Microaggregate stability and storage of organic carbon is affected by clay content in arable Luvisols

Lars Krause[a*], Andrei Rodionov[b], Steffen A. Schweizer[c], Nina Siebers[a], Eva Lehndorff[b], Erwin Klumpp[a], Wulf Amelung[a,b]

[a] Forschungszentrum Jülich GmbH, Agrosphere (IBG-3) Institute of Bio- and Geosciences, Wilhelm Johnen Straße, 52425 Jülich, Germany

[b] University of Bonn, Institute of Crop Science and Resource Conservation - Soil Science and Soil Ecology, Nussallee 13, D-53115 Bonn, Germany

[c] Technical University of Munich, Department Ecology and Ecosystem Management, Chair of Soil Science, Emil-Ramann-Straße 2, D-85354 Freising-Weihenstephan, Germany

[*] Corresponding author, e-mail: l.krause@fz-juelich.de

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Abstract

As soil microaggregates (<250 µm, SMA) usually withstand long-term tillage, we hypothesized that i) elevated clay contents in arable soil support aggregation already at microaggregate level, leading to ii) increasing organic carbon (SOC) enrichment in smaller SMA size fractions. To test these hypotheses we sampled the topsoil (5-20 cm) of Luvisols with a long history of agricultural management at the Scheyern experiment station (Germany) in quintuplicates from each of five subsites with different clay contents (19-34%). The field-fresh topsoil was fractionated into macroaggregates (8000-250 µm), large SMA (250-20 µm), and small SMA (<20 µm) and the mass distribution was recorded. In addition, the water stable macroaggregates (>250 µm) were dispersed ultrasonically to study occluded SMA and single building units. Finally, we analyzed the size distribution of the small SMA by laser diffraction analysis. The total mass distribution of free and occluded SMA grouped soils into those with small (19, 22, and 24%) and large (32 and 34%) clay contents. The finer textured soils exhibited larger portions of occluded SMA, with a gamma size distribution of small SMA peaking at 6 µm. Yet the occluded small SMA in the finer textured soils showed an additional enrichment of colloids <1 µm. The SOC was indeed enriched in finer fractions, but more in the small SMA of the coarse textured sites than in the finer textured ones, whereas the opposite was true for the large SMA. We conclude, therefore, that elevated contents of clay-sized particles promote SMA formation and stabilization, therewith shifting SOC enrichment from small to larger SMA.

1 Introduction

Soil microaggregates (SMA) having a size <250 µm are one of the key structures that influence soil properties such as the storage and stabilization of carbon (C), diversity of microbial habitats, diffusion of nutrients and gases, cycling of trace elements, or water retention as recently reviewed by Totsche et al. (2017). Hence, quantifying SMA and their properties has become an essential task in order to predict dynamic changes in soil ecosystems due to management and global change (Six et al., 2004).

Despite the wealth of information on SMA and their role for SOC protection or formation of organo-mineral-associations as building units (Totsche et al., 2017), we still lack information to systematize microaggregate formation across gradients in soil properties. Up to 40% of organic matter may be stored within SMA (Carter, 1995), and the turnover of organic matter is associated with SMA stability (Goebel et al., 2009; Mueller et al., 2012). The stability of microaggregates increases with decreasing diameter of SMA (Golchin et al., 1994; Six et al., 2002; Tisdall, 1996). As a result, SMA are divided into two size fractions, large (250-20 µm) and small (<20 µm) SMA (Oades and Waters, 1991; Tisdall and Oades, 1982; Totsche et al., 2017). Small SMA are believed to be mainly stabilized by short-range van-der-Waals forces and electrostatic binding as reviewed in Totsche et al. (2017). They are stable against a range of forces, and cannot be further disintegrated into smaller building units (Kaiser and Berhe, 2014; Kaiser et al., 2012). This is different for larger aggregate units (Kaiser and Berhe, 2014), which contain small SMA as subunits, held together by roots, hyphae, or extracellular polymeric substances as temporary binding agents (Kleber et al., 2007; Six et al., 2000).

