

## Sodium hypochlorite separates an older soil organic matter fraction than acid hydrolysis

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Received 23 March 2006; received in revised form 11 January 2007; accepted 28 January 2007

Available online 12 March 2007

### Abstract

Different chemical treatments can be used to isolate an old and chemically resistant soil organic matter fraction from soils. Here, we compared the residues after acid hydrolysis (6 N HCl) with those obtained after NaOCl treatment (6 wt%) of the silt + clay fractions from 48 soil samples. The samples were taken at sites differing in climate and land use across Switzerland. To determine the influence of the two treatments on soil organic matter and mineral structures, we examined infrared spectra and isotopic signatures of carbon ( $^{14}\text{C}$  and  $\delta^{13}\text{C}$ ) of the residues. Treatment with NaOCl removed more (63 to 91%) organic carbon (OC) than did treatment with HCl (35 to 66%), and it had no effect on mineral structures, whereas treatment with HCl converted crystalline minerals to more amorphous ones. Increases in specific soil surface area (SSA) did not correlate with the amount of OC removed. The amount of OC removed by each treatment was not (NaOCl) or only weakly (HCl) related to the initial OC content of the silt + clay fraction, suggesting the presence of a relatively constant fraction of chemically resistant OC.  $^{14}\text{C}$  activities of NaOCl-resistant residues were lower than those of HCl residues, indicating that soil organic matter residues isolated by NaOCl treatment were older than the residues obtained by acid hydrolysis. It is concluded that oxidation with NaOCl is the better way than hydrolysis with HCl to obtain an operationally-defined stable organic matter fraction from soils.

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**Keywords:** Acid hydrolysis; Oxidation with NaOCl; Stable soil organic matter fractions

### 1. Introduction

Organic carbon (OC) in soils can be separated into fractions with different decomposition rates. In models used to describe the dynamics of soil organic matter (SOM) typically two to five conceptual carbon pools are defined by their specific turnover rates (Smith et al., 1997). It has been shown that consideration of one stable or even inert pool is appropriate for modelling long-term SOM dynamics. Under steady-state conditions, this pool would have a turnover time of several hundreds to thousands of years (e.g., Paul et al., 1997). However, its physico-chemical characterization is challenging because no single mechanism has been identified for the long-term stabilization of SOM (von Lützow et al., 2006).

The most important mechanisms of SOM stabilization are: i) selective degradation, i.e. accumulation of biochemically resistant materials relative to labile ones, ii) incomplete combustion of plants, iii) encapsulation into stable micro-aggregates, and iv) association with mineral surfaces (Krull et al., 2003). Isolation and quantification of SOM that is protected by any one of these four mechanisms is difficult because they all operate simultaneously. To cope with the complexity of mechanisms involved in the long-term stabilization of SOM, various approaches are used to isolate an old and chemically resistant OC fraction from soils. If successful, quantification and characterization of resistant SOM could help to parameterize stable OC in models. Here, we compare two frequently used methods namely acid hydrolysis with hydrochloric acid (HCl) and oxidation with sodium hypochlorite (NaOCl) with respect to the amount and characteristics of OC residues.

Hydrolysis with HCl has been widely used to obtain a slowly cycling and old SOM fraction (Trumbore and Zheng, 1996; Leavitt

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et al., 1996; Paul et al., 1997). In these studies, treatment with HCl removed between 30 and 77% of OC and the remaining OC was on average 1500 years older than OC in bulk soils. Acid hydrolysis in combination with  $^{14}\text{C}$  dating has been applied to estimate the inert OC pool in the RothC model (Falloon et al., 1998).

NaOCl was proposed by Anderson (1963) for removal of SOM from clay mineral surfaces. Organic matter treated with NaOCl produces chlorinated compounds that cleave benzene rings (Mikutta et al., 2005a). Kaiser et al. (2002) and Kleber et al. (2005) used NaOCl to obtain a chemically resistant SOM fraction that is bound to mineral surfaces. NaOCl removed between 16 and 86% of OC, and the radiocarbon age of the resistant fraction increased by 75 to 1880 years relative to that of bulk soil samples.

The results from these studies indicated differences in the removal efficiency between acid hydrolysis with HCl and oxidation with NaOCl, but since both methods were applied to different samples, results cannot be compared directly. In order to determine the potential of the two treatments to isolate a stable SOM fraction from the same soils, we compared their chemical removal efficiency, the composition and age of the remaining OC, and the effect of the treatments on mineral structures using the same soil samples from 32 sites after fractionation. The focus of the study was to test for differences in the resistant SOM fraction obtained after acid hydrolysis with HCl or oxidation with NaOCl and to compare the properties of these fractions across samples. Measurement of total OC and analysis with infrared spectroscopy were used to distinguish the effect of both methods on the amount and composition of resistant SOM. To determine changes in the chemical structure of organic matter from that of mineral matter, pure clay minerals were treated with HCl and NaOCl and infrared spectra of the clays before and after the treatments were compared. Age and isotopic composition of the residues were determined using  $^{14}\text{C}$  activities and  $\delta^{13}\text{C}$  signatures of selected samples. Results were also discussed in the light of biochemical stability vs. chemical recalcitrance.

