Biogeochemistry (2017) 132:307–324
DOI 10.1007/s10533-017-0304-2

Aggregation controls the stability of lignin and lipids in clay-sized particulate and mineral associated organic matter

Gerrit Angst · Kevin E. Mueller · Ingrid Kögel-Knabner ·
Katherine H. Freeman · Carsten W. Mueller

Received: 30 August 2016 / Accepted: 1 February 2017 / Published online: 10 February 2017

Abstract Physical separation of soil into different soil organic matter (SOM) fractions is widely used to identify organic carbon pools that are differently stabilized and have distinct chemical composition. However, the mechanisms underlying these differences in stability and chemical composition are only partly understood. To provide new insights into the stabilization of different chemical compound classes in physically-separated SOM fractions, we assessed shifts in the biomolecular composition of bulk soils and individual particle size fractions that were incubated in the laboratory for 345 days. After the incubation, also the incubated bulk soil was fractionated. The chemical composition of organic matter in bulk soils and fractions was characterized by ^{13}C-CPMAS nuclear magnetic resonance spectroscopy and sequential chemical extraction followed by GC/MS measurements. Plant-derived lipids and lignin were abundant in particulate organic matter (POM) fractions of sand-, silt-, and clay-size and the mineral-bound, clay-sized organic matter. These results indicate that recent conceptualizations of SOM stabilization probably understate the contribution of plant-derived organic matter to stable SOM pools. Although our data indicate that inherent recalcitrance could be important in soils with limited aggregation, organo-mineral interactions and aggregation were responsible for long-term SOM stabilization. In particular, we

Responsible Editor: Susan E. Crow.

Gerrit Angst and Kevin E. Mueller equally contributed to the manuscript.

Electronic supplementary material The online version of this article (doi:10.1007/s10533-017-0304-2) contains supplementary material, which is available to authorized users.

G. Angst · I. Kögel-Knabner · C. W. Mueller
Chair of Soil Science, Technical University of Munich
(TUM), Emil-Ramann-Strasse 2, 85345 Freising,
Germany

K. E. Mueller
United States Department of Agriculture, Agricultural
Research Service, Rangeland Resources Research Unit,
Ft. Collins, CO 80526, USA

I. Kögel-Knabner
Institute for Advanced Study, Technical University of
Munich (TUM), Lichtenbergstraße 2a, 85748 Garching,
Germany

K. H. Freeman
Department of Geosciences, Pennsylvania State
University, University Park, PA 16802, USA

Present Address:
G. Angst (✉)
Institute of Soil Biology and SoWa RI, Biology Centre, Czech
Academy of Sciences, Na Sádkách 7, 37005 České Budějovice,
Czech Republic
e-mail: gerrit.angst@gmail.com

observed consistently higher concentrations of plant-derived lipids in POM fractions that were incubated individually, where aggregates were disrupted, as compared to those incubated as bulk soil, where aggregates stayed intact. This finding emphasizes the importance of aggregation for the stabilization of less 'recalcitrant' biomolecules in the POM fractions. Because also the abundance of lipids and lignin in clay-sized, mineral-associated SOM was substantially influenced by aggregation, the bioavailability of mineral-associated SOM likely increases after the destruction of intact soil structures.

Keywords Incubation · Physical fractionation · GC/MS · ^{13}C NMR · CuO · Soil organic matter

Introduction

Soil organic matter (SOM) is composed of a multitude of biomolecules with different residence times, ranging from a few minutes to more than thousands of years (Schimel et al. 1994; Torn et al. 1997; von Lützow et al. 2007; Amelung et al. 2008). The turnover time of SOM components has been shown to be influenced by different stabilization mechanisms, including: (i) resistance against decomposition because of the inherent chemical characteristics of biomolecules, (ii) decreased bioavailability due to the development of soil aggregates separating organic matter (OM) from decomposers, and (iii) stabilization of OM in organo-mineral associations by chemical surface reactions (Sollins et al. 1996; Christensen 2001; von Lützow et al. 2006).

In studies investigating the mechanisms of organic C (OC) stabilization and sequestration in soils, it has become common practice to separate bulk soil by physical means (e.g., density and size of particles or aggregates) into fractions that have different OM characteristics (e.g., residence times, turnover times, and chemical composition) (e.g., Angst et al. 2016b; Hassink et al. 1997; Mueller et al. 2009). Based largely on these studies, it is often asserted that: (i) the persistence of SOM is due mostly to physico-chemical interactions like selective stabilization by organo-mineral association and occlusion of OM inside aggregates (Schmidt et al. 2011), and (ii) inherent recalcitrance of certain organic biomolecules is not important for the long-term stabilization of SOM (Marschner et al. 2008; Kleber 2010). For example, lignin constitutes a major plant input to SOM (Kögel-Knabner 2002) and has commonly been regarded as relatively resistant to decomposition because of its aromaticity (Krull et al. 2003; Rasse et al. 2005). However, it does not appear to be selectively preserved in many mineral soil horizons, evidenced by a depletion of lignin and aromatic C forms in fine-sized mineral fractions (Rumpel et al. 2004; Mikutta et al. 2006) and no relative accumulation of lignin inside aggregates (Poirier et al. 2005). In contrast, lipids/aliphatics (Rumpel et al. 2004) and commonly easily degradable carbohydrates (Rumpel et al. 2010) were shown to accumulate in mineral fractions. Particularly, plant-derived bound lipids such as cutin and suberin might be selectively preserved in low-density, particulate OM fractions (POM), due to a possible inherent recalcitrance of those biopolymers (Filley et al. 2008a, b; Pisani et al. 2014), or in mineral-associated OM fractions, due to selective interactions with mineral surfaces (Rumpel et al. 2004; Clemente et al. 2011; Carrington et al. 2012). Recalcitrance might play a greater role, as compared to physico-chemical mechanisms of stabilization, in particulate organic matter fractions and in coarse textured soils with lower levels of aggregation and reactive mineral surfaces. These soil type specific differences are probably one reason why a consensus about the relative importance of the different stabilization mechanisms is still absent (e.g., Moni et al. 2010; Schrumpf et al. 2013). Another reason for the persistence of uncertainties about the roles of recalcitrance vs. organo-mineral association and aggregation derives from the fact that there are very few published observations on the dynamics of different biomolecule types during SOM mineralization (e.g., Feng and Simpson 2008). Commonly, SOM mineralization and bioavailability are evaluated by measuring respired CO_2 of incubated soils over time (e.g., Alvarez et al. 1998; Stemmer et al. 1999; Spaccini et al. 2000). The sole consideration of CO_2 respiration rates, however, does not allow insights into mechanisms responsible for the stabilization or respiration of certain OM constituents. This lack of molecular-level studies hinders the ability to model and predict the response of soil C pools to environmental change reliably (Campbell and Paustian 2015).

Here, to provide new insights into SOM stabilization and the role of inherent recalcitrance and aggregation, we uniquely combined physical soil fractionation, soil incubations, and comparative chemical analyses of SOM composition. In a previous study by Mueller et al. (2014), both naturally aggregated bulk soil and individual particle-size fractions from the same soil were simultaneously incubated. The incubated bulk soil was subsequently separated into fractions resembling those used for the individual particle-size incubations (Mueller et al. 2014). By quantifying various plant-derived biomolecules in the fractions separated by Mueller et al. (2014), we were able to study the role of inherent recalcitrance and bioavailability for the chemical composition of each individual fraction, where aggregates were disrupted prior to incubation, and the'combined' fractions within the bulk soil, where aggregation was intact. We especially aimed to track the fate of lignin and aliphatics like cutin- and suberin-derived monomers because these plant-derived biomolecules are major inputs to SOM, which are considered 'recalcitrant' over at least short time scales (from days to months), and are often used to characterize and trace the sources of SOM (Kögel-Knabner 2002; Jansen et al. 2006; Crow et al. 2009; Angst et al. 2016c). To the best of our knowledge, these compounds have not been studied before in a comparative incubation of bulk soil and individually incubated SOM fractions, an approach that also has great potential for investigating stabilization mechanisms of other biomolecule compounds in SOM, such as amino or neutral sugars.

Materials and methods

The experimental design of the present study, including methodological details for the fractionation, incubation, C, N, and NMR measurements, has been published by Mueller et al. (2014). That study focused on heterotrophic respiration of $^{13}CO_2$ and $^{14}CO_2$. Here, to set the analyses used in this study into a comprehensive context and enable the reader to interpret the data properly, we include a brief description of the experiment and previously conducted analyses.

Soil material

Soil was sampled in 2005 at the Höglwald located in Southern Germany (48°17′24″N, 11°04′24″E), an even-aged Norway spruce forest (*Picea abies* (L.) Karst) established in 1925. The soils at the site were mainly composed of Quartz, while Illites and primary Chlorites were also abundant; Smectites and Kaolinites were only found in trace amounts. The soil samples were randomly taken from the Ah horizon (40.7% sand, 39.4% silt, 19.9% clay; 75.3 ± 19.2 mg C/g soil) of an acidic Albic Luvisol (IUSS Working Group WRB, 2014) at ten subplots (within a 20 × 20 m area) to account for natural variability. The samples were air dried, sieved to <2 mm, and combined to a composite sample for further analyses. To test representativeness, the samples from the ten subplots were analysed for C/N ratio (21.1 ± 1.2) and pH value (pH in H_2O: 3.5 ± 0.1; pH in $CaCl_2$: 2.8 ± 0.1). The coefficients of variation were always ≤5.7%, suggesting the subsamples and composite sample are representative of the typical soil conditions at the site (Mueller et al. 2014).

