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Determination of dissolved organic carbon in soils with UV spectroscopy, ultrasonic dispersion pre-treatment and separation with size exclusion chromatography

Determinación de carbono orgánico disuelto con espectroscopía ultravioleta, pretratamiento con dispersión ultrasónica y separación mediante cromatografía de exclusión por tamaño
Determinação do COD por espectroscopia UV, pré-tratamento com dispersão ultrasónica e separação por cromatografía de exclusão de tamanho

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ABSTRACT

This study aimed to reveal differences in UV (ultraviolet) -absorbing dissolved organic carbon (DOC) between three prominent Austrian soil types: a Cambisol and a Chernozem developed from Tertiary marl, both under agricultural management, and a Podzol from a mixed coniferous beech forest stand. Topsoil samples (0–300 mm) were pre-treated, air-dried, sieved and four grams of each probe was added to 80 cm^3 of de-ionized water and subjected to ultrasonic treatment with specific energies of 6.7 J cm^{-3} and 161 J cm^{-3}, respectively, which dispersed the macroaggregates and released formerly occluded soluble carbon. The soils were investigated for morphological differences with a scanning electron microscope after sonication. The suspensions were filtered < 0.45 μm and UV-spectroscopy at 254 nm was performed after the dispersion pre-treatment. In addition the suspension was separated by high performance size exclusion chromatography linked to an UV-vis detector measuring at 254 nm and 210 nm and dissolved organic carbon (DOC) was determined. More DOC was released with higher specific energies for all soil types in the sequence Podzol > Cambisol > Chernozem but the differences in SOM/DOC ratio became less significant with increasing ultrasonic energy. The detected molecules were in the range of 1300-1600 Da for Cambisol, 1500-5400 Da for Chernozem and 1700-10400 Da for Podzol. The different energy levels reached different carbon pools. Based on a model according to von Lützow et al. (2008), the applied energy levels of 6.7 J cm^{-3} reached the active carbon pool consisting of plant residues and exudates, and microbial/faunal biomass and residues. Sonication with 161 J cm^{-3} dispersed more aggregate fractions and released carbon from the intermediate carbon pool where biogenic aggregation preserves the organic matter pool.

RESUMEN

Este estudio tiene como objetivo mostrar las diferencias de carbono orgánico disuelto y absorbente de UV entre tres tipos de suelos predominantes en Austria: un Cambisol y un Chernozem, cultivados y formados sobre margas terciarias, y un Podzol de un bosque de tipo mixto. Las muestras de suelo superficial (0-300 mm) fueron pretratadas, secadas y tamizadas. A continuación, cuatro gramos de cada muestra se añadieron a 80 cm^3 de agua desionizada y se sometieron a un tratamiento de ultrasonido con energías específicas de 6,7 J cm^{-3} y 161 J cm^{-3}, respectivamente, que dispersó los macroagregados y liberó el carbón soluble anteriormente ocluido. Las diferencias morfológicas entre estos suelos fueron investigadas mediante microscopía electrónica de barrido tras sonicación. Las suspensiones se filtraron a < 0.45 μm y la espectroscopía-UV se realizó a 254 nm después del pretratamiento de dispersión. Adicionalmente se separó la suspensión usando cromatografía de exclusión por tamaño vinculado a un detector UV-vis, midiendo a 254 nm y 210 nm, y se determinó el carbono orgánico disuelto (COD). Mayores cantidades de COD fueron liberadas bajo energías específicas más altas en todos los suelos analizados en el siguiente orden: Podzol > Cambisol

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> *Chernozem, pero las diferencias en la relación MOS/COD se mostraron menos significativas a medida que iba aumentando la energía de ultrasonido. Las moléculas detectadas estaban en rangos entre 1300-1600 Da en el Cambisol, 1500-5400 Da en el Chernozem y 1700-10400 Da en el Podzol. Los distintos niveles de energía alcanzaron diferentes reservas de carbono. De acuerdo con el modelo de Lützow et al. (2008), la aplicación de niveles de energía de 6,7 J cm^{-3} alcanzó la reserva de carbón activo formada por residuos y exudados de plantas, y biomasa y residuos provenientes de microbios y animales. La sonicación a 161 J cm^{-3} dispersó más agregados y liberó carbono de las reservas intermedias donde la agregación biogénica preserva la fracción de materia orgánica.*

RESUMO

Este estudo pretende detectar diferenças de carbono orgânico dissolvido absorvido por radiações UV entre três tipos de solos predominantes na Áustria: um Cambisol e um Chernozem cultivados, ambos com origem em margas terciárias, e um Podzol de uma floresta tipo misto. As amostras da camada superficial do solo (0-300 mm) foram pré-tratadas, secas e crivadas. A quatro gramas de cada amostra adicionaram-se 80 cm^3 de água desionizada sendo posteriormente submetidas a um tratamento de ultra-sons com energias específicas de 6,7 J cm^{-3} e 161 J cm^{-3}, respetivamente, para dispersão dos macroagregados e libertação do carbono solúvel previamente ocluso. As diferenças morfológicas destes solos foram investigadas após sonicação utilizando um microscópio eletrónico de varrimento. As suspensões foram filtradas < 0,45 µm e utilizou-se a espectroscopia de UV a 254 nm após pré-tratamento por dispersão. Para além disso, as suspensões foram separadas por cromatografia de exclusão de tamanho ligada a um detector de UV-Vis, tendo a determinação do carbono orgânico dissolvido (COD) sido efetuada a 254 nm e 210 nm. Observou-se a libertação de maiores quantidades de COD para energias específicas mais elevadas em todos os solos analisados de acordo com a seguinte ordem: Podzol > Cambissolo > Chernozem, mas as diferenças na relação MOS/COD tornaram-se menos evidentes com o aumento da energia ultrasónica. As moléculas foram detectadas nas gamas entre 1300-1600 Da no Cambissolo, 1500-5400 Da no Chernozem e 1700-10400 Da no Podzol. Os diferentes níveis de energia alcaçaram diferentes reservatórios de carbono. Com base num modelo de acordo Lützow et al. (2008), a aplicação de níveis de energia de 6,7 J cm^{-3} atingiu o reservatório que consiste em resíduos e exudados de plantas bem como resíduos de biomassa de micróbios e animais. A sonicação a 161 J cm^{-3} dispersou mais frações de agregados de carbono e libertou carbono do reservatorio intermediário, onde a agregação biogénica preserva o reservatorio de matéria orgânica.

