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Lignin is preserved in the fine silt fraction of an arable Luvisol

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Abstract

Knowledge about the fate of individual biomolecules during the decomposition process in soil is limited. We used the natural isotopic label introduced by 23 years of continuous maize cropping, together with compound specific ^{13}C isotope analysis, to study lignin monomers in particle size fractions of a Luvisol. Isotope data indicated apparent decadal turnover times for lignin. A kinetic model suggests the existence of a fast and a slow decomposing lignin pool in the soil, reconciling a low stock-to-input ratio with decadal turnover times. We found new, maize-derived lignin primarily in the 63–2000 μm fraction, whereas old, C_3-derived lignin from the pre-maize vegetation had accumulated mainly in the silt (2–20 μm) fraction. This distribution of lignin differed from that of total organic carbon, which was concentrated in the <2 μm fraction. Old, C_3-derived carbon in all the soil fractions was depleted in lignin compared to new, maize-derived carbon. The observation that the 2–20 μm fraction was less depleted than the others indicates that lignin preservation is particle size specific, but the underlying mechanism controlling its preservation is not clear.
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1. Introduction

The prediction of carbon stock dynamics in soil requires a thorough understanding of the mechanisms by which organic molecules are stabilized in soil. One method for following the fate of individual molecules uses isotopically labelled compounds. Biomass from C_4 plants (e.g., maize) differs in its $^{13}C/^{12}C$ ratio from that from C_3 plants (e.g., wheat). Hence, after a vegetation change from C_3 to C_4 plants, isotope analysis of soil organic matter (OM) enables discernment of the contribution of C_3-derived carbon from that of C_4-derived carbon (Balesdent and Mariotti, 1996). Compound specific isotope analysis (CSIA) has gone a step further and yields this type of information on a molecular level. Using this approach, it can be shown that unaltered biomolecules may persist in soil from years to decades. In arable soils, plant *n*-alkanes, *n*-carboxylic acids and lignin monomers turned over within a few decades, which was always faster than bulk soil organic carbon (C_{org}; Wiesenberg et al., 2004; Dignac et al., 2005). Recently, we showed that rapid lignin turnover also occurs in grasslands and can be demonstrated in the absence of a vegetation change if the isotopic label is introduced, e.g., by fumigation with labelled CO_2 (Heim and Schmidt, 2007). In order to describe the dynamics of lignin decomposition in an agricultural soil, Rasse et al. (2006b) developed a two-pool model based on a

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time series of one to nine years after change from wheat to maize cultivation. This model suggests that 92% of the lignin input is in a fast cycling pool, with turnover times <1 y, while only 8% reaches a protected soil carbon pool, where it is stabilized for substantially longer periods (decades). Several attempts have been made to relate measurable soil fractions to modelled soil pools, especially to the stable soil pool (Lopez-Capel et al., 2005; Poirier et al., 2005). Several authors have tried to correlate the stable, or refractory, soil organic carbon pool in soil with hydrolysis residues (Poirier et al., 2003), oxidation residues (Plante et al., 2004), density fractions (Sohi et al., 2001), or particle size fractions (Kiem et al., 2002). All these fractionation schemes produce fractions that vary in chemical composition (e.g., C, N, lignin, carbohydrate). However, relatively little is known whether these variations in molecular composition between fractions actually reflect differences in carbon dynamics between the fractions or not.

Particle size fractions of a soil differ in specific surface area and mineralogy. Sand size fractions are composed mainly of primary mineral grains, while finer fractions are dominated by secondary minerals (clay minerals and oxides) with a high specific surface area and thus a high sorption capacity. It is well documented that sorption of soil OM to the clay size fraction is significantly greater than to other size fractions (Christensen, 2001). Lignin is, however, not enriched in clay fractions to the same extent as bulk OM. This has been shown by several authors, who consistently report decreasing lignin/C_{org} ratio values with decreasing particle size (Guggenberger et al., 1994; Amelung et al., 1999; Kiem and Kögel-Knabner, 2003). Thus, although field data (Dignac et al., 2005; Heim and Schmidt, 2007) and a modelling approach (Rasse et al., 2006b) indicate the existence of medium term lignin stabilization in soil, it remains unclear by which mechanism this stabilization occurs and if the stable lignin is preferentially present in a specific physical fraction.

The aim of this paper was to determine the distribution of old and new lignin in particle size fractions of an arable soil in order to test the hypothesis that the stability of lignin is related to particle size. In particular, we wanted to know if stability is related to a specific size class and in which size class old (i.e., stabilized) lignin is mainly located.

The test site at Rotthalmünster (Germany) is one of several sites analyzed within the priority program SPP 1090 on carbon stabilization in soil. For this site, various other data are available, e.g., depth distribution of ^{14}C, carbon distribution in aggregates and density fractions and composition of soluble OM (Rethemeyer et al., 2004; Ellerbrock and Kaiser, 2005; John et al., 2005; Ludwig et al., 2005; Rethemeyer et al., 2005). The site was chosen because part of it has received a continuous isotopic label for 23 years by way of input from residues from maize cropping, while another part has been cropped with wheat for the same period.

