Age distribution, extractability, and stability of mineral-bound organic carbon in central European soils

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Abstract. The largest share of total soil organic carbon (OC) is associated with minerals. However, the factors that determine the amount and turnover of slower versus faster cycling components of mineral-associated carbon (MOC) are still poorly understood. Bioavailability of MOC is thought to be regulated by desorption, which can be facilitated by displacement and mobilization by competing ions. However, MOC stability is usually determined by exposure to chemical oxidation, which addresses the chemical stability of the organic compounds rather than the bonding strength of the OC-mineral bond. We used a solution of NaOH, a strong agent for desorption due to high pH, and NaF, adding F$^-$, a strongly sorbing anion that can replace anionic organic molecules on mineral surfaces, to measure the maximum potentially desorbable MOC. For comparison, we measured maximal potential oxidation of MOC using heated H_2O_2. We selected MOC samples (>1.6 g cm^3) obtained from density fractionation of samples from three soil depth increments (0-5 cm, 10-20 cm, 30-40 cm) of five typical soils of the mid-latitudes, with a range of clay and pedogenic oxide contents, and under different ecosystem types (one coniferous forest, two deciduous forests, one grassland, one cropland). Extracts and residues were analyzed for OC and ^{14}C contents, and further chemically characterized by CPMAS-^{13}C-NMR. We expected to remove less MOC by NaF/NaOH extraction than by H_2O_2 oxidation, and for the NaF/NaOH extracted OC to be relatively younger. We expected subsoils and soils with high contents of pedogenic oxides to have relatively less and relatively older NaF/NaOH extractable OC.

The results showed that a surprisingly consistent proportion of 58±11% (standard deviation) of MOC was extracted with NaF/NaOH across soils, independent of depth, mineral assemblage, or land use. NMR spectra revealed strong similarities of the extracted organic matter, with more than 80% of OC in the O/N alkyl and alkyl C region. Total MOC amounts were correlated with the content of pedogenic oxides across sites, independent of variations in total clay, and the same was true for OC in extraction residues. Thus, the uniform extractability of MOC may be explained by dominant interactions between OC and pedogenic oxides across all study sites.

While Δ^{14}C values of bulk MOC suggested differences in OC turnover between sites, these were not linked to differences in MOC extractability. As expected, OC contents of residues had more negative Δ^{14}C values than extracts (average difference

between extracts and residues: 78±36‰), suggesting that non-extractable OC is older. Δ^{14}C values of extracts and residues were strongly correlated and proportional to Δ^{14}C values of bulk MOC, but not dependent on mineralogy. Neither MOC extractability nor differences in Δ^{14}C values between extracts and residues changed with depth along soil profiles, where declining Δ^{14}C values might indicate slower OC turnover in deeper soils. Thus, the ^{14}C depth gradients in the studied soils were not explained by increasing stability of organic-mineral associations with soil depth.

Although H_2O_2 removed 90±8% of the MOC, the Δ^{14}C values of oxidized OC were similar to those of OC extracted with NaF/NaOH (on average -50±110‰ for oxidized MOC and -51±122‰ for OC in NaF/NaOH extracts). Oxidation residues were much more ^{14}C-depleted than OC in NaF/NaOH extraction residues (average of -345±227‰ for H_2O_2 and -130±121‰ for NaF/NaOH residues). Different chemical oxidation treatments removed OC from the same continuum, leaving increasingly older residues behind the more OC is removed. In contrast to the NaF/NaOH extractions, higher contents of pedogenic oxides slightly increased the oxidation-resistance of MOC, but this higher H_2O_2-resistance did not coincide with more negative Δ^{14}C values of MOC or its oxidation residues.

Our results indicate that total MOC was dominated by OC interactions with pedogenic oxides rather than clay minerals, as we detected no difference in bond strength between clay-rich and clay-poor sites. This suggests that site-specific differences in Δ^{14}C values of bulk MOC and depth profiles are driven by the accumulation and exchange rates of OC at mineral surfaces.

1 Introduction

The persistence of organic matter (OM) in soil is a key control of atmospheric CO_2 concentrations. Association of OM with minerals is considered an effective pathway of stabilizing otherwise degradable OM from microbial mineralization (Schmidt et al., 2011; Lehmann and Kleber, 2015; Hemingway et al., 2019) and in many soils the vast majority of organic carbon (OC) is bound to minerals (Kleber et al., 2015; Kögel-Knabner et al., 2008; Cotrufo et al., 2019). Despite the abundance of mineral-bound OC (MOC), we lack fundamental knowledge on the drivers of stability and turnover of MOC along soil profiles and across sites.

The formation of MOC involves sorption to reactive minerals such as phyllosilicate clays and pedogenic aluminum (Al) and iron (Fe) oxi-hydroxides (Schrumpf et al., 2013; Kögel-Knabner et al., 2008; Kaiser and Guggenberger, 2000; Khomo et al., 2017). Along with plant-derived decomposition products, microbial residues and metabolites are important precursors and sorbates for MOC formation (Avneri-Katz et al., 2017; Chenu and Stotzky, 2002; Cotrufo et al., 2015; Kalbitz et al., 2005; Kallenbach et al., 2016). Experimental studies have shown that sorption to minerals reduces OC mineralization (e.g. Kalbitz et al., 2005; Jones and Edwards, 1998; Eusterhues et al., 2014; Porras et al., 2018). Radiocarbon (^{14}C) analyses confirmed the greater stability of MOC in soils, showing that average MOC age is older than OC not bound to minerals (Schrumpf et al., 2013; Kögel-Knabner et al., 2008; Hemingway et al., 2019; Heckman et al., 2018). Several field and incubation studies suggested that the total amount and stability of MOC increases with the amount of pedogenic Al and Fe oxi-hydroxides (Bruun et al., 2010; Torn et al., 1997; Porras et al., 2017; Rasmussen et al., 2006). Other studies found correlations between

65   Al and Fe oxi-hydroxides and MOC concentrations but not with the ^{14}C content of MOC or average age (Herold et al., 2014; Khomo et al., 2017; Schrumpf et al., 2013).

Studying MOC turnover and its drivers in soil is complicated because, similar to bulk soil OC, it is a mixture of younger and older carbon with a range of levels of stabilization (Trumbore et al., 1989; Swanston et al., 2005; Schrumpf and Kaiser, 2015; Koarashi et al., 2012). Various chemical methods, including acid hydrolysis and chemical oxidation, are commonly

70   used to distinguish faster and slower cycling fractions (e.g. Mikutta et al., 2006; Jagadamma et al., 2010; Helfrich et al., 2007; Six et al., 2002; Paul et al., 2001; Eusterhues et al., 2003). All methods separate bulk soil OC or MOC into younger and older fractions but differ in the extent of OC removal and the ^{14}C contents of obtained residues. The oxidants H_2O_2 or $Na_2S_2O_8$ were more effective in removing OC from samples than NaOCl, and their residues were older (Jagadamma et al., 2010; Helfrich et al., 2007). With increasing presence of Al and Fe oxi-hydroxides in soils, larger amounts of OC resisted

75   chemical oxidation, suggesting that binding to those minerals provides some protection against oxidative attack (Mikutta et al., 2006; Kleber et al., 2005; Eusterhues et al., 2005). However, there are indications that such chemically defined fractions are not causally related to MOC bioavailability or persistence in soils (Poirier et al., 2003; Mikutta and Kaiser, 2011; Helfrich et al., 2007; Jagadamma et al., 2010; Paul et al., 2008).

Release of mineral-bonded OC, either by desorption or upon mineral dissolution under changing environmental conditions,

80   can support or even be prerequisite for its microbial degradation (Keil et al., 1994; Mikutta et al., 2007). Desorption of OM, although typically small under conditions similar to those during the formation of MOC (e.g. Gu et al., 1994), increases in the presence of competing ions such as SO_4^{2-} or $H_2PO_4^-$ and is maximized by increasing the alkalinity of solution (e.g. Kaiser and Zech, 1999; Kaiser and Guggenberger, 2007). While some studies observed an increased release of OC from minerals into alkaline solutions with greater mineral OC loading (Kaiser and Guggenberger, 2007, Kaiser et al., 2007), others showed

85   increased OC desorption despite smaller OC concentrations (Kaiser and Zech, 1999; Mikutta et al., 2009). Greater desorption and biodegradation of OM bonded to phyllosilicates than of OM bonded to Al and Fe oxi-hydroxides has been attributed to differences in bonding strength. The sorption between OM and phyllosilicates are largely a result of the weaker cation bridges and van der Waals bonds, while the sorption of OM to Al and Fe oxi-hydroxides involves surface complexation, which results in strong chemical bonds (Singh et al., 2016; Mikutta et al., 2007). Thus, the presence and

90   abundance of Al and Fe oxi-hydroxides typically decreased both desorption and mineralization rates (Oren and Chefetz, 2012; Saidy et al., 2012; Singh et al., 2017). Unfortunately, OC desorption has primarily been studied using model minerals in laboratory experiments, and observation times for desorption or mineralization were much shorter than carbon residence times in soil.

Mikutta et al. (2010) analyzed the ^{14}C contents of MOC after removing all potentially desorbable OC by extracting soil with

95   a combination of NaF and NaOH. This extraction enables the study of the potential displacement of complexed organic functional groups by introducing OH^{-} and F[-] anions to compete for and displace mineral-bound OC, and by increasing the pH (Kaiser et al., 2007). Consistently younger OM was extracted from MOC of topsoils, supporting the idea that desorbable OC turns over faster than more strongly bonded OC. Results for subsoils were more variable (Mikutta et al., 2010). Along

with ^{14}C contents, the chemical composition of bulk soil OC extractable into alkaline solutions also changes with soil depth,

100   with subsoils containing less lignin-derived aromatics but more O-alkyl C, possibly of microbial origin (Möller et al., 2000; Mikutta et al., 2009). Desorption of OC from MOC has, to our knowledge, not yet been studied systematically across soil types. If potential OC desorption better represents the mechanism of mineral protection of soil OC than the abovementioned oxidative or hydrolytic extractions, then further research must focus on factors controlling amount, age, and composition of desorbable OC in soils. Potential factors affecting MOC desorption include soil mineralogy, chemical composition of OM

105   and thus vegetation type, and OM loading on minerals.

Based on the literature review we expect that:

(1) extraction in NaF/NaOH releases a weaker bound fraction of total MOC, which is younger than the stronger bound residue fraction.

(2) MOC of soil samples with higher proportions of extractable carbon is younger than MOC with more extraction

110   resistant OC.

(3) the proproportion of NaF/NaOH-extractable MOC decreases with increasing contents of pedogenic oxides, which form strong bonds with OC.

(4) the chemical composition of extractable MOC varies between study sites due to differences in vegetation composition, and thus litter chemistry.

115   (5) the proportion of NaF/NaOH-extractable MOC declines with depth due to declining OC loading of minerals.

(6) the chemical composition of extractable MOC changes with depth due to declining contributions of plant and increasing contributions of microbial derived OC.

(7) MOC should be less prone to desorption and accordingly of older ^{14}C age when organic molecules forming MOC have many carboxyl groups that enable strong bonds with minerals surfaces.

120   (8) the strong oxidizing agent H_2O_2 removes more OC from MOC than NaF/NaOH.

(9) oxidizable and non-oxidizable OC should both be older than the extractable and non-extractable OC fractions if the harsher oxidation treatment removes more, and thus presumably better stabilized OC from mineral surfaces, leaving OC residues of even older ^{14}C age behind.

To test if maximum desorption with NaF/NaOH is a suitable indicator for the labile proportion of MOC, we took advantage

125   of a previous experiment in which MOC was isolated from soils by density fractionation (heavy fraction at a density cutoff of >1.6 g cm^{-3}, HF) for a range of sites across Europe (Schrumpf et al., 2013). We selected five sites across ranges of clay content, type of clay and pedogenic oxides, and land use, with associated differences in amount and quality of litter input. Samples from three soil depths were extracted with a mixture of NaF and NaOH, and the extracted OC was analyzed for amount, composition, and ^{14}C content. We compared results from the extraction to the amount and age of OC oxidizable by

130   heated H_2O_2, which was shown to remove the most MOC and isolate the oldest MOC (Helfrich et al., 2007; Jagadamma et al., 2010). The in NaF/NaOH extraction experiment seeks to elucidate mineral protection as a stabilization mechanism, while the oxidation treatment explores the chemical recalcitrance of MOC.

