

Extractable lipid contents and colour in particle-size separates and bulk arable soils

G. L. B. WIESENBERG^a, L. SCHWARK^a & M. W. I. SCHMIDT^b

^aDepartment of Geology and Mineralogy, University of Cologne, Zùlpicher Str. 49a, 50674 Cologne, Germany, and ^bDepartment of Geography, University of Zürich, Winterthurerstr. 190, 8057 Zürich, Switzerland

Summary

Chemical alteration of plant biomass to soil organic matter is often accompanied by characteristic trends, e.g. with decreasing particle size and increasing depth organic carbon and nitrogen concentrations and stable carbon isotope values ($\delta^{13}\text{C}$) often increase. In agricultural soils, systematic studies of soil organic carbon (SOC) distribution in bulk soils and particle-size separates of depth profiles are scarce. In this study, three soil profiles from one site with different monoculture crops were analysed for organic carbon and nitrogen concentrations, stable carbon isotopes, bulk extractable lipids, and soil colour. In contrast to most previous observations, stable carbon isotope values were constant over soil depth and within particle-size separates, probably as a result of little biomass input due to the harvesting techniques applied and the presence of fossil carbon. Bulk extractable lipids contributed 1–10% to the total SOC. Significantly more lipids could be extracted from rye- than from maize-derived SOC. Lipid yields normalized to soil mass increased with decreasing particle size and decreased with depth. When normalized to organic carbon concentration, sand-size fractions had the largest lipid yields. Soil colour, expressed as Munsell values, was lightest in sand- and silt-size separates. A cross-plot of Munsell values and their SOC concentrations revealed characteristic, non-overlapping areas for each particle-size class and the bulk soils. Clay-size separates and bulk soils were almost identical in Munsell values, although for clay-size separates SOC concentrations were much larger than for bulk soils. Thus, the SOC-rich clay-size separates exerted the dominant influence on the colour of the bulk soils. Determination of colour and extractable lipid contents could be useful additional parameters for soil characterization.

Introduction

Soil lipids constitute a major part of the organic components of fresh plant materials and soils (e.g. Gregorich *et al.*, 1996). Lipids play an important role in the incorporation of plant material into soil organic carbon (SOC) (Kögel-Knabner, 2002), and contain specific biomarkers used for taxonomic classifications (Gleixner *et al.*, 2001). SOC contents in separated size fractions show several distinct trends in the order sand > silt > clay and with increasing depth (Christensen, 1996). Concentrations of organic carbon and nitrogen increase, while C:N ratios decrease, thus leaving silt- and clay-associated SOC rich in nitrogen. In the same direction, individual chemical compound classes including polysaccharides, carbohydrates and lignin show increasing degrees of chemical alteration in arable soils (e.g. Guggenberger *et al.*, 1995; Amelung *et al.*, 1997). Investigations of soil lipids, however, are scarce for particle-size separates and profiles of agricultural

soils, although several studies exist on forest soils (e.g. Marseille *et al.*, 1999). Most molecular studies of agricultural soils have focused on the distribution patterns of individual lipid fractions of ploughed horizons, e.g. *n*-alkanes (Lichtfouse *et al.*, 1994, 1997, 1998; Wiesenberg *et al.*, 2004a,b), or bulk lipid extracts (e.g. van Bergen *et al.*, 1998). Bulk lipid extract yields of arable soils have only rarely been discussed, e.g. by Amblès *et al.* (1994). Similarly, distribution patterns of lipid fractions or single lipids have rarely been analysed in particle-size separates of ploughed horizons from arable soils (Cayet & Lichtfouse, 2001), and never for deeper soil horizons.

For stable carbon isotopes ($\delta^{13}\text{C}$), several trends have previously been observed within soil profiles and particle-size separates. With increasing soil depth, SOC $\delta^{13}\text{C}$ values increase within most soils by 1–2‰ (e.g. Desjardins *et al.*, 1994). These trends have been observed when overlying vegetation uses the C_3 photosynthetic pathway (e.g. most plants of temperate climates in forests, grassland and agriculture; Desjardins *et al.*, 1994; Balesdent & Mariotti, 1996; Boutton *et al.*, 1998). However, the opposite trend has also been observed, usually when C_4 plants with large $\delta^{13}\text{C}$ values

Correspondence: G. L. B. Wiesenberg. E-mail: guido.wiesenberg@uni-koeln.de

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replaced C₃ vegetation (e.g. Balesdent & Mariotti, 1996) or when a mixed culture of C₃ and C₄ plants had grown on soils which were exclusively labelled by C₃ biomass, e.g. after clearing of a virgin forest (Krull & Skjemstad, 2003). In particle-size separates, $\delta^{13}\text{C}$ values often show a systematic increase from coarse to fine separates (e.g. Balesdent & Mariotti, 1996; Bird & Pousai, 1997; Boutton *et al.*, 1998). Most soil carbon isotope analyses for particle-size separates have been carried out on soils under pasture and forest (e.g. Desjardins *et al.*, 1994; Bird & Pousai, 1997; Bird *et al.*, 2003), but, to the best of our knowledge, only one study has focused on arable soil (Cayet & Lichtfouse, 2001), and there have been no studies on the isotopic composition of agricultural subsoils.

