which had decreased to an average of $21 g kg^{-1}$ in November 2004. As described by Djurhuus

and Olesen (2000), the experiment was laid out over an area covering two fields with different long-term management histories. Thus, the SOC content measured at the start of the experiment in the topsoil varied from 19 to $25 g kg^{-1}$ soil in different areas of the field experiment. This was reflected in the statistically significant effect of block and sub-block on SOC and soil N observed in 2019. Based on the results from spring 2019, at the end of the fifth cycle of the crop rotation experiment we observed small and inconsistent differences in SOC and soil N between long-term treatments, mostly reflecting the initial soil variation (Fig. 3). The temporal development in SOC showed small variations for all treatments; the relatively constant SOC concentration throughout the period indicates a situation of steady state for all managements. Based on the low clay/SOC ratio at the beginning of the experiment, we expected only a limited potential for increase in SOC (Six et al., 2002). Importantly, none of the treatments we investigated led to a significant decline in SOC after 14 years, with even the conventional treatment without cover crops being able to maintain its initial SOC concentration. As highlighted by Schjøning et al. (2002), the comparison between conventional and organic systems should not be treated as a “black box”, since the observed effects are the result of several interacting management factors. In our study, all the treatments had diversified crop

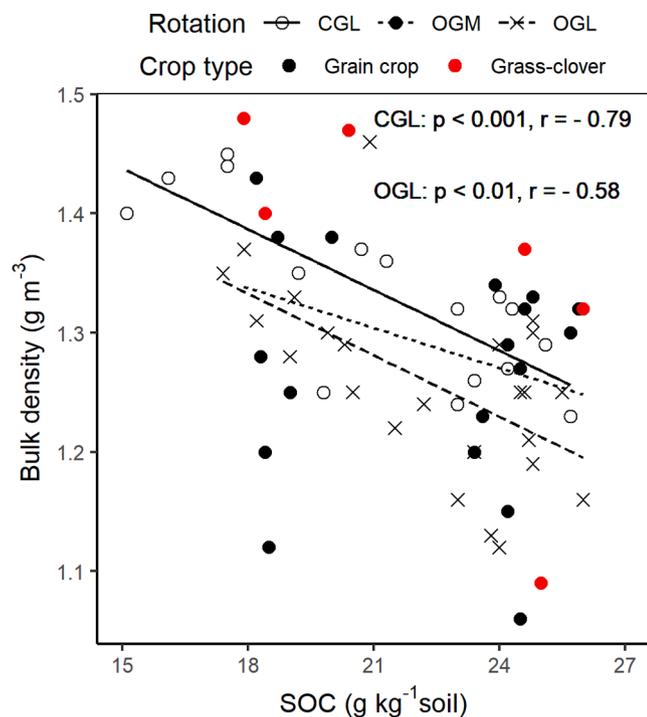


Fig. 6. Soil bulk density (g cm^{-3}) as a function of soil organic C (SOC, g kg^{-1} soil). Each data point represents one observation. For statistically significant correlations, p values and correlation coefficients are indicated (Pearson's correlation). OGM = Organic with Green Manure; OGL = Organic with Grain Legume; CGL = Conventional with Grain Legume.

rotations, and the reduced soil disturbance in conventional treatments (weed control was not performed mechanically) may have counterbalanced the lower input of organic matter in those systems. In line with [Hu et al. \(2018\)](#), different microbial activity in conventional and organic treatments could further contribute to explaining the observed results. Within the time frame investigated in this study, a lower microbial activity under conventional compared to organic management in OGM, associated with the reduced soil disturbance, could have contributed to the stability of SOC in those treatments, as also shown by [Petersen et al. \(2013\)](#) for the microbial biomass in this experiment.

4.2. Effect of management on soil physical properties

Soil physical properties are closely connected to the SOM status and, in particular, to the content of C interacting with clay, which is crucial in determining soil physical behavior. In our experiment, the initial clay/SOC ratio was approximately 4, which is well below the threshold of 10 identified by [Dexter et al. \(2008b\)](#) for a critical change in soil physical behavior. As discussed above, all our treatments maintained a fairly constant SOC concentration for 14 years, explaining the similar and high WSA we observed in 2019 across all treatments and the lack of a general correlation between WSA and SOC, which otherwise can be generally expected ([Tisdall and Oades, 1982](#)).

