Comparison of different methods of obtaining a resilient organic matter fraction in Alpine soils

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Abstract

Soil organic matter (SOM) may give precious information about the age of soil landscapes and, thus, can contribute to decipher geomorphic surface dynamics. We tested five methods to isolate the oldest possible stable organic matter of 2 soil profiles developed on a Pleistocene morainic paragneiss substratum in an Alpine environment in northern Italy. Before and after the individual treatments, the organic fraction was dated with $^{14}$C. The first two methods consist in the oxidation of organic matter with 10% hypochlorite (NaOCl), followed by dissolution of minerals with 10% hydrofluoric acid (HF) or 1M Aqua Regia. Methods 3 and 4 were similar to the first two but with a changed order of the treatments (chemical oxidation as the last step). Method 5 included only a treatment with hydrogen peroxide ($\text{H}_2\text{O}_2$) for 7 days. Methods 1 to 4 enabled, in theory, the separation of labile, mineral-protected and recalcitrant SOM fractions. With method 5 only two different fractions can be discerned, i.e. a labile one and a stable one. The lowest ages for each soil were obtained with methods 1 to 3 (5176 and 8835 cal BP). Higher ages were obtained with method 4 (Aqua Regia and NaOCl). Methods 1 - 4 showed increasing ages with increasing soil depth. The $\text{H}_2\text{O}_2$ treatment, however, left behind an organic fraction with the highest ages of up to 17000 cal BP in the topsoil and decreasing ages with soil depth. In general, the $^{14}$C ages of the treatment-resistant fraction increased in the order: untreated samples < method 3 (HF + NaOCl) ≤ method 1 (NaOCl + HF) ≈ method 2 (NaOCl + Aqua Regia) < method 4 (Aqua Regia + NaOCl) < method 5 ($\text{H}_2\text{O}_2$). Before and after the individual treatments, the fractions were analysed for their C
and N content, functional groups were measured with FT-IR and complementary information was obtained with SEM-EDS. The changes in functional groups before and after the treatment with methods 1 and 3 were not very distinct. NaOCl was a too weak reagent to oxidise all labile organic matter in soils. It seems to work more or less properly only in soils with a low OC content. The use of HF produced artefacts such as the precipitation of cryolite. This could also influence organic matter (co-precipitation) and finally its age. HF treatment destroyed kaolinite, attacked quartz but did not affect that much other phyllosilicates like mica. The subdivision of OM into a labile, mineral-protected and recalcitrant fraction using NaOCl and HF was consequently far from being precise. Consequently, these methods might be questioned. About 90% of organic C was oxidised with H$_2$O$_2$ (method 5) but only 60% of N removed. The remaining organic matter was enriched in nitrogen with a C/N ratio between 3 to 10, aliphatic, aromatic and amide compounds. H$_2$O$_2$ isolated the oldest and most stable OM pool. Our results indicate that the isolated organic matter after the H$_2$O$_2$ treatment can probably be used to estimate the age of the earliest formation of Holocene/Pleistocene-aged Alpine soils. The obtained ages corresponded well with the maximum age of charcoal fragments and the geomorphological settings - in particular the end of the Egesen-equivalent glacial state and the oldest Dryas.

**Keywords**: Dating, soil organic matter, $^{14}$C, organic fractions, hydrogen peroxide, hydrofluoric acid, aqua regia

1. **Introduction**

Easily recognisable traces of dramatic climatic variations make high mountain areas unique geotopes and "storytellers" about past as well as potential future climate change effects on landscape dynamics and living conditions in regions of rugged topography. The general pattern of the glacial deposits in Alpine valleys can be associated with distinct readvance phases of the retreating ice-stream systems after the LGM (Last Glacial Maximum; 25'000 – 20'000 cal BP). However, the chronology of these lateglacial fluctuations is still poorly established so far and is based only on a few, often questionable minimum dates (e.g. basal samples of peat bogs), selected pollen profiles and the analysis of lake sediments. $^{14}$C dates with varve counting indicated that alpine valleys (e.g., Upper Engadine, Switzerland) became ice-free prior at approximately 13'500 $^{14}$C yBP (Ohlendorf, 1998). Most of the stadials older than Egesen (Younger Dryas; 11000 – 10000 $^{14}$C yBP), i.e. equivalents of the Daun, Clavadel and Gschnitz stadials, still need direct links to an absolute time scale (e.g., Schlüchter, 1988; Kerschner, 2000; Ivy-Ochs et al., 2004).
Several methods exist which use soils to distinguish surfaces of different ages. However, the methods are far beyond of being all suitable in the Alps (Fitze, 1982; Veit, 2002) and they give, furthermore, rather relative instead of absolute ages. Soil pH, translocation of organic matter, and development of typical spodic horizons all seem to attain a steady state within a few hundred to thousands of years (Egli et al., 2001). The dating of soil organic matter with $^{14}$C could give a numerical value, if humified and stable substances which were produced almost at the beginning of soil formation were found and dated.

Soil organic carbon is known to contain a stable fraction with an old radiocarbon age, even though the stabilisation processes leading to the formation of this old soil carbon pool are not entirely clear (e.g. Eusterhues et al., 2003; Helfrich et al., 2007). Part of the organic carbon in soils is easily mineralised, whereas another carbon pool is known to degrade slowly at timescales from hundred to thousands of years (Oades, 1995). Since the conventional soil organic matter (SOM) fractionation in humic acids, fulvic acids and humin produce SOM pools that hardly differ with respect to turnover rates (Gamper, 1985; Balesdent, 1996) and contents of functional groups (Krosshavn et al., 1992), physical fractionation methods such as particle size and density fractionation have been proposed to analyse the processes of organic matter stabilization in soils (Christensen, 1992; Golchin et al., 1997). Although the concept of OM fractionation into humic and fulvic acids and humin can widely be applied, the technique is operational and does not separate necessarily different types of molecules with a characteristic age (Hayes et al., 1989; Sutton and Sposito, 2005).