Soil colour depends strongly on mineral assemblages and soil texture, whereas soil lightness can also reflect SOC content (Schulze *et al.*, 1993; Konen *et al.*, 2003). To the best of our knowledge, there have been no studies concerning soil colour measurements of particle-size fractions within soil profiles.

Here, a set of three very similar arable soils from one site was studied, where vegetation has always been C₃ vegetation, i.e. rye monoculture since 1878, which was divided into several smaller plots in 1961. While a part of the plot was kept under rye monoculture, on another part rye was replaced by silage-maize cropping, and on another part of the plot grain-maize cropping was introduced in 2001.

The main objectives of this study were to answer the following questions:

- How are masses of extractable soil lipids distributed within the soil profiles and between particle-size separates?
- Do soil lipid yields follow similar decomposition trends with soil depth, and particle size, as other chemical compound classes (e.g. lignin, polysaccharides)?
- Are extractable lipids soil colouring agents?
- Does soil colour (lightness) reflect the carbon contents in soil profiles and particle-size separates?

Materials and methods

Samples

The soil profiles originated from the long-term agricultural 'Eternal Rye' trial in Halle/Saale, Germany (Stumpe *et al.*, 1990; Merbach *et al.*, 1999, 2000; Flessa *et al.*, 2000), classified as a Haplic Phaeozem (FAO–UNESCO, 1994). The 'Eternal Rye' plot was established in 1878 and has been cropped continuously with rye (*Secale cereale* L.) until present. One part of the plot was converted to continuous silage-maize (*Zea mays* L.) cropping in 1961. Since 1961 all plots with different croppings have been separated from each other and the surrounding plots by fallow land, which was a part of the formerly rye-cropped 'Eternal Rye' plot. Additionally, grain-maize cropping was introduced on fallow land in 2001 directly adjacent to the silage-maize cropped soil. The different soil textures

of maize- and rye-cropped plots have been described previously (Stumpe *et al.*, 1990).

During silage-maize cropping, most of the above-ground biomass is removed, leaving only the lowermost parts of stems (up to 15 cm height) and all of the root biomass on the field. During grain-maize cropping only cobs are harvested, leaving most of the above-ground biomass on the field. Rye and silage-maize cropped soils with mineral nitrogen, phosphorus and potassium (NPK) fertilization were sampled in March 2001. The grain-maize cropped soil was sampled in September 2001. From each plot, three to five soil profiles were taken using an Eijkelkamp core sampler with an inner diameter of 6.7 cm. Depth of the plough horizon (Axp) varied between 22 and 28 cm for all plots. The underlying Axh horizon reached a depth of approximately 45 cm and the Bv horizons were sampled down to 70 cm depth. Subsamples from each horizon were combined and homogenized. Texture and horizon depths appeared very similar for all profiles. Contrastingly, soil colour showed some variations especially between the Axh horizons of the three profiles. Generally, soil colour changed in the profiles with depth from grey (Munsell 10YR 5/1) in the Axp horizons to pale reddish grey (4YR 5/2) in the Bv horizons. While the soil colour of the Axp and Axh horizons of both maize-cropped soil profiles was uniform (10YR 5/1), the colour of the Axh horizon from the rye-cropped soil (4YR 6/2) was similar to that of the Bv horizon.

The 'Eternal Rye' trial is situated in an urban area and is thus prone to input from fossil sources, especially, until the 1990s, from the brown coal mines around Halle, and from nearby railway lines which surround the experimental trial at a distance of 100 m to 400 m, and along which brown coal was transported. We found, upon macroscopic inspection, brick, charcoal and brown coal fragments in all soils.

Soil samples were stored in a freezer (−27°C) until further treatment. After freeze-drying (Steris Lyovac GT-2), aggregates of bulk soil samples were carefully crushed with a pestle and mortar, dry-sieved over a 2-mm aperture sieve and macroscopically visible plant debris and root fragments were removed.

Particle-size separation

Three particle-size separates (clay, silt, sand) were isolated from the Axp, Axh and Bv horizons of the rye and maize plots from the 'Eternal Rye' trial, by combining wet sieving and sedimentation after sequential ultrasound dispersion. The ultrasonic energy of a Branson ultrasonic titanium probe with a diameter of 12.7 mm was calibrated and 60 J ml^{−1} of suspension were applied to destroy macro-aggregates (63–2000 µm) (Ludwig *et al.*, 2003). Sand (63–2000 µm) was separated from the suspension by wet sieving. Micro- and meso-aggregates were disrupted by application of an ultrasonic energy of 440 J ml^{−1}. Coarse silt (20–63 µm) was removed

from the suspension by wet sieving. The remaining clay and silt were separated by gravity sedimentation, and coarse clay (0.45–2 µm) was recovered by filtration (cellulose nitrate, 0.45 µm; Schmidt & Kögel-Knabner, 2002). The suspension containing soluble components, fine and medium clay (< 0.45 µm) was not recovered. All separates were oven-dried at 40°C. Particle-size separation was repeated at least 10 times for each soil horizon and separates were combined and homogenized after drying.