It is well established that the amount of clay-sized particles affects the stability and hierarchy of such larger aggregate units (Denef and Six, 2005; Goldberg, 1989; Wagner et al., 2007). Furthermore extensive studies were made about the influence of clay content on the composition and distribution of free and occluded organic matter. These studies showed that clay has an positive effect on the storage of organic carbon in occluded particulate organic matter, which has been linked with increased aggregation in the soils with larger clay content (Kölbl and Kögel-Knabner, 2004). In arable soils, stable organic carbon is mainly associated with fine particle size fractions (Kiem et al., 2002). In turn, organic carbon contributes to the stabilization of microaggregates as well (Kölbl et al., 2005). In soils without tillage and soil management these effects are stronger resulting in an increased soil aggregation and stability as well as improved storage of organic carbon (Ayoubi et al., 2012; Karchegani et al., 2012), which can breakdown during land use change (Schweizer et al., 2017). Furthermore, the stabilization of SOC can also be governed by the surface area of oxides influencing the interaction of organic compounds with minerals (Kiem and Kögel-Knabner, 2002). However, detailed studies on the role of clay for the properties of the small SMA (< 20 µm) as major building units are largely lacking.

We hypothesize that for a given arable land-use system i) elevated clay contents support aggregation not only at macro- but already at microaggregate level, and that ii) stabilization of the smaller size fractions through increased amounts of SOC shifts the overall particle-size distribution to larger equivalent mean weight diameter than at subsites with lower clay contents. In order to test these hypotheses we collected topsoil samples with increasing clay contents from a heterogeneous arable field at Scheyern experiment station, Germany, and investigated the size and mass distribution of small SMA as major building units of their larger counterparts.

2 Materials and Methods

2.1 Soil sampling and sample characteristics

We selected five adjacent sample sites on the same arable plot (Table A.1; Supplementary Materials) at the research station Scheyern (48° 29' 36'' N, 11° 26' 15'' E) situated in the rural area of the hill slopes north of Munich (Germany) for sampling. Plot selection was facilitated by using gamma spectrometry for sensing textural variations within a heterogeneous field site (Heggemann et al., 2017). The study site was characterized by a mean annual temperature of 7.4 °C and a mean annual precipitation of 803 mm (Schröder et al., 2002). The soils were sandy to loamy textured Luvisols, developed on sandy molasse sediments with silty to clayey molasse deposits. All samples were free of carbonates and dominated by illitic clay mineralogy, which did not show systematic differences across the texture gradient (K.

U. Totsche, personal communication, 2018). Other clay minerals detected were Fe-chlorite, kaolinite and smectite, the latter however only in samples with 12 and 18.5% clay, the other samples contained vermiculite instead (K. U Totsche, personal communication).

Overall 25 samples were used for analysis from each of five sampling sites with five replicates each, taken in a radial sampling scheme (1 m around each subsite; Wilding (1985)). Samples were taken after removal of the upper Ap layer prone to crust formation (first 4-5 cm) with a large core cutter (diameter: 16 cm, length: 15 cm, volume: ~3000 cm^2). Clay contents were determined based on texture analysis using H_2O_2 oxidation. About 10 g of each sample was treated with an excess of H_2O_2 (30%) until no further oxidation could be observed. The oxidized samples were put into an oven at 60 °C with additional 500 mL H_2O for 12 hours to remove excess H_2O_2. Then, the samples were sieved <63 µm, freeze-dried and 3 g were mixed with 80 mL 0.0125 M $Na_4P_2O_7$. The clay content was determined by X-ray attenuation in a Sedigraph III Plus (Micromeritics, Georgia, USA). The clay contents were 19, 22, 24, 32, and 34%, respectively. Additionally 100 cm^3 cores were sampled for field moisture and bulk density determination. The fresh soil samples were gently sieved <8 mm and stored at 4 °C. The Fe, Al, and Mn phases (Fe_D, Al_D, Mn_D) were extracted using dithionite-citrate-bicarbonate (DCB) according to Mehra and Jackson (1958), serving as a measure for the abundance of pedogenic oxides. The extracts were analyzed with inductively coupled plasma optical emission spectroscopy (ICP–OES) Vista-Pro (Varian, California, USA). The total C contents in each aggregate fraction were determined using elemental analysis (Vario MICRO cube, Elementar Analysesysteme GmbH, Langenselbold, Germany), according to ISO 10694 (1995).

