

## Short Communication

# Andosol clay re-aggregation observed at the microscale during physical organic matter fractionation

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## Abstract

The high aggregate stability of Andosols and the direct effects of sample drying led to several inconsistencies during physical soil organic matter fractionation. We have determined that NaCl addition displayed little influence on clay dispersion. At the microscale, we observed the re-aggregation of the clay fraction caused by freeze-drying. This issue was avoided by analyzing aliquots of soil suspension. Thus, we recommend reserving a small soil liquid aliquot to be subjected to microscopy analysis.

**Key words:** allophane / carbon / microscopy / short range order minerals / soil dispersion

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## 1 Introduction

Soil organic matter (SOM) fractionation is an important tool used to investigate the distribution and composition of differently stabilized C pools. Thus, it provides important insight into its persistence and turnover (Golchin et al., 1994; Six et al., 2002). Fine mineral soil fractions (*i.e.*, fine silt and clay size) are used to separate long-term protected OM, as they contain the majority of organo-mineral associations (Kögel-Knabner et al., 2008). However, separating fine fractions especially from allophanic Andosols by physical fractionation was shown to be critical because the dispersion of such soils is hampered by the high stability of microaggregates (< 63  $\mu\text{m}$ ), which is derived from strong interactions between organic matter, Al oxides and Fe oxides (Asano and Wagai, 2014; Matus et al., 2014). Silva et al. (2015) demonstrated that high levels of ultrasonic dispersion (*i.e.*, up to 1600 J mL<sup>-1</sup>) were necessary to achieve clay (< 2  $\mu\text{m}$ ) dispersion depending on the soil weathering stage and concentration of Al and Fe oxides. Studies that sought to obtain even smaller fractions such as fine clay (< 0.2  $\mu\text{m}$ ) (Asano and Wagai, 2014) or nano-clay (< 10 nm) (Calabi-Floody et al., 2011) required even higher levels of dispersion. In addition, soil saturation with sodium (*i.e.*, “Na saturation”) has been used to increase dispersion (Kyuma et al., 1969; Yonebayashi et al., 1974). However, studies examining the combined effects of Na saturation with ultrasonic dispersion are still relatively rare.

In addition, clay flocculation after soil dispersion is a common artifact reported in studies that focused on the fractionation of Andosols, and performing the procedure within a single day has been recommended to avoid biases in SOM distribution

between fractions (Asano and Wagai, 2014; Asano et al., 2018; Wagai et al., 2018). However, this effect becomes even more important for analyses at the microscale because it can potentially preclude spectromicroscopic analysis (*e.g.*, nano-scale secondary ion mass spectrometry–NanoSIMS) when the aim is to investigate clay-size particles due to the formation of sample topography (Mueller et al., 2013). However, there is still a lack of studies evaluating this flocculation effect at the microscale.

Therefore, SOM fractionation was performed with and without Na saturation prior to ultrasonic dispersion in an allophanic Andosol to evaluate the effects of fractionation on clay dispersion. Two different approaches were employed to avoid clay flocculation at the microscale and analyzed using scanning electron microscopy (SEM), including the standard method of using freeze-dried samples and taking a liquid aliquot from the clay suspension during the final step of fractionation.

## 2 Material and methods

### 2.1 Soil samples

We focused on obtaining clay-size fractions to study the composition and amount of organo-mineral associated OC in Andosols. Therefore, a subsoil sample with a lower content of light particulate fractions (to reduce possible bias from particulate OM) from an Andosol localized in the Kohala region in northern Hawaii (20°4′14.16″N, 155°43′21.94″E) was used in this study. The soil was collected from a B horizon at a depth of approximately 0.5–0.6 m that contained 50.4 g kg<sup>-1</sup>



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of total C (SOC). It was sieved to 4 mm mesh size and maintained under field moisture conditions at 4°C until the analyses were performed.

