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Changes in litter chemistry and soil lignin signature during decomposition and stabilisation of ^{13}C labelled wheat roots in three subsoil horizons

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ABSTRACT

Despite their importance for C sequestration, especially in the subsoil, little is known about decomposition and stabilisation processes affecting root litter in soil horizons at different depths. In particular the influence of specific conditions at depth on molecular alterations of degrading root litter is unknown. We took advantage of a decomposition experiment, which was carried out at different soil depths under field conditions and sampled litterbags with ^{13}C-labelled wheat roots, incubated in subsoil horizons at 30, 60 and 90 cm depth for up to 36 months. Changes of bulk root chemistry were studied by solid-state ^{13}C NMR spectroscopy, and lignin content and composition was assessed after CuO oxidation. Compound-specific isotope analysis allowed assessment of the role of root lignin for soil C storage at the different soil depths. Results indicated that decomposition proceeded in a similar way at all three depths, but at a different rate. The alkyl/O-alkyl C ratio was a meaningful indicator to assess the degree of root litter degradation within the mineral soil. After three years, the greatest increase of this ratio, corresponding to the most advanced degradation degree, occurred at 30 cm compared to the lower depths despite a similar carbon loss. The greater proportion of O-alkyl C persisting in deeper subsoil horizons was consistent with their higher clay content. Root derived lignin-C concentration decreased at all soil depths and soil lignin content reached a similar level after 12 months, suggesting that microbial communities in all subsoil depths had capability to degrade lignin. However, the intensity of degradation appeared to be different at different soil depths, with lignin being less transformed at 60 and 90 cm depth. We conclude that chemistry of subsoil organic matter is determined by horizon-specific conditions, which have to be fully understood in order to explain the long residence times of subsoil C. In our study physico-chemical parameters only partly explained the observations.

1. Introduction

Considering the source/sink function of soils for atmospheric CO_2, the fate of root litter and its influence on soil organic matter (SOM) formation throughout the soil profile is of great interest (Rasse et al., 2005). The evaluation of root litter contribution to stable SOM in the soil profile is necessary because root carbon input and turnover could be affected by land-use (Carter and Gregorich, 2010; Post and Kwon, 2000; Chang et al., 2012) and climate change (Sanaullah et al., 2012). Despite the importance of organic matter in subsoils for carbon storage, the decomposition and stabilisation pathways of root carbon are unknown (Rumpel and Kögel-Knabner, 2011). Within a soil profile, root litter decomposition and stabilisation was thought to be affected by soil abiotic (e.g. oxygen level, moisture) and biotic conditions (e.g. microbial biomass, diversity and community structure) (Salomé et al., 2010),

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which vary with depth. With increasing soil depth, a reduction of microbial biomass, activity, and diversity, and changes in microbial community structure were reported (Blume et al., 2002; Taylor et al., 2002; Eilers et al., 2012). However, oxidative enzyme activities could even increase with soils depth (Kramer et al., 2013).

During a 3 year field experiment with ^{13}C labelled root litter, quantitative differences in SOM decomposition and stabilisation processes with depth were significant but small (Sanaullah et al., 2011). For the present study we hypothesised that the contrasting conditions in different subsoil horizons may have an effect on the quality of the decomposition residue. In particular, lack of oxygen and easily degradable compounds, prerequisites for the co-metabolic degradation of structural plant litter compounds, may contribute to their preferential accumulation in subsoil. The accumulation of these compounds in subsoil horizons could contribute to long-term carbon storage and thus carbon sequestration (Kell, 2012).

We took advantage of a litterbag experiment with ^{13}C labelled wheat roots which were kept in subsoil at three different depths under temperate grassland for up to three years. Via measurement of the ^{13}C label, we were able to analyse changes over time in (1) bulk chemical composition by solid-state ^{13}C nuclear magnetic resonance spectroscopy and (2) quantity and composition of soil and wheat root lignin by cupric oxide (CuO) oxidation and compound-specific isotope analysis of lignin-derived phenols. The aim of the study was to examine if chemical changes during decomposition of root litter are dependent on specific physico-chemical parameters of different soil horizons, and lead to SOM of contrasting quality.