KEY WORDS
DOC, HPSEC, SOM, soil aggregates, molecular weight

PALABRAS CLAVE
COD, HPSEC, SOM, agregados de suelo, peso molecular

PALAVRAS-CHAVE
COD, HPSEC, SOM, agregados do solo, massa molecular

1. Introduction

A better understanding of soil organic matter (SOM) dynamics is needed to improve management of soil organic carbon. SOM contains more than three times as much carbon as either the atmosphere or terrestrial vegetation (Schmidt et al. 2011) and dissolved organic matter (DOM) is the most mobile humus fraction. It is the link between soils and other ecosystem properties (Akagi and Zsolnay 2008).

Schmidt et al. (2011), who investigated the persistence of SOM, pointed out that organic matter should be described by quantifiable characteristics governing stability, such as solubility, molecular size and functionalization. To a large extent this is due to the chemical complexity of high molecular weight (HMW) components which are thought to form a large proportion of DOM in soil (Jones et al. 2012). The concentration of DOM is usually measured via dissolved organic carbon (DOC). Dissolved organic carbon is operationally defined as the organic carbon that can pass through a 0.45 µm membrane filter. But the passages between dissolved, high molecular and colloid substances are fluent and the dissolved fraction can also include colloid particles < 0.45 µm (Blume et al. 2010). DOC consists of a wide range of molecules ranging from simple amino acids, sugars and lipids to complex humic substances with HMW which may be of allochthonous or autochthonous origin (Egeberg et al. 1999). Due to its mobility, DOC plays an important role in transport of nutrients and pollutants (van den Berg et al. 2012).

Dissolved organic carbon is an aggregate parameter and subsequently, it does not indicate the character of organic matter (Marhaba and Pu 2000). Size exclusion chromatography (SEC) has prominently been used for characterising DOC in water samples (Matilainen et al. 2011; Korshin et al. 2009) and significant progress has been achieved in the development of efficient SEC separation systems and detectors. SEC is a physical fractionation method based on molecular size: the bigger the molecules, the shorter the retention time. This can give an insight into the compounds mobility. The bigger molecules generally have slow diffusion rates and a greater affinity for sorption sides. The smaller organic compounds show weaker sorption affinities and are more mobile (Jones et al. 2012). The most widely used method for estimating the apparent molecular weight (AMW) of DOC is SEC with ultraviolet (UV) absorbance detection. UV spectroscopy describes the potential energy of molecules generally represented as a sum of its electronic, vibrational and rotational energies. These sum parameters are responsible for the unspecific absorbance of UV radiation and the limitations of this technique for chemical analysis. Most absorption by organic compounds results from the present of π bonds (Burgess and Knowles 1984). The combination of UV spectroscopy and high performance liquid chromatography (HPLC) separation techniques reduces unspecific aspects of the method and the quantification of molecular size fractions is possible.

The objective of this study was to compare SOM fractions and DOC of three soils of the temperate zone with a specific focus on the effect of aggregate disruption under well-defined conditions using ultrasonic dispersion technique. We assumed a morphological and chemical difference between the soils' solid and mobile SOM fractions and that sonication would mobilize protected water-soluble organic matter and consequently highlight the anticipated differences.

2. Materials and Methods

2.1. Study site

Samples were taken from two arable soils and one forest soil in Lower Austria in spring 2012. The arable soils, a Cambisol on Tertiary marl (*Robolus schlier*) and a Chernozem on Tertiary marl with loess (*Molasse zone*) have been under conventional tillage (CT) management with mechanical weed control. CT involved a short disc harrow (working depth 5.5 cm), a conventional mouldboard plough (23 cm) and a crop growing combination grubber (12.5 cm). The crop rotation included winter-wheat, oat, sunflower and maize. The two fields are part of a long-term tillage experiment (14 years) studied by the University of Natural Resources and Life Sciences in Vienna, designed to test the response of plants and soils to different tillage regimes (Klik et al. 2010). The forest soil samples were taken from an overgrown plot on a flysch silica sandstone (*Greifenstein formation*) in the Vienna Woods, a mixed coniferous beech forest stand adjacent to the town of Pressbaum. The soils have been classified according to WRB (IUSS 2007):

i) a loamy clay Cambisol from Tulln at 48°18'N and 16°02'E.
ii) a degraded loamy silt Chernozem from Pixendorf at 48°16'N and 15°58'E.
iii) a Podzol from the Vienna Woods at 48°11'N and 16°03'E.

The mean annual precipitation at the sites is 600-700 mm with a mean annual temperature of 9.4 °C. Tulln has a slope of 0-2%, Pixendorf 5-6% and the Podzol has a hillside location. The Ap horizon of the Pixendorf Chernozem is severely eroded due to its slope and therefore the soil type is addressed as physically degraded. The soil pHs in $CaCl_2$ are shown in **Table 1**.

2.2. Soil sampling and analysis

Topsoil samples were taken representatively in April 2012 and characterized for their main chemical and physical parameters according to ISO (International Organisation for Standardization) standards (**Table 1**). All textural analyses were made with the pipette method using $Na_4P_2O_7$ for dispersion and H_2O_2 to destroy organic matter (ISO11277 2009). Carbon and nitrogen were measured using dry combustion with a Carlo Erba NA 1500 (ISO10694 2009; ISO13878 1998) determined with flash combustion and chromatographic analysis of carbon dioxide. The difference between total carbon and inorganic carbon is expressed as organic carbon. Determination of calcium carbonate was in accordance with the Scheibler volumetric method (ISO10693 1995). SOM was calculated with C_{org} x 1.724 assuming average C-concentration of organic matter of 58% (Blume et al. 2010). Each sample was air-dried, homogenously mixed and sieved to gain the aggregate fraction with size between 2000 µm and 1000 µm. This fraction is used to determine soil aggregate stability (SAS) according to OENorm L 1072 (2003).

