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Organic Geochemistry 34 (2003) 1591–1600
Organic Geochemistry
www.elsevier.com/locate/orggeochem

Stabilisation of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation

Karin Eusterhues[a,*], Cornelia Rumpel[a,1], Markus Kleber[b], Ingrid Kögel-Knabner[a]

[a] *Lehrstuhl für Bodenkunde, Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt, Technische Universität München, D-85350 Freising-Weihenstephan, Germany*

[b] *Institut für Bodenkunde und Pflanzenernährung, Martin-Luther-Universität Halle-Wittenberg, D-06099 Halle (Saale), Germany*

Received 6 January 2003; accepted 28 August 2003
(returned to author for revision 3 April 2003)

Abstract

Soil organic matter is known to contain a stable fraction with an old radiocarbon age. Size and stabilisation processes leading to the formation of this old soil carbon pool are still unclear. Our study aims to differentiate old organic matter from young and labile carbon compounds in two acid forest soils (dystric cambisol, haplic podzol). To identify such fractions soil samples were exposed to oxidation with $Na_2S_2O_8$ and to dissolution by hydrofluoric acid (HF). A negative correlation between ^{14}C activity and carbon release after dissolution of the mineral matrix by HF indicates a strong association of stabilised carbon compounds with the mineral phase. A negative correlation between the ^{14}C activity and the relative proportion of carbon resistant to oxidation by $Na_2S_2O_8$ shows that young carbon is removed preferentially by this treatment. The fraction remaining after oxidation represents a certain stabilised, long residence time carbon pool. This old fraction comprises between 1 and 30% of the total soil organic carbon in the surface horizons, but reaches up to 80% in the sub-surface horizons. Old OC is mainly stabilised by organo-mineral associations with clay minerals and/or iron oxides, whereas intercalation in clay minerals was not found to be important.

1. Introduction

The global carbon cycle is strongly dependent on the turnover of soil organic matter (SOM) (Schlesinger, 1991; Lal, 2001). Part of the organic carbon in soils is easily mineralised, whereas another carbon pool is known to degrade slowly at timescales from hundreds to thousands of years (Oades, 1995). Three key processes are proposed to explain the formation of this passive or long-residence-time SOM fraction: (i) chemical recalcitrance, i.e. stabilisation due to the structural properties of the organic matter, (ii) inclusion of organic matter into aggregates or micropores, leading to physical protection of organic matter from microbial attack and (iii) interaction of carbon compounds with soil minerals (Sollins et al., 1996; Baldock and Skjemstad, 2000; Anderson and Paul, 1984). The contribution and relative importance of these stabilisation mechanisms in different soils are not fully understood.

Physical as well as chemical techniques have been used to recover old carbon components (Skjemstad et al., 1996; Falloon et al., 1998; Falloon and Smith, 2000; Christensen, 2001). The chemical approaches include,

* Corresponding author. Tel.: +49-8161-714195; fax: +49-8161-714466.
E-mail address: eusterhues@wzw.tum.de (K. Eusterhues).
[1] Present address: CNRS, Laboratoire de Biogéochimie des Milieux Continentaux, Centre INRA Versailles-Grignon, F-78820 Thiverval-Grignon, France.

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doi:10.1016/j.orggeochem.2003.08.007

among others, acid hydrolysis, defining a resistant non-hydrolysable carbon fraction (Trumbore and Zheng, 1996; Leavitt et al., 1996; Paul et al., 1997; Augris et al., 1998; Derenne and Largeau, 2001). Acid hydrolysis was shown to separate resistant carbon fractions 1300–1500 years older than that in bulk soils (Paul et al., 1997). In contrast, Balesdent (1996) did not find a relationship between age of organic matter in agricultural soils and chemical fractions obtained by acid hydrolysis, wet oxidation with H_2O_2, thermic oxidation, pyrolysis or alkaline extraction. Poirier et al. (2000, 2002) isolated a refractory carbon fraction by successive chemical treatments including extraction of lipids, fulvic and humic acids, followed by base and acid hydrolysis and by demineralisation using HF/HCl. They found that even in a very old soil with a ^{14}C age of 8000 years BP only a small carbon fraction of about 5% was chemically resistant.

Acid hydrolysis preferentially removes proteinaceous- and polysaccharide-type OM and isolates organic matter that is enriched in alkyl and aromatic C components (Augris et al., 1998; Kiem et al., 2000). Both protein and polysaccharide components in soils may originate from rather young plant remains, but also from relatively old, stable C fractions (Kiem et al., 2002; Knicker and Hatcher, 2001). On the other hand, recently-deposited lignin and waxes may resist acid hydrolysis. Thus, the acid hydrolysis approach is able to concentrate an old C pool, but may produce biased results, due to its non-specific nature.