Physical fractionation

The soil was fractionated by particle size and density to obtain distinctively different SOM fractions. A detailed description of the fractionation protocol is given elsewhere (Mueller et al. 2014). Briefly, the air dried and sieved (<2 mm) bulk soil was saturated with deionized water and subjected to a two-step ultrasonication. According to Amelung and Zech (1999), a two-step soil structure break up scheme was used to avoid the rupture and translocation of larger OM particles of coarse and medium sand size into finer fractions. In the first step, 60 J/ml were applied to the soil–water mixture and subsequently the coarse- and medium-sized SOM fractions (200–2000 µm) were separated via wet-sieving (mesh-size of 200 µm). In the second step, 440 J/ml were applied to break up stronger bound smaller aggregate structures. The fine sand fraction (≥63 µm, ≤200 µm) was separated after the second ultrasonication step with a sieve of 63 µm mesh-size. After the fractionation, the sand fractions were recombined. The combined fine silt and clay fraction (<6.3 µm) was separated from the coarse and medium silt fractions (>6.3–63 µm) via sedimentation. We combined the fine silt and clay fraction because their chemical composition and degree of organo-mineral association is similar (Mueller et al. 2014; Angst et al. 2016c), whereas the time for sedimentation is reduced by a factor of 10 compared to the sedimentation of the clay fraction (<2 μm) only. The obtained sand (63–2000 μm), silt (6.3–63 μm), and clay (<6.3 μm) fractions and the bulk soil were used for the subsequent incubation experiment. The incubated bulk soil was fractionated after the incubation as described above.

At the end of the incubation, aliquots (replicated) of each fraction were saturated with a density solution (sodiumpolytungstate 1.8 g/cm^3), shaken, and centrifuged. The floating particulate POM was collected using a water jet pump. These steps were repeated until no additional POM was floating in the density solution. To remove excessive salt from the remaining soil matrix, the soil fractions with a density greater than 1.8 g/cm^3 (i.e., the 'mineral' fractions) were repeatedly washed with de-ionized water until the electrical conductivity of the eluted water fell below 5 μS/cm. By applying this procedure, we obtained sand-, silt-, and clay-sized POM (termed 'sand POM', 'silt POM', and 'clay POM') and mineral fractions (termed 'sand MIN', 'silt MIN', and 'clay MIN'). Because the C content of the sand MIN and silt MIN were negligibly small (Table 1; Mueller et al. 2014), we focused our analyses and interpretations on the sand POM, silt POM, clay POM and clay MIN fractions.

Incubation experiment

Bulk soil (n = 3) was incubated for 345 days and subsequently fractionated according to the procedure described in the "Physical fractionation" section. Simultaneously, the three SOM fractions (sand, silt, and clay fraction, n = 3 for each) obtained from fractionation of non-incubated bulk soil were individually incubated for the same amount of time (345 days). This approach enabled us to investigate different stabilization mechanisms separately (individually incubated fractions) and in interaction with each other (incubated bulk soil). The bulk soil and SOM fractions were incubated under the same conditions. A detailed description of the experimental setup is given by Mueller et al. (2014). Briefly, the bulk soil and the three SOM fractions were mixed with acid-washed (10% HCl) and annealed quartz sand (ratio 1:2.5, w:w) to increase the air-filled pore space. The maximum water-holding capacity was determined by draining steel rings (9 cm^3) filled with aliquots of each sample on a porous plate for 2 h at 10 hPa. The maximum water-holding capacity was then calculated by difference of the dry and drained soil materials. Each sample was inoculated with 100 μl of a soil solution obtained from the pre-incubation of a mixture of 10 soils to assure a wide microbial variety. For an optimal nutrition, a nutrient solution according to Ingestad and Lund (1986) was added to the samples containing both macro- and micronutrients. The samples were incubated in 1 L air-tight glass bottles for 345 days in triplicate at a constant temperature of 20 °C. The water content was initially adjusted to 70% of maximum water holding capacity and, if needed, water was added to sustain this level of water saturation. We thus studied the fate of SOM in different particle-size fractions under optimal conditions for soil microorganisms, to some extent

Table 1 Content of the sand, silt, and clay POM and sand, silt, and clay mineral fractions (MIN) per g dry soil and respective SOC contents before and after the incubation for bulk soil (incub. bulk) and individually incubated fractions (incub. fract.)

		Content mg/g dry soil	SOC mg/g before	SOC mg/g after	
				Incub. bulk	Incub. fract.
Fractions	Sand POM	29.6 ± 10.6	315.5 ± 23.0a**	183.1 ± 15.3b**	182.3 ± 14.7b**
	Sand MIN	331.9 ± 4.9	bdl	0.8 ± 0.04a	0.8 ± 0.03a
	Silt POM	47.0 ± 0.2	344.3 ± 6.2a	294.0 ± 27.4a	291.9 ± 26.1a
	Silt MIN	298.8 ± 6.8	2.2 ± 0.5a	2.4 ± 0.2a	2.4 ± 0.1a
	Clay POM	53.2 ± 4.5	289.3 ± 12.2a	254.2 ± 25.3a	256.6 ± 24.9a
	Clay MIN	239.6 ± 18.1	100.7 ± 28.9a	99.1 ± 6.5a	97.7 ± 5.5a

Letters (a, b) indicate significant differences between initial, incub. bulk, and incub. fract

** $p < 0.05$

bdl Below detection limit

Data partly taken from Mueller et al. (2014)

offsetting possible nutrient limitations encountered in soils such as investigated in the present study.

The SOM fractions obtained from the incubated bulk soil are termed ‘bulk soil fractions’ and the individually incubated SOM fractions are termed ‘individually incubated fractions’ in the following chapters.

Determination of C and N contents and ^{13}C NMR spectroscopy

Aliquots of the individually incubated and bulk soil fractions, including both incubated and non-incubated material, were ground and C and N contents were measured in duplicate via dry combustion (Vario EL CN Analyzer). All samples were free of carbonates (Mueller et al. 2014), thus the measured C concentrations equaled the organic C concentrations. Ground aliquots of the incubated and non-incubated fractions were also measured by solid-state ^{13}C CPMAS NMR spectroscopy on a Bruker DSX 200 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). The samples were spun in zircon oxide rotors around a magic angle at a speed of 6.8 kHz. The contact time was set to 1 ms. Chemical shifts were referenced to tetramethylsilane (TMS = 0 ppm) and shift regions were integrated as follows: −10 to 45 ppm (alkyl C), 45–110 ppm (O/N-alkyl C), 110–160 ppm (aryl C), and 160–220 ppm (carboxyl C).

Soil OM characterization by sequential extraction and GC–MS

Solvent-extractable lipids were recovered from all samples by using a Dionex ASE 200 to perform accelerated solvent extraction. The extraction cells were filled with sand that was previously ashed, then the cells were pre-extracted with a mixture of dichloromethane (DCM):methanol (MeOH) (9:1, v:v) at a pressure of 1200 psi and a temperature of 100 °C. After the pre-extraction, enough sand to fit the sample was poured out of the cell. Then, the sample was placed in the cell between two pre-extracted glass fiber filters. Free space between the top of the sample and the cell cap was filled again with pre-extracted sand. The samples were extracted 4× with DCM:MeOH (9:1, v:v), 3× at a pressure of 1000 psi and a temperature of 75 °C and then 1× at a pressure of 1200 psi and a temperature of 100 °C. All samples were dried by applying a gentle flow of nitrogen gas to the headspace of sample vials. Extracted lipids were then stored in DCM and refrigerated (~4 °C) until further analysis.

We used alkaline cupric oxide to release lignin-derived compounds from incubated and non-incubated soil fractions. Our methods were similar to those developed by Hedges and Ertel (1982), Goñi and Montgomery (2000), and Filley et al. (2008a, b). The sample material (containing at least 2–5 mg OC) was weighed into 10-ml Monel reaction vessels (“mini-bombs”) together with 50 mg of ammonium iron (II) sulfate hexahydrate ($Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$), 300 mg of pre-extracted cupric oxide and enough 2 M sodium hydroxide (NaOH) solution to almost fill the reaction vessel. The bombs were sealed with Teflon lined lids, shaken to mix the sample, and subsequently heated to 155 °C at 4.2 °C per minute held for 150 min. After cooling, 100 µl of trans-cinnamic acid recovery standard were added to each sample and the whole content of each bomb was transferred to centrifuge tubes by repeated washing of the bombs with NaOH solution. The tubes were centrifuged for 5–10 min at 4000 rpm. The supernatant NaOH was pipetted into separate vials and acidified with 6 M, DCM extracted HCl. The solution was 4× extracted with ethyl acetate, dried under N, and stored in the fridge after the addition of 500 µl pyridine.

Lipids and phenols in the ASE extracts and those recovered after the alkaline cupric oxide oxidation were measured on a Hewlett-Packard (HP) 6890 GC coupled to a HP 5973 quadrupole mass spectrometer with electron impact ionization. Aliquots of each sample in pyridine (50 µl) were combined with 10 µl of internal standards in pyridine (3,4-dimethoxybenzoic acid and 12-hydroxyoctadecanoic acid). Acids and alcohols were converted to their trimethylsilyl derivatives by adding 50 µl of a derivatizing agent (bistrimethylsilyltrifluoroacetamide, BSTFA) and heating for 15–30 min. at 60 °C. Analytes were separated on a 30 m fused silica column with a diameter of 0.25 mm (Agilent J&W DB-5). Helium was used as carrier gas with a flow of 1.5 ml/min for the ASE extracts and 1.3 ml/min for the analytes recovered after alkaline cuprix oxide oxidation. All analytes were injected in pulsed splitless mode with an injector temperature of 320 and 300 °C, respectively. The temperature program of the GC oven for the analytes in ASE extracts was as follows: 100 °C initial temperature (held for 2 min), to 170 °C at 8 °C/min, to 200 °C at 1.5 °C/min, to 200 °C at 1.5 °C/min, to 250 °C at 4 °C/min, to 300 °C at 6 °C/min, to 320 °C (held for 10 min) at 10 °C/min. The temperature program of the GC oven for the analytes recovered after alkaline cuprix oxide oxidation was as follows: 100 °C initial temperature (held for 2 min.) to 172 °C at 8 °C/min, to 184 °C at 4 °C/min, to 320 °C (held for 10 min.) at 10 °C/min. The scan range was *m*/*z* 50–550 and ionization energy was 70 eV.