## 2. Materials and methods

### 2.1. Soil samples

Soil samples were selected from the archive of the Swiss national soil survey program, which contains material from 102

agricultural and forest sites across Switzerland. This material consists of composite soil samples from 50 cores taken between 1985 and 1991 at all sites in plots of  $10 \times 10$  m. From sites with agriculturally disturbed topsoils, soil profiles were separated into layers of 20 cm. Undisturbed topsoils from grasslands were divided into horizons of 0–5 cm, 5–10 cm, 10–20 cm, 0–10 cm or 0–20 cm. A detailed description of the sampling technique together with individual site characteristics such as climate, geology and land use can be found in Vogel et al. (1992) and Desaules and Studer (1993).

For our analysis, 48 topsoil samples from 0 to 20 cm were selected from agricultural (cropland, meadow or alpine pasture), carbonate-free mineral soils with OC contents of less than 8.7%. Selected sites vary in altitude from 265 to 2400 m a.s.l., with corresponding mean annual temperature ranging from  $-1.6$  °C to  $10.6$  °C and mean annual precipitation from 722 to 2327 mm.

Textural and chemical bulk soil properties were available from the laboratory of the Swiss national soil survey program determined using standard methods (FAC, 1989). Data for total OC, texture, pH, oxalate-extractable Al- and Fe-oxides and potential cation exchange capacity (CEC) for the selected bulk soil samples are summarized in Fig. 1. In brief, samples were dried at  $40$  °C, crushed and particles  $>2$  mm were removed. Silt and clay contents were determined by the pipette method and sand contents were calculated by difference. Amounts of oxalate-extractable Al- and Fe-oxides were measured by ICP-AES (inductively coupled plasma atomic emission spectrometry) after extraction of amorphous oxides with oxalate solution for 2 h in the dark at room temperature (pH 3). To determine the potential cation exchange capacity (CEC), samples were saturated with buffered  $\text{BaCl}_2$  (pH 8.1) and barium was then exchanged by  $(\text{NH}_4)_2\text{Cl}_2$  at pH 8. Barium in solution was measured with an atomic absorption spectrometer and the potential CEC was calculated. pH (in 0.01 M  $\text{CaCl}_2$ ) was measured with a glass electrode in the supernatant of a 2.5:1 suspension of water to soil. Carbon content was measured with an elemental analyzer (Vario EL, Elementar).

### 2.2. Fractionation procedure

Soil samples were fractionated by means of physical and chemical procedures to obtain a physically non-aggregated

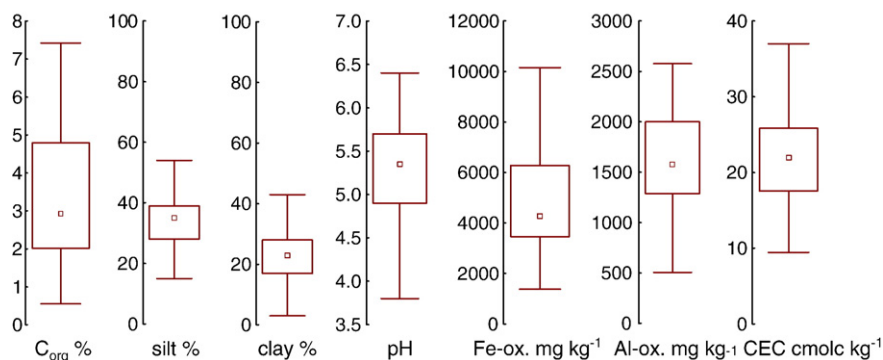


Fig. 1. Box plots for organic carbon, silt, clay, pH, oxalate-extractable Fe- and Al-oxides and cation exchange capacities with the minimum, maximum, and median values, as well as the 25% percentiles.

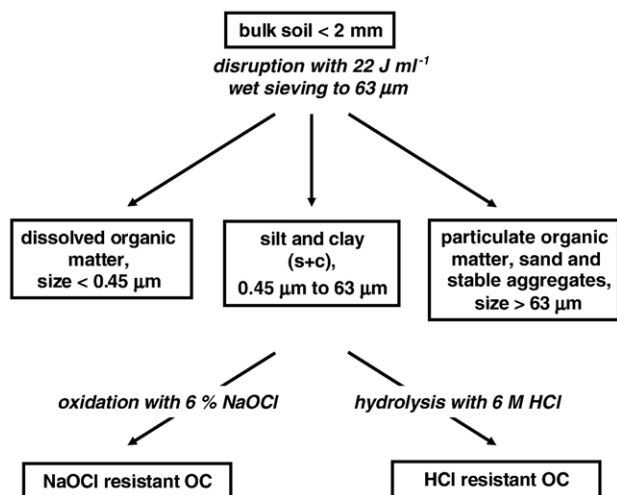


Fig. 2. Fractionation procedure.

