Management effects on composition and dynamics of cutin and suberin in topsoil under agricultural use
C. M. Armas-Herrera, M.-F Dignac, C. Rumpel, C. D. Arbelo, A Chabbi

To cite this version:

HAL Id: hal-01306768
https://hal.sorbonne-universite.fr/hal-01306768
Submitted on 25 Apr 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Management effects on composition and dynamics of cutin and suberin in topsoil under agricultural use

C.M. Armas-Herrera\textsuperscript{a}, M.-F. Dignac\textsuperscript{a}, C. Rumpe\textsuperscript{a,b}, C.D. Arbelo\textsuperscript{c}, A. Chabbi\textsuperscript{a,b,d}

\textsuperscript{a}Unité Mixte de Recherche Institut d’écologie et des sciences de l’environnement de Paris, CNRS-UPMC-UPEC-INRA-IRD, Campus ParisAgroTech, Thiverval-Grignon, France,

\textsuperscript{b}Unité Mixte de Recherche Écologie fonctionnelle et écotoxicologie des agroécosystèmes, INRA-AgroParisTech, Campus ParisAgroTech, Thiverval-Grignon, France,

\textsuperscript{c}Departamento de Biología Animal, Edafología y Geología, Universidad de La Laguna, Spain, and

\textsuperscript{d}Unité de Recherche Prairies et Plantes Fourragères, INRA Poitou-Charentes, Lusignan, France

Correspondence: C.M. Armas-Herrera; E-mail: cmarmas@unizar.es

Running title: Cutin and suberin in agricultural soil

Keywords: shoots, roots, biomarkers, C\textsubscript{3}/C\textsubscript{4} chronosequence, \textsuperscript{13}C, bare soil

Highlights:

- Cutin and suberin monomers should meet certain criteria to be shoot and root markers in SOM studies
- Shoot and root markers should be characterised for each plant species to study their dynamic in soil
- The concentrations of cutin and suberin in agricultural soil depended on the amount of organic inputs
- Roots contributed more to SOM accumulation, but had a shorter residence time in soil than shoots
Summary

We identified and quantified specific biomarkers of shoots and roots (cutin and suberin-derived compounds, respectively) of three grassland species (*Dactylis glomerata* L., *Festuca arundinacea* Schreb. and *Lolium perenne* L.) in soil under different land use (grass, crop and bare soil) of the SOERE-ACBB experimental site in Lusignan (France). We also investigated the fate of these markers in soil after conversion from grassland (*C_3* plants) to *Zea mays* L. (maize) (*C_4* plant) from natural $^{13}$C isotope abundances. Our results indicated that 9-hydroxy hexadecanedioic acid and 8(9)(10),16-dihydroxy hexadecanoic acid may be used as biomarkers for aboveground biomass, whereas 1,22-docosandioic acid, 22-hydroxy docosanoic acid and 24-hydroxy tetracosanoic acid might be the best belowground biomarkers for the plants investigated under the experimental conditions studied. The presence, concentration and shoot–root allocation pattern of these markers were different from those described for other species, which demonstrates the importance of verifying biomarker specificity for each species. Concentrations of cutin and suberin were largest in soil under maize and smallest under bare soil; this corresponded to the biomass added to the two soils. Suberin decreased by 40–64 % and cutin by 24–40 % during a 6-year bare fallow, which indicates that root markers were more sensitive than shoot markers to degradation. Changes in $^{13}$C isotopic signatures of specific biomarkers after 6 years of maize showed a faster turnover of root than shoot biomarkers, in spite of the much smaller root inputs from maize than from grasses. The sequestration of suberin in soil was more rapid, but less durable than that of cutin.
**Introduction**