2. Material and methods

2.1. Experimental set up

The field experiment was set up at Lusignan (France) on a Cambisol with loamy texture (Table 1) (Chabbi et al., 2009) and grassland vegetation. Briefly, litterbags containing root material and soil were prepared for each of three different soil depths. Soil cores were taken by an auger sampler (Ø 7.5 cm) from 30, 60 and 90 cm depth, respectively. All three depths correspond to well defined pedogenic horizons, i.e. A (ploughing horizon), B1 (brunification horizon), B2 (horizon containing red clay) with different physico-chemical properties (Table 1). Labelled wheat had been grown hydroponically in a growth chamber under 2 atom% $^{13}C{-}CO_2$ atmosphere for 16 weeks. After harvest, the roots had been dried at 40 °C before they were cut into 1 cm pieces (initial wheat roots). One hundred gram of soil from each soil depth were mixed with 2 g of ^{13}C labelled wheat roots. The soil root mixture was put in 10 × 10 cm litter bags (mesh size 100 μm). Those were placed at the different soil depths before the original soil core was used to cover them.

We excavated the litterbags after 6, 12, 20, 29 and 36 months of incubation. The soil root mixture was air-dried and ground for the characterization of the bulk SOM and lignin composition.

2.2. Solid-state ^{13}C-NMR analyses

Solid-state ^{13}C NMR spectroscopy was performed on initial wheat roots and soil root mixtures after 6, 20 and 36 months of incubation at the different depths. All three soil root mixture replicates of one depth were pooled. Non-replicated analysis is generally used in NMR spectroscopy due to the low sensitivity of the technique. Replicates generally yield the same quantitative results because they represent the accumulation of several hundred or thousands of signals. The samples were pre-treated with 10% hydrofluoric acid (HF) to remove paramagnetic minerals and to enrich organic C in order to ensure better resolution of the NMR spectra (Rumpel et al., 2006).

Solid-state ^{13}C-NMR analyses were performed on a Bruker DSX 200 NMR spectrometer (Germany) operating at a ^{13}C resonance frequency of 50.3 MHz. Samples were packed in a cylindrical zirconia rotor and spun at 6.8 kHz with a contact time of the standard cross-polarisation (CP) pulse sequence of 1 ms. Recycle delays were 2 s (wheat) and 0.8 s (soil root mixtures), respectively. For the wheat spectrum a total of 400 scans and for soil root mixture samples 1000–60,000 scans were collected. We applied a line broadening of 50 Hz (wheat) and 50–75 Hz (soil root mixtures). The chemical shift was externally referenced to the methyl resonance of tetramethylsilan at 0 ppm. The spectra were integrated across four chemical shift regions assigned to the C-types mainly representative for that region: carboxyl (220-160 ppm), aryl (160-110 ppm), O-alkyl (110-45 ppm), alkyl (45- −10 ppm).

2.3. CuO oxidation

Phenol monomers from lignin were released from soil and wheat roots by alkaline CuO oxidation (Hedges and Ertel, 1982; Kögel and Bochter, 1985) using 500 mg soil root mixtures and 50 mg wheat roots. Ethylvanillin was added as internal standard before precipitation of humic acids. After purification by sorption onto C_{18}-columns (1 g; Isolute®, International Sorbent Technology, UK) and drying under N_2, monophenols were eluted by ethyl acetate. Dried samples were taken up in pyridine and the internal standard phenylacetic acid. Monophenols were derivatised by BSTFA (N,O-Bis(trimethylsilyl)trifluoroacetamide) and quantified using a Hewlett Packard gas chromatograph (HP GC 6890) equipped with a flame ionisation detector (GC-FID) and a SGE BPX-5 column (65.0 m × 320 μm ID, 0.25 μm film thickness, SGE, Australia). Helium was used as carrier gas. Temperature specifications were as follows: injector and detector of 350 °C, temperature program: 100 °C hold for 2 min, 8 °C min^{-1} to 172 °C, 4 °C min^{-1} to 184 °C, 10 °C min^{-1} to 310 °C, hold for 5 min.

Monophenol concentration was calculated from monophenol standard mixes containing vanillyl (V), syringyl (S) and cinnamyl (C) −type lignin units. V-units were represented by vanillin, acetovanillone, vanillic acid, S-units by syringaldehyde, acetosyringone, syringic acid, and C-units by p-coumaric acid and ferulic acid. The sum of V-, S- and C-type units (VSC) is considered to

Table 1
Characteristics of the Cambisol of the present experiment (data from Sanaullah et al., 2011).

