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Application of δ^{13}C and δ^{15}N isotopic signatures of organic matter fractions sequentially separated from adjacent arable and forest soils to identify carbon stabilization mechanisms

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Abstract. Identifying the chemical mechanisms behind soil carbon bound in organo-mineral complexes is necessary to determine the degree to which soil organic carbon is stabilized belowground. Analysis of δ^{13}C and δ^{15}N isotopic signatures of stabilized OM fractions along with soil mineral characteristics may yield important information about OM-mineral associations and their processing history. We analyzed the δ^{13}C and δ^{15}N isotopic signatures from two organic matter (OM) fractions along with soil mineral proxies to identify the likely binding mechanisms involved. We analyzed OM fractions hypothesized to contain carbon stabilized through organo-mineral complexes: (1) OM separated chemically with sodium pyrophosphate (OM(PY)) and (2) OM occluded in micro-structures found in the chemical extraction residue (OM(ER)). Because the OM fractions were separated from five different soils with paired forest and arable land use histories, we could address the impact of land use change on carbon binding and processing mechanisms. We used partial least squares regression to analyze patterns in the isotopic signature of OM with established mineral and chemical proxies indicative for certain binding mechanisms. We found different mechanisms predominate in each land use type. For arable soils, the formation of OM(PY)-Ca-mineral associations was identified as an important OM binding mechanism. Therefore, we hypothesize an increased stabilization of microbial processed OM(PY) through Ca_{2+} interactions. In general, we found the forest soils to contain on average 10 % more stabilized carbon relative to total carbon stocks, than the agricultural counter part. In forest soils, we found a positive relationship between isotopic signatures of OM(PY) and the ratio of soil organic carbon content to soil surface area (SOC/SSA). This indicates that the OM(PY) fractions of forest soils represent layers of slower exchange not directly attached to mineral surfaces. From the isotopic composition of the OM(ER) fraction, we conclude that the OM in this fraction from both land use types have undergone a different pathway to stabilization that does not involve microbial processing, which may include OM which is highly protected within soil micro-structures.

1 Introduction

Forest and agricultural soils are potential carbon sinks that can help mitigate the current trajectories of climate change effects on the terrestrial biosphere. Carbon storage belowground is balanced by carbon losses and inputs, hence, soil carbon stocks will accumulate by increasing the mean residence time of carbon sent belowground (Smith et al., 1997; Lal, 2004) or by increasing inputs while minimizing priming effects. Organic matter (OM) is a complex mixture of

organic compounds at different stages of decomposition posing a significant problem of characterizing the residence time of carbon belowground based on an understanding of chemical and physical properties (Kleber and Johnson, 2010). Ongoing challenges facing soil scientist and biogeochemists are to define and quantify which organic molecules are stabilized, how long carbon molecules persist in soil, and to identify the underlying stabilization and destabilization mechanisms.

Currently, OM is considered stabilized in soil when it is protected from microbial oxidation by (1) occlusion in aggregates (Bachmann et al., 2008), (2) interactions with polyvalent cations (OM-cation complexes), (3) interactions via polyvalent cations with soil mineral surfaces (OM-mineral associations) (von Lützow et al., 2006) or OM is preserved due to freezing temperatures, low O_2 content or water saturation (climatic stabilization; Trumbore, 2009). To characterize binding mechanisms, soil OM is generally divided into operationally defined fractions that are hypothesized to contain carbon stabilized by the mechanisms previously described (Mikutta et al., 2006; von Lützow et al., 2007; Sollins et al., 2009). Specifically, an extraction with Na-pyrophosphate solution separates soil OM that interacts with polyvalent cations (forming OM-cation complexes) (Masiello et al., 2004) and OM that interacts via polyvalent cations with soil mineral surfaces (forming OM-mineral associations) (Wattel-Koekkoek et al., 2003; Kögel-Knabner et al., 2008). Consequently, Na-pyrophosphate soluble fractions are primarily comprised of OM stabilized through complexes formed with soil mineral compounds (Kaiser et al., 2011).

Experiments using changes in C3/C4 vegetation, have interpreted the stable isotopic signature (δ^{13}C and δ^{15}N) of OM fractions to determine mean residence times (Balesdent and Mariotti, 1996; Liao et al., 2006; Haile-Mariam et al., 2008; Ellerbrock and Kaiser, 2005), the impact of vegetation change (Solomon et al., 2002) and mining disturbance (Wick et al., 2009). However, the potential to use the isotopic signature of soil OM fractions to reveal OM binding mechanisms that lead to stabilization has not been fully realized. Studies that have analyzed the isotopic signature of soil OM fractions (beyond C3/C4 labeling techniques) have found patterns of enrichment of δ^{13}C and δ^{15}N with increasing density of sequentially separated OM fractions (Huygens et al., 2008; Sollins et al., 2009; Marin-Spiotta et al., 2010). They attributed these patterns to isotope discrimination during microbial processing whereby microbes consume OM, respire the light isotope (carbon and nitrogen) and incorporate the heavy isotope (carbon and nitrogen) into biomass that is subsequently deposited in the soil OM complex. Indeed, Huygens et al. (2008) found a high degree of microbial biomarkers in soil micro-aggregates, providing strong evidence that microbial processing of OM is an important step towards OM stabilization.

Analysis of δ^{13}C and δ^{15}N isotopic signatures of stabilized OM fractions along with soil mineral characteristics may yield important information about OM-mineral associations and their processing history. For example, oxalate extractable Al and Fe contents are established proxies for poorly crystalline minerals, which form stable complexes with OM via ligand exchange reactions (Kleber et al., 2005; Mikutta et al., 2006), while polyvalent cations such as Ca^{2+} and Fe^{3+} play an important role in bridging OM to mineral surfaces (Oades, 1988; Baldock and Nelson, 2000; Wuddivira and Camps-Roach, 2006). Thus, analyses of these proxies along side with patterns in stable isotopes can be used to characterize OM fractions of different land use types and potentially identify which binding mechanisms predominate.

Breaking down soil OM into different fractions is necessary to identify which OM is stabilized, but we need a method of re-assembly to understand how OM and the different binding mechanisms are arranged in the organo-mineral complex. Kleber et al. (2007) provided such a tool by formulating a model that incorporates different binding mechanisms into a zonal, structural model specific to organo-mineral interactions. While a detailed discussion of the model is beyond the scope of this paper, the model does provide a framework to interpret the exchange and isotopic signatures of the OM directly interacting with mineral surfaces or present in the subsequent layers. The model describes a zone of direct interaction between OM and mineral surfaces (contact zone), a zone dominated by hydrophobic interactions and a kinetic zone of OM crosslinked via polyvalent cations. Each zone represents different levels of stabilization, the strongest being the contact zone while weak stabilization occurs in the kinetic zone.

