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Master Thesis

Aggregate-associated carbon as influenced by management system and site characteristics

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Affirmation

I hereby certify that this thesis was written only by me, not using sources other than permitted and without use of any other illegitimate support.

Furthermore, I confirm that I have not submitted this thesis elsewhere, either nationally or internationally in any form.

Vienna, 17 December 2021

David Fabio Luger (*manu propria*)

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Abstract

Improving the soil structure is a key measure for soil management to enhance physical stabilization of soil organic carbon (SOC) in aggregates. However, there is a need to evaluate soil management effects in relation to site-specific benchmarks of soil structural quality and aggregate associated SOC. This study assessed the potential of innovative soil management (*Pioneer*) to improve selected aggregate stability indicators and SOC in distinct size fractions in comparison to common soil management (*Standard*) and a reference ecosystem (*Reference*).

Our results revealed a consistent effect of *Pioneer* to improve soil structure and aggregate SOC in different soils. A preliminary analysis across 21 study sites showed that *Pioneer* relatively increased soil aggregate stability (SAS) by 11.4 % (± 3.9 %) compared to *Standard*. This effect was highest in light soils with +16.8 % (± 7.3 %). Ultrasonication of aggregates helped to detect differences in the aggregate breakdown behavior and showed that *Pioneer* maintains higher aggregate stability across a wide range of disruptive energies. Higher proportion of stable SOC in microaggregates was reflected by increased release of dissolved organic carbon (DOC) at total aggregate breakdown.

Macroaggregate C was most responsive to *Pioneer* management. This was however only relevant for higher total SOC in a heavy soil with increased macroaggregate mass. Contrary, in a medium and light soil SOC was stored in microaggregates resulting in lower total aggregate SOC contents. Generally, the effect of *Pioneer* on both soil structure and aggregate SOC was strongest in light soils indicating higher improvement potential. However, despite the positive effect of *Pioneer*, it could only approach the level of *Reference* in the heavy soil. This indicated limitations for *Pioneer* to promote SOC stabilization in aggregates and suggests to focus more on soil fertility co-benefits provided through a better soil structure rather than measurable SOC pools.

Kurzfassung

Die Verbesserung der Struktur von ackerbaulichen Böden gilt als Schlüsselmaßnahme, um organischen Kohlenstoff (SOC) in Aggregaten zu speichern. Der Einfluss von Ackerbausystemen auf die Bodenstruktur und SOC sollte jedoch auf Basis von Standortpotenzialen erfolgen. Der Effekt von einem bodenaufbauenden Ackerbau (*Pioneer*) auf Aggregatstabilität und SOC in verschiedenen Größenfraktionen wurde im Vergleich zu einem herkömmlichen Bodenmanagement (*Standard*) und einem naturnahen Ökosystem (*Referenz*) bewertet.

Die Ergebnisse zeigten einen starken Effekt von *Pioneer* auf die Verbesserung der Bodenstruktur und Erhöhung von aggregat-gebundenen SOC. *Pioneer* erhöhte die Aggregatstabilität im Vergleich zu *Standard* um 11,4 % ($\pm 3,9$ %) auf 21 Standorten. Auf leichten Böden war dieser Effekt mit +16,8 % ($\pm 7,3$ %) am höchsten. Die Anwendung von Ultraschall zeigte einen verzögerten Aggregatzerfall und eine höhere Aggregatstabilität von *Pioneer* über einen weiten Bereich von Ultraschallenergien.

Der Effekt von *Pioneer* auf aggregat-gebundenen SOC war am stärksten in Makroaggregaten. Dies führte jedoch nur auf einem schweren Boden zu höheren totalen SOC Werten, da die hohe Aggregatstabilität die Speicherung von SOC in Makroaggregate ermöglichte. Im Gegensatz dazu wurde SOC in einem mittleren und leichten Boden vor allem in Mikroaggregaten gespeichert. Der aggregat-gebundene SOC und die Aggregatstabilität in *Pioneer* grenzte sich vor allem in leichten Böden von *Standard* ab. Eine Annäherung an das *Referenz* System war jedoch nur in schweren Böden ersichtlich. Dies zeigt, dass das Verbesserungspotential für Bodenstrukturqualität und SOC Speicherung in Aggregaten standortabhängig ist. Die Bewertung von bodenaufbauender Bewirtschaftung (*Pioneer*) sollte daher nicht nur auf Basis messbarer Potenziale erfolgen, sondern auch auf positive Nebeneffekte in der Bodenfruchtbarkeit bezogen werden.

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1. Introduction

1.1. The relationship between soil aggregates and soil organic carbon

Soils constitute the largest terrestrial C reservoir on earth. However, historically the conversion of native soils to agricultural land resulted in a loss of soil organic carbon (SOC) in the range of 36 to 78 % in the upper 30 cm (Sanderman, Hengl and Fiske, 2018). The protection of existing soil carbon stocks and promotion of SOC stabilization in croplands is thus imperative for improving soil fertility and ensuring soil ecosystem services upon climate change (Du et al., 2013; Lal, 2004). Processes controlling the synthesis, transformation and stabilization of SOC are closely linked to the quality of the soil structure and related level of aggregation and spatial arrangement of aggregates at a given time. Soil aggregate stability (SAS) is a fundamental soil property indicating the ability of the soil structure to withstand mechanical stresses through soil management or climate events while maintaining its physical arrangement (Six et al. 2000; von Lützow et al. 2008). Soil organic matter (SOM) compounds act as binding agents of soil mineral particles promoting thus stability and aggregation of the soil structure. Soil aggregates provide a complex spatial structure of solids and voids enhancing SOM persistence through spatial isolation and reduced microbial activity related to anaerobic conditions in fine pores (Kravchenko et al., 2015). This is reflected by a reduced SOM decomposition rate in aggregates compared to unprotected SOM (Sheng et al. 2020; Kong et al. 2005). Yet, the degree of SOM accessibility to soil decomposer varies with aggregate fraction size leading to a hierarchical stability of SOC within aggregates (J. Six et al. 2004; Totsche et al. 2018). This highlights the need to evaluate soil management effects on SOC stocks in distinct aggregate fractions indicative in their capacity to stabilize SOC.

According to the aggregate hierarchy concept of Tisdall and Oades (1978) the arrangement of aggregates is not simply a random mix of mineral particles and organic compounds but follows a hierarchical order where different binding agents act on certain stages of aggregate formation and stabilization. Macroaggregate (> 250µm) formation is induced by the input of fresh particulate organic matter (POM) that stimulates microbial activity and the production of microbial binding agents such as mucilage (Golchin et al. 1994; Jastrow, 1996; Six et al. 1999). Progressive decomposition of POM in macroaggregates is associated with the formation of microaggregates within macroaggregates due to gradual encrustation of decomposed POM with mineral and microbial binding agents (Six et al. 2002). This process is characterized by a time-increasing redistribution of C from macro-to microaggregates and was first observed by Angers et al. (1997) when tracing ¹³C-labelled wheat straw in soil aggregates. C content and quality differ across aggregate size class with macroaggregates having higher C contents composing of plant-derived labile POM (Elliott, 1986; Beare et al. 1994). In contrast, C content

decreases towards microaggregates but consists of more fine particulate and dissolved organic matter complexed via clay particles and microbial-derived binding agents. Based on this, Six et al. (2004) proposes two indicative characteristics for soils with present aggregate hierarchy: (i) lability and concentration of C increases with aggregate size whereas (ii) decomposition stage/age and stability of C is higher in microaggregates. The concept of formation of microaggregates within macroaggregates was repeatedly corroborated by several studies (Six et al. 1998; Gale et al. 2000; Puget et al. 2000; Deneff et al. 2007) and helped to model SOC losses influenced by soil management-induced destruction of macroaggregates.

- ✚ The persistence of SOC in aggregate size fractions differs due to different spatial accessibilities for decomposers. While big pores of macroaggregates provide sufficient space and air for decomposers to enter small anaerobic pores in microaggregates increasingly limit decomposer activity.

Soil management influences the dynamics of SOC and aggregate turnover by controlling the quantity and quality of organic matter inputs and the level of soil disturbance. Several studies reported higher SOC contents and aggregation in management systems with low tillage intensity and high organic matter input (Haddaway et al. 2016; Kong et al. 2005). High soil disturbance is related with frequent soil perturbation and accelerates the turnover of macroaggregates and related release of unprotected organic matter and microaggregates. According to Tisdall and Oades (1979) aggregate breakdown occurs when mechanical stress overcomes the attractive forces within aggregates. Short macroaggregate lifetime impairs the formation of stable microaggregates via binding of clay particles and microbial mucilage and limits thus the potential of C stabilization in microaggregates. In line with this, studies from Six et al. (2000) and Deneff et al. (2004, 2007) have shown that increases in SOC under reduced soil tillage regimes are not solely attributable to larger macroaggregate-C levels but also to slower macroaggregate turnover.

- ✚ Although, SOC stabilization occurs predominantly in microaggregates the turnover of macroaggregates needs to be focused from a soil management perspective. The longevity of macroaggregates controls SOC storage by enhancing the formation of microaggregates.

Several studies have postulated the concept of C saturation based on the observation that C pools saturate in a hierarchical order with microaggregates saturating at smaller C input than macroaggregates (Hassink, 1997; Stewart et al. 2007; O'Rourke et al. 2015). This is explained by a finite number of mineral surfaces for long-term SOC adsorption and limited aggregate

interspace for effective SOC protection at a given time (Hassink, 1997; Kleber et al. 2015). Yet, whereas the amount of mineral surfaces is given by site-specific soil pedological properties the protective capacity of aggregates is largely controlled by the turnover and formation rate of aggregates (Johan Six & Paustian, 2014). While SOC stored in microaggregates is considered as rather stable due to mineral-association and reduced microbial activity SOC in macroaggregates is largely composed of labile POM (Totsche et al. 2018; Toosi et al. 2017; Beare et al. 1994). Long-lived mineral associated organic matter (MAOM) is generally focused with regard to climate mitigation strategies in agriculture. It is suggested to define SOC storage potentials given by the maximum content associable with the < 20 μm fraction where SOC is predominantly stabilized through mineral-surface bonding (Hassink, 1997; Barre et al. 2017a). While this approach enables to derive site-specific SOC storage potentials and deficits according to the proportion of the < 20 μm fine-silt clay fraction it is conceptionally inappropriate in that it refers only to a fraction of total SOC (Barre et al. 2017b). Several studies found that land-use induced increases in SOC stocks were largely due to increased SOC stocks in the > 20 μm fraction where C is increasingly present as labile POM (Feng et al. 2014; Cardinael et al. 2015; Chimento et al. 2016). It may be thus important to focus also on labile POM in macroaggregates as it serves as a responsive indicator of soil management intensity and strongly contributes to total SOC levels (Jastrow et al. 1996; Barre et al. 2017). Having this in mind, diagnostic aggregate fractions may be used as indicators for potential losses of SOC upon changes in soil management intensity (Denef et al. 2007). In this sense, macroaggregates may be a good proxy for early SOC responses to soil management change whereas microaggregates can indicate SOC stabilization potential among different soil management systems (Angers and Giroux. 1996 ; Jastrow et al. 1996; Kong et al. 2005; Denef et al. 2004; Six and Paustian, 2014). Site specific SOC storage potentials can be related to SOC levels in natural vegetation - so called "reference SOC stocks" - representing maximum SOC levels under specific pedoclimatic conditions and land-use. This may help to evaluate the potential of best management practices to close SOC deficits in contrasting soils (Batjes, 2011; Barre et al. 2017; Henin and Dupuis, 2006).

- ✚ Individual aggregate size fractions may serve as diagnostic indicators for SOC responses to changes in soil management intensity. Macroaggregates having much labile organic matter may be an early indicator for the effect of management changes in SOC whereas microaggregates can serve as a predictor of the stabilization potential of different management systems. However, the improvement potential of soil management practices should be related to site-specific SOC stabilization potentials.

1.2. Study of aggregate breakdown behavior via ultrasonic dispersion

Ultrasound application is a widely accepted method to study aggregate stability as it allows for precise quantification of the disruptive energy input applied to soil aggregates (North, 1976; Amézketa, 1999; Mentler et al. 2004; Schomakers et al. 2011). Aggregate breakdown behavior gives information about the interplay of the main structural elements such as mineral and organic particles and organic molecules. Different chemical and physical composition of these aggregate building blocks result in a certain intensity of interaction and cohesiveness which influences the ability to resist disruptive forces. This variation of aggregate components cause different responses to disruptive energies and may result in a characteristic breakdown behavior for specific soils (Cerli et al. 2012; Kaiser and Berhe, 2014). While low disruptive energy may be enough to disintegrate coarse POM from macroaggregates it may be far too low to disperse silt-and clay sized SOM of microaggregates (Pronk, Heister and Kögel-Knabner, 2011). Application of ultrasonication energy is therefore meaningful as it allows to study aggregate breakdown over a wide range of disruptive energies and helps to detect fine-scale differences in aggregate stability (Schomaker et al. 2011). However, there is still no clear consensus about energy levels to extract discrete functional units of soil aggregates such as microaggregates or POM (Kaiser and Berhe, 2014). During aggregate dispersion the size of aggregates gradually decreases. Given the higher cohesive forces in microaggregates means that aggregate breakdown requires progressively more energy towards full aggregate dispersion (Kaiser and Berhe, 2014). Macroaggregate breakdown can start already at low sonication energy input ($< 2 \text{ J mL}^{-1}$) (North, 1976; Mentler et al. 2004; Six et al. 2004). Major part of the breakdown occurs at low ultrasonic energies up to 10 J mL^{-1} (Schomaker et al. 2011). Previous studies reported a wide range of energy needed for total disruption of aggregates. Total breakdown of aggregates is defined as the point where no further change in particle size distribution occurs (Kaiser & Berhe, 2014). So far, soil specific energy input to achieve total aggregate dispersion were observed to be below 800 J mL^{-1} (Hunter and Busacka. 1989; Oorts et al. 2005; Kaiser et al. 2012; Pronk et al. 2011; Schmidt et al. 1999; Yang et al. 2009). However, particle size distribution may not be a good indicator for total dispersion as clay-sized microaggregates have been observed to remain even after high energy input of $< 800 \text{ J mL}^{-1}$ (Chenu and Plante, 2006). Generally, high ultrasound energy application may cause chemical and physical modification of aggregate compounds and thus result in artefacts (Kaiser and Berhe, 2014). Low ultrasonic treatment is therefore recommended by Schomaker et al. (2011) for more refined studies of aggregate breakdown dynamics and associated release of organic carbon compounds.

- ✚ Ultrasound application is a widely accepted method to study the stability of aggregates over a range of disruptive energies. The application of different ultrasonic energies allows to isolate aggregate fractions with distinct stabilities. Low ultrasonic treatment ($< 10 \text{ J mL}^{-1}$) is recommended to maintain the composition of carbon release products that are important for the evaluation of breakdown behavior and account for soils with poor soil structure.

1.3. Hypothesis and objectives

This study aims to assess the potential of innovative soil management systems to improve soil aggregate stability indicators and SOC contents in distinct aggregate fractions.

Objectives

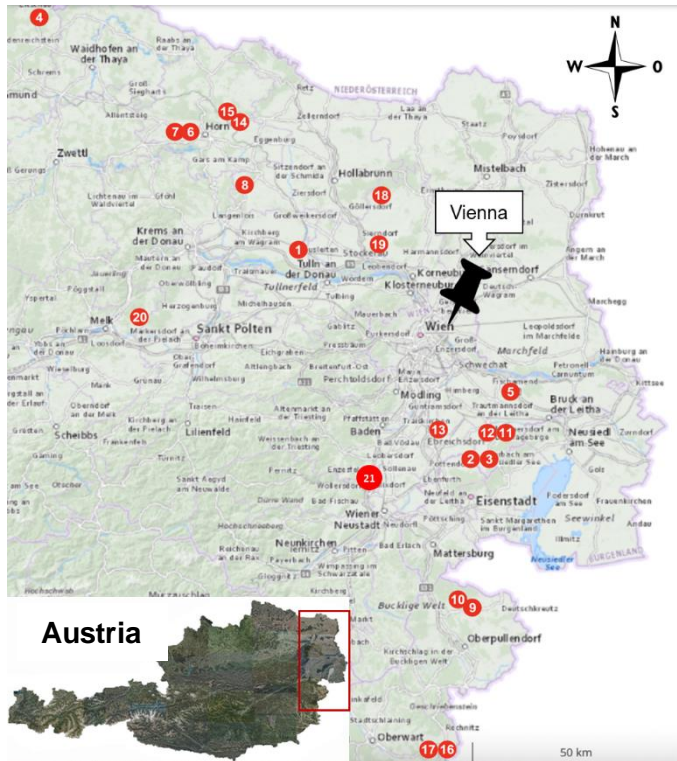
- 1) Investigating the relationship between soil aggregates and the soil organic carbon as influenced by the type of management systems and site effects
- 2) Refined study of soil aggregate stability under different soil management systems by characterizing the breakdown behavior of aggregate along a range of disruptive energies.
- 3) Evaluating the potential for increasing soil organic carbon and aggregate stability in contrasting soil types

Hypothesis:

- ❖ Soil organic carbon (SOC) promotes higher soil aggregate stability (SAS) and differs among management systems with Pioneer having higher SOC and SAS compared to Standard ([H1](#))
- ❖ Pioneer management improves SOC in distinct aggregate fractions indicative in their stabilization potential ([H2](#))
- ❖ The improvement of soil structure parameter and aggregate SOC under Pioneer management is limited by site-specific potentials to store SOC ([H3](#))

2. Methods

2.1. Experimental set-up



	Sites	Soil texture	Soil type (WRB)
1.	Absdorf	Heavy clay	Calcic Phaeozem
2.	Au am Leithagebirge 1	Light sandy soil	Leptosol
3.	Au am Leithagebirge 2	Light sandy soil	Leptosol
4.	Eisgarn	Light sandy soil	Leptic Cambisol
5.	Fischamend	Medium silty soil	Chernozem
6.	Groß Burgstall 1	Light sandy soil	Leptic Cambisol
7.	Groß Burgstall 2	Light sandy soil	Leptic Cambisol
8.	Grübern	Medium silty soil	Chernozem
9.	Lackendorf 1	Light sandy soil	Eutric Cambisol
10.	Lackendorf 2	Medium silty soil	Gleysol
11.	Mannersdorf 1	Light sandy soil	Haplic Phaeozem
12.	Mannersdorf 2	Medium silty soil	Fluvisol
13.	Moosbrunn	Medium silty soil	Phaeozem
14.	Rodingersdorf 1	Medium silty soil	Stagnosol
15.	Rodingersdorf 2	Medium silty soil	Luvisol
16.	Schachendorf 1	Medium silty soil	Stagnic Cambisol
17.	Schachendorf 2	Heavy clay	Gleysol
18.	Steinabrunn	Medium silty soil	Regosol/Colluvium
19.	Stockerau	Medium silty soil	Chernozem
20.	Umbach	Medium silty soil	Luvisol
21.	Theresienfeld	Light sandy soil	Leptosol

Figure 1: Austrian soil map with 21 study sites and the respective soil characteristics. Grey colored sites were chosen for in depth analysis of aggregate breakdown behavior and SOC distribution in aggregate fractions.

Soil samples for this study were taken from 21 study sites that are located in the semi-arid Eastern Austria (Figure 1). Besides common agricultural practices the farms of the study sites apply different innovative soil management measures aiming to improve soil fertility properties and increase SOC stocks. Transfer and generation of knowledge on innovative soil management measures of the study sites is partly obtained through science-farmer hub initiatives (“Boden.Leben”, www.bodenistleben.at, “Humusbewegung”, www.humusbewegung.at) during soil workshops and field excursions. On all study sites the following management systems are present:

- (i) Pioneer agro-ecosystem (Pioneer): High level of innovative soil conservation practices. High input of organic matter via compost, organic manures and cover crops, diverse crop rotations and reduced to no-till regimes. Not all measures are applied on all sites.

- (ii) Semi-natural reference ecosystems (Reference): Undisturbed semi-natural vegetation plot next to arable fields. The vegetation composes largely of grass but also shrubby vegetation.
- (iii) State-of-the art cropping system (Standard): Application of common agricultural practices. Relatively higher soil disturbance through tillage. Few input of organic matter and soil cover throughout the year.

Based on soil textural data and a pilot test assessing soil aggregate stability of all study sites topsoil samples (0-5 cm) of three contrasting soil types have been selected for this study aiming for high representativeness of the variability between the sample sites (see Table 1).

Table 1: Soil and site characteristics of three selected study sites are listed. The three sites were selected from 21 study sites to represent contrasting soil properties among all sites. Following soil management measures are characteristic for the selected sites: NT - No till, CC - intense cover cropping, OM - organic manure.

Site characteristics	Heavy soil	Medium soil	Light soil
Coordinates	48.405609° 15.972181°	48.512088° 16.206748°	47.585173° 16.498775°
MAP (mm)	577	564	667
Soil type	Chernozem	Regosol/Kolluvium	Eutric Cambisol
Soil texture	Clay	Silty loam	Loamy sand
Clay (%)	48.1	28.7	11.3
Silt (%)	38.7	45	28.8
Sand (%)	13.2	26.3	59.9
pH	7.8	7.9	7
EC (S/m)	142	137	90

The description of the management practices is not complete and serves only for rough differentiation in this study. A detailed analysis and evaluation of specific management practices of Pioneer, Reference and Standard systems is conducted by Scharf, B (in preparation). Detailed analysis of site-specific characteristics of the 21 study sites can be found in the master thesis of Steiner, P. (in preparation).

2.2. Soil sampling and processing

Soil sampling and on-field analysis of soil moisture and temperature were conducted between March and October 2020. On each of the study sites samples were taken for three at following sampling depths: 0-5 cm, 5-20 cm and 20-35 cm. The fields of the management system were located close to each other at < 200 m on all sites to ensure similar soil characteristics. Four subsamples were taken for each management system and sampling depth resulting in a total number of 21 x 3 x 3 x 4 (Sites x Systems x sampling depths x subsamples/replicates) – 756 – samples.

All samples were sieved to < 2mm under field moisture condition and filled into plastic bags. Air-dried subsamples were then pooled in equal amounts resulting in one mixed sample per sample site, soil depth and management system. From each mixed sample around 70-90 g of 2-1 mm sieved soil was collected for detailed analysis on aggregate stability and breakdown behavior at different disruptive energy levels. All samples were stored at room temperature for further analysis.

2.3. Lab analysis

2.3.1. Soil aggregate Stability (SAS)

Soil aggregate stability was assessed for all samples following the method of wet sieving as described in DIN-Norm 19683-16. 4 g of soil sieved to < 2 mm was put on 250 μ m sieves and dipped in steel beakers filled with 80 mL of deionized water. Through the rotating movement of the steel beakers the soil material on the sieves was repeatedly submerged over a time period of 5 min. This caused the breakdown of some aggregates that passed through the sieves. The remaining soil on the sieves was collected in porcelain dishes and dried overnight at 105 ° C. The dried samples were weighed and suspended with around 25 mL tetrasodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7 \times 10 \text{H}_2\text{O}$) to allow for full dispersion of soil aggregates during 2 h. Subsequently, the dispersed samples were rinsed through the 250 μ m sieve till the rinsing water was visually free of organic compounds. The remaining material (sand) was transferred to porcelain cups and dried overnight at 105° C for dry mass determination. The percentage of stable aggregates (SAS -%) was calculated using Eq. (1):

$$SAS -\% = (m_{A,S} - m_S) / (EW - m_S) \quad (1)$$

where $m_{A,S}$ is the mass (g) of stable aggregates and sand, m_S is the mass of sand and EW is the mass of the sample.

2.3.2. Aggregate breakdown – Method procedure

Aggregate breakdown curves were produced by following the change in mass of selected aggregate size classes when applying discrete sonication energy levels. A pilot test with low dispersive sonication energies and simultaneous continuous DOC release measurement was conducted with the samples of three sites (Heavy clay soil, Medium silty soil, Light sandy soil). This should help to establish a method procedure that is reproducible for all sites. Based on the characterization of DOC release curves of all study sites five sonication energy levels were selected for investigation of mass distribution at discrete levels of disruptive energies.

Table 2: Discrete energy levels were defined representing the onset, peak and point of no further DOC release of the continuous DOC release curves.

Energy level	Sonication time (s)	Phase of DOC release	Experiment
E1	0	Spontaneous decay	DOC release curve
E2	80	Onset of DOC release	DOC release curve
E3	140	Peak of DOC release	DOC release curve
E4	500	No further DOC release	DOC release curve
E5	1200	Total aggregate decay	Approximation of total decay

2.3.2.1. Ultrasonic energy determination

In a sonication pilot experiment with continuous DOC measurement conducted by Orracha et al. (in preparation) DOC release curves were produced for all study sites (n= 21). Based on this five energy levels expressed as the duration (s) of sonication treatment were identified (Figure 2).

