

Lignin turnover in arable soil and grassland analysed with two different labelling approaches

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Summary

When modelling the carbon dynamics of temperate soils, soil organic carbon (SOC) is often represented by three kinetic pools, i.e. fast, slow and passive/inert. Lignin is often considered to be relatively resistant to decomposition, thus possibly contributing to the passive SOC pool. One way to assess SOC turnover under natural conditions is to follow the fate of ^{13}C -labelled biomass in soils. We used compound-specific isotope analysis to analyse CuO oxidation products of lignin from grassland topsoils and from an arable topsoil that had received a natural (by C3-C4 vegetation change) or an artificial (by fumigation with labelled CO_2) isotopic label for 9–23 years. Results indicate faster apparent turnover for lignin (5–26 years in grassland, 9–38 years in arable soil) compared with bulk SOC (20–26 years in grassland, 51 years in arable soil). Although these calculated lignin turnover times cannot be extrapolated to the whole soil profiles, this paper provides isotopic evidence that lignin in soils is not preferentially preserved, which is a consistent result from both ways of isotopic labelling. It also demonstrates, however, that a considerable proportion of lignin in temperate soils can be stabilized for at least a few decades.

Introduction

Soils store about twice as much carbon than exists in the atmosphere as CO_2 (Prentice *et al.*, 2001). Global warming will probably affect soil organic carbon (SOC) decomposition and may affect the ratio of carbon transferred to the atmosphere and carbon sequestered in soil, although the magnitude of this effect is still uncertain (Jones *et al.*, 2005). In models of carbon dynamics in temperate soils, e.g. CENTURY (Parton *et al.*, 1987) or Roth-C (Jenkinson & Rayner, 1977; Coleman & Jenkinson, 1996), SOC is often represented by three kinetic pools with different turnover times: an active, or fast, pool (turnover time 1–5 years), a slow, or intermediate, pool (approximately 20–40 years), and a passive, or (nearly) inert, pool (200–1500 years or more). So far, these pools are purely operationally defined and it is not clear which chemical compounds contribute to which pool.

Lignin is the main constituent of the cell walls of woody plants. Beside polysaccharides, it is the second most abundant biopolymer in terrestrial plants (Killops & Killops, 2005) and contributes significantly to the organic carbon input into soils. Lignin degradation is driven by consortia of decomposer microorganisms such as fungi (Haider, 1992; Kögel-Knabner, 2002). Soft-rot and brown-rot fungi induce structural changes in lignin

but not complete mineralization, whereas white-rot fungi are able to completely decompose lignin to CO_2 . Upon biodegradation, lignin undergoes a gradual oxidative transformation, which introduces carboxyl groups into the molecule. The molecule then becomes extractable with sodium hydroxide and thus can be found in the humic acid fraction.

Classical litter decomposition studies generally find that lignin decomposes more slowly than most other litter compounds and is relatively enriched during the decomposition process (Berg & McClaugherty, 2003). Consequently, lignin residues have been considered to form a large proportion of the stock of old, slowly degradable organic carbon in the soil. The resistance of lignin to strong acid attack in the laboratory, which is the basis of proximate lignin analyses (e.g. Klason lignin; Effland, 1977), has been considered as supporting evidence for the chemical recalcitrance of lignin to decomposition.

Recently, there is increasing circumstantial evidence that lignin is not particularly stable in soils. From a qualitative point of view, several studies showed preferential loss of lignin from soils. Analysis of lignin in C-depleted agricultural soils (Kiem & Kögel-Knabner, 2003) showed that lignin is relatively more depleted than bulk SOC. In a chronosequence of cultivated semiarid grasslands, lignin was found to be lost at the same rate as total organic carbon upon cultivation (Lobe *et al.*, 2002). Indications for preferential loss of lignin compared with other compound classes in forested soils were found by

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Rumpel & Kögel-Knabner (2002) and Nierop & Verstraten (2003). In pyrolysates of solvent-insoluble residues from Rothamsted Experimental Station, van Bergen *et al.* (1997) did not find signals for the lignin of the vegetation growing on these soils and concluded that the lignin must be rapidly altered diagenetically, which was supported by a later paper, where they found that the lignin signature is mainly retained in the humic layer and much less in mineral soils (van Bergen *et al.*, 1998). In addition to these studies, evidence has accumulated that stability against hydrolysis in the laboratory does not correlate with stability under natural conditions. As an example, Poirier *et al.* (2003) found only traces of lignin in the non-hydrolysable fraction of two soils, whereas the same fraction in maize leaves was mainly composed of lignin and condensed tannins.

Quantitative information on lignin decomposition rates is provided either by short-term (days to weeks) *in vitro* experiments (Freer & Detroy, 1982; Martin *et al.*, 1982; Martinez *et al.*, 1991; Steffen *et al.*, 2000) or by long-term litterbag studies performed mainly in forest ecosystems. In a boreal forest, Berg *et al.* (1982) reported 48% loss of the proximate lignin fraction after 5 years. Similarly, in Mediterranean forest stands, Rutigliano *et al.* (1996) observed between 60% and 87% lignin loss after 5 years. However, both *in vitro* studies and litterbags cannot fully reflect natural conditions.