We analyzed the isotopic signal of OM fractions sequentially separated from a range of soil types under arable and forest land use to investigate patterns of isotopic enrichment in different OM fractions and to determine the type of interaction between OM and soil minerals. We focused on the δ^{13}C and δ^{15}N of (1) OM sequentially extracted by a Na-pyrophosphate solution (OM(PY)) after separating organic particles and water-extractable OM (Kaiser et al., 2011) and (2) OM remaining in the extraction residue (OM(ER)); both fractions are hypothesized to contain stabilized carbon. We compared common soil mineral parameters (i.e. specific surface area, contents of clay, oxalate soluble, and exchangable cations) with isotopic data using a partial least squares regression analyses (PLS), which enabled us to draw conclusions about mechanisms behind OM stabilization. We then used the zonal model, which provides molecular resolution to OM stabilization, and the molecular characterization of the OM present in the fractions as determined by stable isotopes, to characterize the structure of the organo-mineral interaction for each land use type.

Table 1. Soil classification, coordinates, altitude, and climatic parameters for the different study sites.

Site name	Soil classification	Geographical position			Climatic parameters	
		Longitude (° E)	Latidue (° N)	Altitude (m a.s.l.)	Precipitation (mm yr^{-1})	Temperature (°C)
Rowa	AL: Albic Luvisol	13°16′20″	53°29′47″	90	536[d]	7.9[d]
Elmenhorst	HSt: Haplic Stagnosol	13°02′19″	54°12′14″	23	566[e]	8.0[e]
Simmringen	HL: Haplic Luvisol	09°52′57″	49°34′47″	338	577[f]	9.4[f]
Nellingen	HC: Haplic Cambisol	09°46′18″	48°33′16″	710	1069[g]	6.8[g]
Herrenberg	VC: Vertic Cambisol	08°56′13″	48°33′59″[a]	420	795[i]	8.3[i]
Herrenberg	VC: Vertic Cambisol	08°56′31″	48°33′12″[b]	420	795[i]	8.3[i]

Coordinates and altitude were taken from topographical maps with a scale of 1:25 000; [a] coordinates for the agricultural soil; [b] coordinates for the forest soil. Soil classification was done according to WRB (2006). Climatic parameters are mean values recorded from the following climatic stations and years: [d] Neubrandenburg: 1961–1990; [e] Greifswald: 1961–1990; [f] Würzburg: 1971–2000; [g] Geislingen-Stötten: 1961–1990; [i] Rottenburg: 1961–1990.

2 Methods

2.1 Site selection and soil sampling

We selected 5 sites in Germany characterized by different soil types (Table 1) and mineral properties (Table 2). Two land use types, arable and forest, were present in close proximity at each site. The land uses have been practiced for at least 100 yr. Management practices, including crop rotation and fertilization regime, for the different sites are shown in Table 2. The selected soils were classified according to World Reference Base for Soil Resources (2006) as Albic Luvisol (AL), Haplic Stagnosol (HSt), Haplic Cambisol (HC), Haplic Luvisol (HL), and Vertic Cambisol (VC). Kaiser et al. (2009), provides further details on soil sampling description.

2.2 Physicochemical characterization of soil samples

The pH values, and SOC, clay, silt, and sand contents were analysed as given in Kaiser et al. (2009). The amount of exchangeable cations (Ca_{ex}) were determined from 5 g soil according to Deutsche Idustrie Norm (DIN) 19684 (1977) using Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Type 138, Jobin Yvon Ltd, München, Germany) (DIN EN ISO 11885 (1998)) and corrected by using data from blank solutions. The oxalate soluble Al and Fe (Fe_{ox}, Al_{ox}) were extracted according to Schlichting et al. (1995), and the contents of Al and Fe in solution were determined using ICP-OES (DIN EN ISO 11885 (1998)). All analyses were done in duplicates and the data were normalized to 105 °C dry soil. To assess the specific surface area (SSA) of the soil mineral phase, the OM was oxidized (Kaiser and Guggenberger, 2003) using a NaOCl solution (6 %, adjusted to pH 8.0 with concentrated HCl) at a soil-to-solution ratio of 1:10 at 25 °C for 6 h (Siregar et al., 2005). The samples were centrifuged, and the supernatants were removed. The NaOCl-treatment was repeated five times (Kaiser and Guggenberger, 2003). The remaining solid residues were then washed once with de-ionised water and centrifuged. The supernatant was removed, and the solid residue was shaken with de-ionised water overnight. Following overnight storage, the NaOCl treated topsoil samples were dialysed and then freeze-dried (Siregar et al., 2005). The SSA of the freeze-dried solid residue was determined by N_2 adsorption (Quantasorb, QUANTACHROME CORP., Syosset, NY, USA). The NaOCl treatment did not remove the OM completely from the soil so we corrected the SSA values as determined after the NaOCl treatment according to Mikutta et al. (2005). The corrected SSA values are given in Table 3.

2.3 Sequential separation of Na-pyrophosphate ($Na_4P_2O_7$) soluble OM (OM(PY)) and OM remaining in extraction residue OM(ER)

1. Following the methods of Kaiser et al. (2009, 2010) we sequentially separated the physically uncomplexed, macro- and micro-aggregate occluded organic particle and water-extractable OM from air-dried (<2 mm) soil sample by a combination of electrostatic attraction, ultra-sonication (60 and 440 J ml^{-1}), sieving, and water extraction (Fig. 1).

2. Following the methods of Ellerbrock and Kaiser (2005), the solid residue of (1) was mixed with 50 ml 0.1 m $Na_4P_2O_7$ solution (pH 9–10) and shaken for 6 h with a rock and roll shaker. The sample was centrifuged and the supernatant decanted. The decanted supernatant was filtered through a 0.45 μm polyamide filter (Schleicher and Schuell, Dassel, Germany) and denoted as OM(PY)$_{total}$. The pH of the filtrate - OM(PY)$_{total}$ – was adjusted with 1 M HCl to pH 2 and cooled overnight in a refrigerator to precipitate organic matter. Then the mixture was centrifuged (35 min, 1400 × g) to separate the HCl soluble from the HCl insoluble (OM(PY): subfraction of OM(PY)$_{total}$) fraction. The reason for the separation of $Na_4P_2O_7$ soluble and HCl insoluble OM is

Table 2. Management practices, including duration of recorded period, type and rotation of cover crops, and fertilization regime, for the different arable sites.

