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Contribution of maize root derived C to soil organic carbon throughout an agricultural soil profile assessed by compound specific ^{13}C analysis

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ABSTRACT

Cutin and suberin structural units might be stabilized in subsoils and contribute to the aliphatic structures observed in stabilized soil organic matter (SOM). We studied their dynamics in subsoils by measuring the concentrations and ^{13}C contents of cutin and suberin markers in soil profiles under wheat (C_3) and after 9 years of maize cropping (C_4 plant). Alkandioic acids were considered as markers for roots, mid-chain hydroxy acids were only present in shoots and ω-hydroxy acids were identified in both roots and shoots. The diacid concentrations greatly increased below the ploughed layer after 9 years of maize cropping, possibly due to a higher root density of maize compared to wheat or to a faster turnover of fine roots and increased exudation of maize compared to wheat. From 0–75 cm, 9 years of maize cropping did not affect the distribution of shoot biomarkers but increased their concentrations. By contrast, below 75 cm, the shoot marker concentrations drastically decreased from the wheat control to the 9 year maize soil. The difference of $\delta^{13}C$ observed for shoot markers was always lower than that observed for ω-hydroxy acids, and below 15 cm, it was close to that observed for SOC. The difference in $\delta^{13}C$ of diacids was much more variable along the profile. Since the concentrations of the different markers were not at equilibrium, it was not possible to estimate their turnover. This study suggests several caveats for the use of molecular markers of roots and shoots to study the dynamics of SOM in deep soils: the higher heterogeneity compared to the ploughed layer, the presence of long history record of past vegetation that may hinder the short time scale changes tracked with the ^{13}C isotope technique, and the difficulty in evaluating root inputs in the soil systems.

1. Introduction

Soil organic matter (SOM) can act as a carbon sink in the global carbon cycle, which generated interest in its dynamics. The turnover of SOM is generally studied in the topsoil horizons, where the highest concentrations of SOM are found and where rapid exchanges occur between microbial and plant biomass, SOM and atmospheric CO_2. It has been recognized only recently that SOM stored in subsoils, although displaying low amounts of organic carbon compared to topsoils, greatly contributes to terrigenous carbon stocks (Batjes, 1996; Rumpel et al., 2002; Schöning et al., 2006; Chabbi et al., 2009). Furthermore, high radiocarbon ages of SOM in subsoils (Scharpenseel et al., 1989; Paul et al., 1997; Rumpel et al., 2004) suggest important proportions of stabilized SOM. Deep soil carbon may thus be a key component of the terrigenous C cycle (Rumpel and Kögel-Knabner, 2011). Most probably, carbon in deep soil is protected by mineral interactions (Eusterhues et al., 2005), which restrict access to SOM for microbial decomposers (Xiang et al., 2008). As compared to topsoil, the microbial biomass might be less abundant and less active in subsoil (Taylor et al., 2002). Its energy supply might be limited (Garcia-Pausas et al., 2008) by the scarcity of fresh plant litter input (Fontaine et al., 2007), although locally input of fresh organic matter might occur through rhizodeposition (Wiesenberg et al., 2010). Processes leading to the formation and protection of stabilized SOM in deep soils remain largely unknown.

Aliphatic moieties have been shown to be preserved during SOM degradation (Christensen, 1996; Six et al., 2002; Schöning et al., 2005; Mikutta et al., 2006), enriched in soil as compared to aromatic lignin (Dignac and Rumpel, 2006) and particularly to be stabilized in deep soils (Kögel-Knabner et al., 1992; Rovira and Vallejo, 2002; Lorenz et al., 2007). Protective layers of plant tissues contain alkyl structures (cutins and suberins), which could be the precursors of stable C in subsoils (Rumpel et al., 2004; Winkler et al., 2005).

Cutins and suberins are plant biopolyesters constituted of saturated and unsaturated *n*-carboxylic acids and diacids, the aliphatic

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chain bearing epoxy and alcohol groups (Kolattukudy, 1984; Bernards, 2002). In non-woody plants, cutins are mainly present in leaf cuticles protecting plants from transpiration losses (Kolattukudy and Walton, 1972; Heredia, 2003), whereas suberins are found at the outside of roots. Using saponification of aliphatic biopolyesters in wheat and maize soils, we recently documented that diacids originate specifically from wheat and maize root suberized tissues, whereas mid-chain hydroxy acids were specific for above ground cutin polyesters (Mendez-Millan et al., 2010a). In this study, we used compound specific ^{13}C analysis to determine the fate of cutin and suberin biomarkers in the topsoil horizon after a C_3/C_4 vegetation transition. Changes from C_3 (C_4) to C_4 (C_3) vegetation allows for the study of SOM dynamics through the contrasting ^{13}C signature that is specific to each plant photosynthetic type. Indeed, plants discriminate against $^{13}CO_2$ depending on their photosynthesis pathway (C_3, C_4, CAM) (Bender, 1971; Smith and Epstein, 1971). The ^{13}C signatures of C_3 and C_4 plants are contrasted enough to relate the changes of ^{13}C signatures of SOM to the amount of C_3 and C_4 derived carbon present in SOM. In the ploughed horizon of a wheat/maize transition field experiment, we showed a higher incorporation of maize biomarkers originating from roots than from shoots. The latter were mainly degraded within one year (Mendez-Millan et al., 2010a). Similarly, in a forest soil, the dynamics of the shoot and root markers obtained from FACE experiments were in the same order of magnitude (Feng et al., 2010).

Using the C_3/C_4 isotopic technique to evaluate the dynamics of SOM at depth, Rasse et al. (2006) showed that 2% of SOC was corn derived after 10 years and Collins et al. (1999) found 4–15% of new SOC at 50–100 cm after 30 years. Since cutins and suberins contain structural units that can be considered as biomarkers for above and below ground plant tissues (Mendez-Millan et al., 2011), and since SOM in deep soils may mainly originate from roots (Balesdent and Balabane, 1996; Puget and Drinkwater, 2001; Rasse et al., 2005; Rees et al., 2005), studying the dynamics of these biomarkers might help us understand how SOM is formed and preserved in subsoils. The aim of the present study was to better understand the mechanisms leading to the formation of stable SOM in subsoils by following the dynamics of root and shoot markers in deep soil horizons 9 years after a C_3/C_4 transition. We followed the changes in the ^{13}C natural abundance of cutin and suberin molecular biomarkers, from 0–105 cm depth in a soil after 9 years of maize cropping at the "Les Closeaux" experiment.

2. Material and methods

2.1. Experimental site and soil sampling

The INRA experimental field "les Closeaux", located in the park of the Château de Versailles, near Paris in France, is a chronosequence of maize (C_4 plant) cropping, replacing previous C_3 vegetation (wheat or forest). The soil is an eutric Cambisol (FAO-WRB, 2006) with a silt loam texture (33% sand, 50% silt, and 17% clay) and a carbonate content <1 g/kg (Dignac et al., 2005). A major proportion of the above ground biomass is returned to soil after manual harvesting of grain maize. All plots were mouldboard ploughed to a depth of 30 cm in October of each year. A control plot under wheat (*Triticum aestivum* L.) and a plot sown to maize (*Zea mays* L.) for 9 years were sampled in April 2003 (wheat control and 9 year maize soils, respectively). Four 105 cm deep cores were taken in each plot with a 2 cm ID hydraulic probe. Cores were then divided into 15 cm depth increments. Each of the four subsamples were pooled together to obtain a composite sample for each depth at plot scale. Samples were air dried, sieved at 2 mm and ground to 200 μm.