We propose two other chemical procedures to identify and separate old and/or stable organic matter from labile carbon fractions. To obtain organic matter fractions of different chemical stability, bulk soil samples were exposed to oxidation by disodium peroxodisulfate ($Na_2S_2O_8$) and to mineral dissolution by hydrofluoric acid (HF). The oxidation approach is based on the idea that chemical oxidation will imitate the natural oxidative processes of microorganisms and therefore enrich stable organic compounds. Disodium peroxodisulfate has been reported to be an effective oxidant for organic matter in clay sediments, which preserves clay minerals and Fe oxides (Meier and Menegatti, 1997; Menegatti et al., 1999).

Through demineralisation we intended to release organic matter associated with soil minerals, because this fraction is thought to be part of the stabilised carbon pool in soils. The stable fraction of a soil will include both old and stabilised organic matter, as well as chemically recalcitrant, but possibly young, organic species, like, e.g., recently deposited black carbon. However, we expected the stable fraction to contain a significantly higher amount of old organic carbon than the labile fraction and vice versa.

Samples were collected from surface and sub-surface horizons from two acid forest soils, which contain organic matter with a large range of mean residence times (Rumpel et al., 2002). The residual organic carbon, resistant to the above treatments, was related to the radiocarbon activity as an indicator of SOM age. Special attention was paid to clay minerals and Fe-oxyhydroxides (Fe_{DCB}, Fe_{OX}) in order to investigate their relevance for SOM stabilisation.

2. Material

The soil profiles are located within the Fichtelgebirge (Waldstein) and the Steigerwald (Steinkreuz) in Northern Bavaria, Germany. The climate is humid, with annual rainfall ranging from 700–800 mm at Steinkreuz and from 950 to 1250 mm at Waldstein. The soil at Waldstein, under Norway spruce (*Picea abies (Karst.) L.*) forest, is classified as a highly acidic haplic podzol (FAO, 1998) and developed from a granitic solifluction layer. The forest floor is moder- to mor-type and about 8–10 cm thick. The soil at Steinkreuz is covered by a mixed stand of European beech (*Fagus sylvatica L.*) and European oak (*Quercus robur L.*). It is classified as a dystric cambisol (FAO, 1998) and developed from a triassic sandstone with clayey and silty intersections. The forest floor is moder with a thickness of 3 cm. Selected properties of the horizons of both soils are shown in Table 1. Both soils have a sandy texture.

3. Methods

3.1. Sample pre-treatment

Bulk samples of the mineral soil were taken by horizon from soil pits, air dried at room temperature and passed through a 2 mm sieve. Visible root fragments were removed by hand-picking. For chemical analysis an aliquot of the soil was ground in a ball mill for 10 min.

3.2. Determination of clay content

Clay content was determined by particle size fractionation, which was intended to provide a complete recovery of all fractions. In this paper only the amounts of the total clay fractions (<2 μm) are reported. Complete dispersion of the aggregates was achieved in two steps by using an ultrasonic probe (Amelung and Zech, 1999). The macroaggregates of all samples were disrupted using an energy input of 60 kJ ml^{-1}. The fractions >20 μm were removed by wet sieving. The amount of energy needed to disperse soil particles in aggregates <20 μm was calibrated against standard particle size analysis (Schmidt et al., 1999), using a sedigraph. The results of the calibration procedure showed that 440 kJ ml^{-1} had to be applied for dispersion of the

Table 1
Selected properties of soils: pH_{CaCl}, CEC_{pH7}—cation exchange capacity at pH 7, total organic carbon concentration, ^{14}C activity in percent modern carbon, ^{14}C age in years before 1950, Fe_{OX}—oxalate extractable Fe, Fe_{DCB}—dithionite-citrate-bicarbonate extractable Fe and clay content

Horizon	Depth (cm)	pH_{CaCl_2}	CEC_{pH7} ($mmol_c\ kg^{-1}$)	Total OC ($g\ kg^{-1}$)	^{14}C activity (pMC)	^{14}C age (yrs BP)	Fe_{OX} ($g\ kg^{-1}$)	Fe_{DCB} ($g\ kg^{-1}$)	Clay ($g\ kg^{-1}$)
Steinkreuz (dystric cambisol)									
A	0–5	3.2	365	82.6	112.3	Modern*	1.3	1.4	100
Bw1	5–24	3.7	142	9.8	101.3	Modern*	1.6	2.7	88
Bw2	24–50	4.0	105	3.0	92.1	655±25	0.5	1.5	90
Bw3	50–80	4.0	129	1.4	80.9	1700±30	0.6	1.1	132
3C	85–115	4.1	210	1.1	80.6	1758±56	0.3	0.7	137
4C1	115–140	4.3	208	0.5	76.3	2165±30	0.1	0.4	78
Waldstein (haplic podzol)									
EA	0–10	2.9	248	38.1	93.6	525±30	0.6	1.7	147
Bh	10–12	3.2	887	92.8	98.5	120±25	3.3	24.5	143
Bs	12–30	3.9	663	52.0	91.1	745±40	10.1	16.4	130
Bw	30–55	4.3	279	7.7	82.2	1570±25	1.6	6.4	122
C1	55–70	4.2	125	1.7	62.0	3840±70	0.6	3.7	102
C2	70–80	4.1	99	1.9	62.0	3840±70	0.6	4.5	107