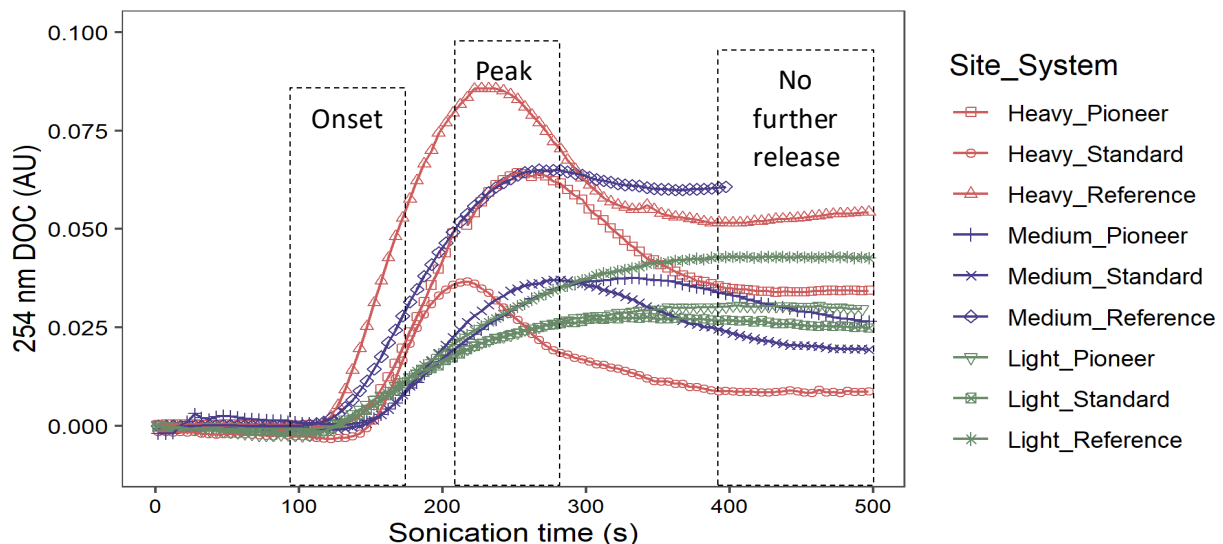


Figure 2: Continuous DOC release measurement during sonication over time. DOC was measured with UV-VIS at 254 nm. Discrete energy levels were derived from the Onset, the peak and the point of no more DOC release. AU = Absorbance unit. Heavy = Heavy clay soil; Medium= Medium silty soil, Light= Light sandy soil

Subsequently, a calorimetric calibration procedure was used to determine the energy input of the ultrasonic treatment over time (Figure 3): The ultrasonic probe was inserted with constant vibration amplitude of 1 μm into an aluminum beaker with 100 mL of water (mass m_w is 0.1 kg and specific heat capacity c_w is 4.18 J kg⁻¹). The increase in water temperature, ΔT was measured in constant time intervals of 20 s during the sonication time period Δt . The change of thermal energy of water is a function of the emission of acoustic pressure waves and the heat exchange between the sonication probe, sample beaker and the ambient air temperature. The heat exchange is caused by a temperature gradient given by the difference between the temperature of the water and ambient temperature. The ultrasonic power output can be directly linked with ΔT when the water temperature is equal to the ambient temperature. The sonication power P_{US} , per time unit, Δt , can be calculated with Eq. (2) in Schomaker et al. (2011):

$$P_{US} = m_w * c_w * \Delta T \Delta t^{-1} \quad (2)$$

The total sonication energy, E (J mL⁻¹), emitted to a soil-water suspension is determined calorimetrically and given by Eq. (3):

$$E = P * t \quad (3)$$

where P (W) is the power output in of the ultrasonic probe, t (s) is the time of sonication and V (mL) the volume of the soil-water suspension. With regard to our experimental conditions the ultrasonic power emitted into the soil-water suspension for the respective energy level is summarized in Table 3.

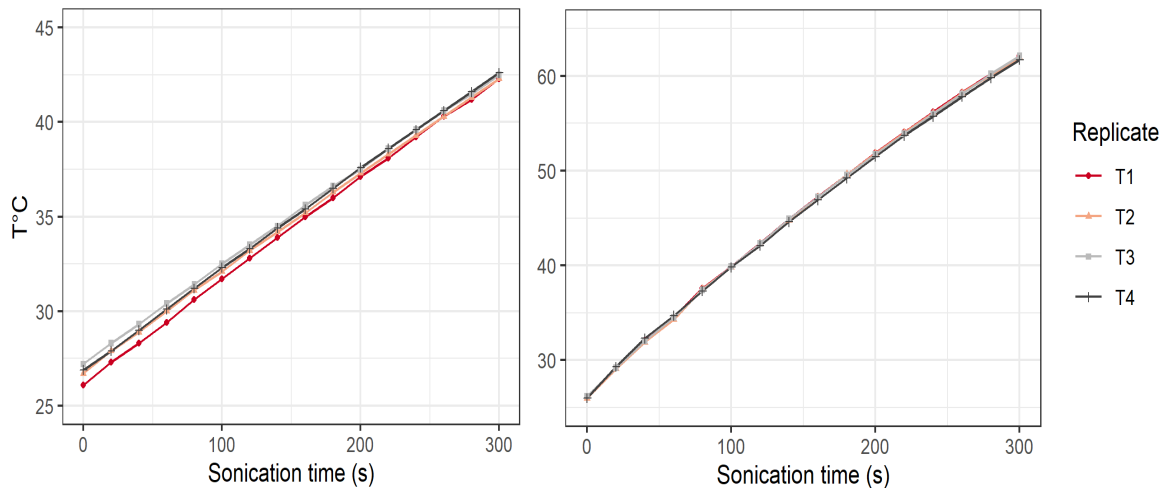


Figure 3: Calorimetric energy determination. Increase of water temperature with time of sonication. Temperature was measured at time intervals of 20 s with for replications T1, T2, T3, T4.

Table 3: Sonication energy levels derived from the continuous DOC release experiment. Energy in J mL⁻¹ was derived from a calorimetric energy experiment. Soil-water suspension refers to the volume that was used for sonication.

Energy levels	Sonication time (s)	Energy (J mL ⁻¹)	Soil-water volume (mL)
E1 (Spontaneous decay)	0	0	200
E2	80	1.87	200
E3	140	3.27	200
E4	500	11.66	200
E5 (Total breakdown)	1200	597	100

2.3.2.2. Calibration of high ultrasonic energy

The energy level E5 for total breakdown of aggregates was identified assuming that with sufficient disruptive energy the soil is dispersed entirely into single particles due to complete aggregate disruption. It is further assumed that this decay is associated with the release of strongly bounded DOC in microaggregates (Kaiser & Berhe, 2014; Mueller et al. 2012; Totsche et al. 2018). Therefore, three soils with known particle size distribution were used to identify the sonication power needed to reach up to 90 % of the actual particle size distribution of aggregate size fractions < 250µm.

For approximation of the total decay of soil aggregates a Bandelin Sonoplus HD 2200 ultrasonic equipment was used. The ultrasonic probe was shaped cylindrically and with a diameter of 30 mm. Ultrasonication was conducted at 50 % performance with a vibration amplitude of 1 µm. Insertion depth of the ultrasonic probe was 1 cm. 100 mL⁻¹ of deionized water and four grams of three homogenized test soils (1000-2000 µm) with known particle size distribution were placed in an aluminum beaker (Ø 44 mm). Two replicates were used for each soil. The aluminum beaker was wound with a copper tube connected with a peristaltic pump. Water was pumped through the tube to cool the soil-water suspension in the beaker and prevent the ultrasonic coil from overheating. Ultrasonication was applied for 2 x 600 seconds. Mass fractions of 2000-1000 µm, >250 µm, > 63 µm and > 20 µm were collected via the sieving tower. For comparison with the actual mass distribution only the microaggregate fractions 63 – 20 µm and < 20 µm were used because single sand grains may have biased the weight of bigger fraction significantly. Figure 4 shows the mass distribution of the microaggregate fractions for three contrasting soil types (heavy, medium and light soil). The mass distribution from the ultrasonication treatment (“approximated”) of all test soils deviated within a range of < 15 % of the actual mass distribution (“measured”) (Figure 4). Given an operational method

error of 10 -15 % during wet sieving we regarded this accuracy as sufficient to assume complete dispersion of all soil aggregates.

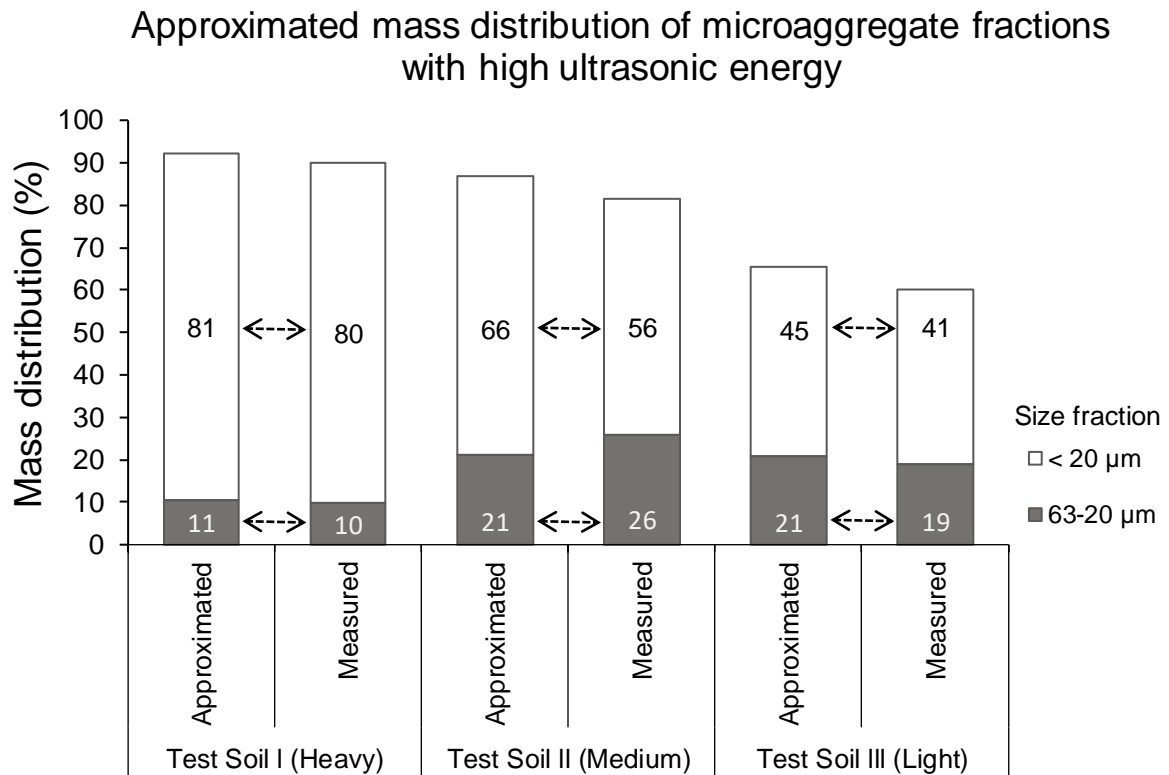


Figure 4: Comparison of the mass distribution after sonication treatment (“Approximated”) with the actual particle size distribution (“Measured”) of three test soils. Rounded values of mass distribution of the fractions are indicated in stacked bars in %. A heavy, medium and light test soil with known particle size distribution was used to evaluate the effect of high ultrasonic energy (597 J mL^{-1}) to cause total breakdown of microaggregate fractions.

2.3.2.3. Ultrasonic equipment

The aggregate breakdown behavior is characterized by the release of DOC at certain increase rate in response to the disruptive energy emitted by the ultrasonic probe. Ultrasonic dispersion treatments were performed with a self-modified ultrasonic dispersion device (Mayer, 2006). A titanium alloy probe with a cylindrical shape ($\varnothing 30 \text{ mm}$) was immersed into the soil-water mixture and vibrated at 20 kHz. Low vibration amplitudes ($1 \text{ }\mu\text{m}$) were chosen for comparison of samples with contrasting soil texture and aggregate stability to avoid immediate dispersion of weakly aggregated soils. Commercial ultrasonic dispersion devices have rather high-power settings and use voltage and current signals to control the magnitude of acoustic pressure waves emitted in the soil-water suspension Schomaker et al. (2011). According to Oorts et al. (2005) this leads to substantial differences between the displayed and the actual power output. In contrast, the extent of acoustic pressure waves at the self-modified ultrasonic device is controlled via the vibration amplitude. The selected vibration amplitude is measured by an

induction coil and highly correlates with the magnitude of the acoustic pressure waves that causes the dispersion of the soil material (Schomakers et al. 2015). The cumulative energy loading in J mL^{-1} is controlled with the time of ultrasonication.

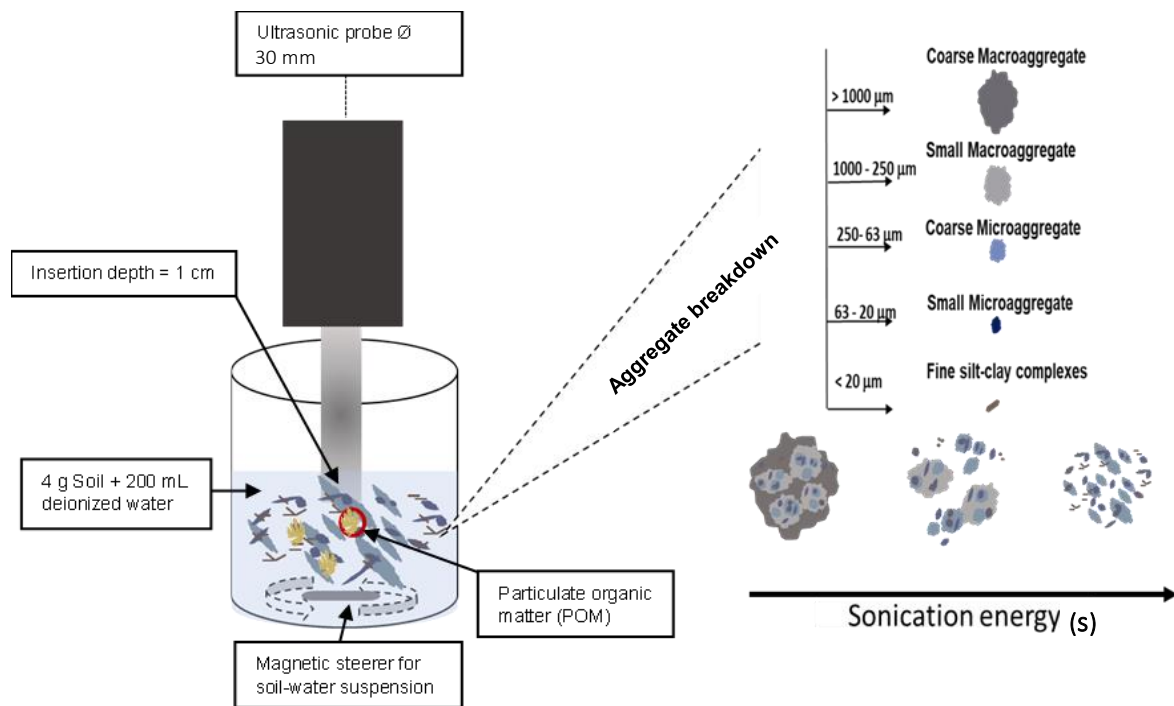


Figure 5: Set up of ultrasonic dispersion of soil aggregates and schematic aggregate breakdown along increasing sonication energy as a function of time (s). Energy amplitude was kept constant.

2.3.2.4. Ultrasonic dispersion

Four grams of air-dried and sieved soil (1000-2000 μm) were placed in a plastic beaker with 200 mL of deionized water. A magnetic steering device on the bottom of the beaker was used for homogeneous suspension throughout the whole ultrasonication treatment. Vibration amplitude was set constantly at 1 μm. Insertion depth of the ultrasonic probe was adjusted to 1 cm in the soil-water suspension. All samples were subjected to five defined energy levels (E1-E5). Spontaneous decay (E1) was conducted without sonication power. Samples were gently shaken in deionized water to achieve homogeneous suspension. Total decay (E5) was done in a separate experiment due to higher sonication power requirements and the need of continuous cooling during ultrasonication (see 2.3.2.2. Calibration of high ultrasonic energy).

2.3.2.5. Mass distribution

After sonication the soil-water suspension was transferred to a wet sieving tower (Fritsch Analysette 3 Pro). Standardized sieves were used to obtain the mass fractions of 2000-1000 μm > 250 μm , > 63 μm and > 20 μm . To limit the operational error of the remaining soil material the 20 μm fraction was collected separately in a small 20 μm sieve from the rinsing water. Ethanol ($\text{C}_2\text{H}_5\text{OH}$) was put in few amounts on the small sieve to reduce the water surface tension and avoid clogging. Sieving duration was set at 120 seconds with 1600 mL water. The collected mass fractions were transferred to aluminum cups and dried in an oven at 60°C for 24 h. The dry mass of the fractions was recorded precisely to three decimal places. The mass of the < 20 μm fine-silt clay fraction was derived from the difference between masses of all collected fractions and the sample mass of 4 g.

2.3.2.6. Ultrasonic soil aggregate stability (USAS)

Ultrasonic aggregate stability (USAS) was assessed for the macroaggregate fractions of > 250 μm to be equivalent with the SAS method (250 μm sieves). The percentage of stable aggregates at discrete absorbed sonication energy was determined according to Mentler et al. (2004) Eq. (4):

$$\text{USAS-}\% (E) = (m_{<250\mu\text{m}}(E) - m_s) / (EW - m_s) * 100 \quad (4)$$

where $m_{<250\mu\text{m}}(E)$ is the mass fraction > 250 μm that remained at the specific energy E . m_s is the mass of sand (> 250 μm) that refers to the sand collected in the SAS experiment for the same samples. EW is the original sample mass sieved to 1000 – 2000 μm .

The mean weight diameter (MWD) was determined to indicate the average dry aggregate size distribution (Youker and McGuinness, 1957). The calculation was done according to Kemper and Rosenau (1986) Eq. (5):

$$\text{MWD} = \sum_{i=j}^n X_i W_i \quad (5)$$

Where X_i is the mean diameter of each aggregate size fraction on sieve i , and W_i is the mass of the respective aggregate fraction on sieve i .

2.3.2.7. Dissolved organic carbon (DOC)

After each sonication treatment 2 mL were extracted from the dispersed soil-water suspension and transferred to Eppendorf tubes. Extraction ratio of DOC was 1:50 for E1-E4 and 1:25 for E5. This produced 3x3x5 (3 sites x 3 systems x 5 energy levels) samples for DOC measurement. All Eppendorf tubes were centrifuged for 10 min (15 000 g) at 10°C with a Heraeus Multifuge 3 S centrifuge. 100 µL were then collected with a micropipette and transferred to micro well plates. Spectral absorption was analyzed with a microplate reader at 254, 400 and 600 nm. DOC absorbance was quantified at 254 nm (A_{254nm}) according to the method of (Brandstetter et al., 1996) (Eq.6):

$$DOC = 0.449 \times A_{254nm} + 1.0 \quad (6)$$

Eq.6 quantifies DOC in the unit of mg L⁻¹ whereas the unit of absorbance (A_{254nm}) is given in m⁻¹.

2.3.3. Elementary analysis of soil organic carbon (SOC)

Elementary analysis C was conducted for all aggregate fraction that were produced after each sonication energy level. The dried soil material of the collected fractions was homogenized in a ball mill (Retsch MM 200). 2 mg of soil was weighed on a Sartorius fine weight scale (Sartorius AG, Germany) and transferred to small tin cups. Hydrochloric acid (HCl) was added for correction of inorganic carbon with an exposure time of 24 hours. The tin cups were then put to a Thermo scientific Flash smart Elementar Analyzer (Thermo Fischer Scientific. Inc. USA). Organic C in the samples was combusted at near 1000 T°C and quantified by a thermal conductivity detector. All values were given as %-w/w.

2.4. Statistical analysis

Statistical significance tests were performed with IBM SPSS Statistics 20 Software package for Windows XP. Graphical illustrations and correlation analysis were done with R Studio using the `ggplot2` and `ggpubr` package. Normality of data was assessed via visualization of data distribution and a Shapiro-Wilkinson's test. In case of non-normality data were log-transformed before statistical analysis. Figures were however not made with log-transformed data for graphical readability. For SAS, SOC and DOC of bulk soil differences in mean values were tested with a two-way analysis of variance with 1) management system and site and 2) with management system and soil depth as independent factors. Differences in breakdown parameters (MWD, USAS, DOC and mass distribution) were assessed using a MANCOVA with management system, site and energy level as independent factor.

SOC levels in aggregates were compared between systems and size fractions using a two-way ANOVA. Differences between factor levels were tested with a tukey post-hoc test. The level of significance was set to $p < 0.05$. N data were modified due to extreme outliers and not considered in the discussion. This was related to very small sample masses of some aggregate fractions after ultrasonication.

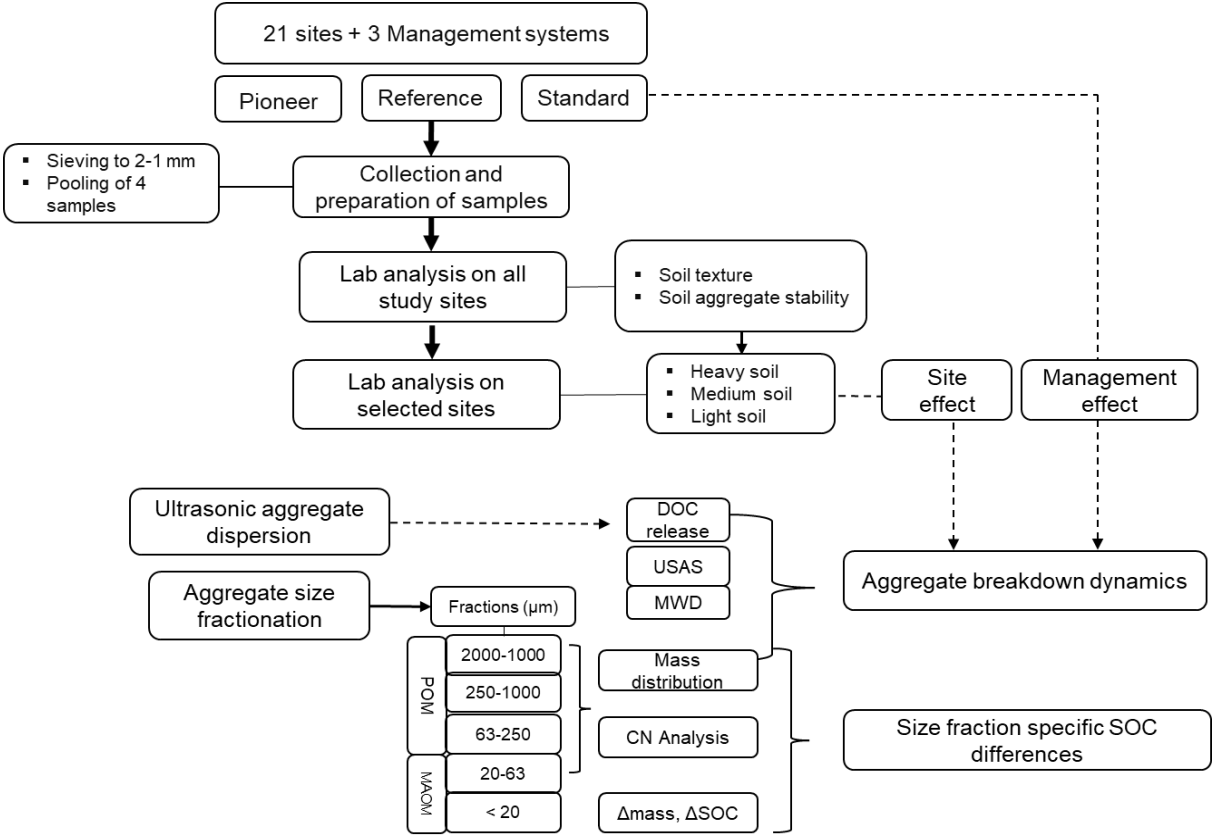


Figure 6: Flowchart of the working procedure of this study

3. Results

3.1. Soil aggregate Stability

A soil aggregate stability (SAS) test was conducted across 21 study sites with each having the three management systems: Standard, Pioneer and Reference. Management systems and soil depth did both influence the level of SAS significantly ($p < 0.05$) as single factor but without combined effect. The SAS analysis revealed the highest aggregate stabilities for Reference followed by Pioneer and Standard with a mean SAS of 73.1 %, 55.1 % and 48.8 % respectively, across all study sites. Reference differed significantly from both Pioneer and Standard only in the topsoil (0-5 cm) whereas in the lower soil depths (5 – 35 cm) it was significantly higher only than Standard. The comparison of mean SAS between the two arable systems showed lower aggregate stability for Standard compared to Pioneer in all depths although not significantly ($p > 0.05$). Generally, the management system differentiation was highest in the topsoil and decreased with soil depth. Pioneer systems had substantially lower SAS values than Reference only in the topsoil ($p = 0.048$) whereas this difference leveled out in the lower soil depths (Figure 7a). To assess the site effect on the level of SAS the 21 study sites were grouped according to USDA soil texture classes in heavy (clayey), medium (silty) and light (sandy) soils. Generally, soil types varied in SAS in the order of heavy > light > medium soils. The trend of higher SAS for Reference and Pioneer was the same in all soil type groups, although the difference between the management systems changed. It can be seen in Figure 6b that in light soils Pioneer is significantly higher than Standard. However, there is still a big difference between Pioneer and Reference that reaches its highest SAS level of all soil types (82.2 %). In medium soils there seems to be no effect of Pioneer on SAS given the small difference to Standard (42.4 % and 40.4 %) while Reference is much higher (65.2 %). In the heavy soils all management systems reach rather high SAS values with Reference and Pioneer both differing significantly from Standard (Figure 7b).

