

Identification of soil organic carbon thresholds for sustaining soil functions in arable mineral soils from the humid temperate region

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Preface

This thesis has been submitted to the Graduate School of Science and Technology (GSST), Aarhus University (AU), in partial fulfilment of the requirements for the Doctor of Philosophy (Ph.D.) degree at the Faculty of Science and Technology, AU. The Ph.D. study was scheduled for four years and was carried out at the Department of Agroecology, AU Foulum, from 1 September 2014 to 11 January 2020 including six months' leave for doing research-based policy support and 10.5 months' paternity leave.

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Summary

Soil organic matter (SOM) of which carbon (C) is the primary component is important for soil properties and functions. Accordingly, loss of SOM is considered a main threat to sustained soil functions. Loss of soil organic C (SOC) reduces soil structural stability, which in turn increases the risk of soil erosion, surface crusting and loss of fine particles carrying pollutants. However, a major unresolved issue is to define the lower level of SOC at which essential soil functions are compromised.

The overall objective of the work presented in this thesis was to: (i) investigate whether the ratio of fine mineral particles to SOC can be used as an indicator of critically low SOC content for soil functions. A prerequisite for quantifying such threshold values is reliable values for soil texture as well as SOC and SOM. Therefore, the investigation looked at (ii) whether the conversion of loss-on-ignition (LOI) to SOC as well as the estimation of the SOC to SOM conversion factor can be improved by accounting for clay content and (iii) whether removal of SOM prior to texture analysis affects the estimation of clay- and silt-sized particles ($<2\ \mu\text{m}$ and $2\text{--}20\ \mu\text{m}$, respectively). In addition, short-term effects of changes in soil management were investigated by exploring (iv) whether soil physical properties can be restored by converting soil with low SOC levels under arable and bare fallow management to grass, and, conversely, whether soil physical properties may be compromised by converting grass with high SOC levels to arable and bare fallow management. Finally, it was investigated whether (v) the soil contents of permanganate-oxidizable C (POXC) and hot water-extractable C (HWC) were more closely related to soil physical properties than to SOC content and the ratio of mineral fines to SOC.

To achieve objective (i) soil was sampled from unique long-term field experiments at Askov (Denmark) and Highfield (Rothamsted Research, UK) with a gradient in SOC within the same field due to contrasting management but with a relatively small range in texture. These research platforms were also utilized to investigate (ii) and (iii), but were supplemented with data from a cultivated field with large variations in clay content situated at Lerbjerg (Denmark) and the long-term field experiment at Bad Lauchstädt (Germany), respectively. To achieve objective (iv) soil was sampled six years after the initiation of the Highfield Land-Use Change Experiment (Highfield-LUCE). A range of soil physical properties were determined including structural stability measured at different scales, varying in degree of disturbance and pretreatment, aggregate strength and pore-size distribution. Fractions of SOM – SOC, POXC and HWC – were also determined.

Accurate estimates of SOC can be achieved by high-temperature ($\geq 900\ ^\circ\text{C}$) dry combustion, and consequently this method is recommended. If the indirect analytical approach LOI is used for estimating SOC, the conversion is considerably improved by accounting for clay content in the conversion model. The SOC to SOM conversion factor could also be estimated by relating LOI to

SOC and clay content. The results showed that the conversion factor was close to 2 and thus larger than the conventional conversion factor 1.724. Therefore, a conversion factor of 2 is recommended.

The thesis showed that it was critical to remove SOM prior to soil texture analysis to avoid systematic errors in the estimation of clay- and silt-sized particles. Further, the importance of expressing soil texture and SOC on an oven-dry basis (105 °C, 24 h) as well as defining units on soil properties explicitly were also emphasized.

The results from the long-term field experiments showed that SOC was not a good predictor of proper soil functioning across different soil types, but that the soil-type-independent critical ratios of fine mineral particles (clay and Fines₂₀ (<20 µm)) to SOC were better indicators for soil physical condition. The critical threshold values were, however, not exactly defined and ranged from 8-13 and 16-27 for clay/SOC and Fines₂₀/SOC, respectively, and were in some cases not apparent. The Ph.D. study illustrated that threshold values depended on the measured soil physical property. The concept was more suitable for soil structural stability parameters than for aggregate strength or parameters derived from the pore-size distribution.

Six years after the initiation of the Highfield-LUCE, soil structure was affected beyond what is expected based on changes in SOC content and the ratio of mineral fines to SOC. This was presumably related to the interaction between changes in soil tillage and organic matter input, including binding agents such as plant roots and fungal hyphae. It further illustrated that critical threshold values based on the ratio between fine mineral particles and SOC should be used cautiously.

Quantification of rates of change in SOC, structural stability and soil structure in restoration and degradation scenarios showed that it was faster to lose than to restore SOC and a complex soil structure. In contrast, it was faster to restore macro-aggregate stability than to degrade it.

The results from the long-term (>50 years) and the short-term (six years) changes in management showed that POXC and HWC were neither more sensitive to management changes nor able to describe changes in soil structural stability better than contents of total SOC.

Sammendrag (Danish summary)

Jordens indhold af organisk materiale, repræsenteret ved kulstof (C), er vigtig for jordens egenskaber og funktioner. Derfor anses tab af organisk materiale fra jord som en af de alvorligste trusler mod jordens frugtbarhed. En reduktion i jordens C indhold mindsker jordens strukturstabilitet, hvilket øger jordens følsomhed over for erosion, skorpedannelse og tab af partikelbårne fremmedstoffer. Det er dog uklart, hvor meget C, der er tilstrækkelig for at sikre en god funktion af jorden.

Formålet med denne Ph.D.-afhandling var (i) at undersøge om forholdet mellem jordens indhold af mineralpartikler og C kan benyttes til at fastsætte en nedre grænseværdi for jordens funktioner. En forudsætning for at fastsætte sådanne grænseværdier er pålidelige værdier for jordens tekstur samt indhold af C og organisk materiale. Derfor var formålet også at undersøge, om (ii) inddragelse af jordens lerindhold kan forbedre omregningen af glødetab til C-indhold samt estimeringen af omregningsfaktoren fra C til organisk materiale, og om (iii) fjernelse af organisk materiale før teksturanalysen kan påvirke bestemmelsen af jordens ler- og siltpartikler (hhv. $<2\ \mu\text{m}$ og $2\text{-}20\ \mu\text{m}$). Desuden blev korttidseffekter af ændringer i landbrugets driftsforanstaltninger undersøgt. Herunder om (iv) jordens fysiske egenskaber kan genoprettes ved at omlægge ensidigt kornsædskifte og sortjordsbrak med lavt C-indhold til permanent græs, og omvendt om jordens fysiske egenskaber vil blive bragt i fare ved at omlægge permanent græs med højt C-indhold til ensidigt kornsædskifte og sortjordsbrak. Det blev også undersøgt, om (v) jordens indhold af permanganat-oxiderbart C (POXC) og varmtvands-ekstraherbart C (HWC) var bedre relateret til jordens fysiske egenskaber end C-indhold og forholdet mellem mineralpartikler og C.

For at undersøge (i) blev der udtaget jordprøver i unikke langvarige markforsøg fra Askov (Danmark) og Highfield (Rothamsted Research, England) med stor dyrkningsbetinget forskel i C-indhold inden for den samme mark med relativt ensartet tekstur. Disse forskningsplatforme blev også benyttet til at undersøge (ii) og (iii), men blev suppleret med data fra hhv. en dyrket mark med stor variation i lerindhold beliggende i Lerbjerg (Danmark) og det langvarige markforsøg fra Bad Lauchstädt (Tyskland). For at undersøge (iv) blev der udtaget jordprøver seks år efter igangsættelsen af omlægningseksperimentet fra Highfield. En række jordfysiske egenskaber blev bestemt, herunder strukturstabilitet målt på forskellig skala med varierende grad af forstyrrelse samt forbehandling, aggregatstyrke og porestørrelsesfordeling. Ud over jordens C indhold blev POXC og HWC, som er andre fraktioner af jordens organisk materiale, bestemt.

Jordens C-indhold kan bestemmes nøjagtigt ved tørforbrænding ved høj temperatur ($\geq 900\ ^\circ\text{C}$), hvorfor denne metode bør benyttes. Hvis den indirekte analysetilgang glødetab benyttes til at bestemme C-indholdet, forbedres omregningen af glødetab til C markant ved at inddrage ler i omregningsmodellen. Ligeledes kunne omregningsfaktoren fra C til organisk materiale estimeres ved at relatere glødetab til

C og ler. Resultaterne viste, at omregningsfaktoren var tæt på 2, og dermed højere end den sædvanligt benyttede omregningsfaktor på 1,724, hvorfor en omregningsfaktor på 2 anbefales.

Ph.D.-studiet viste, at det var afgørende at fjerne jordens organiske materiale inden teksturanalyse for at undgå systematiske fejl i ler- og siltbestemmelsen. Desuden blev det tydeliggjort, hvor vigtigt det var at udtrykke jordens tekstur og C-indhold på basis af ovntør (105 °C, 24 t) jord samt at definere enheder på jordegenskaberne eksplicit.

Resultaterne fra de langvarige markforsøg viste, at jordens C-indhold i sig selv ikke var et tilstrækkeligt mål for jordens tilstand på tværs af forskellige jordtyper. Derimod var de jordtypeuafhængige forhold mellem mineralpartikler (ler og ler+silt(<20 µm)) og C bedre indikatorer for jordens fysiske tilstand. Kritiske nedre grænseværdier for jordens strukturstabilitet varierede fra 8-13 og 16-27 for hhv. ler/C- og (ler+silt)/C-forholdet, og i nogle tilfælde kunne en nedre grænseværdi ikke udpeges. Ph.D.-studiet viste, at kvantificeringen af grænseværdier afhang af den målte jordfysiske egenskab. Forholdet mellem mineralpartikler og C-indhold var mere anvendeligt for jordstrukturstabilitets-parametre end for aggregatstyrke eller de afledte parametre fra porestørrelsesfordelingen.

Resultaterne fra omlægningseksperimentet i Highfield seks år efter omlægningen viste, at kombinationen af ændringer i jordbearbejdning og organisk stof tilførsel, herunder bindemidler som planterødder og svampetråde, kunne påvirke jordstrukturen ud over, hvad der ville forventes af ændringer i C-indhold og forholdet mellem mineralpartikler og C. Dette tydeliggør, at kritiske grænseværdier baseret på forholdet mellem mineralpartikler og C bør benyttes med forsigtighed.

Kvantificeringen af hvor hurtigt jordens C-indhold, strukturstabilitet og struktur ændrer sig i genopretnings- og nedbrydnings-scenarier viste, at det var hurtigere at nedbryde C og en veludviklet jordstruktur end at genoprette disse. Derimod var det hurtigere at genoprette makroaggregatstabiliteten end at nedbryde den.

Resultaterne fra de langvarige (>50 år) og de kortvarige (seks år) ændringer i driftsforanstaltninger viste, at hverken POXC eller HWC var mere påvirkelige over for ændringer i driftsforanstaltninger, og de var heller ikke i stand til at beskrive ændringer i jordens strukturstabilitet bedre end jordens totale C-indhold.

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In accordance with GSST rules, parts of this thesis were also used in the progress report for the qualifying examination.

List of supporting papers

The thesis is based on seven published papers. Throughout the thesis, the papers will be referred to by their Arabic numbers. The papers comprise **Chapter 7**.

1. **Jensen, J. L.**, Christensen, B. T., Schjønning, P., Watts, C. W., Munkholm, L. J. 2018. Converting loss-on-ignition to organic carbon content in arable topsoil: pitfalls and proposed procedure. *European Journal of Soil Science*, **69**, 604-612. doi: 10.1111/ejss.12558.
2. **Jensen, J. L.**, Schjønning, P., Watts, C. W., Christensen, B. T., Munkholm, L. J. 2017. Soil texture analysis revisited: Removal of organic matter matters more than ever. *PLoS One*, **12**, e0178039. doi: 10.1371/journal.pone.0178039.
3. **Jensen, J. L.**, Schjønning, P., Christensen, B. T., Munkholm, L. J. 2017. Suboptimal fertilisation compromises soil physical properties of a hard-setting sandy loam. *Soil Research* **55**, 332-340. doi: 10.1071/SR16218.
4. **Jensen, J. L.**, Schjønning, P., Watts, C. W., Christensen, B. T., Peltre, C., Munkholm, L. J. 2019. Relating soil C and organic matter fractions to soil structural stability. *Geoderma*, **337**, 834-843. doi: 10.1016/j.geoderma.2018.10.034.
5. **Jensen, J. L.**, Schjønning, P., Watts, C. W., Christensen, B. T., Munkholm, L. J. 2019. Soil water retention: Uni-modal models of pore-size distribution neglect impacts of soil management. *Soil Science Society of America Journal*, **83**, 18-26. doi: 10.2136/sssaj2018.06.0238.
6. **Jensen, J. L.**, Schjønning, P., Watts, C. W., Christensen, B. T., Obour, P. B., Munkholm, L. J. 2020. Soil degradation and recovery – Changes in organic matter fractions and structural stability. *Geoderma*, **364**, 114181. doi: 10.1016/j.geoderma.2020.114181.
7. **Jensen, J. L.**, Schjønning, P., Watts, C. W., Christensen, B. T., Munkholm, L. J. 2020. Short-term changes in soil pore size distribution: Impact of land use. *Soil and Tillage Research*, **199**, 104597. doi: 10.1016/j.still.2020.104597.

In addition, I have contributed to four papers within the subject of my thesis. The four supplementary papers are listed in **Chapter 8** together with other papers I have contributed to during my Ph.D. study, conference contributions and dissemination on other platforms.

In relation to printing of the thesis, **Papers 6 and 7** have been replaced by the published manuscripts.

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List of abbreviations

AIC	Akaike's information criterion
BD	Bulk density
CEC	Cation exchange capacity
COC	Complexed organic carbon
DI	Clay-SOM disintegration
DispClay _{FM}	Clay dispersibility on field-moist soil
DispClay 1-2 mm	Clay dispersibility on 1-2 mm aggregates rewetted to -100 hPa
DispClay 8-16 mm	Clay dispersibility on 8-16 mm aggregates rewetted to -100 hPa
DispFines20	Fines20 (<20 µm particles) dispersibility
<i>E</i>	Young's modulus
<i>E_{sp}</i>	Mass-specific rupture energy
<i>f</i>	Rate of change
Fines20	Mineral particles <20 µm
FTIR-PAS	Fourier transform mid-infrared photoacoustic spectroscopy
H ₂ O ₂	Hydrogen peroxide
HWC	Hot water-extractable carbon
KMnO ₄	Potassium permanganate
LFSOC	Light fraction soil organic carbon
LOI	Loss-on-ignition
LTE	Long-term experiment
LUCE	Land-Use Change Experiment
N	Nitrogen
NCC	Non-complexed clay
PAWC _{eq}	Plant-available water capacity based on a soil mass equivalent to 20 cm in the grassland soil
PD	Particle density
POXC	Permanganate-oxidizable carbon
PSD	Pore-size distribution
R ²	Coefficient of determination
RWC	Residual water content
SOC	Soil organic carbon
SOM	Soil organic matter
SSA	Specific surface area
SSS	Soil structural stability
<i>V</i> ₂	Structural void ratio
WSA _{FM}	Water-stable aggregates on field-moist soil
WSA 1-2 mm	Water-stable aggregates on 1-2 mm air-dry aggregates
<i>Y</i>	Tensile strength

1. Introduction

Soil ecosystem services are defined as “the aspects of ecosystems utilized (actively or passively) to produce human well-being” (Fisher et al., 2009). Food, biofuel and biomaterials are hence highly important ecosystem services provided by soil, on which the human population is dependent. Besides the production of these fundamental resources, the soil delivers important services such as protection of water resources by acting as a filter and mitigation of greenhouse gas emissions by sequestering CO₂ from the atmosphere. The provision of soil ecosystem services relies on the ability of the soil to perform its functions. Soil organic matter (SOM) decline is a main threat to sustained soil functions and services on a global (Amundson et al., 2015), European (Stolte et al., 2015) and Danish scale (Schjønning et al., 2009). Loss of SOM may increase in the future due to increased temperatures and stagnating yields (Wiesmeier et al., 2016). In addition, increased pressure to produce higher yields from arable land to feed the growing population poses a threat to the preservation of SOM stocks.

Assessments of changes in soil organic carbon (SOC) stocks have been conducted in several countries (e.g., Reynolds et al., 2013; Taghizadeh-Toosi et al., 2014). Under Danish conditions, the SOC stock (0-50-cm depth) on clayey agricultural mineral soils (>15 % clay) has on average decreased with 0.86 Mg C ha⁻¹ year⁻¹ in the period 1986-2009 (Taghizadeh-Toosi et al., 2014). Clayey soils are often more prone to structure-related problems so this trend is alarming. Based on measurements in the Askov long-term experiments (LTEs) in the period 1924-2016, Hu et al. (2019) and Christensen et al. (2019) showed that arable soils lost between 0.10 and 0.18 Mg C ha⁻¹ year⁻¹ (0-20-cm depth). Results from LTEs in England also show that arable soils of similar texture and with similar management lose small amounts of C annually (Poulton et al., 2018). Hence, in arable mineral soils in humid temperate regions, SOC is being lost, and without political intervention this decline is expected to continue due to increased pressures from rising global temperatures and human population growth.

1.1 Relating soil properties to soil organic matter

Soil structural stability (SSS) is an important soil property, which is highly affected by SOM. Soil structural stability is defined as the ability of soil structure to resist external stresses, mechanical and/or from water (Dexter, 1988). The effect of SOC on SSS is clearly illustrated in **Fig. 1**, where 1-2 mm air-dry aggregates with contrasting SOC contents were subjected to a simulated rainfall event. For soil with a low SOC content, the aggregates disintegrated and a surface crust was formed when the soil dried. In contrast, aggregates remained intact in the soil with a high SOC content (Jensen and Obour, unpublished data).

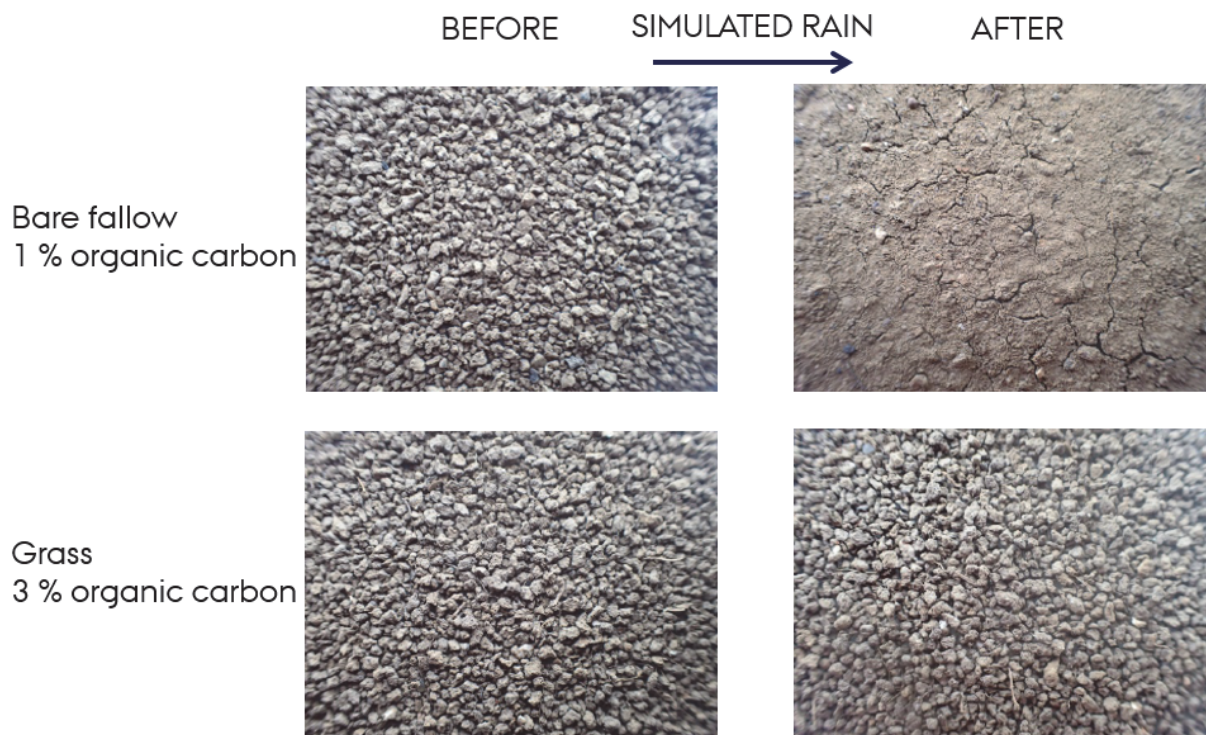


Fig. 1. The effect of soil organic carbon (SOC) on aggregate stability. The 1-2 mm air-dried aggregates are from the bare fallow (BF) and grass (G) treatment with a low and high SOC content, respectively (Highfield-LTE). The aggregates were subjected to 50 mm of simulated rain after which they were allowed to dry (Jensen and Obour, unpublished data). Photos: Johannes L. Jensen.

Hence, a low SOC content frequently results in a poor SSS, which in turn may reduce infiltration, increase surface runoff and soil erosion (Le Bissonnais, 1996) as well as the transport of fine particles carrying pollutants to the water environment (de Jonge et al., 2004; Nørgaard et al., 2013). The dispersed fine particles may also be deposited in macropores (Kjaergaard et al., 2004), which may cause internal crusting and reduce the drainage capacity of the soil. Deposition of fine particles at the soil surface and on aggregate surfaces may result in soil cementing and on drying result in hard and non-friable aggregates (Schjønning et al., 2012). Such conditions impair the potential for crop establishment and early growth and adversely affect soil aeration. Further, a seedbed with hard and non-friable aggregates requires additional energy to break down the aggregates to a desirable size, which in turn leads to further dispersion and loss of SSS. A critically low SOC content may therefore result in a vicious circle, where more and more energy is required for seedbed preparation leading to progressive deterioration of SSS.

Based on the decline in SSS at low SOC levels described above, the capacity of the soil to support crop production could be expected to be compromised. Schjønning et al. (2018) found that the amount of nitrogen (N) needed to reach optimal grain yield for winter wheat was slightly reduced at high SOC contents. However, SOC did not have any positive effect on crop yield besides this reduction in N use.

This is supported by an analysis of long-term field experiments distributed throughout Europe showing an insignificant effect of SOC on crop yield not restricted by any nutrient (Hijbeek et al., 2017). However, Hijbeek et al. (2017) found that for specific crops such as potatoes, maize and spring-sown cereals a high SOC content was related to increased optimal yield. Further, Díaz-Zorita et al. (1999) found that crop yield was compromised at low SOM levels in dry climates.

1.2 Identification of a critically low SOC content

A major unresolved issue is to define the lower level of SOC at which essential soil functions are compromised. Loveland and Webb (2003) found little evidence for any general threshold based on a review of mainly British literature on SOC and soil quality. Based on N response trials in cereal crops in Denmark, Oelofse et al. (2015) and Schjønning et al. (2018) failed to identify a critical SOC threshold. Van-Camp et al. (2004) were also unable to identify a general, lower critical SOC threshold across soil types, whereas Murphy (2015) in a comprehensive review of SOM effects on functional soil properties suggested a critically low SOC level of 0.7%. Schjønning et al. (2009) reviewed Danish studies and noted satisfactory tilth characteristics in soils with a SOC content as low as 1.2%, while another soil with 1.4% SOC showed poor tilth conditions.

The various suggestions on critically low SOC levels based on the C content *per se* illustrate very clearly the need for identifying a critical SOC threshold across soil types. Identification of a critically low SOC content would make it possible to identify risk areas and thus prevent exhaustion of vulnerable soil and instead secure soil functions and services by sustainable farm management.

Increasing evidence suggests that the fine mineral fraction (<20 or <50 μm) defines the SOC storage capacity of the soil (Wiesmeier et al., 2019). This was first observed in the seminal work by Hassink (1997), who found that although arable soils contained less SOC than corresponding grassland soils, the amounts of SOC associated with Fines₂₀ (<20 μm particles) were identical. Thus, the grassland soils seemed to have reached their SOC storage capacity. More recently, a range of studies have worked with this approach (Chen et al., 2019; McNally et al., 2017; Robertson et al., 2019; Six et al., 2002; Stewart et al., 2009). However, none of the studies have examined the potential impact of SOC saturation on soil properties and functions.

Dexter et al. (2008b) found that the amount of SOC interacting with clay (<2 μm) influenced the SSS measure, clay dispersibility, rather than the SOC content *per se*. They identified a critical clay/SOC ratio close to 10, which means that SSS was reduced when clay/SOC>10. Later studies have indicated that a clay/SOC ratio of 10 is a reasonable threshold, beyond which changes in SSS and quality may occur (Getahun et al., 2016; Johannes et al., 2017; Schjønning et al., 2012; Soinne et al., 2016). Schjønning et al. (2012) found that a Fines₂₀/SOC ratio of 20 served as a similar critical threshold value. Pieri (1992) suggested a structural stability index (S_t) defined as organic matter (OM) / Fines₂₀

$\times 100$, and by using this approach he arrived at a S_t index of 9 as a critical threshold value for tropical soils (Pieri, 1992). The S_t index has also been used in more recent papers (Pulido Moncada et al., 2015; Reynolds et al., 2009).

The actual distribution of OM on particle surfaces and in aggregates is highly complex due to the heterogeneous nature of these interactions and the various associations at different scales (Chenu and Plante, 2006; Kopittke et al., 2020; Peth et al., 2014; Schweizer et al., 2018). On top of this, OM encompasses a wide range of different components that are important at different scales (Bronick and Lal, 2005; Six et al., 2004; Tisdall and Oades, 1982). At micro-aggregate scale ($<250\ \mu\text{m}$), flocculation of clay and SOM and the gluing effect of bonding agents, e.g. excreta such as polysaccharides from plants, soil fauna and microbes, contribute to SSS. At this scale, other SOM components have been suggested as useful indicators for soil quality (Haynes, 2005). They are known as labile SOM fractions, and among them are particulate OM, dissolved OM and various extractable OM fractions such as hot water-extractable C (HWC) and permanganate-oxidizable C (POXC) (Bongiorno et al., 2019; Ghani et al., 2003). At macro-aggregate scale ($>250\ \mu\text{m}$), fungal hyphae and plant roots act as binding agents by cross-linking and enmeshing the micro-aggregates (Degens, 1997). Plant roots and fungal hyphae are hence the main contributors to SSS in macro-aggregates.

The mineral fines/carbon ratios are based on total SOC and clay or Fines₂₀ in bulk soil, and it is hence an empirical approach to quantify critical carbon levels. The concept seems useful for identifying thresholds across soil types, and it can be used operationally since existing databases contain information on mineral fines and SOC, making it possible to produce maps (Adhikari et al., 2014; Adhikari et al., 2013; Merante et al., 2017). The ratio of mineral fines to SOC can also be predicted by spectroscopy with a relatively high prediction accuracy (Hermansen et al., 2016).

1.3 Objectives and hypotheses

The overall objective of this thesis is to improve our knowledge of the mechanisms that determine the effects of SOC on soil properties and functions. More specifically, the thesis aims at quantifying the critically low SOC content required to maintain proper soil functioning of agricultural mineral soils. As discussed in **section 1.2**, a single critically low SOC threshold cannot be applied across soil types. Consequently, the overall hypothesis of this thesis is that the amount of SOC in relation to fine mineral particles – expressed as the ratio of mineral fines to SOC - constitutes a better basis than the SOC content *per se* for identifying a functional threshold for soil properties.

A prerequisite for defining critically low SOC contents in agricultural soils based on the clay (mineral particles <2 µm)/SOC and Fines₂₀ (mineral particles <20 µm)/SOC ratios is reliable values for clay, silt (2-20 µm), SOC and SOM contents. Thus, a critical evaluation and selection of appropriate methods to measure these properties will also be covered.

The following hypotheses were formulated:

1. Accounting for clay content improves the conversion of loss-on-ignition (LOI) to SOC content as well as the estimation of the SOC to SOM conversion factor (**Paper 1**).
2. Lack of removal of SOM before texture analysis induces underestimation of clay-sized particles and overestimation of silt-sized particles (**Paper 2**).
3. The ratio of mineral fines to SOC can be used as an indicator of critically low SOC contents for soil physical properties (**Papers 3, 4 and 5**).
4. Permanganate-oxidizable C (POXC) and HWC are more closely related to soil physical properties than total SOC content and the ratio between mineral fines and SOC (**Papers 4 and 6**).
5. For soils with a low SOC content, soil physical properties can be restored by converting arable and bare fallow management to grass, while for soils with a high SOC content soil physical properties may be compromised by converting grass to arable and bare fallow management (**Papers 6 and 7**).

2. Methodology

2.1 Study sites

Changes in SOC content due to soil management are a slow process. Therefore, soil samples from LTEs with contrasting fertilization and management systems were used to obtain gradients in SOC content in fields with a homogeneous topsoil texture. An advantage of using samples retrieved from LTEs is that confounding effects of variations in soil type, soil texture and climate are eliminated. Hence, quantification of various effects of SOC on soil properties becomes more reliable. The study sites used are summarized in **Table 1** and are described briefly in the following sections. The Askov Long-Term Experiment on Animal Manure and Mineral Fertilizers (Askov-LTE), the Highfield Ley-Arable Long-Term Experiment (Highfield-LTE), and the Highfield Land-Use Change Experiment (Highfield-LUCE) are the main study sites used in the thesis and will be described in more detail than the other sites.

Table 1. Overview of study sites used in the Ph.D. study.

Study site	Coordinates	Soil class (WRB)	<i>n</i>	Clay	SOC	Involvement	Paper
				Range (kg kg ⁻¹ minerals)			
Askov-LTE, Denmark	55°28’N, 09°07’E	Aric Haplic Luvisol	12	0.09-0.10	0.009-0.014	Sampling & analysis	1, 2 and 3
Highfield-LTE, UK	51°80’N, 00°36’W	Chromic Luvisol	16/48	0.23-0.34	0.008-0.043	Sampling & analysis	1, 2, 4 and 5
Highfield- LUCE, UK	51°80’N, 00°36’W	Chromic Luvisol	27	0.23-0.30	0.008-0.040	Sampling & analysis	6 and 7
Bad Lauchstädt, Germany ^a	51°24’N, 11°23’E	Haplic Chernozem	6	0.27-0.28	0.016-0.026	Received data	2
Lerbjerg, Denmark ^b	56°22’N, 09°59’E	-	16	0.10-0.72	0.011-0.042	Received data	1
Typical Danish soils ^c	Throughout Denmark	-	32	0.02-0.27	0.002-0.034	Retrieved from Jacobsen (1989)	5
Flakkebjerg, Denmark ^d	55°19’N, 11°23’E	Glossic Phaeozem	8	0.15	0.012-0.013	Received data	5

^aData from Eden et al. (2012).

^bSee Schjøning et al. (1999) for additional information.

^cData from Jacobsen (1989).

^dData from Abdollahi and Munkholm (2017).

The selected treatments in the Askov-LTE, Highfield-LTE and Highfield-LUCE are summarized in **Table 2** and are described in more detail in the following sections.

Table 2. Overview of treatments and treatment codes from the Askov-LTE, Highfield-LTE and Highfield-LUCE.

Askov-LTE		Highfield-LTE		Highfield-LUCE	
Treatment	Abbreviation	Treatment	Abbreviation	Treatment	Abbreviation
Unfertilized	UNF	Bare fallow	BF	Bare fallow converted to grass	BFG
½ mineral fertilizer	½NPK	Continuous arable rotation	A	Arable converted to grass	AG
1 mineral fertilizer	1NPK	Ley-arable rotation	LA	Grass converted to bare fallow	GBF
1½ animal manure	1½AM	Grass	G	Grass converted to arable	GA

2.1.1 Askov-LTE

The Askov-LTE was established in 1894 on a sandy loam soil at Askov Experimental Station. The field carries a four-year crop rotation of winter wheat (*Triticum aestivum*), silage maize (*Zea mays*), and spring barley (*Hordeum vulgare*) undersown with a grass-clover mixture used for cutting in the subsequent production year. Four different fertilization treatments were selected to obtain the widest gradient possible in SOC content: unfertilized (UNF), ½ mineral fertilizer (½NPK), 1 mineral fertilizer (1NPK) and 1½ animal manure (1½AM). Since 1973, 1NPK and 1AM have corresponded to 100 kg total-N ha⁻¹, 20 kg phosphorus (P) ha⁻¹ and 90 kg potassium (K) ha⁻¹ (annual mean of rotation) and animal manure has been cattle slurry with 65% of its total-N being ammoniacal-N. The four treatments were embedded in a block design with three field replicates providing a total of 12 samples (Fig. 1 in **Paper 3**). More details are given in Christensen et al. (2019).

2.1.2 Highfield-LTE

The Highfield-LTE was established in 1949 on a silt loam soil at Rothamsted Research, Harpenden, UK, in a field that had been under permanent grass for centuries. Three treatments were selected in the ley-arable experiment: (i) continuous arable rotation (A) consisting of winter cereals (winter wheat, *Triticum aestivum* and winter oats, *Avena sativa*), (ii) ley-arable rotation (LA) consisting of three-year grass/clover ley (meadow fescue, *Festuca pratensis*; timothy-grass, *Phleum pratense*; white clover, *Trifolium repens*) followed by three years of arable and (iii) grass (G), ploughed and reseeded to grass (predominantly rye grass, *Lolium perenne*) when the experiment was established. The bare fallow (BF) treatment located adjacent to the experiment was also selected. The BF treatment has been maintained free of plants by regular tillage since 1959. The A, LA and G treatments were embedded in a randomized block design with four field replicates, whereas the four BF plots were located at one

end of the experiment (Fig. 1 in **Paper 4**). **Fig. 2** shows the treatments at sampling. For more details, see **Paper 4**.

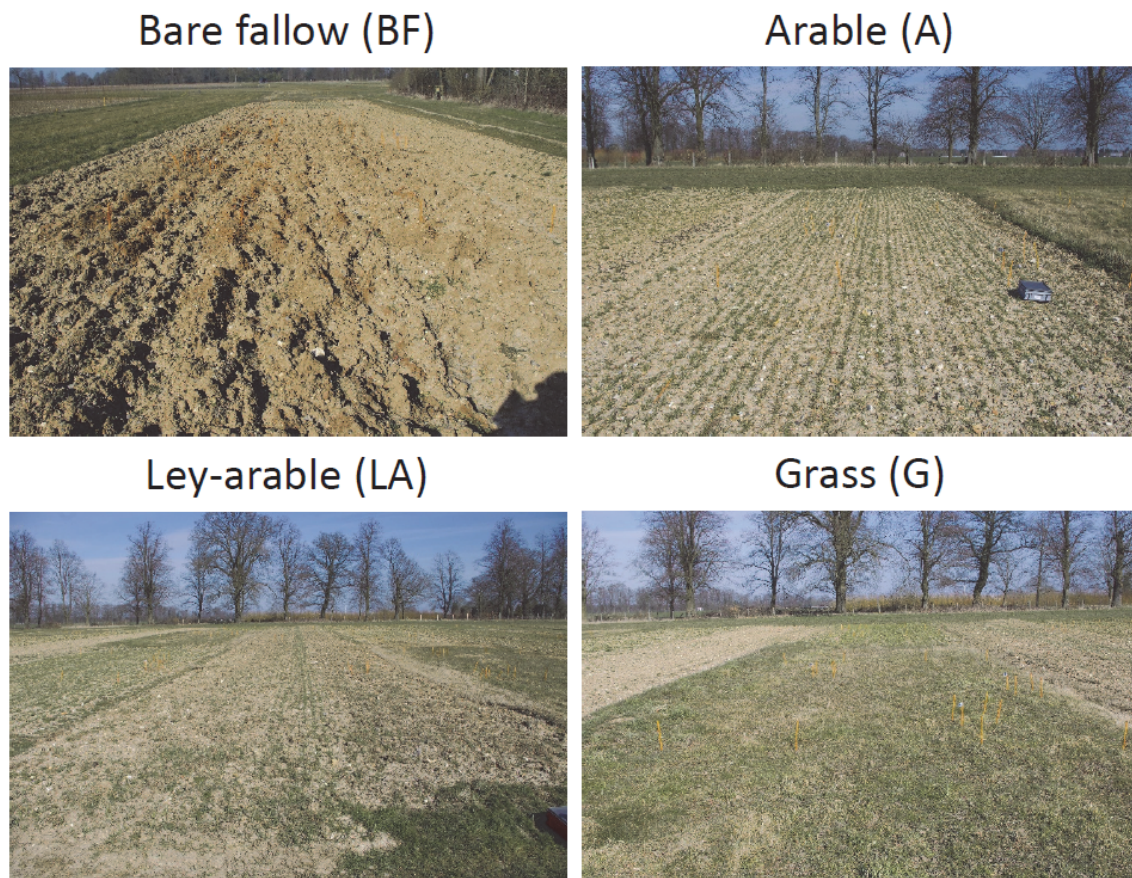


Fig. 2. The bare fallow (plot 3), arable (plot 33), ley-arable (plot 1516) and grass (plot 17) treatments from the Highfield-LTE at sampling. Photos: Johannes L. Jensen (11-3-2015).

2.1.3 Highfield-LUCE

In 2008, areas within the existing A and G plots on the Highfield-LTE site were converted to bare fallow, arable or grass. Likewise, areas within the existing BF plots were converted to arable or grass. The following degradation treatments were selected: Conversion of grassland to arable (GA) and bare fallow (GBF) management (**Fig. 3**). The following restoration treatments were selected: Introduction of grassland in bare fallow (BFG) and arable (AG) soil (**Fig. 4**). The A, AG, G, GA and GBF treatments were embedded in a randomized block design with four field replicates, whereas the four BF and three BFG plots were located at one end of the experiment (Fig. 1 in **Paper 6**). More details are given in **Paper 6**.

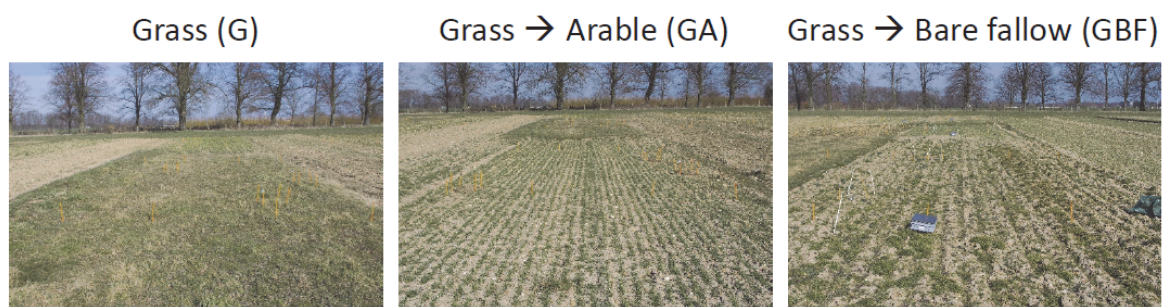


Fig. 3. Degradation scenarios: The grass (plot 17), grass converted to arable (plot 16) and grass converted to bare fallow (plot 28) treatments from the Highfield-LUCE at sampling. Photos: Johannes L. Jensen (11-3-2015).

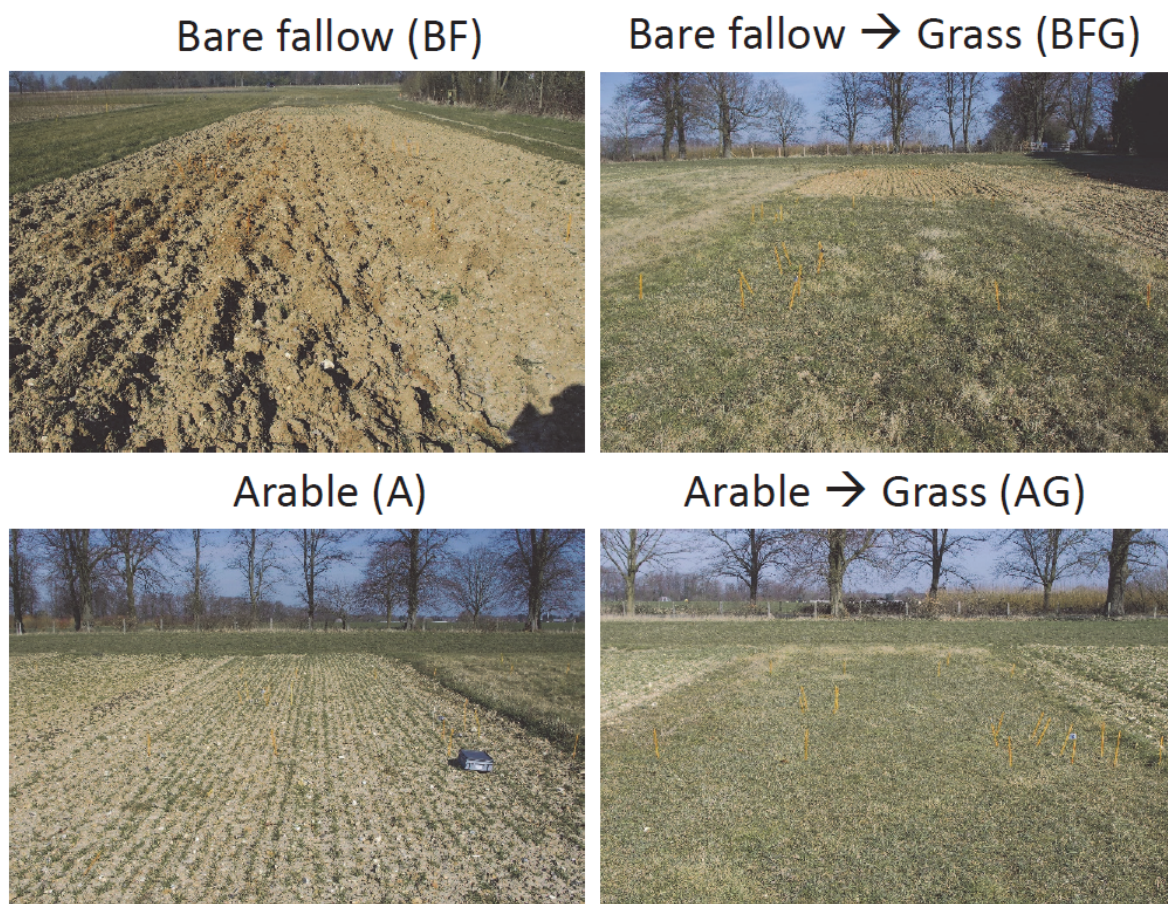


Fig. 4. Restoration scenarios: The bare fallow (plot 3), bare fallow converted to grass (plot 9), arable (plot 33) and arable converted to grass (plot 21) treatments from the Highfield-LUCE at sampling. Photos: Johannes L. Jensen (11-3-2015).

2.1.4 Other soils used

The Bad Lauchstädt long-term static fertilizer experiment was established in 1902 on a silt loam soil. The data used in **Paper 2** originate from soil sampled in spring 2008 from six different fertilization treatments. The treatments ranged from unfertilized to treatments receiving 30 t animal manure ha⁻¹ every second year, resulting in a gradient in SOC. More details are given in Eden et al. (2012). The

arable field near Lerbjerg, Denmark, has a naturally occurring textural gradient. The data used in **Paper 1** originate from soil sampled in autumn 2015 at 16 locations along the clay gradient. In **Paper 1**, archived samples of soil particle-size fractions from the same field were used (Schjønning and de Jonge, 1999). The Jacobsen data set was used in **Paper 5** since it encompasses 16 Danish agricultural soils sampled in top- and subsoil layers varying in soil textural composition and SOC content. Details are provided in Jacobsen (1989) and **Paper 5**. The conservation tillage experiment at Flakkebjerg Experimental Station, Denmark, was established in 2002 on a sandy loam soil. The data used in **Paper 5** originate from soil sampled in autumn 2013 from mouldboard ploughed and direct-drilled treatments, giving rise to differences in pore-size distribution. Further details are given in Abdollahi and Munkholm (2017).

2.2 Soil sampling and processing

Soil was sampled on 23 September 2014 in Askov following a winter wheat crop. At Highfield, soil was sampled on 10 and 11 March 2015. Soil was sampled at a soil water content close to field capacity. At both sites, undisturbed soil cores (100 cm³) were extracted from the 6-10 cm soil layer and soil blocks were carefully extracted from the 6-15 cm soil layer using a spade (**Fig. 5**). At Askov, six cores and four blocks (4000 cm³ each) were extracted within each plot, whereas the sampling procedure was as follows at Highfield: Three sampling sites in each experimental plot were randomly chosen and two cores and one block (2750 cm³) were extracted from each subplot adding up to six cores and three blocks at plot level.



Fig. 5. Extraction of soil blocks in Askov on 23 September 2014 (left) and soil cores in Highfield on 11 March 2015 (right). Photos: Johannes L. Jensen.

The samples were sealed with plastic lids, kept in sturdy containers to prevent soil disturbance during transport and stored in a 2 °C room until required for analyses. Soil from the blocks was spread out on the floor or in steel trays at room temperature, carefully fragmented by hand in several sittings along natural planes of weakness, and finally left to air-dry (**Fig. 6**).



Fig. 6. Fragmentation and air-drying of bulk soil from Askov (left) and Highfield (right). Photos: Johannes L. Jensen.

2.3 Analytical methods

The following sections contain a brief description of basic chemical and physical analyses, SOM characteristics and a range of soil physical analyses related to soil functioning used in this Ph.D. thesis. An assessment/discussion of the applied methodologies will be included when appropriate. An overview of the analyses and on which study sites they were applied can be seen in **Table 3**.

Table 3. Overview of the methods used at plot level and on which study sites they were applied (Askov-LTE, Highfield-LTE and Highfield-LUCE).

	Askov-LTE	Highfield-LTE	Highfield-LUCE
Basic chemical and physical analyses			
Soil texture	X	X ^a	X
Clay (<2 µm) and silt (2-20 µm) without SOM removal	X	X ^a	
Soil organic carbon (SOC)	X	X ^a	X
Loss-on-ignition (LOI)	X	X ^a	
Specific surface area (SSA)	X	X	X
Cation exchange capacity (CEC)		X	X
Soil pH	X	X	X
Soil organic matter characteristics			
Permanganate-oxidizable C (POXC)	X	X ^a	X
Hot water-extractable C (HWC)	X	X ^a	X
Light fraction SOC (LFSOC)		X ^a	
Fourier transform mid-infrared photoacoustic spectroscopy (FTIR-PAS)		X	X
Soil structural stability			
Clay-SOM disintegration (DI)		X ^a	X
Clay dispersibility on field-moist soil (DispClay _{FM})	X		
Clay dispersibility on 1-2 mm aggregates rewetted to -100 hPa (DispClay 1-2 mm)		X ^a	X
Clay dispersibility on 8-16 mm aggregates rewetted to -100 hPa (DispClay 8-16 mm)		X	X
Fines20 dispersibility, time series on field-moist soil (DispFines20)		X	
Water-stable aggregates on field-moist soil (WSA _{FM})	X		
Water-stable aggregates on 1-2 mm air-dry aggregates (WSA 1-2 mm)	X	X	
Soil strength			
Aggregate strength; different water content; 8-16 mm	X	X ^b	X ^c
Aggregate strength; remoulded aggregates; 8-16 mm	X	X ^b	X
Aggregate strength; air-dry; 1-2, 2-4, 4-8, 8-16 mm		X ^b	
Shear strength	X		
Soil pore characteristics			
Particle density (PD)		X ^d	X ^d
Bulk density (BD)	X	X	X
Pore-size distribution (PSD)	X	X	X
Air permeability	X	X	X

^aDetermined at subplot level.

^bCarried out together with Peter B. Obour.

^cAir-dry aggregates only.

^dDetermined at treatment level.

2.3.1 Basic chemical and physical analyses

Soil texture of air-dried bulk soil (crushed and passed through a 2-mm sieve) was determined by the hydrometer method for clay ($<2\ \mu\text{m}$) and silt ($2\text{--}20\ \mu\text{m}$) content and the sieve method for mineral particles $>63\ \mu\text{m}$ (Gee and Or, 2002). The soil was tested for the existence of CaCO_3 by adding a few droplets of 10% HCl , but none was found. Soil organic matter was oxidized with hydrogen peroxide (H_2O_2) before estimation of clay and silt. Additionally, soil samples from the Askov-LTE and the Highfield-LTE at plot and subplot level, respectively, were analyzed without pretreatment. **Paper 2** provides a detailed description of the hydrometer method and removal of SOM. The SOC content was determined on ball-milled subsamples using dry combustion. Loss-on-ignition was determined on oven-dried ($105\ ^\circ\text{C}$ for 24 h) subsamples of bulk soil ($<2\ \text{mm}$). The experimental procedures were based on recommendations by Hoogsteen et al. (2015). **Paper 1** provides a detailed description of the LOI method used. Specific surface area (SSA) was determined by the ethylene glycol monoethyl ether method (Petersen et al., 1996), and cation exchange capacity (CEC) was determined after Kalra and Maynard (1991). Soil pH was determined in 0.01 M CaCl_2 (1:2.5, w/w).

2.3.2 Soil organic matter characteristics

Permanganate-oxidizable C (POXC), hot water-extractable C (HWC), light-fraction soil organic C (LFSOC) and Fourier transform mid-infrared photoacoustic spectroscopy (FTIR-PAS) were measured to analyse if they were more closely related to SSS than the SOC content and the ratio of mineral fines to SOC.

Permanganate-oxidizable C, HWC, LFSOC and FTIR-PAS were determined on air-dry, 2-mm sieved soil as described in detail in **Paper 4**. Determination of POXC: Briefly, soil was shaken in a potassium permanganate (KMnO_4) solution and allowed to settle after which the supernatant was transferred, absorbance measured and finally converted to a POXC quantity. Determination of HWC: Briefly, soil was shaken in water at $20\ ^\circ\text{C}$, centrifuged, and the supernatant decanted. The soil was re-suspended in water, shaken for 16 h at 200 rpm and $80\ ^\circ\text{C}$, centrifuged, and the supernatant was filtered after which HWC was determined. Determination of LFSOC: To determine the light fraction, soil was fractionated based on density using a NaI solution with a density of $1.8\ \text{g cm}^{-3}$. Based on LFSOC, light-fraction-free-SOC (LF-free-SOC) was calculated by subtracting LFSOC from SOC. Determination of FTIR-PAS: The soil samples were ball-milled and packed in 10 mm diameter cups, and functional groups of soil components were recorded using a Nicolet 6700 FTIR spectrometer (Thermo Scientific) equipped with a PA301 photoacoustic detector (Gasera Ltd., Turku, Finland). For further details, see Peltre et al. (2014).

2.3.3 Soil structural stability

A range of stability tests were employed varying in pretreatment, degree of disturbance and spatial scale. To quantify the stability of SOM-mineral interactions (primary level, $<20\ \mu\text{m}$) an extreme clay-SOM disintegration test was applied. The clay-SOM disintegration measure was developed based on **Paper 2**, where soil samples were analysed for clay content without and with H_2O_2 -removal of SOM. Clay-SOM disintegration was calculated as the ratio between clay content estimated without and with SOM removal. The test was considered extreme since the soil is end-over-end shaken for 18 h in sodium pyrophosphate.

On the larger scale, wet stability tests were performed on soil or aggregates of different sizes with different moisture statuses or by measuring dispersed clay or dispersed particles $<20\ \mu\text{m}$ (DispFines₂₀). Field-moist bulk soil samples from Askov were analysed for dispersed clay (DispClay_{FM}) and water-stable aggregates (WSA_{FM}) (**Paper 3**) based on the method suggested by Pojasok and Kay (1990). Likewise, the Highfield soil samples were analysed for dispersed clay (**Paper 4**), but due to the combination of the clayey soil and treatments low in SOC (e.g. bare fallow), it was difficult to fragment the soil to $<8\ \text{mm}$ without disturbing it. Further, the aggregate size distribution of the $<8\ \text{mm}$ soil would likely be different for bare fallow (BF) and grass (G) with G having a larger proportion of small-sized aggregates compared to BF since G is more friable. To get a more homogeneous basis for the tests, 1-2 mm and 8-16 mm aggregates were isolated from air-dry $<2\ \text{mm}$ sieved soil and air-dry bulk soil, respectively (**Fig. 7a**). The aggregates were adjusted to a matric water potential of -100 hPa on tension tables (**Fig. 7bc**, described in detail in **Paper 4**) to mimic field moisture under humid temperate conditions, i.e., extreme rainfall event on field-moist rather than air-dry soil.

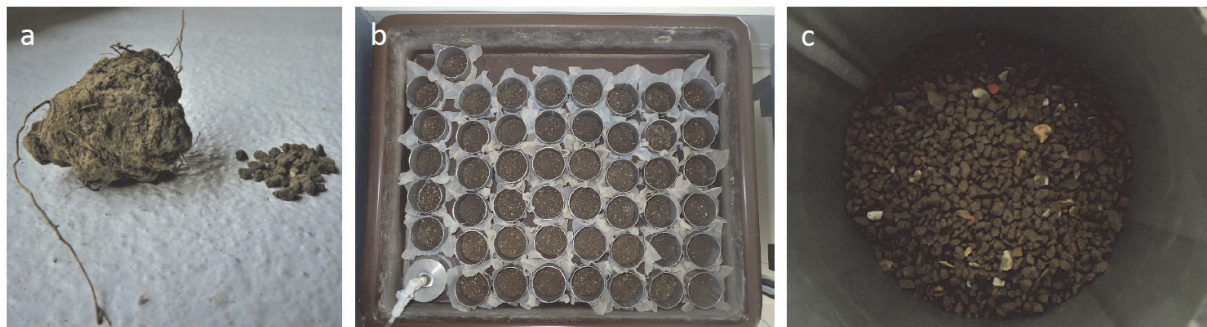


Fig. 7. (a) An 8-16 mm aggregate and 1-2 mm aggregates from the grass treatment (Highfield-LTE), (b-c) rewetting of 1-2 mm aggregates from Highfield-LTE. Photos: Johannes L. Jensen.

Clay dispersion involved shaking soil or aggregates end-over-end in artificial rainwater for 2 min after which the suspension was left to stand (**Fig. 8**) and $\leq 2\ \mu\text{m}$ particles were siphoned off. The weight of dispersed clay was determined after oven-drying ($105\ ^\circ\text{C}$ for 24 h), and the sediment was corrected for particles $>250\ \mu\text{m}$ for 1-2 mm aggregates and for particles $>2\ \text{mm}$ for field-moist $<8\ \text{mm}$ soil and 8-16

mm aggregates. Dispersed particles $<20\ \mu\text{m}$ was measured at different time steps on field-moist $<8\ \text{mm}$ soil as described in **Paper 4**.

