


Wet sieving versus dry crushing: Soil microaggregates reveal different physical structure, bacterial diversity and organic matter composition in a clay gradient

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Abstract

Soil microaggregates contain particles of different sizes, which may affect their potential to store organic carbon (OC). A variety of methods can be used to isolate microaggregates from the larger soil structures, among which wet sieving approaches are widely employed. We developed a novel dry crushing method that isolates microaggregates along failure planes due to mechanical stresses rather than hydraulic pressures and compared the mechanical stability, OC contents and microbial community composition between dry-crushed and wet-sieved samples with contrasting clay contents. Dry-crushed samples exhibited a higher stability and bacterial diversity compared to wet-sieved samples. As a result, the dry-crushed microaggregates had different size distributions when analysed dry and after wetting. In the dry state, dry-crushed microaggregates were larger and contained more sand-sized primary particles within the aggregate structures. The wetting of dry-crushed aggregates caused a disintegration of larger microaggregates and sand-sized primary particles into smaller microaggregates that contained finer particles. In the soils with lower clay contents, the diameter of dry-crushed microaggregates was 40 µm larger due to more sand-sized primary particles remaining within the aggregates. Depending on how much volume in microaggregates is occupied by large primary particles, the OC concentration increased in the soil with higher clay content. Wet-sieved size fractions also showed a similar pattern of OC distribution, whereas more primary particles were observed outside of aggregates. Wet sieving approaches disperse the soil into OC-rich aggregates and might be preferable if OC dynamics are investigated. Differences in bacterial community composition in dependence on clay content were more pronounced in dry-crushed microaggregates. If intact aggregate architectures are of interest for the

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isolation of soil structural units, the presented dry crushing method might provide an advantageous alternative that also better preserves bacterial diversity.

KEYWORDS

aggregate breakdown dynamics, aggregate carbon, aggregate separation, aggregate stability, aggregation, microaggregate, microbial community structure, SOM distribution

1 | INTRODUCTION

The physical structure of soil is of primary importance for most of its functions and is often expressed as the degree of aggregation (Bronick & Lal, 2005; Dexter, 1988). The aggregation of soil components is a critical process that regulates C sequestration (Six, Paustian, Elliott, & Combrink, 2000b) and determines physical support, hydraulic properties, aeration and the accessibility of biogeochemical interfaces, which are essential for nutrient exchange and microbial processes in soils (Bronick & Lal, 2005; Totsche et al., 2010). Soil microaggregates with a size smaller than 250 µm have been proposed as stable compound soil structures (cf. Totsche et al., 2018). Microaggregates are themselves comprised of various smaller building units, such as coarse and fine mineral primary particles, phyllosilicates, Fe and Al (hydr)oxides and diverse organic materials (Totsche et al., 2018). In contrast to macroaggregates, microaggregates are more stable and have longer turnover times, which is why aggregate size plays an important role in long-term C storage in soils (Angers, Recous, & Aita, 1997; Six et al., 2000a; Trivedi et al., 2017).

Under field conditions, various mechanisms cause soil (micro)aggregates to break apart. These can be either mechanical forces, originating from field traffic and tillage implements, or radial pressures from roots and earthworms causing point loads and shear failure (Horn & Peth, 2012; Ruiz, Schymanski, & Or, 2017). Mechanical forces are also introduced during raindrop impact (Fu, Li, Zheng, Li, & Zhang, 2017) or differential swelling of clay minerals; however, the resulting disintegration of aggregates is a mixture of mechanical and hydraulic stresses (pore water pressures). These mechanical forces can create and sustain preconditioned failure planes within soil aggregates that may be reactivated when repeatedly exposed to stresses. Further mechanisms for aggregate breakdown can be slaking and dispersion, which occur during wetting and drying of the soil and are related to hydraulic stresses (Le Bissonnais, 1996).

Various approaches are used to break down aggregates. The most common method to isolate microaggregates from bulk soil is wet sieving, which was mostly employed to investigate the stabilization of aggregate structures through organic matter (OM) and OM

turnover (Bach, Williams, Hargreaves, Yang, & Hofmockel, 2018; Six, Bossuyt, Degryze, & Denef, 2004). The impact of water immersion on soil aggregates has been shown to depend on the size, structure, shrinking and swelling behaviour, wettability of soil components, porosity, and the spatially heterogeneous distribution of these properties within aggregates (Baumgartl & Horn, 1993; Chenu, Le Bissonnais, & Arrouays, 2000; Kaiser, Kleber, & Berhe, 2015). The super-saturation of aggregates during wet sieving can increase the gas pressure inside aggregates, causing their disruption (Baumgartl & Horn, 1993). Previous studies have shown that water-stable microaggregates are stabilized by various agents, such as OM, phyllosilicates, Fe and Al (hydr)oxides and CaCO₃ (Amézqueta, 1999; Bronick & Lal, 2005; Oades & Waters, 1991; Totsche et al., 2018). In other studies (using glass beads to increase the dispersion energy), it was postulated that microaggregates are formed while being occluded in macroaggregates (Angers et al., 1997; Six et al., 2002). Likewise, sonication of soil suspensions can be used to liberate microaggregates from macroaggregates (Amelung & Zech, 1999).

The dry sieving of soil (field-fresh or after air-drying) is based on the mechanical impact on soil structure during shaking the samples in a sieve tower, either by hand (Bach & Hofmockel, 2014; Blaud, Menon, van der Zaan, Lair, & Banwart, 2017) or using a mechanical sieve shaker (Nahidan & Nourbakhsh, 2018; Panettieri, Berns, Knicker, Murillo, & Madejón, 2015). It is used to differentiate microhabitats that are associated with aggregates of various size fractions (Bach & Hofmockel, 2014; Blaud et al., 2017) or to gain information about the impact of wind erosion on aggregates (Chepil, 1962). Apart from dry sieving to a certain size (typically 8 or 4 mm), the aggregates in the aforementioned studies are usually not treated by dispersion agents before the dry sieving.

Another approach, which is able to liberate more microaggregates that are trapped within macroaggregates (Six et al., 2000), can be to mechanically crush the macroaggregates down to microaggregate sizes, using a uniaxial crushing procedure. Uniaxial crushing enables the breaking down of dry aggregates along “natural planes of mechanical weakness” (Kristiansen et al., 2006) that are likely to fail under mechanical forces in the field as well.

If intact aggregate structures are of interest, for example, their internal spatial architecture creates a habitat for microorganisms or storage site for OM, dry separation protocols such as dry crushing have the advantage that they avoid potential structural artifacts that are likely to occur during water immersion and subsequent oven drying (Kaiser et al., 2015; Panettieri et al., 2015; Siebers, Abdelrahman, Krause, & Amelung, 2018). Because different isolation methods are based on different breakdown mechanisms, differences between the isolated size fractions can be expected (Six & Paustian, 2014). In addition to the existing studies that compared mainly microbial parameters of wet and dry-sieved aggregates (Bach & Hofmockel, 2014; Blaud et al., 2017), more studies are needed to better understand how isolation methods such as wet sieving and dry crushing affect the structures, size distribution and physicochemical, mechanical and biological properties of soil aggregates.

During aggregation, primary particles are combined to form larger soil structures. The individual contribution of soil particles to properties of the bulk soil therefore depends on its arrangement within the aggregate architecture. Especially fine mineral soil particles can affect the surface adsorption of OM and formation of microaggregates through organo-mineral associations (Baldoek & Skjemstad, 2000; Wagner, Cattle, & Scholten, 2007). Clay content is positively correlated with aggregate tensile strength (Imhoff, da Silva, & Dexter, 2002; Kavdir, Özcan, Ekinici, Yigini, & Yüksel, 2004; Kay & Dexter, 1992) and was found to stabilize larger aggregate structures that drive the arrangement and the distribution of OM in aggregate fractions (Dexter et al., 2008; Krause et al., 2018; Schjønning et al., 2012; Schweizer, Bucka, Graf-Rosenfellner, & Kögel-Knabner, 2019). Because larger primary particles do not play a dominant role in OM storage inside soil aggregates, our current conception of aggregate structures has mostly focused on structures that are comprised of many fine particles. Aggregates with a coarse texture component were, hitherto, not at the centre of scientific attention. One of the first studies looking at the functional role of sand grains inside microaggregates by Paradiš, Brueck, Meisenheimer, Wanzek, and Dragila (2017) described sandy microaggregates with a solid sand grain nucleus that is surrounded by mineral fines. They conceptualized a model where water menisci forces draw fine soil constituents towards the solid surface of larger particles after multiple wetting-drying events. This may also lead to the coalescence of multiple sand grains within aggregates (Ghezzehei & Or, 2000). Because higher clay contents are mostly correlated with lower sand contents, many aggregate properties are likely to be modified by soil texture (Schweizer et al., 2019; Wagner et al., 2007). Detailed size analyses of aggregates

and the particles they are composed of are needed to reveal the size composition and the role of the individual particles within microaggregates.

