

Evaluation of an ultrasonic dispersion procedure to isolate primary organomineral complexes from soils

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Summary

Soil organic matter can be intimately associated with mineral particles of various sizes. For structural studies, soil organic matter can be isolated in particle size fractions after complete dispersion of the aggregates by ultrasonication. The ultrasonic dispersion energy necessary for complete dispersion was investigated in three A and two B horizons originating from four soils differing in pedogenesis (Gleysol, Phaeozem, Podzol, Alisol), organic C (4.2–34.5 g kg⁻¹) and clay content (24–294 g kg⁻¹). Calorimetric calibration of five probe-type ultrasonifiers revealed that the actual energy output from an instrument can depart widely from its nominal output, and that this discrepancy varies from instrument to instrument. Calorimetric calibration is therefore essential for consistency and comparisons between laboratories. Between 450 and 500 J ml⁻¹ of ultrasonic dispersion energy was enough to disperse completely all samples investigated. The particle size distributions obtained were close to those from standard analysis, except for smaller yields (–20 to –80 g kg⁻¹) of sand size fractions, which suggests that dispersion by ultrasound is more effective. Based on total C, C:N ratio and distribution of dissolved C, no detachment of soil organic matter from primary organomineral complexes and no redistribution between particle size fractions could be detected in the range 30–590 J ml⁻¹ of dispersion energy.

Introduction

Organic matter can be intimately associated with mineral matter in soil. Its dynamics can be followed by fractionating it physically in density and particle size fractions, which have been related to structure and function of organic matter *in situ* (Andreux *et al.*, 1995). Physical fractionation is considered to be less destructive to soil organic matter (SOM) than chemical fractionation, which may alter the chemistry of SOM (Morra *et al.*, 1991; Ladd *et al.*, 1993). The SOM can be fractionated physically by dispersing aggregates with ultrasound, and the resulting organomineral complexes (OMCs) can be separated using liquids of different density or by particle size fractionation.

Many authors have expressed concern about the lack of standardization of the ultrasonic energy of probes used for dispersion (Watson, 1971; North, 1976; Christensen, 1992; Raine & So, 1993, 1994). Amongst other factors (i.e. diameter of probe, depth of immersion and shape of vessel), the dispersion efficiency was significantly affected by the output of ultrasonically induced cavitation energy produced by the

probe (Raine & So, 1994). Because this energy cannot be measured directly, indirect effects were used to assess it, for example sonochemical, sonobiological, sonoluminescence and calorimetric measurements. The last have been used in soil studies to calibrate instruments using demineralized water as the reference (North, 1976; Gregorich *et al.*, 1988, 1989; Morra *et al.*, 1991; Preston *et al.*, 1994). In many studies, however, the ultrasonic dispersion energy applied has not been calibrated, making direct comparison between studies impossible. For example, particle size fractionation on the same soil resulted in different distributions of particle size and C and N content, probably as a result of variations in the efficiency of dispersion (Schnitzer & Ivarson, 1982; Schnitzer & Kodama, 1992; Schulten *et al.*, 1993). This shows that without calibration of the applied energy, comparisons between laboratories can be misleading.

The experimental set up should be optimized for any range of individual soil types to ensure that dispersion is neither excessive nor incomplete. Excessive dispersion might cause abrasion of OMCs and a redistribution of detached SOM between size fractions, whereas incomplete dispersion can yield particle size fractions comprising an unknown mixture of primary and secondary OMCs as defined by Christensen (1992). Primary OMCs are related to the primary structure of soils, as determined by standard soil texture analysis (Gee &

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Bauder, 1986), and can be separated after complete dispersion. Secondary OMCs relate to the aggregate structure of soils obtained after limited dispersion, and consist of aggregates of smaller primary OMCs.

The concept of primary and secondary OMCs may be considered simplistic, and probably cannot be applied to all soils *per se*. However, this concept provides a first approach to evaluating the necessary ultrasonic dispersion energy, which seems essential considering the enormous variation in dispersion energy (90–5350 J ml⁻¹), summarized by Christensen (1992), applied in previous studies.