2. Material and methods

2.1. Experimental site

Samples were taken from a long term field trial of the Höhere Landbauschule Rotthalmünster (Germany), described in detail by Ludwig et al. (2005). The site is located in the lower Rottal (48°21′47″ N, 13°11′46″ E), 360 m above sea level. The mean annual precipitation is 886 mm and mean annual temperature 8.7 °C. The soil type is a stagnic Luvisol developed from loess. Soil samples were taken from two plots, referred to as ‘maize plot’ and ‘wheat plot’. The maize plot was established in 1979. Since then, grain maize (*Zea mays* L.) has been cultivated on it, and stover plus roots were incorporated into the soil at harvest. Before 1979, the maize plot had been cultivated grassland until 1960, and cropped with different C_3 crops between 1961 and 1978. It has been ploughed regularly to a depth of 30 cm. The wheat plot serves as a reference soil without any C_4 input. The plot was established in 1969 on former grassland and has been cropped continuously with wheat since; in most years, mustard (*Sinapis alba* L.) was grown as an intercrop. On the wheat plot, conservation tillage (grubbing, 15 cm depth) was introduced in 1998 and has been practiced since. Soil samples from the 0 to 30 cm layer were taken from both plots on September 4 and 5, 2002. To characterize the biomass input, wheat straw was sampled after harvest and maize stems and maize roots close to the harvest date. Wheat root samples are from a later resampling in June 2005.

2.2. Sample preparation and analysis

Soil samples were oven dried at 40 °C and sieved to 2 mm. Particle size fractionation was done after a two step dispersion, as suggested by Amelung and Zech (1999). Briefly, samples (soil:water 1:5)

were dispersed at low ultrasonic energy (60 J ml^{-1}), after which the >63 μm fraction was separated by wet sieving. In a second step, the <63 μm fraction was dispersed at higher ultrasonic energy (440 J ml^{-1}) and the 20–63 μm fraction was separated by wet sieving. The 2–20 μm and <2 μm fractions were separated by sedimentation in an Atterberg cylinder. All fractions were centrifuged and freeze dried. Concentration and isotopic composition of C_{org} in the soil samples were determined after carbonate removal with 10% HCl. Carbon concentrations were measured with a CHN analyser (Vario EL, Elementar Analysensysteme, Hanau, Germany). The carbon isotopic composition of the soil and plant samples was determined in duplicate using a Europa Scientific Roboprep-CN elemental analyser, coupled to a Europa Scientific 20–20 isotope ratio mass spectrometer (Iso-Analytical Ltd., Sandbach, Cheshire, UK).

For lignin analysis, the samples were oxidized in a microwave oven using the CuO oxidation method and cleaned up using solid phase extraction over C_{18} columns (for more details see Heim and Schmidt (2007)). Samples were derivatised with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide/tetramethylchlorosilane (BSTFA/TMCS, 99:1) immediately before gas chromatography (GC) analysis. Identification and quantification was carried out using an Agilent GC–mass spectrometry (MS) system (HP 6890N Plus gas chromatograph, connected to a 5973N MSD detector), with anisic acid (added before derivatisation) as internal standard for quantification and ethylvanillin and cinnamic acid (added after CuO oxidation) to correct for losses during sample preparation. Analysis conditions were: 1 μl injection volume; injector temperature 290 °C; column DB5MS (50 m × 0.20 mm × 0.33 μm); 5 m pre-column (fused silica); He flow rate 1.2 ml min^{-1}; temperature programme: 100–160 °C at 3 °C min^{-1} (held 5 min), 160–250 °C at 3 °C min^{-1}, to 320 °C at 10 °C min^{-1} (held 10 min).

Compound specific isotope analysis (CSIA) of the CuO oxidation products (Goñi and Eglinton, 1996) was performed at least in duplicate for each sample using a Trace GC coupled to a GC Combustion III interface (maintained at 940 °C) and a Delta^{PLUS}XL (Thermo Finnigan, Bremen, Germany) isotope ratio mass spectrometry (IRMS) instrument. The same column and temperature programme as above were used. C_{24} *n*-alkane was added to the samples as an internal standard for CSIA. Its off line δ^{13}C value from an elemental analyser coupled to an IRMS instrument was used to correct for isotopic shift during analysis.