Soil aggregates are important habitats for microorganisms (Blaud et al., 2017) depending on various aggregate properties, such as their size and structure. Higher clay contents correlate positively with microbial biomass (Wei et al., 2014), which can lead to larger and more stable aggregates (Wang, Li, & Zheng, 2017). Soil aggregation increases microhabitat heterogeneity and thus bacterial diversity in soils (Davinic et al., 2012; Nunan, Leloup, Ruamps, Pouteau, & Chenu, 2017). Different microaggregate sizes are likely to show differences between their pore systems, leading to differences in the accessibility of OM sources, which strongly affects microbial activity (Bimüller, Kreyling, Kölbl, von Lütow, & Kögel-Knabner, 2016; Ebrahimi & Or, 2018; Gupta & Germida, 2015). Similarly, differences in soil texture are expected to influence microbial activity depending on how they affect OM accessibility. The rewetting of dried samples during wet sieving was shown to affect the characterization of microbial communities in soil aggregates (Bach et al., 2018). For this reason, Bach and Hofmockel (2014) have stated that wet sieving should be used to evaluate long-term changes in microbial activity and OM, whereas dry separation without rewetting would allow capturing short-term (inter-annual) dynamics of soil microbial activity, too. For all these reasons, the characterization of the microbial community provides important information to compare the structure, size and other properties of aggregates as influenced by the isolation method.

Here, we compared the mechanical stability of individual microaggregates and OM concentrations as well as microbial community composition between wet fractionated and dry-crushed size fractions. The dry-crushed fractions were further characterized regarding their size distributions and organic matter content. To investigate the effect of soil texture on dry-crushed aggregates, we used samples from a clay content gradient of 19–34%. We developed a novel method to isolate microaggregates from macroaggregates by mechanically crushing them with a loading frame prior to dry tap sieving. The particle and aggregate size distribution of the dry-crushed size fractions was precisely analysed using dynamic image analysis. In addition to the differentiation into several size fractions by sieving, the dynamic image analysis allowed detecting size changes at a resolution of several μm for the whole microaggregate scale $<250 \mu\text{m}$.

We hypothesized that dry crushing isolates aggregate size fractions shaped by different failure planes and yields different properties compared with wet-sieved aggregate size fractions (with and without sonication). In this comparison, dry-crushed size fractions

were expected to have a lower mechanical stability, retain less OM and exhibit differences in microbial community composition compared to wet-sieved ones (both with and without sonication). For the investigation of the soil texture gradient, we hypothesized that a higher clay content would increase the mechanical stability, mean aggregate size, OM content and bacterial diversity of microaggregates.

2 | MATERIAL AND METHODS

2.1 | Study site

We studied a soil toposequence at an agricultural research station in Scheyern (Germany) that was previously described by Schröder, Huber, Olazábal, Kämmerer, and Munch (2002). The mean annual temperature is 7.4°C, with an average annual precipitation of 803 mm at an altitude of approximately 470 m above sea level. Soils developed on Miocene Upper Freshwater Molasse covered by several metres of Quaternary loess and were classified according to the WRB (FAO, 2015) as Cambisol (Schröder et al., 2002). Soils were managed by reduced tillage (harrowing and chiselling). Samples were taken at clay contents of 19, 24 and 34% ($n = 5$ each). More detailed information on the sampling of the toposequence can be found in Krause et al. (2018). Briefly, the top 5–20 cm were sampled in late 2015 in a field-fresh state (water content approximately 16 wt% or 21 vol.%), sieved to <8 mm and stored at 4°C until

further processing. An overview of the complete sample analysis process is given in Figure 1.

2.2 | Fractionation by dry crushing

We produced microaggregates with diameters of <250 μm by crushing macroaggregates under uniaxial compression in a mechanical loading frame (Zwick Roell AllroundLine, Zwick Roell, Ulm, Germany). Briefly, we used air-dried large macroaggregates with a diameter of <2 mm. We weighed approximately 5 g and measured the matric potential in a WP4C dew point potentiometer (Decagon devices, Pullman, WA, USA) to make sure the sample was completely dry. All samples had pF values of >6. Next, the samples were crushed under the loading frame to separate differently sized microaggregates, which are constituents of the macroaggregates. For this, the distance between the loading frame table and piston was slowly reduced from 3 mm to 250 μm at a constant speed of 250 $\mu\text{m min}^{-1}$. On average, the samples contained 33.6, 20.0 and 10.2% sand grains of >250 μm in the soils with 19, 24 and 34% clay. These grains might have been crushed during the dry separation, which would result in sand grain fragments in the size fraction. To minimize this, the crushing speed of the loading frame was deliberately chosen to be slow so that the sand grains could reorient themselves during the process (the slow speed was chosen as a result of preliminary experiments). No high-pitched sounds related to the rupture of sand grains or any sharp peaks in the load–displacement curves that

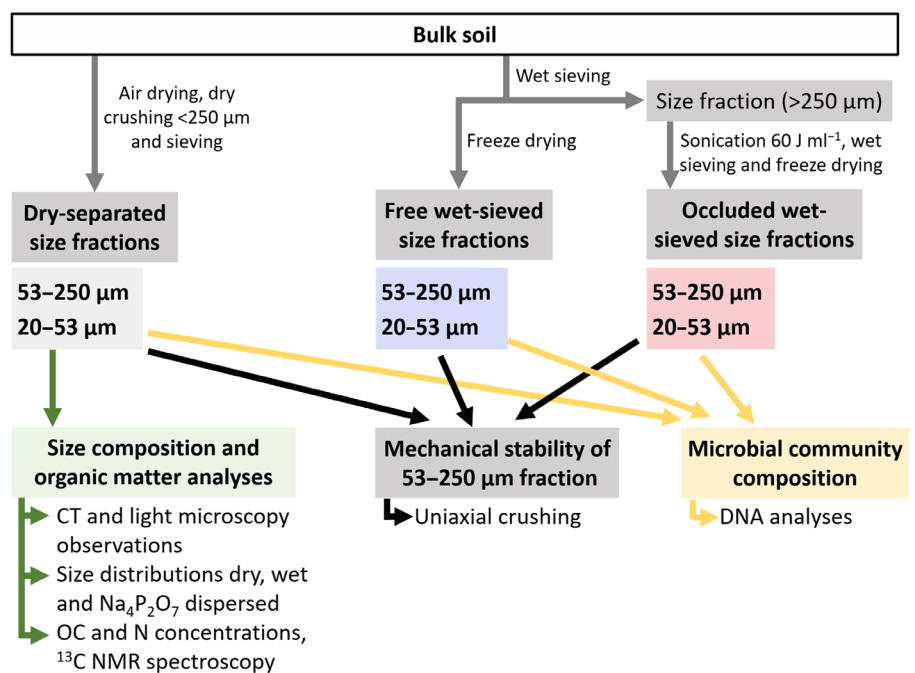


FIGURE 1 Overview of isolation methods and analyses [Color figure can be viewed at wileyonlinelibrary.com]

would indicate crushed sand grains were observed (Figure S1). During light microscopy and tomography scans, we also did not observe any sand grain fragments.

After crushing, we transferred the crushed sample into a modified Casagrande apparatus (Mennerich Geotechnik, Hannover, Germany), consisting of three sieves that rest on a collecting vessel. Here, the sample was exposed to the purely mechanical forces of repeated tap-sieving cycles at a frequency of 2 Hz. Tap sieving was chosen to prevent abrasion of soil material from the aggregate surfaces through circular shaking, which would have biased aggregate fractions. In this process, microaggregates were separated under air-dry conditions into the following size fractions: small microaggregates and primary particles ($< 20 \mu\text{m}$), medium (20–53 μm) and large ones (53–250 μm), similar to size limits applied in previous studies (Jastrow, 1996; Tisdall & Oades, 1982; Virto, Barré, & Chenu, 2008). The smallest fraction ($< 20 \mu\text{m}$) was not analysed in this study due to the very low amount that could be isolated (Figure S2). In a preliminary experiment, we determined the number of cycles in the Casagrande apparatus that are required to get a relatively steady aggregate size distribution, which was about 1,000 cycles (Figure S3). Hence, after 1,000 cycles we collected the residue on all three sieves. The crushing and tapping procedure can be seen in the following videos: <https://doi.org/10.6084/m9.figshare.10327397.v1> and <https://doi.org/10.6084/m9.figshare.10327529.v1>.