2.2. Bulk C analysis

Bulk analyses of the carbon content (OC) and the ^{13}C isotope signature of soil samples were carried out with a CHN autoanalyser Carlo Erba Instruments CHN NA 1500 coupled with an isotopic ratio mass spectrometer VG Sira 10. The isotopic ratio ($^{13}C/^{12}C$) was expressed as $\delta^{13}C$ (in ‰) calibrated against the internal standard Vienna Pee Dee Belemnite (VPDB). The total carbon and ^{13}C contents of the soil samples are reported in Rasse et al. (2006) and reproduced here in Table 1.

2.3. Preparation of cutin and suberin structural units

The cutin and suberin structural units were released from the insoluble residues left after free lipid extraction, using the saponification method recommended by Mendez-Millan et al. (2010b). Before saponification, free lipids were removed from soil samples with a solvent extractor Dionex ASE 200. Approximately 10 g of soil were extracted three times in 30 ml stainless steel extraction vessels. The first extraction was carried out at 75 °C in a solvent mixture 3:1 (v:v) dichloromethane:methanol (DCM:MeOH). The extraction was repeated changing the temperature to 140 °C. Finally, the sample was extracted with DCM at 140 °C. For each extraction, the heating time was 5 min, the static extraction lasted 20 min at a pressure of 5×10^6 Pa (Wiesenberg et al., 2004).

The extracted samples (1 g for horizons of the ploughed layer and 3 g for deeper horizons) were refluxed for 18 h in an aqueous solution of potassium hydroxide in MeOH (6% KOH/MeOH) (Cardoso and Eglinton, 1975; Ray et al., 1995). The solution was filtered and the residue washed with a MeOH:water mixture ($MeOH:H_2O$) (9:1, v:v). To obtain acidic bound lipids, 150 ml of distilled water was added to the mixture and the solution adjusted to pH 2 with 6 M hydrochloric acid (HCl) (Naafs and Van Bergen, 2002). The aqueous solution was finally extracted with DCM (three times 50 ml). The volume of the recombined extracts was reduced with a rotary evaporator under low pressure and transferred into a vial until the sample was prepared for analysis. Just before analysis, depolymerisation extracts were dried under nitrogen and redissolved in pyridine. Silylation with N,O-Bis (trimethylsilyl)-trifluoroacetamide) containing 1% of trimethylchlorosilane from Altech (BSTFA) was performed at 70 °C for 1 h. Silylation transformed hydroxy and carboxylic acid functions into their trimethylsilyl ether and ester derivatives (TMS ether/TMS ester).

2.4. Identification and quantification of cutin and suberin structural units

Identification and quantification of the biomarkers obtained after depolymerization were performed with a HP GC 6890 gas chromatograph equipped with an SGE BPX-5 column (50 m long, diameter 0.25 mm, coating 0.32 μm). The GC oven temperature was programmed at 100 °C for 2 min, then from 100 °C to 150 °C at 10 °C/min, from 150 °C to 200 °C at 5 °C/min, and finally at a rate of 2 °C/min from 200 °C to 350 °C and 5 min at 350 °C. Compounds

Table 1
Properties of the different soil layers (from data reported from Rasse et al., 2006).

Bulk density 10^6 g/m^3	Total SOC 10^{-3} g OC/g soil	Layer thickness 10^{-2} m	SOC stocks g SOC/m^2
1.50	12.7	15	2862
1.47	13.2	15	2901
1.49	10.4	15	2335
1.52	5.0	15	1145
1.61	3.9	15	951
1.60	3.7	15	880
1.61	3.5	15	843

were identified with GC coupled to an Agilent 5973 Electron Impact (70 eV, scan range *m*/*z* 40–700, 1.2 scan/s) mass spectrometer (MS). Compounds were identified according to their fragmentation pattern supported by comparison with published mass spectra (Eglinton et al., 1968; Holloway and Deas, 1971; Hunneman and Eglinton, 1972) and with a mass spectra library (Wiley). Compounds were quantified with GC coupled to a flame ionization detector (FID). The monomers were quantified by using an internal standard, the nonadecanoic acid, added prior to derivatisation. An external calibration with the 16-hydroxyhexadecanoic acid was used to calculate the concentrations. The response factor between the nonadecanoic acid and the 16-hydroxyhexadecanoic acid was close to 1.

The depolymerisation procedure was performed in triplicate, on three solvent extracted soil samples. Quantification results are presented as the mean of the three depolymerizations. The standard deviations were obtained from the three analyses.

2.5. Compound specific ^{13}C isotopic analysis

Molecular isotopic analyses were carried out with a GC HP5890 coupled with an Isochrom III Isotopic mass spectrometer (Micromass-GVI Optima). Samples were injected in the splitless mode. The same column and chromatographic conditions were used as for the identification and quantification. The $\delta^{13}C$ values were measured for the silylated monomers of the molecules of interest. These values were corrected for the ^{13}C contents of carbon atoms introduced by silylation, measured off-line with an Elemental Analyser (EA) coupled to an isotopic ratio mass spectrometer (Micromass NA 1500 NC). The accuracy of the correction was verified for lignin molecules by comparison of isotopic measurements of sylilated standards with off-line analysis of the ^{13}C content of BSTFA and of the non-derivatized standards. Results suggested that all C atoms of BSTFA have the same isotopic ratio (Dignac et al., 2005). Compound specific $\delta^{13}C$ values (mean and standard deviation) were calculated from several GC-C-IRMS analyses of one or two replicates of soil depolymerization mixtures.

For quantification as well as for isotopic signature measurements, the 9,10-epoxy, 18-hydroxy octadecanoic acid (9,10-epoxy,ω-$C_{18:0}$) was analyzed as the isomeric mixture of the 9-methoxy,10,18-dihydroxy octadecanoic and the 9-hydroxy,10-methoxy,18-hydroxy octadecanoic acids. Indeed, the epoxy function reacts upon basic hydrolysis to be converted into vicinal diols and vicinal methoxy groups (Holloway and Deas, 1973; Goñi and Hedges, 1990). The 9,10,18-trihydroxy octadecanoic acid (9,10,ω-$C_{18:0}$) might thus also derive from the 9,10-epoxy,ω-$C_{18:0}$ but since it can also be present as such in the samples, it was analysed separately.

3. Results

3.1. Concentrations of cutin and suberin structural units throughout the agricultural soil profiles

3.1.1. Root markers

In a recent study (Mendez-Millan et al., 2011), we have shown that the hexadecan-1,16-dioic acid ($C_{16:0}$DA), the docosan-1,22-dioic acid ($C_{22:0}$DA) and the tetracosan-1,24-dioic acid ($C_{24:0}$DA) were present in wheat and maize roots but not found in their above ground tissues. In wheat and maize cultivated soils, these three diacids can thus be considered as biomarkers specific for roots (Mendez-Millan et al., 2010a). In the wheat control soil, the total concentration of the diacids decreased from the surface (82 ± 11 μg OC/g SOC) to the 60–75 cm depth (16 ± 5 μg OC/g SOC) and then increased to 52 μg OC/g SOC at 90–105 cm (Fig. 1A). From 15–90 cm, diacid distribution was very similar in the different layers. The average concentration over the five intermediate depths (15–90 cm) was 12 ± 2 μg OC/g SOC for $C_{16:0}$DA, 9 ± 3 μg OC/g SOC for $C_{24:0}$DA, and 7 ± 1 μg OC/g SOC for $C_{22:0}$DA. Surprisingly, in the ploughed layer (0–30 cm), higher contents of $C_{22:0}$DA and $C_{24:0}$DA were observed in the 0–15 cm layer (38 ± 4 and 25 ± 5 μg OC/g SOC, respectively) compared to the 15–30 cm one (7 ± 2 and 9 ± 3 μg OC/g SOC, respectively). The three diacids had higher concentrations in the 90–105 cm depth (22 ± 1, 18 ± 1 and 11 ± 1 μg OC/g SOC for the C_{16}, C_{22} and C_{24} counterparts, respectively) compared to their average concentrations measured between 15 and 90 cm.