Depth	pH	Carbon content[b]			Labelled C remaining	Sand	Silt	Clay	Total oxides (DCB)[a] (g kg^{-1})
	(H_2O)	Before (g kg^{-1})	Start (g kg^{-1})	End (g kg^{-1})	% of initial	(%)	(%)	(%)	
30 cm	6.3	8.7 ± 1.5	14.1	9.6 ± 0.6	30.7 ± 4.6	10.5	72.7	16.8	25.8
60 cm	7.6	3.5 ± 0.6	9.3	6.0 ± 0.3	44.8 ± 3.5	9.5	59.4	31.1	34.5
90 cm	8.0	3.2 ± 0.3	9.1	5.3 ± 0.3	37.6 ± 1.3	10.3	52.0	37.7	54.8

[a] After dithionith-citrate-bicarbonate (DCB) extraction.
[b] Carbon content: before (soil C before root litter addition); start (soil C after root litter addition at the beginning of the experiment); end (soil C after 36 months of incubation).

reflect total lignin content. As indicators for lignin degradation the acid to aldehyde mass ratios $(Ac/Al)_V$, $(Ac/Al)_S$ as well as the mass ratios of S/V and C/V were used (Hedges and Ertel, 1982).

2.4. Stable carbon isotope analysis

Isotopic composition was analysed in initial substrates and soil root mixtures after 36 months of incubation. For accurate determination of highly ^{13}C enriched monophenols, extracts were diluted 1:4 (wheat amended samples) or 1:10 (wheat itself) using natural abundance standard mixtures (Baumann et al., 2012). The diluted sample was analysed by gas chromatography/combustion/isotopic ratio mass spectrometry (GC/C/IRMS) using a Hewlett–Packard 5890 gas chromatograph coupled to an isotope ratio mass spectrometer (IRMS) (Isoprime, Micromass, UK) via a combustion interface (CuO, combustion reactor set to 850 °C). Separation of monophenols was performed using the same column as described for quantitative analysis. Temperature specifications were set to an inlet temperature of 260 °C and the temperature program 100 °C for 2 min, 5 °C min^{-1} to 250 °C, 15 °C min^{-1} to 350 °C, hold for 10 min.

GC/C/IRMS derived isotopic values were corrected for exogenous carbon addition during silylation reaction before calculating the amount of compound-C originating from wheat and soil, respectively (Dignac et al., 2005).

2.5. Statistical analyses

Normal data distribution was tested by the Kolmogorov–Smirnov test, the homogeneity of variances by the Levene test. Analyses of variance (ANOVA) with subsequent Tukey or least significant difference (LSD) tests were performed to analyse the effects of depth and time on lignin concentration using the statistical software R (R software 2008, version 2.13.1). Analysis of covariance (ANCOVA) was performed using Excelstat (XLSTAT Version 2013.4.03) to examine the relationship between lignin content and root C loss using depth as a covariate. Unless otherwise noted, significant differences refer to $P \leq 0.05$.

3. Results

3.1. Solid-state ^{13}C-NMR spectroscopy

Compared with the initial plant material, O-alkyl-C (40–110 ppm, Fig. 1) of wheat roots, which is most likely assigned to hemicelluloses and cellulose constituents (Kögel-Knabner, 1997), decreased during incubation in all three depths. The contribution of carboxyl-C (160–220 ppm) most likely representing proteins and carboxylic acids, aryl-C (110–160 ppm) with lignin specific signals as well as alkyl-C (0–45 ppm), which is most probably assigned to lipids and other aliphatic biomolecules (Baldock et al., 2004) increased. During incubation, similar changes in chemical composition were observed in all three soil depths, although at different intensity. The alkyl/O-alkyl ratio increased in all depths and this increase was most rapid for root material incubated at 30 cm. After 36 months, this ratio was highest at 30 cm depth compared with the other two depths, where similar values were found (Fig. 1). Likewise the aryl/O-alkyl ratio increased in all three depths. After 36 months of incubation, it was lowest at 90 cm depth and similar at 30 and 60 cm.