The identification and quantification of the analytes was done following Mueller KE et al. (2012b) and Filley et al. (2008a, b). For quantification of most analytes ($n = 73$), the peak areas of target ions were normalized to an internal standard (3,4-dimethoxybenzoic acid or 12,*ω*-hydroxyoctadecanoic acid) and the concentrations estimated by comparison with peak areas of external standards (quantified separately at seven different concentrations). Analytes that could not be directly quantified by a standard compound of the same identity were either quantified by extrapolation or interpolation using equations fit to the response factors of homologous compounds (Chaurasia et al. 1995) or by comparison with standard compounds of similar structure; this method of quantification was used for 6 compounds present in the ASE extracts and 12 compounds recovered after alkaline cuprix oxide oxidation. The average recovery of the trans-cinnamic acid was 81% ($SD = 3\%$) with a minimum recovery of 75%. Cutin monomers, suberin monomers, other plant-derived lipids, and lignin monomers were identified according to previously published data (Mueller KE et al. 2012b; Otto et al. 2005; Angst et al. 2016a, b; Otto and Simpson 2006; Spielvogel et al. 2014). Briefly, plant-derived lipids in the ASE extracts were *n*-alkanoic acids (*n*-acids) $> C_{20}$ with an even chain length, α,*ω*-alkanedioic acids (diacids) C_{12}–C_{22} with an even chain length, *ω*-hydroxy alkanoic acids and mid-chain substituted hydroxy alkanoic acids (subsumed under hydroxyacids), *n*-alcohols $> C_{20}$ with an even chain length, and *n*-alkanes C_{25}–C_{31} with an uneven chain length. Plant-derived lipids in the CuO extracts were hydroxyacids and α,*ω*-alkanedioic acids with an even chain-length $\geq C_{16}$.

Statistics and calculations

From the NMR spectra, the alkyl C to O/N-alkyl C ratio was calculated as a proxy for the degree of decomposition of a sample because more resistant aliphatic components (alkyl C) tend to relatively increase during decay compared to more easily degradable cellulose, hemicellulose and proteins (O/N-alkyl C) (Baldock et al. 1997). Further, the molecular mixing model (Nelson and Baldock 2005) was applied to the spectra to infer the relative contribution of different biomolecules (carbohydrates$_{NMR}$, proteins$_{NMR}$, lignin$_{NMR}$, and lipids$_{NMR}$).

To evaluate the origin and alteration of lignin during incubation, we calculated different proxies from lignin derived compounds: the ratios between syringyl to vanillyl (S/V) and cinnamyl to vanillyl (C/V) type monomers (Ertel and Hedges 1984; Otto and Simpson 2006), the ratios between lignin-derived phenolic acids and their corresponding aldehydes for vanillyl $(Ac/Al)_V$ and syringyl $(Ac/Al)_S$ (Ertel and Hedges 1985), the ratio between ρ-coumaric and ferulic acids (F/C) (Ziegler et al. 1986), and the ratio between 3,5-dihydroxybenzoic acids and vanillyls (Prahl et al. 1994).

To identify if the chemical composition of SOM in each soil fraction changed significantly during the incubation experiment, we used one-way analysis of variance (ANOVA) followed by TukeyHSD tests to assess differences in SOM chemical composition between the initial, non-incubated samples and the individually incubated and bulk soil fractions. The ANOVA models were run using R 3.0.3 (R Core Team 2015). The Bartlett-test was used to test the variables for homoscedasticity. Differences between treatment means are reported as significant ($p < 0.05$) or marginally significant ($p < 0.1$).

Results

Carbon content and chemical composition as derived from NMR spectra

The SOC contents before the incubation were highest for the POM fractions, followed by the clay MIN fraction (Table 1). The decrease in SOC at the end of the incubation was only significant ($p < 0.05$) for the sand POM, while the silt and clay POM only showed a trend of decreasing SOC contents. The SOC content of the clay MIN fraction did not change at all. Notably, there were no significant differences between individually incubated and bulk soil fractions (Table 1).

The NMR spectra of all samples (Online Resource 1) showed a dominance of alkyl and O/N-alkyl C compared to aryl and carboxyl C (Fig. 1). In the sand POM, O/N-alkyl C clearly dominated the spectra, indicating the relative freshness of the material, which was also supported by high amounts of carbohydrates$_{\mathrm{NMR}}$ present in this fraction (Table 2). The degree of OM degradation progressed to the silt and clay POM, evidenced by increasing alkyl/O/N-alkyl C ratios (Fig. 1) and high amounts of aliphatic lipids$_{\mathrm{NMR}}$ with concurrently lower amounts of carbohydrates$_{\mathrm{NMR}}$ (Table 2). In contrast, the clay MIN showed a relatively

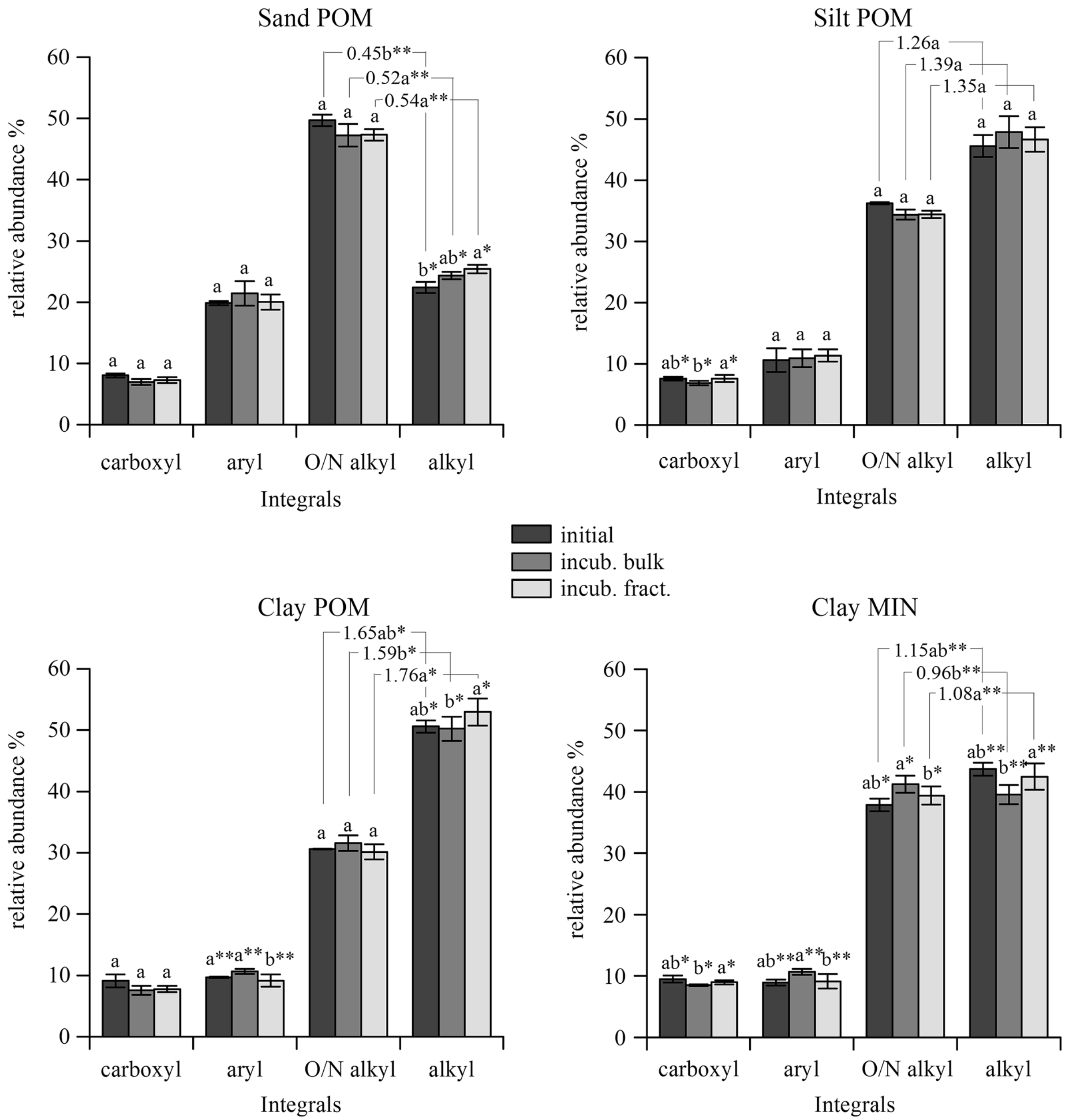

Fig. 1 Integration regions (carboxyl, aryl, O/N-alkyl, and alkyl C) obtained from ^{13}C NMR spectra for the initial, non-incubated SOM fractions (initial), the bulk soil fractions (incub. bulk), and individually incubated fractions (incub. fract.). The numbers above the bars depict the alkyl/O/N-alkyl C ratio for the respective treatment. Significant differences between the initial, non-incubated fractions and the different incubation treatments are indicated by different letters above the bars (*a*, *b*). $^{*}p < 0.1$, $^{**}p < 0.05$, n = 3 for each bar

Table 2 Relative amounts of carbohydrates, proteins, lignin, and lipids for initial, bulk soil (incub. bulk) and individually incubated fractions (incub. fract.)

	Relative amount [%]			
	$\text{Carbohydrates}_{NMR}$	Proteins_{NMR}	Lignin_{NMR}	Lipids_{NMR}
Sand POM				
initial	31.7 ± 0.5a	15.0 ± 0.1a	34.8 ± 0.7a	17.1 ± 0.9b*
incub. bulk	27.4 ± 2.2a	12.8 ± 1.2a	38.7 ± 3.8a	20.0 ± 0.5ab*
incub. fract.	29.0 ± 0.4a	15.2 ± 0.4a	32.4 ± 0.6a	21.8 ± 0.3a*
Silt POM				
initial	21.2 ± 0.5a*	9.6 ± 0.2a**	14.5 ± 1.4a	52.0 ± 1.0a
incub. bulk	19.0 ± 0.4b*	7.2 ± 0.6ab**	15.1 ± 2.6a	55.8 ± 2.9a
incub. fract.	18.5 ± 0.3b*	5.9 ± 0.1b**	16.0 ± 0.1a	54.0 ± 0.0a
Clay POM				
initial	15.7 ± 0.1a	7.7 ± 0.1a	12.1 ± 0.2a**	59.3 ± 1.4ab*
incub. bulk	15.4 ± 0.7a	7.8 ± 1.2a	14.0 ± 0.7a**	58.0 ± 2.9b*
incub. fract.	14.7 ± 0.1a	5.4 ± 0.3a	8.0 ± 0.8b**	67.2 ± 1.1a*
Clay MIN				
initial	21.8 ± 1.1a	18.9 ± 0.0a	9.3 ± 0.5ab*	44.9 ± 0.7ab**
Incub. bulk	24.8 ± 1.0a	19.0 ± 0.5a	13.7 ± 1.0a*	40.0 ± 1.9b**
incub. fract.	22.7 ± 0.4a	18.4 ± 0.6a	6.6 ± 1.6b*	48.4 ± 0.8a**

The data was derived from the application of the molecular mixing model to the NMR spectra of each fraction. Significant differences between initial, bulk soil, and individually incubated fractions are indicated by letters (a, b). * $p < 0.1$; ** $p < 0.05$

balanced contribution of alkyl and O/N-alkyl C (Fig. 1), which was also mirrored by lower amounts of lipids_{NMR} and higher amounts of $\text{carbohydrates}_{NMR}$ in this fraction compared to the silt and clay POM (Table 2). The relative amount of lignin_{NMR} decreased from the sand POM to the silt POM, clay POM, and clay MIN. Interestingly, the relative amount of the chemical compound classes and biomolecule components did not significantly change after 345 days of incubation compared to the initial material; this was true for both the individually incubated and bulk soil fractions (Fig. 1; Table 2) with only few exceptions: the alkyl C and the relative amount of lipids_{NMR} in the sand POM increased significantly (by 13 and 4.7%, respectively; $p < 0.1$) in the individually incubated fraction and the relative amount of lignin_{NMR} decreased significantly ($p < 0.05$) in the individually incubated clay POM (Fig. 1; Table 2).