2 Materials and Methods

Composition and age structure of MOC were studied on heavy fraction (HF) material obtained at five of the sites presented
135 by Schrumpf et al. (2013). The sites include two deciduous forests developed on loess at Hesse (France, Luvisol) and loess
over limestone at Hainich (Germany, Cambisol). Soils at the grassland site Laqueuille (France, Andosol) and the coniferous
site Wetzstein (Germany, Podzol) are characterized by large contents of pedogenic oxides. The soil at the fifth site, a
cropland at Gebesee (Germany, Chernozem), reveals a plow layer down to 30 cm, and large contributions of old OC
throughout the profile (Schrumpf et al., 2013). The MOC fraction was separated using two-step sequential density flotation
140 in sodium-polytungstate solution (1.6 g cm^{-3}). After removal of the unprotected free light fraction in a first flotation step,
samples were sonicated (site-specific energy application) to disrupt aggregates and separate the occluded light fraction from
the targeted MOC in the HF (for details see Schrumpf et al. (2013)). Concentrations of OC in MOC are shown in Figure 1
and ranged from 16.8 g kg^{-1} at Gebesee to 108 g kg^{-1} at Laqueuille in the uppermost layer, and from 4.6 g kg^{-1} at Hesse to 44
g kg^{-1} at Laqueuille in the deepest studied layer. Selected bulk soil properties of respective samples were adopted from
145 Schrumpf et al. (2013) and are summarized in Table 1.
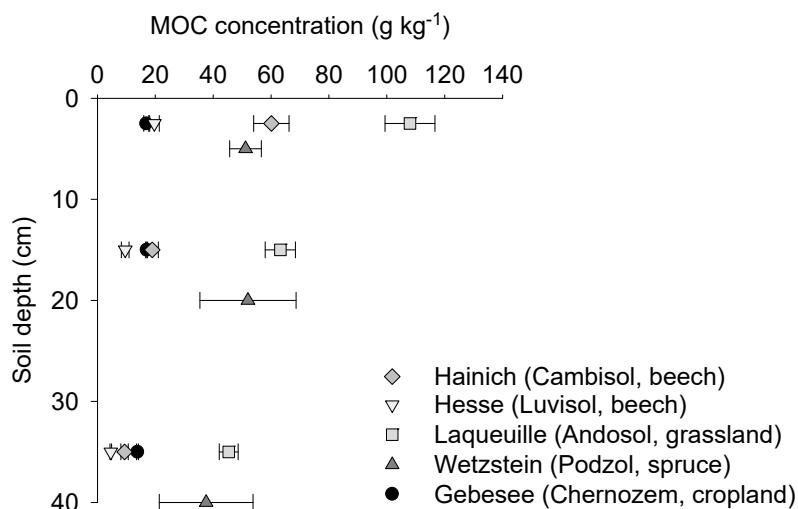
Figure 1: Original concentration of OC in mineral association (MOC, means and standard deviation) of the studied soil samples
150 **from 5 sites (data adopted from Schrumpf et al. 2013). Symbols indicate mid-points of integrated sampled depth intervals (Table 1).**

Table 1: Basic soil properties of the study sites (means of the three replicated samples analyzed for mineral associated carbon per soil depth with standard deviations in brackets). Values of selected cores are adopted from Schrumpf et al. 2013 (Ci: inorganic carbon, CN: OC-to-total nitrogen ratio, Feo: acid oxalate-extractable Fe, Alo: acid oxalate-extractable Al, Fed: dithionite-
155 **extractable Fe).**

Site and soil depth	pH (H$_2$O)	OC g kg^{-1}	CN	Ci g kg^{-1}	Sand g kg^{-1}	Clay g kg^{-1}	Feo g kg^{-1}	Fed g kg^{-1}	Alo g kg^{-1}
Hainich (Cambisol, beech)									
0-5	6.1 (0.9)	73 (11)	13.0 (0.5)	0	22 (4)	546 (5)	3.0 (1.0)	14.0 (0.8)	1.9 (1.1)
10-20	6.7 (0.5)	27 (6)	10.7 (0.4)	0	28 (4)	514 (5)	2.0 (0.4)	15.3 (1.0)	1.8 (0.6)
30-40	7.4 (0.2)	11 (1)	9.0 (0.4)	0	23 (7)	731 (7)	n.d.	n.d.	n.d.
Hesse (Luvisol, beech)									
0-5	4.6 (0.7)	31 (6)	13.9 (0.6)	0	68	342	1.8 (0.3)	11.8 (1.3)	1.1 (0.2)
10-20	4.5 (0.3)	14 (4)	11.8 (0.5)	0	56 (12)	315 (11)	1.4 (0.5)	11.5 (1.1)	1.0 (0.1)
30-40	n.d.	6 (0)	8.3 (0.0)	0	55(3)	371 (22)	n.d.	n.d.	n.d.
Laqueuille (Andosol, grassland)									
0-5	5.3 (0.2)	126 (11)	11.1 (0.2)	0	186 (36)	263 (28)	12.7 (0.1)	24.0 (0.6)	19.3 (0.7)
10-20	5.6 (0.3)	66(7)	10.2 (0.2)	0	259 (58)	215 (8)	16.4 (3.0)	20.3 (0.9)	24.0 (2.6)
30-40	n.d.	50 (4)	10.7 (0.3)	0	236 (21)	225 (22)	15.8 (1.7)	20.6 (1.1)	27.6 (2.3)
Wetzstein (Podzol, spruce)									
0-10	3.5 (0.0)	76 (15)	24.6 (2.2)	0	264 (164)	250 (165)	9.2 (6.2)	17.0 (10.4)	1.5 (0.8)
10-30	3.8 (0.3)	60 (29)	22.0 (3.6)	0	219 (40)	344 (123)	24.9 (15.5)	37.0 (12.6)	4.4 (0.8)
30-50	4.2 (0.3)	45 (24)	19.1 (2.3)	0	221 (46)	364 (63)	17.4 (16.6)	27.4 (16.1)	7.8 (2.6)
Gebesee (Chernozem, cropland)									
0-5	6.8 (0.1)	26 (2)	11.3 (0.9)	0	28 (5)	345 (5)	1.4 (0.1)	6.9 (0.3)	1.3 (0.1)
10-20	7.0 (0.4)	22 (2)	10.5 (0.1)	0	26 (3)	336 (2)	1.4 (0.2)	6.9 (0.3)	1.4 (0.1)
30-40	n.d.	17 (1)	11.1 (0.2)	1.6 (0.9)	21 (1)	368 (1)	n.d.	n.d.	n.d.

We randomly selected three out of the original ten replicated soil cores per site and analyzed the soil layers 0-5 cm, 10-20 cm, and 30-40 cm (excluding Wetzstein, where 0-10 cm, 10-30 cm and 30-50 layers were analyzed from soil pits instead of cores, see Schrumpf et al. 2013) for (1) extractable MOC and (2) oxidation-resistant MOC.

NaF-NaOH-extractable MOC was determined as an indicator for maxial potential desportion by weighing 25 g of heavy fraction (MOC) material of each sample into 250-ml polypropylene centrifuge bottles and adding 125 ml of a 1:1 solution containing 0.8 M NaF and 0.2 M NaOH. Containers were then closed and agitated overnight (at least for 18 hours) in an end-over shaker. A few drops of magnesium chloride were added as flocculant to the solution, which was then centrifuged for 15 minutes at 4000 × g. Then supernatants were decanted into 1000-ml PE bottles and stored in the refrigerator. Another 125 ml

of the extraction solution was added to settled soil material in the centrifuge tube, stirred and mixed well to repeat the
165 extraction for a total of four times. Finally, the combined extract solution from each sample was passed through glass fiber filters with a pore size of 1.6 μm and stored in a 4° C room until transfer into deionized water-rinsed, 75 cm long SERVAPOR 29-mm cellulose-acetate tubing for dialysis. The ~2/3 full tubes were placed into clean 10-l buckets filled with deionized water, which was frequently replaced until the electrical conductivity of the external solution was <2 μS. The content of the dialysis tubes was then freeze dried and analyzed for total C, N, and ^{14}C signatures (as described below). The
170 extracted residual soil containing the non-extractable OC was washed three times with deionized water to minimize remaining fluoride content before freeze drying and analyses of total C and N contents, and ^{14}C signature (as described below).

Oxidation-resistant MOC was obtained following a slightly modified procedure from Jagadamma et al. (2010). After letting 2 g of soil soak in 20 ml of Millipore DI water for ten minutes, 60 ml of 10% hydrogen peroxide (H_2O_2) was gradually added
175 to the soil. After the frothing had subsided from the reaction of wet samples with 60 ml of H_2O_2 at room temperature, the samples were heated and stirred regularly in a 50°C water bath in order to catalyze the oxidation of organic matter. Because H_2O_2 decomposes with exposure to light and temperature, the samples were centrifuged, the supernatant decanted, and fresh H_2O_2 added to continue the oxidation. Each sample was oxidized for two periods of 24 hours and one period of 72 hours. After the final oxidation, samples were centrifuged at $3500 \times g$ for at least 15 minutes, and then washed three times with 80
180 ml of deionized water. Magnesium chloride solution was added to enhance flocculation during centrifugation. After the final oxidation each sample was washed three times with DI water and afterwards freeze dried. The samples were then homogenized using a ceramic ball mill and measured for total carbon and nitrogen content by dry combustion with the Vario EL CN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). All soils analyzed in this study were free of carbonate. Therefore, total carbon measurements are equivalent to total organic carbon in the soil.
185 Radiocarbon contents of the samples were measured on graphitized samples at the ^{14}C laboratory in Jena, Germany (Steinhof et al., 2017). Soil samples were weighed into tin capsules and combusted in an elemental analyzer. The evolved CO_2 was transferred into a glass tube cooled by liquid nitrogen, and reduced to graphite at 600 °C under hydrogen gas atmosphere, using iron as catalyst. The graphite was analyzed by ^{14}C AMS (3MV Tandetron 4130 AMS ^{14}C system: High Voltage Engineering Europe, HVEE, The Netherlands). Samples with low OC concentrations from the H_2O_2 residues were
190 combusted with CuO wire in quartz tubes and graphitized using a sealed zinc reduction method, then analyzed at the WM Keck Carbon Cycle AMS facility at UC Irvine (Xu et al., 2007).

Radiocarbon data are reported as Δ^{14}C in per mille [‰], which is the relative difference in parts per thousand of the ^{14}C/^{12}C ratio of the sample with respect to an absolute standard (0.95 times the oxalic acid standard NBS SRM 4990C decay corrected to 1950), after normalization of the sample ^{14}C/^{12}C for mass dependent fractionation by normalizing to a δ^{13}C of -
195 25‰ (Trumbore et al., 2016). The average measurement precision of the Δ^{14}C values was 2.8‰. Reported in this way, Δ^{14}C values close to zero indicate that most of the C in the sample is close to that of the standard, i.e. it was fixed from the preindustrial atmosphere (up to ~350 years prior to 1950). Negative Δ^{14}C values indicate lower ^{14}C/^{12}C than the standard and

indicate that most of the C in the sample had been isolated from the atmosphere long enough for detectable radioactive decay to deplete ^{14}C; samples with negative Δ^{14}C values have been isolated longer. Positive Δ^{14}C values indicate enrichment with bomb-derived ^{14}C from nuclear weapon testing in the early 1960ies, which suggests that OC from these samples is younger and mostly fixed in the last century. In 2004, when samples in this study were collected, atmospheric Δ^{14}C values (about 70 ‰ (Levin et al., 2010)) were still declining from the peak bomb C in 1963 of about +900 ‰. Thus, comparing oxidized or extracted C with the original MOM depends on its original Δ^{14}C values. For total MOM with negative Δ^{14}C values, we assume the extracted or oxidized C is 'younger' if it has Δ^{14}C values that are either less negative or positive. For total MOM that initially has positive Δ^{14}C values, we might expect 'older' C residues to have either higher Δ^{14}C values (closer to the bomb peak) or negative Δ^{14}C values; in both cases we expect 'younger' extracted Δ^{14}C values to have positive Δ^{14}C values. Our previous results based on temporal changes of Δ^{14}C values of MOC at the Hainich site (Schrumpf and Kaiser, 2015), indicate that total MOC in our soils will have turnover times > 20 years. Therefore, if extracted/oxidized OC has Δ^{14}C values that are higher than unextracted or residual MOM, we refer to these as younger throughout the manuscript.