Soil organic matter (SOM) has beneficial effects on soil physical structure, water-retention capacity and plant nutrient availability. It consists of a heterogeneous mixture of substances that have a wide range of decomposability. Organic matter enters the soil from litter fall, root turnover and root exudates. Once incorporated into the mineral soil matrix, a major part of the organic matter is metabolized and mineralized by microorganisms. Another portion (approximately 30%) remains in soil for longer because of transformation and stabilization processes (von Lützow *et al.*, 2006). The amount of mineralized and stabilized organic matter may differ according to the environment and land use (Marschner *et al.*, 2008). Precise quantification and identification of the origin of labile and more stable SOM pools is necessary to improve the understanding of carbon cycling and the response of SOM to changing environmental conditions (Marschner *et al.*, 2008), in particular climate or land-use change. The conversion from native forest or pasture to arable crops has caused losses that range from –40 to –60% of original C stocks in soil, whereas an increase in soil C stocks has been reported after a change from crop to pasture and forest (+20–60%) (Guo & Gifford, 2002). The preservation of soil C stocks has focused on management practices, such as tillage (conventional or reduced) or no tillage, and soil cover (crop residues, catch crops, intercrops) or none. Many studies have concentrated on bulk C to investigate the effects of management on the formation of SOM (Dungait *et al.*, 2013). It is only recently that the relative contributions of shoots and roots to the SOM pool (Rasse *et al.*, 2005), the root and shoot turnover (Mendez-Millan *et al.*, 2011) or the pattern of biodegradation of different plant tissues (Clemente *et al.*, 2013) have been addressed at the molecular level.

Cutins and suberins are aliphatic plant biopolymers that occur in vascular plants and could be among the most recalcitrant plant macromolecules in soil. As a result, they might play an important role in enriching the slower cycling pool of SOM (Riederer *et al.*, 1993; Nierop *et
Cutins are embedded within intracuticular waxes and covered with epicuticular waxes to form the plant cuticle. The cuticle covers all aerial parts (leaves, fruits, flowers, seeds, and so on) of vascular plants and protects them from desiccation. Cutins are composed mainly of derivatives of saturated C$_{16}$ (palmitic) acid and C$_{18}$ acids, such as di- and tri-hydroxy and epoxy fatty acids, interlinked through ester bonds. Although cutin from most plants contains both the C$_{16}$ and C$_{18}$ groups, their individual composition varies according to the plant species, specific plant tissue, stage of development and environmental conditions (Kolattukudy, 2001; Kögel-Knabner, 2002; Otto & Simpson, 2006). Suberins are wall components of cork cells from which all protective and wound-healing layers of bark, woody stems and roots are composed. They are also in the endodermis and in the bundle sheath of grasses. Suberins are composed of an aliphatic polyester and a polyphenolic domain that are spatially segregated (Kolattukudy, 2001; Bernards, 2002; Kögel-Knabner, 2002). The most characteristic compounds of the aliphatic domain are a mixture of $\alpha,\omega$-dioic acids, $\omega$-hydroxy acids, very long chain fatty acids, mid-chain-oxidized fatty acids and esterified hydroxycinnamic acids; there is emerging evidence that glycerol is a major component of the aliphatic domain. In turn, the polyphenolic domain has been related to lignin, and more recently has been considered to be composed of a large amount of hydroxycinnamic acids and their derivatives, and monolignols (Bernards, 2002).

The dynamics of cutins and suberins may be studied by analysing their stable carbon isotope composition after extraction from soil under C$_3$–C$_4$ succession (Mendez-Millan et al., 2010a). Isotopic analyses have shown that shoot biomarkers in soil under continuous cropping are degraded rapidly, whereas root biomarkers are incorporated into SOM, which suggests their selective preservation (Mendez-Millan et al., 2011). Root biomarkers also contributed considerably to SOM from soil under pasture after forest conversion (Hamer et al., 2012). This suggests that the introduction of ley grasslands into cropping systems could increase
SOM content by the accumulation of root-derived C (Rasse et al., 2005). In this research, we addressed the effect of grassland management on the composition and turnover of root and shoot biomarkers in soil. The research took advantage of a long-term agricultural experiment investigating SOM dynamics after the introduction of grassland into the cropping cycle.

The aim of this research was to evaluate the dynamics of specific root and shoot biomarkers after land-use changes from grass to an arable land. For this we (i) determined the composition of cutins and suberins in above- and below-ground biomass of the three dominant grassland species, Dactylis glomerata L., Festuca arundinacea Schreb. and Lolium perenne L., (ii) investigated the composition of cutin and suberin in soil under different land uses (continuous and temporary grassland, arable and bare soil) and (iii) used natural $^{13}$C isotope abundances to follow the fate of specific markers in the cutins and suberins in soil after conversion from grassland ($C_3$ plants) to arable land ($C_4$ plants).