Table 1. Site conditions at the respective locations (sample depth 0-300 mm); Data represent mean values of six measurements during growing season (sand 2000-63 µm, silt 63-2 µm, clay < 2 µm). SOM: soil organic matter; SAS: Soil aggregate stability: STD: Standard deviation

	Sand	Silt	Clay	N_{tot}	C_{org}	$CaCO_3$	SOM	SAS	C/N	pH
				%						$CaCl_2$
Cambisol	10.6	36.2	53.2	0.3	3.5	1.2	6.0	80.1	14	7.3
STD	±1.5	±2.1	±2.6	±0.1	±0.1	±0.3	±0.1	2.5	±0.2	±0.02
Chernozem	24.9	61.3	13.9	0.2	1.9	13.2	3.3	6.8	13	7.3
STD	±1.6	±1.8	±1.1	±0.1	±0.1	±0.2	±0.3	±2.1	±0.2	±
Podzol	61.5	35.0	3.5	1.1	29.4	<0.5	50.7	25.1	26	2.6
STD	±1.9	±1.4	±0.9	±0.1	±0.3	±0.1	±0.4	±2.5	±0.3	±0.01

2.3. Ultrasonic dispersion equipment

The ultrasonic dispersion equipment used in the present study is an adapted ultrasonic fatigue testing equipment (Mayer 2006). The probe is inserted into a soil water suspension and performs resonance vibrations at 19.1 kHz. This led to emission of pressure waves into the soil water suspension. The vibration amplitude was measured with an induction coil. Under well-defined geometrical conditions, the vibration amplitude strongly correlates with the magnitude of the acoustic pressure waves emitted into the suspension that cause dispersion of soil particles (Kuttruff 1988; Millner 1987). The amplitude and resonance frequency was controlled and kept constant with very high accuracy in a closed-loop electronic circuit. Deviation of pre-selected and actual vibration amplitude was maximum ±1%. The equipment can be run in pulsed mode to limit the temperature increase of the soil-water suspension.

Commercially available equipment use ultrasonic power as a control parameter where the power setting is rather high and accuracy is low. Since ultrasonic power is derived from voltage and current signals, the efficiency of the ultrasonic transducer and other electrical and mechanical parameters of the system it is prone to errors. Subsequently, the power of these systems can be quantified with 10-20% accuracy only (Zhu et al. 2009; Schmidt et al. 1999).

The cylindrical probe used in this study had a diameter of 30 mm. The rather large diameter of the probe improves the homogeneity of the pressure field. During the experiments, the probe was dipped into de-ionized water. The insertion depth was four millimetres, which set the distance between probe and beaker bottom at about 50 mm (**Figure 1**). This distance to the beaker bottom was chosen to avoid resonance of the acoustic waves in water (half wavelength of sound waves of 20 kHz frequency in water is 37 mm).

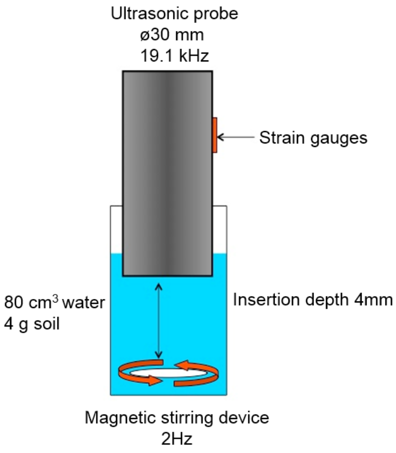


Figure 1. Principle of the ultrasonic dispersion equipment.

2.4. Calibration procedure to determine power of the ultrasonic equipment

The system operated close to the cavitation threshold of gas saturated de-ionized water, which is 0.5-0.6 µm at 19.1 kHz frequency (Schomakers et al. 2011b). The following procedure was developed and applied to determine ultrasonic power at low vibration amplitudes. The probe was inserted in water (mass of water is m_w and specific heat capacity is c_w) and vibrates at constant amplitude. The increase of water temperature, ΔT during the time period Δt was measured. Changes of thermal energy of water are caused by ultrasonic vibrations and by heat exchange with probe, beaker and ambient air. With the ultrasonic power, P_{us} and the heat exchange per second, $\Delta Q_{Exchange}/\Delta t$, change of thermal energy of water per second, $m_w c_w \Delta T/\Delta t$ is given by **Eq. 1** in Schomakers et al. 2011b:

$$m_w c_w \frac{\Delta T}{\Delta t} = P_{US} + \frac{\Delta Q_{Exchange}}{\Delta t} \qquad \textbf{Eq. 1}$$

A temperature gradient drives heat flow between water and environment due to convection or thermal conduction. Heat flow from thermic conduction (ultrasonic probe, beaker) and convection (ambient air) is proportional to the respective temperature difference. At the start of the power measurements, all mechanical components of the system are at room temperature. Only water measures a few degrees less: $\Delta Q_{Exchange}/\Delta t > 0$ and the increase of thermal energy of water per unit time is the sum of ultrasonic power and heat flow into the water. During sonication, the water temperature increases until it is greater than the ambient temperature and $\Delta Q_{Exchange}/\Delta t < 0$. When temperature of water and ambiance coincide $\Delta Q_{Exchange}/\Delta t = 0$ and the ultrasonic power is directly correlated to the increase of water temperature.

Ultrasonic power was determined at different vibration amplitudes for the used setup, i.e. an ultrasonic probe with diameter ø 30 mm, insertion depth 4 mm and vibration frequency 19.1 kHz. At vibration amplitude 1 µm mean ultrasonic power was 2.9 W. At vibration amplitude 2 µm it was 8.9 W, at 3 µm the mean power was 14.9 W, at 4 µm it was 20.8 W and at 5 µm it was 26.8 W. Standard deviation was mean ±0.9 W.