2.3 | Light microscopy and tomography

The size fractions were analysed with optical microscopy using a Zeiss Axio Imager M2. Explorative computed microtomography scans were performed on one dry-crushed microaggregate for each clay content at the CT laboratory of the University of Kassel with a Zeiss Xradia 520 Versa (Carl Zeiss AG, Oberkochen). For each of the three scans, we acquired 1,600 projections during a full rotation with 3-s acquisition time at 80 keV and a resulting voxel resolution of 570 nm, 482 nm and 656 nm, respectively.

2.4 | Dynamic image analysis of dry-crushed soil size fractions

Size distributions were determined by dynamic image analysis with rear illumination from a pulsed laser light (450 fps) using a QICPIC (Sympatec GmbH, Clausthal-Zellerfeld, Germany) as described previously (Kayser, Graf-Rosenfellner, Schack-Kirchner, & Lang, 2019). In the dry state, we analysed the size fractions (60 mg to 80 mg) with the GRADIS drop tower (Sympatec GmbH,

Clausthal-Zellerfeld, Germany) (50 cm height) using a constant flow of compressed air. The medium size fraction (20–53 μm) was analysed with the M4 lens (2–682 μm measuring range) and the large size fraction (53–250 μm) with the M6 lens (5–1705- μm measuring range; ISO 13322-1).

To test how the failure planes during dry crushing and wet sieving differ and what the underlying primary particle size distribution was, we submerged the dry-crushed fractions in water and subsequently dispersed them further using $\text{Na}_4\text{P}_2\text{O}_7$. It should be noted that the size analysis was carried out with the fractions obtained from dry crushing in order to get further information on the size distribution after their dispersion. Detailed information on the particle and microaggregate size distribution of the samples after wet sieving can be found in a previous study by Schweizer et al. (2019), which is used for comparisons in the discussion section.

For this, we determined the size distributions of the dry-crushed fractions after submersion in deionized water and subsequently in $\text{Na}_4\text{P}_2\text{O}_7$ solution. After slow wetting of the size fractions with deionized water for 30 min, the suspensions were measured with a different setup in the same QICPIC machine. A closed pumping cycle using the LIXELL flow cell and SUCCELL homogenization unit with a 0.2-mm and 1-mm cuvette allowed transportation of the suspension by the optical unit of the machine.

To determine the size distribution and mass contribution of primary particles in the size fractions, we measured the dry-crushed samples after dispersion of 100 mg sample material in 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ (Kemper & Koch, 1966; Murer et al., 1993). After dispersion, the suspension was transferred to the pump unit and measured as described above.

The data obtained by dynamic imaging were analysed using the automatic WINDOX software (Sympatec GmbH, Clausthal-Zellerfeld, Germany). By relation to a density of 2.65 g cm^{-3} , the normalized volumetric distribution density q_3 was calculated according to the minimum distance of two tangents to the edges of the objects, the so-called Feret diameter (Allen, 1981). The median diameter was used to compare the distributions of different clay contents statistically. To compute distributions of the whole size range we used class sizes of 6 μm for the medium fraction and 15 μm for the large fraction. Shape criteria (Table S1) were chosen based on a gallery function within the image analysis algorithm, which enables verifying that a low number of individual odd-shaped objects like root pieces (low circularity) or overlapping particles (high optical density) do not distort the computed size distribution (Kayser et al., 2019). The influence of bubbles and root hairs on the size distributions was minimized by specific shape criteria for both size

fractions using the circularity of the objects (Table S1). The circularity is the ratio of the perimeter of an equivalent circle to the perimeter of the object, which decreases when particles are rounder.

Due to optical limits of the applied camera lenses, some particles might have been too small to be detected by the dynamic image analysis. To relate the volumetric distribution to the actual mass proportions, we corrected the size distributions for particles $<20\ \mu\text{m}$ and $<53\ \mu\text{m}$ by sieving the particles after dispersion with $\text{Na}_4\text{P}_2\text{O}_7$. We observed that 19.1, 21.2 and 24.8% of the particles in the soils with 19, 24 and 34% clay were $<20\ \mu\text{m}$ in the aggregate size fraction 20–53 μm . In the size fraction 53–250 μm , 3.1, 1.5 and 7.1% of the particles were $<53\ \mu\text{m}$ after dispersion with $\text{Na}_4\text{P}_2\text{O}_7$ in the soils with 19, 24 and 34% clay. We subtracted the total contribution of the whole size distribution of the respective size fraction accordingly. Both size fractions, 20–53 μm and 53–250 μm , were merged according to their respective mass contribution to the bulk soil and are displayed as one size distribution across the whole size range.

2.5 | Carbon and nitrogen analysis and solid state ^{13}C nuclear magnetic resonance spectroscopy

The C and N contents were measured by dry combustion using a Vario EL CN analyser (Elementar, Langensfeld, Germany). The contributions of the size fractions to the bulk OC were computed according to their mass contributions and OC concentrations.

The chemical composition of the OM in the size fractions 20–53 μm and 53–250 μm was measured using a Bruker Avance III 200 spectrometer (Bruker, Billerica, USA) at a resonance frequency of 50.3 MHz using the cross-polarization magic angle spinning technique at a speed of 6.8 kHz. The contact time was 1 ms and the recycle delay time was 0.4 s. The spectra were processed with a line broadening of 100 Hz, phase adjusted and baseline corrected. The spectra were separated into the four integration areas: carboxyl-C at 220–160 ppm, aryl-C at 160–110 ppm, O/N-alkyl-C at 110–45 ppm and alkyl-C at 45–(–10) ppm. We used the ratio between alkyl-C and O/N-alkyl-C as an indicator for the degree of decomposition of the OM (Baldock et al., 1997).

2.6 | Fractionation by wet sieving

The isolation of aggregates using wet sieving was carried out according to Krause et al. (2018). Briefly, the pre-wetted field-fresh aggregates were isolated on a sieve tower

under constant shaking (Kösters, Preger, Du Preez, & Amelung, 2013). The wet-sieved fractions $<250\ \mu\text{m}$ will be further referred to as free wet-sieved size fractions. The fraction $>250\ \mu\text{m}$ was sonicated at $60\ \text{J mL}^{-1}$ according to Amelung and Zech (1999) to break down macroaggregates into microaggregates, which will be further referred to as occluded wet-sieved size fractions. To avoid re-aggregation, the fractions were shock frozen in liquid N_2 and freeze-dried after wet sieving (Siebers et al., 2018).

2.7 | Microaggregate stability (tensile strength)

The mechanical stability of individual dry-crushed and wet-sieved (free and occluded) microaggregates was determined using the same high-resolution loading frame for material testing that was used for crushing the macroaggregates of $<2\ \text{mm}$ (Zwick Roell AllroundLine, Zwick Roell, Ulm, Germany). The method is similar to the one described by Skidmore and Powers (1982). The loading frame was equipped with a high-resolution load cell with a capacity of 100 N (Xforce HP 100 N). We randomly selected 50 individual microaggregates of the size fraction 53–250 μm from each treatment and measured their weight with a high-precision balance (precision: 0.01 mg). After weighing the samples, they were placed on the table of the loading frame and a custom-made piston with a width of 500 μm was attached to the load cell of the frame. The location of the sample was checked with a Canon Eos 2000D digital camera, equipped with a macro lens. Subsequently, the distance between piston and table was reduced from 300 μm to 25 μm at a constant speed of $250\ \mu\text{m min}^{-1}$ to ensure that the microaggregate is completely crushed (Figure S4). The force required to crush each microaggregate was recorded and the work (the area of the load–displacement curves) was finally normalized by the aggregate's mass (mJ mg^{-1}). Because the aim of this study was to measure the stability of microaggregates (not of single particle grains), we only used the randomly selected specimen when they could reliably be identified as an actual microaggregate under the stereo microscope. If a specimen was identified as a large primary particle, it was omitted.

2.8 | Molecular microbial community analysis

DNA was extracted using 300 mg of soil from the different aggregate size fractions with the Macherey Nagel NucleoSpin® Soil Kit (Macherey Nagel, Düren, Germany) following the manufacturer's instructions with minor modifications, which are outlined as follows. The

mechanical disruption of the bacterial cells was performed for 60 s at 1200 rpm using the Fastprep96 instrument (MP Biomedicals, Santa Ana, CA, USA). In the final step, DNA was resuspended in 30 μ L of PCR-grade water. PCR amplification of the 16S rRNA gene, purification of PCR products, sample pooling and sequencing of the PCR products was carried out on the Illumina Hi Seq platform (2 \times 250 bp reads) as described by Maarastawi, Frindte, Linnartz, and Knief (2018).