Elemental analysis

All bulk soil samples and particle-size separates were analysed for organic carbon and nitrogen concentrations (OC, TN, Table 1). The organic carbon concentration of dried samples was determined, after removal of carbonate with HCl (10% v/v), by means of a Leco CS-225 analyser, and nitrogen was determined with a Heraeus Vario EL analyser.

Isotopic analyses

Carbon dioxide was obtained by combustion of the samples in a continuous flow sample preparation system (Robo Prep-CN), and the stable carbon isotope composition of the purified CO₂ was analysed with a Europa Scientific Tracer Mass Classic mass spectrometer. Carbon isotope compositions are expressed as:

$$\delta^{13}\text{C} [\text{‰}] = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3, \quad (1)$$

where R_{sample} is the ratio of ¹³C to ¹²C in the sample and R_{standard} is the ratio of the two stable carbon isotopes in the international Vienna Pee Dee Belemnite standard (V-PDB), with $R_{\text{standard}} = 0.0112372$.

Lipid extraction

The complete extraction procedure for soil lipids has been previously described in detail (Wiesenberg *et al.*, 2004a). Briefly, one to five stainless-steel extraction vessels for each sample were filled with approximately 30 g of dried soil. Free lipids were extracted with dichloromethane/methanol (93/7; v/v) at 5×10^6 Pa at a temperature of 75°C in an accelerated solvent extractor (Dionex ASE 200). The heating phase was 5 minutes and static extraction time was 20 minutes. Extraction was then repeated under identical conditions except a higher temperature was used (140°C) and both extracts of several vessels and replicates from the same sample were combined.

Colour spectrometry

Aliquots of all samples before and after lipid extraction, except for the silt-size separate of the Axh horizon from the rye-cropped soil before extraction, were crushed in a mortar with a pestle until aggregates were destroyed. Non-destructive

colour measurements were performed in triplicate using a Minolta CM-2002 colour photo spectrometer, which determines the colour of areas with a diameter of 1 cm. Colours were measured in the L*a*b*-System, with L* as the lightness of a colour, a* as the red-green proportion and b* as the yellow-blue proportion. The results were converted to the Munsell system (Schulze *et al.*, 1993).

Statistical analysis

We tested Munsell values of 35 samples before and after lipid extraction for statistically significant differences. Differences were not normally distributed and thus we selected the Wilcoxon rank sum test, a non-parametric test comparing differences for paired samples by calculating differences (Δ) of absolute values as

$$\Delta x = x_{\text{before}} - x_{\text{after}}, \quad (2)$$

where x stands for the Munsell value before and after lipid extraction.

Results and discussion

Particle-size distribution

Particle-size separation recovered most of the bulk material (> 99%) for all samples (Table 1). Sand dominated all soils and horizons (64–75 mass %), with maize-cropped soils containing 3–8% less sand than the rye-cropped soil, consistent with previous results (e.g. Ludwig *et al.*, 2003). In the maize-cropped soils, sand contents were uniform throughout the profile, but increased with depth in the rye-cropped soil. For all maize-cropped soil profiles, clay contents were least in the ploughed horizons.

Carbon and nitrogen concentrations and distributions

Carbon and nitrogen concentrations (Table 1) were typical for Haplic Phaeozems (Batjes, 1996). In the maize-cropped soils, carbon and nitrogen concentrations were only marginally greater than in the rye-cropped soil. In bulk soils, carbon and nitrogen concentrations decreased systematically with increasing soil depth (A_{xp} > A_{xh} > B_v), and consistently decreased with increasing particle size (clay > silt > sand). The recoveries of carbon and nitrogen concentrations in the particle-size separates (Table 1) generally were large ($\geq 92\%$), when compared with bulk soils. Proportions of carbon and nitrogen stored in the clay-size separates increased consistently with soil depth, at the expense of silt- and sand-size separates. The contributions of the clay-size separates to total organic carbon were less than observed for other arable soils of similar textures (Christensen, 1996). The smaller contributions of the clay-size separates were most likely due to larger contents of carbon-enriched brown coal, charcoal and soot particles in the