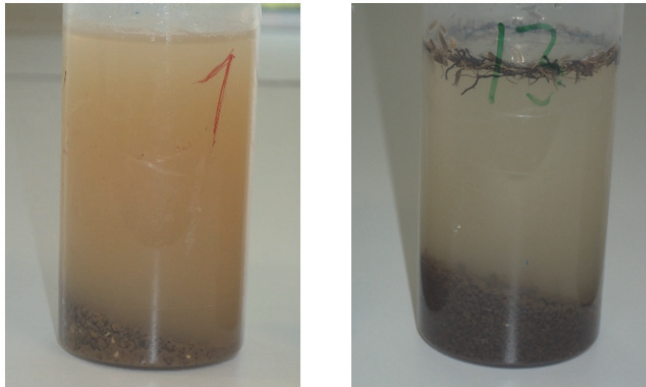


Fig. 8. Tests on soil from the bare fallow (left) and grass treatment (right) at Highfield-LTE with a high and low amount of dispersed clay, respectively. Photos: Johannes L. Jensen.

Wet aggregate stability on field-moist soil (WSA_{FM}) involved a gentle wet-sieving in artificial rainwater for 2 min using a sieve with $250\ \mu\text{m}$ apertures (**Paper 3**). Finally, WSA on 1-2 mm air-dry aggregates ($\text{WSA}_{1-2\ \text{mm}}$) was determined on Askov and Highfield-LTE samples using another wet sieving apparatus (Eijkelkamp Agrisearch Equipment, the Netherlands). The method is not included in the supporting papers and will therefore be described in more detail here: 4 g of 1-2 mm air-dry aggregates was

transferred to a sieve with $250\ \mu\text{m}$ openings and rewetted with a vaporizer. The sieve was moved up and down in artificial rainwater for 3 min ($34\ \text{cycles min}^{-1}$; stroke length 13 mm). Wet aggregate stability on 1-2 mm air-dry aggregates was calculated as the stable soil aggregate fraction remaining on the sieve and corrected for mineral particles $>250\ \mu\text{m}$ (sand particles).

2.3.4 Soil strength

At Askov, aggregate strength was determined on 8-16 mm aggregates with different moisture statuses: air-dry, adjusted to $-100\ \text{hPa}$ and field-moist state (**Paper 3**). At Highfield-LTE, aggregate strength was determined on air-dry aggregates in four size classes (1-2, 2-4, 4-8 and 8-16 mm) and on 8-16 mm aggregates adjusted to matric potentials of -100 , -300 and $-1000\ \text{hPa}$ (Obour et al., 2018). At Highfield-LUCE, only aggregate strength of 8-16 mm air-dry aggregates was determined. At Askov and Highfield, aggregate strength of remoulded aggregates was determined (**Paper 3**). The remoulded aggregates were based on air-dry 8-16 mm aggregates (Askov and Highfield) and field-moist 8-16 mm aggregates (Askov only).

Briefly, tensile strength (Y) was determined for 15 randomly selected aggregates at plot level by subjecting them to an indirect tension test comprising crushing between parallel plates. The point of failure for each aggregate was detected when a continuous crack or sudden drop in force was reached. An air-dry and a remoulded 8-16 mm aggregate following a successful test can be seen in **Fig. 9**.

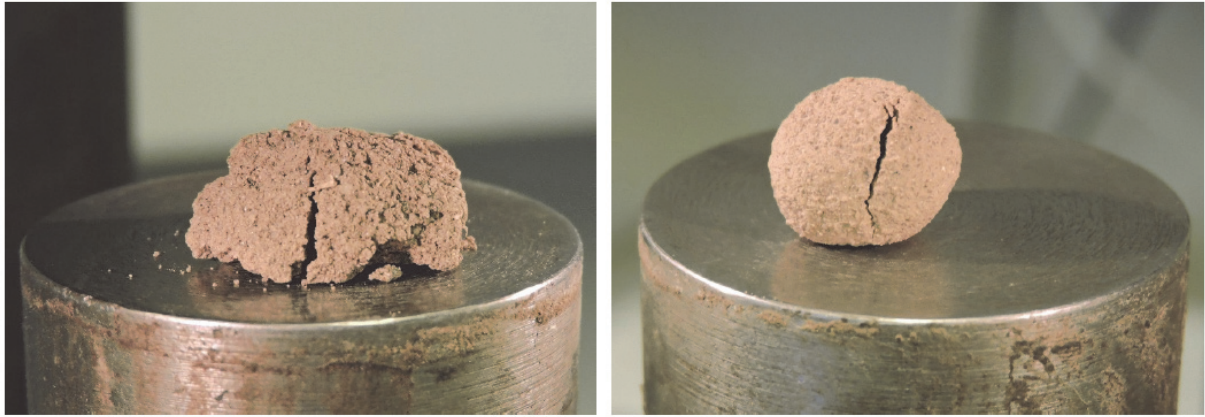


Fig. 9. An air-dry and remoulded 8-16 mm aggregate (left and right, respectively) from Askov with continuous cracks. Photos: Johannes L. Jensen.

The test results allowed the calculation of Y , mass-specific rupture energy (E_{sp}), Young's modulus (E) and friability (**Papers 3 and 6**, Obour et al., 2018). Shear strength on soil cores adjusted to -300 hPa was determined on samples from Askov by an annulus shear test (**Paper 3**).

2.3.5 Soil pore characteristics

Undisturbed soil cores (100 cm³) were placed on top of a tension table and saturated with water from beneath. Soil water retention was determined at -10, -30, -100, -300, and -1000 hPa (-1000 hPa not measured at Askov) matric potential using tension tables and pressure plates (Dane and Hopmans, 2002). The soil cores were oven-dried and bulk density (BD) calculated. At Highfield, BD was corrected for weight and volume of >2 mm particles since the soil contained a significant amount of stones (see **Paper 7** for a detailed description of stone correction). Soil porosity was estimated from BD and particle density (PD). At Askov, PD was estimated by the pedotransfer function of Schjønning et al. (2017). At Highfield, PD was measured for one plot from each treatment, i.e. seven analyses in total, by the pycnometer method (Flint and Flint, 2002) and predicted for the remaining plots based on Eq. [1] in **Paper 7**. Water content at -1.5 MPa was determined on <2 mm air-dry soil using a WP4-T Dewpoint Potentiometer (Scanlon et al., 2002). Porosity in the different pore size classes was calculated, and the water retention data were fitted to the van Genuchten (1980) and the double-exponential model (Dexter et al., 2008a). The pore-size distribution predicted by the two models was visualized by differentiating the equations with respect to the logarithm of matric potential (**Paper 5**). At Askov, air permeability was measured according to Iversen et al. (2001). At Highfield, air permeability was also measured, but the results were unreliable due to stones and are accordingly not presented.

2.3.6 Calculations and statistics

The soil properties presented in this thesis are expressed as an oven-dry mass fraction (105 °C for 24 h) of the mineral fraction. The properties include particle size fractions, SOC, POXC, HWC, DispClay_{FM}, DispClay 1-2 mm, DispClay 8-16 mm, WSA_{FM} and WSA 1-2 mm. In **section 3.1**, however, the particle size fractions, SOC and LOI are expressed as an oven-dry mass fraction of bulk soil since this was required for that analysis.

For the statistical analysis, the R-project software package Version 3.4.0 (R Foundation for Statistical Computing) was used. Treatment effects were analysed with a linear model including block as fixed effect for Askov and with a linear mixed model including block as random effect for Highfield. The criterion used for statistical significance of treatment effects was $P < 0.05$. When the treatment effect was significant, further analyses were made to isolate differences between treatments (pairwise comparisons) using the general linear hypothesis (*glht*) function implemented in the R *multcomp* package. For Askov, Fischer's protected *t*-test was applied and at Highfield the Kenward-Roger method was used to calculate degrees of freedom. Treatment differences for the comparison of the BF and BFG treatments with other treatments were based on a pair-wise *t*-test since the treatments were located at one end of the experiment in its own design (Fig. 1 in **Paper 6**). I acknowledge that this is a less robust test and that treatment differences potentially could be due to soil variation since the BF and BFG treatments are not part of the original ley-arable experiment.

The broken-stick model was fitted using the *segmented* function and the significance of the change point was assessed using *davies.test* implemented in the *segmented* package in R. A piece-wise linear model was used:

$$y = \beta_0 + \beta_1(x) + \beta_2(x - c)^+ + e \quad [1]$$

where y is the dependent variable, x is the independent variable, c is the change point and e is the residual standard error (Toms and Lesperance, 2003). The $^+$ sign indicates that the last term is only valid when $x > c$.

The coefficient of determination (R^2) for models without intercept was calculated as $1 - SS_{\text{res}}/SS_{\text{tot}}$, where SS_{res} was taken from the model without intercept and SS_{tot} from the model with intercept. Akaike's information criterion (AIC) was used to compare models with different numbers of parameters, and a smaller or more negative AIC indicates better model performance.

3. Results and discussion

3.1 Soil organic carbon (**Paper 1**)

Accurate estimates of SOC content are essential in many aspects. For instance, when assessing potentials for mitigating CO₂ emissions (Smith et al., 2020) and when calculating the ratio of mineral fines to SOC as in this study. Loss-on-ignition is a widely used approximation for SOC and therefore the conversion of LOI to SOC was revisited (**Paper 1**). In addition, estimates of SOM content is required when determining soil texture (**section 3.2**) and when developing pedotransfer functions based on SOM contents (e.g., Jensen et al., 2015; Schjønning et al., 2017). Estimates of SOM content can be achieved by multiplying SOC with a conversion factor. The SOC to SOM conversion factor can be estimated by LOI and was compared to the conventional conversion factor of 1.724.

*In the following, analyses including clay and SOC content from Lerbjerg have been revised since I first assumed that data were expressed as an air-dry weight mass proportion, and therefore I did a residual water correction (as described in **section 3.2**). Data were already expressed as an oven-dry weight mass proportion for which reason the properties in **Paper 1** erroneously were corrected for residual water twice. However, this did not change the conclusions of **Paper 1**.*

A direct measure of SOC content can be achieved by high-temperature (≥ 900 °C) dry combustion methods with detection of evolved CO₂ by infrared or thermal conductivity detectors (Nelson and Sommers, 1996). Indirect analytical approaches such as LOI also exist (method described in **Paper 1**). To convert LOI to SOC content, a widely used approach is to multiply LOI with the conventional conversion factor of 0.58. Without constraints, this approach is recommended in the Soil Organic Carbon Mapping manual issued by the UN-FAO (Olmedo and Baritz, 2018). However, application of the conventional conversion factor overestimated SOC grossly compared to direct measures of SOC by high-temperature dry combustion (**Fig. 10a**). Further, the error increased with increasing contents of clay. Applying a regression of LOI against measured SOC underestimated SOC at small clay contents and overestimated SOC at larger contents (**Fig. 10b**). The significant effect of clay on overestimation of SOC in **Fig. 10ab** could be ascribed to structural water loss from clay minerals (Sun et al., 2009). Accounting for clay content improved the conversion of LOI to SOC substantially (**Fig. 10c**), but the accuracy of prediction was still not satisfactory (**Paper 1**). To obtain precise estimates of SOC, high-temperature dry combustion methods are therefore highly recommended.

Contents of SOM are routinely calculated by multiplying SOC by the so-called “Van Bemmelen factor” of 1.724 (Nelson and Sommers, 1996). The Van Bemmelen factor rests on an analysis of the C content of humic acid extracted from peat in the 19th century and is therefore not recommended as a universal conversion factor for soils (Pribyl, 2010). Soil organic matter can be estimated by LOI or oxidation of OM with H₂O₂. However, both methods are subject to a high degree of uncertainty due to

losses other than OM when using LOI, and incomplete oxidation of OM when using H_2O_2 (Nelson and Sommers, 1996). In this project, the SOC to SOM conversion factor was estimated in the same way as the conversion of LOI to SOC (**Paper 1**), i.e., by taking clay content into account. The SOC to SOM conversion factors for Highfield, Askov and Lerbjerg were 1.92, 2.02 and 2.09, respectively. Differences in the conversion factors across sites may be ascribed to differences in SOM quality (ISO, 1995; Pribyl, 2010) resulting from different management practices. The conversion factor was close to 2, which corroborates the review by Pribyl (2010). If a SOC to SOM conversion factor for arable topsoil is needed, a factor of 2 is therefore more appropriate than the conventional conversion factor of 1.724. However, site-specific conversion factors may be estimated by multiple regression analysis as done above.

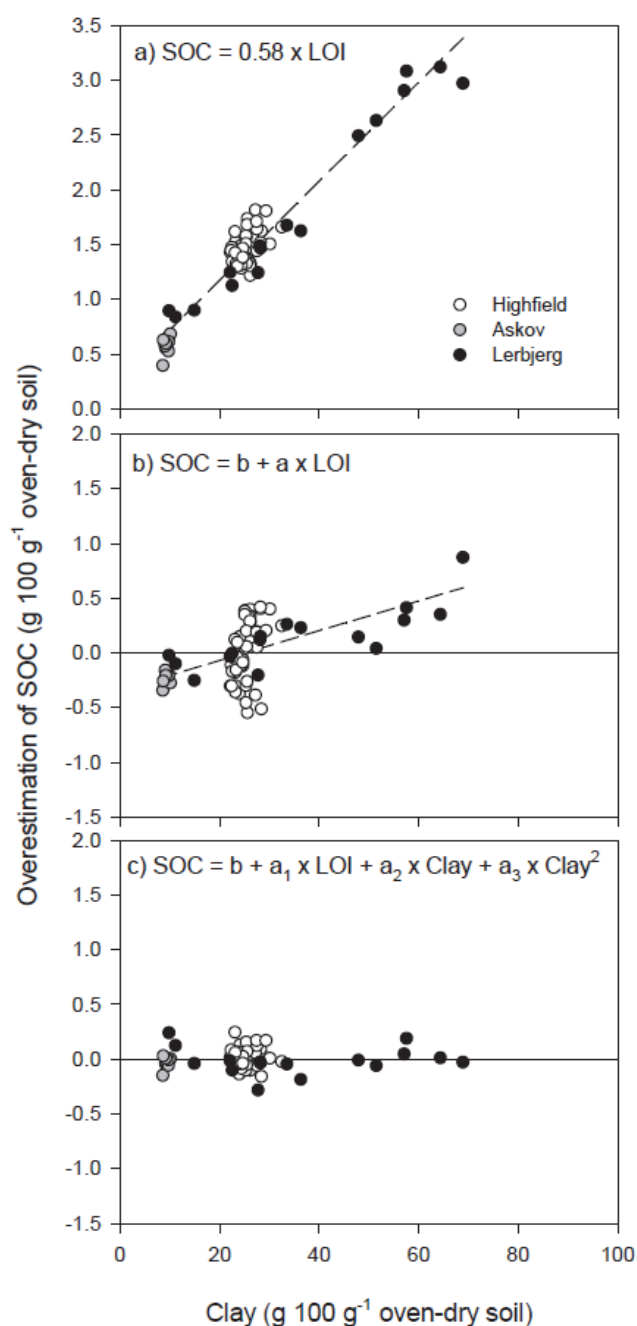


Fig. 10. Overestimation (predicted-measured values) of soil organic carbon (SOC) as a function of clay when (a) multiplying loss-on-ignition (LOI) with the conventional conversion factor 0.58, (b) when estimating SOC by a model including measured LOI, and (c) when estimating SOC by a model including LOI and the quadratic clay expression. Short-dash regression lines are indicated if clay had a significant effect on the overestimation of SOC (modification of Fig. 3 in **Paper 1**).

3.2 Clay and silt (**Paper 2**)

Accurate estimates of clay (<2 μm) and silt (2-20 μm) content are also essential when calculating the ratio of mineral fines to SOC. In addition, accurate clay and silt contents are necessary when using soil spectroscopy and pedotransfer functions to predict soil textural composition since both approaches rely on precise reference values from conventional texture analysis. Highfield is characterized by having a homogeneous soil texture and a wide gradient in SOC (Watts and Dexter, 1997).

Surprisingly, the results on clay and silt received from Analyseenheden (the analytical lab unit at Department of Agroecology, Aarhus University) deviated from that assumption (**Fig. 11**).

Analyseenheden is following the Danish standard protocol for texture analysis (Danish Plant Directorate, 1994), which only includes removal of SOM before texture analysis if the SOC content is above 3 g 100 g⁻¹ air-dry bulk soil.

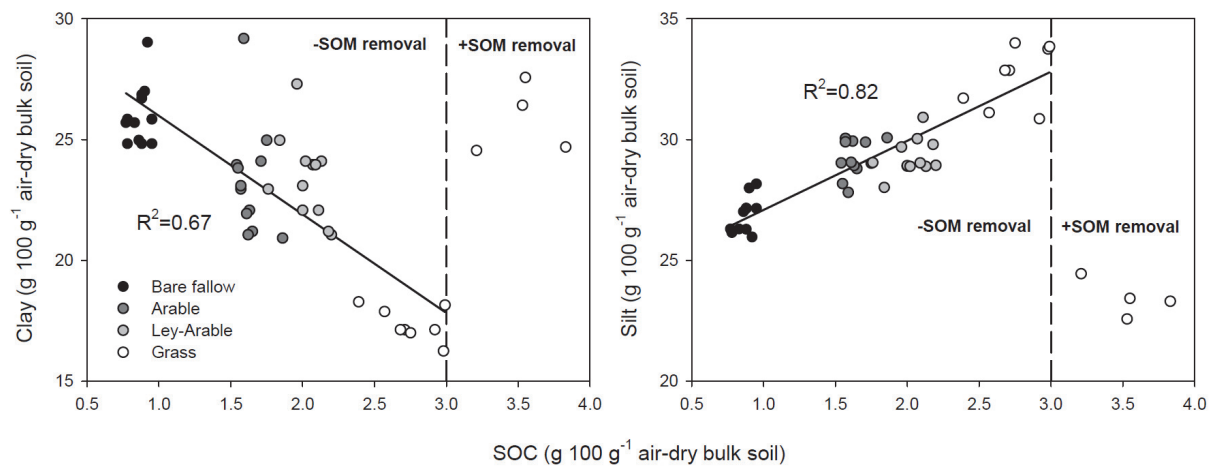


Fig. 11. Clay (<2 μm) (left) and silt (2-20 μm) (right) content as a function of SOC content for soil samples from the Highfield-LTE. Samples with more than 3 g SOC 100 g⁻¹ air-dry bulk soil were pretreated with hydrogen peroxide, while samples with less than 3 g SOC 100 g⁻¹ air-dry bulk soil were not pretreated (the limit is indicated by the dashed line). The linear regression line and R^2 -value for non-pretreated soil samples are indicated.

There was a strong negative relationship between SOC and clay estimates for soil samples without SOM removal ($R^2=0.67$, $P<0.001$), and a corresponding positive relationship between SOC and silt estimates ($R^2=0.82$, $P<0.001$). Obviously, a systematic error was induced by SOM for the soils without pretreatment, suggesting increasingly incomplete dispersion of soil aggregates smaller than <20 μm with increasing SOC.

To quantify the effect of SOM removal on the determination of clay and silt-sized particles, the soil samples from Highfield were reanalysed with SOM removal irrespective of SOC content (**Paper 2**). The reanalysis of soils from Highfield made it possible to study the effect of SOM removal without confounding effects such as mineralogy and textural composition. The results from the reanalyses

showed that the systematic error disappeared (Fig. 1a and b, **Paper 2**). However, reviewing the standard protocol revealed that two hydrometer readings were taken to estimate clay content (2 and 18 h), but only one reading was taken to estimate Fines20 (<20 µm particles) content (6.5 min). This procedure may be problematic since the reading after 6.5 min is not necessarily equal to a particle diameter of 20 µm. Further, when interpolating the clay content by relating the hydrometer readings to the calculated particle diameters (D), D is used as default, even though $\log(D)$ is recommended (American Society for Testing and Materials, 2000). Re-calculating the clay and Fines20 contents using $\log(D)$ and interpolating the Fines20 content by using the readings after 6.5 and 120 min resulted in an increase in clay and silt content of 0.3 and 0.6 g 100 g⁻¹ air-dry bulk soil, respectively (**Table 4**). Moreover, it was also possible to correct for differences in particle density (PD) in the calculation of D by using Table 1 and 3 in American Society for Testing and Materials (2000). After SOM removal, the PD of all samples from Highfield was estimated to 2.724 g cm⁻³ using Eq. [1] in **Paper 7**. The default value is 2.65 g cm⁻³ and correcting for the larger PD-value resulted in a small decrease in clay and silt content (**Table 4**).

Within soil science literature, the units of the soil particle fractions are ambiguously defined since it is often not stated whether the properties are expressed on an air- or oven-dry basis or in relation to minerals or bulk soil weight. Internationally, it is stated that particle size fractions should be expressed in relation to oven-dry soil weight (Gee and Or, 2002). However, Poeplau et al. (2015) stressed that it is often unclear whether this is the case. Even in ISO (2009) it is not clear if the particle size fractions should refer to oven-dry soil weight. At our local lab the calculations of particle size fractions are based on air-dry soil. Thus, the residual water content (RWC) had to be estimated by oven-drying the soil (105 °C, 24 h) (ISO, 1993). The RWC was calculated as the difference between the air-dry and oven-dry weight and related to oven-dry soil. The RWC for the Highfield long-term treatments ranged from 1.8-3.0 g 100 g⁻¹ oven-dry bulk soil, and omitting the RWC correction resulted in an underestimation of 0.4-0.9 g clay 100 g⁻¹ oven-dry bulk soil (on average 0.5 and 0.6 g clay 100 g⁻¹ oven-dry bulk soil for bare fallow and grass, **Table 4**). The RWC systematically increased with increases in SOC and clay content:

$$\text{RWC} = 0.275 (P<0.001) \times \text{SOC} + 0.068 (P<0.001) \times \text{Clay}, s=0.120, R^2=0.82, n=48 \quad [2]$$

where s is the standard deviation of the predicted value. To avoid systematic underestimation of the particle size fractions, particle size fractions should be expressed on an oven-dry basis.

Table 4. Refining clay (<2 µm) and silt (2-20 µm) estimation. The clay and silt contents are given as average values for the 12 subplots from the bare fallow and grass treatment from the Highfield-LTE. The average SOC contents for bare fallow and grass are 0.90 and 3.29 g 100 g⁻¹ oven-dry minerals, respectively.

	Unit	Clay (<2 µm)				Silt (2-20 µm)			
		Bare fallow		Grass		Bare fallow		Grass	
Analyseenheden	g 100 g ⁻¹ air-dry bulk soil	26.0		23.9		23.5		24.5	
log(<i>D</i>) + interpolation of Fines20	g 100 g ⁻¹ air-dry bulk soil	26.3	0.3	24.2	0.3	24.1	0.6	25.1	0.6
Particle density correction	g 100 g ⁻¹ air-dry bulk soil	26.0	-0.3	23.9	-0.3	23.9	-0.1	25.0	-0.2
Residual water correction	g 100 g ⁻¹ oven-dry bulk soil	26.5	0.5	24.6	0.6	24.4	0.5	25.6	0.7
Soil organic matter correction	g 100 g ⁻¹ oven-dry minerals	27.0	0.5	26.1	1.6	24.9	0.4	27.2	1.6
Omitting SOM removal	g 100 g ⁻¹ oven-dry minerals	27.4	0.4	19.2	-6.9	28.5	3.6	36.2	9.0

Particle size fractions are internationally expressed in relation to minerals (Gee and Or, 2002; ISO, 2009), but to do that the SOM content is needed since one of the particle size fractions is often calculated by summing up to 100%.

At Analyseenheden the coarse silt fraction is calculated as:

$$\text{Coarse silt (20-63 } \mu\text{m)} = 100 - \text{SOM} - \text{Clay (<2 } \mu\text{m)}^* - \text{Silt (2-20 } \mu\text{m)}^* - \text{Fine sand (63-200 } \mu\text{m)}^* - \text{Coarse sand (200-2000 } \mu\text{m)}^*$$

where SOM is calculated as $1.70 \times \text{SOC}$, and * indicates a direct measure of the mineral fraction.

At AGROLAB, which is an accredited laboratory and carries out texture analysis for AU and Seges (<https://www.seges.dk/en>) among others, the fine sand fraction is calculated as:

$$\text{Fine sand (20-200 } \mu\text{m)} = 100 - \text{SOM} - \text{Clay (<2 } \mu\text{m)}^* - \text{Silt (2-20 } \mu\text{m)}^* - \text{Sand (200-2000 } \mu\text{m)}^*$$

where SOM is calculated as $1.724 \times \text{SOC}$, and * indicates a direct measure of the mineral fraction.

An estimate of the SOM content is also needed when the particle size fractions are determined by the hydrometer method following ISO (2009) since the fractions are expressed in relation to the total mass of dry, pretreated soil minus SOM, where SOM is calculated as $1.7\text{-}2.0 \times \text{SOC}$ (ISO, 1995).

However, the SOC to SOM conversion factor used at Analyseenheden and Agrolab (1.70 and 1.724, respectively) is not in agreement with findings presented in **section 3.1** and **Paper 1**. For Highfield, a conversion factor of 1.92 was used (**Paper 1**) after which the particle size distribution was normalized (i.e., the particle size fractions were expressed in relation to minerals). The SOM correction resulted in an increase in clay content of 0.5 and 1.6 g 100 g⁻¹ oven-dry minerals for the bare fallow and grass treatment, respectively (**Table 4**). To overcome the need for SOM content when estimating particle size fractions, each mineral fraction can be estimated directly by the pipette method for particles ≤ 63 µm and sieving for particles >63 µm (Gee and Or, 2002; ISO, 2009).

Without removal of SOM prior to texture analysis, the content of clay was underestimated by 6.9 g 100 g⁻¹ minerals, and silt was overestimated by 9.0 g 100 g⁻¹ minerals for the grass treatment (**Table 4**). Such a marked error would reduce the clay/SOC ratio by 2. Soil organic matter removal became unnecessary for determination of clay content when the soil contained less than 2 g SOC 100 g⁻¹ minerals (Fig. 2a, **Paper 2**). For silt content determination, SOM removal was crucial regardless of the SOC content (Fig. 2b, **Paper 2**). Consequently, SOM removal prior to soil texture analysis is needed to obtain precise estimates of soil particles <20 µm.

In a Danish perspective, the findings question the validity of the Danish soil classification (a survey conducted in the 1970s) with regard to soil texture. Samples with more than 10% CaCO₃ or SOM were not analysed for texture, but classified as unusual and humus soils, respectively. The remaining samples were analysed without removal of SOM (Madsen et al., 1992) because including SOM removal was too time-consuming. The Danish soil classification resulted in a division of Danish soil into different soil types, denoted the JB-system. The rules and recommendations regarding fertilization, irrigation and tillage in Denmark are based on the JB-system (see, e.g., Dijk and Berge, 2009). As an example, a soil could change its JB-class from JB 3 (5-10% clay) to JB 5 (10-15% clay) due to SOM removal (Borggaard et al., 2011), resulting in an increase in the N quota for winter wheat from 139 to 153 kg N ha⁻¹. Thus, the findings may directly influence farm management.

The findings presented in **Paper 2** have also resulted in changes to procedures at Analyseenheden at the Department of Agroecology: On the requisition forms for soil texture analysis, the person ordering should now tick off if SOM should be removed or not. In addition, the hydrometer method has been refined, and determination of RWC as part of soil analysis is now default.

In summary, omitting removal of SOM in texture analysis can introduce systematic errors, especially on soils with high SOC contents. Likewise, lack of RWC correction may also introduce a systematic error, especially for soils with a wide range in SOC or clay content. Erroneous estimation of SOM, lack of PD and SOM correction may further influence the results. I encourage my colleagues within soil science to use explicitly defined units and detailed method descriptions for texture analysis. Soil texture is considered a basic soil property, but the soil texture analysis can be subject to several pitfalls, which we need to be aware of.

3.3 Critical carbon levels (Papers 3, 4 and 5)

The overall hypothesis of this work is presented in **Fig. 12**, where the SOC content is plotted against the clay and Fines20 content for all plots at Highfield and Askov. The hypothesized critical threshold values, clay/SOC=10 and Fines20/SOC=20 are indicated. For plots below the lines, i.e., with ratio values above 10 or 20, a deterioration of the soil physical properties is expected.

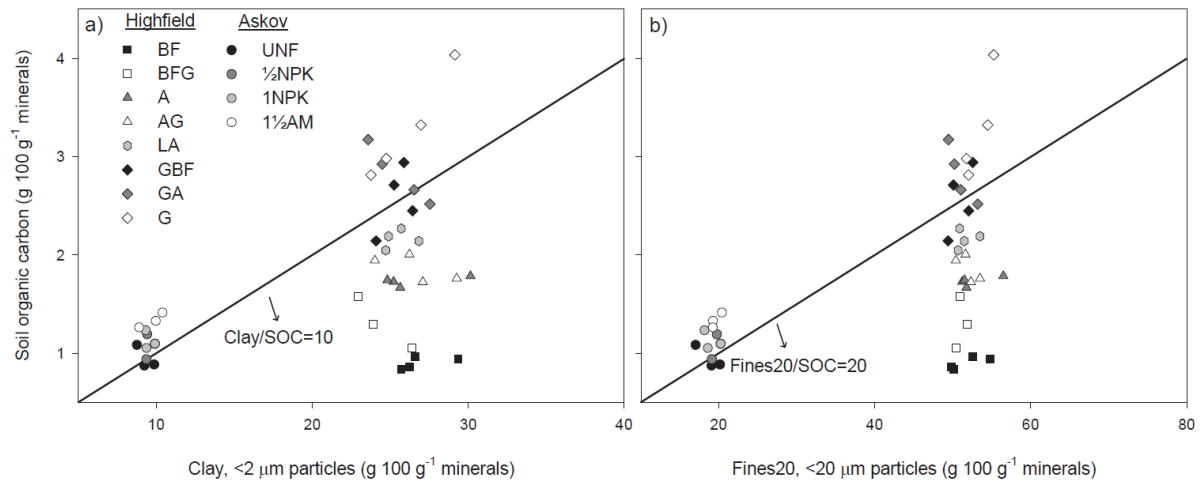


Fig. 12. Soil organic carbon plotted against (a) clay and (b) Fines20 content for the 12 experimental plots from Askov and the 31 experimental plots from Highfield. The hypothesized critical ratio values are indicated. For treatment abbreviations see **Table 2**.

The soils at Askov ranged from 9-10 g clay 100 g⁻¹ minerals and 0.87-1.41 g SOC 100 g⁻¹ minerals, while the soils at Highfield ranged from 23-30 g clay 100 g⁻¹ minerals and 0.83-4.04 g SOC 100 g⁻¹ minerals. Soil structural stability can be affected by both SOC and clay content (Schjønning et al., 2012), but since the ranges in mineral fines content were relatively narrow in the present study, it is reasonable to mainly attribute changes in SSS to the SOC content. At Askov, only two UNF plots had clay/SOC values >10, while all plots had clay/SOC values >10 at Highfield except the G plots, three GA plots and two GBF plots. Hence, the plots were distributed above and below the hypothesized critical ratio values, which is a prerequisite for testing whether the soil physical properties deteriorate when the clay/SOC value >10.

3.3.1 Soil structural stability, aggregate strength and pore characteristics

At Askov, the 1NPK and 1½AM soils had a lower clay dispersibility and larger content of water-stable aggregates (assessed on field-moist soil; DispClay_{FM} and WSA_{FM}, respectively) than the UNF and ½NPK soils (**Fig. 13ab**). Hence, a change point in SOC between ½NPK and 1NPK for these SSS measures could be suggested, which corresponds to a SOC content of 1.10 g C 100 g⁻¹ minerals (**Fig. 13ab**). Relating this SOC content to the mineral fines content resulted in clay/SOC and Fines20/SOC ratios of 9 and 18, respectively (**Table 6**). The 1½AM treatment had a larger proportion of water-

stable aggregates of 1-2 mm air-dry aggregates (WSA 1-2 mm) than the other treatments (**Fig. 13c**), suggesting a change point in SOC between 1NPK and 1½AM for this measure, which corresponds to a SOC content of 1.23 g C 100 g⁻¹ minerals. Relating this SOC content to the mineral fines content resulted in clay/SOC and Fines20/SOC ratios of 8 and 16, respectively (**Table 6**). However, the Askov treatments are considered very stable with respect to WSA 1-2 mm according to Pulido Moncada et al. (2015) since WSA 1-2 mm was above 0.7 for all the treatments. The markedly greater WSA 1-2 mm compared to WSA_{FM} is consistent with Dexter et al. (2011), who found lower clay dispersibility for initially drier soil, which implies greater WSA. In addition to differences in initial water content, the differences may be explained by composition of basis material (bulk soil <8 mm vs. 1-2 mm aggregates), and differences in the analytical method (e.g. different durations of sieving).

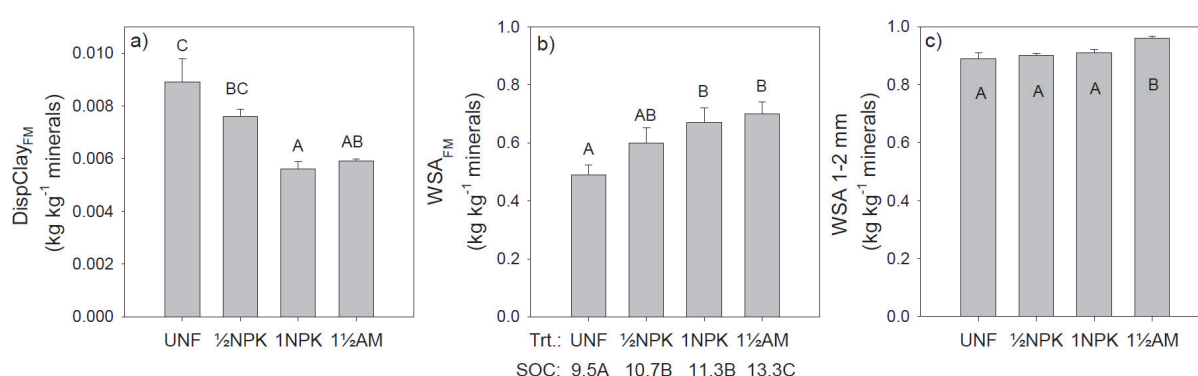


Fig. 13. Long-term fertilization effects on (a) clay dispersibility of field-moist soil (DispClay_{FM}), (b) water-stable aggregates of field-moist soil (WSA_{FM}), and (c) water-stable aggregates of 1-2 mm aggregates (WSA 1-2 mm). The soil organic carbon (SOC, g kg⁻¹ minerals) contents of the treatments are indicated below Fig. b. Letters denote statistical significance at $P < 0.05$. Error bars represent the standard error of the mean. For treatment abbreviations see **Table 2**. Fig. 13a and b are a modification of Fig. 2 in **Paper 3**, while Fig. 13c shows unpublished data.

Even though change points for SSS measures could be estimated based on treatment differences, it was not possible to identify change points when relating the SSS measures to SOC or SOC/Clay at plot level (data not shown).

At Highfield-LTE, the four treatments were significantly different in clay dispersibility of 1-2 mm and 8-16 mm rewetted aggregates (DispClay 1-2 mm and DispClay 8-16 mm, respectively) and WSA 1-2 mm, with the G treatment having the highest stability followed by the LA, A and BF treatments (**Table 5**).

Table 5. Soil structural stability for the Highfield-LTE treatments. Clay dispersibility of 1-2 mm and 8-16 mm aggregates rewetted to -100 hPa, water-stable aggregates of 1-2 mm air-dry aggregates and clay-SOM disintegration (the ratio between clay content estimated without SOM removal and with removal). Within rows, letters denote statistical significance at $P < 0.05$ for the comparison of arable, ley-arable and grass. An asterisk (*) indicates that bare fallow is significantly different from the other treatments based on a pairwise t -test.

		Bare fallow (BF)	Arable (A)	Ley-Arable (LA)	Grass (G)
Clay dispersibility 1-2 mm ¹	(kg kg ⁻¹ minerals)	0.0115	0.0074 ^{c*}	0.0051 ^{b*}	0.0034 ^{a*}
Clay dispersibility 8-16 mm ²	(kg kg ⁻¹ minerals)	0.0108	0.0060 ^{c*}	0.0040 ^{b*}	0.0021 ^{a*}
1-2 mm/8-16 mm ²	-	1.09	1.24	1.32	1.59 [*]
Water-stable aggregates ²	(kg kg ⁻¹ minerals)	0.49	0.58 ^{c*}	0.72 ^{b*}	0.96 ^{a*}
Clay-SOM disintegration ¹	(kg kg ⁻¹ minerals)	1.02	0.96 ^b	1.00 ^b	0.74 ^{a*}

¹ From **Paper 4**. ² Unpublished data.

For clay-SOM disintegration (DI) (**Table 5**) and dispersion of particles $< 20 \mu\text{m}$ (DispFines20) (**Paper 4**), the G treatment was more stable than the other treatments. Since the four treatments were very different in DispClay 1-2 mm, DispClay 8-16 mm and WSA 1-2 mm, it is difficult to define a change point for these properties. For DI and DispFines20, only the G treatment deviated from the other treatments, and hence a change point in SOC between G and LA could be suggested.

Clay dispersibility of 1-2 mm rewetted aggregates and DI was also measured at subplot level at Highfield-LTE. These SSS measures were related to SOC contents, and a change in DispClay 1-2 mm and DI was quantified at around $2.30 \text{ g C } 100 \text{ g}^{-1} \text{ minerals}$ (Table 3 and Fig. 4a in **Paper 4**). The clay content differed between the subplots, and since clay can affect clay dispersibility (Schjønning et al., 2012), the SOC content was normalized to identical soil clay contents in a revised data analysis (**Fig. 14**).

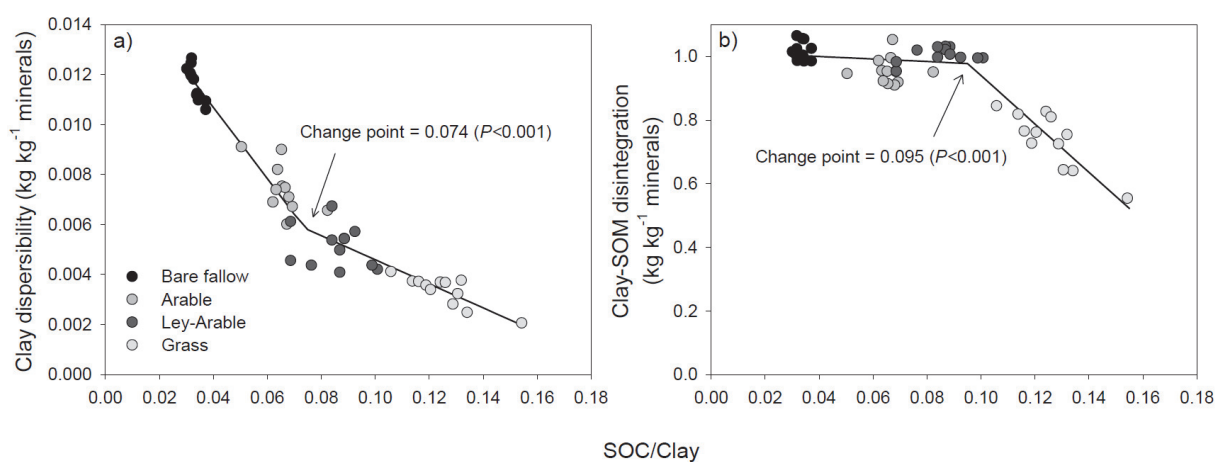


Fig. 14. (a) Clay dispersibility of 1-2 mm aggregates rewetted to -100 hPa and (b) Clay-SOM disintegration as a function of SOC/Clay for the four treatments at Highfield-LTE at subplot level. The broken-stick models (Eq. [1]), change points and significance of change points are indicated.

Three models were employed using the approach in **Paper 4** (see Table 3 in **Paper 4**), and the broken-stick model explained more of the variation in DispClay 1-2 mm than linear and semi-logarithmic models (linear: $R^2=0.884$, $AIC=-501.7$; semi-log: $R^2=0.952$, $AIC=-542.8$; broken-stick: $R^2=0.953$, $AIC=-543.3$). The change point of the broken-stick model was at a SOC/Clay value of 0.074 (**Fig. 14a**) corresponding to 1.95 g C 100 g⁻¹ minerals and clay/SOC and Fines20/SOC ratios of 13 and 27, respectively (**Table 6**). It is noteworthy that this change point was less confounded with management than the change point found when relating DispClay 1-2 mm to SOC at subplot level (Fig. 4a in **Paper 4**). Likewise, the broken-stick model explained more of the variation in DI than linear and semi-logarithmic models (linear: $R^2=0.629$, $AIC=-107.9$; semi-log: $R^2=0.475$, $AIC=-91.3$; broken-stick: $R^2=0.866$, $AIC=-154.9$). The change point of the broken-stick model was at a SOC/Clay value of 0.095 (**Fig. 14b**) corresponding to 2.50 g C 100 g⁻¹ minerals and clay/SOC and Fines20/SOC ratios of 11 and 23, respectively (**Table 6**). Making the same relations at plot level showed that the change point for DI did not change (**Table 6**), whereas the change point for DispClay 1-2 mm was at a larger SOC/Clay value (**Fig. 15, Table 6**). So, the final threshold for DispClay 1-2 mm was affected by whether the broken-stick analysis was based on subplot or plot-observations. The subplot analyses are preferred since the points are distributed throughout the range in SOC/Clay as compared to plot level, making the quantification of the change point more trustworthy.

Clay dispersibility of 8-16 mm rewetted aggregates was only measured at plot level and was also related to SOC/Clay (**Fig. 15**). The broken-stick model identified a change point in SOC/Clay at 0.092, corresponding to 2.42 g C 100 g⁻¹ minerals and clay/SOC and Fines20/SOC ratios of 11 and 22, respectively (**Table 6**).

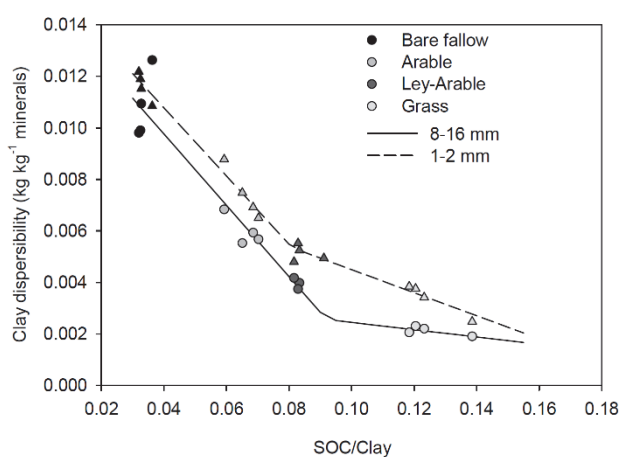


Fig. 15. Clay dispersibility of 1-2 mm (triangle symbols) and 8-16 mm (circle symbols) aggregates rewetted to -100 hPa as a function of SOC/Clay for the four treatments at Highfield-LTE at plot level. The broken-stick model (Eq. [1]) is indicated. **Unpublished data.**

Interestingly, the 8-16 mm aggregates were more stable than the 1-2 mm aggregates, especially for the G treatment (**Table 5**), which may indicate that additional stabilizing agents were at play in larger aggregates for G as compared to BF.

The critical lower content in SOC across the SSS measures was lower for Askov-LTE than for Highfield-LTE (**Table 6**), illustrating that the SOC content *per se* was not a good indicator across the two different soil types. This is also exemplified in **Fig. 16a**, where WSA 1-2 mm is related to the SOC content for both soil types. A higher content of SOC was

required to gain a high amount of WSA 1-2 mm for the silt loam soil at Highfield than for the sandy loam at Askov.

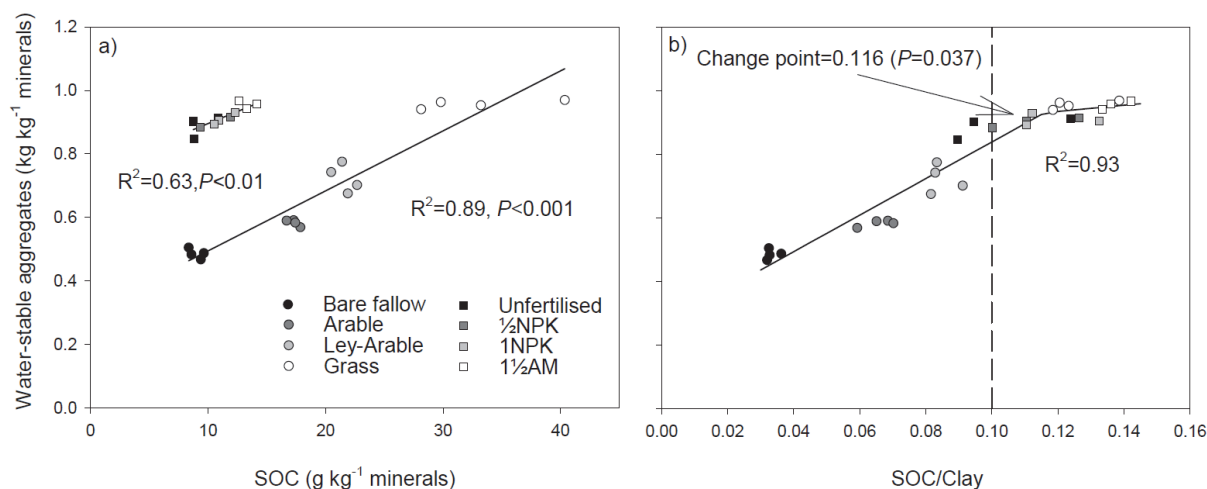


Fig. 16. Water-stable aggregates of 1-2 mm aggregates as a function of (a) soil organic carbon (SOC) and (b) SOC/Clay for the treatments at Highfield-LTE and Askov-LTE at plot level. (a) Linear regressions within sites, significance and R^2 are indicated. (b) The broken-stick model (Eq. [1]) for all sites, change point and significance of change point as well as R^2 are indicated. In addition, the SOC/Clay=0.10, corresponding to clay/SOC=10 is indicated. For treatment abbreviations see **Table 2. Unpublished data.**

Water-stable aggregates of 1-2 mm air-dry aggregates was also plotted against SOC/Clay for the Highfield and Askov soils. When the clay content was accounted for, it became clear that there was a change point and a broken-stick model was fitted (**Fig. 16b**). A significant change point in SOC/Clay was found at 0.116, corresponding to a clay/SOC value of 9 (**Table 6**).

Table 6 summarizes the suggested critical lower mineral fines/SOC ratios based on the different SSS measures for Askov- and Highfield-LTE. The critical cut-off values ranged from 8-13 and 16-27 for clay/SOC and Fines20/SOC, respectively, depending on the SSS measure, and whether the statistical analysis was based on plot or subplot level or derived from treatment differences. Interestingly, the critical clay/SOC and Fines20/SOC ratios found in this Ph.D. study are close to the ratios proposed by Dexter et al. (2008b) and Schjønning et al. (2012), i.e. critical clay/SOC and Fines20/SOC ratios of 10 and 20, respectively.

Table 6. An overview of quantified and estimated critical soil organic carbon (SOC, g 100 g⁻¹ minerals), clay/SOC and Fines20 (<20 µm particles)/SOC values based on different soil structural stability measures for Askov- and Highfield-LTE.

Soil structural stability measure	Basis	Askov-LTE			Highfield-LTE		
		SOC	Clay/ SOC	Fines20/ SOC	SOC	Clay/ SOC	Fines20/ SOC
Clay-SOM disintegration (DI)	Plot				2.52	10	23
	Subplot				2.50	11	23
Clay dispersibility on field-moist soil (DispClay _{FM})	Treatment	1.10	9	18			
Clay dispersibility on 1-2 mm aggregates rewetted to -100 hPa (DispClay 1-2 mm)	Plot				2.13	12	24
	Subplot				1.95	13	27
Clay dispersibility on 8-16 mm aggregates rewetted to -100 hPa (DispClay 8-16 mm)	Plot				2.42	11	22
Fines20 dispersibility, time series on field-moist soil (DispFines20)	Treatment				2.52	10	23
Water-stable aggregates on field-moist soil (WSA _{FM})	Treatment	1.10	9	18			
Water-stable aggregates on 1-2 mm air-dry aggregates (WSA 1-2 mm)	Treatment	1.23	8	16			
	Plot	NI ²	9 ¹	18 ¹	NI ²	9 ¹	18 ¹

¹Based on Askov-LTE and Highfield-LTE.

²Not identified.

It was also tested whether a critical lower SOC threshold could be quantified for tilth characteristics such as soil aggregate strength and soil pore functionality, as defined by Schjønning et al. (2007). Tensile strength of air-dry 8-16 mm aggregates for the Askov and Highfield soils was not significantly related to either SOC content or the SOC/Clay ratio, and thus no threshold value could be identified (Fig. 17).

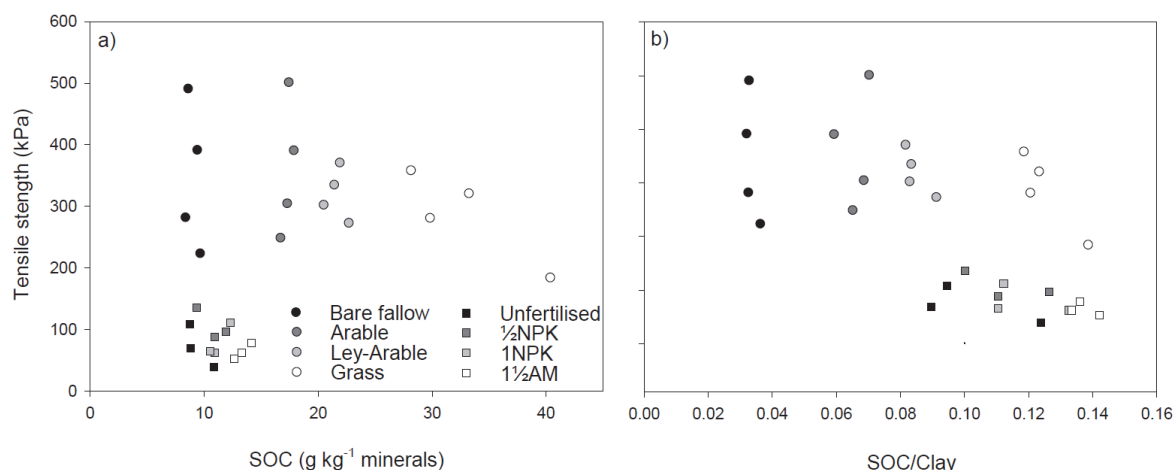


Fig. 17. Tensile strength of 8-16 mm air-dry aggregates as a function of (a) soil organic carbon (SOC) and (b) SOC/Clay for the treatments at Highfield-LTE and Askov-LTE at plot level. For treatment abbreviations see **Table 2**. From **Paper 3** and Obour et al. (2018). None of the relationships were significant.

The pore-size distribution of the four treatments at Highfield-LTE was affected by SOC and tillage (**Fig. 18**). The well-structured soil in treatment G had a large SOC content, and the soil was not tilled which was reflected in a more distinct bi-modality of the pore-size distribution compared to the other treatments (**Fig. 18b**). The textural and structural void ratio was positively affected by increasing SOC and decreasing tillage intensity rather than being affected at a specific cut-off value (**Paper 5**). Similarly, total porosity for Highfield-LTE treatments (data not shown) and the range in water contents for tillage at both sites (Fig. 4 in Obour et al., 2018) were positively related to SOC.

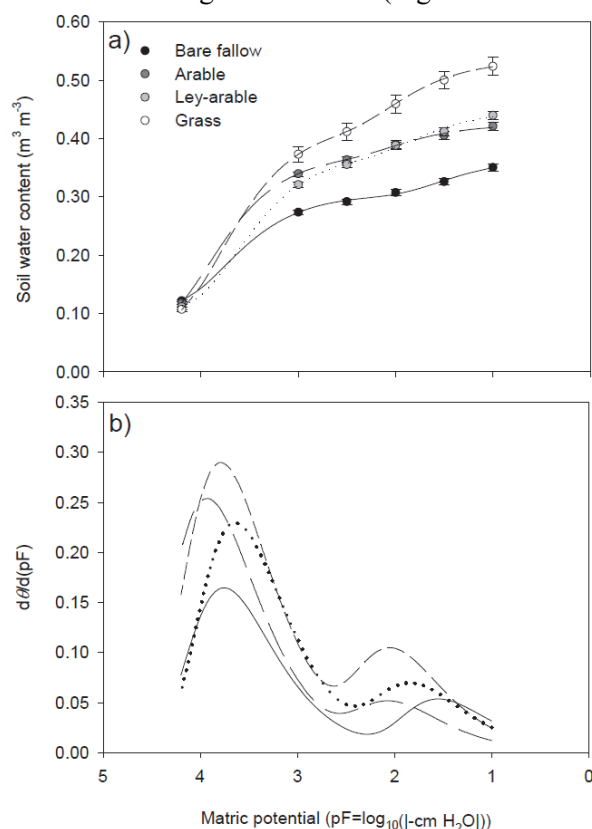


Fig. 18. (a) Measured volumetric water content for the four Highfield-LTE treatments and fits of the double-exponential model as a function of matric potential. The standard error of the mean is indicated ($n=4$). (b) Pore-size distribution $[d\theta/d(pF)]$ as a function of matric potential for the four treatments. Eq. [5] in **Paper 5** was used to obtain the pore-size distributions. From **Paper 5**.

The critical mineral fines/SOC ratios were thus less influential on tilth characteristics than on tilth forming processes. This is in accord with Schjønning et al. (2012) and Getahun et al. (2016), who found that clay dispersibility was more sensitive to differences in the clay/SOC ratio than aggregate strength. Schjønning et al. (2012), however, based their study on soil samples with clay/SOC values above 10, which

makes it difficult to assess whether SSS was less affected below the hypothesized critical threshold value. In addition, clay content explained almost as much of the variation in clay dispersibility as the content of non-complexed clay (NCC) (defined by Dexter et al., 2008a) did in Schjønning et al. (2012). Dexter et al. (2008b) based their study on French and Polish soils, sampled from different horizons and pits, differing in structural states and analysed using different methods for SOC and clay determination. However, relying on such a diverse dataset may be problematic since confounding factors such as differences in management history, climatic conditions and soil type can affect the quantification of critical mineral fines/SOC ratios. The study sites in this thesis were characterized as having a well-known management history, distribution of soils above and below the hypothesized critical ratio values (**Fig. 12**) and did not have any confounding effects derived from differences in climate and soil type, making the quantification of critical ratio values more reliable. In addition, Dexter et al. (2008a) relied on the NCC or complexed-organic carbon (COC) approach to estimate the clay/SOC threshold value. This approach introduces bias with respect to the representation of the entire sample population, as also noted by Johannes et al. (2017).

3.3.2 Soil organic matter fractions

Permanganate-oxidizable C and HWC are known as labile SOM fractions (Haynes, 2005) and have been shown to be more sensitive to management changes than total SOC (Bongiorno et al., 2019). In addition, POXC has been suggested as a comprehensive soil quality indicator (Bongiorno et al., 2019) and the best single predictor for soil health (Fine et al., 2017). Therefore, it was investigated whether POXC and HWC were better at explaining changes in SSS than SOC content. At both sites, contents of POXC and HWC followed the same pattern as the content of SOC (Table 2 in **Paper 4** and **Table 7**).

Table 7. Soil organic matter fractions in the Askov treatments. Within rows, letters denote statistical significance at $P < 0.05$. For treatment abbreviations see **Table 2**.