Overall, bulk density and porosity were affected by cropping system and use of cover crops. In line with previous studies ([Hossain et al., 2015](#); [Williams et al., 2017](#)), bulk density was negatively correlated with SOC concentration, but it tended to be greater in CGL compared to OGL at similar levels of SOC ([Fig. 6](#)). The main difference between OGL and CGL was observed for -CC treatments, which were harrowed more often in OGL for weed control in autumn compared to CGL. This could have contributed to lower soil bulk density as well as lower aggregate stability in OGL-CC compared to + CC, while increasing pores in the $> 30 \mu\text{m}$ class, in agreement with what reported for other field experiments ([Abdollahi et al., 2014](#); [Li et al., 2019](#)). However, contrasting results

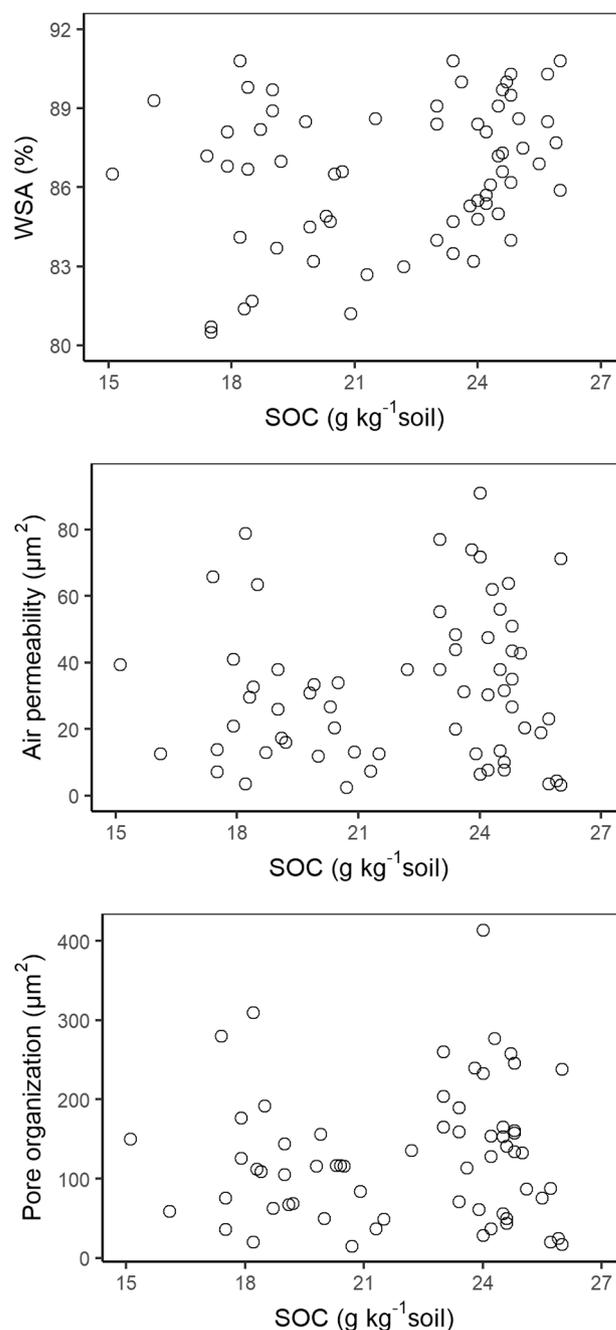


Fig. 7. (a) Water stable aggregates (WSA, %), (b) air permeability (μm^2) and (c) pore organization (μm^2) as a function of soil organic C (SOC, g kg^{-1} soil). Each data point represents one observation.