Soil	Recorded period of management practices	Type and rotation of cover crops		Fertilizer type and application rate (kg ha^{-1} yr^{-1})
AL	1990–2005	canola, winter wheat, winter barley, triticale, pea, corn	canola in a four year rotation	Farmyard manure: 300 NPK: 150
HSt	1991–2005	canola, winter wheat, winter barley	annual rotation	Urea: 350 NS: 100 K: 250
HL	1950–1980	alfalfa, winter wheat, spring barley, sugar beets	annual rotation	Farmyard manure: 300 N: 150
	1980–2005	winter wheat, spring barley, sugar beets	annual rotation	P: 100 K: 150
HC	1988–2006	winter barley, canola, winter wheat, spring barley	annual rotation	N: 150 P: 60 K: 70
VC	1970–1985	spring barley, winter wheat, winter barley	annual rotation	NH$_4$NO$_3$ (75 %) + CaCO$_3$ (25 %):
	1985–2006	canola, winter wheat, spring barley	annual rotation	150 kg^{-1} ha^{-1} (first and second year)

to concentrate high molecular OM containing carboxylate functional groups in the OM(PY) fraction (Kaiser et al., 2011). We dialyzed and freeze dried OM that was Na$_4$P$_2$O$_7$ soluble and insoluble in HCl (we analyzed this fraction's isotopic composition and refer to it as OM(PY)) as well as the OM that was Na$_4$P$_2$O$_7$ soluble and soluble in HCl.

3. The solid residues of (2) were washed with 0.1 m HCl and the Na$_4$P$_2$O$_7$ extraction was repeated as described for step (2) to remove the Na$_4$P$_2$O$_7$ soluble OM as complete as possible. The remaining extraction residue (ER) was washed with distilled water and freeze dried. The OM residing in the ER (OM(ER)) might be unextractable despite the use of H$_2$O and Na$_4$P$_2$O$_7$ (step 1 and 2) due to the chemical nature of the OM (less ionizable oxygen containing functional groups) and occlusion in aggregates not dispersed by ultrasonication (60 and 440 J ml^{-1}). The ER can contain organic particles <63 µm that cannot be distinguished by eye from mineral particles. All extractions steps were done in 3 replicate samples.

2.4 Determination of the organic C contents separated by the OM(PY) and OM(ER) fractions from the soil samples

The organic C (OC) content in the OM(PY) fraction was determined (Formacs TOC Analyser, SKALAR, Breda, Netherlands) from the OC contents of the OM(PY)$_{total}$ fraction minus the OC content of the OM fraction that is Na$_4$P$_2$O$_7$ and HCl soluble (Kaiser et al., 2011). This method was used because the precipitated Na$_4$P$_2$O$_7$ soluble and HCl insoluble OM(PY) can not be homogenized and directly measured. The freeze dried ER was homogenized by grinding in an agate mortar. The total C content in the ER was determined by elemental analysis (vario EL, ELEMENTAR, Hanau, Germany) and was assumed to be equivalent to the OC content because the ER are free of carbonates. The data were normalized to 105 °C dry soil and given in g OC kg^{-1} soil.

2.5 Determination of δ^{13}C and δ^{15}N of OM(PY) and OM(ER)

The isotope composition of the OM(PY), and the OM(ER) fractions were analyzed at the Center for Agricultural Landscape Research Stable Isotope Laboratory. A Thermo-Finnegan Flash HT elemental analyzer flash combusted the samples converting carbon and nitrogen to CO$_2$ and N$_2$ respectively, which were separated on a gas chromatograph column. The sample gas was flushed via a con-flow III to a Thermo-Scientific, Delta V advantage isotope ratio mass spectrometer. Calibration at this facility was to IAEA-CH-6 (sucrose) and IAEA-N-1 (ammonium sulphate). The isotopic values are expressed in delta notation (in ‰ units), relative to VPDB (Vienna Pee Dee Belemnite) for carbon and N$_2$ in air for nitrogen. Analysis of internal laboratory standards ensured that the estimates of the organic isotopic values were accurate to within 0.1 ‰.