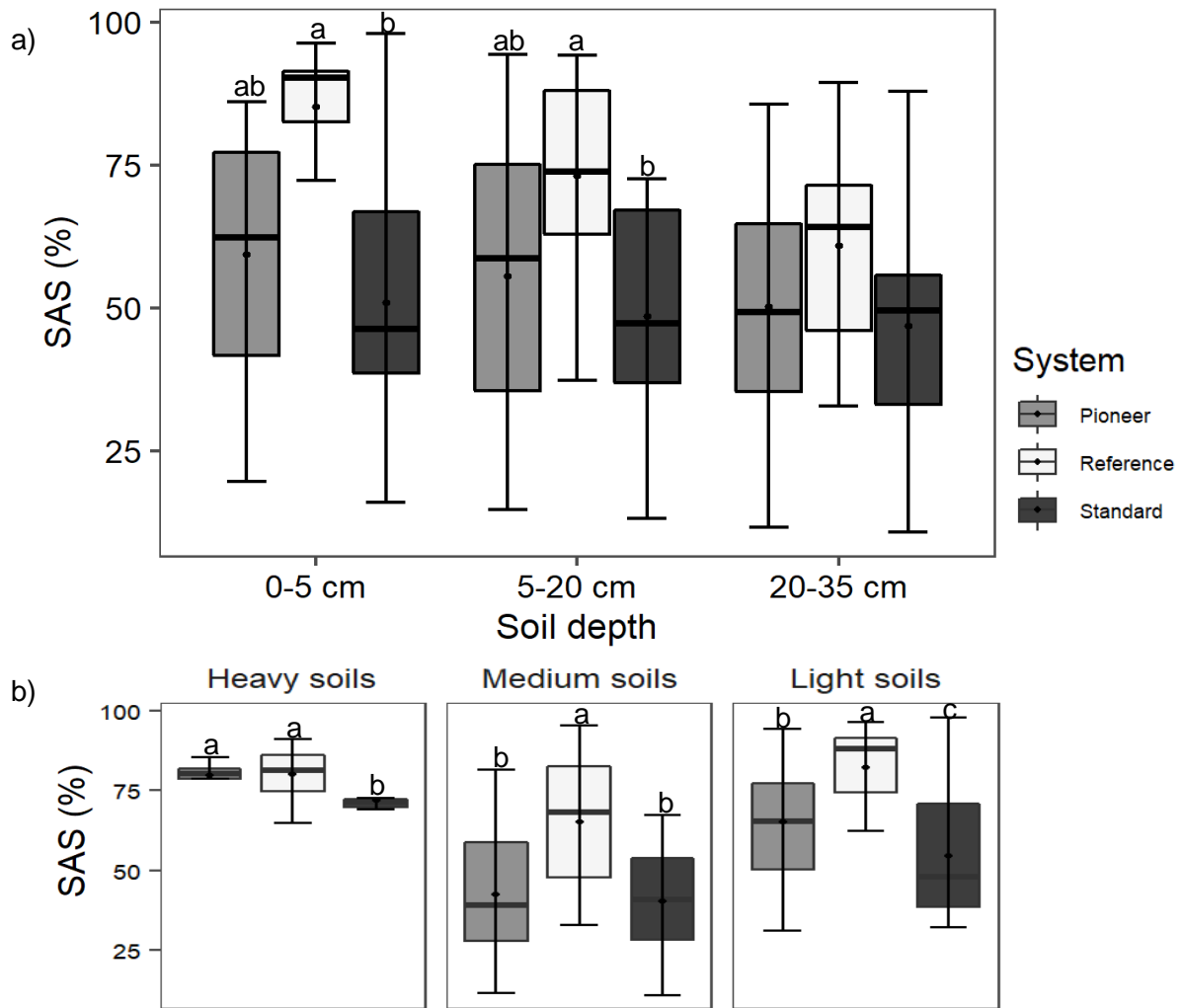


Figure 7: a) SAS comparison between management systems per each sample depths; b) Management differences in SAS among three soil type groups. Significant effects of the independent variables management systems and soil depth was tested with a two-way anova. Management differences at each soil depths were assessed via a tukey hsd post-hoc test. Significant difference between management systems within each soil depth is indicated by different lowercase letters. Main effects: System, $p < 0.01$; Site, $p > 0.01$; System*Soil depth, $p > 0.05$.

2.1.2. Soil aggregate stability relates to dissolved and soil organic carbon

Soil organic carbon (SOC) was highest in Reference soils with a mean value of 3.7 % across all sites and soil depths. A two-way analysis of variance was conducted for management systems with either soil depth or soil type as factor. This revealed intermediate to strong effect size of management system ($\eta^2 = 0.54$) and site ($\eta^2 = 0.74$) on the level of SOC. Following a Tukey HSD post-hoc analysis showed that the SOC content of Reference, 3.4 %, differed significantly from Pioneer, 2.4 %, ($p < 0.05$) and from Standard, 2 %, ($p < 0.01$). The comparison of means of SOC between the two arable systems revealed no significant difference although Pioneer showed higher SOC than Standard in all depths approaching the

Reference system in lower soil depths ($p > 0.05$). In contrast, Standard systems differed in all soil depths significantly from Reference systems (Appendix, Figure Ap.4).

The general relationship between SAS and SOC was tested in a correlation analysis which revealed an intermediate strong correlation ($r = 0.73$, $p < 0.01$). The degree of correlation between SAS and SOC increases slightly from Standard - Pioneer - Reference. It can be seen from the fitted regression line in Figure 8a that the increase rate of SAS levels off at lower SOC values at Standard systems whereas it increases further in Pioneer and Reference up to higher SOC values. While in Pioneer the SAS rises continuously with higher SOC values it stagnates clearly at Standard around 2 % SOC and at Reference around 3.7 % SOC. The correlation of SAS and SOC was also tested among soil type groups. It can be seen that the strength of correlation with SOC differs between soil types. While SAS increases most strongly with higher SOC in medium and light soils, there is only a weak insignificant correlation in heavy soils. In medium and light soils SAS rises continuously up to 6 % SOC (Figure 8b). Correlation coefficients of SAS and other soil physical and chemical parameters are summarized in a correlation matrix in Figure Ap.7 (Appendix).

For Dissolved organic carbon (DOC) the management systems differed in the same order as SAS and SOC with Reference showing the highest DOC with a mean concentration of 0.34 mg g^{-1} followed by Pioneer with 0.22 mg g^{-1} and Standard with 0.17 mg g^{-1} . The effect of management system ($\eta^2 = 0.63$) and site ($\eta^2 = 0.40$) was both significant ($p < 0.05$) with management system being the strongest explanatory factor. Multiple comparison of DOC between management systems revealed significant differences between all systems with the strongest difference of Reference to Pioneer and Standard. Management differentiation was strongest in the upper soil depth. Standard had significantly lower DOC concentration in all soil depths than Reference which was approached by Pioneer in the lowest soil depth ($p > 0.05$). Heavy soils had in average the highest DOC content with 0.24 mg g^{-1} compared to light soils with 0.25 mg g^{-1} and medium soils with 0.22 mg g^{-1} ($p > 0.05$). In medium soils the effect of Pioneer on the DOC content was lowest indicated by the small difference to Standard systems but much lower DOC values than Reference. Generally, the DOC content of Standard hardly differed between the sites while Pioneer increased from heavy to light soils (see Appendix for bulk DOC results, Figure Ap.5). A correlation analysis between SAS and DOC showed an intermediate positive relationship over all sites and management system. This correlation was found also within all management systems and soil type groups (heavy, medium and light soils). Whereas SAS had a clear positive relationship with DOC in Pioneer and Reference systems, it did not clearly increase with higher DOC content in Standard systems (Figure 9a). In soil type groups SAS correlated with DOC most strongly in medium and light soils while in

heavy soils there was no clear trend indicated. However, the correlation strength of heavy soils is not comparable due to the low number of sites in this soil type group ($n = 18$) (Figure 9b).

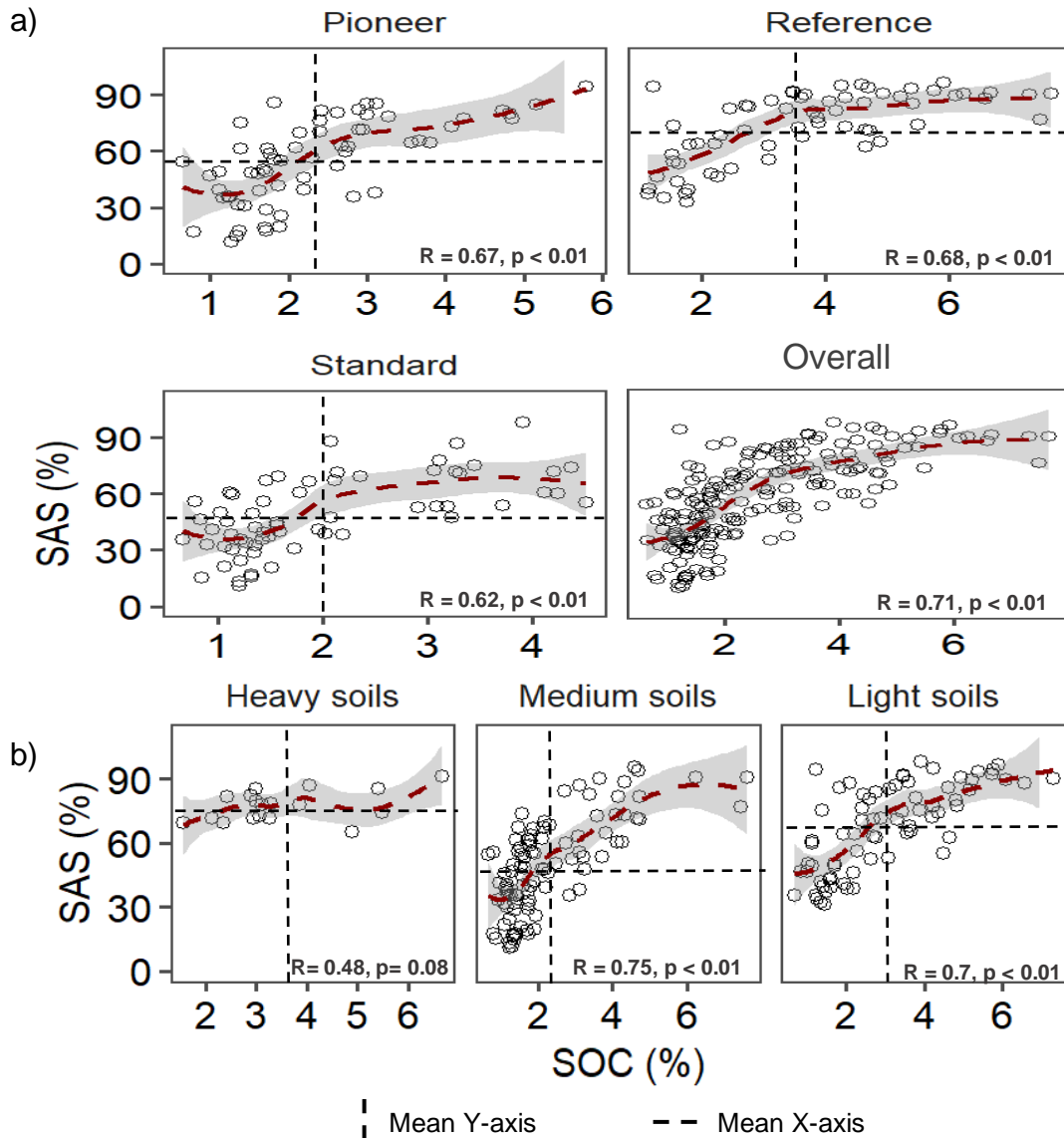


Figure 8: a) The relationship between SAS and SOC is depicted in scatterplots. Correlation coefficients and fitted regression lines are shown for each single management system and for overall b) the relationship between SAS and SOC is shown in a scatterplot for three groups of soils. Vertical dashed lines indicate arithmetic mean values of x-axis variables. Horizontal dashed lines show arithmetic mean of the y-axis variable

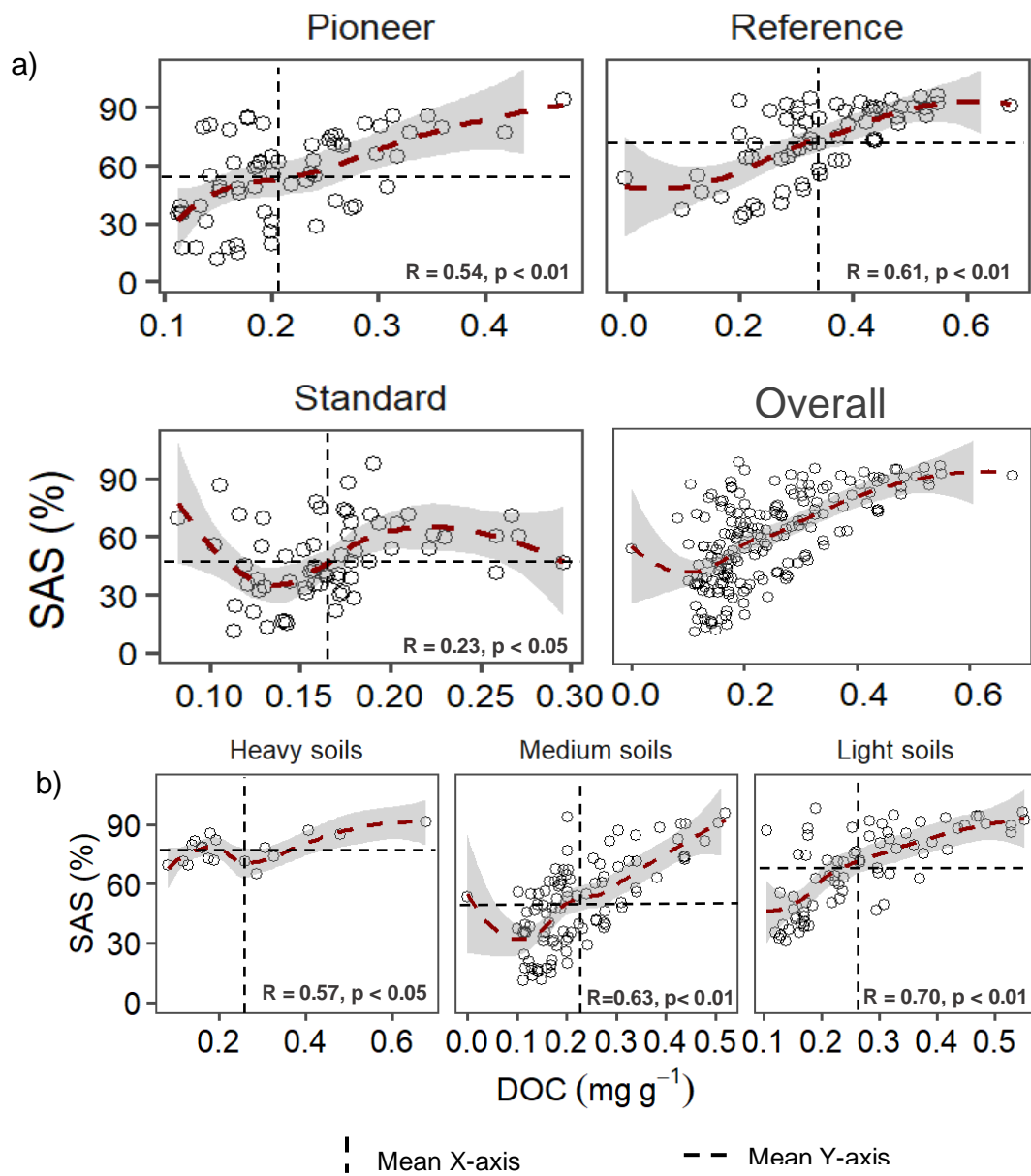


Figure 9: a) The relationship between SAS and DOC is depicted in scatterplots. The correlation between SAS and SOC is shown for each single management system and for all systems ("Overall"); b) the relationship between SAS and SOC is shown in a scatterplot for three groups of soils. Vertical dashed lines indicate arithmetic mean values of x-axis variables. Horizontal dashed lines show arithmetic mean of the y-axis variable.

3.2. Aggregate breakdown behavior

Samples of three soil types, clay soil (heavy), silty soil (medium) and a sandy soil (light) were subjected to dispersion at five different sonication energy levels to assess the aggregate breakdown behavior by means of selected breakdown parameters: Ultrasonic aggregate stability (USAS) in %, Mean weight diameter (MWD) in μm , release of dissolved organic matter (DOC) in mg L^{-1} and the ratio of total DOC to SOC (DOC:SOC). The combined and single effect of management system and soil type on all breakdown parameter was tested via an one- and two way anova. This revealed a strong significant effect of soil type on all parameters except the release of DOC ($p < 0.01$). The effect size of soil type as factor was biggest for the change of MWD during aggregate dispersion ($\eta^2 = 0.75$). Management systems as factor significantly influenced the variation of all breakdown parameter with the highest effect size for the release of DOC ($p < 0.01$; $\eta^2 = 0.5$). As there were no replicates for the combination management system*soil type*energy level statistical differences in breakdown parameters within soil type are not indicated but only described.

3.2.1. Ultrasonic aggregate stability (USAS)

The measurement of breakdown behavior during dispersion via sonication energy showed the highest USAS for Reference with a mean value of 41.5 % compared to 24.4 % of Pioneer and 17.5 % of Standard. Generally, USAS was negatively correlated with increasing energy level illustrating the progressive breakdown of soil aggregates ($r = -0.6$) (see Figure 10a). Comparison of USAS between management systems showed significant difference of Reference compared to both Pioneer ($p = < 0.05$) and Standard ($p = < 0.01$) while the USAS of Pioneer and Standard did not differ significantly from each other ($p > 0.05$). The difference in USAS between all management systems increased slightly till E4 (11.66 J mL^{-1}), although not significantly ($p > 0.05$). For soil types the USAS was highest in the heavy soil (40.89 %) followed by the light soil (25.51 %) and medium soil (17 %). Within soil types the management systems differed in their USAS in the order of Reference > Pioneer > Standard. The difference in USAS between Reference and both arable systems is highest in the medium soil, while in the heavy soil all management systems have rather high USAS values. In the light soil Standard (9.14 %) is substantially lower than both Pioneer (28.32 %) and Reference (39.07 %) (Appendix, Figure Ap.9, Table Ap.17).

When comparing the change of USAS among energy levels till E5 (597 J mL^{-1}) the strongest change can be observed between E1-E2 ($1.87 - 3.27 \text{ J mL}^{-1}$) overall systems and soil types. Regarding management systems, Standard is facing the strongest change with 37 % (32 to 20 %) compared to Pioneer with 31 % (42 to 29 %) and Reference with 18 % (60 to 49 %) (see Table 4). In contrast, the change in USAS of all management systems is rather low till E4 (11.6

J ml⁻¹) ($p > 0.05$). This was particularly observed in Reference systems where there is hardly any change with increasing dispersion energy up to E5. The mean relative change of USAS differed among soil types in the order of light > heavy > medium soil (48.49 %; 37.75 % and 36.5 %, respectively). USAS values at maximum sonication energy level were not used for statistical comparison due to very low values near zero.

3.2.2. Mean weight diameter (MWD)

As a result of the progressive breakdown during aggregate dispersion the MWD decreased with increasing sonication energy. Differences between management systems can be seen till E4 with Reference maintaining the highest MWD (115.8 μm) compared to Pioneer (72.7 μm) and Standard (63.7 μm). The change in MWD was most pronounced between E1-E2 at all management systems. Pioneer and Standard showed higher changes with 13 % (88 to 76.1 μm) and 11 % (74 to 66 μm) compared to Reference with 3 % (133 to 129 μm) (see Table Ap.13). It can be seen in Figure 10b, that there is hardly any change of the MWD of Reference systems prior the total breakdown of aggregates while the MWD of Pioneer and Standard decreases by 12 % and 27 %, respectively. Besides, it can be seen that both Reference and Pioneer show a greater drop in MWD with the maximum dispersion energy than Standard (Figure 10b).

The MWD of the light soil remained at the highest level with a mean value of 136 μm over all energy levels and management systems. This was in contrast to the MWD of medium soil which was in average the lowest with 40.22 μm . However, the relative change of MWD prior total aggregate breakdown was greatest in the heavy soil with a decrease of 25 % while there was no change in the medium and sandy soil. Pioneer systems did not much influence the MWD in the medium soil. This is indicated by the fact that it does not differ much from Standard (19 μm ; 16.8 μm , respectively) while the MWD of Reference is much higher (84.9 μm) (see Appendix, Figure Ap.9)

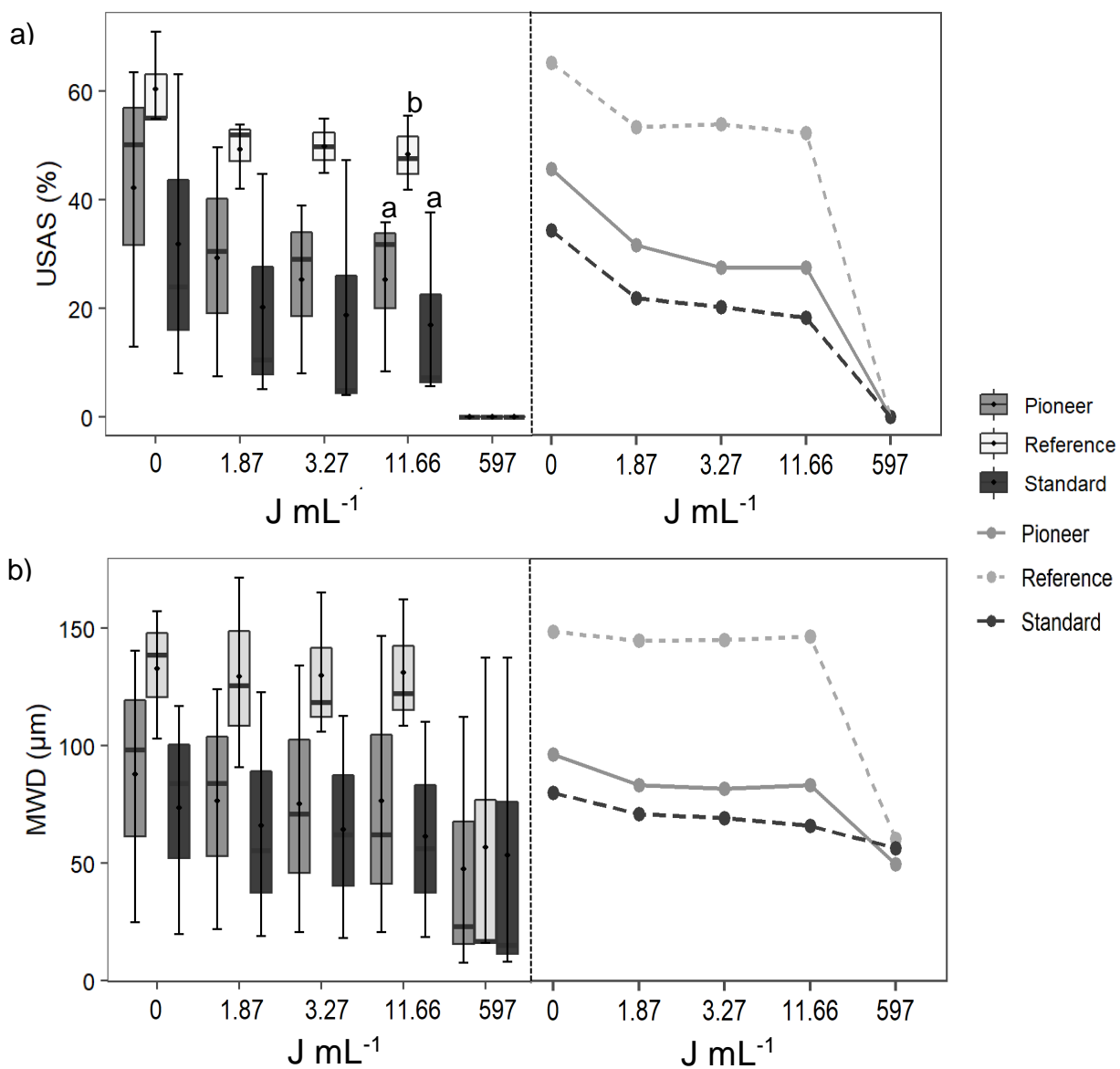


Figure 10: a) Boxplots of ultrasonic aggregate stability (USAS) and b) mean-weight diameter (MWD) compared between three management systems for three discrete disruptive sonication energy levels. Significant differences between management systems are shown as different lowercase letters following a one-way anova ($p < 0,05$) and tukey hsd post-hoc test. All values are listed in Table Ap.17, Appendix

3.3.1. Release of dissolved organic carbon (DOC)

DOC was released during aggregate dispersion in a positive relationship with increasing energy level ($r = 0.5$) at all management systems (Figure 11a) and soil types (Appendix, Figure Ap.9). The average release of DOC was highest at Reference with 6.84 mg L^{-1} and differed significantly only from Standard with 3.2 mg L^{-1} ($p < 0.01$). The DOC release of Pioneer was with 4.4 mg L^{-1} not significantly different from Reference. When comparing the DOC release between the systems for each energy level it reveals that management differentiation occurs with increasing level of dispersion energy. The DOC release of Reference differed significantly from Standard at E4 whereas it did from Pioneer only at E5 ($p < 0.05$) (see Figure 11a). The relative change of DOC release prior total aggregate breakdown of Reference system was strongest between E1 and E2 where the DOC release increased by around 50 %. This occurred latter in Pioneer systems between E2-E3 and E3-E3 with 23.6 % and 18.7 % increment of DOC release. Contrary, in Standard systems the strongest increment in DOC release prior total decay was between E3-E4 (see Table 4). Generally, the release of DOC of Standard occurred at lower level and with higher sonication energy. While in Reference and Pioneer systems the DOC release was more evenly distributed over all energy levels. Additionally, it can be seen in Figure 11a that the increment of DOC release at E5 increases in the order of Reference > Pioneer > Standard. This indicates that there was more DOC in aggregates of Reference and Pioneer retained and only released upon total aggregate breakdown. The average DOC release across all energy levels and management systems differed weakly between soil types and was highest in the heavy soil followed by medium and light soils (5.21 , 4.87 and 4.36 mg L^{-1}). Accordingly, the factor site had no significant influence on the amount of DOC release ($p < 0.05$). The level of DOC release decreased at all soil types in the order of Reference>Pioneer>Standard. While in the light soil there was not much difference between all systems at E5, the Reference systems stands out with its total DOC release being three times higher than of Pioneer and Standard (17.87 , 5.43 and 5.56 mg L^{-1}). The relative change of DOC release was especially strong at E5 in the light soil for all systems. Contrary in the medium soil the relative change of DOC release at E5 was only high for Reference with an increment of around 200 %. In the heavy soil there Reference and Pioneer show a strong pulse DOC release by 225 and 122 % respectively (Figure Ap.8).