In order to overcome this limitation, isotopic signatures can be used to trace the origin and fate of SOC in soils (Balesdent & Mariotti, 1996; Gerzabek *et al.*, 2001). Natural labelling exploits the isotopic difference between C4 plants (e.g. maize) and C3 plants (e.g. wheat); the latter have a mean $\delta^{13}\text{C}$ value of around -27‰ whereas C4 plants are isotopically heavier (around -12‰). This isotopic difference is maintained at both the bulk and molecular level during decomposition of plant biomass (Balesdent & Mariotti, 1996). When C4 vegetation replaces C3 vegetation, the new, isotopically heavier C4-derived carbon progressively replaces the old, decaying C3 carbon in SOC. Thus increasing $\delta^{13}\text{C}$ values are directly related to the proportion of the new, C4-derived biomass, and can be used to estimate the residence time and pool size of individual SOC components (Balesdent & Mariotti, 1996). This approach has been successfully applied to bulk soil organic carbon, and, at the molecular level, to solvent-extractable compounds such as soil lipids (Cayet & Lichtfouse, 2001; Wiesenberg *et al.*, 2004).

So far, there is only one study on lignin residence times in soils exploiting this approach in an arable soil (Dignac *et al.*, 2005). Rasse *et al.* (2006) subsequently developed a lignin turnover model using data from the same site. After 9 years of maize cropping, 47% of the original lignin had been replaced by maize-derived lignin, but only 9% of bulk SOC originated from maize. Gleixner *et al.* (2002) provided additional molecular-level information from a long-term agricultural trial with naturally labelled biomass. Combining analytical pyrolysis with isotopic information, these authors found almost no pyrolysis products that could be related to

lignin precursors and concluded that lignin must be severely biodegraded.

The natural labelling technique requires a change in vegetation, which is a potential source of error if the new vegetation differs from the previous vegetation in terms of carbon input into soil or degradability. In arable soils, a change from wheat to maize has mostly been employed and yielded satisfying results (Balesdent & Mariotti, 1996; Cayet & Lichtfouse, 2001; Wiesenberg *et al.*, 2004; Dignac *et al.*, 2005). Artificial labelling could extend the potential of the isotopic labelling technique. As it can produce an isotopic label without the need for a vegetation change, it can be applied to sites and ecosystems where no suitable pair of C3 and C4 species is available and reduces the potential error associated with a vegetation change. An artificial label is produced in fumigation experiments where plants take up ^{13}C -labelled CO_2 and form labelled biomass. This allows investigation of perennial cultures like grasslands that have not been investigated so far due to lack of a natural label. Here, we use compound specific isotope analysis (CSIA) on lignin oxidation products in order to determine lignin input into grasslands and an arable soil after labelling periods between 9 and 23 years and the stability of lignin in these soils. The purpose of this paper is: (i) to test the applicability of artificial labelling in order to provide data for permanent cultures like grassland where no natural labelling experiment is available; (ii) to compare the stability of individual lignin compounds with that of bulk soil organic carbon in order to provide data on lignin stability in different ecosystems; and (iii) to estimate preservation of lignin in soils on a decadal scale.

Materials and methods

Soil and plant samples

Boigneville site. The experimental field of the Institut Technique des Céréales et des Fourrages (ITCF) is located at Boigneville ($48^\circ 45'\text{N}$, $2^\circ 05'\text{E}$) in the Département of Essonne, France. This site was established in 1970 to compare different tillage practices (Balesdent *et al.*, 1990) under various crops. From 1970, there was continuous cropping of maize or wheat, respectively, on the subplots for 23 years. Before 1970, the entire field had been ploughed and sown to C3 crops, except for three maize crops in 1962, 1964 and 1966 (Balesdent *et al.*, 1990). The soil is a Typic Hapludalf (USDA) developed from loess. The mean annual temperature is 10.5°C , with monthly means of 3.0°C in January and 18.4°C in July, and a mean rainfall of 600 mm year^{-1} . Samples were taken in 1993 by the Institut National de Recherche Agronomique (INRA) from the 0–30 cm layer of conventionally tilled plots under wheat and maize. The sampling depth corresponds to the depth of the plough layer. One 10-kg sample was taken from the four side walls of one soil pit per plot (Puget *et al.*, 1995). Only soil samples were available from the Boigneville site. Isotope data for plant material had to be taken from the literature. For the isotopic difference

between bulk maize and wheat carbon in Boigneville a value of 13.8‰ was used, as published by Balesdent (1996). For individual phenols from wheat and maize lignin, the only available data are those of Dignac *et al.* (2005). The isotopic difference between maize and wheat lignin varies between the phenols, with the largest difference for syringaldehyde (isotopic difference $\delta_{\text{maize}} - \delta_{\text{wheat}} = 16.8\text{‰}$) and the smallest for syringic acid ($\delta_{\text{maize}} - \delta_{\text{wheat}} = 13.0\text{‰}$).

Derivatization reduces the isotopic difference by a factor of $n_{\text{underderiv}} / (n_{\text{underderiv}} + n_{\text{TMS}})$, where $n_{\text{underderiv}}$ is the number of C atoms in the underivatized phenol and n_{TMS} is the number of derivative C atoms added. This was taken into account individually for each compound. For our estimation of the new lignin fraction, we assumed that the isotopic difference between the end-members (wheat and maize plant lignin) is 16.8‰ for each underivatized phenol (according to the biggest difference found by Dignac *et al.* 2005). This introduces a certain error, but can be considered a conservative estimate. Consequently, for all monomers that show a smaller isotopic difference between the end-members, turnover is faster than estimated here.