Table 1 Soil properties

Horizon	Size separate / μm	Mass \pm SE ^a /% of bulk ^b	Organic C		$\delta^{13}\text{C}$ /‰ ^d	N		Lipid extract yield	
			/g kg ⁻¹ c	% of bulk ^b		/g kg ⁻¹ c	% of bulk ^b	/g kg ⁻¹ c	/g kg ⁻¹ OC ^e
<i>Rye-cropped soil</i>									
Axp	Sand 63–2000	70.3 \pm 0.1	3.0	18	-24.5	0.1	9	0.03	83
	Silt 2–63	19.9 \pm 0.2	21.7	36	-25.6	0.4	23	1.37	63
Axx	Clay 0.45–2	9.2 \pm 0.1	47.5	37	-25.7	4.0	52	1.56	33
	% recovery	99.4		92			84		
Axx	Bulk soil		11.8		-25.7	0.7		0.55	47
	Sand 63–2000	73.3 \pm 0.2	0.6	13	-23.8	< 0.1	10	0.20	313
Axx	Silt 2–63	17.6 \pm 0.1	4.5	22	-25.7	0.4	16	0.44	97
	Clay 0.45–2	8.6 \pm 0.1	26.1	62	-25.4	3.7	74	1.44	55
Bv	% recovery	99.5		96			100		
	Bulk soil		3.6		-25.0	0.4		0.35	96
Bv	Sand 63–2000	75.1 \pm 0.1	0.3	11	-25.3	< 0.1	11	0.01	33
	Silt 2–63	16.4 \pm 0.0	2.0	15	-25.8	0.3	16	0.05	23
Bv	Clay 0.45–2	7.6 \pm 0.1	21.5	74	-24.8	3.1	68	0.37	17
	% recovery	99.1		99			95		
Bv	Bulk soil		2.2		-25.1	0.3		0.02	9
	Sand 63–2000	67.0 \pm 0.2	3.1	18	-22.9	0.1	9	0.11	37
Axp	Silt 2–63	22.0 \pm 0.2	19.5	37	-24.2	0.9	25	0.60	31
	Clay 0.45–2	10.5 \pm 0.1	41.8	38	-23.9	4.6	58	1.41	34
Axp	% recovery	99.5		93			93		
	Bulk soil		11.6		-24.0	0.8		0.38	33
Axx	Sand 63–2000	67.0 \pm 0.6	0.5	5	-25.0	< 0.1	5	0.03	53
	Silt 2–63	20.8 \pm 0.3	5.6	16	-24.9	0.6	16	0.23	41
Axx	Clay 0.45–2	12.1 \pm 0.1	34.9	56	-25.1	4.4	72	0.81	23
	% recovery	99.9		76			93		
Axx	Bulk soil		7.5		-25.1	0.8		0.13	17

Table 1 Continued

Horizon	Size separate / μm	Mass \pm SE ^a /% of bulk ^b	Organic C		$\delta^{13}\text{C}$ / ^d	N		Lipid extract yield		
			/g kg ⁻¹ c	/% of bulk ^b		/g kg ⁻¹ c	/% of bulk ^b	/g kg ⁻¹ c	/g kg ⁻¹ OC ^e	
Bv	Sand 63–2000	66.6 \pm 0.5	0.4	5	-20.1	< 0.1	6	- ^f	0.03	86
	Silt 2–63	20.8 \pm 0.0	3.7	14	-24.8	0.4	13	9	0.08	21
	Clay 0.45–2	12.5 \pm 0.0	34.7	75	-25.4	4.0	76	9	0.57	17
	% recovery	99.9		93			95			
	Bulk soil		5.8		-24.8	0.7		9	0.09	15
<i>Grain-maize cropped soil</i>										
Axp	Sand 63–2000	64.3 \pm 0.2	3.3	17	-24.3	0.1	7	26	0.08	23
	Silt 2–63	23.7 \pm 0.3	18.9	36	-25.2	0.9	20	20	0.62	33
	Clay 0.45–2	11.3 \pm 0.1	48.6	45	-25.1	4.9	49	10	1.71	35
	% recovery	99.3		98			76			
	Bulk soil		12.3		-24.6	1.1		11	0.35	28
Axh	Sand 63–2000	63.7 \pm 0.3	0.8	6	-24.2	< 0.1	4	- ^f	0.02	29
	Silt 2–63	22.4 \pm 0.3	6.9	20	-25.7	0.5	15	14	0.14	20
	Clay 0.45–2	13.0 \pm 0.2	39.3	67	-25.3	4.3	76	9	0.89	23
	% recovery	99.1		98			96			
	Bulk soil		7.7		-25.7	0.7		10	0.13	17
Bv	Sand 63–2000	65.0 \pm 0.4	0.3	5	-26.0	< 0.1	5	- ^f	0.01	34
	Silt 2–63	21.5 \pm 0.2	2.5	15	-26.3	0.3	14	9	0.05	19
	Clay 0.45–2	12.7 \pm 0.2	22.9	80	-25.5	2.8	78	9	0.39	17
	% recovery	99.2		100			96			
	Bulk soil		3.6		-25.8	0.4		8	0.04	11

^aStandard error of the mean. Number of analyses varied between 10 and 13.^bParticle-size separates obtained after ultrasonic dispersion.^cExpressed as g kg⁻¹ of size separate.^dAs ‰, with reference to V-PDB.^eExpressed as g kg⁻¹ organic carbon of size separate or bulk soil.^fNitrogen concentrations too small to calculate.

coarse size separates, leading to greater relative contributions of sand- and silt-size separates to total carbon.