2.9 | Statistical analyses

We used SigmaPlot 11 and SPSS 25 to compute normality and equal variance tests and to analyse the variances. When significant at $p < 0.05$ a Tukey post-hoc test was applied to compare the means. Statistical analysis of the bacterial community data was performed using R (R Core Team, 2017) with the Vegan (Oksanen et al., 2018) and Phyloseq packages (McMurdie & Holmes, 2013) as described earlier (Maarastawi et al., 2018). Sequences that were not assigned to the domains *Bacteria* or *Archaea* were excluded from the operational taxonomic unit (OTU) table, which was built using a 97% sequence identity cut-off value for the classification of OTUs.

Alpha-diversity was analysed as species richness based on the Chao1 index using a rarefied OTU table based on 7,057 reads per sample. Significant differences between samples were tested in the complete dataset using the non-parametric Kruskal-Wallis test with Nemenyi post-hoc tests (as a Shapiro–Wilk test revealed non-normal data distribution).

Beta-diversity is presented in non-metric multi-dimensional scaling (NMDS) plots based on a Bray–Curtis dissimilarity matrix, which was calculated from the OTU table. Significant differences between the groups of samples were evaluated by an analysis of similarity (ANOSIM) with 999 permutations, also based on the Bray–Curtis distance matrix. ANOSIM provides R-values in the range of 0 to 1, whereby 1 indicates clear differences in microbial community composition between predefined groups of samples, whereas 0 indicates the absence of differences. The reliability of the R-values is assessed based on p -values.

3 | RESULTS

3.1 | Microaggregate structure as observed with microscopy and tomography

In the dry-crushed microaggregates from the soils with 19% and 24% clay, we found large sand grains

contained within the aggregate structures in the large size fraction (Figure 2a–d). Exploratory CT scans of the dry-crushed samples confirmed this observation, namely that the content of large sand grains decreased with increasing clay content. These sand grains had diameters between 80 and 200 μ m (Figure 2b,d,f). This finding was confirmed for 20 additional aggregates for each clay content, of which CT scans were also made (data not shown here).

The aggregates in the 34% clay soil mainly contained silt-sized primary particles although some sand-sized particles were also found (Figure 2e,f). The amount of free primary particles that were not integrated in a micro-aggregate was minor in all size fractions of the dry-crushed samples. In the samples isolated by wet sieving (with and without sonication), more free primary particles were found (Figure 3). Their number increased in the order dry crushing < wet sieving < wet sieving plus sonication (occluded) (Figure 3).

3.2 | Particle and aggregate size distributions

The soil with 34% clay content contained more macroaggregates >250 μ m than the soils with lower clay contents (Figure S2). The microaggregate size fraction of <53 μ m was much lower for the soil with 19% clay content compared to the soils with higher clay contents (Figure S2). The high mass proportion of macroaggregates >250 μ m after the crushing procedure is likely to be explained by the uniaxial crushing. During uniaxial crushing, only one dimension of the aggregates can be controlled (here: height/the Y-axis), but in the X and Z axes, the samples can still be larger than 250 μ m. The size distributions of the combined dry-crushed fractions (20–53 μ m and 53–250 μ m) showed a bimodal pattern with peaks at approximately 50 μ m and 150 μ m (Figure 4a). The mean object diameter of dry-crushed size fractions decreased significantly with increasing clay content from 163 μ m to 125 μ m (Table 1). When immersing these dry-crushed aggregates in water, the mean aggregate diameters decreased by approximately 60 μ m (Table 1). The size distribution shifted to a more unimodal pattern with a peak at approximately 25 μ m (Figure 4b). The soil with 34% clay showed the smallest objects by comparing the dry to the wet measurement, which indicated most aggregate breakdown by water immersion (Figure 4b, Table 1). When dispersing the aggregates further, we found almost no sand-sized primary particles in the soil with highest clay content (34%), whereas the soil with 19% clay also showed less silt-sized particles (Figure 4c).

FIGURE 2 (a, c, e) Incident light microscopy images and (b, d, f) tomograms of the dry-crushed microaggregate size fraction 53–250 μm from soils with different clay contents. With increasing clay contents, fewer (sand-sized) primary particles were observed. Resolution of the tomograms is 570 nm (b), 482 nm (d) and 656 nm (f) [Color figure can be viewed at wileyonlinelibrary.com]

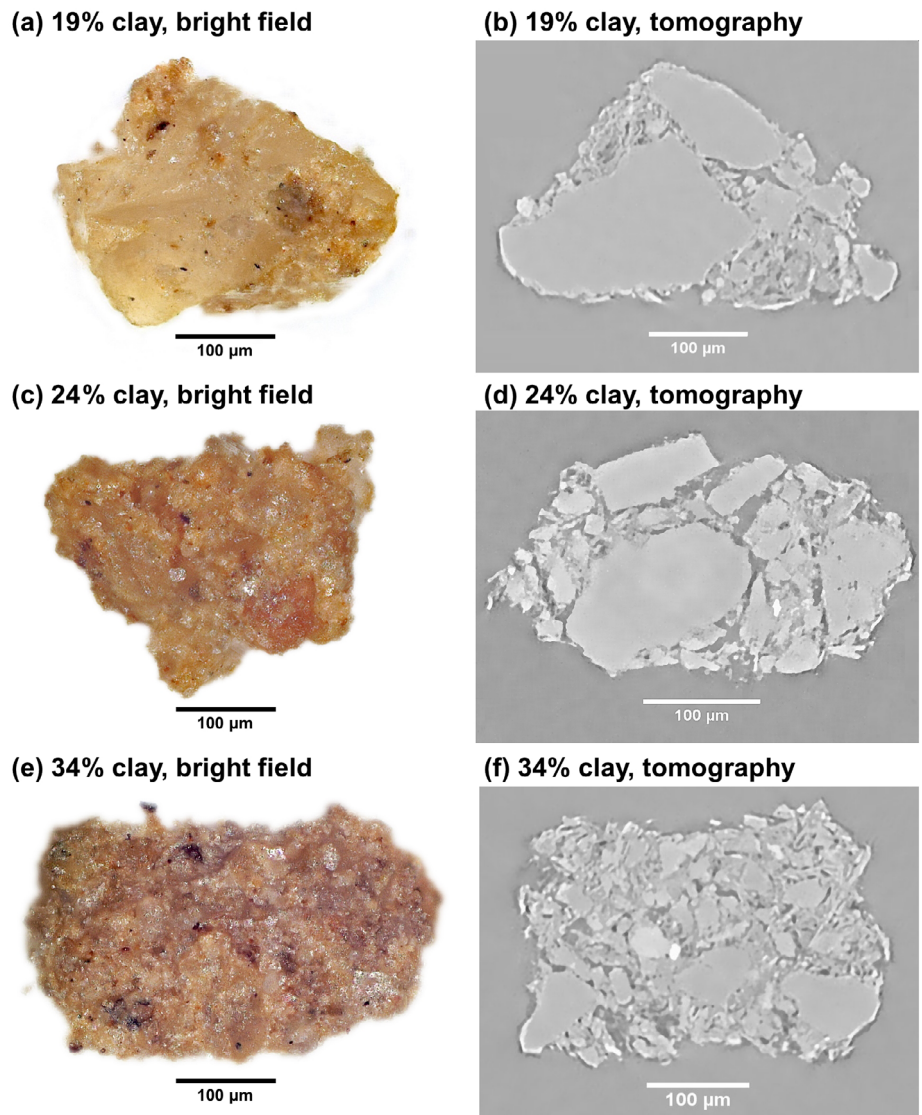
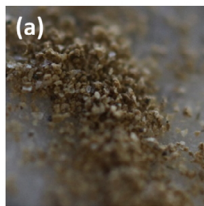
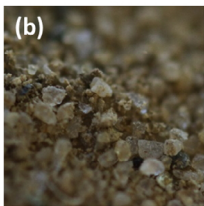
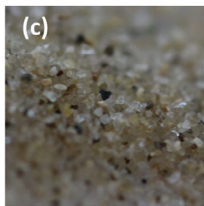
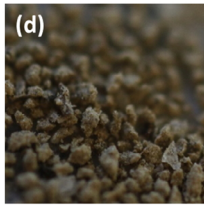
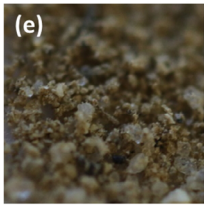
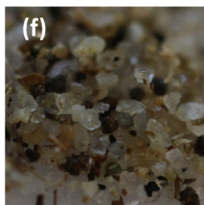


FIGURE 3 Macroscopic images of large microaggregates (53–250 μm) with a low (19%, a–c) and high (34%, d, e) clay content. Dry-crushed microaggregates (a, d) clearly exhibit a higher degree of aggregation of smaller particles, whereas microaggregates that were isolated by wet sieving without sonication (b, e) or with sonication (c, f) exhibited more free primary particles [Color figure can be viewed at wileyonlinelibrary.com]