2.6 Statistics

We used analysis of variance to test for differences in δ^{13}C and δ^{15}N signatures of OM(PY) and OM(ER) fractions between land use and soil type. We used partial least squares regression (PLS) to explain the variation in δ^{13}C and δ^{15}N of the different fractions attributed to the soil variables measured. PLS is commonly used to eliminate the problem of multicolinearity that occurs in regression when the number of independent variables is large compared to the number of the observations. Furthermore, PLS creates components that explain as much as possible the covariance in dependent and independent variables, unlike principle components analysis, which reduces the dimensionality only i independent variables (Abdi, 2003; Geladi and Kowalski, 1986). While PLS is often used to create predictive models (Ekblad et al., 2005), we are primarily interested in using PLS to: (1) outline land use effects, and (2) identify mineral characteristics relevant for organo-mineral interactions across different soil types. Thus, in our analysis we grouped soil texture variables (contents of sand, silt, and clay particle-size fractions) to address
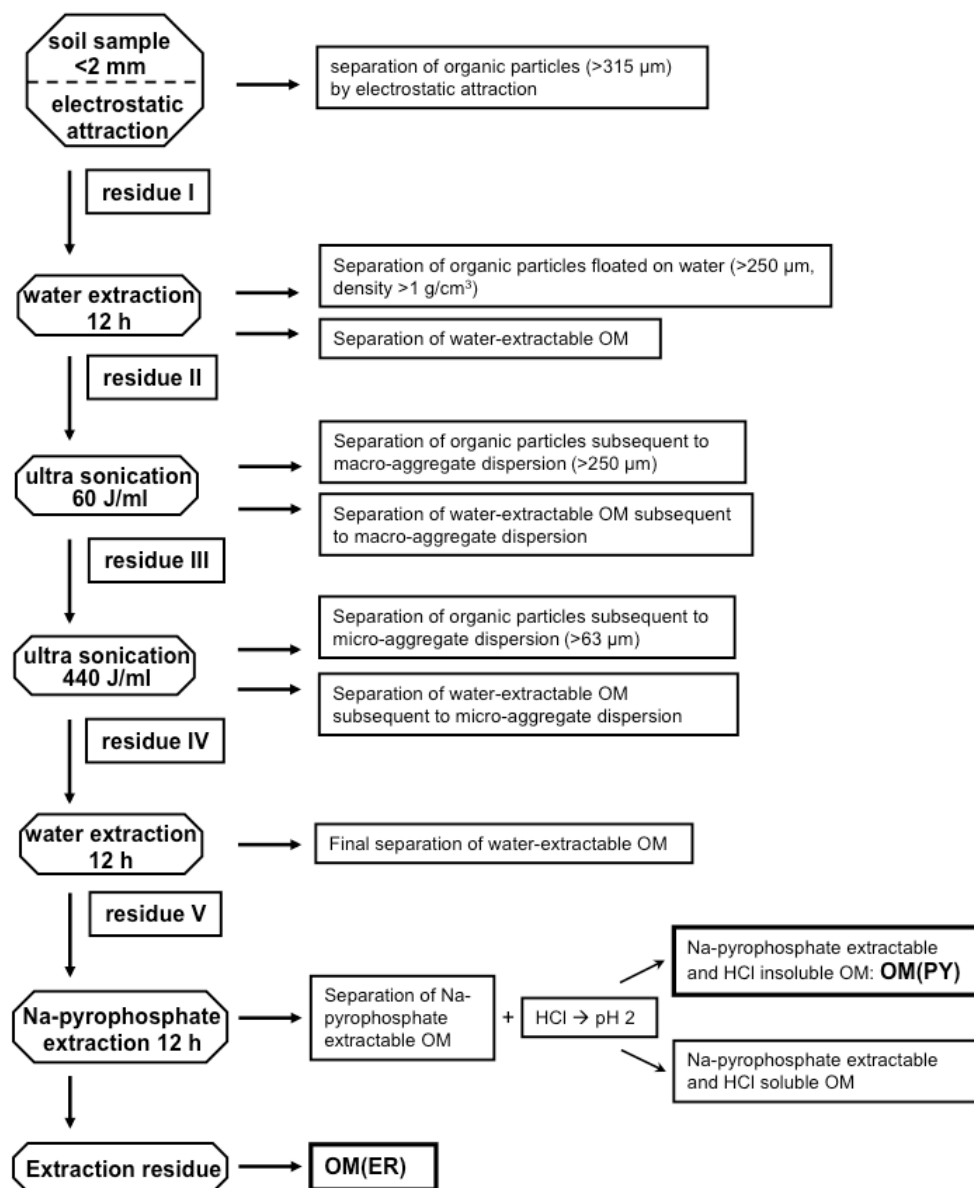
Fig. 1. Organic matter fractionation scheme.

differences between soil types. From the PLS analysis we report percent of variance explained by the first three components, weights of independent variables on the third component, and regression coefficients of the PLS model to indicate magnitude and direction of each independent variable on the variability in the isotopic data.

3 Results

The amount of OC separated by organic particle and water-extractable OM fractions relative to the bulk soil OC amount for the forest sites was 23.9 % (mean) ±6.6 (s.d.) and, when

the HC soil was omitted (5.4 %), 13.6 % ±0.41 % for the arable sites. The stablized carbon in the forest OM(PY) fraction was nearly twice that (20.4 % ±4.8 %) of the arable sites (10.4 % ±4.4 %). Differences in extractable cations were also seen between land use types. Arable sites tended to have greater exchangeable Ca(Ca$_{ex}$) and Mg(Mg$_{ex}$) content which corresponded with higher pH values as well. In general, forest sites exhibited a higher oxalate-soluble Fe and Al content than the arable sites.

For OM(ER) fractions, land use did have an impact on the isotopic composition. Arable soil OM(ER) fractions were primarily depleted in δ^{13}C and enriched in δ^{15}N while forest soil OM(ER) fractions were enriched in δ^{13}C and depleted

Table 3. Land use, and depth, as well as mean values of pH, and contents of sand, silt, clay, soil organic carbon (SOC; determined after the separation of organic particles by electrostatic attraction and aggregate occuled organic particles were removed), oxalate soluble Fe, Al (Fe$_{ox}$, Al$_{ox}$) as well as exchangeable Ca (Ca$_{ex}$) of the arable (Ap) and forest (Ah) topsoil samples from the Albic Luvisol (AL), Haplic Stagnosol (HSt), Haplic Luvisol (HL), Haplic Cambisol (HC), and Vertic Cambisol (VC) sites.

Soil	Land use	Horizon	Horizon Depth (cm)	pH[a] CaCl$_2$	Texture Sand[b] g kg^{-1}	Silt[c] g kg^{-1}	Clay[d] g kg^{-1}	SOC g kg^{-1}	SSA m^2 g^{-1}	Fe$_{ox}$ mg kg^{-1}	Al$_{ox}$ mg kg^{-1}	Ca$_{ex}$[e] cmol$_c$ kg^{-1}
AL	Arable	Ap	0–25	6.7	592	348	69	7.4 (±0.18)	2.92	1372 (±2)	500 (±2)	3.4
	Forest	Ah	1/2–5/10[f]	3.4	616	341	63	39.0 (±0.97)	0.79	1886 (±10)	782 (±16)	0.5
HST	Arable	Ap	0–30	7.4	610	290	113	10.4 (±0.02)	4.09	1591 (±33)	451 (±15)	7.6
	Forest	Ah	2/3–10	3.6	650	279	90	26.4 (±0.01)	1.41	2256 (±38)	1115 (±7)	1.3
HL	Arable	Ap	0–20	7.5	45	799	174	9.1 (±0.05)	10.97	3252 (±174)	588 (±21)	11.2
	Forest	Ah	1/2–15/20	3.7	46	833	143	30.7 (±0.12)	5.87	4048 (±120)	1121 (±5)	4.0
HC	Arable	Ap	0–8/12	7	45	619	380	25.4 (±0.34)	17.52	5086 (±73)	2139 (±8)	25.1
	Forest	Ah	0.5/1–7/10	4.3	24	624	399	29.7 (±0.05)	21.40	4052 (±22)	2906 (±40)	8.9
VC	Arable	Ap	0–5/8	7.1	108	384	561	21.5 (±0.57)	34.41	2848 (±105)	1382 (±9)	20.8
	Forest	Ah	1/3–25/30	4.5	80	599	352	13.4 (±0.17)	23.68	4006 (±120)	1343 (±24)	4.2

Values in parenthesis are single standard errors ($n = 2$).
[a] SD ($n = 2$) is less than or equal to ±0.14.
[b] SD ($n = 2$) is less than or equal to ±11.
[c] SD ($n = 2$) is less than or equal to ±11.
[d] SD ($n = 2$) is less than or equal to ±3.
[e] SD ($n = 2$) is less than or equal to ±0.08.
[f] Means here, for example, upper boundary of the horizons between 1 and 2 cm depth and lower boundary of the horizons between 5 and 10 cm depth.

in δ^{15}N (Fig. 2). Despite the trends in the data only the δ^{13}C between land use was significantly different ($p(F) < 0.03$) when the OM(ER) and OM(PY) fractions were grouped; however, the differences between OM fractions were not significant when compiled by land use type (Supplement Fig. S1.1) or soil type (Supplement Fig. S1.2).