2.5. Ultrasonic dispersion

Four grams of air-dried soil (2 000-1 000 µm) were placed in a plexiglass beaker (Ø 44 mm), 80 cm^3 of de-ionised water was added and the suspension was subsequently sonicated. To obtain a homogeneous distribution, the soil water suspension was stirred with a magnetic device (2 Hz, cylindrical shape with length 25 mm and thickness 8 mm), immediately prior and during the experiment (**Figure 1**). The soil samples were subjected to a combination of ultrasonic dispersion, in accordance with OENorm L 1092 (2005). One treatment included a sonication time of 60 s at 2 µm, the other treatment included a sonication time of 480 s at 5 µm. With the ultrasonic power of 8.9 W and 26.8 W, respectively, the ultrasonic energies absorbed in 80 cm^3 were 0.53 kJ (i.e. 6.7 J cm^{-3}) in the experiments at vibration amplitude 2 µm, and 12.9 kJ (i.e. 161 J cm^{-3}) in the experiments at vibration amplitude 5 µm. Pulsed loading was applied to limit maximum temperature of the soil-water suspension to maximum 30 °C.

2.6. Scanning electron microscope

To describe the morphology of the organic particles and coating on mineral surfaces, scanning electron microscope (SEM) investigations were carried out. In addition, we studied the difference in SOM morphology between the three soil classes; and also the morphology of SOM after the ultrasonic treatment. After sonication, the soil water suspension was sieved (Fritsch Analysette 3 Pro, ø 10 cm) with vibration amplitude set at 0.1 µm and frequency at 50 Hz. A standardized sieve of aperture 63 µm was used. Sieving lasted one minute with 700 cm^3 water. The remaining soil fraction was transferred onto a Petri dish, lyophilized (Christ Alpha, vacuum 0.024 mbar, -55 °C) and subsequently stored in an exsiccator for further microscopic analysis. The scanning electron microscope used to obtain images of the different soil fractions was a Philips XL 30 Environmental Scanning Electron Microscope (ESEM). Sputtering system was a Scancoat Six Sputter Coater (Edwards). The ESEM was operated in high vacuum mode at an accelerating voltage between 3-5 kV.

2.7. Dissolved organic carbon

Immediately after sonication, the remaining soil water suspension samples were centrifuged for 10 min (3000 g) at 10 °C using a Heraeus Multifuge 3 S centrifuge with a swivel-out rotator. The suspensions were then pressed through a 0.45 µm membrane filter. To achieve a better quantitative and qualitative evaluation, both photometric and chromatographic analyses were performed.

2.8. Ultraviolet-visible spectroscopy

The samples were analysed with an UV-Vis diode array spectrometer (Agilent 8453). The spectral absorption was tested at 254 nm with a 1 cm quartz cuvette. The amount of DOC was calculated using a 254 nm (A_{254nm}) analysis, with the method of Brandstetter et al. (1996) for detecting substances which have an absorption maxima within the UV-range (**Eq. 2**).

$$DOC = 0.449 \times A_{254nm} + 1.0 \qquad \text{Eq. 2}$$

Unit of DOC in **Eq. 2** is mg dm^{-3}, unit of absorbance A_{254nm} is m^{-1} and coefficient of determination, $R^2 = 0.930$.

2.9. Molecular size of doc compounds

Selection of proper standards for HPSEC was based on hypothesised sizes of DOC molecules. Amino acids are of rather small size (< 500 Da) and a few typical examples, such as arginine and tryptophane, are listed in **Figure 2**. Other non-humic substances such as fats, waxes, alkane, peptides, proteins, lipids and organic acids have distinct MW below < 15 kDa. Fulvic acid (FA) molecules (about 2500 Da) are of small size and can easily enter the plant and are effective carbon-containing chelating compounds. Humic acid (HA) molecules (> 15 kDa) function as important ion exchange and chelating systems. The podzolization process,

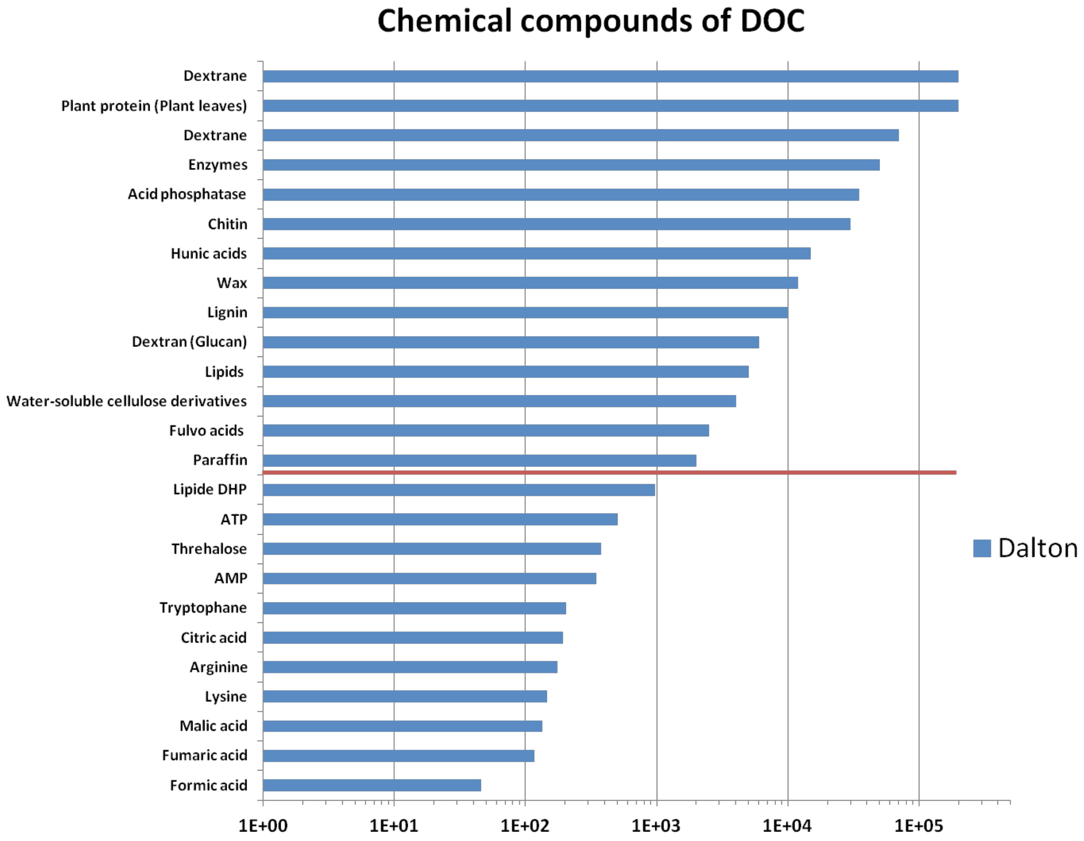


Figure 2. Horizontal bars show defined (below red line) or maxima apparent (above red line) molecule sizes (Da) of different substances of DOC. This figure compiles published data.