## 2.2 Soil organic matter fractionation

The procedure was adapted from the SOM fractionation method described by *Golchin* et al. (1994), who tested three different dispersion agents followed by ultrasonic dispersion. All analyzes were performed in triplicate, and a schematic diagram of the method is presented in Fig. 1.

We used 10 g of field-moist soil (*i.e.*, dry equivalent, sieved to 4 mm), which were gently saturated with the specific fractionation solutions at a solid-to-liquid ratio of 1 to 2.5, and two different solutions were as follows: (1) 1 M solution of NaCl and (2) deionized water. After allowing the saturated samples to equilibrate overnight, the free particulate organic matter (fPOM) was removed from the supernatant using an electric vacuum pump.

Thereafter, ultrasonic dispersion (SonopulsHD2200, Bandelin, Berlin, Germany) was performed using an energy input of 1500 MJ m<sup>-3</sup>. The chosen energy level was considered sufficient for providing clay (< 2 μm) dispersion of the highly-stable Andosol without causing damage to the primary mineral structure (*Silva* et al., 2015).

To remove the residual salt, each fraction was rinsed with deionized water until the electrical conductivity dropped below 0.001 S m<sup>-1</sup>. Later, the soil was sieved with 20-μm sized mesh to separate the sand and coarse silt fractions; subse-

quently, the residue was subjected to sedimentation to separate the medium silt and clay fractions of 20–2 μm and < 2 μm, respectively, and SOC recovery was 99.4 and 98.0% for the NaCl and H<sub>2</sub>O treatments, respectively. OC content in the bulk soil sample and each fraction were measured in duplicate by dry combustion (CHNSO Elemental Analyzer, Hekatech, Wegberg, Germany).

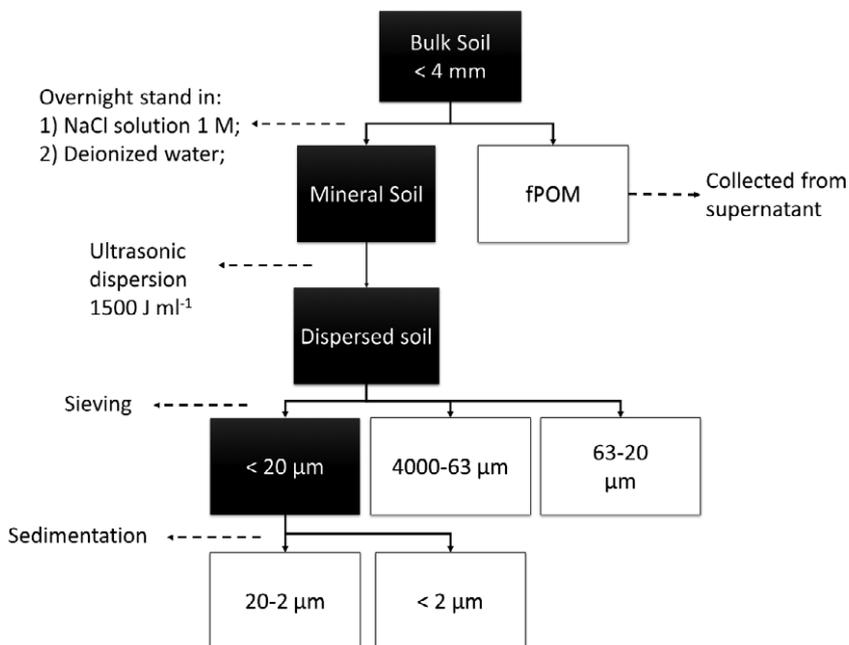
## 2.3 Microscopy analysis

To observe clay flocculation artifacts at the microscale on samples with and without freeze-drying, the clay-size fraction (< 2 μm) was analyze using both fractionation procedures (*e.g.*, NaCl and H<sub>2</sub>O) using SEM (JSM 5900LV, JEOL, Tokyo, Japan). Two different sample preparation approaches were employed, including: (1) freeze-drying of the clay-size fraction, and (2) direct deposition of the clay-size mineral suspension from the last step of physical fractionation. Samples were suspended in water at a proportion of 1 × 10,000, pipetted onto a silica wafer, and dried in a desiccator at room temperature for subsequent SEM analyzes.

