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# Bias in aggregate geometry and properties after disintegration and drying procedures

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ABSTRACT

Isolation and drying soil microaggregates and their building units are of crucial importance when studying their structure and function within different soil management systems. Our aim was to evaluate how different drying techniques preserve small aggregate building units after different disintegration steps. After applying fast wetting, slaking, or ultrasonic dispersion at 440 J $mL^{-1}$ to Cambisol topsoils under either long-term forest, grassland, or arable soil management, aggregate-size distributions were assessed using fast image analyses after optical particle-size assessment prior and after air- and freeze-drying. Microaggregates isolated by dry-sieving served as control. While ultrasonic dispersion significantly disintegrated soil aggregates into smaller units, slaking in water did not. Intriguingly, freeze-drying preserved the aggregate size distribution fairly well, with a reaggregation ranging between 1.2 and 10.1%. In contrast, air-drying led to substantial reaggregation of particles ranging between 20.4 and 44.9%. However, freeze-drying also led to slight deformation of particles and also to a redistribution of elements between size-fractions, the extent of which being different for the samples under different land-use. We conclude that ultrasonic treatment followed by freeze-drying is suitable to preserve the correct aggregate size of at least Cambisols, but the properties of the secondary particles may still not reflect true geometric forms and chemical properties.

## 1. Introduction

A healthy and functioning soil must store nutrients efficiently and provide them to plants when needed. The physical structure of the soil plays a crucial role in these processes and thus in maintaining soil functions. Most soils are composed of so-called aggregates, secondary structural units which itself may be hierarchically composed of smaller units, separated by persistent planes of weakness or glued together by a range of different binding agents (Oades and Waters, 1991). Within the soil aggregates system, two kinds of soil aggregate sizes are usually distinguished: soil microaggregates < 250 μm, and soil macroaggregates > 250 μm (Oades and Waters, 1991).

Soil microaggregates usually contain stable organic matter, persist slaking in water and persist changes in tillage regime (Six et al., 2000). They are composed of mineral and organic components arranged in a heterogeneous but rather unknown pattern. Soil microaggregates are also major building units of soil macroaggregates (Oades and Waters, 1991; Six et al., 2000), stabilized by temporary binding agents, such as, roots, fungal hyphen (Six et al., 2000; Kleber et al., 2007). Eventually, soil microaggregates are even formed within stable macroaggregates (Six et al., 2004). During decomposition of organic fragments inside the macroaggregate, microbial gums are produced, which might interact with clay-sized minerals. These clay particles might then encrust the organic fragment (Oades, 1984). Over time, the binding agents in macroaggregates degrade, resulting in a loss of macroaggregate stability and the release of stable microaggregates, which become the building blocks of the next cycle of macroaggregate formation (Six et al., 2000).

While soil macroaggregates were in the focus of many studies in regard to the response to different parent material, management, or environmental changes (Jastrow, 1996; Bronick and Lal, 2005), comparable information on the stability, turnover and composition of soil microaggregates is much less available. In order to obtain such information, it is a priori necessary to isolate soil microaggregates in a

*Abbreviations:* CCD, charge coupled device; MaA, Macroaggregates; MANOVA, Multivariate analyses of variance; MiA, Microaggregates; SEM-EDX, scanning-electron microscopy coupled to energy-dispersive X-ray spectroscopy; SOM, Soil organic matter; XPT, micro particle detector; ZAF, Z = atomic number, A = absorption, F = fluorescence

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representative manner from soil. This is not easy, because many methods available for microaggregate characterization, such as elemental analyses, scanning electron microscopy, or X-Ray diffractometry require a dried-sample, while the disintegration of macroaggregates is usually performed under wet conditions. Re-drying microaggregates from wet state, however, includes the risk that they reaggregate (Edwards and Bremner, 1967; Amelung et al., 2002) and thus change their properties.

Past studies used various sets of methods for the disintegration of soil aggregates including ultrasonication (Edwards and Bremner, 1967), slaking (Panabokke and Quirk, 1957; Grieve, 1980), and related mechanisms like breakdown by differential swelling, breakdown by raindrop impact, and physico-chemical dispersion (for review see Bissonnais, 1996). Slaking in water may also lead to disaggregation (Bissonnais, 1996), but mainly of macroaggregates (Six et al., 2000), possibly leaving behind microaggregates of different properties. When biological properties are to be preserved, soil disintegration is usually stopped at ultrasonic energy input of 60 J $mL^{-1}$ (Stemmer et al., 1998). For complete dispersion of particles, currently, a two-step ultrasonic treatment has become most widely with minimum detachment or redistribution of organic matter (Amelung and Zech, 1999; Kaiser and Berhe, 2014). Thus method builds on results of Stemmer et al. (1998) in that the soil is dispersed first at 60 J $mL^{-1}$ ultrasonic energy, thereafter coarse particulate organic matter is removed prior to prolonged ultrasonic treatment (440 J $mL^{-1}$ in the method of Amelung and Zech, 1999). While biological analyses was then usually continued on the moist fractions, the released microaggregates were usually dried and even ground prior to most subsequent chemical analyses in order to characterize the chemical properties of a whole aggregate-size fraction. In doing so, potential reaggregation mechanisms have not been of relevance for the underlying research questions. This, however, is different when information on the single aggregate level is to be achieved. Here, we are not aware of any systematic study that recorded changes in individual microaggregate properties by the re-drying procedure. Therefore, the aims of this study were to i) evaluate changes in microaggregate properties released by different disintegration procedures in comparison to microaggregates that were wetted or dry-sieved only, and ii) to record changes in macro- and microaggregate properties by the drying step. For this purpose, we isolated the < 250 μm fraction (microaggregates and/or primary particles) by dry-sieving, wetting, slaking, and ultrasonic dispersion from Cambisols under different management and thus likely of different soil organic matter (SOM) composition and aggregation dynamics (e.g., Allen, 1985; Guggenberger et al., 1995). In addition to microaggregate-size distribution we analyzed the shape of samples relative to the fractions obtained after the different disintegration steps prior to drying as well as after different drying procedures. Furthermore, we assessed surface element contents of individual aggregates using scanning-electron microscopy coupled to energy-dispersive X-ray (SEM-EDX) spectroscopy.