Separation method	dry	wet	
		free (no sonication)	occluded (sonication)
19% clay content			
34% clay content			

3.3 | Mechanical soil aggregate stability (tensile strength)

The mechanical stability of the microaggregates was in a similar range across all clay contents within one isolation method. No significant differences were observed for microaggregates obtained by different isolation methods or between soils of different clay contents using the same method. Despite the lack of statistically significant differences, the mechanical stability showed a clear tendency

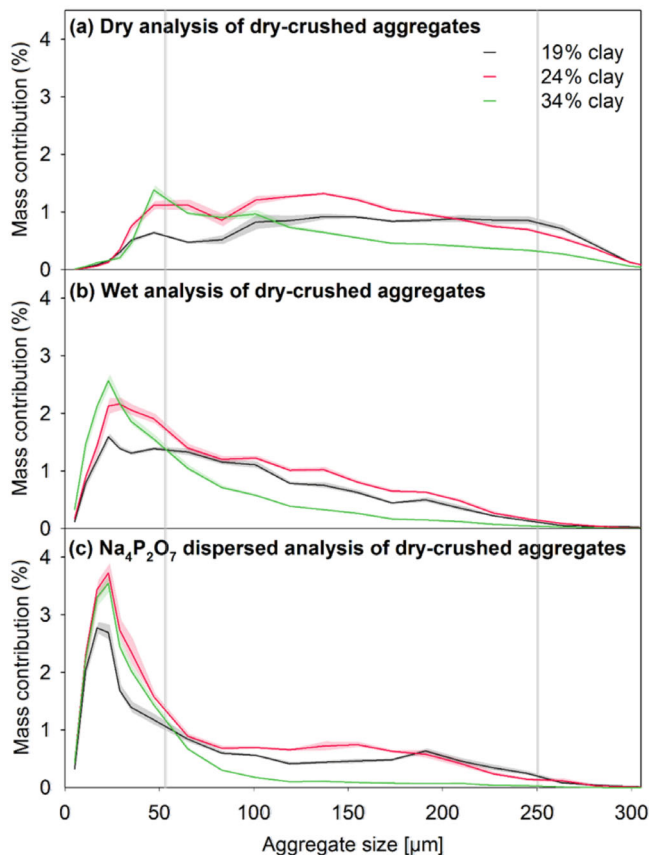


FIGURE 4 Mass contributions of dry-crushed microaggregates to the bulk soil analysed (a) in a dry state, (b) in a wet state and (c) in a wet and dispersed state. The size distribution was merged from the two individual size distributions of the size fractions 20–53 μm and 53–250 μm according to their relative mass contribution to the bulk soil (mean \pm standard error) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Mean object sizes for the combined size fractions 20–53 μm and 53–250 μm (in μm ; mean \pm standard error; $n = 5$). Mean values of different clay contents that have the same lower-case letter were not significantly different at $p < 0.05$. All analyses of variance were significant at $p < 0.001$

Treatment	19% clay	24% clay	34% clay			
Dry-crushed, dry analysis	163.4 \pm 3.2	a	145.4 \pm 2.1	b	124.5 \pm 1.8	c
Dry-crushed, wet analysis	96.2 \pm 0.8	a	96.3 \pm 1.4	a	65.7 \pm 1.1	b
Dry-crushed, wet and $\text{Na}_4\text{P}_2\text{O}_7$ -dispersed analysis	90.9 \pm 1.7	a	83.5 \pm 0.5	b	45 \pm 0.7	c

to decrease from dry-crushed to wet-sieved (free) to occluded microaggregates (Figure 5). This indicates that the structure of the samples becomes more homogeneous after increased aggregate breakdown, which is shown by the variability of tensile strengths that decreases in the same direction. It should be noted that although we measured approximately 50 replicates per isolation method and clay content, these samples were hand-picked under a stereo microscope. For a truly representative result, it would be necessary to measure the stability of all microaggregates that are isolated from a given number of macroaggregates and that is much higher than 50. However, the high number of replicates makes us confident that the high variability of our results actually represents the real degree of structural heterogeneity of the microaggregates that we investigated.

3.4 | Organic matter content and chemical composition of microaggregates

The OC concentration in the medium-sized fractions (20–53 μm) was generally lowest in the soils with 34% clay compared to higher OC concentrations in the soils with 19% clay for all isolation methods. In contrast, the OC in the large fractions (53–250 μm) increased with increasing clay content for all isolation methods (Figure 6a). When comparing the OC concentrations between aggregates obtained based on the different isolation methods, they decreased in the order dry-crushed > wet-sieved free > wet-sieved occluded (Figure 6a).

In the dry-crushed fractions, the OC contribution to the bulk OC was higher in the large size fraction of the 19% and 24% clay soil compared to the 34% clay soil (Figure 6b). In the wet-sieved free fractions, the OC contributions of the medium and large size fractions were higher with lower clay contents (Figure 6b). In the wet-sieved occluded fractions, however, the OC contributions of the medium size fraction was higher in the 34% clay soil compared with the 19% and 24% clay soil (Figure 6b). The mass contributions of the dry-crushed and wet-separated fractions (free aggregates) were in a comparable range but deviated for the wet-separated

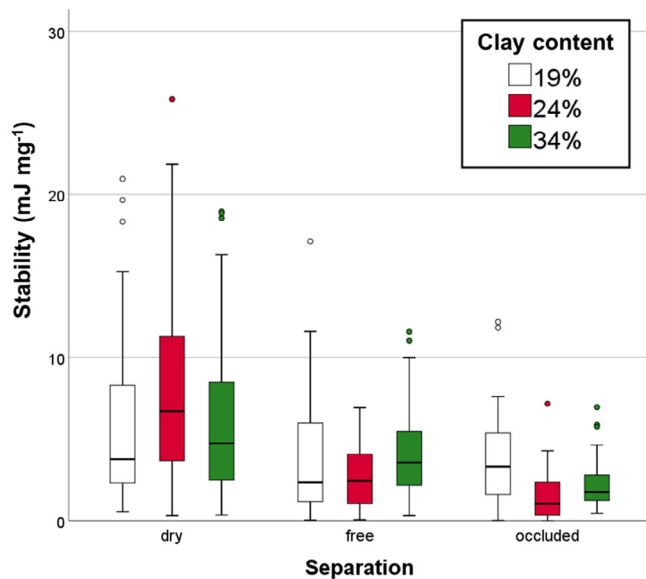


FIGURE 5 Mechanical stability of microaggregates with different clay contents obtained with different isolation methods ($n = 43$ to 50). Circles represent outliers. Extreme outliers (> 3 SD) were omitted [Color figure can be viewed at wileyonlinelibrary.com]

and sonicated fractions (occluded aggregates, Figure S5). The OC concentrations of the size fractions were similar when the OC concentrations of the medium and large size fractions were calculated, while excluding the medium to coarse silt and sand grains according to their mass contributions (Table S2).

The C:N ratios were higher for the large size fraction compared with the medium size fraction (Figure 6c). This difference was, however, smallest between the dry-crushed fractions, which did not show any differences across the clay content. The wet-sieved fractions showed lower C:N ratios in the 19% clay content compared with 34% clay. When analysing the OM composition by ^{13}C NMR, we observed more O/N-alkyl C in the dry-crushed size fraction 53–250 μm than in the size fraction 20–53 μm (Table S4).

3.5 | Composition and diversity of bacterial communities in the soil microaggregates

We compared the bacterial community composition of the large and medium size fractions that were obtained from the soils of the lowest (19%) and highest (34%) clay content with the different isolation methods. Here, we focused on the highest and lowest clay content because the generation of sufficient dry-crushed material for the molecular work was rather laborious and because sample material was limited. Analysis of the alpha-diversity

based on the Chao1 index revealed that the bacterial diversity was lower in the wet-sieved occluded fraction compared to the dry-crushed fraction ($p < 0.01$), whereas no significant difference was seen between the dry-crushed fraction and wet-separated free fraction (Figure 7). Neither clay content nor size of the fraction affected alpha-diversity significantly. Differences in bacterial community composition between size fractions (i.e., beta-diversity) were assessed in NMDS plots. This revealed that the isolation method had a significant effect on the bacterial community composition, evident from the clustering of size fractions in the plot according to the isolation methods (Figure 8a). The dependence on the isolation method was statistically confirmed by an analysis of similarity (ANOSIM), resulting in an R-value of 0.51 ($p < 0.001$). Based on this finding, the effects of clay content and fraction size were further compared between dry-crushed and wet-fractionated samples. This comparison revealed a much stronger effect of clay content on bacterial community composition in the dry-crushed fractions (ANOSIM $R = 0.767$, $p = 0.001$; Figure 8b) compared to the wet-separated fractions, where a weaker effect was seen in the wet-sieved free fraction and no effect in the wet-sieved occluded fraction (ANOSIM $R = 0.157$, $p = 0.005$). An effect of the fraction size on the bacterial community composition was again not detectable, regardless of the isolation method (Figure 8a, Table S3).