2.2 Wet fractionation and density fractionation

The fractionation was conducted on field-moist soil in triplicates, using a combination of wet sieving and pressure filtration (Fig. 1). In order to avoid re-aggregation, the samples were kept submerged in deionized water during the whole process of fractionation until they were shock frozen in liquid nitrogen and lyophilized. In short, 50 g of 8 mm field fresh sieved samples were placed on a glass fiber filter being on the top of a sieve tower and pre-wetted for five minutes. The sample was then sieved in a sieve-tower using 6 sieves having a mesh size between 2800 to 20 µm (Fig. 1) under constant shaking (30 U min^{-1}) for 10 minutes. Collected fractions were categorized into free and occluded SMA. Free SMA (250-53, 53-20, and 20-0.22 µm) were collected without further treatment. In order to isolate occluded SMA, remnants of wet sieving being >250 µm were merged, suspended in H_2O and subjected to an ultrasonic treatment using 60 J mL^{-1} (Branson Ultrasonics corp., Connecticut, USA) as recommended by Amelung and Zech (1999). After ultrasonic treatment the suspension was wet sieved using the sieve tower as described above starting with the 2800 µm sieve and obtained size fractions being >250, 250-53, 53-20, and <20 µm using the sieving tower described by Kösters et al. (2013).

Wet sieving resulted in approximately 50 L suspensions each containing <20 µm fractions of free and occluded SMA. The collection of small SMA was performed through pressure filtration. Homogenized aliquots were pressure filtered (Pressure filter Holder Type 16274, Sartorius, Göttingen, Germany) with 2-3 bar N_2 through a 0.22 µm PVDF filter (Membrane filter PVDF hydrophilic, 1201322, Berrytec GmbH, Oberbayern, Germany). After filtration the remnants were removed from the membrane filter with H_2O, the resulting suspension was shock frosted in liquid nitrogen and freeze-dried. The collected <0.22 µm fraction was considered as nanoparticle fraction. The process of fractionation resulted in an average total mass recovery of 97%.

Since the collected large SMA and macroaggregates still contained primary mineral particles, a density fractionation with sodium metatungstate was performed with pooled field replicates according to Virto et al. (2008). With this method, primary sand and silt particles with a density of >2.5 g cm^{-3} were removed from the affected size fractions. Particulate organic matter (POM) with a density of 1.6-1.8 g cm^{-3} was not removed in an additional step since the mass contribution of the POM fractions was limited to a minor proportion (Kölbl and Kögel-Knabner, 2004).

2.3 Particle size distribution

The size distribution of small SMA (<20 µm) was measured with a laser particle-size analyzer (LA-950, Horiba, Kyōto, Japan). Before each measurement the sample was suspended in water and ultrasonicated (13 J mL^{-1}) using the internal ultrasonic finger of the instrument. During the measurement the SMA were kept in suspension through stirring. The scattered light data was transformed into a volume based size distribution by a software algorithm using the mie-theory provided by the manufacturers’ software.

2.4 Statistical tests

Calculation of Pearsons correlation coefficients between clay content and measured elements was performed with Excel (Microsoft Excel 2010, Microsoft corporation, Washington, USA). Statistical analysis was performed with Origin (OriginPro 2017, Originlab, Massachusetts, USA). The Shapiro Wilk test was used for testing the normal distribution. With the one-way ANOVA the statistical significance was tested at a significance level of α=0.05 and in a post-hoc test the means were compared with Tukey's method. The tests were applied for the SOC and mass percentage data, each pooled into soils with small (19-24%) and large (33-34%) clay content. Overall four levels of significance were defined and indicated by asterisks (not significant, p>0.05, no symbol; significant, p≤0.05, *; very significant, p≤0.01, **; highly significant, p≤0.001, ***; extremely significant, p≤0.0001, ****).

3 Results

Generally, the subsampling of the sites was successful in yielding samples with different texture: as clay content increased from 19-34%, mass proportions of sand decreased respectively (Fig. 2). When NaOCl was used instead of H_2O_2, the respective clay contents were slightly lower, but changed in similar direction (Table A.2; Supplementary Materials). Intriguingly, the Fe_D content did not show significant differences along this transect, being in average 8.3 g kg^{-1} in soils with 19-24% clay, and 8.5 g kg^{-1} in soils with larger clay content (32-34%), respectively (Table A.2; Supplementary Materials). In turn, correlation coefficients of 0.83 and 0.87 indicate that the concentrations of Al_D and Mn_D increased with clay content (Table A.2; Supplementary Materials). With density fractionation the mass of primary mineral particles with a density of >2.5 g cm^{-3} was evaluated. For the 8000-250 µm fractions, the average mass percentage of primary mineral particles reached 94% and 96% soil mass, being slightly larger in soils with larger clay content compared to soils with smaller clay content (Table A.2; supplementary materials). For all 250-20 µm fractions, i.e., the large SMA fractions, the mass percentage of primary mineral particles averaged 80% without noticeable differences between subsites of different clay contents. Intriguingly, the amount of primary particles in "free" fractions was lower (75%) than in the occluded fractions (84%; data not shown). The small SMA fraction was not corrected for primary particle contents.