2.3. Calculations

As a biomarker for lignin, the sum of the eight lignin monomers (vanillin, vanillic acid, acetovanillone, syringaldehyde, syringic acid, acetosyringone, p-coumaric acid, ferulic acid) was calculated and is referred to as the VSC concentration. VSC stocks for the ploughed horizon (0–30 cm depth) were calculated assuming a bulk density of 1.38 g cm^{-3} (Ludwig et al., 2005). The amounts of new and old VSC were calculated as follows:

(I) For each monomer *i* in particle size fraction *j*, the maize-derived proportion F_{ij} is calculated by the isotopic mass balance approach given for soil organic carbon by Dignac et al. (2005). When applied to lignin monomers the mass balance equation is

$$F_{ij} = \frac{c_{ij,\text{new, maize soil}}}{c_{ij,\text{maize soil}}} = \frac{(\delta_{ij,\text{maize soil}} - \delta_{ij,\text{wheat soil}})}{(\delta_{ij,\text{new, maize soil}} - \delta_{ij,\text{new, wheat soil}})} - \frac{c_{ij,\text{maize soil}} - c_{ij,\text{wheat soil}}}{c_{ij,\text{maize soil}}} \cdot \frac{(\delta_{ij,\text{new, wheat soil}} - \delta_{ij,\text{wheat soil}})}{(\delta_{ij,\text{new, maize soil}} - \delta_{ij,\text{new, wheat soil}})}, \tag{1a}$$

where δ_{ij}, represents the δ^{13}C value [‰ V-PDB (Vienna Pee Dee Belemnite)] of monomer *i* in particle size fraction *j* of a soil sample and c_{ij}, its concentration, while $\delta_{ij,\text{new}}$ is the isotopic value of newly added monomers. This value cannot be analytically determined, so the denominator is usually approximated by the isotopic difference between the plant inputs where δ_i represents the δ^{13}C value (‰ V-PDB) of monomer *i* in a plant sample (Balesdent and Mariotti, 1996; Heim and Schmidt, 2007). The equation becomes

$$F_{ij} = \frac{(\delta_{ij,\text{maize soil}} - \delta_{ij,\text{wheat soil}})}{(\delta_{i,\text{maize plant}} - \delta_{i,\text{wheat plant}})} - \frac{c_{ij,\text{maize soil}} - c_{ij,\text{wheat soil}}}{c_{ij,\text{maize soil}}} \cdot \frac{(\delta_{ij,\text{new, wheat soil}} - \delta_{ij,\text{wheat soil}})}{(\delta_{i,\text{maize plant}} - \delta_{i,\text{wheat plant}})}. \tag{1b}$$

The only unknown is the numerator of the last term, describing potential isotope

discrimination during isotope selective degradation of monomers in the control soil. The extent of this discrimination is unknown, but was approximated to be identical to the difference between above ground biomass (−27.03‰) and soil OM (−26.46‰) on this plot.

(II) The total maize-derived amount $m_{i,j}$ of a monomer i in a specific particle size fraction j of the maize soil is then calculated as

$$m_{i,j} = F_{i,j} * c_{i,j,\text{maize soil}} * p_{j,\text{maize soil}}, \quad (2)$$

where $c_{i,j,\text{maize soil}}$ is the concentration of the monomer i in particle size fraction j of the maize soil and $p_{j,\text{maize soil}}$ is the relative proportion of this particle size fraction to the bulk soil.

(III) New (maize-derived) and old (C_3-derived) VSC is calculated as the sum of new and old monomer amounts, respectively. C_{VSC} (i.e., the C concentration attributable to VSC monomers) is calculated by multiplying each monomer concentration with its specific C content before summation. Vanillic acid is excluded from the calculation of new and old VSC, because in plant samples no isotopic value could be determined for this compound due to coelution with an interfering compound.

Apparent turnover times for individual monomers were estimated from the maize-derived fraction with the following formula, which assumes mono-exponential decay:

$$T_{ij} = -t/\ln(1 - F_{ij}), \quad (3)$$

where T_{ij} = apparent turnover time of monomer i in particle size fraction j, t = duration of maize cultivation and F_{ij} = fraction of maize-derived carbon of monomer i in particle size fraction j.

3. Results and discussion

3.1. Lignin concentration in plants and soil fractions

Lignin yields from CuO oxidation were 90, 93, 70 and 64 mg VSC (g dry matter^{-1}) for maize roots, maize shoots, wheat roots and wheat straw, respectively. As the total carbon content of these samples was 26%, 43%, 32% and 34%, respectively, this corresponds to proportions of 22% C_{VSC} for maize roots, 14% for maize stems, 14% for wheat roots and 12% for wheat straw (Table 1). The value of 22% C_{VSC} in maize roots is higher than reported for roots (10% in maize, 13% in wheat (Dignac et al., 2005)). In a related study, we analyzed lignin concentration during plant development and observed a threefold increase in concentration in maize roots during the last month before harvest (Abiven et al., unpublished results). Thus, lower lignin concentration found in maize biomass by Dignac et al. (2005) could reflect sampling less developed plants. The proportion of C attributable to lignin was similar for maize stems and wheat straw in our study, while Dignac et al. (2005) found a greater lignin content in wheat stems (18% vs. 11% in maize stems). Again, we assume that differences in the state of maturity of the plants at the sampling dates can explain these variations. The concentration in the bulk soil was found to be 0.33 mg VSC (g dry soil)$^{-1}$ for the wheat soil and 0.44 mg VSC (g dry soil)$^{-1}$ for the maize soil. This corresponds to 18 mg C_{VSC} (g C_{org})$^{-1}$ in the wheat soil and 24 mg C_{VSC} (g C_{org})$^{-1}$ in the maize soil.