described in detail by Lundström et al. (2000) depends on the activity of organic acids which include HA, FA or low molecular weight (LMW) aliphatic and aromatic acids in soil solution and contribute to mineral weathering and formation of the eluvial horizon. Approximate molecular weights of these acids range from > 3000 Da for HA, 1000–3000 Da for FA, and < 1000 Da for LMW acids.

2.10. High performance size exclusion chromatography

High performance size exclusion chromatography, called HPSEC in the following, has been performed. Agilent HPLC Separation by size exclusion was performed using a silica-based column (Zorbax GF-250, particle diameter 4 µm). The stainless steel HPLC column (4.6 mm inner diameter, 250 mm length) was employed with a phosphate buffer eluent (0.2 M Na_2HPO_4, pH 7). The Agilent 1200 SL HPLC system was equipped with a diode array detector (UV-DAD).

HPSEC instrumental settings are:

- SEC column (column: ZORBAX GF250, 9.4 mm ID 25 cm (from 4 to 4.5 µm));
- Mobile phase: 1 G1312A binary pump, 30 mM NaCl, 20 mM Na_2HPO_4, pH 7.0, flow of mobile phase: 1.0 $cm^3 min^{-1}$, temperature: 23 °C;
- Sample handling: G1367B autosampler, injection volume 10 µl;
- Detector: G1315B diode array UV detector, measurements performed at 210 and 254 nm.

2.11. Calibration of the hpsec system

Three protein standards with MW of 42.7 kDa, 67 kDa and 660 kDa were purchased from Sigma-Aldrich (USA). These macromolecules were used as standards with known molecular weights, assuming no chromatographic difference between these proteins and the respective soils samples. Column efficiency was measured using sodium azide (65.01 Da, Fluka Chemika, CH) and albumin from chicken egg white (42.7 kDa) dissolved in mobile phase. All chemicals and solvents used were HPLC or higher grade.

2.12. Sec standards and calibration curve

The data sets of retention time vs. known MWs of the chosen protein standards and azide were used to establish calibration curves for the HPSEC system. Calibration equation with R^2 > 0.96 between apparent molecular weight, AMW (in Da) and retention time, T_R (in seconds) is shown in **Eq. 3**:

$$AMW = -25.6 \times 10^3 \times T_R + 150.5 \times 10^3 \qquad \textbf{Eq. 3}$$

The calibration equation was used to calculate the apparent molecular weights of the fractionised DOC samples.

2.13. Ultraviolet absorbance ratio index

Absorbance at 254 nm is typical for aromatic groups with varying degree of activation (Korshin et al. 2009). Her et al. (2004) obtained an ultraviolet absorbance ratio index (URI) for water samples measuring at 210 and 254 nm (Abs_{210}/Abs_{254}) for estimating relative contributions of FAs, HAs and microbial biopolymers to varying organic fractions. URI values were found to be the lowest for HA, intermediate for FA and highest for proteins.