Three key processes have been proposed to explain the formation of passive or long-residence-time SOM fractions: (i) specific protection due to chemical recalcitrance, i.e. stabilisation due to the structural properties of the organic matter such as condensed and lignin-derived aromatic carbons, melanoidins, some tannins or aliphatic compounds (Sollins et al., 1996; Baldock and Skjemstad, 2000; Anderson and Paul, 1984; Krull et al., 2003; Poirer et al., 2003), (ii) spatial inaccessibility: inclusion of organic matter into aggregates or micropores, leading to physical protection of organic matter from microbial attack and (iii) sorption processes on minerals and interaction with metal ions which are known to play an important role in SOM preservation (Wiseman and Püttman, 2006) and consequently influence the mean residence time of SOM (Saggar et al., 1996). Metal oxides have been demonstrated to be particularly effective in adsorbing and stabilising organic matter in soils (Kaiser and Zech, 1999; Wiseman and Püttmann, 2006). Nevertheless, the contribution and relative importance of these stabilisation mechanisms are not fully understood. Several authors have used different chemical treatments to extract the most stable OM (organic matter), followed by $^{14}$C dating and in some cases also by comparing the ages of SOM with those of charcoal fragments naturally found in the same soils (Mikutta et al., 2006; Kleber et al., 2005; Eusterhues et al., 2005;
Sodium hypochlorite (NaOCl) has been used to isolate a chemical resistant OM fraction that is bound to mineral surfaces (Kaiser and Guggenberger, 2003). Acid hydrolysis is one of the procedures most commonly used to isolate stable OM because it preferentially removes young, potentially biodegradable compounds (e.g. proteins, nucleic acids and polysaccharides) and leaves behind an old C fraction (Paul et al., 1997, 2001). Acid hydrolysis is, however, inappropriate to distinguish between individual factors of OM stabilisation (e.g. Balesdent, 1996).

Mikutta et al. (2006) performed the oxidation (first step) with NaOCl and used HF to liberate mineral-protected OM by dissolution of the mineral phase. The chemically resistant organic matter showed in most cases the oldest $^{14}$C age. The formation of carbon pools with a long residence time was studied by Eusterhues et al. (2005) using H$_2$O$_2$. This treatment allowed to isolated an organic matter fraction with an age of up to 6000 years which represents an actively stabilised fraction of soil organic matter.

A better understanding of $^{14}$C age profiles in soils is, furthermore, rendered possible by the analysis of SOM fractions in the horizons and comparing them to fossil charcoal naturally buried at similar depths (Pessenda et al., 2001) or in the close neighbourhood of the studied site. Charcoal fragments found in the soils are often considered biologically inert and physically stable in relation to isotopic changes in the environment. Charcoal pieces are carbonised fragments of wood. They can give indication about past fire frequency and paleobotanical proxies to reconstruct past vegetation composition and structure (Berli et al., 1994; Carcailllet and Thinon, 1996; Figueral and Mosbrugger, 2000; Ali et al., 2005;) and, since charcoal fragments > 0.4 mm are not mass-transported by air more than a few meters from the source of ignition, they can be used to get additional information about landscape dynamics and soil evolution during the last thousands of years BP. In Alpine environments, charcoal fragments cannot be time-stratified, because of the possible soil bioturbation by soil fauna, soil reworking by uprooted trees and freeze-thaw processes (Carcaillet et al., 2001). The identification and dating of charcoal gave, however, additional information about soil evolution, landscape history and SOM storage (Figueral and Mosbrugger, 2000).

A comparison of this type of methodologies on the same soil profiles has rarely been done and consequently only a little data about comparable $^{14}$C ages is available. Our aim was to determine the oldest possible SOM-fraction in soils to obtain information about the minimum age of soil formation. We compared the techniques according to Mikutta et al. (2006) where NaOCl and HF were used and the one according to Eusterhues et al. (2005) and Plante et al. (2004) where H$_2$O$_2$ was used. In a third conceptual approach, we hypothesised that a changed order of NaOCl and HF application isolates more organic C that will be oxidised in a second step. We assumed that more
aggregates will (would?) be destroyed using HF as a first step and, consequently, more mineral-protected OM will (would?) be liberated. This procedure should then left behind an older organic C fraction. To reduce risks in handling samples, we substituted in an additional experiment HF by Aqua Regia (3 w/v HCl 32% + 1 w/v HNO₃ 65%).

2. Materials and Methods

2.1. Study area and investigation sites

The investigation area is located in Val di Rabbi, Trentino, in the south Alpine belt in northern Italy (Fig.1). The climate of the valley ranges from temperate to alpine (above the timberline). Mean annual temperature varies from 8.2° C in the valley floor to around 0° C at 2400 m a.s.l., and mean annual precipitation approximately from 800 to 1300 mm/year (Servizio Idrografico, 1959). The timberline is close to 2000-2200 m a.s.l. and the forests are dominated by the conifers Larix decidua and Picea abies (Pedrotti et al., 1974). Above the timberline, alpine meadow is the typical vegetation type. The two investigated sites (Table 1; Fig. 1) were at 2100 m a.s.l. and at 2083 m a.s.l., therefore, close to the timberline. The whole landscape near the investigation sites was strongly influenced by glaciers and both profiles developed on moraines (paragneiss). The investigated sites were covered by ice during the last glaciation period. According to the geomorphological studies of Baroni and Carton (1990) or Filippi et al. (2007), surface ages can be estimated to be around 14000 to 16000 years.

The soil profiles were selected during a soil cartographic inventory and can be considered as undisturbed and representative of the elevation zones. Both soils can be classified as Entic Podzols (Skeletic) according to the WRB (IUSS Working Group, 2006). According to Soil Taxonomy (Soil Survey Staff, 2006), the soil moisture regime is udic (humid conditions, <90 days/year with a dry soil) at all sites and the soil temperature regime is cryic (mean annual temperature < 8 °C, no permafrost). Maximum precipitation occurs during the summer months. Special attention was given to insure that the soils showed an undisturbed evolution with almost no signs of erosion or burial. Soil material was collected from excavated pits and undisturbed soil samples were taken, where possible, down to the BC horizon. Around two to four kilograms of soil material (Hitz et al., 2002) were collected per soil horizon.

2.2. Soil chemistry and physics

The samples were air-dried, large aggregates were gently broken by hand and sieved to < 2 mm. Total C and N contents of the soil were measured with a C/H/N analyser (Elementar Vario EL,
elementar Analysensysteme GmbH) using oven-dried and ball-milled fine earth. Total C corresponds in this case to organic C due to the absence of any carbonates in the soil. Soil pH (in 0.01 M CaCl₂) was determined on air-dried samples of fine earth using a soil solution ratio of 1:2.5. After a pre-treatment of the samples with H₂O₂ (3%), particle size distribution of the soils was measured by a combined method consisting of sieving the coarser particles (2000 - 32 µm) and the measurement of the finer particles (< 32 µm) by means of an X-ray sedimentometer (SediGraph 5100). The dithionite- (Feₐ, Alₐ) and oxalate-extractable (Feₒ, Alₒ) fractions were measured according to McKeague et al. (1971). The elemental concentrations were determined by atomic absorption spectroscopy.