3.3.2. DOC:SOC ratio

The ratio of DOC:SOC generally increased with higher energy reflecting the higher DOC release upon progressive aggregate breakdown. It can be seen from Figure 11b that the management systems differ mainly at lower energy levels while they approach each other at E5. Standard holds the highest DOC:SOC across all energy levels meaning more DOC release relative to the SOC content of the bulk soil. Although, the DOC release of Reference was

highest its DOC:SOC (5.91) is the lowest compared to Pioneer (8.11) and Standard (9.17) meaning less DOC relative to its SOC content. Soil types differed in their DOC:SOC in the order of medium>light> heavy soil (8.68, 8.12, 6.36). Generally, it can be seen that the management systems differentiate more in DOC:SOC than in of the DOC release. This shows the effect of different SOC levels of the management systems. The DOC:SOC of management systems was higher for Standard in the medium and light soil. The difference in DOC:SOC between management systems was high across all energy levels in the light soil. In the heavy soil management differentiation occurred only at E5 where the DOC:SOC of Standard decreased contrary to Reference and Pioneer. This was caused by the low increment of DOC release of Standard at E5.

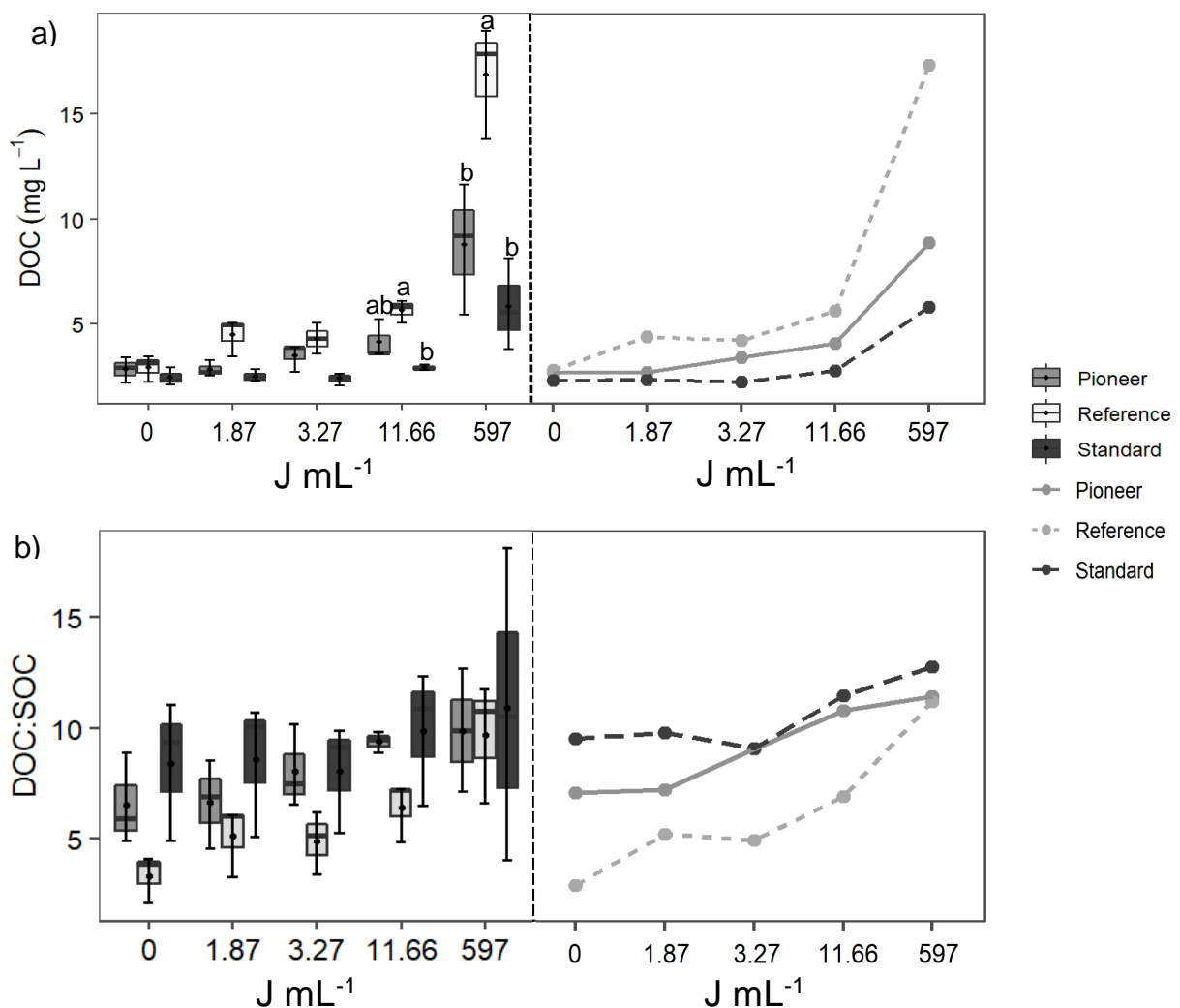


Figure 11: a) Boxplots of released dissolved organic matter (DOC) and b) the ratio of DOC:SOC indicating the relative release of DOC during aggregate dispersion compared between three management systems for five discrete disruptive sonication energy levels. Significant differences between management systems are shown as different lowercase letters following a one-way anova ($p < 0,05$) and tukey hsc post-hoc test. In case of no significance there are no lowercase letters indicated. All values are listed in Table Ap.17, Appendix.

Table 4: Relative change energy levels (E1-E5) of the breakdown parameter USAS, MWD, DOC release and DOC:SOC ratio are averaged over three sites (n=3) for the management systems Pioneer, Standard and Reference. Values for relative change are given in percent. Energy levels (J mL⁻¹): E1= 0; E2=1.87; E3= 3.27; E4= 11.66; E5= 597.

Parameter	Energy	Pioneer		Reference		Standard		Total	
		Mean	SD(±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)
ΔUSAS (%)	E1-E2	30.72	11.01	18.20	13.6	36.62	14.01	28.51	8.15
	E2-E3	13.30	9.103	1.05	6.95	7.13	33.19	7.16	5.30
	E3-E4	0.01	2.601	3.02	2.882	9.93	39.55	4.32	4.40
	E4-E5	100	0	100	0	100	0	100	0
	Total	36.01	40.23	30.57	42.44	38.42	39.03	35.00	39.56
ΔMWD (%)	E1-E2	12.78	1.573	2.59	1.364	10.56	16.88	8.64	4.64
	E2-E3	1.66	5.508	0.26	6.781	2.32	3.559	1.41	0.91
	E3-E4	1.59	5.947	0.96	0.884	4.33	4.331	2.29	1.55
	E4-E5	37.73	22.65	56.65	40.49	13.23	24.58	35.87	18.85
	Total	13.44	15.40	15.11	25.06	7.61	4.64	12.05	17.01
ΔDOC (%)	E1-E2	0.27	6.643	52.48	5.983	2.15	2.375	18.30	25.65
	E2-E3	23.63	18.05	3.69	6.672	4.81	3.317	10.71	9.70
	E3-E4	18.68	15.45	31.55	14.73	22.11	12.44	24.12	5.77
	E4-E5	111.65	54.84	198.77	26.17	101.13	85.15	137.18	46.42
	Total	38.56	45.00	71.62	78.78	32.55	42.12	47.58	58.72
ΔDOC/SOM (%)	E1-E2	5.56	6.643	52.48	5.983	2.19	2.375	20.08	24.35
	E2-E3	5.65	18.05	4.04	6.672	6.23	3.317	5.31	0.98
	E3-E4	7.05	15.45	30.74	14.73	22.60	12.44	20.13	10.43
	E4-E5	8.36	9.311	50.34	13.09	10.05	23.11	22.92	20.58
	Total	6.66	1.20	34.40	20.33	10.27	7.98	17.11	17.51

3.4. Management and soil type effects on SOC in aggregate fractions

Following a two-way ANOVA revealed a significant single effect of the factors management system and soil type on the level of aggregate SOC ($p < 0.01$). Generally, the management systems differed in the order of Reference>Pioneer>Standard (3.36, 2.29, 1.42 % SOC). Reference showed in average the highest level of aggregate SOC which was significantly higher than Pioneer and Standard ($p < 0.01$). The difference between Pioneer and Standard was not significant ($p= 0.051$) (Figure 12a).

The level of aggregate SOC among soil types differed in the order of heavy > medium > light soil (2.96, 2.52, 1.59 %). Generally, the order of management system differentiation in aggregate SOC was the same in all soil types with Reference showing higher SOC values compared to Pioneer and Standard. Reference differed from Standard significantly on all soil

types but from Pioneer only in the light soil. Contrary, in the heavy and medium soil the effect of Pioneer on aggregate SOC is more prominent and indicated in that Reference does not differ substantially from Pioneer ($p > 0.05$) while it does from Standard ($p > 0.05$). The difference between Pioneer and Standard in aggregate SOC is lowest in the light soil. However, both Pioneer and Standard differ the most from Reference. Accordingly, Reference shows higher aggregate SOC (3.02 %) by at least 2 percent points compared to both Pioneer (1.1 %) and Standard (0.65 %) (see Figure 12b).

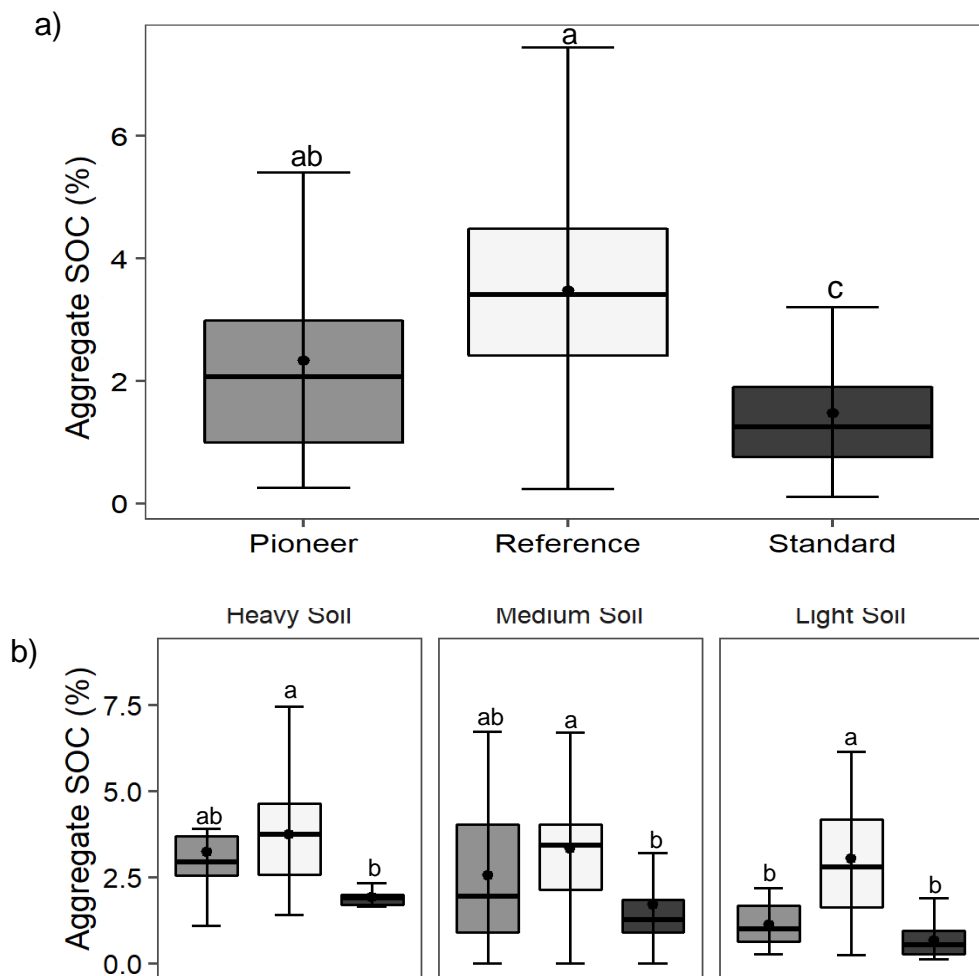


Figure 12: Boxplots of total SOC in all collected aggregate fraction (< 20 μm not included) a) shows the comparison of total level of SOC in aggregates between management systems b) shows management system differences among contrasting soil types. Differences between factor levels were assessed conducting a tukey hsd post-hoc test. Significant differences are indicated by lowercase letters.

3.4.1. Management effect within aggregate fractions

Management system as factor had a significant effect on the distribution of SOC among aggregate fractions and system within each fraction ($p < 0.05$). As we did not collect the $< 20 \mu\text{m}$ fine-silt clay fraction the respective SOC contents and aggregate mass of this fraction were derived from difference calculation between the total SOC in aggregates and total SOC in bulk soil. The distribution of SOC differed across aggregate size fraction in the order of $1000 \mu\text{m} > 250 \mu\text{m} > 20 \mu\text{m} > 63 \mu\text{m}$ when averaging it over all three sites. Analysis of variance showed however no difference of means among aggregate fractions ($p > 0.05$). This was in line with very low effect size and insignificant influence of aggregate size fractions as factor ($p > 0.05$) (Figure 13b). The content of aggregate SOC between management systems showed the same order in all aggregate fractions with Reference $>$ Pioneer $>$ Standard. However, management system differentiation varied between aggregate fractions. Statistical difference was found between Reference and Standard in the $>1000 \mu\text{m}$ and $>20 \mu\text{m}$ fraction where Reference showed its highest SOC contents ($p < 0.01$). Pioneer did not show significantly lower SOC in any fraction and could best approach Reference in the $1000 \mu\text{m}$ fraction (see Figure 13a).

Figure 13c depicts the distribution of SOC g per kg fraction mass (SOC mass) among management systems within aggregate fractions. SOC mass was calculated by multiplying SOC contents (%) with the mass of the respective aggregate fraction. It can be seen that aggregate mass of Reference is especially high in $1000 \mu\text{m}$ followed by Pioneer and Standard. This trend reverses in smaller aggregate fractions where Standard accounts for higher aggregate mass. SOC mass is highest for Reference in all aggregate fractions with the biggest difference to Pioneer and Standard in the $1000 \mu\text{m}$ and $<20 \mu\text{m}$ fraction. The SOC mass of Pioneer is generally higher than Standard ($p > 0.05$) and differs most strongly in the $1000 \mu\text{m}$ fraction.

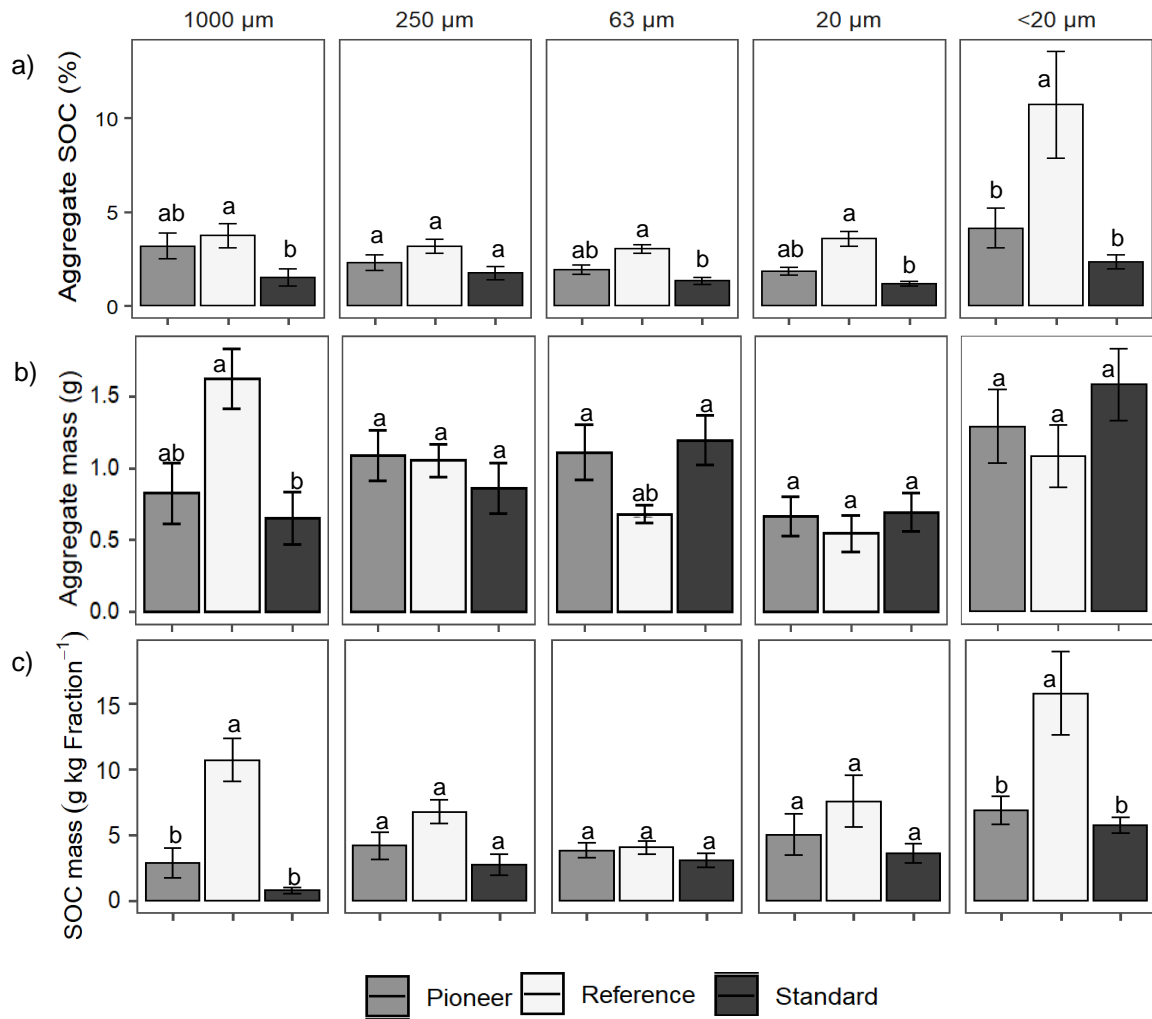


Figure 13: a) Management system comparison of SOC mass at aggregate fraction level, b) SOC in % in aggregate fractions and c) Mass distribution of aggregate fractions. Aggregate mass in g is referred to 4 g sample mass. Error bars on bar plots indicated standard deviation. Fractions are indicated by the numbers on top of each plot. Significance was tested with a one-way anova and tukey hsd. Significant differences are indicated by different lowercase letters. * <20 μm fraction was not physically collected. SOC content was derived by difference calculation between the total SOC in aggregates and bulk soil. Aggregate mass was determined as the difference to the sample mass of 4 g.

3.4.2. Management effect within sites

The relative share of SOC in aggregate fractions was compared separately between soil types and between management systems to illustrate their individual effects. It can be seen in Figure 15 that among soil types the SOC level in the $< 20 \mu\text{m}$ fraction increases from heavy to light soils. Accordingly, the relative share of SOC of the $< 20 \mu\text{m}$ fraction in light soils is significantly higher compared to the one of the medium and heavy soil ($p < 0.05$). The inverse trend can be observed with macroaggregate fractions ($>1000 \mu\text{m}$, $>250 \mu\text{m}$) where SOC levels increase in the order of light $<$ medium $<$ heavy soil ($p > 0.05$). Between management systems there is a clear trend of macroaggregates storing relatively more SOC in Reference and Pioneer systems.

The distribution of SOC among aggregate fractions varies between the soil types. Accordingly, there is a clear pattern in light soils of storing more SOC in smaller aggregate fractions. This is in contrast to medium and heavy soil where macroaggregate fractions account for the highest SOC levels. In the heavy soil Pioneer and Reference differed most clearly from Standard in coarse macroaggregates owing to high SOC contents of 5 % and 5.34 % respectively ($p < 0.01$). A similar picture can be seen in the medium soil where Reference and Pioneer showing much higher aggregate SOC contents in contrast to Standard (4.6 %, 4.1 %, 2.5 % SOC, respectively) ($p > 0.05$). In the light soil Pioneer and Reference had higher aggregate SOC levels in all fractions and differed significantly from Standard in the 1000 μm , 250 μm and 63 μm fraction ($p < 0.05$) (Figure 14a).

Reference and Pioneer hold the higher total levels of SOC mass (g SOC in kg Fraction) compared to Standard in all soil types. However, the distribution of SOC mass among aggregate fractions differed from the distribution of SOC contents (%) in aggregate fractions (Figure 14c) due to the different mass proportions. In the light soil relatively high abundance of macroaggregate mass (1000 and 250 μm) did not result in higher SOC mass. In contrast, in the medium soil the SOC mass of macroaggregate fractions was mainly driven by very high aggregate SOC contents. In the heavy soil Standard accounted for the highest SOC mass of the $< 20 \mu\text{m}$ fraction (7.25 g) compared to Reference (5.4 g) and Pioneer (1.61 g). Among aggregate fractions the SOC mass of the $< 20 \mu\text{m}$ fraction was generally highest in the light soil where Reference showed the highest value (30.01 g) compared to Pioneer (9.96 mg) and Standard (6.2 mg). Having this in mind, the very high SOC mass of Reference and its comparable mass of the $< 20 \mu\text{m}$ fraction to Pioneer and Standard indicates very high SOC stocks in the $< 20 \mu\text{m}$ fraction of Reference in the light soil.

Table Ap. 18,19 summarizes all values of SOC, aggregate mass and SOC mass in aggregate fractions.