In studying the methodology, we have evaluated the ultrasonic dispersion procedure on five horizons from four soils, which we chose because they are common in Central Europe, and varied in pH, texture and C content. Our objectives were (i) to compare the power output of different ultrasonic devices by calorimetric calibration, (ii) to determine the energy necessary for complete dispersion, and (iii) to assess a potential redistribution of SOM between particle size fractions at different levels of ultrasonic energy.

Soils and methods

Soils

We sampled and described the soils according to established procedures (FAO, 1994). We designated horizons according to the German Soil Survey Description (AG-Boden, 1994). Table 1 gives a short description of the soil properties. Samples were chosen to represent different horizons from soils varying in pedogenesis, vegetation and texture. The Ap horizon of a Dystric Gleysol (0–20 cm) was sampled from a field that had been under agriculture for 100 years (Wageningen, The Netherlands). During this period mineral fertilizers and cattle manure had been applied. The other samples were from Germany. A spodic horizon (Bh) from a Haplic Podzol (25–31 cm) under forest south of Haltern, Nordrhein-Westfalen was sampled along with an Ap horizon of a Haplic Phaeozem (0–20 cm) from an agricultural plot north of Halle/Saale. The Haplic Alisol originated from the eastern hills in Siggen, Schleswig-Holstein under forest. From this soil, the eluvial (Ah, 0–14 cm) and the illuvial (Bvt, 47–91 cm) horizons were selected.

Soil pretreatment and chemical analysis

Roots and visible plant remains were removed manually, and samples were freeze-dried (at –60°C and air pressure 8 Pa). Soil aggregates were carefully crushed, and the fraction >2 mm was removed by dry-sieving. For chemical analysis, a subsample was ball-milled for 10 min. The pH was measured with a glass electrode in the supernatant suspension of a 2.5:1 (water:soil by weight). The contents of C and N were

Table 1 Soil characteristics

Soil group ^a and horizon ^b	Depth /cm	pH /H ₂ O	Organic C /g kg ⁻¹	Total N /g kg ⁻¹	C:N	DOC ^c /g kg ⁻¹ of total soil C	Particle size distribution ^d /g kg ⁻¹						
							2000–630 µm	630–200 µm	200–63 µm	63–20 µm	20–6 µm	6–2 µm	2–0.45 µm
Dystric Gleysol, Ap	0–20	5.2	18.8	1.2	17	30	24	332	468	103	29	25	33
Haplic Podzol, Bh	25–31	3.5	34.5	1.0	35	29	8	270	603	60	20	15	24
Haplic Phaeozem, Ap	0–20	6.5	22.6	2.0	11	10	20	106	184	288	160	51	193
Haplic Alisol, Ah	0–14	4.1	19.4	1.6	12	54	59	175	237	161	123	78	177
Haplic Alisol, Bvt	47–91	5.3	4.2	0.5	8	42	44	158	206	90	115	93	294

^a Classification according to FAO (1994).

^b Horizon designation according to AG-Boden (1994).

^c After dispersion with 440 J ml⁻¹ and filtration, the content of dissolved organic carbon was determined for the fraction <0.45 µm with an aliquot of the solution and from this data g kg⁻¹ of total organic C was calculated.

^d Results obtained by standard particle size analysis as described in the Soils and methods section.

determined in duplicate with an Elementar Vario EL (minimum detection level for C and N: $0.1 \pm 0.3 \text{ g kg}^{-1}$). Dissolved organic carbon was determined with a Shimadzu TOC 5050 analyser.

Calibration of the power output of the ultrasonicators

We assessed the cavitation energy applied by ultrasonication calorimetrically with demineralized water (North, 1976) and calculated the power output using the equation:

$$P = m_w c_w \Delta T / t + H, \quad (1)$$

where P is the power (W), m_w is the mass of water (g), c_w is the specific heat capacity of water ($4.18 \text{ J g}^{-1} \text{ K}^{-1}$), ΔT is the difference in temperature (K), t is the sonication time (s) and H is the energy loss by conduction (J s^{-1}). The power P is that consumed by heating the water plus that lost by conduction. Conduction can be reduced by placing water in a Dewar vessel and covering its surface with plastic chips so that H becomes negligible. The power applied was determined by monitoring the rising temperature (resolution 0.1 K) with increasing energy as indicated by the instrument. The mass of water varied between 200 and 600 g, and the sonication time was between 60 and 150 s.