A, Bh, and Bs horizons, 250 kJ ml^{-1} for the Bw horizons, and 100 kJ ml^{-1} for the C horizons. Clay sized particles (<2 μm) were separated by sedimentation and then recovered by pressure filtration through 0.22 μm polyvinylidene fluoride (Durapore) membranes. Clay-particle-size separates were then freeze-dried and weighed.

3.3. OC content, Fe and Al extraction

Total C content was measured with a CN analyser (Vario EL, Elementar GmbH, Hanau, Germany) on (i) bulk samples, (ii) after removal of the mineral phase with HF and (iii) after oxidation of organic matter with $Na_2S_2O_8$. Inorganic C is not present in any sample, thus the total C analysed corresponds to organic C.

Aluminium and iron from poorly crystalline oxides and bound to organic matter were extracted from bulk soil samples with 0.2 M NH_4 oxalate (pH 3) according to Schwertmann (1964). Total iron oxides were estimated by the dithionite–citrate–bicarbonate (DCB) method (Mehra and Jackson, 1960). Aluminium and Fe in the extracts were measured with inductively coupled plasma optical emission spectroscopy (ICP-OES).

3.4. HF-treatment (demineralisation)

The HF treatment was performed by adding 10 ml of 10% HF to 1 g of ground bulk sample. The suspension was shaken for 2 h at room temperature, centrifuged and the supernatant removed. This procedure was repeated five times and then the sediment was washed five times with deionised H_2O to remove salts and residual HF. Afterwards, the samples were freeze-dried and weighed. The concentration of OC was determined (see above) and the amount of OC lost upon this treatment was obtained by mass balance calculation.

3.5. Wet oxidation

Oxidation of the organic matter was achieved by $Na_2S_2O_8$ (Meier and Menegatti, 1997; Menegatti et al., 1999). Briefly, 500 mg of bulk soil were dispersed in 250 ml bidistilled water and allowed to react with 20 g $Na_2S_2O_8$, buffered with 22 g $NaHCO_3$ for 48 h at 80 °C. The reaction time of 2 h as proposed by Meier and Menegatti (1997) was found to be too short. Therefore reaction times of 2, 3 and 4 d were tested. Generally, no additional decrease in the bulk soil carbon content was observed after 2 d. However, to obtain the same effect, one organic-rich sample needed to be treated twice (Steinkreuz A), whereas even three repetitions did not lead to lower concentrations for the Waldstein Bh, a sample with a similarly high TOC concentration. After oxidation the samples were washed twice with bidistilled water, freeze-dried and analysed for the remaining OC concentration.

3.6. Radiocarbon analysis

The ^{14}C activity was determined on ground bulk soil samples at the accelerator mass spectrometer (AMS)

facility of the Leibniz laboratory, Kiel, Germany (Nadeau et al., 1998). The measured ^{14}C activity was corrected for isotope fractionation and is given in percent modern carbon (pMC). Radiocarbon ages in years before 1950 (years BP) were calculated after Stuiver and Polach (1977).

3.7. Clay mineral assemblage

Phyllosilicate clay minerals were identified using X-ray diffraction on an orientated specimen of the clay (<2 μm) fractions. We used a Siemens/Bruker AXS D5005 instrument with CuK_α-radiation (40 kV; 30 mA). Sample pre-treatment for mineralogical analysis included removal of organic matter (30% H_2O_2) and iron oxides according to Tributh and Lagaly (1986). After pre-treatment, the clay suspension was divided, saturated with Mg or K and freeze-dried. Mg- and K-saturated, orientated clay mineral aggregates were prepared using porous ceramic tiles; 100 mg of clay were resuspended in 4 ml of distilled water and deposited on rectangular tiles (4×5 cm; Diapor G30; Schumacher Umwelt- und Trenntechnik GmbH, Crailsheim, Germany) of 8 mm thickness. Particle-size segregation effects were avoided by pre-drying the tiles at 250 °C to ensure rapid ingestion (less than 1 min) of the suspension liquid into the tile. The following treatments were prepared: Mg^{2+}-air dry; Mg^{2+}- glycerol; K^+ air dry and K^+ heated to 550 °C. Clay mineral identification was carried out according to Whitton and Churchman (1987). Peak areas were determined with the software WinFit! V1.2 (Krumm, 1999). Estimates of clay mineral abundance were obtained by multiplying peak areas with empirical mineral intensity factors as suggested by Gjems (1967) and Laves and Jähn (1972).