## 2. Materials and methods

### 2.1. Sample characteristics

Surface soil samples (0 to 10 cm) were collected in spring 2013 from three different TERENO experimental test sites in Germany. TERENO spans an Earth observation network across Germany, whose observatories supply important data for responding to the impact of long-term climate change on ecosystems, land-use, and infrastructure at the regional level (www.TERENO.net) (Zacharias et al., 2011). The three TERENO test sites under study were Rollesbroich (50°37′25″N/6°18′16″E, grassland soil, Cambisol, pH 5.8, 20.8% sand, 59.2% silt, 20.0% clay), Wüstebach (50°30′15″N/6°18′15″E, forest soil, Cambisol, pH 3.6, 18.1% sand, 60.6% silt, 21.3% clay), and Selhausen (50°52′10″N/6°27′4″E, arable soil, Cambisol, pH 7.1, 23.1% sand, 58.8% silt, 15.2% clay). These experimental sites were selected in order to incorporate a maximal variety of Cambisol characteristics as it is generally accepted that land-use has a great impact on soil properties and aggregation. The soil pH was determined in water (Blume et al., 2011), texture was assessed according to Rowell (1994). The C and N analysis was performed after dry combustion with a Fisons NA 2000 elemental analyzer applying the ISO 10694 procedure. At the grassland site, mineral soil samples were taken below the turf. The dominant vegetation at the grassland site is a ryegrass society, particular perennial ryegrass (*Lolium perenne*) and smooth meadow grass (*Poa pratensis*) and at the forest site Norway spruce (90% coverage). The arable site was used for maize cropping at time of sampling. For each of these three land-uses three subplots were sampled being approximately 15 m apart from each other. For each subplot, again four samples were taken but pooled in order to reduce soil sample heterogeneity. Overall, we thus had three composite samples for each land-use. All soil samples were air-dried, 2 mm sieved, and then stored for subsequent analyses.

### 2.2. Disintegration and subsequent drying experiment

The soil samples were treated in different ways, namely: (1) dry-sieving, (2) slaking (breakdown of large, air-dry soil aggregates), and (3) ultrasonic treatment in suspension using a two-step approach. The detailed experimental approach and subsequent analyses were visualized in Fig. 1. For each treatment three analytical replicates were used for each land-use sample.

As a control, we dry-sieved the soil samples over a 250 μm aperture sieve. Subsamples of soil macro- (> 250 μm) and microaggregates (< 250 μm) were stored for subsequent analyses and served as a reference for the other treatments. Additionally, the obtained soil microaggregates by dry-sieving were carefully but abruptly wetted with deionized water using a soil:solution ratio of 1:5 (w:v). Obtained soil microaggregates were then air-dried under the hood for three days at an average temperature of 25 °C or freeze-dried in order to obtain reaggregated particles > 250 μm by dry-sieving as control. After drying, the samples were gently sieved with a mesh size of 250 μm. Sieving always holds the risk of abrasion and mechanical break-down of aggregates; in order to minimize this risk we gently placed the dried sample on top of the sieve and slowly shaked it for about 20 s. Soil macroaggregates (on top of the sieve) and soil microaggregates (within the collection container) were collected as sum and the weights were recorded. We are aware that due to the dry-sieving the surface properties might be altered and do then not reflect the true properties of undisturbed aggregates in the field; however, in order to judge alterations of aggregates due to isolation and drying we have to compare them to some kind of control aggregates. Therefore, we labeled this variant as control even though it might not truly reflect the aggregate properties of undisturbed aggregates in the field. To minimize a bias from this final abrasion in data interpretation, also the freeze-dried aggregates were sieved in similar manner after freeze-drying was completed.

For slaking we preferred initial dry samples as the moisture of the samples is another factor which influences the subsequent breakdown of aggregates (Truman et al., 1990). Hence, the air-dried bulk soil samples were shaken in deionized water at 1:5 (w:v) for 16 h on a reciprocal shaker at 250 rpm to promote the gentle breakdown of all larger macroaggregates into microaggregates. After shaking, the samples were sieved (mesh size 250 μm), and only the microaggregates were transferred into pre-weighed tins and either air-dried or also freeze-dried. After drying the samples were dry-sieved again as described above.

For complete disintegration of the samples we applied ultrasonic energy using a Branson 450 W sonifier that was calibrated thermally according to North (1976). The probe tip was placed 15 mm below the water surface. In short, 10 g dried and sieved soil (2 mm) was suspended in 50 mL deionized water and ultrasonicated using 60 J $mL^{-1}$ in a first step. After the first ultrasound treatment, samples were wet sieved (mesh size 250 μm) and soil macroaggregates and