**Material and methods**

**Experimental site**

The fieldwork was conducted at Lusignan (46°25'12.91N"; 0°07"29.35'E) in western France. The site is part of a long-term field experiment initiated in 2005 (SOERE ACBB, Systems of Observation and Experimentation in Environmental Research- Agro-ecosystem, Biogeochemical Cycles and Biodiversity, [http://www.soere-acbb.com/](http://www.soere-acbb.com/)), which was designed to increase our understanding of the effects of temporary grassland management on the environmental outputs of mixing arable cropping and grasslands systems. This site had been under agricultural use for at least 200 years and before being cultivated, it supported an oak forest.

The experimental treatments (4000 m$^2$ plot size) were established in 2005 in a randomized block design with four blocks. They consisted of continuous grassland, continuous cropping
and temporary grassland including crop rotations of maize (*Zea mays* (L.)), wheat (*Triticum aestivum* (L.)) and barley (*Hordeum vulgare* (L.)) (Figure 1). Continuous cropping treatments were fertilized with N at rates adjusted to achieve the potential yield for each crop in this region. The two treatments with temporary grasslands consisted of rotations of maize, wheat and barley alternating with three or six years of grassland with a large application of N adjusted to achieve near maximum forage production. In addition, a continuous grassland composed of a mixture of *Festuca arundinacea* (Cv Soni), *Lolium perenne* (Cv Milca) and *Dactylis glomerata* (Cv Ludac) established with treatments that included large applications of N and no applications of N on a bare soil. Management of the crop rotation followed agricultural practices to achieve a yield close to the potential determined for the region by soil and climate. The rate and timing of N fertilizer application were adjusted every year with PC-AZOTE software (http://www.i-cone.fr/front/viewnode.aspx?typnode=4&idnode=60).

The soil at the site is a Plinthic Cambisol (IUSS Working Group WRB 2014) developed under a temperate climate from loess material over a Mesozoic tropical palaeosol. It has five soil horizons: a plough layer that overlies two red-brown upper horizons characterized by a loamy texture and two lower red clayey horizons rich in kaolinite, iron nodules and iron oxides. More detailed information about the soil and site characteristics is in Chabbi et al. (2009) and Moni et al. (2010).

**Sampling design and preparation of samples**

**Soil sampling.** Samples were taken in November 2011 after the conversion of 3- and 6-year grasslands into arable land (Figure 1). We sampled soil under (i) 6 years of continuous grassland, (ii) converted ley grassland of 6 years, (iii and iv) 6 years of continuous cropping maize, wheat and barley rotation or continuous maize, (v) converted ley grassland of 3 years after 3 years of crop rotation and (vi) 6-years of continuous bare soil. Three cores of soil were sampled at 0–30-cm depth from each plot and were mixed to obtain bulked samples.
Samples were air-dried, sieved at 2 mm and ground to pass a 100-µm sieve. The contents of organic carbon (OC) and nitrogen (N), and carbon isotopic signature ($\delta^{13}$C) of the soil samples are given in Table 1.

**Plant sampling.** Samples of the three grass species were collected in triplicate in November 2011. Shoots and roots were separated, dried at 60 °C and ground at 100 µm. Roots were taken in the field by manual separation and subsequently washed with deionized water. The contents of organic carbon (OC) and nitrogen (N), and carbon isotopic signature ($\delta^{13}$C) of the shoots and roots are listed in Table 1. The plant inputs to soil under different land use are summarized in Table 2.

**Analytical procedures**

**Extraction of free lipids.** To remove free lipids before the depolymerization of cutin and suberin biopolymesters, plant samples of 1 g or soil samples of 3 g were extracted with dichloromethane:methanol (DCM:MeOH) (1:2, volume:volume) at a 1:10 sample:extractant ratio. The suspensions were mixed with a vortex mixer for 30 s, agitated overhead for 2 hours and subsequently centrifuged at 2200 g for 10 minutes. This extraction was repeated. Thereafter, the samples were rinsed with DCM:MeOH (1:2, volume:volume) by means of mixing and centrifugation. The lipid-free samples were air-dried and kept until analysis.