Functional groups and compounds of organic matter as well as some mineralogical features were determined by FT-IR measurement (Bruker, Tensor 27). Spectra were recorded from 4000 to 250 cm⁻¹ on pellets made with 1mg of sample and 250mg of KBr heated at 150°C. Major IR absorption bands and functional groups assignments are given in Table 2. Relative peak intensities were used for quantitative analysis using the software OPUS 6.

2.3. Charcoal
Both soil profiles were sampled for charcoal. Only in one of them large charcoal fragments were found. The identification and dating of charcoal gives a rough idea about fire events, plant communities and landscape evolution. Charcoal was separated from the soil material and dried at 40°C. Being fragile, the charcoal was handled very carefully to reduce any mechanical stress as much as possible. To identify the sources of charcoal, the individual particles were analysed microscopically. The charcoal was investigated in the dry state, micro sections were cut by hand with a razor blade to see the transversal section. Charcoal classification was restricted to black, completely opaque, angular fragments, a conservative criterion necessary to ensure origin by fire (Clark, 1988). The charcoal fragments were separated into coniferous and broad-leaved tree species (Schoch, 1986) with a stereomicroscope (magnification 6.4-40x, Wild M3Z Leica, Germany). The charcoal fragments from the coniferous trees were divided further at the genus level using a reflected-light microscope (objective 5x, 10x, and 20x, Olympus BX 51, Japan). The observations were compared with a histological wood-anatomical atlas, using an identification key (Schweingruber, 1990). Charcoal samples were treated with hydrochloric acid, followed by a treatment with sodium hydroxide to remove humic acids formed during the rotting process.

2.4. Radiocarbon dating
The samples (soil or charcoal fragments) were heated and catalytically reduced over cobalt powder at 550°C to elemental carbon (graphite). This mixture was pressed into a target and the ratios $^{14}$C:$^{12}$C were measured by Accelerator Mass Spectrometry (AMS) using the tandem accelerator of the Institute for Particle Physics at the Swiss Federal Institute of Technology Zürich (ETH). The calendar age was obtained using the CalibETH calibration program (Niklaus, 1991).

2.5. Fractionation of organic matter

Our conceptual approach was based on the finding that partial oxidative degradation of OM leaves behind intrinsically resistant as well as mineral-protected organic materials. We treated the soils using five different methodologies (Table 3) to eliminate more labile organic material from more refractory organic matter having a longer mean residence time (Eusterhues et al., 2005; Mikutta et al., 2006; Plante et al., 2004).

i) The first two methods were based on the oxidation of “fresh” OM by 10% NaOCl followed by mineral dissolution using 10% HF (method 1; Mikutta et al., 2006, modified) or 1M Aqua Regia (method 2). 8 grams of air-dried soil samples were treated with 80 ml of 10 wt% NaOCl, which was adjusted at pH 8.0 by adding 32% HCl. Three treatment cycles of 6h were performed at 25 ± 1°C. Samples were then centrifuged ($2574 \times g$; 5 min), decanted, and the residues washed three times with 60 ml 1M NaCl by hand stirring, then centrifuged and the supernatant discarded. The residues were then washed from NaCl with deionised water to reach an electrical conductivity of < 40 µs cm$^{-1}$ and then freeze-dried. The NaOCl-treated samples were subsequently extracted with 10% HF or 1M Aqua Regia in order to dissolve minerals and release stabilised OM. After the NaOCl treatment, weights were recorded, C and N analyses done and $^{14}$C dating performed. In the subsequent step, three grams of freeze-dried samples were transferred into pre-weighted plastic bottles and treated four times with 20 ml 10% HF or 20 ml 1M Aqua Regia (experiment in parallel on the same sample). The samples were shaken for 2 h at 150 rpm, centrifuged ($2574 \times g$; 5 min), and the supernatant discarded. The residues were washed five times with 20 ml deionised water and freeze-dried. The same types of analyses as performed after the NaOCl treatment were done on both residues.

After the HF or Aqua Regia treatment, the amount of organic C was related to the initial content with a mass-balance approach to obtain the corresponding recoveries with

\[
\frac{(gC_{after} / gC_{before}) \times 100}{(1)}
\]

The same recoveries were calculated for the amounts of N and of dry matter.
ii) The approach for methods 3 and 4 is similar to that of methods 1 and 2 but with a changed order of treatments, i.e. chemical oxidation as the last step. Briefly, 7.5 g of untreated soil were treated four times with 50 ml of 10% HF (= method 3) or with 1 M Aqua Regia (= method 4). The samples were shaken for 2 h at 150 rpm, centrifuged (2574 × g; 5 min), and the supernatant discarded. The residues after the HF treatment were transferred into centrifuge tubes and washed with 50 ml deionized water five times. The samples were then freeze-dried and the weight recorded. Parts of the HF and Aqua Regia residues were used for 14C dating and total C and N. Four grams of the freeze-dried residues were transferred into 250 ml plastic bottles to perform the oxidation. Using 40 ml of 10% NaOCl in three replicates, the samples were shaken after three treatments (cycles of 6 h), centrifuged (2574 × g; 5 min) and the supernatant discarded. The residues were then washed using 30 ml of 1 M NaCl by hand stirring in centrifuge tubes for 3 times. Subsequently, the removal of NaCl was performed with 30 ml of deionised water until the electrical conductivity was < 40 µs cm⁻¹. The samples were then freeze-dried and the weight recorded, 14C dated and analysed for C and N.

iii) The third conceptually different technique (= method 5) is based on the oxidation of the OM by 10% H2O2 (Eusterhues et al., 2005; Plante et al., 2004, modified). 1 gram of air-dried soil was wetted for 10 min with distilled water in a 150-ml beaker. Afterwards, 90 ml of 10% H2O2 were added. The procedure was run at a minimum temperature of 50° C throughout the treatment period. The beakers were closed with two layers of parafilm to avoid evaporation of the reagent. Peroxide treatments were performed for 168 h (7 days). At the end of the treatment the samples were washed three times with 40 ml deionised water and freeze-dried, the weight recorded, analysed for total C and N and 14C dated.

2.6. SEM and EDS analyses
Selected treated and untreated soil samples were analysed by scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS). The samples were prepared for the SEM analysis by first sonicating 10 µg of material for 30 s in 10 ml of deionised water and then transferring 60 µl of the solution onto standard aluminium Al-SEM stubs covered with aluminium foil. The analysis was performed using a Philips XL20 SEM operating at an accelerating voltage of 20 kV. Identification of minerals and organic matter was aided by the use of EDS which provides an elemental composition of the solid phases analysed. The EDS detector was equipped with an ultra thin window allowing detection of elements down to carbon.