The concentrations and the distributions of diacids in the wheat control and 9 year maize soils were very different (Fig. 1B). In the 9 year maize soil, the concentration of the diacids in the ploughed layer was 70 ± 4 μg OC/g SOC, similar to their concentration in the 0–15 cm layer of the soil under wheat. In the 15–30 cm layer, the $C_{24:0}$DA could not be quantified due to the occurrence of an overlapping peak in the chromatogram. From 30–90 cm depth, concentration of the diacids increased from 58 ± 3 to 94 ± 1 μg OC/g SOC and was only 71 ± 1 μg OC/g SOC in the 90–105 cm layer. From 30–105 cm, the concentrations of the root markers were much higher in the 9 year maize soil compared to the wheat control soil, especially in the 60–75 cm layer. In the top 15 cm, the $C_{24:0}$DA was the major root marker, accounting for 44% of the diacids, whereas the $C_{16:0}$DA dominated at 45–60 cm, and the $C_{22:0}$DA in the layers deeper than 60 cm. The concentration of the $C_{16:0}$DA was rather homogenous from 15–105 cm, with an average over these 6 layers of 29 ± 1 μg OC/g SOC.

3.1.2. Shoot markers

Six mid-chain hydroxy acids have been identified mostly in the shoots of maize and wheat by Mendez-Millan et al. (2011) and can thus be considered as shoot markers in the wheat and maize soils: the isomeric mixture of *x*,16-dihydroxy hexadecanoic acid (*x*,ω-$C_{16:0}$; with *x* = 8, 9 and 10), the 9-hydroxy hexadecanedioic diacid (9-$C_{16:0}$DA), three dihydroxy octadecenoic acids (11,ω-$C_{18:1}$ and *x*,ω-$C_{18:1}$; with *x* = 9 and 10), the 9,10-epoxy,ω-$C_{18:0}$ and the 9,10,ω-$C_{18:0}$. In the wheat plant they represented the major class of compounds, with a concentration of 1.9 mg OC/g SOC (54% of the total released monomers). In maize their concentration was lower than in wheat (0.6 mg OC/g SOC, 23% of total monomers) (Mendez-Millan et al., 2011). Their concentrations in the profiles of the wheat control and 9 year maize soils are presented in the Fig. 2. The concentrations of the two dihydroxy octadecenoic acids were estimated separately but added for graphical representation, such as those of the trihydroxy and the epoxy hydroxy octadecanoic acids (Fig. 2).

The total concentration of shoot markers was much higher compared to those of root markers in soil under wheat and maize (Figs. 1 and 2). In the wheat control soil (Fig. 2A) the total concentration of mid-chain hydroxy acids slightly decreased (from 578 ± 8 to 390 ± 64 μg OC/g SOC) between 0 and 75 cm. This decrease was mainly due to the decrease of the dihydroxy octadecenoic acids (from 51 ± 4 to 11 ± 4 μg OC/g SOC) and of the trihydroxy and epoxy octadecanoic acids (from 95 ± 4 to 18 ± 4 μg OC/g SOC). The concentration of the shoot markers greatly increased again in the wheat control soil below 75 cm and was even higher than in the top soil (756 ± 5 and 1148 ± 202 μg OC/g SOC in the 75–90 and 90–105 cm layers, respectively). This increase can mainly be attributed to the increase in the concentrations of the two C_{16} acids (*x*,ω-$C_{16:0}$ and 9-$C_{16:0}$DA). The *x*,ω-$C_{16:0}$ was the major mid-chain hydroxy acid of the wheat soil all along the soil profile, representing from 51% (at 0–15 cm and at 90–105 cm) to 69% (60–75 cm) of the total shoot markers. The 9-$C_{16:0}$DA diacid represented from 20% (at

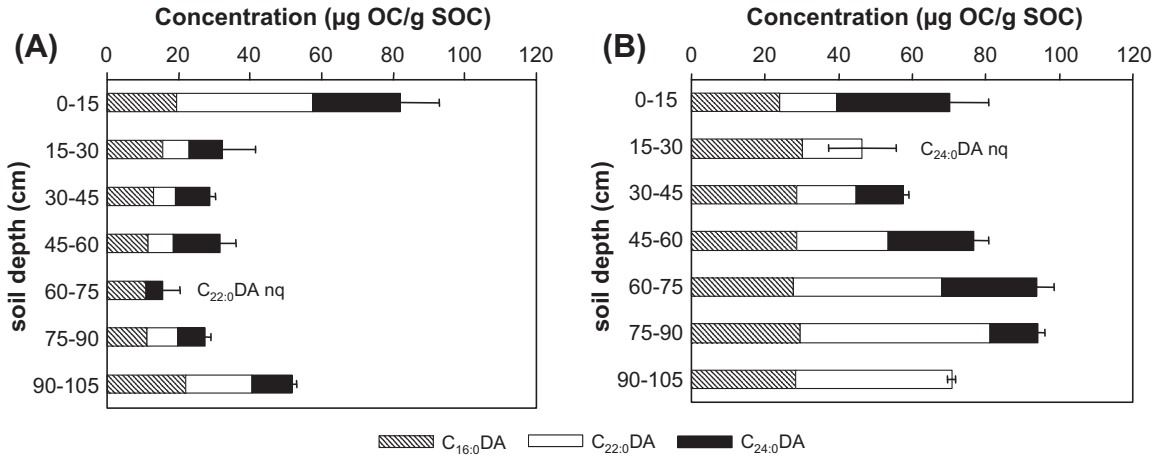

Fig. 1. Concentration (µg OC/g SOC) of the biomarkers only found in maize and wheat roots. The changes of the concentrations with soil depth are presented for wheat control (A) and 9 year maize (B) soils. nq: means that the molecule was present but could not be quantified. Error bars represent the standard deviation obtained from three depolymerization procedures applied on each soil sample.

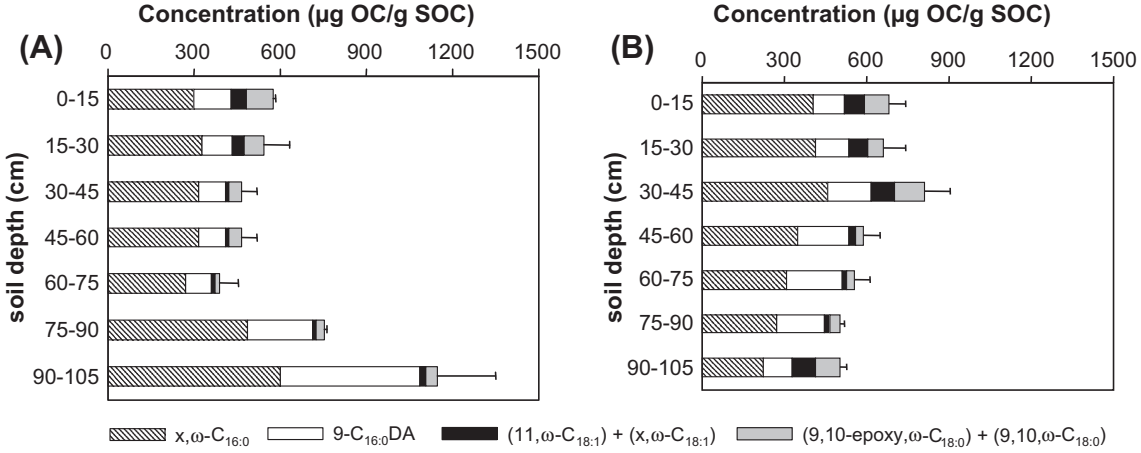

Fig. 2. Concentrations (µg OC/g SOC) of the mid-chain hydroxy acids, only found in maize and wheat shoots. The changes of the concentrations with soil depth are presented for wheat control (A) and 9 year maize (B) soils. Error bars represent the standard deviation obtained from three depolymerization procedures applied on each soil sample.