4. Results and discussion

4.1. Radiocarbon dating

Radiocarbon activities and the calculated radiocarbon ages of the soils are given in Table 1. Radiocarbon age increases with increasing soil depth in both soils, consistent with work of Rumpel et al. (2002) and Kaiser et al. (2002). The top soil at the Steinkreuz site shows the highest ^{14}C activity of 112 pMC, close to the recent atmospheric isotopic composition (Levin and Kromer, 1997). An old carbon fraction, if present, seems to be completely masked by a high input of young plant material. The lowest value of 62 pMC is observed in the Waldstein C1 and C2 horizons. This corresponds to a ^{14}C age of 3840±70 years BP. The EA horizon is relatively old compared to other surface horizons because the podzolisation process led to selective removal of young carbon compounds, which are translocated to the Bh horizon. Old radiocarbon ages in lower soil horizons have been explained by a lower input of young organic material and/or translocation of old organic carbon by dissolved organic matter (Schiff et al., 1997; Kaiser and Zech, 1999).

4.2. HF treatment

Table 2 gives the percentage of OC solubilised after dissolution of the mineral phase with 10% HF. Only 10 and 19% of the total OC are released in the A and EA horizons, but values increase with soil depth up to 92 and 84%, respectively. Carbon loss after HF treatment is reported to be in the range from 10 to 30% of total OC for surface horizons, plant litter or compost (Skjemstad et al., 1994; Schmidt et al., 1997; Mathers et al., 2002). Dai and Johnson (1999) treated organic horizons as well as surface and sub-surface horizons with HF and observed an increasing OC loss with increasing soil depth. While they found an OC loss of 12–38% in the Oa horizons, 47–56% of total OC was released by HF in the lower Bs2 horizons. This is in general accordance with our data, showing high carbon loss due to HF treatment for sub-soil material.

HF reacts with silicates by forming soluble fluoride complexes. It is widely used to demineralise refractory material like coal and kerogen (e.g. Durand and Nicaise, 1980), or to enrich soil and sediment samples in OC for NMR measurements by removing mineral matter, especially paramagnetic substances (Preston and Newman, 1992; Skjemstad et al., 1994; Schmidt et al., 1997; Gélinas et al., 2001; Dai and Johnson, 1999). Schmidt et al. (1997) observed little compositional change in SOM on treatment with HF and recommend the technique as a routine chemical pre-treatment. However, other authors report changes in the organic matter composition. Preferential removal of *O*-alkyl C structures and carboxyl C groups was observed by Dai and Johnson (1999), who further mention the possibility of chemical alteration of either the extracted or the residual organic matter. As pointed out by Gélinas et al. (2001), a large fraction of chemically or biologically labile organic matter may be adsorbed on, and protected by, the mineral matrix. Dissolution of the minerals by HF treatment will lead to a release of this organic carbon into solution.

A preliminary study on mineral-free litter layers of different degrees of decomposition showed that carbon loss due to the treatment was in the range of 5–10%. This can partly be explained by leaching of water soluble compounds, but may mainly be due to the handling procedure. The ^{13}C CPMAS NMR spectra from HF-treated and untreated samples were almost identical (data not shown). Thus, we assume that the HF treatment only

Table 2
Carbon loss due to HF treatment, OC fraction resistant to oxidation by $Na_2S_2O_8$ and carbon balance for these OC fractions. Carbon storage values taken from Rumpel et al. (2002)

Horizon	Depth (cm)	HF-soluble OC (% of total OC)	OC not removed by $Na_2S_2O_8$ (% of total OC)	HF-soluble OC (g kg^{-1})	OC not removed by $Na_2S_2O_8$ (g kg^{-1})	OC storage (kg m^{-2})	Storage of HF- soluble OC (kg m^{-2})	Storage of OC not removed by $Na_2S_2O_2$ (kg m^{-2})
Steinkreuz (dystric cambisol)								
A	0–5	10	0.7	8.26	0.58	4.29	0.43	0.03
Bw1	5–24	54	7.0	5.29	0.69	2.41	1.30	0.17
Bw2	24–50	55	18.3	1.65	0.55	0.79	0.43	0.14
Bw3	50–80	58	67.1	0.81	0.94	0.4	0.23	0.27
3C	85–115	65	83.6	0.72	0.92	0.14	0.09	0.12
4C1	115–140	92	82.0	0.46	0.41	0.07	0.06	0.06
Waldstein (haplic podzol)								
EA	0–10	19	2.3	7.24	0.88	2.9	0.55	0.07
Bh	10–12	45	2.2	41.76	2.00	1.04	0.47	0.02
Bs	12–30	61	2.1	31.72	1.08	5.47	3.34	0.11
Bw	30–55	70	8.8	5.39	0.68	2.09	1.46	0.18
C1	55–70	84	27.6	1.43	0.47	0.25	0.21	0.07
C2	70–80	84	28.9	1.60	0.55	0.19	0.16	0.06

dissolves soil minerals and mineral-associated organic matter, while the non-associated soil organic matter remains nearly unaffected.