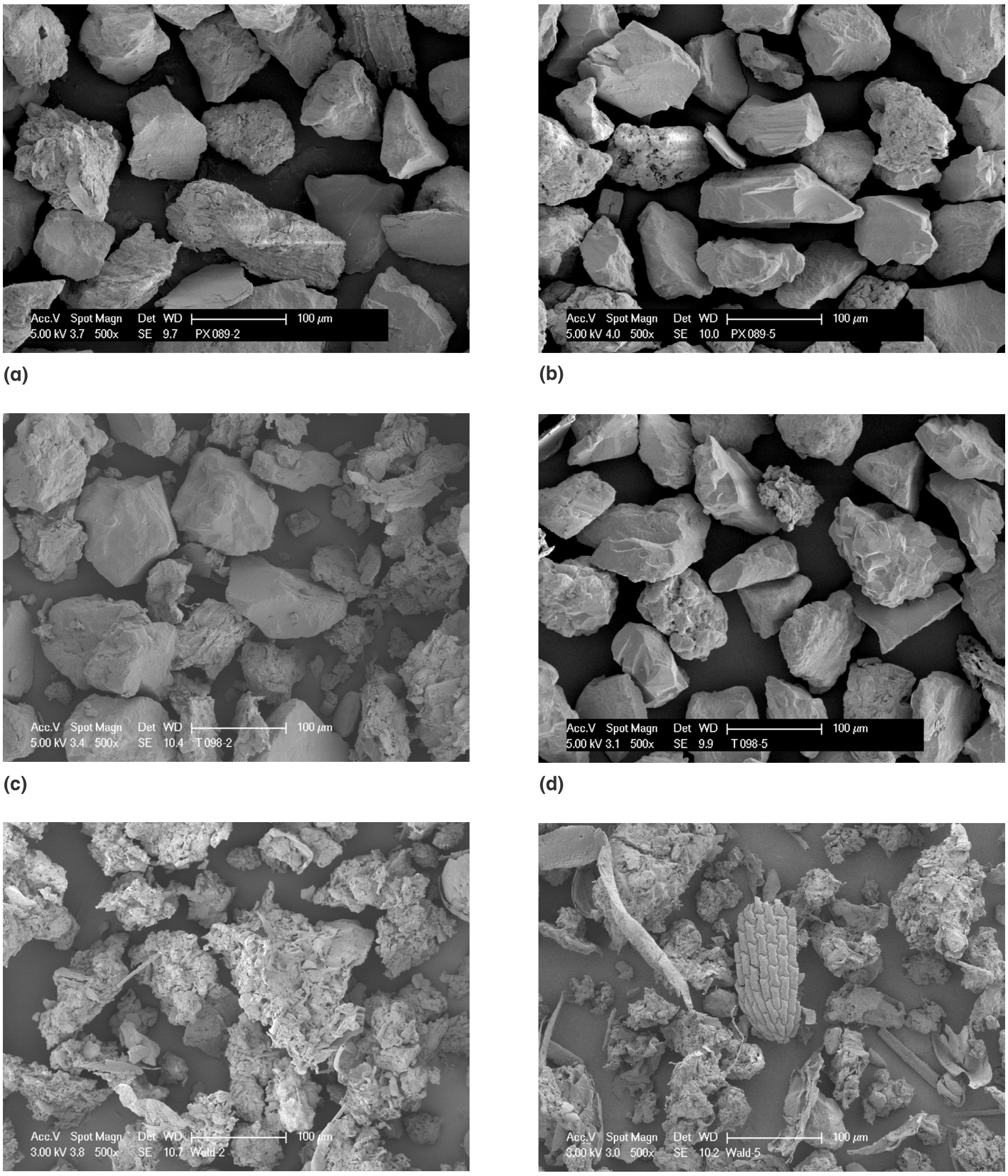
2.14. Statistics

Differences in DOC properties between sites were tested using an ANOVA procedure (SPSS for Windows 8.0), followed by the Duncan test ($P < 0.05$) to compare means.

3. Results and Discussion

3.1. SEM

For analysis of aggregation, microstructure changes after ultrasonic application of 2 µm (6.7 J cm^{-3}) and 5 µm (161 J cm^{-3}) were investigated based on SEM images. Exemplarily, six ESEM images at a magnification factor of 500 are shown in **Figures 3a-f**. SEM figures show a very high depth of focus, which is one benefit of this electron microscopy compared with optical microscopy. Another benefit is the possibility of very high magnification investigation that allows the study of



(a) **(b)** **(c)** **(d)** **(e)** **(f)**

Figure 3a-f: Scanning electron micrographs of different soils after application of different ultrasonic amplitudes: **(a)** Chernozem (2 µm); **(b)** Chernozem (5 µm); **(c)** Cambisol (2 µm); **(d)** Cambisol (5 µm); **(e)** Podzol (2 µm); **(f)** Podzol (5 µm).

hyphen or biofilms, for example. A shortcoming is the lack of colour information, and it is not possible, for example, to differentiate amorphous organic matter from amorphous humic substances.

A common problem with interpretation of ESEM results is the selection of an appropriate field of view over the specimens (Koliji et al. 2010). In this study, the comparison of approximately 90 close-ups of the specimens resulted in the conclusion that there was a significant difference in aggregation status, coating and presence of organic matter, particularly between the forest and the agricultural soils. The surface of the degraded Chernozem (**Figures 4a-b**) was smooth, indicating that these particles were either uncoated or had a very thin coating of organic matter. Aggregates were not evident. No great difference between **Figures 3a** and **3b** was visible. This indicates that particles can be disaggregated with ultrasonic dispersion at already low ultrasonic energies of 6.7 J cm^{-3}. The SEM images of the Cambisol fractions (**Figures 3c** and **3d**) revealed numerous small aggregates scattered among isolated mineral particles. Also, particles with organic coatings as well as particles, which were made of organic matter only, could be detected. These features were less frequent in **Figure 3d**, after sonication with a higher ultrasonic energy than in **Figure 3c**.

By contrast, the Podzol's surface (**Figures 3e** and **3f)** appeared rougher than the Cambisol and Chernozem specimens. The particles were aggregated in clusters, and non-decomposed or partially decomposed organic debris of possibly plant or animal origin could be clearly observed. This characterization applied notably for **Figure 3f** after sonication with 5 µm reflecting the high aggregate stability of this forest soil.

3.2. Dissolved organic carbon

Brandstetter et al. (1996) showed that there is a close correlation between DOC concentration in natural waters and UV absorbance at 254 nm. The non-UV absorbing DOC averaged 1 mg l^{-1} and it was suggested that this was due to saturated aliphatic compounds such as acetate or formate. This is reasonable since the focus of UV spectra interpretation is on the detection of conjugated systems. Ultraviolet absorbance is not an adequate detector for quantitative analysis of molecular weight estimation but it can be used for qualitative analysis (Her et al. 2002).

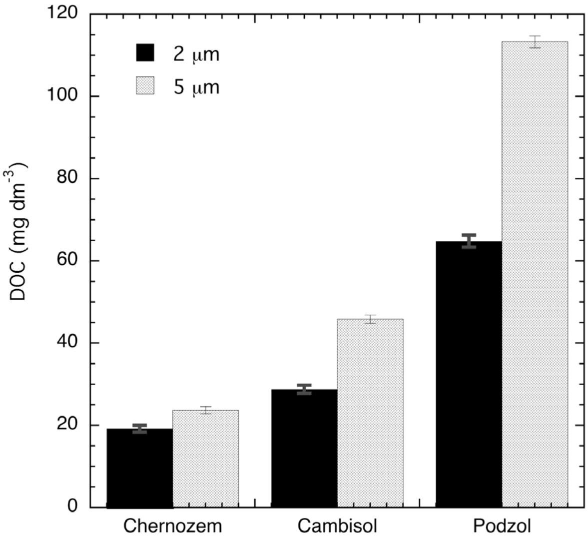


Figure 4. DOC (mg l^{-1}) release at 254 nm versus sonication amplitudes 5 µm (hatched bars) and 2 µm (black bars) for Chernozem, Cambisol and Podzol (mean and standard error).