have been reported so far on the effect of tillage on soil bulk density and other parameters, with time frame and local conditions being crucial factors to consider ([Soane et al., 2012](#); [Fiorini et al., 2020](#)). Previous studies have reported mixed effects of cover crops on soil physical properties as well, depending on soil characteristics, environmental conditions and time under different management ([Blanco-Canqui and Ruis, 2020](#)). The relative distribution of different pore sizes in a given volume of soil provides a detailed indication of soil structure, as textural ($< 30 \mu\text{m}$) and structural ($> 30 \mu\text{m}$) pores are related to different aspects of soil quality. For example, structural pores are generally related to air exchange, while textural pores provide habitat for organisms and plant available water ([Liang et al., 2017](#); [Rabot et al., 2018](#)). We observed similar total porosity across different treatments but a greater

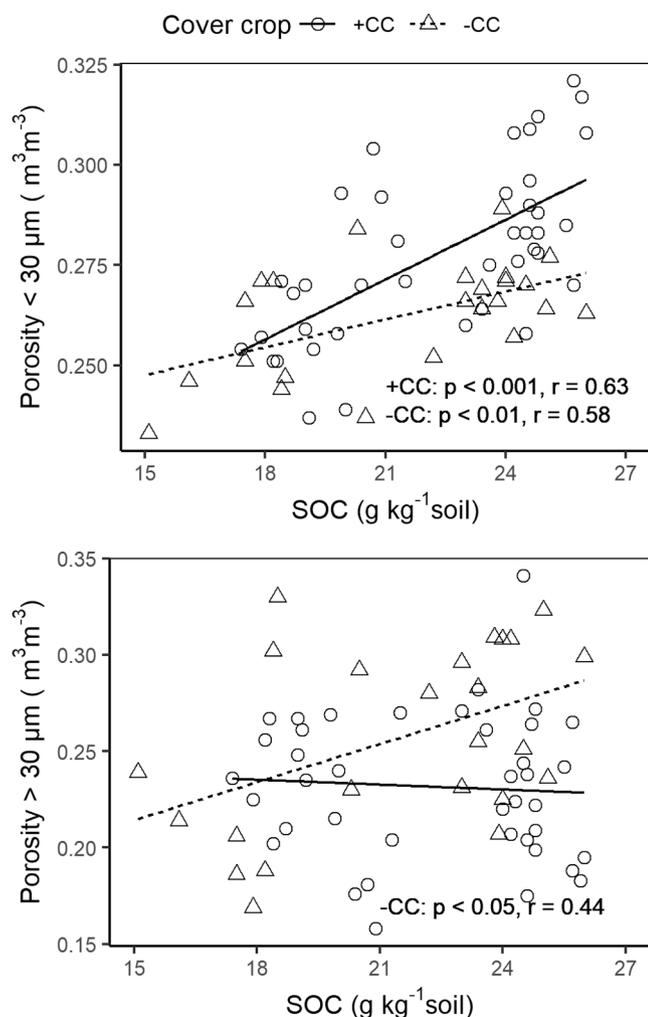


Fig. 8. (a) Soil textural porosity ($<30 \mu\text{m}$, $\text{m}^3 \text{m}^{-3}$) and (b) structural porosity ($>30 \mu\text{m}$, $\text{m}^3 \text{m}^{-3}$) as a function of soil organic C (SOC, g kg^{-1} soil). Each data point represents one observation. For statistically significant correlations, p-values and correlation coefficients are indicated (Pearson's correlation). CC = Cover Crop.

proportion of pores $< 30 \mu\text{m}$ with cover crops than without, corresponding to an increase in $> 30 \mu\text{m}$ pores in -CC treatments. However, due to the different tillage frequency in +CC and -CC treatments in the organic systems, it is not possible to isolate the effect of cover crop and tillage. Nonetheless, it has been documented by previous studies that the use of cover crops exerts a positive effect on soil porosity as well as aggregation (e.g., Villamil et al., 2006), with a synergistic effect by reduced soil disturbance (Blanco-Canqui and Ruis, 2020). In addition, methodological issues, such as structural pores being occluded by cover crop roots in intact soil cores, could have masked the positive effect of cover crops on total porosity in our study.