**Saponification and derivatisation.** Saponification was used to release biomarkers of cutins and suberins, as suggested by Mendez-Millan et al. (2010b). A lipid-free plant sample of 100 mg or of 1 g of soil was refluxed for 18 hours in a solution of water:methanol (MeOH) (1:9, volume:volume) containing 6% of potassium hydroxide (KOH) (Cardoso & Eglinton, 1975). These conditions lead to the depolymerization of cutin and suberin. Hydroxylated fatty acids are released, and epoxy functions only are transformed into methoxy functions. Then, the solution was filtered (GF/A Whatman glass microfibre filters, 1.6 µm) with a Millipore vacuum filtration system (Darmstadt, Germany) and the residue was washed with
water:MeOH (1:9, volume:volume). The pH of the filtrate was adjusted to 2 with 6 N HCl after the addition of 150 ml of distilled water to isolate the acidic products (Naafs & Van Bergen, 2002). The acidified solution was extracted three times with 50 ml of dichloromethane (DCM; CH₂Cl₂). The volume of the extracts was reduced with a rotary evaporator and dried completely in a nitrogen atmosphere. All dried extracts were redissolved in 2 ml of DCM containing nonadecanoic acid (C₁₉₀₉) as an internal standard and then kept in the freezer until analysis. Prior to analysis, samples were derivatized by silylation to transform hydroxyl and carboxylic acid functions into their trimethylsilyl (TMS) ether and ester derivatives (TMS ether and TMS ester). An aliquot of each sample (0.2–1 ml) was dried in a nitrogen atmosphere, redissolved in 40 µl of pyridine and 10 µl of BSTFA (N,O-bis(trimethylsilyl)-trifluoroacetamide) that contained 1% TMCS (trimethylchlorosilane) and heated at 70 °C for 1 hour.

**Identification and quantification of cutin and suberin monomers**

The silylated monomers of cutin and suberin were identified according to their fragmentation pattern after analysis with an Agilent HP6890 gas chromatograph (Santa Clara, CA, USA) coupled to an Agilent HP5973 mass spectrometer (Santa Clara, CA, USA) (GC/MS) and compared with published mass spectra and with a mass spectral library (G1035B Wiley Mass Spectral Database) (Mendez-Millan et al., 2010a). One µl was injected in splitless mode at a temperature of 300 °C. The GC oven temperature was set at 100 °C for 2 minutes, then from 100 to 150 °C at 10 °C minute⁻¹, from 150 to 200 °C at 5 °C minute⁻¹ and finally at a rate of 2 °C minute⁻¹ from 200 to 350°C and kept for 5 minutes at 350 °C. Quantification of the monomers was done with a flame ionisation detector (FID) using the internal standard C₁₉₀₉ and an external calibration with 16-hydroxyhexadecanoic acid (ωOH C₁₆₀₉). The chromatographic conditions were the same as for the GC/MS analysis. We obtained a
response factor for the external standard $\omega$OH-C$_{16:0}$ relative to the internal standard C$_{19:0}$ close to 1.

**Compound specific isotopic analysis**

We measured the $\delta^{13}$C values (expressed in ‰ relative to Vienna PeeDee Belemnite) of individual compounds in the plant and soil samples from the 6-year grassland, 6-year maize and 6-year bare soil treatments. We did the analysis with an isotopic ratio mass spectrometer (Micromass-GVI Optima, Manchester, UK) coupled with a combustion interface to a GC (GC-C-IRMS), and used the same chromatographic conditions as for the identification and quantification of the monomers. The carbon atoms of BSTFA were assumed to have the same isotopic ratio as that reported by Dignac et al. (2005) in a previous study of lignin-derived phenols. We corrected the $\delta^{13}$C of the C introduced by the derivatisation process (C atoms from the trimethylsilyl groups) by measuring the $\delta^{13}$C off-line with an Elemental Analyser (NA 1500, Carlo Erba) coupled to the IRMS. The value obtained was used to correct the $^{13}$C concentrations of the cutin and suberin monomers in the samples, according to a mass balance equation, following the procedure of Dignac et al. (2005).

**Calculations**

**Suberin:cutin ratios.** We selected monomers that were specific to cutin (C), to suberin (S) or specific to both molecules (SC), and calculated the respective sums of their contents ($\Sigma$C, $\Sigma$S, $\Sigma$SC). We calculated the following suberin:cutin ratio adapted from Otto & Simpson (2006):

$$\text{Suberin:cutin ratio} = (\Sigma S + \Sigma SC/2) / (\Sigma C + \Sigma SC/2) \quad (1)$$

and the sum of suberin and cutin

$$\Sigma SC = \Sigma S + \Sigma C + \Sigma SC \quad (2)$$
Carbon isotopic signature (δ\(^{13}\)C)

We computed the δ\(^{13}\)C of each compound class (\(\delta_{\text{class}}\)) for both shoots and roots following Mendez-Millan et al. (2011). This δ\(^{13}\)C of individual compounds with similar chemical structure were weighted by their concentrations with the following equation:

\[
\delta_{\text{class}} = \frac{\sum_{i=1}^{N} (\delta_{i\text{comp}} \times C_{i\text{comp}})}{\sum_{i=1}^{N} C_{i\text{comp}}},
\]

(3)

where \(C_{i\text{comp}}\) is the concentration of the \(i\)th compound of the chemical class, \(\delta_{i\text{comp}}\) is the isotopic ratio of this compound and \(N\) is the number of individual compounds within each class.