To calibrate the output of ultrasonic energy for different devices five probe-type ultrasonifiers operating at 20 kHz were compared in the non-pulse mode. They were as follows.

1 Branson Sonifier 250 (Branson, CT, USA) with 200 W output to the converter, equipped with a titanium probe (12.5 mm diameter). A model at the Technical University of Munich (a) and another model at the University of Adelaide (b) were compared.

2 Branson B-30 (Branson) 350 W equipped with a titanium probe (12.5 mm diameter) at the CSIRO in Adelaide.

3 Labsonic U (Braun Melsungen, Germany) equipped with a titanium probe (19 mm diameter) located at the Technical University of Cottbus (a) and the University of Bochum (b).

The rated power output of the instruments was displayed in watts (Labsonic) or determined with an inbuilt meter and a conversion chart supplied by the manufacturer (Branson). Calibration lines were obtained by linear regression analysis, and the correlation coefficient was calculated according to Pearson. Variation of the power output was <5%, even after 31 months' use as determined calorimetrically for the Braun Labsonic U (b).

Dispersion techniques

Three procedures for dispersion of soil samples were used.

1 Standard textural analysis included a treatment of 20 g of the sample with H_2O_2 (10%) initially at room temperature. After gas development decreased, samples were heated for a maximum of 24 h. Subsequently 0.5 l of demineralized water

and 0.4 M sodium polyphosphate were added, and the vessels were shaken horizontally over night.

2 Dispersion by shaking in water was performed with 30 g soil in 150 ml demineralized water on a horizontal shaker for 30 min.

3 For ultrasonic dispersion, a soil suspension (30 g soil in 150 ml water) was prepared in a 250-ml glass beaker (diameter 70 mm, height 130 mm) and allowed to stand for 12 h. The probe tip of the ultrasonic device was immersed 15 mm into the suspension. The temperature of the suspension was kept below 35°C using a water cooling jacket. For this procedure, the Braun Labsonic U ultrasonifier was used and operated at a constant 75 W determined calorimetrically. Treatment times varied between 1 and 20 min to produce a range of energies between 30 and 590 J ml^{-1} . Between treatments the instrument was allowed to cool for 15 min. The energy E dissipated into the suspension was calculated by

$$E = P t / V, \quad (2)$$

where P is the power output (W), t the sonication time (s) and V is the volume of the suspension (ml). The last was calculated using an average particle density of 2.6 g cm^{-3} and water density of 1.0 g cm^{-3} . These values were also applied to convert data from other authors.

Particle size fractionation

The dispersed samples were separated into seven particle size fractions according to the German classification (AG-Boden, 1994). Three sand fractions (630–2000 μm , 200–630 μm and 63–200 μm), three silt fractions (20–63 μm , 6–20 μm and 2–6 μm) and a clay fraction (<2 μm) were obtained. Two separation procedures were used.

1 For standard particle size analysis and to investigate the effect of different ultrasonic dispersion energy on the particle size distribution, the soils were fractionated in cylinders following the standard pipette sieve method (Gee & Bauder, 1986). The samples were transferred into the cylinders, topped to 1.0 l, shaken end-over-end and then allowed to settle. Settling times for 2, 6, 20 and 63- μm particles for temperatures between 18 and 28°C were calculated according to Stokes' law, applying a particle density of 2.65 g cm^{-3} . The temperature of the water was measured in an additional cylinder. Particle size limits refer to equivalent spherical diameter, signifying the diameter of a spherical particle with the same density and settling velocity as those analysed. Aliquots of silt and clay fractions were decanted in preweighed beakers, dried (105°C), after which the beakers were weighed again. The remaining fraction was washed over a nest of sieves (630, 200 and 63 μm), dried (60°C) and the mass determined gravimetrically.