Figure 1 shows the relationship between ^{14}C activity and carbon loss after treatment with hydrofluoric acid. In both soils a high proportion of old carbon, as indicated by a low ^{14}C activity, is associated with high carbon loss after HF treatment. These results suggest a strong association of stable carbon compounds with the mineral phase. Carbon compounds which are adsorbed on mineral surfaces are dissolved. This HF-soluble fraction contains high amounts of old, stabilised organic matter and the observed negative correlation supports the assumption that the association of OM with soil minerals leads to a sequestration of OC in soils. However, the HF-soluble fraction does not necessarily include the whole stable C-pool, nor does this interpretation exclude additional stabilisation mechanisms such as chemical recalcitrance. Stabilisation due to physical protection by aggregates seems unlikely owing to the sandy texture of the soils. Because of the strong relationship observed between the ^{14}C activity and the carbon loss after HF, we suggest that interaction with the mineral phase is the most important process for carbon stabilisation in these soils.

If the HF-soluble fraction is taken as the OM fraction stabilised through interaction with the mineral matrix, only a small proportion (about 10–20%) of the total OM is stabilised by this process in the top soils. In the sub-surface horizons up to 92% of the OM is associated with soil minerals (Fig. 1; Table 2). It is interesting to note that although the *relative* proportions of HF-soluble OC increase with depth, the *total* amounts of this fraction (g kg^{-1} dry weight) are highest in the upper soil horizons. If storage values (kg m^{-2}) are calculated, highest carbon storage for the HF-soluble fraction is found in the Steinkreuz Bw1 and the Waldstein Bs and Bw horizons (Table 2).

For the sub-soils, correlations between the clay content and the HF-soluble OC fraction indicate interactions

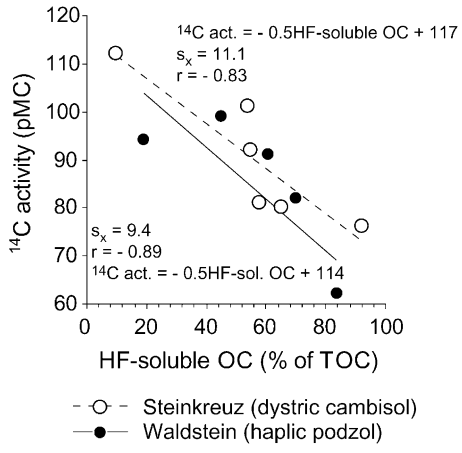

Fig. 1. ^{14}C-activity in % modern carbon (pMC) vs. the HF-soluble OC fraction; correlation coefficients (r) and standard errors (s_x) are given for the Waldstein and the Steinkreuz site separately.

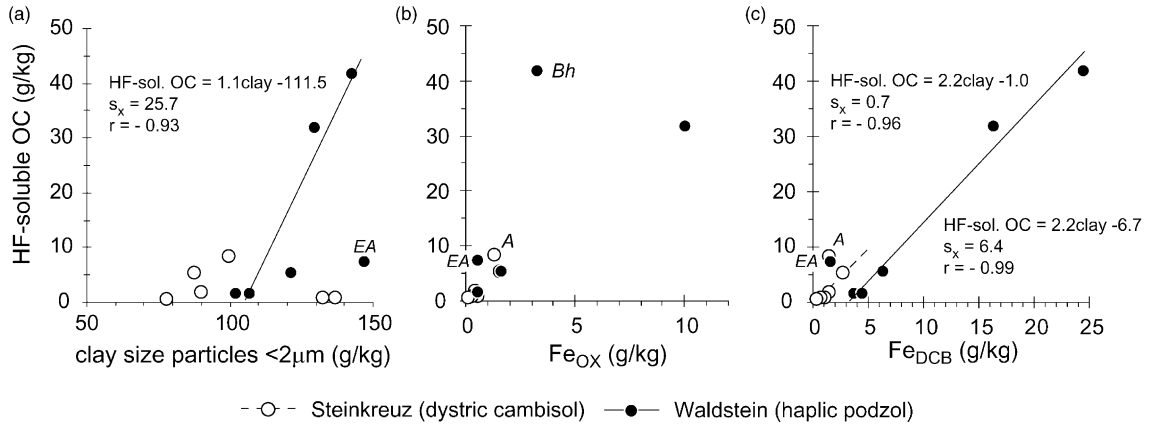

Fig. 2. Relationships between concentration of HF-soluble fraction and clay content (a), amount of poorly ordered Fe oxyhydroxides (Fe_{OX}) (b) and content of total Fe oxides (Fe_{DCB}) (c); r = correlation coefficient, s_x = standard error. Both top soil horizons (Steinkreuz A and Waldstein EA) are excluded from the correlations.