4 | DISCUSSION

4.1 | Comparing dry crushing and wet sieving on the basis of mechanical stability, organic carbon concentrations and bacterial diversity

Depending on the isolation method, the tensile strength and variability of soil fractions in the size range of microaggregates decreased in the order dry-crushed $>$ wet-sieved free $>$ wet-sieved occluded (Figure 5). When comparing the isolation methods, the organic carbon (OC) concentrations and OC contributions of the size fractions exhibited a similar tendency and decreased in the same order (Figure 6). The mass contributions of dry-crushed and wet-sieved free fractions were in a similar range (Figure S5). The differences in the OC content are related to different aggregate failure mechanisms addressed by dry crushing in comparison to wet sieving, resulting in different isolated subunits. Although dry crushing led to the isolation of size fractions where primary particles were still observed within aggregate structures (Figure 2), wet sieving produced size fractions containing free primary

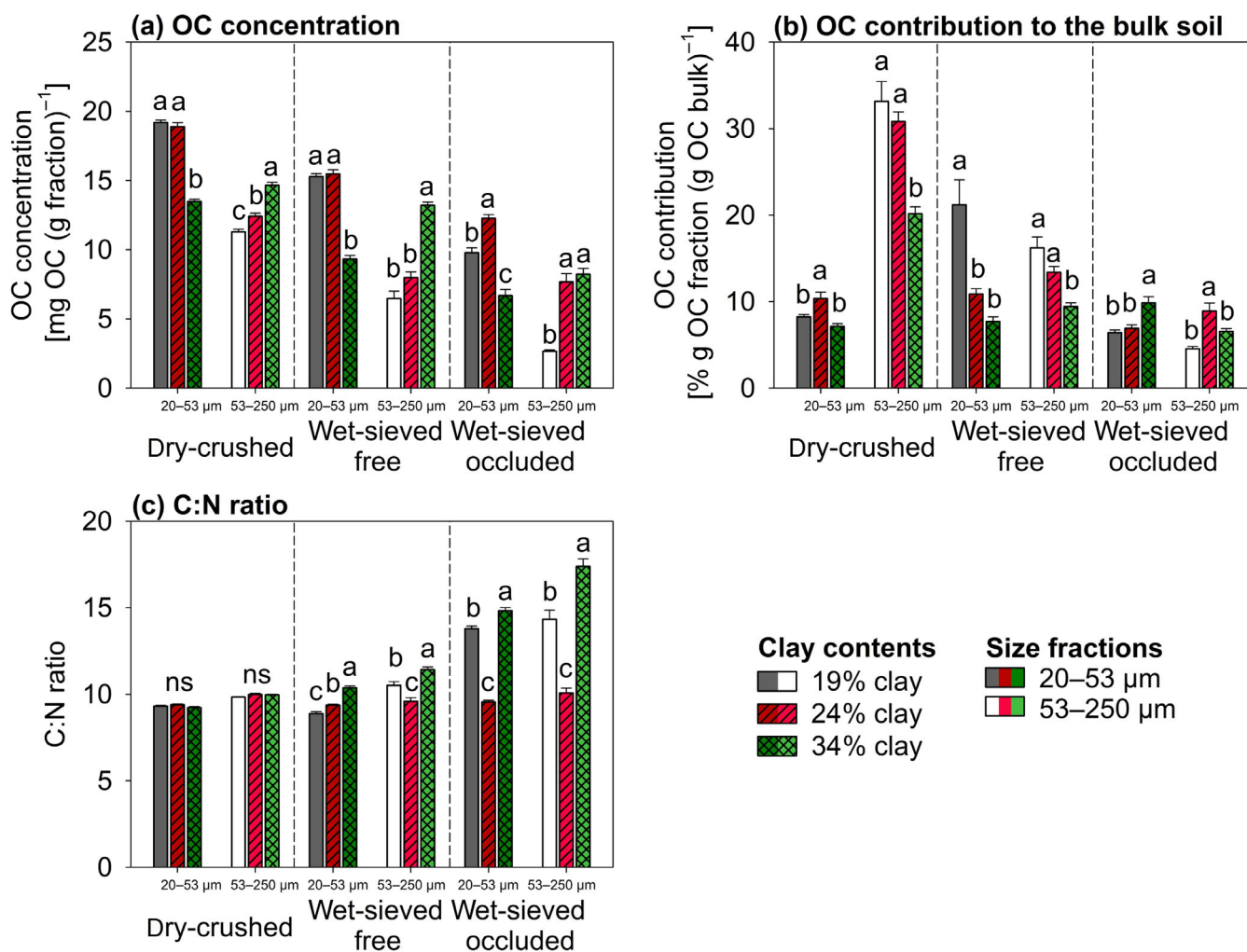


FIGURE 6 (a) Organic carbon (OC) concentration, (b) OC contributions and (c) C:N ratios comparing dry-crushed size fractions 20–53 μm and 53–250 μm with wet-sieved free and wet-sieved occluded size fractions (mean ± standard error; $n = 5$). Means with the same letter in the same isolation method and size fractions were not significantly different. Data of the wet-sieved free and wet-sieved occluded fractions adapted from Krause et al. (2018) [Color figure can be viewed at wileyonlinelibrary.com]

particles outside of aggregates (Figure 3). For the mechanical stability measurements, individual aggregates were manually selected, whereas free primary particles were not included. The higher content of sand-sized particles in the dry-crushed fractions led to a higher variability of the tensile strength (although they were absent in the wet-sieved samples, sand grains still contributed to the overall aggregate stability in the dry-crushed samples). Hence, with respect to the effect of isolation method on aggregate stability, our hypothesis could not be confirmed.

The isolation of defined size fractions from larger aggregates necessitates disaggregation and breakdown forces for the separation into smaller units. Mechanical separation by uniaxial dry crushing or dry sieving appears to generate different aggregate subunits to wet sieving, due to the different failure mechanisms

(mechanical vs. hydraulic stresses). This has a great influence on aggregate properties. The size composition of wet-sieved size fractions was shown to be highly reproducible by Graf-Rosenfellner et al. (2018). This homogeneity, however, appears to be the product of the wet sieving procedure with sonication.

Dry separation avoids a change in physical, chemical and biological characteristics that can be observed during drying and wetting. These can be (as reviewed by Kaiser et al., 2015)) the formation of new interactions between minerals and OM, the killing of microorganisms and shifts in their community structure and an increase of mineral surface acidity and hydrophobicity (possibly affecting aggregate stability; cf. Ibrahim, Mowrer, Amami, and Belaid, 2019)). The degree to which these characteristics are altered by wetting and drying depends on various factors, such as soil depth (Kaiser et al., 2015),

land use (Fierer & Schimel, 2003), texture and OM content (Albalasmeh & Ghezzehei, 2014), sodium and calcium cation configuration (Aquino et al., 2011), pH value (Kang & Xing, 2008) and drying speed (Kemper & Rosenau, 1986). All of these influences alone are difficult to quantify, which is why (repeated and/or unnecessary) wetting and drying should be avoided to preserve the natural properties of soil aggregates and to prevent the creation of artifacts.