2 For complete isolation of the particle size fractions, the soil suspension was washed through a nest of sieves (630, 200 and

63 µm) and made up to 0.71 in a glass cylinder (diameter 60 mm) with a siphon attached. The prerequisites for gravity sedimentation were fulfilled by keeping clay and silt content in the suspension less than 5% (Genrich & Bremner, 1974) and the ratio of cylinder to those of the particles was > 100 (Elonen, 1971). The cylinders were filled to the 25-cm mark, shaken end-over-end and left to settle at room temperature. When the appropriate settling time for the clay fraction (<2 µm) was reached, the supernatant was siphoned into a glass flask and kept for further treatment. The treatment was repeated until the supernatant appeared clear. Between 8 and 12 sedimentation cycles were necessary for the complete isolation of any particular fraction. Gravity sedimentation of the clay fraction was followed by separation of fine, medium and coarse silt size fractions. The separation for the clay and silt fractions took up to 3 weeks. To reduce possible alteration of suspended SOM as a result of microbial activity, the separated fractions were stored in the dark at 5°C and filtered as soon as the complete fraction was recovered. The fractions were filtered through prewashed cellulose nitrate filters (0.45 µm), using stainless steel pressure filtration cylinders with a pressure of about 5×10^6 Pa. The filter pellet, easily removed from the filter, was freeze-dried, weighed and ball-milled. The standard deviations for the ultrasonic dispersion-particle size fractionation were determined on a total of 12 samples. They varied for individual fractions between ± 1 and 6 g kg^{-1} of soil. To determine the amount of dissolved organic carbon in the <0.45-µm fraction, the filtrate of all clay and silt size fractions was collected and the concentration of organic carbon was determined in an aliquot with a Shimadzu TOC 5050 analyser.

Results and discussion

Calibration of ultrasonic instruments

The output of ultrasonic power was calibrated calorimetrically for five ultrasonic instruments. The power dissipated to the water was assessed by monitoring the increase in temperature produced on irradiating a known mass of water. The calorimetrically determined power was plotted against the displayed power (Figure 1). All instruments revealed linear relations (Pearson correlation coefficient $r \geq 0.97$), and the lines in the figure are of calorimetrically determined power predicted by displayed power. These were calculated by regressing the calorimetrically determined power on a fixed set of displayed power for each instrument. As shown in the example in Figure 1, to obtain 75 W of calorimetrically determined power dissipation instruments needed to be adjusted to settings between 88 W (Branson 250 (a)) and 260 W (Branson B-30). These results demonstrate the variation of power output among the models. The differences between identical models were somewhat smaller but still substantial. For example, the settings for the two Branson 250 instruments

varied between 88 and 130 W and the Braun Labsonic U between 185 and 235 W, respectively, in the given example. These different performances of the same models might result from different adjustments of the instruments and tolerances in production.

Thus calorimetric calibration of the instruments revealed that calorimetrically determined power output (i) was always less than the power displayed by the instruments and (ii) varied widely for different models. In numerous studies of SOM in particle size fractions, however, only the type of instrument and rated power output were reported, and calorimetric measurements were not made (summarized by Christensen, 1992). The differences in power actually imparted to the soil suspension probably partly explain variations in dispersion efficiency as well as chemical and physical properties of the fractions such as those observed by Christensen (1992). This shows that calorimetric calibration of ultrasonic instruments is crucial to ensure reproducibility and allow comparison between laboratories.

Determination of ultrasonic energy for complete dispersion

The ultrasonic dispersion energy needed to disperse aggregates completely was determined in two steps. First, the yields of the particle size fractions were monitored while ultrasonic dispersion energy was increased, so that the optimum energy could be determined where changes in yield level off (Figure 2). Secondly, the particle size distribution at this energy was calibrated against yields obtained from standard textural analysis. For better comparison all ordinates are adjusted to cover a range of 350 g kg^{-1} . For display purposes, the three sand fractions were shown as one group. Owing to large differences in sand content, sand yields for loamy soils (Phaeozem, Alisol) and sandy soils (Podzol, Gleysol) are displayed in two separate graphs.

Increasing ultrasonic dispersion resulted in consistently decreasing yields for the sand and coarse silt fractions (-20 to -30 g kg^{-1} whole soil). The yields of clay fractions increased ($+20$ to $+30 \text{ g kg}^{-1}$), while yields of medium and fine silt fractions varied (maximum $\pm 20 \text{ g kg}^{-1}$). For sand, coarse silt and clay fractions, changes in yield level off between 450 and 500 J ml^{-1} . The data indicate that after application of small amounts of energy the sand and coarse silt size fractions still comprised secondary OMCs composed of finer primary OMCs. With increasing input of ultrasonic energy, secondary OMCs disintegrated into smaller secondary OMCs or primary OMCs or both, which resulted in a continuous accumulation of clay-sized OMCs. These results confirmed the utility of the concept of aggregate hierarchy in soils in which aggregate stability is controlled by organic materials (Oades & Waters, 1991). Between 450 and 500 J ml^{-1} , the yields reached a plateau, suggesting that more energy caused no additional disintegration of sand-sized OMCs, and only primary OMCs