15–30 cm) to 41% (at 90–105 cm). The *x*,ω-$C_{18:1}$ acid was not detected below the 0–15 cm layer in the wheat soil.

The concentrations of the shoot biomarkers in the 9 year maize soil showed a similar pattern as in the wheat soil but only in the first 30 cm. The increase observed below 75 cm in the wheat soil was not observed in the maize soil. The concentrations were decreasing from 680 ± 61 µg OC/g SOC at 0–15 cm to 504 ± 23 µg OC/g SOC at 90–105 cm. The shoot markers in the profile of the maize cropped soil were dominated by the *x*,ω-$C_{16:0}$ and the 9-$C_{16:0}$DA, which displayed a relative contribution to total shoot markers close to that observed in the wheat control soil: the contribution of the *x*,ω-$C_{16:0}$ ranged from 44% at 90–105 cm to 60% in the top horizons, and that of the 9-$C_{16:0}$DA varied between 17% and 38% of the total mid-chain hydroxy acids. The *x*,ω-$C_{18:1}$ acid was not detected below 45 cm in the maize soil.

From 0–75 cm, the 9 year maize and the wheat control soils displayed similar distributions of shoot biomarkers, but with different concentrations. Concentrations of the shoot markers were particularly high in the 30–45 cm layer of the 9 year maize soil (810 ± 93 µg OC/g SOC) compared to the wheat control soil (467 ± 63 µg OC/g SOC). Below 75 cm, the concentrations of the shoot markers was higher in the wheat control (756 ± 5 µg OC/g SOC at the 75–90 cm, 1148 ± 202 µg OC/g SOC at 90–105 cm) compared to the 9 year maize soil (515 ± 17 µg OC/g SOC at 75–90 cm, 504 ± 23 µg OC/g SOC at 90–105 cm). This difference was mainly due to a lower concentration of the two C_{16} acids in the maize soil. Their concentrations were very high in the two deeper layers of the wheat control soil compared to their concentrations in the upper layers (Fig. 2A), while in the maize soil, their concentrations regularly decreased from 30 cm to 105 cm (Fig. 2B). This is probably due to a high spatial heterogeneity of SOM composition in the deep layers.

3.1.3. ω-Hydroxy carboxylic acids

The ω-hydroxy carboxylic acids from ω-$C_{20:0}$ to ω-$C_{26:0}$ are plant specific, but not organ specific, as reported in a previous study on roots and shoots of wheat and maize (Mendez-Millan et al., 2011). Here, the concentrations of the ω-$C_{16:0}$, ω-$C_{18:1}$ and ω-$C_{20:0}$ were estimated separately but added for graphical representation (Fig. 3).

In the wheat control soil (Fig. 3A), the concentration of these plant biomarkers decreased from the top soil (638 ± 10 µg OC/g SOC) to 60–75 cm (106 ± 10 µg OC/g SOC), and then increased to reach 318 ± 24 µg OC/g SOC at 90–105 cm. Their distribution remained similar throughout the soil profile. The ω-$C_{22:0}$ acid

represented from 40–60% of this class of compounds, and the ω-$C_{24:0}$, 20 to 30%.

In the 9 year maize soil, the concentrations of the ω-hydroxy acids (Fig. 3B) did not vary between 0 cm and 45 cm (average 685 ± 30 μg OC/g SOC over the three upper layers). They decreased to 326 ± 45 μg OC/g SOC at 75–90 cm, and remained at a similar level (353 ± 2 μg OC/g SOC) at 90–105 cm. Their distribution was similar through the soil profile: the ω-$C_{22:0}$ was the major compound (43–57% of the total ω-hydroxy acids), followed by the ω-$C_{24:0}$ (25–36%).

In the 9 year maize soil the concentrations of the ω-hydroxy acid between 15–90 cm were higher than at the same depth of the wheat control soil. As already observed for the diacids, the larger difference in the ω-hydroxy acids was observed at 60–75 cm (106 ± 10 in the wheat control and 427 ± 10 μg OC/g SOC in the 9 year maize soil). The concentrations of all the 6 monomers were larger, and especially those of ω-$C_{24:0}$ and ω-$C_{22:0}$.

3.2. Stocks of cutin and suberin structural units in the wheat and maize soils

The stocks (mg OC/m^2) of root markers, shoot markers and ω-hydroxy acids (Fig. 4) were calculated by using the total SOC stocks reported by Rasse et al. (2006) (Table 1) for the depths of the maize soil. The stocks of total SOC did not change with the duration of maize cropping after wheat in any of the soil layers and decreased from 2858 g SOC/m^2 in the 0–15 cm layer to 845 g SOC/m^2 at 90–105 cm (Rasse et al., 2006).

In the wheat control soil, the stocks of ω-hydroxy acids and shoot markers followed the same pattern from 0–60 cm (Fig. 4A). The stocks followed a regular decrease from around 1900 mg OC/m^2 in the ploughed layer to 204 and 532 mg OC/m^2 at 45–60 cm for ω-hydroxy acids and shoot markers, respectively. Between 60 and 105 cm the stocks of both classes of compounds increased. This increase was more marked for shoot markers (from 367 mg OC/m^2 at 60–75 cm to 970 mg OC/m^2 at 90–105 cm) than for ω-hydroxy acids (from 100–269 mg OC/m^2). In the 9 year maize soil (Fig. 4B), the stocks of these two classes of compounds followed the same pattern in the entire profile, with rather constant stocks between 0 and 45 cm (average 1839 ± 201 and 1911 ± 30 mg OC/m^2 for ω-hydroxy acids and shoot markers, respectively), a large decrease between the 30–45 and 45–60 cm layers, and a slight decrease from 45–105 cm.

In the wheat control soil, total amounts of ω-hydroxy acids in the ploughed layer (3330 mg OC/m^2) were almost twice those present in the 30–105 cm subjacent soil (1729 mg OC/m^2). By contrast total amounts of shoot markers were similar in the ploughed layer (3225 mg OC/m^2) and in the 30–105 cm profile (3625 mg OC/m^2). In the 9 year maize soil, stocks of ω-hydroxy acids and shoot markers were both equally distributed between the ploughed layer (3887 and 3850 mg OC/m^2, respectively) and in subjacent 30–105 cm profile (3173 and 3969 mg OC/m^2, respectively).

The stocks of root markers were always much lower than those of the other two compound classes (Fig. 4). In the ploughed layer (0–30 cm) they were of the same order of magnitude in the wheat control and the 9 year maize soils (386 and 335 mg OC/m^2, respectively). In the wheat control soil, the stocks decreased progressively to 15 mg OC/m^2 at 60–75 cm and then slightly increased to 44 mg OC/m^2 at 90–105 cm. In the 9 year maize soil, the stocks of diacids constantly decreased to 60 mg OC/m^2 at 90–105 cm. In the wheat control soils, the stocks of diacids were much lower in the 30–105 cm soil (186 mg OC/m^2) compared to the ploughed layer. The stocks of root markers below the ploughed layer were largely increased after nine years of maize cropping and they were even higher than in the ploughed layer in the 9 year maize soil (453 mg OC/m^2).

3.3. ^{13}C isotopic signatures of cutin and suberin structural units in soils

3.3.1. Changes in ^{13}C contents of individual markers with depth and maize cropping

The ^{13}C content of the plant biomarkers in the different depths of the wheat control and 9 year maize soils are presented in Table 2. Under the wheat crop, $\delta^{13}C$ ranged from −27.7‰ to −33.4‰ for shoot markers, from −25.4‰ to −34.7‰ for root markers and from −26.0‰ to −37.4‰ for ω-hydroxy acids (Table 2). These compounds were always slightly ^{13}C enriched in the wheat control soil horizons compared to the wheat plant (from −33.5 to −35.9‰ for shoot markers, from −34.7 to −37.0‰ for root markers and from −30.9 to −37.3‰ for ω-hydroxy acids, Mendez-Millan et al., 2010a). No trend was observed for the isotopic signature with soil depth.