Given that the difference in δ^{13}C of OM between arable and forest soils was significant, we grouped the data set by land use type for the Partial Least Square (PLS) analysis. The first three components of the PLS analysis explained 36–82 % of the variance in the δ^{13}C data and 78–80 % of the variance in δ^{15}N data for arable soils (Fig. 3). Contents of sand, silt and clay (i.e. texture) were strongly related to the first two components of the PLS analysis (Supplement S2), thus, the texture explained much of the variation in the isotopic data of OM(PY) and OM(ER) for the arable soils; however, this was not the case for the forest soils except for the δ^{13}C of the OM(PY) fraction. Exchangable Ca$_{ex}$ heavily influenced the third component for all OM fractions of arable soils and explained between 3 % and 25 % of the variation of δ^{13}C and δ^{15}N. While the third component for the forest sites explained much more (36–82 %) of the variation in the δ^{13}C and δ^{15}N data (Fig. 3).

The impact of the measured variables on component three was analyzed through the weights calculated during PLS (Fig. 4a, b). The third component of the OM(ER) fraction for each of the two isotopes was impacted by the measured soil variables in a similar way: the weights of the variable on the component were either both positive or both negative for δ^{15}N and δ^{13}C. The opposite occurred in the OM(PY) fraction where the weights of the soil variables on the third com-

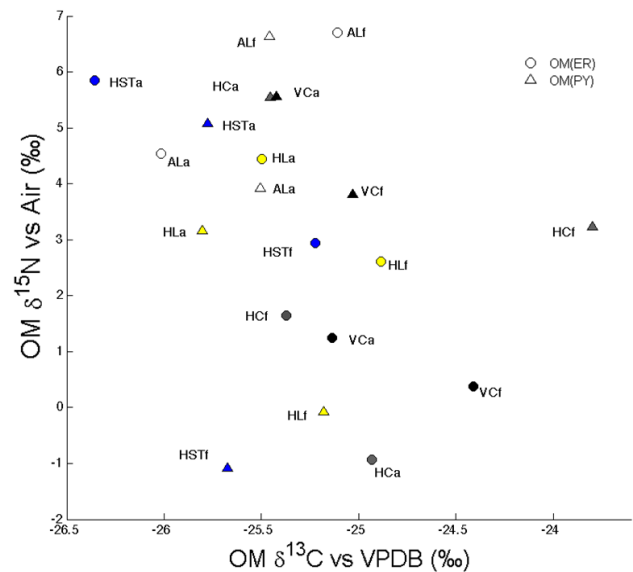**Fig. 2.** Isotopic composition (δ^{15}N, δ^{13}C) of the OM(PY) and OM(ER) fractions sequentially separated from the arable and forest soils. Each soil type is represented by a single color and an abbreviation (see Table 1 for abbreviations) that appear next to each data point along with a land use type abbreviations (a = arable, f = forest).

ponent were consistently opposite from each other: when the third component of the δ^{15}N of the OM(PY) fraction was impacted negatively, the δ^{13}C of this fraction was impacted positively.
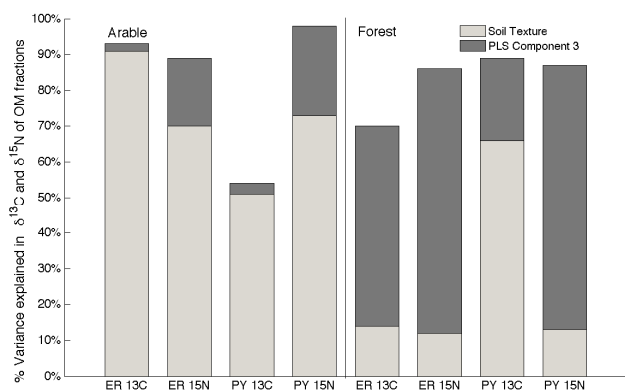
Fig. 3. Variance in the isotopic data (y-axis) in OM fractions (x-axis) explained by the first three components of the PLS analysis. The first two components were highly correlated with soil texture (distribution of sand, silt, clay) and were combined. PLS component 3 is orthogonal to the first two components, therefore, the variation explained and the subsequent models are related to soil mineral proxies.

The regression coefficient of the PLS analysis reports the direction of the correlation to the isotopic data of the different OM fractions. For arable soils, the δ^{15}N$_{ER}$ signatures became more depleted with Ca$_{ex}$ (Fig. 5a). This is in contrast to the δ^{15}N$_{PY}$ which became more enriched with an increase in Ca$_{ex}$. The regression coefficient associated with soil texture, primarily contents of silt and sand, was less than 0.01 but given the high variation in texture among soils, the impact on the ensuing isotopic composition could be large. For δ^{15}N$_{ER}$, an increase in silt and sand contents resulted in depleted values whereas for δ^{13}C$_{ER}$, an increase in the silt content led to depleted values while an increase in the sand content led to enriched values.

For forest soils, δ^{15}N$_{ER}$ and δ^{15}N$_{PY}$ signatures became more enriched with an increase in the ratio of SOC and specific surface area (SOC/SSA), (Fig. 5b). And, while the SOC/SSA ratio explained most of the variation of the third component, soil texture also played a role. Based on the regression coefficients, the degree of clay, silt or sand contents resulted in enriched δ^{15}N$_{ER}$ and δ^{13}C$_{PY}$ signals whereas decreased silt or sand contents resulted in depleted δ^{15}N$_{PY}$ and δ^{13}C$_{ER}$ signals.

4 Discussion

In this research, we set out to explore whether or not the isotopic signal of OM fractions sequentially separated from a range of soil types under arable and forest land use would yield additional information about OM isotopic enrichment and insights into the type of interaction between OM and soil minerals. The investigated OM(PY) and OM(ER) fractions are both hypothesized to contain stabilized OM, but given

their differences in extractability we expected differences in interaction with the various compounds present in the soil.