Despite the absence of significant changes to the initial, non-incubated fractions in most cases, there were some small but notable differences in SOM composition between individually incubated and bulk soil fractions. Aryl C and the relative amount of lignin_{NMR} were significantly lower (by at least 14 and 6%, respectively; $p < 0.05$ and $p < 0.1$) and alkyl C and lipids_{NMR} were significantly higher in individually incubated clay POM (by 5 and 7.9%, $p < 0.1$) and MIN (by 7 and 8.4%, $p < 0.05$) compared to the corresponding bulk soil fractions (Fig. 1; Table 2). A trend of higher alkyl C (4% higher) in the individually incubated fraction was also present in the sand POM (Fig. 1). The O/N-alkyl C was significantly lower in individually incubated clay MIN (by 4%, $p < 0.1$) compared to that of bulk soil fractions. Consequently, alkyl to O/N-alkyl C ratios were also significantly higher in individually incubated clay MIN but also in the individually incubated clay POM compared to the corresponding bulk soil fractions (Fig. 1).

Solvent-extractable lipids

The ASE procedure released a number of *n*-alkanoic acids (C_{12} to C_{23}), α,ω-alkanedioic acids (C_{12} to C_{22}), ω-hydroxy alkanoic acids (C_{12} to C_{24}), mid-chain substituted hydroxy alkanoic acids (dihydroxy C_{16} and trihydroxy C_{18}), *n*-alcohols (C_{16} to C_{30}) and *n*-alkanes (C_{25} to C_{31}). Most of these compounds were plant-derived (70.6 ± 1.1%), independent of the fraction or incubation treatment (individually incubated or bulk soil fraction; Fig. 2). The other compounds could not be unambiguously ascribed to a source and may be either plant- and/or microbial-derived. The concentration of lipids was highest in the sand and clay POM (8.3–11.6 mg/g C) followed by the clay MIN (4.3–5.3 mg/g C), and silt POM (3.4–3.7 mg/g C; Fig. 2). The ω-hydroxy alkanoic acids and mid-chain

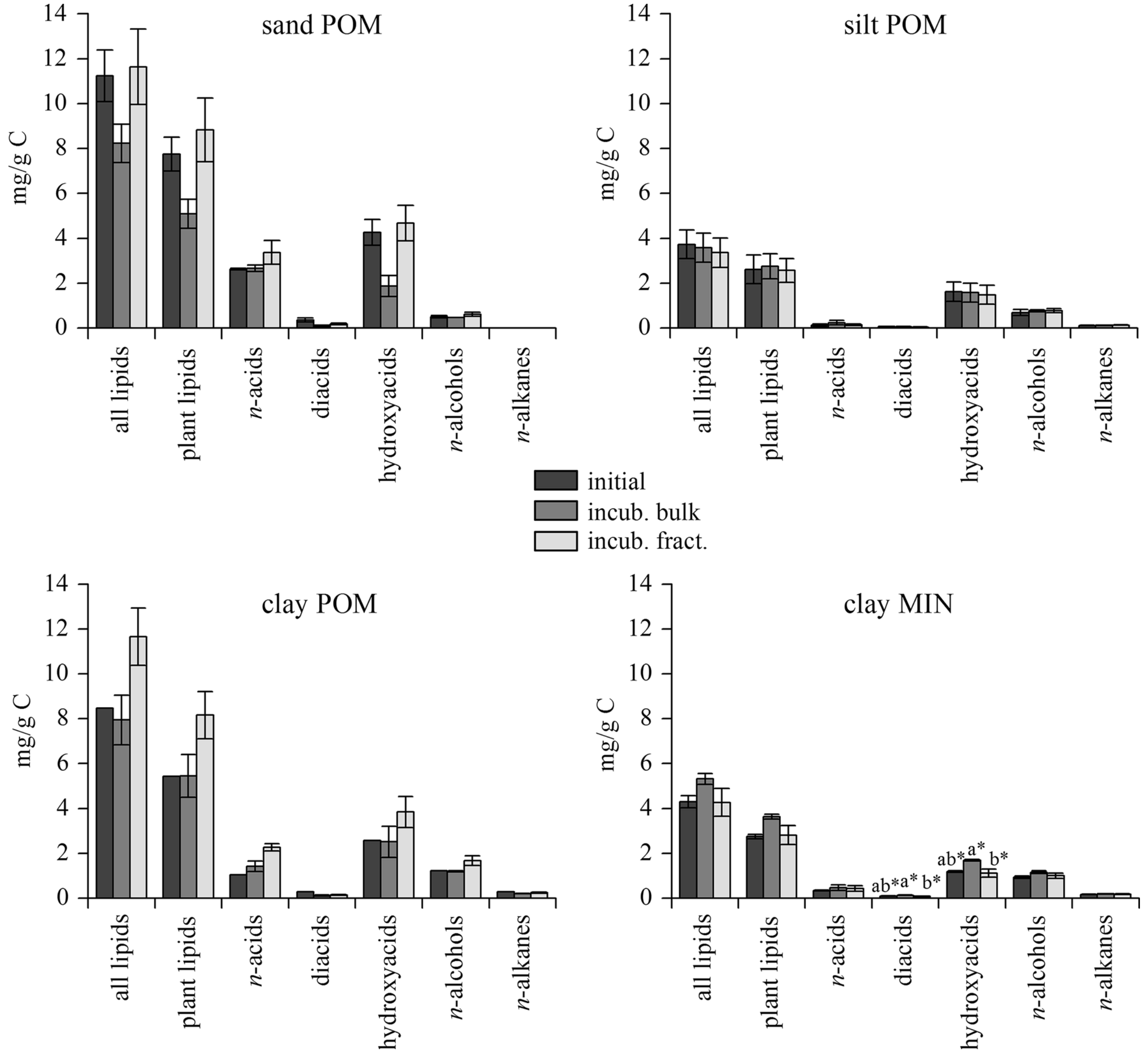

Fig. 2 Total lipid concentrations (all lipids) and plant lipid concentrations (summed and split into different lipid types) for the initial, non-incubated SOM fractions (initial), the bulk soil fractions (incub. bulk), and individually incubated fractions (incub. fract.). Since outliers were removed from the initial, non-incubated clay POM fractions, we could not calculate any statistics between that data point and the incubation treatments. However, the lipid data obtained for the initial, non-incubated clay POM conform to the general observed trend. Significant differences between the treatments are indicated by different letters above the bars (*a*, *b*). *$p < 0.1$

substituted hydroxy alkanoic acids (subsumed under 'hydroxyacids') were the most abundant lipid type in all fractions, followed by *n*-alcohols and *n*-alkanoic acids. The *n*-alkanes and α,ω-alkanedioic acids were not abundant (<0.4 mg/g C). There were no significant differences between the initial, non-incubated fractions and the fractions from the different incubation treatments. However, there were some trends of potential interest. In the sand POM, the concentration of total extractable lipids and plant-derived lipids tended to be lower in bulk soil fractions (by at least 27%) compared to initial, non-incubated and individually incubated fractions, predominantly due to lower concentrations of hydroxyacids (Fig. 2). In the clay POM, concentrations of hydroxyacids, *n*-alkanoic acids and *n*-alcohols tended to be higher in individually incubated fractions (by at least 13%). In contrast, concentrations of plant lipids in the clay MIN tended to be higher in bulk soil fractions compared to initial, non-incubated and individually incubated fractions (by 29%). Here, concentrations of hydroxyacids and α,ω-alkanedioic acids were significantly lower (at least 34%, $p < 0.1$) in individually incubated fractions (Fig. 2).

CuO oxidation products

In addition to the single-ring phenol compounds vanillyl (V), syringyl (S), cinnamyl (C) (sum = VSC), and their aldehyde, ketone, and acid substitutions, the CuO oxidation released several aliphatic *n*-alkanoic acids (C_{12} to C_{18} with even chain-length), α,ω-alkanedioic acids (C_{12} to C_{18} with even chain-length), and hydroxyacids (ω-hydroxy alkanoic acids C_{14} to C_{18} and mid-chain substituted hydroxy alkanoic acids C_{15} to C_{18}) (Fig. 3). The larger part of these compounds (63 ± 1.8%), especially the hydroxyacids and α,ω-alkanedioic acids with an even chain-length $\geq C_{16}$, were plant-derived (Otto and Simpson 2006; Mueller KE et al. 2012b; Angst et al. 2016a, b). The α,ω-alkanedioic acids $<C_{16}$ and the *n*-alkanoic acids could not be unambiguously assigned to a plant origin and may also derive from microorganisms. The concentration of total CuO oxidation products was highest in the silt and clay POM (102.3–130.2 mg/g C), followed by sand POM (69.2–85.0 mg/g C) and clay MIN (59.1–76.2 mg/g C). All fractions were dominated by VSC phenols and hydroxyacids. Similar to the solvent-extractable lipids, the concentrations of compounds released upon CuO oxdation did not show significant differences between initial, non-incubated fractions and the fractions from the different incubation treatments. Exceptions to this general pattern were observed for the silt POM, where concentrations of *n*-alkanoic acids were significantly higher after the incubation (by at least 16%, $p < 0.1$), and the clay MIN, where concentrations of hydroxyacids were significantly lower (by on average 43%, $p < 0.05$) after the incubation (Fig. 3). Further, although the differences were not significant, POM in individually incubated sand, silt and clay fractions had consistently higher concentrations of total CuO oxidation products (at least 10% higher) compared to the corresponding bulk soil fractions. The concentrations of VSC and/or hydroxyacids that tended to be higher in individually incubated fractions were mainly responsible for this trend.