3.2. Lignin dynamics assessed via harvest mass balance

When the soil carbon pool is in equilibrium, the mean residence time of any compound can be determined by calculating the ratio between its stock in the soil and its annual input to the soil. The annual input of carbon from harvest residues to the maize soil was estimated by Ludwig et al. (2005) using two independent methods. When above ground harvest residues were collected and a root/(residues + grains) ratio of 0.2 was assumed, estimated input was 0.63 kg C m^{-2} a^{-1} (0.46 kg C m^{-2} a^{-1} from above ground residues and 0.17 kg C m^{-2} a^{-1} from roots). When the input was modelled with the RothC model in order to fit the actual soil carbon pool, the total annual input rate was only 0.41 kg C m^{-2} a^{-1}.

With average C_{VSC}/C_{org} values of maize residues given in Table 1, the harvest-based value results in an annual lignin input of 99 g lignin C m^{-2} a^{-1}. Lignin stocks in the ploughed horizon amount to 111 g lignin C m^{-2} (30 cm)$^{-1}$. From the ratio of stocks to annual input, the mean residence time (MRT) of lignin C in the soil is estimated to be 1.1 y. Such a short MRT contrasts strongly with MRTs for lignin C in the decadal range as suggested by stable isotope studies (see below and Dignac et al., 2005; Heim and Schmidt, 2007). A possible explanation for this apparent discrepancy is that in the soil at

Table 1
Panels (a): Concentration of C_{org}, N and lignin monomer units in soil, soil fractions and plants and (b): Lignin quality indicators in soil, soil fractions and plants

	Weight fraction ($g\ g^{-1}$)	C_{org} ($mg\ g^{-1}$)	N ($mg\ g^{-1}$)	Cinnamyl units ($\mu g\ g^{-1}$)	Syringyl units ($\mu g\ g^{-1}$)	Vanillyl units ($\mu g\ g^{-1}$)	C_{VSC}/C_{org} ($mg\ g^{-1}$)
Panel (a)							
Maize soil							
Bulk		11.3	1.0	88	219	135	23.8
>63 μm	0.11	12.3	0.8	224	341	191	37.6
20–63 μm	0.36	2.8	0.2	16	54	49	25.9
2–20 μm	0.35	5.1	1.0	37	130	102	31.9
<2 μm	0.17	29.3	4.1	67	202	96	7.5
Maize roots		260	2.5	45×10^3	32×10^3	13×10^3	216
Maize shoots		426	2.3	45×10^3	31×10^3	17×10^3	135
Wheat soil							
Bulk		11.2	1.3	44	152	129	17.7
>63 μm	0.09	12.0	1.0	264	500	386	58.7
20–63 μm	0.35	3.0	0.3	24	86	76	37.5
2–20 μm	0.37	6.4	0.8	28	86	99	20.4
<2 μm	0.19	28.4	4.2	43	171	114	7.0
Wheat roots		319	n.a.[a]	23×10^3	31×10^3	16×10^3	135
Wheat straw		336	4.3	12×10^3	30×10^3	21×10^3	116

	Acid/aldehyde ratio			
	Vanillic ($g\ g^{-1}$)	Syringic ($g\ g^{-1}$)	C/V ($g\ g^{-1}$)	S/V ($g\ g^{-1}$)
Panel (b)				
Maize soil				
Bulk	0.36	0.56	0.65	1.62
>63 μm	0.26	0.52	1.18	1.79
20–63 μm	0.47	0.54	0.33	1.11
2–20 μm	0.43	0.58	0.36	1.28
<2 μm	0.54	0.70	0.71	2.12
Maize roots	0.08	0.17	3.44	2.44
Maize shoots	0.08	0.27	2.70	1.89
Wheat soil				
Bulk	0.36	0.64	0.34	1.18
>63 μm	0.21	0.43	0.69	1.30
20–63 μm	0.34	0.63	0.32	1.13
2–20 μm	0.38	0.54	0.28	0.86
<2 μm	0.36	0.61	0.37	1.50
Wheat roots	0.06	0.05	1.47	1.88
Wheat straw	0.12	0.25	0.59	1.45

[a] Not available.

least two lignin pools with different kinetics exist (Rasse et al., 2006b) and that the two approaches measure different kinetic pools, as explained in the next section.

3.3. Lignin dynamics assessed from stable carbon isotope studies

The isotopic differences for individual lignin monomers between C_3 (wheat) and C_4 (maize) soil and between wheat and maize plants are given in Table 2a. Assuming mixing of the two sources "C_3 lignin" and "C_4 lignin" and taking into account differences in lignin concentration between the wheat and maize soils (Eq. (1b)), these isotopic differences result in fractions of C_3- and C_4-derived carbon as given in Table 2b. As input of C_3-derived lignin to the maize plot occurred only before 1979, any lignin biomarkers with C_3 isotopic signature detected at the sampling in 2002 must have been conserved in the soil for at least 23 years.