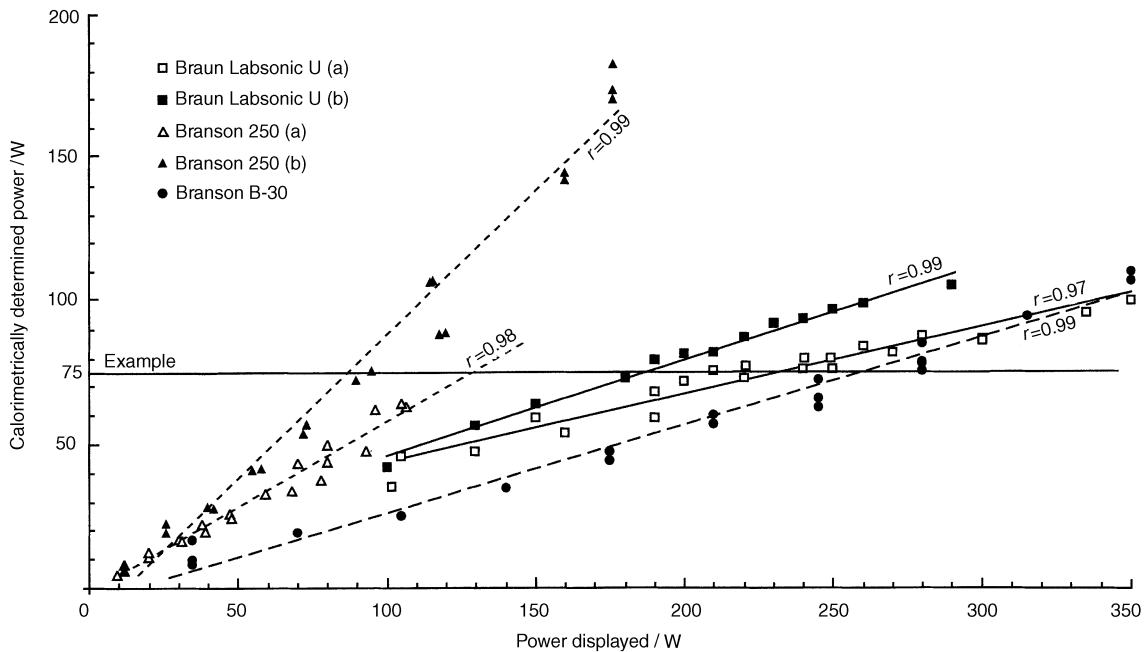


Figure 1 Calorimetric calibration of the ultrasonic devices. The power displayed by the instruments is compared with the calorimetrically determined power. Calibration lines were obtained by linear regression analysis, and r is the Pearson correlation coefficient.

were left in these fractions. The fact that medium and fine silt fractions showed only small changes could be explained by gains from sand and coarse silt size fractions balancing the losses to clay-sized fractions (Gregorich *et al.*, 1988). Another possible explanation is that silt fractions contain more stable OMCs than coarse fractions do (Morra *et al.*, 1991).

The particle size distribution after complete dispersion with 450–500 J ml⁻¹ was calibrated against conventionally determined texture. The results of standard particle size analysis were also included in Figure 2. Yields of the sand fractions were 20–30 g kg⁻¹ less after ultrasonic dispersion than found by standard textural analysis, except for the spodic horizon (Bh) which was 80 g kg⁻¹ less. In spodic horizons, organic and sesquioxide cementing material accumulated. In standard analysis, a large percentage of the organic cementing material was removed by the pretreatment with hydrogen peroxide, but sesquioxides probably were not destroyed. The remaining sesquioxides could explain the larger yields of sand-sized particles in the standard analysis of the Bh horizon than obtained by ultrasonic dispersion. As a trend, pretreatment with ultrasound seemed to be more effective in dispersion of sand-sized secondary OMCs than standard analysis. Similar observations were reported in previous studies (Gregorich *et al.*, 1988; Christensen, 1992). Yields of the coarse silt fractions accorded with results obtained from standard analysis, again except for the Bh horizon. The larger yield (40 g kg⁻¹) after ultrasonication probably resulted from a gain of OMCs derived from the more effective breakdown of sand-sized OMCs. The yields of medium silt, fine silt and clay