To determine the amount of carbon in the residues from both extraction procedures, we multiplied the measured OC content in the recovered residues with its mass. The amount of carbon lost by the treatments was determined as difference between the original OC content of the HF sample and OC in the residues. The radiocarbon contents of the H_2O_2 residues were directly measured, and the ^{14}C fraction of the OC lost/extracted ($^{14}C_{extract}$) was determined by mass balance as follows:

$^{14}C_{HF} = (OC_{extract}/OC_{HF})\cdot {}^{14}C_{extract} + (OC_{residue}/OC_{HF})\cdot {}^{14}C_{residue}$

$^{14}C_{extract}= ({}^{14}C_{HF} - (OC_{residue}/OC_{HF})\cdot {}^{14}C_{residue})/(OC_{extract}/OC_{HF})$

The same formula was used to determine the ^{14}C fraction of OC extracted by NaF/NaOH. Since OC extracted by NaF/NaOH extraction was also measured directly, the mass balance results allow for identifying potential bias in measured ^{14}C data caused by losses of extracted OC during dialyses.

Solid-state cross-polarization magic angle spinning ^{13}C-nuclear magnetic resonance (CPMAS ^{13}C-NMR) spectra of NaF/NaOH extracted, dialyzed, and freeze-dried OM were recorded on an Avance III spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) at a resonance frequency of 100.5 MHz, with a proton spin-lock and decoupling frequency of 400 MHz. The proton nutation frequency was 80 kHz, corresponding to a $\pi/2$ pulse duration of 3.12 μs. The cross-polarization time was 500 μs. Samples were weighed into 4-mm zirconium oxide rotors that were spun at 10 kHz around an axis declined by the 'magic angle' of 54.74° against the static magnetic field; contact time was 1 ms and the recycle delay time was set to 0.4 s. Depending on the sample, between 4,000 and 35,000 scans were recorded. The spectra were processed with a line broadening of 100 Hz. Chemical shifts are given relative to the resonance of tetramethylsilane. After baseline correction, the intensities of spectral regions were corrected for different cross-polarization efficiencies in different spectral regions. To do so, ^{1}H $T_{1\rho}$ as well as T_{CH} were estimated selectively for the regions. Since OM samples with C contents >30% were analyzed, no treatment for removal of paramagnetic mineral phases was required and the obtained spectra were well resolved and showed no indications of paramagnetic interferences. In addition, we analyzed one bulk MOC sample low in pedogenic

oxides without removal of paramagnetic phases. In all cases, spectra were reasonably well resolved and without indications of paramagnetic interferences.

Resonance areas were calculated by electronic integration: alkyl region (0–50 ppm), mainly representing C atoms bonded to other C atoms (methyl, methylene, methine groups); O/N-alkyl region (50–110 ppm), mainly representing C bonded to O and N (carbohydrates, alcohols, and ethers) and including the methoxyl C (peak centered around 56 ppm); aromatic region (110–160 ppm), representing C in aromatic systems and olefins, and (d) the carbonyl region (160–220 ppm), including carboxyl C (160–190 ppm). Further information on the assignment of ^{13}C-NMR regions are given by Wilson (1987) and Orem and Hatcher (1987).

3 Results

3.1 Mineral-associated organic carbon extracted by NaF/NaOH

The NaF/NaOH extraction removed on average 58±11% of bulk MOC across sites and soil depths (Figure 2). Extraction efficiency was not explained by soil depth, as average extraction removal values were 57±7% in the uppermost, 60±15% in the intermediate, and 56±11% in the deepest analyzed soil layer. Average extraction efficiency was smaller at the Gebesee site compared to other sites (41±10% vs. 62±11% on average across depths). The amounts of extracted and residual OC, which represent similar proportions of bulk MOC, were strongly correlated to bulk MOC concentrations across sites and soil depths (r=0.96, p<0.01; Figure 3, Figure A1). Neither contents of pedogenic oxides, OC loadings of minerals, nor soil pH affected the proportion of extractable MOC. However, since bulk MOC concentration was positively related to contents of pedogenic oxides (Schrumpf et al. 2013), the same relationship applied to OC concentrations in extraction residues (Figure 4).
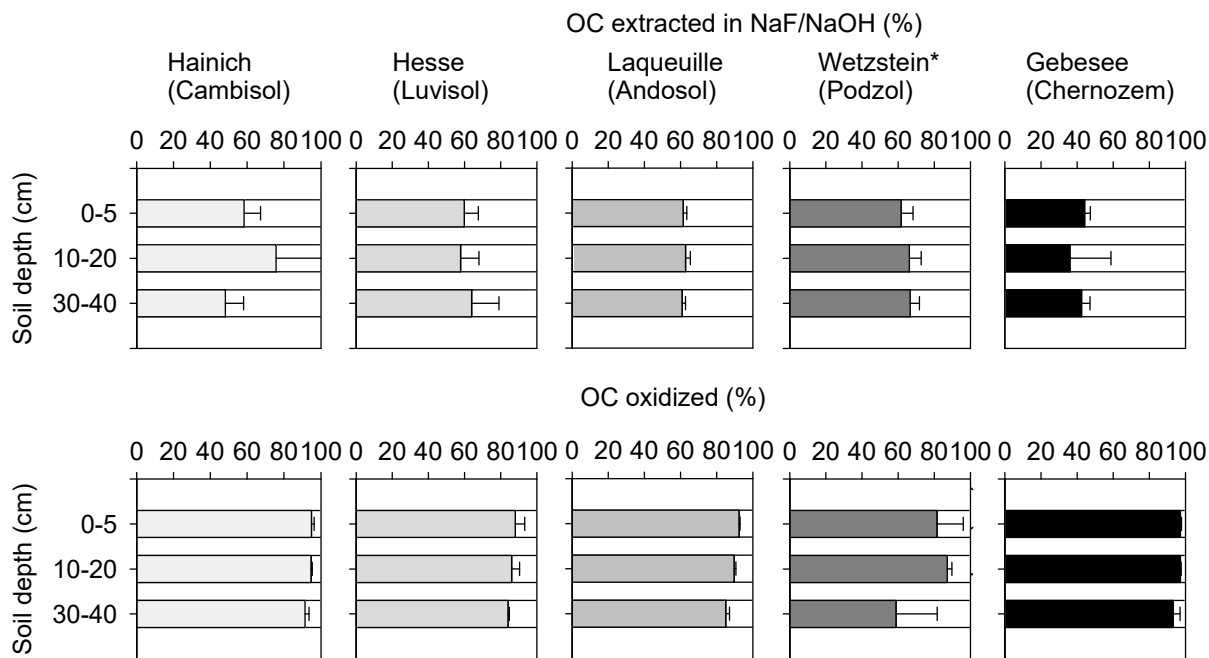
Figure 2: Proportion of OC extractable with NaF/NaOH or oxidized with heated H$_2$O$_2$ from the mineral associated OC fractions of soil samples from five different sites (Hainich, Hesse, Laqueuille, Wetzstein, Gebesee) and three soil depths (0-5 cm, 10-20 cm, 30-40 cm); for Wetzstein the studied soil depths were 0-10 cm, 10-30 cm, and 30-50 cm.

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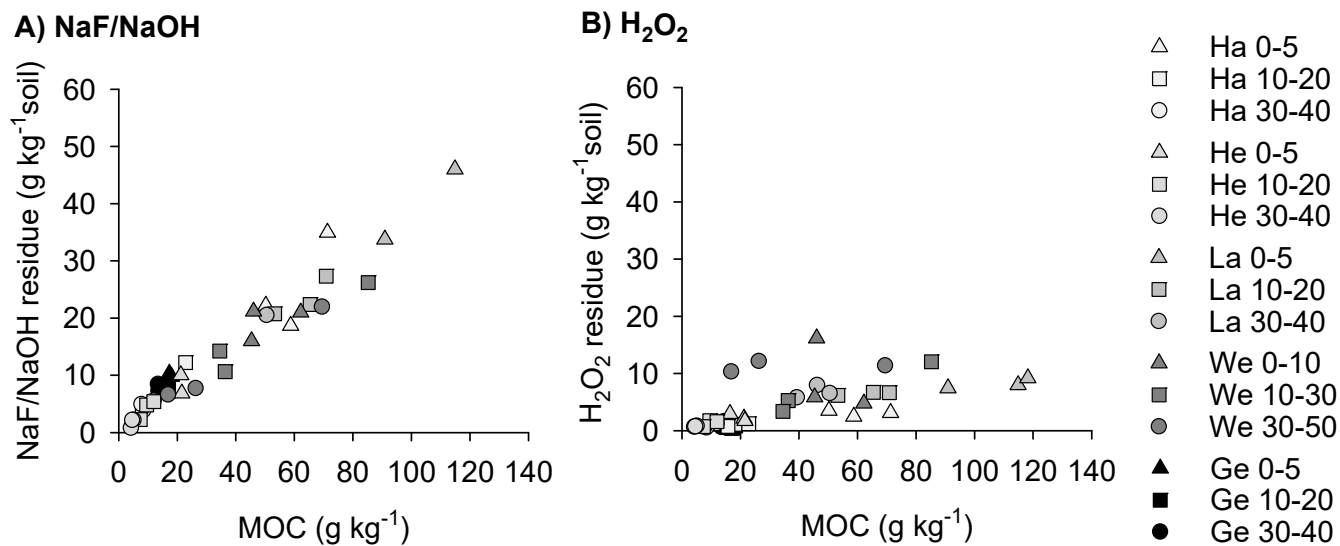
Figure 3: Residual OC depended on original concentrations of mineral associated OC for A) NaF/NaOH extraction (left) and B) the H$_2$O$_2$ oxidation (right) for all study sites (Hainich (Ha), Hesse (He), Laqueuille (La), Wetzstein (We), Gebesee (Ge)).

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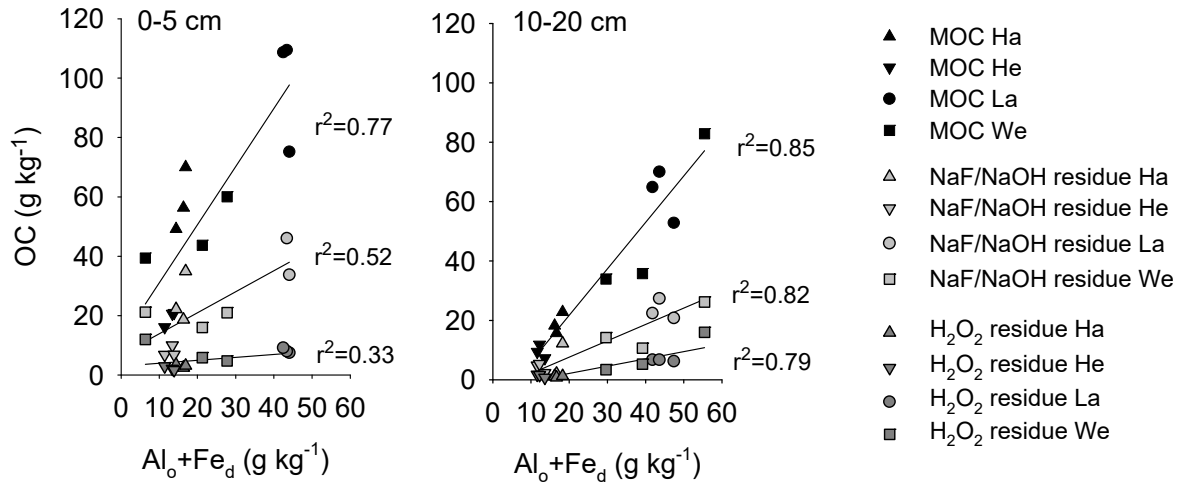
Figure 4: OC concentrations in each fraction (bulk MOC, NaF/NaOH extraction residue, and oxidation residue) was related to the pedogenic oxide content of the sample, which is expressed as the sum of the acid oxalate-extractable Al and dithionite-extractable Fe.