Figure 4 shows the extinction rates at 254 nm with ultrasonic amplitudes of 2 µm and 5 µm, respectively, for water extracts from the three investigated soil classes. The Podzol had the highest DOC concentration (mean 65 ± 3.0 and 113 ± 3.8 mg l^{-1} respectively), as was expected from its higher soil organic matter content (average 50.7%) and C:N ratio (25.8; **Table 1**). In agricultural soils, concentrations were 29 ± 1.5 and 46 ± 2.1 mg l^{-1} for the Cambisol, and 19 ± 0.8 and 27 ± 1.5 mg l^{-1} for the Chernozem. Published data from agricultural and forest soils under a temperate climate showed DOC concentrations ranging from 15 to 50 mg l^{-1} (agriculture) and 50 to 150 mg l^{-1} (forest) which is in the range we found in our study (Blume et al. 2010). van den Berg et al. (2012) also determined a higher DOC concentration in Podzol than in Cambisol and Lundström et al. (2000) pointed to the importance of DOC for Podzol formation. Our results indicate, as expected, that more organic substances are released when ultrasonic energy is increased. The increased releases of DOC after sonication of aggregated soil indicate, according to (Mueller et al. 2012), the detachment of formerly protected SOM. **Table 2** shows the relation between SOM and DOC. At an ultrasonic vibration amplitude of 5 µm, the difference between the agricultural soils is not significant, whereas with 2 µm there is a significant difference between all soil types detectable with the Cambisol displaying a higher SOM/DOC ratio than the Chernozem. This is in line with soil genesis where the Cambisol has stored more carbon than the eroded Chernozem in East Austria.

Dissolved organic carbon concentration was larger in forest than in agricultural soils and lowered in the sequence Podzol > Cambisol > Chernozem. This result is in line with Chantigny (2003) who measured a decrease of DOM concentrations in order forest soils > grassland soils > arable soils.

Table 2. Relation of soil organic matter (SOM) and dissolved organic carbon (DOC) according to sonication with vibration amplitudes of 2 µm and 5 µm for Cambisol, Chernozem and Podzol (including standard deviation STD)

Vibration amplitude		2 µm	5 µm	2 µm	5 µm
SOIL TYPE	SOM [w/w%]	DOC [mg kg^{-1}]	DOC [mg kg^{-1}]	SOM/DOC	
Cambisol	6.0	290	460	207	130
STD	±0.1	±0.2	±1.3	±0.5	±6.7
Chernozem	3.3	190	270	174	122
STD	±0.3	±0.3	±1.9	±0.9	±5.8
Podzol	50.7	650	1130	780	449
STD	±0.4	±0.4	±3.1	±1.1	±7.7

3.3. URI

A higher density of UV-absorbing functional groups corresponds to a higher absorption at 210 nm, producing higher URI (UVA_{210}/UVA_{254}). URI values for Podzol are 1.6, for Cambisol 1.4 and Chernozem 0.9. All three soils show low URI values which indicate high aromaticity of the specimens. The URI values imply a low proportion of functional groups with high proportions of aromatic rings (Her et al. 2004).

3.4. HPSEC

The HPSEC system detected molecules in the range of 1300-1600 Da for the Cambisol, 1500-5400 Da for the Chernozem and 1700-10.4 kDa for the Podzol (**Table 3**). This is in line with Jones et al. (2012) who filtered soil specimens and found most of the UV-detectable DOC (87%) present in a high MW form > 1 kDa. With the ultrasonic energies applied and UV detection, it was possible to detect molecules in the range of alkanes, low molecular weight humic acids, derivate, lipids and lignin (**Figure 2**).

The system detected the highest AMW for the Podzol specimens after sonication with a low vibration amplitude of 2 µm compared to 5 µm. Published data show that large molecules and organo-metal complexes are more abundant in DOM from forest floors (Strobel et al. 1999), whereas agricultural soils contain a greater proportion of smaller molecules, such as fulvic and hydrophilic acids, carbohydrates and amino acids. At 2 µm, the first Podzol peak showed the highest AMW (10 422 Da). At 5 µm, the first peak is slightly bigger and higher but AMW is lower (9731 Da). The second eluted peak showed higher AMW at 5 µm (2186 Da) compared to 1726 Da at 2 µm. The two peaks' signatures are more similar at 5 µm than 2 µm.

The agricultural samples also showed molecules of higher AMW after an ultrasonic treatment with vibration amplitude of 2 µm and the Chernozem showed molecules with a higher AMW than the Cambisol. On the other hand the peaks' respective height and area were smaller at 2 µm compared to 5 µm. The Chernozem's AMW difference after sonication of 2 µm and 5 µm is significant whereas the Cambisol's AMW differed in AMW and peakedness just slightly (**Figure 5**).

It was shown that SOM structures contain a range of chromophores with varying molar absorptivities (Matilainen et al. 2011) which means that the AMW calculated may not involve all of DOC compounds present. Her et al. (2002) called the estimation of molecular weights with UV absorbance detection inaccurate because UV absorbance only detects limited components (mostly π bonded molecules) of natural organic matter. In addition, water-soluble organic matter has a high bioavailability due to its small molecular size (Wagai and Sollins 2002) but when it is physically uncomplexed or occluded

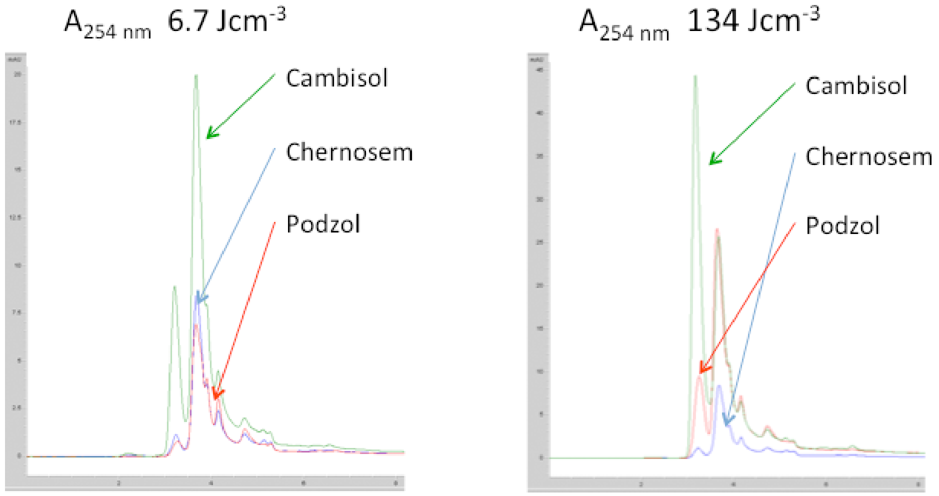


Figure 5. Chromatograms at 254 nm after ultrasonic dispersion pre-treatment with absorbed specific energy of 6.7 J cm^{-3} (left figure) and 134 J cm^{-3} (right figure), respectively. Molecular sizes are shown in Table 3.

in macro- and microaggregates it seems to differ in lability and accessibility for microbial decomposition (Kaiser et al. 2010).