Hohenheim site. The *Miscanthus giganteus* field in Hohenheim (48°43'N, 9°13'E, Germany) was planted in 1994 on a loamy Haplic Luvisol (FAO). The reference soil is a mixed pasture with various C3 grasses, *Trifolium*, *Taraxacum* and other herbs. The mean annual temperature is 8.8°C; mean annual precipitation is 698 mm year⁻¹. Soil samples (0–10 cm) were taken in autumn 2003 in the *Miscanthus* field and the reference grassland (2–3 kg from a single pit each). Leaves from the *Miscanthus* plant and from a mixture of the pasture plants were used to study the isotopic shift between the C3 and C4 vegetation.

Eschikon site. The experimental plot of the Institute of Plant Sciences of the Swiss Federal Institute of Technology (ETH) Zürich (Switzerland) is located at Eschikon (47°27'N, 8°41'E), near the Field Station, at an altitude of 550 m above sea level. The soil is a Eutric Cambisol (FAO). The mean annual temperature between 1978 and 1995 was 8.6°C, with monthly means of -0.7°C in January and 17.3°C in July, and a mean rainfall of 1138 mm year⁻¹. A detailed description of the free-air CO₂ enrichment experiment can be found in Hebeisen *et al.* (1997) and van Groeninge *et al.* (2002). Briefly, ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) were grown in CO₂-enriched (60 Pa CO₂, $\delta^{13}\text{C}$ approximately -18‰) and ambient atmosphere (35 Pa CO₂, $\delta^{13}\text{C}$ approximately -8‰), resulting in a different ¹³C label in the plant biomass. Soil samples (0–10 cm) were taken at the end of the experimental period (1993–2002) in duplicate from each of the three replicate plots per treatment and plant species, and pooled to yield one sample per treatment and plant species (total of four soil samples). The respective plant samples were harvested during the 1998 vegetation period and pooled from four cuttings to yield one plant sample per treatment and species (total of four

plant samples). Further information on the sites is given in Table 1.

Chemical analyses

Concentrations and isotopic composition of bulk organic carbon in soils were determined after carbonate removal by 10% HCl (by volume). Carbon concentrations were measured on a CHN analyser (Vario EL, Elementar Analysensysteme, Hanau, Germany). The isotopic composition of the bulk organic carbon 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).

Dried and milled soil and plant samples were subjected to the alkaline CuO oxidation procedure introduced by Hedges & Ertel (1982) in order to break down the lignin molecule into its monomers (Figure 1). We used microwave digestion as described in Goñi & Montgomery (2000), which we adapted slightly in our laboratory. All samples were oxidized at least in triplicate as follows. The microwave system used was an ETHOS EM-2 (1000 W nominal power; Egrolyt AG, Oberwil, Switzerland). Soil or plant samples equivalent to 2–5 mg C were weighed into vessels and oxidized with 500 mg of CuO powder, 50 mg of ferrous ammonium sulfate and 20 ml of 2 M NaOH solution in the microwave at 150°C for 90 minutes. After the vessels had cooled, an internal standard of 500 µl of a cinnamic acid + ethylvanillin mixture (50 mg litre⁻¹ each) was added to the samples. Humic acids were removed by precipitation after acidification of the solution to a pH of approximately 2 with concentrated HCl. The samples were collected on preconditioned (ethyl acetate, methanol, water) C-18 columns, from which they were eluted with 5 × 500 µl of ethyl acetate and dried under a stream of nitrogen. The dried sample was redissolved in 400 µl of a solution of anisic acid in ethylacetate (50 mg litre⁻¹).

In each solution, the individual lignin monomers were quantified in triplicate after derivatization of 70 µl of sample with 70 µl of BSTFA/TMCS 99:1 derivatizing agent (Fluka, Buchs, Switzerland) for 15 minutes at 60°C. Analysis was performed by gas chromatography–mass spectrometry (GC-MS; HP 6890 N Plus gas chromatograph, connected to 5973 N MSD detector, Agilent Technologies, Palo Alto, USA; column, HP 5 MS (Agilent Technologies), length 50 m, internal diameter 0.20 mm, film thickness 0.33 µm); temperature programme – start at 100°C, ramp to 160°C at 3°C minute⁻¹, hold for 5 minutes, ramp to 250°C at 3°C minute⁻¹, ramp to 320°C at 10°C minute⁻¹, hold for 10 minutes). Individual peak sizes were quantified using external calibration curves. The data were corrected for losses during sample preparation. For each sample, the averaged recovery of the internal standards ethylvanillin and cinnamic acid (typically 60–80%) was determined individually, and the raw data divided by the recovery. Compound specific isotope analyses (CSIA) (Goñi & Eglinton, 1996) were performed at least in duplicate for each solution. Samples were separated on