	UNF	½NPK	1NPK	1½AM
Soil organic carbon (SOC, g kg ⁻¹ minerals) ¹	9.5 ^a	10.7 ^b	11.3 ^b	13.3 ^c
Permanganate-oxidizable carbon (POXC, g kg ⁻¹ minerals) ²	0.202 ^a	0.261 ^b	0.275 ^b	0.400 ^c
% of SOC	2.1 ^a	2.4 ^b	2.4 ^b	3.0 ^c
Hot water-extractable carbon (HWC, g kg ⁻¹ minerals) ²	0.413 ^a	0.472 ^{ab}	0.531 ^b	0.681 ^c
% of SOC	4.4	4.4	4.7	5.1

¹From **Paper 3**. ²Unpublished data.

Hot water-extractable C correlated positively with the SOC content and represented a constant proportion of the SOC content for all treatments (approx. 5%, **Fig. 19b**). In contrast, POXC constituted a higher proportion of total SOC with increasing SOC content at Askov (**Table 7**). For

Highfield-LTE treatments, the broken-stick model indicated that POXC responded less to increased SOC for the G treatment than the other treatments (**Fig. 19a**).

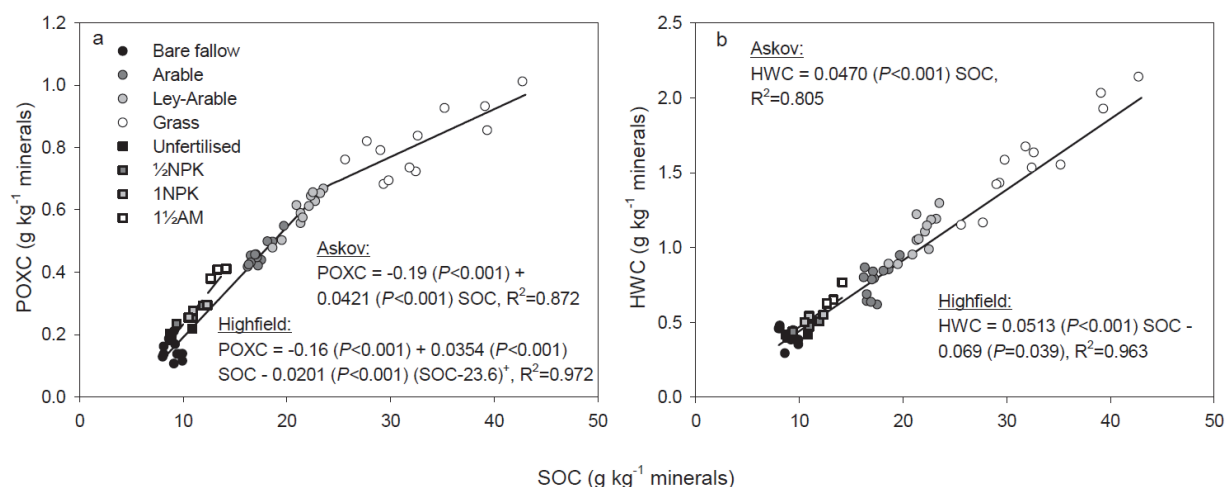


Fig. 19. (a) Permanganate-oxidizable carbon (POXC) as a function of soil organic carbon (SOC) and (b) hot water-extractable carbon (HWC) as a function of SOC for the four Highfield-LTE treatments at subplot level and the four Askov treatments at plot level. The broken-stick (Eq. [1]) and linear regression models are indicated. For treatment abbreviations see **Table 2**. Highfield data from **Paper 4** combined with **unpublished data** from Askov.

Relating POXC/Clay and HWC/Clay to DispClay 1-2 mm revealed overall that the SOM fractions were not better at explaining changes in SSS than SOC/Clay (data not shown). Clay-SOM disintegration was better described by SOC than by POXC and HWC (**Paper 4**). At Askov, SOC, POXC and HWC explained changes in DispClay_{FM} equally well (data not shown). Hot water-extractable carbon was positively correlated with WSA_{FM} ($R^2=0.35$), whereas SOC and POXC were not significantly related to WSA_{FM} (data not shown). Permanganate-oxidizable C explained marginally more of the variation in WSA 1-2 mm than SOC and HWC (POXC: $R^2=0.70$, SOC: $R^2=0.63$, HWC: $R^2=0.62$). The long-term treatments are under steady-state conditions, and this may explain why POXC and HWC were neither better to explain changes in SSS nor more sensitive to management changes than SOC. Instead, POXC and HWC might be more sensitive to short-term effects (six years) of management changes than SOC, which will be discussed in **section 3.4**.

3.3.3 Management system effects

The 1NPK and 1½AM treatments at Askov had similar WSA_{FM} and DispClay_{FM}, but different contents of SOC, POXC and HWC, indicating that the similar SSS was not related to the SOM fractions. As discussed in **Paper 3**, the similar WSA_{FM} and DispClay_{FM} could be related to a supposedly similar number of plant roots in the two treatments, which enmesh aggregates (Miller and Jastrow, 1990) and thus potentially stabilize them to a similar extent.

In the treatments at Askov, only fertilization differed, while the treatments at Highfield encompassed

contrasting management systems varying in tillage intensity, fertilization and cropping. For some of the SSS analyses, the thresholds for changes in SOC or SOC/Clay at Highfield were confounded with management. For instance, the G treatment had a very stable structure in terms of DI, DispFines20, DispClay 8-16 mm and WSA 1-2 mm (**Table 5** and **Paper 4**). This may be ascribed to the management system with greater input of above- and belowground plant residues, an increased abundance of mesofauna and fungal hyphae and a markedly greater number of roots (Hirsch et al., 2009; Hirsch et al., 2017). Plant roots and fungal hyphae bind aggregates together and stabilize them, making them more resistant to mechanical damage (Miller and Jastrow, 1990). In the G treatment, roots and hyphae might be persistent since they are replaced annually and remain undisturbed due to the absence of tillage. The nearly constant DispFines20 (Fig. S4 in **Paper 4**), DispClay 8-16 mm (**Fig. 15**) and WSA 1-2 mm (**Fig. 16b**) across the four G plots, despite varying SOC/Clay values, may be related to a markedly greater presence of binding agents in the G treatment, supposed to be similar for the four plots. The DI test involved extreme disruptive energy, and the ability of the G treatment to resist this was not expected to be related to binding agents but rather to stable organo-mineral associations (**Paper 4**), i.e., the SOM quality in the G treatment may be different from the tilled treatments.

3.3.4 Limitations regarding the use of critical threshold values

As mentioned above, other drivers, such as tillage and root density, may be more important for soil physical properties than the ratio of mineral fines to SOC content. Further, machinery-induced soil compaction, cropping and harvest operations may also be more crucial. As an example, Schjønning et al. (2012) found that potato harvest and tillage in late autumn overshadowed the effects of mineral fines and SOC content on SSS in the preceding year. Soil physical properties related to the mineral fines/SOC ratios are dynamic properties and may be affected by seasonal and annual conditions (Daraghmeh et al., 2009; Perfect et al., 1990). The concentration of exchangeable cations and contaminants such as copper may also overshadow effects of mineral fines and SOC content on SSS (Arthur et al., 2012; Kumari et al., 2017).

The relatively permanent and inert structure of sand particles suggests that the critical mineral fines/SOC ratios are not applicable on coarse sandy soil. A conservative estimate for a lower clay content limit for the use of the concept could be 5% clay.

3.4 Soil degradation and recovery (**Papers 6 and 7**)

The Highfield-LUCE provided a suitable research platform for studying soil degradation and recovery. Briefly, the degradation managements comprised conversion of grassland plots on the Highfield-LTE site to arable and bare fallow plots (treatment GA and GBF, respectively), while the restoration managements included introduction of grassland in arable and bare fallow plots (treatment AG and BFG, respectively). The simultaneous conversion of selected long-term contrasting plots that had reached steady-state conditions to new soil degradation and restoration management gave a unique opportunity to compare short-term effects (six years) with existing long-term plots. In addition, this setup makes quantification of the rate of change in SOM fractions, SSS measures and pore-size distribution possible.

Conversion of grassland to arable and bare fallow management resulted in a decrease in SOC by 14% and 22%, respectively, and accordingly an increase in the clay/SOC ratio (**Table 8**).

Table 8. Soil organic carbon (SOC) and clay/SOC ratio. Within rows, letters denote statistical significance at $P < 0.05$ for the comparison of G with GA and GBF, BF with BFG, and A with AG. For treatment abbreviations see **Table 2**. Partly from **Paper 6**.

	Degradation managements			Restoration managements			
	G	GA	GBF	BF	BFG	A	AG
Soil organic carbon (SOC, g kg ⁻¹ minerals)	32.9 ^b	28.2 ^a	25.6 ^a	9.0	13.1	17.3	18.6
Relative change (%)		-14%	-22%		+46%		+8%
Clay (<2 µm)/SOC	8.0 ^a	9.2 ^{ab}	10.0 ^b	30.1 ^b	19.4 ^a	15.3	14.4
Relative change (%)		+15%	+25%		-36%		-6%

The SSS measures (**Fig. 20a-c**) and pore characteristics (**Fig. 21ab**) were affected differently by the degradation managements. Thus, SOC or the clay/SOC ratio were, in general, not able to explain the changes in the soil properties, presumably because of the interaction between increased tillage intensity and loss of binding agents following conversion of grassland, as discussed in **Papers 6 and 7** and summarized below. Clay-SOM disintegration increased by around 20% for the GA and GBF treatments (**Fig. 20c**). The reduced stability of extremely stable organo-mineral associations was ascribed to the decrease in SOC concentration with a change towards total disintegration at levels below 2 g C 100 g⁻¹ minerals irrespective of management (**Fig. 4a** in **Paper 4**, **Fig. 6g** in **Paper 6**). The persistent stability for DispClay 1-2 mm (**Fig. 20a**) was supposedly due to formation of <2 mm aggregates caused by microbial decomposition products offsetting the tillage-induced breakdown of aggregates in the former grassland (**Paper 6**). In contrast, the increase in DispClay 8-16 mm for the GA and GBF treatments (**Fig. 20b**) might be related to the increase in tillage intensity destroying the stabilizing enmeshment of aggregates by plant roots and fungal hyphae, which are known to be sensitive to tillage and important for stability in larger soil aggregates (Miller and Jastrow, 1990). The

nearly constant DispClay 8-16 mm across the G and GA plots indicated that the SOC/Clay value was less important for these treatments (Fig. 7d in **Paper 6**), and possibly that binding agents present in a similar quantity were more important. For the GA and GBF treatments, tillth characteristics derived from the pore-size distribution were related to a decrease in SOC content, tillage-induced increase in DispClay 8-16 mm and rearrangement of the soil pore system (**Paper 7**). However, plant-available water capacity based on identical soil quantities ($PAWC_{eq}$, see **Paper 7** for a detailed description of how $PAWC_{eq}$ was calculated) followed the relative decrease in SOC concentration (**Table 8** and **Fig. 21a**), whereas the relative decrease in structural void ratio (V_2) was more pronounced (**Fig. 21b**).

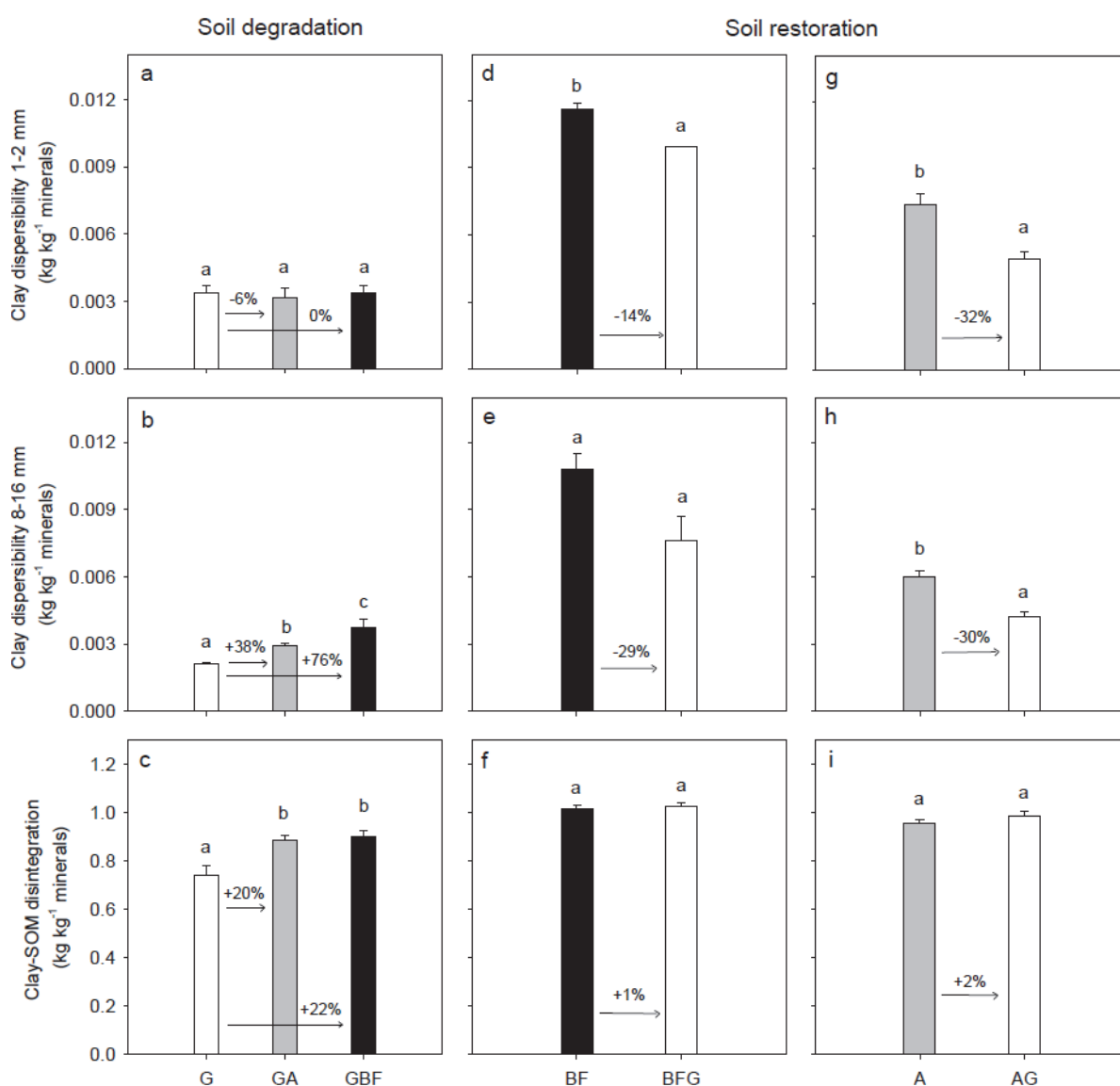


Fig. 20. Management system effects in degradation (a-c) and restoration (d-i) scenarios on clay dispersibility of 1-2 mm aggregates rewetted to -100 hPa, clay dispersibility of 8-16 mm aggregates rewetted to -100 hPa, and clay-SOM disintegration (the ratio between clay particles retrieved without SOM removal and with removal). White, grey and black bar-fills represent grass, arable and bare fallow treatments, respectively, at time of sampling. Letters denote statistical significance at $P < 0.05$. The numbers denote relative differences. For treatment abbreviations see **Table 2**. Modified from **Paper 6**.

Changes in soil physical properties following conversion from grassland to arable and bare fallow management could not exclusively be explained by changes in SOC and clay/SOC, but content of SOC and the clay/SOC ratio were associated with a degradation of the SSS measures and pore-size distribution, except for DispClay 1-2 mm.

Introduction of grassland in bare fallow soil resulted in an increase in SOC by 46% (although only marginally significant, $P=0.053$) and accordingly a decrease in the clay/SOC ratio (**Table 8**). The increase in SOC and corresponding decrease in clay/SOC after introducing grassland in arable soil was small (**Table 8**). The SSS measures (**Fig. 20d-i**) and pore characteristics (**Fig. 21c-f**) for the treatments under restoration management were less related to SOC and clay/SOC than were the treatments under degradation management. This presumably relates to the combination of grassland establishment with associated positive impacts and the absence of tillage, as discussed in **Papers 6** and **7** and summarized below.

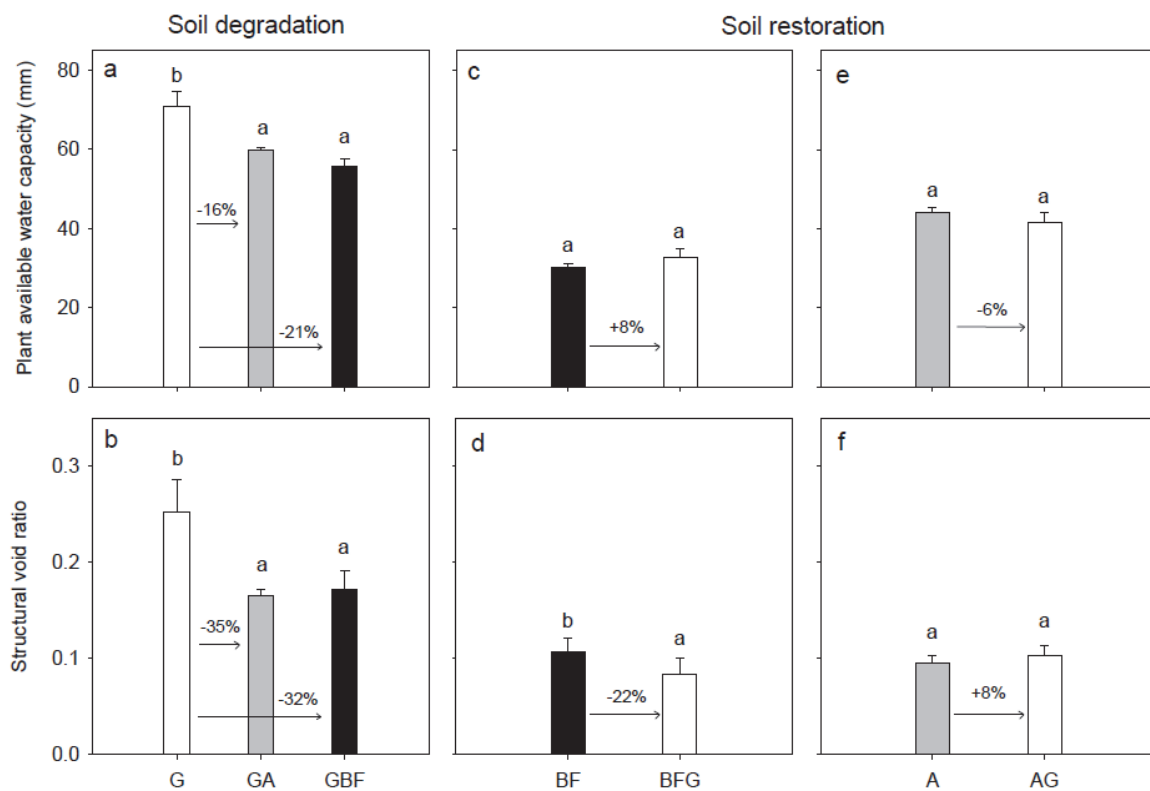


Fig. 21. Management system effects in degradation (a-b) and restoration (c-f) scenarios on plant-available water capacity calculated based on a soil mass equivalent to 20 cm in the G soil, and structural void ratio. White, grey and black bar-fills represent grass, arable and bare fallow treatments, respectively, at time of sampling. Letters denote statistical significance at $P < 0.05$. The numbers denote relative differences. For treatment abbreviations see **Table 2**. Modified from **Paper 7**.

Clay dispersibility of 1-2 mm rewetted aggregates and DispClay 8-16 mm decreased when grassland was introduced (**Fig. 20d-e, g-h**). Hence, SSS increased for AG despite the limited increase in SOC.

Other studies have also found that small increases in SOC may have disproportionately large and beneficial effects on SSS (e.g., Poulton et al., 2018). However, in this case other drivers may be affecting SSS rather than the small increase in SOC, namely, the introduction of grassland which is associated with increases in aggregate-binding agents (Attard et al., 2016; Schjønning et al., 2007) and bonding agents arising from increased microbial and mesofauna abundance (Hirsch et al., 2017) as well as the absence of tillage enabling the aggregate-forming agents to persist. Despite the increase in macro-aggregate stability, tilth characteristics derived from the pore-size distribution of the soils for BFG and AG did not indicate recovery (**Fig. 21c-f**). The lack of an effect on $PAWC_{eq}$ and V_2 may be related to an increase in density of the previously intensively tilled and degraded soils (**Paper 7**), illustrating that self-organization of the pore network may be a slow process. The structural void ratio even *decreased* when grassland was introduced in bare fallow soil, implying that SOM-depleted and highly degraded soil may experience a period with increasing density when discontinuing tillage.

The rate of change (f) in SOM fractions, SSS measures and pore-size distribution was calculated as $f = x/y \times 100$, where x and y denote the changes in the properties after six years and at steady-state condition, respectively. The process of loss of SOC and degradation of a complex structure was faster than their restoration and development (Fig. 2d and g and Fig. 3a and d in **Paper 6**; Fig. 2c-f and Fig. 3 in **Paper 7**). In contrast, it was faster to restore than to degrade SSS at macroscale (Fig. 4d-e and g-h and Fig. 5a-b and d-e in **Paper 6**). The different responses of SOC, SSS measures and measures of pore-size distribution to soil degradation and restorative managements have implications for SOC modelling which is discussed in **Paper 6** and show that the effect of soil management changes depends on the particular soil property being considered.

3.4.1 Soil organic matter fractions

As already mentioned, POXC and HWC have been suggested as more sensitive indicators of soil management change than total SOC. Bongiorno et al. (2019), for example, assessed a range of labile SOM fractions and found that POXC was the most sensitive to tillage and OM additions. In the present study, however, SOC, POXC and HWC showed similar sensitivities to management changes for both the degradation and restoration managements (**Paper 6**). The similar response to management for SOC and POXC agrees with the results of Romero et al. (2018), who studied management effects in semiarid drylands. Permanganate-oxidizable C has also been termed as a multifunctional soil quality indicator (Bongiorno et al., 2019), but it did not describe changes in SSS any better than SOC (**Paper 6**). Permanganate-oxidizable C is not a well-defined fraction of OM as it is best described as a complex mixture of $KMnO_4$ -reactive OM (Romero et al., 2018). Permanganate-oxidizable C is thus defined by the extraction procedure and is not based on a mechanistic understanding of its role in soil. This may partly explain why it was no better than SOC at explaining changes in SSS.

4. Conclusions

The main conclusions of the Ph.D. work are:

- Accurate estimates of soil organic carbon (SOC) content can be achieved by high-temperature (≥ 900 °C) dry combustion methods. If indirect analytical approaches such as loss-on-ignition (LOI) are used, accounting for clay content considerably improves the conversion of LOI to SOC.
- The SOC to soil organic matter (SOM) conversion factor for arable top-soil can be estimated by relating LOI to SOC and clay. A conversion factor of 2 is more appropriate than the conventional conversion factor of 1.724.
- Without the removal of SOM prior to soil texture analysis, clay (< 2 μm) was underestimated and silt (2-20 μm) overestimated, and the systematic errors increased with increasing SOC. Consequently, removal of SOM prior to soil texture analysis is needed to obtain precise estimates of soil particles < 20 μm .
- Particle size fractions and SOC should be expressed on an oven-dry basis to avoid systematic underestimation of these properties.
- The use of explicitly defined units for soil properties and detailed method descriptions for soil texture analysis and SOC are strongly recommended.
- The content of SOC was not a good predictor of proper soil functioning across different soil types. Instead, mineral fines/SOC ratios were more applicable for identifying a critically low carbon threshold.
- For soil structural stability measures, the critical ratio values for clay/SOC and Fines₂₀ (< 20 μm)/SOC ranged from 8-13 and 16-27, respectively. For aggregate strength and pore-size distribution measures, it was not possible to identify critical clay/SOC and Fines₂₀/SOC ratios.

- In the degradation scenarios, conversion of grassland to arable or bare fallow management reduced the SOC content and soil physical properties, except for clay dispersibility of 1-2 mm rewetted aggregates. Changes in soil physical properties could not solely be ascribed to the loss of SOC, most likely due to the interaction between increased tillage intensity and binding agents.
- In the restorative scenarios, introduction of grassland in bare fallow and arable soils increased the macro-aggregate stability, whereas the structural void ratio and the plant-available water capacity based on identical soil quantities showed no sign of recovery. Thus, self-organization of the pore network may be a slow process. Changes in soil physical properties were related less to changes in SOC content, which may be due to the combined positive effects of grassland and the absence of tillage.
- Loss of SOC and the degradation of a complex soil structure were more rapid than the restoration of SOC and development of a complex soil structure. However, it was faster to restore macro-aggregate stability than to degrade it.
- Permanganate-oxidizable C (POXC) and hot water-extractable C (HWC) were neither more sensitive to management changes nor able to better describe changes in soil structural stability than total SOC. This was true for both the long-term (>50 years) and the short-term management changes (six years).

5. Perspectives and implications

The mineral fines/SOC ratios hypothesis has only been tested to a limited extent and only on arable mineral soils from the temperate region in Europe. Therefore, there is a need to evaluate the concept on soils from other climate regions, and on different soil types and clay mineralogies. Study sites where the content of clay and silt (2-20 μm) differs are also needed to reveal whether the Fines20 (<20 μm)/SOC ratio is better than the clay/SOC ratio. Moreover, relating the mineral fines/SOC ratios to other soil functions would be a key step towards identification of a critical mineral fines/SOC ratio.

A refinement of the mineral fines/SOC ratios for more weakly structured sandy soils is also needed. A way forward could be to sort out non-texture-stabilized C as defined by Springob and Kirchmann (2010). This would be an advantage on arable soils with a land use history of e.g. heathland or waterlogging (Schjønning et al., 2018; Springob and Kirchmann, 2010).

An indirect approach to investigate the mineral fines/SOC ratios further would be to carry out physical fractionation of clay and silt (Christensen, 1996) and measure the SOC content of each particle size fraction. In this way, soil structural stability (SSS) measures could be related to the actual SOC content in the mineral particle fractions rather than to the SOC content in bulk soil.

The mineral fines/SOC ratios were more suitable for identifying critically low carbon levels across soil types than the content of SOC in itself. This indicates that coverage of C on mineral particle surfaces influences SSS. Inspired by this, the spatial arrangement of SOM on mineral particle surfaces and in aggregates may be of vital importance for particle interaction and aggregation, and thus protection and stabilization of soil structure. Therefore, an interesting way forward would be to merge our present understanding of SOM effects on SSS at various scales with novel approaches for assessing SOM intensity and spatial distribution (quantification of coverage and connectivity). The spatial arrangement of SOM can be measured non-invasively at μm - and mm-scale (Arai et al., 2019; Kopittke et al., 2020; Peth et al., 2014; Rawlins et al., 2016; Schweizer et al., 2018). However, the methodologies have not been adapted and related to stabilization of soil structure. Merging non-invasive assessment of the spatial distribution of SOM with SSS at the scale of interest may potentially lead to scientific advancements in the underlying processes controlling soil structural development as well as C sequestration. In addition, it would be possible to investigate how SOM coverage and connectivity change according to the mineral fines/SOC ratios. From this we might be able to assess whether mineral particles have a finite C storage capacity, and whether an abrupt change in SOM coverage can be quantified at clay/SOC>10 and Fines20/SOC>20.

Additional studies investigating rates of change in degradation and restoration scenarios initiated simultaneously are needed to examine whether the findings in this Ph.D. study are generally applicable. Based on this study, a next step could be to refine SOC models by implementing the asymmetric response in SOC to degradation and restoration management in the models.

Prospectively, the agricultural sector in Denmark is expected to contribute to mitigation of climate change, by, for example, abandoning peatland or including more long-term grass in the crop rotation. Conversion of arable land to grassland is considered an important means of increasing SOC sequestration. The findings in this thesis show that long-term grassland has positive effects on both SSS, soil structure and SOC sequestration. However, converting agricultural land to permanent grassland would be impractical or uneconomic for many farmers and thus requires political intervention. Further, a widespread adoption of such a management change may pose a threat to global food security since the area used for growing crops for use as food would be reduced. In a Danish context, it may be possible to increase the proportion of grass in the crop rotation on dairy farms and potentially increase SOC sequestration. It is, however, important to consider other greenhouse gas emissions as well since, e.g., nitrous oxide emissions can be substantial following grassland cultivation (Brozyna et al., 2013).

6. References

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7. Supporting papers

Paper 1

***Converting loss-on-ignition to organic carbon content in arable topsoil:
pitfalls and proposed procedure***

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Converting loss-on-ignition to organic carbon content in arable topsoil: pitfalls and proposed procedure

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Summary

Assessments of changes in soil organic carbon (SOC) stocks depend heavily on reliable values of SOC content obtained by automated high-temperature C analysers. However, historical as well as current research often relies on indirect SOC estimates such as loss-on-ignition (LOI). In this study, we revisit the conversion of LOI to SOC using soil from two long-term agricultural field experiments and one arable field with different contents of SOC, clay and particles <20 µm (Fines20). Clay-, silt- and sand-sized fractions were isolated from the arable soil. Samples were analysed for texture, LOI (500°C for 4 hours) and SOC by dry combustion. For a topsoil with 2 g C and 30 g clay 100 g⁻¹ soil, converting LOI to SOC by the conventional factor 0.58 overestimated the SOC stock by 45 Mg C ha⁻¹. The error increased with increasing contents of clay and Fines20. Converting LOI to SOC by a regression model underestimated the SOC stock by 5 Mg C ha⁻¹ at small clay and Fines20 contents and overestimated the SOC stock by 8 Mg C ha⁻¹ at large contents. This was due to losses of structural water from clay minerals. The best model to convert LOI to SOC incorporated clay content. Evaluating this model against an independent dataset gave a root mean square error and mean error of 0.295 and 0.125 g C 100 g⁻¹, respectively. To avoid misleading accounts of SOC stocks in agricultural soils, we recommend re-analysis of archived soil samples for SOC using high-temperature dry combustion methods. Where archived samples are not available, accounting for clay content improves conversion of LOI to SOC considerably. The use of the conventional conversion factor 0.58 is antiquated and provides misleading estimates of SOC stocks.

Highlights

- Assessment of SOC contents is often based on less accurate methods such as LOI.
- Reliable accounts of changes in SOC stocks remain high on the agenda (4% initiative).
- Conversion of LOI to SOC is considerably improved by accounting for clay content.
- Converting LOI to SOC by the conventional factor 0.58 leads to grossly overestimated SOC stocks.

Introduction

Accounting for changes in soil organic carbon (SOC) induced by changes in climate, land use and soil management remains high on the agenda, as exemplified by the 4 per mille initiative launched at the recent COP-21 conference in Paris (Minasny *et al.*, 2017). This global research initiative aims at a relative annual increase in SOC of 0.4% in the top 40 cm of soil. Changes in SOC stocks occur slowly and over long periods, therefore verification of changes involves present as well as historical

accounts of SOC. Verification of changes in SOC stocks on global scales are not always well described in terms of sources of SOC content data and methods used for determination of SOC (Stockmann *et al.*, 2015; Hengl *et al.*, 2017). Accurate and precise determination of SOC contents is fundamental for reliable estimates of SOC stocks (Goidts *et al.*, 2009; Conant *et al.*, 2011; Schrumpf *et al.*, 2011); this can be obtained by automated, high-temperature dry combustion methods (Chatterjee *et al.*, 2009).

Loss-on-ignition (LOI), however, remains a widely used method for assessing SOC in agricultural and forest soils, with LOI being converted to SOC either by a fixed conversion factor or by

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regression analyses (Konen *et al.*, 2002; De Vos *et al.*, 2005; Salehi *et al.*, 2011; Reynolds *et al.*, 2013). The basic assumption is that LOI is due only to combustion of soil organic matter (SOM) and that the content of SOC in SOM is constant (Christensen & Malmros, 1982). No standard protocol exists for LOI analysis, but it is well documented that LOI is affected by ignition temperature, duration of ignition and ignited sample mass (Abella & Zimmer, 2007; Salehi *et al.*, 2011; Hoogsteen *et al.*, 2015). Further, structural water loss (SWL) from soil minerals may contribute significantly to LOI (Sun *et al.*, 2009; Hoogsteen *et al.*, 2015) and the validity of the conventional LOI-to-SOC conversion factor of 0.58, although widely used, remains dubious (Pribyl, 2010). When LOI and SOC are both measured, regression models for converting LOI to SOC have been proposed (Grewal *et al.*, 1991; De Vos *et al.*, 2005; Abella & Zimmer, 2007). Regression models based on less accurate analytical approaches, such as dichromate oxidation followed by titration, and soils with confounding effects from differences in clay mineralogy have been found to be less reliable (Howard & Howard, 1990).

In our current research attempting to define critical small SOM contents for soil structural properties based on the clay content ($<2\mu\text{m}$)/SOC and particles $<20\mu\text{m}$ (Fines20)/SOC ratios (Schjønning *et al.*, 2012; Getahun *et al.*, 2016; Jensen *et al.*, 2017a), it is essential to have access to reliable values of SOC content. The combined fraction of clay plus silt (particles $<20\mu\text{m}$) is denoted Fines20. As a 'spin-off' from this research, we revisited the conversion of LOI to SOC. Data for temperate zone arable topsoil with different contents of SOC were collected from long-term agricultural field experiments with contrasting management at Askov (Denmark) and Rothamsted (UK), and from a texture gradient in a farmer's field at Lerbjerg (Denmark) with uniform management and mineralogy. These fields had large ranges in LOI, SOC, clay and Fines20, making them representative of arable soils with respect to these properties. We also included clay-, silt- and sand-sized fractions isolated from Lerbjerg soil samples.

Materials and methods

Rothamsted Highfield ley–arable experiment

Soil texture and SOC data for the Highfield experiment at Rothamsted Research, UK ($51^{\circ}80'\text{N}$, $00^{\circ}36'\text{W}$), were extracted from Jensen *et al.* (2017b). This experiment is on a silt loam soil belonging to the Batcombe series; the parent material includes a silty (loess-containing) deposit overlying and mixed with clay-with-flints (Avery & Catt, 1995). The soil was classified as an Aquic Paludalf (Soil Survey Staff, 2014) and Chromic Luvisol (IUSS Working Group WRB, 2015). The clay fraction is dominated by smectite, mica and kaolinite, with traces of feldspar, chlorite and crystalline and amorphous ferric oxides (Avery & Catt, 1995). Bulk soil was taken in spring 2015 from the 6–15-cm layer of four different treatments: bare fallow maintained free of vegetation since 1959, arable rotation with

winter cereals (winter wheat (*Triticum aestivum* L.) and winter oat (*Avena sativa* L.)) since 1948, ley–arable rotation with 3-year grass–clover ley (meadow fescue (*Festuca pratensis* L.), timothy-grass (*Phleum pratense* L.) and white clover (*Trifolium repens* L.)) followed by 3 years under arable management (as arable rotation) since 1948 and grassland ploughed and reseeded to grass (predominantly rye grass, *Lolium perenne* L.) in 1948. Soil was sampled from three positions within each of four replicate plots, providing 48 samples. Jensen *et al.* (2017b) provide further details.

Askov long-term experiment on animal manure and mineral fertilizers (Askov-LTE)

Data on soil texture and SOC for the Askov-LTE in southern Denmark ($55^{\circ}28'\text{N}$, $09^{\circ}07'\text{E}$) were retrieved from Jensen *et al.* (2017a). This experiment was established in 1894 on a sandy loam soil. The parent material comprises terminal morain deposits from the Weichselian glaciation stage. The soil was classified as a Ultic Hapludalf (Soil Survey Staff, 2014) and Aric Haplic Luvisol (IUSS Working Group WRB, 2015). The clay fraction is dominated by illite, kaolinite, quartz and smectite, with traces of vermiculite, Al-Fe-oxyhydroxides, feldspar and chlorite. Following harvest of winter wheat (*Triticum aestivum* L.), bulk soil was sampled in autumn 2014 from the 6–15-cm layer of four different treatments in the B5 field: unfertilized, $\frac{1}{2}$ mineral fertilizer (since 1923), 1 mineral fertilizer and $1\frac{1}{2}$ animal manure. Nutrient addition rate 1 corresponds to $150\text{ kg total-N ha}^{-1}$, 30 kg P ha^{-1} and 120 kg K ha^{-1} . Three replicate plots of each treatment were sampled, providing 12 samples. Further details are given in Jensen *et al.* (2017a).

Lerbjerg textural gradient

Soil was sampled from a naturally occurring textural gradient located in an arable field at Lerbjerg, Denmark ($56^{\circ}22'\text{N}$, $09^{\circ}59'\text{E}$). The Lerbjerg field has a uniform parent material (Weichselian morainic deposits) but varies widely in both texture and SOC content. The clay fraction is dominated by illite, smectite and vermiculite, with traces of kaolinite, quartz and feldspar (Schjønning *et al.*, 1999). Bulk soil from the 5–10-cm layer was sampled in autumn 2015 at 16 locations along the texture gradient following harvest of oil-seed rape (*Brassica napus* L.).

Lerbjerg soil particle-size fractions

Archived samples of soil particle-size fractions from Lerbjerg (Schjønning & de Jonge, 1999) were used to estimate soil mineral structural water loss (SWL) from clay- ($<2\mu\text{m}$), silt- ($2\text{--}63\mu\text{m}$) and sand-sized ($63\text{--}2000\mu\text{m}$) soil components. Soil samples were fully dispersed with an ultrasonic probe (300 W for 15 minutes), and the size fractions were isolated by a combination of sedimentation in water and dry sieving. Schjønning & de Jonge (1999) describe the protocol for particle-size fractionation in detail.

Determination of clay, silt, loss-on-ignition and soil organic carbon

Clay (< 2 µm) and silt (2–20 µm) contents of air-dried soil (< 2 mm) were determined by the hydrometer method for Highfield and Askov, and the pipette method for Lerbjerg, both described by Gee & Or (2002). Samples for determination of clay and silt were treated with hydrogen peroxide to remove SOM. The presence of carbonates was tested by adding a few droplets of 10% HCl, but none was found. Loss-on-ignition was determined on the oven-dried subsamples of bulk soil and soil size fractions. Five grams of air-dry soil was added to previously ignited and weighed porcelain crucibles, dried at 105°C for 24 hours in a ventilated oven, cooled in a desiccator and weighed again. Residual water content (RWC) was calculated as the difference between the air-dry and oven-dry weights and related to oven-dry soil. Finally, the crucibles were ignited at 500°C for 4 hours in a muffle furnace (Thermolyne Largest Tabletop Muffle Furnace, Thermo Fisher Scientific, Waltham, MA, USA). After ignition, the crucibles were cooled in a desiccator and weighed. The LOI was calculated as the difference between the oven-dry weight before and after ignition and related to oven-dry soil. The SOC content was determined by high-temperature dry combustion using ball-milled subsamples of air-dried soil (< 2 mm). A Thermo Flash 2000 NC Soil Analyser (Thermo Fisher Scientific, Waltham MA, USA) was used for soil size fractions from Lerbjerg and bulk soil from Highfield and Askov, and an ELTRA Helios C-Analyser (ELTRA GmbH, Haan, NRW, Germany) was used for bulk soil from Lerbjerg. Results for SOC, LOI and soil size fractions are expressed as g 100 g⁻¹ oven-dry soil (105°C for 24 hours).

Evaluation dataset

The regression model developed to convert LOI to SOC was evaluated using a study reporting data on LOI-450 (450°C for 5 hours), LOI-550 (550°C for 5 hours), SOC (high-temperature dry combustion) and clay content (Grewal *et al.*, 1991). This study was used because it focused on arable soil, measured SOC by high-temperature dry combustion, reported clay content, used an LOI protocol close to ours and reported raw data in tabulated form. The study was based on 40 samples from cultivated soils and grasslands in New Zealand, including topsoil and subsoil. The samples were from eight different fields, of which five differed in parent material. Our study was based solely on data from topsoil; therefore, subsoil samples (> 35 cm depth) in the evaluation dataset were not considered, reducing the evaluation dataset to 31 samples. This subset of data had a range of values for SOC, clay, LOI-450 and LOI-550 from 0.75 to 6.33, 17 to 57, 2.64 to 15.19 and 3.35 to 15.94 g 100 g⁻¹, respectively.

Statistics

Linear regression was applied using the R-project software package version 3.1.1 (R Core Team, 2017). The variance inflation factor (VIF) was calculated when more than one predictor was used in

the regression. The VIF expresses the degree of multicollinearity among the predictors. The upper threshold value for non-erroneous conclusions from multiple regressions has been set previously to 5 (Rogerson, 2001) or 10 (Kutner *et al.*, 2004). For models with more than one predictor and an intercept term, the adjusted coefficient of determination (R^2) is reported. The R^2 was calculated as $1 - SS_{\text{res}}/SS_{\text{tot}}$ for models without intercept, where SS_{res} is from the model without intercept and SS_{tot} from the model with intercept. Akaike's information criterion (AIC) was used to compare models with different numbers of parameters (Akaike, 1973). A smaller or more negative AIC indicates better model performance. The root mean square error (RMSE) and mean error (ME) were calculated to evaluate model performance:

$$\text{RMSE} = \sqrt{\frac{1}{m} \sum_{i=1}^m d_i^2}, \quad (1)$$

$$\text{ME} = \frac{1}{m} \sum_{i=1}^m d_i, \quad (2)$$

where d_i is the difference between the predicted and measured SOC content and m is the sample size.

Results

The soils differed in SOC content because of long-term contrasting management at Highfield and Askov, and soil topography at Lerbjerg (Table 1). Clay and Fines20 contents varied little at Highfield and Askov, whereas samples from Lerbjerg reflected a wide texture gradient.

The VIF, calculated for the combination of LOI and clay, was 1.02, 1.18 and 6.83 for Highfield, Askov and Lerbjerg, respectively. For LOI and Fines20, the corresponding VIF was 1.00, 1.06 and 6.58. Although the use of VIF threshold values has been questioned (O'Brien, 2007), the degree of multicollinearity among predictors in the regressions was small for Highfield and Askov and allowed the use of both in the regression analysis. We recognize that the VIF value for Lerbjerg was on the limit of multicollinearity.

The RWC ranged from 0.9 to 6.2 g 100 g⁻¹ oven-dry soil and increased linearly with increasing contents of SOC and clay content (data not shown). For the soil with the largest content of clay (69 g clay 100 g⁻¹) and SOC (4.14 g C 100 g⁻¹), neglecting the correction for RWC underestimates SOC by 0.26 g C 100 g⁻¹.

There was a strong positive relation between LOI and SOC ($\text{SOC} = 0.39 \times \text{LOI} - 0.28$; Figure 1). In general, the sandy soils are above the regression line, whereas the clayey soils are below. The clay (< 2 µm), silt (2–63 µm) and sand (63–2000 µm) fractions were not included in the linear regression. They were used only for determination of SWL.

Table 2 gives the results from tests of various linear models of the relation between SOC and LOI, clay and Fines20. Fines20 was tested in addition to clay because silt-sized separates may

Table 1 Soil organic carbon (SOC), loss-on-ignition (LOI), clay (< 2 µm) and mineral particles < 20 µm (Fines20) for bulk soils and particle-size fractions from Lerbjerg

Sample	n	SOC	LOI	Clay	Fines20
		Mean, minimum and maximum / g 100 g ⁻¹ soil			
Highfield (UK)	48	1.93 (0.78–3.94)	5.84 (3.62–9.80)	25 (22–32)	50 (47–57)
Askov (Denmark)	12	1.09 (0.86–1.37)	2.90 (2.40–3.55)	9 (9–10)	19 (17–20)
Lerbjerg (Denmark)	16	2.37 (1.06–4.14)	7.11 (3.34–12.08)	38 (10–73)	49 (15–91)
Clay fraction < 2 µm	4	1.81 (1.44–2.17)	7.63 (7.12–8.08)	98 (98–99)	NA
Silt fraction 2–63 µm	4	2.00 (1.82–2.27)	4.76 (4.57–4.92)	0	NA
Sand fraction 63–2000 µm	4	0.14 (0.04–0.20)	0.42 (0.22–0.65)	0	0

n, number of samples; NA, not applicable.

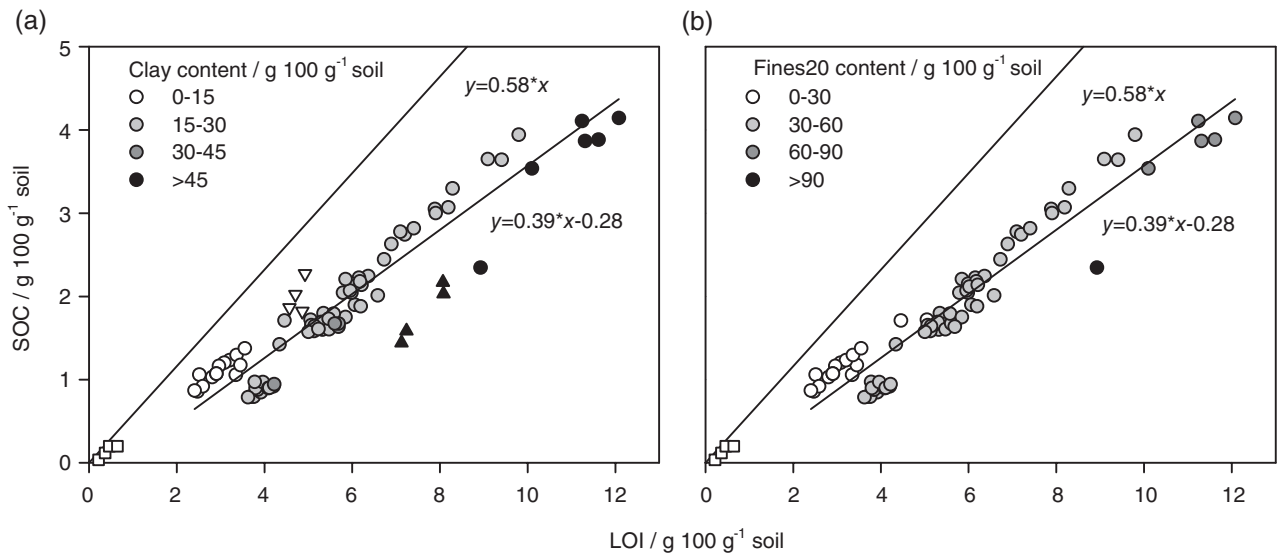


Figure 1 Soil organic carbon (SOC) as a function of loss-on-ignition (LOI) for (a) samples grouped by soil clay content ($n = 88$) and (b) samples grouped by soil mineral particles < 20 µm (Fines20) content ($n = 80$). The clay- (< 2 µm), silt- (2–63 µm) and sand-sized (63–2000 µm) fractions from the Lerbjerg site are shown with triangle up, triangle down and square symbols, respectively. Because of different size limits for silt isolated from Lerbjerg, panel (b) does not include Fines20 from Lerbjerg. The line representing the conventional relation between LOI and SOC ($\text{SOC} = 0.58 \times \text{LOI}$) is also shown.

also contain clay minerals. The interaction between LOI and clay for the individual sites was not significant (Highfield, $P = 0.995$; Askov, $P = 0.193$; Lerbjerg, $P = 0.301$). Similarly, the interaction between LOI and Fines20 was not significant (Highfield, $P = 0.125$; Askov, $P = 0.248$; Lerbjerg, $P = 0.086$). Quadratic clay or Fines20 terms were not significant when included in the models for Highfield (clay², $P = 0.937$; Fines20², $P = 0.581$) and Lerbjerg (clay², $P = 0.439$; Fines20², $P = 0.137$). For Askov the quadratic clay term was not significant (clay², $P = 0.439$). However, the quadratic Fines20 term was significant (Fines20², $P = 0.009$), but the assumption of homoscedasticity for the linear regression model was not fulfilled, so the quadratic term was not included in the model. When the intercept of a given model was non-significant, it was disregarded and the regression forced through the origin. In general, the regression coefficient for LOI was positive, whereas clay and Fines20 had negative regression coefficients when both LOI and clay or Fines20 were included in the models. The best model for each site was taken as the model with the largest R^2 and smallest AIC. If the intercept

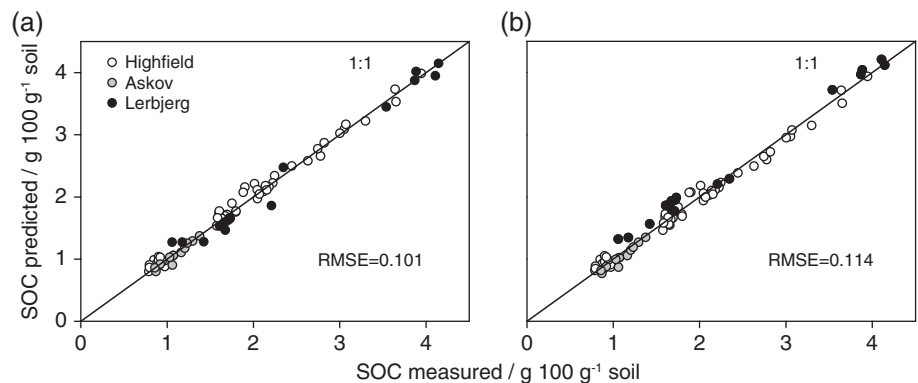
was non-significant, the model without intercept was selected as the best model. Models differing by < 2 in AIC values are not considered significantly different (Burnham & Anderson, 2002). The best models for Highfield included clay, whereas the best models for Askov and Lerbjerg included Fines20. To find the best overall model based on data from all three sites, a model including a linear effect of LOI and clay was tested. However, the plot of residuals for this model showed that it was not fully able to capture the effect of clay across individual sites. Therefore, we tested a model that included a quadratic clay term, and the residual plot revealed a better prediction of clay effect across individual sites. Thus, the best overall model included a quadratic clay expression (model O2.1, Table 3):

$$\text{SOC} = 0.513 \text{ LOI} - (0.047 \text{ Clay} - 0.00025 \text{ Clay}^2). \quad (3)$$

The inclusion of clay as a predictor increased the variation explained by 7% compared with the simpler model including LOI only (Table 3). The model including Fines20 (O3, Table 3) was

Table 2 Parameter estimates, R^2 and the Akaike information criterion (AIC) for linear models of the relation between soil organic carbon (SOC) and loss-on-ignition (LOI), clay ($< 2 \mu\text{m}$) and mineral particles $< 20 \mu\text{m}$ (Fines20) for individual sites

Model	Intercept	P-value	LOI / g 100 g ⁻¹	P-value	Clay / g 100 g ⁻¹	P-value	Fines20 / g 100 g ⁻¹	P-value	R^2	AIC
Highfield										
H1	-1.145	< 0.001	0.526	< 0.001					0.981	-66.6
H2	-0.164	0.322	0.519	< 0.001	-0.037	< 0.001			0.990	-94.9
H2.1	0		0.515	< 0.001	-0.043	< 0.001			0.990	-95.8
H3	0.579	0.083	0.525	< 0.001			-0.034	< 0.001	0.988	-88.2
H3.1	0		0.528	< 0.001			-0.023	< 0.001	0.988	-86.9
Askov										
A1	-0.155	0.244	0.432	< 0.001					0.910	-33.2
A1.1	0		0.379	< 0.001					0.896	-33.5
A2	0.314	0.301	0.461	< 0.001	-0.059	0.107			0.919	-34.8
A2.1	0		0.461	< 0.001	-0.026	0.080			0.925	-35.3
A3	0.440	0.103	0.453	< 0.001			-0.035	0.025	0.939	-38.2
A3.1	0		0.465	< 0.001			-0.013	0.046	0.942	-36.5
Lerbjerg										
L1	-0.103	0.501	0.347	< 0.001					0.959	3.2
L1.1	0		0.335	< 0.001					0.957	1.8
L2	-0.230	< 0.001	0.506	< 0.001	-0.026	< 0.001			0.992	-23.0
L3	-0.150	0.028	0.505	< 0.001			-0.022	< 0.001	0.993	-25.0

Figure 2 Soil organic carbon (SOC) content predicted by (a) the linear model including loss-on-ignition (LOI) and the quadratic clay expression (model O2.1, Table 3 (Equation (3))) and (b) the linear model including LOI and the quadratic mineral particles $< 20 \mu\text{m}$ (Fines20) expression (model O3, Table 3) as a function of the measured SOC content. RMSE, root mean square error.

almost as good as the model including clay. The interaction term in the models was not significant (O2.1, $P = 0.172$; O3, $P = 0.991$). The clay and Fines20 models predicted SOC with an RMSE of only 0.101 and 0.114 g C 100 g⁻¹, respectively, and the predicted versus measured SOC corresponded closely to the 1:1 line (Figure 2).

The mineral structural water loss (SWL) was estimated by calculating the SOC content as $\text{LOI} \times 0.513$ (Equation (3)) and then subtracting the measured SOC content. The SWL from Lerbjerg clay ($< 2 \mu\text{m}$), silt ($2-63 \mu\text{m}$) and sand ($63-2000 \mu\text{m}$) fractions was 2.11, 0.45 and 0.08 g 100 g⁻¹, respectively, with standard deviations of 0.10, 0.18 and 0.04 g 100 g⁻¹. The SWL was mainly from the clay fraction, emphasizing the need to include clay or Fines20 in the regression models. When the conventional conversion factor of 0.58 was used, the overestimation of SOC increased significantly with increasing contents of clay (Figure 3a) and Fines20 (Figure 3b).

For soils with large clay and Fines20 contents, the SOC content was overestimated by up to 2.86 g C 100 g⁻¹. Predicting SOC from LOI by a regression model overestimated SOC at

large clay and Fines20 contents, and underestimated SOC at small contents (Figure 3c,d). Clay and Fines20 had a significant effect on the overestimation of SOC for Highfield (clay, $R^2 = 0.46$, $P < 0.001$; Fines20, $R^2 = 0.39$, $P < 0.001$) and for all sites (clay, $R^2 = 0.31$, $P < 0.001$; Fines20, $R^2 = 0.33$, $P < 0.001$). Fines20 had a significant effect on the overestimation of SOC for Askov ($R^2 = 0.42$, $P < 0.022$). When the regression model was based on LOI only (O1, Table 3), SOC was underestimated by 0.37 g C 100 g⁻¹ and overestimated by 0.81 g C 100 g⁻¹ for soils with 9 and 73 g clay 100 g⁻¹, respectively. The systematic error disappeared when quadratic clay or Fines20 expressions were included in combination with LOI (Figure 3e,f). The best overall model including LOI and a quadratic clay expression (Equation (3)) predicted SOC with an RMSE of 0.101 g C 100 g⁻¹ (Figure 2a).