fraction corresponded well with results obtained after standard dispersion except for minor variations (+20 to +30 g kg⁻¹) in the medium silt fractions of the sandy soils and the fine silt fraction of the Phaeozem Ap. Summarizing, for all the soils investigated, dispersion with 450–500 J ml⁻¹ resulted in (i) complete dispersion and (ii) a particle size distribution that corresponded well with standard analysis.

This observation accords with several other recent sets of results. Gregorich *et al.* (1988) calibrated the ultrasonic device calorimetrically (46 W), but reported results relative to the displayed energy (120 W). Referring to the calorimetrically determined energy, dispersion with 450 J ml⁻¹ seemed to be sufficient to obtain similar clay yields and slightly smaller sand yields (–60 g kg⁻¹) than standard analysis. Also, for several prairie soils a similar total ultrasonic energy (calorimetrically determined 500 J ml⁻¹) was determined for complete dispersion and a particle size distribution similar to results from standard texture analysis (Amelung *et al.*, 1998). Lower dispersion energy (334 J ml⁻¹ calorimetrically determined ultrasonic energy) resulted in evidently smaller yields for silt and clay compared with standard analyses, suggesting incomplete disruption of aggregates and dispersion of the soil (Preston *et al.*, 1994). The same was true for the even smaller dispersion energy (56–93 J ml⁻¹ calorimetrically determined) suggested by Morra *et al.* (1991) to obtain adequate dispersion and to limit creation of artefacts. However, calibration of their data against standard analysis revealed larger sand yields and smaller clay yields, suggesting incomplete disruption of aggregates. This conclusion was supported by linear plateau