between certain minerals and the stabilised organic matter (Fig. 2). In the dystric cambisol the Fe_{DCB} is related to the OC lost by demineralisation, whereas in the haplic podzol the clay content and the Fe_{DCB} concentration are correlated. This shows that iron oxides and/or clay minerals are important sorbents for this stabilised OC fraction. The concomitant correlation between the clay content and the HF-soluble fraction for the podzol is due to a correlation of Fe_{DCB} and the clay content ($r = 0.92$), which in turn can be explained by a linkage of both mineral phases or an enrichment in Fe oxides in the clay-sized soil fraction. The dystric cambisol has, however, comparably high amounts of clay, but shows no relation between clay content and HF-soluble OC. These results are in accord with studies of Kiem and Kögel-Knabner (2002), which show that iron oxides play an important role for carbon stabilisation in acid soils. Translocated and subsequently adsorbed DOC may be a major source of organic matter in sub-soils (Kaiser and Guggenberger, 2000; Guggenberger and Kaiser, 2003). Root exudates may contribute as well to the organic fraction associated with the soil minerals.

In the top soils, high amounts of organic matter are present in comparison to the iron oxides. Therefore, the relationship with iron is not evident, possibly due to (i) higher organic loadings on the iron oxides and/or (ii) greater importance of other minerals for organic matter stabilisation.

4.3. Resistance to wet oxidation

Table 2 gives the proportion of organic C which was not removed by $Na_2S_2O_8$. In the top soils only small proportions of OC are resistant to oxidation by $Na_2S_2O_8$, whereas the resistant fraction increases with depth to about 30% in the haplic podzol and to more than 80% of total OC in the dystric cambisol.

A non-oxidisable fraction can be explained by aromatic C structures like charcoal or black carbon in the original material (Skjemstad et al., 1998; Wu et al., 1999; Schmidt et al., 2001). Leifeld and Kögel-Knabner (2001) found that alkyl C compounds showed the greatest resistance to oxidative treatment with H_2O_2. In Steinkreuz and Waldstein, aryl C increases with increasing soil depth (from 13% of TOC in EA to 15% of TOC in Bs of Waldstein and 14% of TOC in A to 20% of TOC in Bw2 of the Steinkreuz soil), but does not represent a major fraction of the soil organic matter (Rumpel et al., 2002). An increase in alkyl C from 33 to 44% of TOC with increasing age occurs in the Steinkreuz soil only. Thus we do not assume chemical composition alone to be a major factor controlling the amount of resistant OC.

Figure 3 shows the correlation between ^{14}C activity and the percentage of OC which is not removed by $Na_2S_2O_8$. Both soils show a negative correlation, indicating that chemically-resistant organic matter is enriched in old organic carbon. A steeper slope as observed for the Waldstein data set, indicates a higher contribution of young and labile organic matter in the refractory organic matter fraction of this soil. Since the Waldstein soil has equal or lower ^{14}C activity at depth compared to the Steinkreuz soil, we conclude that the younger OC of this soil is not as susceptible to oxidation as the younger OC of the Steinkreuz soil. This could be related to differences in the organic matter composition or to different stabilisation mechanisms, which could provide different levels of protection against chemical oxidation.

Except for three horizons (Steinkreuz Bw3, 3C and 4C1) in both soils the OC resistant to chemical oxidation is present in much lower amounts than the carbon assumed to be stabilised by soil minerals (HF soluble OC, Table 2). In agreement with Poirier et al. (2002), our data show that only a small portion of the stabilised carbon is also resistant to chemical

oxidation. Poirier et al. (2002) interpreted this "conspicuous uncoupling" between stable and refractory carbon as being due to mineral protection. This may be different in the three above mentioned horizons, where the amounts of both carbon fractions are similar.