Table 2a
Isotopic difference ($\delta^{13}C_{maize} - \delta^{13}C_{wheat}$) for derivatised individual lignin monomers between wheat and maize soil and between wheat and maize plants

	Fd[a] (‰ V-PDB)	pCd[a] (‰ V-PDB)	Sd[a] (‰ V-PDB)	Sl[a] (‰ V-PDB)	Sn[a] (‰ V-PDB)	Vd[a] (‰ V-PDB)	Vl[a] (‰ V-PDB)	Vn[a] (‰ V-PDB)
Soil fraction								
Bulk soil	10.6	10.9	8.9	11.3	10.0	5.7	7.8	7.6
>63 μm	9.6	10.8	12.2	14.1	11.9	11.1	10.9	10.7
20–63 μm	n.d.[b]	11.2	9.9	11.0	8.6	4.9	8.8	7.0
2–20 μm	n.d.	8.8	5.8	7.7	6.3	3.4	3.8	4.0
<2 μm	5.2	9.0	7.1	10.2	9.2	3.0	3.7	7.1
Plants[c]	9.3	10.0	13.7	16.0	12.9	n.d.	14.4	13.5

[a] Fd – ferulic acid, pCd – para-coumaric acid, Sd – syringic acid, Sl – syringaldehyde, Sn – acetosyringone, Vd – vanillic acid, Vl – vanillin, Vn – acetovanillone.
[b] Not determined.
[c] For maize, the average between stems and roots was used; for wheat, only a value for the straw was available.

Using this information in a mono-exponential decay model results in MRTs for C_3 lignin in the range of several decades (Table 2b). The following patterns can be observed:

1. Individual structural units exhibit specific dynamics. Cinnamyl units are replaced faster by maize-derived moieties than syringyl units, while the highest proportions of C_3 origin are found in vanillin and acetovanillone units. This order of degradability (cinnamyl > syringyl > vanillyl) is in line with a recent report by Bahri et al. (2006), who observed the same trend over a 9 y experiment with a loamy Cambisol. They suggest as possible explanations differences between the monomers in terms of localization in the plant tissue, linkages within the lignin macromolecule and susceptibility to microbial degradation.
2. For all the monomers, proportions of maize-derived monomers are highest in the >63 μm fraction. Probably relatively labile particulate OM contributes to this fraction.
3. At the other end of the range, the 2–20 μm fraction contains the greatest proportion of C_3 origin for all the lignin monomers. Exceptions are ferulic and vanillic acids, where we could not measure isotopic signatures.

At first glance, MRTs in the decadal range seem to contradict the MRT of 1.1 y calculated from the lignin balance approach in Section 3.2. However, such an apparent discrepancy can be explained if two soil pools with different decomposition rates are considered, as suggested by Rasse et al. (2006b). For the arable soil they studied, their model predicted that 92% of the lignin input from plants was degraded within less than one year, whereas 8% was stabilized and accumulated in the soil with a mean residence time of approximately 20 y. We checked the plausibility of our data with a simplified two-pool model based on the following equations:

$$P_1(t+1) = (P_1(t) + J * \alpha) * \exp(-k_1), \quad (4)$$

$$P_2(t+1) = (P_2(t) + J * (1-\alpha)) * \exp(-k_2), \quad (5)$$

where P_1, P_2 are pools 1 and 2 (g m^{-2}), respectively, k_1, k_2 their decay constants (a^{-1}), t is the time in years, J the total input (g m^{-2}) and α the fraction of the input reaching P_1 (g g^{-1}). These equations imply that the pool size is measured immediately before the annual input and that the annual input occurs at a single point in time. Though the latter is a simplification, it is justifiable given the fact that in

Table 2b
Fraction of maize-derived carbon in lignin monomers based on isotopic mass balance and estimated apparent turnover times

	Fd[a]	pCd	Sd	Sl	Sn	Vd	Vl	Vn
Fraction of new VSC[b] *(g g^{-1})*								
Bulk soil	1.00	1.00	0.64	0.69	0.76	n.d.[c]	0.54	0.56
>63 μm	1.00	1.00	0.90	0.90	0.94	n.d.	0.80	0.82
20–63 μm	n.d.	1.00	0.76	0.71	0.69	n.d.	0.64	0.53
2–20 μm	n.d.	0.87	0.41	0.47	0.46	n.d.	0.26	0.35
<2 μm	0.53	0.87	0.51	0.64	0.70	n.d.	0.27	0.54
Apparent turnover time[d] *(years)*								
Bulk soil	undef.[e]	undef.	22	19	16	n.d.	30	28
>63 μm	undef.	undef.	10	10	8	n.d.	14	14
20–63 μm	n.d.	undef.	16	19	20	n.d.	22	30
2–20 μm	n.d.	11	43	36	37	n.d.	77	54
<2 μm	30	11	33	23	19	n.d.	73	30

[a] Abbreviations as in Table 2a.
[b] Calculated using Eq. (1b); calculated values >1 set to 1.00.
[c] Not determined.
[d] Calculated with Eq. (3).
[e] For fractions ⩾1, turnover time undefined.