The data extracted from Grewal *et al.* (1991) for evaluation did not include silt contents and was used only to evaluate the model including the quadratic clay expression (Equation (3)). The range in LOI and SOC contents in the soils evaluated was similar to that of

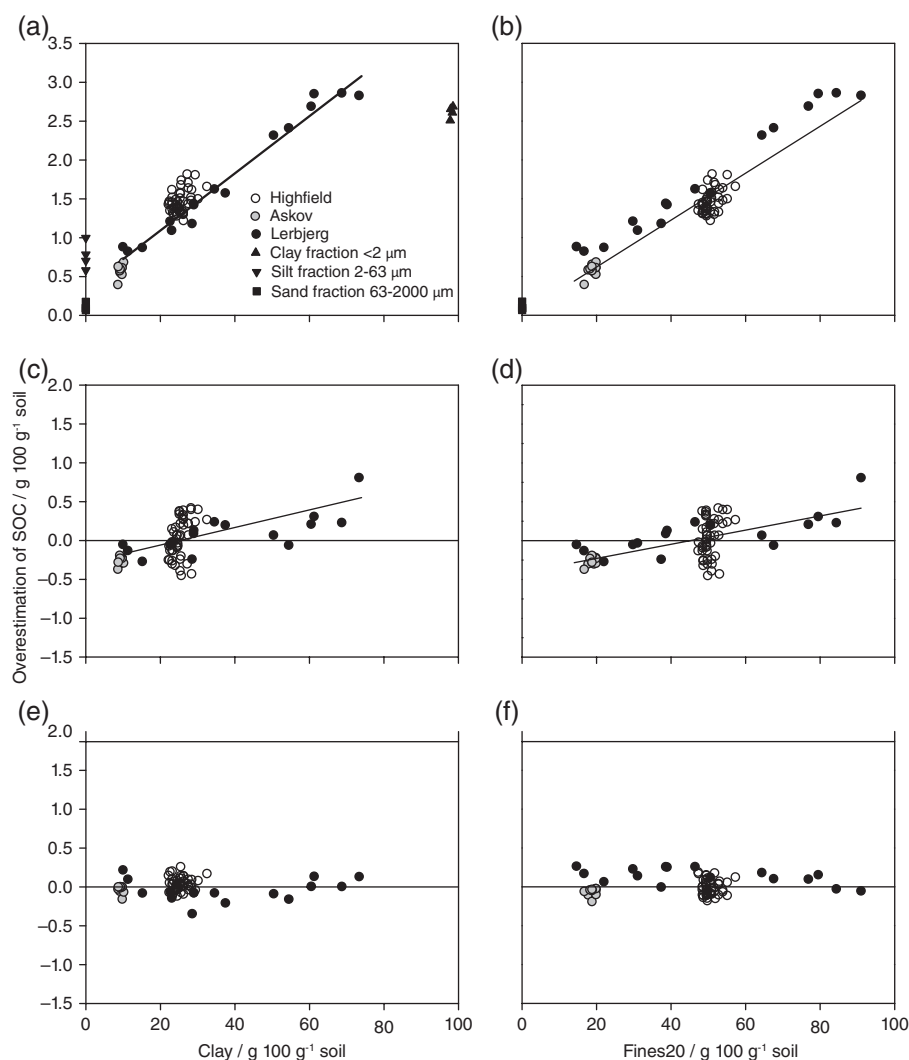


Figure 3 Overestimation (predicted minus measured values) of soil organic carbon (SOC) as a function of clay or mineral particles <20 μm (Fines20) (a, b) when multiplying loss-on-ignition (LOI) with the conventional conversion factor 0.58, (c, d) when estimating SOC by a model including measured LOI (model O1, Table 3) and (e) when estimating SOC by a model including LOI and the quadratic clay expression (model O2.1, Table 3 (Equation (3)) or (f) LOI and the quadratic Fines20 expression (model O3, Table 3). Solid regression lines are indicated if clay or Fines20 had a significant effect on the overestimation of SOC.

our soils, whereas the range in clay was smaller. Prediction accuracy and bias of Equation (3) were better when the dataset based on LOI-450 (RMSE = 0.295, ME = 0.125) was used rather than that based on LOI-550 (RMSE = 0.402, ME = 0.348). Soil organic carbon in the evaluation soils was predicted with an accuracy of $\pm 0.295 \text{ g C } 100 \text{ g}^{-1}$ at 450°C with Equation (3), and this model had similar predictive capability for small and large contents of LOI and clay (Figure 4).

Discussion

Pitfalls

Historical as well as recent estimates of SOC have relied on less accurate analytical approaches such as dichromate oxidation followed by titration and LOI (Bellamy *et al.*, 2005; Xie *et al.*, 2007; Reynolds *et al.*, 2013; Aitkenhead & Coull, 2016). Although these methods involve conversion factors of uncertain scientific foundation (Lettens *et al.*, 2007; Pribyl, 2010), they have recently been reported unreservedly as methods for SOC analysis in the Soil

Organic Carbon Mapping manual issued by the UN-FAO (Olmedo *et al.*, 2017).

In accordance with Poeplau *et al.* (2015), we found that correcting for RWC is critical to avoid systematic underestimation of SOC. Without correction for RWC, the SOC stock will be underestimated by 2 Mg C ha^{-1} for a topsoil (0–20-cm depth) with a bulk density of 1.5 g cm^{-3} , 2 g C and $30 \text{ g clay } 100 \text{ g}^{-1}$. Converting LOI data by multiplication with the conventional conversion factor 0.58 (Figure 3a,b) overestimates the SOC stock by 45 Mg C ha^{-1} for the same soil. Predicting SOC from LOI with regression model O1 (Table 3) underestimates the SOC stock by 5 Mg C ha^{-1} for a soil with a small clay content ($10 \text{ g } 100 \text{ g}^{-1}$) and overestimates the SOC stock by 8 Mg C ha^{-1} for a soil with a large clay content ($50 \text{ g } 100 \text{ g}^{-1}$). Predicting SOC content from LOI by a regression model that accounts for clay, increases the accuracy of prediction of SOC stock to $\pm 3 \text{ Mg C ha}^{-1}$ regardless of the clay or Fines20 content. This accuracy could be compared with management-induced differences in SOC sequestration in an agricultural context, which vary from 0.1 to $1.0 \text{ Mg C ha}^{-1} \text{ year}^{-1}$

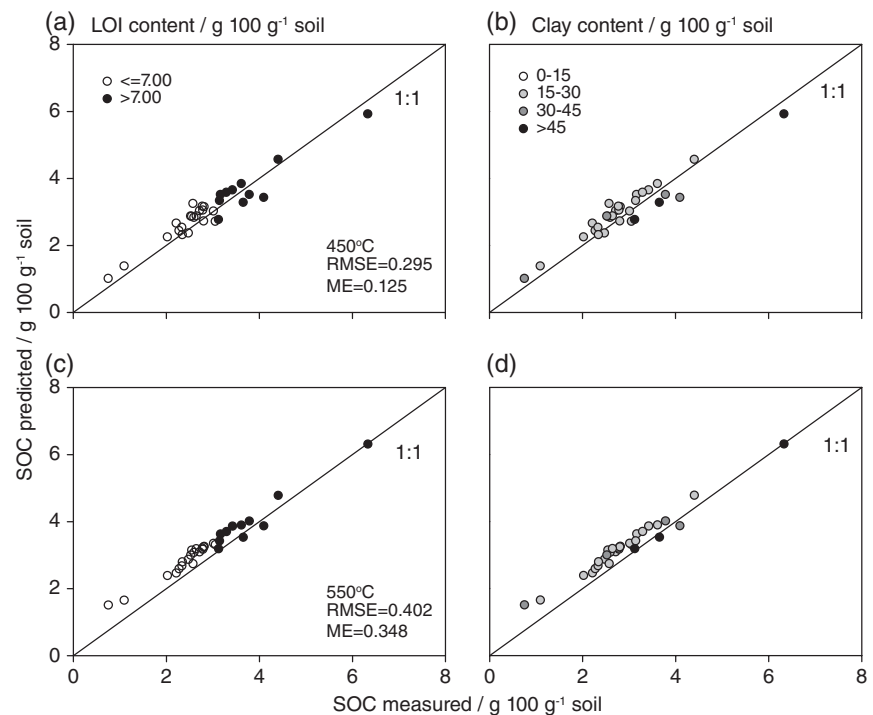


Figure 4 The relation between measured soil organic carbon (SOC) in the evaluation dataset and SOC predicted by a linear model including loss-on-ignition (LOI) and the quadratic clay expression (Equation (3)). The SOC predictions were tested with data on LOI based on an ignition temperature of (a, b) 450°C and (c, d) 550°C. Samples are grouped by LOI content (a, c) and clay content (b, d). Based on data published by Grewal *et al.* (1991). ME, mean error; RMSE, root mean square error.

Table 3 Parameter estimates, R^2 and the Akaike information criterion (AIC) for linear models of the relation between soil organic carbon (SOC) and loss-on-ignition (LOI), clay (< 2 μm) and mineral particles < 20 μm (Fines20). The models are based on data from all three sites

Model	Intercept	<i>P</i> -value	LOI / g 100 g ⁻¹	<i>P</i> -value	Clay / g 100 g ⁻¹	<i>P</i> -value	Clay ² / g 100 g ⁻¹	<i>P</i> -value	Fines20 / g 100 g ⁻¹	<i>P</i> -value	Fines20 ² / g 100 g ⁻¹	<i>P</i> -value	R^2	AIC
O1	-0.280	< 0.001	0.385	< 0.001									0.921	14.9
O2	-0.018	0.720	0.513	< 0.001	-0.046	< 0.001	0.00024	< 0.001					0.987	-123.5
O2.1	0		0.513	< 0.001	-0.047	< 0.001	0.00025	< 0.001					0.988	-125.3
O3	-0.206	0.006	0.507	< 0.001					-0.00956	0.003	-0.00014	< 0.001	0.984	-105.1

(Paustian *et al.*, 2016), illustrating that if LOI data are used uncritically the error in the estimate of SOC could easily exceed any management-induced difference even when adjusted for SWL and RWC. Our study was restricted to arable topsoil from the temperate zone with clay and SOC contents that ranged from 9 to 73 and 0.78 to 4.14 g 100 g⁻¹ soil, respectively. Thus, the relations established with the dataset might not be valid for soils under different land use, with different clay mineralogy, subsoils, soils rich in carbonates, and soils with clay and SOC contents outside these ranges (Christensen & Malmros, 1982; Jolivet *et al.*, 1998).

Proposed procedure

Previous studies have shown that if clay content was included in the prediction of SOC by LOI the variance explained was increased (Grewal *et al.*, 1991; De Vos *et al.*, 2005; Abella & Zimmer, 2007), which corroborates our findings. The difference in the regression coefficients for clay or Fines20 between sites (Table 2) could

possibly be a result of differences in clay mineralogy causing differences in structurally bound water. The larger regression coefficient for clay at Highfield than Lerbjerg might relate to a larger kaolinite content in the clay fraction from Highfield. Kaolinite shows a larger loss of water when ignited at 550°C for 4 hours (Sun *et al.*, 2009). The presence of negative intercepts for Lerbjerg, when both clay and Fines20 were included (Table 2), might be related to losses other than SOM and mineral structural water loss (e.g. certain salts or free iron) (Pribyl, 2010). The models including clay or Fines20 accounted for structural water loss from clay minerals (Sun *et al.*, 2009), which improved the models substantially.

For all sites, the models accounting for clay or Fines20 improved the conversion of LOI to SOC compared with models based on LOI alone. Equation (3) included a quadratic clay expression, which can be interpreted as a decrease in the effect of clay with increasing clay content. Similarly, Spain *et al.* (1982) included a quadratic clay expression in their prediction model. However, further research is needed to explain these observations. The regression model based on the Danish and British soils of the present study (Equation (3))

was able to predict the SOC contents in the New Zealand soils of the evaluation dataset with satisfactory accuracy (LOI-450, RMSE = 0.295; LOI-550, RMSE = 0.402). Differences in temperature, sample size, clay mineralogy and SOM characteristics between the evaluation dataset and our dataset might affect model performance.

Where archived soil samples are available, SOC should be determined directly by high-temperature dry combustion methods, with detection of evolved CO₂ by infrared or thermal conductivity detectors. However, Arrouays *et al.* (2012) reported that some 40% of the monitoring programmes in the European Union do not archive soil samples. Where LOI has been used to estimate SOC contents and soil samples are no longer available, Equation (3) provides more reliable estimates of SOC stocks for agricultural topsoil provided that LOI data are accompanied by information on soil texture. Equation (3) is valid for the conversion of LOI data that meet the following criteria: ignition temperature of approximately 500°C, ignition duration of 3–6 hours and preferably a sample mass of at least 5 g soil. Additional research that includes a wider range of soil types will increase the applicability of Equation (3).

The regression coefficient for LOI, interpreted as SOM, was similar for all sites when the regression equation accounted for the effects of clay or Fines₂₀ (Table 2). The regression coefficients ranged from 0.45 to 0.52 (=45–52% SOC in SOM), confirming that the conventional conversion factor of 0.58 is too large (Pribyl, 2010). Nevertheless, the so-called van Bemmelen factor of 1.724 (1/0.58) is still used to convert SOC to SOM (Olmedo *et al.*, 2017). The SOC to SOM conversion factor for Highfield, Askov and Lerbjerg was 1.92, 2.02 and 1.94, respectively. Estimating the conversion factor based on all soils gave 1.92. In accord with previous reports (Christensen & Malmros, 1982; Abella & Zimmer, 2007; Chatterjee *et al.*, 2009; Pribyl, 2010), we conclude that the conventional LOI-to-SOC conversion factor 0.58 is antiquated and leads to grossly overestimated SOC contents and misleading accounts of SOC stocks.

We acknowledge that other potential sources of error, in addition to the accuracy of the analytical approach, have to be considered when estimating SOC stocks. These potential sources of error include sampling design and intensity, information on the depth of the respective soil layers, and adjustment for stone content and bulk density (Poeplau *et al.*, 2017). However, precise estimates of SOC concentrations remain a key issue when establishing credible accounts of SOC stocks (Goidts *et al.*, 2009; Schrumpp *et al.*, 2011).

Conclusions

Converting LOI to SOC by the conventional conversion factor 0.58 led to grossly overestimated SOC stocks in agricultural topsoil. When SOC data are based on LOI conversion, we recommend re-analysis of archived soil samples for SOC by high-temperature dry combustion methods. Where archived soil samples are not available, accounting for clay content improves the conversion of LOI to SOC considerably.

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Paper 2

Soil texture analysis revisited: Removal of organic matter matters more than ever

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RESEARCH ARTICLE

Soil texture analysis revisited: Removal of organic matter matters more than ever

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Abstract

Exact estimates of soil clay (<2 µm) and silt (2–20 µm) contents are crucial as these size fractions impact key soil functions, and as pedotransfer concepts based on clay and silt contents are becoming increasingly abundant. We examined the effect of removing soil organic matter (SOM) by H₂O₂ before soil dispersion and determination of clay and silt. Soil samples with gradients in SOM were retrieved from three long-term field experiments each with uniform soil mineralogy and texture. For soils with less than 2 g C 100 g⁻¹ minerals, clay estimates were little affected by SOM. Above this threshold, underestimation of clay increased dramatically with increasing SOM content. Silt contents were systematically overestimated when SOM was not removed; no lower SOM threshold was found for silt, but the overestimation was more pronounced for finer textured soils. When exact estimates of soil particles <20 µm are needed, SOM should always be removed before soil dispersion.

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Introduction

Reliable estimates of clay- (<2 µm) and silt-sized (2–20 µm) particles in soil are now more important than ever as the use of pedotransfer functions are becoming increasingly abundant. Based on clay and silt contents, pedotransfer functions include attempts to predict soil water characteristics [1, 2], solute transport [3] and particle density [4]. Using reference values from conventional soil texture analysis, soil spectroscopy has been adopted as rapid methods to predict clay and silt contents [5–7]. Prediction of soil clay content from soil water characteristics is another rapidly progressing line of research based on pedotransfer concepts [8, 9].

For our ongoing research on the potential of soil clay/carbon and Fines₂₀ (mineral particles <20 µm)/carbon ratios in defining critical low soil organic carbon (SOC) contents in agricultural soils [10, 11], it is essential to have access to exact values for clay and silt contents. This need is amplified by a recent study attempting to incorporate clay/SOC ratios to map the impact of management on soil quality at European scale [12].

Removal of soil organic matter (SOM) is recommended as a pretreatment before particle size analysis (e.g., [13]) to ensure effective dispersion of micro-aggregates. The internationally published studies originally underpinning the effect of SOM removal on estimates of clay and

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silt contents date back many decades [14–16]. These studies were based on a limited number of samples retrieved from contrasting sites, which prevent quantification of the effect of SOM *per se* on clay and silt estimates. Moreover, the historic studies applied less reliable methods for determination of SOC such as dichromate oxidation/titration and loss-on-ignition converted to SOC by division with the factor 1.724, a factor with a dubious scientific foundation [17]. Thus, we found it necessary to revisit this fundamental issue of soil texture analysis and examine in more detail the quantitative significance of SOM removal on clay and silt estimates.

This study quantifies the effect of SOM removal by H₂O₂ on the determination of clay and silt-sized particles using samples covering a wide range of SOC and clay contents. The samples were retrieved from three long-term field experiments each with a uniform mineralogical and textural composition.

Materials and methods

To obtain soils with a gradient in SOC but with a uniform mineralogical and textural composition, samples were retrieved from three long-term field experiments in plots with contrasting management.

Highfield ley-arable experiment

In the Highfield Experiment at Rothamsted Research, UK (51°80'N, 00°36'W), four treatments were sampled: BF, bare-fallow maintained free of vegetation since 1959; A, arable rotation with winter cereals since 1948; LA, ley-arable rotation with three-year grass/clover ley followed by three years arable since 1948; RG, grassland ploughed and reseeded to grass in 1948. The A, LA and RG treatments were embedded in a randomized block design with four field replicates, whereas the four BF plots were not part of the original design and are located at one end of the experiment. The soil is a silt loam soil belonging to the Batcombe series ([18]; Chromic Luvisol (WRB) or Aquic Paleudalf (USDA Soil Taxonomy System)). Details of the experiment are given in the electronic Rothamsted Archive (www.era.rothamsted.ac.uk). Sample request and transfer of Highfield soils were issued by the Farm and Field Experiment Committee (FFEC) at Rothamsted Research.

In spring of 2015, bulk soil (6–15 cm depth) was sampled at three positions within each of four replicate plots providing a total of 48 samples (4 treatments x 4 replicates x 3 sampling positions).

Bad Lauchstädt static fertilizer experiment

We revisited previously published data on soil texture and SOC for the Bad Lauchstädt long-term static fertilizer experiment, Germany (51°24'N, 11°23'E). Bulk soil (2–15 cm depths) was sampled in spring 2008 from six different fertilization treatments in a field grown with a 4-year crop rotation (winter wheat (*Triticum aestivum*), sugar beet (*Beta vulgaris*), spring barley (*Hordeum vulgare*), and potato (*Solanum tuberosum*)) [19]. Animal manure (AM) was applied every 2 years in rates of 0, 20 or 30 Mg ha⁻¹. Half of the plots addressed received no additional fertilizer, while the other half was dressed with nitrogen, phosphorus and potassium depending on the nutrients in the applied AM. There were no field replicates in the experiment. The experiment was established in 1902 on a silt loam soil and is classified as a Haplic Chernozem (WRB). More details are given in [19].

Askov animal manure and mineral fertilizers experiment

Previously published data on soil texture and SOC for the Askov long-term experiment on animal manure and mineral fertilizers, Denmark (55°28'N, 09°07'E) was revisited. Bulk soil (6–15 cm depth) was sampled in autumn 2014 from four different fertilization treatments in a field grown with a 4-year crop rotation (winter wheat (*Triticum aestivum*), silage maize (*Zea mays*), and spring barley (*Hordeum vulgare*) undersown with a grass-clover mixture that is used for cutting in the subsequent production year) [20]. The nutrient treatments were: unfertilized; ½ mineral fertilizer (initiated in 1923); 1 mineral fertilizer; 1½ animal manure. The treatments were embedded in a block design with three replicates providing a total of 12 samples. The experiment was established in 1894 on a sandy loam soil and is classified as an Aric Haplic Luvisol (WRB) and Ultic Hapludalf (USDA Soil Taxonomy System). More details are given in [20].

Clay, silt and soil organic carbon

Contents of clay (<2 µm) and silt (2–20 µm) was determined on air dry bulk soil (< 2 mm) by the hydrometer method [13] using the ASTM 152H hydrometer. First, the soils were tested for CaCO₃ by adding a few droplets of 10% HCl, but none was found. Then one subsample (50 g soil) was treated with 35% H₂O₂ in an acidic solution under heating to remove SOM while another subsample was left untreated. After removal of H₂O₂ by boiling, the sample was washed with demineralized water until pH 6. Subsequently, the two sets of subsamples were dispersed by the same procedure. All lab work took place at 20°C. Each subsample was placed in 500-mL plastic bottles and 50 mL of 0.08 mol L⁻¹ sodium pyrophosphate (Na₄P₂O₇) and 200 mL demineralized water were added and the solution shaken end-over-end for 18 h. After transfer to 1000-mL sedimentation glass cylinder, demineralized water was added until 1000 mL and hydrometer readings were taken after 6.5 and 120 min to determine the <20 µm fraction, and after 2 and 18 h to determine the <2 µm fraction. The SOC content was determined on separate ball-milled sub-samples using dry combustion (Flash 2000 NC Soil Analyzer, Thermo Fisher Scientific).

Contents of SOC, clay and silt are related to oven-dry weight (105°C for 24 h) of the SOM-free mineral fraction. Correction factors for particle density were applied in calculating clay and silt contents (Table 1 and 3 in [21]). Values for individual soil samples are shown in [S1 Dataset](#).

Statistics

Linear regressions and ANOVA were applied using the R-Project software package Version 3.1.1 (R Foundation for Statistical Computing). The broken-stick model was fitted using the *segmented* function and the significance of the change point was assessed using the *davies.test* implemented in the *segmented* package in R. A simple piece-wise linear model was used:

$$y = \beta_0 + \beta_1(x) + \beta_2(x - c)^+ + e \quad (1)$$

where y is the dependent variable, x is the independent variable, c is the change point and e are the residual error [22].

Results and discussion

The soils at Highfield ranged from 0.80 to 4.27 g C 100 g⁻¹ minerals, with the smallest SOC content under BF and the highest under RG. The soils at Bad Lauchstädt and Askov ranged from 1.63–2.57 and 0.87–1.41 g C 100 g⁻¹ minerals, respectively, with the smallest SOC content under the unfertilized treatments and the highest under the treatments receiving the highest

Table 1. Average values (g 100 g⁻¹ minerals) of clay, silt and Fines20 for hydrogen peroxide treated soils from Group 1 and Group 2 at Highfield. P-values for testing differences between the two groups are indicated and were calculated by a one-way ANOVA.

	Group 1 ¹⁾	Group 2 ²⁾	p-value
Clay (<2 µm)	27.0	26.1	0.21
Silt (2–20 µm)	24.9	26.5	<0.001
Fines20 (<20 µm)	51.9	52.6	0.34

¹⁾ Group 1 –Bare-fallow.

²⁾ Group 2 –Arable, Ley-Arable and Reseeded Grass.

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amount of animal manure. A stringent test of the effect of SOC on clay and silt estimates requires that differences between treatments in contents of clay and silt are insignificant. Because the BF treatment was located only at one end of the field experiment, it was tested whether clay and silt in pretreated soil samples differed between BF and the other treatments. For clay, no significant difference was found (Table 1), whereas silt contents differed with 1.6 g 100 g⁻¹ minerals (6% less silt for the BF treatment). It was not possible to test for differences between treatments at Bad Lauchstädt due to the lack of replicates, but in general the texture of the plots were similar [19], although the clay and silt in pretreated soil samples were slightly correlated with treatments having the highest SOC content. Contents of clay and silt between treatments were insignificant at Askov [20].

There was a strong negative relationship between SOC and clay estimates for soils without SOM removal at Highfield ($R^2 = 0.66$, $p < 0.001$), whereas the relationship was non-significant for samples pretreated with H₂O₂ ($R^2 = 0.00$, $p = 0.84$) (Fig 1A). Similarly, there was a strong positive relationship between SOC and silt estimates when SOM was not removed ($R^2 = 0.72$, $p < 0.001$) and no significant relationship for H₂O₂ treated samples ($R^2 = 0.11$, $p = 0.053$) (Fig 1B). The linear regression analysis for silt excluded the BF soils due to the significant difference in silt content between BF and the other treatments. There was a non-significant negative relationship between SOC and clay estimates both without SOM removal ($R^2 = 0.61$, $p = 0.06$) and for samples pretreated with H₂O₂ ($R^2 = 0.36$, $p = 0.20$) at Bad Lauchstädt (Fig 1C). The SOM effect was close to significant and more pronounced for soils without SOM removal being in agreement with the results from Highfield. There was a non-significant relationship between SOC and silt estimates when SOM was not removed ($R^2 = 0.12$, $p = 0.52$), whereas there was a negative relationship for H₂O₂ treated samples ($R^2 = 0.72$, $p < 0.05$) (Fig 1C). This is in line with the results from Highfield; since the silt content with SOM removal decreased slightly with an increase in SOC, a more or less unaffected silt content without SOM removal was expected. For Askov there was a non-significant relationship between SOC and clay estimates both when SOM was not removed ($R^2 = 0.09$, $p = 0.36$) and for samples pretreated with H₂O₂ ($R^2 = 0.10$, $p = 0.31$) (1C). The SOM effect on silt estimates was in agreement with results from Highfield and Bad Lauchstädt with a positive relationship between SOC and silt estimates when SOM was not removed ($R^2 = 0.37$, $p < 0.05$) and a non-significant relationship for H₂O₂ treated samples ($R^2 = 0.00$, $p = 0.84$) (Fig 1D). In general it can be seen, that the presence of SOM caused a systematic error in clay and silt estimates.

We suggest that the underestimation of clay and overestimation of silt with increasing SOC is due to incomplete dispersion of soil aggregates smaller than 20 µm. Silt-sized micro-aggregates made up of SOM-clay complexes will settle faster and be quantified as silt even though, if fully dispersed, they should be classified as clay. Alternatively, SOM and clay particles may flocculate after dispersion and be classified as silt although flocculation is less likely in a sodic solution with a low particle concentration [23].

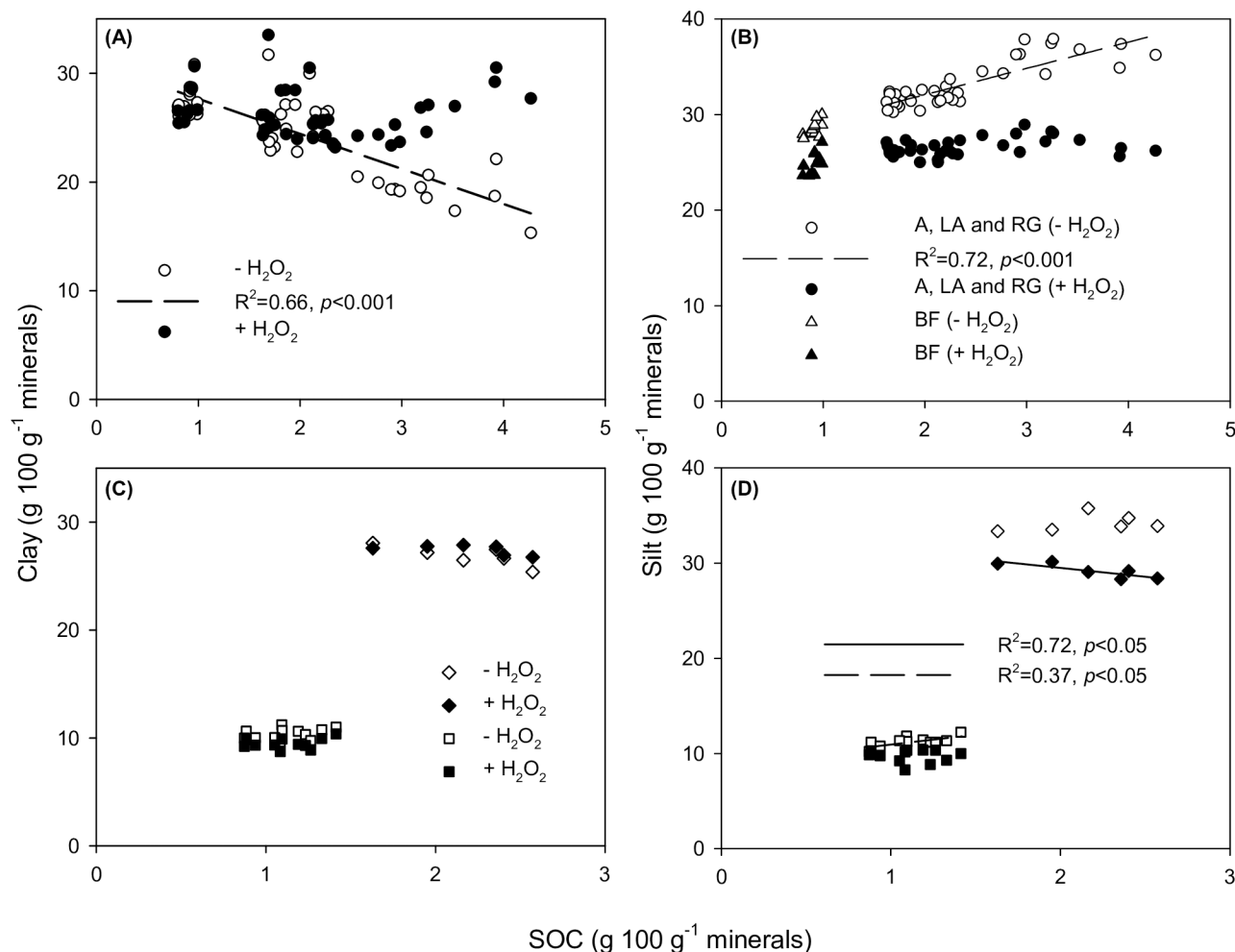


Fig 1. Clay (<2 μm) and silt (2–20 μm) content as a function of SOC content for soil samples pretreated with hydrogen peroxide and without pretreatment. (A) Clay content plotted against SOC content for soil samples pretreated with hydrogen peroxide (black symbols) and without pretreatment (white symbols) at Highfield. The linear regression line, R^2 and p -value for non-pretreated soil samples are indicated ($n = 48$). (B) Silt content plotted against SOC content for soil samples pretreated with hydrogen peroxide and without pretreatment at Highfield. The linear regression line, R^2 and p -value for non-pretreated arable (A), ley-arable (LA) and reseeded grass (RG) soil samples are indicated (white circles, $n = 36$). The bare-fallow (BF) soil samples are shown with triangle symbols ($n = 24$). (C) Clay content plotted against SOC content for soil samples pretreated with hydrogen peroxide and without pretreatment at Bad Lauchstädt (diamond symbols) and Askov (square symbols). (D) Silt content plotted against SOC content for soil samples pretreated with hydrogen peroxide and without pretreatment at Bad Lauchstädt and Askov. The linear regression line, R^2 and p -value for pretreated and non-pretreated soil samples for Bad Lauchstädt ($n = 6$) and Askov ($n = 12$), respectively, are indicated.

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The underestimation of clay caused by omitting the H_2O_2 treatment increased with increasing SOC content (Fig 2A). To establish the SOC content below which SOM removal becomes unnecessary, a broken-stick model was fitted to the Highfield data. This threshold value for clay was 2.27 g C 100 g⁻¹ minerals (95% confidence interval, 2.03–2.52 g C 100 g⁻¹ minerals). This point of change was highly significant ($p < 0.001$), and the following piecewise linear regression equation can be used to model the underestimation of clay (UnClay) at Highfield:

$$\text{UnClay} = -0.78 (p = 0.19) + 0.66 (p = 0.07) \text{SOC} + 5.22 (p < 0.001) (\text{SOC} - 2.27)^+, R^2 = 0.90 \quad (2)$$

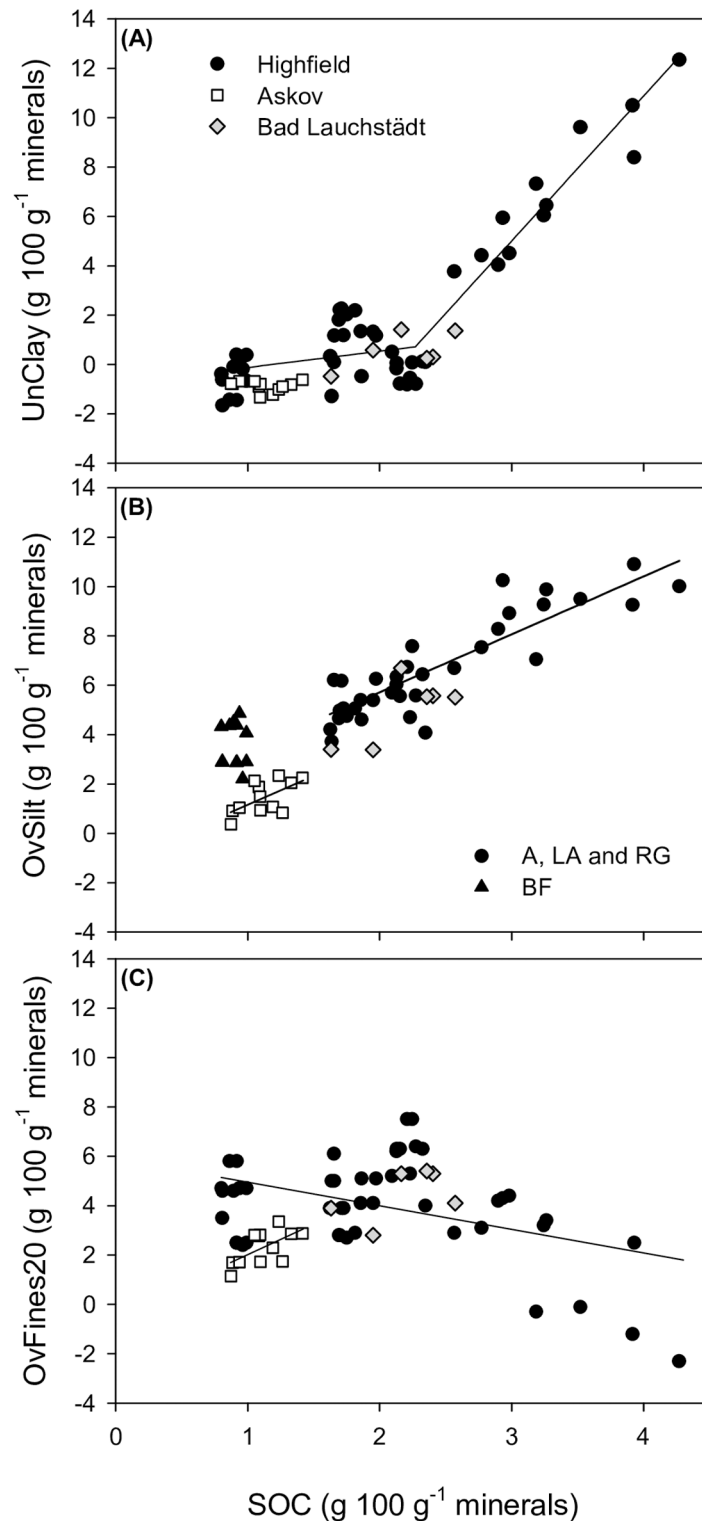


Fig 2. Underestimation of clay content (<2 μ m), overestimation of silt content (2–20 μ m) and overestimation of Fines20 content (<20 μ m) caused by omitting hydrogen peroxide (H_2O_2) pretreatment as a function of SOC content. (A) Underestimation of clay content (UnClay) caused by omitting H_2O_2 pretreatment plotted against SOC content. The soil samples from Highfield, Bad Lauchstädt and Askov are shown with black, grey and white symbols, respectively. The broken-stick model is indicated for Highfield ($n = 48$). (B) Overestimation of silt content (OvSilt) caused by omitting H_2O_2 pretreatment plotted

against SOC content. The linear regression line for Askov ($n = 12$) and for the Highfield arable (A), ley-arable (LA) and reseeded grass (RG) soil samples are indicated (circle symbols, $n = 36$). The bare-fallow (BF) soil samples are shown with triangle symbols ($n = 12$). (C) Overestimation of Fines20 content (OvFines20) caused by omitting H_2O_2 pretreatment plotted against SOC content. The linear regression lines for Askov ($n = 12$) and Highfield ($n = 48$) are indicated.

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The last term in the equation is only applicable for SOC contents above $2.27 \text{ g C } 100 \text{ g}^{-1}$ minerals. No threshold was observed for Bad Lauchstädt ($p = 0.75$) and Askov ($p = 0.10$), and the linear relationship between UnClay and SOC was non-significant (Bad Lauchstädt; $R^2 = 0.37$, $p = 0.19$ and Askov; $R^2 = 0.01$, $p = 0.77$). The overestimation of silt increased with an increase in SOC (Fig 2B) and was linearly related to SOC at Highfield and Askov and close to significant at Bad Lauchstädt ($R^2 = 0.52$, $p = 0.11$). No threshold was observed at Highfield ($p = 0.62$), Askov ($p = 0.70$) and Bad Lauchstädt ($p = 0.61$). The following linear regression can be used to model the overestimation of silt (OvSilt) at Highfield:

$$\text{OvSilt} = 1.03(p < 0.001) + 2.35(p < 0.001)\text{SOC}, R^2 = 0.76 \quad (3)$$

And the following linear regression can be used to model OvSilt at Askov:

$$\text{OvSilt} = -1.19(p = 0.30) + 2.35(p < 0.05)\text{SOC}, R^2 = 0.37 \quad (4)$$

The models show that SOM removal is critical for estimating clay in soils with more than $2 \text{ g C } 100 \text{ g}^{-1}$ minerals, whereas an unbiased estimate of silt requires SOM removal regardless of SOC content. For example, with no SOM removal, clay will be underestimated by 19% and silt overestimated by 30% in a soil with $3 \text{ g C } 100 \text{ g}^{-1}$ minerals (using Eqs 2 and 3; based on Highfield data). The data from Bad Lauchstädt and Askov are in general agreement with the broken-stick model on Highfield (Fig 2A), whereas the OvSilt for Askov is lower (Fig 2B). Interestingly, the slope estimate for Eqs 3 and 4 is identical indicating a similar effect of SOM on OvSilt between the two sites. The lower OvSilt for Askov compared with Highfield and Bad Lauchstädt may be due to textural differences with Askov being coarser textured.

Standard protocols for estimation of clay- and silt-sized particles are the hydrometer and pipette methods, both based on gravitational sedimentation following soil dispersion [13]. We relied on the hydrometer approach. The results obtained for clay and silt in H_2O_2 treated soils from Highfield are consistent with previous estimates based on the pipette method [18].

Most protocols propose the use of 30% H_2O_2 for SOM removal before soil dispersion. We recognize that the prescribed H_2O_2 treatment does not remove all SOM from the soil. Typically, 80 to 90% of the initial SOC content is removed by the prescribed protocol (e.g., [24–26]). We also recognize that the H_2O_2 treatment may dissolve mineral constituents including vermiculite, mica and smectite in particular [27]. The clay fraction of Highfield and Bad Lauchstädt soil do have a higher content of smectite and vermiculite than Askov [28–30], which may explain the slightly higher loss of Fines20 for Highfield and Bad Lauchstädt than Askov (Fig 2C).

We also acknowledge that our study was based on a coarse sandy soil and two silt loams each having different clay mineralogies. However each of the soils considered here encompassed a reasonably wide range of SOC contents resulting from contrasting long-term agricultural treatments. We advocate similar studies based on long-term field experiments located on other soil types to examine any additional effects of differences in soil textural composition and mineralogy. Studies on soils dominated by low-activity clays such as kaolinite and Fe and Al oxide minerals are in particular needed.

Conclusions

The presence of SOM induced systematic errors in the estimation of clay and silt contents. For soil with less than 2 g C 100 g⁻¹ minerals, clay estimates were little affected by SOM. An overestimation of silt occurred at all SOC contents considered here. The overestimation of the silt fraction was greater for the silt loams compared to the sandy loam. Consequently, SOM should always be removed before soil dispersion when exact estimates of particles <20 µm are needed.

Supporting information

S1 Dataset. Data used in Fig 1, Table 1 and for developing the models in Fig 2. The soil characteristics are expressed in relation to oven-dry weight of the SOM-free mineral fraction. (PDF)

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Author Contributions

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Paper 3

Suboptimal fertilisation compromises soil physical properties of a hard-setting sandy loam

Johannes L. Jensen, Per Schjønning, Bent T. Christensen, and Lars J. Munkholm

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Paper 4

Relating soil C and organic matter fractions to soil structural stability

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Lars J. Munkholm

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Supplementary material is included

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Relating soil C and organic matter fractions to soil structural stability

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ABSTRACT

Soil organic matter (SOM) is important for maintaining soil structural stability (SSS). This study quantified the influence of soil organic carbon (SOC) and different organic matter components on various SSS measures. We used a silt loam soil with a wide range of SOC (8.0–42.7 g kg⁻¹ minerals) sampled in spring 2015 from the Highfield Ley-Arable Long-Term Experiment at Rothamsted Research. Four treatments were sampled: Bare fallow, continuous arable rotation, ley-arable rotation, and grass. Soils were tested for clay dispersibility (DispClay), clay-SOM disintegration (DI, the ratio between clay content without and with SOM removal) and dispersion of particles < 20 µm. The SSS tests were related to SOC, permanganate oxidizable carbon (POXC), hot water-extractable carbon (HWC), mid-infrared photoacoustic spectroscopy (FTIR-PAS) and mineral fines/SOC ratio. SSS increased with increasing content of SOM components. The relationships between SOM components and SSS followed a broken-stick regression with a change point at ~23.0 g SOC kg⁻¹ minerals (clay/SOC~10) coinciding with a change from the tilled treatments to the grass treatment. We found a greater influence of SOC, POXC and HWC on SSS at contents below the change point than above. A stronger linear relation between POXC and DispClay compared to SOC and HWC suggests that POXC was a better predictor of the variation in DispClay. POXC and HWC were less related to DI than SOC. The grass treatment had a very stable structure, shown in all SSS tests, probably due to the absence of tillage and large annual inputs of stabilizing agents. This suggests that a change in management from arable rotation to permanent grass is one effective tool for improving SSS.

1. Introduction

The importance of soil organic matter (SOM) on key soil functions is well known (e.g., Johnston et al., 2009), and consequently loss of SOM is considered a major threat to sustained soil functions (Amundson et al., 2015). One soil property affecting key soil functions is soil structure. Soil structure is the relative arrangement of particles and pores (Dexter, 1988), and the ability of soil structure to resist external stresses both mechanical and or from water is termed soil structural stability (SSS). Greater SSS is essential for minimizing the risk of downward transport of fine particles carrying pollutants to the water environment (de Jonge et al., 2004), soil erosion (Le Bissonnais, 1996), soil cementing, and seedbeds with hard and non-friable aggregates (Kay and Munkholm, 2004). SOM content is an important factor affecting

SSS (Bronick and Lal, 2005), and a range of studies have shown that an increase in SOM content increases SSS (e.g., Jensen et al., 2017a; Watts and Dexter, 1997).

Soil organic carbon (SOC) is the main constituent of SOM and may serve as a proxy for SSS. Labile organic compounds are potentially better indicators for soil functions (Haynes, 2005). For example, permanganate oxidizable carbon (POXC) is considered a labile component of SOM and has been found to be more sensitive to differences in management than total SOC (Culman et al., 2012). POXC is an easy and low-cost method and POXC has been suggested the best single predictor of soil health (Fine et al., 2017) and a better predictor of crop productivity than total SOC (Hurisso et al., 2016). Similarly, hot water-extractable carbon (HWC) is highlighted as a soil quality indicator more sensitive to management changes than total SOC (Ghani et al., 2003).

Abbreviation: A, Continuous arable rotation; BF, Bare fallow; CEC, Cation exchange capacity; DI, Clay-SOM disintegration; DispClay, Clay dispersibility; DispFines20, Dispersion of particles < 20 µm; Fines20, Mineral particles < 20 µm; FTIR-PAS, Mid-infrared photoacoustic spectroscopy; G, Grass; HWC, Hot water-extractable carbon; LA, Ley-arable rotation; LF-free-SOC, Light fraction-free-SOC; LFSOC, Light fraction organic carbon; NTU, Nephelometric turbidity unit; PCA, Principal component analysis; POXC, Permanganate oxidizable carbon; SSA, Specific surface area; SSS, Soil structural stability

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HWC is considered a labile component of SOM consisting of microbial and plant derived material (Hirsch et al., 2017; Villada et al., 2016). Another measure, which potentially could better explain changes in SSS than total SOC, is light fraction (LF) free SOM. This can be obtained by subtracting light fraction organic carbon (LFSOC) from total SOC since LFSOC is a fraction not closely associated with mineral particles (Gregorich et al., 2006). Finally, mid-infrared photoacoustic spectroscopy (FTIR-PAS) can be used to assess differences in SOM quality (Peltre et al., 2014; Peltre et al., 2017), which potentially could improve the explanatory power in predicting SSS.

Increasing evidence suggest that soils exhibit a capacity factor for SOC stabilization also known as saturation state (Hassink, 1997; McNally et al., 2017). The saturation state of the soil has been found to influence the SSS measure, clay dispersibility, more than SOC per se (Dexter et al., 2008). The saturation state can be expressed by the clay/SOC ratio. A critical value close to 10, corresponding to a soil where the mineral particles are considered to be saturated with SOC, has been found in several studies (Dexter et al., 2008; Getahun et al., 2016; Jensen et al., 2017a; Schjønning et al., 2012). Soils with clay/SOC above 10 are considered SOC-unsaturated, and for such soils, SSS may be reduced. A corresponding threshold has been found for mineral particles < 20 µm (Fines20) where the ratio of Fines20/SOC above 20 indicates reduced SSS. Consequently, these mineral fines to SOC ratios may serve as soil type independent threshold values for SSS.

The quantitative importance of various SOM components on SSS tested using different pretreatments and energy inputs remains elusive. Previous studies often rely on samples retrieved from contrasting sites with different soil types and textures making quantification of the effect of SOM components on SSS dubious.

The objective of this study was to quantify the influence of SOC on several soil structural stability parameters and to test whether SOM characteristics proposed in the literature improve the predictive ability. A wide range of measures for the determination of SSS exists ranging in sample preparation, pretreatment, degree of disturbance and quantification (Le Bissonnais, 1996; Pojasok and Kay, 1990; Pulido Moncada et al., 2015). We applied stability tests varying in pretreatment and ranging from low to very high degree of disturbance focusing on SOC effects on clay dispersibility of 1–2 mm rewetted aggregates, dispersion of particles < 20 µm in < 8 mm field-moist soil, and clay-SOM disintegration of < 2 mm air-dry soil. Soil was from the Highfield Ley-Arable Long-Term Experiment at Rothamsted Research (Highfield-LTE), a silt loam with a wide gradient in SOC. The gradient has developed during at least 56 years of contrasting management practices. We thereby eliminated confounding effects of variations in soil type, soil texture and climate. Soil was from treatments that ensured the widest possible gradient in SOC content and differences in SOM quality.

2. Materials and methods

2.1. The Highfield-LTE and treatments

The Highfield-LTE was established in 1949 on a silt loam soil (Table 1) at Rothamsted Research, Harpenden, UK (51°80'N, 00°36'W) in a field that had been under permanent grass for centuries. The soil belongs to the Batcombe series, and the parent material includes a relatively silty (loess-containing) superficial deposit overlying and mixed with clay-with-flints (Avery and Catt, 1995). The soil is classified as an Aquic Paludalf (USDA Soil Taxonomy System) and Chromic Luvisol (WRB) (Watts and Dexter, 1997). Average annual temperature and precipitation are 10.2 °C (mean of 1992–2014) and 718 mm (mean of 1981–2010), respectively (Scott et al., 2014).

We selected three treatments in the ley-arable experiment:

Continuous arable rotation (A), winter cereals (winter wheat, *Triticum aestivum* L. and winter oats, *Avena sativa* L.) fertilized with 220 kg N ha⁻¹ y⁻¹ and maintained under standard Rothamsted farm

Table 1

Soil characteristics. Within rows, letters denote statistical significance at $P < 0.05$ for the comparison of A, LA and G. An asterisk (*) indicates if BF is significantly different from A, LA and G based on a pairwise t -test. For treatment abbreviations, see Fig. 1.

	BF	A	LA	G
Texture ^a				
Clay < 2 µm	0.270	0.264	0.255	0.261
Silt 2–20 µm	0.249	0.263	0.261	0.272*
Silt 20–63 µm	0.335	0.318	0.324	0.319
Sand 63–2000 µm	0.146	0.155	0.160	0.148
Specific surface area (m ² g ⁻¹ minerals) ^b	56.7	67.9 ^a	68.4 ^a	78.4 ^b
Exchangeable cations and CEC				
Na ⁺ (mmol _c kg ⁻¹ minerals)	0.4	0.5 ^a	0.4 ^a	0.7 ^b
K ⁺ (mmol _c kg ⁻¹ minerals)	3.3	6.3	17.7	5.8
Ca ²⁺ (mmol _c kg ⁻¹ minerals)	93.7	113.3 ^a	128.7 ^a	155.6 ^b
Mg ²⁺ (mmol _c kg ⁻¹ minerals)	5.4	4.0	4.1	4.6
Sum of bases (mmol _c kg ⁻¹ minerals)	102.9	107.1 ^a	120.7 ^{ab}	142.0 ^b
CEC (mmol _c kg ⁻¹ minerals)	145.8	173.8	171.3	209.9*
Base saturation (%)	72.8	65.5	74.8	74.4
pH (CaCl ₂)	5.7	5.1	5.1	5.4

^a kg kg⁻¹ of mineral fraction and based on oven-dry weight.

^b Clay is included as a co-variable as it is significant and makes the treatment effect significant.

practice with straw removed. Ley-arable rotation (LA), three-year grass/clover ley (meadow fescue, *Festuca pratensis* L.; timothy-grass, *Phleum pratense* L.; white clover, *Trifolium repens* L.) followed by three years arable (managed as A). The grass/clover ley received no N fertilizer and the biomass was cut and removed in early summer. The small amount of regrowth was topped in early autumn and left on the plots. Two of the sampled plots were drilled with winter cereals following three years of grass/clover, whereas the other two were drilled with grass/clover following three years of winter cereals. Grass (G), ploughed and reseeded to grass (predominantly rye grass, *Lolium perenne* L.) when the experiment was established (1949). The grass was managed as the grass/clover ley in LA.

We also selected the bare fallow (BF) treatment, which is not part of the original ley-arable experiment but located adjacent to the experiment (denoted Highfield bare fallow and Geescroft bare fallow). The BF treatment has been maintained free of plants by regular tillage (ploughed or rotavated two to four times a year) since 1959. The ploughing depth in BF, A, and LA was 0.23 m. The A, LA and G plots were fertilized with 65 kg P ha⁻¹ and 250 kg K ha⁻¹ every three years.

The A, LA and G treatments were part of a randomized block design with four field replicates, whereas the four BF plots were located at one end of the experiment (Fig. 1). The dimensions of the LA plots were 50 m × 7 m, whereas they were 10 m × 6 m for the other plots. The A, LA and G plots were smaller since they were part of a reversion experiment initiated in 2008. For more details see Johnston (1972) and the electronic Rothamsted Archive (www.era.rothamsted.ac.uk).

2.2. Soil sampling

Soil was sampled in March 2015 at field capacity corresponding approximately to a soil water potential of −100 hPa. Soil blocks (2750 cm³) were carefully retrieved from the 6–15-cm soil layer by use of a spade. Three sampling sites in each experimental plot were randomly chosen and labelled subplot. One block was extracted from each subplot adding up to three blocks per plot. The soil was kept in sturdy containers to prevent soil disturbance during transport and stored in a field-moist condition at 2 °C until required for analyses. Soil from the blocks at subplot level was spread out in steel trays at room temperature, carefully fragmented by hand in several sittings along natural planes of weakness, and finally left to air-dry.

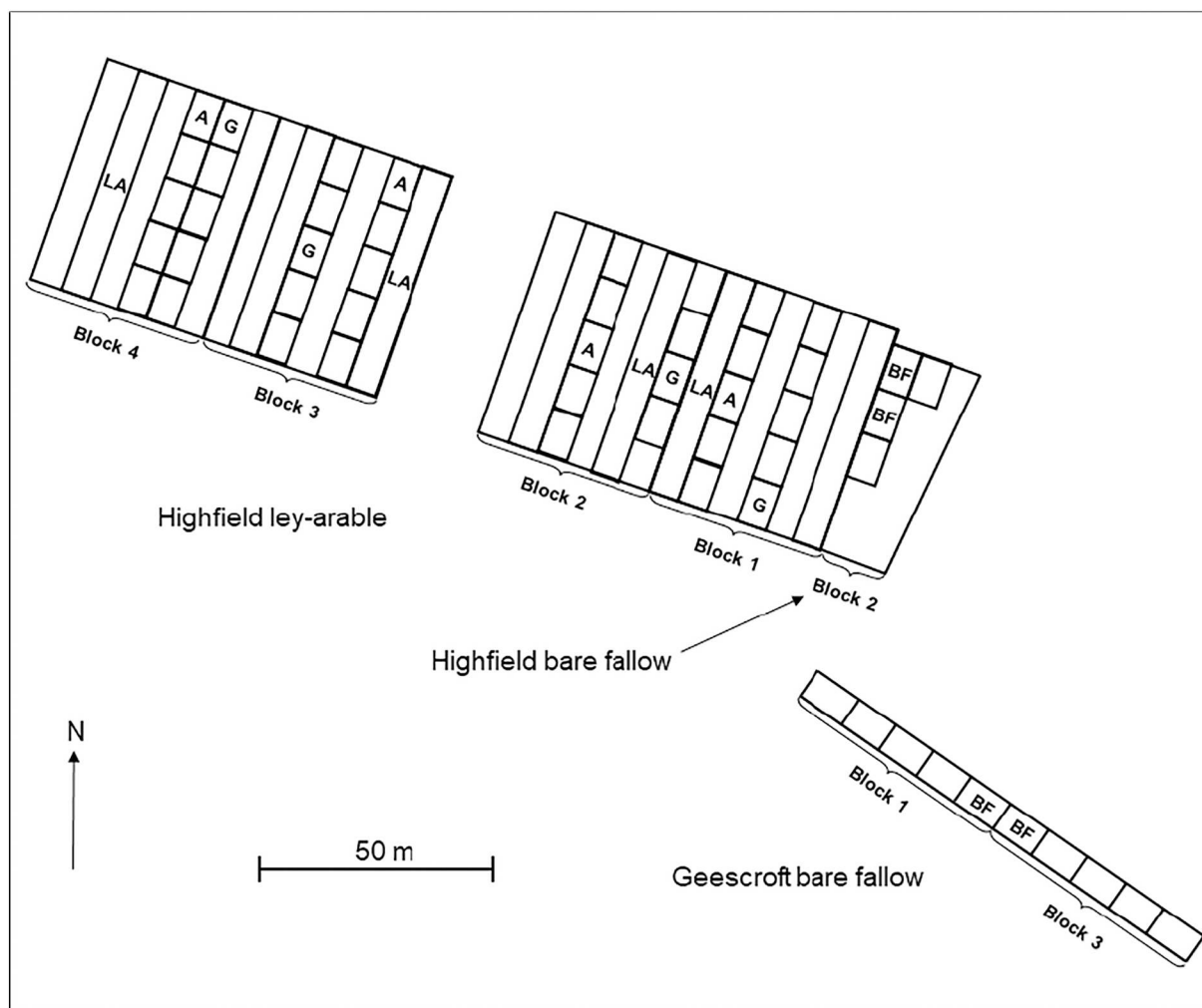


Fig. 1. Distribution of plots in Highfield showing the arable (A), ley-arable (LA) and grass (G) treatments in blocks 1–4 of the ley-arable experiment, and the bare fallow (BF) treatment in blocks 1–3 of the bare fallow experiments.