To investigate if changes during decomposition of root material are occurring in a similar manner in all three depths, we established relationships between NMR parameters and root carbon loss during incubation as an indicator of increasing state of decomposition

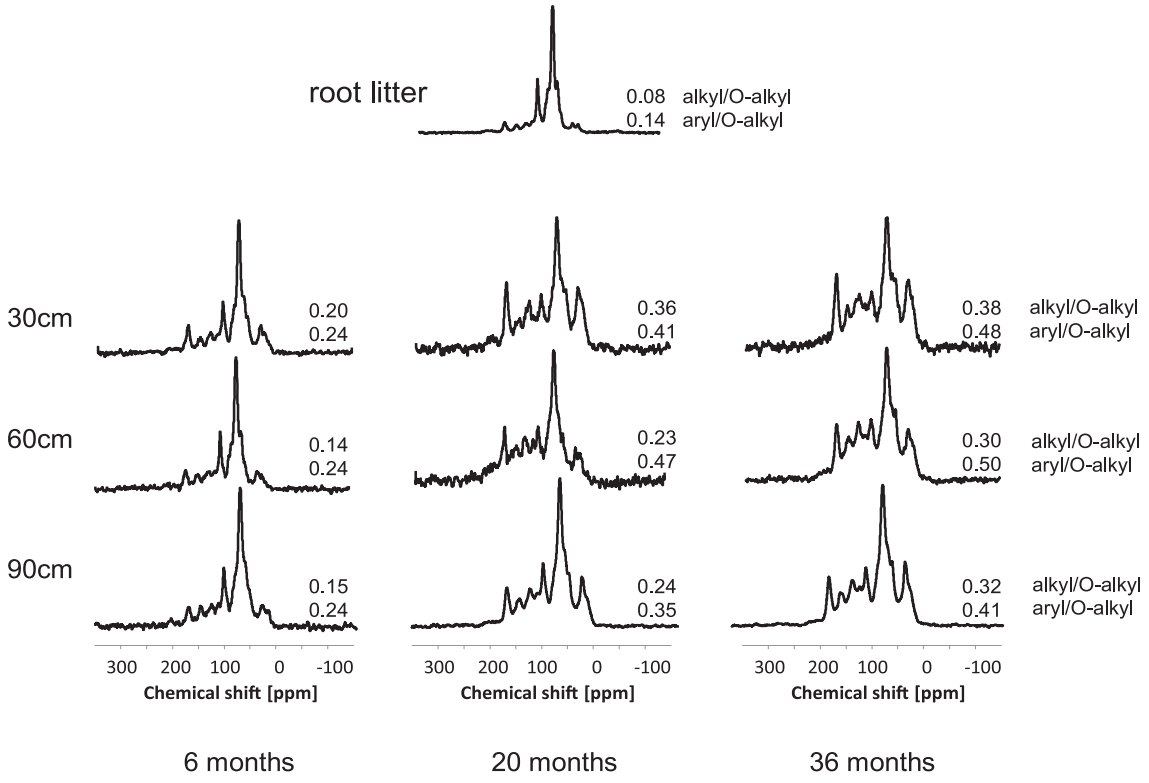

Fig. 1. Solid-state ^{13}C NMR spectra of initial wheat root-litter and soil root mixtures at different depths after 6, 20 and 36 months of incubation. Each spectrum was obtained from a mixture of three treatment replicates. C-type mainly representative for a chemical shift region: carboxyl (220-160 ppm), aryl (160-110 ppm), O-alkyl (110-45 ppm), alkyl (45-−10 ppm). Numbers give alkyl/O-alkyl (upper value) and aryl/O-alkyl ratio (lower value) of each spectrum.