There were also few significant differences between fractions for the ratios calculated from CuO oxidation products (Table 3, $p < 0.05$). The $(Ac/Al)_S$ ratio of sand POM that was significantly higher in the individually incubated fraction and the F/C ratio that was significantly lower in the individually incubated clay POM and significantly higher in the clay MIN incubated within bulk soil. The S/V and C/V ratios were always below a value of 0.5.

Discussion

Initial chemical composition of the SOM fractions

The concentrations of compounds released by the CuO oxidation were approximately tenfold the concentrations released upon ASE, indicating a higher contribution of bound lipids and lignin-derived compounds to SOM compared to solvent-extractable lipids. All initial, non-incubated soil fractions were dominated by plant-derived lipids, especially hydroxyacids (Figs. 2 , 3). This was also the case for individually incubated and bulk soil fractions. The bound hydroxyacids were derived from the lipid biopolymers cutin and/or suberin (Kolattukudy et al. 1975; Kolattukudy 1980, 1981). A dominance of cutin- over suberin-derived compounds can be inferred from the fact that spruce needles have been found to be enriched in ω-hydroxy alkanoic and mid-chain substituted hydroxy alkanoic acids, whereas spruce roots were depleted in mid-chain substituted hydroxy alkanoic acids and low in ω-hydroxy alkanoic acids (Angst et al. 2016a). The concentrations of VSC phenols in our samples were similar to, but slightly higher than those observed by Guggenberger et al. (1994) for particle size separates under spruce. The dominance of vanillyl units in VSC confirmed the recent spruce vegetation as source for the lignin compounds (Sjöberg et al. 2004; Thevenot et al. 2010). The S/V and C/V values of the POM and MIN fractions were similar to those reported for gymnosperms (Goni and Hedges 1992), suggesting the characteristic lignin pattern of the source vegetation has to some extent been preserved in the SOM of the present soil, as also observed by Otto and Simpson (2006).

Apart from those similarities, distinct chemical differences between the fractions were particularly evident in the NMR data (Fig. 1; Table 2) that depict the overall chemical composition of the SOM fractions. The sand POM was dominated by O/N-alkyl C

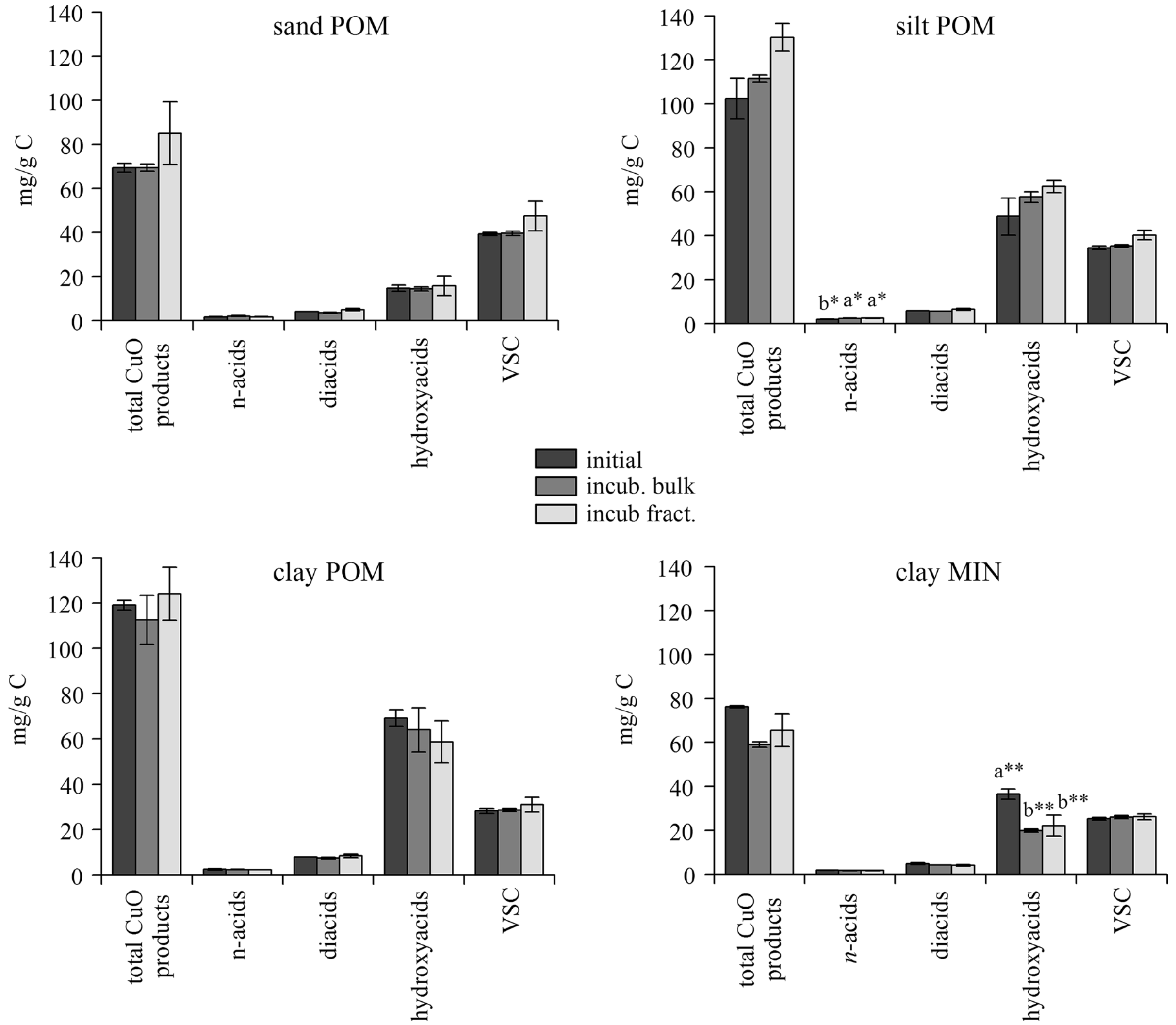

Fig. 3 Concentrations of total CuO oxidation products along with concentrations of *n*-alkanoic acids (*n*-acids), α,ω-alkanedioic acids (diacids), ω-hydroxy alkanoic acids and mid-chain substituted hydroxy alkanoic acids (subsumed under hydroxyacids), and VSC for the initial, non-incubated SOM fractions (initial), the bulk soil fractions (incub. bulk), and individually incubated fractions (incub. fract.). Significant differences between the treatments are indicated by different letters above the bars (*a*, *b*). $^*p < 0.1$, $^{**}p < 0.05$

and carbohydrates$_{\mathrm{NMR}}$, whereas amounts of alkyl C and lipids$_{\mathrm{NMR}}$ were considerably higher in the silt and clay POM. This finding is also mirrored by the alkyl/O/N-alkyl C ratios that increased from the sand to the silt and clay POM, indicating an increasing degree of degradation and a lower bioavailability for smaller POM (Wagai et al. 2009; Mueller et al. 2014). The clay MIN showed a lower degree of aliphaticity compared to the silt and clay POM that was probably caused by a higher amount of microbial-derived compounds (Golchin et al. 1996), also mirrored by a higher amount of carbohydrates$_{\mathrm{NMR}}$ and proteins$_{\mathrm{NMR}}$ in the clay MIN compared to the silt and clay POM. This inference was also supported by low C/N ratios around 15 (Mueller et al. 2014), approximating the C/N ratio of the microbial biomass. However, considerable amounts of plant-derived lipids along with VSC in the clay MIN (Figs. 2, 3) indicate that plant-derived compounds substantially contribute to the mineral-associated SOM. Recently, many authors have suggested that SOM, or mineral associated OM in particular, is dominated by microbial-derived OM

Table 3 Ratios for the different fractions (initial = non-incubated, incub. bulk = bulk soil fractions, incub. fract. = individually incubated fraction) calculated from the CuO oxidation products ± SE: syringyl/vanillyl (S/V), cinnamyl/vanillyl (C/V), ratio of phenolic acids and their corresponding aldehydes for vanillyl $(Ac/Al)_V$ and syringyl $(Ac/al)_S$, ferulic acid/coumaric acid (F/C), and 3,5-dihydroxybenzoic acid/vanillyls (3,5-OHdB/V)

		Ratio					
		S/V	C/V	$(Ac/Al)_V$	$(Ac/Al)_S$	F/C	3,5-OHdB/V
Sand POM	*initial*	0.04 ± 0.004	0.11 ± 0.004	2.3 ± 0.06	0.7 ± 0.02b	2.20 ± 0.10	0.05 ± 0.006
	incub. bulk	0.04 ± 0.002	0.09 ± 0.001	2.0 ± 0.09	0.7 ± 0.02b	2.30 ± 0.17	0.06 ± 0.004
	incub. fract.	0.03 ± 0.003	0.11 ± 0.004	2.3 ± 0.07	0.8 ± 0.01a	2.30 ± 0.17	0.07 ± 0.003
Silt POM	*initial*	0.05 ± 0.003	0.36 ± 0.008	2.8 ± 0.04	0.9 ± 0.03	0.30 ± 0.00	0.07 ± 0.005
	incub. bulk	0.05 ± 0.001	0.35 ± 0.009	2.8 ± 0.01	0.8 ± 0.06	0.30 ± 0.01	0.04 ± 0.010
	incub. fract.	0.04 ± 0.002	0.34 ± 0.001	2.8 ± 0.01	0.9 ± 0.03	0.30 ± 0.00	0.06 ± 0.002
Clay POM	*initial*	0.05 ± 0.002	0.34 ± 0.040	2.8 ± 0.02	0.9 ± 0.10	0.42 ± 0.01a	0.06 ± 0.010
	incub. bulk	0.06 ± 0.003	0.30 ± 0.010	2.8 ± 0.03	0.8 ± 0.02	0.40 ± 0.01a	0.05 ± 0.005
	incub. fract.	0.05 ± 0.003	0.36 ± 0.020	2.8 ± 0.11	1.0 ± 0.12	0.37 ± 0.01b	0.06 ± 0.009
Clay MIN	*initial*	0.05 ± 0.005	0.15 ± 0.010	3.1 ± 0.02	0.9 ± 0.00	0.73 ± 0.03b	0.07 ± 0.002
	incub. bulk	0.05 ± 0.000	0.12 ± 0.007	3.1 ± 0.02	1.0 ± 0.04	0.90 ± 0.03a	0.07 ± 0.004
	incub. fract.	0.04 ± 0.002	0.12 ± 0.005	2.9 ± 0.04	1.0 ± 0.03	0.77 ± 0.01b	0.07 ± 0.007