3.1 SMA mass distribution

Unlike the textural gradient, the evaluation of isolated fractions indicated that the mass distribution of soil aggregates was not linearly related to the amount of clay-sized particles, but rather grouped into soils having small and large clay content, respectively (Fig. 3a). Samples with large clay content showed large amounts of occluded small SMA, while samples with small clay contents showed less occluded small SMA and higher content of free SMA (Fig. 3b). The main differences between samples with small and large clay content were thus the amount of occluded small SMA, the proportion of which was significantly larger in soils with ≥32% clay (Fig. 4). The mass-proportion of free small SMA was also affected by clay content and significantly larger in soils with smaller clay contents.

3.2 Size distribution of the small SMA

The volume weighted size distribution of small SMA followed mainly the gamma distribution (Fig. A.1; Supplementary Materials). More than 90% of the measured fraction was ≤10 µm (Fig. 5). The most abundant size class of small SMA was found in the range of 5 to 7 µm (Fig. 5a). Intriguingly, this size distribution was similar for free and occluded small SMA, which can be clearly seen by comparison of the cumulative percentages (Fig. 5b). In addition, however, soils with larger clay content (≥32%) showed an additional abundance of particles ≤1 µm within the occluded small SMA (Fig. 5c, d), pointing to a potential role of colloids in SMA occlusion. To better understand the underlying mechanisms, we assessed the concentrations of SOC inside the different SMA fractions.

3.3 SOC content of different SMA fractions

The SOC contents did not strongly correlate with the gradient of clay content but showed some differences between small (19-24%) and large (32-34%) clay content. Apparently, the relationship between SOC-enrichment in clay and clay-content was not linear (see also Christensen (1992)). Compared to the SMA fraction with small clay content the total SOC concentration of SMA from soils with larger clay content had increased significantly in the free and decreased in occluded SMA (Fig. 6a). There were no significant differences in SOC contents between the free and occluded SMA from soils with small clay content. However, the relationship of the SOC content of the SMA to the clay content was not a linear, i.e., the data grouped similarly as did the mass distribution of SMA, we keep the comparison of SOC within SMA of sites with low (≤ 24% clay) and large clay content (≥ 32%), respectively.

Overall in the soil sequence, larger SOC concentrations were found in the small SMA fraction than in the large SMA. It contained larger amounts of SOC in occluded SMA than in the free ones, and larger amounts of SOC in the coarser than in the finer textured soils (Fig. 6b). In contrast to the observations from small SMA, the large SMA fractions had significantly larger SOC concentrations in the free than in the occluded SMA, with now also larger SOC contents in the samples with larger clay contents than in the coarser textured ones (Fig. 6c). Hence, the distribution of SOC into occluded and free aggregates as well as among the differently textured soils just behaved opposite in the small SMA relative to the large SMA.

4 Discussion

The applied fractionation scheme enabled us deeper insights into the dynamic aggregation processes at different scales ranging from macroaggregates to small SMA. The disruption energy of 60 J mL^{-1} has been considered as suitable for evaluating the SMA size distribution and associated organic matter (Kölbl et al., 2005) preserving largely their natural state (Amelung and Zech, 1999). Differentiation between occluded and free aggregates supported our first hypotheses that elevated clay contents support aggregation not only at macro- but already at microaggregate level. Specifically, elevated clay contents promoted the formation of occluded SMA (Fig. 4), likely by higher aggregate stability through interactions with more abundant colloids < 1µm (Fig. 5). In this regard, our data are in support of the concept of aggregate hierarchy (Oades and Waters, 1991), in that macroaggregates are built from microaggregates which can be formed from smaller building units. Here we show a novel size distribution measurement of the smaller building units (0.1 to 1 µm), which was significant higher at sites with elevated clay content. The occluded and free small SMA reveals a gamma like size distribution (Fig. A.1; Supplementary Materials) peaking at 4-6 µm (Fig. 5). The gamma distribution model showed the best fit in comparison to other models which is in agreement with the work of Yang et al. (2012).