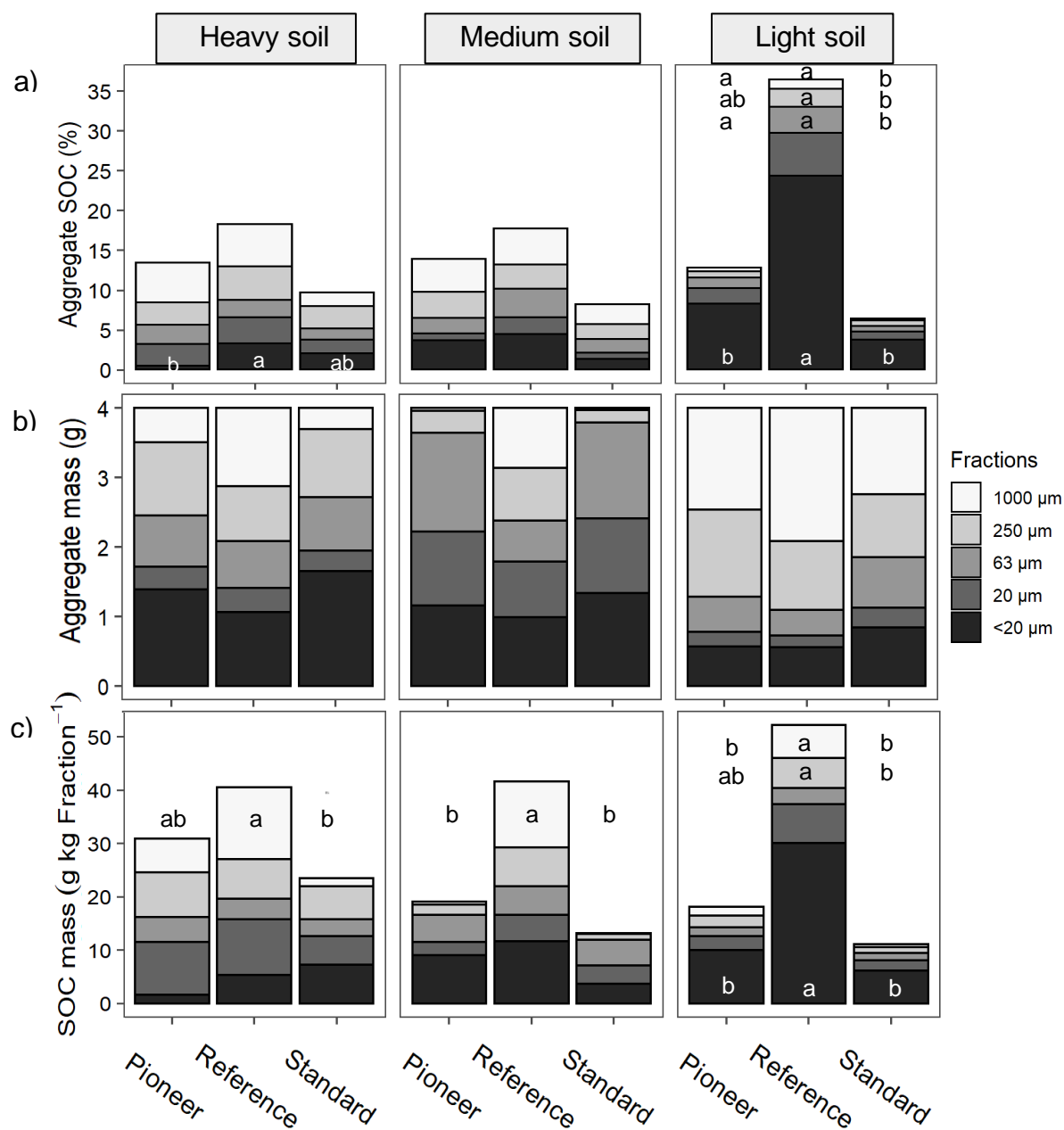


Figure 14: a) Aggregate SOC mass in g per kg Fraction mass, b) aggregate SOC in % and c) aggregate mass in g of the respective aggregate fraction is depicted in stacked bar plots for all soil types and management systems. Mean values were averaged over five energy levels. Significant difference between management systems within the soil types was tested with an one-way anova ($p < 0.05$). Significant difference is indicated by different lowercase letters. Significance letters on top of each other are ordered in the respective sequence of aggregate fractions from top to down if they exceed the margins of the stacked bars. All values are listed in Table Ap.18.

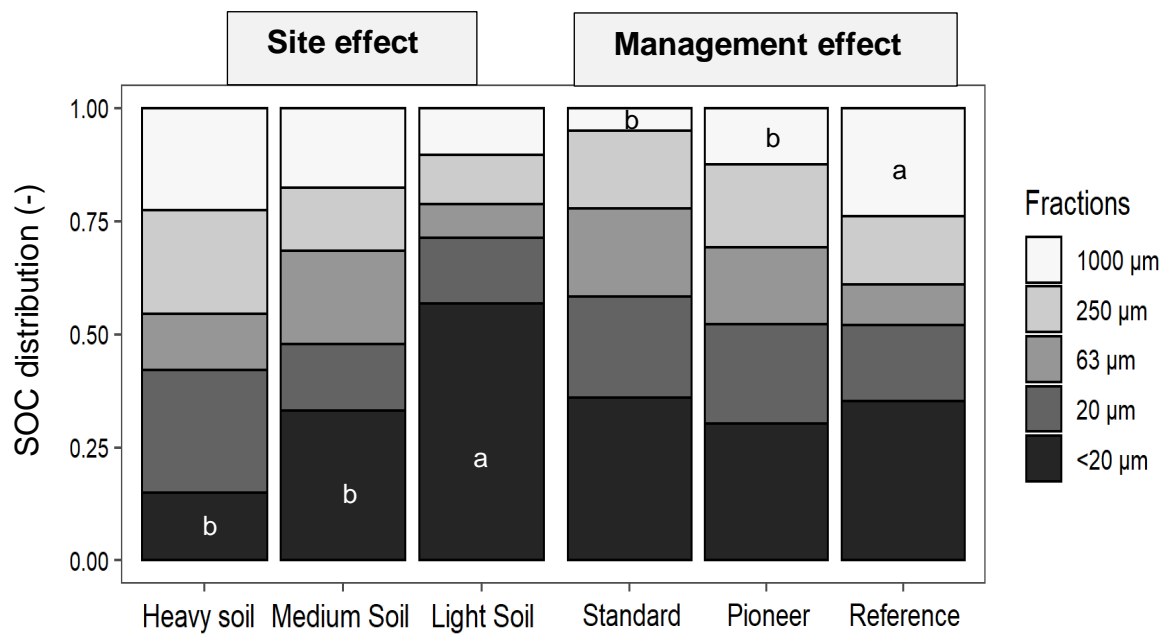


Figure 15: Relative SOC mass contribution of aggregate fractions compared between three sites and three management systems. Relative shares of SOC mass of each aggregate fractions are presented in stacked bars. Significant differences were tested with a one-way anova between management systems and between soil types ($p < 0.05$). Significance is indicated by lowercase letters in the respective aggregate fraction.

3.4.3. Aggregate mass and SOC distribution at different energy levels

Differences in the aggregate breakdown and related mass loss of aggregate fractions were greater between sites than between management systems. This was reflected by a significant factor effect of site on the change in mass distribution during ultrasonication ($p < 0.01$). Generally, heavy and light soils could maintain a higher mass of coarse macroaggregates ($> 1000 \mu\text{m}$) across all energy levels. Interestingly, the aggregate breakdown prior total breakdown was most strongly at E2 indicated by a high relative change in aggregate mass. The relative loss of aggregate mass was in average across all management systems and sites highest at E2 (25.7 % mass loss). This could be largely observed in all soil types with the strongest breakdown in the heavy soil (34.32 % mass loss) followed by the medium (25.86 %, mass loss) and light soil (17.2 %, mass loss). However, different aggregate size fractions accounted for a substantial mass loss at E2 among soil types. Generally, the decrease of coarse macroaggregate mass was most strongly pronounced for Standard in the heavy soil, where coarse macroaggregates were most abundant. In the medium soil where macroaggregates were hardly present coarse microaggregates (63-250 μm) responded most strongly at E2 with the highest change in Standard (30 % mass loss). In the light soil large macroaggregate mass was highest among the soil types for all management systems.

However, a substantial mass loss was only observed in Pioneer system at E2 and E5 (22.5 %, 38.5 mass loss, respectively) and in Reference only at E5 (30.1 % mass loss). Contrary, in Standard coarse macroaggregate mass hardly changed across all energy levels in the light soil. A substantial breakdown occurred only at E5. While in Standard systems the strong increase of the < 20 μm fine-silt clay fraction mass in the light soil was mainly related with the decay of small macroaggregates (250-1000 μm) and coarse microaggregates (63-250 μm) in Pioneer and Reference this was mainly due to the breakdown of coarse macroaggregates. The SOC content increased in coarse and small microaggregates with increasing sonication energy in the heavy and medium soil. In the heavy soil the SOC enrichment occurred mainly in small microaggregates and changed between E1-E5 from 2.06 % to 2.95 %. In the medium soil the SOC enrichment occurred mainly in coarse microaggregates and increased from 1.9 % to 3.53 %. In the light, the SOC content of both coarse and small microaggregates decreased which indicates a C-transfer to the fine-silt clay fractions < 20 μm that was not analyzed for C in this study. A clear loss of SOC at total aggregate breakdown was only observed in large macroaggregates in the medium and light soil for all systems. In the heavy soil SOC was completely lost in large macroaggregates in Standard systems. This is also in line with the complete decay of large macroaggregates in Standard indicated by the complete mass loss. Contrary, in Pioneer and Reference large macroaggregates showed very high SOC contents (6.24 %, 7.45 % SOC, respectively).

4. Discussion

4.1. Management and site effect on soil aggregate stability

In this study, the effect of innovative soil management practices (Pioneer) on soil aggregation and stability was compared in relation to undisturbed semi-natural vegetation (Reference) and common soil management practices (Standard). It was observed that overall 21 study site the level of SAS, SOC and DOC decreased in the sequence of Reference > Pioneer > Standard. Management differentiation in SAS was mostly pronounced in the upper 5 cm and decreased towards the lowest soil depths (5-35 cm) where Reference was not significantly higher than Pioneer and Standard. A decrease of SAS with soil depth was previously observed and mainly related to higher bulk density and a lack of root activity (Deneff et al., 2013; Liu & Han, 2019). Moreover, the difference in SAS with depth maybe related with the different soil management depths between Pioneer and Standard. Whereas in Standard typical tillage depth is around 20 cm, the soil management depth in Pioneer is usually conducted rather shallow if at all (no-till practices). This may also explain the greater difference between management systems in the upper soil depths (0-20 cm). Across all study sites the Reference system differed significantly from Standard in SAS while the difference to Pioneer was not significant. It was previously observed that the degree of soil aggregation and stability is strongly related to tillage intensity and the input of diverse organic matter (Tisdall and Oades, 1980). Soil turning tillage practices such as ploughing is known to affect the soil structure by mechanical disruption of soil aggregates whereas the input of diverse organic matter is assumed to promote aggregate formation processes by acting as binding agents and energy source for soil biota (Dorji et al. 2020; Kumar et al. 2013; J. Six et al. 2004) .

This study found a positive correlation of SAS with bulk levels of SOC and DOC ($R=0.74$, $p < 0.01$; $R=0.63$, $p < 0.01$, respectively). SOC is inherently linked with soil aggregation processes. Coarse particulate organic matter (POM) of crop residues builds up an organic skeleton where mineral particles are gradually encrusted by microbially derived C (Bronick and Lal, 2005, 2009; Six et al. 2004; Tisdall and Oades, 1980). Since DOC composes largely of root exudates and microbial decomposition products, the high level of DOC in Pioneer and Reference indicates higher microbial activity and root-derived C inputs (Bolan et al. 2018; Jackson et al. 2017). Semi-natural vegetation systems such as present in Reference are known to provide favorable conditions for soil aggregation due to a dense root system and a permanent soil cover not affected through soil tillage (Abera & Wolde-Meskel, 2013). The higher aggregate stability and bulk SOC content of Pioneer maybe related to the combined effect of lower soil disturbance and higher input of organic matter through diverse living plant species compared to Standard. This is comparable with Huang et al. (2020) who observed

synergistic effects of low tillage intensity combined with high input of organic matter on the SOC content. Low soil disturbance in Pioneer systems is mainly realized through reduced and no-till regimes. The higher input of organic matter is due to the use of cover crops, diverse crop rotations and input of organic manures and compost. The correlation analysis showed that in Standard systems the increase of SAS levels off at lower SOC contents in contrast to Pioneer and Reference (Figure 8a). This indicates that the positive effect of SOC on soil aggregation varies between soil management systems and may be counteracted by higher tillage intensity as was observed in previous studies (Cania et al. 2019; Dorji et al. 2020).

The positive effect of Pioneer systems on SAS was differently pronounced among soil type groups of the 21 study sites. It was shown that medium soils pose a strong site-specific limit for the improvement of the soil structure while in heavy and light soils the SAS of Pioneer differed much stronger from Standard. Medium soils contain a high proportion of silt and fine-sand particles that are known to disaggregate easily and have a high risk of erosion (Baruah et al. 2019). Additionally, the lowest total SOC content was observed in medium soils (2.27 ± 1.58 % SOC) which can be another factor causing the lower SAS level (Lal and Shukla, 2013; Zhou et al. 2020). The higher SAS in light soils stands in contrast to findings of previous studies where a high sand proportion is generally thought to affect aggregate stability (Bronick and Lal, 2005; Bazzoffi and Mbagwu and Chukwu, 1995; Hyun et al. 2007). However, given that the DOC and SOC content was substantially improved by Pioneer systems and correlated most strongly with SAS may explain the higher SAS level in light soils. In contrast in heavy soils, Pioneer systems substantially improved the SAS from an already high level (> 70 % for all systems) compared to Standard while the difference in SOC and DOC was small. Additionally, the weak correlation between SAS and SOC in heavy soils does not indicate a strong SOC effect on higher SAS ($r = < 0.4$, $p > 0.05$). Depending on the type of clay mineralogy heavy soils naturally tend to have higher level of soil aggregation and SOC due to higher proportion of fine-silt and clay particles (Almajmaie et al. 2017; Denef et al. 2004). The effect of low soil disturbance and higher root biomass in Pioneer systems may therefore be higher compared to medium soils where the high content of silt particles generally impairs aggregate stability (Suraj et al. 2019).

4.2. Aggregate breakdown behavior

Differences in aggregate breakdown behavior was tested for three management systems (Reference, Pioneer and Standard) of three selected sites, where each site should represent one soil type group of the 21 study sites (heavy, medium and light soil). Ultrasonic aggregate stability (USAS) and Mean weight diameter (MWD) were used as aggregate stability indicators. The release of dissolved organic carbon (DOC) at discrete disruptive energy was regarded as indicator for the proportion of formerly protected C in aggregates (Lützow et al. 2006). Management differentiations were seen in particular for USAS and DOC with higher sonication energy level. The higher SAS of Reference and Pioneer was reflected by a higher USAS and MWD compared to Standard. With regard to the effect of management intensity the results are comparable with Kasper et al. (2009) who observed higher USAS and MWD for no-till and reduced tillage systems. However, the positive relationship of USAS with bulk SOC ($r = 0.63$, $p < 0.01$) suggests also a positive effect of SOC in Pioneer and Reference systems.

The USAS analysis enabled a refined study of aggregate stability dynamics upon different disruptive energies (Schomaker et al. 2011). This revealed fine-scale differences in the time and magnitude of aggregate breakdown among management systems. The relative change of USAS and MWD was used as additional proxy of soil aggregate stability by indicating the magnitude of aggregate breakdown. This showed that the strongest breakdown prior total decay occurred at the second energy level E2 in all systems and soil types. However, the magnitude of breakdown was much lower at Reference compared to Pioneer and Standard (Table 4). This indicates a step-wise rather than continuous aggregate breakdown whereby the magnitude and course of the breakdown is influenced by the type of management system.

Ultrasonic aggregate breakdown is caused by the gradual abrasion of aggregate surface layers causing eventually the detachment of organic binding compounds and the related breakdown. (Kaiser and Berhe. 2014). However, according to the aggregate hierarchy model of Tisdall and Oades (1982) stable microaggregates form within macroaggregates via the transfer of highly processed C of decomposed organic matter in macroaggregates. Microaggregates are thought to have much higher stability due to different SOC stabilizing processes such as electrostatic interaction with mineral surfaces (Eriksen et al. 1995; North, 1979). This leads to a hierarchical disintegration of different aggregate subunits in the course of complete aggregate breakdown owing to different aggregate stabilization mechanism related to aggregate size (Totsche et al. 2018). A step-wise breakdown such as most present in Reference soils may thus indicate the presence of more stable microaggregates requiring more energy to disintegrate once the outside macroaggregate layer is detached. Despite the higher SAS of Pioneer there was only a slight trend of lower magnitude of breakdown compared to Standard system. The process of formation and stabilization of microaggregates

is a function of the turnover of macroaggregates which in turn is controlled by the degree of soil disturbance in agroecosystems (Six et al. 1998). In contrast to semi-natural vegetation sites such as Reference with no soil cultivation processes the level of soil disturbance in arable systems such as Pioneer and Standard is expected to be higher due to frequent agricultural crop and soil management practices (Or, Keller and Schlesinger, 2021).

However, it was shown that the effectiveness of Pioneer management to improve aggregate stability indicators differed between soil types. While Pioneer management hardly improved USAS and MWD in the medium soil, it approached the level of Reference in light and heavy soils. Accordingly, the MWD of Reference decreased only substantially by 84.31 % at the highest sonication energy while the MWD of Pioneer and Standard hardly changed at a low level ($< 25 \mu\text{m}$, Figure 14). This highlights a clear differentiation between the site-specific potential of soil aggregate stability (Reference) and the two arable systems. It may indicate that the positive effect of Pioneer management on aggregate stability indicators is limited in medium soils due to the poor soil structure (Suraj et al. 2019). This is also in line with the poor effect of Pioneer on SAS in the medium soil type group of the 21 study sites. Contrary, in light and heavy soils the effect of Pioneer was clearly indicated by a strong differentiation in USAS and MWD compared to Standard. However, considering that Pioneer still differed strongly from Reference in the light soil indicates site-specific limitations for improving soil structural properties. In the heavy soil Reference systems seem to be a more realistic target level for improving structure given the small difference to the USAS of Pioneer. This may be related with the improved soil structure in heavy soils owing to greater proportions of fine-silt and clay particles (Almajmaie et al. 2017; Jarvis et al. 2012).

The spatial separation of substrate and decomposer is a key mechanism of soil aggregates enhancing the stabilization of SOM (von Lützow et al. 2006). The stability of soil aggregates determines the degree of SOM protection by withstanding disruptive forces on the aggregate structure (Kaiser & Berhe, 2014; Johan Six & Paustian, 2014). The breakdown of aggregates during ultrasonication was associated with increased DOC release which indicates according to (Mueller et al., 2012) the detachment of formerly protected SOM. As mentioned before, the breakdown of aggregates occurs in a hierarchical manner owing to the higher stability of smaller microaggregates within macroaggregates. Higher DOC release after ultrasonication was observed previously by Fuller and Goh (1992) and related to increased detachment of organic matter from mineral surfaces (MAOM). Hence, the level of DOC release at higher ultrasonic energy reflects the proportion of stable SOC on mineral-surfaces. The level of DOC release differed increasingly with higher energy level among management systems. However, this increase occurred at higher magnitude for Reference and Pioneer compared to Standard. The higher increase of DOC release in Pioneer and Reference at total aggregate breakdown

indicates thus more strongly bound MAOM in microaggregates compared to Standard (Totsche et al. 2018; von Lützow et al. 2006). Similar results were reported from Kasper et al. (2009) where the level of DOC release during ultrasonication was higher for minimal and reduced tillage systems compared to conventional tillage regimes. Yet, in this study, the higher DOC release of Pioneer system must be linked with the long period of soil cover that is known to promote higher DOC contents by prolonged interaction of roots with the soil. This results in higher inputs of root exudates and microbial activity which are two important drivers of DOC levels (Austin et al. 2017; Tiemann et al. 2015; Sokol et al. 2019). On the other hand, the better soil structure in Pioneer systems can enhance the undisturbed occlusion of DOC in aggregates which maybe reflected by the higher magnitude of increased DOC release after each energy level (Six and Paustian, 2014).

Generally, the DOC release curves of the management systems were rather similar among soil types. Although, the type of management system was the dominant factor controlling the level of DOC release the effect of soil types was especially present at the total aggregate breakdown. While in light soils the DOC release increased for all systems in the same magnitude in medium soils only Reference showed a strong pulse (Table Ap.3). In heavy soils Pioneer systems differentiated most strongly from Standard. Given that strongly protected SOC is detached at higher sonication energy the small difference between Pioneer and Standard in DOC release indicates that the effect of Pioneer management on SOC stabilization may be limited in medium soils (Mueller et al. 2012). Despite a rather high level of aggregate SOC in medium soils (2.02 SOC %) the SOC storage may be limited due to a poor soil structure. Hence, the level of soil disturbance maybe a particularly critical lever in medium soils to promote SOC stabilization (Ogle et al. 2019). The strong but similar pulse of DOC release at total aggregate breakdown among management systems in the light soil suggests a trend of higher proportion of highly stabilized SOM regardless of the type of management system (Mueller et al. 2012). Considering that small microaggregates require higher disruptive energy to decay, the preferential SOC distribution in the < 20 µm fine silt-clay fraction in light soils may explain the strong pulse of DOC at total aggregate breakdown for all management systems (Totsche et al. 2018).

4.2.1. Effect of sonication energy level on the distribution of SOC and aggregate mass

The disruptive energy of ultrasound significantly influences the distribution of mass and C in aggregate size fractions during aggregate breakdown (Kaiser & Berhe, 2014; Poeplau & Don, 2014). In this study we applied four ultrasonic energy levels (E2-E5) to detect differences in the mass and SOC distribution among management systems and sites. It was shown that increasing ultrasonic energy was related with an increase of the SOC content in coarse and small microaggregates (63-250 μm , 20-63 μm) in the heavy and medium soil. The enrichment of SOC in smaller aggregates during ultrasonic dispersion was also observed by John et al. (2005) and Poeplau and Don (2015) and explained with higher SOC contents in macroaggregates. In contrast, in the light soil the SOC content of coarse and small microaggregates decreased which may indicate a C transfer to the < 20 μm fine-silt clay fraction. However, as we did not analyze the C content of the < 20 μm fine-silt clay fraction we could not obtain a complete picture of the C transfer from macro-to microaggregates. The aggregate mass loss prior total breakdown was at all sites and management systems strongest at E2 (1.87 J mL⁻¹) mainly due to the breakdown of macroaggregates. This was also reflected by the strongest change in USAS and DOC release at E2 in all sites and management systems prior total breakdown. This confirms the results of Mentler et al. (2004) and Schomakers et al. (2015) that observed strong initial aggregate breakdown at low energy inputs at < 2 J mL⁻¹. Low sonication energy < 2 J mL⁻¹ maybe therefore an useful energy level for a refined study of management differences in aggregate stability across contrasting soil types.

Total aggregate breakdown was approached at E5 (597 J mL⁻¹) and was related with a strong increase of the < 20 μm fine-silt clay fraction mass in all sites with small differences among the management systems. Management differences could be best observed in the heavy soil at E5 where the SOC content of large macroaggregates did not decrease in Pioneer and Reference contrary to Standard systems. Given that macroaggregate mass of Pioneer and Reference was almost completely lost in the heavy soil at E5 (Figure Ap.7) the SOC content in macroaggregates may reflect light-weight POM that was formerly occluded (Kaiser et al. 2012; Mueller et al. 2012). Management differentiation in the DOC release was also best seen at E5 in all three sites. It was previously mentioned that the amount of DOC release at higher sonication energies reflects the level of formerly protected SOC (Mueller et al. 2012). Therefore, the application of high sonication energy inputs maybe also important to reveal management differences in highly stabilized SOC (Kaiser and Berhe, 2014).

4.3. Management and site effect on SOC in aggregates

The mass distribution of macroaggregates (2000-250 μm) differed significantly among the management systems in the sequence of Reference > Pioneer > Standard. The proportion of large macroaggregates (200-250 μm) was especially elevated by Reference (+ 100 %) and Pioneer (+ 40%) compared to Standard systems. This can be linked to the lower turnover of macroaggregates and the high input of organic matter that promotes aggregate formation (Six et al. 2004). In contrast, in Standard systems the impact of wind and water erosion on macroaggregate turnover is more severe due to the lower soil cover compared to Pioneer and Reference (Hao et al. 2015; Yan et al. 2008). Moreover, high intensity and frequency of tillage regimes are well known to destroy primarily large macroaggregates (Sheng et al. 2020; Yoo and Wander, 2008). Microaggregate (250 – 20 μm) mass was generally lowest for Reference compared to Pioneer and Standard. High macroaggregate proportion coupled with low microaggregate fractions were also observed by Sekaran, Sagar, and Kumar (2021) for a low soil disturbance ecosystem and explained by the binding of small macroaggregates with microaggregates. Generally, it is believed that a high level of DOC such as present in Reference systems facilitates the encrustation of small microaggregates to small macroaggregates leading to a higher proportion of large macroaggregates than microaggregates (Qiu. et al. 2015; Six and Paustian. 2014).