The ^{13}C content of the plant biomarkers in the 9 year maize soil ranged from −24.1‰ to −34.1‰ (9,10,ω-$C_{18:0}$ at 0–15 cm) for shoot markers, from −24.5‰ to −32.9‰ for root markers and from −22.9‰ to −33.8‰ for ω-hydroxy acids. The most ^{13}C depleted root marker was always the $C_{22:0}$DA, with an average $\delta^{13}C$ value along the soil profile of −32.6 ± 1.1‰ in the wheat soil and −31.5 ± 1.1‰ in the maize soil (Table 2). The most enriched root marker was the $C_{16:0}$DA, except in the 15–30 cm layer of the wheat soil, where it had a low $\delta^{13}C$ value (−32.8‰). The average $\delta^{13}C$ of the $C_{16:0}$DA was −28.4 ± 2.8‰ in the wheat soil and

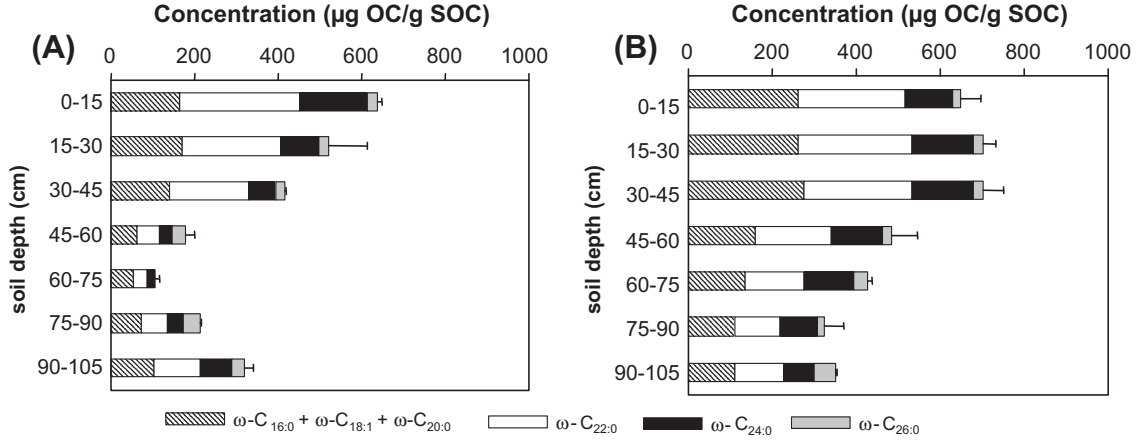

Fig. 3. Concentration (μg OC/g SOC) of ω-hydroxy carboxylic acids. The changes of the concentrations with soil depth are presented for wheat control (A) and 9 year maize (B) soils. Error bars represent the standard deviation obtained from three depolymerization procedures applied on each soil sample.

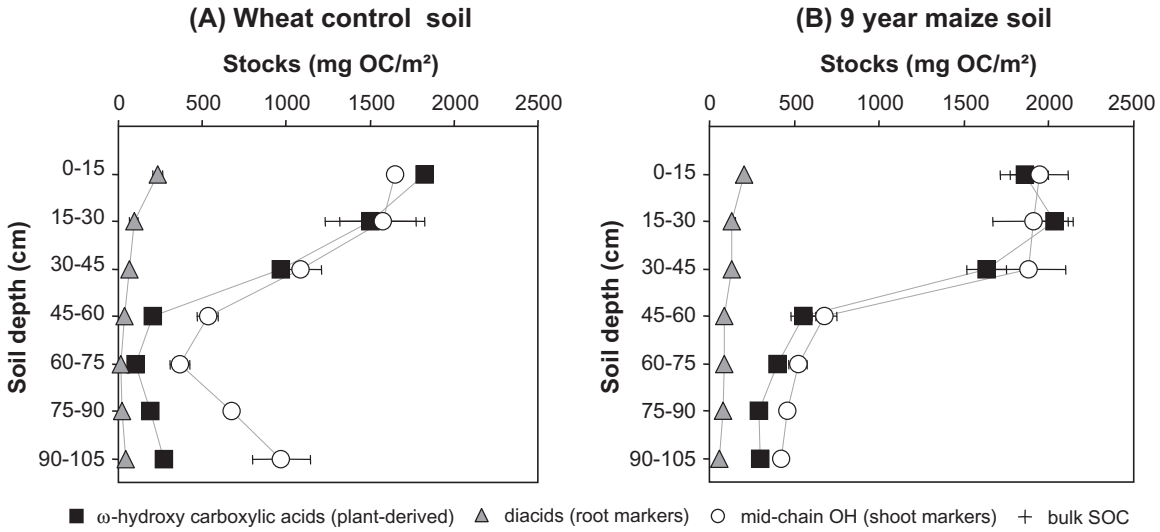

Fig. 4. Stocks (mg OC/m^2) of ω-hydroxy carboxylic acids, diacids (root markers) and mid-chain hydroxy acids (shoot markers) in the wheat control (A) and 9 year maize soil (B).

−25.5 ± 0.6‰ in the maize soil. After 9 years of maize cropping, the ^{13}C enrichments of the root markers were highly variable. In the three deeper layers (60–105 cm), the higher enrichment was observed for the $C_{16:0}$DA (2.3–3.3‰) then for the $C_{24:0}$DA (1.1–2.6‰) and the $C_{22:0}$DA (0.2–2.1‰).

The most ^{13}C depleted shoot marker was the 9,10,ω-$C_{18:0}$ in the ploughed layer of the wheat control soil (−33.2‰) and in the 0–15 cm layer of the 9 year maize soil (−34.1‰). No clear tendency was observed for changes in δ^{13}C of the mid-chain hydroxy acids below the ploughed layer after 9 years of maize cropping. The highest ^{13}C contents were observed in various compounds depending of the depth, with no clear tendency either (Table 2). In the 45–60 and 60–75 layers, the 9,10-epoxy,ω-$C_{18:0}$ was largely ^{13}C depleted (by −4.8 to −5.9‰) in the 9 year maize soil compared to the wheat control soil. By contrast, the 11,ω-$C_{18:1}$ was the most ^{13}C enriched, by 3.7–5.0‰ at 0–45 cm in the maize compared to the wheat soil. The three other shoot markers followed similar patterns, with average ^{13}C enrichments in the maize soil ranging from 0.4–0.7‰.

The most ^{13}C depleted of the ω-hydroxy acids was the ω-$C_{22:0}$ in the entire profile of the 9 year maize soil, with an average value of −32.1 ± 0.7‰. The ω-$C_{22:0}$ was also the most ^{13}C depleted ω-hydroxy acid between 0 and 60 cm of the wheat control soil, with an average δ^{13}C of −34.6 ± 0.7‰. However, in the three deeper layers the ω-$C_{20:0}$ was even more depleted, with a δ^{13}C of −37.2 ± 0.2‰. The most ^{13}C enriched ω-hydroxy acid was the ω-$C_{18:1}$ from 0–75 cm in the wheat control soil (average −27.3 ± 1.1‰). It was also the most depleted ω-hydroxy acid in the 9 year maize soil, between 30 cm and 75 cm, with an average value of −26.0 ± 1.2‰ at 0–75 cm. The δ^{13}C values of this compound could not be measured in the two deepest layers of both soils (Table 2). The largest enrichment after 9 years of maize cropping was observed for the ω-$C_{16:0}$, which δ^{13}C increased on average by 5.9‰ in the profile. The ω-$C_{20:0}$ followed a different pattern, with an increase of 0.1‰ at 15–30 cm, reaching 8.3‰ at 60–75 cm and remaining above 7‰ for the two deepest layers.