The impact of soil texture (i.e. clay, silt, and sand content) was overwhelming in explaining the isotopic variation in OM(PY) and OM(ER) of arable soils in our study. In contrast, texture explained little of the isotopic variation in OM fractions of the forest soils except for δ^{13}C$_{PY}$. The Relationship between soil texture and stabilized OM is well established (Chenu and Plante, 2006; Six et al., 2002) and the driving question behind this research is to reach beyond this empirical relationship and determine whether or not we can identify how OM is bound to soil mineral particles. This explains why we used PLS analysis. The variation in our data that can be attributed to soil particle size distribution is accounted for by the first two PLS components. Thus, the third component is orthogonal to the first two components and allows us to investigate further the relationship between isotopic patterns and proxies for soil mineral characteristics. We analyzed five arable and five forest topsoil samples, but the different soil types could obscure significant land use patterns. By using PLS, we were able to factor out the influence of soil type and focus on the analysis of OM that is most susceptible to land use impacts.

4.1 Isotopic patterns in arable soils

We found that variation in δ^{15}N$_{ER}$ and δ^{15}N$_{PY}$ of the arable soils was related to Ca$_{ex}$ content. Interestingly, the Ca$_{ex}$ level correlated differently to each fraction in the regression model: a negative correlation with δ^{15}N$_{ER}$ and a positive correlation with δ^{15}N$_{PY}$, an indication of different nitrogen processing or sources. From a soil biological perspective, the relationship between Ca and N is largely thought of in terms of the specific activity of microbial cells: the more Ca^{2+} cations the more microbial activity due to higher pH values (Groffman et al., 2006). Thus, the pattern of δ^{15}N$_{PY}$ enrichment with an increase of Ca$_{ex}$ is consistent with the hypothesis of enhanced microbial transformation (Böstrom et al., 2007; Sollins et al., 2009). Moreover, Ca$_{2+}$ plays an important role in cation mediated interactions between organic molecules and mineral surfaces (Clough and Skjemstad, 2000; Wuddivira and Camps-Roach, 2007) and other organic molecules through a process decribed as "crosslinking" (*sensu* Subramaniam et al., 2004). According to Oades (1988), the effect of adding Ca$_{2+}$ to soil is a transient acceleration of OM decomposition and a long-term effect of stabilization. Indeed, evidence exists of less labile, stabilized material in OM bound by Ca relative to OM fractions removed by NaOH in a range of agricultural soils (Zech et al., 1997; Olk, 2006). Therefore, we hypothesize an increased stabilization of microbial processed OM(PY) through the following Ca interactions: OM(PY)-Ca-mineral, OM(PY)-Ca (chelates) and/or OM(PY)-Ca-OM(PY) "crosslinking" (Subramaniam et al., 2004; Yang et al., 2001).
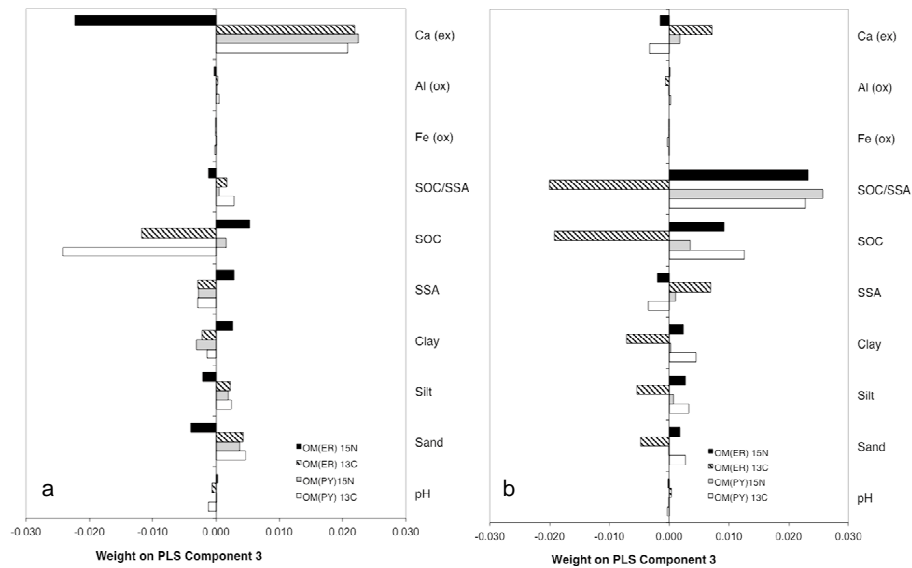
Fig. 4. PLS weights of soil parameter of each soil parameter of arable soil **(a)** and forest soil **(b)** on the isotopic signature of different OM fractions represented in component 3.
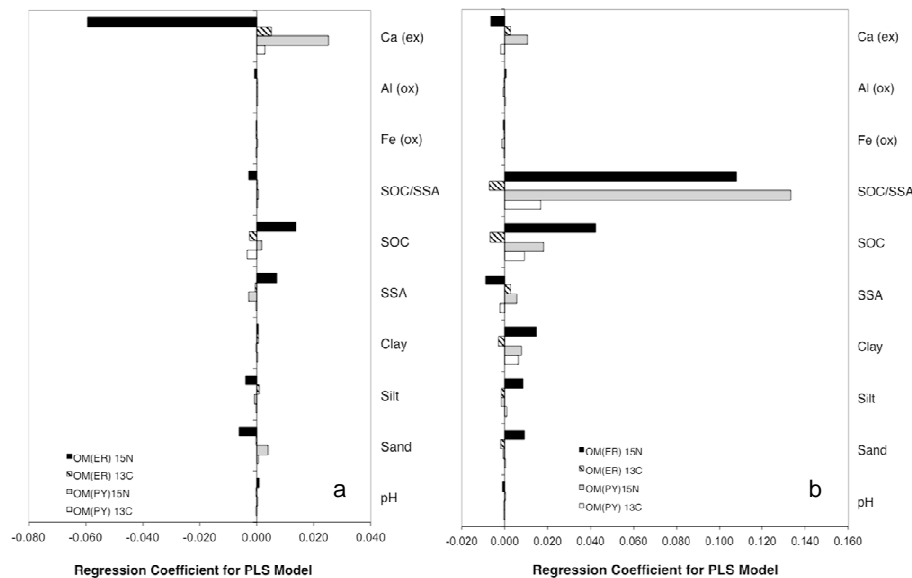

Fig. 5. PLS regression coefficients of each soil parameter of arable soil **(a)** and forest soil **(b)** on the isotopic signature of different OM fractions represented in component 3.