2.7. Isotopic analysis of organic matter fractions
Following archaeological protocol, conventional radiocarbon ages were calculated using the Libby
half-life (5568 years; mean life 8033 years) rather than the correct value of 5730 years (Stuiver and
Polach, 1977) and were referenced to 1950. Conventional radiocarbon age provides a qualitative
indication of C residence time and stability (Mikutta et al., 2006). The isotopic content \( I \) of labile
OC pools could not be measured directly. The \( pMC \) (percent of modern carbon) of NaOCl (\( \text{H}_2\text{O}_2 \))-removable OC (LOC), HF (Aqua Regia)-removable OC (MOC) were calculated from mixing
models (Mikutta et al., 2006):

\[
I - C_L = \frac{((I - C_U) \cdot \text{UOC} - (I - C_S) \cdot \text{SOC})}{\text{LOC}}
\]

\[
I - C_M = \frac{((I - C_S) \cdot \text{SOC} - (I - C_R) \cdot \text{ROC})}{\text{MOC}}
\]

where \( I-C_U, I-C_L, I-C_S, I-C_M \) and \( I-C_R \) are, respectively, the values of untreated, NaOCl removable,
NaOCl-resistant (stable), mineral-protected and chemically resistant OM. \( \text{UOC} \) (untreated OC),
\( \text{LOC} \) (labile OC), \( \text{SOC} \) (stable OC), \( \text{MOC} \) (mineral-protected OC) and \( \text{ROC} \) (resistant OC)
correspond to the respective OC contents.

3. Results

3.1 Soil characteristics

The soils are acidic and have a high SOM content in the surface horizon. They are furthermore
characterised by a high proportion of soil skeleton which is typical for Alpine soils on a morainic
substratum. The soils exhibited a sandy loam to loamy texture; the proportion of sand decreased
towards the soil surface, and correspondingly silt and clay increased (Table 4). The upward
decrease of the grain sizes is a concomitant effect of weathering. The soils are well developed and
have a bleached horizon followed by a horizon with an accumulation of sesquioxides.
Consequently, the soils are acid and show an eluviation and illuviation of Fe and Al (Table 4).

3.2. Fractionation of organic matter

Qualitative aspects of organic matter fractions

Depending on the soil and horizon, the NaOCl treatment decreased the OC content by about 36-
62% of the initial OC and ranged between 8.4- 71.6 g kg\(^{-1}\) soil (Table 5). NaOCl resistant N was
with 0.3 – 2.2 g kg\(^{-1}\) very low and represented around 25-50% of the initial content. HF treatment
released 25% of the NaOCl-resistant organic carbon which corresponds to the mineral protected
organic carbon (MOC) and around 14% of N. Aqua Regia treatment, after NaOCl, reduced the
sample weight less than with HF, but released 26% of the stable OC and 12% of the NaOCl-stable N (both values almost identical to the HF treatment) (Table 5). After the HF treatment (method 3), a distinct increase in the OC and N concentration was measured in both profiles in the upper horizons (Table 5). These results are consistent with findings of Schmidt and Gleixner (2005). HF reacts with silicates and oxides to form soluble fluoride complexes, but is expected to leave organic matter mostly unaffected (Eusterhues et al., 2007). It is used to enrich soils and sediments in organic matter before pyrolysis (Zegouagh et al., 2004). In the second step of the treatment, hypochlorite oxidised a part of the OM and generally decreased the OM concentration. HF as the first step reduced the dry matter by about 50% in both profiles and hypochlorite as the second step by about 10%. The Aqua Regia treatment led to a decrease in OC of about 65-70% and 17-30% for N. The NaOCl treatment after Aqua Regia decreased the C content additionally from about 7% in the first profile to 15% in second profile and the N content from about 40 to 55%, respectively (Table 5). The differences between the procedures using HF or Aqua Regia were distinct with respect of the OC and N content as well as the C/N ratio (Table 5). The concentrations of OC and N after the different steps were much lower with the Aqua Regia procedure. Aqua Regia did not dissolve that much silicate minerals. In addition, the C/N ratios were different with lower values, especially in the subsoil, with the Aqua Regia procedure. In the subsoil, N-rich organic compounds that are strongly associated with (clay)minerals are less attacked by Aqua Regia and by the subsequent oxidation with NaOCl. In contrast, HF seems to dissolve such relatively stable mineral – organic matter complexes due to a partial dissolution of phyllosilicates. The hydrogen peroxide (H$_2$O$_2$) method was the most effective in reducing the C content. Profile 1 had a carbon concentration ranging from 2.1 to 15 g/kg after the treatment. This corresponds to an oxidized percentage of about 90% (Table 5). The highest resistance to oxidation with 15g/kg soil or 25% of the initial total organic carbon was found in the BE horizon. In both profiles, the decrease in total N was less expressed compared to C giving rise to low C/N ratios. This finding is in agreement with Schulten et al. (1996) and Cheshire et al. (2000) who found oxidation-resistant organic matter enriched in N-compounds.

Recovery of dry matter
The recovery of mass after methods 1 and 3 (using HF) was always around 30-40% (Table 6). More mass, usually between 62 and 74%, was recovered using Aqua Regia (methods 2 and 4) instead of HF. The difference is mainly due to the dissolution of silicatic bonds (Si-O) with HF (Rumpel et al., 2006). Mass recovery with H$_2$O$_2$ was in a similar range with those obtained by the other methods using Aqua Regia.
Recovery of org. C

Methods 1 and 2 (hypochlorite followed by HF or Aqua Regia): about 50% of the initial C mass was oxidised after the NaOCl treatment. Additional organic C was removed after the application of HF or Aqua Regia, so that at the end about 7-18% of the initial amount of C remained. In profile Rabbi 38, the recovery generally decreased with soil depth while in the other profile no trend with depth could be observed. In most cases, the procedure with Aqua Regia gave similar results to HF, except for the AE horizon of LAX 1.

Methods 3 and 4 (HF or Aqua Regia followed by hypochlorite): In contrast to our expectations, the changed order of reagents with HF or Aqua Regia as the first step led to a higher recovery of C. With HF and NaOCl, up to 77% of the original amount of C remained unaffected. Especially in the topsoil, the differences to method 1 were large while in the subsoil almost the same values were obtained.

Method 5 (H$_2$O$_2$): C recovery was very low after the hydrogen peroxide treatment. In contrast to the other two methods, the H$_2$O$_2$ treatment led generally to an increase of the recovery value with soil depth. Much more organic matter was oxidised in the topsoil than with the other two methods. In the subsoil, less organic matter was removed compared to the other methods.