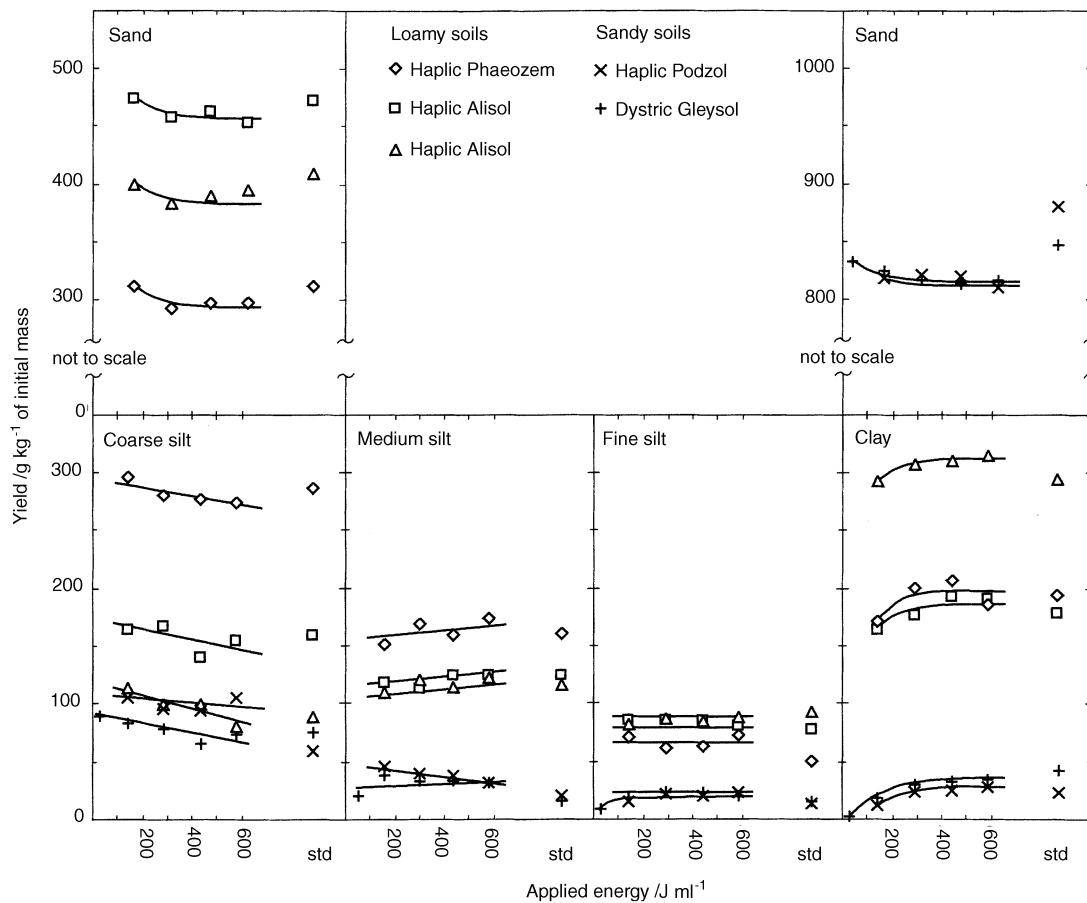


Figure 2 Influence of ultrasonic dispersion energy on yields of particle size fractions compared with standard texture analysis. Yields of particle size fractions after ultrasonic dispersion (30–590 J ml⁻¹) are connected by eye-fitted curves, and results from standard texture analysis are displayed as std. For better comparison all ordinates are adjusted to cover a range of 350 g kg⁻¹. Owing to large differences in sand content, data for loamy soils (Phaeozem, Alisol) and sandy soils (Podzol, Gleysol) are displayed in two separate graphs, where origins of the ordinates are not to scale. Standard deviation for the ultrasonic dispersion-particle size fractionation procedure was determined for 12 samples, and varied for individual fractions between ± 1 and 6 g kg⁻¹ whole soil.

regression analysis performed by the authors. Again, ultrasonic dispersion with 450–500 J ml⁻¹ seemed to be sufficient for a complete dispersion of samples from a range of soils and horizons, whereas less energy resulted in a limited dispersion of aggregates.

Redistribution of SOM between particle size fractions

To assess a possible redistribution of SOM between size fractions, the C content, the C:N ratio and the C distribution were monitored in the Gleysol at energies considerably less (30 J ml⁻¹) and more (590 J ml⁻¹) than the suggested ultrasonic dispersion energy of 450–500 J ml⁻¹ (Table 2). The Gleysol offered the best evidence for a possible redistribution of organic matter between size fractions. Owing to its sandy texture, this soil was rich in coarse SOM which could be redistributed between size fractions.

A comparison of the C contents in the fractions after dispersion with 30 and 590 J ml⁻¹ ultrasonic energy revealed only minor variations. With higher dispersion energy, C contents decreased only slightly in the coarse fractions (sand, coarse and medium silt fraction, factor 0.5–0.9) and slightly increased in the fine fractions (fine silt, clay, factor 1.1). Also, the C:N ratios varied little between the different fractions as well as between different dispersion energies with no consistent trend. Differences between the fractions became more obvious by calculating the contribution of each fraction to the C in the whole soil. With increasing dispersion energy, contributions of coarse fractions (sand, coarse and medium silt) to the C in the whole soil decreased (factor 0.4–0.8), whereas contributions of fine silt (factor 1.9) and clay fractions (factor 6.9) increased markedly. The increasing yields, as well as the increasing C and N contents of the fine fractions, could indicate a detachment of SOM from coarse primary OMCs by

Table 2 Effect of ultrasonic dispersion energy on C and N content and C distribution of a Dystric Gleysol

Particle size / μm	Applied energy / J ml^{-1}							
	30				590			
	Mass recovered / g kg^{-1} ^a	C		C:N	Mass recovered / g kg^{-1} ^a	C		C:N
	/g kg^{-1} ^b	/g kg^{-1} of C ^c		/g kg^{-1} ^b	/g kg^{-1} of C ^c			
630–2000	902	7.7	537	12	853	4.4	260	14
20–63	52	24.9	92	11	53	12.3	38	11
6–20	23	118.9	196	13	26	101.7	160	12
2–6	10	159.7	113	13	21	169.3	221	14
<2	3	177.5	42	13	24	192.7	291	13
<0.45 ^d	–	–	20	–	–	–	30	–
Sum	963				976			–

–, Not determined.

^a Standard deviation for the ultrasonic dispersion-particle size fractionation was determined for 12 samples, and varied for individual fractions between ± 1 and 6 g kg^{-1} whole soil.