Table 1 Soils used in the study

Site	References	Labelling method	Time since start of labelling/ years	Climate	Location	pH ^a	Particle size distribution			C content /mg g ⁻¹	N content /mg g ⁻¹
							Silt /%	Clay /%			
Boigneville	#1 Balesdent <i>et al.</i> (1990)	Vegetation conversion C3 to C4 (maize)	23	MAT ^b : 10.5°C, MAP ^c : 600 mm (#1)	France, 48°45' N, 02°05' E	6.5 (#3)	68.7 (#2)	24.9 (#2)	Maize	1.0	
	#2 Puget <i>et al.</i> (1995)						9.8	Wheat			
	#3 Balesdent (1996)						11.6				
Hohenheim		Vegetation conversion C3 to C4 (<i>Miscanthus</i>)	9	MAT 1961–90: 8.8°C, MAP 1961–90: 698 mm ^d	Germany, 48°43' N, 09°13' E	5.6–5.7	<i>Miscanthus</i>	57	39	16.2	1.6
Eschikon	#4 Hebeisen <i>et al.</i> (1997)	Fumigation with labelled CO ₂	10	MAT 1978–95: 8.6°C, MAP 1978–95: 1138 mm (#4)	Switzerland, 47°27' N, 08°41' E	7.0–7.1 (#5)	63	33	24.2	2.6	
	#5 van Kessel <i>et al.</i> (2000)						33 (#4)	28 (#4)	24.0–28.7		
	#6 van Groeningen <i>et al.</i> (2002)										

^aDetermined in CaCl₂ (Hohenheim samples); for the other sites no information is given.

^bMAT, mean annual temperature.

^cMAP, mean annual precipitation.

^dClimate data were provided by the Institute of Physics and Meteorology at the University of Hohenheim.

a HP 6890 gas chromatograph (Palo Alto, USA) (column: RTX 5 MS (Restek Corporation, Bellefonte, USA), length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm; temperature programme as for GC-MS analysis), combusted at 850°C and the CO₂ was analysed in an isotope-ratio mass spectrometer (IRMS; Isoprime, Micromass (now GV Instruments), Manchester, UK) with Mass Lynx Inorganic software (version 4.0i). Combustion water was trapped by a liquid nitrogen water trap held at –110°C. We added C24 *n*-alkane to the samples as an internal standard for CSIA. The off-line δ¹³C value of this alkane from an elemental analyser (EA) coupled to an IRMS was used to correct for isotopic shift during analysis. An alkane was used for this purpose because it requires no derivatization, and thus the isotopic value determined by GC-C-IRMS can be directly compared with the value from EA-IRMS.

For calculation of the fraction of new carbon (F_{new}), only isotopic differences between labelled samples and their corresponding reference samples were used. The isotopic shift introduced during derivatization of the compounds is identical for the labelled sample and its corresponding reference sample. As it is eliminated when the difference is calculated, it was not necessary to correct the isotope data of the derivatized monomers for the isotopic composition of the trimethylsilyl carbon.

Calculating lignin residence time

As shown by Dignac *et al.* (2005), the complete isotopic mass balance to calculate the proportion of carbon derived from labelled plants (i.e. C4 or artificially labelled plants) in the labelled soil can be written as

$$F_{\text{new}} = \frac{OC_{\text{new,L}}}{OC_{\text{L}}} = \frac{(\delta_{\text{L}} - \delta_{\text{C}})}{(\delta_{\text{new,L}} - \delta_{\text{new,C}})} - \frac{OC_{\text{L}} - OC_{\text{C}}}{OC_{\text{L}}} \cdot \frac{(\delta_{\text{new,C}} - \delta_{\text{C}})}{(\delta_{\text{new,L}} - \delta_{\text{new,C}})}, \quad (1)$$

where $OC_{\text{new,L}}$ is the new organic carbon in the labelled plot, OC_{L} is the total organic carbon in the labelled plot, OC_{C} is the total organic carbon in the control plot, δ_{L} is the delta value of soil organic matter in soil under labelled vegetation (‰ V-PDB, where V-PDB is the Vienna Pee Dee Belemnite reference standard), δ_{C} is the delta value of organic matter in control soil (‰ V-PDB), $\delta_{\text{new,L}}$ is the delta value of new organic matter from labelled vegetation (‰ V-PDB), and $\delta_{\text{new,C}}$ is the delta value of new organic matter from control vegetation (‰ V-PDB).

If there is no change in soil organic carbon stocks after the vegetation change (i.e. the system is at steady state), ($OC_{\text{L}} - OC_{\text{C}}$) is zero, and Equation (1) reduces to

$$F_{\text{new,C}} = \frac{OC_{\text{new,L}}}{OC_{\text{L}}} = \frac{(\delta_{\text{L}} - \delta_{\text{C}})}{(\delta_{\text{new,L}} - \delta_{\text{new,C}})}. \quad (2)$$