4.4. Interaction of resistant organic matter with soil minerals: clay minerals and Fe oxides

Waldstein and Steinkreuz are sandy soils and contain between 8 and 14% clay-sized minerals <2 μm (Table 1). Iron from poorly ordered oxides as extracted with the oxalate method varies from 0.1 to 1.6 g kg^{-1} in the Steinkreuz soil and from 0.6 to 10.1 g kg^{-1} in the Waldstein haplic podzol. As expected, the highest amounts of poorly ordered ferrihydrite are found in the podzol Bs horizon. Fe from crystalline oxides ($Fe_{DCB} - Fe_{OX}$) is found to be in the range of 0.1–1.1 g kg^{-1} at the Steinkreuz site and between 1.1 and 21.2 g kg^{-1} at Waldstein. Goethite is the most common crystalline iron oxide in such acid soils from temperate climates (Cornell and Schwertmann, 1996).

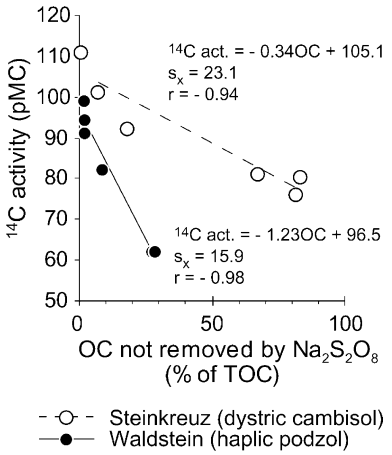

Fig. 3. ^{14}C-activity in % modern carbon (pMC) vs. amount of OC not removed by oxidation with $Na_2S_2O_8$ (a); r = correlation coefficient, s_x = standard error.

Organic matter can become trapped within the interlayer spaces between individual sheets of smectites (Schnitzer and Kodama, 1967) and may thus be protected against wet oxidation (Righi et al., 1995; Theng et al., 1986). We therefore analysed the clay mineral composition of both soils by X-ray diffraction for the presence of expandable clay minerals (Table 3). At the Steinkreuz and Waldstein sites the phyllosilicate clay mineral assemblage is characterised by the presence of kaolinite (0.7 nm), micaceous minerals (1.0 nm), a continuum of mixed layer minerals with broad reflections in the 1.15–1.25 nm region, vermiculite (1.4 nm) and hydroxy-interlayered vermiculites. The presence of chlorite was inferred from a 1.4 nm signal, which neither responded to glycerol treatment nor to heating to 550 °C (data not shown). Smectitic phases, known to supply internal surfaces, are not present (no expansion to 1.8 nm after glycerol treatment). Expandable vermiculite occurs in significant amounts in the A and B horizons of both soils, but is absent from the sub-soil horizons that contain high proportions of stabilised OC (Table 3). The vermiculite abundance is neither related to ^{14}C activity (Table 1) nor to the amount of carbon which is resistant to wet oxidation (Table 2). Experimental evidence that vermiculite and hydroxy-interlayered vermiculite can intercalate significant amounts of organic matter under natural conditions is missing. Thus, intercalation of

Table 3
Relative signal intensities of phyllosilicate clay mineral phases in clay (<2 μm) fraction. Estimated uncertainty (Whitton and Churchman, 1987) of quoted values for the 20–100% abundance range is ±10%, and ±20% for the 0–20% abundance range

Horizon	Smectite (%)	Chlorite (%)	Vermiculite (%)	HIV[a] (%)	Interstratified[b] (%)	Illite (%)	Kaolinite (%)
Steinkreuz (dystric cambisol)							
Ah	0	4	7	0	21	63	4
Bw1	0	31	9	15	14	27	3
Bw2	0	29	20	9	7	30	4
Bw3	0	23	10	7	10	46	4
3C	0	20	0	0	1	78	2
4C1	0	29	0	0	0	68	3
Waldstein (haplic podzol)							
EA	0	0	36	0	24	14	24
Bh	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bs	0	31	12	10	6	28	13
Bw	0	20	1	6	0	54	18
C1	0	12	0	4	0	60	23
C2	0	21	0	2	0	58	19

[a] HIV = Hydroxy-interlayered vermiculite.
[b] Interstratified 1.0 and 1.4 nm phases.