3.1 Radiocarbon contents of NaF/NaOH extracts and residues

Directly measured $\Delta^{14}C$ values of dialyzed NaF/NaOH extracts were comparable to calculated $\Delta^{14}C$ values in extracts using the mass balance approach (Figure 5), suggesting that there were no systematic losses of older or younger C during the extraction and subsequent dialysis procedure. The only exceptions were the results from Gebesee, where the mass balance approach suggests that some young carbon was lost during dialysis of the extracts.
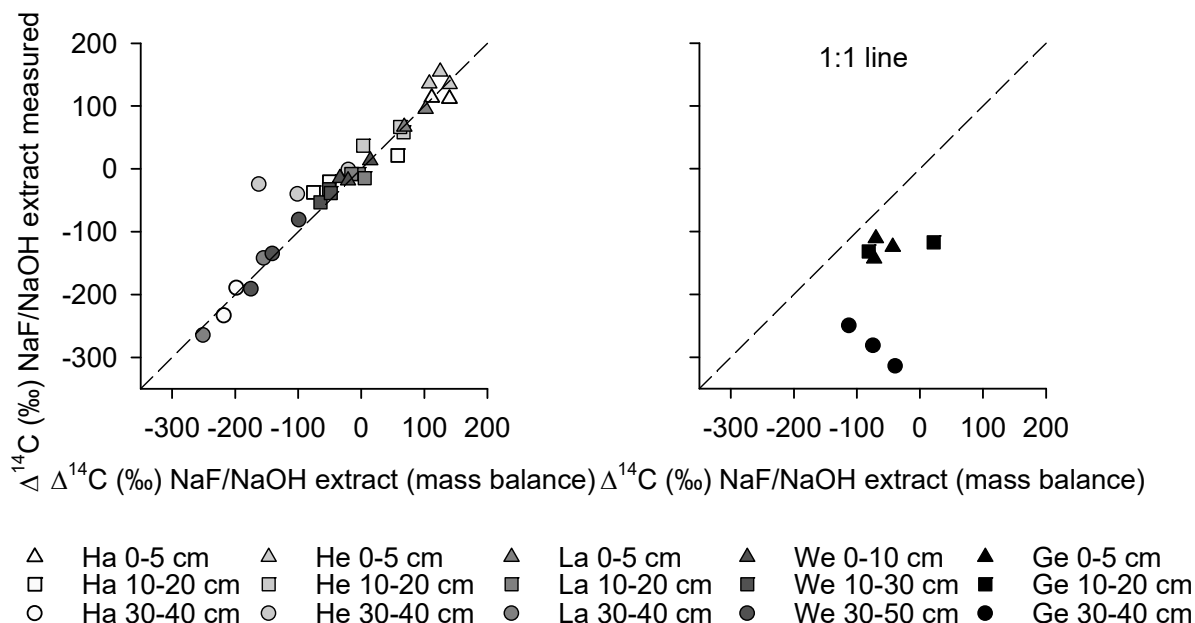
Figure 5: Comparison of ^{14}C contents of NaF/NaOH extracted OC obtained using a mass balance approach with direct measurements of the extracts after dialyses (study sites: Hainich (Ha), Hesse (He), Laqueuille (La), and Wetzstein (We) on the left, Gebesee (Ge) on the right).

275

Extracted fractions had consistently higher Δ^{14}C values than extraction residues, and Δ^{14}C values decreased with depth at all sites (Figure 6). The Δ^{14}C values of the OC extracted from the uppermost layers increased in the order Gebesee (Chernozem) < Wetzstein (0-10 cm, Podzol) < Laqueuille (Andosol) < Hainich (Cambisol) < Hesse (Luvisol) from -126‰ to 142‰. The average difference in Δ^{14}C between extracted and residue OC was 79±36‰ across sites and increased in the order Gebesee

280 (34±4‰) < Laqueuille (38±6‰) < Hainich (63±3‰) < Hesse (84±5‰) < Wetzstein (100±15‰). As indicated by the almost parallel shifts in Δ^{14}C values, there was no general trend for increasing or declining differences in ^{14}C contents with soil depth (Figure 6). Instead, Δ^{14}C values of extracts and extraction residues were highly correlated (r^2=0.91, p<0.01, supplementary Figure S2). However, Δ^{14}C values of bulk MOC, extractable or residual MOC were all unrelated to total MOC or its extractability (results not shown).

285
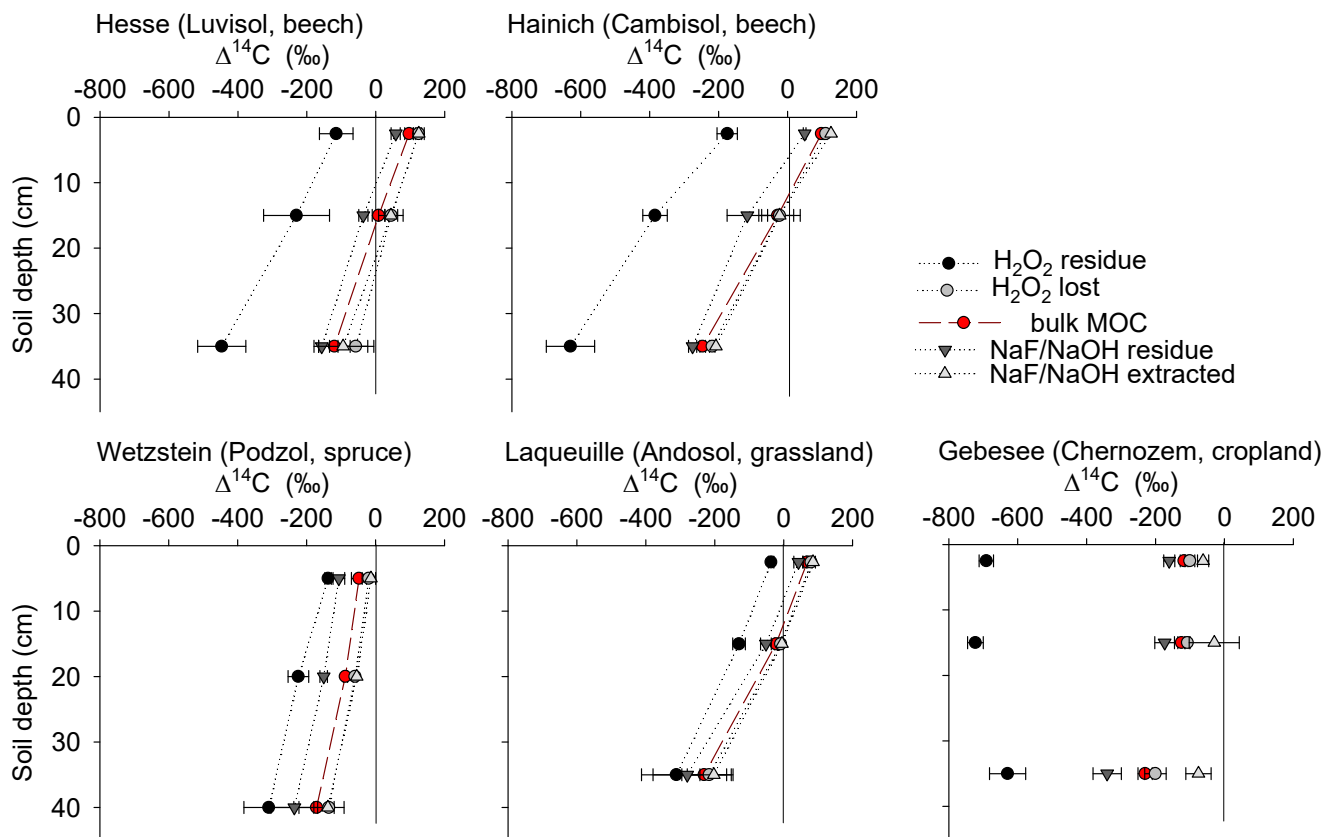
Figure 6: Depth profiles of radiocarbon (Δ^{14}C) in bulk mineral associated OC (bulk MOC), as well as in OC removed from mineral surfaces using either NaF/NaOH or H_2O_2, and in the respective OC residues remaining on mineral surfaces.

3.3 NMR spectroscopy of NaF/NaOH treated samples

The NMR spectra of extracted OM from soils of the sites Hainich, Hesse, and Laqueille were remarkably similar and dominated by signals in the O/N-alkyl C region (62-74% of total peak area across sites and depths) and the alkyl C region (18-25%), suggesting a strong contribution of carbohydrates and aliphatic compounds to the extracted OM (Figure 7, Table 2). All six spectra also reveal a distinct peak centered around 174 ppm, due to carboxyl C, which is in line with the fact that the alkaline extraction tends to preferentially release acidic compounds (5-9%). All spectra show small signals in the aromatic regions centered around 150 ppm (phenols) and 130 ppm (non-substituted aromatic systems). All six spectra featured signals, some even well-resolved, around 56 ppm, indicating the presence of methoxy C.
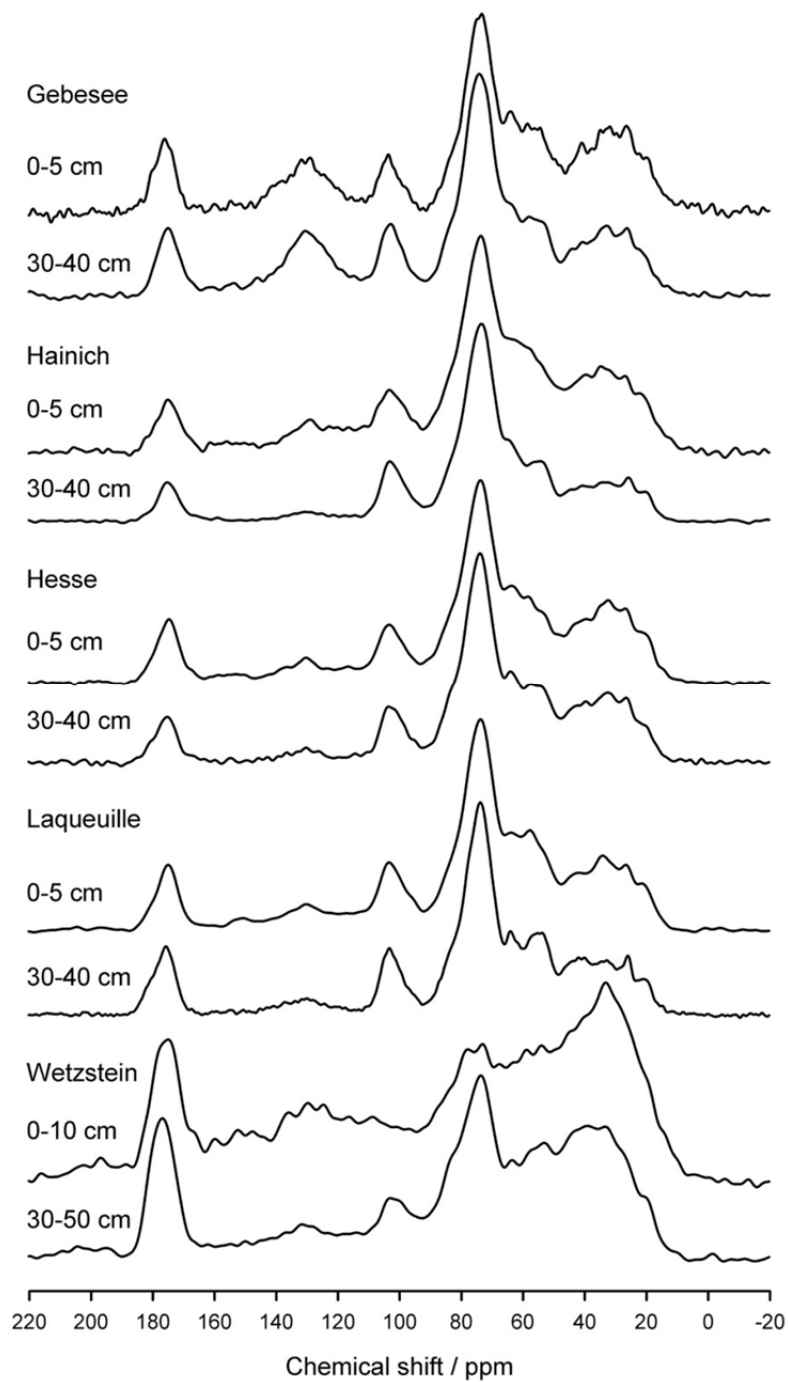
Figure 7: NMR spectra of OM extracted into NaF/NaOH from the mineral associated fraction of two soil depths from the five study sites Gebesee (Chernozem, cropland), Hainich (Cambisol, beech), Hesse (Luvisol, beech), Laqueuille (Andosol, grassland), and Wetzstein (Podzol, spruce).