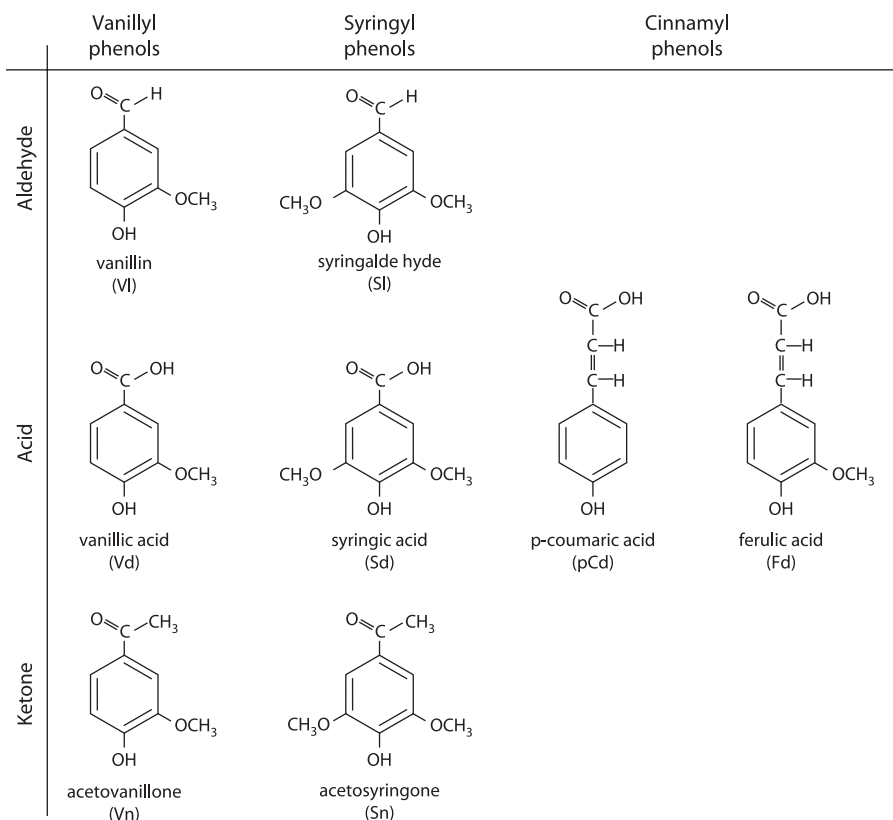


Figure 1 Lignin monomers produced by CuO oxidation.

Equation (2) is normally used for calculating SOC turnover (Balesdent & Mariotti, 1996). It has also been applied to determine the proportion of new lignin monomers in a soil by replacing bulk organic carbon concentrations with those of individual compounds (Dignac *et al.*, 2005).

The term $(\delta_{\text{new,L}} - \delta_{\text{new,C}})$ cannot be determined directly. As an estimate, we used the isotopic difference between both plant materials. Dignac *et al.* (2005) pointed out that this does not mean that we assume that there is no isotopic discrimination during biodegradation or sample preparation. It is, however, assumed that such discrimination is the same for both labelled and control plant material. This is a reasonable assumption for bulk materials with similar biochemical composition, and even more for individual molecules like the lignin monomers, which are chemically identical in both plants, except for carbon isotopy.

For the Boigneville experiment, no plant samples were available. Isotopic difference between bulk maize and wheat plants was assumed to be 13.8‰, in accordance with Gleixner *et al.* (2002), who worked with samples from the same site.

SOC decomposition is assumed to follow first order kinetics. When a system is at steady state, i.e. the carbon content (lignin

content in our case) is constant, the percentage of new carbon can be translated into turnover times, defined as the inverse of the decay constant, k (Balesdent *et al.*, 1990):

$$T = 1/k = -t/(\ln(1 - F_{\text{new}})), \quad (3)$$

where T is the turnover time (years), k is the decay constant (year^{-1}), t is the time since start of labelling (years), and F_{new} is the fraction of new carbon in soil (g g^{-1}).

Propagation of errors

Uncertainty (e) of the fraction F_{new} depends on the error of $\delta^{13}\text{C}$ measurements made on the sample from the labelled soil (δ_{L}) and from the C3 soil (δ_{C}), as well as on the error of the $\delta^{13}\text{C}$ measurements made on the end members, i.e. the C3 and C4 plants. We used the error propagation formula (Equation 4) to calculate errors for the isotopic differences between plant pairs and soil pairs in a first step, and errors for the proportion of new carbon in a second step,

$$e_y = \sqrt{\left(\frac{\partial y}{\partial a}\right)^2 e_a^2 + \left(\frac{\partial y}{\partial b}\right)^2 e_b^2}, \quad (4)$$

where y is the dependent variable, a and b are independent variables, e_y is the error of y , and e_a , e_b are the errors of a and b , respectively.

When estimating uncertainty for the calculated residence times (T), we used the upper and lower limit of the error interval of F_{new} to calculate minimum and maximum residence times in order to account for the logarithmic scale.

Results and discussion

Suitability of the sites for turnover time calculations

The calculation of the percentage of new carbon and of turnover times from carbon isotope data relies on several requirements:

- 1 There has to be a reference plot that had the same history as the labelled plot before the start of the experiment, and has not experienced a vegetation change since then.
- 2 Crops on the reference plot and the labelled plot must be biochemically similar.
- 3 Carbon stocks in the soil should have reached a steady state situation.
- 4 Isotopic compositions, and thus the isotopic differences between both end-members (i.e. the sources of carbon input into soil) are known.

The number of field sites fulfilling all of these requirements is very limited worldwide. If some of these conditions are not completely met, the calculated absolute turnover time value has a certain error. This error can be estimated for each site individually (below). It has to be stated, however, that for the purpose of the present study, *relative* differences between turnover times of lignin and bulk SOC are more important than the *absolute* turnover times.