3.3.2. Changes in ^{13}C contents of compound classes with depth and maize cropping

The δ^{13}C values of the different compound classes (root marker diacids, shoot marker mid-chain hydroxy acids, and ω-hydroxy acids) were computed using the concentration and δ^{13}C of each monomer and compared to the δ^{13}C values of bulk SOC (from Rasse et al., 2006) in wheat-control (Fig. 5A) and 9 year maize soils (Fig 5B). In both soils the three organic families were largely ^{13}C depleted compared to SOC. In the wheat control soil, the ω-hydroxy acids were always the most ^{13}C depleted class of compounds with values ranging from −33.8‰ in the 15–30 cm layer to −32.4‰ in the 30–45 cm layer (Fig. 5A). The δ^{13}C values of this class of compounds were rather homogeneous along the entire profile, with an average δ^{13}C of −32.9 ± 0.5‰. Between 0 and 45 cm, in the wheat control soil, the δ^{13}C of the root and shoot markers had similar values. Below 45 cm, the diacids were ^{13}C enriched compared to the mid-chain hydroxy acids. The δ^{13}C of the shoot markers were rather homogeneous below the ploughed layer, with an average of −31.8 ± 0.4‰. By contrast, the δ^{13}C of the diacids greatly increased between 15 and 75 cm, reaching a maximum of −27.9‰ in the 60–75 cm layer, and decreased at 75–90 cm.

The order of the δ^{13}C values of the mid-chain hydroxy and the ω-hydroxy acids was reverse in the 9 year maize soil (Fig. 5B) compared to the wheat control soil (Fig. 5A). The shoot markers were generally the most ^{13}C depleted compounds, with an average value below 45 cm of −31.3 ± 0.7‰. Except in the 75–90 cm layer, the diacids were the most ^{13}C enriched compounds in the maize soil, such as in the wheat control soil. The ω-hydroxy acids had intermediate δ^{13}C averaging −30.5 ± 0.1‰ in the ploughed layer and −29.0 ± 0.3‰ below 30 cm. In both wheat control and 9 year maize soil, the δ^{13}C of the root markers displayed the largest variability with depth compared to that of the shoot markers and the ω-hydroxy acids.

We demonstrated the validity of the simplified isotopic mass balance equation to calculate a proportion of maize derived compounds in the ploughed layer of the Closeaux experiment (Dignac et al., 2005). However, in the present study, the multiple origin of OM in the deeper layers of soils makes it impossible to use this simplified equation. According to Rumpel and Kögel-Knabner (2011), downward movement of OM through transport of dissolved organic matter (DOM) and bioturbation are major sources of OM at depth along with inputs of dead root and root exudates. All these inputs are not occurring homogeneously throughout the soil profile and may have a specific isotopic signature distinct from the original C_4 plant signature. Furthermore the concentrations of the different markers were different in the profiles of the wheat control and the 9 year maize soils (Figs. 1–3). For these reasons,

Table 2
^{13}C isotopic signatures ($\delta^{13}C$‰) of the monomers specific for plants (ω-hydroxy acids), for plant roots (diacids) and for plant shoots (mid-chain hydroxy acids) in the wheat control (WS, bold) and the 9 year maize (9YS) soils.

	0–15 cm		15–30 cm		30–45 cm		45–60 cm		60–75 cm		75–90 cm		90–105 cm	
	WS	9YS	**WS**	9YS	**WS**	9YS	**WS**	9YS	**WS**	9YS	**WS**	9YS	**WS**	9YS
ω-hydroxy acids														
ω-$C_{16:0}$	**−32.0 ± 1.3**	−26.3 ± 1.7	**−32.2 ± 0.7**	−26.6 ± 0.4	**−29.9 ± 0.9**	−25.8 ± 0.4	**−30.7 ± 0.6**	−24.8 ± 2.1	**−30.4 ± 1.5**	−24.6 ± 0.5	**−30.0 ± 1.3**	−22.9 ± 0.9	**−30.9 ± 0.4**	−23.9 ± 0.4
ω-$C_{18:1}$	**−26.0 ± 0.1**	−27.1 ± 0.5	**−28.9 ± 0.1**	−27.0 ± 0.9	**−26.7 ± 1.6**	−24.8 ± 0.7	**−27.3 ± 0.5**	−24.7 ± 1.4	**−27.6 ± 0.7**	−26.6 ± 0.5	**nd**	nd	**nd**	nd
ω-$C_{20:0}$	**−32.1 ± 1.6**	−33.8 ± 0.5	**−33.0 ± 0.9**	−33.0 ± 0.2	**−32.2 ± 0.8**	−30.9 ± 0.9	**−34.6 ± 1.2**	−30.8 ± 0.2	**−37.0 ± 0.5**	−28.7 ± 0.5	**−37.4 ± 0.5**	nd	**−37.1 ± 0.5**	−30.0 ± 0.5
ω-$C_{22:0}$	**−34.3 ± 1.0**	−32.9 ± 0.5	**−35.7 ± 0.3**	−33.4 ± 0.1	**−34.5 ± 1.0**	−32.2 ± 0.6	**−35.0 ± 0.4**	−31.5 ± 1.1	**−34.7 ± 1.4**	−31.7 ± 0.5	**−34.4 ± 0.7**	−31.8 ± 0.9	**−33.4 ± 1.1**	−31.5 ± 1.3
ω-$C_{24:0}$	**−32.7 ± 1.6**	−30.7 ± 0.6	**−34.4 ± 0.2**	−30.6 ± 0.6	**−33.2 ± 1.4**	−30.1 ± 0.3	**−34.4 ± 1.5**	−29.1 ± 1.6	**−33.0 ± 1.3**	−29.8 ± 0.5	**−31.9 ± 0.5**	−28.0 ± 0.6	**−32.0 ± 0.6**	−28.0 ± 1.4
ω-$C_{26:0}$	**−29.4 ± 1.7**	−26.5 ± 0.5	**−30.2 ± 0.5**	−25.8 ± 0.5	**−30.0 ± 0.8**	−26.0 ± 0.2	**nd**	−28.7 ± 0.4	**−30.2 ± 0.5**	−28.7 ± 0.5	**−30.8 ± 0.8**	−29.8 ± 0.9	**−31.5 ± 0.8**	−28.4 ± 1.1
α,ω-diacids (root markers)														
$C_{16:0}$DA	**−25.4 ± 2.1**	−26.0 ± 0.5	**−32.8 ± 0.9**	−25.8 ± 0.5	**nd**	−25.2 ± 0.8	**nd**	−25.8 ± 0.7	**−26.8 ± 0.5**	−24.5 ± 0.5	**−28.0 ± 0.5**	nd	**−29.0 ± 0.6**	−25.7 ± 0.5
$C_{22:0}$DA	**−33.3 ± 1.5**	−32.9 ± 0.5	**−34.7 ± 0.7**	−32.5 ± 0.5	**−32.2 ± 1.9**	−31.2 ± 0.3	**−32.3 ± 0.5**	−30.3 ± 0.5	**−31.4 ± 0.5**	−31.3 ± 0.5	**−31.9 ± 0.6**	−32.2 ± 0.5	**−32.3 ± 0.3**	−30.1 ± 2.0
$C_{24:0}$DA	**−31.0 ± 1.5**	−30.7 ± 0.5	**−30.3 ± 0.3**	−28.2 ± 0.4	**−31.2 ± 0.5**	−27.7 ± 0.5	**−29.5 ± 0.5**	−28.9 ± 0.5	**−30.1 ± 0.5**	−29.1 ± 0.5	**−30.3 ± 0.5**	−29.1 ± 1.3	**−30.5 ± 0.8**	−27.9 ± 1.9
mid-chain hydroxy acids (shoot markers)														
x,ω-$C_{16:0}$	**−30.2 ± 0.7**	−31.7 ± 0.2	**−33.1 ± 1.0**	−32.1 ± 0.6	**−31.4 ± 0.5**	−30.0 ± 0.6	**−31.4 ± 0.7**	−31.7 ± 0.9	**−32.7 ± 1.0**	−32.0 ± 0.5	**−32.5 ± 0.5**	−30.6 ± 0.4	**−32.2 ± 0.1**	−31.4 ± 0.6
9-$C_{16:0}$DA	**−30.3 ± 1.2**	−31.5 ± 0.9	**−33.0 ± 1.3**	−32.3 ± 0.4	**−32.8 ± 0.8**	−30.6 ± 0.4	**−32.8 ± 1.1**	−31.4 ± 1.7	**−32.0 ± 0.3**	−32.1 ± 0.5	**−31.4 ± 0.0**	−30.6 ± 0.0	**−30.8 ± 0.1**	−31.4 ± 1.5
11,ω-$C_{18:1}$	**−32.5 ± 0.5**	−28.9 ± 0.5	**−33.0 ± 0.4**	−29.0 ± 0.5	**−29.0 ± 0.0**	−24.1 ± 0.4	**−29.0 ± 0.5**	nd	**nd**	nd	**nd**	nd	**nd**	−29.4 ± 0.4
x,ω-$C_{18:1}$	**nd**	nd	**nd**	nd	**nd**	nd	**nd**	nd	**nd**	nd	**nd**	nd	**nd**	−25.9 ± 0.5
9,10-epoxy,ω-$C_{18:0}$	**−31.3 ± 0.7**	nd	**nd**	−28.4 ± 0.5	**nd**	−25.1 ± 0.8	**−28.1 ± 0.3**	−34.0 ± 0.5	**−27.7 ± 0.9**	−32.5 ± 0.5	**nd**	−30.4 ± 0.5	**nd**	−30.2 ± 0.5
9,10,ω-$C_{18:0}$	**−33.4 ± 0.4**	−34.1 ± 0.1	**−33.1 ± 0.9**	−30.8 ± 0.3	**−31.7 ± 0.4**	−29.9 ± 0.1	**−32.0 ± 0.9**	nd	**−32.0 ± 0.5**	nd	**−32.5 ± 1.2**	nd	**−33.0 ± 1.1**	nd