Table 2: Distribution of C species in organic matter extracted into NaF/NaOH from heavy fractions of mineral topsoil (0–5 cm depth) and subsoil (30–40 or 30–50 cm depth) layers as revealed CPMAS-^{13}C-NMR.

Sample	Carbonyl/carboxyl C 220–160 ppm	Phenolic/aromatic C 110–160 ppm	O/N-alkyl C 45–110 ppm	Alkyl C −10–45 ppm
			%	
Hainich (Cambisol, beech)				
0–5 cm	6	8	63	24
30–40 cm	5	3	74	18
Hesse (Luvisol, beech)				
0–5 cm	8	6	62	24
30–40 cm	5	3	67	25
Laqueuille (Andisol, grassland)				
0–5 cm	9	8	62	21
30–40 cm	9	6	63	22
Wetzstein (Podzol, spruce)				
0–10 cm	14	17	35	34
30–50 cm	14	9	47	30
Gebesee (Chernozem, cropland)				
0–5 cm	9	14	52	25
30–40 cm	6	15	60	19

305

The spectra obtained on OM extracted from the Chernozem-type soil at site Gebesee resembles those of the sites Hainich, Hesse, and Laqueille, except for that they indicate more non-substituted aromatic systems, which is consistent with the occurrence of pyrogenic OM in this soil type. When comparing the spectra of OM from top- and subsoils, differences were surprisingly small at Hainich, Hesse, Laqueille, and Gebesee, with proportions of alkyl and aromatic C tending to decrease in favor of increased signals of O/C-alkyl C. The contribution of carbonyl C remained constant with soil depth.

The spectra obtained on extracted OM from the Podzol-type soil at Wetzstein showed the strongest deviation in spectral features. The signals due to carbonyl/carboxyl, aromatic, and alkyl C were much more prominent than in all other spectra. Also the change in composition with depth was much more evident for the Podzol site Wetzstein than the others. Here, aromatic and alkyl C decreased while O/C-alkyl increased, reflecting the strong re-distribution of OM along the profile during podzolization. The composition of the subsoil OM at Wetzstein approached that of the OM at the sites Hainich, Hesse, and Laqueille.

To understand how the extract differed from the bulk and residual fractions, we also analyzed the bulk MOM and the NaF/NaOH extraction residue of the Hainich 0-5 cm sample. The signal-to-noise ratio of these spectra was lower than in the extracted OM due to the presence of paramagnetic minerals (Figure 8). Nevertheless, the results reveal larger proportions of carbonyl/carboxyl C and especially aromatic C but less O/N-alkyl C for the extraction residue than in the extracted OM (Figure 8, Table 3).
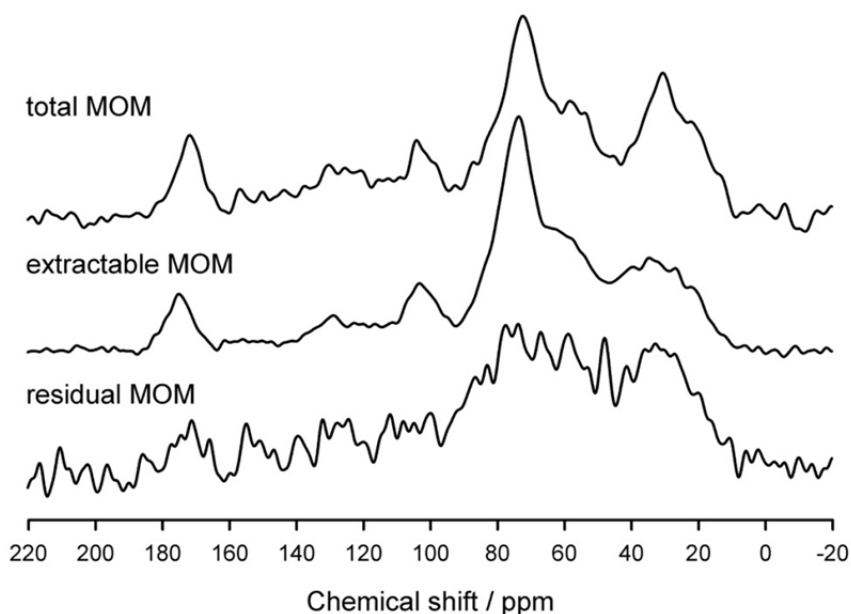
Figure 8: NMR spectra of OM from the 0-5 cm layer of the site Hainich (Cambisol, beech). Top: total mineral associated OM (MOM), middle: MOM-fraction extracted into NaF/NaOH, bottom: residual OM after extraction of MOM into NaF/NaOH.

Table 3: Distribution of C species in total, NaF/NaOH-extractable and residual organic matter of the heavy fraction of the mineral topsoil layer (0–5 cm depth) at Hainich (Cambisol, beech) as revealed by CPMAS-^{13}C-NMR.

Sample	Carbonyl/carboxyl C 220–160 ppm	Phenolic/aromatic C 110–160 ppm	O/N-alkyl C 45–110 ppm	Alkyl C −10–45 ppm
	——————————— % ———————————			
Total OM	8	14	52	26
Extracted OM	6	8	63	24
Residual OM	9	15	50	26

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3.4 MOC oxidation in heated H_2O_2

On average, only 11±6% of MOC resisted treatment with heated H_2O_2, with 90±8% of MOC oxidized in the uppermost layer, 90±6% oxidized in the intermediate layer, and 87±5% oxidized in deepest studied soil layer (Figure 2). The proportion

335   of MOC resisting the H_2O_2 treatment differed between study sites. The largest proportion of H_2O_2-resistant MOC was found at the Wetzstein site (Podzol, on average 19±4%), especially in the deepest layer, while the smallest proportions of H_2O_2-resistant MOC occurred at Gebesee (Chernozem, 5±3%). The absolute amount of H_2O_2-resistant OC was correlated with the bulk MOC concentration (r=0.76, p<0.01; Figure 3) but the correlation was weaker than the one found for residual OC after NaF/NaOH extraction and bulk MOC.

340   Residues of the H_2O_2 treatment had much lower Δ^{14}C values than residues of the NaF/NaOH extractions (Figure 6). The average Δ^{14}C values of H_2O_2 residues ranged between -36±8‰ (Laqueuille) and -691±21‰ (Gebesee) in the uppermost, and between -310±73‰ (Wetzstein) and -630±70‰ (Hainich) in the deepest layer (Figure 6). The difference between Δ^{14}C values of oxidized and residual OC in the uppermost layer increased in the order Laqueuille = Wetzstein (115‰) < Hesse (239‰) < Hainich (290‰) < Gebesee (591‰), and increased slightly with soil depth at Hesse, Hainich, and Wetzstein.

345   When comparing Δ^{14}C values of OC removed by either NaF/NaOH or H_2O_2 treatments, we were surprised to find no ^{14}C-difference for most sites (Figure 9), despite different amounts of OC being removed by the individual procedures. The almost parallel shift in Δ^{14}C values of H_2O_2 residues from NaF/NaOH residues in all soil profiles indicate that both were highly correlated within depth profiles (Figure 9). For the sites rich in pedogenic oxides, Wetzstein and Laqueuille (Podzol and Andosol), Δ^{14}C values in NaF/NaOH residues deviated from H_2O_2 residues by only 62±26‰. In contrast the difference

350   in extraction and oxidation residual Δ^{14}C values were 258±99‰ for the Luvisol and Cambisol sites Hesse and Hainich (Figure 9). The only exception was again the Chernozem site (Gebesee), where OC extracted by NaF/NaOH was on average

younger than OC removed by H_2O_2, and ^{14}C contents of the two residues differed on average by 456±135‰ and were not correlated.

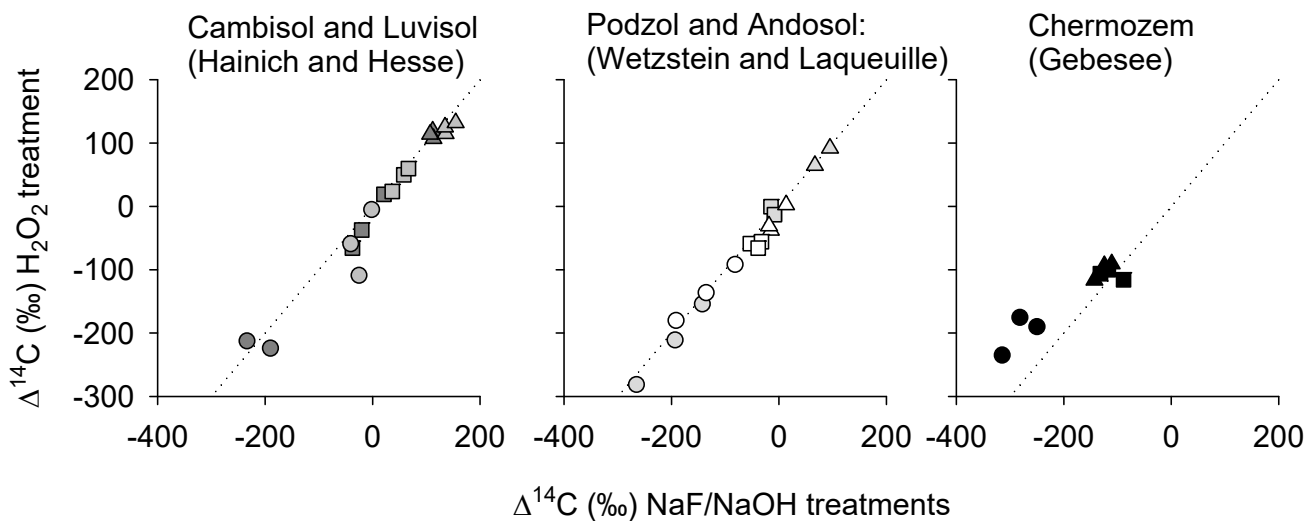
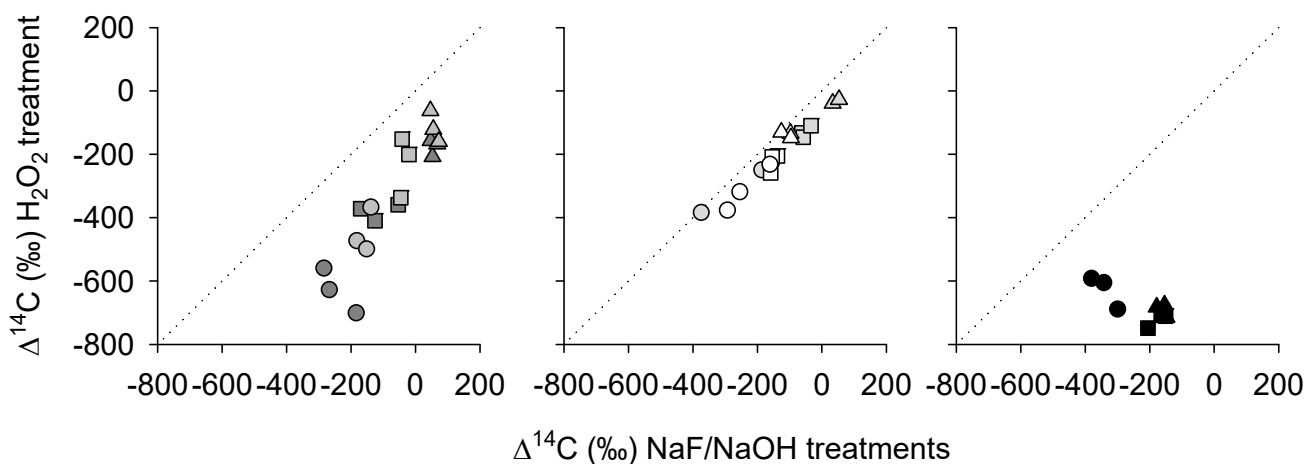
Comparison of ^{14}C in extracts

Comparison of ^{14}C in residues

△ Ha 0-5 cm	△ He 0-5 cm	△ La 0-5 cm	△ We 0-10 cm	▲ Ge 0-5 cm
▪ Ha 10-20 cm	▫ He 10-20 cm	▫ La 10-20 cm	▫ We 10-30 cm	▪ Ge 10-20 cm
● Ha 30-40 cm	● He 30-40 cm	○ La 30-40 cm	○ We 30-50 cm	● Ge 30-40 cm

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Figure 9: Relationships between the radiocarbon content of OC removed from mineral surfaces by H_2O_2 and NaF/NaOH (upper graphs) and the radiocarbon content of OC residues after treatment with H_2O_2 and NaF/NaOH (lower graphs) for the study sites Hainich (Ha), Hesse (He), Laqueuille (La), Wetzstein (We), and Gebesee (Ge).