## 3 Results and discussion

### 3.1 Effectiveness of sodium saturation on clay dispersion

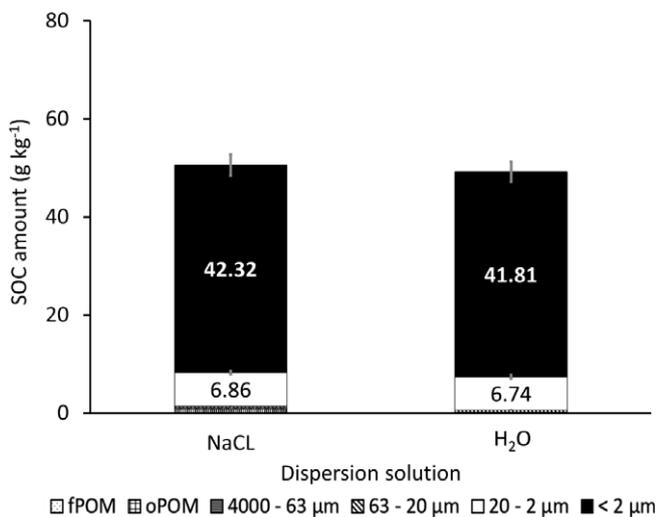
Regardless of the fractionation solution used, for both soils, the greatest amount (*i.e.*, more than 95%) of SOC was concentrated in the mineral fractions of < 2 μm and 20–2 μm (Fig. 2). Such distribution was likely due to the sampling depth (*i.e.*, below 0.4 m). In subsoil layers, there is normally a lower concentration of light fractions recovered as particulate organic matter and the greatest amount of OC was concentrated in the mineral fractions (*Golchin* et al., 1994).



**Figure 1:** Schematic figure of the soil organic matter fractionation procedure. White boxes represent the different fractions separated by the procedure (*i.e.*, light particulate organic matter–fPOM; mineral fractions of 4000–64 μm, 63–20 μm, 20–2 μm, and < 2 μm). Dashed arrows and observations represent the procedures performed for conducting each step.

Overall, the dispersion treatment resulted in a comparable SOC distribution among fractions. We expected that NaCl would increase the < 2 μm clay dispersion, but the difference in the SOC amount of this fraction between the NaCl and the H<sub>2</sub>O treatments was lower than 2%. The addition of NaCl solution prior to fractionation, also referred to as “Na saturation”, has been a common procedure used to obtain higher soil dispersion (*Yonebayashi* et al., 1974). The weakening of Ca and Mg cation bridges and the expansion of the double layer on the soil particle surface are the primary reasons for the enhanced capacity of NaCl to disperse soils (*Edwards* and *Bremner*, 1967; *Kato* and *Fujisawa*, 1973).

When evaluating Andosol aggregation using different fractionation procedures, *Asano* and *Wagai* (2014) also concluded that saturation with NaCl was necessary to