The distribution of aggregate SOC among different size fractions entails critical information about the sequestration and dynamics of SOC as influenced by the land use system (Six et al. 2004; Ananyeva et al. 2013). In line with previous findings SOC in aggregates differed both between aggregate size fraction and management system (Sandén et al. 2017; Sheng et al. 2020). The aggregate hierarchy concept of Six et al. (2004) was reflected by decreasing contents of SOC from coarse macroaggregates to small microaggregates. This pattern was mostly pronounced in Reference and Pioneer. Macroaggregate formation starts with the input of fresh plant-derived POM. Microaggregates in turn contain less POM but more labile highly processed MAOM (Totsche et al. 2018; Virto et al. 2010). Reference and to smaller extent Pioneer increased the SOC content in all aggregate fractions compared to Standard. The effect of Pioneer on the level of aggregate SOC was mostly pronounced in large macroaggregates (>1000 μm) where it differed most strongly from Standard. This may reflect the high level of POM both in Reference and Pioneer that may derive from a higher level of crop and plant residues. POM is known to be rich in C and plays a critical role in the commence of macroaggregate formation and fast cycling nutrient provision for microorganisms (Six et al. 2000; Dungait et al. 2012). The strong difference between Pioneer and Standard in large macroaggregates highlights the important role of macroaggregates as early indicator of SOC responses upon management change which was observed previously (Sheng et al. 2020; Six

et al. 2004). In contrast, changes in SOC of microaggregate (< 250 μm) are thought to indicate a long-term SOC stabilization potential (Totsche et al. 2017; Deneff et al. 2007) owing to strong physical and chemical protection of SOC in small anoxic pores (Kravchenko et al. 2015; Ananyeva et al. 2013; Toosi et al. 2017). In this study, the SOC content of Reference was substantially elevated in the 20-63 μm microaggregate fraction compared to Pioneer (-120 %) and Standard (-180 %). This may be related to the lower macroaggregate turnover which supports the processing of POM in macroaggregate and related C transfer to small microaggregates in the long-term (Six et al. 2004).

The effect of Pioneer management to increase the level of aggregate-SOC in all size fraction in relation to Standard could be observed across three contrasting soil types. However, given that the difference of Pioneer to Standard and to Reference changed among soil types indicated site-specific patterns for the accrual of SOC in aggregates. The high bulk SOC content of the heavy soil was also reflected in the highest content of SOC in aggregates among soil types. The high aggregate mass and SOC content of macroaggregates showed a trend of preferential SOC storage in macroaggregates in heavy soils where Pioneer systems differed strongly from Standard system. This may be explained with the naturally high level of soil aggregation and SOC that correlate with the high proportion of fine-silt and clay particles in heavy soils (Arrouays et al. 2006; Zinn et al. 2007). The high SOC content in macroaggregates in the medium soil was mainly driven by Reference and Pioneer. However, the very low macroaggregate mass resulted in a low effective SOC mass for both Pioneer and Standard. Macroaggregate SOC in the medium soil may be therefore not relevant for higher SOC stocks. Given the poor effect of Pioneer on improving soil aggregate stability points out that the accrual of SOC in macroaggregates conflicts with a poor soil structure (Six and Paustian, 2014) in medium soils. However, the trend of Pioneer and Reference to store more SOC in the < 20 μm fine silt-clay fraction in the medium soil indicates a higher SOC stabilization potential given that small microaggregates store SOC mainly in a highly stable mineral-associated organic matter (MAOM) pool (Wiesmeier et al. 2014; Beare et al. 2014). In the light soil the small differentiation of Pioneer to Standard regarding the total SOC content in aggregates may be related with the site-specific preferential storage of SOC in the 20-63 μm and <20 μm microaggregate fractions. SOC in these microaggregate fractions (< 63 μm) occurs mainly in the form of MAOM which is thought to have a much slower turnover time (Poeplau et al. 2018; Kong et al. 2005). Although, Pioneer increased the SOC level of the 20-63 μm fraction significantly compared to Standard, higher total SOC levels are thought to be achieved only via SOC in macroaggregates. Macroaggregate SOC is primarily present in a particulate organic matter (POM) pool (Six et al. 2004). While POM is generally related with higher total SOC levels in the short-term, the contribution of MAOM may be limited due to saturation on mineral surfaces (Angers and Giroux, 1996; Chenu et al. 2019). It was shown by Cotrufo et al.

2019 that grassland ecosystems consistently store more SOC in the form of MAOM in microaggregates. In this study, the semi-natural vegetation of Reference systems has similar characteristics as grassland ecosystems as it composes besides shrubs largely of grass vegetation. This may explain the higher SOC level of Reference in microaggregate fractions in the light soil. It may be therefore concluded that in light soils MAOM in microaggregates is a diagnostic indicator for SOM stabilization (Denef et al. 2007). However, given that MAOM saturates at a certain point due to limited number of mineral surfaces, suggests that the increase of SOC may be only realized in combination with higher POM stocks in light soils (Cotrufo et al. 2019; Lavalley et al. 2020).

4.4. Implications for SOC stabilization in Pioneer systems

Given that the persistence of SOM is associated with the degree of physical protection from decomposers, highlights the potential of stable soil aggregates to enhance higher SOM stability under Pioneer management (Six et al. 2002). While higher bulk SOC contents are not surprising under Pioneer management given the high input of organic matter through crop residues, changes in distinct aggregate size fractions can inform about a SOM stabilization potential (Kong et al. 2005; Six and Paustian, 2014). It was recently requested by several studies to evaluate the effect of agricultural management on SOM stability on the basis of pools with unique formation pathways and functioning in the soil. In this context, the notion of short-lived POM and long-lived MAOM was introduced as two physical soil fractions with contrasting responses to agricultural management change and related climate implications (Cotrufo et al. 2015; Lavalley et al. 2020; Poeplau et al. 2018). MAOM consists largely of highly processed soluble microbial- and root-derived C products that can strongly bind to mineral-surfaces in small microaggregates. Contrary, POM composes of larger insoluble plant-derived C occurring primarily in macroaggregates. This results in different degrees of physical protection as a function of aggregate size and leads to a much higher persistence of MAOM compared to POM (Poeplau et al. 2018; Kleber et al. 2015). However, while microaggregate-C is included in the definition of MAOM, macroaggregate-C should not be referred simply to POM, as macroaggregates compose of a mixture of MAOM and POM (Six et al. 2000, Lavalley et al. 2020). For the evaluation of Pioneer management to increase SOM stability, changes in MAOM will thus only be referred to the 20-63 and < 20 μm fraction.

The positive effect of Pioneer management on the soil structure was not only seen in contrasting soil types but also across a wide range of disruptive energies which suggests a consistent positive effect of Pioneer management systems on the soil structure. It was further shown that the aggregate stability was positively related with higher SOC levels in the bulk soil and in soil aggregates. SOC contents were improved by Pioneer in all aggregate fractions with the greatest difference to Standard systems in coarse macroaggregates (> 1000 μm) and to

smaller extent in small microaggregates (20-63 μm). Previous studies observed significant SOC persistence via physical protection only below the level of $< 63 \mu\text{m}$ (Virto et al. 2010). This indicates a positive influence of Pioneer management on stable MAOM pools. However, given the high difference to Reference in the 20-63 μm fraction indicates a limited effect of Pioneer to increase MAOM across different soil types. On the other hand, the small difference of Pioneer and Reference in macroaggregate SOC suggests a predominant effect on a less stable pool for SOC stabilization. This is in accordance with previous studies that observed stronger SOC responses upon management change in macroaggregate fractions (Gartzia-Bengoetxea et al. 2009; Six et al. 2000; Angers and Giroux, 1996). However, considering that the effect of Pioneer management to improve SOC in macro-and microaggregates varied strongly among soil types indicates site-specific potentials and limitations for SOM stabilization. Knowing this maybe important to tailor soil management practices to site-specific SOC storage mechanisms (Mayes et al. 2014; Wiesmeier et al. 2015)

4.4.1. Site-specific limitations and potentials regarding SOC stabilization

In the heavy soil SOC was primarily stored in macroaggregates which resulted in the highest contents among soil types. The strong effect of Pioneer on the SOC content in macroaggregates can be associated with the high aggregate stability that promoted the storage of SOC in macroaggregates. It was mentioned before that macroaggregates contain a mixture of labile POM and stable MAOM in microaggregates (Six et al. 2000; Lavalee et al. 2020). Considering the high aggregate stability and high SOC contents, macroaggregates may be also used as indicative fraction for long-term stabilization in heavy soils (Pronk et al. 2012). Additionally, the high proportion of the $< 20 \mu\text{m}$ fine silt-clay fraction suggests a higher saturation level of MAOM compared to the other soil types. However, the small difference between Pioneer and Reference may indicate a small potential for further improvements of the soil structure and SOM stabilization. This is because it is assumed that the level of aggregate stability and SOC storage in a semi-natural vegetation systems such as Reference is generally more enhanced compared to arable systems (Martin et al. 2011; Meersmans et al. 2011).

In the medium soil the effective SOC mass was primarily present in the $< 20 \mu\text{m}$ fine silt-clay fraction despite a rather high SOC content in macroaggregates under Pioneer and Reference. This was primarily related with the high mass proportion of the $< 20 \mu\text{m}$ fine silt-clay fraction that may be related with the poor soil structure in silty soils limiting the formation of stable macroaggregates. Although, the SOC content in the $< 20 \mu\text{m}$ fraction is regarded as highly stable, the contribution to SOM stabilization maybe not relevant given the high risk of erosion

in medium soils (Baruah et al. 2019; Feng, Plante and Six, 2013). Management practices targeting higher bulk SOC levels in medium soils should be thus tailored to very low soil disturbance to enhance also SOC storage in macroaggregates (Six et al. 2004).

The light soil showed a clear pattern of increasing SOC contents in smaller aggregate fractions. The effect of Pioneer was clearly indicated by significant higher SOC contents in macroaggregate fractions than Standard. However, this did not result in clear differences in the total SOC content in aggregates because macroaggregates were generally low in SOC. Conversely, the strong difference of Pioneer to Reference indicates a site-specific limitation to raise total SOC stocks in aggregates. This can be related with two conflicting processes: 1) The preferential storage of SOC in MAOM fractions (20-63 and $< 20 \mu\text{m}$), 2) the relatively low mass proportion of MAOM fractions. It was already observed that light soils with low C content in aggregates predominantly increase MAOM but only to a low saturation limit that is set by a low number of mineral-surfaces of the $< 20 \mu\text{m}$ fine silt-clay fraction (Cotrufo et al. 2019; Angers et al. 2011). Despite the positive effect on stable MAOM fractions of Pioneer in the light soil the relevance for increased SOM stabilization maybe therefore limited. The low capacity to store SOC in macroaggregates and the low saturation limit can be thus seen as the main constraints for Pioneer management to increase SOC storage in light soils.

While the focus on stable MAOM in microaggregates is meaningful for SOM stabilization the management of short-lived POM in macroaggregates is especially important from a soil fertility perspective. The high lability of POM and its low physical protection in macroaggregates make it to a readily available nutrient source fueling microbial activity and contributing to agricultural productivity (Lavalee et al. 2020). In soils close to the saturation limit (light soil) the increase of SOC contents maybe realized via the accrual of POM as long as there is a net increase over time (Cotrufo et al. 2019). This approach must however also account for potential nutrient losses that may be caused through the excessive input of nutrient rich products such as organic manures or compost. In this context it may be necessary for agricultural management to manage SOC not only from a persistence perspective but also focusing on soil fertility effects of single management practices (e.g. higher water storage). This can help to reconcile both SOC stabilization and soil fertility requirements (Janzen, 2006).

4.4.2. Pioneer management practices related to SOC stabilization

SOC responses upon soil management change are studied mainly with regard to specific soil management practices (Tiefenbacher et al. 2021; Xu et al. 2020). Pioneer management however is characterized by a combination of agricultural practices such as mixed cover cropping, diverse crop rotations, organic manure and compost application and conservation tillage practices. Increases in SOC must be therefore related to synergistic effects of the mentioned agricultural practices of Pioneer. The low tillage intensity in Pioneer systems may be not directly linked with higher SOC storage as there is evidence that this leads only to a redistribution of SOC in the soil matrix (Powlson et al. 2014). It is rather thought to improve the soil structure by less mechanical disruption of soil aggregates contributing in this way to the conservation of existing SOC (Tiefenbacher et al. 2021). However, there is increasing evidence that higher SOC storage is best achieved when combining both low tillage intensity with higher OM inputs (Poeplau and Don, 2015; Virto et al. 2012; Autret et al. 2016). Also, agricultural systems with high crop rotation diversity and long period of soil cover through cover crops were associated with higher SOC storage mainly driven by increased root- and microbial derived C coupled with higher formation of aggregates (Tiemann et al. 2015; Jarecki and Lal, 2003; Wiesmeier et al. 2019). Previous studies found that below-ground C inputs from roots and microbes contribute much more to stable SOC in agricultural systems (Angst et al. 2018; Kong and Six, 2010; Liang and Balsler, 2011). The long period of soil cover through the use of diverse cover crop mixtures with different rooting systems in Pioneer systems may result not only in higher quality of crop residues but involves also a diversification of belowground C inputs (Kallenbach et al. 2016; Austin et al. 2017). This is in line with the concept of Cotrufo et al. (2013) which claims that high quality residues and belowground C inputs can be processed more efficiently by microbes leading to greater production of microbial C products and related formation of stable SOC. Also, diverse rooting systems and high microbial activity are related with higher formation and stabilization of soil aggregates and better allocation of C in the soil matrix (Six et al. 2000; von Lützow et al. 2008; Martins and Angers, 2015). Besides, the application of organic manures and compost under reduced tillage and no-till regimes has been repeatedly observed to increase SOC in the topsoil (Gross and Glaser, 2021; Bogužas et al. 2018). To summarize, Pioneer management practices are characterized by a high quantity and quality of organic matter inputs coupled with lower soil disturbance through tillage. This may contribute to both higher storage and stabilization of SOC.

A detailed analysis and description of Pioneer management practices can be found in the work of Scharf, B. (in preparation).

4.4.3. Research limitations

Assessing SOM responses to management change at discrete soil aggregate fractions gives a more detailed picture of how soil management measures may contribute to SOM stabilization. However, the turnover rates of aggregate fractions strongly depend on the method to disrupt and isolate soil aggregates (Rabot et al. 2017; von Lützow et al. 2007). This study combined ultrasonic aggregate dispersion with wet-sieving and particle-size fractionation to investigate management differences in aggregate stabilities and mass distribution across a range of disruptive energies.

An arguable limitation of this study is that soil aggregates were only fractionated by size and not by density. We cannot therefore not differentiate occluded POM within aggregates (oPOM) from free POM outside aggregates (fPOM) (Moni et al. 2012). The high SOC level and mass of macroaggregates in Reference and Pioneer may thus provoke a biased conclusion of the management impact on macroaggregate SOC given the high level of biomass input in these systems. Density separation of POM is a highly laborious procedure and was as a preliminary analysis not suitable for the broad scope of this study. However, for precise quantification and interpretation of SOC changes in aggregates free POM should be separated from aggregate fractions (Peoplau et al. 2018; Moni et al. 2018). It was previously observed that the disruption of aggregate POM and related redistribution of SOC can be strongly reduced when dispersing aggregates at $\leq 60 \text{ J mL}^{-1}$ (Amelung and Zech, 1999). We used five sonication energy levels for aggregate dispersion whereof four (E1-E4) were of low intensity $< 12 \text{ J mL}^{-1}$ to account for the contrasting soil textures of the study sites (Schomaker et al. 2011). For the total breakdown of aggregates we applied an energy of 597 J mL^{-1} which may have caused substantial redistribution of C among aggregate fractions and overestimated the level of DOC release. Our results regarding SOC contents in aggregates and DOC release during aggregate breakdown must be therefore treated with caution and should not be generalized beyond the scope of this study.

In this study we did not collect the $< 20 \mu\text{m}$ fine-silt clay fraction physically. Our results regarding the SOC in the $< 20 \mu\text{m}$ fine-silt clay fractions were estimated by the difference between SOC in aggregates and in the bulk soil. While this served as a proxy for the SOC level present in the respective mass of the $< 20 \mu\text{m}$ fraction and helped to obtain a complete picture of the transfer of mass and SOC during aggregate breakdown we can not infer on the actual SOC concentration in this fraction. Given that the $< 20 \mu\text{m}$ fine-silt clay fraction mainly reflects a highly stable MAOM pool (Hassink, 1997) limits thus the interpretability of the SOC stabilization potential of Pioneer systems. To obtain a complete picture of the potential of Pioneer systems to increase SOC in a highly stable MAOM pool the $< 20 \mu\text{m}$ fraction should be included in future aggregate fractionation.

We derived five discrete sonication energy levels from a previous experiment where the continuous DOC release curves were produced during ultrasonication (Orracha et al. in preparation). The sample mass for ultrasonication was relatively small (2 g) due to high sensitivity of the filtering system of the experimental setup. In order to mimic the same disruptive forces we used a comparable sample mass of 4 g. Given the wide soil:water ratio of 1:50 we regarded this sample mass (4 g) as adequate to not alter the disruption energy due to limited propagation of cavitation waves (Kaiser and Berhe et al. 2014). However, this caused very low sample masses in the mass distribution experiment subsequent to the ultrasonication treatment. We could therefore not fully use the results of the elementary analysis of N in different fractions and consider this data as not robust. This was related to the insufficient sample mass for elementary analysis (< 2 mg).

5. Conclusion

The approach of this study was to assess the effect of Pioneer management on different soil structure parameter (MWD, USAS, SAS) and aggregate associated SOC on various scales. This included 1) a preliminary SAS analysis across 21 study sites, 2) the analysis of aggregate breakdown across a range of disruptive energies 3) the determination of SOC and mass distribution in five aggregate fractions collected after each sonication energy level. The broad scope of this study should help to identify meaningful experimental setups and indicative parameters for the evaluation of soil management effects on soil aggregate stability and aggregate associated SOC that can be applied to the whole data set of the research project.

Overall, this study proved a consistent effect of Pioneer management to enhance better soil structure and increased SOC contents in all aggregate fractions compared to Standard management. Regarding the effect of Pioneer on aggregate SOC strong differences to Standard systems were mainly observed in macroaggregates where much of the C is stored in labile POM. This highlights their important role to serve as a good predictor of SOC responses upon soil management change. On the other hand, the increase of SOC in microaggregates was associated with higher stabilization potential under Pioneer management given that SOC in microaggregates is largely stored as stable MAOM. However, this effect was differently pronounced among soil types indicating site-specific limitations for the stabilization of SOC in aggregates. It was shown, that a heavy soil stores SOC primarily in macroaggregates which resulted in generally high SOC contents but also enhanced the stabilization in microaggregates. In contrast, light soils showed the highest potential for Pioneer management to improve aggregate stability (SAS, USAS) and bulk SOC levels. The stabilization of SOC in aggregates maybe however limited by the preferential storage of SOC in a saturating MAOM pool in microaggregates. In medium soils the SOC stabilization potential under Pioneer management seems to be limited due to a poor soil structure resulting in low protection of SOC in small microaggregates. Regarding the analysis of aggregate breakdown behavior, ultrasonication appeared to be a useful technique to detect fine-scale management differences in soil structure indicators in different soils. For future studies of aggregate breakdown we recommend to include sonication energy levels below 2 J mL^{-1} (E2) as the breakdown of aggregates occurred mainly in this energy range regardless of the soil type. High ultrasonic energy (E5) maybe applied to infer on the proportion of highly stabilized SOC reflected by the level of DOC release at total aggregate breakdown. This revealed a strong differentiation between Pioneer and Standard in their DOC release indicating much higher level of protected SOC in microaggregates in Pioneer systems. Higher DOC release maybe related with the long period of soil cover in Pioneer and Reference system enhancing higher input of root inputs and microbial activity.

To conclude, our results support following hypothesis

- 1) Hypothesis [H1](#) and [H2](#) are supported by the fact that Pioneer management consistently enhanced higher aggregate stability and increased SOC levels not only on a bulk level but also in distinct aggregate fractions with higher protection capacity. Soil aggregate stability is appeared to be a dynamic parameter and associated with SOC enhancing its physical stabilization. It should be assessed via different soil structure parameters (USAS, MWD, SAS) and across different disruptive energies. Ultrasonication of aggregates can be a viable technique to detect fine-scale differences in aggregate stability among different management systems and contrasting soil types
- 2) With regard to hypothesis, [H3](#), it must be noted that the effect size of Pioneer systems strongly varied among soil types indicating the highest improvement potential of the soil structure and SOC in light soils. The great difference to Reference systems in the medium and light soil in both soil structure indicators and SOC in stable aggregates indicates the limits of Pioneer management to increase SOC stabilization. Reference ecosystems maybe therefore not a realistic target level for SOC improvements in this soils. The potential of Pioneer systems to promote higher SOC storage should be thus not only evaluated with regard to measurable SOC pools but also focus on soil fertility co-benefits of management practices. While in medium soils the stabilization of SOC is strongly limited by the poor soil structure the predominant incorporation in a saturating MAOM pool must be considered in light soils. Increased SOC stocks maybe thus only achieved by cumulative POM inputs. The labile nature of POM requires however constant input of organic matter and low soil disturbance in medium and light soils. Contrary in heavy soils Reference ecosystems seem to be a realistic target level for Pioneer management. SOC storage is mainly enhanced by stable macroaggregates and relies not solely on net inputs of organic matter.

6. References

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9. Appendix

9.1. Complementary Figures

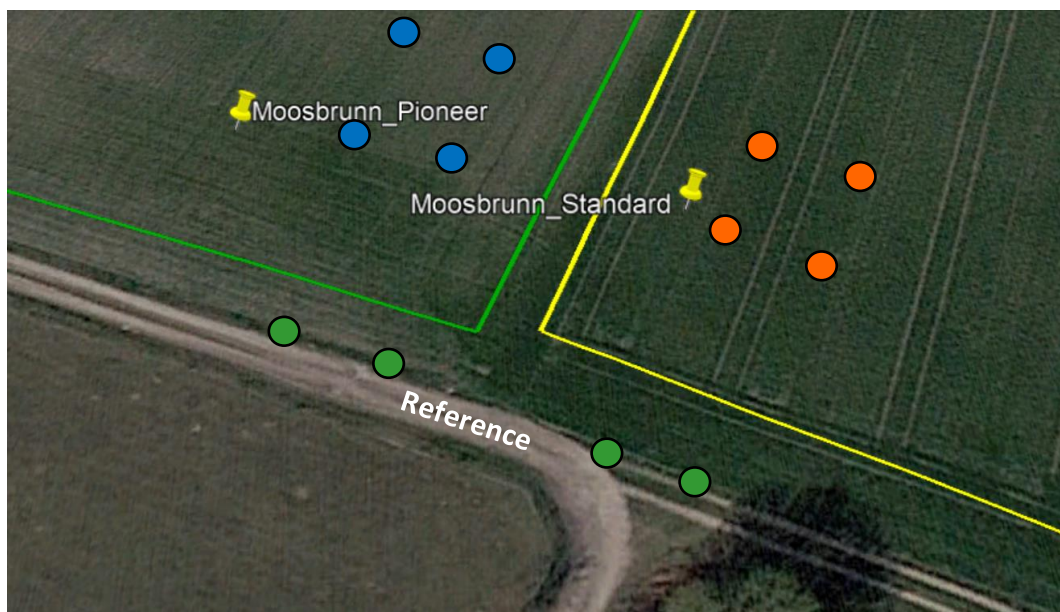


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Figure Ap.2: Collected aggregate fractions from left to right: $> 1000 \mu\text{m}$ - $> 250 \mu\text{m}$ - $> 63 \mu\text{m}$ - $> 20 \mu\text{m}$. From top to down: Pioneer – Standard – Reference of the heavy soil. Ultrasonic energy level, E1 = Spontaneous decay!