4 Discussion

4.1 Unexpected similarity of the NaF/NaOH-extractable MOC

Strong hysteresis, rendering part of adsorbed OC resistant to desorption, is a common phenomenon found in sorption-desorption experiments with OM (Gu et al., 1994; Oren and Chefetz, 2012). Given that desorption rates in ambient soil solutions are low, we consider NaF/NaOH extractability an indicator for maximum potential desorption. Because the NaF/NaOH extraction offers ideal desorption conditions of the combination of competing OH^- and F^- anions and alkaline conditions, the method targets OC bound to minerals by Coulombic forces and surface complexation. The OC desorbed likely also includes OC held by different weaker forces, such as hydrogen bonds, cation bridges, or hydrophobic interactions. We expected that the proportion of extractable C would increase with OC loading of minerals and thus be higher in topsoils with larger MOC concentrations than in subsoil layers with smaller concentrations. Since minerals have different characteristic dominant binding modes for OC at a given pH (Mikutta et al., 2007), we further assumed that extractability would depend on mineral composition and soil pH, with the lowest desorption in acidic soils with larger amounts of pedogenic oxides (i.e. the Podzol or the Andosol site in our study). And finally, we expected that land use- and site-specific differences in OM quality would influence MOC extraction. Our results showed, however, that for our four uncultivated test sites a surprisingly constant proportion of on average 62±11% of the MOC was extracted by NaF/NaOH, irrespective of soil depth, study site, and original OC concentration. Thus, despite the presumed variation in the chemical composition of litter input and the mineral assemblage between sites, actual interactions between soil OC and minerals were uniform.

Laboratory experiments showed similar proportions of extracted OC by NaF/NaOH from two Fe-oxides, despite differences in the absolute amounts of OC sorbed by goethite and ferrihydrite (Kaiser et al., 2007). The amounts extracted from these model MOC were surprisingly similar to results of this study (around 65%, Kaiser et al., 2007). Results from another experiment in which OC was extracted with NaOH from experimentally produced MOC on goethite also extracted a similar fraction of MOC (57-60%, Kaiser and Guggenberger, 2007). Thus, we expected to observe more extractable MOC at sites poor in Fe- and Al-oxides but rich in clay minerals (like for the Cambisol at Hainich with 50-70% clay), which we expected would have a larger share of weakly bound OC on MOC. It is possible that some of the weakly bonded OC was already lost in the preceding density fractionation with Na polytungstate solution (Schrumpf et al., 2013). However, the uniform extractability suggests that the extracted OC was bound to minerals by the same mechanism, irrespective of mineral composition. This indicates that either the same dominant bond mechanism operates for different minerals, or that extracted OC originated predominantly from one mineral type. While OC adsorbed by covalent bonds is usually associated with pedogenic oxides, covalent bonds have also been shown to bind OC on the edges of clay minerals. (e.g. Chen et al., 2017; Gu et al., 1994). In our study, the linear relation between MOC and extraction residues with the sum of oxalate-extractable Al and dithionite-extractable Fe indicates that pedogenic Al and Fe oxi-hydroxides were important for OC binding across the

study sites (Figure 4, A2). Even very high clay contents (>50%), such as at the Hainich site, could not compensate for lower pedogenic oxide content, resulting in lower MOC storage. The measured uniform extractability of MOC is therefore likely an immediate result of the pedogenic oxides controlling MOC accumulation.

The similar chemical composition of MOC extracted from the sites Hainich, Hesse, and Laqueuille further supports a uniform mechanism dominating association of organic C with minerals, especially given the lack of variation with pedogenic oxide content. One explanation could be that the type of sorptive interaction targeted by the NaF/NaOH extraction selects for a specific composition of the extracted MOC. Alternatively, the similar MOC composition may reflect uniform microbial processing of the organic input (Liang et al., 2017). However, differing chemical composition at the sites Gebesee and Wetzstein suggest that site-specific properties and pedogenic properties can indeed influence MOC composition. For the Chernozem at Gebesee, higher contents of non-substituted aromatic systems are likely due to a different vegetation history, where also fire played a role. Differences in OM composition between the sites Wetzstein and Laqueille were surprising, since the pedo-environmental conditions (acidity and mineralogy) of OM accumulation in Podzol-type and Andosol-type soil are often considered similar (Aran et al., 2001; Young et al., 1980). The unique MOC composition in the podzol-type soil are likely due to the unique decomposition conditions at that site (conifer-dominated vegetation, pH, microbial community, soil climate), which subsequently uniquely influences the dissolved organic carbon production and chemistry at that site.

Experiments on model minerals suggested that desorption increases with increasing OC loading of minerals, i.e. when more OC is bound per mineral content of a sample (Kaiser and Guggenberger, 2007). This is consistent with our observation of reduced NaF/NaOH extraction at the agricultural soil Gebesee, where OC loading of minerals is reduced relative to undisturbed soils due to lower inputs, increased mineralization, and soil mixing by plowing (Plante et al., 2005; Helfrich et al., 2007). Although Figure 4 shows that for the same amount of pedogenic oxides, less OC was bound to MOM in subsoils, soil depth did not affect MOC extractability in this study. This result is supported by studies by Mikutta et al. (2010) and Möller et al. (2000), which observed no significant decrease in NaF/NaOH extractability of OC with soil depth. One explanation for the uniform extraction with soil depth could be that DOC input to subsoils occurs mostly along specific flow paths (Bundt et al., 2001), enabling exposed mineral surfaces in subsoils to have similar OC loads as topsoils. Additionally, increasing pH with soil depth could reduce the sorption capacity of minerals in subsoils. Assuming that pH reduces the total number of available sorption sites at depth, the same proportion of available sorption sites could be occupied in subsoils than in topsoils, despite a smaller ratio of OC-to-pedogenic oxide contents. Whatever the mechanism, our findings confirm that a decline in potential desorption is not responsible for greater subsoil OC stability (Rumpel and Kögel-Knabner, 2011) and cannot explain increased carbon ages of MOC with depth.

4.2 MOC desorption and $\Delta^{14}C$ values

Results of ^{14}C analyses of NaF/NaOH extracts and residues confirmed our hypothesis of preferential extraction of younger carbon, with lower $\Delta^{14}C$-values in residues compared to extracts or unextracted MOM across soil types and depths. These findings indicate that desorption facilitates the exchange of old OC for new OC on mineral surfaces, while non-extractable

OC is less frequently exchanged. Using Na pyrophosphate as extractant, Heckman et al. (2018) also observed consistently

younger OC in MOC extracts and older OC in extraction residues across different soils and soil depths. We expected Na pyrophosphate would have a similar effect on MOC as NaF/NaOH due to the comparable increase in pH and because both pyrophosphate and fluoride act as chelating agents to strongly compete with OC for binding sites on mineral surfaces.

Some MOC may be resistant to extraction due to multiple chemical bonds binding the OC with greater strength to mineral surfaces (Kaiser and Guggenberger, 2007). Since multiple bonds require a higher number of involved functional groups, OC of extraction residues of multiply bonded OC should be enriched in carboxylic groups. Our NMR data support the resistance mechanism of multiple bonds, showing a higher share of carbonyl/carboxyl groups in non-extractable than in extractable MOC from the Hainich site. Phenolic and aromatic groups, which are also known to bind preferentially and strongly to mineral surfaces (Chorover and Amistadi, 2001; Kothawala et al., 2012; Avneri-Katz et al., 2017), were also enriched in the residues, while O/N-alkyl-C was depleted. Given this mechanism, we expect the non-desorbable OC to be composed of aromatic and other compounds strongly bound to mineral surfaces by multiple functional groups, but this remains to be tested in future studies.

Our results did not confirm our original expectation, that the average age of MOC is inversely related to its extraction in NaF/NaOH, due to the strength of the stabilizing chemical bonds in non-desorbable MOC. This may be a direct consequence of the small variation in OC extractability between samples and shows that extraction in NaF/NaOH is not able to explain between-site variation in $\Delta^{14}C$ values.

4.3 Comparison between NaF/NaOH extraction and H_2O_2 oxidation results

As expected, residues of H_2O_2 treatments were older than residues of NaF/NaOH extractions. We further hypothesized that the heated H_2O_2 treatment would remove a larger and on average more stable fraction, and therefore the oxidized OC would have an older mean age than the extracted OC. While H_2O_2 removed on average 89% of bulk HF-OC and NaF/NaOH removed only 62%, the ^{14}C ages of MOC removed by oxidation were surprisingly comparable to those extracted by NaF/NaOH. This result indicates that NaF/NaOH residues contain oxidizable OC of similar or only slightly older age as the extracted material. We found that both NaF/NaOH and H_2O_2 mostly removed OC from a younger, ^{14}C-enriched pool, resulting in increasingly old residual OC as more OC is removed. Jagadamma et al. (2010) similarly observed that sequentially stronger oxidants removed C with similar ^{14}C contents, irrespective of the extent of OC removal. Applying a mass balance approach to the results of the extraction procedures used by Helfrich et al. (2007) showed similar soil ^{14}C contents of removed OC, irrespective of the extracted OC amounts. Accordingly, a large proportion of MOC is more homogenous in ^{14}C contents than previously thought, while only a small proportion is very old.

The $\Delta^{14}C$ difference between NaF/NaOH and oxidation residues was unexpectedly smaller for the two soils rich in pedogenic oxides (Laqueuille, Wetzstein) compared to the Luvisol and the Cambisol. The soils rich in pedogenic oxides had slightly higher amounts of OC left in oxidation residues, suggesting that they protected a larger proportion of OC against oxidation. Eusterhues et al. (2005) similarly observed that more OC resisted H_2O_2 oxidation in subsoils rich in pedogenic

oxides, and older residues in the Dystristic Cambisol than the Haplic Podzol studied. This study, in combination with our results, suggests that high contents of pedogenic oxides in soils increase oxidation resistance of MOC but do not increase residue ages. Accordingly also oxidation is likely not the dominant mechanism for exchange of OC on these mineral surfaces. The relatively young oxidation residues at Wetzstein and Laqueuille in our study could be due to high DOC fluxes, and thus, overall faster OC replacement of all MOC components at the Podzol site Wetzstein, and the younger soil age of the Andosol soil at Laqueuille. It is further possible that at some of the sites, old oxidation-resistant OC was inherited from the parent material (e.g., the loess layer or limestone residues at Hainich and Hesse) or a specific fire history (e.g., at the Chernozem site Gebesee, where only small, but very old amounts of OC were left after the H_2O_2 treatment).

4.4 Changes in OC turnover along soil profiles

The close correlations between $\Delta^{14}C$ values of NaF/NaOH extracts and residues along the soil profiles (see also Figure A4) result in a parallel decline in $\Delta^{14}C$ values of both fractions with soil depths. Not only was the same proportion of OC extracted across soil depths, but also the absolute differences between $\Delta^{14}C$ values of extracts and residues remained constant. The observed constant extractability indicates that changes in $\Delta^{14}C$ with depth are not driven by an increase in the stability of residual or extractable OC. This suggests that (1) the distribution fast and slower cycling MOC is constant with depth and that (2) the shape of the ^{14}C distribution of MOC (if we consider ^{14}C in MOC to be a continuum) remains constant with depth. This is supported by the observation that residues of the H_2O_2 treatment, though on average much older, declined almost parallel to NaF/NaOH residues with soil depth. The factor that causes the decline of $\Delta^{14}C$ values with soil depth is shifting the entire ^{14}C distribution of MOC. This, along with the uniform extractability, suggests that subsoil and topsoil MOC are at comparable equilibriums with their environment, and the reasons for overall ^{14}C declines with depth in soils are not due to changes in the character of mineral- organic matter stabilization mechanisms.