The comparison of isolation methods demonstrated that although wet-sieved fractions were found to contain

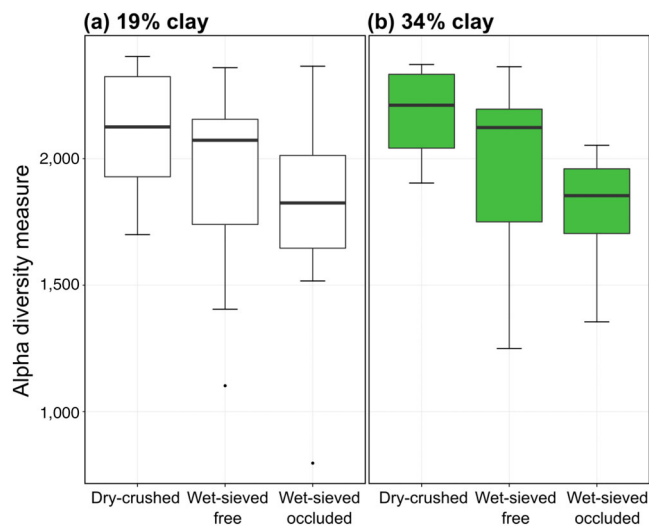


FIGURE 7 Comparison of the alpha diversity based on the Chao1 diversity index between dry-crushed and wet-separated (wet-sieved free and wet-sieved occluded) fractions (including the size fractions 20–53 μm and 53–250 μm) for two soils with (a) 19% clay and (b) 34% clay [Color figure can be viewed at wileyonlinelibrary.com]

more free primary particles and fine-sized aggregates, dry-crushed fractions contained more aggregates with inclusions of primary particles. This leads to more heterogeneous aggregate architectures within the size fractions isolated by dry crushing. This heterogeneous architecture is reflected by a higher bacterial alpha-diversity in the dry-crushed fractions (Figure 7). Significant differences in dependence on the isolation method were also observed in the bacterial community composition (Figure 8a). It is known that the use of classical methods for wet sieving and dry sieving (not comprising dry crushing) comes with specific influences on the bacterial community composition (Wilpiszeski et al., 2019). These differences are likely to be related to habitat heterogeneities of the respective aggregate fractions. In addition, differences in the bacterial community composition may result from the different methodological procedures, that is the immersion in water during wet sieving, which may cause the dislodgement of bacterial cells from microaggregates. The loss of bacterial cells by wet-sieving steps is likely to affect different species to a different extent, considering that the interactions between bacterial cells and soil constituents such as mineral particles are known to vary between species or even strains (Ams, Fein, Dong, & Maurice, 2004; Hong et al., 2012; Huang, Wu, Cai, Fein, & Chen, 2015; Krause et al., 2019). Specific strains may be more easily lost during the wet-sieving process compared to others, which may have contributed to a reduced diversity on wet-sieved microaggregates, especially for the wet-sieved occluded microaggregates, which are exposed to wet sieving twice and additionally treated by ultrasound. In addition, the wet-sieving protocol includes a lyophilization step, but we consider this a minor source of bias, as we observed no significant effects

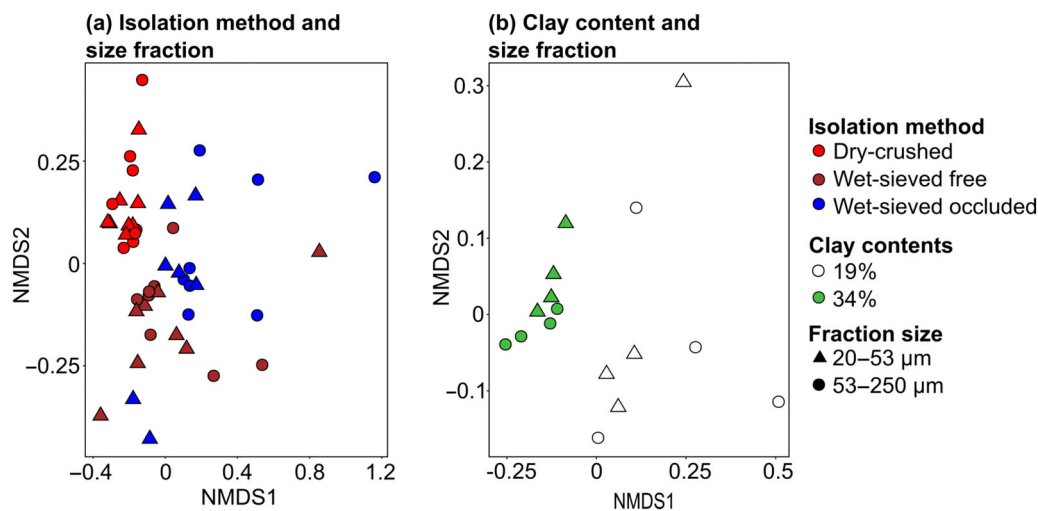


FIGURE 8 Non-metric multidimensional scaling (NMDS) plots comparing (a) isolation method and size fraction and (b) clay content and fraction size of dry-crushed fractions. NMDS plots were calculated based on Bray-Curtis distance matrices, derived from 16S rRNA gene amplicon data [Color figure can be viewed at wileyonlinelibrary.com]

on alpha- or beta-diversity when testing the effect of lyophilization on bacterial community composition with a subset of bulk soil samples independently (see Figure S7). Undoubtedly, the air-drying step for the soil prior to the dry crushing procedure also has an influence on the bacterial community, but this can be regarded as less intrusive for DNA-based studies and causes less bias compared to the full wet-sieving protocol. Dry crushing might therefore be a useful alternative when microaggregates need to be isolated while maintaining bacterial community composition. It should be noted that the methodological impact of the different fractionation procedures may have different effects on microbial respiration or enzyme activities, which remain to be evaluated in detail. Likewise, future studies should be performed to assess systematically the identity of the taxa that are primarily affected by different fractionation methods.

Based on our findings, that is the isolation of different structures during dry crushing and a potential loss of cells due to the wet sieving procedure, our initial hypothesis of a higher bacterial diversity in the dry-crushed samples was confirmed. This is in accordance with studies that compared the impact of wet sieving with dry sieving on microbial parameters (Blaud et al., 2017; Nahidan & Nourbakhsh, 2018; Trivedi et al., 2017). Likewise, as in this study, Blaud et al. (2017) found major differences in the microbial community composition between wet-sieved and dry-sieved fractions, whereas the effect of the size of a fraction was minor. Besides the reduction of sources introducing methodological bias, aggregate properties, that is the differences in failure mechanisms and structures observed in this study, might explain some of the previously observed differences in the microbial community between dry-separation and wet-sieving techniques (Bach & Hofmockel, 2014; Blaud et al., 2017). The recommendation of earlier studies to employ wet sieving when focusing on long-term changes in OM and enzyme activity is, therefore, related to a preferential isolation of fine particle and OM-rich aggregates (Nahidan & Nourbakhsh, 2018). Our results show that the dispersion and loss of sand-sized particles by wet sieving from intact aggregate structures may distort the transfer of findings from the isolated microhabitats and their associated microbial communities to the intact bulk soil.

4.2 | How clay content affects the soil microstructure of dry-crushed microaggregates

Because differences in texture may lead to different aggregate architectures, we analysed the microaggregates isolated by dry crushing from soils with different clay

contents. A bimodal size distribution of dry-crushed microaggregates reveals the existence of preferential microaggregate sizes with diameters of approximately 50 μm , which were more abundant in the 34% clay soil, and approximately 150 μm , which were more abundant in the 19% clay soil (Figure 4a). The smaller aggregates in the soil with 34% clay compared to those in the soil with 19% clay can be explained by a smaller size of the primary particles in soils with high clay content. The mechanical breakdown of the 34% clay soil into smaller structural units indicates a multitude of failure planes compared to the 19% clay soil, which broke down into larger structures as a result of having fewer failure planes.

Increasing aggregate diameters in dry-crushed fractions of low-clay soils are in contrast to the aggregate relationships found by wet sieving. In a previous study using wet sieving on the same gradient, most water-stable microaggregates were found to be of approximately 30- μm diameter independent of differences in clay content (Schweizer et al., 2019). In the high-clay soils, more water-stable macroaggregates $>250 \mu\text{m}$ were found, whereas microaggregates were larger in the size range of 50–180 μm (Schweizer et al., 2019). Accordingly, various earlier studies that applied wet sieving found larger water-stable aggregates in soils with higher proportions of 2:1 clay minerals (Amézqueta, 1999; Bronick & Lal, 2005; Six, Paustian, et al., 2000b). During wet sieving, soil structures are mostly dispersed through slaking and the swelling of phyllosilicates. By contrast, the dry crushing method breaks soil structures along mechanical planes of weakness (Kristiansen et al., 2006).

The size distribution measurements of dry-crushed fractions after water immersion and $\text{Na}_4\text{P}_2\text{O}_7$ dispersion show which sizes of water-stable units and primary particles are contained in dry-separated aggregates (Figure 4b, c). The dry-crushed microaggregates in the lower clay soils (19 and 24% clay) consisted of more sand-sized particles with sizes around 100–250 μm , in contrast to the 34% clay soil. The comparison of the particle size distributions in dry, wet and dispersed states indicates that a majority of these sand-sized particles formed parts of dry-crushed soil aggregates. This was also visible in both microscopy images and tomography scans (Figure 2), suggesting that the inclusion of sand-sized particles increased the microaggregate diameter in the soils with lower clay contents. In an earlier study using wet-sieved fractions, free primary particles $>100 \mu\text{m}$ were observed not to form water-stable microaggregates (Schweizer et al., 2019). In this study, we found such sand-sized primary particles integrated in aggregates of dry-crushed size fractions. Accordingly, primary particles with diameters $>100 \mu\text{m}$ are stable aggregate compounds when samples are

subjected to dry crushing but may be detached from fine particles during wet-sieving procedures.