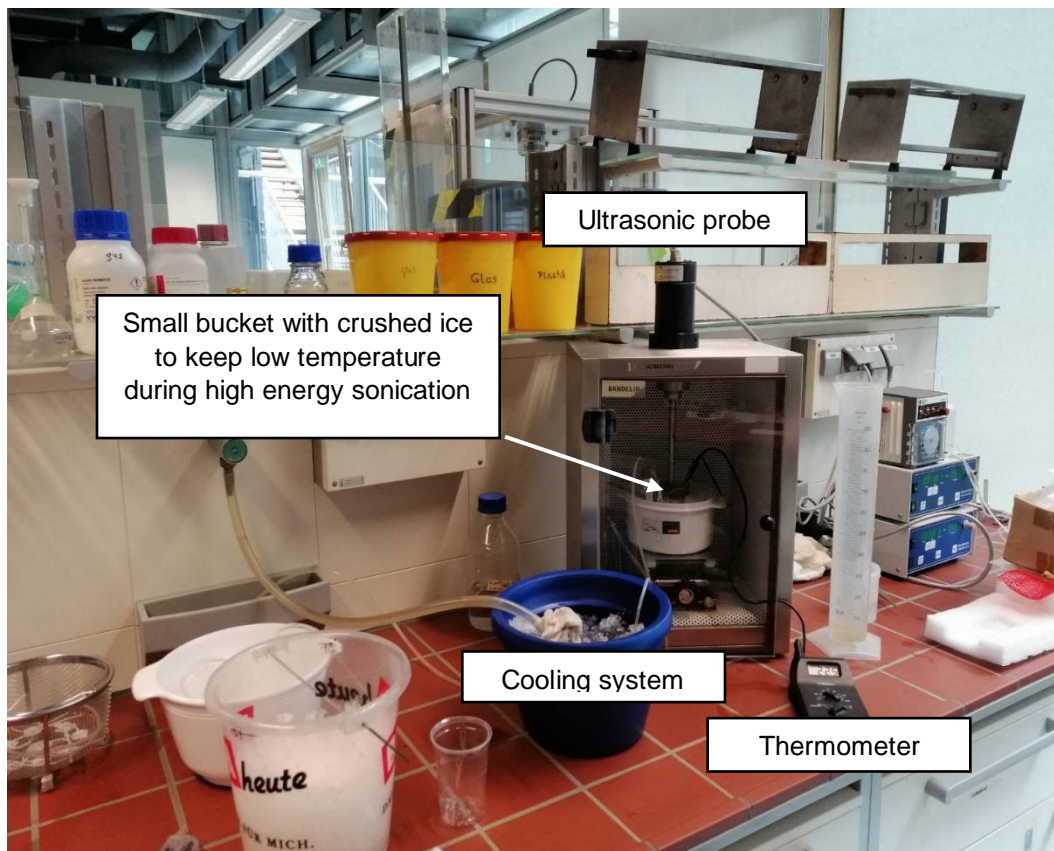


Figure Ap.3: Experimental setup for total aggregate breakdown at 597 J mL^{-1} . Cold water from the blue bucket was constantly pumped around the sample cup to prevent excessive heating during high energy ultrasonication

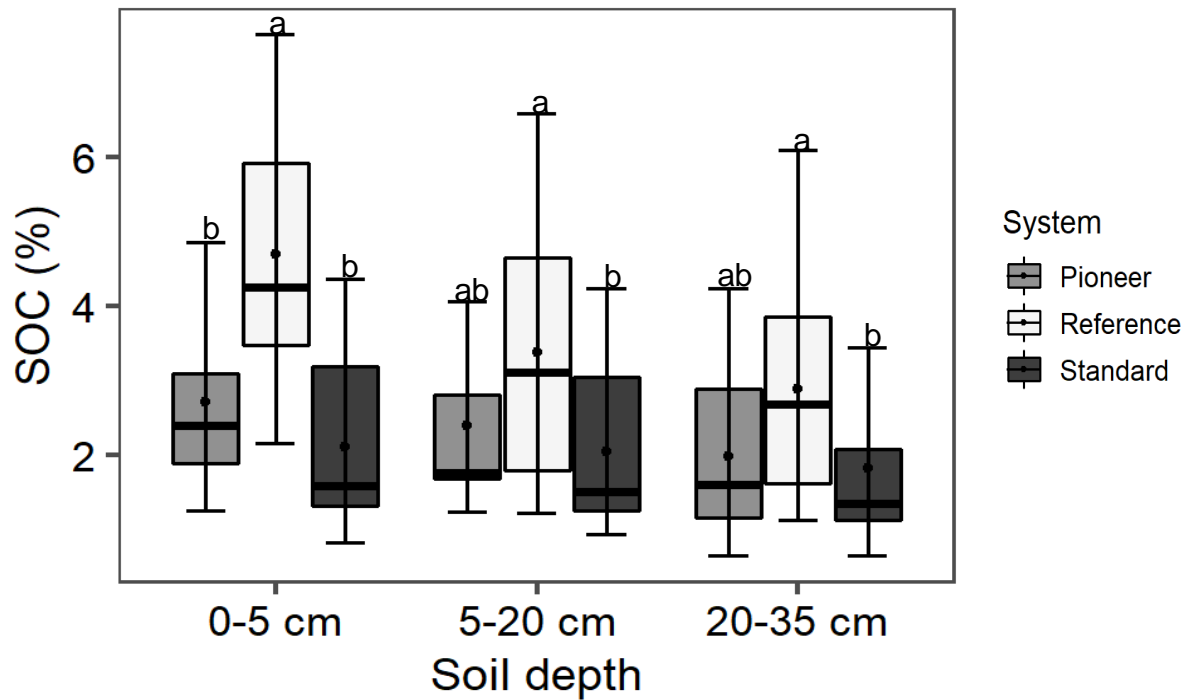


Figure Ap.4: Soil organic carbon in different soil depths compared between three management systems. Differences between management systems were tested with a one-way anova ($p < 0,05$). Significant differences between management systems are indicated by different lowercase letters. Steiner, P. (in preparation)

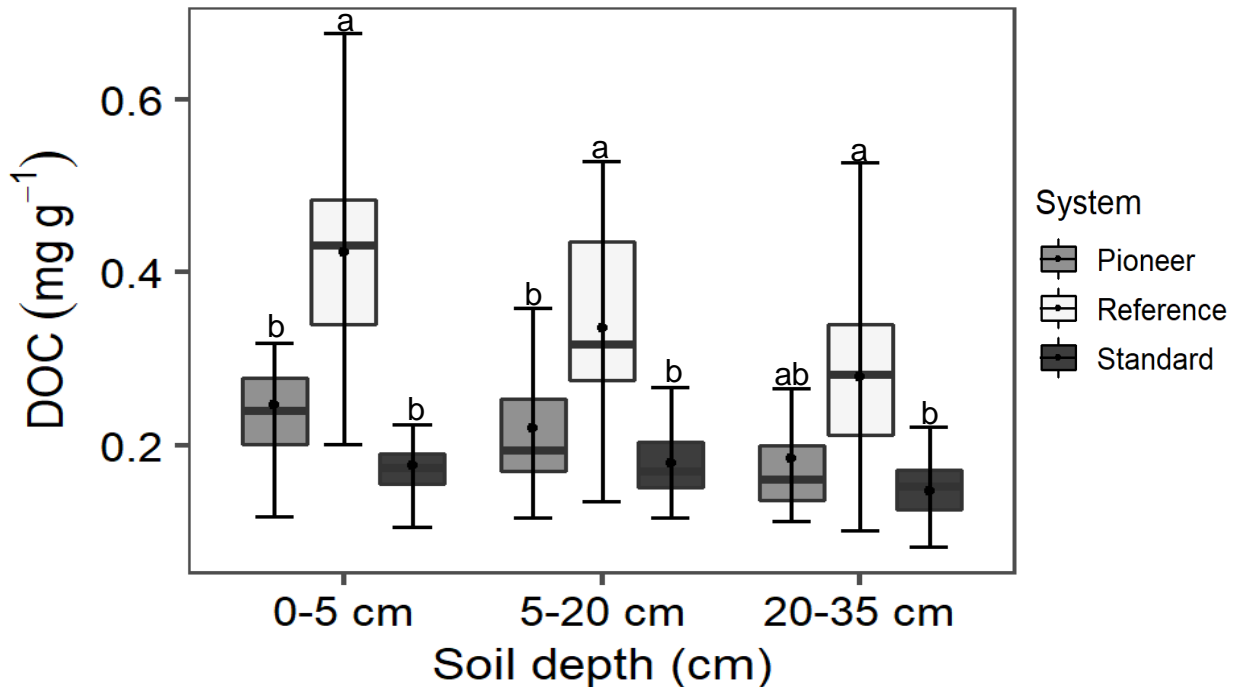


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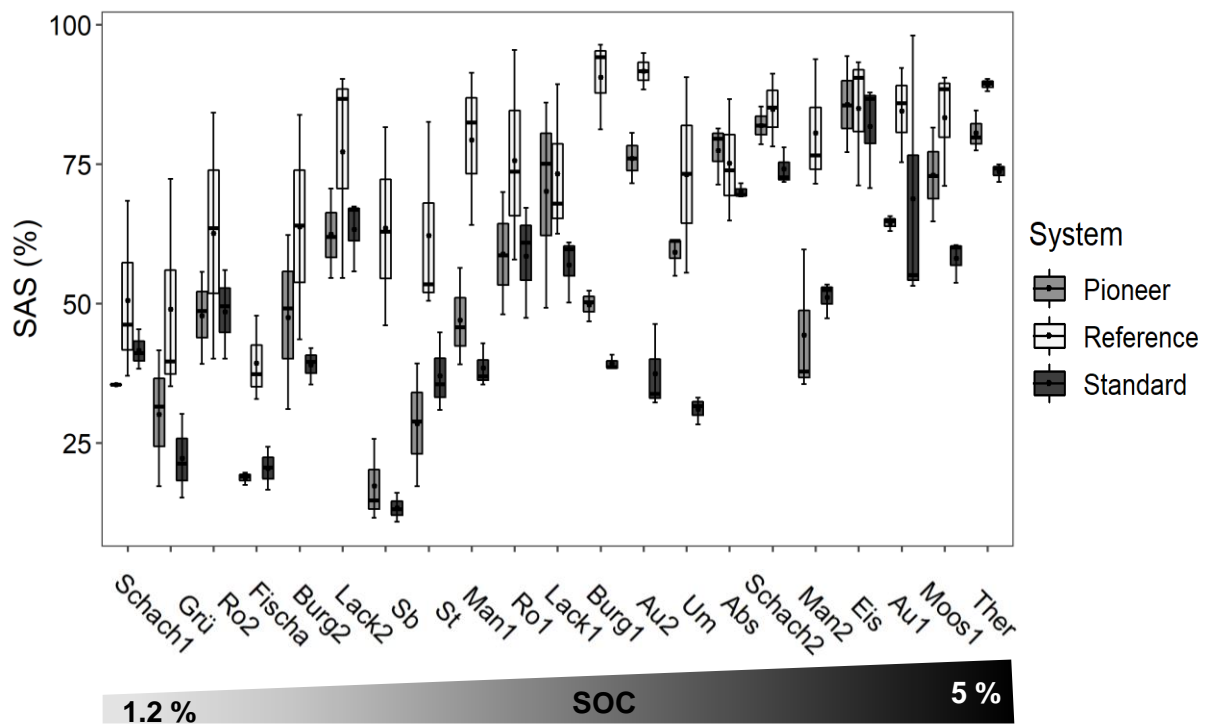


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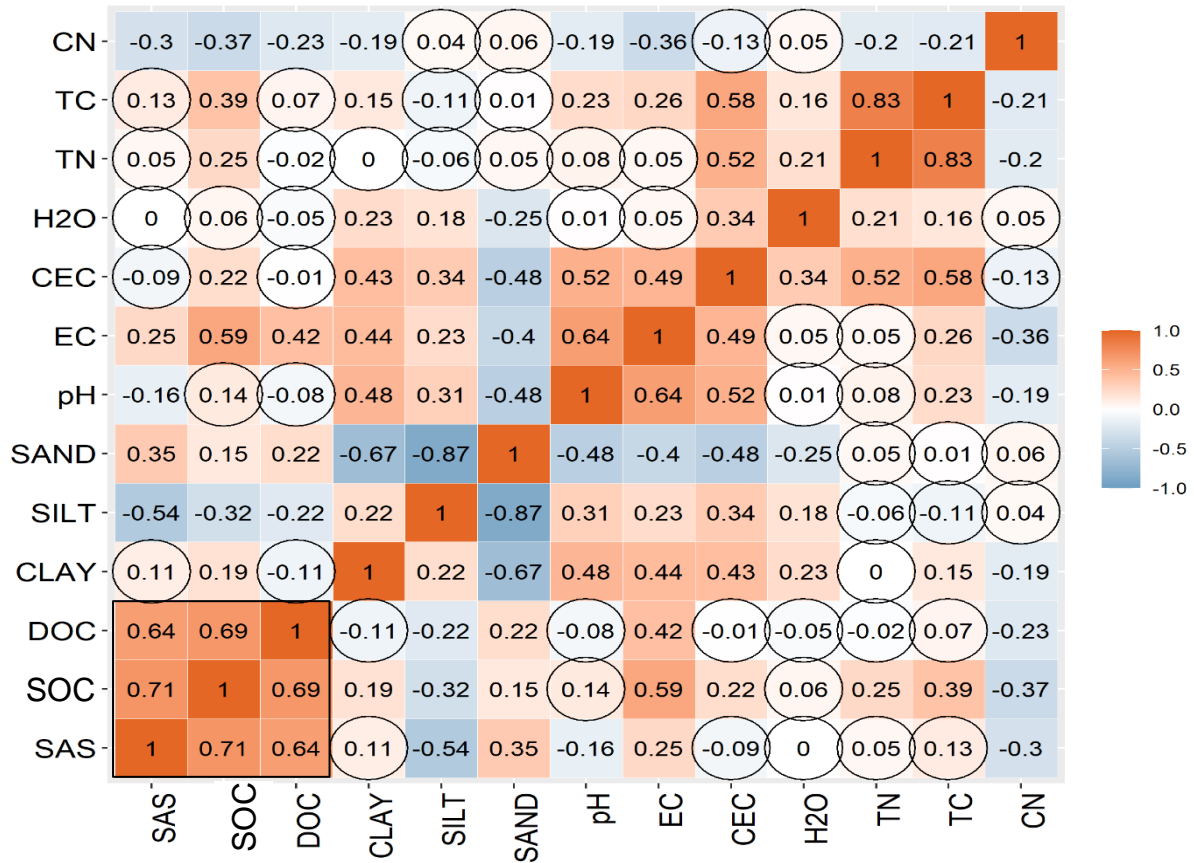


Figure Ap.7: Correlation plot indicating Pearson correlation coefficients between SAS, basic soil properties (Electric conductivity – EC, Moisture content – H2O, pH, Cation exchange capacity – CEC, sand, silt and clay, total nitrogen – TN, total carbon – TC, Carbon:Nitrogen ratio – CN, Dissolved organic carbon – DOC, organic Carbon – OC. Insignificant values are marked with a circle.

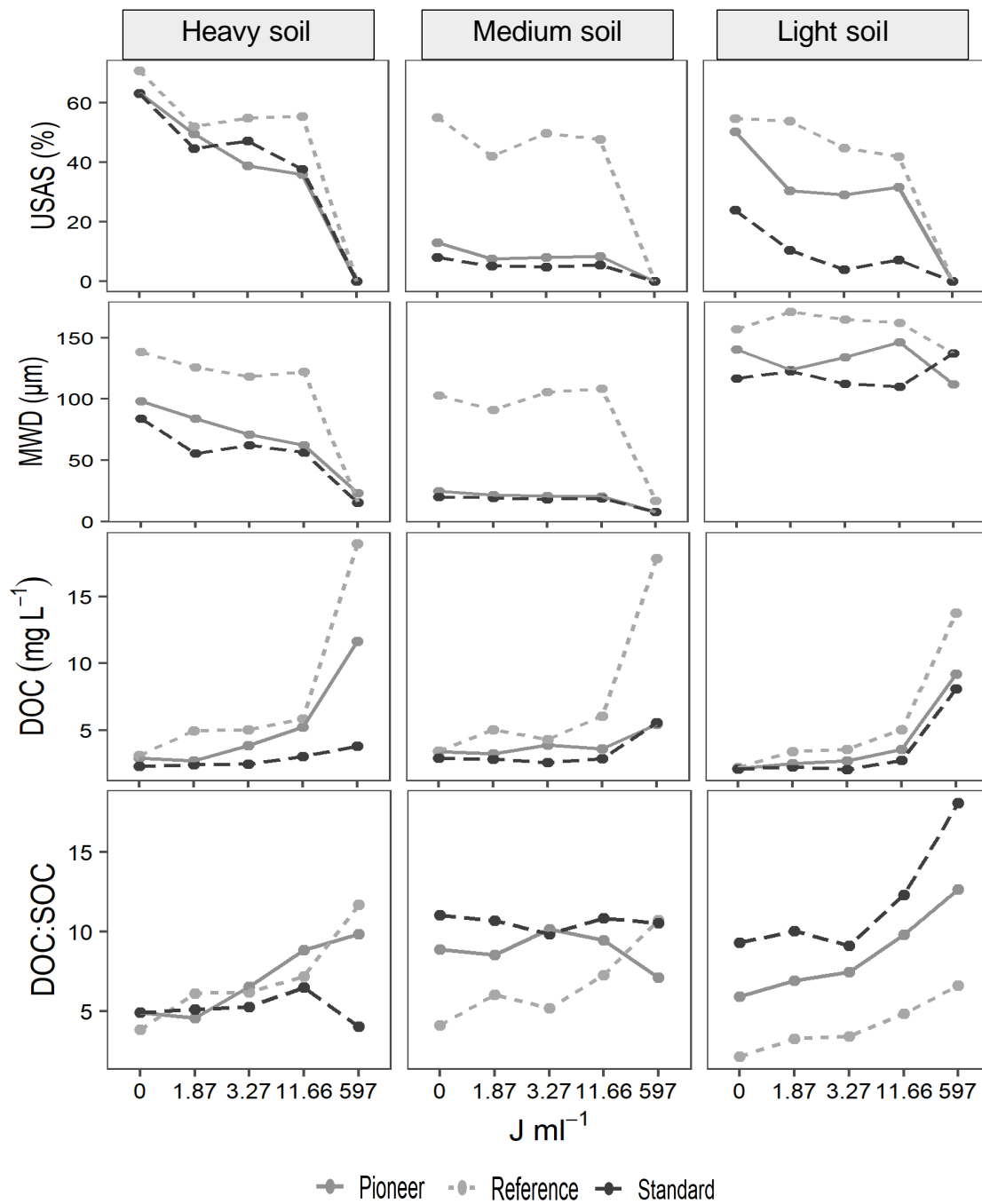


Figure Ap.8: Change of USAS, MWD, DOC and DOC:SOC ratio along increasing sonication energy for all sites and systems. Due to low number of replicates no statistical evaluation has been made. See absolute values for each site and management system in table x in the appendix.

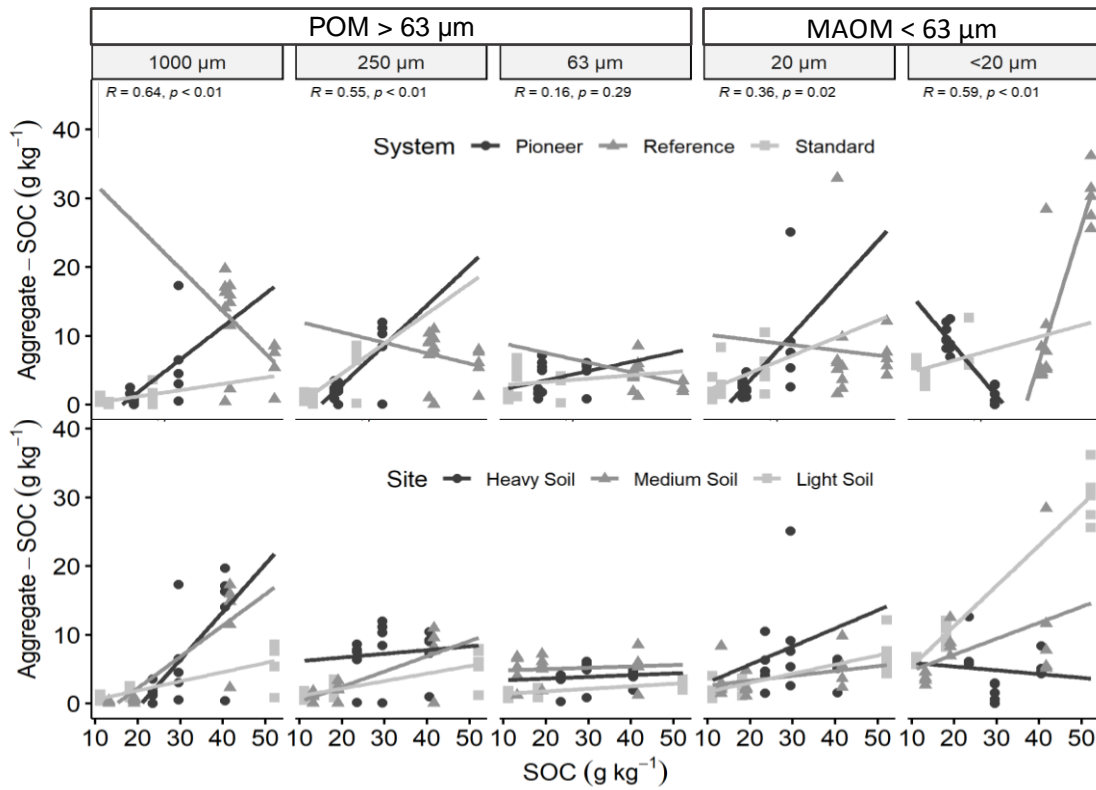


Figure Ap.9: Relationship between soil organic carbon (SOC) content and SOC in aggregate fractions. This relationship is given for sites (top) and management systems (bottom). Correlation coefficients are indicated only for each fractions. Correlation coefficients are not given for each system and site due to low number of replicates per SOC value ($n=2$).

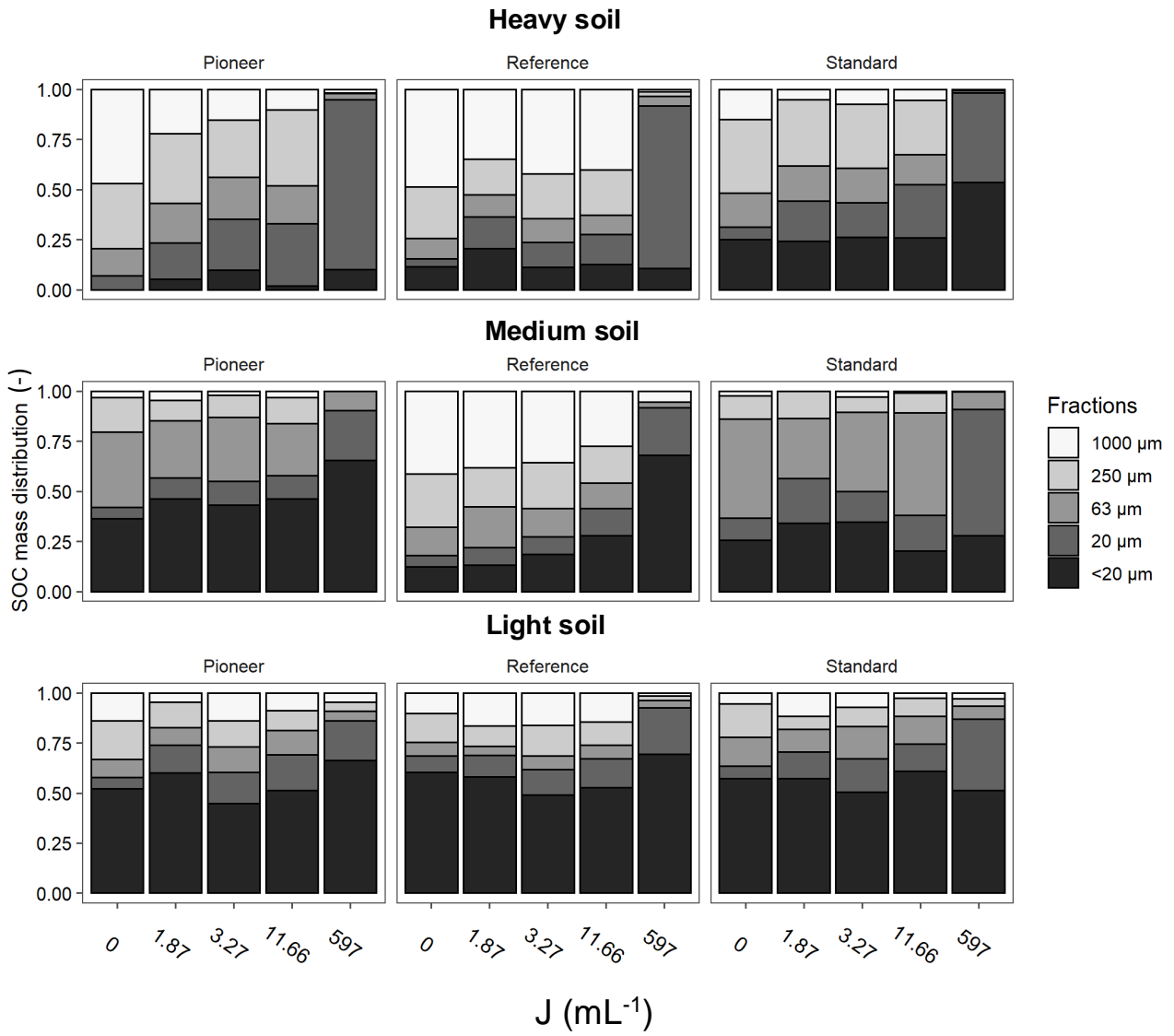


Figure Ap.11: Relative distribution of SOC mass in aggregates fractions during ultrasonication.