The pattern of a depleted δ^{15}N$_{ER}$ signal with higher soil Ca$_{ex}$ content has not been previously observed and the processes that lead to this pattern are unclear. We hypothesize that the isotopic composition of OM(ER) is influenced by δ^{15}N depleted OM of previous forest ecosystems still present in soils due to occlusion in soil micro-structures. We base this hypothesis on the methodology of OM separation we used. We sequentially separated at first organic particles (>63 µm) and water-extractable OM in combination with a

stepwise dispersion of macro- and micro-aggregates (using ultrasonic energy: 60 and 440 J ml^{-1}) followed by an extraction of OM(PY) from soil samples. The extraction residue after this treatments can contain highly stable clay and silt sized micro-structures, dispersible only by ultrasonic energy amounts >440 J ml^{-1}) (Chenu and Plante, 2006, Zhu et al., 2009; Moni et al., 2010), preserving OM occluded in such structures from separation. The δ^{15}N$_{ER}$ patterns show little sign of degradation or microbial transformation in the

OM(ER) fraction, indicating that nitrogenous compounds in this fraction are highly protected from microbial processing or the energy cost of microbes to release the N compounds is too high.

The arable soils are from actively managed sites and past land management effects are difficult to assess; however, tillage practices are generally thought to destabilize OM occluded in aggregates thus freeing OM for microbial decomposition. In this study, we separated the more labile, physically uncomplexed organic particles occluded in macro- and micro-aggregate as well as water extractable OM (Kaiser et al., 2011) prior to separating the OM(PY) fraction. Thus, the effect due to plowing should be negligible. Management practices extended to fertilization application at our sites. There were different fertilizers applications over the past 100 yr (Table 2) that could lead to a misinterpretation of the data. However, the differences between the agricultural fertilization regime and crop rotation had a small impact on the variability of the bulk isotopic signatures in the arable soils as a whole. Thus, the isotopic signatures of the separated organic matter fractions are a result of different processing and binding mechanisms of organic matter.

Effects due to different land use practices are often unavoidable with investigations that attempt to understand processes that occur over multiple time scales, such as OM stabilization in soil. We sought to limit these effects by centering our hypothesis around the organo-mineral interactions that occur on two very specific OM fractions. This approach reduces the uncertainty associated with the analysis of multiple isotopic sources represented in bulk OM. Furthermore, our results are similar to previous studies that found a consistency in isotopic signals within OM fractions that identified microbial processing as a precursor to deposition (Bol et al., 2005; Lobe et al., 2005). Nitrogenous compounds are increasingly seen as important for OM stabilization and only with further study can we realize the impact of varying nitrogen fertilization practices on the subsequent ^{15}N isotopic signature of stabilized OM.

4.2 Isotopic patterns in forest soils

In forest soils, the third component for all OM fractions, which explained up to 55 % and 80 % of the variation in δ^{13}C and δ^{15}N respectively, was largely driven by SOC content and SOC/SSA ratio. Reports in the literature suggest that an increase in the SOC/SSA ratio indicates an increase in the number of OM layers covering mineral surfaces (Keil et al., 1994; Koegel-Knaber et al., 2008). The SOC/SSA ratios in soils of this study ranged from 0.61 to 59.62 g m^{-2}, with all soils exceeding 1 mg OC m^{-2} SSA, the theoretical lower threshold for multi-layering of OM on mineral surfaces. The isotopic signatures of δ^{13}C$_{ER}$ and δ^{13}C$_{PY}$ were influenced by SOC and SOC/SSA ratios in contrasting directions. The δ^{13}C$_{PY}$ signature tended to become enriched with an increase in SOC levels while δ^{13}C$_{ER}$ incorporated less of the heavy

isotope, reflected by a depleted isotopic signature. The pattern of enrichment in δ^{13}C$_{PY}$ with SOC/SSA levels is an indication of microbial processing of OM. This pattern is shared with both δ^{15}N$_{PY}$ and δ^{15}N$_{ER}$ thus, reinforcing the interpretation of microbially processed organic matter sequentially layered on soil mineral surfaces (Kleber et al., 2007; Huygens et al., 2008; Sollins et al., 2009). However, this did not occur with OM(ER) where δ^{13}C values decreased with increasing SOC levels. It is likely, that the OM in the ER fraction has undergone a different pathway to stabilization that does not involve microbial processing or perhaps the OM is highly protected within soil micro-structures, similar to the OM(ER) of the arable soil. Bachmann et al. (2008) posit that "there are several lines of evidence that organic matter covers minerals in a patchy manner and that even at the nanoscale organic matter and minerals aggregate". This is confirmed by findings of Chenu and Plante (2006) who found that many of so called "clay particles" were nanometer to micrometer-sized micro-aggregates in which OM was encrusted by minerals. The authors concluded that these very small micro-aggregates protect OM from decomposition through physical entrapment.

4.3 Molecular model application

We can infer relationships between the isotopic signatures of OM fractions and soil mineral characteristics. Applying the isotopic patterns within the context of the conceptual zonal model proposed by Kleber et al. (2007) an overall picture of OM dynamics and stabilization in soils under arable and forest land use may be achieved. The model of Kleber et al. (2007) describes OM interactions with minerals within three zones: a contact zone, a hydrophobic zone, and a kinetic zone. Within each zone the force of attraction is different: the contact zone represents the strongest attraction while in the kinetic zone organic matter is loosely bound. Within each zone the authors describe potential mechanisms that may lead to the binding of OM. In the arable soils, Ca$_{ex}$ played a large role in driving the δ^{15}N patterns of OM(PY). The δ^{15}N$_{PY}$ enrichment with increasing Ca$_{ex}$ can be a result of separating OM(PY) from the contact zone where OM can bound to mineral surfaces via cation bridging by Ca^{2+} ions. In contrast, the δ^{15}N$_{ER}$ became depleted with increasing Ca$_{ex}$ which suggests that the OM in the ER fraction was not from the contact zone. The OM in the ER fraction may be located within micro-aggregates rendering the N in this fraction inaccessible to microorganisms or, potentially, the N in this fraction could be proteinaceous material covering the mineral surface in the contact zone (Kleber et al., 2007; Sollins et al., 2009). If the OM is inaccessible by physical or chemical means (Knicker, 2004), then the OM will be less processed by microbes resulting in a depleted signature relative to OM that is highly processed.