2.3. Basic chemical and physical analysis

The texture of air-dried bulk soil (crushed and passed through a 2-mm sieve) was determined by the hydrometer method for clay ($< 2 \mu\text{m}$) and silt ($2\text{--}20 \mu\text{m}$) content and the sieve method for mineral particles $> 63 \mu\text{m}$ (Gee and Or, 2002). The soil was tested for CaCO_3 by adding a few droplets of 10% HCl, but none was found. SOM was removed with H_2O_2 before estimation of clay and silt as described in Jensen et al. (2017b). The SOC content was determined on ball-milled subsamples using high-temperature dry combustion (Thermo Flash 2000 NC Soil Analyzer, Thermo Fisher Scientific, Waltham Massachusetts, USA). Specific surface area (SSA) was determined by the ethylene glycol monoethyl ether method (Petersen et al., 1996), and cation exchange capacity (CEC) was determined after Kalra and Maynard (1991). Soil pH was determined in 0.01 M calcium chloride (CaCl_2) solution (1:2.5, w/w). Clay, silt and SOC contents were determined at subplot level, whereas the other properties were determined at plot level.

2.4. Soil organic matter characteristics

Permanganate oxidizable carbon (POXC) was determined at subplot level following Culman et al. (2012). Air-dry 2-mm sieved soil equivalent to 2.5 g oven-dry weight was weighed into a 50 ml falcon tube and shaken end-over-end (33 rpm for 2 min) in 18.0 ml of distilled water and 2.0 ml 0.2 M potassium permanganate (KMnO_4) adjusted to

pH 7.2. After shaking, the soil was allowed to settle for 10 min after which 0.5 ml of the supernatant was transferred to falcon tubes containing 49.5 ml of water. The absorbance of the diluted solution was measured at 550 nm using a spectrophotometer (Thermo Electron Spectronic Helios Alpha Beta UV–visible). The absorbance of four standard stock KMnO_4 solutions was measured to create a standard curve, and sample absorbance was converted to POXC using the equation of Weil et al. (2003).

Hot water-extractable carbon (HWC) was determined at subplot level following Ghani et al. (2003). Briefly, air-dry 2-mm sieved soil equivalent to 3 g oven-dry weight was weighed into a 50 ml falcon tube and shaken end-over-end (33 rpm for 30 min) in 30 ml distilled water at 20°C . After centrifugation (3500 rpm, 20 min) the supernatant was decanted (water soluble carbon), and the soil was re-suspended in distilled water and shaken for 16 h at 200 rpm and 80°C . After centrifugation, the second supernatant (hot water-extractable carbon) was transferred to 50 ml maxi-spin filter tubes equipped with a cellulose acetate membrane filter ($0.45 \mu\text{m}$ pore size) and filtered by centrifugation for 10 min at 3000 rpm. The extracted carbon was determined by wet oxidation using a Shimadzu TOC-V analyzer.

Fractionation based on density was determined at subplot level using a modification of the method described by Sohi et al. (2001). Briefly, 10 g of air-dried 2-mm sieved soil was weighed into a 50 ml falcon tube, 35 ml of NaI solution with a density of 1.8 g cm^{-3} was added, and the solution was shaken at 33 rpm for 2 h. The suspension was centrifuged for 30 min after which floating particles was

Table 2

Soil organic matter characteristics, clay dispersibility of 1–2 mm aggregates rewetted to -100 hPa and clay-SOM disintegration (the ratio between clay content estimated without SOM removal and with removal). Within rows, letters denote statistical significance at $P < 0.05$ for the comparison of A, LA and G. An asterisk (*) indicates if BF is significantly different from A, LA and G based on a pairwise *t*-test. For treatment abbreviations, see Fig. 1.

	BF	A	LA	G
Soil organic matter characteristics				
Soil organic carbon (SOC, g kg ⁻¹ minerals)	9.0	17.3 ^a	21.6 ^a	32.9 ^b
Permanganate oxidizable carbon (POXC, g kg ⁻¹ minerals)	0.161	0.458 ^a	0.600 ^b	0.818 ^c
% of SOC	1.7	2.6 ^{ab}	2.8 ^b	2.5 ^a
Hot water-extractable carbon (HWC, g kg ⁻¹ minerals)	0.437	0.777 ^a	1.082 ^b	1.611 ^c
% of SOC	4.6	4.5 ^a	5.0 ^b	4.9 ^{ab}
Light fraction carbon (LFSOC, g kg ⁻¹ minerals)	0.167	1.285 ^a	1.732 ^a	2.579 ^b
% of SOC	1.9	7.4 [*]	8.0 [*]	7.8 [*]
Aliphatic peak area	58	99 ^a	121 ^a	159 ^b
Aliphatic peak area/SOC	6.5	5.7	5.6	4.9 [*]
Soil structural stability				
Clay dispersibility (DispClay, kg kg ⁻¹ minerals)	0.0115	0.0074 ^c	0.0051 ^b	0.0034 ^a
Clay-SOM disintegration (DI, kg kg ⁻¹ minerals)	1.02	0.96 ^b	1.00 ^b	0.74 ^a

transferred to a glass fiber filter (type GF/A, 110 mm diam., 1.6 μ m retention, Whatman International, Kent, UK), and filtered under suction in a vacuum filtration unit (Büchner funnel). The light fraction organic matter (LFOM) retained on the filter was washed carefully and transferred to a crucible. To ensure a quantitative removal of LFOM the procedure was repeated. The remaining heavy fraction (HF) was washed three times and transferred to a large crucible. The oven-dry weight of the LF and HF was estimated by drying (105 °C for 24 h). The amount of OM recovered was estimated by loss-on-ignition (LOI; 500 °C for 4 h) for both the LFOM and HF. A 5 g air-dry bulk soil sample was dried to allow expressing results on an oven-dry basis after which LOI was determined. The LOI of bulk soil was used to make a model to predict the SOC of the HF based on a multiple regression of SOC against LOI and clay (Model H2.1, Table 2 in Jensen et al., 2018):

$$\text{SOC} = 0.515 \text{ LOI } (P < 0.001) - 0.043 \text{ Clay } (P < 0.001), (n = 48, R^2 = 0.990) \quad (1)$$

Ten tests without soil (blind tests) were performed. The blind test value was subtracted from the LFOM estimate. The LFOM was converted to LFSOC by multiplying with 0.515 (Eq. 1), expressed as percentage of the sum of LFSOC and HFSOC, and normalized to the measured SOC content. Light fraction-free-SOC (LF-free-SOC) was calculated by subtracting LFSOC from SOC.

Fourier transform mid-infrared photoacoustic spectroscopy (FTIR-PAS) was determined at plot level following Peltre et al. (2014). Air-dry 2-mm sieved soil samples were ball-milled and packed in 10-mm diameter cups and the functional groups of soil components recorded using a Nicolet 6700 FTIR spectrometer (Thermo Scientific) equipped with a PA301 photoacoustic detector (Gasera Ltd. Turku, Finland). Spectra were recorded with an average of 32 scans within the range 4000–600 cm⁻¹ and with 2 cm⁻¹ intervals. A flow of helium was used as purge gas to remove noise produced by ambient moisture and CO₂ as well as moisture from the sample after insertion of the cup in the photoacoustic detector chamber. We focused on the 1700–1300 cm⁻¹ region to reduce overlapping bands arising from soil minerals. The spectral peak area between 3000 and 2800 cm⁻¹ was integrated as described in Peltre et al. (2017) and taken to reflect the amount of aliphatics in the soil (Leifeld, 2006).

2.5. Soil structural stability

Clay dispersibility (DispClay) was determined at subplot level on 1–2 mm aggregates isolated from the air-dry 2-mm sieved soil. The aggregates were adjusted to a matric water potential of -100 hPa as described in Schjønning et al. (2012). In short, the aggregates were placed on a tension table at -100 hPa, gradually exposed to reduced suctions until -3 hPa, and finally equilibrated at -100 hPa by gradually increased suctions. The rewetting was with great caution to avoid air explosion (aggregate slaking). Artificial rainwater was added to cylindrical plastic bottles containing 10 g of aggregates in order to obtain a soil:water ratio of 1:8 by weight. After end-over-end rotation (33 rpm, 23-cm diameter rotation) for 2 min, the bottles were left to stand for 230 min, after which the upper 50 mm (60 ml) containing particles $\leq 2 \mu$ m was siphoned off. The weight of dispersed clay was determined after oven-drying (105 °C for 24 h) and corrected for particles $> 250 \mu$ m isolated by chemical dispersion.

Dispersion of particles $< 20 \mu$ m (DispFines20) was measured at different time steps at plot level using field-moist soil. Soil was retrieved with a small corer (22-mm diameter) from the minimally-disturbed soil cubes and gently crumbled by hand to pass an 8-mm sieve. Artificial rainwater was added to a cylindrical bottle containing soil equivalent to 1 g oven-dry weight to obtain a soil:water ratio of 1:100 by weight. The bottle was shaken end-over-end (33 rpm, 23-cm diameter rotation) for 2, 4, 8, 16, 32, 64 and 128 min. At each time step the bottle was left to stand for 67 s, after which the upper 30 ml containing particles $< 20 \mu$ m was siphoned off and the turbidity of the suspension was measured on a Hach 2100AN turbidimeter (Hach, Loveland, CO). After each of the turbidity measurements taken at time steps 2–64 min, the soil suspension was returned to the bottle. Thus, the measurements at the different time steps were on the same sample. After the final measurement (128 min), the 30 ml was transferred to a beaker and bulked at treatment level. For each treatment, correlations between nephelometric turbidity unit (NTU) and particle concentration were established from dilution series. Calibration curves are shown in Fig. S1 in Supplementary material. The results were corrected for particles $> 250 \mu$ m isolated by chemical dispersion.

Soil samples at subplot level were analyzed for clay content without H₂O₂-removal of SOM (Jensen et al., 2017b), and clay-SOM disintegration (DI) was calculated as the ratio between clay content estimated without and with SOM removal. Soil with DI values $< 1 \text{ kg kg}^{-1}$ can be interpreted as being extremely stable since they have resisted disintegration after end-over-end shaking for 18 h in sodium pyrophosphate.

2.6. Calculations and statistics

The soil components measured in this study refer to fractions of oven-dry weight (105 °C for 24 h) of the SOM-free mineral fraction. The components include particle size fractions, SOC, POXC, HWC, LF-free-SOC, SSA, CEC and DispClay. DispFines20 is given as a fraction of SOM-free mineral fraction $< 20 \mu$ m.

For the statistical analysis and processing of spectral data, the R-project software package Version 3.4.0 (R Foundation for Statistical Computing) was used. Treatment effects were analyzed with a linear mixed model including block as a random effect when comparing A, LA and G. The criterion used for statistical significance of treatment effects was $P < 0.05$. When the treatment effect was significant, further analyses were made to isolate differences between treatments (pairwise comparisons) using the general linear hypotheses (*glht*) function implemented in the R *multcomp* package and the Kenward-Roger method to calculate degrees of freedom (Kenward and Roger, 2009). Treatment differences for the comparison of BF and the other treatments were based on a pairwise *t*-test, acknowledging that this is a less robust test, and that the treatment differences could be due to soil variation since the BF treatment is not a part of the ley-arable experiment. Inverse

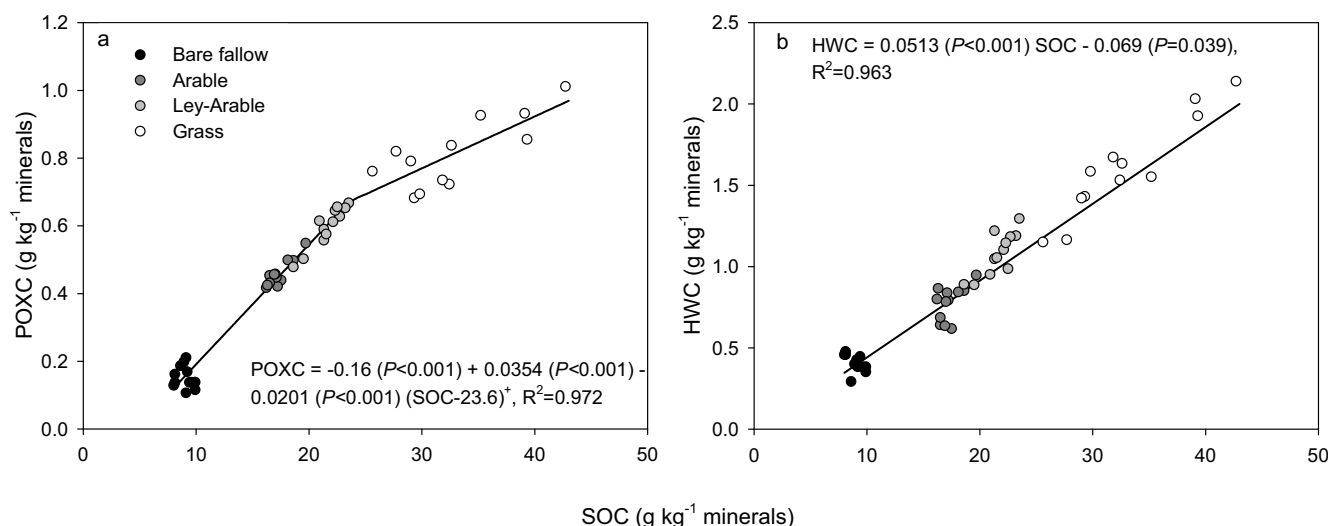


Fig. 2. (a) Permanganate oxidizable carbon (POXC) as a function of SOC and (b) hot water-extractable carbon (HWC) as a function of SOC for the four treatments at subplot level. The broken-stick and linear regression models are indicated.

transformation was performed on DispFines20 to stabilize the variance. The broken-stick model was fitted using the *segmented* package in R. A piece-wise linear model was used:

$$y = \beta_0 + \beta_1(x) + \beta_2(x - c)^+ + e \quad (2)$$

where y is the dependent variable, x is the independent variable, c is the change point and e is the residual standard error (Toms and Lesperance, 2003). The $^+$ sign indicates that the last term only is valid when $x > c$.

Spectral data processing included baseline correction, smoothing using a Savitzky-Golay filter calculated on three data points on each side with a zero-order polynomial, and normalization by the average absorbance of the whole spectra. Principal component analysis (PCA) on the FTIR-PAS spectra was performed using the *ade4* package in R.

3. Results

3.1. Basic soil characteristics

Generally, contents of clay, silt and sand did not differ significantly between treatments (Table 1) allowing the effect of contrasting management to be examined without confounding effects related to soil texture. SSA differed significantly following the same pattern as SOC. CEC was significantly higher for G than for BF treatment, and the amount of exchangeable Ca^{2+} was significantly higher for G compared to other treatments. Soil pH was not affected by the contrasting management practices.

3.2. Soil organic matter characteristics

Concentrations of SOC differed significantly and decreased in the order $G > LA = A > BF$ (Table 2). POXC, HWC, LFSOC, and the aliphatic C-H peak area ($3000\text{--}2800\text{ cm}^{-1}$) followed differences in SOC. Supplementary material (Figs. S2 and S3) shows the full spectral range as well as the SOM fingerprint region ($1700\text{--}1300\text{ cm}^{-1}$) at plot level.

The aliphatic peak area normalized by SOC content was higher in the BF than in the G treatment indicating that SOM in G soil was depleted in aliphatics. The POXC accounted for 1.7, 2.6, 2.8 and 2.5% of total SOC in BF, A, LA and G treatments, respectively, while HWC contributed 4.6, 4.5, 5.0 and 4.9%. The increase in POXC with an increase in SOC was different for the G treatment compared to A and LA (Fig. 2a). The narrow SOC range in the BF soil did not allow an evaluation of the POXC-SOC relation, but the first slope of the broken-stick

model was similar to a linear regression including A and LA (0.0354 compared to $0.0339\text{ g kg}^{-1}\text{ SOC}$). This indicates that the level of POXC for BF aligned with that of the other tilled treatments (A and LA). The x-intercept of the broken-stick model in Fig. 2a was $4.6\text{ g SOC kg}^{-1}\text{ minerals}$ suggesting that no POXC at or below this SOC content was oxidized. For the wide range of SOC in this study, HWC correlated linearly to SOC with an intercept value close to zero (Fig. 2b). Our data thus indicates that HWC represents a fixed proportion of SOC ($\sim 0.05\text{ g HWC per g SOC}$ or $\sim 5\%$).

The PCA analysis based on the $1700\text{--}1300\text{ cm}^{-1}$ region clearly separated the treatments on the first principal component (PC1) explaining 84.6% of the spectral variance (Fig. 3a). Field plots from the G and BF treatment were located to the left and right side of the PCA plane, respectively. The A and LA treatments were in the center of the PCA plane and did not differ much. The loading of PC1 indicated that BF soils were enriched in organic compounds vibrating between 1700 and 1580 cm^{-1} with a peak at 1625 cm^{-1} (Fig. 3b). Absorption in this region is attributed aromatics and carboxylate at $1600\text{--}1570\text{ cm}^{-1}$, amine at 1610 cm^{-1} , clay-bound water at 1640 cm^{-1} , aromatics at $1660\text{--}1600\text{ cm}^{-1}$ and amides at $1670\text{--}1640\text{ cm}^{-1}$ (Table 1 in Peltre et al., 2017). In contrast, G soils were relatively enriched in organic compounds vibrating between 1580 and 1495 cm^{-1} and $1475\text{--}1325\text{ cm}^{-1}$ with peaks at 1550 , 1510 and 1385 cm^{-1} (Fig. 3b). These regions are attributed nitrate at 1380 cm^{-1} , carboxylate at 1390 cm^{-1} , amide III at 1420 cm^{-1} , carbonates at 1430 cm^{-1} , aliphatic methyls at $1445\text{--}1350\text{ cm}^{-1}$, lignin rings at $1505\text{--}1515\text{ cm}^{-1}$ and amide II at $1570\text{--}1540\text{ cm}^{-1}$ (Table 1 in Peltre et al., 2017).

3.3. Soil structural stability

The amount of dispersible clay differed significantly, decreasing in the order $BF > A > LA > G$, and the disintegration of soil without SOM removal was significantly lower for the G treatment compared to the other treatments (Table 2). Linear, semi-logarithmic and broken-stick models were employed to describe the correlations of SOC, POXC and HWC to DispClay and DI (Table 3). The coefficient of determination (R^2) peaked when DispClay and DI were related to SOC with a broken-stick model (see relation on Fig. 4a). Similar relationships were found when relating POXC and HWC to DispClay and DI (Fig. 4b and c).

Relating changes in DispClay to LF-free-SOC did not improve R^2 compared to SOC (Fig. 5a), whereas LF-free-SOC increased the explained variation in DI by 1%-unit (Fig. 5b).

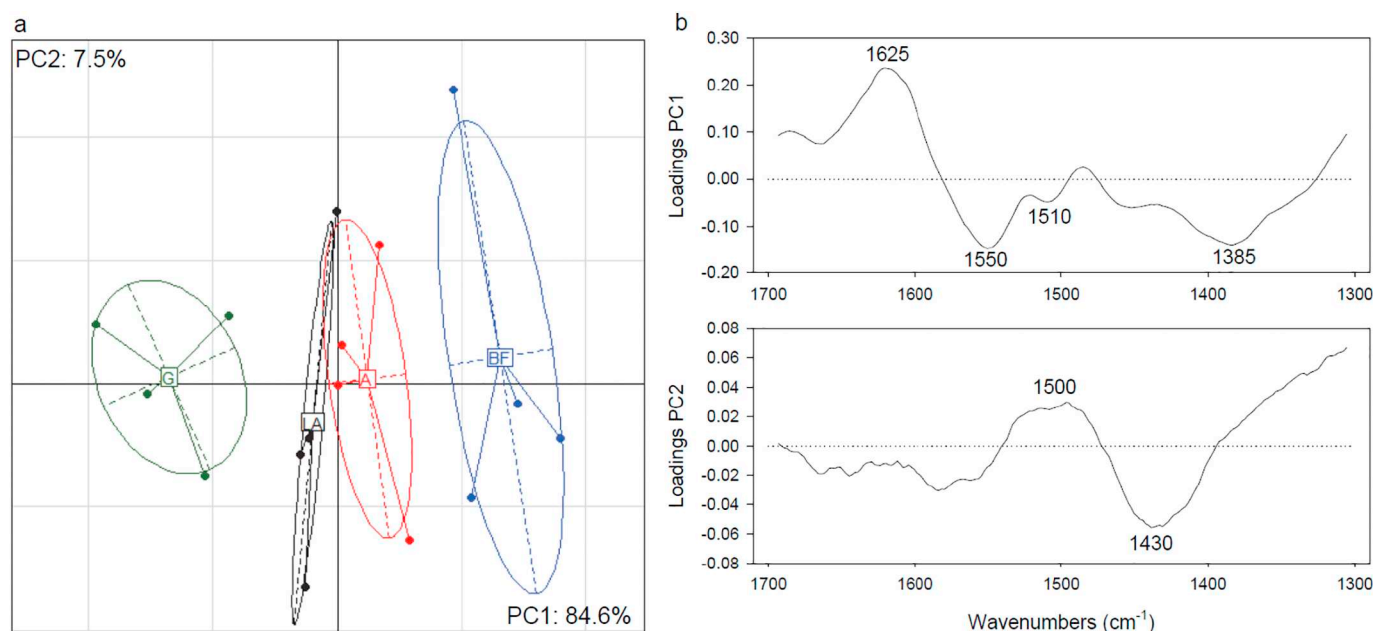


Fig. 3. Principal component analysis (PCA) based on FTIR-PAS spectra for the different treatments. The dots indicate the four plots of each treatment. For treatment abbreviations, see Fig. 1. (a) Scores plot in the plane defined by principal component 1 (PC1, explaining 84.6% of the variance) and principal component 2 (PC2, explaining 7.5% of the variance) of the PCA. (b) Loadings of the PCA for PC1 and PC2.

DispFines20 was significantly lower for the G treatment compared to the other treatments at every time step (Fig. 6a), and the release-curve had a contrasting shape compared to that of the other treatments. DispFines20 was significantly lower for LA than for A treatment after both 64 and 128 min. The release rate was markedly higher in the beginning for BF, A and LA compared to G (Fig. 6b). After c. 24 min, G showed a higher release rate than the other treatments. At every step, DispFines20 was virtually constant across the four G treatment plots despite a range in SOC, while considerable variation was observed for the narrower SOC ranges of the other three treatments (Fig. S4 in Supplementary material).

4. Discussion

4.1. Linking soil organic matter components to soil structural stability

POXC has been advocated as a partly processed but reactive pool of SOM (Culman et al., 2012), able to support biological functions (Idowu et al., 2008), while HWC has been promoted as an indicator of soil biochemical quality (Ghani et al., 2003). Both fractions are supposedly sensitive indicators for the assessment of management-induced changes in SOM quality (Culman et al., 2012; Ghani et al., 2003). Labile organic compounds are known to bond primary mineral particles into differently

Table 3

Parameters of the linear, semi-logarithmic and broken-stick models for clay dispersibility (DispClay; kg kg⁻¹ minerals) and clay-SOM disintegration (DI; kg kg⁻¹ minerals) as a function of soil organic carbon (SOC; g kg⁻¹ minerals), permanganate oxidizable carbon (POXC; g kg⁻¹ minerals), and hot water-extractable carbon (HWC; g kg⁻¹ minerals). The change point of the broken-stick model and the corresponding 95% confidence interval is indicated. The relation between the first and second slope estimate of the broken-stick model (Slope1/Slope2) was calculated if both slopes were significant. The coefficient of determination (R²) is indicated.

Predictor	Model		Equation	Change point	Slope1/Slope2	R ²
SOC	Linear	DispClay	0.0134*** – 0.00032*** SOC			0.839
		DI	1.16*** – 0.0116*** SOC			0.723
	Semi-log	DispClay	0.0255*** – 0.0148*** log(SOC)			0.930
		DI	1.49*** – 0.442*** log(SOC)			0.555
	Broken-stick	DispClay	0.0160*** – 0.00049*** SOC + 0.00039*** (SOC-23.5) ⁺	23.5*** [20.9;26.0]	4.6	0.940
		DI	1.03*** – 0.0029 ^{NS} SOC – 0.018*** (SOC-22.5) ⁺	22.5*** [19.9;25.1]		0.880
POXC	Linear	DispClay	0.0131*** – 0.0122*** POXC			0.907
		DI	1.11*** – 0.366*** POXC			0.550
	Semi-log	DispClay	0.0029*** – 0.011*** log(POXC)			0.891
		DI	0.83*** – 0.261*** log(POXC)			0.364
	Broken-stick	DispClay	0.0136*** – 0.0138*** POXC + 0.0084* (POXC-0.694) ⁺	0.694* [0.564;0.824]	2.6	0.927
		DI	1.02*** – 0.087 ^{NS} POXC – 1.00*** (POXC-0.628) ⁺	0.628*** [0.573;0.683]		0.819
HWC	Linear	DispClay	0.0128*** – 0.0061*** HWC			0.815
		DI	1.13*** – 0.21*** HWC			0.648
	Semi-log	DispClay	0.0059*** – 0.0133*** log(HWC)			0.900
		DI	0.90*** – 0.378*** log(HWC)			0.490
	Broken-stick	DispClay	0.0156*** – 0.0105*** HWC + 0.00760*** (HWC-0.970) ⁺	0.970*** [0.833;1.107]	3.6	0.913
		DI	1.02*** – 0.049 ^{NS} HWC – 0.341*** (HWC-1.104) ⁺	1.104*** [0.921;1.288]		0.788

NS: Not significant.

* Indicates significance level at $P < 0.05$.

*** Indicates significance level at $P < 0.001$.

⁺ Indicates that the last term is valid only when the content of SOC, POXC or HWC are larger than the change point.

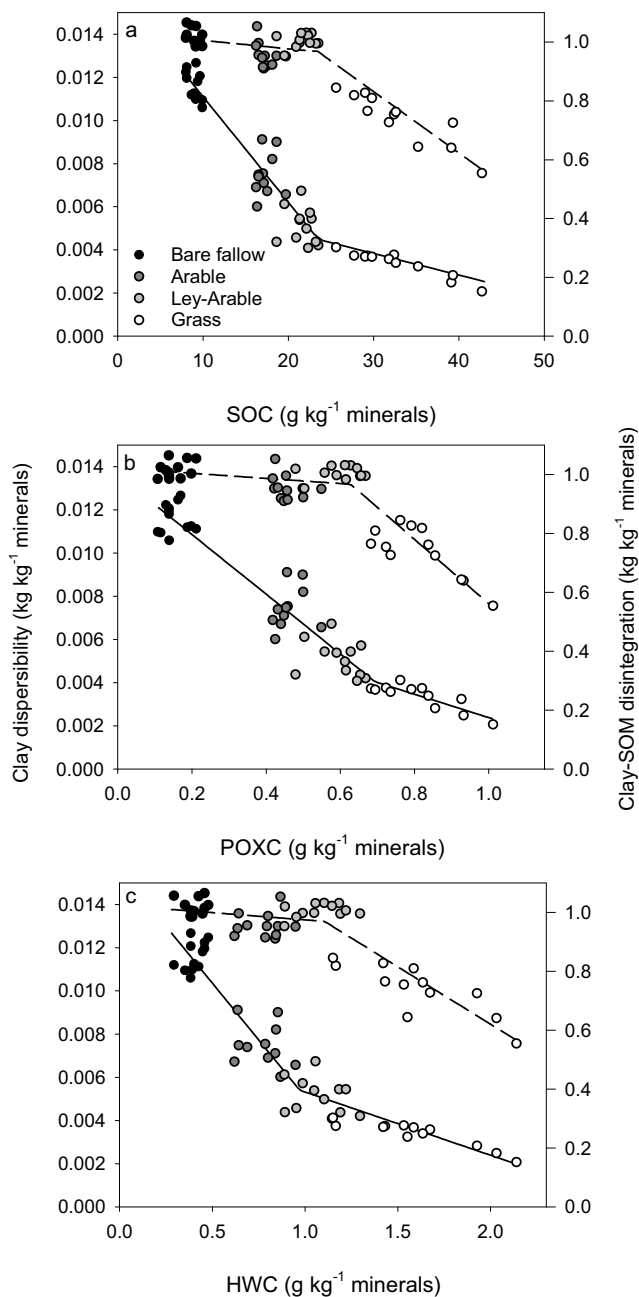


Fig. 4. Clay dispersibility of 1–2 mm aggregates rewetted to -100 hPa (solid lines) and disintegration (the ratio between clay content estimated without SOM removal and with removal) (dashed lines) as a function of (a) soil organic carbon (SOC), (b) permanganate oxidizable carbon (POXC), and (c) hot water-extractable carbon (HWC) for the four treatments at subplot level. The broken-stick models (Table 3) are indicated. See Table 3 for equations and R^2 -values.

sized organo-mineral complexes and stabilize these against mechanical damage (Degens, 1997). However, the clay-SOM disintegration (DI) test involves extreme disruptive energy (end-over-end shaking for 18 h in sodium pyrophosphate solution) and is not expected to be affected by variations in labile SOC components. In accordance with this, we noted a higher coefficient of determination in the broken-stick regression relating DI to SOC ($R^2 = 0.88$) than in those for POXC and HWC ($R^2 = 0.82$ and $R^2 = 0.79$, respectively). One may speculate that stable organo-mineral associations (i.e. at sub-micro-aggregate scale) are responsible for the extreme stability at high SOC contents. The similar pattern observed for POXC and HWC (broken-stick) relates to the near linear relations observed between total SOC and these two fractions (Fig. 2).

For the DispClay measure of SSS, we observed nearly identical coefficients of determination in the broken-stick models describing the data: R^2 equals 0.94, 0.93 and 0.91 with SOC, POXC and HWC as predictor (Table 3). We further noted that the broken-stick was “less broken” especially when using POXC as predictor (slope ratios, Table 3). This observation is supported by the higher ability of POXC to describe data in a linear model ($R^2 = 0.91$) compared to HWC and SOC ($R^2 = 0.82$ and $R^2 = 0.84$, respectively). Overall, this may indicate that POXC is superior to SOC and HWC in describing the variation in DispClay.

Our study does not reveal mechanisms leading to SSS. The indication that POXC is superior in describing the variation in DispClay suggests a link to bonding agents such as polysaccharides, assumed to be important bonding agents at micro-aggregate scale (Tisdall and Oades, 1982). However, the composition of POXC remains unknown as it is destroyed by oxidation. The lower predictive ability of HWC to explain SSS data and its close correlation to SOC may indicate that it is a less relevant quality characteristic of SOC. Other studies have emphasized that hot water-extractable carbohydrate-C is a better predictor of SSS than SOC (Haynes, 2005; Haynes and Swift, 1990).

Changes in DispClay and DI may relate better to LF-free-SOC than total SOC. However, the difference in using LF-free-SOC compared to SOC was marginal (Fig. 5). The decrease in DispClay and DI when going from BF to G may relate to an enrichment in aliphatics and lignin, and a decrease in carboxylic groups and amides (Table 2, Fig. 3). The higher amount of carboxyls and amides in BF soils suggest that SOM is more oxidized in these soils. This agrees with the findings of Barré et al. (2016), and carboxyls and amides may be related to microbial processed and stable SOM in organo-mineral associations (Kleber et al., 2015). The higher proportion of aliphatics in SOM from the BF soils also supports the presence of a more decomposed SOM. The results indicate that plant residues, initially present in the BF soil, decomposed rapidly leaving behind SOM enriched in microbial-derived OM. In contrast, less oxidized plant residues accounts for a larger fraction of the SOM in the G soils.

4.2. Management system effects on soil structural stability

The four treatments reflect three management systems comprising the BF treatment with intensive tillage, no plants or carbon input, the A and LA treatments with plants and tillage, and the G treatment with plants and absence of tillage. The G treatment differed from the other treatments by having a very stable structure and a better ability to resist physical disturbance. Consequently, the potential maximum SSS was reached only in the long-term G treatment. This was reflected in the DispClay and DI tests, where the change point of the broken-stick model for SOC content was confounded with treatment and represented a change from the LA to G treatment (Fig. 4a). Also, the results on DispFines20 support a difference in stability between the G treatment and the other treatments illustrated by the contrasting release-curve, release rate and higher stability at every time step (Fig. 6). Permanent grass stands out from the other treatments by having a greater input of above- and belowground plant residues. Hirsch et al. (2009, 2017) found markedly greater numbers of roots and mesofauna in G compared to BF and A, and a larger abundance of fungi. Roots and fungal hyphae can act as binding agents, enmeshing aggregates (Elmholt et al., 2008; Tisdall and Oades, 1982), and potentially increasing SSS, while mesofauna fecal pellets can contribute by stabilizing decomposition products (Oades, 1993). In addition, the effects of these stabilizing agents persist since they are continuously replaced and the resulting structure remains undisturbed due to the absence of tillage. The limited effect of SOC on DispFines20 within the G treatment at every time step (Fig. S4 in Supplementary material) may relate to the larger scale applied in the test, i.e. whole-soil samples not broken down to more than 8 mm. At this scale, management derived drivers such as macro-aggregate stabilizing agents appear more important, while SOC may play a minor

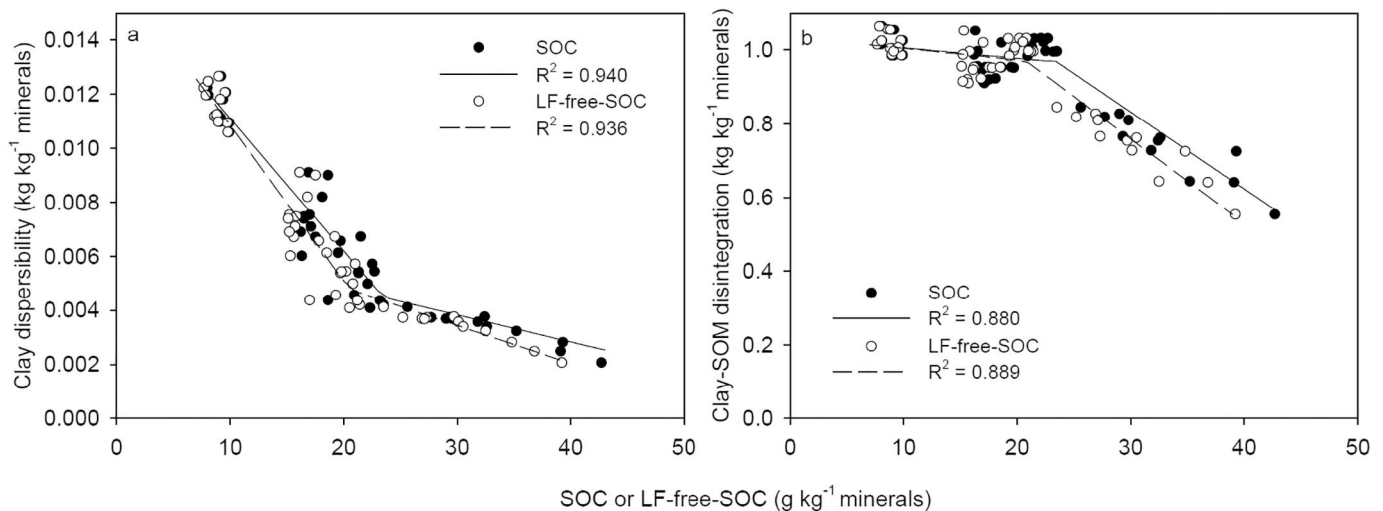


Fig. 5. (a) Clay dispersibility and (b) Clay-SOM disintegration as a function of soil organic carbon (SOC; black symbols) and LF-free-SOC (Light fraction-free-SOC; white symbols). The broken-stick models and R²-values are indicated.

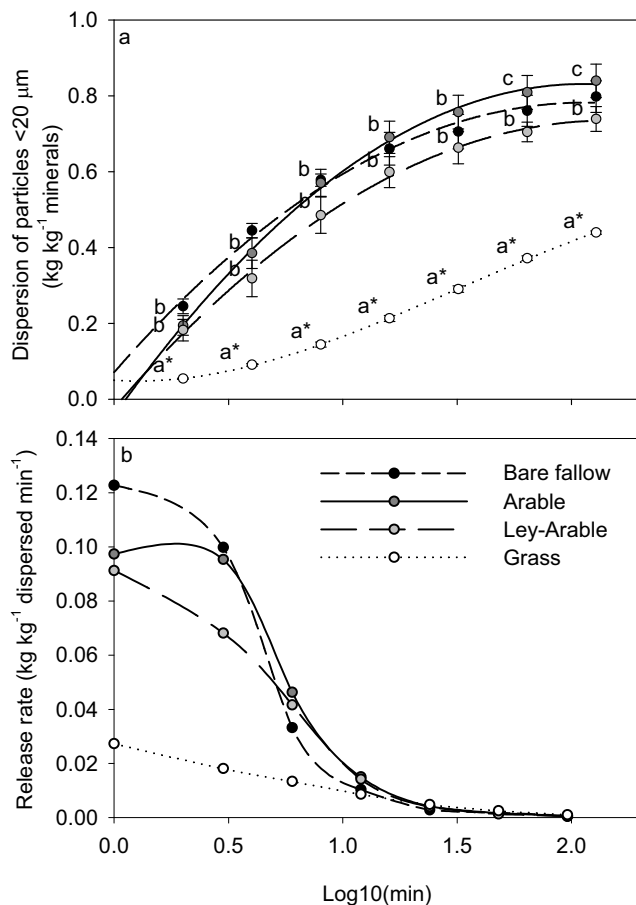


Fig. 6. (a) The ratio between dispersed particles < 20 μm and the total content of particles < 20 μm as a function of Log10(min) at treatment level. The standard error of the mean is indicated ($n = 4$). The polynomials are fitted to the four replicates time's seven data points per treatment. Letters denote statistical significance at $P < 0.05$ for the comparison of A, LA and G. An asterisk (*) indicates if BF is significantly different from A, LA and G based on a pairwise t -test. (b) Release rate (kg kg⁻¹ minerals dispersed min⁻¹) as a function of Log10(min) at treatment level. A stepwise, simple calculation of slope from time step to time step was employed to calculate the release rate, and a smoothed spline curve was added.

role. The greater stability of the G treatment may also link to the absence of tillage, known to be detrimental to the preservation of stabilizing agents.

The higher concentration of soluble Ca²⁺ ions in the G treatment may promote flocculation of clay particles (Le Bissonnais, 1996) contributing to the higher structural stability. However, the importance of cations for aggregate stability is considered less important in soils high in clay or SOC (Bronick and Lal, 2005). Matthews et al. (2008) found a decrease in wettability for G, whereas wettability was similar for BF, A and LA treatments. Thus, decreased wettability may contribute to the very stable structure of the G soil.

4.3. Critical carbon levels

Our SSS measures DI and DispClay showed a change in the relation to SOC at around 23.0 g kg⁻¹ minerals (broken-stick change point; Fig. 4a). The carbon saturation concept (Six et al., 2002; Stewart et al., 2007) implies the existence of a SOC concentration that for a given soil provides a full “coverage” of the surface of soil minerals with SOC. This concept of a finite SOC storage capacity (Ingram and Fernandes, 2001) was supported by results for a range of grassland soils assumed to be saturated with SOC (Hassink, 1997). Thus, the SOC concentration of ~23.0 g kg⁻¹ minerals found in this study may reflect a potential storage capacity for this soil. The broken-stick pattern for DispClay indicates that SOC influences SSS more for soils with SOC below the change point than above (Fig. 4a). DispClay increases more with reduction in SOC when the soil is unsaturated (below the change point) than when it is saturated. This is in line with Jensen et al. (2017a), who found SOC to be important for SSS in SOM-depleted soil. Interestingly, the DI test with extreme energy input showed that all unsaturated soil samples behaved similarly and showed complete disintegration (slope not significantly different from zero; Fig. 4a).

The threshold for a change in SOC effects on SSS differ from soil to soil, e.g. around 11.0 g SOC kg⁻¹ minerals for a sandy loam in Denmark (Jensen et al., 2017a). Thus, the SOC level critical to SSS seems soil type dependent. Dexter et al. (2008) and Jensen et al. (2017a) found increasingly compromised SSS when the clay/SOC ratio was above 10. Schjøning et al. (2012) and Jensen et al. (2017a) found that a Fines20/SOC ratio of 20 serve as a similar critical threshold value. The clay/SOC and Fines20/SOC ratios for a change in DispClay and DI were calculated by dividing the average clay or Fines20 content with the change point, giving values of 11 and 23, respectively. Thus, our results support the soil clay/SOC ~10 or Fines20/SOC ~20 as defining factors for SSS.

In this study, the thresholds for changes in SOC (as well as POXC and HWC) effects on SSS are confounded with management (Fig. 4). Thus the calculated soil mineral fines/SOC thresholds may relate to a quantity of SOC as well as to management system (as discussed in Section 4.2). This was unavoidable since creating systems with a wide range in SOC often involves the use of contrasting management. However, confounding effects derived from differences in soil type, soil texture and climate were eliminated in our study. We encourage further studies on soils with a gradient in SOC and little variation in texture, and where the mineral fines/SOC thresholds do not coincide with a shift in management system.

5. Conclusions

We exploited the unique range in SOM within the Highfield-LTE developed from contrasting long-term managements. Soil structural stability (SSS) increased with an increase in SOM components. However, the relationships followed a broken-stick regression with the greater effect occurring when SOM components were low. The SOM fractions permanganate oxidizable carbon (POXC) and hot water-extractable carbon (HWC) were less related to clay-SOM disintegration than was SOC. However, POXC seemed superior in describing the variation in clay dispersibility compared to SOC and HWC. The permanent grass had a very stable structure, even when exposed to a high degree of disturbance. This may relate to the management system supporting large concentrations of stabilizing agents due to large annual inputs of above- and belowground plant residues as well as the absence of tillage. This management system facilitates high abundance of soil microbiota and mesofauna. For the present soil, SOM promoting management increased SSS up to a specific threshold coinciding with a change to permanent grass. For soils low in SOC, increasing the SOC content or changing the management from arable rotation to permanent grass improve SSS. Further, this study supports the existence of critical soil mineral fines/SOC ratios for SSS with change points at clay/SOC~10 and Fines20/SOC~20.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2018.10.034>.

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Supplementary material for the article entitled: "Relating soil C and organic matter fractions to structural stability" by Jensen et al.

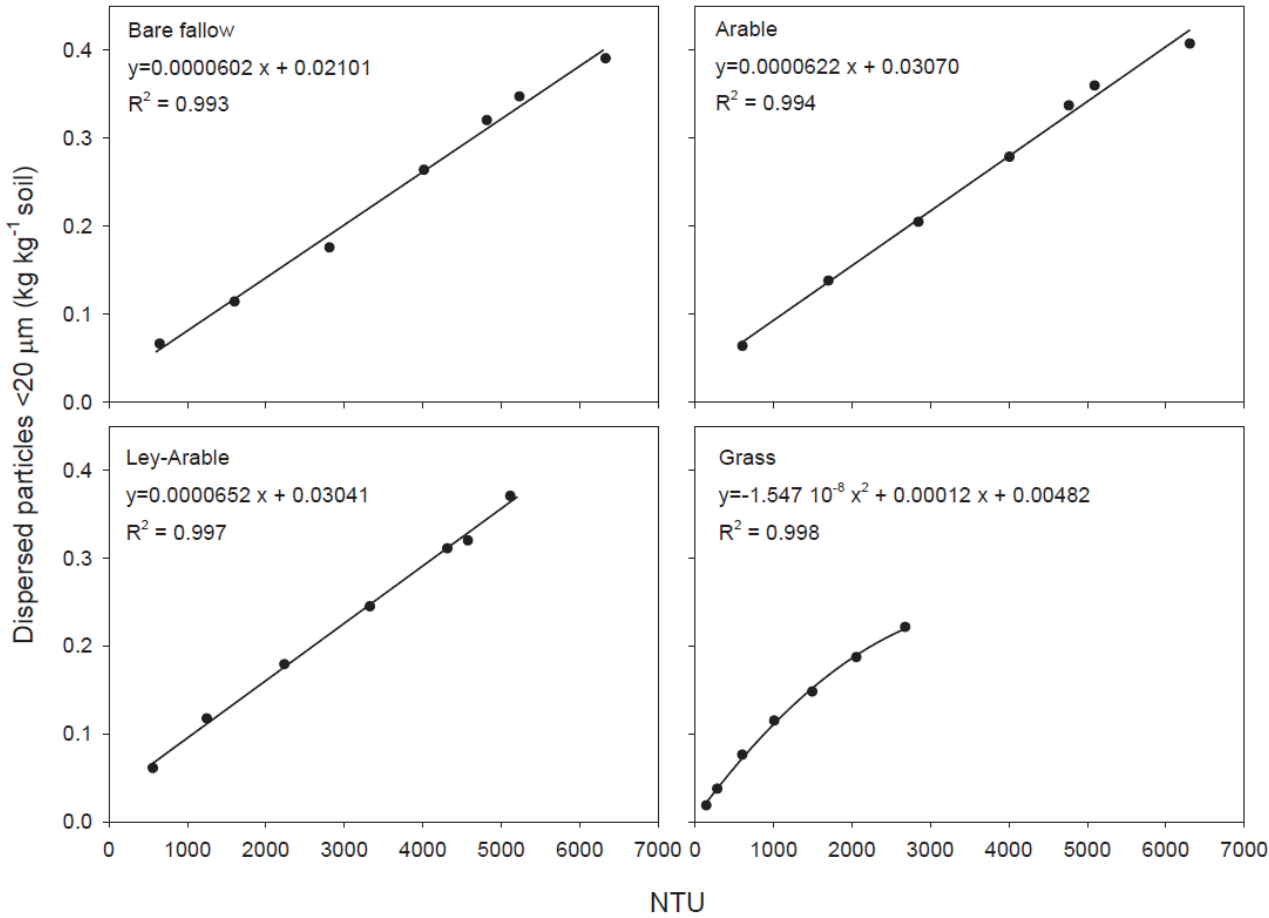


Fig. S1. The correlation between nephelometric turbidity (NTU) and dispersed particles <20 μm (kg kg⁻¹ soil) for the four different treatments.

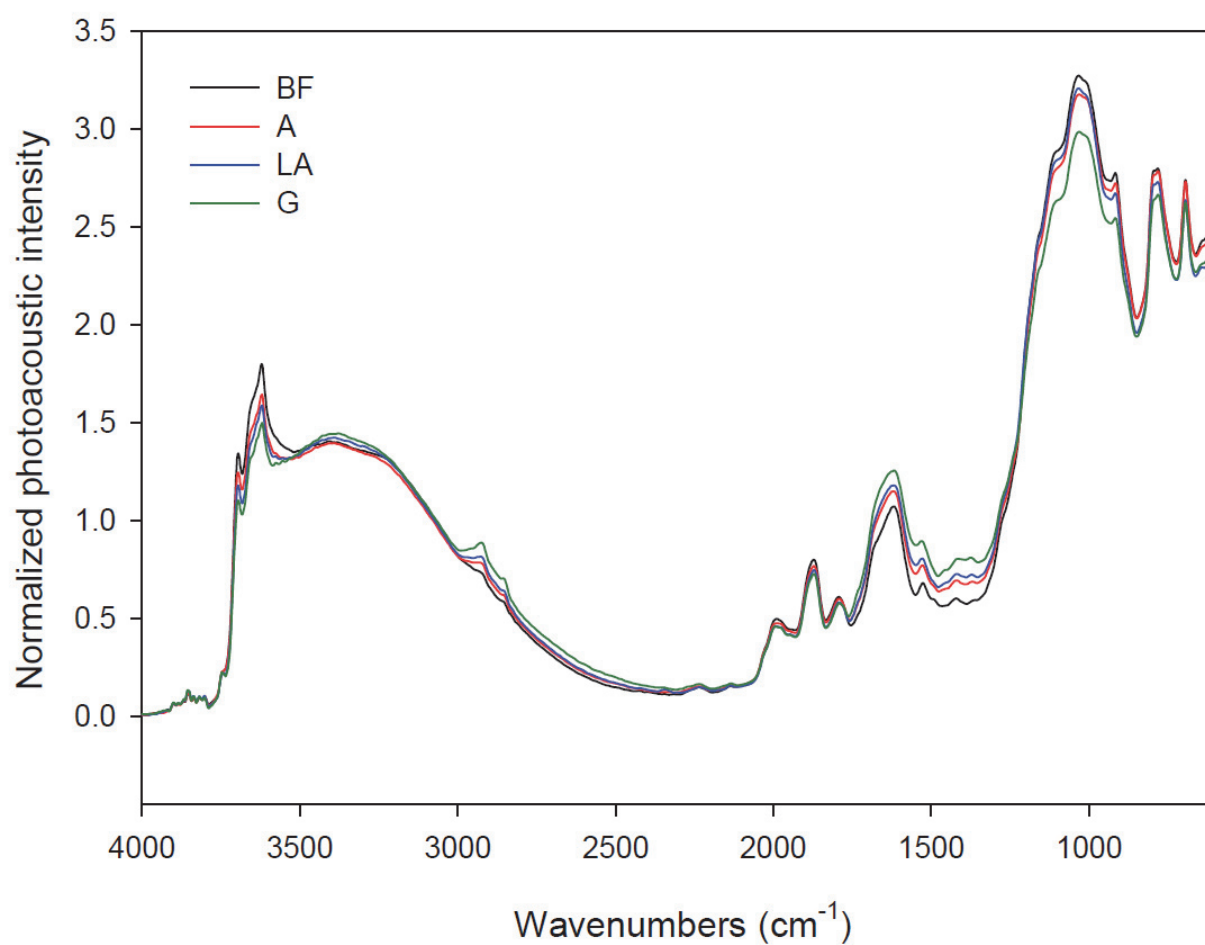


Fig. S2. Spectra of the different treatments from Highfield over the selected FTIR region 4000-600 cm⁻¹. The spectra are presented as the average of the spectra from the four field plots. BF, Bare fallow; A, Arable; LA, Ley-arable; G, Grass.

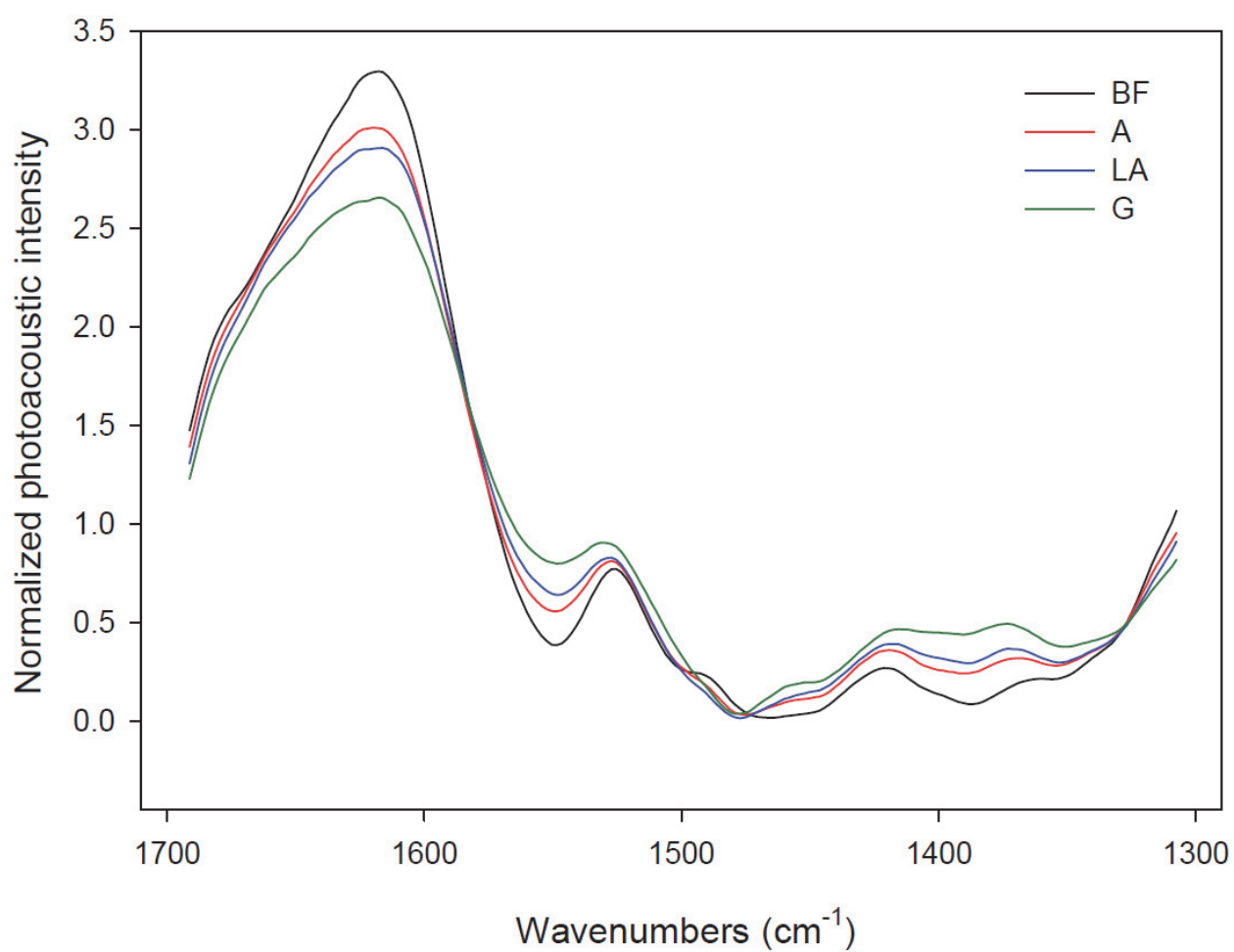


Fig. S3. Spectra of the different treatments from Highfield over the selected FTIR region 1700-1300 cm^{-1} . The spectra are presented as the average of the spectra from the four field plots. BF, Bare fallow; A, Arable; LA, Ley-arable; G, Grass.