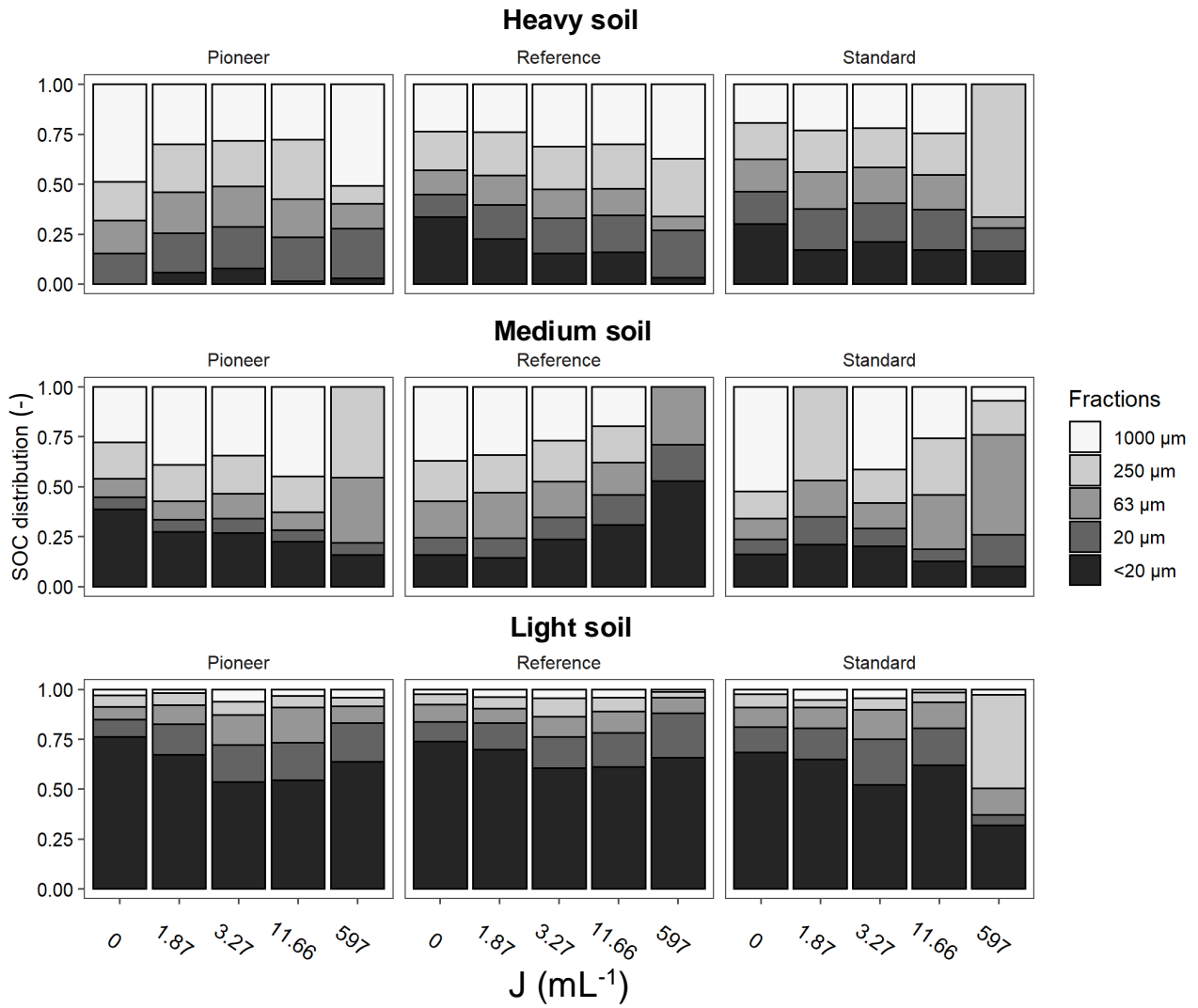


Figure Ap.12: Relative distribution of SOC content in aggregate fractions during ultrasonication. The SOC content of the < 20 μm is derived from the difference calculation between total SOC in aggregates and in the bulk soil.

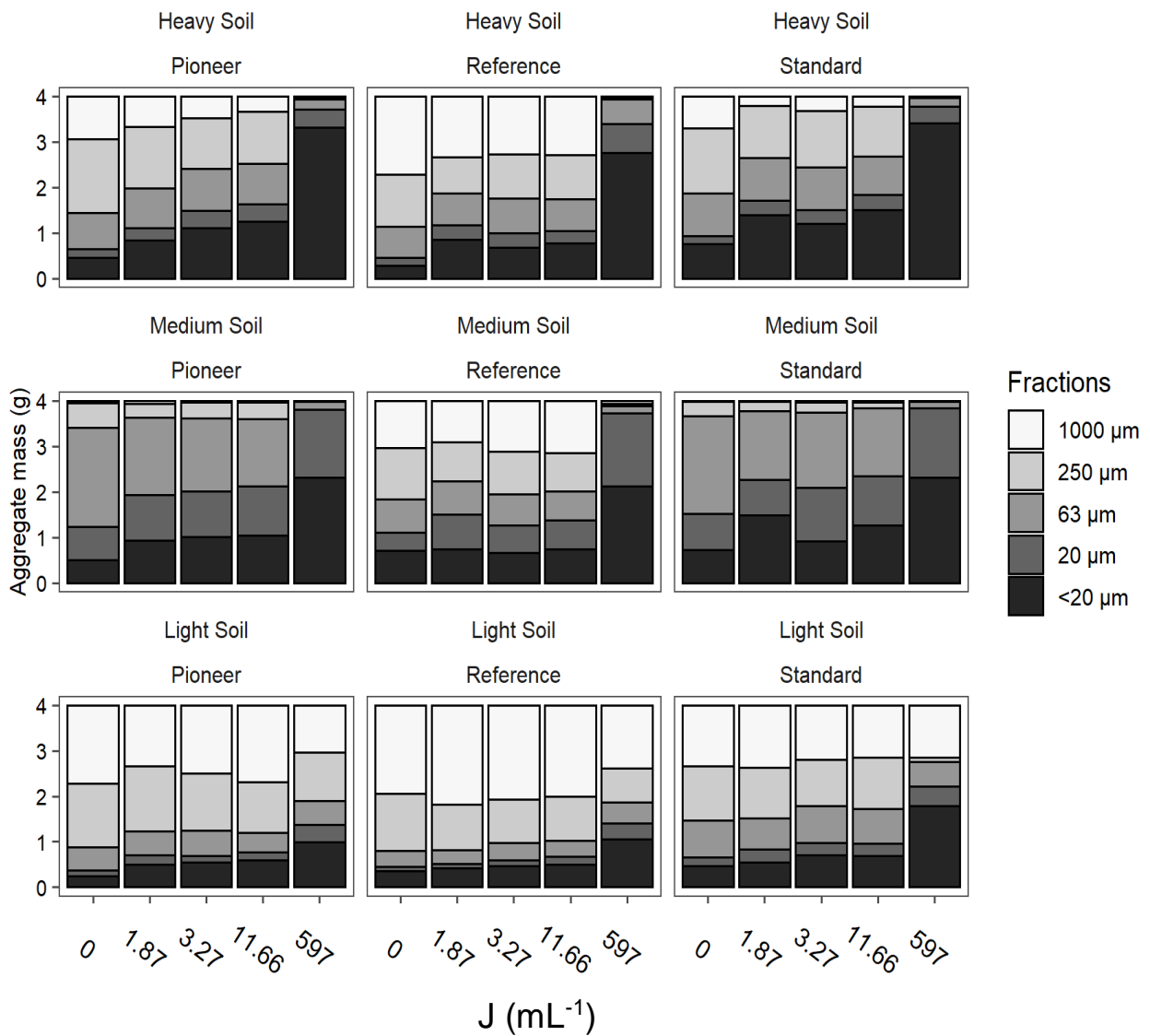


Figure Ap.13: Mass distribution in aggregate fractions during ultrasonication. 4 g indicates 100 % of the mass of all fractions.

Table Ap.14: Mean values of SAS, SOC and DOC (bulk soil) averaged over 21 study sites.

System	Soil depth	SAS (%)			SOC (%)			DOC mg g ⁻¹		
		Mean	SD(±)	n	Mean	SD(±)	n	Mean	SD(±)	n
Pioneer	0-5 cm	59.35	19.94	21	2.72	1.08	21	0.25	0.07	21
	5-20 cm	55.64	22.43	21	2.40	1.23	21	0.22	0.08	21
	20-35 cm	50.24	23.17	21	1.99	1.04	21	0.18	0.07	21
	Total	55.08	21.86	63	2.37	1.14	63	0.22	0.08	63
Reference	0-5 cm	85.24	11.37	21	4.70	1.72	21	0.42	0.11	21
	5-20 cm	73.09	18.16	21	3.39	1.60	21	0.34	0.10	21
	20-35 cm	60.93	17.31	21	2.90	1.46	21	0.27	0.11	21
	Total	73.09	18.57	63	3.66	1.75	63	0.34	0.12	63
Standard	0-5 cm	51.01	21.59	21	2.12	1.10	21	0.18	0.05	21
	5-20 cm	48.52	18.50	21	2.05	1.04	21	0.18	0.04	21
	20-35 cm	46.88	20.27	21	1.83	1.08	21	0.15	0.03	21
	Total	48.81	19.91	63	2.00	1.07	63	0.17	0.04	63
Total	0-5 cm	65.20	23.16	63	3.18	1.72	63	0.28	0.13	63
	5-20 cm	59.08	22.07	63	2.61	1.41	63	0.25	0.10	63
	20-35 cm	52.68	20.95	63	2.24	1.28	63	0.20	0.09	63
	Total	58.99	22.55	189	2.68	1.52	189	0.24	0.11	189

Table Ap.15: Relative and absolute effect of Pioneer compared to Standard for SAS and SOC

Soil type	SAS				SOC			
	Absolute	SD	%	SD	Absolute	SD	%	SD
Heavy	7.58	2.85	9.51	3.19	0.34	0.30	11.74	9.76
Medium	1.92	3.56	4.53	7.71	0.18	0.21	9.13	9.10
Light	10.97	5.19	16.78	7.28	0.59	0.31	21.23	8.50
Total	6.27	2.60	11.39	3.95	0.36	0.22	15.40	7.14

Table Ap.16: Mean values for breakdown parameter USAS, MWD, DOC and the ratio of DOC:SOC. Values were averaged over three sites (n=3).

Parameter	J mL ⁻¹	Pioneer		Reference		Standard		Total	
		Mean	SD(±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)
USAS (%)	0	42.18	26.1	60.26	9.2	31.72	28.3	44.72	23.4
	1.87	29.22	21.1	49.29	6.4	20.10	21.5	32.87	20.1
	3.27	25.33	15.7	49.81	5.0	18.67	24.7	31.27	20.5
	11.66	25.33	14.8	48.31	6.8	16.82	18.0	30.15	18.6
	597	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
	Total	24.41	20.7	41.53	22.6	17.46	20.6	27.80	23.2
MWD (µm)	.00	87.67	58.5	132.70	27.6	73.58	49.3	97.98	48.7
	1.87	76.47	51.4	129.26	40.3	65.82	52.6	90.52	51.2
	3.27	75.21	56.7	129.60	31.1	64.29	47.2	89.70	50.2
	11.66	76.40	64.1	130.84	28.0	61.50	45.8	89.58	52.4
	597	47.57	56.3	56.72	69.6	53.37	72.6	52.55	57.7
	Total	72.66	50.6	115.82	47.1	63.71	46.5	84.07	52.3
DOC (mg L ⁻¹)	.00	2.81	0.6	2.92	0.6	2.44	0.4	2.72	0.5
	1.87	2.82	0.4	4.46	0.9	2.49	0.3	3.25	1.0
	3.27	3.48	0.7	4.29	0.7	2.37	0.3	3.38	1.0
	11.66	4.14	0.9	5.65	0.5	2.89	0.1	4.23	1.3
	597	8.75	3.1	16.87	2.7	5.82	2.2	10.48	5.5
	Total	4.40	2.6	6.84	5.4	3.20	1.6	4.81	3.8
DOC/SOC	.00	6.56	2.1	3.36	1.1	8.41	3.2	6.11	3.0
	1.87	6.65	2.0	5.13	1.6	8.60	3.0	6.79	2.5
	3.27	8.05	1.9	4.92	1.4	8.06	2.5	7.01	2.3
	11.66	9.36	0.5	6.43	1.4	9.88	3.0	8.56	2.3
	597	9.86	2.8	9.67	2.7	10.88	7.0	10.14	4.1
	Total	8.10	2.2	5.90	2.6	9.17	3.6	7.72	3.1

Parameter	J mL ⁻¹	Heavy soil					Medium soil					Light soil				
		Pioneer	Reference	Standard	Total	SD(±)	Pioneer	Reference	Standard	Total	SD(±)	Pioneer	Reference	Standard	Total	SD(±)
USAS (%)	0	63.32	70.86	63.13	65.77	4.4	37	55.10	8.04	25.37	25.9	50.23	54.81	23.99	43.01	16.6
	1.87	49.58	52.00	44.68	48.75	3.7	7.52	42.02	5.13	18.22	20.6	30.56	53.85	10.50	31.64	21.7
	3.27	38.86	54.88	47.17	46.97	8.0	8.06	49.70	4.90	20.89	25.0	29.08	44.85	3.94	25.96	20.6
	11.66	35.86	55.40	37.63	42.96	10.8	8.39	47.68	5.54	20.53	23.6	31.75	41.85	7.28	26.96	17.8
	597	0.00	0.00	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.0
	Total	37.53	46.63	38.52	40.89	23.3	7.39	38.90	4.72	17.00	20.2	28.32	39.07	9.14	25.51	20.6
MWD (µm)	0	98.15	138.45	84.02	106.87	28.2	24.62	102.68	19.93	49.08	46.5	140.25	156.96	116.79	138.00	20.2
	1.87	83.94	125.68	55.57	88.40	35.3	21.74	90.84	19.10	43.89	40.7	123.73	171.27	122.78	139.26	27.7
	3.27	70.94	118.16	62.27	83.79	30.1	20.72	105.78	18.16	48.22	49.9	133.95	164.84	112.44	137.08	26.3
	11.66	62.20	122.15	56.12	80.16	36.5	20.55	108.19	18.59	49.11	51.2	146.45	162.16	109.80	139.47	26.9
	597	23.18	16.11	15.20	18.17	4.4	7.62	16.97	7.86	10.82	5.3	111.90	137.08	137.05	128.68	14.5
	Total	67.68	104.11	54.64	75.48	39.8	19.05	84.89	16.73	40.22	38.9	131.26	158.46	119.77	136.50	20.4
DOC (mg L ⁻¹)	0	2.89	3.12	2.31	2.77	0.4	3.39	3.43	2.92	3.24	0.3	2.15	2.22	2.09	2.15	0.1
	1.87	2.69	4.94	2.40	3.34	1.4	3.25	5.03	2.83	3.70	1.2	2.51	3.41	2.24	2.72	0.6
	3.27	3.86	5.03	2.47	3.78	1.3	3.88	4.31	2.60	3.60	0.9	2.71	3.54	2.04	2.77	0.8
	11.66	5.23	5.83	3.05	4.70	1.5	3.61	6.06	2.87	4.18	1.7	3.57	5.05	2.76	3.79	1.2
	597	11.63	18.97	3.79	11.46	7.6	5.43	17.87	5.56	9.62	7.1	9.20	13.78	8.10	10.36	3.0
	Total	5.26	7.58	2.80	5.21	4.5	3.91	7.34	3.36	4.87	3.8	4.03	5.60	3.45	4.36	3.4
DOC/SOM	0	4.90	3.84	4.91	4.55	0.6	8.86	4.12	11.02	8.00	3.5	5.91	2.13	9.31	5.78	3.6
	1.87	4.56	6.09	5.10	5.25	0.8	8.51	6.03	10.68	8.41	2.3	6.90	3.27	10.01	6.73	3.4
	3.27	6.53	6.20	5.24	5.99	0.7	10.15	5.17	9.83	8.38	2.8	7.46	3.39	9.11	6.65	2.9
	11.66	8.85	7.19	6.49	7.51	1.2	9.45	7.27	10.85	9.19	1.8	9.80	4.84	12.32	8.98	3.8
	597	9.84	11.69	4.03	8.52	4.0	7.10	10.71	10.52	9.44	2.0	12.64	6.60	18.08	12.44	5.7
	Total	6.93	7.00	5.15	6.36	2.2	8.81	6.66	10.58	8.68	2.2	8.54	4.04	11.77	8.12	4.2

Table Ap.18: Values of SOC (%), mass (g) and SOC mass (g kg Fraction⁻¹) are listed for all management systems within three soil types. Mean values are derived from 5 sonication energy levels

System	Parameter	Fraction	Heavy soil		Medium soil		Light soil		Total		
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	N
Pioneer	SOC (%)	1000 µm	5.00	1.73	4.07	2.52	0.44	0.18	3.17	2.61	15
		250 µm	2.81	1.04	3.28	1.66	0.74	0.32	2.28	1.56	15
		63 µm	2.39	0.48	1.99	1.39	1.39	0.52	1.92	0.93	15
		20 µm	2.72	0.28	0.86	0.03	1.89	0.29	1.82	0.81	15
		Total	3.23	1.43	2.55	1.98	1.12	0.66	2.30	1.69	60
	Mass (g)	1000 µm	0.49	0.34	0.04	0.02	1.46	0.28	0.66	0.66	15
		250 µm	1.06	0.61	0.32	0.18	1.25	0.17	0.87	0.55	15
		63 µm	0.74	0.29	1.42	0.75	0.51	0.04	0.89	0.59	15
		20 µm	0.33	0.09	1.06	0.28	0.21	0.10	0.53	0.42	15
		Total	0.65	0.45	0.71	0.68	0.86	0.55	0.74	0.57	60
	SOC mass g kg ⁻¹	1000 µm	6.38	6.50	0.50	0.32	1.67	0.85	2.85	4.39	15
		250 µm	8.40	4.84	1.96	1.22	2.17	0.97	4.18	4.11	15
		63 µm	4.68	2.17	5.11	2.01	1.73	0.58	3.84	2.24	15
		20 µm	9.96	8.81	2.47	1.38	2.65	0.99	5.03	6.00	15
		Total	7.35	5.94	2.51	2.12	2.05	0.89	3.97	4.35	60
Reference	SOC (%)	1000 µm	5.34	1.26	4.60	2.83	1.21	0.59	3.72	2.51	15
		250 µm	4.17	0.91	3.04	1.71	2.28	0.99	3.16	1.41	15
		63 µm	2.23	0.48	3.55	0.68	3.26	0.98	3.01	0.90	15
		20 µm	3.23	0.95	2.11	0.55	5.33	0.65	3.56	1.54	15
		Total	3.74	1.46	3.32	1.82	3.02	1.73	3.36	1.68	60
	Mass (g)	1000 µm	1.13	0.64	0.86	0.45	1.92	0.31	1.30	0.65	15
		250 µm	0.79	0.43	0.76	0.42	0.99	0.18	0.84	0.35	15
		63 µm	0.68	0.08	0.59	0.24	0.37	0.06	0.54	0.19	15
		20 µm	0.34	0.18	0.80	0.47	0.17	0.10	0.44	0.39	15
		Total	0.73	0.47	0.75	0.38	0.86	0.72	0.78	0.54	60
	SOC mass g kg ⁻¹	1000 µm	13.52	7.59	12.37	6.01	6.17	3.27	10.69	6.40	15
		250 µm	7.37	3.74	7.27	4.27	5.63	2.71	6.76	3.46	15
		63 µm	3.84	1.13	5.39	2.66	2.94	0.77	4.06	1.91	15
		20 µm	10.41	12.71	4.99	2.93	7.28	2.99	7.56	7.52	15
		Total	8.79	7.93	7.50	4.88	5.51	2.91	7.27	5.70	60
Standard	SOC (%)	1000 µm	1.74	0.98	2.49	2.61	0.21	0.12	1.48	1.79	15
		250 µm	2.74	1.81	1.80	0.60	0.67	0.69	1.74	1.39	15
		63 µm	1.47	0.55	1.71	0.88	0.76	0.14	1.31	0.70	15
		20 µm	1.71	0.39	0.81	0.23	0.99	0.45	1.17	0.53	15
		Total	1.91	1.11	1.70	1.44	0.66	0.48	1.43	1.20	60
	Mass (g)	1000 µm	0.30	0.25	0.02	0.01	1.24	0.11	0.52	0.56	15
		250 µm	0.98	0.56	0.18	0.12	0.91	0.46	0.69	0.54	15
		63 µm	0.77	0.32	1.39	0.74	0.72	0.11	0.96	0.54	15
		20 µm	0.30	0.07	1.07	0.30	0.29	0.08	0.55	0.42	15
		Total	0.59	0.44	0.67	0.70	0.79	0.42	0.68	0.53	60
	SOC mass g kg ⁻¹	1000 µm	1.56	1.29	0.17	0.18	0.67	0.41	0.80	0.94	15
		250 µm	6.10	3.41	1.12	0.67	1.02	0.53	2.75	3.09	15
		63 µm	3.17	1.66	4.74	2.29	1.39	0.42	3.10	2.08	15
		20 µm	5.40	3.33	3.42	2.79	1.92	1.24	3.58	2.83	15
		Total	4.06	3.02	2.37	2.51	1.25	0.83	2.56	2.56	60
Total	SOC (%)	1000 µm	4.03	2.10	3.72	2.63	0.62	0.56	2.79	2.47	45
		250 µm	3.24	1.39	2.71	1.47	1.23	1.02	2.39	1.54	45
		63 µm	2.03	0.62	2.42	1.26	1.80	1.25	2.08	1.09	45
		20 µm	2.55	0.87	1.26	0.70	2.74	1.99	2.18	1.45	45
		Total	2.96	1.53	2.53	1.85	1.60	1.50	2.36	1.72	180
	Mass (g)	1000 µm	0.64	0.55	0.31	0.47	1.54	0.37	0.83	0.70	45

	250 μm	0.94	0.51	0.42	0.36	1.05	0.32	0.80	0.48	45
	63 μm	0.73	0.24	1.13	0.70	0.53	0.16	0.80	0.50	45
	20 μm	0.32	0.11	0.98	0.36	0.22	0.10	0.51	0.40	45
	Total	0.66	0.45	0.71	0.60	0.84	0.57	0.73	0.54	180
SOC mass g kg ⁻¹	1000 μm	7.15	7.41	4.35	6.70	2.84	3.07	4.78	6.17	45
	250 μm	7.29	3.87	3.45	3.70	2.94	2.56	4.56	3.88	45
	63 μm	3.90	1.70	5.08	2.18	2.02	0.89	3.67	2.07	45
	20 μm	8.59	8.77	3.63	2.52	3.95	3.05	5.39	5.89	45
	Total	6.73	6.21	4.13	4.12	2.94	2.58	4.60	4.80	180

Table Ap.19: Values of SOC (%), mass (g) and SOC mass (g kg Fraction⁻¹) are listed for all management systems within three soil types. Mean values are derived from 5 sonication energy levels. Only for < 20 μm fine-silt clay fractions. This fraction was not physically collected. All values are derived by difference calculation between SOC in aggregates and SOC in bu

System	Fraction	Parameter	Heavy soil		Medium soil		Light soil		Total		
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	N
Pioneer		Mass (g)	1.39	1.12	1.16	0.68	0.57	0.27	1.04	0.80	15
		SOC (%)	0.47	0.42	3.63	1.22	5.18	3.23	3.09	2.75	15
		SOC mass g kg ⁻¹	1.59	1.33	9.07	2.07	9.98	1.52	6.88	4.19	15
Reference	< 20 μm	Mass (g)	1.07	0.97	0.99	0.63	0.56	0.29	0.87	0.68	15
		SOC (%)	3.28	2.17	4.43	1.50	24.35	8.10	10.69	11.00	15
		SOC mass g kg ⁻¹	5.40	1.69	11.68	9.71	30.17	4.06	15.75	12.28	15
Standard		Mass (g)	1.65	1.02	1.34	0.62	0.84	0.54	1.28	0.78	15
		SOC (%)	1.98	0.67	1.32	0.61	3.71	1.61	2.34	1.44	15
		SOC mass g kg ⁻¹	7.29	2.99	3.77	0.81	6.20	0.50	5.75	2.26	15
Total		Mass (g)	1.37	0.99	1.16	0.61	0.66	0.38	1.06	0.75	45
		SOC (%)	1.91	1.72	3.13	1.74	11.08	10.82	5.37	7.49	45
		SOC mass g kg ⁻¹	4.76	3.14	8.17	6.32	15.45	11.14	9.46	8.70	45