Recovery of N

Methods 1 and 2: In the soil Rabbi 38, already a substantial amount of the initial N was eliminated after the NaOCl treatment while in the other soil a large part remained unaffected. The following treatment with HF gave slightly lower recoveries than with Aqua Regia. In contrast to C, the recoveries increased with soil depth for both treatments – HF and Aqua Regia. The resistant organic matter in the subsoil was consequently richer in nitrogen.

Methods 3 and 4: The changed order of the reagents led to much higher recoveries. Especially with HF, the removal was – except for the subsoil – very ineffective. Compared to HF, Aqua Regia eliminated more N. In contrast to method 1, a decrease of recoveries was measured with soil depth using HF. With Aqua Regia a slight increase can be measured, but the recoveries were distinctly higher than with method 1.

Method 5 (H$_2$O$_2$): Low N recoveries were recorded for the topsoil. In the subsoil, however, only a minor part of N was oxidised and recoveries values were up to 60-70% (Tables 5 and 6).

3.3. Radiocarbon ages of SOM fractions
Methods 1 and 2: A distinct increase in age was measurable from untreated to NaOCl-treated (1st step) and to HF- or Aqua Regia treated (2nd step) samples. Although the fractionation of organic carbon with HF and Aqua Regia was different, the obtained ages at the end of the treatment procedure were almost identical (Table 7). The obtained ages after the treatments ranged from “modern” to 8835 ± 116 cal BP in the first profile, and from 609 ± 41 to 5781 ± 87 cal BP in the second one (Table 7).

Methods 3 and 4: The distribution of the different C fractions showed a greater recalcitrant pool, together with a distinct decrease in the C/N ratio, especially after the application of Aqua Regia. Although less carbon was removed compared to methods 1 and 2, the obtained ages were higher. They went up to 10946 ± 142 cal BP in the first profile, and up to 7981 ± 6 cal BP in the second one (Table 7). In both profiles, an increase in age with soil depth was measured, similar to methods 1 and 2. The changed order of reagents obviously destroys less organic matter, but eliminates more young organic matter. The Aqua Regia can be used as a reagent that clearly leaves behind an old OM pool. Aqua Regia is in this respect at least equally powerful as HF in obtaining a stable organic fractions.

Method 5: The obtained ages of OM that resisted H$_2$O$_2$ were definitively the highest compared to the other methods. They varied from 11068 ± 147 to 17729 ± 237 cal BP in the first profile, and from 8498 ± 64 to 10851 ± 174 cal BP in the second one (Table 7). Additionally, the age trend within the soil profile was different to the other methods (cf. also Mikutta et al., 2006; Eusterhues et al., 2003, 2005; Rumpel et al., 2006). Surprisingly, the highest ages were measured in the topsoil. This would, however, be logic as soil organic matter which derives from organic litter and dead roots is produced first in the topsoil and not in the subsoil. In contrast to the opinion of many authors (e.g. Scharpenseel and Becker-Heidmann, 1992; Rumpel et al., 2002), the oldest organic matter fraction can under certain circumstances still be found in the topsoil – if a suitable reagent is used that allows its detection.

3.4. Charcoal

Charcoal pieces can occasionally be found in soils of the investigated area. Dating of charcoal fragments from the horizons of the second profile (LAX 1) gave increasing $^{14}$C ages with soil depth varying from 3271 ± 65 cal BP in the upper horizon to 10353 ± 86 cal BP in the lower one (Table 7). Because the oldest charcoals were found in the lower horizons, the increase of age with increasing soil depth can be explained by a downward migration of charcoal caused by soil fauna or soil water (Carcaillet, 2001). The charcoal fragments could be identified as Larix and Pinus - the same kind of trees which actually dominate the forest. After about 150-300 years of soil formation,
first Larix-trees are able to grow at such an altitude according to the plant succession model of Burga (1999). The measured age of 10353 yBP of the charcoal and the minimum time necessary for tree-growth would give a minimum age of soil formation of about 10600 yBP which corresponds very well with the measured age of the resistant organic matter fraction in the surface horizon after the H$_2$O$_2$ extraction.

3.5. **FT-IR and SEM analyses**

FT-IR spectra of the H$_2$O$_2$-treated samples showed that the oxidation-resistant OM is relatively enriched in aliphatic, aromatic and amide compounds (Figs. 2 and 3) as found also by Theng et al. (1986) and Leifeld and Kögel-Knabner (2001). The other methods did not show a great difference before and after the treatment. The aromatic band shifts measured on the H$_2$O$_2$-resistant fraction are most probably due to the presence of charcoal-like substances. H$_2$O$_2$ was very effective in removing organic matter which was clearly visible with SEM (Fig. 4). However, charcoal particles remained. SOM in between soil minerals and, consequently, adsorbed on mineral surfaces partially remained. SEM analyses demonstrated that H$_2$O$_2$ did not attack minerals but destroyed almost completely the soil organic matter, leaving a few unaltered charcoal fragments (Fig. 4).

NaOCl and HF (method 1) were not very effective in removing organic matter: larger, single organic tissues having most probably a low $^{14}$C age, small roots, etc. remained (Fig. 4). Many organic particles also remained in the soil sample when HF is first used and then NaOCl (method 3). HF seemed to attack particularly quartz and dissolve phytoliths. Quartz grains had many and quite strong fissures whereas other minerals like mica seemed to be much less affected (Fig. 4). Quartz in the untreated sample showed no distinct fissures. This effect can, thus, be definitely attributed to the HF-treatment. The untreated sample had, not surprisingly, a higher amount of OM. After HF treatment, FT-IR analyses showed the dissolution of silicate minerals and the formation of artefacts (cryolite, a sodium aluminium fluoride) (Fig. 3).

3.6. **Correlation analyses**

The untreated soil material showed a positive correlation between OC and the clay content (Fig. 5). This is not necessarily a proof that SOM is stabilised and protected from decay by clay minerals because both parameters also are a function of soil depth. Except for the H$_2$O$_2$ treatment, a positive correlation of SOM with the clay fraction was found after each treatment. This could mean that the higher the amount of clays, the more organic matter remains protected from degradation. This is, however, not fully true. The clay content correlated with the $^{14}$C age, i.e. the higher the clay content the lower was in general the age of the remaining SOM (Fig. 5). Such a trend was evident after each
treatment, except for the H$_2$O$_2$ method. The more organic C is present in the soil, the more fresh material also adsorbs onto surfaces (Fig. 6). Methods 1 - 4 were consequently not able to fully eliminate this young material. Only in soil horizons with a low C content, i.e. in the subsoil, the destruction of young SOM material was effective enough to give rise to very high $^{14}$C ages. The pMC of the removed OM correlated positively with the clay fraction and the SOM content after each treatment (Fig. 7), except with the H$_2$O$_2$ technique and the usage of NaOCl as the first treatment step. The oxidation with either NaOCl or H$_2$O$_2$ removes primarily young OM. Hypochlorite was not efficient enough to remove all labile C. The higher the OM concentration in the soil, the more labile C was removed by HF and Aqua Regia.