^b Content is expressed as g C per kg size fraction; minimum detection level is $0.1 \pm 0.3 \text{ g kg}^{-1}$.

^c Mass of recovered organic C (g kg^{-1}) calculated by [C content of fraction (g kg^{-1}) \times mass of recovered fraction (g kg^{-1})]/total C recovered (g kg^{-1}).

^d See Table 1.

ultrasonic dispersion, or it could be an effect of the breakdown of large secondary OMC into primary OMC which are rich in associated SOM.

Another indication for detachment and redistribution of SOM could be a large contribution of the soluble carbon ($<0.45 \mu\text{m}$) to the whole soil C. Small particles of SOM eventually released from silt-sized OMCs would accumulate in the clay and $<0.45\text{-}\mu\text{m}$ fraction, because they have a small density and settle slowly. The contribution of the soluble carbon to the whole soil C varied only slightly with different dispersion energies, i.e. 30 J ml^{-1} : 20 g kg^{-1} , 440 and 590 J ml^{-1} : 30 g kg^{-1} (Tables 1 and 2). Also, for the other soils (Table 1) the amount of solubilized carbon ($<54 \text{ g kg}^{-1}$) represented only a small proportion of the whole soil C. This accords with previous studies in which soluble C accounted for $10\text{--}110 \text{ g kg}^{-1}$ of whole soil C (compilation by Christensen, 1992). If detachment and redistribution of SOM occurred then larger amounts of soluble C would be expected, unless organic matter was rapidly reabsorbed (Christensen, 1992; Golchin *et al.*, 1994). Another indication that redistribution of SOM did not occur to a greater extent could be that the silt-sized fractions of the Gleysol still retained large proportions of the whole SOM (Table 2). This implies that OMCs remained intact in these fractions, supporting results obtained by Gregorich *et al.* (1988). More evidence that detachment and abrasion of OMCs by ultrasonic dispersion was less important comes from previous structural studies. Structural alterations due to high energy ultrasonic dispersion were not evident for

SOM in the presence of quartz and soil material (Schmidt *et al.*, 1997), minerals in the presence of SOM (Watson, 1971) and the surface area of clays (Gregorich *et al.*, 1988). However, to further reduce a potential redistribution of SOM in some soils, coarse particle size fractions can be removed after limited dispersion and subsequently ultrasonic dispersion can be completed (Andreux *et al.*, 1980; Balesdent *et al.*, 1991; Amelung *et al.*, 1998). As a result, ultrasonic dispersion with $30\text{--}590 \text{ J ml}^{-1}$ did not result in major alterations of the bulk chemical composition of SOM in the investigated particle size fractions, as detectable by elemental analysis. This provides evidence that a redistribution of SOM as an effect of ultrasonic dispersion with $450\text{--}500 \text{ J ml}^{-1}$ was quantitatively not important.

Conclusions

We evaluated an ultrasonic dispersion and particle size fractionation procedure for isolating primary organomineral complexes from soils. The power output of several ultrasonic devices can vary considerably between instruments and even between identical models. We strongly recommend calorimetric calibration of ultrasonic instruments as a simple and effective way to ensure reproducibility and comparison between laboratories.

Increasing ultrasonic dispersion ($30\text{--}590 \text{ J ml}^{-1}$) of three A and two B horizons resulted in consistently decreasing yields of the sand and coarse silt fractions and concomitantly increasing clay yields while the yields of medium and fine

silt fractions revealed variable trends. The increasing disintegration of aggregates into smaller particles reached a plateau between 450 and 500 J ml⁻¹ indicating complete dispersion. This was confirmed by conventional particle size analysis, except for smaller yields of the sand size fractions (-20 to -80 g kg⁻¹).

The application of 450–500 J ml⁻¹ ultrasonic energy was sufficient for the complete dispersion of samples from a variety of soils and pedogenetic horizons. Dispersion with energy in the range 30–590 J ml⁻¹ revealed no evidence for detachment and redistribution of organic matter. This implied that with increasing ultrasonic energy, the disintegration of aggregates releases primary organomineral complexes with their associated organic matter retained, and artefacts caused by ultrasonication with 450–500 J ml⁻¹ are of little importance.

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