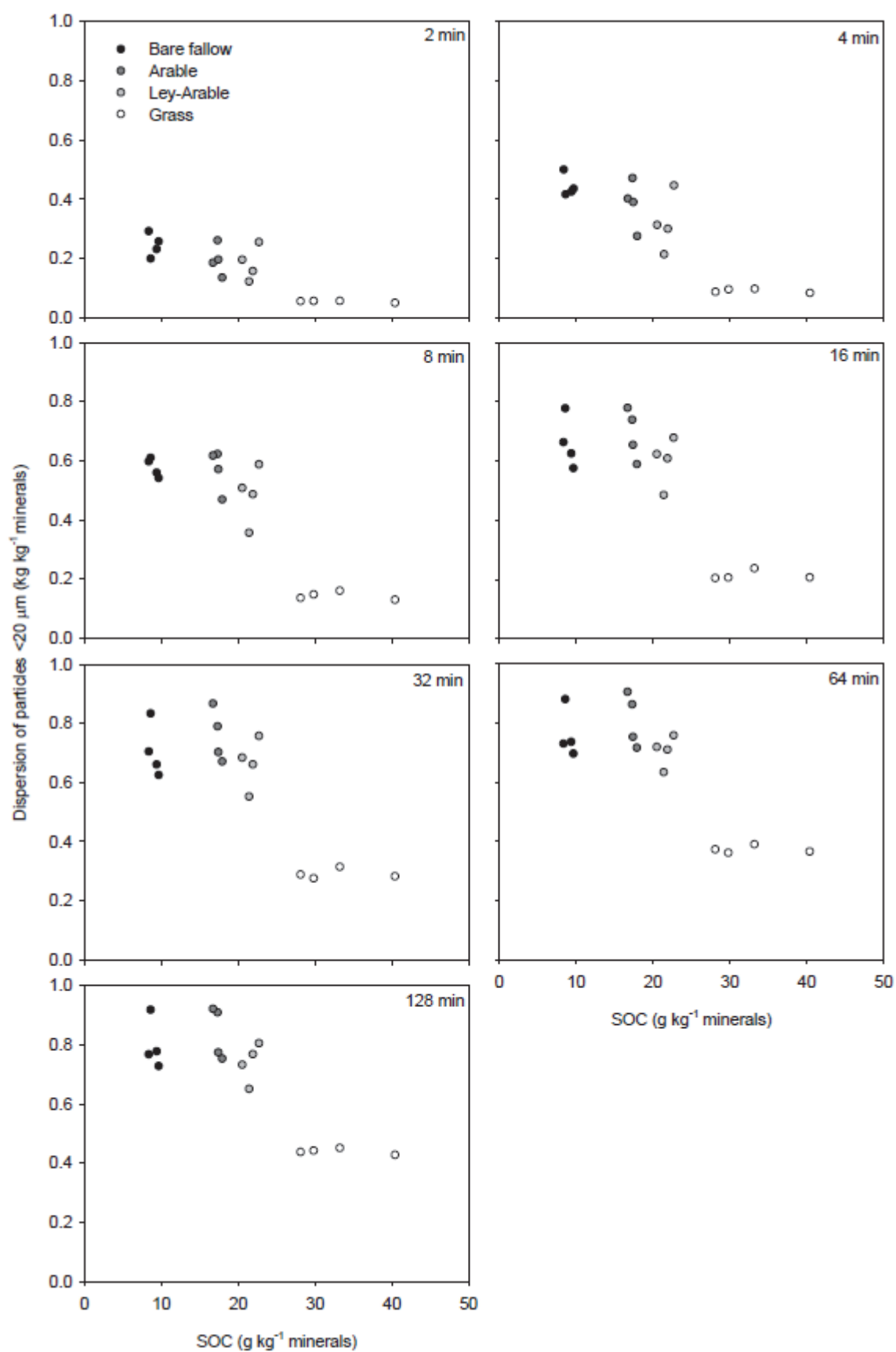


Fig. S4. The ratio between dispersed particles $<20\ \mu\text{m}$ and the total content of particles $<20\ \mu\text{m}$ as a function of soil organic carbon (SOC) at plot level for the seven time steps.

Paper 5

*Soil water retention: Uni-modal models of pore-size distribution
neglect impacts of soil management*

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Supplementary material is included

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Soil Water Retention: Uni-Modal Models of Pore-Size Distribution Neglect Impacts of Soil Management

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Most models describing soil water retention imply a uni-modal pore-size distribution (PSD). The uni-modal model presented by van Genuchten (termed vanG) is widely used although double-exponential models (termed Dex) implying a bi-modal PSD may better reflect reality. We tested the ability of vanG and Dex models to represent water retention in sandy top- and subsoils with different texture, in soil with contrasting management (Highfield), and in soil exposed to different tillage (Flakkebjerg). Soils were subjected to matric potentials from -10 hPa to -1.5 MPa. For all soils, the bi-modal Dex model showed a better fit to water retention data than the uni-modal vanG model. Neither of the models worked well for highly sorted soils. The vanG model gave a poorer fit for topsoils than for subsoils because of a more pronounced bi-modality of the PSD in topsoils caused by larger soil organic carbon (SOC) content and tillage. For Highfield soils, the root mean squared error (RMSE) of the vanG fit increased from long-term bare fallow (low C content, intensive tillage) to permanent grass (high C content, no tillage) reflecting a more distinct bi-modality of the PSD for well-structured soils. We conclude that uni-modal models should be used with great caution when describing effects of texture and management on PSD and that bi-modal models may provide a better fit to PSD.

Abbreviations: AIC, Akaike's information criterion; d_2 , dominating pore size of the structural peak; Dex, double-exponential; PSD, pore-size distribution; RMSE, root mean squared error; SOC, soil organic C; SOM, soil organic matter; V_1 , textural void ratio; V_2 , structural void ratio; vanG, van Genuchten; VIF, variance inflation factor.

The availability of water in soil is crucial for plant growth, microbial activity, and percolation (Rabot et al., 2018). Water storage and availability link intimately to soil PSD and reliable models describing soil water retention become vital when simulating water and solute movement in soil, and availability of water for crop development and soil organic matter (SOM) turnover.

Numerous models for describing soil water retention has been suggested, most of which are uni-modal analytical expressions (Cornelis et al., 2005) such as the one proposed by Brooks and Corey (1964). The uni-modal model suggested by van Genuchten (1980) is probably the most widely used model for describing soil water retention. By May 2018, this publication has received ~10,000 citations of which ~4000 are within the last 5 yr (Clarivate Analytics, 2018). The fitting parameters of the van Genuchten model (here termed vanG) are often used to estimate the unsaturated hydraulic conductivity of soils (Mualem, 1986). Many pedo-transfer functions have been developed to predict the vanG parameters from basic soil properties (Cornelis et al., 2001; Minasny et al., 1999; Patil and Singh, 2016). Unsaturated hydraulic conductivity predicted by the vanG parameters are used in simulation models such as Daisy (Hansen et al., 2012) and HYDRUS (Šimunek et al., 2012) when predicting crop production and associated environmental impacts.

Core Ideas

- A uni- and a bi-modal soil water retention model were evaluated.
- The bi-modal double-exponential model fitted better to soil pore-size distribution.
- The uni-modal model fit was affected by texture, soil organic C, and tillage.
- Uni-modal models are not well suited for describing the pore-size distribution.

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Table 1. Soil textural composition and organic C (SOC) content for the 16 Danish soils at ~0.10-m depth of the Jacobsen (1989) data set listed in order of increasing clay content. The Rosin-Rammler parameters (α and β) were calculated by Eq. [6] and are based on the seven particle-size fractions listed in Jacobsen (1989).

Site	SOC	Clay,	Silt,	Silt,	Sand,	α	β
		<2 μm	2–20 μm	20–63 μm	63–2000 μm		
		g 100 g ⁻¹ minerals				μm	–
Hals	2.36	2.6	3.4	7.9	86.0	150	1.76
Tylstrup	1.30	3.7	4.9	17.2	74.2	88	3.58
Jyndevad	1.36	4.2	3.9	3.2	88.8	367	1.41
Borris	1.31	5.7	7.8	22.8	63.7	131	0.96
Hornum	1.86	5.8	8.4	13.3	72.5	180	0.93
Travsted	3.38	7.7	6.8	16.2	69.3	189	0.86
Foulum	1.49	7.9	10.1	15.6	66.4	176	0.75
Ødum	1.49	10.1	15.5	20.2	54.3	104	0.71
Årslev	1.36	10.6	14.9	21.1	53.4	95	0.79
Roskilde	1.43	10.8	17.3	19.3	52.7	93	0.74
Askov	1.55	11.0	12.6	16.5	59.9	124	0.77
Rønhave	1.24	14.5	15.6	27.5	42.4	65	0.78
Tystofte	1.18	14.7	16.4	19.5	49.4	75	0.73
Ø. Ulslev	1.38	15.8	15.5	16.5	52.2	102	0.58
Kalø	0.82	17.7	14.4	15.9	52.0	102	0.55
Højer	1.73	18.6	15.4	39.9	26.0	42	1.00

Uni-modal models implicitly assumes a maximum volume of pores at a given tube equivalent pore size. However, presenting the size distribution of pores by frequency for example by numerical differentiation of the water retention curve has documented important deviations from a uni-modal PSD (Eden et al., 2011; Pulido-Moncada et al., 2019; Schjønning, 1992). This calls for a more adequate description of the soil pore system than that obtained by uni-modal expressions. Several non-uni-modal models have been proposed (e.g., Durner, 1994; Poulsen et al., 2006; Ross and Smettem, 1993), including a dual-porosity model for simulating the preferential movement of water and solutes

Table 2. Soil textural composition and organic C (SOC) content for the 16 Danish soils at ~0.50-m depth of the Jacobsen (1989) data set listed as in Table 1. The Rosin-Rammler parameters (α and β) were calculated by Eq. [6] and are based on the seven particle-size fractions listed in Jacobsen (1989).

Site	SOC	Clay,	Silt,	Silt,	Sand,	α	β
		<2 μm	2–20 μm	20–63 μm	63–2000 μm		
		g 100 g ⁻¹ minerals				μm	–
Hals	0.17	2.0	0.5	1.0	96.5	190	3.31
Tylstrup	0.29	3.1	2.4	12.8	81.7	82	5.96
Jyndevad	0.35	3.5	1.9	1.0	93.6	359	2.25
Borris	0.29	11.2	7.3	14.9	66.6	136	0.90
Hornum	0.17	7.2	6.3	13.7	72.8	200	0.88
Travsted	0.35	10.8	6.7	10.8	71.7	194	0.84
Foulum	0.17	13.4	9.6	13.4	63.5	166	0.64
Ødum	0.17	16.5	12.6	16.4	54.4	106	0.60
Årslev	0.17	20.4	12.6	15.9	51.0	78	0.63
Roskilde	0.29	23.8	16.3	11.9	48.0	72	0.49
Askov	0.35	24.5	11.6	14.3	49.6	72	0.55
Rønhave	0.29	19.6	16.5	25.1	38.8	53	0.67
Tystofte	0.29	22.8	15.3	17.9	44.0	58	0.58
Ø. Ulslev	0.23	15.6	13.5	14.1	56.8	120	0.59
Kalø	0.29	26.8	12.4	14.3	46.6	77	0.43
Højer	0.24	7.9	6.4	35.6	50.1	69	3.08

in structured porous media developed by Gerke and van Genuchten (1993). This model involves two overlaying continua reflecting a macropore system and a matrix pore system.

Dexter et al. (2008) presented a double-exponential model (here termed Dex) that provides a bi-modal PSD, that is, a pore-size distribution with two peaks. The two peaks represent the pore space defined by soil texture and that defined by soil structure. By providing parameters with physical meaning, the Dex model may provide a better and more mechanistic description of the PSD of soil in situ (Dexter, 1988; Dexter et al., 2008).

Relying on previously published data, this study evaluates the ability of the uni-modal vanG and bi-modal Dex model to fit water retention data for (i) sandy top- and subsoils with different texture, (ii) soil with contrasting long-term crop rotations, and (iii) a soil subject to different tillage practices.

MATERIALS AND METHODS

The Jacobsen Data Set

Jacobsen (1989) reported hydraulic properties of 16 Danish agricultural soils sampled from topsoil (~0.10 m) and subsoil (~0.50 m) layers. Topsoils ranged from loamy sand to silt loam with the main part being sandy loam or loam soils. The Jyndevad and Tylstrup soils were extremely sorted with 51.2 g 100 g⁻¹ minerals in the 200- to 500- μm fraction and 51.8 g 100 g⁻¹ minerals in the 63- to 125- μm fraction, respectively. Table 1 and 2 show the soil textural composition of top- and subsoils.

All soils were from long-term arable fields derived from the Weichsel glacial stage (glacial deposits: 10 soils; glaciofluvial deposits: Jyndevad), the Saale glacial stage (glacial deposits: Borris and Travsted), the raised Holocene sea floor (Tylstrup and Hals), and one soil sampled on recently reclaimed marine marshland (Højer).

At each site, topsoil was sampled in six plots of about 1 m², whereas subsoil was sampled only in one of the plots. For topsoil, three 100-cm³ soil cores (61-mm diam., 34-mm height) were sampled providing 18 cores per site. For subsoil, nine cores were sampled at each site.

Rothamsted Highfield Ley-Arable Experiment

Data on soil texture, SOC, and pore characteristics for the Highfield long-term ley-arable experiment at Rothamsted Research, UK (51°80' N, 00°36' W) was recently published by Jensen et al. (2019) and Obour et al. (2018). Here we use data for four treatments: (i) Bare fallow maintained free of plants by frequent tillage since 1959; (ii) Continuous arable rotation with winter cereals since 1948; (iii) Ley-arable rotation—a 3-yr grass-clover ley followed 3 yr

arable since 1948; and (iv) Grassland plowed and reseeded to grass in 1948. The bare fallow treatment was cultivated three to five times per year, arable once a year, ley-arable once in 2 yr (in 6-yr cycle) while grass had not been cultivated since 1947. The arable, ley-arable, and grass treatments were embedded in a randomized block design, whereas the bare fallow plots were not part of the original design and located at one end of the experiment. The soil is silt loam and classifies as Aquic Paludalf (USDA Soil Taxonomy System; Soil Survey Staff, 2014). The parent material includes a silty (loess-containing) deposit overlying and mixed with clay-with-flints (Avery and Catt, 1995).

Soil was sampled in spring 2015. Six 100-cm³ soil cores (61-mm diam., 34-mm height) were extracted from ~0.10-m depth in each of four replicate plots providing 24 cores per treatment. Further details are given in Jensen et al. (2019) and Obour et al. (2018).

Flakkebjerg Tillage Experiment

We used previously published data on SOC and pore characteristics for the long-term conservation tillage experiment at Flakkebjerg Experimental Station, Denmark (55°19' N, 11°23' E). This experiment compares moldboard plowing to 0.20-m depth and direct drilling using a split-plot experiment with four replicates. Soil was sampled in autumn 2013 after 11 yr of different tillage practices. The soil is a sandy loam with 15% clay (<2 μm), 14% silt (2–20 μm), 43% fine sand (20–200 μm), and 27% coarse sand (200–2000 μm) and classifies as Oxyaquic Agriudoll (USDA Soil Taxonomy System; Soil Survey Staff, 2014). The rotation included autumn and spring sown crops (mainly cereals) with all aboveground residues removed.

Six 100-cm³ soil cores (61-mm diam., 34-mm height) were extracted from the 0.12- to 0.16-m soil layer of each plot providing 24 cores per treatment. Further details can be found in Abdollahi and Munkholm (2017).

Laboratory Measurements

Soil texture was determined on air dry bulk soil (<2 mm) with a combined hydrometer/sieve method (Gee and Or, 2002). Samples from Highfield were treated with hydrogen peroxide to remove soil organic matter (SOM), while this was not done for Flakkebjerg and Jacobsen soils. The content of SOC was measured by dry combustion using a Thermo Flash 2000 NC Soil Analyzer (Thermo Fisher Scientific) for Highfield and Flakkebjerg, and a LECO CNS-1000 analyzer (LECO Corp.) for the Jacobsen soils.

Before measuring soil water retention, the soil cores were placed on the top of a tension table and saturated with water from beneath. For the Jacobsen data set, soil water retention was measured at –4 (Højer only), –10-, –16-, –50-, –100-, –160-, and –500-hPa matric potential using tension tables and pressure plates (Dane and Hopmans, 2002). Samples from the Highfield and Flakkebjerg experiments were drained to –10-, –30-, –100-, –300-, and –1000-hPa matric potential. The soil cores were oven-dried (105°C for 24 h), and bulk density calculated. The Highfield soil contained a significant amount of stones, and bulk density was corrected for weight and volume of >2-mm mineral particles. Soil

porosity was estimated from bulk density and particle density. Particle density was measured by the pycnometer method (Flint and Flint, 2002). For Highfield, particle density was measured on one plot from each treatment, and the particle density for the remaining plots were predicted from SOC by a linear regression model. For Flakkebjerg, a particle density of 2.65 g cm^{–3} was used (Abdollahi and Munkholm, 2017).

Water retention at –1.5 MPa was determined on <2-mm air-dry soil for each site and depth for the Jacobsen soils and at plot level for Highfield. For the Jacobsen and Highfield soils a pressure plate system and a WP4-T Dewpoint Potentiometer, respectively, was used (Scanlon et al., 2002). For Flakkebjerg, water retention at –1.5 MPa was predicted based on clay and SOC content using Eq. [1] in Hansen (1976).

Pore-water suction was assumed to relate to an average pore size by the approximate relation:

$$d = -3000/h \quad [1]$$

where d is the tube-equivalent pore diameter (μm) and h is the soil matric potential (hPa). Equation [1] derives from the physics-based capillary rise equation of Young-Laplace.

Soil Water Retention Models

The water retention data was fitted to the van Genuchten (1980) model (termed vanG):

$$\theta = (\theta_{\text{sat}} - \theta_{\text{res}}) \left[1 + (\alpha h)^n \right]^{-m} + \theta_{\text{res}} \quad [2]$$

where θ_{sat} and θ_{res} are the water contents at saturation and the residual water content, respectively, h is the soil matric potential, a is a scaling factor for h , and n and m are parameters that control the shape of the curve. The widely used Mualem (1976) restriction ($m = 1 - 1/n$) was used to prevent over-parametrization (Dexter et al., 2008) and unstable results (van Genuchten et al., 1991). The Mualem restriction is also recommended, when only measured values in the wet range are used (van Genuchten et al., 1991). The PSD predicted by the vanG model was obtained by differentiating Eq. [2] with respect to matric potential:

$$\frac{d\theta}{d(\log_{10} h)} = (\theta_{\text{sat}} - \theta_{\text{res}}) \left\{ \alpha n (\alpha h)^{n-1} (-m) \left[1 + (\alpha h)^n \right]^{-m-1} \right\} h \ln 10 \quad [3]$$

The double-exponential model proposed by Dexter et al. (2008) was fitted to water retention data (termed Dex):

$$\theta = C + A_1 e^{(-h/h_1)} + A_2 e^{(-h/h_2)} \quad [4]$$

where C is the residual water content, A_1 and h_1 describe the textural pore space, and A_2 and h_2 describe the structural pore space. The PSD predicted by the Dex model was obtained by differentiating Eq. [4] with respect to matric potential:

$$\frac{d\theta}{d(\log_{10} h)} = -\frac{A_1}{h_1} e^{(-h/h_1)} h \ln 10 - \frac{A_2}{h_2} e^{(-h/h_2)} h \ln 10 \quad [5]$$

The parameters of the vanG model were obtained using the curve-fitting program RETC (van Genuchten et al., 1991), which is based on a nonlinear least-squares optimization approach. Similarly, the parameters of the Dex model were obtained by nonlinear regression analysis to achieve the smallest residual sum of squares.

Calculations and Statistics

The Rosin-Rammler equation (Eq. [2] in Rosin and Rammler, 1933) was fitted to the seven chemically dispersed particle-size fractions listed in Jacobsen (1989), that is, <2, 2 to 20, 20 to 63, 63 to 125, 125 to 200, 200 to 500, and 500 to 2000 μm , for each soil. It can be written as:

$$P(X < x) = 1 - \exp\left[-\left(\frac{x}{\alpha}\right)^\beta\right] \quad [6]$$

where $P(X < x)$ is the fraction by weight of particles less than size x , α indicates the coarseness of particles and β indicates the spread of particle sizes. Equation [6] described the particle-size distribution of the soils well, with coefficients of determination (R^2) from 0.95 to 1.00.

For the statistical analysis, the R-project software package Version 3.4.0 (R Foundation for Statistical Computing) was used. Treatment effects for Highfield was analyzed as described in Jensen et al. (2019). The key indices of goodness of fit were Akaike's information criterion (AIC), which was used to compare models with different number of parameters (Akaike, 1973), and the RMSE:

$$\text{RMSE} = \sqrt{\frac{1}{N} \sum (\theta_{\text{meas}} - \theta_{\text{fitted}})^2} \quad [7]$$

where N is the number of matric potentials. A smaller or more negative AIC indicates better model performance.

Multiple linear regression was used to identify how structural void ratio (V_2) related to SOC, soil texture, and void ratio. Structural void ratio was calculated as follows:

$$V_2 = A_2 / (1 - P) \quad [8]$$

where A_2 is the Dex model estimate of structural pore space, and P is porosity. Likewise, textural void ratio (V_1) was calculated by relating A_1 to $(1 - P)$.

The variance inflation factor (VIF) was calculated when more than one predictor was used in the regression. The VIF expresses the degree of multicollinearity among the predictors. Upper value limits of VIF for non-erroneous conclusions from multiple regressions has been set to five (Rogerson, 2001) or ten (Kutner et al., 2004).

RESULTS

Jacobsen Data Set

The soils differed in their textural composition and SOC content (Tables 1 and 2). In the topsoil, clay ranged from 2.6 to 18.6 g 100 g⁻¹ minerals and SOC from 0.82 to 3.38 g 100 g⁻¹ minerals, whereas the range in the subsoil was from 2.0 to 26.8 g clay 100 g⁻¹ minerals and from 0.17 to 0.35 g SOC 100 g⁻¹ minerals. The α -parameter for topsoil ranged from 42 to 367 μm and for subsoil from 53 to 359 μm . The Jyndevad soil, however, stand out being very coarse textured ($\alpha_{\text{topsoil}} = 367 \mu\text{m}$, $\alpha_{\text{subsoil}} = 359 \mu\text{m}$), and the range changed to 42 to 200 μm if omitting Jyndevad. The β -parameter describes the spread of particle sizes, with large values indicating that the soil is well sorted (a narrow range of particle sizes), and small values indicating that the soil is graded with an evenly distributed mass of particles across size classes. The β -parameter for topsoil ranged from 0.55 to 3.58 and for subsoil from 0.43 to 5.96. The 12 glacial till soils, however, had a narrow range from 0.43 to 0.96, whereas Hals, Tylstrup, Jyndevad, and Højer were highly sorted with $\beta > 1$ (Tables 1 and 2).

For topsoil, mean values of AIC were -58.6 and -70.6 and mean RMSE were 0.011 and $0.005 \text{ m}^3 \text{ m}^{-3}$ using vanG and Dex, respectively. For subsoil, mean values of AIC were -63.2 and -75.1 and mean RMSE were 0.008 and $0.005 \text{ m}^3 \text{ m}^{-3}$. However, the four highly sorted soils ($\beta > 1$) had relatively poor goodness of fit measures both when using the vanG and Dex models (Fig. 1, Tables S1 and S2).

When omitting the highly sorted soils in the calculation of mean AIC and RMSE values, the vanG model gave AIC values of -59.6 and -66.8 and RMSE values of 0.009 and $0.006 \text{ m}^3 \text{ m}^{-3}$ in top- and subsoil, respectively. The Dex model gave AIC values of -75.1 and -80.9 and RMSE values of 0.003 and $0.002 \text{ m}^3 \text{ m}^{-3}$ in top- and subsoil, respectively. The lower AIC and RMSE values obtained for the Dex compared with the vanG model indicate that the Dex model provides a better description of data.

We tested the correlation between the structural void ratio (V_2) and the variables α , β , void ratio, SOC, and clay content. This was done for both top- and subsoils and with and without exclusion of the highly sorted soils ($\beta > 1$). For topsoil samples V_2 could be well predicted by $\log(\beta)$ and clay content:

$$V_2 = 0.558^{***} (\pm 0.118) \times \log(\beta) - 0.011^* (\pm 0.005) \times \text{clay} + 0.424^{***} (\pm 0.048), \quad s = 0.068, R^2 = 0.84 \quad [9]$$

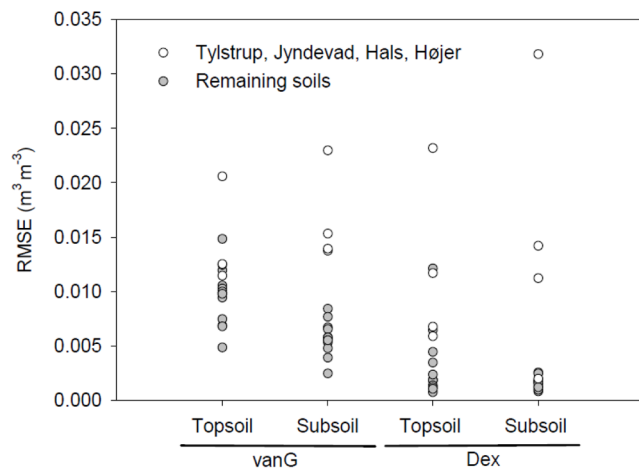


Fig. 1. The root mean squared error (RMSE) value for the Danish top- and subsoil of the Jacobsen data set using the van Genuchten (vanG) or double-exponential (Dex) model.

Excluding the highly sorted samples from topsoil gave:

$$V_2 = 0.878^{***}(\pm 0.143) \times \beta - 0.441^{**}(\pm 0.110), s = 0.057, \\ R^2 = 0.79 \quad [10]$$

For subsoil samples V_2 could be well predicted by $\log(\beta)$ and α :

$$V_2 = 0.592^{***}(\pm 0.078) \times \log(\beta) - 0.001^{**} \\ (\pm 0.0003) \times \alpha + 0.184^{**}(\pm 0.050), \\ s = 0.100, R^2 = 0.85 \quad [11]$$

Excluding the highly sorted samples from subsoil gave:

$$V_2 = 0.289^{***}(\pm 0.025) \times \beta, s = 0.057, R^2 = 0.55 \quad [12]$$

In Eq. [9]–[12], the numbers in parentheses are standard errors of estimate, and s is the standard deviation of the predicted value. When developing the four models, we tested for multicollinearity and interaction among the predictors, but found only low VIF values and no significant interactions.

Rothamsted Highfield Ley-Arable Experiment

The soils at Highfield ranged from 0.84 to 4.04 g SOC 100 g⁻¹ minerals and soil texture was in general not significantly different between treatments (Table 3). Thus, the effect of contrasting long-term management could be investigated without confounding effects related to variations in soil type. The Dex model generally fitted the water retention data for the contrasting treatments well (Fig. 2a).

Mean values of AIC, when using the vanG and Dex models were -43.8 and -69.1, respectively. Similarly, mean values of RMSE were larger when using the vanG compared with the Dex model with values of 0.016 and 0.002 m³ m⁻³, respectively. The RMSE when using the vanG model increased from 0.010 to 0.028 m³ m⁻³ with an increase in SOC from 0.84 to 4.04 g 100 g⁻¹ minerals (Fig. 3), whereas no systematic error was observed when using the Dex model ($P = 0.532$). Parameter estimates and goodness of fit measures for the 16 individual plots can be seen in Table S3.

Table 3. Soil textural composition and organic carbon (SOC) content of the four treatments from Highfield. Within rows, letters denote statistical significance at $P < 0.05$ for the comparison of Arable, Ley-arable, and Grass. Data from Jensen et al. (2019).

Treatment	SOC	Clay, <2 μm	Silt, 2–20 μm	Silt, 20–63 μm	Sand, 63–2000 μm
g 100 g ⁻¹ minerals					
Bare fallow	0.90	27.0	24.9	33.5	14.6
Arable	1.73a†	26.4	26.3	31.8	15.5
Ley-arable	2.16a†	25.5	26.1	32.4	16.0
Grass	3.29b†	26.1	27.2†	31.9	14.8

† Indicates if bare fallow is significantly different from the other treatments based on a pairwise t test.

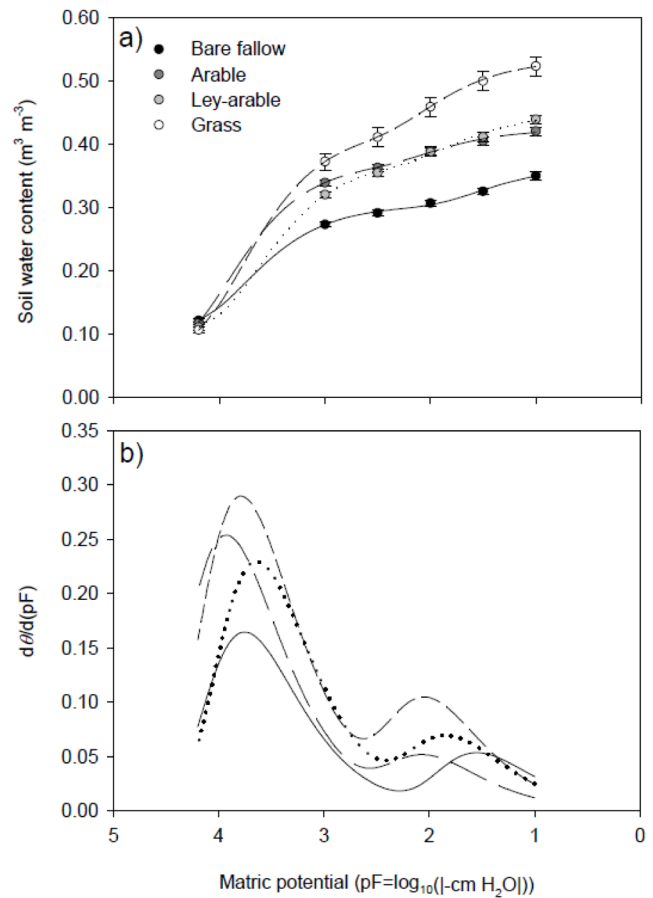


Fig. 2. (a) Measured volumetric water content for the four treatments at Highfield and fits of the double-exponential (Dex) model as a function of matric potential. The standard error of the mean is indicated ($n = 4$). (b) Pore-size distribution [$dq/d(pF)$] as a function of matric potential for the four treatments. Equation [5] was used to obtain the pore-size distributions.

Textural (V_1) and structural void ratio (V_2) increased with increasing SOC content and decreasing tillage intensity (V_1 : $R^2 = 0.91$, $P < 0.001$, V_2 : $R^2 = 0.74$, $P < 0.001$). The dominating pore size of the structural peak (d_2) was 86 μm for the bare fallow

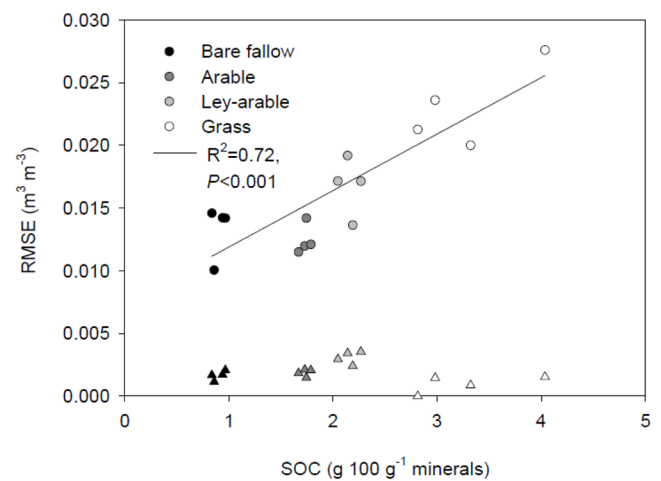


Fig. 3. The root mean squared error (RMSE) value as a function of soil organic carbon (SOC) for the four treatments at Highfield using the van Genuchten (vanG) model (circle symbols) and the double-exponential (Dex) model (triangle symbols).

Table 4. Estimated parameters of the double-exponential model (Dex) of the four treatments from Highfield. Within rows, letters denote statistical significance at $P < 0.05$ for the comparison of Arable, Ley-arable and Grass. d_1 and d_2 indicate the dominating pore size of the textural and structural peak, respectively, and were estimated by Eq. [1].

Treatment	Parameters of the Dex model						
	C	A_1	h_1	d_1	A_2	h_2	d_2
	$\text{m}^3 \text{m}^{-3}$	$\text{m}^3 \text{m}^{-3}$	hPa	μm	$\text{m}^3 \text{m}^{-3}$	hPa	μm
Bare fallow	0.110	0.195	5729	0.5	0.061	35	86
Arable	0.068a†	0.305ab†	8707b†	0.3	0.051a	97b†	31
Ley-arable	0.104b	0.271a†	4379a	0.7	0.073a	63a	48
Grass	0.080ab†	0.345b†	6216a	0.5	0.110b†	102b†	29

† Indicates if bare fallow is significantly different from the other treatments based on a pairwise t test.

treatment, while it was significantly lower for the arable and grass treatments, and in between for ley-arable treatment (Table 4).

Flakkebjerg Tillage Experiment

Moldboard plowing to 0.20-m depth and direct drilling had contents of 1.25 and 1.15 g SOC 100 g^{-1} minerals, respectively, in the 0.12- to 0.16-m layer. The Dex model fitted the two treatments well (Fig. 4), and better compared with the vanG

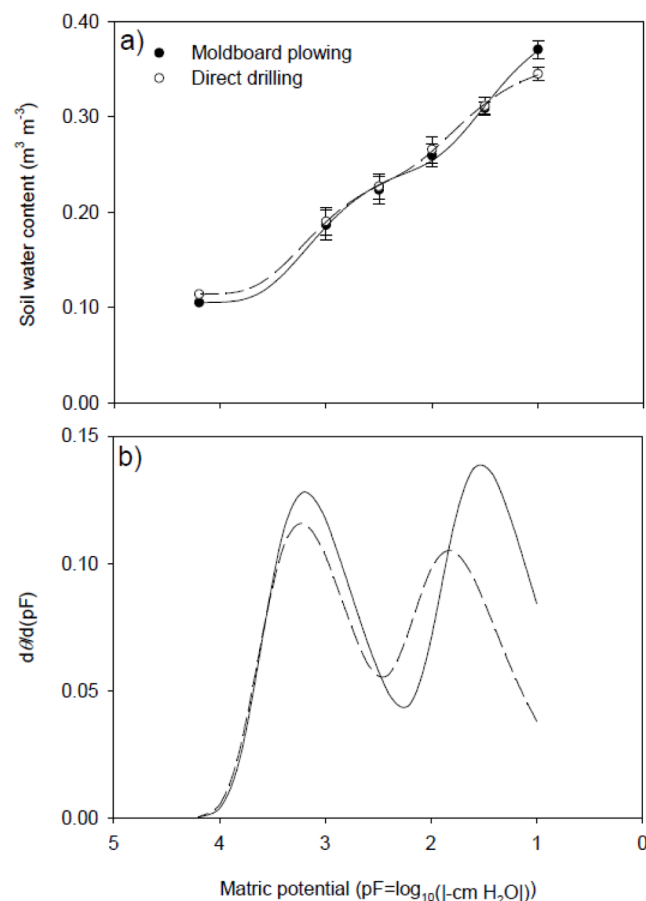


Fig. 4. (a) Measured volumetric water content for moldboard plowing to 0.20-m depth and direct drilling and fits of the double-exponential (Dex) model as a function of matric potential. The standard error of the mean is indicated ($n = 4$), except for $\text{pF} 4.2$ which is predicted based on Eq. [1] in Hansen (1976). (b) Pore-size distribution [$d/d(\text{pF})$] as a function of matric potential for the two treatments. Equation [5] was used to obtain the pore-size distributions.

model as revealed by lower mean AIC and RMSE values (Plowing: $\text{AIC}_{\text{vanG}} = -56.7$ and $\text{AIC}_{\text{Dex}} = -59.5$, $\text{RMSE}_{\text{vanG}} = 0.007 \text{ m}^3 \text{m}^{-3}$ and $\text{RMSE}_{\text{Dex}} = 0.003 \text{ m}^3 \text{m}^{-3}$; Direct drilling: $\text{AIC}_{\text{vanG}} = -58.0$ and $\text{AIC}_{\text{Dex}} = -69.5$, $\text{RMSE}_{\text{vanG}} = 0.005 \text{ m}^3 \text{m}^{-3}$ and $\text{RMSE}_{\text{Dex}} = 0.001 \text{ m}^3 \text{m}^{-3}$).

Structural void ratio (V_2) for moldboard plowing and direct drilling was 0.30 and 0.19, respectively. The dominating pore size of the structural peak (d_2) was 52 μm for direct drilling and 96 μm for moldboard plowing. Parameter estimates and goodness of fit measures for the eight individual plots can be seen in Table S4.

DISCUSSION

Model Fit

The Dex model provided a better fit to soil water retention data than the vanG model for the Jacobsen glacial till top- and subsoils. This was also true for contrasting treatments from Highfield and Flakkebjerg. Thus, the PSD for these soils was better described with a bi- rather than a uni-modal model. This is in accordance with Schjønning (1992) who found the vanG model unable to describe the double-peak pattern for PSD in glacial till soils. Further, Dexter et al. (2008), Berisso et al. (2012), and Zhou et al. (2017) found that the Dex model fitted their data better than the vanG model. Dexter et al. (2008) based their analysis on 42 Polish soils (26 topsoils, 6 samples from 0.30- to 0.35-m depth, and 10 subsoils) ranging from 2 to 25 g clay 100 g^{-1} , Berisso et al. (2012) focused on a sandy clay loam ranging from 19 to 27 g clay 100 g^{-1} , and Zhou et al. (2017) investigated clay loam paddy soil ($\sim 20 \text{ g clay } 100 \text{ g}^{-1}$). Our study included soils ranging in clay content from 2.0 to 30.0 g 100 g^{-1} minerals, substantiating that the Dex model is superior for soils $< 30 \text{ g clay } 100 \text{ g}^{-1}$ minerals. Thus, uni-modal models seem too simplistic for describing the size distribution of pores in most soils with less than 30 g clay 100 g^{-1} minerals. A range of papers comparing other bi-modal models with uni-modal models reached similar conclusions (e.g., Durner, 1994). However, the Dex model is less parameter demanding than other proposed bi-modal models, making it easier to apply to datasets with a restricted number of measurement points.

Neither of the models worked well for highly sorted soils ($\beta > 1$). This finding calls for alternative water retention models for soils with a narrow distribution of pore sizes. Dexter et al. (2008) mentioned the problems associated with the use of the Dex model for uniform sands. However, we emphasize that the Dex as well as the vanG model cannot describe highly sorted soils well regardless of the dominating particle size.

Pitfalls using Uni-Modal Models—Effects of Soil Organic Carbon and Tillage

For the Jacobsen data set, the vanG model provided a better description of subsoils than of topsoils (Fig. 1). This was ascribed to a more distinct bi-modal PSD for topsoil. The bi-modality re-

flects that topsoils were larger in SOC content and exposed to tillage (Fig. 5).

Tillage increases the amount of large structural pores. The effect of structure forming agents in subsoil are much reduced and limits the structural pore space at depth. Similarly, the systematic increase in RMSE with increasing SOC content for Highfield (Fig. 3) could be ascribed to a more pronounced bi-modal behavior (Fig. 2b), most clearly seen for soils from the grass treatment (Fig. 6a and 6b).

However, the vanG model overestimated the pore volume in the size range 10- to 30- μm (pF 2.5–2) and underestimated the pore volume at pF 3 and 1 markedly for both treatments, although more pronounced for the grass treatment (Fig. 6c and 6d).

Due to its absorptive capacity for water, the presence of SOC may increase the textural pore space especially in soils with <20 g clay 100 g⁻¹ (Rawls et al., 2003). The structural pore space is mainly affected by SOC through improved aggregation (Bronick and Lal, 2005). At Highfield, SOC affected both V_1 and V_2 positively whereas the estimate of the mean size of structural voids (d_2) decreased with increasing SOC content. For Flakkebjerg, where plowing was compared with direct drilling, both V_2 and d_2 increased with tillage intensity. The limited effect of tillage on V_2 when comparing grass and bare fallow at Highfield suggests that the effect of SOC on soil structure outweighed any effect of tillage. Interestingly, d_2 was larger for bare fallow than for grass suggesting that tillage introduces large pores as also observed for soils from Flakkebjerg. Zhou et al. (2017) found that SOC promoting management increased the structural porosity when comparing organic manure with unfertilized and inorganically fertilized treatments being in correspondence with our results from Highfield.

Structural Void Ratio

The structural void ratio (V_2) is an important parameter for soil functions such as air exchange and

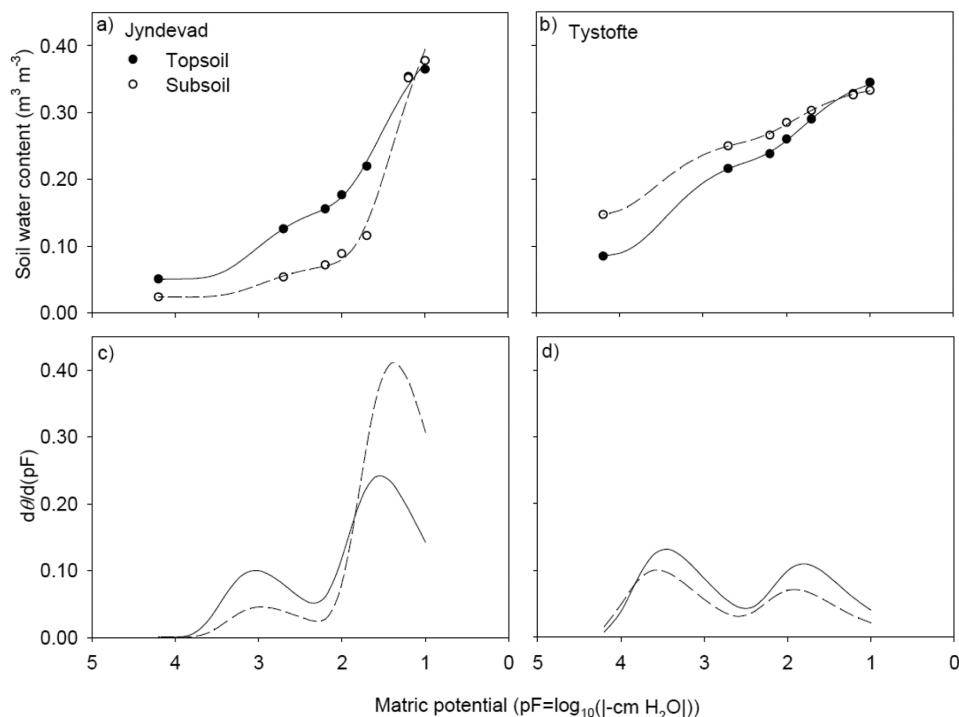


Fig. 5. (a, b) Measured volumetric water content for Jyndeved and Tystofte top- and subsoils of the Jacobsen data set and fits of the double-exponential (Dex) model as a function of matric potential. (c, d) Pore-size distribution [dq/d(pF)] as a function of matric potential for Jyndeved and Tystofte top- and subsoils. Equation [5] was used to obtain the pore-size distributions.

water availability for plants. We used V_2 rather than volumetric water content to allow for comparisons across soils with different bulk density. As discussed above SOC contents and tillage inten-

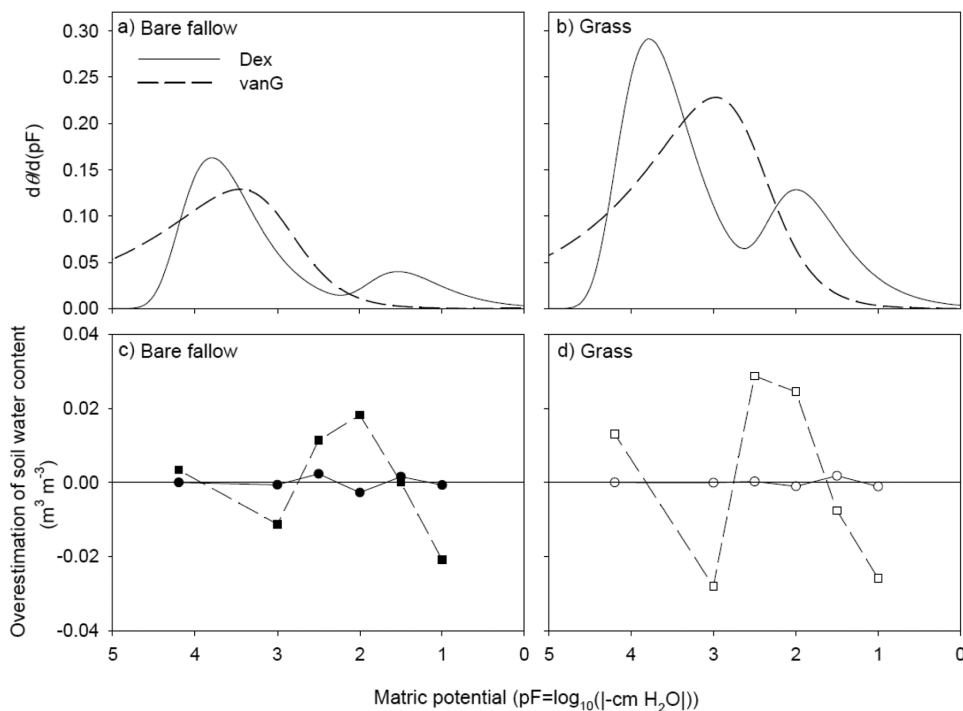


Fig. 6. Pore-size distribution [dq/d(pF)] as a function of matric potential for (a) the bare fallow and (b) the grass treatment at Highfield either obtained by differentiating the double-exponential (Dex) model (solid line) or the van Genuchten (vanG) model (dashed line). Equation [3] and [5] were used to obtain the pore-size distributions predicted by the vanG and the Dex models, respectively. Overestimation of soil water content (fitted-measured values) as a function of matric potential for (c) the bare fallow and (d) the grass treatment when fitted to the vanG model (square symbols) and the Dex model (circle symbols).

sity are important drivers for V_2 , but soil texture also affects V_2 through a positive relation to β (Eq. [9] to [12]) indicating that the more sorted soils have larger V_2 than graded soils. This agrees with Ehlers and Claupein (1994), who reported that graded coarse-textured soils readily compact to high densities. Similarly, Schjønning and Thomsen (2013) found that graded soils low in SOC showed a hard-setting behavior. A low V_2 may affect root growth negatively and reduce soil gas exchange. Therefore, SOC promoting management should target graded soil low in SOC.

CONCLUSIONS

Predicting soil water retention by the uni-modal vanG model is likely to introduce larger error in top—than in subsoils and errors is likely to be larger for well-structured soils than for structurally degraded soils. Ignoring management-derived effects (e.g., derived from changes in SOC and tillage) on PSD may compromise modeling of key soil processes and simulations based on pedotransfer functions. We found that the more flexible bi-modal Dex model provides an adequate description of the PSD and we discourage uncritical use of uni-modal models.

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SUPPLEMENTAL MATERIAL

Supplemental material is available with the online version of this article. The supplemental document presents parameter estimates of the van Genuchten and double-exponential model for the 16 Danish top- and subsoils of the Jacobsen data set, the 16 plots at Highfield, and the 8 plots at Flakkebjerg.

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Supplemental Material for SSSAJ manuscript “Soil Water Retention: Uni-modal Models of Pore Size Distribution Neglect Impacts of Soil Management”

Tables S1 to S4 follow on the next pages.

Table S1. Estimated parameters of the van Genuchten (vanG) and the double-exponential (Dex) model for the 16 soils at ~0.10 m depth of the Jacobsen (1989) data set listed as in Table 1.

	Parameters of the vanG model						Parameters of the Dex model						
Site	Θ_{sat}	Θ_{res}	α	n	$RMSE$	AIC	C	A_1	h_1	A_2	h_2	$RMSE$	AIC
	($\text{m}^3 \text{m}^{-3}$)	($\text{m}^3 \text{m}^{-3}$)	-	-	($\text{m}^3 \text{m}^{-3}$)	-	($\text{m}^3 \text{m}^{-3}$)	($\text{m}^3 \text{m}^{-3}$)	(hPa)	($\text{m}^3 \text{m}^{-3}$)	(hPa)	($\text{m}^3 \text{m}^{-3}$)	-
Hals	0.443	0.058	0.017	1.66	0.012	-55.4	0.009	0.155	15275	0.301	117	0.012	-52.3
Tylstrup	0.423	0.064	0.013	2.19	0.021	-48.4	0.000	0.126	15565	0.334	116	0.023	-42.7
Jyndevad	0.457	0.044	0.101	1.47	0.011	-54.6	0.051	0.119	1100	0.277	34	0.007	-60.0
Borris	0.410	0	0.022	1.39	0.015	-53.0	0.000	0.165	13296	0.254	120	0.012	-51.8
Hornum	0.387	0	0.026	1.28	0.010	-58.8	0.000	0.197	14970	0.194	112	0.006	-60.6
Travsted	0.437	0	0.021	1.26	0.012	-54.0	0.079	0.227	2057	0.145	67	0.002	-78.3
Foulum	0.369	0	0.009	1.30	0.007	-63.8	0.075	0.188	2752	0.114	139	0.003	-69.3
Ødum	0.383	0	0.006	1.32	0.007	-62.6	0.084	0.244	1792	0.066	102	0.002	-78.2
Årslev	0.378	0	0.027	1.22	0.011	-57.7	0.081	0.169	4474	0.135	75	0.002	-78.3
Roskilde	0.451	0	0.106	1.23	0.009	-57.3	0.072	0.161	2247	0.195	50	0.001	-91.2
Askov	0.390	0	0.022	1.23	0.010	-58.1	0.083	0.177	4983	0.138	86	0.001	-82.4
Rønhave	0.414	0	0.052	1.21	0.010	-58.5	0.085	0.195	1465	0.142	40	0.004	-65.8
Tystofte	0.355	0	0.036	1.21	0.010	-58.8	0.084	0.156	2876	0.121	60	0.002	-74.8
Ø. Ulslev	0.337	0	0.002	1.33	0.005	-68.7	0.108	0.214	3571	0.023	85	0.001	-84.5
Kalø	0.318	0	0.005	1.23	0.007	-63.9	0.111	0.179	2170	0.041	56	0.001	-86.4
Højer	0.459	0	0.037	1.19	0.013	-64.1	0.124	0.238	1269	0.118	25	0.006	-72.2

Table S2. Estimated parameters of the van Genuchten (vanG) and the double-exponential (Dex) model for the 16 soils at ~0.50 m depth of the Jacobsen (1989) data set listed as in Table 2.

	Parameters of the vanG model						Parameters of the Dex model						
Site	Θ_{sat}	Θ_{res}	α	n	$RMSE$	AIC	C	A_1	h_1	A_2	h_2	$RMSE$	AIC
	($\text{m}^3 \text{m}^{-3}$)	($\text{m}^3 \text{m}^{-3}$)	-	-	($\text{m}^3 \text{m}^{-3}$)	-	($\text{m}^3 \text{m}^{-3}$)	($\text{m}^3 \text{m}^{-3}$)	(hPa)	($\text{m}^3 \text{m}^{-3}$)	(hPa)	($\text{m}^3 \text{m}^{-3}$)	-
Hals	0.347	0.000	0.026	2.18	0.014	-52.0	0.000	0.040	8604	0.362	58	0.011	-52.9
Tylstrup	0.441	0.000	0.014	2.87	0.023	-44.8	0.000	0.043	23172	0.465	95	0.032	-38.3
Jyndevad	0.406	0.047	0.044	2.77	0.014	-51.9	0.024	0.054	930	0.483	24	0.014	-49.6
Borris	0.369	0.044	0.045	1.50	0.005	-67.3	0.054	0.095	1425	0.224	63	0.002	-79.0
Hornum	0.357	0.000	0.057	1.29	0.004	-71.6	0.048	0.135	1379	0.167	63	0.002	-79.7
Travsted	0.352	0.000	0.020	1.27	0.007	-62.1	0.067	0.155	3362	0.137	100	0.001	-84.0
Foulum	0.359	0.000	0.112	1.20	0.002	-78.1	0.075	0.148	914	0.123	36	0.003	-73.6
Ødum	0.311	0.000	0.018	1.19	0.006	-66.5	0.104	0.156	1477	0.062	47	0.002	-80.1
Årslev	0.357	0.000	0.025	1.15	0.007	-64.4	0.135	0.152	1949	0.078	53	0.001	-89.8
Roskilde	0.317	0.000	0.044	1.12	0.008	-60.9	0.132	0.117	3606	0.076	41	0.002	-78.0
Askov	0.436	0.000	0.144	1.15	0.008	-62.2	0.132	0.155	1843	0.132	38	0.002	-74.2
Rønhave	0.350	0.000	0.029	1.17	0.006	-66.2	0.119	0.166	1338	0.078	38	0.001	-87.4
Tystofte	0.339	0.000	0.029	1.13	0.006	-66.3	0.146	0.119	3673	0.078	75	0.002	-79.3
Ø. Ulslev	0.326	0.000	0.030	1.19	0.005	-68.8	0.094	0.152	1470	0.086	57	0.002	-80.9
Kalø	0.356	0.000	0.011	1.15	0.006	-66.8	0.164	0.146	2542	0.054	75	0.001	-84.3
Højer	0.478	0.000	0.007	1.38	0.015	-60.9	0.000	0.328	9611	0.164	142	0.002	-89.7

Table S3. Estimated parameters of the van Genuchten (vanG) and the double-exponential (Dex) model for the 16 plots from Highfield. Θ_{res} was changed to zero in all cases during the optimization procedure.

Treatment	Plot	Parameters of the vanG model					Parameters of the Dex model						
		Θ_{sat}	α	n	$RMSE$	AIC	C	A_1	h_1	A_2	h_2	$RMSE$	AIC
		($\text{m}^3 \text{m}^{-3}$)	-	-	($\text{m}^3 \text{m}^{-3}$)	-	($\text{m}^3 \text{m}^{-3}$)	($\text{m}^3 \text{m}^{-3}$)	(hPa)	($\text{m}^3 \text{m}^{-3}$)	(hPa)	($\text{m}^3 \text{m}^{-3}$)	-
Bare fallow	3	0.322	0.001	1.322	0.010	-49.2	0.113	0.192	6244	0.044	32	0.001	-71.4
Bare fallow	4	0.319	0.002	1.277	0.015	-44.7	0.109	0.182	5905	0.069	32	0.002	-66.7
Bare fallow	7	0.336	0.002	1.295	0.014	-45.1	0.109	0.196	5319	0.064	45	0.002	-64.1
Bare fallow	8	0.340	0.001	1.334	0.014	-45.0	0.107	0.209	5450	0.066	29	0.002	-66.4
Arable	14	0.393	0.001	1.456	0.012	-47.1	0.059	0.307	9415	0.048	93	0.002	-64.0
Arable	20	0.398	0.001	1.477	0.011	-47.6	0.054	0.320	9660	0.045	86	0.002	-65.6
Arable	24	0.424	0.001	1.415	0.012	-47.0	0.100	0.290	6407	0.053	116	0.002	-64.2
Arable	33	0.397	0.001	1.427	0.014	-45.1	0.060	0.301	9346	0.060	92	0.001	-68.2
Ley-arable	11/12	0.418	0.002	1.382	0.019	-41.4	0.098	0.276	4470	0.083	56	0.003	-58.1
Ley-arable	15/16	0.397	0.002	1.402	0.017	-42.8	0.095	0.269	4257	0.072	46	0.004	-57.7
Ley-arable	25/26	0.416	0.002	1.370	0.014	-45.5	0.112	0.267	4395	0.060	83	0.002	-62.4
Ley-arable	45/46	0.430	0.002	1.353	0.017	-42.8	0.112	0.272	4395	0.078	69	0.003	-59.9
Grass	10	0.528	0.003	1.359	0.028	-37.1	0.090	0.344	6055	0.137	89	0.002	-67.8
Grass	17	0.465	0.003	1.357	0.024	-39.0	0.085	0.303	6169	0.115	87	0.001	-68.4
Grass	26	0.481	0.002	1.466	0.021	-40.2	0.078	0.343	5371	0.094	100	0.000	-125.3
Grass	30	0.515	0.001	1.493	0.020	-40.9	0.066	0.389	7268	0.091	129	0.001	-74.6

Table S4. Estimated parameters of the van Genuchten (vanG) and the double-exponential (Dex) model for the eight plots from Flakkebjerg.