grain maize cropping the majority of the biomass input into soil occurs at harvest. In a steady state system, the pool sizes at time $t+1$ equal the pool sizes at time t, and the total pool size can be calculated as

$$P = P_1 + P_2 = J * \alpha/(\exp(k_1) - 1) + J * (1-\alpha)/(\exp(k_2) - 1) \quad (6)$$

Thus, for a given P and J, α can be calculated from the decomposition constants. Using the data given in Section 3.2 ($P = 111.2$ g C_{VSC} m^{-2} (30 cm)$^{-1}$ and $J = 98.8$ g C_{VSC} m^{-2} a^{-1}) with the decomposition constants ($k_1 = 1.88$ a^{-1}; $k_2 = 0.052$ a^{-1}) given by Rasse et al. (2006b), α is calculated to be 94.9%.

When this model is used to calculate the size of the maize-derived pool after 23 y of maize cultivation, it gives a value of 74.4%. This corresponds well with the measured value of 72.5% given the analytical uncertainty. The residual error may be explained by differences in climate and soil properties resulting in different decomposition rates at the site in south-eastern Germany compared with the site in the Paris Basin for which Rasse et al. (2006b) had originally determined the decomposition rates. Our site receives about 250 mm more precipitation per annum and has an annual mean temperature that is 1.5–2 °C lower than the French site when compared to data from the nearest climate station available on www.worldclimate.com. At our site, the climate is wetter and cooler and the soil contains more silt and clay (90%) than the French site (70%) (Rasse et al., 2006a), which might reduce decomposer activity. In summary, modelled and measured proportion of maize-derived lignin in our soil are in good agreement. The combination of data for lignin input, lignin stock in the soil and lignin isotope values confirms the existence of two lignin pools. Although, according to our estimate, the slower pool receives only about 5% of the input, it is about 5.6 times larger than the fast pool and controls the amount of C_3-derived lignin stabilized in the soil. In contrast, in the fast pool maize-derived lignin already had replaced all of the pre-existing C_3 lignin within two years (Fig. 1).

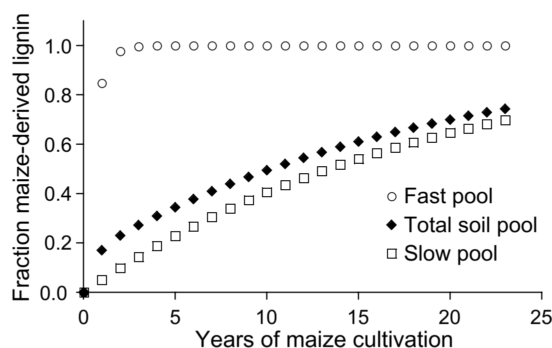

Fig. 1. Modelled fraction of maize-derived lignin as a function of duration of maize cultivation on a former C_3 soil. The soil lignin pool is divided into two sub-pools: a fast cycling pool ($k = 1.88$ a^{-1}) receiving 94.9% of the annual input and a slow cycling pool ($k = 0.052$ a^{-1}) receiving 5.1% of the annual input. Further details are given in the text.

3.4. Distribution of new and old lignin in particle size fractions

In this section, we discuss whether the slow lignin pool can be related to a specific soil fraction or not. Due to the nature of the analytical CuO procedure, this interpretation is limited to the fraction of relatively unaltered lignin molecules that can be detected as phenolic monomers. Strongly modified lignin degradation products may behave differently, and in the following discussion we explicitly exclude these degradation products. Fig. 2 shows the distribution of C_3-derived and maize-derived C_{VSC} as well as of total C_{org} in the soil particle size fractions. While 43% of maize-derived C_{VSC} recovered after fractionation was found in the sand fraction (>63 μm), the remainder is about equally distributed among the 20–63 μm, 2–20 μm and <2 μm fractions. In contrast, total maize-derived C_{org} preferentially accumulates in the finest (<2 μm) fraction (41%) and only 26% of the maize-derived C_{org} can be found in the coarse (>63 μm) fraction. C_3-derived C_{VSC} is distributed differently from maize-derived C_{VSC}: More than half of the recovered C_3-derived C_{VSC} is found in the 2–20 μm fraction, 27% in the <2 μm fraction and ca. 10% each in the 20–63 μm and >63 μm fractions. Total C_3-derived C_{org} preferentially accumulates in the <2 μm fraction, as was observed for total maize-derived C_{org}. Such an accumulation of C_{org} in the finest fractions is typical of soils (Christensen, 2001).