Table 4. Contents of organic carbon as well as $\delta^{13}C$ and $\delta^{15}N$ signatures of bulk organic matter and organic matter sequentially separated by Na-pyrophosphate solution (OC$_{PY}$, $\delta^{13}C_{PY}$, $\delta^{15}N_{PY}$) and remaining in the extraction residue (OC$_{ER}$, $\delta^{13}C_{ER}$, $\delta^{15}N_{ER}$), as well as the relative proportion of OC$_{PY}$ and OC$_{ER}$ contents in soil organic carbon (SOC) contents for the arable (Ap) and forest (Ah) topsoil samples from the Albic Luvisol (AL), Haplic Stagnosol (HSt), Haplic Luvisol (HL), Haplic Cambisol (HC), and Vertic Cambisol (VC) sites.

Soil	Horizon	$\delta^{13}C_{bulk}$ (‰)	$\delta^{15}N_{bulk}$ (‰)	OC$_{PY}$ (g kg^{-1})	OC$_{PY}$/SOC (%)	$\delta^{13}C_{PY}$ (‰)	$\delta^{15}N_{PY}$ (‰)	OC$_{ER}$ (g kg^{-1})	OC$_{ER}$/SOC (%)	$\delta^{13}C_{ER}$ (‰)	$\delta^{15}N_{ER}$ (‰)
AL	Ap	−26.3	5.0	0.58 (±0.04)	7.8	−25.5	3.9	4.4 (±0.10)	59.2	−26.0	4.5
	Ah	−25.7	1.3	9.14 (±0.55)	23.5	−25.5	6.6	16.8 (±1.37)	43.1	−25.1	6.7
HST	Ap	−26.2	6.5	1.13 (±0.02)	10.9	−25.8	5.1	5.5 (±0.12)	53.1	−26.4	5.9
	Ah	−26.2	0.5	4.14 (±0.22)	15.7	−25.7	−1.1	13.3 (±0.23)	50.4	−25.2	2.9
	Ap	−26.8	5.3	0.57 (±0.02)	6.3	−25.8	3.2	5.4 (±0.13)	59.6	−25.5	4.4
	Ah	−24.7	0.2	10.95 (±1.05)	35.6	−25.2	−0.1	16.6 (±0.47)	54.0	−24.9	2.6
HC	Ap	−24.6	5.7	4.47 (±0.18)	17.6	−25.5	5.5	17.9 (±0.32)	70.6	−24.9	−0.9
	Ah	−25.4	1.8	5.2 (±0.42)	17.5	−23.4	3.2	19.0 (±0.32)	64.0	−25.4	6.5
VC	Ap	−25.2	6.2	2.01 (±0.1)	9.3	−25.4	5.6	16.3 (±0.29)	75.7	−25.1	1.2
	Ah	−23.4	2.7	1.29 (±0.04)	9.6	−25.0	3.8	7.9 (±0.07)	58.9	−24.4	0.4

Values in parenthesis are standard errors ($n = 3$).

The absence of a strong correlation of component 3 and the $\delta^{13}C_{PY}$ or $\delta^{13}C_{ER}$ patterns in the arable soils, indicates that the small amount of carbon in these fractions is not interacting with mineral surfaces or is not occluded in microstructures (represented by PLS component 1) and is, therefore, readily available for exchange. The carbon could be derived from organic particles <63 µm not separated during soil fractionation. Alternatively, the carbon could be derived from OM present in the kinetic zone. Evidence for carbon exchanging in the kinetic zone is also found in ^{14}C studies where labeled C was identified in organo-mineral complexes, which are long thought to be stable based on long residence times (Swanston et al., 2005; Bruun et al., 2008).

Interestingly, in the forest soil, both the $\delta^{13}C_{PY}$ and $\delta^{15}N_{PY}$ values become enriched with the increase in the ratio of SOC/SSA. A SOC/SSA ratio >1 mg m^{-2} implies multiple layers of OM attached to mineral surfaces, and as indicated by isotopic signature of the OM(PY), the OM in these layers is likely highly processed by microorganisms. The pattern in the enriched isotopic signals suggests that OM in these layers exhibit slow exchange kinetics most likely due to the crosslinking of OM via polyvalent cations.

5 Conclusions

The isotopic signatures of OM fractions from arable soils were related to contents of the clay and silt size particles and Ca$_{ex}$, while forest soils were related to SOC/SSA ratios. Thus, we infer different binding mechanisms predominate in each land use type. For arable soils, the formation of OM(PY)-Ca-mineral associations was a relevant OM stabilization mechanism while the OM(PY) of forest soils was separated from layers of slower exchange not directly attached to mineral surfaces. This means there is a potential to build multiple OM layers on mineral particles in the arable soil and thus the potential for carbon accumulation. Caution must be exercised when comparing the two land use types; for example, the soil depths were different between the sites, which could adversely affect decomposition conditions especially when considering different soil horizons. However, we went through extensive measures to ensure similar soils between the two land use types (i.e. paired plot design) and we did not observe differences in aeration or soil water status, therefore, we expect the conditions in the top 30 cm of soil for a given land use pair to be similar.

The $\delta^{13}C_{PY}$ and $\delta^{13}C_{ER}$ values of the arable soils were generally found to be depleted (except HC, $\delta^{13}C_{ER}$) as compared to the respective forest soils. A greater number of microorganisms or an increased level of microbial metabolic activity in the forest soils (Kaiser et al., 2010) could explain this pattern. Although, the carbon fixed by trees and deposited in the soil was likely carboxylated at an earlier date than the arable vegetation. This would result in forest OM having a more enriched isotopic signal due to the Suess effect (the depletion in atmospheric CO$_2$ over time as a function of an increase in fossil fuel combustion). Future studies, with higher replications among soil types and assessing sites where land use change occurred at different time points will be necessary to elucidate these patterns.

The application of the OM fraction isotopic composition and soil mineral proxies with the molecular model yielded specific information about the binding mechanisms of OM in each land use. Open questions still remain concerning the molecular characteristics of OM in organo-mineral associations. These questions might be resolved with knowledge of the isotopic signatures of specific molecules using advanced methods such as compound-specific isotopic analysis (Bol et al., 2009), nanoSIMS (Herrmann et al., 2007) or through methods that identify organic functional groups in organo-mineral microaggregates (Kleber et al., 2010).

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