For the Boigneville field site, 3 years of maize cropping were documented in the 1960s before the beginning of the experiment (Balesdent *et al.*, 1990). This cropping increased the ^{13}C content of the later maize and wheat fields in the same way. As the extent of this increase is known from the isotope data of the reference plot, it is taken into account in the calculation and will not affect the results. The Boigneville field experiment has been terminated and no more plant samples were available, so we had to rely on literature data that have been published for this site in order to get information on the isotopic composition of the soil carbon input. This is not an ideal situation, but as the literature data are from plants growing on exactly the same site, the potential error is minimized.

For individual phenols from wheat and maize lignin, we used a conservative estimate of the isotopic difference between wheat and maize, as explained above. Consequently, for all monomers that show a smaller isotopic difference between the end-members, F_{new} is greater and the apparent turnover is faster than estimated.

The history of the Hohenheim site is less well known, but the smaller carbon content of the *Miscanthus* soil indicates that the

two plots had a different history before the experiment. This introduces some uncertainty about the starting conditions into the calculation, and the apparent turnover times calculated for the Hohenheim site might be the least precise in absolute terms, but still valid for the purpose of comparing lignin relative to bulk SOC turnover.

Although winter wheat had been grown on part of the Eschikon field the year before the start of the experiment, and grass/white clover on the other part, the block design accounted for these differences, so that each fumigated subplot had a reference subplot with the same history (Hebeisen *et al.*, 1997).

The most important advantage of the Eschikon site is that fumigation with ^{13}C -depleted CO_2 allowed isotopic labelling of vegetation and soil organic matter without a vegetation change. This excludes any errors associated with a vegetation change, such as variations in organic matter input or in degradability. The additional CO_2 in the fumigated subplots did not lead to a measurable accumulation of carbon in the soil (van Groeningen *et al.*, 2002), so that steady state requirements were fulfilled.

Isotopic composition and residence time of bulk soil organic carbon

Table 2 shows bulk isotopic data of SOC and above-ground plant parts of the three experimental sites. The C3 grassland soils were more depleted in ^{13}C (-26.9 to -28.1‰) than the wheat soil (-26.1‰), which could be either due to input of C4 (maize) carbon to the wheat soil before 1970 (Balesdent *et al.*, 1990) or to slight differences in isotopic composition between wheat plants and pasture species. Balesdent *et al.* (1990) report a $\delta^{13}\text{C}$ value for wheat straw at Boigneville of -26.3‰ , whereas the values for the pasture plants varied between -27.2‰ and -28.3‰ (Table 2). The input of C4 carbon to the soils increased the $\delta^{13}\text{C}$ values to -21.1‰ and -22.5‰ in arable soil and the *Miscanthus* soil, respectively. Input of ^{13}C -depleted biomass in the Eschikon experiment reduced bulk $\delta^{13}\text{C}$ values of the SOC to -29.8‰ (clover) and -30.8‰ (ryegrass).

Following Equation (2), these isotopic differences translate into fractions of 32–34% of new organic C in the grassland soil after 10 years, 37% in the *Miscanthus* soil after 9 years, and 36% in the arable soil after 23 years. The data for the arable soil are similar to results reported earlier (41% by Cayet & Lichtfouse, 2001; 44% by Puget *et al.*, 1995).

The *Miscanthus* soil differs in C content from its control site, which indicates that the second term in Equation (1) cannot be neglected. We therefore estimated the contribution of the second term to the calculation of F_{new} . The expression $(\delta_{\text{new,C}} - \delta_{\text{C}})$ describes the isotopic difference between new organic matter and soil organic matter in the control plot, i.e. a potential discrimination during decomposition. The extent of this discrimination is unknown, but in an approximation can be assumed to be identical to the difference between

Table 2 Carbon and lignin characteristics of the soils used in this study (standard errors in parentheses)

	$\delta^{13}\text{C}$ vegetation ‰	$\delta^{13}\text{C}$ soils ‰	Fraction of new C g g^{-1}	Cinnamyl units mg (kg soil)^{-1}	Syringyl units mg (kg soil)^{-1}	Vanillyl units mg (kg soil)^{-1}	VSC ^a mg (kg soil)^{-1}	VSC/C _{org} $\text{mg (g C}_{\text{org}})^{-1}$	(ac/al) _S g g^{-1}	(ac/al) _V g g^{-1}	S/V
<i>Arable soil</i>											
Boigneville											
Wheat	NA ^b	-26.14 (0.04)		27 (4)	138 (5)	138 (9)	303 (11)	26 (1.2)	0.57	0.72	1.0
Maize	NA	-21.11 (0.04)	0.36 ^c	32 (5)	135 (11)	132 (16)	299 (20)	31 (2.0)	0.89	0.60	1.0
<i>Grassland sites</i>											
Hohenheim											
Pasture		-27.24 (0.06)		111 (12)	341 (23)	337 (22)	789 (34)	32	0.45	0.55	1.0
<i>Miscanthus</i>		-11.85 (0.05)	0.37	144 (16)	301 (17)	342 (22)	787 (33)	49	0.49	0.50	0.9
Eschikon											
Ryegrass control		-28.25 (0.03)		133 (14)	339 (16)	334 (17)	806 (27)	31	0.48	0.44	1.0
Ryegrass labelled		-38.00 (0.05)	0.34	169 (31)	388 (31)	347 (34)	904 (55)	34	0.38	0.34	1.1
Eschikon											
Clover control		-27.43 (0.03)		73 (13)	219 (19)	343 (19)	636 (30)	25	0.45	0.47	0.6
Clover labelled		-36.26 (0.04)	0.32	85 (18)	232 (21)	346 (18)	663 (33)	30	0.41	0.41	0.7

^aVSC, sum of vanillyl, syringyl and cinnamyl units; C_{org}, organic carbon; (ac/al), acid/aldehyde ratio; S, syringyl units; V, vanillyl units.
^bNA, not available.