Previous studies showed that the oxidation resistant proportion of bulk soil samples increased with soil depth (Butnor et al., 2017; Eusterhues et al., 2005). While the MOC analyzed in this study showed only a small and insignificant trend in oxidation resistance with depth, we observed a stronger decline in $\Delta^{14}C$ values of oxidation residues with depth relative to bulk MOC. Thus, oxidation resistance (i.e., chemical recalcitrance), rather than desorption resistance could contribute to some extent to the formation of stable MOC with soil depth. The divergence of oxidized and residual $\Delta^{14}C$ values as more OC is oxidized occurred across sites (Figure A3), suggesting an overall trend of increasingly older OC with smaller amounts of OC remaining on the minerals.

5 Synthesis and implications

5.1 Suitability of chemical fractionation schemes to study MOC stability

NaF/NaOH extraction and H₂O₂ oxidation results suggest that, rather than averaging over a wide distribution of ages, the majority of total MOC has very similar ^{14}C contents, with only a small proportion (on average <12%) of total MOC being much older than the mean of the extracted or oxidized fractions. The different chemical fractionation schemes removed OC from the same pool, leaving increasingly old OC behind. Neither chemical oxidation nor NaF/NaOH extractability of MOC were related to the Δ^{14}C values of the removed OC. The combined application of several chemical fractionation schemes can therefore help identify ^{14}C distributions of samples, but their value for explaining site-specific differences in Δ^{14}C values is limited.

5.2 Similarity of MOC extraction results across sites

The initial assumption of this study was that the stability of mineral-bound OC would be related to desorption, and thus, vary between soils with different mineral composition and under different land use. We found that OC extracted in NaF/NaOH was indeed consistently younger than bulk MOC, suggesting that desorbed OC was more frequently exchanged than the older residue. The chemical uniformity of extracted MOC for three sites (two beech forests and one grassland) suggests a certain selection for specific OC molecules during MOC formation or that microbial processing of OC on mineral surfaces homogenizes MOC composition relative to original OM differences from litter and vegetation types. However, the Podzol site and the Chernozem site deviated from the pattern of the other results, indicating that factors including decomposition conditions affecting DOC or a specific fire history can affect the organic matter composition of MOC.

Despite differences in mineral and chemical composition, extractability of MOC was uniform across non-cultivated soils and depths for the acid to neutral central European soils studied. Based on these results, most extractable MOC is formed from specific interactive functional groups, including metal-coordinated hydroxyl groups on the minerals and carboxyl groups of OM. The minerals or OM molecules that these groups are attached to are interchangeable. Since total MOC amounts were controlled by contents of pedogenic Al and Fe oxides across sites, extraction results may mostly reflect the response of oxides, which would overshadow potential mineral-specific differences in binding strengths observed in pure minerals in the laboratory. This supports the paradigm that oxides are more important than clay for OC storage in soil (Rasmussen et al., 2018) and could facilitate modelling of MOC formation and turnover in the future.

5.3 Subsoil MOC stability

We observed no indication of the presence of stronger, less desorbable bonds between OC and minerals in subsoils than topsoils, and declines in the ^{14}C content of extracts and extraction residues with depth paralleled each other. Consequently, subsoil OC was not better protected against desorption, and thus, potential subsequent degradation than topsoil OC. Although older, subsoil MOC can be equally vulnerable to changes in soil environmental conditions like temperature. Reduced NaF/NaOH-extraction at the cropland site indicates that desorption may be explained in part by OC loading of minerals. A possible explanation for the missing depth gradient in MOC extraction is that subsoil MOC is typically at a

similar equilibrium with the local conditions as the topsoil. This could be the case if OC input to undisturbed subsoils occurs mostly in hotspots, for example via root inputs or along preferential flow paths, where OC binds to exposed mineral surfaces but bypasses surfaces located inside aggregates or away from such hot spots.

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5.4 Alternative hypothesis for site- and depth-specific differences in ^{14}C

Despite the overall similarity in MOC composition and extractability across sites, Δ^{14}C values of extracted MOC (and of extraction residues) were proportional to Δ^{14}C values in bulk MOC and exhibited site-specific differences. Since Δ^{14}C values of bulk MOC and its fractions are independent of OC resistance to desorption and soil mineralogy, site specific differences

525 are likely driven by factors driving MOC accumulation and displacement, rather than intrinsic differences in bond stabilities. Assuming that MOC is the product of DOC entering and leaving mineral surfaces, Δ^{14}C values of MOC should be sensitive to site-specific differences in fluxes, composition and Δ^{14}C values of DOC (Figure 10). These metrics should reflect differences in overall ecosystem properties and decomposition conditions, including litter chemistry, soil pH, microbial community composition or climate, rather than soil mineralogy. Differences in depth profiles of Δ^{14}C values would then be

530 caused variations in OC input by roots and DOC transport. Under conditions of lower direct litter input, vertical OC transport e.g. the "cycling downwards" concept (Kaiser and Kalbitz, 2012) would shape the trends of Δ^{14}C values with depth. Alternatively, differences in the stability of pedogenic oxide minerals themselves are also shaped by soil environmental factors.

For example, at the Podzol site Wetzstein, the thick organic layer (8-14 cm) overlying the topsoil could have contributed to

535 old Δ^{14}C values of total and extractable MOC in the uppermost mineral layer relative to the other undisturbed sites via input of pre-aged DOC. Kindler et al. (2011) measured much higher DOC leaching rates from topsoils at Wetzstein than at Laqueuille or Hainich, despite only slightly higher subsoil leaching rates, suggesting that a large proportion of this mobilized topsoil DOC at Wetzstein was adsorbed in the subsoil. The absorption of leachate in the subsoil explains the shallower depth decline of ^{14}C compared to the other two sites.. Future research on the role of DOC formation, composition and Δ^{14}C values

540 for accumulation and exchange rates of OC on mineral surfaces along soil profiles under field conditions will contribute to the understanding of the age distribution of MOC in soils.
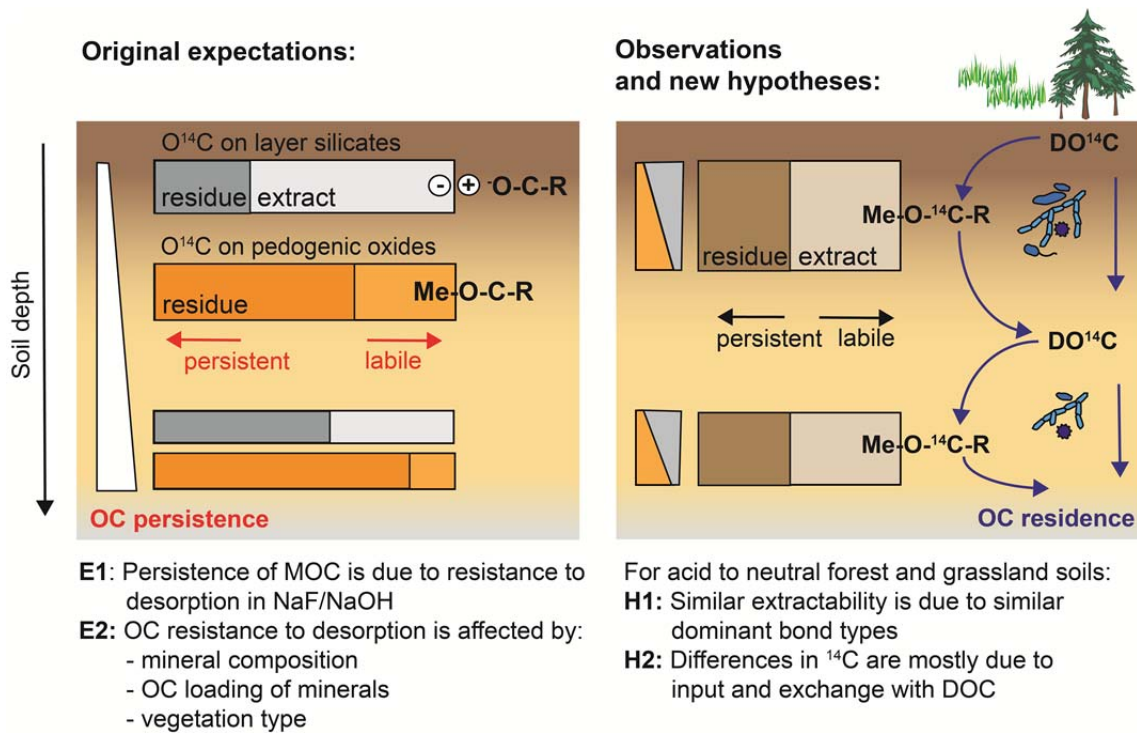
Figure 10: Graphical summary of (1) original expectations (left) where potential MOC desorption explains MOC persistance and varies with mineral composition and soil depth; (2) the observation of site-independent desorption (right), and alternative hypotheses explaining Δ^{14}C values of MOC across sites and with depth.