SOM fraction that is bonded to clay- and silt-sized particles, as shown in Fig. 2. Thirty grams of soil (<2 mm) were suspended in 150 ml water and dispersed using an ultrasonic probe with a calibrated output-energy of 22 J ml<sup>-1</sup>. Higher energy application may disrupt coarse sand-sized SOM (Amelung and Zech, 1999). This suspension was then wet-sieved (63 μm) until the rinsing water was clear, and afterwards the rinsing water was filtered through a 0.45-μm nylon mesh to quantify dissolved OC. Particles >63 μm consist of sand, stable aggregates and particulate organic matter (POM), and particles between 63 and 0.45 μm of silt and clay (s+c).

We isolated chemically resistant OC from the s+c fraction by means of acid hydrolysis and by oxidative treatment with NaOCl. Acid hydrolysis was performed with 2 g of s+c suspended in 50 ml of 6 M HCl following a modified method of Leavitt et al. (1996). The suspension was heated during 18 h at 105 °C in a sealed glass bottle and then centrifuged at 1000 g for 20 min. The supernatant was decanted, the residue washed with 50 ml deionised water, and the centrifugation repeated twice. Oxidation with NaOCl was done at room temperature following a modified method of Kaiser et al. (2002). One gram of s+c was oxidised during 18 h at 25 °C with 50 ml of 6% (wt/wt) NaOCl adjusted to pH 8 with concentrated HCl. The residue was centrifuged at 1000 g for 20 min, decanted, and washed with deionised water. Oxidation was repeated three times. Carbon and nitrogen contents of all dried fractions (40 °C) before and after chemical treatment were measured with an elemental analyzer (Vario EL, Elementar) after dry combustion.

### 2.3. Standard clay minerals

In addition to soil samples we treated six standard clay minerals with HCl and NaOCl, as described above, to examine the effect of the treatments on soil minerals. Samples of pure ground clay minerals, which are typical components of Swiss soils (M. Plötze, personal communication), were provided by the Institute of Geotechnical Engineering of ETH Zurich. These were a high-defect (KGa 1) and low-defect (KGa 2) kaolinite, a

montmorillonite (SWy 1), a smectite (STx 1), an illite (ILp), and a vermiculite (VRu).

### 2.4. Soil surface area

We measured the specific surface area (SSA) of the s+c fraction before and after chemical treatment with a Quantachrome Nova 2200 surface analyser. Samples were degassed under vacuum (pressure <0.01 mbar) for 16 h at 40 °C to remove adsorbed water from particle surfaces. Tests indicated that all water was removed after degassing, and that degassing under vacuum was more efficient in removing water than degassing under continuous N<sub>2</sub> flow (data not shown). The SSA was estimated by 5-point N<sub>2</sub> adsorption at 77 K in the relative pressure range of 0.1–0.5, using the Brunauer–Emmett–Teller equation

$$\frac{p/p_0}{n(1-p/p_0)} = \frac{1}{n_m C} + \frac{C-1}{n_m C} \frac{p}{p_0}, \quad (1)$$

where  $p_0$  is the saturation vapour pressure at the measurement temperature,  $p/p_0$  the relative gas pressure,  $n$  the amount of gas adsorbed per mass material,  $n_m$  the monolayer adsorption capacity and  $C$  is a constant related to the enthalpy of the gas adsorption (Kaiser and Guggenberger, 2003).

### 2.5. DRIFT-spectroscopy

Infrared spectra of s+c fractions from selected soil samples and of pure clay minerals were recorded with a Perkin Elmer Spectrum One spectrometer with DRIFT (Diffuse Reflectance Infrared Fourier Transformation) inlet. Thirty milligrams of dried (40 °C) samples were diluted with 970 mg KBr to reduce scatter light intensity, homogenized for 30 s in a ball mill and then scanned 16 times in the range of 4000 to 500 cm<sup>-1</sup> (mid-infrared region) at a resolution of 4 cm<sup>-1</sup>. KBr background spectra were subtracted from measured spectra. DRIFT spectra were corrected against atmospheric CO<sub>2</sub> and water vapour and automatically adjusted to an internal CH<sub>4</sub> cell. Baseline-corrected results are shown as %-reflectance.

### 2.6. <sup>14</sup>C and <sup>δ</sup><sup>13</sup>C measurements

In samples from four sites varying in land use, soil depth and climatic conditions we analysed <sup>14</sup>C activities and <sup>δ</sup><sup>13</sup>C concentrations in bulk soil and in s+c, HCl-resistant and NaOCl-resistant fractions. <sup>14</sup>C activities were measured at the accelerator mass spectrometry (AMS) laboratory of ETH Zurich, Switzerland. Samples were cleaned chemically with an acid (0.5 M HCl at 60 °C for 1 h)–base (0.1 M NaOH at 60 °C for 1 h)–acid (0.5 M HCl at 60 °C for 1 h) treatment and dried at 60 °C (Hajdas, 1993). The dried material was weighed into small quartz glass tubes with a CuO and silver wire and then oxidized to CO<sub>2</sub> under vacuum at 950 °C. Resulting CO<sub>2</sub> was reduced to graphite on cobalt in the presence of hydrogen in a steel extraction line and pressed onto disk-shaped copper targets (Bonani et al., 1994). The graphite targets were analyzed together with standards made from oxalic acids and blanks

Table 1  
Pearson's correlation coefficients ( $r$ ) and errors of probability ( $p$ ) between bulk soil properties and organic carbon contents in the s+c fractions

OC in s+c <sup>a</sup>	$r$	$p$	$N$
Clay (%)	0.05	0.14	47
Silt (%)	-0.19	0.23	47
pH	<0.01	0.89	48
Oxalate-extractable Fe <sup>b</sup>	0.52	<0.01	47
Oxalate-extractable Al <sup>b</sup>	0.22	0.15	47
Potential CEC (pH 8) <sup>c</sup>	0.63	<0.01	48

<sup>a</sup> mg g<sup>-1</sup> soil.