In untreated soils, nitrogen did not correlate very well with the clay fraction. This relationship was better after the treatments (except after H$_2$O$_2$). N-containing components were obviously more resistant to the treatments and were partially protected by clays.

No or negative correlations were found between the SOM of untreated samples SOM after the individual treatments and Fe-Al mineral phases. Only method 3 gave a positive correlation between the HF-extracted amount of SOM with oxyhydroxides. HF was obviously able to release additional organic matter by destroying oxyhydroxides.

**Discussion**

The methods that included HF (1 and 3) treatment led, in general, only to a small decrease in OM which agrees well with findings of other authors (i.e. Schmidt and Gleixner, 2005; Eusterhues et al., 2007). The overall loss in weight was, however, greatest using HF due to the dissolution of silicate minerals. Purification of SOM using HF includes the partial breakdown of Si-O bonds leading to the solubilisation of silicate minerals (Rumpel et al., 2006). HF and Aqua Regia have different strengths in breaking such bonds which explains the different weight of the samples after the treatments. The differences between the HF or Aqua Regia procedure (methods 1 and 2) were in general small with respect to the OM fractions and $^{14}$C ages. The changed order of the reagents showed that Aqua Regia was, in contrast to HF, a more powerful reagent to eliminate organic matter leaving behind an older resilient fraction. The H$_2$O$_2$ treatment (method 5) was the most efficient method in isolating stable OM. This is in agreement with the results of Helfrich et al. (2007). Independent of the org. C concentration, H$_2$O$_2$ removed the easily degradable and, thus, labile OM fraction. N-containing compounds were less affected by oxidation. The other methods (methods 1 - 4) partially destroyed the old organic fraction and, consequently, gave rise to lower ages of the resistant fractions. Especially in the topsoil, where a high amount of organic matter was
found, considerable proportions of young SOM remained unaffected. The high concentrations of org. C in the topsoil obviously hindered an effective dissolution of minerals and the subsequent oxidation. The subdivision of organic matter into a labile, mineral-protected and recalcitrant fraction using the NaOCl and HF method produces, therefore, many uncertainties and is far from being precise.

According to the obtained results, the $^{14}$C ages increased in the order: untreated samples $<$ method 3 (HF + NaOCl) $\leq$ method 1 (NaOCl + HF) $\approx$ method 2 (NaOCl + Aqua Regia) $<$ method 4 (Aqua Regia + NaOCl) $<$ method 5 (H$_2$O$_2$).

Except for the H$_2$O$_2$ method, a negative correlation was found between the OC content and the $^{14}$C age. The higher the content of SOM, the lower was the determined age (Fig. 6). This is in agreement with the results of Rumpel et al. (2002). They presumed that young carbon is located in horizons with a high carbon content. Only in the subsoil or in horizons with a low organic C concentration, NaOCl (methods 1 - 4) was effective enough in removing soil OC with a relatively short residence time, leaving behind a more stable fraction (Kleber et al., 2005; Mikutta et al., 2006). In contrast, H$_2$O$_2$ was able to oxidise all labile OM and to isolate old and stable organic matter from soils having a high organic C content.

Calibrated radiocarbon ages of the treatment resistant fraction of methods 1-4 showed an increase with soil depth in both profiles which agrees well with results from other authors (e.g. Mikutta et al., 2006; Rumpel et al., 2002). The first oxidation process step leaves behind intrinsically resistant organic materials (Theng et al., 1992; Cuypers et al., 2002). In contrast to all other methods, the highest ages of SOM after the treatment with H$_2$O$_2$ were measured in the topsoil. Young, contemporary carbon is transported by percolation in highly acidic soils, via root growth, animal transport and through soil turbation processes, such as cryoturbation, bioturbation, downward peloturbation, inflecting also the subsoil to rejuvenation (Scharpenseel and Becker-Heidmann, 1992; Rumpel et al., 2002). Most techniques used so far in dating “resistant fractions” of SOM showed an increase of age with soil depth. This increase in age was explained by a continuous rejuvenation of SOM from the soil surface. Most of modern carbon is less than 100 years old and decreases exponentially with increasing depth leading to an increase of the percentage of old carbon present (O’Brien and Stout, 1978; Mikutta et al., 2006). In the investigated Alpine soils (Holocene/Pleistocene), 3-15% of SOM in the surface horizons must be attributed to a very old OM fraction obtained after the H$_2$O$_2$ treatment. Although the proportion of this fraction increases with soil depth – which would fit with the findings of O’Brien and Stout (1978) – its mean age decreases. This trend would in theory, however, fit with the theoretical expectances. Soil formation starts at the surface and proceeds with time in greater depths. Organic matter from plants and
animals is incorporated into soil material and mixed with the mineral part. Because the investigated soils are podzols and consequently have a low biological activity and no earthworms, the ages of the old and stable SOM with increasing soil depth should decrease. Humic compounds may contract close and strong associations with the mineral phase, especially with clay minerals (Righi and Meunier, 1995). Clay organic complexes, once formed, do not easily exchange the organic component with infiltrated younger humus components (Scharpenseel and Becker-Heidmann, 1992; Fig. 5). In the investigated soils, some of the initially formed OM seems still to be present and detectable using the H$_2$O$_2$ treatment.

After methods 1 to 4, a lower C/N ratio was found in the subsoil where most probably N-rich compounds interact with the mineral phase. Stabilisation of soil organic matter in soils is predominantly due to its interaction with mineral phases. According to Kleber et al. (2007) interaction occurs with polar organic functional groups of amphiphiles via ligand exchange with singly coordinated mineral hydroxyls forming stable inner-sphere complexes, proteinaceous materials adsorbed on charged surfaces (“contact” zone; Kleber et al., 2007) and association of hydrophobic substances with noncharged mineral surfaces (“zone of hydrophobic interactions”; Kleber et al., 2007). The higher C/N ratio of the resistant organic matter in the topsoil after methods 1 to 4 indicates that N-rich material was preferentially digested which would agree with results of Schmidt and Gleixner (2005) who noted a preferential loss of N over C during the HF treatment. Either N-rich organic matter which is strongly bound to clay minerals (= organic fraction of the “contact zone”; Kleber et al., 2007) or fresh material from the outer region (kinetic zone according to Kleber et al. (2007)) was eliminated. Because NaOCl was not efficient enough to remove all young OM, we must assume that HF and Aqua Regia also removed relatively easily degradable N-rich compounds from the outer zones (discrete zonal model according to Kleber et al., 2007).