C:N ratios were similar for all the soils (Table 1), and slightly smaller for the grain-maize cropped soil, most likely because this plot was fallow land, which did not receive fresh biomass for four decades before conversion to grain-maize cropping in the year of sampling. The C:N ratios of the bulk soils decreased from the Axp horizon (11–14) down to the Bv horizon (6–8) – a typical trend for many agricultural Phaeozems (Christensen, 1996; Marseille *et al.*, 1999). C:N ratios in the Axp horizons were large for sand-size (26–28) and silt-size (20–21) separates – typical of plant fragments, whereas in the clay-size separates C:N ratios were small (7–9) – typical of microbial biomass (Cayet & Lichtfouse, 2001; Gleixner *et al.*, 2001). Nitrogen concentrations were too small to calculate reliable C:N ratios for sand-size separates of Axx and Bv horizons.

Stable carbon isotope contents ($\delta^{13}\text{C}$)

In the plots studied here, the stable carbon isotope ratios of the plant biomass differed due to their different photosynthetic pathways. Rye (C_3 pathway) was ^{13}C -depleted (-31%) compared with maize (-13% ; C_4 pathway: Wiesenberg *et al.*, 2004b). Thus, due to the different cropping histories of the individual plots, isotope ratios of bulk soils and particle-size separates did not reveal the uniform trends that were found for carbon and nitrogen concentrations (Table 1).

Two opposite isotope trends were observed with soil depth for bulk soils. Rye-cropped soil became ^{13}C -enriched below the ploughed horizon, typical for C_3 -cropped soils (e.g. Desjardins *et al.*, 1994). In contrast, bulk soils of the ploughed (Axp) horizons of both maize-cropped soils were ^{13}C -enriched (-24.0% to -24.6%), relative to the subsoil horizons (Axx and Bv) (-24.8% to -25.8%). These decreasing $\delta^{13}\text{C}$ values with depth were due to less C_4 biomass input in the deeper soil horizons (e.g. Balesdent & Mariotti, 1996). For the subsoil horizons (Axx and Bv) of the rye and grain-maize plots, similar $\delta^{13}\text{C}$ values were expected because the grain-maize plot was cropped with rye, then (1961) converted to fallow land and converted to grain-maize in the year (2001) of sampling. However, the grain-maize cropped soil was ^{13}C -depleted by 0.7% compared with the rye-cropped soil. The lesser value could either reflect small-scale differences of carbon isotope properties or could result from the fact that for 40 years no new root biomass entered the fallow soil or that microorganisms decomposed the existing SOC, leaving SOC with smaller $\delta^{13}\text{C}$ values.

Silt- and clay-size separates had relatively uniform $\delta^{13}\text{C}$ values, differing only up to 1.5% , whereas ratios for sand-size separates were more divergent (Table 1). Bird & Pousai (1997) reported similar trends for particle-size separates of forest soils. The greatest $\delta^{13}\text{C}$ values (-20.1%) occurred in the sand-size separate of a subsoil (Bv) of the silage-maize plot. This probably indicates the presence of fragments from maize

roots developed in the year of sampling. Generally, particle-size separates of individual horizons followed the pattern that silt-size separates were more $\delta^{13}\text{C}$ -depleted than sand- and clay-size separates (except for the Axx horizon of the silage-maize cropped soil).

In summary, the data set did not support the postulated general trend for a greater ^{13}C enrichment with increasing depth or decreasing size, as discussed in the introduction. One obvious explanation, which holds for the maize plots, is that the input of isotopically different parent biomass changed the isotopic pattern. However, Krull & Skjemstad (2003) and Bird *et al.* (2003) summarized the major potential processes which can alter the $\delta^{13}\text{C}$ values of soil organic carbon with depth and decreasing particle size: (i) microbial degradation would result in a ^{13}C depletion with depth or decreasing particle size, and (ii) kinetic fractionation processes, translocation of soluble compounds or the Suess effect would yield the opposite trend. Thus, in the soils studied here, several competing processes could have produced the observed result. Additionally, a detailed explanation for the observed isotope contents is complicated by the fact that the soils investigated here are situated in a highly industrialized area and thus contain lignite dust and charred fragments from mining and combustion (Wiesenberg *et al.*, 2004b). In the ploughed horizons, lignite particles present in the sand-size separates were rich in organic carbon (480 g kg^{-1}), and $\delta^{13}\text{C}$ values (-25.4%) were between rye (-30%) and maize biomass (-13%) (Wiesenberg *et al.*, 2004b). Thus, we believe that the presence of fossil organic matter contributed to the elemental and isotopic ratios, yielding values different from corresponding soils without fossil carbon.

Lipid concentration and distribution

Lipids are operationally defined by their extractability in non-polar solvents. Lipid yields from bulk soils and particle-size separates (Table 1) were normalized to soil mass (g lipids kg^{-1} dry soil), and to total organic carbon (g lipids kg^{-1} OC). Where concentrations of extractable lipids were small, especially in coarse particle-size separates and subsoil horizons, weighing errors were larger than for those separates with larger lipid yields. Thus, the calculated contributions of lipids to bulk soil mass and to bulk organic carbon (OC) may have larger uncertainties. During lipid extraction a part of coarse aggregates was broken down into smaller aggregates as a result of the treatment with a high temperature and pressure. Thus, a significant effect of the lipid extraction procedure on the particle-size distribution can be excluded.