The proportion, \(F\), of maize-derived C\(_4\)-C in soil after 6-years of conversion was calculated for each compound class according to the following equation from Balesdent & Mariotti (1996):

\[
F = \frac{(\delta^{13}C_{\text{maize-soil}} - \delta^{13}C_{\text{grassland-soil}})}{(\delta^{13}C_{\text{maize}} - \delta^{13}C_{\text{grasses}})}.
\]

(4)

The \(\delta^{13}C_{\text{grasses}}\) is the average value of \(\delta^{13}C\) of the three grasses studied.

Results and discussion

Identification of characteristic compounds for shoots and roots

The monomers obtained after saponification of the cutin and suberin biopolyesters were grouped into six chemical classes: \(n\)-carboxylic acids, \(n\)-alcohols, \(\alpha\)-hydroxy carboxylic acids, \(\omega\)-hydroxy carboxylic acids, mid-chain hydroxy acids and \(\alpha,\omega\)-alkanedioic acids. Because the \(n\)-carboxylic acids, \(n\)-alcohols and \(\alpha\)-hydroxy carboxylic acids (Appendix 1) can appear in plant polymers other than cutins and suberins (Otto et al., 2005; Amelung et al., 2008), they were not considered appropriate plant biomarkers.
We detected seven \(\omega\)-hydroxy carboxylic acids in the range \(C_{16}\) to \(C_{26}\) (Figures 2, 3). This chemical class was most abundant in grass roots, with a contribution of 46–49% to the total released monomers. Their abundance and contribution (13–17%) in the plant shoots were much smaller. The \(\omega\)-hydroxy carboxylic acids showed differences in their shoot–root allocation patterns. For example, the \(\omega C_{16:0}\) was almost twice as abundant in roots than in shoots; the \(\omega C_{18:0}\) was detected only in the shoots at very small concentrations, and the \(\omega\)-hydroxy carboxylic acids from \(C_{20:0}\) to \(C_{26:0}\), dominated by the \(\omega C_{22:0}\) and \(\omega C_{24:0}\), were about three to ten times more abundant in roots than in shoots. The \(\omega\)-hydroxy carboxylic acids with more than 20 atoms of C are generally considered to be more frequent in the suberins than in the cutins (Kolattukudy, 2001; Kögel-Knabner, 2002; Otto & Simpson, 2006), which is consistent with our results. Some authors also found a larger abundance of these compounds in the roots than in the shoots of other species (Spielvogel et al., 2014; Bull et al., 2000). However, irregular or non-significant plant allocation patterns have been reported (Hamer et al., 2012; Andreetta et al., 2013) or an even larger content of \(\omega\)-hydroxy carboxylic acids in shoots than in roots (Mendez-Millan et al., 2011, for wheat and maize, Figure 2).

We detected three \(\alpha,\omega\)-alkanedioic acids (\(C_{18:1}\), \(C_{20:0}\) and \(C_{22:0}\)) in grass roots, but none in shoots (Figures 2,4). Their concentrations and contribution to the total of monomers (5–7 %) were small. In maize, \(\alpha,\omega\)-alkanedioic acids were also exclusively present in roots and their concentrations were smaller than in the grasses (Mendez-Millan et al., 2010a). The \(C_{18:1}\) diacid was by far the most abundant \(\alpha,\omega\)-alkanedioic acid, which is the case for several plants (Otto & Simpson, 2006; Mendez-Millan et al., 2010a, 2011; Hamer et al., 2012; Spielvogel et al., 2014), whereas the \(C_{22:0}\) diacid was only slightly more abundant than \(C_{20:0}\). For this chemical class, there is general agreement about the exclusive presence of the \(\alpha,\omega\)-
alkanedioic acids in the suberins of plants (Kolattukudy, 2001; Kögel-Knabner, 2002; Otto & Simpson, 2006; Amelung et al., 2008; Mendez-Millan et al., 2010a).