 7 Appendices

Appendix A: Additional Figures.
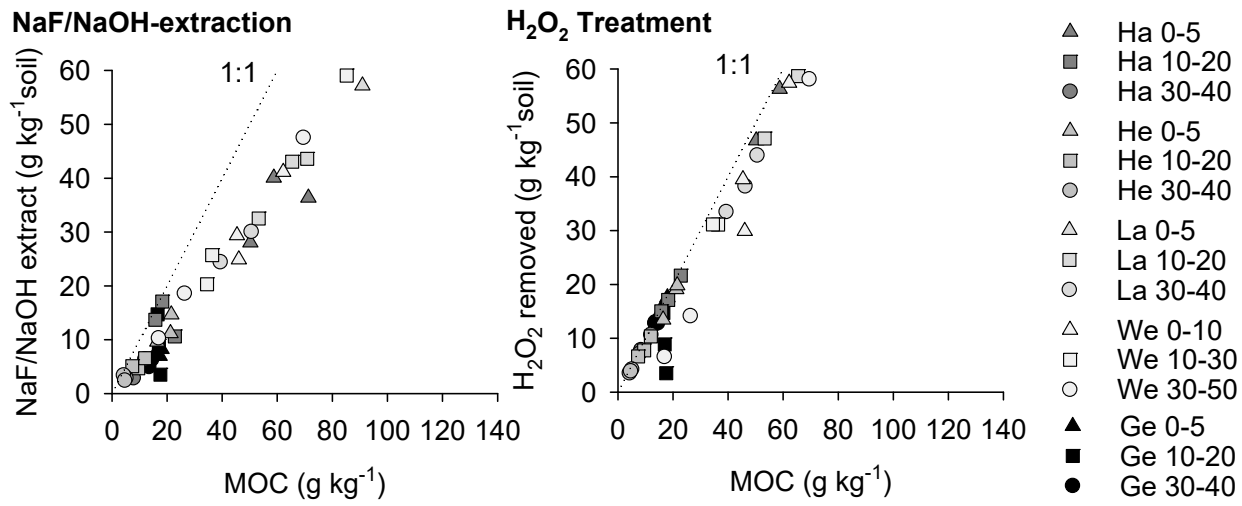
Figure A1: Dependency of removed OC from original concentrations of mineral-associated OC for the NaF/NaOH extraction (left)
555 **or the H$_2$O$_2$ oxidation (right).**
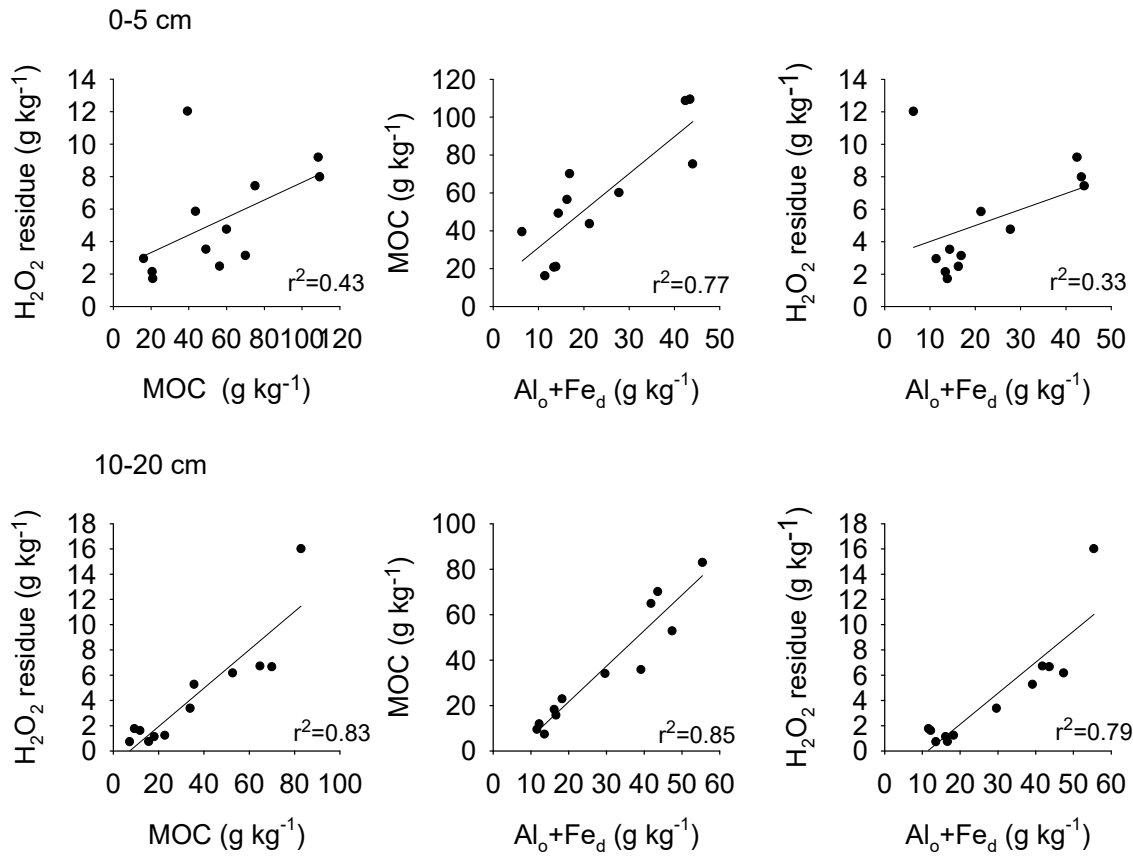
Figure A2: Relations between OC in mineral association (MOC), the amount of OC left from MOC after the H₂O₂ treatment
560 **(H₂O₂ residue) and the content of oxalate-extractable Al (Alₒ) and dithionite-extractable Fe (Fe_d) for two soil depths (Alₒ and Fe_d data were taken from Schrumpf et al. (2013)).**
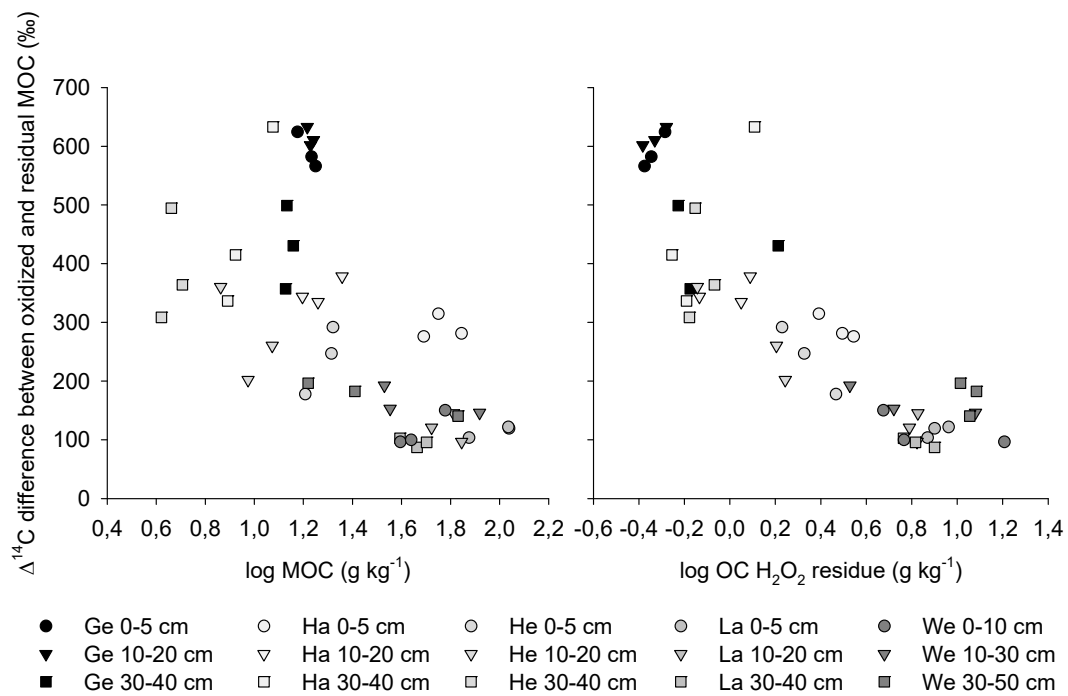
Figure A3: Decline in ^{14}C differences between oxidized and residual OC after H_2O_2 treatment with increasing OC amounts in total MOC and OC left in H_2O_2 residues across sites and depths.
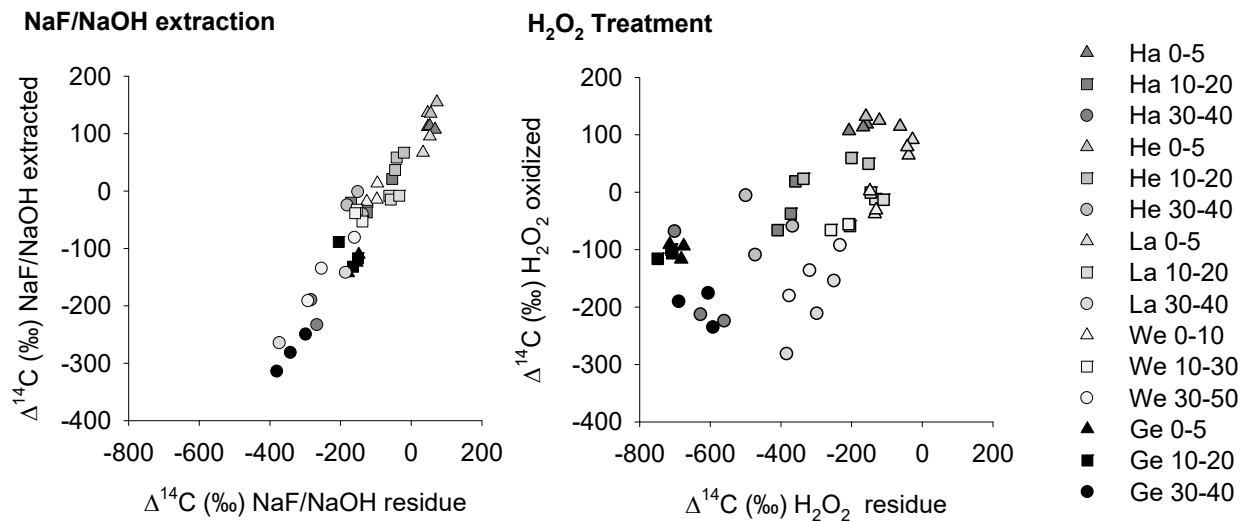
Figure A4: Correlation between ^{14}C contents of NaF/NaOH extracts and extraction residues of HF-OC (left) and between oxidized OC and residue OC following treatment of HF-OC with heated H_2O_2 (right).
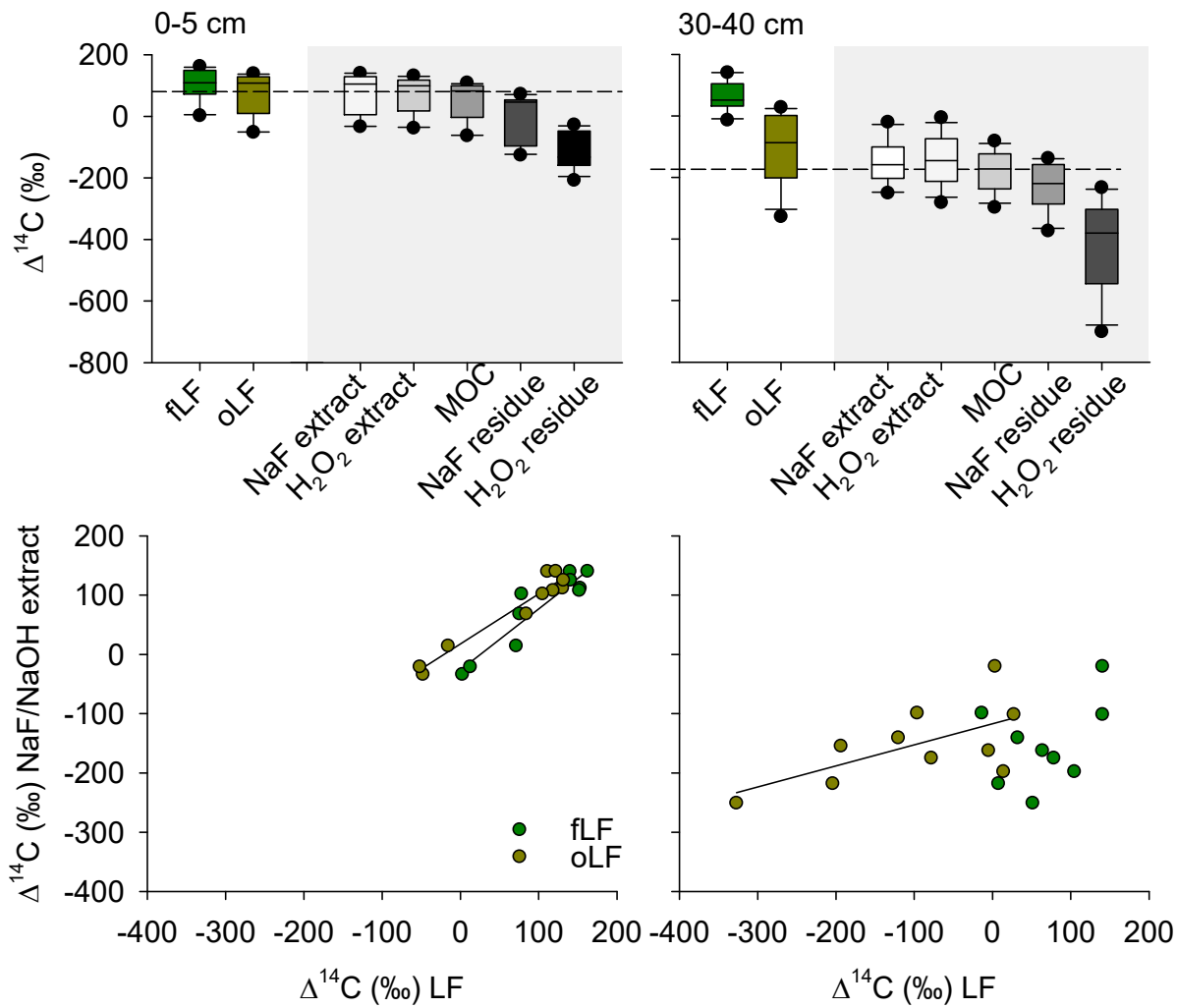
Figure A5: Top: box-plots giving an overview of ^{14}C contents in all OC fractions separated from bulk samples in 0-5 cm (left) and 30-40 cm (right) depth for the non-arable samples (dashed line denotes the median of the ^{14}C in bulk MOC). Bottom: relation between ^{14}C contents of OC in NaF/NaOH extracts of MOC and OC in the two light fractions in 0-5 cm (left) and 30-40 cm (right) depths for the non-arable samples. fLF: free light fraction, oLF: occluded light fraction, values adopted from Schrumpf et al. (2013).

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8 Code availability: not applicable

9 Data availability: we intend to publish the data in the ISRAD database

10 Sample availability: all remaining material of original MOC samples and the obtained fractions is stored at the MPI-BGC and can be made available upon request**.**

11 Video supplement: none

12 Supplement link: not applicable unless primary data should be provided for review in the supplement

13 Team list: who should be mentioned in addition to people contributing as authors or added to the acknowledgement?

14 Author contributions

MS, KK, AM and ST designed the experiments and AM carried them out. GH conducted the NMR analyses. MS prepared the manuscript with contributions from all co-authors.

15 Competing interests: none

16 Disclaimer The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

17 not applicable

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