With increasing particle size and increasing soil depth, extractable lipid concentrations decreased systematically (Table 1). Concentrations of extractable lipids followed the same trend already observed for organic carbon concentrations. The yields were similar to free lipid extract yields observed by Amblès *et al.* (1994) and Lichtfouse *et al.* (1995) for ploughed horizons of arable soils. Lipid yields increased linearly with increasing SOC concentrations, both in bulk soils

and in particle-size separates (Table 1, Figure 1). Lipid yields for the rye-cropped soil were larger than for the silage-maize cropped soil (Figure 1). These results are consistent with previous observations, in that lipid extract yields of rye plant parts are approximately twice as great as the corresponding parts of maize plants, although bulk organic carbon concentrations are similar (Wiesenberg, 2004). Consequently, yields from the grain-maize cropped soil (a mixture of pre-1961 rye biomass and one year of maize cultivation) were intermediate between rye and silage-maize cropped soils.

The distribution of the extractable lipids within the soil profile and between the particle-size separates became clear when lipid yields were normalized to organic carbon concentrations. In the bulk soils, extractable lipids contributed 1–10% to the organic carbon present in the bulk sample (9–96 g lipids kg⁻¹ OC). Lipid contributions decreased consistently with depth for all soil profiles investigated (Axp > Axh > Bv) except for the Axh horizon of the rye-cropped soil, which showed unusually great lipid contributions that we cannot explain. Also in the particle-size separates, there seemed to be a general decrease of yields from large contributions from sand-size (3–31% of the total organic

carbon) to clay-size (2%) separates (sand > silt > clay), with few exceptions from this general trend (grain-maize Axp and Axh horizons). Thus, it seems that from sand- to clay-size separates the proportions of extractable lipids (e.g. relatively fresh plant material) decreased whereas proportions of less extractable, more altered, biomass increased, although the small number of samples investigated could limit general conclusions. The observed trend of increasing decomposition of soil organic matter with soil depth and decreasing particle size is consistent with previous observations based on bulk chemical parameters, and individual compound classes, including lignin (e.g. Christensen, 1996; Gleixner *et al.*, 2001).

A mass balance of particle-size separates revealed that the sum of the lipid yields of the particle-size separates was larger than the lipid yields of the bulk soils. This lack of agreement might result from two effects. First, lipid extract yields were determined gravimetrically, and especially for samples with small OC and lipid contents (i.e. coarse-size separates from deeper soil horizons) weighing errors could have added up compared with smaller errors for the bulk soil extracts. Second, particle-size separation might have made lipids extractable that remained locked inside aggregates in the bulk soils.