<sup>b</sup> mg g<sup>-1</sup> soil.

<sup>c</sup> cmolc kg<sup>-1</sup> soil.

according to the standard procedure used at the ETH/PSI AMS radiocarbon facility (Bonani et al., 1987). Results were corrected for isotopic fractionation and are given as values of percent modern carbon (pMC).  $\delta^{13}\text{C}$  values were measured by Iso-Analytical (Sandbach, UK) by use of EA-IRMS (elemental analyser isotope ratio mass spectrometer). Reference material used during the analysis was wheat flour (IA-R001 standard) with a  $\delta^{13}\text{C}$  value of -26.4‰ relative to the Pee Dee Belemnite standard.

### 2.7. Statistical analysis

Correlations between variables are expressed as Pearson's correlation coefficients ( $r$ ). Significance of differences between groups was tested at  $p < 0.05$  using a two-sided  $t$ -test. Results are given with corresponding errors of probability ( $p$ ).

## 3. Results and discussion

### 3.1. Correlation between bulk soil properties and OC content of the s+c fraction

Between 14 and 88% of OC in bulk soil was present in the s+c fraction. The amount of OC in s+c decreased in the order of croplands (77±8% of the total OC in the s+c fractions) > meadows (57±17%) > soils under alpine pastures (36±17%), which reflects the stronger aggregation and a higher proportion of particulate organic matter in soils under less intensive agricultural practices. The amount of OC lost as dissolved OC during sieving was on average 2.7% (±1.4) of total OC.

We related the concentration of OC in the s+c fraction to different chemical and textural properties of bulk soil samples (Table 1). Significant correlations between OC in s+c and bulk soil attributes were found for potential CEC ( $r = 0.63$ ,  $p < 0.01$ ) and oxalate-extractable Fe ( $r = 0.52$ ,  $p < 0.01$ ). Potential CEC was auto-correlated with oxalate-extractable Fe ( $r = 0.48$ ,  $p < 0.01$ ), indicating that Fe-oxides contribute significantly to the negative surface charge of the soil. Oxalate-extractable Fe was also correlated with oxalate-extractable Al ( $r = 0.80$ ,  $p < 0.01$ ) and with clay content ( $r = 0.53$ ,  $p < 0.01$ ), whereas these properties were not correlated with the content of OC in the s+c fraction.

These results indicate that the amount of oxalate-extractable Fe has a strong effect on storage of OC in the s+c fraction,

while the total amount of clay has almost no effect. This is in agreement with Wiseman and Püttmann (2005) and Mikutta et al. (2006) who found that oxalate-extractable Fe and Al had a greater influence than the clay mineral composition on OC concentrations of five topsoils from forest and agricultural sites.

### 3.2. Removal efficiency

HCl removed between 35 and 66% of OC in the s+c fraction, and NaOCl removed between 63 and 91%. The removal efficiency of HCl and NaOCl for all samples is illustrated in Fig. 3. Treatment with NaOCl removed significantly more OC than treatment with HCl ( $p < 0.01$ ) with the efficiency being independent of the amount of OC in the s+c fraction ( $r = 0.14$ ,  $p = 0.31$ ). Conversely, the relative amount of OC removed by treatment with HCl in the s+c fraction tended to decrease with the OC content ( $r = 0.55$ ,  $p < 0.01$ ).

The fraction of OC removed by HCl corresponded well to results from similar studies. Leavitt et al. (1996) reported removal efficiencies between 37 and 65% of OC in soils taken at 0–30 cm. These authors sieved soil samples to <1 mm, removed all particles having a density of less than 1.2 g cm<sup>-3</sup>, removed plant and root fragments under a microscope, and heated 1 to 3 g of the remaining sample to boiling for 18 h with 50 ml 6 N HCl. Paul et al. (1997) used the same method and removed 41 to 61% of OC. Trumbore and Zheng (1996) reported an efficiency of OC removal of 39 to 94% after heating of soil fractions with a density of >2.1 g cm<sup>-3</sup> with 6 N HCl to 95 °C. The fraction of OC removed by NaOCl in the present study is in the same range as reported previously by Mikutta et al. (2005b) who treated clay fractions from acid subsoils four times with 6% NaOCl (pH 8) at 25 °C during 6 h and removed between 49 and 81% of OC. A wider range of removal efficiencies was observed by McDowell and Condron (2001) who removed between 26 and 80% OC in bulk soil samples by repeated treatment with 6% NaOCl (pH 8.5) at room temperature overnight.