The similar size distribution of free and occluded particles in soils with low clay content (Fig. 5a) indicated that there is no preferential accumulation of specific sized free small SMA during aggregation. The evaluation of the total masses of soil aggregate fractions showed that the macroaggregate/SMA ratios became narrower when moving to subsites with larger clay content. This agrees with Yu et al. (2015), who found similar changes in macroaggregate/SMA ratios as a function of silt and clay content. In soils with large clay content the abundance of occluded small SMA increased, which indicates larger amounts of water stable aggregates and thus an essential role of the clay-sized fraction not only for the formation but also for the stability of SMA.

It has been suggested that a minimum clay content of 15% is required for the abiotic formation of aggregates (Horn, 1990; Oades, 1993). The percentage of the analyzed low clay content soils (19-24%) was above this minimum value. An additional factor contributing to more occluded SMA in soils with large clay content could be the significantly larger concentrations of Al- and Mn-bearing phases (Appendix Table A.2); at least for aluminum oxides it is known that they promote the stabilization of clay minerals and organic matter on mineral surfaces (Goldberg, 1989; Kögel-Knabner et al., 2008).

Another essential factor promoting SMA formation is organic matter. Our results showed that especially small SMA were rich in SOC (Fig 6b, c), which confirms previous studies showing that organic carbon mainly accumulates in the smallest fractions with the largest specific surface area (Kahle et al., 2002; Kennedy et al., 2002). In addition, the SOC concentrations were higher in occluded small SMA than in the free ones (Fig. 6b), confirming previous findings that only clayey soils supplied with organic matter were able to aggregate and that there is a strong correlation between SOC accrual and aggregate stability (Al-Kaisi et al., 2014; Imhoff et al., 2002; Wagner et al., 2007). In turn, more stable aggregates also protect SOC better from degradation as aggregates that disintegrate. Through occlusion, for instance, SOC is better protected against microbial decomposition in the <20 µm fraction than in larger SMA (Hassink, 1997). Physical stabilization affects the turnover of labile molecules like carbohydrates and proteins (Krull et al., 2003) that can act as gluing agents for aggregates. Previous studies showed that this SOC protection works in addition to chemical and biochemical stabilization (Six et al., 2002) due to the preservation of polysaccharides and phenolic C in the clay fraction (De Nobili et al., 2008; Schöning et al., 2005). The microbial cycling of SOC nearby mineral surfaces fosters the accrual of carbohydrate and other microbial residues (Amelung et al., 1997; Zhang et al., 1999), which might act as glue within microaggregate cores (Ladd et al., 1993; Lynch and Bragg, 1985; Rodionov et al., 2001). As a result, soil organic matter composition may vary with aggregate size (Golchin et al., 1994; Yu et al., 2015). In this regard, a higher SOC concentration in occluded small SMA relative to the free SMA (Fig. 6b) might be explained by an enrichment of microbial-altered SOC (Bingham and Cotrufo, 2016; Cotrufo et al., 2013; Rodionov et al., 2001), whereas fungal hyphae and fine root debris might be part of free SOC, enriched within the free large SMA fraction (Fig. 6c). According to Amelung et al. (2002), SOC accumulation in microaggregates may well happen at the outer surface, i.e., it does not necessarily have to result in an additional occlusion process. Nevertheless, this different direction of SOC enrichment hardly altered the size distribution of SMA with increasing clay content (Fig. 5).

5 Conclusion

Results indicated that the abundance of clay-sized particles as building unit is a superior requirement for aggregate formation and stability. Elevated clay contents promote the formation and stability of SMA with different size, resulting in larger concentrations of occluded SMA than of free ones as clay contents rose. Notably, SOC enrichment followed the opposite direction, i.e., the additional clay mostly trapped SOC in free, large SMAs. We attribute this to an interaction loop: the preferred formation of occluded aggregates with elevated clay contents leads to lower amounts of “free” particles, which therewith concentrate the remaining SOC at their surface.