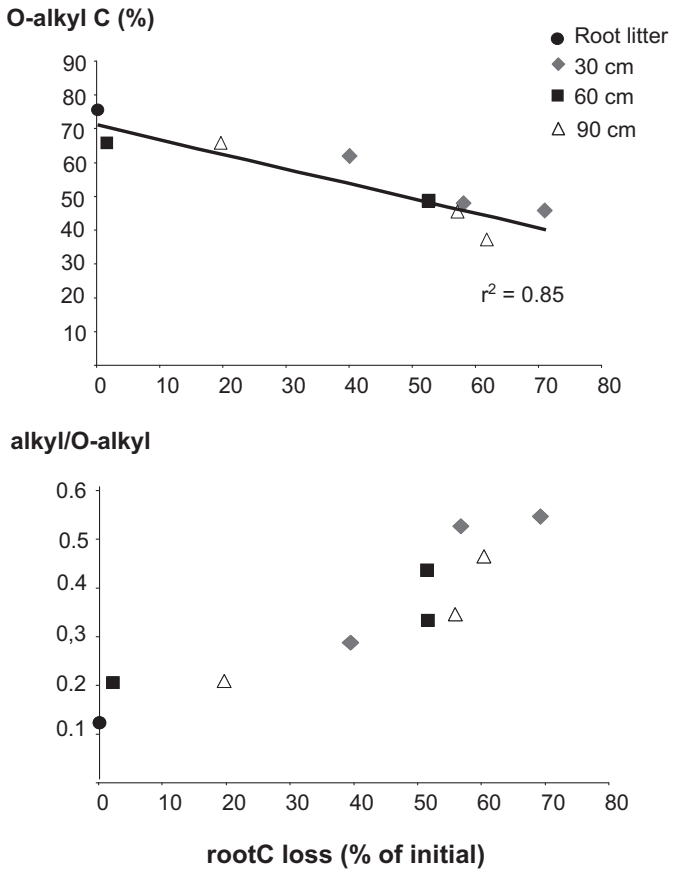

Fig. 2. Evolution of (a) O-alkyl-C and (b) alky/O-alkyl ratio of root litter at all three depths related to root-C loss.

(Fig. 2). The O-alkyl C contribution was linearly related to root carbon loss ($r^2 = 0.85$, Fig. 2A), whereas an exponential trend was observed with the alkyl/O-alkyl ratio (Fig. 2B).

3.2. Lignin

Total lignin-C concentration ranged between 70 and 20 μg lignin-C g^{-1} soil at month 0 before root litter addition (Fig. 3). This corresponded to 8 to 5 mg lignin-C g^{-1} soil C (data not shown). Six months after root litter addition, lignin content was significantly increased at all three soil depths without difference between them. After 20 months, lignin content stabilised on a lower level at all three depths, remaining significantly higher than initial soil lignin values (Fig. 3). Soil lignin content was correlated to root-C loss during the incubation (Fig. 4). ANCOVA analyses using depth as a covariate showed that correlations between root loss and lignin-C content were similar in 60 and 90 cm depths, whereas in 30 cm at similar root-C loss higher lignin contents were present in soil (Fig. 4). Lignin parameters representing its decomposition (S/V, C/V, $(Ac/Al)_V$ and $(Ac/Al)_S$) are presented as a function of time in Fig. 5. Unlike all other parameters the S/V ratio did not show any dynamics throughout the incubation experiment (Fig. 5A). The C/V ratio was highest at 60 and 90 cm and decreased during 36 months (Fig. 5B). Acid to aldehyde ratios of vanillyl and syringyl showed distinct dynamics with a decrease at 6–12 months after root litter addition and an increasing trend thereafter (Fig. 5C and D). Highest acid to aldehyde ratios and lowest C/V were recorded for material incubated at 30 cm depth throughout the incubation. Acid to aldehyde ratios of vanillyl and syringyl, which are usually used to assess the state of lignin decomposition, were strongly but differently related to each other in the three horizons (Fig. 6).

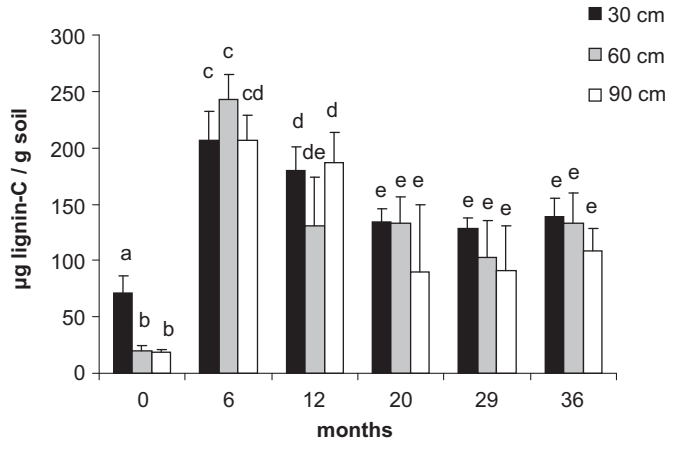

Fig. 3. Total soil lignin content in three soil depths during the incubation period. Data are presented as mean ± standard deviation ($n = 3$); different letters indicate significant differences; the data for month 0 represent soil lignin content before root litter addition.

After 36 months of incubation, the amount of wheat derived lignin-C remaining was highest at 60 cm depth (Table 2). Compared with the concentration of initial wheat derived lignin-C, 66% of it was left at 60 cm depth after 36 months of incubation. This was significantly more compared with the wheat derived lignin-C remaining at 30 cm and 90 cm (41% and 43%, respectively). Lignin parameters of wheat showed changes during incubation with preferential degradation of wheat derived cinnamyl lignin-units over wheat derived vanillyl units (Table 2); soil depth had no significant effect. S/V ratios of added wheat roots were not significantly influenced over time or by soil depth. During incubation, wheat derived vanillin decreased faster than vanillic acid leading to an $(Ac/Al)_V$ ratio, which was about twice as high as that of initial wheat; there was no influence of soil depth on the increase of this ratio. The $(Ac/Al)_S$ ratio of wheat lignin did not change during incubation and did not differ with soil depth.