Lignin quality and quantity differ between the particle size fractions and between old and new carbon, which is consistent with the different accumulation of lignin compared with total OM in the size fractions. First, maize-derived C_{org} in the >63 μm fraction contains about 3.5 times more VSC units (58 mg C_{VSC} (g C_{org})$^{-1}$) than maize-derived C_{org} in the clay fraction (16 mg C_{VSC} (g C_{org})$^{-1}$). This difference in relative VSC content indicates that considerable lignin degradation already occurs before the new, maize-derived organic matter is found in the clay fraction. This finding is supported by several previous studies, which show that carbon in the clay size fraction of many soils is generally poor in VSC units (Amelung et al., 1999; Lobe et al., 2002; Kiem and Kögel-Knabner, 2003). Second, the older, C_3-derived carbon generally contains a lower proportion of VSC units than the maize-derived carbon (Fig. 3), clearly indicating a relative

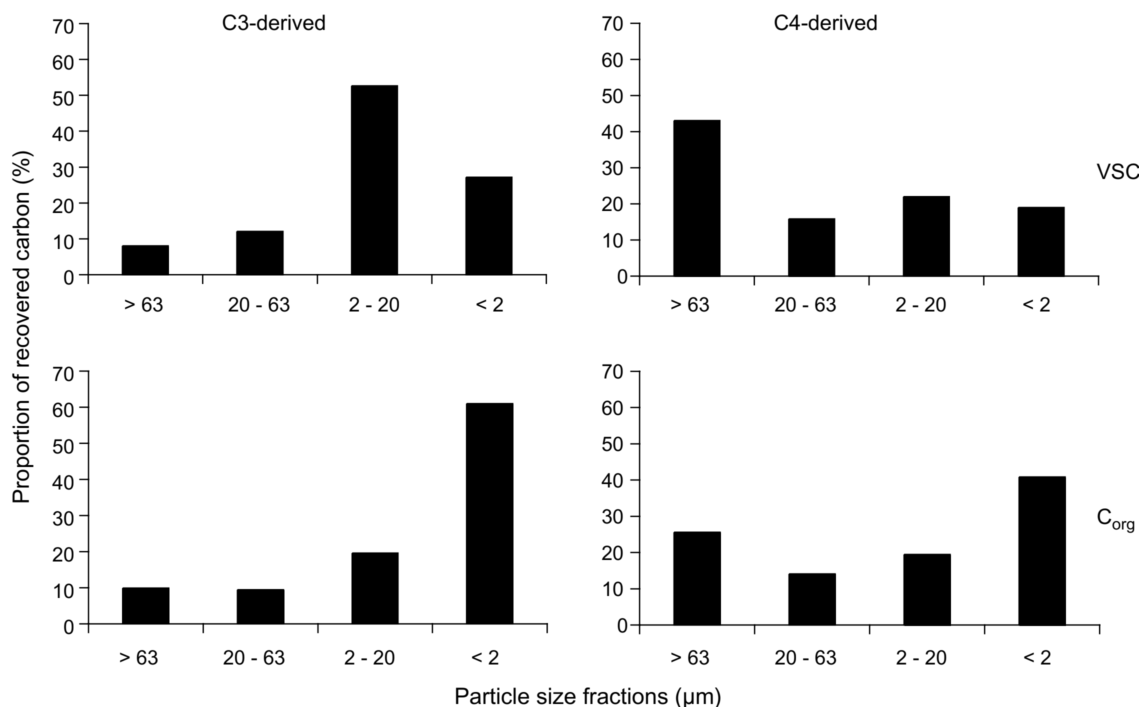

Fig. 2. Distribution of C_3 derived and maize-derived lignin and carbon in particle size fractions. Vanillic acid was excluded from the calculation of total VSC, because its isotope value could not be determined, so its maize-derived proportion is unknown.

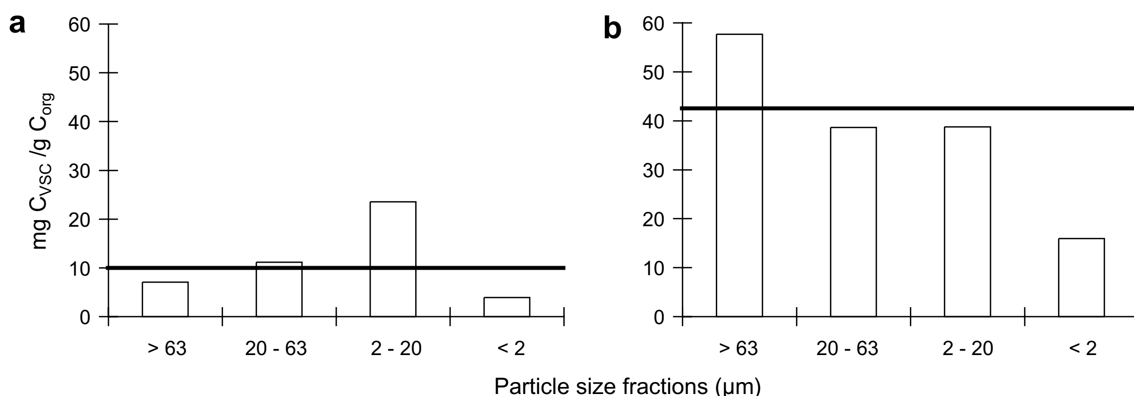

Fig. 3. Proportion of (a) old and (b) new carbon in particle size fractions attributable to lignin monomer units. The horizontal line represents the value for unfractionated soil.