The distribution of organic functional groups changed only slightly from the untreated to the NaOCl-treated and HF-treated soils (Fig. 2; highest proportion of C=O stretching of amide groups). This means that the treatments did not preferentially attack some specific functional groups or components. Methods that include Aqua Regia led to a relative enrichment of aliphatic and aromatic compounds. Furthermore, the oxidation- and Aqua Regia-resistant organic matter fraction was enriched in N when compared to the untreated samples (Table 5). Similarly, the H$_2$O$_2$ treatment led to a resistant fraction which had not only a distinctly higher aliphatic content, but also more aromatic functional groups. The aromatic groups are indicative of charcoal-like substances. Several authors (e.g. Eusterhues et al. 2005; Cheshire et al., 2000) found oxidation-resistant organic matter enriched in aliphatic C and N-containing compounds. Aliphatic material can be refractory in nature (e.g., fatty acids, waxes) and can bind to minerals by a variety of mechanisms. Specifically, the
alkyl chain interacts with hydrophobic parts of the mineral matrix via van der Waals forces, whereas the carboxylic, alcoholic or amino functional groups may form hydrogen, ionic or coordination bonds with polar sites on mineral surfaces (Deng and Dixon, 2002). In addition, aliphatic compounds are believed to penetrate the interlayer space of expandable phyllosilicates at low pH (Theng, 1974).

The HF-method partially destroyed mineral surfaces and liberated consequently strongly adsorbed N-rich compounds, while Aqua Regia affected the minerals less and did not liberate that many N-rich compounds. The H$_2$O$_2$ treatment (method 5) was the most efficient method in eliminating organic matter. After treatment, a N-rich and aliphatic organic fraction remained in the soil – in every horizon. The low C/N ratio after treatment does, however, not necessarily confirm that stabilisation of the H$_2$O$_2$-resistant soil organic matter is only due to its interaction with mineral phases. As the residual fraction (after treatment) did not show a good correlation with the clay content, we must assume that also refractory organic material exists with almost no interaction with minerals. Charcoal fragments which were not dissolved by H$_2$O$_2$ and were detected with SEM must therefore have a high age and be very stable. The other methods (methods 1 - 4) partially destroyed and mixed this old fraction with younger ones.

The FT-IR analyses confirmed the dissolution of kaolinite and other silicates with HF (Fig. 3). After the HF-treatment, the peaks in the band region 650 cm$^{-1}$ and near 515 cm$^{-1}$ had, however, a higher intensity (Fig. 3). These peaks (Anghel et al., 1999) are typical for the mineral cryolite (Na$_3$AlF$_6$). Cryolite was obviously formed during the HF-treatment by a reaction between Al and Na (deriving from minerals) and F from HF. The precipitation of Al-F-phases could also have caused a co-precipitation of (young?) SOM which could have affected the age of the resistant organic matter.

All methods used gave a higher age for soil Rabbi 38 than LAX 1. This was also confirmed by the higher evolitional status of the former with a more expressed podzolisation (i.e. expressed by the differences between subsoil and surface horizon regarding Al$_o$, Al$_d$ and Fe$_o$). There is a good agreement with the age obtained from dating of charcoal and H$_2$O$_2$-resistant organic matter in soil profile LAX 1. The soil age derived from charcoal gives 10700 cal BP and the one derived from OM (H$_2$O$_2$ resistant) 10851 cal BP. This age corresponds well with the end of the Egesen-equivalent glacial state (Maisch et al., 1999; Kerschner, 2000). Soil formation could have started approximately 11000 yBP. The soil profile Rabbi 38 would consequently have an age of 17729 cal BP (oldest age of the H$_2$O$_2$-resistant OM fraction). This age fits to the documented deglaciation occurred worldwide during the three millenia 18 – 15 ka BP, obtained by ice cores and global sea level (Fairbanks, 1989; Johnsen et al., 1992; Maisch et al., 1999) (). LAX 1 is on a moraine within a glacial cirque where a small, alpine glacier probably re-advanced during the Egesen-equivalent.
Rabbi 38, however, seems to be on an older part of the moraine which most probably became ice-free after the decay of the main valley-glacier. Consequently, the morphological position of the soil profiles supports the findings of the dating.

Helfrich et al. (2007) obtained very large ages for SOM when using H\textsubscript{2}O\textsubscript{2} or Na\textsubscript{2}S\textsubscript{2}O\textsubscript{8}. The \(^{14}\text{C}\) age of the H\textsubscript{2}O\textsubscript{2}-resistant organic matter from soils developed on loess was in the range of 13000 y BP, which would correspond to a calibrated age of around 16000y BP. These authors suggested that some of the old, stable SOC was not formed in situ, but was already present in the loess deposits. The measured age would, however, fit quite well with the Lateglacial when loess was deposited.

Conclusions

We tested five different methods to extract the oldest possible organic matter that potentially could be used as a tool for dating soils or the start of soil formation. Methods 1 and 2 consisted of a NaOCl oxidation followed by a treatment with either HF or Aqua Regia. In methods 3 and 4, the order of the treatments was changed with the application of HF or Aqua Regia as the first step and the oxidation with NaOCl as the second. In method 5, only H\textsubscript{2}O\textsubscript{2} was used. We obtained the following main findings:

- the H\textsubscript{2}O\textsubscript{2} treatment leaves behind an organic fraction with the highest ages.
- in contrast to the other methods, the highest \(^{14}\text{C}\) age was measured in the topsoil using H\textsubscript{2}O\textsubscript{2}.
- The treatments with Aqua Regia gave similar results to that using HF with respect of the ages and size of the different organic matter pools. HF is a dangerous reagent and can probably be substituted by Aqua Regia, at least when Alpine soils developed on a silicate parent material are investigated.
- The changed order of the NaOCl and HF / Aqua Regia treatments produced only different results for Aqua Regia, where higher ages were obtained with Aqua Regia as the first step. Reversal of HF and NaOCl treatments gave identical results.
- Methods 3 and 4 leave behind a N-richer OM after the treatment and, in general, higher ages than methods 1 and 2.
- NaOCl is too weak a reagent to oxidise all labile organic matter in soils. It seems to work more or less properly only in soils with a low OC content.
- The use of HF (in method 1) produced artefacts (precipitation of cryolite). This could also influence organic matter (co-precipitation) and finally its age.
- HF treatment destroyed kaolinite, attacked quartz but did not affect that much other phyllosilicates (like mica).
- The subdivision of organic matter into a labile, mineral-protected and recalcitrant fraction using the NaOCl and HF method produces many uncertainties and is far from being precise. A clear separation of these SOM fractions is not possible when the soils have a large content of organic matter.