4. Discussion

Our experimental setup included the addition of the same amount of labelled root material to all three depths. As a result, carbon content almost tripled at 90 cm depth, whereas it not even

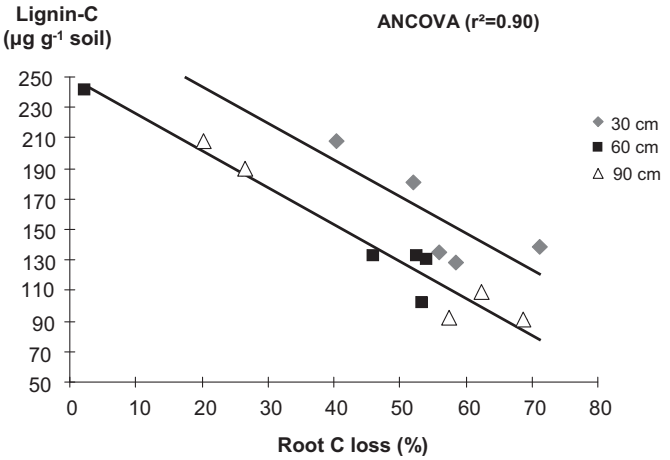

Fig. 4. Relationship between soil lignin-C content, root C loss and soil depth analysed by analysis of covariance (ANCOVA).

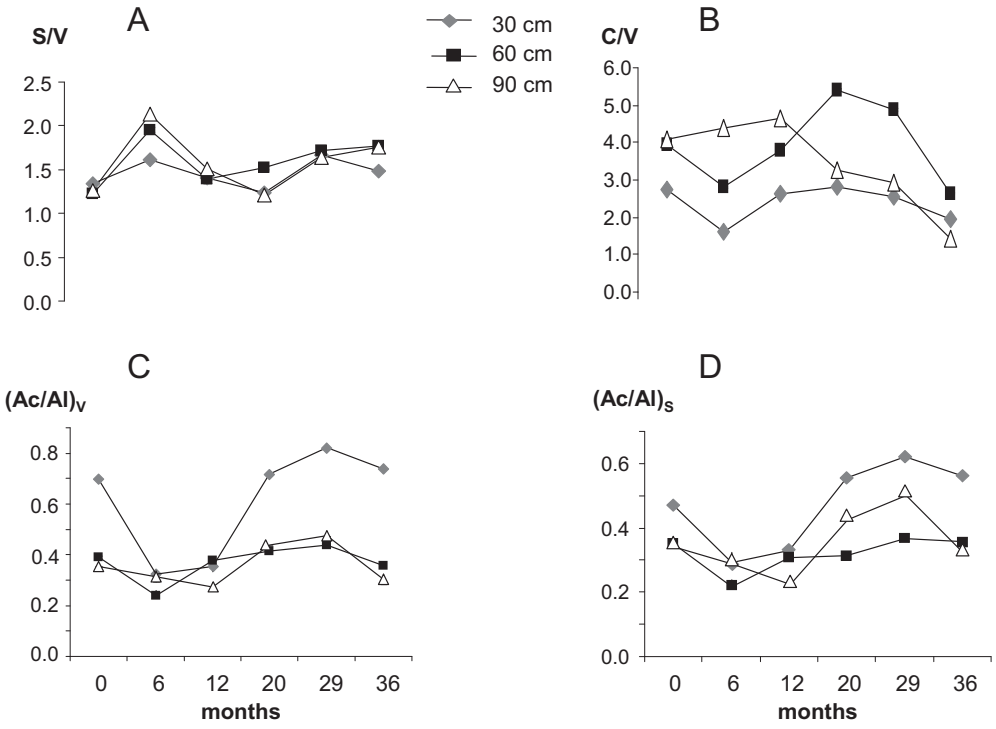

Fig. 5. Dynamics of lignin parameters during incubation. Panels represent syringyl to vanillyl (A), cinamyl to vanillyl (B), acid to aldehyde ratio of vanillyl (C) and acid to aldehyde ratio of syringyl (D).

doubled at 30 cm depth (Table 1). The proportions of added root carbon remaining after the experiment were within a similar range for all three depths (Table 1). This resulted in similar carbon contents at 30 and 90 cm depth but slightly increased C contents at 60 cm depth. Physico-chemical parameters such as clay and iron oxide content were quite different at all three soil depths. At 90 cm, highest concentrations of clay and iron oxides were found (Table 1). Due to the strong physico-chemical differences in the three depths, our results do not reflect the influence of depth only. We therefore investigated how the different physico-chemical conditions at all three depths influenced changes in chemical composition of the added root litter.