^cAn isotopic difference of the end-members of 13.8‰ was used to calculate the fraction of new C (Gleixner *et al.*, 2002).

above-ground biomass (-27.24‰) and soil organic matter (-28.13‰) on this plot.

This correction increases the calculated fraction of new bulk C from 37 to 39% (a relative increase of 7.8%). The lignin concentrations in the two soils differ less than the bulk C concentration. Consequently, the effects of the correction are smaller for lignin than for bulk C (< 5%, Table 3).

From the proportion of new, labelled organic carbon we can calculate the mean residence time of organic carbon in that soil, if the requirements listed above are met. These data are shown in Figure 2, together with mean residence times of the lignin monomers.

Lignin yields, isotopic composition and apparent turnover of lignin monomers compared with bulk SOC

The sum of vanillyl-, syringyl- and cinnamyl-type (VSC) monomers for each soil is given in Table 2. The values range from 25 to 49 mg g^{-1} organic carbon and are slightly larger than values found by other authors in arable soils (Lobe *et al.*, 2002; Kiem & Kögel-Knabner, 2003) and grasslands (Amelung *et al.*, 1999). This may be partly caused by differences in the quantification method. Kiem & Kögel-Knabner (2003) state an average recovery of 70% of their internal standard, but did not correct for losses. Consequently, if their data are corrected for 70% recovery, their soils contain on average 29.8 mg VSC g^{-1} organic carbon, which compares well with our data. Most authors, unfortunately, do not state if recovery was taken into account.

Acid/aldehyde ratios of vanillyl and syringyl units (Table 2) are indicators of the degree of lignin alteration and usually increase with increasing decomposition (Kögel, 1986). In the grassland sites, this ratio ranged from 0.34 to 0.55 for vanillyl units and 0.38–0.49 for syringyl units. This is within the range found by Amelung *et al.* (1999) for North American prairie soils. Values for the arable soil are slightly greater (0.60 and 0.72 for the vanillyl units and 0.57 and 0.89 for syringyl units), which is close to the values reported by Dignac *et al.* (2005) in a soil under similar climate and bearing the same crops. In comparison with other arable soils, for which smaller acid/aldehyde ratios were reported, e.g. Lobe *et al.* (2002) and Kiem & Kögel-Knabner (2003), lignin seems to be in a slightly more oxidized state. Syringyl/vanillyl (S/V) ratios (Table 2) reflect preferential degradation of syringyl moieties (Kögel, 1986) and lie between 0.9 and 1.1, except for the *Trifolium* soil at Eschikon (0.6 and 0.7), which was probably due to the smaller proportion of syringyl phenols in the *Trifolium* plants (data not shown). All of these values are within the range reported by other authors, e.g. 0.53–1.45 for prairie soils (Amelung *et al.*, 1999), or 0.77–1.45 (Lobe *et al.*, 2002) and 1.1–2.0 (Kiem & Kögel-Knabner, 2003) for various arable soils. From these comparisons, we draw the conclusion that the degree of lignin decomposition in our soils is representative for these types of land use.

Table 3 Effect of correction for variable C and monomer concentrations at the Hohenheim site on calculated fraction of new C. Abbreviations for the monomers as in Figure 1

	Fd	pCd	Sd	Sl	Sn	Vd	Vl	Vn	Bulk organic matter
F_{new} calculated by									
Equation 2	0.66	0.83	0.49	0.58	0.45	0.44	0.48	0.47	0.37
Equation 1	0.69	0.79	0.50	0.58	0.46	0.44	0.48	0.47	0.39
Absolute difference	0.023	-0.041	0.012	0.005	0.011	-0.005	0.000	0.001	0.029
Relative difference/%	3.5	-5.0	2.5	0.8	2.4	-1.2	0.1	0.1	7.8

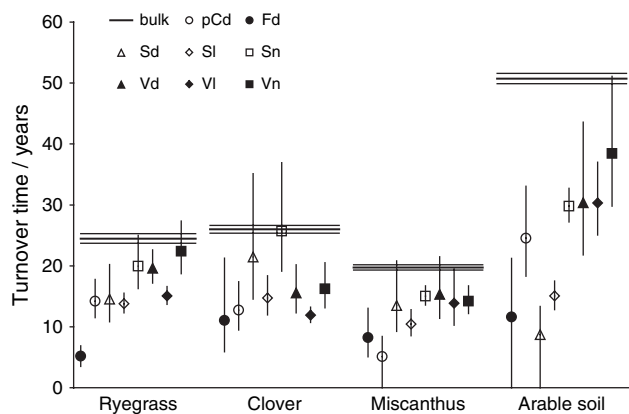
Determination of the absolute isotopic composition of lignin monomers is complicated by the fact that derivatization of the phenols adds additional carbons from the derivatization reagent to the molecule and only the isotopic composition of the derivative can be determined after gas-chromatographic separation. However, the calculation of turnover times is based on the isotopic differences between treatments and controls. As the isotopic shift caused by derivatization depends only on the type of phenol but not on its origin, it is identical for a phenol from a control sample and a sample originating from any of the treatments. Thus, an isotopic shift introduced by derivatization will affect neither the isotopic difference nor the calculated apparent turnover times. For each of the lignin monomers, apparent turnover times can thus be calculated in the same way as for bulk carbon (Figure 2). Thus, lignin turnover can be compared with bulk SOC turnover for each site individually. The apparent lignin turnover times are generally shorter than for bulk SOC, and range from 5 to 26 years in grasslands. Similarly, Wiesenberg *et al.* (2004) recently reported relatively fast turnover times for *n*-alkanes in soil, which had also been assumed to be rather resistant against degradation (Huang *et al.*, 1999).