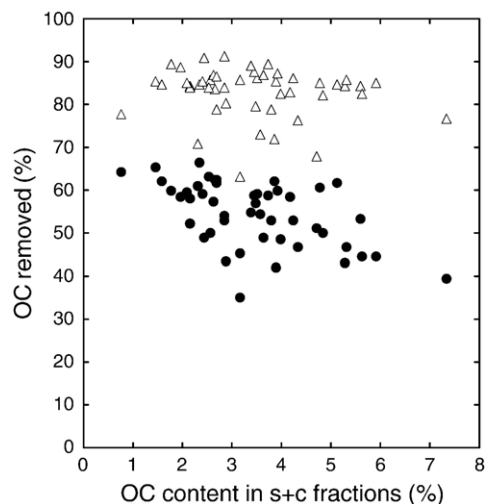


Fig. 3. Organic carbon removed from silt and clay fractions by hydrolysis with HCl (●) or oxidation with NaOCl (Δ).

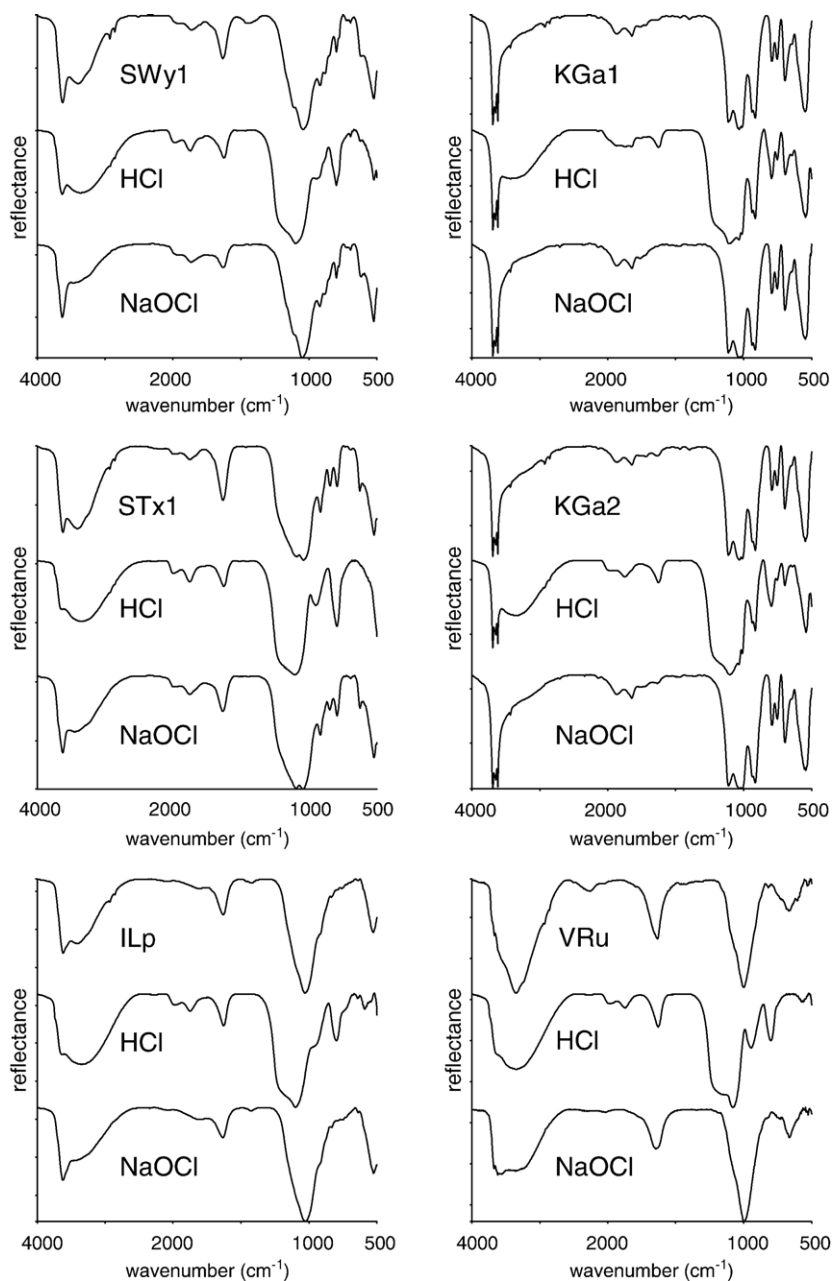


Fig. 4. DRIFT spectra of standard clay minerals before and after chemical treatments. SWy 1 = montmorillonite, KGa1 = high-defect kaolinite, STx 1 = smectite, KGa 2 = low-defect kaolinite, ILp = illite, VRu = vermiculite.