4.1. Changes in bulk chemical composition at different soil depths due to root litter input

For all three depths solid-state ^{13}C-NMR analyses showed increasing alkyl/O-alkyl ratios over time indicating decomposition of the root litter (Fig. 1). The alkyl/O-alkyl ratio was introduced as an indicator for chemical changes occurring during decomposition of organic material from aboveground litter layers (Baldock et al., 1997). This ratio usually increases during leaf litter decomposition outside the mineral soil, because of loss of polysaccharides and selective preservation of plant-derived alkyl compounds and/or neoformation of microbial-derived alkyl decomposition products (Golchin et al., 1996). This ratio was thought to relate to the state of degradation of pure OM only (Baldock et al., 1997), because in mineral soil changes in chemical composition are usually soil-type specific (Rumpel et al., 2002; Spielvogel et al., 2008). In our experiment, the litterbags contained root litter mixed to mineral soil allowing for the occurrence of stabilisation processes in addition to decomposition (Sanaullah et al., 2011). Thanks to the ^{13}C label, solid-state ^{13}C NMR signals may be mainly related to root-derived material. Therefore, even if the alkyl/O-alkyl ratio was introduced to characterise the degree of decomposition of pure organic matter, our results show that this ratio may also be a meaningful parameter for the characterisation of the degree of decomposition of organic materials added to the mineral soil. We suggest that general changes in the bulk chemical composition of root litter within the mineral soil are similar to those occurring for leaf litter outside the soil.

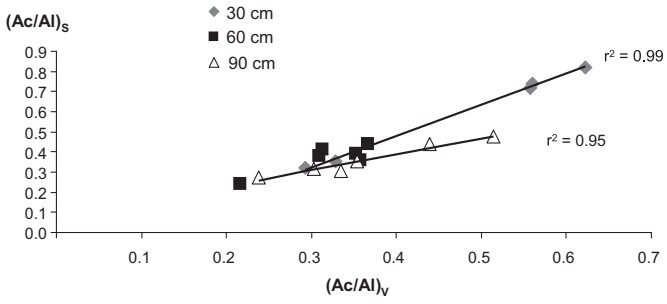

Fig. 6. Relationship between acid/aldehyde ratios of syringyl and vanillyl.

Table 2
Lignin signature of initial wheat and wheat remaining after 36 months of incubation at different depths determined by compound specific isotope analyses. Means ± standard deviation, $n = 3$, different letters indicate significant differences within one parameter.

	VSC (μg g^{-1} soil)	C/V	S/V	(Ac/Al)$_V$	(Ac/Al)$_S$
Initial Wheat added	127 ± 9.9^a	5.8 ± 0.5^a	1.2 ± 0.1^a	0.3 ± 0.0^a	0.4 ± 0.0^a
Wheat remaining					
30 cm	51.4 ± 13.5^c	3.0 ± 1.3^b	1.7 ± 0.4^a	0.7 ± 0.1^b	0.4 ± 0.0^a
60 cm	84.3 ± 13.2^b	2.7 ± 1.6^b	1.8 ± 0.2^a	0.7 ± 0.1^b	0.4 ± 0.1^a
90 cm	54.9 ± 5.6^c	1.3 ± 0.3^b	1.4 ± 0.3^a	0.7 ± 0.2^b	0.5 ± 0.1^a