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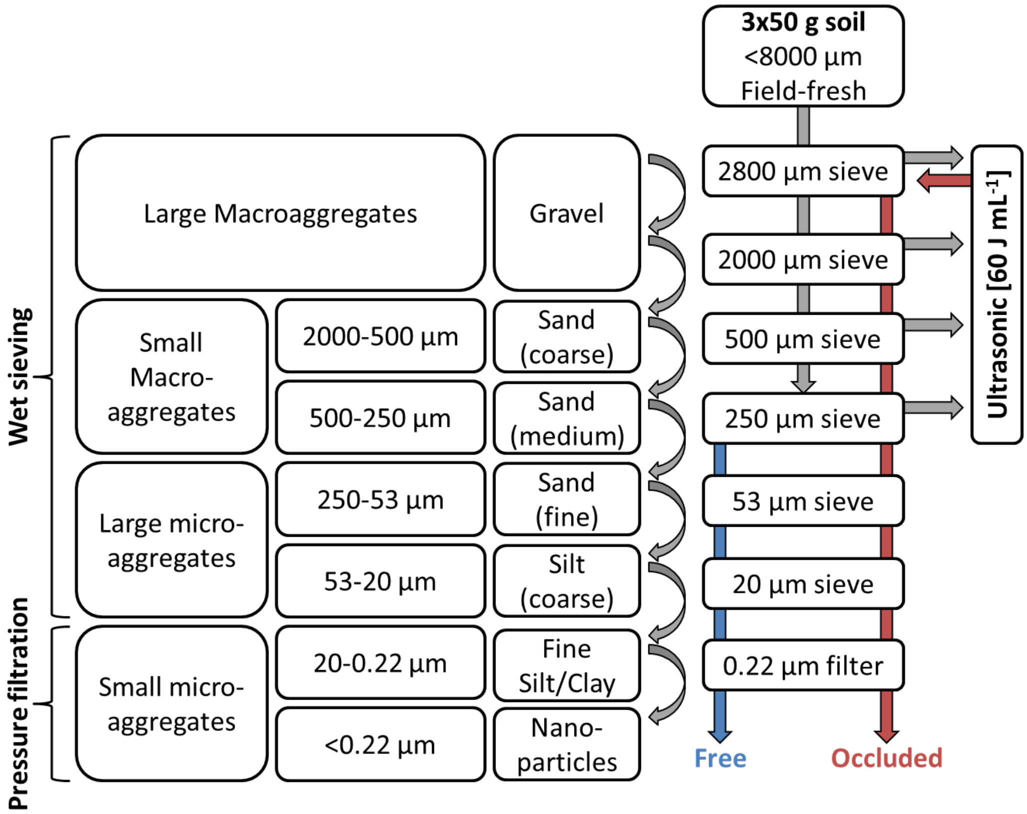

Fig. 1. Overview about the fractionation process using wet sieving and pressure filtration. According to the different size fractions the samples were divided into macroaggregates, large, and small SMA. Mechanical disaggregation via ultrasonication allowed a differentiation between occluded (ultrasonic treatment, red arrow) and free (no treatment, blue arrow) SMA.

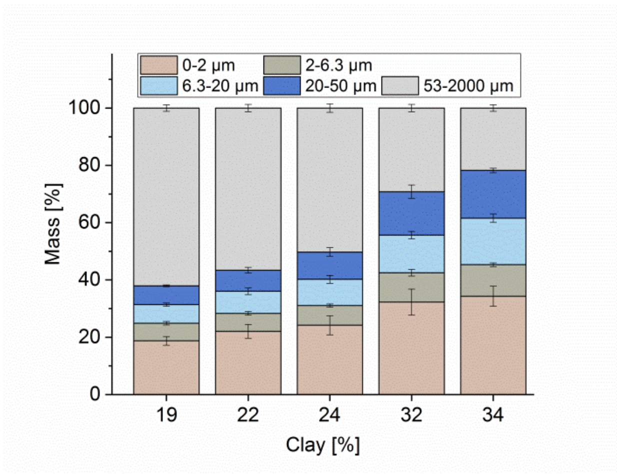

Fig. 2. Texture of H_2O_2 oxidized soil samples obtained from the toposequence at an agricultural site.