We identified six mid-chain hydroxy acids, which were about five to seven times more abundant in the shoots than in the roots (relative abundance of 32–39 % and 9-11 %, respectively) (Figures 2,5). The 9,10-epoxy, 18-OH C$_{18:0}$ was predominant and was one of the major monomers released in the shoots of the three grasses. These compounds were detected in the shoots of maize only (Mendez-Millan et al., 2010a), and they were less abundant than in the grasses. The 9,10-epoxy, 18-OH C$_{18:0}$, the 9(10),16-diOH C$_{16:0}$ and the 9,10,18-triOH C$_{18:0}$ are considered the most common monomers in cutins (Kolattukudy, 2001). In our study, only the 9,10,18-triOH C$_{18:0}$ showed no differences in its concentration between shoots and roots in Dactylis glomerata. This is not an exception in the literature, other authors have also found an irregular pattern of distribution in certain mid-chain hydroxy acids, including the 9,10,18-triOH C$_{18:0}$, which has been found to be more abundant in the roots than in the shoots of several plant species (Hamer et al., 2012; Spielvogel et al., 2014). Moreover, our results showed that Dactylis glomerata and Lolium perenne released more aliphatic monomers and had larger suberin/cutin ratios than Festuca arundinacea (Table 3). The amount of monomers in these grasses contrasted with those found for different species (Mendez-Millan et al., 2010a; Hamer et al., 2012; Andreetta et al., 2013; Spielvogel et al., 2014). These findings emphasize the need to identify specific cutin and suberin biomarkers for each plant species in order to use them as indicators of relative fluctuations in above- and below-ground biomass in soil.

Differences in the $^{13}$C isotopic content of aliphatic monomers in plants

There were small differences in the $^{13}$C content between the classes of aliphatic monomers in the three grasses (Table 4, Figure 6): $^{13}$C content of the $\alpha$,ω-alkanedioic acids in Dactylis glomerata and Festuca arundinacea was slightly smaller than for the mid-chain hydroxy
acids and ω-hydroxy carboxylic acids, whereas *Lolium perenne* showed no clear differences. Most of the monomers in *Lolium perenne* were more $^{13}$C depleted than in the other two grasses. We found the biopolyesters of the grasses investigated to be $^{13}$C-depleted compared to the bulk plant tissues (Figure 6). Similar results were obtained by Mendez-Millan *et al.* (2011) and Hamer *et al.* (2012) for several plant species. This $^{13}$C depletion is common for lignins and lipids because of isotopic fractionation along transport pathways (Hobbie & Werner, 2004). The same explanation might be applied here to the monomers of cutin and suberin; they have similar biosynthetic pathways to extractable lipids. The $^{13}$C of the bulk samples of the grasses agree with those for some C$_3$ plants (Wiesenber & Schwark, 2006; Mendez-Millan *et al.*, 2011), such as ryegrass, oats, barley or wheat (from −28.1 to −32.3 ‰ on average). The isotopic signature of maize (C$_4$ plant) reported by the same authors was much larger (12.5 ‰ on average) than for C$_3$ plants. To the best of our knowledge, the $^{13}$C isotopic signature of different cutin and suberin biopolyesters has been determined for a few plant species only: wheat and maize (Mendez-Millan *et al.*, 2010a, 2011), the grass *Setaria sphaelata* (Schumach.) and the bracken fern *Pteridium arachnoideum* (Kaulf.) (Hamer *et al.*, 2012) and in the grasses investigated here. The differences in $^{13}$C content of the aliphatic monomers for different C$_3$ plants also demonstrates the need to study each plant species independently.