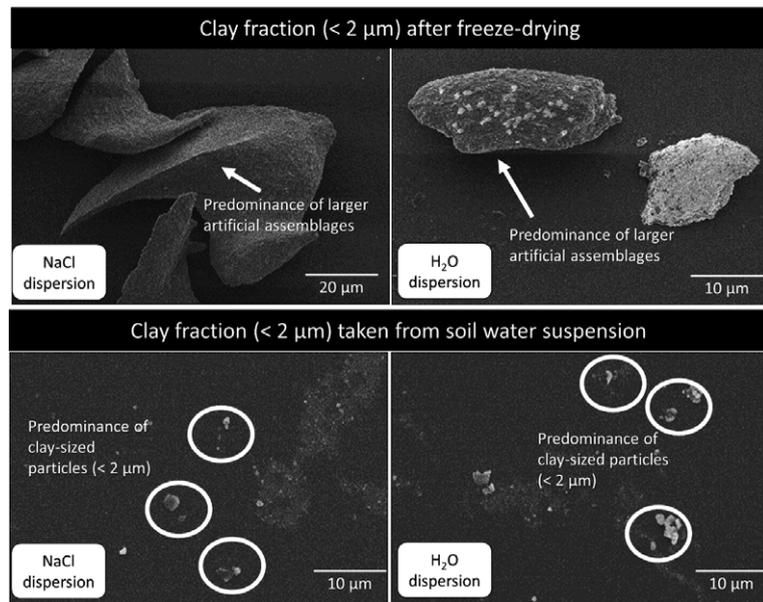


**Figure 2:** Soil organic matter fractions in subsoil B horizon (0.4–0.6 m) of an Andosol with two dispersion solutions prior to ultrasound sonication, including 1 M NaCl solution and deionized water (H<sub>2</sub>O). Numbers in the columns represent the amount of SOC (g kg<sup>-1</sup>) retained in each fraction. Error bars represent the standard deviation of three laboratory replicates.

achieve maximum dispersion and to obtain fractions smaller than 0.2 μm. However, in this study, for clay particles < 2 μm, the NaCl addition did not cause a significant difference when compared with dispersion only in water. The latter method has the main advantage of being less time consuming, as salt washing is unnecessary.

### 3.2 Re-aggregation effects at the microscale

At the microscale, we observed a clay re-aggregation effect in freeze-dried samples, while this was avoided when sampling directly from the soil suspension (Fig. 3). Although the sam-



**Figure 3:** Scanning electronic microscopy (SEM) images of samples taken from a clay fraction (< 2 μm) water suspension and the same fraction freeze-dried.

ples were diluted in the same proportion, the freeze-drying procedure created assemblages larger than 20 μm that did not represent the size of the respective fraction. On the other hand, aliquots from the soil suspension were predominantly composed of clay-size particles that were 2 μm or smaller. This re-aggregation was very likely a result of the strong binding effects of Fe and Al oxides in Andosols (Asano et al., 2018; Wagai et al., 2018).

Freeze-drying is a common procedure used in different methods including SOM fractionation but also general soil analyzes protocols due to its efficiency in sample preservation (Miller et al., 1999). However, our experimental results demonstrated that for the Andosol used in this study, this procedure created sample artifacts that can clearly affect the analysis when the objective is to isolate the clay-size fraction, especially with the intent to examine the microscale organic matter surface coverage or the specific surface area (Vogel et al., 2014; Schweizer et al., 2018).

Over the last decade, investigating microenvironments using spectromicroscopy has provided important discoveries with respect to SOM turnover and C sequestration mechanisms (Lehmann et al., 2008; Mueller et al., 2012; Steffens et al., 2017). Among the different processes, the formation of organo-mineral associations occurring at a small spatial scale are considered as key for C protection in soils (Kögel-Knabner et al., 2008). In this way, Andosols are a particularly ideal soil for investigating such interactions, given their high concentration of short-range order minerals (Dahlgren et al., 2004). Therefore, avoiding artifacts at the microscale is extremely important to fully explore microenvironments and understand their enhanced OC stabilization capacity in soils.

Results of this study indicated that artifacts such as re-aggregation could easily be avoided by taking liquid aliquots from the soil suspension at the final step of fractionation. Therefore, we recommend the separation of a small liquid portion for microscopic analysis to avoid biases when exploring microenvironments.

## 4 Conclusions

This study demonstrated that Na saturation through the addition of 1 M NaCl solution caused low (less than 2% in SOC amount) clay dispersion (< 2 μm) compared with the dispersion when water alone was used. Direct effects of freeze-drying on Andosol clay fractions at the microscale were also observed, which caused the formation of assemblages larger than 20 μm. The presence of strong binding effects of the poorly crystalline mineral phases in Andosols is likely the primary reason for this effect. Therefore, in future studies, we recommend reserving a small liquid aliquot from soil suspensions after sonication to proceed with microscopy analyzes.

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