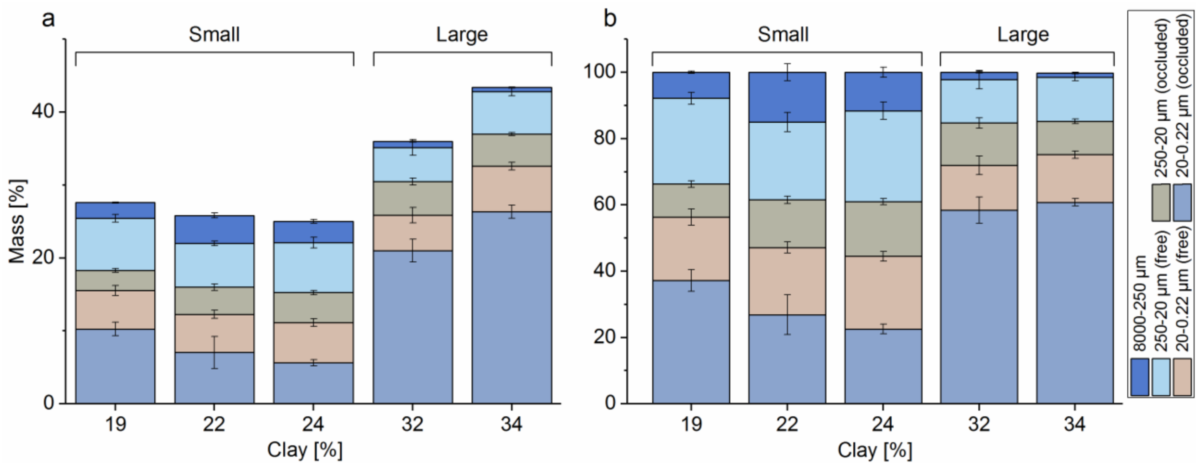

Fig. 3. Mass percentage of macroaggregates and SMA in bulk soil (a) and within SMA (b) of soils with different clay content (small and large).

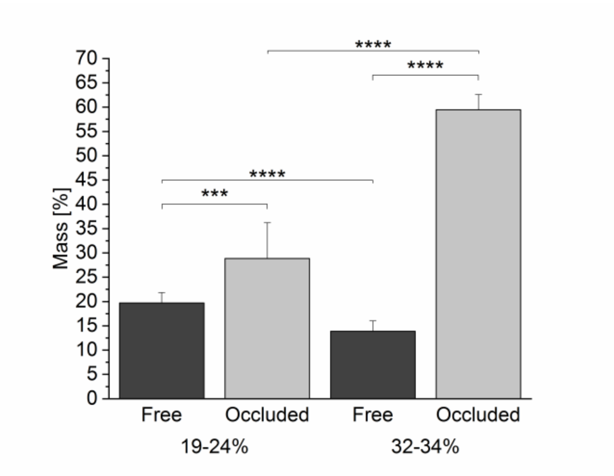

Fig. 4. Mass percentage of free and occluded small SMA; significant differences are indicated with asterisks.