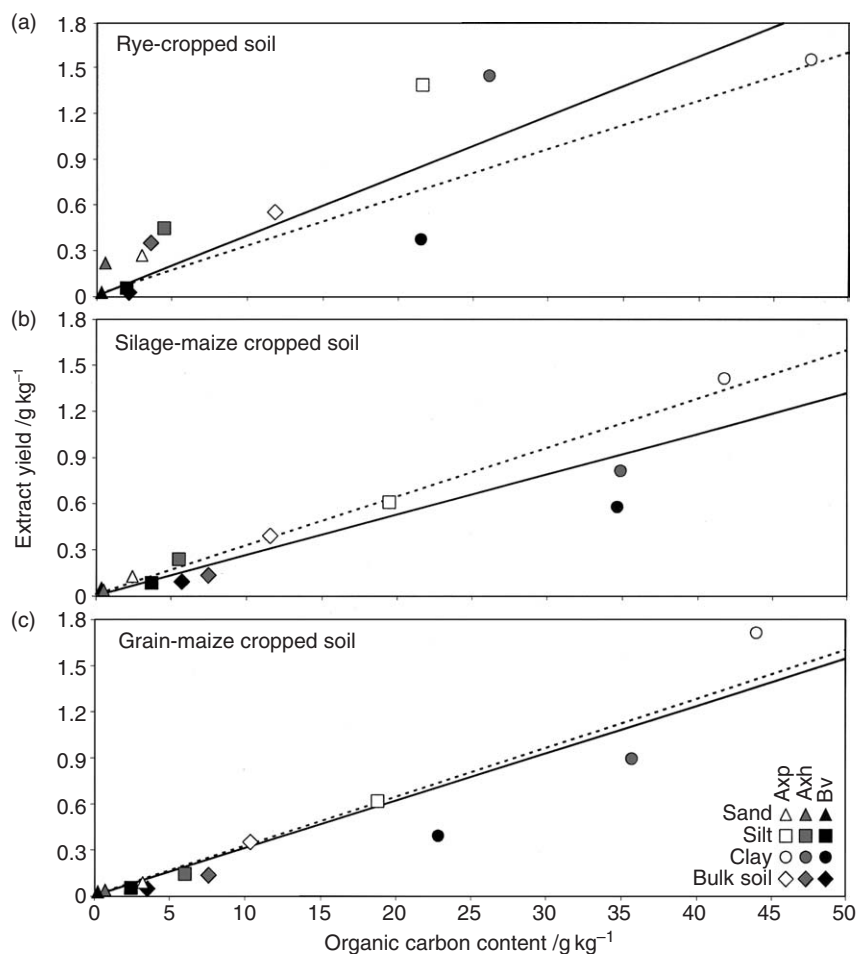


Figure 1 Extract yields of total lipids vs. total organic carbon concentrations for particle-size separates and bulk soils of (a) rye, (b) silage-maize, and (c) grain-maize cropped soils. Correlations (black lines) for (a) rye-cropped soil: $y = 0.039x$ ($r = 0.88$), (b) silage-maize cropped soil: $y = 0.026x$ ($r = 0.93$), and (c) grain-maize cropped soil: $y = 0.031x$ ($r = 0.95$). The overall correlation (dashed line): $y = 0.032x$ ($r = 0.89$).

Colour spectrometry

Spectrometric measurements confirmed the visual observations that bulk soils and particle-size separates become significantly lighter ($P = 0.0001$) after lipid extraction (Figure 2). Munsell values for bulk soils and clay-size separates were very similar and always increased after extraction. This suggests that lipids extracted from clay-size separates were important soil colouring constituents. Furthermore, a uniform trend with particle size was observable for all soils. In ploughed (Axp) horizons, values decreased systematically with decreasing

particle size, whereas in subsoil horizons (Axx, Bv) values peaked in silt-size separates.

To test if the presence of organic matter can explain the different Munsell values, both parameters were combined in one graph (Figure 3). No obvious relation between these two parameters could be found for the whole sample set. Instead, bulk soils and the three particle-size separates plotted in distinct, individual areas. Although bulk soils and clay-size separates both had the least lightness, they differed in organic carbon concentrations. We could find a clear correlation between smaller organic carbon contents and greater lightness