nd: not determined, either due to a too low concentration, coelution with the chromatographic peak, or absence of the compound.

we just computed the differences in $\delta^{13}C$ values in the wheat control and 9 year maize soils for the different classes of compounds (Fig. 5C). For the ω-hydroxy acids, the changes in $\delta^{13}C$ after 9 years of maize cropping slightly increased with depth from 2.3‰ at 0–15 cm to 4.2‰ at 90–105 cm (Fig. 5C), with an average difference over the entire profile of 3.5 ± 0.7‰. The $\delta^{13}C$ of shoot markers was always lower than that observed for the ω-hydroxy acids, and below 15 cm was close to that observed for SOC. The difference in $\delta^{13}C$ of diacids was much more variable. Except for the depths 60–75 and 75–90 cm, where the $\delta^{13}C$ of diacids was higher in the wheat soil compared to the maize soil, the differences for diacids were higher and followed the same pattern as those observed for shoot markers (Fig. 5C).

4. Discussion

In the wheat control soil, the concentrations of the C_{16} shoot markers (*x*,ω-$C_{16:0}$ and 9-$C_{16:0}$DA), greatly increased below 75 cm, where they were even higher than in the top soil (Fig. 2). By contrast, the contribution of the unsaturated and epoxy shoot biomarkers (11,ω-$C_{18:1}$, *x*,ω-$C_{18:1}$, 9,10-epoxy,ω-$C_{18:0}$) was 25% of total shoot markers at the top of the profile and decreased down to 5% at the bottom. The decrease in the contribution of the unsaturated compounds might be due to the fact that labile functions such as double bonds and epoxy groups are preferentially degraded in soil (Nierop et al., 2003). By contrast compounds with several reactive groups (diacids, several OH groups) might be more stabilized through physico-chemical protection.

The high concentration of some shoot markers (*x*,ω-$C_{16:0}$ and 9-$C_{16:0}$DA) in deeper horizons can have different origin. These markers could be added to deeper horizons through bioturbation or through downward movement of small particles in DOM (Rumpel and Kögel-Knabner, 2011). These biomarkers could also originate from former vegetation, in which they might have been present in roots. Before this experimental field was cropped (more than 50 years before the setup of the chronosequence), the vegetation was a C_3 forest. Tree species contain different types of cutin and suberin biomarkers than those present in maize and wheat cereal crops (Feng et al., 2010; Mendez-Millan et al., 2011). Some of the structural units originating from the roots of the old forest might have remained in the soil. The occurrence of biomarkers from the old forest vegetation was already suggested in the ploughed layer (0–25 cm) of the same experimental field to explain that the contribution of maize shoot markers to the soil shoot marker pool was low after 12 years of maize cropping (Mendez-Millan et al., 2010a). Small and random variations of the ^{13}C contents of these compounds were observed over the soil profile of the wheat control soil (Table 2), which corroborates the contribution of biomarkers from different plant sources. The different plant sources of the biomarkers might also explain the ^{13}C depletion of the 9,10-epoxy,ω-$C_{18:0}$ in the 9 year maize soil at the 45–75 cm layers. The ^{13}C content of the *x*,ω-$C_{16:0}$ and 9-$C_{16:0}$DA were only slightly modified by 9 years of maize cropping (Table 2). As already suggested in the ploughed layer (0–25 cm) of this experimental field (Mendez-Millan et al., 2010a), maize shoot markers might be mainly degraded within one year after addition to soil. We thus suggest that a pool of *x*,ω-$C_{16:0}$ and 9-$C_{16:0}$DA present in the subsoils originates from the former forest vegetation and might thus be stabilized in deep soils on the long term. This is consistent with the unexpected observation of slight concentration changes of shoot markers after 9 years of maize cropping below 30 cm. The only ^{13}C enrichment of shoot markers after maize cropping was observed in the 15–45 cm depth. Below 45 cm, direct input of shoot biomass is unlikely, which might explain the lack of ^{13}C changes at these depths.

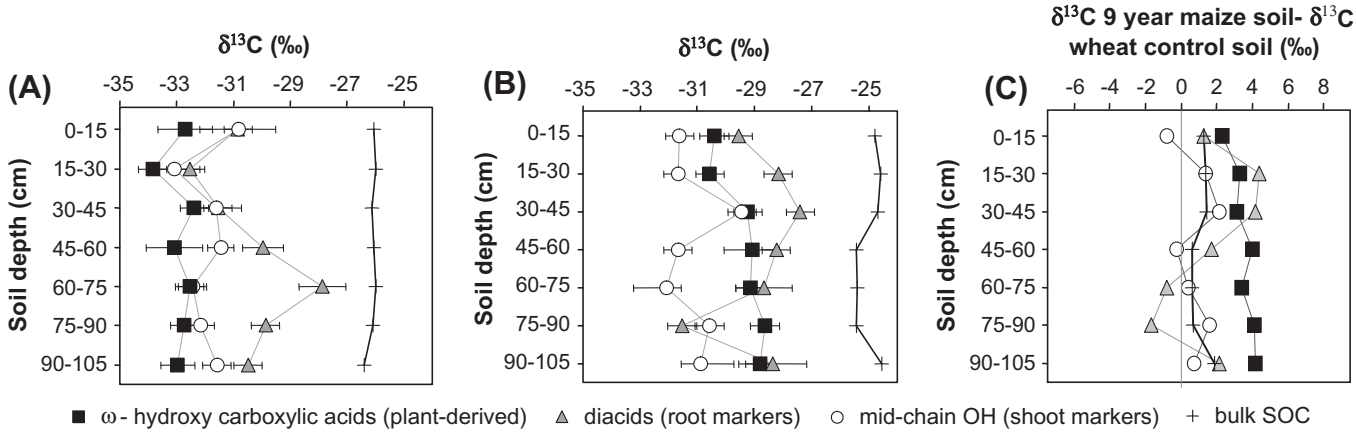

Fig. 5. Changes with soil depth of the ^{13}C isotopic signatures ($\delta^{13}C‰$) of the compound classes ω-hydroxy carboxylic acids, diacids (root markers) and mid-chain hydroxy acids (shoot markers) in the wheat control (A) and 9 year maize soil (B). The differences between the maize and the wheat soils are also presented (C). Data for aliphatic biomarkers are compared to data for bulk SOC reported from Rasse et al. (2006).