To increase the selectivity for molecules of DOC in a wide range the use of more specific detector systems is needed such as fluorescence detectors. For example, the use of refractometer index detectors would help to detect more details of molecules with non-UV absorbance. Detectors working in the near infrared range give more specific molecular information and mass sensitive detectors have the highest selectivity but separation techniques reach their limits.

Table 3. Detected peaks at DAD Absorbance at 254 nm; Apparent Molecular weights (AMW) are approximations since the selection of proper standards are based mostly on hypothesised structures of DOC. Amplitude: Ultrasonic pre-treatment with vibration amplitudes 2 µm and 5 µm

	Amplitude [µm]	Retention-time [min]	Area	Height	Width	Area [%]	Symmetry	AMW [Da]
Cambisol	2	5.8	217.9	23.6	0.14	100	0.88	1547
	5	5.8	282.5	29.7	0.16	100	0.94	1342
Chernozem	2	5.7	21.2	4.50E-01	0.79	100	1.67	5358
	5	5.8	200.3	21.7	0.14	100	0.86	1572
Podzol	2	5.5	14.4	5.90E-01	0.30	7.66	3.81	10422
	5	5.8	173	19.4	0.15	92.33	0.87	1726
	2	5.5	31.4	1.3	0.41	56.01	3.74	9731
	5	5.8	24.6	3.3	0.12	43.98	0.83	2186

3.5. Influence of sonication and application of a conceptual model

Process-orientated conceptual models demonstrate that pedogenetic conditions and soil management influence the importance of stabilisation mechanisms of SOM pools (von Lützow et al. 2008). The soil forming factors of the here-investigated soils showed differences in the release of DOC and molecular weight distribution. The composition of DOC reflects the selective preservation and re-synthesis of transport and transformation processes of DOM in these soils. The high molecular weight fraction of the Podzol of 10422 DA is related to the source of the accumulated and weakly humified plant litter material. The driving force of podzolic dynamics reflects the production and leaching of DOM. Intercalated OM and interactions with mineral surfaces are shown in **Figures 3 e-f**, **Table 3**). The leaching of DOM in Podzols under these low pH conditions is a principal alternative to soil respiration as a loss route for carbon. Tillage increased the importance of organo-mineral interactions for OM stabilisation in Ap-horizons with high microbial activity and carbon turnover for the Chernozem and Cambisol (5358 DA; 1547 DA).

von Lützow et al. (2008) developed a carbon model, based on Sollins et al. (1996), which integrates three groups of mechanisms stabilizing DOM pools in soils: i) primary and secondary recalcitrance, ii) spatial inaccessibility and iii) organo-mineral interactions. Sonication treatments aim to characterize surface structures and the functional architecture of soil aggregates. Different sonication energy levels reach different carbon pools depending on the physical and chemical structure of soil aggregates. Low sonication energy levels with vibration amplitude 2 µm (i.e. 6.7 J cm^{-3}) can break down the surface of soil aggregates related to the active carbon pool which contains plant residues and exudates, microbial and faunal biomass and residues. Dominant processes of these surfaces are selective preservation and re-synthesis. The turnover time of this pool was estimated to be 1-10 years (von Lützow et al. 2008). The molecule mass size fraction at 2 µm was bigger than the mass size fraction after an ultrasonic application with 5 µm vibration amplitude i.e. 161 J cm^{-3}. This higher energy level reached surfaces of soil aggregates, which belong to the intermediate carbon pool. This compartment is generated by soil processes and is dominated by decomposed residues, microbial/faunal biomass and residues. The mechanisms responsible are spatial inaccessibility due to occlusion in aggregates and organo-mineral interactions and complexation of OM with metal ions. The turnover time of these specific aggregate surfaces is postulated to be between 10 and 100 years.

Various authors have determined a critical dispersive energy range. Zhu et al. (2009) determined one in the range of 100-600 J g^{-1} for relatively stable soils, Raine and So (1993) reported values < 40 J cm^{-3}. Mueller et al. (2012) studied leptic Cambisols and lithic Eutrodepts and used an energy input of 200 J cm^{-3} which led to complete disruption of macroaggregates but left the major proportion of microaggregates intact. Chenu and Plante (2006) concluded that true primary organo-mineral complexes must be regarded as conceptual entities because the energy required to fully disperse the clay-sized fraction are likely to render the results meaningless in natural systems. With the energy applied in this study we cannot access the passive carbon pool in soil aggregate fractions (Schomakers et al. 2011a). These aggregate fractions are much smaller and their dissolved organic matter contains humic polymers, pseudo-macromolecules, intercalated OM and OM in clay microstructures with high molecular size.

4. Conclusions

Soil aggregation separates substrate and decomposer and is a major mechanism for SOM stabilization (von Lützow et al. 2006). Sonication and breakdown of soil aggregates is a promising tool to release DOC under energy-controlled conditions from the surfaces of soil aggregates and reveal chemical and morphological differences between soil types. The HPSEC system was a useful technique to characterize DOM by providing some interesting information on AMW. However, due to the complexity of SOM and inferential uncertainty of the results interpretation, combined analytical methods and separation methods are needed to better characterize the physicochemical composition of SOM pools and thus the functional significance of its components.

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