Different letters (a, b) following SE-values indicate significant differences ($p < 0.05$) between initial, incub. bulk, and incub. fract

(Grandy and Neff 2008; Schmidt et al. 2011; Miltner et al. 2012; Cotrufo et al. 2013; Kallenbach et al. 2016), but our results and others show that cutin- and suberin-derived lipids might be selectively preserved in relatively stable mineral associated OM pools (Carrington et al. 2012; Clemente et al. 2011; Filley et al. 2008a, b; Pisani et al. 2014; Rumpel et al. 2004). We thus suggest not to undervalue the contribution of plant-derived compounds to stabilized SOM. Further, the relative amounts of $lignin_{NMR}$ decreased from the sand to the silt and clay POM and clay MIN, which is in line with decreasing yields of VSC for the same fractions (Fig. 3; Table 2). These outcomes indicate that CuO oxidation and NMR spectroscopy yield comparable results and lignin does not necessarily accumulate relative to other biomolecules in POM or MIN fractions investigated in this study. These findings correspond to recent conceptualizations stating that supposedly recalcitrant OM (like lignin) is not necessarily more stable than other, more labile OM in soil (Marschner et al. 2008; Cotrufo et al. 2013). However, our study and others likely underestimate the contribution of phenols to mineral-associated OM because substantial amounts of lignin may not be released during CuO oxidation due to strong interactions with mineral surfaces (Hernes et al. 2013).

Minimal changes in chemical composition of SOM fractions after long-term incubation

Notably, statistically significant shifts in the chemical composition of OM during the year-long incubation were absent (Figs. 2 and 3; Table 1). This suggests all the investigated compounds were mineralized with similar rates, independent of chemical characteristics like aromaticity or aliphaticity. These results are, at least to some extent, inconsistent with assertions of higher inherent recalcitrance of aliphatic (Baldock et al. 1997; Lorenz et al. 2007; Bonanomi et al. 2013) and aromatic compounds (Krull et al. 2003; Schöning et al. 2005) compared to e.g., carbohydrates. A different stability of aliphatic compounds and carbohydrates is also responsible for changes in the alkyl/O/N-alkyl C ratio calculated from NMR spectra; with one exception, this ratio did not change significantly in the two incubation treatments compared to the initial, non-incubated SOM fractions (Fig. 1). The only significant increase of the alkyl/O/N-alkyl C ratio was detected in the sand POM, which is assumed to be the most bioavailable fraction (Mueller et al. 2014). Here, also SOC contents significantly decreased during the incubation (Table 1). However, the change in chemical composition of the sand POM was probably minor because the increase in the alkyl/O/N-alkyl C ratio did not correspond to the relative amount of carbohydrates$_{NMR}$, which did not significantly change and the relative amounts of lipids$_{NMR}$ only slightly increased (Table 2). Similarly, with few exceptions, ratios of lignin-derived phenols did not significantly change during the incubation (Table 3), indicating the chemical composition of residual lignin was mostly unaltered. A similar result was obtained by Sjöberg et al. (2004), who did not find a major lignin alteration of spruce litter after 559 days of incubation. The acid to aldehyde ratio of syringyl lignin monomers increased slightly for the sand POM when the sand-sized fraction was incubated alone, providing further evidence that only the composition of sand POM was altered by further decomposition during the incubation.

The properties of the soil used for this study seem to be at least partly responsible for the minimal changes in SOM composition during the incubation. Since we investigated an acidic soil under Norway spruce, before the incubation the SOM was likely already at an advanced stage of decomposition with only minor changes in chemical composition over time, similar to the late stages of litter decay (Berg 2000; Berg and McClaugherty 2008). This is supported by the high initial alkyl/O/N-alkyl C ratios of sand POM (0.45) compared to those commonly observed for fresh Norway spruce needles (0.27–0.29; Angst et al. 2016a; Sjöberg et al. 2004), indicating an advanced stage of decomposition where bioavailable carbon for further degradation of lignin and other OM might be limited (Klotzbücher et al. 2011). Further supporting evidence for this hypothesis comes from the findings of Mueller et al. (2014), who measured CO_2 respiration rates on the same SOM fractions used for this study. They found a comparatively low cumulative respiration rate that resulted in losses of 1 (silt and clay) to 3% (sand) of total C during incubation over almost one year. In contrast, for a less acidic, base-rich soil derived from a European beech stand (Rendzic Leptosol), the C respired from sand, silt, and clay fractions after 280 days of incubation were higher by a factor of 3–8 (Bimüller et al. 2014). Similarly, Creamer et al. (2013) incubated aggregate and free POM fractions from a Phaeozem vegetated with grass and woody plant species and found a 5–8 times higher respiration rate after one year compared to the findings by Mueller et al. (2014) for the present incubation. Thus, although our results suggest that inherent recalcitrance had little impact on the composition of the relatively stable SOM pools we studied, it is possible that recalcitrance could play a larger role during earlier stages of plant litter decomposition, in systems where nutrients are limited, in less stable SOM pools, or in different soil types as compared to that investigated in the present study (Riederer et al. 1993; Clemente et al. 2013; Angst et al. 2016a).

The role of aggregation for POM

Although there were only minor changes in OM composition during the incubation, in the bulk soils, there were clear indications of a restricted bioavailability of SOC on the molecular level, as was also observed for the SOC mineralization in intact soil structures of the same soils (Mueller et al. 2014). Regarding the solvent-extractable lipids, concentrations of especially hydroxyacids, but also *n*-alkanoic acids and *n*-alcohols, tended to be higher in individually incubated POM fractions (sand and clay POM) compared the corresponding bulk soil fractions (Fig. 2). A similar trend was detected in the CuO oxidation products, where concentrations of VSC (sand POM, silt POM) and/or hydroxyacids (silt POM) tended to be higher in individually incubated POM fractions compared to bulk soil fractions (Figs. 2, 3). Consistent with these trends in molecular-level OM composition, the NMR data showed a higher relative amount of alkyl C and thus lipids$_{NMR}$ in individually incubated sand (trend) and clay POM (significant) and a higher alkyl/O/N-alkyl C ratio (significant) in individually incubated clay POM. Collectively, these findings indicate that if a soil is aggregated (as for the incubation of bulk soils), compounds that have relatively low inherent recalcitrance (polysaccharides, carbohydrates (von Lützow et al. 2006)) are similarly stable compared to aliphatic and aromatic compounds due to an occlusion within aggregates. As a result, no selective preservation occurs. This applies predominantly to clay-sized POM, since this fraction has exclusively been found occluded within aggregates (Mueller et al. 2009; Wagai et al. 2009). However, if aggregates are disrupted (as in the individually incubated fractions), inherent recalcitrance determines the SOC stability. This results in the selective preservation of plant-derived lipids. These interpretations are consistent with the results of Mueller CW et al.

(2012a), who showed that bioavailability is smaller in intact soils compared to soils after aggregate dispersion. It has to be noted that formation of new aggregates may have taken place during the incubations due to microbial activity (e.g., Blankinship et al. 2016). However, any supply of additional OM possibly promoting aggregate formation, such as root exudates or particulate OM (Six et al. 2004; De Gryze et al. 2005), was omitted. Thus, the formation of new aggregates within the fractions should have been marginal. Interestingly, the $\text{lignin}_{\text{NMR}}$ significantly decreased relative to $\text{lipids}_{\text{NMR}}$ in individually incubated clay POM (Table 2), pointing to a lower inherent recalcitrance of lignin as compared to lipids and other aliphatics. This observation substantiates recent evidences that lignin may not be specifically recalcitrant, at least under favorable conditions for decomposition (e.g., low pH and optimal laboratory conditions) and its stabilization in small POM fractions is to some extent dependent on occlusion within aggregates.

In accordance with previous assumptions that the relevance of different mechanisms of SOM stabilization varies with soil type and land-management (Six et al. 2002), we suggest that inherent recalcitrance could be important for the selective preservation of OM compounds in soils or soil fractions with limited aggregation (e.g., organic horizons or sandy mineral soils; see also Castellano et al. 2015).

The role of aggregation for mineral-associated SOM

The changes in chemical composition of the clay MIN showed different dynamics as compared to the POM fractions. In the ASE extracts, the concentrations of lipids tended to be higher in bulk soil fractions compared to those in individually incubated fractions. More soluble lipids may be leached from the POM fractions (especially sand-sized POM; Fig. 2) and stabilized in the clay MIN through interactions with mineral surfaces. This process is absent in individually incubated fractions due to a missing supply of potentially mobile lipids from other fractions, here recovered as solvent-extractable lipids. This process might also apply to carbohydrates/microbial-derived carbon, since O/N-alkyl C was significantly higher in bulk soil clay MIN compared to individually incubated clay MIN (Fig. 1). Notably, the concentrations of bound hydroxyacids, which tended to increase or stay constant for other soil fractions, decreased significantly in clay MIN during incubations of bulk soils and individual soil fractions, perhaps due to a strong binding of a considerable amount of hydroxyacids to mineral surfaces making them non-extractable by base hydrolysis or CuO oxidation (Feng et al. 2010; Hernes et al. 2013; Lin and Simpson 2016). The latter inference is supported by the NMR data, where $\text{lignin}_{\text{NMR}}$ as well as $\text{lipids}_{\text{NMR}}$ did not significantly change during the incubation in individually incubated and bulk soil fractions as compared to the initial fractions.