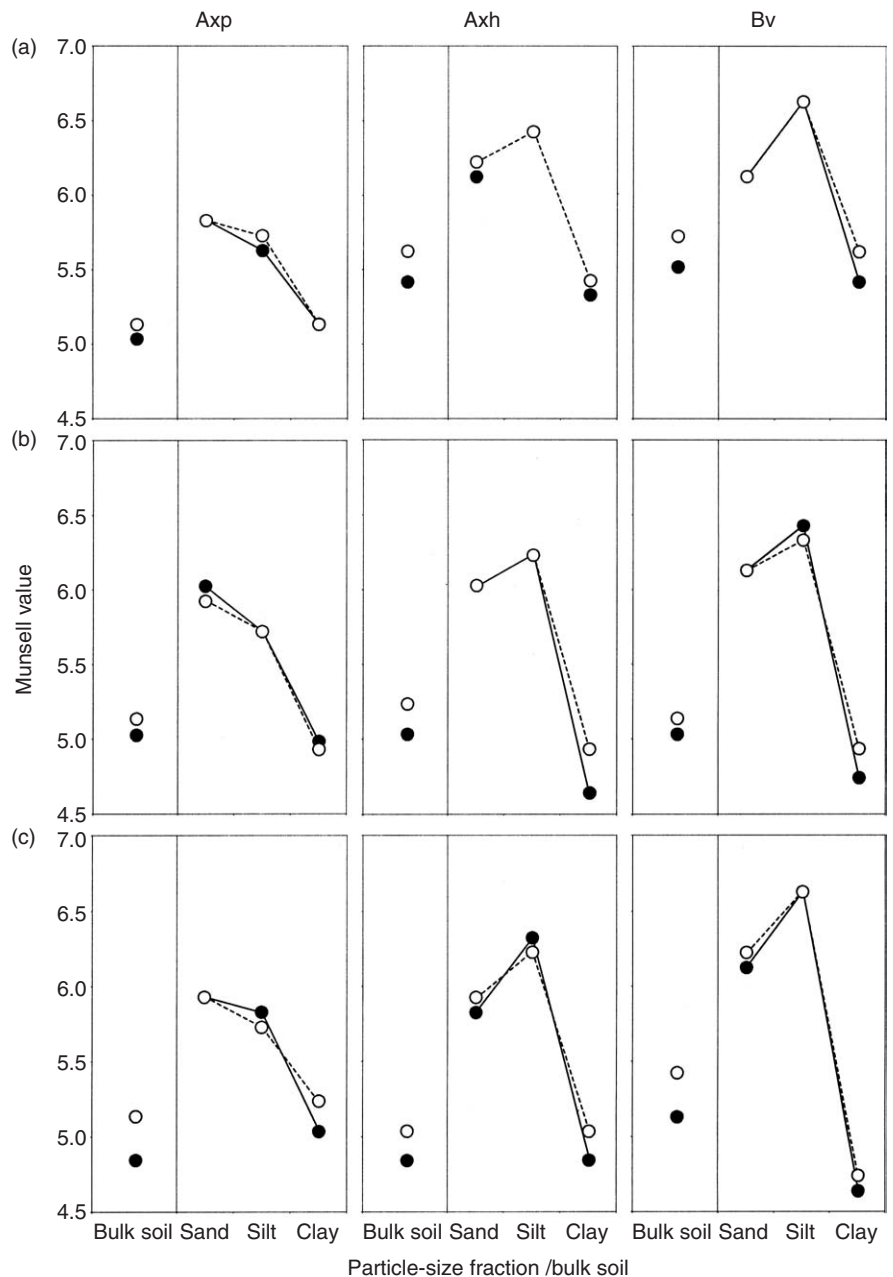


Figure 2 Munsell values of particle-size separates and bulk soils for the three soil horizons (Axp, Axh, Bv), before (●) and after (○) extraction: (a) rye-cropped soil, (b) silage-maize cropped soil, and (c) grain-maize cropped soil. The silt-size separate of the rye-cropped Axh horizon before extraction was not measured, because not enough material was available for colour measurements. Standard errors were always smaller than 0.05 for Munsell values and thus smaller than the symbol size.

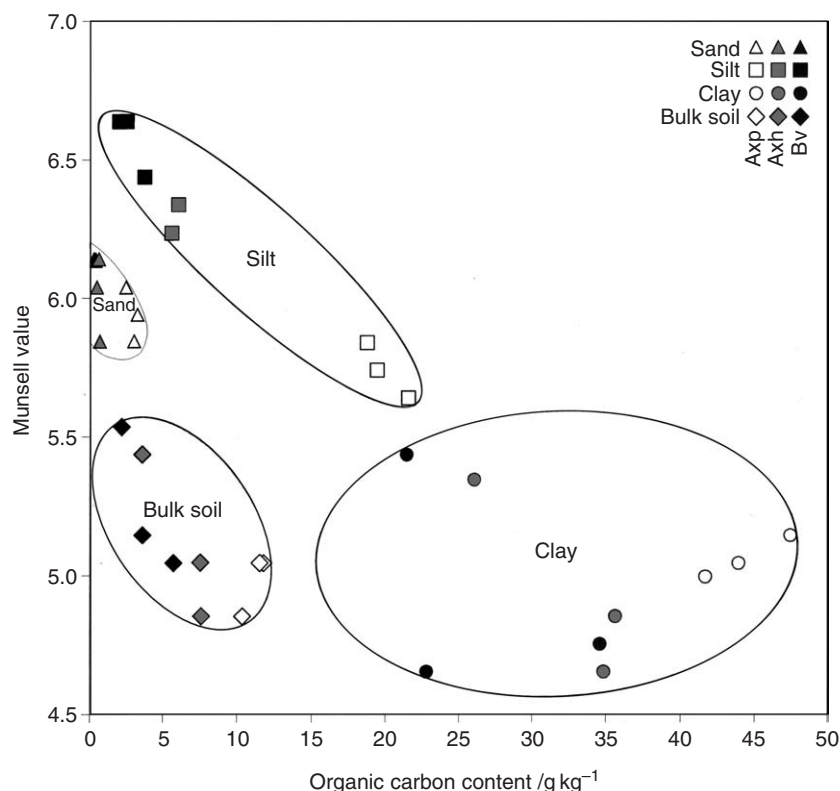


Figure 3 Munsell values vs. total organic carbon concentrations of particle-size separates and bulk soils of three soils with different croppings. Standard deviations were always smaller than 0.05 and thus smaller than the symbol size.

only for silt-size separates, but the correlation of both parameters was weaker for bulk soils and sand-size separates.

Another interesting fact was that sand-size separates for all soils, and bulk soils as well as particle-size separates for all ploughed horizons, plotted close together for each sample type, whereas subsoil horizons and other particle-size separates scattered more. One explanation for the uniform properties of the ploughed horizons could be the constant homogenization due to ploughing. Variation in lightness and carbon contents in subsoil horizons could be explained by lateral variations in soil properties like texture, mineralogy and organic carbon contents.

Conclusions

The three soil profiles originated from the same long-term agricultural experimental station, where vegetation was the main difference between profiles. Some small-scale lateral heterogeneities could be observed when comparing soil textures, carbon and nitrogen concentration and distributions, and extractable lipid concentrations between soil profiles. However, lipid extract yields and colour determinations of bulk soils and particle-size fractions of arable soils showed:

- 1 Soil lipid yields (although mostly < 10% of the bulk organic matter) differed with the species of the parent biomass (rye biomass > maize biomass).

- 2 Yields of bulk extractable lipids normalized to mass increased with decreasing depth and particle size, and when

normalized to organic carbon, sand-size fractions showed the largest lipid yields. These data suggested that the degree of chemical alteration increased for (i) bulk soils with depth and (ii) particle-size separates with decreasing particle size.

- 3 After lipid extraction, bulk soils and size separates became significantly lighter, demonstrating that extractable soil lipids were an important soil colouring agent.

- 4 Soil colour of bulk soils and of clay-size separates was similar, whereas sand- and silt-size separates were lighter. Thus, clay-size separates dominated soil colour.

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