The differences in sampling depth and the sampling strategy do not allow direct comparison of turnover times between the

different sites on a statistical basis. In particular, the longer apparent turnover times that are calculated for lignin monomers (< 9–38 years) and bulk SOC (51 years) in the arable soil may be attributable to the difference in sampling depth and the ploughing of the soil, which dilutes new SOC at the top of the soil profile with older SOC from lower parts.

Additionally, the data presented here do not claim to be valid throughout the entire soil profile. They can only describe the lignin dynamics in the topsoil horizon investigated. Depending on the rooting depth of the different species, subsoil horizons might experience substantially different carbon inputs and different rates of turnover.

However, the analysis of four isotopic labelling experiments on three completely independent sites yielded one common result: at all three sites, we observed a stronger incorporation of the isotope label into the lignin fraction than into the bulk soil, which implies that lignin turns over faster than bulk SOC in all soil horizons studied, independent of the labelling technique (natural through C3-C4 shift, or artificial through fumigation with ^{13}C -labelled CO_2). Together with the data reported by Dignac *et al.* (2005), our results suggest that relatively rapid lignin turnover in temperate soils may be widespread and irrespective of the land use types investigated so far. Other types of land use (e.g. forests) and climatic conditions, however, remain to be tested.

**Figure 2** Turnover times of lignin monomers as compared with bulk SOC. Monomers were determined in three laboratory replicates and at least two isotope determinations per replicate. Error bars for monomers and thin lines for bulk SOC represent standard errors. Abbreviations as in Figure 1.

Limitations

It must be considered here that the calculation of turnover times by following the gradual replacement of one isotopic label by another inherently assumes that any substance that can no longer be found analytically, has been decomposed. While this is correct when bulk SOC is analysed (except for a presumably negligible error caused by leaching of dissolved organic carbon), it only provides apparent turnover times when specific compounds, like lignin monomers, are analysed. This is caused by the fact that decomposition processes could alter the lignin molecule in such a way that the analytical procedure no longer detects the typical lignin monomers. With the present method, it is not possible to discern between lignin that has been altered in such a way and lignin that has been completely mineralized to CO_2 . Therefore, the calculated residence times refer to unaltered lignin molecules and thus provide a minimum turnover

time of lignin in soils. The residence time of modified lignin-derived C in soils could be substantially longer. This hypothesis, however, can neither be proved nor be disproved by the present method.

Despite these limitations, our finding that apparent turnover of lignin in soil is faster than that of bulk SOC agrees with earlier circumstantial evidence that lignin is not part of the refractory carbon pool (Kiem & Kögel-Knabner, 2003) and may disappear rapidly from some soils (van Bergen *et al.*, 1997; Nierop & Verstraten, 2003). Consequently, lignin can be regarded as a good example of the uncoupling between resistance of organic molecules to laboratory hydrolysis and their resistance against degradation in the field as observed in various studies (Poirier *et al.*, 2002, 2003; Nierop & Verstraten, 2003; Plante *et al.*, 2004) and postulated to be generally valid (Poirier *et al.*, 2006).

Conclusions

The present study provides the first *in situ* measurement of lignin residence times in soils under different land uses: an arable soil and two permanent cultures (*Miscanthus* plantation and grassland).

1 Two different labelling approaches were used (C3-C4 conversion, and artificial labelling), which yielded very similar results. This indicates that the vegetation changes that are inevitably associated with the C3-C4 conversion, do not substantially affect the result of the turnover time calculation.

2 The residence time of lignin structures, as detectable by the alkaline CuO oxidation method, is shorter than for bulk SOC in soils. This supports earlier work (see text), which suggested that selective preservation of intact lignin does not contribute significantly to the inert pool of stable organic carbon in soils. On the other hand, we demonstrated that even 23 years after a vegetation change, more than 60% of lignin in an arable soil derives from the previous vegetation. Lignin therefore seems to contribute to the slow or intermediate pool in carbon turnover models. With the method employed here, however, we cannot discern if lignin that is no longer found in the soil has been mineralized to CO₂, or has been transformed into more recalcitrant degradation products that are no longer detectable.

3 Neither lignin nor *n*-alkanes or *n*-carboxylic acids contribute to the inert soil organic carbon pool. There is increasing evidence that chemical recalcitrance of primary organic matter, such as lignin or lipids, does not significantly contribute to the stabilization of organic carbon in soils.

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