The organic system with a ley crop included (OGM) had the lowest air permeability and pore continuity, especially the +M +CC treatment and in plots with grass-clover ley. At sampling, in March 2019, the grass-clover ley had last experienced being plowed two years before, whereas plowing had occurred a year before in the other crops. Other studies have observed that topsoil may experience increased density when changing to a system with less disturbance, and that especially the fraction of soil volume represented by pores $> 30 \mu\text{m}$ may be reduced (Jensen et al., 2020).

4.3. Effects of management on soil biological properties, productivity and nutrient availability

Earthworms influence soil quality and structure in several ways (Bottinelli et al., 2015), and a high abundance of earthworms can be associated with good soil quality. In our study, the greatest density of earthworms was observed in OGM +CC, especially following the year of grass clover ley. This is in line with previous studies (Riley et al., 2008; Bai et al., 2018), and can be explained by the greater input (i.e. via mulching) of high quality organic matter (i.e. legumes) and the reduced soil disturbance compared to other treatments.

In terms of crop productivity, the conventional system had the greatest grain yield, with the gap between conventional and organic systems being reduced by the use of animal manure and cover crops, as also shown by previous studies (e.g., De Notaris et al., 2018). The availability of nutrients in the soil was affected in various ways by cropping system, rotation, cover crops as well as type of nutrient source (Table 3). However, a correct interpretation of these results would require long-term field nutrient balances (difference between nutrient inputs and outputs), which can be expected to drive variations in available P, K and Mg. Available P was 27 and 28 mg P kg^{-1} in the two treatments that had not received input of P in animal manure since 1997 in the OGM and OGL systems, respectively. These treatments are approaching the critical level of 20 mg P kg^{-1} guiding fertilizer recommendations in Denmark (Jordan-Meille et al., 2012). Overall, available soil P in 2019 had declined in all treatments from 54 mg P kg^{-1} , measured in 1997, when the experiment was initiated (Djurhuus and Olesen, 2000). Further studies are needed to clarify whether changes over time in available P can be explained by the P balance alone, and the potential role of other factors related to cropping system, use of cover crops and source of P.

4.4. Perspectives

All treatments investigated in this study maintained a good soil quality status, with SOC being relatively constant during 14 years under different management. As discussed above, no extreme treatments were included in the long-term experiment, which may explain why none of the soil quality parameters we assessed was compromised. In particular, all our treatments were based on crop rotations and had limited soil disturbance, and -M treatments in the organic systems were only tested with sowing of CC. However, we have little information on soil biological properties, such as macro- and microorganisms diversity and abundance. As previous studies suggested, crop and soil management can lead to different microbial composition and activity (e.g., Wang et al., 2017), which may play a crucial role in C and N dynamics in the soil, affecting nutrient availability and C stabilization (Lavallee et al., 2020). Thus, further studies should focus on microbial diversity and activity, as well as on the characterization of SOM pools, to assess how different treatments may affect C and N dynamics in our long-term field

Table 3

Analysis of variance (ANOVA) for management factors effect on soil chemical properties. For balanced comparisons, only treatments +M were included when comparing OGL and CGL and when testing the effect of cover crops, and only treatments +CC where included when testing the effect of manure (relevant for the organic systems only).

	SOC	STN	pH	P	K	Mg
System (OGL vs CGL)	ns	*	***	***	ns	***
Rotation (OGL vs OGM)	ns	ns	***	ns	***	ns
Cover crop	*	*	ns	*	0.06	ns
Manure	ns	ns	ns	***	**	*

OGL = Organic with Grain Legume; CGL = Conventional with Grain Legume; OGM = Organic with Green Manure; SOC = Soil Organic Carbon; STN = Soil Total Nitrogen. Statistical significance is indicated as * when $p < 0.05$, ** when $p < 0.01$ and *** when $p < 0.001$.

Table 4

Soil physical properties for the different treatments. Data reported are mean values, and numbers in brackets are standard errors (n = 8).