However, aggregation also seemed to play an important role for the stabilization of lipids and lignin in the mineral associated OM. Similar to the clay POM, the $\text{lignin}_{\text{NMR}}$ significantly decreased and the $\text{lipids}_{\text{NMR}}$ significantly increased in individually incubated clay MIN as compared to the respective bulk soil fraction (Table 2). First, these data indicate that the stability of lignin associated with clay-sized minerals is to some extend dependent on the incorporation of the respective organo-mineral association inside aggregates. Second, the relative increase in lipids after the disruption of aggregates (in individually incubated fractions) supports the notion of strong organo-mineral interactions between clay-sized minerals and lipids (e.g., Lin and Simpson 2016).

Conclusions

Although inherent recalcitrance could be important during earlier stages of SOM decomposition or in soils with low aggregation, in our study, occlusion of OM within aggregates and preferential preservation of cutin/suberin-derived lipids via organo-mineral or organo–organo interactions were responsible for long-term SOM stabilization. The importance of aggregation was particularly highlighted by consistently higher concentrations of plant-derived biomolecules in individually incubated fractions (disrupted aggregation) as compared to POM fractions from incubated bulk soils (intact aggregates), where less recalcitrant biomolecules were protected against decomposition. Our results indicate that lignin is not specifically recalcitrant because a higher abundance in the clay-sized POM was dependent on an intact aggregation. As also the abundance of lignin and lipids in mineral-associated OM was substantially influenced by aggregation, our results demonstrate the strong interrelation of organo-mineral interactions and aggregation for the stabilization of SOM. Thus, the bioavailability of mineral-associated SOC likely increases after the destruction of intact soil structures.

These results are consistent with recent conceptualizations that emphasize the significance of mineral matrix interactions for long-term stabilization of SOM. However, these same conceptualizations of SOM stabilization probably understate the contribution of plant-derived OM to stable SOM pools. Future studies should evaluate the possibility that mineral-associated OM contains substantial amounts of plant-derived biomolecules, some of which might not be released using typical methods such as base hydrolysis or CuO oxidation due to strong organo-mineral interactions.

Acknowledgements For their help in the laboratory we thank Livia Urbanski and Maria Greiner (TUM Freising, Germany). We also thank David Eissenstat, Professor of Woody Plant Physiology at the Pennsylvania State University, for his assistance, Dr. Werner Häusler (TUM Freising, Germany) for mineralogical analyses, and two anonymous reviewers whose comments helped to greatly improve the quality of the manuscript. The project was generously funded by the Helmholtz Association in the joint virtual institute VH-129 “Centre for Stable Isotope Analysis in Ecosystem Research”, the U.S. National Science Foundation (DEB-0816935, OISE-0754731), the U.S. Department of Energy (Global Change Education Program), the European Association of Organic Geochemists, and the Deutsche Forschungsgemeinschaft (DFG) within the research unit FOR1806 “SUBSOM - The Forgotten Part of Carbon Cycling: Organic Matter Storage and Turnover in Subsoils”.

References

Alvarez CR, Alvarez R, Grigera MS, Lavado RS (1998) Associations between organic matter fractions and the active soil microbial biomass. Soil Biol Biochem 30:767–773. doi:10.1016/S0038-0717(97)00168-5

Amelung W, Zech W (1999) Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons. Geoderma 92:73–85. doi:10.1016/s0016-7061(99)00023-3

Amelung W, Brodowski S, Sandhage-Hofmann A, Bol R (2008) Chapter 6 combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. Adv Agron 100:155–250

Angst G, Heinrich L, Kögel-Knabner I, Mueller CW (2016a) The fate of cutin and suberin of decaying leaves, needles and roots: inferences from the initial decomposition of bound fatty acids. Org Geochem 95:81–92. doi:10.1016/j.orggeochem.2016.02.006

Angst G, John S, Mueller CW et al (2016b) Tracing the sources and spatial distribution of organic carbon in subsoils using a multi-biomarker approach. Sci Rep. doi:10.1038/srep29478

Angst G, Kögel-Knabner I, Kirfel K et al (2016c) Spatial distribution and chemical composition of soil organic matter fractions in rhizosphere and non-rhizosphere soil under European beech (Fagus sylvatica L.). Geoderma 264:179–187. doi:10.1016/j.geoderma.2015.10.016

Baldock JA, Oades JM, Nelson PN et al (1997) Assessing the extent of decomposition of natural organic materials using solid-state ^{13}C NMR spectroscopy. Soil Res 35:1061–1084. doi:10.1071/S97004

Berg B (2000) Litter decomposition and organic matter turnover in northern forest soils. For Ecol Manage 133:13–22. doi:10.1016/S0378-1127(99)00294-7

Berg B, McClaugherty C (2008) Plant litter: decomposition, humus formation, carbon sequestration, 2nd edn. Springer, Berlin

Bimüller C, Mueller CW, von Lützow M et al (2014) Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil. Soil Biol Biochem 78:263–273. doi:10.1016/j.soilbio.2014.08.001

Blankinship JC, Fonte SJ, Six J, Schimel JP (2016) Plant versus microbial controls on soil aggregate stability in a seasonally dry ecosystem. Geoderma 272:39–50. doi:10.1016/j.geoderma.2016.03.008

Bonanomi G, Incerti G, Giannino F et al (2013) Litter quality assessed by solid state 13C NMR spectroscopy predicts decay rate better than C/N and Lignin/N ratios. Soil Biol Biochem 56:40–48. doi:10.1016/j.soilbio.2012.03.003

Campbell EE, Paustian K (2015) Current developments in soil organic matter modeling and the expansion of model applications: a review. Environ Res Lett 10:123004. doi:10.1088/1748-9326/10/12/123004

Carrington EM, Hernes PJ, Dyda RY et al (2012) Biochemical changes across a carbon saturation gradient: lignin, cutin, and suberin decomposition and stabilization in fractionated carbon pools. Soil Biol Biochem 47:179–190. doi:10.1016/j.soilbio.2011.12.024

Castellano MJ, Mueller KE, Olk DC et al (2015) Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Glob Chang Biol 21:3200–3209. doi:10.1111/gcb.12982

Chaurasia CS, Williams TD, Judson CM, Hanzlik RP (1995) Quantitation of fatty acids and hydroxy fatty acids by gas chromatography/mass spectrometry. Predictively useful correlations of relative response factors with empirical formula. J Mass Spectrom 30:1018–1022. doi:10.1002/jms.1190300711

Christensen BT (2001) Physical fractionation of soil and structural and functional complexity in organic matter turnover. Eur J Soil Sci 52:345–353. doi:10.1046/j.1365-2389.2001.00417.x

Clemente JS, Simpson AJ, Simpson MJ (2011) Association of specific organic matter compounds in size fractions of soils under different environmental controls. Org Geochem 42:1169–1180. doi:10.1016/j.orggeochem.2011.08.010

Clemente JS, Simpson MJ, Simpson AJ et al (2013) Comparison of soil organic matter composition after incubation with maize leaves, roots, and stems. Geoderma 192:86–96. doi:10.1016/j.geoderma.2012.08.007

Cotrufo MF, Wallenstein MD, Boot CM et al (2013) The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob Chang Biol 19:988–995. doi:10.1111/gcb.12113

Creamer CA, Filley TR, Boutton TW (2013) Long-term incubations of size and density separated soil fractions to inform soil organic carbon decay dynamics. Soil Biol Biochem 57:496–503. doi:10.1016/j.soilbio.2012.09.007

Crow SE, Lajtha K, Filley TR et al (2009) Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. Glob Chang Biol 15:2003–2019. doi:10.1111/j.1365-2486.2009.01850.x

De Gryze S, Six J, Brits C, Merckx R (2005) A quantification of short-term macroaggregate dynamics: influences of wheat residue input and texture. Soil Biol Biochem 37:55–66. doi:10.1016/j.soilbio.2004.07.024

Ertel JR, Hedges JI (1984) The lignin component of humic substances: distribution among soil and sedimentary humic, fulvic, and base-insoluble fractions. Geochim Cosmochim Acta 48:2065–2074. doi:10.1016/0016-7037(84)90387-9

Ertel JR, Hedges JI (1985) Sources of sedimentary humic substances: vascular plant debris. Geochim Cosmochim Acta 49:2097–2107. doi:10.1016/0016-7037(85)90067-5

Feng X, Simpson MJ (2008) Temperature responses of individual soil organic matter components. J Geophys Res 113(G3). doi:10.1029/2008JG000743

Feng X, Xu Y, Jaffé R et al (2010) Turnover rates of hydrolysable aliphatic lipids in Duke Forest soils determined by compound specific ^{13}C isotopic analysis. Org Geochem 41:573–579. doi:10.1016/j.orggeochem.2010.02.013

Filley TR, Boutton TW, Liao JD, et al (2008a) Chemical changes to nonaggregated particulate soil organic matter following grassland-to-woodland transition in a subtropical savanna. J Geophys Res Biogeosci. doi:10.1029/2007JG000564

Filley TR, McCormick MK, Crow SE et al (2008b) Comparison of the chemical alteration trajectory of Liriodendron tulipifera L. leaf litter among forests with different earthworm abundance. J Geophys Res. doi:10.1029/2007JG000542

Golchin A, Clarke P, Oades JM (1996) The heterogeneous nature of microbial products as shown by solid-state ^{13}C CP/MAS NMR spectroscopy. Biogeochemistry 34:71–97. doi:10.1007/BF02180974

Goni MA, Hedges JI (1992) Lignin dimers: structures, distribution, and potential geochemical applications. Geochim Cosmochim Acta 56:4025–4043. doi:10.1016/0016-7037(92)90014-A

Goñi MA, Montgomery S (2000) Alkaline CuO oxidation with a microwave digestion system: lignin analyses of geochemical samples. Anal Chem 72:3116–3121. doi:10.1021/ac991316w

Grandy AS, Neff JC (2008) Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. Sci Total Environ 404:297–307. doi:10.1016/j.scitotenv.2007.11.013

Guggenberger G, Christensen BT, Zech W (1994) Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. Eur J Soil Sci 45:449–458. doi:10.1111/j.1365-2389.1994.tb00530.x

Hassink J, Whitmore AP, Kubát J (1997) Size and density fractionation of soil organic matter and the physical capacity of soils to protect organic matter. Eur J Agron 7:189–199. doi:10.1016/S1161-0301(97)00045-2