The alkyl/O-alkyl ratio began to change rapidly after a carbon loss of around 40% (Fig. 2 B). These observations are in agreement with the results of Chabbi and Rumpel (2004), showing that changes in chemical composition of organic matter are only evident at advanced stages of decomposition because in the beginning of the process plant material is lost as a whole and it is only in later decomposition stages, that compounds are lost selectively (Chabbi and Rumpel, 2004). The most rapid increase as well as highest ratio after 36 months was found at 30 cm depth. This suggests that in the upper 30 cm the second stage of decomposition was reached faster than in deeper subsoil layers. However, in mineral soil stabilisation processes may interfere with decomposition and lead to further changes in chemical composition. The high clay content at 90 cm depth was therefore expected to favour the preservation of O-alkyl compounds over alkyl compounds. Consistent with this expectation O-alkyl loss was smaller at 90 cm depth compared to 30 and 60 cm depths despite a similar total C loss, which may be related to selective preservation of O-alkyl C. These results might be explained by accumulation of microbial sugars consistent with observations of high contribution of microbial sugars interacting with minerals in subsoil horizons (Rumpel et al., 2010). On a longer time scale these rather small differences observed over three years may become more important and may lead to the observed contrasting chemical composition of SOM in different soil horizons (Rumpel et al., 2012; Vancampenhout et al., 2012).

4.2. Lignin signature and fate of root-derived litter at different soil depths

Before root litter addition (0 months) lignin content in soil was lower compared to all other sampling dates, where lignin content was measured after root litter addition (Fig. 3). From 6 to 12 months, lignin content decreased at all soil depths until stabilisation of similar lignin content occurred (Fig. 3). Between 59 and 34% of initial wheat lignin were lost after 36 months. This implies that microorganisms, which were able to degrade this structural plant compound, were present and active at all soil depths. This is consistent with studies showing that enzyme activities did not decrease with increasing soil depths despite lower biomass content (Kramer et al., 2013). Despite the similar lignin content, depth specific differences in lignin characteristics were noted. At 30 cm depth we recorded the most degraded lignin signature with the highest acid to aldehyde ratio and lowest C/V ratio (Fig. 5). The increase of acid to aldehyde ratios and decrease of cinnamyl compounds are common changes observed during lignin degradation in soil (Thevenot et al., 2010). In our study, the relationship between the acid to aldehyde ratios of syringyl and vanillyl were different for 30 and 90 cm depth (Fig. 6). This may be another indicator for the horizon dependent composition changes of organic matter. Interestingly, we could not relate the differences in lignin decomposition to physico-chemical soil characteristics, such as pH, clay or iron oxide content (Table 1).

Several studies reported a strong influence of depth specific water regimes on organic matter decomposition (Rovira and Vallejo, 1997; Garcia-Pausas et al., 2012). During the three year field experiment, water content and temperature in all three horizons were similar (Sanaullah et al., 2011). Therefore we suggest that changes of the soil lignin signature are likely due to horizon-specific microbial communities having different lignin degradation abilities. We observed strong relationships between soil lignin content and total root C loss at all three depths (Fig. 4). ANCOVA analyses showed at similar root-C loss a higher soil lignin content at 30 cm compared to 60 and 90 cm. Together with the highest degradation state of lignin at 30 cm (Fig. 5), this might indicate a faster, more efficient, biochemical evolution leading to selective preservation at this depth. However, the higher lignin content at 30 cm might also be related to the higher initial lignin at this depth compared to the lower ones. This second hypothesis is strengthened by ^{13}C tracing of added root-derived lignin. Lignin parameters recorded in the three depths after 36 months of incubation do not indicate selective preservation of root-derived lignin at 30 cm (Table 2). Highest root-derived lignin was observed at 60 cm depth, where most root-C was remained (Table 2), suggesting that lignin contributed to increased C storage at this depth. Considering that lignin of plants grown under hydroponic conditions is probably less recalcitrant than those of plants grown in soil (Abiven et al., 2005), the lignin contribution to C storage in specific soil horizons was likely underestimated during our experiment.

5. Conclusion

We studied chemical changes in root litter decomposing in subsoil horizons at different depths. In agreement with our hypothesis, the decomposition pattern was horizon specific and resulted in SOM of different quality. General decomposition patterns of root litter were similar at all soil depths. However, we observed horizon specific alterations in bulk chemical composition as well as in lignin content and composition. ^{13}C labelling revealed molecular changes during litter decomposition, in particular decreasing contribution of O-alkyl compounds and increasing contribution of alkyl compounds, which are general changes also observed for shoot litter decomposing outside the mineral soil. In particular the decomposition and/or stabilisation rate of specific organic compounds may depend on the location within the soil profile and thus may lead to accumulation of O-alkyl material and/or lignin. In the long term these differences may lead to contrasting SOM bulk chemical composition. The study further indicates that lignin from root addition can contribute to carbon storage in subsoil horizons. However, we were unable to relate these chemical differences to specific physico-chemical parameters.

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