### 3.3. Influence of chemical treatments on standard clay minerals

Six clay minerals were treated with HCl or NaOCl to distinguish by infrared spectroscopy effects of both treatments on organic and mineral compounds (Fig. 4). The most obvious changes caused by HCl consisted in broadening of the OH-stretching peaks at  $3350\text{ cm}^{-1}$ , an increase in the OH-deformation peaks at  $1630\text{ cm}^{-1}$  for the kaolinite clays, appearance of broad shoulders at  $1235\text{ cm}^{-1}$ , and an increase in the peaks at  $800\text{ cm}^{-1}$ . Peaks at  $1235\text{ cm}^{-1}$  and at  $800\text{ cm}^{-1}$  can be ascribed to changes in the Si–O structure of the clay minerals. According to Gates et al. (2002) all these changes may be caused by the conversion of crystalline mineral structures to

more amorphous ones, and by simultaneous adsorption of  $\text{H}_2\text{O}$  on newly structured mineral surfaces. This conversion dissolves the central Al-ions of octahedral layers in clay minerals and the central Si-ions of tetrahedral structures, as reflected by the relative decrease in signal intensity between  $650$  and  $500\text{ cm}^{-1}$  (Madejova and Komadel, 2001). On the contrary, treatment of these clay minerals with NaOCl caused almost no change in the infrared spectra.

### 3.4. Increase in SSA of s+c fraction caused by chemical treatments

SSA of untreated s+c fractions ranged between  $1.1$  and  $14.2\text{ m}^2\text{ g}^{-1}$ . This surface is largely provided by clay minerals,

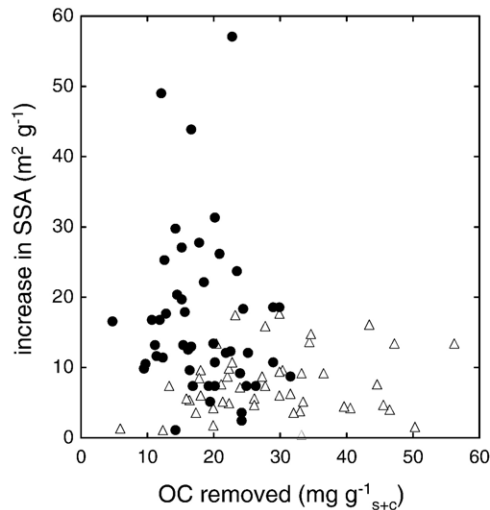


Fig. 5. Comparison of dissolved organic carbon after hydrolysis with HCl (●) or oxidation with NaOCl ( $\Delta$ ), and increase in specific surface area (SSA) of the s+c fraction caused by treatments.

oxides and to a smaller extent by SOM with a much smaller  $N_2$ -accessible SSA than clay minerals and oxides (Chiou et al., 1990). Treatment with HCl increased SSA on average by a factor of 3.7 and oxidation with NaOCl by a factor of 2.4.

An increase in SSA is expected to result from removal of SOM that encapsulates clay minerals and oxide surfaces thereby reducing access of  $N_2$  to surfaces (Kaiser and Guggenberger, 2003). Because oxidation with NaOCl had almost no effects on mineral structures, it is likely that removal of SOM by this treatment was responsible for the observed increase in SSA. Another explanation for this increase may be the addition of sodium. In the case of hydrolysis with HCl a combination of changes in the chemical structures of minerals and removal of SOM might be responsible for the increase in SSA. However, this increase was not related to the amount of OC removed by NaOCl or by HCl, as shown in Fig. 5. Hydrolysis with HCl removed less OC than did oxidation with NaOCl but it caused a larger increase in SSA. This could be explained by the destruction of the mineralogical structure of clay minerals and the dissolution of Fe- and Al-oxides.

Kaiser and Guggenberger (2003) found that treatment with NaOCl causes a larger increase in SSA in samples with low OC contents than in samples with high OC contents. For their examinations, they used 47 soil samples from forest sites worldwide. Our results do not confirm these findings as increases in SSA did not correlate with the amount of OC removed from the s+c fractions.