		Parameters of the vanG model						Parameters of the Dex model						
Treatment	Block	Θ_{sat}	Θ_{res}	α	n	$RMSE$	AIC	C	A_1	h_1	A_2	h_2	$RMSE$	AIC
		($\text{m}^3 \text{m}^{-3}$)	($\text{m}^3 \text{m}^{-3}$)	-	-	($\text{m}^3 \text{m}^{-3}$)	-	($\text{m}^3 \text{m}^{-3}$)	($\text{m}^3 \text{m}^{-3}$)	(hPa)	($\text{m}^3 \text{m}^{-3}$)	(hPa)	($\text{m}^3 \text{m}^{-3}$)	-
Direct drilling	1	0.348	0.000	0.026	1.172	0.007	-51.0	0.115	0.151	2089	0.093	55	0.002	-67.6
Direct drilling	2	0.354	0.090	0.049	1.371	0.003	-62.4	0.112	0.104	996	0.140	54	0.001	-80.4
Direct drilling	3	0.370	0.000	0.025	1.189	0.006	-53.2	0.111	0.152	2379	0.112	84	0.002	-65.0
Direct drilling	4	0.362	0.000	0.065	1.154	0.002	-65.3	0.122	0.137	1403	0.100	49	0.002	-65.0
Plowing	1	0.356	0.000	0.015	1.195	0.011	-46.0	0.111	0.180	2057	0.091	38	0.003	-61.3
Plowing	2	0.670	0.064	0.814	1.278	0.002	-65.6	0.107	0.131	981	0.223	30	0.003	-58.7
Plowing	3	0.447	0.002	0.214	1.188	0.001	-70.7	0.099	0.145	1193	0.161	38	0.003	-59.4
Plowing	4	0.420	0.000	0.271	1.146	0.012	-44.6	0.104	0.150	2252	0.151	24	0.003	-58.4

Paper 6

Soil degradation and recovery – Changes in organic matter fractions and structural stability

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Lars J. Munkholm

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Supplementary material is included

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Soil degradation and recovery – Changes in organic matter fractions and structural stability

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Soil management

ABSTRACT

The combination of concurrent soil degradation and restoration scenarios in a long-term experiment with contrasting treatments under steady-state conditions, similar soil texture and climate make the Highfield land-use change experiment at Rothamsted Research unique. We used soil from this experiment to quantify rates of change in organic matter (OM) fractions and soil structural stability (SSS) six years after the management changed. Soil degradation included the conversion of grassland to arable and bare fallow management, while soil restoration comprised introduction of grassland in arable and bare fallow soil. Soils were tested for clay dispersibility measured on two macro-aggregate sizes (DispClay 1–2 mm and DispClay 8–16 mm) and clay-SOM disintegration (DI, the ratio between clay particles retrieved without and with SOM removal). The SSS tests were related to soil organic carbon (SOC), permanganate oxidizable C (POXC) and hot water-extractable C (HWC). The decrease in SOC after termination of grassland was greater than the increase in SOC when introducing grassland. In contrast, it was faster to restore degraded soil than to degrade grassland soil with respect to SSS at macro-aggregate scale. The effect of management changes was more pronounced for 8–16 mm than 1–2 mm aggregates indicating a larger sensitivity towards tillage-induced breakdown of binding agents in larger aggregates. At microscale, SSS depended on SOC content regardless of management. Soil management affected macroscale structural stability beyond what is revealed from measuring changes in OM fractions, underlining the need to include both bonding and binding mechanisms in the interpretation of changes in SSS induced by management.

1. Introduction

Soil aggregation and soil structural stability (SSS) play a significant role in soil organic C (SOC) sequestration as stable aggregates protect soil organic matter (SOM) against decomposition (Six et al., 2004). Further, SSS links to loss of particle-associated pollutants (de Jonge et al., 2004), soil erosion (Le Bissonnais, 1996), soil cementation and seedbed quality (Kay and Munkholm, 2004).

The quantity and quality of SOM are main drivers in the formation and stabilization of soil structure in most soils with different SOM bonding and binding agents being important at different soil structural

levels (Abiven et al., 2009; Bronick and Lal, 2005; Tisdall and Oades, 1982). Bonding relates to gluing mineral particles together by decomposition products (e.g. polysaccharides), while binding refers to enmeshment of aggregates by plant roots and fungal hyphae (Tisdall and Oades, 1982). At micro-aggregate level (< 250 µm), flocculation of clay and SOM, cementation of dispersed clay, and bonding agents from plants, soil fauna and microbes add to SSS (Chenu, 1989; Haynes and Swift, 1990). At macro-aggregate level (> 250 µm), cross-linking and enmeshment by fungal hyphae and plant roots are crucial for SSS (Miller and Jastrow, 1990). Micro-aggregates are more stable than macro-aggregates, and less affected by management and SOM, while

Abbreviations: A, Arable; AG, Arable converted to grass; BF, Bare fallow; CEC, Cation exchange capacity; DI, Clay-SOM disintegration; DispClay 1–2 mm, Clay dispersibility of 1–2 mm aggregates; DispClay 8–16 mm, Clay dispersibility of 8–16 mm aggregates; E, Young's modulus; E_{sp} , Mass-specific rupture energy; G, Grass; GA, Grass converted to arable; GBF, Grass converted to bare fallow; HWC, Hot water-extractable carbon; POXC, Permanganate oxidizable carbon; SSA, Specific surface area; SSS, Soil structural stability; Y, Tensile strength

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stabilization of macro-aggregates is controlled mainly by management and SOM levels (Oades, 1984).

Permanganate oxidizable C (POXC) and hot water-extractable C (HWC) have been considered as labile SOM fractions more sensitive to management than total SOC (Culman et al., 2012; Ghani et al., 2003). Bongiorno et al. (2019) found that POXC can provide information about soil physical condition, and suggested POXC as a comprehensive soil quality indicator, while Fine et al. (2017) claimed POXC to be the best single predictor for soil health.

Only a few studies have related changes in land use to changes in SOM fractions and SSS (e.g., Perfect et al., 1990). The quantification of rates of change in SSS and knowledge of links between SSS and SOM fractions is beneficial for restoring degraded soil and identifying sustainable management of soils with adequate SSS. One outstanding issue is whether degradation and restoration occurs at a similar rate in relation to both SOM fractions and SSS.

The objective of this study was to quantify the effects of different SOM fractions on SSS in soil subjected to degradation and restoration managements. Permanent grassland was used as reference treatment. Changes in SOM content due to management affect SSS differently at different spatial scales. At $< 20 \mu\text{m}$ scale, extremely stable SOM-mineral interactions are responsible for SSS. Accordingly, we applied an extreme clay-SOM disintegration test to reveal differences at micro-scale. We hypothesize that SSS at microscale change more slowly than SOM content in both degradation and restoration managements. Further, we applied a clay dispersibility test with low degree of disturbance to 1–2 mm and 8–16 mm rewetted macro-aggregates to investigate if the rate of change in SSS was scale-dependent. We hypothesize that SSS at macroscale changes more rapidly than SOM contents in soil under both degradation and restoration managements.

Soils were from the Highfield land-use change experiment at Rothamsted Research (Highfield-LUCE), sampled six years after changes in managements. This ensured that soil degradation and restoration management were initiated simultaneously on a site with a well-known history, with long-term treatments under steady-state conditions, and without confounding effects of differences in soil type, soil texture and climate. The changes in management were profound making this experiment ideal for investigating shorter-term effects on SOM fractions and SSS.

2. Materials and methods

2.1. The Highfield land-use change experiment and treatments

The Highfield ley-arable experiment at Rothamsted Research, Harpenden, UK ($51^{\circ}80'N$, $00^{\circ}36'W$) was initiated in 1949 (Johnston, 1972). Its purpose was to look at the effects of different cropping systems on yield and SOM. Highfield had been in permanent grass since 1838; on this site some plots stayed in permanent grass, others went into continuous arable cropping and some alternated between leys and arable. It has taken about 60 years for soils to reach a steady-state condition following changes in the management systems (Hirsch et al., 2017; Rothamsted Research, 2018).

In 2008, $10 \times 6 \text{ m}$ areas within the existing arable (A) and grass (G) plots on the Highfield ley-arable site were converted to bare fallow, arable or grass, while other areas remained unchanged. Likewise, in 2008, $10 \times 6 \text{ m}$ areas within the existing bare fallow (BF) plots on the Highfield bare fallow and Geescroft bare fallow sites (located adjacent to the Highfield ley-arable experiment) were converted to arable or grass. The long-term BF treatment was established in 1959. For this study, we selected three conversion treatments in the ley-arable experiment: Arable converted to grass (AG), grass converted to bare fallow (GBF) and grass converted to arable (GA). We also selected the conversion of bare fallow to grass (BFG) in the Highfield bare fallow and Geescroft bare fallow sites (Fig. 1).

The AG treatment is sown with a mixture of meadow fescue (*Festuca pratensis* L.), timothy-grass (*Phleum pratense* L.) and white clover (*Trifolium repens* L.). The grass/clover ley receives no N fertilizer and the biomass is cut and removed in early summer. The small amount of regrowth is topped in early autumn and left on the plots. The GBF treatment is plowed or rotavated two to four times a year to keep any plant regrowth to a minimum. The GA treatment was sown with winter cereals (winter wheat, *Triticum aestivum* L. and winter oats, *Avena sativa* L. in rotation). The winter cereals are fertilized with $220 \text{ kg N ha}^{-1} \text{ y}^{-1}$ and straw is removed. The conversion to grass in BFG was as described for AG. The plowing depth in A, GBF, GA and BF was 23 cm. The A, AG, G, GA and BFG plots were fertilized with 65 kg P ha^{-1} and 250 kg K ha^{-1} every three years.

The A, AG, G, GA and GBF treatments were part of a randomized block design with four field replicates, whereas the four BF and three BFG plots were located adjacent to the experiment (Fig. 1). The soil is a silt loam soil belonging to the Batcombe series and is classified as an Aquic Paludalf (USDA Soil Taxonomy System) and Chromic Luvisol (WRB) (Watts and Dexter, 1997). For a more detailed description of the long-term treatments, see Jensen et al. (2019). Basic soil characteristics for BF, A and G treatments have been reported previously along with SOC, POXC, HWC, clay dispersibility of 1–2 mm aggregates and clay-SOM disintegration (Jensen et al., 2019). Hirsch et al. (2017) and Todman et al. (2018) focused on biological aspects in the Highfield-LUCE.

2.2. Soil sampling

Soil was sampled in March 2015 six years after the initiation of the Highfield-LUCE. Sampling was done at field capacity corresponding approximately to a soil water potential of -100 hPa . Soil blocks ($\sim 2.75 \text{ l}$) were carefully retrieved from the 6–15-cm soil layer by use of a spade. Three soil blocks were sampled from randomly chosen sites within each experimental plot. The soil was kept in sturdy containers to prevent soil disturbance during transport and stored in a field-moist condition at 2°C until required for analysis. Soil from the blocks was spread out in steel trays at room temperature, carefully fragmented by hand in several sittings along natural planes of weakness, and finally left to air-dry.

2.3. Basic chemical and physical analysis

The texture of air-dried bulk soil (crushed and passed through a 2-mm sieve) was determined by the hydrometer method for clay ($< 2 \mu\text{m}$) and silt ($2\text{--}20 \mu\text{m}$) content and the sieve method for mineral particles $> 63 \mu\text{m}$ (Gee and Or, 2002). The soil was tested for carbonates by adding a few droplets of 10% HCl, but none was found. Soil organic matter was removed with H_2O_2 before estimation of clay and silt as described in Jensen et al. (2017). The SOC content was determined on ball-milled subsamples using high-temperature dry combustion (Thermo Flash 2000 NC Soil Analyzer, Thermo Fisher Scientific, Waltham Massachusetts, USA). Specific surface area (SSA) was determined by the ethylene glycol monoethyl ether method (Petersen et al., 1996), and cation exchange capacity (CEC) was determined after Kalra and Maynard (1991). Soil pH was determined in 0.01 M calcium chloride (CaCl_2) solution (1:2.5, w/w). The properties were determined at plot level.

2.4. Soil organic matter fractions

Permanganate oxidizable carbon (POXC) was determined at plot level on air-dry 2-mm sieved soil following Culman et al. (2012) and as detailed in Jensen et al. (2019). In short, soil was shaken in a potassium permanganate (KMnO_4) solution and allowed to settle after which the supernatant was transferred, absorbance measured and finally converted to a POXC quantity.

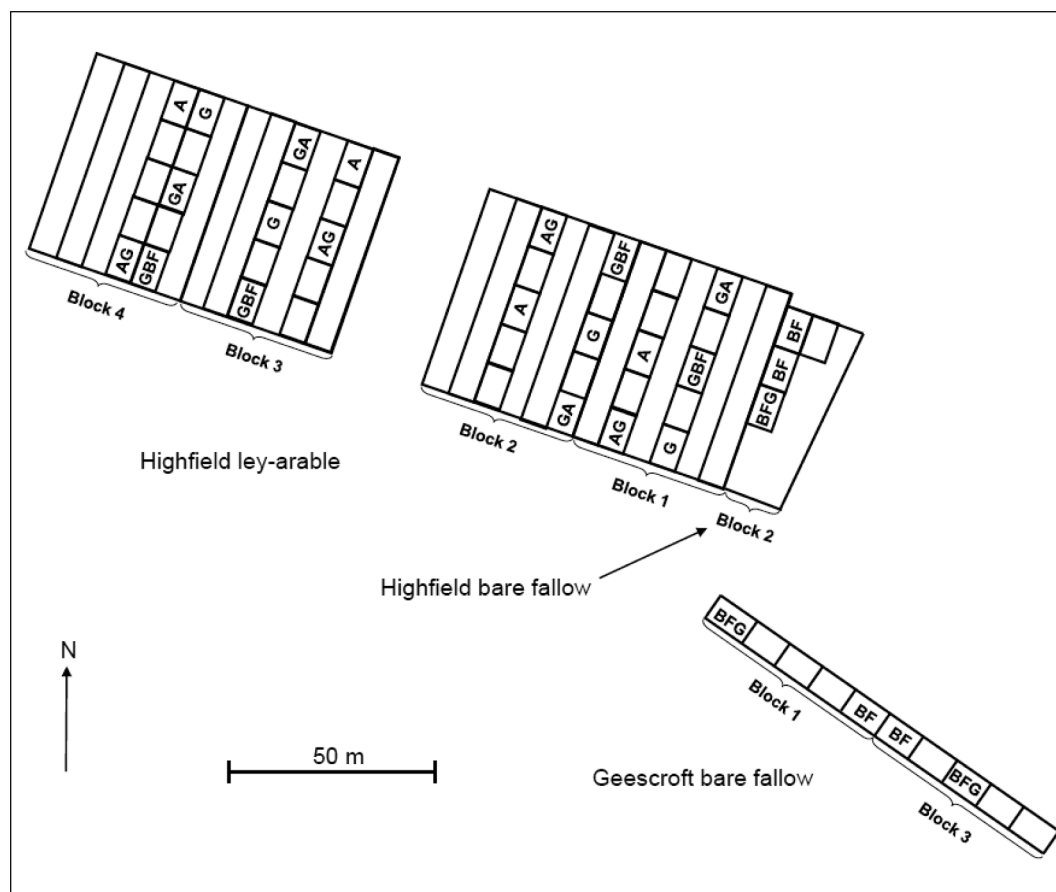


Fig. 1. Distribution of plots in Highfield showing the arable (A), arable converted to grass (AG), grass converted to bare fallow (GBF), grass converted to arable (GA) and grass (G) treatments in blocks 1–4 of the ley-arable experiment, and the bare fallow (BF) and bare fallow converted to grass (BFG) treatments in blocks 1–3 of the adjacent bare fallow experiments.

Hot water-extractable carbon (HWC) was determined at plot level on air-dry 2-mm sieved soil following Ghani et al. (2003) and as detailed in Jensen et al. (2019). Briefly, soil was shaken in water at 20 °C, centrifuged, and the supernatant decanted. The soil was re-suspended in water, shaken for 16 h at 200 rpm and 80 °C, centrifuged, and the supernatant was filtered after which HWC was determined.

2.5. Soil structural stability and strength

Clay dispersibility was determined at plot level on 1–2 mm aggregates (DispClay 1–2 mm) isolated from the air-dry 2-mm sieved soil, and on 8–16 mm aggregates (DispClay 8–16 mm) isolated by sieving the air-dry bulk soil. The method is described in detail in Jensen et al. (2019). In short, the aggregates were adjusted to a matric water potential of -100 hPa on tension tables, shaken in artificial rainwater (0.012 mM CaCl_2 , 0.150 mM MgCl_2 , and 0.121 mM NaCl ; pH 7.82; EC 2.24×10^{-3} S m^{-1}), and the suspension left to stand after which ≤ 2 μm particles was siphoned off. The weight of dispersed clay was determined after oven-drying (105 °C for 24 h). The sediment was corrected for particles > 250 μm for DispClay 1–2 mm and for particles > 2 mm for DispClay 8–16 mm, both isolated by chemical dispersion. This was done to relate clay dispersibility to soil free of particles > 250 μm and stone-free soil for DispClay 1–2 mm and DispClay 8–16 mm, respectively.

Clay-SOM disintegration (DI) and soil aggregate strength were estimated on bulked soil for each plot as outlined as follows. Particles ≤ 2 μm estimated with no H_2O_2 -removal of SOM prior to soil dispersion was measured as described by Jensen et al. (2017), and DI was calculated as the ratio between clay particles retrieved without and

with SOM removal. Soil with DI values < 1 kg kg^{-1} can be regarded as being extremely stable since they have resisted disintegration after end-over-end shaking for 18 h in sodium pyrophosphate.

Aggregate strength was determined on 8–16 mm aggregates isolated from the air-dry bulk soil as detailed in Obour et al. (2018). Briefly, tensile strength (Y) was tested for 15 randomly selected aggregates per plot by subjecting them to an indirect tension test comprising crushing between two parallel plates. The point of failure for each aggregate was automatically detected when a continuous crack or sudden drop in force (40% of the maximum load) was read. After the test, the crushed aggregates were oven-dried at 105 °C for 24 h to determine their gravimetric water content. The calculation of tensile strength (Y), mass-specific rupture energy (E_{sp}) and Young's modulus (E) was as described in Obour et al. (2018) except for the calculation of the effective diameter used in the calculation of Y , where the mean dry mass of all aggregates instead of the mean dry mass of aggregates at plot level was used. Further, E was determined by manually selecting two points on the stress-strain curve for each aggregate.

2.6. Calculations and statistics

The soil properties measured in this study are expressed as an oven-dry weight mass proportion (105 °C for 24 h) of the mineral fraction. The properties include particle size fractions, SOC, POXC, HWC, SSA, CEC, DispClay 1–2 mm and DispClay 8–16 mm.

For the statistical analysis, the R-project software package Version 3.4.0 (R Foundation for Statistical Computing) was used. Treatment effects were analyzed with a linear mixed model including block as a random effect when comparing A, AG, G, GA and GBF. The criterion

used for statistical significance of treatment effects was $P < 0.05$. When the treatment effect was significant, further analyses were made to isolate differences between treatments (pairwise comparisons) using the general linear hypotheses (*glht*) function implemented in the R *multcomp* package and the Kenward-Roger method to calculate degrees of freedom (Kenward and Roger, 2009). Treatment effects for the comparison of BF and BFG were analyzed separately since the BF and BFG treatments were located at one end of the experiment in its own design (Fig. 1). Treatment differences for the comparison of the BF and BFG treatments and the GBF and G treatments were based on a pairwise *t*-test, acknowledging that this is a less robust test, and that the treatment differences could be due to soil variation since the BF and BFG treatments are not a part of the original ley-arable experiment. Logarithmic (ln) transformation was performed on Y , E_{sp} and E to yield normality. For models with more than one predictor, the adjusted coefficient of determination (R^2) is reported. Akaike's information criterion (AIC) was used to compare models with different numbers of parameters (Akaike, 1973).

3. Results

3.1. Basic soil characteristics

Generally, contents of clay, silt and sand did not differ significantly when comparing the converted treatments with its reference (Table 1), allowing the effect of changes in managements to be examined without confounding effects related to soil texture. SSA, the amount of exchangeable Ca^{2+} and pH were significantly larger for AG than for A treatment.

3.2. Soil organic matter fractions

Concentrations of SOC and HWC were significantly lower for GBF and GA than for G (Fig. 2a and c). Similarly, POXC was lower for GBF and GA than for G, but not significantly and the decreases were less as compared to the changes in SOC and HWC (Fig. 2b). Concentrations of SOC, POXC and HWC were approx. 50% larger for BFG compared to BF, and marginally significant (SOC: $P = 0.053$, POXC: $P = 0.055$, HWC: $P = 0.063$), whereas the concentrations were not significantly different for AG compared to A (Fig. 3). POXC and HWC accounted for around 2.4% and 4.6% of total SOC, respectively. Correlations of POXC and HWC to SOC including data from all treatments at plot level can be seen in Fig. S1 in Supplementary material. Both POXC (Fig. S1a; broken stick regression, $R^2 = 0.96$) and HWC (Fig. S1b; broken stick regression, $R^2 = 0.98$) correlated well to SOC.

Table 1

Soil characteristics. In case of statistical significance ($P < 0.05$) letters within rows denote significance for the comparison of G with GA and GBF, BF with BFG, and A with AG. For treatment abbreviations, see Fig. 1.

	G	GA	GBF	BF	BFG	A	AG
Texture ¹							
Clay < 2 μ m	0.261	0.255	0.254	0.270	0.244	0.264	0.266
Silt 2–20 μ m	0.272 ^b	0.255 ^a	0.256 ^a	0.249	0.267	0.263	0.253
Silt 20–63 μ m	0.319	0.335	0.337	0.335	0.338	0.318	0.332
Sand 63–2000 μ m	0.148	0.155	0.153	0.146	0.151	0.155	0.149
Specific surface area ($m^2 g^{-1}$ minerals) ²	78.4	77.4	75.8	59.1	63.3	67.9 ^a	74.4 ^b
Exchangeable cations and CEC							
Na ⁺ ($mmol_c kg^{-1}$ minerals)	0.7 ^b	0.4 ^a	0.5 ^a	0.4	0.4	0.5 ^a	0.6 ^b
K ⁺ ($mmol_c kg^{-1}$ minerals)	5.8	6.9	5.4	3.3	4.5	6.3	5.8
Ca ²⁺ ($mmol_c kg^{-1}$ minerals)	144.4	134.2	142.4	95.0	88.7	102.5 ^a	125.5 ^b
Mg ²⁺ ($mmol_c kg^{-1}$ minerals)	4.6	3.9	4.4	5.4	4.8	4.0	3.9
Sum of bases ($mmol_c kg^{-1}$ minerals)	155.6	145.3	152.6	104.1	98.5	113.3	135.8
CEC ($mmol_c kg^{-1}$ minerals)	209.9	246.6	229.6	140.5	134.4	173.8	186.1
Base saturation (%)	74.4	60.8	67.2	75.5	73.1	65.5	74.6
pH (CaCl ₂)	5.4	5.2	5.4	5.9	5.6	5.1 ^a	5.5 ^b

¹ kg kg⁻¹ of mineral fraction and based on oven-dry weight.

² Clay is included as a co-variable as it is significant and makes the treatment effect significant.

3.3. Soil structural stability and strength

There was no significant differences in the amount of dispersible clay of 1–2 mm aggregates (DispClay 1–2 mm) among G, GA and GBF, whereas DispClay 8–16 mm increased significantly in the order $G < GA < GBF$ (Fig. 4a and b). Clay-SOM disintegration (DI) was significantly lower for the G treatment compared to GA and GBF (Fig. 4c). Tensile strength (Y) and Young's modulus (E) of 8–16 mm aggregates did not differ significantly for G, GA and GBF, whereas rupture energy (E_{sp}) was significantly lower for GA and GBF than for G (Table S1 in Supplementary material).

DispClay 1–2 mm was significantly lower for BFG than for BF (Fig. 5a). A similar marginal significant lowering in DispClay 8–16 mm was seen ($P = 0.072$, Fig. 5b). DispClay 1–2 mm and DispClay 8–16 mm were significantly lower for AG than for A, and the relative reduction was approx. 30% (Fig. 5d and e). DI, Y , E and E_{sp} did not change significantly for BFG compared to BF and AG compared to A (Fig. 5c and f, and Table S1).

Overall, soil structural stability increased with an increase in SOM fractions (Fig. 6). There was a small range in SOC, POXC and HWC within each treatment for BF and BFG and for A and AG, whereas the range in SOM fractions within G, GA and GBF were larger. Accordingly, linear models were employed to describe the correlations of SOC, POXC and HWC to DispClay 1–2 mm, DispClay 8–16 mm and DI for GBF, GA and G treatments only (Fig. 7). The SOM fractions were normalized to identical soil clay contents since this differed within treatments and is known to affect the SSS measures. For all three SSS measures, the coefficient of determination (R^2) was highest when related to SOC/Clay (Table 2). However, there was a significant interaction between treatment and SOC/Clay as well as between treatment and HWC/Clay for DispClay 8–16 mm (Fig. 7d and f). Including the interaction term when describing the relation between SOC/Clay or HWC/Clay and DispClay 8–16 mm increased R^2 from 0.60 to 0.89 and from 0.50 to 0.88, respectively (data not shown). Thus, the best model for describing DispClay 8–16 mm included the interaction term between treatment and SOC/Clay, explained 29%-units more of the variation than the model including SOC/Clay only, and had a lower AIC-value (8.4 vs 23.0). The relationship between SOC/Clay and DispClay 8–16 mm was not significant for G and GA, whereas it was almost significant for GBF ($P = 0.071$). In addition, the slope for GBF was significantly larger than for GA, and slightly larger than for G ($P = 0.09$). The slopes for G, GA and GBF when relating SOC/Clay to DispClay 1–2 mm and DI were not significantly different (no interaction).

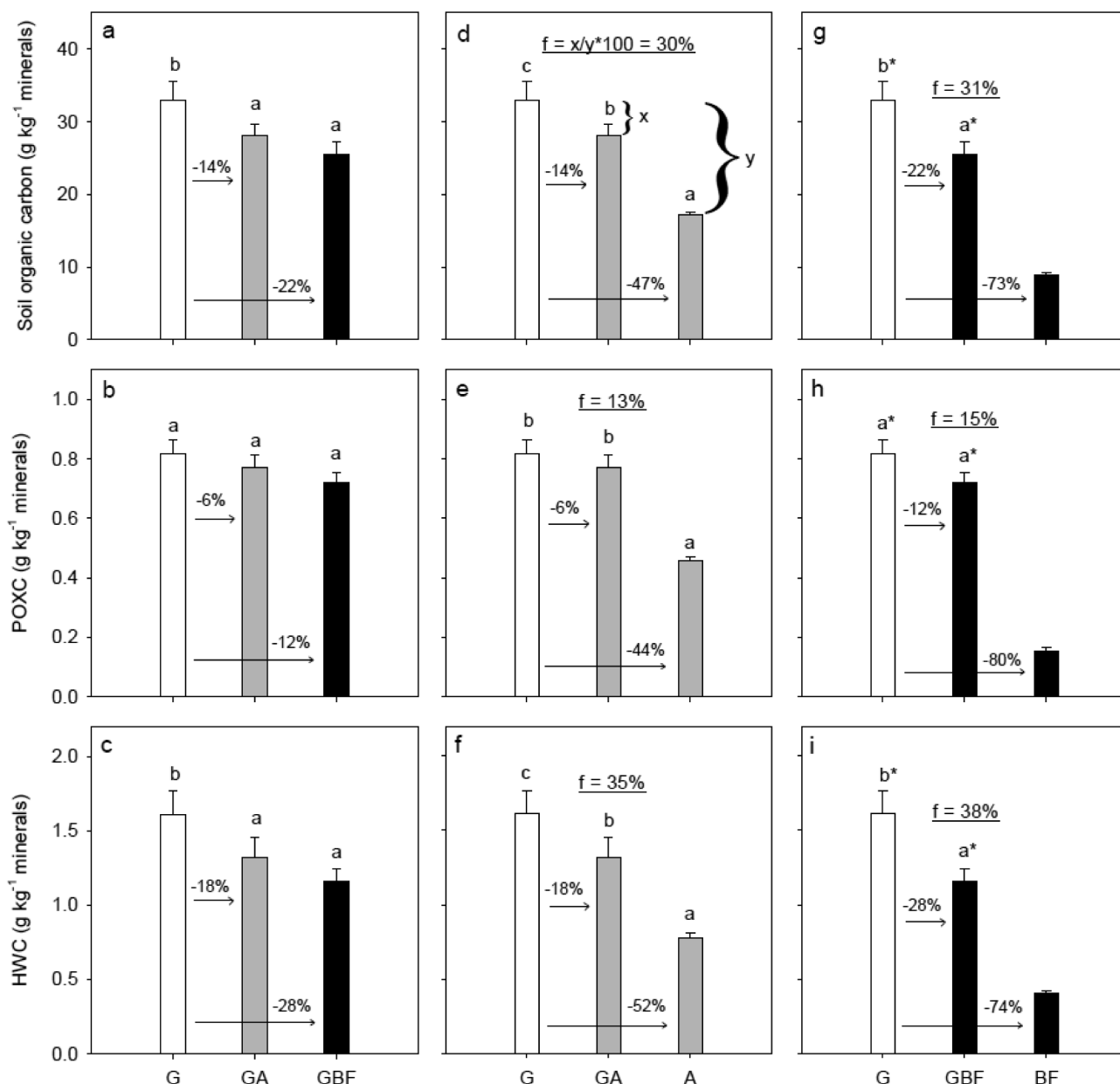


Fig. 2. Degradation scenarios: Management system effects on soil organic carbon, permanganate oxidizable carbon (POXC), and hot water-extractable carbon (HWC). White, gray and black bar fills highlight treatments grass, arable and bare fallow, respectively, at time of sampling. Letters denote statistical significance at $P < 0.05$. An asterisk (*) indicates if BF is significantly different from GBF and G based on a pairwise t -test. The numbers above the arrows denote relative differences. The underlined number in the middle part of the figures denotes the decrease after six years in relation to the long-term decrease, and an example of the calculation is shown in Fig. d. For treatment abbreviations, see Fig. 1.

4. Discussion

4.1. Soil degradation after termination of grassland

Conversion of grassland to arable management (GA) introduces a change from a system with no tillage and permanent plant cover to a system with annual tillage and annual cereals, whereas the conversion of grassland to bare fallow (GBF) introduces a change to a system with intensive tillage and without plants. Consequently, the observed changes are a result of the combined effect of changed OM input and tillage. In this section, we address the effects of these soil degradation mechanisms in terms of changes in OM fractions (Fig. 2) and SSS (Fig. 4).

When grassland was terminated, the SOC content decreased by on average 14% and 22% for GA and GBF, respectively (Fig. 2a), due to

reduced OM inputs and increased tillage intensity. Tillage is known to promote decomposition of SOM as it disrupts micro- and macro-aggregates, releasing entrapped OM, and increase soil aeration (Six et al., 1999). Besides the reduction in OM inputs, the quality of OM may also change in GA and GBF compared to G, and potentially contribute to the decline. The results accord with Attard et al. (2016), who found a rapid reduction in SOC after converting grassland to cropland. Changes in HWC (Fig. 2c) were only slightly higher than changes observed for SOC. This suggests that SOC and HWC show similar sensitivity to changes. This was also true for POXC although the differences were not statistically significant (Fig. 2b). The similar sensitivity to management changes for POXC, HWC and SOC found in this study contrasts with that of Bongiorno et al. (2019), who found that POXC was the most sensitive to changes in tillage and OM input in an analysis based on ten European long-term field experiments. Haynes and Swift (1990) found that the

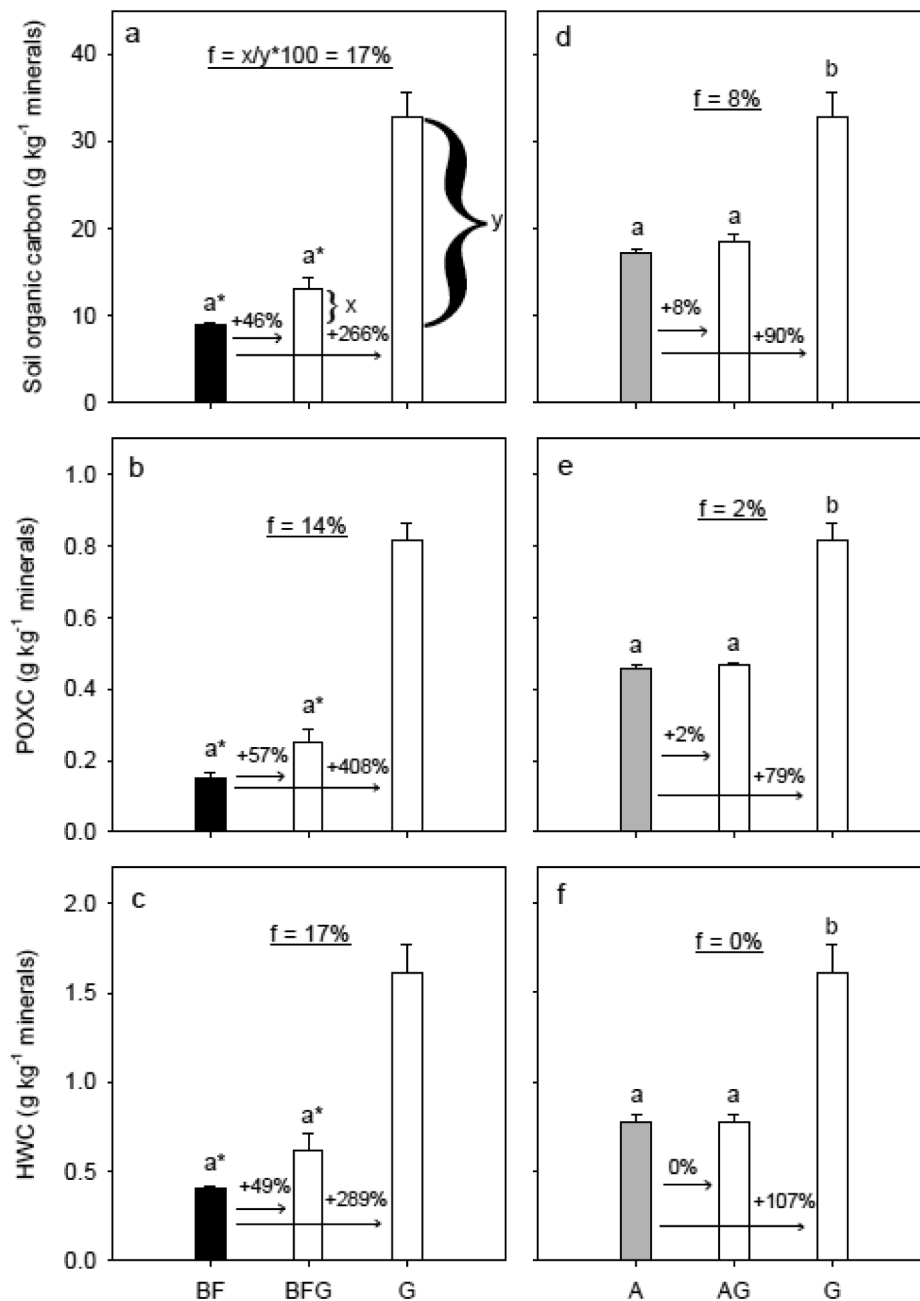


Fig. 3. Restoration scenarios: Management system effects on soil organic carbon, permanganate oxidizable carbon (POXC), and hot water-extractable carbon (HWC). White, gray and black bar fills highlight treatments grass, arable and bare fallow, respectively, at time of sampling. Letters denote statistical significance at $P < 0.05$. An asterisk (*) indicates if G is significantly different from BF and BFG based on a pairwise *t*-test. The numbers above the arrows denote relative differences. The underlined number in the middle part of the figures denotes the increase after six years in relation to the long-term increase, and an example of the calculation is shown in Fig. 1. For treatment abbreviations, see Fig. 1.

hot water-extractable carbohydrate-C was more sensitive to short-term changes in cropping histories than SOC suggesting that it is more relevant to focus on the carbohydrate-C in the hot water extract rather than C.

Changes in SSS due to changes in management depended on the size of macro-aggregates, supporting the theory that different stabilization mechanisms were important for stability of differently sized aggregates (Tisdall and Oades, 1982). The rapid increase in DispClay 8–16 mm and decrease in E_{sp} of similar-sized aggregates retrieved from GA and GBF may relate to destruction and loss of roots and fungal hyphae, these being important for stability at larger scale and both sensitive to tillage (Tisdall and Oades, 1982). Compared to G, DispClay 8–16 mm increased by an average of 38% and 76% for GA and GBF (Fig. 4b), respectively. This is a more dramatic change than the changes observed for SOC, HWC and POXC, indicating that the tillage-induced breakdown of binding agents may have overruled effects of OM fractions on the stability of larger aggregates. Similarly, Sparling et al. (1992) and

Grandy and Robertson (2006) found that macro-aggregate (> 2 mm) stability changed more rapidly than SOC content following conversion of permanent pasture to continuous maize cropping and tilling uncultivated soil, respectively. DispClay 8–16 mm was nearly constant across the four G and GA plots despite a range in the SOC/Clay ratio (Fig. 7d), indicating that management derived drivers such as root density were more important than SOC contents. In contrast, DispClay 8–16 mm increased with decreasing SOC for GBF. This may be due in part to additional tillage energy (Watts and Dexter, 1997) and to the loss of living roots and associated exudates under this management.

In contrast to DispClay 8–16 mm, DispClay 1–2 mm was similar for G, GA and GBF (Fig. 4a). This is surprising since the aggregate hierarchy concept (Oades and Waters, 1991) suggests similar response for > 250 μ m aggregates. The greater stability for smaller sized aggregates may relate to tillage-induced breakdown of larger sized aggregates in the former grassland soil followed by decomposition of OM released from aggregates as well as above- and belowground plant

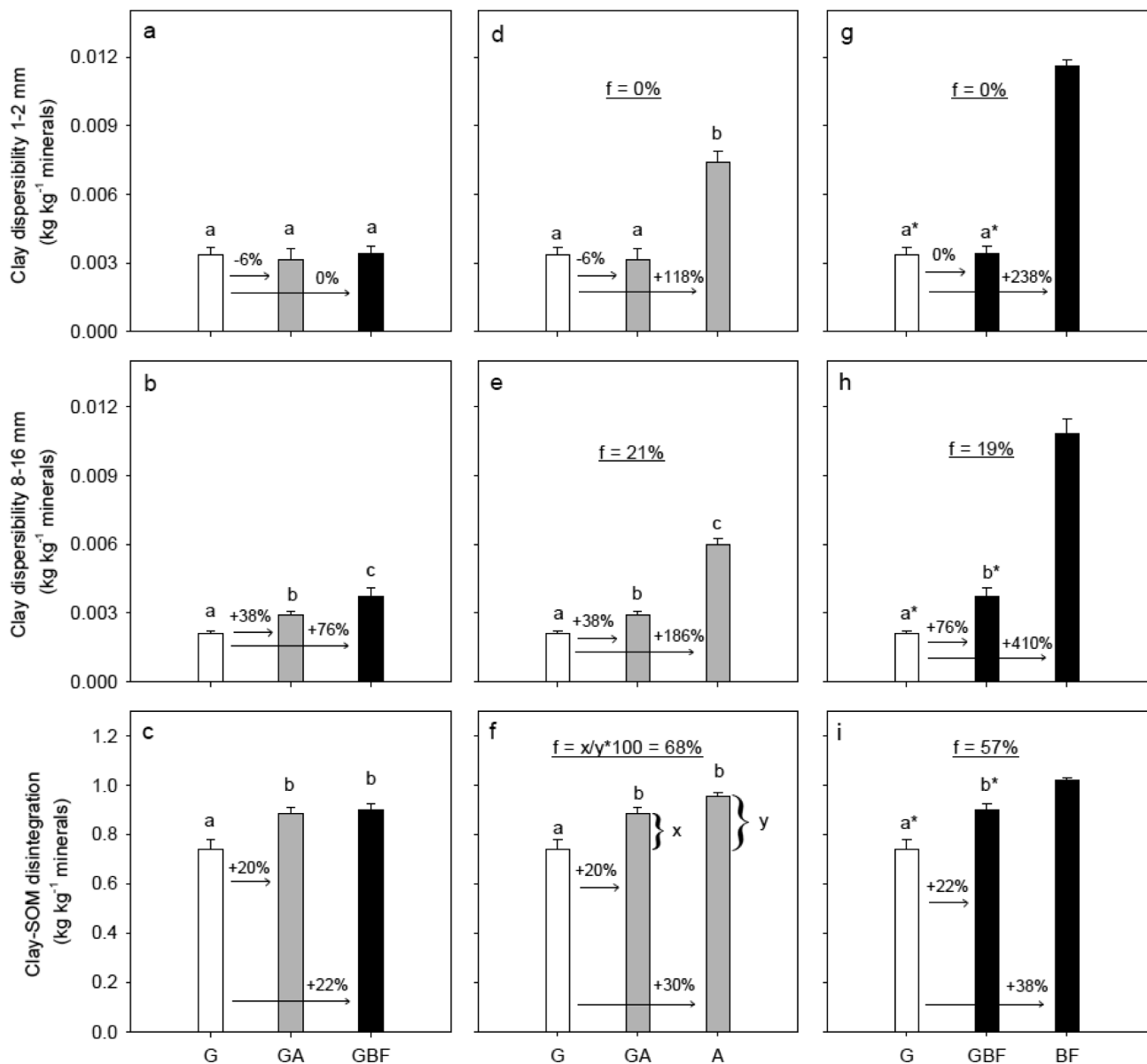


Fig. 4. Degradation scenarios: Management system effects on clay dispersibility of 1–2 mm aggregates rewetted to -100 hPa, clay dispersibility of 8–16 mm aggregates rewetted to -100 hPa, and clay-SOM disintegration (the ratio between clay particles retrieved without SOM removal and with removal). White, gray and black bar fills highlight treatments grass, arable and bare fallow, respectively, at time of sampling. Letters denote statistical significance at $P < 0.05$. An asterisk (*) indicates if BF is significantly different from GBF and G based on a pairwise t -test. The numbers above the arrows denote relative differences. The underlined number in the middle part of the figures denotes the difference after six years in relation to the long-term difference, and an example of the calculation is shown in Fig. f. For treatment abbreviations, see Fig. 1.

residues (Six et al., 1999). Formation of stable < 2 mm aggregates facilitated by microbial decomposition products may thus explain the delay in deterioration in DispClay 1–2 mm. Likewise, Sparling et al. (1992) found that > 2 mm aggregates were more sensitive to grassland termination than 1–2 mm aggregates. DispClay 1–2 mm increased with decreasing SOC/Clay for both G, GA and GBF (Table 2) indicating that roots were less important for SSS in 1–2 mm aggregates than in 8–16 mm aggregates. Soil structural stability at microscale measured as DI increased with approx. 20% for both GA and GBF indicating the partial breakdown in GA and GBF of extremely stable organo-mineral associations that in treatment G ‘survived’ the extreme disturbance (Fig. 4c). SOC/Clay explained more of the variability in DI than both POXC/Clay and HWC/Clay (Table 2), which suggests that changes in DI were not strongly related to the supposed labile compounds. The comparable slopes for the relationship between SOC/Clay and DI for different treatments (Table 2) suggest that stability at microscale relates

to SOC concentrations.

All structural stability measures correlated linearly to POXC/Clay irrespective of treatment (Fig. 7b, e and h). However, SOC/Clay as a sole predictor for all three SSS measures explained more of the variation than POXC/Clay and HWC/Clay as sole predictors. This is add odds with the conclusions of Bongiorno et al. (2019).

For DispClay 8–16 mm we found individual correlations to SOC/Clay and HWC/Clay within each management (Fig. 7d and f). This indicates that other drivers than SOC and HWC, respectively, are in play at this scale. Above, we hypothesize that the additional driver in play in our observations are roots and hyphae acting as stabilizing agents in 8–16 mm aggregates in the GA and G treatments. Our data thus point to the need for focusing on two mechanisms in SSS: 1) binding by roots and hyphae, and 2) bonding supported by microbial activity and residues (Oades, 1984).

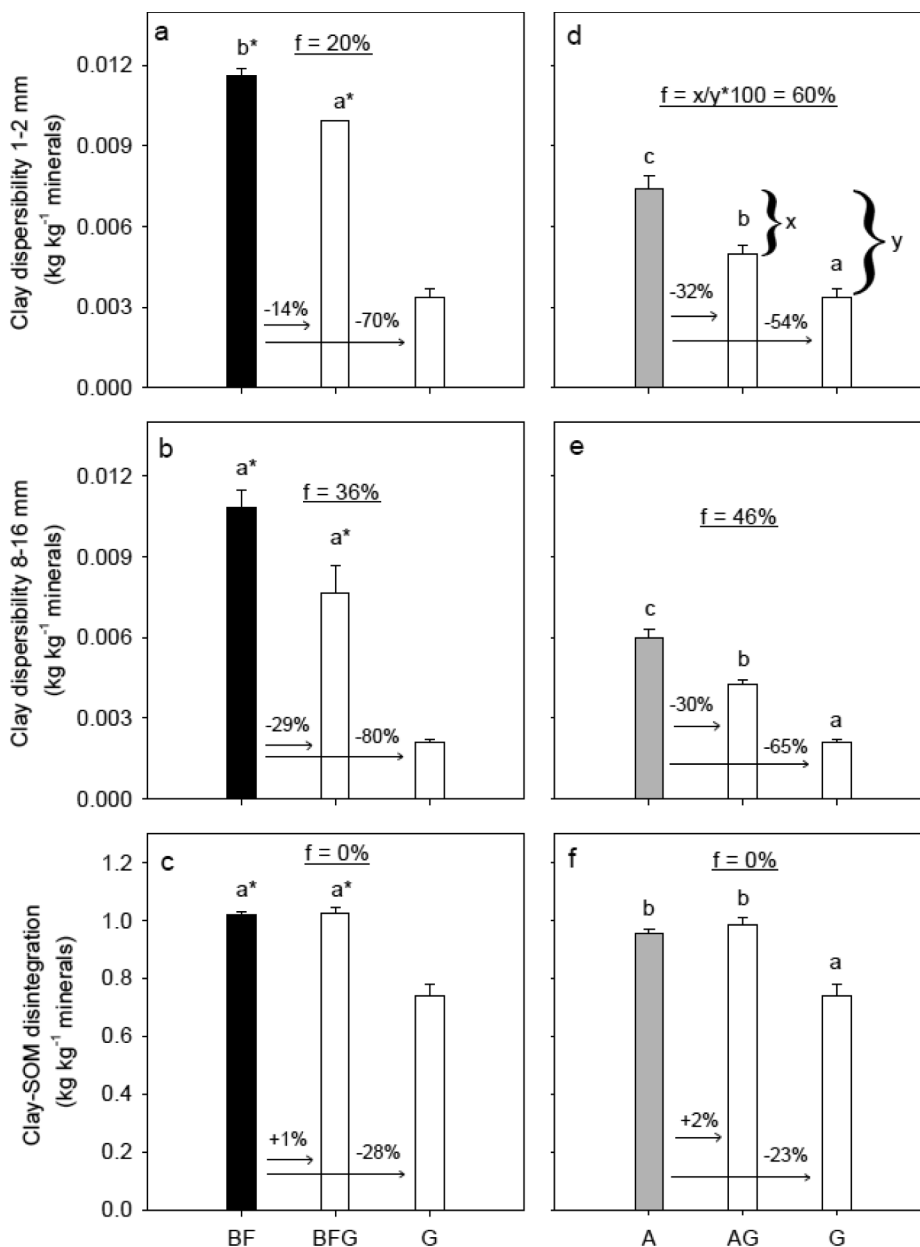


Fig. 5. Restoration scenarios: Management system effects on clay dispersibility of 1–2 mm aggregates rewetted to -100 hPa, clay dispersibility of 8–16 mm aggregates rewetted to -100 hPa, and clay-SOM disintegration (the ratio between clay particles retrieved without SOM removal and with removal). White, gray and black bar fills highlight treatments grass, arable and bare fallow, respectively, at time of sampling. Letters denote statistical significance at $P < 0.05$. An asterisk (*) indicates if G is significantly different from BF and BFG based on a pairwise t -test. The numbers above the arrows denote relative differences. The underlined number in the middle part of the figures denotes the difference after six years in relation to the long-term difference, and an example of the calculation is shown in Fig. d. For treatment abbreviations, see Fig. 1.

4.2. Soil recovery by introduction of grassland

The conversion of bare fallow management to grassland (BFG) introduces a change from intensive tillage and no plant inputs to permanent plant cover and absence of tillage, while the conversion of arable management to grassland (AG) introduces a change from annual tillage and cereals to permanent plant cover and absence of tillage. In this section, we address the effects of these soil restoration mechanisms in terms of changes in OM fractions (Fig. 3) and SSS (Fig. 5).

Compared to the BF treatment, BFG shows similar relative increases in SOC, POXC and HWC (46–57%, Fig. 3a–c). For arable soil converted to grassland, SOC was slightly more responsive to changes in management than HWC and POXC (Fig. 3d–f). This suggests as for the degradation managements, that SOC, POXC and HWC show similar sensitivity to changes in restoration managements, which contrasts with Bongiorno et al. (2019).

DispClay 1–2 mm and DispClay 8–16 mm decreased by an average of 14% and 29% (Fig. 5a and b), respectively, when bare fallow was converted to grassland. However, small non-significant changes in SOC,

POXC and HWC (Fig. 3d–f) had marked effects on both DispClay 1–2 mm and DispClay 8–16 mm when grassland replaced arable management (Fig. 5d and e). The more rapid change in macro-aggregate stability than in SOC content for AG agrees with results of Jastrow (1996) studying conversion of cultivated soil to tallgrass prairie. Poulton et al. (2018) also noted that small increases in SOC might have disproportionately large and beneficial effects on SSS.

Hirsch et al. (2017) found that microbial biomass and numbers of mesofauna increased when bare fallow and arable soils were converted to grassland. Further, the introduction of permanent grass increases root density (Attard et al., 2016) known to increase hyphal length (Schjønning et al., 2007). Roots and fungal hyphae may stabilize macro-aggregates (Tisdall and Oades, 1982), and microbial and faunal products derived from decomposition processes increase aggregate stability (Abiven et al., 2009). For both BFG and AG, the absence of tillage preserves the macro-aggregates and soil structure remains less disturbed, and the stabilizing agents are continuously replaced in soil under permanent grass. Thus, we suggest that the increase in macro-aggregate stability for BFG and AG may be due in part to the absence of

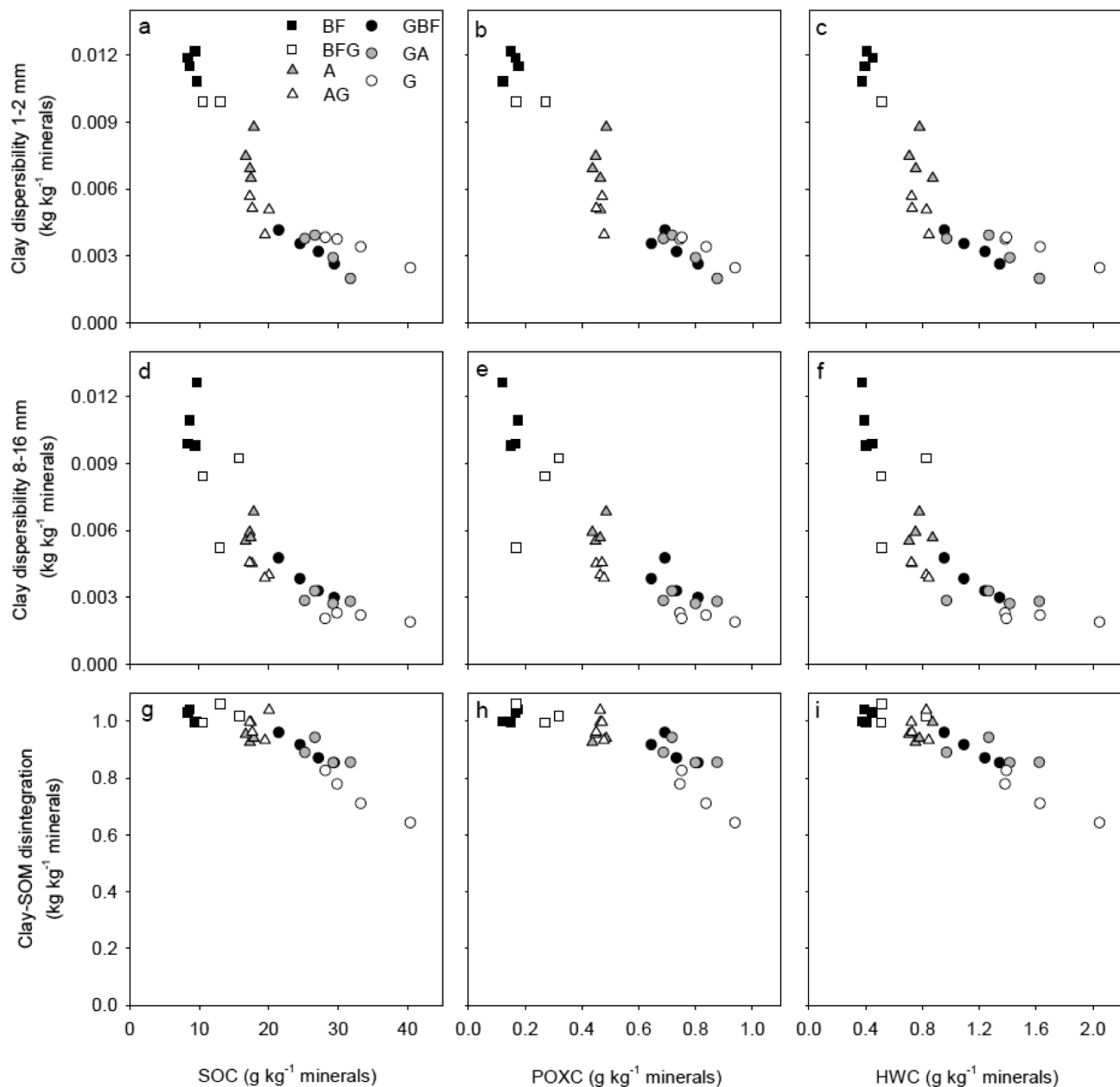


Fig. 6. Soil structural stability measures plotted against soil organic carbon (SOC), permanganate oxidizable carbon (POXC) and hot water-extractable carbon (HWC) for the seven treatments at plot level. White, gray and black symbol fills highlight treatments grass, arable and bare fallow, respectively, at time of sampling. For treatment abbreviations, see Fig. 1.

tillage leading to development of the grass root system with associated positive effects on soil functions (Ajayi et al., 2019) including unrestricted aggregate formation and stabilization. The results from the restoration managements (Figs. 3 and 5) thus align with results from the degradation managements (Figs. 2 and 4) in pointing out the necessity to consider bonding as well as binding mechanisms in soil structural stabilization (Degens, 1997; Elmholt et al., 2008; Schjønnning et al., 2007).