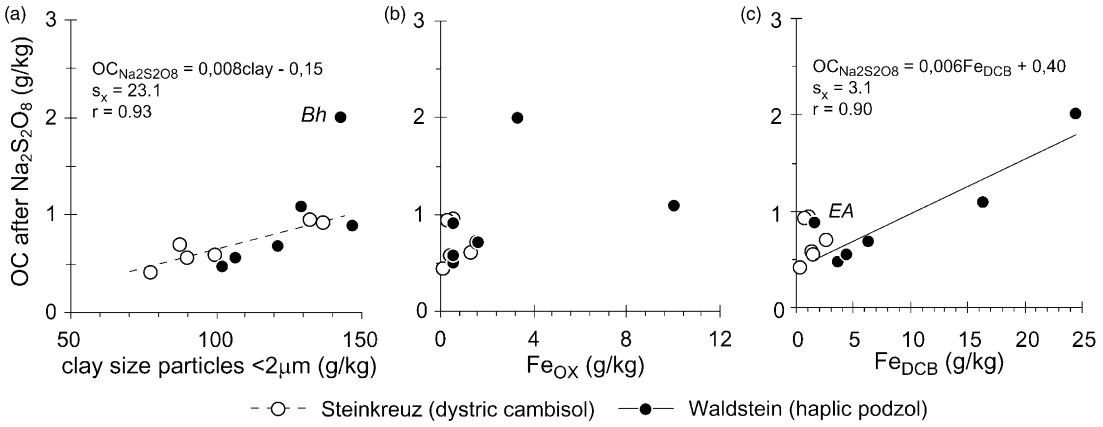

Fig. 4. Relationship between concentration of OC resistant to oxidation by $Na_2S_2O_8$ and clay content (a), amount of poorly-ordered Fe oxyhydroxides (Fe_{OX}) (b) and content of total Fe oxides (Fe_{DCB}) (c); r = correlation coefficient, s_x = is the standard error.

organic matter between internal phyllosilicate surfaces does not seem to contribute significantly to carbon stabilisation in the soils.

Interactions between the $Na_2S_2O_8$-resistant OC and the mineral phase were investigated by relating OC concentration after oxidation to (i) clay content, (ii) the amount of poorly ordered Fe oxyhydroxides (Fe_{OX}) and (iii) the amount of total Fe oxides (Fe_{DCB}; Fig. 4). A positive correlation between the content of $Na_2S_2O_8$-resistant OC and the clay content exists for most samples of both soils (Fig. 4a). However, the sample with the greatest concentration of resistant carbon does not follow this trend (Waldstein Bh).

No correlation is seen for both soils between the C concentration after $Na_2S_2O_8$ treatment and the amount of poorly crystalline Fe oxyhydroxides (Fe_{OX}; Fig. 4b) or the content of poorly ordered Al-phases as indicated by Al_{OX} (data not shown). Minerals like ferrihydrite, though offering high specific surface area and a high density of reactive surficial hydroxyl groups, are obviously not the sole location for organic matter sorption in these soils. For the Waldstein haplic podzol, the best correlation is found between Fe_{DCB} and resistant OC (Fig. 4c). This points to an adsorption of the non-oxidisable, old soil organic matter on to Fe oxides of high and low crystallinity. The EA horizon of the Waldstein soil shows the strongest deviation from this relationship, in the sense that it contains little Fe-oxides compared to its concentration of $Na_2S_2O_8$-resistant organic carbon. This possibly represents a state of supersaturation with regard to C adsorption on Fe oxides.

Where Fe oxides appear frequently, like e.g. in most podzol horizons, they seem to control the storage and possibly also the stabilisation process of old organic matter. The usually small size of the oxides or a linkage between Fe oxides and silicates may explain the general relationship also observed between C and the clay content (Fig. 4a). In soils or horizons with low oxide concentration (Steinkreuz, EA horizon of Waldstein) additional mechanisms for carbon storage and stabilisation must be operative. The role of silicates may be more important here.

5. Conclusions

A strong association of the old and stable carbon fraction with the mineral phase is observed for both forest soils. HF preferentially removes the mineral-bound organic matter. This organic matter fraction seems to be dominated by old carbon compounds, supporting the concept of the stabilising function of minerals.

Oxidation by $Na_2S_2O_8$ selectively removes the younger carbon fraction and allows isolation of a non-oxidisable, highly refractory, most probably slowly cycling soil OC pool. This remaining fraction comprises between 1 and 30% in surface and near surface horizons, but can make up to 80% of the total soil carbon in sub-surface horizons. The technique is proposed to recover a certain long residence time C pool and to enable the determination of the respective pool size and age. The procedure carries the potential for yielding a valuable variable input as required in carbon turnover models.

In both acid forest soils the minerals in the clay fraction seem to be responsible for the storage of old organic matter. In horizons where Fe oxides are sufficiently available, they play the major role in the interaction with old, stabilised soil organic matter within the mineral matrix. Intercalation of the refractory fraction into expandable clay minerals is unlikely in these soils.

Acknowledgements

This project is financially supported by the priority program 1090 "Soils as sinks and sources for CO_2-mechanisms and regulation of carbon stabilisation in soils" of the Deutsche Forschungsgemeinschaft. The radiocarbon analyses were carried out at the Leibniz Laboratory, Universität Kiel. We thank Maria Greiner for support in the laboratory and Kai Uwe Totsche for helpful discussion. Valuable comments were provided by Jeff Baldock, an anonymous reviewer and Claude Largeau.

Associate Editor — **Claude Largeau**

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