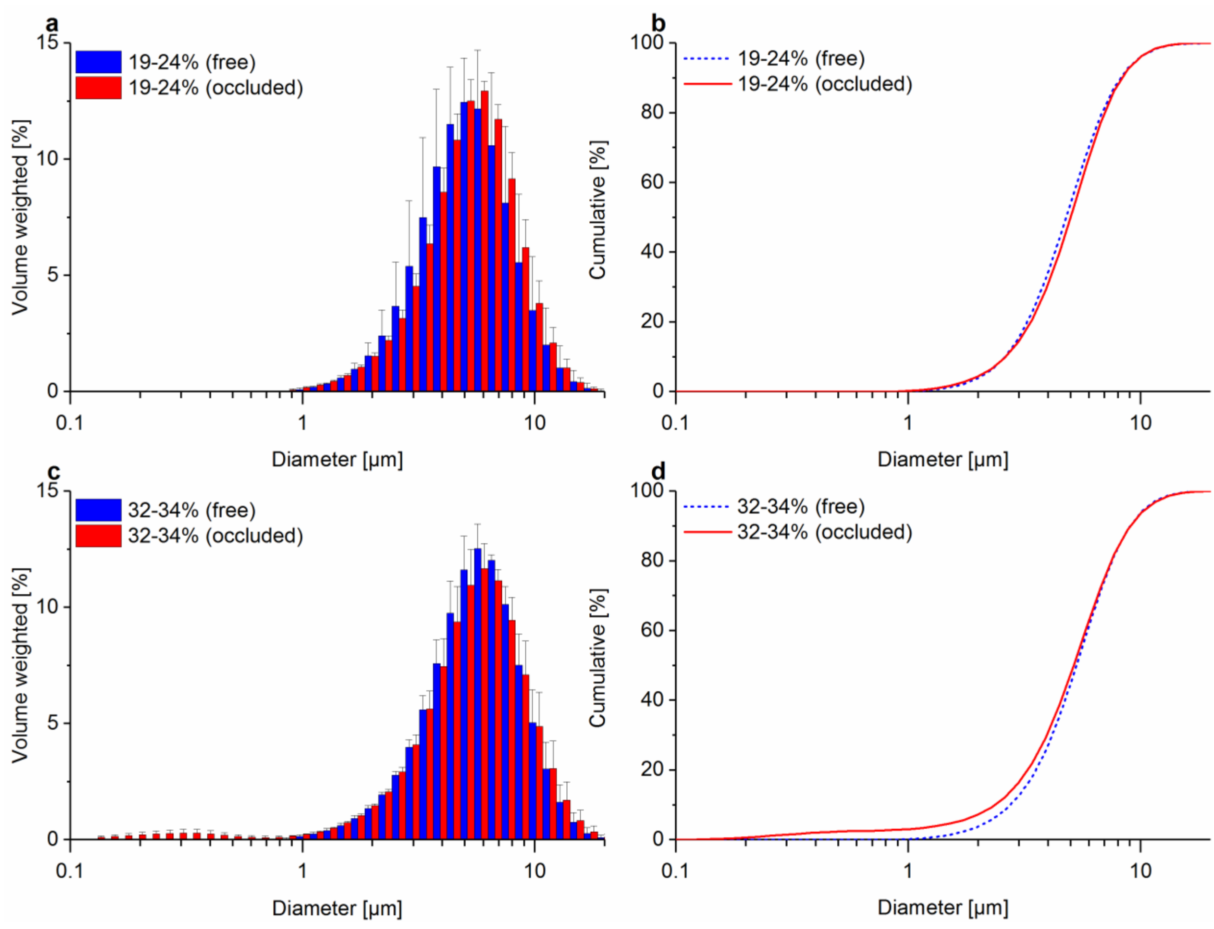

Fig. 5. Volume based size distribution (**a**, **c**) and cumulative percentage (**b**, **d**) of free (blue/dotted) and occluded (red/straight) SMA for soils with small (**a**, **b**) and large (**b**, **c**) clay content.

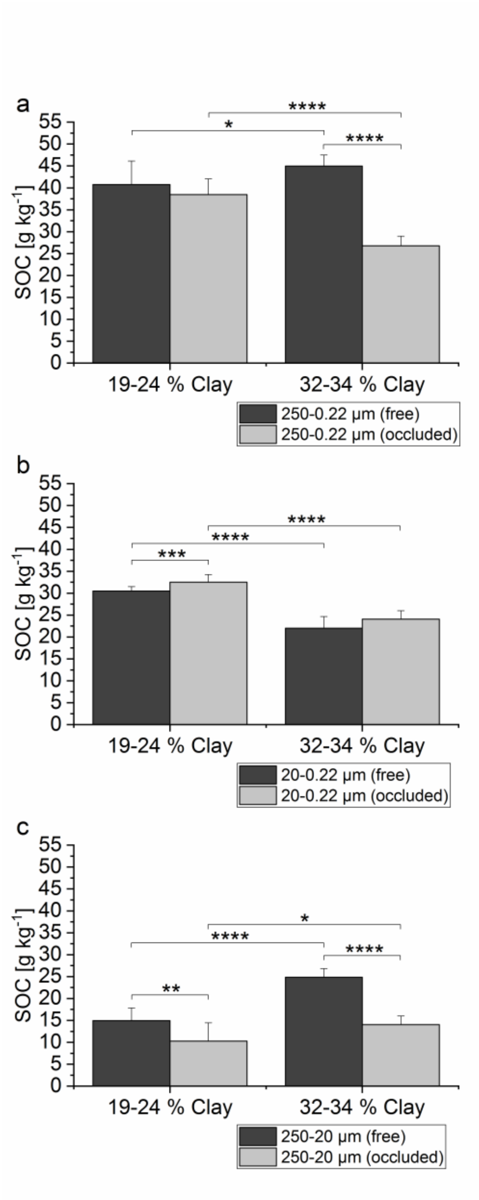

Fig. 6. SOC content in dependence of soil clay content for all SMA (**a**) and their corresponding fractions including small (**b**) and large (**c**) SMA. The proportion of the free form is indicated in black and the occluded form in grey. Significant differences are indicated with asterisks.