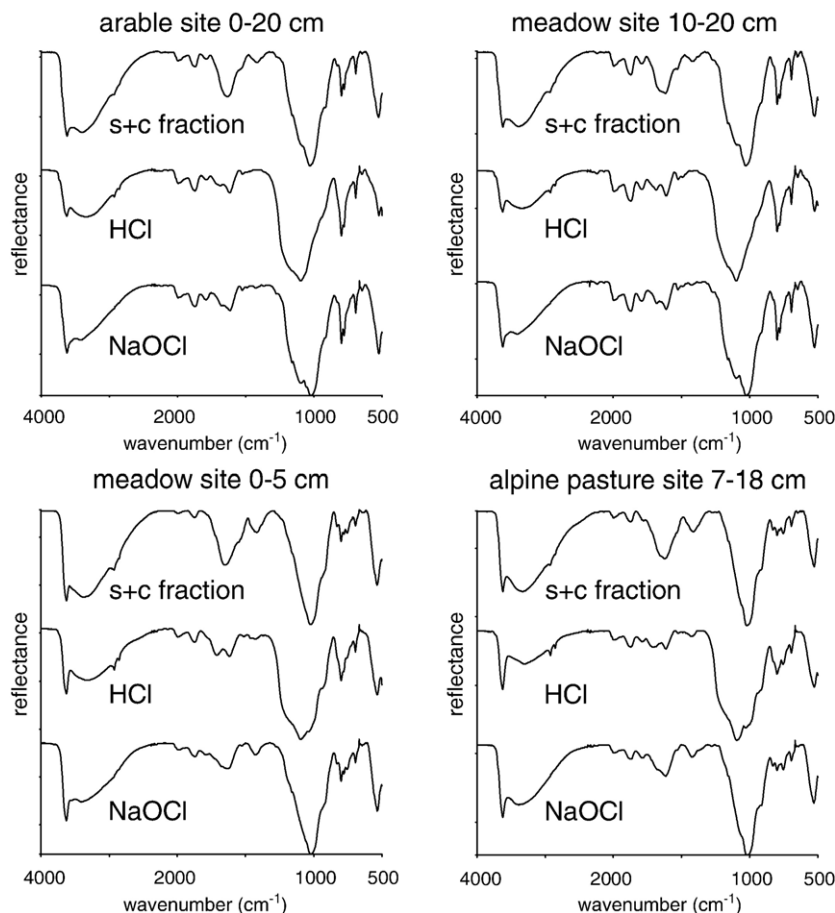


Fig. 6. DRIFT spectra of the s+c fraction from soil samples taken at sites with different land use. Spectra are shown for different depths and before and after chemical treatment.

### 3.5. Changes in functional groups of organic compounds as revealed by DRIFT-spectroscopy

Chemical treatments induced changes in only four regions of the infrared spectra of clay minerals (see above). Therefore, shifts in peaks in other regions are likely to be caused by changes in functional groups of organic compounds. After Baes and Bloom (1989), organic compounds of soil samples can be detected in infrared spectra as aliphatic C–H stretches ( $3000\text{--}2800\text{ cm}^{-1}$ ), carbonylic/carboxylic C=O stretches ( $1720\text{--}1640\text{ cm}^{-1}$ ), aromatic C=C stretches ( $1620\text{--}1525\text{ cm}^{-1}$ ) and aliphatic C–OH and C–C stretches ( $1300\text{--}950\text{ cm}^{-1}$ ). Infrared spectra before and after chemical treatments were used to investigate treatment effects on structural properties of samples from four sites representative of different types of land use (meadow 0–5 cm and 10–20 cm, arable 0–20 cm, and alpine pasture 7–18 cm) (Fig. 6). HCl treatment resulted in a relative increase in alkyl peaks ( $3000\text{--}2800\text{ cm}^{-1}$ ) and a splitting of the broad carboxyl peak at  $1660\text{ cm}^{-1}$  into two smaller peaks at  $1680$  and  $1620\text{ cm}^{-1}$ . This indicated a possible enrichment of alkyl carbon relative to the initial organic matter content and a strong dissolution of components rich in carboxylic groups by acid hydrolysis. Other patterns in these spectra ( $3550\text{--}3200\text{ cm}^{-1}$ ,  $1300\text{--}950\text{ cm}^{-1}$ ) could not clearly be assigned to either changes in mineral structures or changes in aliphatic compounds. Similarly, Paul et al. (1997) found that HCl preferentially hydrolysed proteins, nucleic acids and polysaccharides, corresponding to the changed pattern in infrared spectra, while leaving cellulose, alkyl, and aromatic components largely unaffected.

The main effect of NaOCl on organic compounds was expressed by the disappearance of all alkyl peaks ( $3000\text{--}2800\text{ cm}^{-1}$ ) and a strong decrease in the carboxyl ( $1660\text{ cm}^{-1}$ ) and aromatic ( $1620\text{ cm}^{-1}$ ) peaks. This is in agreement with the conclusion drawn by Mikutta et al. (2005a) that NaOCl cleaves most methine, methylene and methyl groups, as well as most aromatic structures.

### 3.6. Isotopic signatures

Fig. 7 shows the isotopic signatures of the samples previously examined by DRIFT-spectroscopy. Low  $^{14}\text{C}$  values, expressed as percent modern carbon (pMC), indicate a higher mean age of SOM. The untreated s+c fractions had pMC values almost equal to those of the initial bulk soils, thus suggesting similar turnover times. HCl- and NaOCl-resistant fractions were depleted in  $^{14}\text{C}$  relative to the initial s+c fractions and thus appeared to be older. NaOCl-resistant material was consistently older (on average 11.76 units less pMC than the untreated fractions) than HCl-resistant material (on average 4.05 units less pMC than the untreated fractions).

Fractions resistant to HCl and NaOCl were also depleted in  $\delta^{13}\text{C}$ , with the larger depletion of the HCl-resistant fraction. Whether a fraction is enriched or depleted in  $\delta^{13}\text{C}$  depends not on its mean age, but rather on the isotopic signature of the different organic compounds of its organic matter and on isotopic fractionation during decomposition. Cellulose, hemi-cellulose, carboxyl- and amino acids have been shown to be enriched in  $\delta^{13}\text{C}$  (Diels et al., 2001), whereas lignin, lipids, *n*-alkanes, and