VSC loss over time. However, the VSC units are not lost at a uniform rate and the loss rates differ between size fractions. The relative loss from the 2 to 20 µm fraction is least, leading to a preferential enrichment in C_3-derived VSC units in this fraction. For the moment, it remains unclear which process or mechanism prevents VSC units in the 2–20 µm fraction from being lost at the same rate as VSC units from other fractions. Whatever the mechanism, the slow loss rate for this size fraction suggests an important direct or indirect role of the fine silt fraction for the long term preservation of lignin.

Such a particle size dependent preservation supports the view that preservation of old lignin is not caused solely by its intrinsic chemical recalcitrance (i.e., an inherently barely degradable molecular structure; Marschner et al., in press). If mainly recalcitrance controlled lignin preservation, VSC concentrations should be similar in all size classes. Our observations indicate an important role for interactions between the lignin molecules and the mineral phase in the preservation of VSC.

VSC units could be protected by reaction with the surface of poorly crystalline minerals and/or oxides found in the fine fractions (Torn et al., 1997; Kleber et al., 2005), thereby reducing their accessibility to decomposers and their degradability. Such a mechanism depends both on the type of functional groups in the lignin fragments and on the surface characteristics of the minerals. The acid/aldehyde ratio of both syringyl and vanillyl groups is a common proxy of lignin quality. Table 1b shows that the ratio for both of these is larger for the silt and clay fraction (<63 µm) than for the sand fraction >63 µm. Differences between silt and clay are not pronounced. Thus, the highest ratio values in the maize soil are found in the clay fraction (both for vanillyl and syringyl units), but the highest values in the wheat soil are found in the 2–20 µm (vanillyl units) and the 20–63 µm (syringyl units) fraction. In soil, increasing acid/aldehyde values generally reflect increasing oxidation of the phenylpropanoid side chain by lignin-decomposing white rot fungi (e.g., Kögel, 1986; Hedges et al., 1988). Therefore, the observed increase in acid/aldehyde ratio from coarse to fine particle size fractions in arable soil indicates that lignin in the clay fraction is more decomposed and modified than in coarser fractions (Guggenberger et al., 1994; Kiem and Kögel-Knabner, 2003). For our soils, the data indicate that there is no marked difference between silt and clay fractions in the degree of side chain oxidation. Two other lignin quality proxies, the cinnamyl/vanillyl (C/V) ratio and the syringyl/vanillyl (S/V) ratio decrease in the order sand > clay > silt (C/V) or clay > sand > silt (S/V). Both ratios typically decrease from plants to soil due to the relatively high stability of vanillyl units in soil (Kögel, 1986; Hedges et al., 1988). Possible reasons have been compiled by Bahri et al. (2006) and include, among others, localization of the individual monomers in the plant tissue, their linkage with other monomers and differences in reactivity with mineral phases. Bahri et al. (2006) hypothesize that the lower methoxy substitution of the vanillyl phenols favours their reaction with the mineral phase for reasons of geometric accessibility of bonding sites. The low S/V and C/V ratios observed for the fine silt fraction of our soils may thus indicate that a high proportion of vanillyl units contributes to the stabilization of

old lignin in this fraction. On the other hand, the coarse silt (20–63 μm) is characterized by similarly low S/V and C/V ratios but preserved much less old lignin, which demonstrates that lignin quality proxies alone cannot fully explain the preservation mechanism(s).

An alternative or complementary protection mechanism could be inclusion into microaggregates (<250 μm). Although the lifetime of aggregates in soils is difficult to assess, microaggregates are considered fairly stable over long periods of time (Christensen, 2001). While organic substances in the smaller microaggregates (<20 μm) are mainly microbial products or root exudates acting as binding agents between the primary mineral particles, larger microaggregates (20–250 μm) also contain occluded OM (Christensen, 2001). The procedure used to isolate soil particle size fractions uses ultrasonic energy, which is supposed to disperse the sample completely, destroying all microaggregates (Amelung et al., 1998). However, Chenu and Plante (2006) have shown recently that, even after ultrasonic dispersion, clay-sized microaggregates may persist in a soil sample. Thus, both adsorption and entrapment of OM are potential mechanisms for OM stabilization in fine fractions.

4. Conclusions

These preliminary results, relating to lignin stability in particle size fractions using a stable isotope approach, support the hypothesis that stabilization of lignin biomarkers in soil is not due to their inherent chemical recalcitrance but depends on interaction with mineral matter. The higher capacity for VSC stabilization in fine size fractions agrees with hypotheses suggesting surface dependent adsorption processes, but the reason for maximum VSC preservation in the fine silt fraction remains unclear.

Additionally, the results indicate that a large part of the lignin input to soil never reaches this stable fraction, but turns over more rapidly. Consequently, there is not a uniform residence time for lignin in soil. Turnover estimates based on analysis of the stabilized fraction in soil risk underestimating the actual turnover of lignin input.

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