Although the importance of cations for SSS is considered minor in clayey soils (Bronick and Lal, 2005), the higher concentration of soluble Ca²⁺ in AG may potentially have contributed to the increased SSS by promoting flocculation of clay particles (Le Bissonnais, 1996).

4.3. Rate of change

Data from Hirsch et al. (2017) and Rothamsted Research (2018)

show that the levels of SOM in the BF, A and G treatments had reached steady-state conditions when the Highfield-LUCE experiment was initiated. Therefore, changes in SOC and SSS six years after conversion can be related to equilibrium values for SOC and SSS, whereby the rate of change in the scenarios can be revealed (Fig. 2d–i, Fig. 3, Fig. 4d–i, and Fig. 5). The rate of change was calculated as $f = x/y \cdot 100$, where x and y denote the change in SOC and SSS after six years and at steady-state condition, respectively.

The change in SOC from grassland to bare fallow (GBF) and the reverse (BFG) correspond to 31% decrease and 17% increase of the range between the two reference treatments BF and G, respectively (Fig. 2g and Fig. 3a). The change from grassland to arable management (GA) and the reverse (AG) corresponds to 30% decrease and 8% increase of the range between A and G, respectively (Fig. 2d and Fig. 3d). These results agree with Johnston et al. (2009) and Attard et al. (2016), who found that it was faster to lose than to restore SOC by management

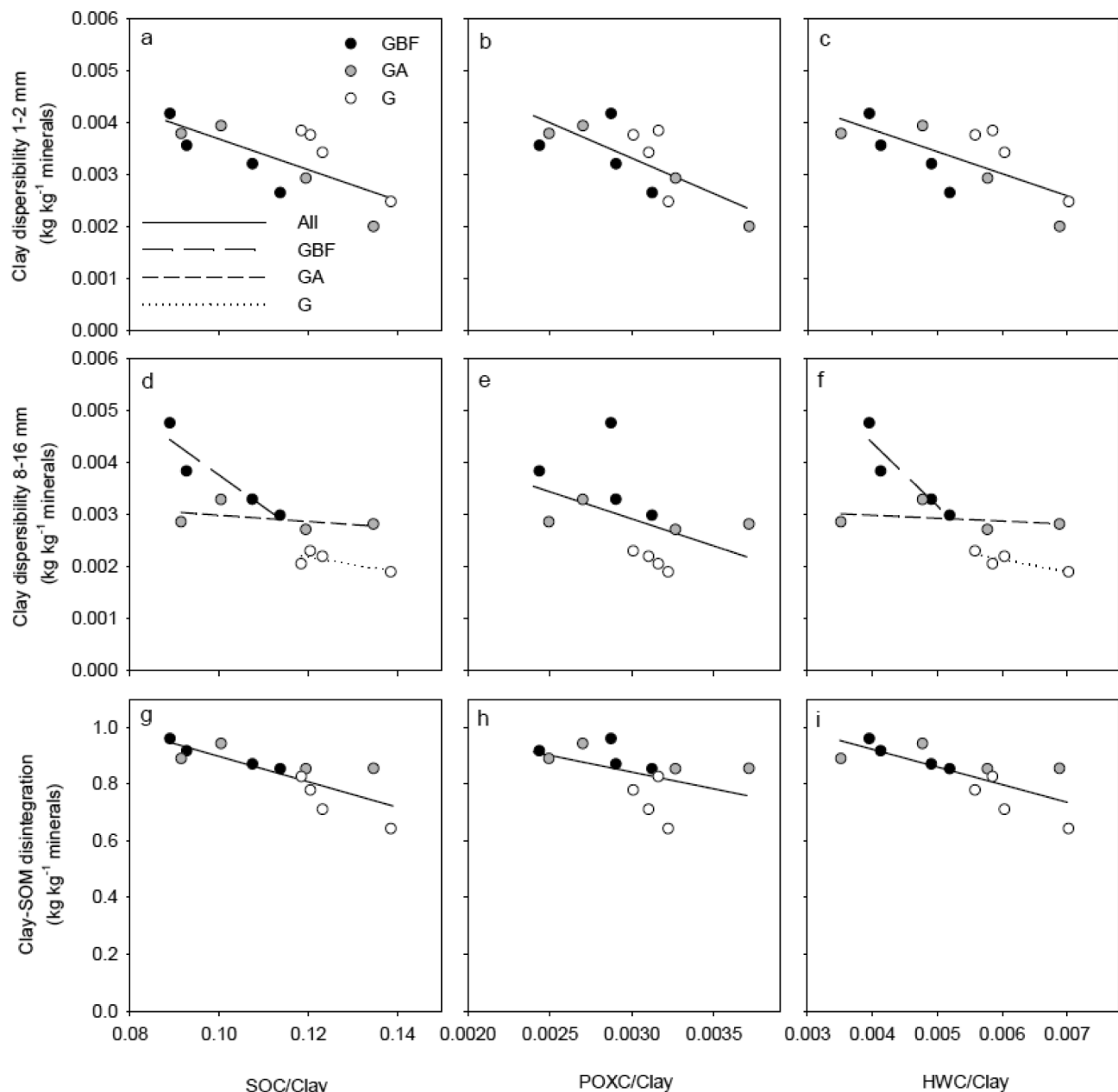


Fig. 7. Correlations between soil structural stability measures and clay-content normalized expressions of soil organic carbon (SOC/Clay), permanganate oxidizable carbon (POXC/Clay) and hot water-extractable carbon (HWC/Clay) for GBF, GA and G at plot level. White, gray and black symbol fills highlight treatments grass, arable and bare fallow, respectively, at time of sampling. The linear regression including data for all treatments are indicated if the slope of linear regressions for individual treatments were not significantly different, whereas the linear regression for individual treatments are shown if the slope of the regressions were significantly different. See Table 2 for slopes, differences between slopes, and R^2 - and P-values. For treatment abbreviations, see Fig. 1.

changes. The greater loss than gain in SOC could be due in part to differences in OM input in restoration and degradation managements. It may be difficult to establish grass in bare fallow and arable soil because of the poor structure, and Attard et al. (2016) found no change in SOC three years after cropland was converted to grassland, which was ascribed to the slow development of the root system. Nevertheless, SOC models such as RothC (Coleman and Jenkinson, 1996) and C-TOOL (Taghizadeh-Toosi et al., 2014) assume rate symmetry, i.e. equal change in both directions. Our findings challenge this assumption, and we encourage additional studies investigating rates of change in both directions.

DispClay 1–2 mm did not change for the grassland terminations. However, the introduction of grassland in bare fallow and arable soil correspond to 20% and 60% of the range between G, respectively

(Fig. 5a and d). The stability of large macro-aggregates (DispClay 8–16 mm) was highly sensitive to management changes in both restoration and degradation scenarios. The decline in stability for grassland terminations correspond to $\approx 20\%$ of the potential range (Fig. 4e and h), whereas the increase in stability in soil subjected to grassland corresponds to $\approx 40\%$ of the range (Fig. 5b and e). Thus, with respect to SSS measures at macroscale, it was faster to restore SSS than to degrade SSS. Based on a compilation of four studies Kay (1990) showed differences in rates of change when planting forages on arable land. However, the studies only focused on restoration managements, and as for SOC knowledge on the rate of change in SSS in both directions are lacking. We encourage similar studies to examine if our findings are generally applicable.

Introduction of grassland did not affect DI at microscale after six

Table 2

Slope, R^2 - and P-value for linear regressions of soil structural stability measures and clay-content normalized expressions of soil C and organic matter fractions individually for G, GA and GBF treatments as well as for all three treatments in combination. In case of statistical significance ($P < 0.05$) letters within rows denote significance for the comparison in slopes for G, GA and GBF. Clay dispersibility of 1–2 mm aggregates rewetted to -100 hPa (DispClay 1–2 mm), clay dispersibility of 8–16 mm aggregates rewetted to -100 hPa (DispClay 8–16 mm), clay-SOM disintegration (DI), soil organic carbon (SOC), permanganate oxidizable carbon (POXC), and hot water-extractable carbon (HWC). For treatment abbreviations, see Fig. 1.

Regression	G	GA	GBF	All
DispClay 1–2 mm vs SOC/Clay	–0.068	–0.044	–0.051	–0.030
R^2 - and P-value	0.99 ($P = 0.005$)	0.92 ($P = 0.039$)	0.90 ($P = 0.051$)	0.53 ($P = 0.007$)
DispClay 1–2 mm vs POXC/Clay	–4.660	–1.571	–1.063	–1.366
R^2 - and P-value	0.46 ($P = 0.323$)	0.94 ($P = 0.029$)	0.23 ($P = 0.521$)	0.52 ($P = 0.008$)
DispClay 1–2 mm vs HWC/Clay	–0.970	–0.564	–1.006	–0.426
R^2 - and P-value	0.95 ($P = 0.027$)	0.82 ($P = 0.096$)	0.89 ($P = 0.058$)	0.49 ($P = 0.011$)
DispClay 8–16 mm vs SOC/Clay	–0.014 ^{ab}	–0.006 ^a	–0.062 ^b	–0.038
R^2 - and P-value	0.54 ($P = 0.312$)	0.22 ($P = 0.536$)	0.86 ($P = 0.071$)	0.60 ($P = 0.003$)
DispClay 8–16 mm vs POXC/Clay	–1.882	–0.224	–0.997	–1.045
R^2 - and P-value	0.96 ($P = 0.021$)	0.24 ($P = 0.511$)	0.14 ($P = 0.632$)	0.21 ($P = 0.139$)
DispClay 8–16 mm vs HWC/Clay	–0.242 ^{ab}	–0.055 ^a	–1.209 ^b	–0.522
R^2 - and P-value	0.75 ($P = 0.131$)	0.10 ($P = 0.687$)	0.86 ($P = 0.074$)	0.50 ($P = 0.010$)
DI vs SOC/Clay	–8.037	–1.508	–3.972	–4.504
R^2 - and P-value	0.83 ($P = 0.086$)	0.48 ($P = 0.310$)	0.94 ($P = 0.031$)	0.64 ($P = 0.001$)
DI vs POXC/Clay	–406.73	–54.39	–81.03	–118.27
R^2 - and P-value	0.21 ($P = 0.539$)	0.51 ($P = 0.288$)	0.23 ($P = 0.518$)	0.20 ($P = 0.143$)
DI vs HWC/Clay	–109.39	–16.54	–78.24	–62.60
R^2 - and P-value	0.73 ($P = 0.144$)	0.31 ($P = 0.439$)	0.94 ($P = 0.032$)	0.55 ($P = 0.006$)

years, whereas termination of grassland increased DI with $\approx 60\%$ of the levels present in the corresponding reference treatments (Fig. 4f and i). To increase SSS at microscale, more than 2% SOC is needed for this soil (Fig. 6g and Fig. 4a in Jensen et al., 2019) regardless of soil management.

5. Conclusions

The Highfield-LUCE enabled us to quantify rates of change in OM fractions and soil structural stability (SSS) six years after the land use changed for soils subjected to contrasting long-term treatments. The loss of SOC in degradation scenarios was greater than the gain in SOC in the corresponding restoration scenarios. However, it was faster to gain SSS than to lose SSS at macro-aggregate scale. Accordingly, soil management affected SSS at macroscale beyond what is revealed from measuring changes in OM fractions. Based on our results, we suggest that the additional driver in play was binding agents. At microscale, SSS appeared to depend solely on the SOC content regardless of soil management. The results underline the need to include both bonding and binding mechanisms in the interpretation of changes in SSS induced by management.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2020.114181>.

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Supplementary material for the article entitled: "Soil degradation and recovery - changes in organic matter fractions and structural stability" by Jensen et al.

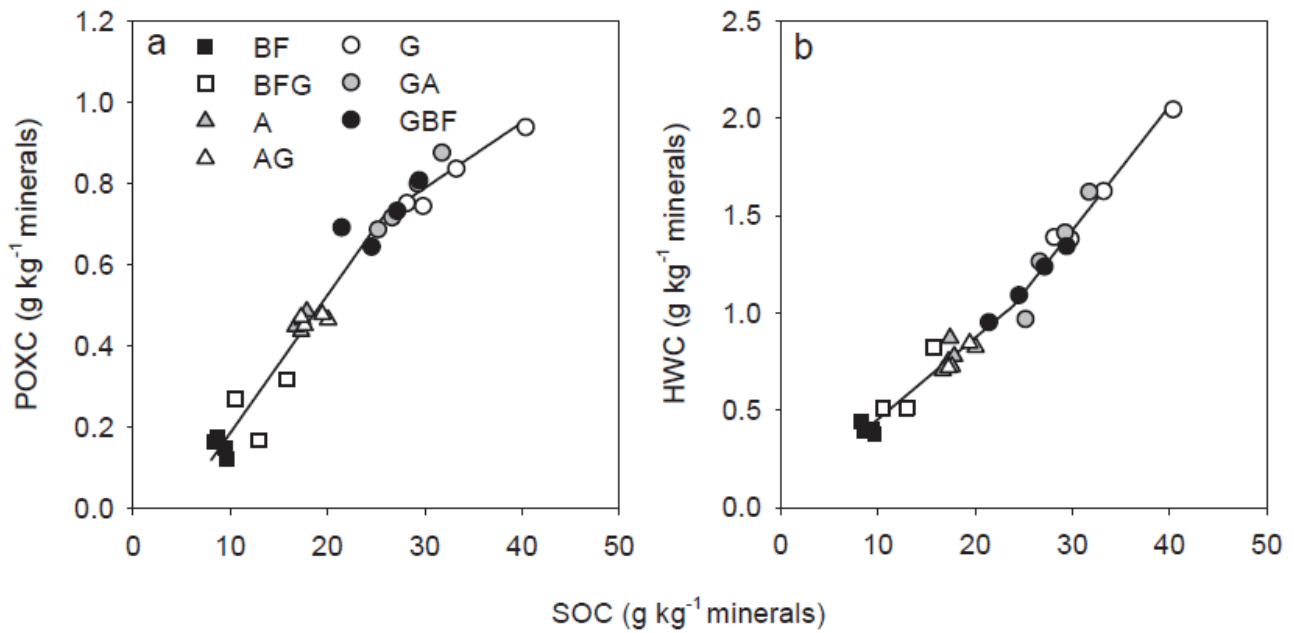


Fig. S1. (a) Permanganate oxidizable carbon (POXC) and (b) hot water-extractable carbon (HWC) as a function of soil organic carbon (SOC) for the seven treatments at plot level. White, gray and black symbol fills highlight treatments grass, arable and bare fallow, respectively, at time of sampling. The broken-stick models are indicated.

Table S1. Soil strength of 8-16 mm air-dry aggregates. In case of statistical significance ($P<0.05$) letters within rows denote significance for the comparison of G, GA and GBF, BF and BFG, and A and AG. For treatment abbreviations, see Fig. 1.

	G	GA	GBF	BF	BFG	A	AG
Tensile strength (Y , kPa)	278	202	191	332	332	349	341
Rupture energy (E_{sp} , J kg ⁻¹)	13.2 ^b	5.5 ^a	5.7 ^a	9.4	8.0	10.8	10.7
Young's modulus (E , MPa)	8.3	9.0	8.7	15.4	20.8	13.7	15.4

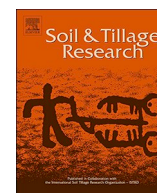
Paper 7

Short-term changes in soil pore size distribution: Impact of land use

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Short-term changes in soil pore size distribution: Impact of land use

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ABSTRACT

Changes in land use affect the pore size distribution (PSD) of the soil, and hence important soil functions such as gas exchange, water availability and plant growth. The objective of this study was to investigate potentially damaging and restorative soil management practices on soil pore structure. We quantified the rate of change in PSD six years after changes in land use taking advantage of the Highfield land-use change experiment at Rothamsted Research. This experiment includes short-term soil degradation and restoration scenarios established simultaneously within long-term contrasting treatments that had reached steady-state equilibrium. The land-use change scenarios comprised conversion to grassland of previously arable or bare fallow soil, and conversion of grassland to arable and bare fallow soils. In the laboratory, we exposed intact soil cores (100 cm³) to matric potentials ranging from −10 hPa to −1.5 MPa. Based on equivalent soil mass, the plant available water capacity decreased after conversion from grassland, whereas no change was observed after conversion to grassland. Structural void ratio decreased after termination of grassland and introduction of grassland in bare fallow soil, while no change was seen when changing arable to grassland. Consequently, it was faster to degrade than to restore a complex soil structure. The study illustrates that introducing grassland in degraded soil may result in short-term increase in soil density.

1. Introduction

The soil-water retention curve, i.e. the relationship between the soil water content and soil matric potential, shows the amount of water retained in the soil at a given matric potential. The tube-equivalent pore size at a given matric potential can be approximated by the physics-based capillary rise equation of Young-Laplace. Management effects on pore size distribution (PSD) have been reported in several papers (e.g., Dexter and Richard, 2009; Reynolds et al., 2009). The PSD of the soil can be derived from the soil-water retention curve either by numerical differentiation (e.g., Pulido-Moncada et al., 2019) or by differentiating fitted water retention models (e.g., Dexter et al., 2008). Changes in land use influence the PSD of the soil and thereby affect a range of important soil functions such as water and nutrient availability essential for plant growth, percolation and microbial activity (Kravchenko and Guber, 2017; Rabot et al., 2018). Previous studies reveal differences in PSDs of contrasting long-term treatments (e.g., Bacq-Labreuil et al., 2018; Jensen et al., 2019). In agricultural cropping systems, land use and

management vary according to the farm type specific crop rotation. In the humid temperate-region, most dairy production systems involve ley-arable rotations. Management includes establishment of grassland on arable soil and conversion of perennial grassland into arable cropping (Eriksen et al., 2015). It is in general anticipated that conversion from arable cropping to grassland has a positive effect on soil structure. Conversely, conversion of grass to arable cropping results in a loss of SOC and is hence expected to negatively affect soil structure (Poulton et al., 2018). To investigate relatively short-term changes in PSD when converting grassland to arable and vice versa a site with well-controlled conditions including well-defined land use history, and without confounding effects from differences in soil type, soil texture and climate is required. The Highfield land-use change experiment at Rothamsted Research (Highfield-LUCE) meets these unique requirements. The land use changes involved conversion to grassland from previously long-term arable or bare fallow management and conversion of long-term grassland into arable or bare fallow management. The bare fallow treatment represented an extreme reference point with regard to soil

Abbreviation: A, Arable; AG, Arable converted to grass; BF, Bare fallow; BFG, Bare fallow converted to grass; Dex, Double-exponential model; G, Grass; GA, Grass converted to arable; GBF, Grass converted to bare fallow; PAWC_{eq}, Plant available water capacity based on identical soil quantities; PSD, Pore size distribution; V₂, Structural void ratio

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degradation. Our objective was to determine short-term soil restoration and degradation on PSD using grassland as focal point.

2. Materials and methods

2.1. The Highfield land-use change experiment

Soil was from the Highfield-LUCE at Rothamsted Research, Harpenden, UK (51°80'N, 00°36'W), a silt loam soil belonging to the Batcombe series, and classifies as an Aquic Paludalf (USDA Soil Taxonomy System) and Chromic Luvisol (WRB) (Watts and Dexter, 1997). Land uses were long-term grass (G), arable (A) and bare fallow (BF) as well as four reversion treatments which had been established as either G or A in 1949 or BF in 1959 and maintained until 2008, where grassland was introduced in arable (AG) and bare fallow soil (BFG), and grassland was converted to arable (GA) and bare fallow (GBF). The G, GA, GBF, A and AG treatments were part of a randomized block design with four field replicates, while the four BF and three BFG plots were located at one end of the experiment. For more details on soil management and the experiment, see Jensen et al. (2020). Pore characteristics for BF, A and G treatments have been reported previously in Obour et al. (2018) and Jensen et al. (2019). Jensen et al. (2020) focused on soil organic matter components and soil structural stability in the Highfield-LUCE. Soil was sampled in spring 2015 at a soil water content close to field capacity. Six 100-cm³ intact soil cores (61-mm diam., 34-mm height) were extracted from 0.06 to 0.10-m depth in each of four replicate plots providing 24 cores per treatment. For BFG there was three replicate plots providing 18 cores in total. The soil cores were retrieved in metal cylinders forced into the soil by means of a hammer. The cylinders were held in position by a special flange ensuring a vertical downward movement into the soil. After careful removal of the soil-filled cylinder, the end surfaces were trimmed with a knife. Subsequently, the soil cores were sealed with plastic lids, kept in sturdy containers to prevent soil disturbance during transport and stored in a 2 °C room until required for analyses.

2.2. Laboratory measurements

Soil texture was determined on air dry bulk soil (<2 mm) with a combined hydrometer/sieve method (Gee and Or, 2002) after removal of soil organic matter (OM) with hydrogen peroxide. The content of soil organic carbon (SOC) was measured by dry combustion using a Thermo Flash 2000 NC Soil Analyzer (Thermo Fisher Scientific). Before measuring soil water retention, the soil cores were placed on top of a tension table and saturated with water from beneath. Soil water retention was determined at -10-, -30-, -100-, -300-, and -1000-hPa matric potential using tension tables and pressure plates (Dane and Hopmans, 2002). The soil cores were oven-dried (105 °C for 24 h), and bulk density (BD) calculated. BD was corrected for mass and volume of >2-mm particles since the soil contained a significant amount of stones. The stone mass of the soil cores varied from 0.0 to 50.6 g and importantly the stone mass for e.g. G, GA and GBF was 5.1, 8.7 and 11.6 g, respectively, and thus different between the treatments. The stone mass was determined after wet sieving and drying. The stone volume was determined by means of a Lenz wide-neck bottle with conical shoulder and NS joint neck, and pycnometer head. The stone volume was calculated as the difference between the stone mass and the weight of displaced water divided by 0.998 g cm⁻³ (density of water at 20 °C). The determination of stone volume derives from Archimedes' principle. Soil porosity was estimated from BD and particle density (PD). PD was measured for one plot from each treatment, i.e. seven analyses in total, by the pycnometer method (Flint and Flint, 2002). For the remaining plots, PD (g cm⁻³) was predicted from SOC (g kg⁻¹ minerals) by a linear regression model based on the seven data points:

$$\text{Particle density} = -0.0041^{***} (\pm 0.0004) \times \text{SOC} + 2.730^{***}$$

$$(\pm 0.008), s = 0.007, R^2 = 0.96 \quad (1)$$

where R^2 is the coefficient of determination, and s is the standard deviation of the predicted value.

Water retention at -1.5 MPa was determined at plot level using a WP4-T Dewpoint Potentiometer (Scanlon et al., 2002) and based on <2-mm air-dry soil. Volumetric water content at each matric potential was calculated from the weight loss upon oven-drying. Pore-water suction was assumed to relate to an average pore size by the approximate relation:

$$d = -3000/h \quad (2)$$

where d is the tube-equivalent pore diameter (μm) and h is the soil matric potential (hPa). The equation derives from the physics-based capillary rise equation of Young-Laplace.

2.3. Double-exponential model, calculations and statistics

The water retention data was fitted to the double-exponential model proposed by Dexter et al. (2008) (termed Dex):

$$\theta = C + A_1 e^{(-h/h_1)} + A_2 e^{(-h/h_2)} \quad (3)$$

where C is the residual water content (m³ pores m⁻³ total soil volume), A_1 (m³ pores m⁻³ total soil volume) and A_2 (m³ pores m⁻³ total soil volume) describe the amount of textural and structural porosity, respectively, and h_1 (hPa) and h_2 (hPa) are the characteristic matric potentials at which the textural and structural porosity empty, respectively. The PSD predicted by the Dex model was visualized by differentiating Eq. 3 with respect to the logarithm of matric potential:

$$\frac{d\theta}{d(\log_{10} h)} = -\frac{A_1}{h_1} e^{(-h/h_1)} h \ln 10 - \frac{A_2}{h_2} e^{(-h/h_2)} h \ln 10 \quad (4)$$

The parameters of the Dex model were obtained by nonlinear regression analysis to achieve the smallest residual sum of squares. Eq. 3 described the water retention data of the soils well (Fig. 1a, c and e), with R^2 ranging from 0.997 to 1.000 and root-mean-square error (RMSE) ranging from 0.00001-0.00638 m³ m⁻³. We used the bi-modal Dex model rather than the widely used uni-modal model proposed by van Genuchten (1980) since the Dex model provided a better fit to the water retention data for the long-term G, A and BF treatments (Jensen et al., 2019). We evaluated the rate of change in plant available water capacity and structural void ratio. Plant available water capacity was calculated based on a soil mass equivalent to 20 cm in the G soil (abbreviated PAWC_{eq}), which is analogous to how changes in SOC stocks are recommended to be assessed (Powlson et al., 2011). PAWC was determined as the difference in volumetric water content at -100 hPa and -1.5 MPa multiplied by the plough layer depth (20 cm). Subsequently, PAWC_{eq} was calculated by first calculating the mass of soil to the designated depth for all plots:

$$M_{\text{soil}} = \text{BD} \times 20 \text{ cm} \times 100 \quad (5)$$

where BD is bulk density (g cm⁻³), and M_{soil} is the mass of soil to 20 cm depth (Mg ha⁻¹). The G treatment was selected as the reference (M_{ref}) since it had the lowest soil mass. Next, the soil mass to be subtracted from the core segment so that mass of soil is equivalent in all sampling sites was calculated:

$$M_{\text{ex}} = M_{\text{soil}} - M_{\text{ref}} \quad (6)$$

where M_{ex} is the excess mass of soil to be subtracted from the core segment. Finally, PAWC_{eq} was calculated:

$$\text{PAWC}_{\text{eq}} = \text{PAWC} \times ((M_{\text{soil}} - M_{\text{ex}}) / M_{\text{soil}}) \quad (7)$$

It is essential to report PAWC on an equivalent soil mass basis to take into account changes in BD and by that allowing a comparison of the quantity of water available to the crop for different cropping

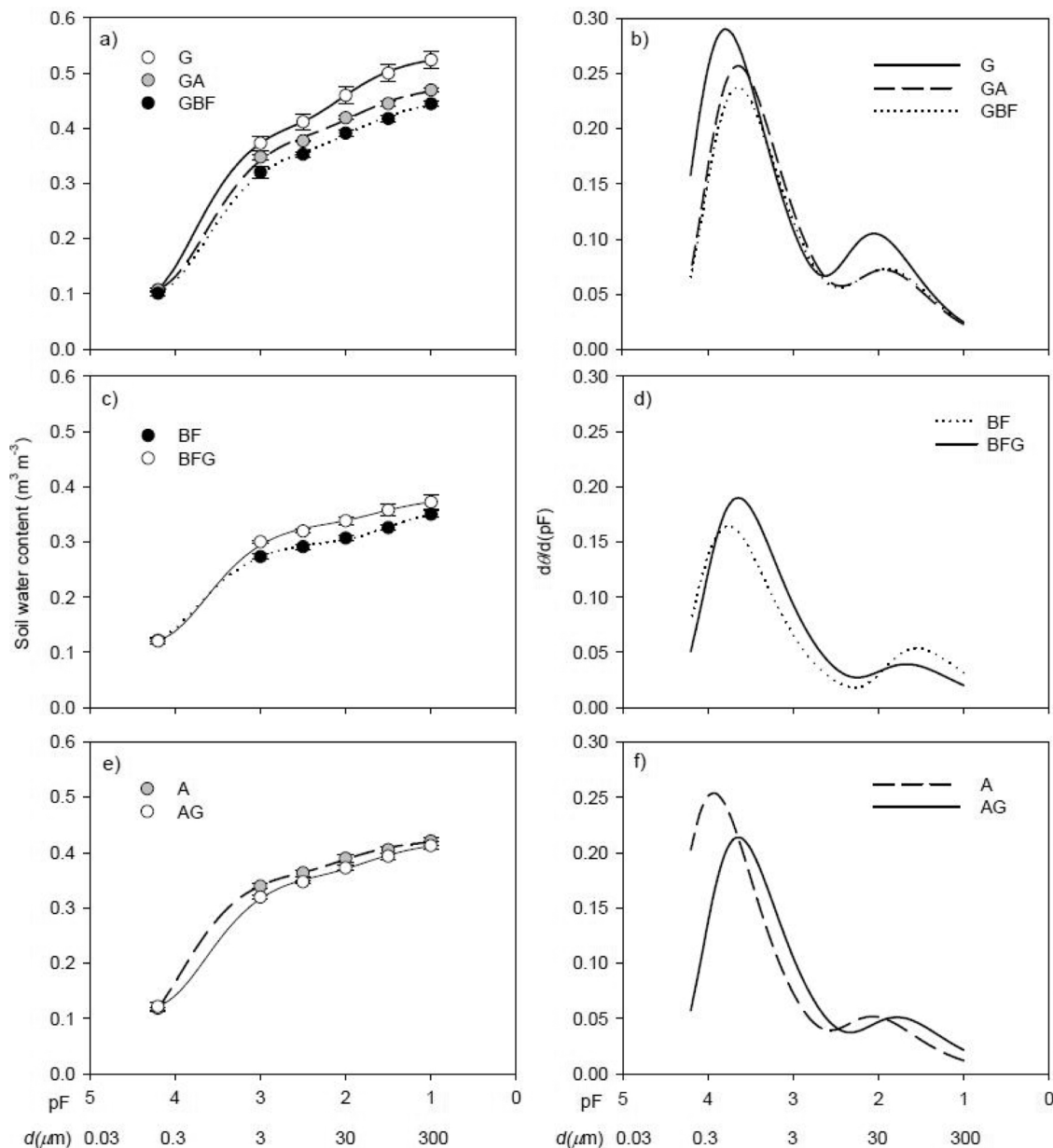


Fig. 1. Measured volumetric water content for the comparison of G with GA and GBF (a), BF with BFG (c) and A with AG (e) and fits of the double-exponential (Dex) model as a function of matric potential ($pF = \log_{10}(-\text{cm H}_2\text{O})$). The standard error of the mean is indicated. Pore size distribution ($dq/d(pF)$) as a function of matric potential for the corresponding comparisons (b, d and f). Eq. 4 was used to obtain the pore size distributions. The equivalent pore diameters are indicated and were estimated by Eq. 2. For treatment abbreviations, see Table 1.

systems.

Structural void ratio (V_2) was calculated as follows:

$$V_2 = A_2 / (1-P) \quad (8)$$

where A_2 (m^3 pores m^{-3} total soil volume) is the Dex model estimate of structural porosity, and P is porosity (m^3 pores m^{-3} total soil volume). V_2 relates the volume of structural pores to the volume of soil solids (i.e. m^3 pores m^{-3} volume of solids), which allows for comparisons across soils with different bulk densities as opposed to A_2 . This expression is analogous to the liquid ratio (Hillel, 1980) and has been used in several studies (e.g., Jensen et al., 2019; Schjønning and Lamandé, 2018).

The long-term BF, A and G treatments had reached steady-state equilibrium with respect to soil OM when the Highfield-LUCE experiment was initiated (Hirsch et al., 2017; Rothamsted Research, 2018). Hence, changes in PAWC_{eq} and V_2 six years after conversion can be

related to equilibrium values for these properties, whereby the rate of change in the scenarios can be revealed. The rate of change was calculated as follows:

$$f = x / y \times 100 \quad (9)$$

where x and y denote the change in PAWC_{eq} and V_2 after six years and at steady-state equilibrium, respectively, and if $x < 0$ then $f = 0$.

For the statistical analysis, the R-project software package Version 3.4.0 (R Foundation for Statistical Computing) was used. Treatment effects were analyzed as described in Jensen et al. (2020).

3. Results

Generally, contents of clay, silt and sand did not differ among soils retrieved from different treatments (Table 1) allowing treatment effects to be examined without confounding effects related to differences in

Table 1

Soil characteristics and bulk density. Within rows, letters denote statistical significance at $P < 0.05$ for the comparison of G with GA and GBF, BF with BFG, and A with AG. Grass (G), grass converted to arable (GA), grass converted to bare fallow (GBF), bare fallow (BF), bare fallow converted to grass (BFG), arable (A) and arable converted to grass (AG). Soil characteristics from Jensen et al. (2020).

	G	GA	GBF	BF	BFG	A	AG
Texture ^a							
Clay <2 μm	0.261	0.255	0.254	0.270	0.244	0.264	0.266
Silt 2–20 μm	0.272 ^b	0.255 ^a	0.256 ^a	0.249	0.267	0.263	0.253
Silt 20–63 μm	0.319	0.335	0.337	0.335	0.338	0.318	0.332
Sand 63–2000 μm	0.148	0.155	0.153	0.146	0.151	0.155	0.149
Soil organic carbon (SOC, g kg ⁻¹ minerals)	32.9 ^b	28.2 ^a	25.6 ^a	9.0	13.1	17.3	18.6
SOC relative change (%)		–14%	–22%		+46 %		+8 %
Bulk density (g cm ⁻³)	1.13	1.19	1.19	1.45 ^a	1.54 ^b	1.39	1.38

^a kg kg⁻¹ of mineral fraction and based on oven-dry weight.

Table 2

Porosity in seven pore size classes and total porosity. Within rows, letters denote statistical significance at $P < 0.05$ for the comparison of G with GA and GBF, BF with BFG, and A with AG. For treatment abbreviations, see Table 1.

			G	GA	GBF	BF	BFG	A	AG
Porosity in pore size classes	> 300 μm	(m ³ m ⁻³)	0.038 ^a	0.075 ^b	0.099 ^b	0.111 ^b	0.049 ^a	0.054	0.067
	100 – 300 μm		0.024	0.025	0.026	0.024 ^b	0.015 ^a	0.016	0.019
	30 – 100 μm		0.041 ^b	0.025 ^a	0.026 ^a	0.024	0.020	0.016	0.021
	10 – 30 μm		0.048 ^b	0.042 ^{ab}	0.038 ^a	0.015	0.019	0.026	0.025
	3-10 μm		0.039	0.029	0.033	0.018	0.017	0.024	0.029
	0.2 – 3 μm		0.266 ^b	0.240 ^{ab}	0.219 ^a	0.159 ^a	0.181 ^b	0.220	0.200
	<0.2 μm		0.101	0.107	0.101	0.116	0.119	0.119	0.119
	Total		0.561	0.544	0.543	0.460 ^b	0.422 ^a	0.475	0.479

soil texture.

Compared with long-term grassland (G), the SOC content decreased by 14 and 22 % (Table 1) in GA and GBF. Total porosity did not change (Table 2), whereas the PSD of the soils changed (Fig. 1b). Plant available water capacity (PAWC; water retained in 0.2–30 μm pores) decreased significantly for GA and GBF compared to G. This was mainly ascribed to 0.2–3 μm and 10–30 μm pore size classes which decreased in the order G > GA > GBF with G and GBF being significantly different (Table 2). The drop in textural porosity was reflected in a significant reduction in A_1 in the GA and GBF treatments (Table 3). Similarly, the fraction of soil volume represented by 30–100 μm pores decreased significantly for GA and GBF compared to G and was reflected by a decrease in A_2 . The fraction of soil volume represented by pores >300 μm was significantly higher for GA and GBF than for G.

Introduction of grassland in bare fallow soil (BFG) led to an increase in SOC by 46 % compared to BF (close to significant, $P = 0.053$), while conversion to grassland in arable soil (AG) increased SOC marginally by 8 % compared to A (Table 1). There were no significant differences in any of the measured pore size classes nor in total porosity when A was compared to AG (Table 2); this was also partly reflected in the PSDs (Fig. 1f). For BFG, however, total porosity decreased significantly compared to BF. The PSD changed towards a significantly greater fraction of soil volume represented by 0.2–3 μm pores, and a significant reduction in 100–300 μm pores as well as >300 μm pores (Table 2). This was reflected in the PSDs (Fig. 1d) and resulted in a greater A_1 for BFG than for BF (close to significant, $P = 0.08$) and a significant reduction in A_2 (Table 3).

For long-term grassland (G), the plant available water capacity based on equivalent soil mass (PAWC_{eq}) was 71 mm water. Conversion of grassland into arable (GA) and bare fallow (GBF) soils reduced PAWC_{eq} to 60 and 56 mm water, respectively. This corresponds to relative reductions of 16 and 21 % (Fig. 2a). PAWC_{eq} for long-term bare fallow (BF) and arable (A) soils were 30 and 44 mm water, respectively. Introduction of grassland did not change these quantities significantly

(Fig. 3a and c). Compared with G, the structural void ratio (V_2) decreased by 35 and 32 % for GA and GBF (Fig. 2b), while V_2 decreased by 22 % for GBF compared to BF and increased by 8 % for AG compared to A (Fig. 3b and d).

The changes in PAWC_{eq} for GA and GBF corresponded to approximately 40 % decrease of the range between A and BF, respectively (Fig. 2c and e). The rate of change in V_2 for the same treatments corresponded to 55 % decrease (Fig. 2d and f). However, PAWC_{eq} and V_2 did not change significantly for BFG compared to BF and AG compared to A (Fig. 3) apart from the reduction in V_2 for BFG.

4. Discussion

Schjønning and Thomsen (2013) advocated expressing PAWC on a

Table 3

Fitted parameters of the double-exponential model (Dex) of the seven treatments. Within columns, letters denote statistical significance at $P < 0.05$ for the comparison of G with GA and GBF, BF with BFG, and A with AG. d_1 and d_2 indicate the dominating pore size of the textural and structural peak, respectively, and were estimated by Eq. 2. For treatment abbreviations, see Table 1.

Treatment	Parameters of the Dex model						
	C	A_1	h_1	d_1	A_2	h_2	d_2
	m ³ m ⁻³	m ³ m ⁻³	hPa	μm	m ³ m ⁻³	hPa	μm
G	0.080	0.343 ^b	6216 ^b	0.5	0.110 ^b	102	29
GA	0.100	0.303 ^a	4396 ^a	0.7	0.075 ^a	74	41
GBF	0.098	0.280 ^a	4396 ^a	0.7	0.078 ^a	72	42
–							
BF	0.110	0.195	5768 ^b	0.5	0.059 ^b	35	86
BFG	0.117	0.223	4398 ^a	0.7	0.047 ^a	39	77
–							
A	0.068 ^a	0.305 ^b	8707 ^b	0.3	0.050	97 ^b	31
AG	0.115 ^b	0.253 ^a	4396 ^a	0.7	0.053	53 ^a	57

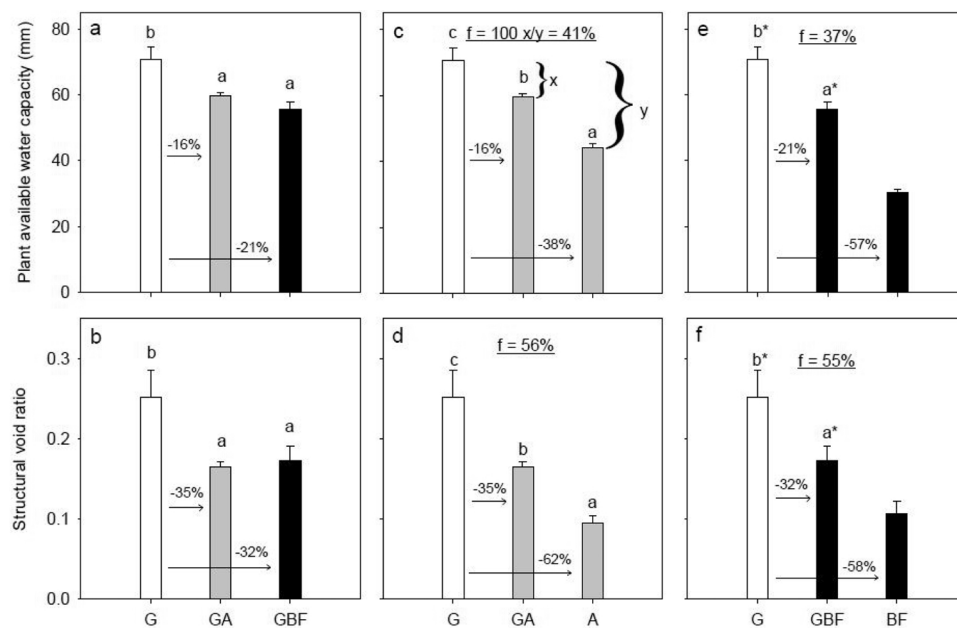


Fig. 2. Degradation scenarios: Land use change effects on plant available water capacity calculated based on a soil mass equivalent to 20 cm in the G soil, and structural void ratio. White, gray and black bar fills represent grass, arable and bare fallow treatments, respectively, at time of sampling. Letters denote statistical significance at $P < 0.05$. An asterisk (*) indicates if BF is significantly different from GBF and G based on a pairwise t -test. The numbers above the arrows denote relative differences. The underlined number in the middle part of the figures denotes the rate of change, and was estimated by Eq. 9. An example of the calculation is shown in Fig. c. For treatment abbreviations, see Table 1.

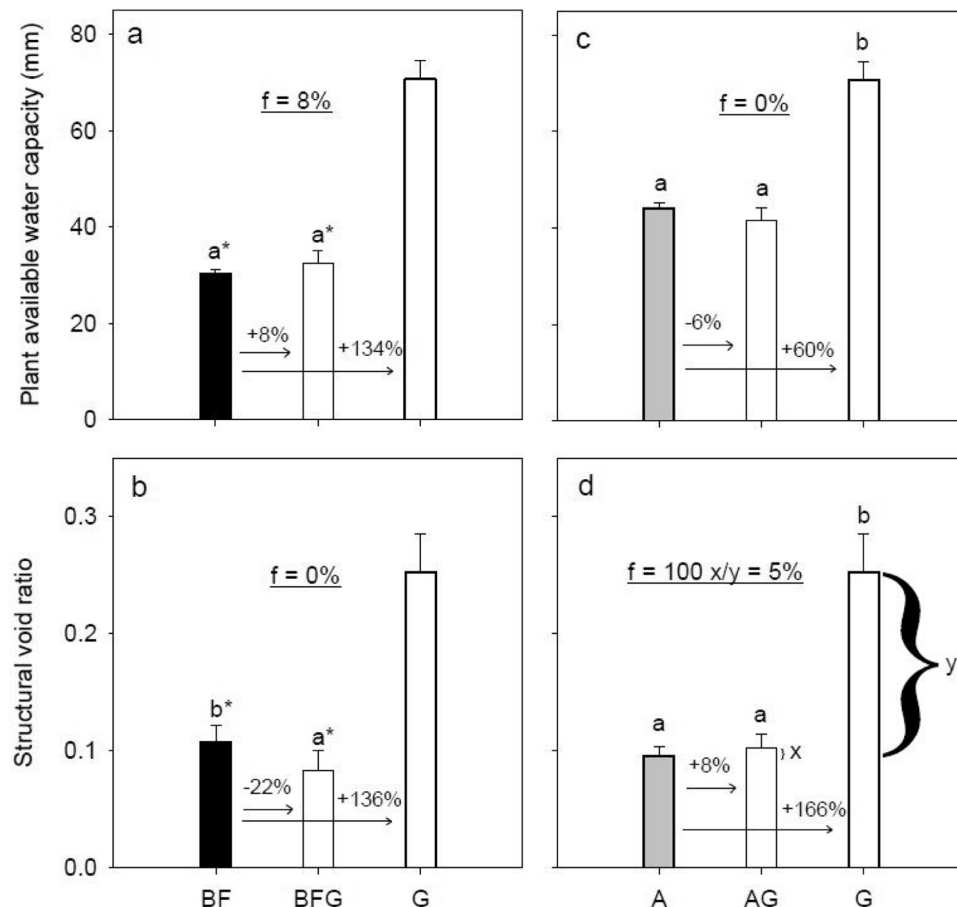


Fig. 3. Restoration scenarios: Land use change effects on plant available water capacity calculated based on a soil mass equivalent to 20 cm in the G soil, and structural void ratio. White, gray and black bar fills represent grass, arable and bare fallow treatments, respectively, at time of sampling. Letters denote statistical significance at $P < 0.05$. An asterisk (*) indicates if G is significantly different from BF and BFG based on a pairwise t -test. The numbers above the arrows denote relative differences. The underlined number in the middle part of the figures denotes the rate of change, and was estimated by Eq. 9. An example of the calculation is shown in Fig. d. For treatment abbreviations, see Table 1.

mass-equivalent basis when comparing tillage systems with significant differences in soil bulk density. In this study, $PAWC_{eq}$ (Figs. 2 and 3) represents the quantity (expressed in mm in analogy with expressions of water balances) of water available to the crop in the 0–20 cm A-horizon in the G treatment and in corresponding soil masses for the other treatments. V_2 relates to the volume of soil solids whereas the corresponding A_2 variable provides the traditional pore volume for a given

soil volume including soil solids and voids.

Converting grassland to arable or bare fallow management decreased $PAWC_{eq}$. This relates to a change in soil structure ascribed to reduced SOC contents and increased tillage intensity. The decrease in $PAWC_{eq}$ for GA and GBF corresponds to a reduction of 11 and 15 mm water. Such a reduction has little impact on plant growth at this specific site because the soil type has a high $PAWC_{eq}$ and an average annual

temperature and precipitation of 10.2 °C (mean of 1992–2014) and 718 mm (mean of 1981–2010), respectively (Scott et al., 2014). However, for light-textured soils in drier regions the reduction would be more significant.

The decrease in V_2 following termination of grassland may be ascribed to tillage-induced breakdown of aggregates (Jensen et al., 2020), including destruction of the enmeshment of aggregates by roots and hyphae and rearrangement of the pore system. The ~30–90 µm pore size class has been shown to enhance microbial activity and the decomposition of OM (Kravchenko and Guber (2017) and references therein). The reduction in this specific pore size class following termination of grassland may thus negatively affect the decomposition of OM and related effects on nutrient supply and soil properties. The increase in >300 µm pores following termination of grassland may be ascribed to the increase in tillage intensity introducing very large pores.

The arrangement of pores did not show any signs of recovery six years after introducing grassland on arable soil. The introduction of grass on bare fallow soil, however, did show signs of recovery with respect to increases in textural pores (only significant for 0.2–3 µm pores), but the effect disappeared when looking at PAWC_{eq} (Fig. 3a) due to the decrease in total porosity for BFG compared to BF (Table 1). The minor changes in PAWC_{eq} caused an insignificant increase of 2 mm in BFG and an insignificant decrease of 3 mm water in AG. Based on a meta-analysis, Minasny and McBratney (2018) found that the SOC related increase in PAWC was 3–4 mm 20 cm⁻¹ per 10 g kg⁻¹ increase in SOC. Our study shows that the effect of SOC on PAWC_{eq} can be even smaller. The marked reduction in >100 µm pores and reduction in V_2 for BFG may be related to an increase in density of the initially intensively tilled and degraded soil. The soil is in a transition phase, where a complex soil structure is developing, and the results indicate that the soil is in its initial phase with regard to the development of such a structure. The marked reduction in V_2 is undesirable as root growth may be negatively affected. Further, gas exchange may be reduced potentially leading to anoxic conditions and increased potential for greenhouse gas emissions (Ball, 2013).

In essence, the results show that it was much faster to degrade both PAWC_{eq} and V_2 than to restore these soil pore properties. Results on macro-aggregate stability for the same treatments, however, showed the opposite, namely that it was faster to gain than to lose stability (Jensen et al., 2020). This implies that even though the aggregates increased in stability, presumably due to the combined effect of an increase in bonding and binding agents and the lack of disturbance (Jensen et al., 2020), the soil pore network did not show signs of self-organization. The results contradict the conceptual model for self-organization of the soil-microbe complex proposed by Young and Crawford (2004). They suggest that as substrate arrives in soil, the respiration will increase and a more open aggregated state will develop, while the structure will collapse in the absence of substrate. They indicated that the rate of change would be similar in both directions. However, our results show that the rate of change is markedly greater during degradation than restoration scenarios (Figs. 2 and 3). Hence, even though the BFG and AG soils show recovery of soil microbial communities (Hirsch et al., 2017), likely an increased root density, and increased structural stability (Jensen et al., 2020) it takes a long time to develop a complex soil structure. Studies on no-till also suggest that it may take a substantial time to develop a good structure when changing from a tilled system to a system with less disturbance, and that topsoil may experience a period with increasing density (e.g., VandenBygaart et al., 1999).

We based our study on a silt loam soil with 0.26 kg clay kg⁻¹ mineral fraction and a relatively evenly distributed soil mass across particle size classes (denoted a graded soil). Graded soils low in SOC may exhibit hard-setting behavior and readily compact to high densities (Jensen et al., 2019; Schjøning and Thomsen, 2013). This may explain why we see little signs of recovery when introducing grassland in degraded soil. However, some soils with >0.35 kg clay kg⁻¹ mineral

fraction and a clay fraction primarily consisting of 2:1 clay minerals exhibit a self-mulching behavior, and rely on natural soil processes for fragmentation (Grant and Blackmore, 1991). We do not expect that our results apply for self-mulching and highly sorted soils.

5. Conclusions

The Highfield-LUCE enabled us to quantify rates of change in pore size distribution six years after the land use changed for soils subjected to contrasting long-term treatments. The results showed that changing land use from long-term grassland to bare fallow or arable decreased both plant available water capacity based on identical soil quantities and structural void ratio. The conversion to grassland from long-term bare fallow or arable soil did not lead to recovery in the short-term. Thus, it was faster to lose than to develop a complex soil structure. The results underline that introducing grassland in degraded soil may induce densification in the short-term with potential negative impacts on gas exchange and root growth.

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.

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8. Supplementary and additional papers

I have contributed to the following four supplementary papers, which are within the subject of my thesis:

1. Peltre, C., Nyord, T., Christensen, B. T., **Jensen, J. L.**, Thomsen, I., Munkholm, L. J. 2016. Seasonal differences in tillage draught on a sandy loam soil with long-term additions of animal manure and mineral fertilizers. *Soil Use and Management*, **32**, 583-593. doi: 10.1111/sum.12312.
2. Schjøning, P., **Jensen, J. L.**, Bruun, S., Jensen, L. S., Christensen, B. T., Munkholm, L. J., Oelofse, M., Baby, S., Knudsen, L. 2018. The role of soil organic matter for maintaining crop yields: Evidence for a renewed conceptual basis. *Advances in Agronomy*, **150**, 35-79. doi: 10.1016/bs.agron.2018.03.001.
3. Obour, P. B., **Jensen, J. L.**, Watts, C. W., Lamandé, M., Munkholm, L. J. 2018. Soil organic matter widens the range of water contents for tillage. *Soil and Tillage Research*, **182**, 57-65. doi: 10.1016/j.still.2018.05.001.
4. Obour, P. B., Keller, T., **Jensen, J. L.**, Edwards, G., Lamandé, M., Watts, C. W., Sørensen, C. G., Munkholm, L. J. 2019. Soil water contents for tillage: a comparison of approaches and consequences for the number of workable days. *Soil and Tillage Research*, **195**, 104384. doi: 10.1016/j.still.2019.104384.

I have contributed to the following additional papers during my Ph.D. study:

1. Christensen, B. T., **Jensen, J. L.**, Thomsen, I. K. 2017. Impact of early sowing on winter wheat receiving manure or mineral fertilizers. *Agronomy Journal*, **109**, 1312-1322. doi: 10.2134/agronj2016.11.0677.
2. Liang, Z., Olesen, J. E., **Jensen, J. L.**, Elsgaard, L. 2019. Nutrient availability affects carbon turnover and microbial physiology differently in topsoil and subsoil under a temperate grassland. *Geoderma*, **336**, 22-30. doi: 10.1016/j.geoderma.2018.08.021.

I have presented my work at conferences:

1. **Jensen, J. L.**, Munkholm, L. J., Schjøning, P. 2015. Long-term effects of fertilizer application rate and type on soil structural properties, Abstract from the 20th International Soil Tillage Research Organization (ISTRO) conference, poster presentation
2. **Jensen, J. L.**, Schjøning, P., Watts, C. W., Christensen, B. T., Munkholm, L. J. 2017. Soil organic matter induced systematic errors in estimation of clay and silt by gravitational sedimentation, Abstract from the 6th International Symposium on Soil Organic Matter, poster presentation

3. **Jensen, J. L.**, Schjønning, P., Watts, C. W., Christensen, B. T., Obour, P. B., Peltré, C., Munkholm, L. J. 2017. Defining critical soil organic matter levels in agricultural soils: Evidence from the Rothamsted Highfield Ley-Arable Experiment, Abstract from the 6th International Symposium on Soil Organic Matter, oral presentation
4. Obour, P. B., **Jensen, J. L.**, Lamandé, M., Watts, C. W., Munkholm, L. J. 2017. Effect of soil organic matter on workability: a case of silt loam soil under long-term management, Abstract from the 6th International Symposium on Soil Organic Matter, poster presentation
5. Obour, P. B., **Jensen, J. L.**, Lamandé, M., Watts, C. W., Munkholm, L. J. 2018. Water retention and consistency approaches for determining the water contents for tillage, Abstract from the 21st International Soil Tillage Research Organization (ISTRO) conference, oral presentation
6. **Jensen, J. L.**, Schjønning, P., Watts, C. W., Christensen, B. T., Munkholm, L. J. 2018. Size distribution of soil pores: a model allowing for bi-modality needed to account for management effects, Abstract from the 21st International Soil Tillage Research Organization (ISTRO) conference, poster presentation
7. **Jensen, J. L.**, Schjønning, P., Christensen, B. T., Munkholm, L. J. 2019. Nedre grænseværdi for kulstof i jord? Abstract from Plantekongres 2019, oral presentation
8. **Jensen, J. L.**, Schjønning, P., Watts, C. W., Christensen, B. T., Obour, P. B., Munkholm, L. J. 2019. Soil degradation and recovery – changes in organic matter and structural stability, Abstract from the 2019 ASA-CSSA-SSSA international annual meeting, oral presentation

The results of my Ph.D. study has been disseminated at other platforms as well:

1. Texture determination: Possibility for following international practice. 2015. Article in AGRO Biweekly, Internal newsletter at the Department of Agroecology
2. Change in analysis method can have far-reaching consequences. 2015. Article in AGRO Biweekly, Internal newsletter at the Department of Agroecology
3. Forholdet mellem ler og kulstof i jorden er vigtig. 2018. Article in the monthly newsletter from the Danish Centre For Food And Agriculture (DCA)
4. Indholdet af kulstof er ikke et tilstrækkeligt mål for jordens tilstand. 2019. Article in Effektivt Landbrug