Treatment			WSA	Bulk density	Porosity			Air per.	Pore org.
					<30 μm	>30 μm	Total		
			%	g cm^{-3}	$\text{m}^3 \text{m}^{-3}$			μm^2	
OGM	+M	+CC	87.6 (1.0)	1.31 (0.04)	0.29 (0.01)	0.22 (0.02)	0.51 (0.02)	15 (6)	60 (19)
		-CC	86.4 (1.0)	1.26 (0.05)	0.26 (0.01)	0.26 (0.02)	0.52 (0.02)	33 (7)	118 (20)
	-M	+CC	87.5 (1.1)	1.31 (0.03)	0.27 (0.01)	0.23 (0.01)	0.51 (0.01)	31 (6)	126 (25)
OGL	+M	+CC	87.0 (0.8)	1.31 (0.02)	0.27 (0.01)	0.24 (0.01)	0.51 (0.01)	45 (11)	192 (46)
		-CC	84.7 (0.4)	1.19 (0.02)	0.26 (0.00)	0.29 (0.01)	0.55 (0.01)	52 (9)	176 (26)
	-M	+CC	86.4 (1.1)	1.28 (0.03)	0.29 (0.00)	0.23 (0.01)	0.52 (0.01)	31 (6)	136 (22)
CGL	+F	+CC	86.8 (0.7)	1.30 (0.02)	0.27 (0.01)	0.24 (0.01)	0.51 (0.01)	29 (7)	117 (31)
		-CC	86.2 (1.3)	1.37 (0.03)	0.26 (0.01)	0.22 (0.01)	0.48 (0.01)	23 (6)	99 (22)

OGM = Organic with Green Manure; OGL = Organic with Grain Legume; CGL = Conventional with Grain Legume; M = Manure; F = mineral Fertilizer; CC = Cover Crop; WSA = Water Stable Aggregates.

Table 5

Analysis of variance (ANOVA) for management factors effect on soil physical properties. For balanced comparisons, only treatments + M were included when comparing OGL and CGL and when testing the effect of cover crops, and only treatments + CC were included when testing the effect of manure (relevant for the organic systems only).

	WSA	Bulk density	Porosity <30 μm	Air permeability	Pore organization
System (OGL vs CGL)	ns	**	ns	***	**
Rotation (OGL vs OGM)	ns	ns	ns	ns	**
Cover crop	0.06	ns	**	*	ns
Manure	ns	ns	ns	ns	ns
Preceding crop	ns	ns	ns	ns	*
Cover crop \times System	ns	*	ns	0.07	ns
Manure \times Rotation	ns	ns	*	ns	*

OGL = Organic with Grain Legume; CGL = Conventional with Grain Legume; OGM = Organic with Green Manure; WSA = Water Stable Aggregates. Statistical significance is indicated as * when $p < 0.05$, ** when $p < 0.01$ and *** when $p < 0.001$.

experiment, as well as resulting effects on soil physical, chemical and biological properties.

5. Conclusions

We investigated the effect of long-term management on soil quality, finding that both the present conventional and organic systems maintained good soil quality under different treatments. The insignificant change in SOC during the 14 years of different organic and conventional management resulted in high aggregate stability, porosity, air permeability and pore organization for all treatments in 2019. The results show that when soil structural stability is high, soil physical parameters, such as bulk density, air permeability and pore organization, are affected by soil and crop management, but only to a limited extent. Several management factors concur in affecting soil quality, and none of our treatments represented an extreme situation, from a system perspective. Nonetheless, we found a clear effect of crop and soil management on earthworms, which were most abundant in the organic system with grass-clover, especially following the ley year. In addition, fertilization and use of cover crops increased crop productivity, which in turn affected the contents of available P, K and Mg in soil, with available P approaching a critically low level in treatments that had not been fertilized since the start of the experiment. We conclude that when

initial soil quality is good, it can be maintained in the long-term by taking into account the combination of different factors, such as crop rotation, use of cover crops, soil cultivation and fertilization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Erling Nielsen for taking good care of the long-term experiment at Foulum during the last 25 years, and the technical staff at AU Foulum for their assistance with analysis. In addition, many thanks go to Zhi Liang for the always inspiring discussions. The study was part of the SoilCare and CCRotate projects. SoilCare was funded by the European Union HORIZON2020 Programme, while CCRotate was funded under the OrganicRD5 programme by the Green Growth and Development programme (GUDP) from the Danish Ministry of Environment and Food and coordinated by International Centre for Research in Organic Food Systems (ICROFS).

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