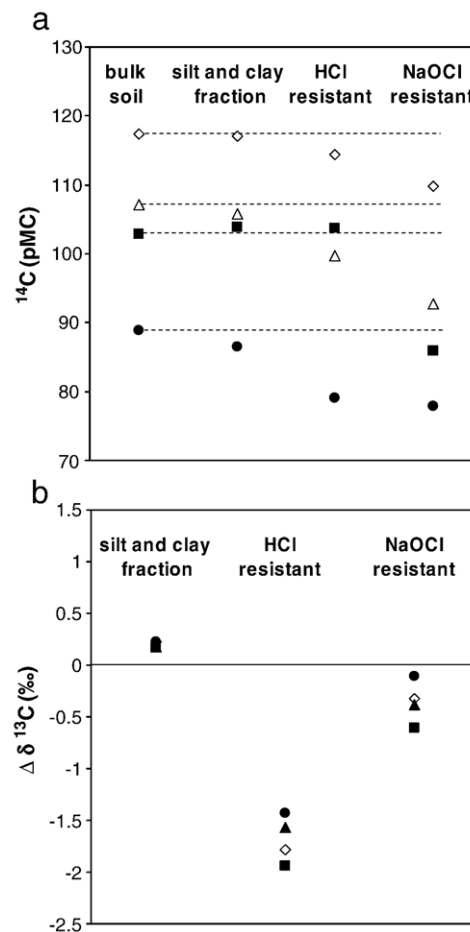


Fig. 7. a)  $^{14}\text{C}$  activities as percent modern carbon of bulk soils and fractions (dotted lines are referred to bulk soil values). Error bars are smaller than symbol sizes. b)  $\Delta \delta^{13}\text{C}$  values as differences between fractions and bulk soils. ( $\diamond$  = meadow site 0–5 cm,  $\triangle$  = arable site 0–20 cm,  $\blacksquare$  = meadow site 10–20 cm, and  $\bullet$  = alpine pasture site 7–18 cm).

some charcoals were found to be depleted (Krull and Skjemstad, 2003; Wiesenberger et al., 2004). Both chemical treatments dissolve  $^{13}\text{C}$ -enriched compounds, thus leaving resistant material depleted in  $^{13}\text{C}$ . The presence of more negative  $\delta^{13}\text{C}$  values in the HCl-resistant fraction may be explained by the fact that HCl does not dissolve lignin and alkyl carbon. This leads to stronger depletion in  $^{13}\text{C}$  than oxidation with NaOCl, as confirmed by infrared spectroscopy. Differences in isotopic signatures thus reflect the specificity of the reactant to particular organic compounds.

### 3.7. Biochemical stability of SOM

While both oxidation with NaOCl and hydrolysis with HCl create residues of older SOM, there is no consensus with regard to the specificity of chemical treatments to produce a single SOM fraction defined by its biochemical stability. Here, we define biochemical stability as resistance against any microbiological decomposition process which might be influenced by a wide range of environmental conditions and land use, as represented by the samples analysed in this study. Thus, differences in site conditions will be reflected in variable contributions to old SOM.

Leifeld and Kögel-Knabner (2001) showed that the removal efficiency for hydrogen peroxide, another common oxidative reactant, was independent of the OC content of the sample, and Plante et al. (2004) using soils from a cultivation sequence concluded that treatment with hydrogen peroxide was inappropriate to estimate a refractory SOM pool because the size of the latter was not related to the history of cultivation. Similarly, here we found no difference in the removal efficiency of NaOCl for soils containing different amounts of carbon. The method is thus suitable to effectively oxidize most of SOM, but its specificity in terms of biochemical stability is to be questioned. In contrast, the amount of HCl-resistant OC was weakly correlated with the initial OC content of the s+c fractions. Paul et al. (2006) found that HCl residues were representative of slowly cycling OC pools, and they reported the existence of a strong correlation between pools and fluxes measured by hydrolysis and  $^{13}\text{C}$  incorporation. The amount of HCl-resistant OC in Paul et al. (2006) was sensitive to the prevailing long-term land use at the sampling site and was also strongly correlated with the amount of total OC, as observed for the HCl-resistant fractions in our study. Therefore, the HCl-resistant fraction may represent a SOM pool of high biochemical stability which, however, may not be necessarily equivalent to chemical resistant SOM, partially due to the contribution of plant compounds such as lignin, crystalline cellulose, or waxes to the non-hydrolysable fraction of SOM.

#### 4. Conclusions

Elemental analysis, DRIFT-spectroscopy, analysis of SSA and isotopic signatures were used to compare residues of mineral soil fractions after acid hydrolysis or oxidation with NaOCl. The results show that i) oxidation with NaOCl removes more OC from soils than acid hydrolysis with HCl, ii) NaOCl has almost no effect on clay minerals, iii) HCl removes  $\delta^{13}\text{C}$ -enriched compounds, and iv) HCl-resistant fractions are younger than NaOCl-resistant ones. We conclude that oxidation with NaOCl is superior to acid hydrolysis with HCl to obtain a chemically resistant and old fraction of SOM. However, the constant ratio of NaOCl-resistant to total OC in the silt+clay fraction in samples from across a wide range of sites implies that a direct relationship between chemical recalcitrance and biochemical stability does not exist.

#### Acknowledgments

This study was financed by the Swiss Federal Office for the Environment. We thank the Swiss national soil survey (NABO) for providing the examined soil samples and the Institute of Geotechnical Engineering of the ETH Zurich for the standard clay minerals. Furthermore, we have to thank two anonymous reviewers for very helpful comments on an earlier draft of the manuscript.

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