Maize shoot markers might be added through ploughing in the 15–45 cm layer of the maize soil, where they might be less degraded than in the upper layer, either due to lower microbial activity or to a higher protection with association with mineral. The shoot biomarkers present in subsoil horizons may be part of the highly stable aliphatic carbon fraction of soil as indicated by Lorenz et al. (2007).

Much higher concentrations of root markers below 15 cm were observed in the 9 year maize soil compared to the wheat control soil (Fig. 1). This difference could reflect a higher input of root biomass from maize compared to wheat, due to contrasting rooting behaviour of maize and wheat. Maize and wheat roots have been shown in many studies to have different densities especially below 30 cm (Aggarwal et al., 2006; Ju et al., 2007; Marx et al., 2007). In this experimental field, Rasse et al. (2006) observed that 70% of the total maize root biomass was in the first 45 cm of soil, while the remaining 30% was homogeneously distributed in the 45–90 cm layers. Higher inputs of root biomass from maize compared to wheat is also suggested by the ^{13}C enrichments of root markers in the 9 year maize soil observed between 15 and 45 cm (around 4‰) and at 45–60 and 90–105 cm (around 2‰) (Fig. 5C). However we cannot exclude that this higher content of root markers at depth in the 9 year maize plot compared to the wheat plot is due to spatial heterogeneity not accounted for by our sampling procedure.

Root rhizodeposition includes exudation, mucilage and progressive root biomass senescence (Rees et al., 2005). A recent study on grass incubations showed that microorganisms are mainly present within the rhizosphere, where they degrade fresh plant material and co-metabolize old organic matter (Wiesenberg et al., 2010). Outside the rhizosphere or far enough from the roots, there might be an accumulation of root markers after root death. The faster turnover of root biomass during plant growth and the possibly higher root density of maize compared to wheat could explain the high contribution of maize C to root markers throughout the soil profile after 9 years of maize cropping.

Marx et al. (2007) showed that maize root rhizodeposition induced a positive priming effect on SOM, when wheat and maize were cropped in experimental greenhouses. The input of maize derived carbon to the 9 year maize subsoils through rhizodeposits might have lead to the decomposition of the compounds of the soil profile originating from C_3 plants and could explain the drastic decrease of the concentrations of the shoot markers below 75 cm after 9 years of maize cropping (Fig. 2). This supposed priming effect induced by root deposition does not seem to affect root markers. This contrasted behaviour of root and shoot markers could arise either from a higher biochemical stability of root residues and/or from their lower accessibility to microorganisms through physical protection in the soil aggregates (Sollins et al., 1996, Rasse et al., 2005).

Between 60 and 90 cm, the concentrations of root markers were much higher in the 9 year maize soil compared to the wheat control soil (Fig. 1), while the $\delta^{13}C$ of the diacids did not increase from the wheat control to the 9 year maize soil (Fig. 5C). In these two layers, the higher concentrations of the diacids cannot be attributed to a high input of maize derived root material, since in the senescent maize plants diacids were less concentrated in maize than in wheat roots (Mendez-Millan et al., 2011) and since it should have induced an increase in the $\delta^{13}C$. This suggests the high heterogeneity of the root input throughout the plant growth. We measured the molecular and isotopic composition of plant biomass collected after the death of the plants (Mendez-Millan et al., 2011) in the state they are after harvest. However, the relative distribution of the structural units found in root suberized tissues as well as their isotopic signatures change with plant growth (Zeier et al., 1999). Contrary to shoot inputs, which are almost exclusively added to the soil after harvest and can be easily characterized and quantified, root contribution added during plant growth, when fine roots die, might vary in molecular and isotopic composition.

The concentrations of the ω-hydroxy acids below 15 cm were higher in the 9 year maize soil compared to the wheat control soil, the largest difference being observed at 60–75 cm. These plant markers were found in both shoots and roots of maize and wheat and could not be used as tracers of above or belowground biomass in the ploughed layer (Mendez-Millan et al., 2010a, 2011). However, these compounds were also major structural units of roots and were often used as root markers (Nierop, 1998). In wheat roots, the ω-hydroxy acids represented 57% of the total released monomers, and the ω-$C_{22:0}$ acid was the major monomer (18% of all monomers). In maize roots, the ω-hydroxy acids represented 50% of the total released monomers, and the concentration of the ω-$C_{24:0}$ acid was higher compared to that of the ω-$C_{22:0}$ acid (Mendez-Millan et al., 2011). The contrasted concentrations of these two major biomarkers in the wheat and maize root biomasses may explain the differences observed in the distribution of these two compounds from 0–90 cm in the 9 year maize and wheat control soils. In the 9 year maize soil, the stocks of ω-hydroxy acids were very similar to those of the shoot markers throughout the profile (Fig. 4B), suggesting that these compounds have a similar origin.

In the wheat control soil (Fig. 4A) the higher stocks of shoot markers below 45 cm suggests a higher input by the old C_3 forest.

5. Conclusions

Between 0 and 60 cm, the differences in $\delta^{13}C$ of the cutin and suberin structural units were in the same order as already observed in the ploughed layer. The cutin biomarkers in the wheat soil might mainly originate from the former C_3 forest present at this site before the set up of the C_3/C_4 chronosequence experiment. The high contribution of maize C to root markers throughout the soil profile after 9 years of maize cropping could be due to faster turnover of fine roots and possibly higher root density of maize compared to wheat. This study indicates that although the cutin and suberin biomarkers provide valuable information on the root and shoot turnover in the upper horizon (Mendez-Millan et al., 2010a; Feng et al., 2010), they are difficult to use in the deeper horizons for several reasons:

(1) The input in root biomarkers is more difficult to evaluate than that of shoot biomarkers because root input occurs continuously during plant growth, with potentially different molecular and isotopic composition from what is measured in the sampled roots.
(2) The rooting behaviour of wheat and maize (root density, fine root turnover, rhizodeposition and exudation) might be very different and induce large variations in the concentrations and distribution of structural units in subsoils, which makes it impossible to estimate turnover for these compounds.
(3) In deeper horizons, the large amounts and large variations with maize cropping of the structural units which were only identified in the shoots of maize and wheat suggests that maize and wheat plant biomasses are not the only sources of these compounds in subsoils. A major contribution might come from the former vegetation, for which biomarkers for roots and shoots might be different and might have contrasting behaviour compared to those of maize and wheat.
(4) In agricultural soils, ploughing makes the top layers of the soil more homogeneous. In deeper horizons, a higher soil heterogeneity, which was not taken into account by the sampling procedure, might be responsible for differences in biomarker concentrations in different soil profiles, even though they are located in the same experimental field.

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