Hedges JI, Ertel JR (1982) Characterization of lignin by gas capillary chromatography of cupric oxide oxidation products. Anal Chem 54:174–178. doi:10.1021/ac00239a007

Hernes PJ, Kaiser K, Dyda RY, Cerli C (2013) Molecular trickery in soil organic matter: hidden lignin. Environ Sci Technol 47:9077–9085. doi:10.1021/es401019n

Ingestad T, Lund A-B (1986) Theory and techniques for steady state mineral nutrition and growth of plants. Scand J For Res 1:439–453. doi:10.1080/02827588609382436

Jansen B, Nierop KGJ, Hageman JA et al (2006) The straight-chain lipid biomarker composition of plant species responsible for the dominant biomass production along two altitudinal transects in the Ecuadorian Andes. Org Geochem 37:1514–1536. doi:10.1016/j.orggeochem.2006.06.018

Kallenbach CM, Grandy A, Frey SD (2016) Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nat Commun. doi:10.1038/ncomms13630

Kleber M (2010) What is recalcitrant soil organic matter? Environ Chem 7:320–332. doi:10.1071/EN10006

Klotzbücher T, Kaiser K, Guggenberger G et al (2011) A new conceptual model for the fate of lignin in decomposing plant litter. Ecology 92:1052–1062. doi:10.1890/i0012-9658-92-5-1052

Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34:139–162. doi:10.1016/S0038-0717(01)00158-4

Kolattukudy PE (1980) Bio-polyester membranes of plants—cutin and suberin. Science 208:990–1000. doi:10.1126/science.208.4447.990

Kolattukudy PE (1981) Structure, biosynthesis, and biodegradation of cutin and suberin. Annu Rev Plant Physiol Plant Mol Biol 32:539–567. doi:10.1146/annurev.pp.32.060181.002543

Kolattukudy PE, Kronman K, Poulose AJ (1975) Determination of structure and composition of suberin from roots of carrot, parsnip, rutabaga, turnip, red beet and sweet-potato by combined gas-liquid-chromatography and mass-spectrometry. Plant Physiol 55:567–573. doi:10.1104/pp.55.3.567

Krull ES, Baldock JA, Skjemstad JO (2003) Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Funct Plant Biol 30:207–222. doi:10.1071/FP02085

Lin LH, Simpson MJ (2016) Enhanced extractability of cutin- and suberin-derived organic matter with demineralization implies physical protection over chemical recalcitrance in soil. Org Geochem 97:111–121. doi:10.1016/j.orggeochem.2016.04.012

Lorenz K, Lal R, Preston CM, Nierop KGJ (2007) Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio(macro)molecules. Geoderma 142:1–10. doi:10.1016/j.geoderma.2007.07.013

Marschner B, Brodowski S, Dreves A et al (2008) How relevant is recalcitrance for the stabilization of organic matter in soils? J Plant Nutr Soil Sci 171:91–110. doi:10.1002/jpln.200700049

Mikutta R, Kleber M, Torn MS, Jahn R (2006) Stabilization of soil organic matter: association with minerals or chemical recalcitrance? Biogeochemistry 77:25–56. doi:10.1007/s10533-005-0712-6

Miltner A, Bombach P, Schmidt-Brücken B, Kästner M (2012) SOM genesis: microbial biomass as a significant source. Biogeochemistry 111:41–55. doi:10.1007/s10533-011-9658-z

Moni C, Rumpel C, Virto I et al (2010) Relative importance of sorption versus aggregation for organic matter storage in subsoil horizons of two contrasting soils. Eur J Soil Sci 61:958–969. doi:10.1111/j.1365-2389.2010.01307.x

Mueller CW, Bruggemann N, Pritsch K et al (2009) Initial differentiation of vertical soil organic matter distribution and composition under juvenile beech (Fagus sylvatica L.) trees. Plant Soil 323:111–123. doi:10.1007/s11104-009-9932-1

Mueller CW, Schlund S, Prietzel J et al (2012a) Soil aggregate destruction by ultrasonication increases soil organic matter mineralization and mobility. Soil Sci Soc Am J 76:1634–1643. doi:10.2136/sssaj2011.0186

Mueller KE, Polissar PJ, Oleksyn J, Freeman KH (2012b) Differentiating temperate tree species and their organs using lipid biomarkers in leaves, roots and soil. Org Geochem 52:130–141. doi:10.1016/j.orggeochem.2012.08.014

Mueller CW, Gutsch M, Kothieringer K et al (2014) Bioavailability and isotopic composition of CO 2 released from incubated soil organic matter fractions. Soil Biol Biochem 69:168–178

Nelson P, Baldock J (2005) Estimating the molecular composition of a diverse range of natural organic materials from solid-state 13C NMR and elemental analyses. Biogeochemistry 72:1–34. doi:10.1007/s10533-004-0076-3

Otto A, Simpson MJ (2006) Evaluation of CuO oxidation parameters for determining the source and stage of lignin degradation in soil. Biogeochemistry 80:121–142. doi:10.1007/s10533-006-9014-x

Otto A, Shunthirasingham C, Simpson MJ (2005) A comparison of plant and microbial biomarkers in grassland soils from the Prairie Ecozone of Canada. Org Geochem 36:425–448. doi:10.1016/j.orggeochem.2004.09.008

Pisani O, Hills KM, Courtier-Murias D et al (2014) Accumulation of aliphatic compounds in soil with increasing mean annual temperature. Org Geochem 76:118–127. doi:10.1016/j.orggeochem.2014.07.009

Poirier N, Sohi SP, Gaunt JL et al (2005) The chemical composition of measurable soil organic matter pools. Org Geochem 36:1174–1189. doi:10.1016/j.orggeochem.2005.03.005

Prahl FG, Ertel JR, Goni MA et al (1994) Terrestrial organic carbon contributions to sediments on the Washington margin. Geochim Cosmochim Acta 58:3035–3048. doi:10.1016/0016-7037(94)90177-5

Rasse DP, Rumpel C, Dignac M-FF (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269:341–356. doi:10.1007/s11104-004-0907-y

Riederer M, Matzke K, Ziegler F, Kogelknabner I (1993) Occurence, distribution and fate of the lipid plant biopolymers cutin and suberin in temperate forest soils. Org Geochem 20:1063–1076. doi:10.1016/0146-6380(93)90114-q

Rumpel C, Eusterhues K, Kogel-Knabner I (2004) Location and chemical composition of stabilized organic carbon in topsoil and subsoil horizons of two acid forest soils. Soil Biol Biochem 36:177–190. doi:10.1016/j.soilbio.2003.09.005

Rumpel C, Eusterhues K, Kögel-Knabner I (2010) Non-cellulosic neutral sugar contribution to mineral associated organic matter in top- and subsoil horizons of two acid forest soils. Soil Biol Biochem 42:379–382. doi:10.1016/j.soilbio.2009.11.004

R Core Team (2015) R: a language and environment for statistic computing, Vienna, Austria.

Schimel DS, Braswell BH, Holland EA et al (1994) Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. Global Biogeochem Cycles 8:279. doi:10.1029/94GB00993

Schmidt MWI, Torn MS, Abiven S et al (2011) Persistence of soil organic matter as an ecosystem property. Nature 478:49–56. doi:10.1038/nature10386

Schöning I, Morgenroth G, Kögel-Knabner I (2005) O/N-alkyl and alkyl C are stabilised in fine particle size fractions of forest soils. Biogeochemistry 73:475–497. doi:10.1007/s10533-004-0897-0

Schrumpf M, Kaiser K, Guggenberger G et al (2013) Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. Biogeosciences 10:1675–1691. doi:10.5194/bg-10-1675-2013

Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241:155–176. doi:10.1023/A:1016125726789

Six J, Bossuyt H, Degryze S, Denef K (2004) A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res 79:7–31. doi:10.1016/j.still.2004.03.008

Sjöberg G, Nilsson SI, Persson T, Karlsson P (2004) Degradation of hemicellulose, cellulose and lignin in decomposing spruce needle litter in relation to N. Soil Biol Biochem 36:1761–1768. doi:10.1016/j.soilbio.2004.03.010

Sollins P, Homann P, Caldwell BA (1996) Stabilization and destabilization of soil organic matter1.pdf. Geoderma 74:65–105. doi:10.1016/S0016-7061(96)00036-5

Spaccini R, Piccolo A, Haberhauer G, Gerzabek MH (2000) Transformation of organic matter from maize residues into labile and humic fractions of three European soils as revealed by 13C distribution and CPMAS-NMR spectra. Eur J Soil Sci 51:583–594. doi:10.1046/j.1365-2389.2000.00341.x

Spielvogel S, Prietzel J, Leide J, Riedel M, Zemke J, Kögel-Knabner I (2014) Distribution of cutin and suberin biomarkers under forest trees with different root systems. Plant Soil 381:95–110. doi:10.1007/s11104-014-2103-z

Stemmer M, Von Lützow M, Kandeler E et al (1999) The effect of maize straw placement on mineralization of C and N in soil particle size fractions. Eur J Soil Sci 50:73–85. doi:10.1046/j.1365-2389.1999.00204.x

Thevenot M, Dignac MF, Rumpel C (2010) Fate of lignins in soils: a review. Soil Biol Biochem 42:1200–1211. doi:10.1016/j.soilbio.2010.03.017

Torn MS, Trumbore SE, Chadwick OA et al (1997) Mineral control of soil organic carbon storage and turnover. Nature 389:170–173. doi:10.1038/38260

von Lützow M, Kögel-Knabner I, Ekschmitt K et al (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions—a review. Eur J Soil Sci 57:426–445. doi:10.1111/j.1365-2389.2006.00809.x

von Lützow M, Kogel-Knabner I, Ekschmittb K et al (2007) SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biol Biochem 39:2183–2207. doi:10.1016/j.soilbio.2007.03.007

Wagai R, Mayer LM, Kitayama K (2009) Nature of the "occluded" low-density fraction in soil organic matter studies: a critical review. J Soil Sci Plant Nutr 55:13–25. doi:10.1111/j.1747-0765.2008.00356.x

Ziegler F, Kögel I, Zech W (1986) Alteration of gymnosperm and angiosperm lignin during decomposition in forest humus layers. J Soil Sci Plant Nutr 331:323–331. doi:10.1002/jpln.19861490309