The effect of isolation method on the particle size compositions is a consequence of the different failure mechanisms of wet sieving (hydraulic) and dry crushing (mechanical). It seems that wet sieving addresses more (and probably other) points of weakness, resulting in different microaggregate subunits. Fine-sized aggregates preferentially remain stable during the isolation of fractions with wet sieving. Such fine aggregates are reflected by the unimodal size distribution at approximately 25 μm after immersing the dry-crushed microaggregates in water (Figure 4b), which is similar to the preferential microaggregate size of wet-sieved microaggregates found in a previous study of the same soils (Schweizer et al., 2019). When upscaling findings based on wet-sieved aggregates, it is therefore important to consider that these do not fully represent the soil structure that was present under field conditions. Therefore, if natural aggregate structures are of interest for the isolation of fractions that include their original sand-sized primary particles, dry crushing shows advantages over wet sieving.

4.3 | Mechanical stability of microaggregates across a texture gradient

The mechanical stability of individual microaggregates from all clay contents showed a similar range and a high variability (Figure 5). The initial hypothesis about the influence of clay content on aggregate stability could therefore not be confirmed. Because microaggregates from the soil with a lower clay content contained more sand-sized primary particles, a similar range of stability suggests that sand-sized particles exerted the same influence on mechanical stability at all clay contents. Alternatively, sand-sized particles within microaggregates might also have less influence on their stability, because aggregates broke along their weakest mechanical failure planes, which might be located in parts of the aggregate with fine particles. The influence of the particle arrangement in a soil aggregate on its mechanical stability warrants further studies to determine the failure planes related to the aggregate breakdown upon crushing. For these future studies, it would be sensible to also use air-dry samples because it is common state for near-surface soils and it is easy to standardize, providing samples with similar matric potentials, which is one of the major influences on soil stability (Horn & Peth, 2012). Our results show, for the first time, that it is possible to determine the stability of an individual microaggregate specimen in a loading frame and give a first overview of the range of

mechanical stability and the relationship of microaggregates with soil clay content and the isolation method.

4.4 | Organic matter allocation in microaggregates

The OC concentrations in large microaggregates (53–250 μm) were found to increase in the soils with higher clay content and were lower in the soil with 34% clay compared to the soil with 19% clay in medium-sized microaggregates (Figure 6a). This was connected to the inclusion of primary particles in size fractions of the low-clay soils and confirmed our initial assumptions. When the sand- and silt-sized primary particles were excluded from the fraction masses included in the measured OC concentrations, both the large and medium size fractions had similar OC concentrations. This suggests that the contribution of fine particles in aggregates is strongly related to the OC contents. Fine mineral particles provide reactive mineral surfaces to stabilize OM or larger structures that occlude OM (Baldoek & Skjemstad, 2000; Ransom, Kim, Kastner, & Wainwright, 1998). In turn, the proportion of larger primary particles occupying space within aggregates reduces the OC concentrations. This can also explain the difference in OC concentrations between the size fractions (Figure 6a): the primary particles contained in the medium size fraction might occupy less space than the larger primary particles in the large size fraction. The OM composition was different between the two dry-crushed size fractions but similar across the clay content (Figure 6c; Table S4). A higher C:N ratio and a lower alkyl:O/N-alkyl ratio indicated OM to be in a less decomposed state in the large compared to the medium size fraction. This corroborates previous studies that used wet-sieved size fractions (Fernández-Ugalde et al., 2013; Schweizer et al., 2019), despite the relatively low signal-to-noise ratio of the NMR spectra in this study (Figure S6, Table S4). Larger fraction sizes might simply include more or larger pore spaces, which could be filled with OM, inhibiting its further decomposition. This assumption has to be confirmed by the analysis of the three-dimensional structure of the aggregate pore systems. The lower C:N ratio of the medium size fraction might also be related to higher amounts of mineral-associated ammonium, which was shown to correlate positively with clay content (Jensen, Christensen, & Sørensen, 1989). The C:N ratios were, however, similar within the size fraction and did not increase in soils with higher clay contents, which contained more clay- and silt-sized primary particles.

Therefore, the C:N ratios were mostly related to the decomposition of OM instead of mineral-associated ammonium. The difference in OM composition between the fraction sizes was not reflected by the microbial community composition, which was similar regardless of the microaggregate size. Although the OC concentrations of the size fractions were mostly related to the proportion of fine primary particles, the OM composition differed between the size fractions depending on its capacity to stabilize OM.

It seems plausible that both wet sieving and dry crushing yield different results for the OC distribution of the different aggregate size fractions. During wet sieving (especially with sonication), large primary particles are dispersed from fine particles (containing the most OC) (Nahidan & Nourbakhsh, 2018). This leads to an underestimation of the OC content in the largest fraction and an overestimation of OC in the smallest fraction. On the other hand, if larger quartz grains are ground into smaller primary particles during dry crushing, their resulting increased abundance in the smaller fractions will probably dilute the smallest fraction, leading to an underestimation of the real OC content. More studies are needed in order to quantify the relevance of each of these two mechanisms for different OC concentrations and substrates, that is sand contents, possibly also comparing dry sieving with and without prior crushing.

4.5 | Microbial community composition in dependence on soil clay content

In the dry-crushed fractions, we observed stronger differences in bacterial community composition in dependence on soil clay content (Figure 8b), whereas no detectable differences between microaggregate sizes were seen regardless of the applied fractionation method. This is likely to be related to our findings on a changing size composition of the microaggregates as indicated by an increasing proportion of sand-sized primary particles in microaggregates from soils with lower clay content. The fact that no differences were seen in bacterial community composition between fraction sizes 20–53 μm and 53–250 μm seems to contradict the findings of some other studies, where distinct bacterial communities in micro- and macroaggregates were seen (Davinic et al., 2012; Fox et al., 2018; Trivedi et al., 2017; Upton, Bach, & Hofmockel, 2019). This effect might therefore be scale dependent, meaning that although differences between micro- and macroaggregates can be found, they do not exist within the microaggregate size fractions that we investigated in this study.

4.6 | Using dry crushing for the isolation of aggregates in future studies

The dry crushing procedure enables addressing different research questions that expand current aggregate separation methods, such as the isolation of aggregates without the dissolution of water extractable OM or the quantification of the mechanical stability of individual aggregates. To adopt the dry crushing procedure, a loading frame is not a necessity, because one does not necessarily need to record the applied force. Any device that can be used to crush soil macroaggregates by reducing the distance of two planar surfaces to a defined threshold (250 μm in this case) would suffice to isolate fractions. For example, a modified bench vice could be used. Alternatively, a metal block with a 250- μm deep cavity could be manufactured. However, this would not allow recording of load–displacement curves, which indicates individual tensile strengths of aggregates and can reveal the crushing of individual primary particles (see 4.3). The utilization of different sized spacers between planar surfaces would allow researchers to isolate any aggregate size that is of interest for a particular study.

5 | CONCLUSIONS

We showed that dry crushing allows the isolation of size fractions that are characterized by a more variable mechanical stability compared to isolation by wet sieving. This is related to a higher content of sand-sized particles in dry-crushed aggregates according to different aggregate failure mechanisms during dry uniaxial mechanical crushing compared to wet sieving. We found that dry-crushed aggregates contained sand-sized primary particles, whereas the wet-sieved fractions contained more free primary particles released from aggregates. These differences in aggregate properties were accompanied by differences in bacterial colonization. Also, the additional re-wetting and sonication during wet sieving probably removed bacterial cells, leading to a lower bacterial diversity in the occluded microaggregate fraction compared to the microaggregates obtained upon dry crushing. We suggest dry crushing as an alternative isolation procedure that better preserves the bacterial associations with minerals. Clearly, the choice of the isolation method is reflected in microbial community composition, which should be considered in future studies and when comparing data between studies.

On average, the inclusion of sand-sized particles in dry-crushed aggregates led to 40- μm larger microaggregate structures when comparing the soils with low and high clay content. The dispersion during wet sieving

equalized the size distribution with most objects at a diameter of approximately 25 μm . The OC concentrations in the size fraction 53–250 μm increased with the clay content of soils. This is explained by a decreasing proportion of sand-sized primary particles in the aggregates from the soils with higher clay contents. The wet-sieved size fractions reflected similar patterns of OC concentration changes across clay content and fraction size. The sand-sized and silt-sized particles within aggregates are dispersed during wet sieving. Therefore, aggregates in wet-sieved size fractions contain more clay-sized and silt-sized particles. If the aim of aggregate isolation is to isolate intact structures including sand-sized primary particles, dry crushing can be recommended.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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