- Clay minerals protect OM from degradation. There is, however, a high competition for adsorption places of OM in surface soils. According to our results, we propose the following conceptual model for resilient OM: one part of OM seems to be very strongly fixed to (clay) minerals and almost no exchange with surrounding environment occurs. In addition, a very resistant recalcitrant OM must be present probably due to resistant charcoal which does not interact with the inorganic part of the soil.

- The application of HF or Aqua Regia destroyed most probably a part of the strongly fixed OM. H$_2$O$_2$, however, is able to remove the younger fractions without affecting the oldest one. The isolated pool of organic matter after the H$_2$O$_2$ treatment is an inert fraction of SOM with a mixture of charcoal and organic materials strongly adsorbed on or trapped in clays (see Frink, 1995; Skjemstad et al., 1996; Falloon et al., 1998).

- The H$_2$O$_2$ treatment seems to enable the dating of undisturbed Holocene/Pleistocene-aged soils. This has, however, to be confirmed with additional absolute dating techniques such as exposure dating of rock surfaces with $^{10}$Be, $^{26}$Al or comparison with moraines of known age.

Acknowledgements
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References


Table 1. Characteristics of the study-site.

<table>
<thead>
<tr>
<th>Profile</th>
<th>Elevation (m a.s.l.)</th>
<th>Aspect</th>
<th>Slope</th>
<th>Parent Material</th>
<th>Vegetation</th>
<th>Land use</th>
<th>WRB (IUSS Working Group, 2006)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbi 38</td>
<td>2100</td>
<td>60° N</td>
<td>32%</td>
<td>Paragneiss</td>
<td>Larix decidua Juniperus spp.</td>
<td>Natural forest</td>
<td>Entic Podzol (Skeletic)</td>
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<tr>
<td>Lax 1</td>
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<td>32%</td>
<td>Paragneiss</td>
<td>Larix decidua Juniperus spp.</td>
<td>Natural forest</td>
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</tr>
</tbody>
</table>

Table 2. Major IR absorption bands and assignments (Piccolo and Mirabella, 1985; Stevenson, 1994; Senesi et al., 2003; Tan, 2003).

*Assignment*

<table>
<thead>
<tr>
<th>Band</th>
<th>Wave number</th>
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Table 3. Overview of the methodologies used for stable organic matter extraction.

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<tr>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 3</th>
<th>Method 4</th>
<th>Method 5</th>
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<td>10% NaOCl</td>
<td>10% HF</td>
<td>1M Aqua Regia (HCl + HNO₃)</td>
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<tr>
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<td>10% HF</td>
<td>1M Aqua Regia (HCl + HNO₃)</td>
<td>10% NaOCl</td>
<td>10% NaOCl</td>
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</table>

Table 4. Selected properties of the investigated soils.

| Profile/ Horizon | Soil depth (cm) | Colour | Skeleton¹ | pH (CaCl₂) | Cₜot (g kg⁻¹) | N (g kg⁻¹) | Sand² (g kg⁻¹) | Silt (g kg⁻¹) | Clay (g kg⁻¹) | Alₐ (g/kg) | F (g/)
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<td>79</td>
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¹ Skeleton = material > 2mm in diameter
2 Size fractions: sand = 2000–62µm, silt = 62–2µm, clay = <2 µm

Table 5. Total organic C (OC), nitrogen (N) and C/N ratios of SOM before and after every single treatment.

<table>
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<tr>
<th>Profile/ Horizon</th>
<th>Untreated samples</th>
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<th>Method 3</th>
<th>Method 4</th>
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<tr>
<td></td>
<td>OC (g kg(^{-1}))</td>
<td>NaOCl N (g kg(^{-1}))</td>
<td>2nd step OC (g kg(^{-1}))</td>
<td>HF N (g kg(^{-1}))</td>
<td>2nd step OC (g kg(^{-1}))</td>
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<tr>
<td>Rabbi 38</td>
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<tr>
<td>AE</td>
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<td>HF N (g kg(^{-1}))</td>
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*AqR = Aqua Regia Treatment

Table 6. Recoveries (ratio of measured amount to the initial amount) of organic C, nitrogen and dry matter after each treatment and used method.
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<th>Method 1 HF mass</th>
<th>Method 2 NaOCl mass</th>
<th>Method 2 AqR mass</th>
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AqR = Aqua Regia treatment

Table 7. Calibrated radiocarbon ($^{14}$C) ages (y calBP) of soil organic matter fractions and charcoal.
Fig. 1. Location of the investigated sites

Fig. 2. Mean distribution (relative proportion) of the IR-bands (and corresponding functional groups) before (untreated) and after the different treatments
Fig. 3 Comparison of FT-IR spectra before any treatment and (A and B) after the HF treatment (method 1) and (C) after the H$_2$O$_2$ treatment (method 5).

Fig. 4. SEM micrographs with EDS analyses of untreated (A), HF + NaOCl-treated (B), and H$_2$O$_2$-treated (C and D) samples of the Bs1 horizon (Rabbi 38)
Fig. 5. Correlation between the organic C before and after the treatments (method 1, 2 and 5) and the clay content (A-D). The organic C-content was recalculated using the recovery values. The measured $^{14}$C ages (calibrated) of SOM in untreated samples, samples treated with NaOCl and Aqua Regia (method 4) and samples treated with H$_2$O$_2$ (method 5) are compared in (E).

Fig. 6. Relationship between the content of soil organic C and the calibrated $^{14}$C ages obtained with the individual treatment techniques. (A) untreated samples, (B) method 1 (NaOCl + HF), (C)
method 2 (NaOCl + Aqua Regia), (D) method 3 (HF + NaOCl), (E) method 4 (Aqua Regia + NaOCl), (F) method 5 (H_2O_2).

Fig. 7. Relationship between the pMC value (percent of modern carbon) of organic matter removed by NaOCl (method 1) and the clay content (A) and the soil organic C content (B).