Criteria for selecting cutin and suberin biomarkers in soil

The fact that these aliphatic monomers are considered to be specific for cutin or suberin is not enough to consider them as adequate above- or below-ground biomass markers in soil. The $\omega$C$_{22:0}$ and $\omega$C$_{24:0}$ were the predominant $\omega$-hydroxy carboxylic acids (Figure 3) in the soils studied here. Their largest contents were in the 6-year maize crop and the smallest in the 6-year bare soil, with no clear pattern in the other soils. The same was true for the $\omega$C$_{16:0}$, $\omega$C$_{18:0}$ and $\omega$C$_{20:0}$. The $\omega$C$_{18:1}$ was not detected in the 6-year maize crop. Four alkanedioic
acids (C16:0, C18:1, C20:0, C22:0) were identified in soil under grass and crops (Figure 4). In the 6-year bare soil C22:0 only was detected. The largest values for the C16:0 diacid were in the 6-year maize crop. This monomer was not detected in the grasses but it is present in the roots of maize and wheat (Mendez-Millan et al., 2010a, 2011), which are the likely sources in our study. The C18:1 diacid, which was predominant among the alkanedioic acids of the grasses, had a much smaller relative abundance in soil. The total concentration of mid-chain hydroxy acids was much smaller in the 6-year bare soil than in the other soils. The 8(9)(10),16-diOH C16:0 diacid and the 9-OH C16:0 diacid were the most abundant compounds (Figure 5).

Moreover, in our study the relative contribution of the different compounds to the total of released monomers often varied considerably from plant to soil. These changes were almost exclusively the result of a drastic decrease in the relative abundance of a few monomers that contained either epoxy functions or double bonds: ωC18:1, C18:1 diacid, 11,18-diOH C18:1, 9(10),18-diOH C18:1 and 9,10-epoxy, 18-OH C18:0. In contrast, the 8(9)(10),16-diOH C16:0 and the 9-OH C16:0 diacid showed a larger relative distribution in soil (4.9–10.5 % and 2.7–4.2 %, respectively) than in the plant tissues (5.0–6.9 % and 0.7–1.1 %). The decrease in the double bond functions from plant to soil might be explained by their preferential degradation with respect to the saturated building blocks (Nierop, 2001; Nierop et al., 2003) because the epoxy groups are considered as first intermediates in the oxidation of double bonds (Watkinson & Morgan, 1990). There is more debate about the preservation of cutin and suberin monomers in soil. Some authors have reported a similar rate of decomposition irrespective of their chemical composition (e.g. Riederer et al. (1993) in an in vitro decomposition experiment of cutin in Fagus sylvatica (L.) leaves; Nierop et al. (2003), for suberin monomers in an oak forest), whereas other authors have proposed different mechanisms for preservation of these monomers in soil. Mendez-Millan et al. (2011) suggested chemical recalcitrance for some cutin monomers and soil physical protection for suberin in soils cultivated with wheat and
maize, whereas Hamer et al. (2012) found that organo-mineral interactions were responsible for the long-term survival of several cutin and suberin monomers in soil under different land use, and chemical recalcitrance for the \(x,16\text{-diOH C}_{16:0}\).

We suggest that for cutin or suberin to be considered as a useful marker to study SOM dynamic, each compound should meet the following criteria: (i) significant differences in their amounts between shoots and roots and (ii) adequate concentrations in the soil. Our results suggested that the \(9\text{-OH C}_{16:0}\) diacid and \(8(9)(10),16\text{-diOH C}_{16:0}\) were the most appropriate aboveground biomarkers for grassland species. On the other hand, the \(\omega\text{C}_{22:0}\) diacid, the \(\omega\text{C}_{24:0}\), the \(\omega\text{C}_{20:0}\) and to a lesser extent the \(\omega\text{C}_{26:0}\) were the most useful belowground biomass biomarkers for Dactylis glomerata, Festuca arundinacea and Lolium perenne.

Dynamics of cutin and suberin biomarkers in soil under different land use

The concentrations of cutin and suberin in soil (Table 3) were related to the amount of organic inputs into the soil (Table 2); their largest concentrations were in soil cultivated with maize and their smallest were in bare soil. The largest amounts of cutin and suberin in the soil under 6-year maize cultivation is easily explained by the larger maize biomass inputs (mostly from shoots) than for wheat, barley and the grasses. The large suberin/cutin ratio in the 6-year maize crop probably results from the larger amounts of \(\omega\)-hydroxy carboxylic acids in the shoots than in the roots of maize (Mendez-Millan et al., 2010a). In general, we found no marked differences in the concentrations of cutin and suberin monomers between soil under continuous and temporary grasslands. Grass species are characterized by a dense root system in the topsoil, therefore most of their residues that enter the soil come from roots (Table 2).

Because the grasses are harvested, the addition of leaf litter to soil is limited to around 20 % (Sanaullah et al., 2010). At the same experimental site, Rumpel et al. (2009) and Rumpel & Chabbi (2010) found a rapid decrease in SOM and a change in its composition three months
after conversion from grassland to cropland. However, this disturbance of SOM composition was transitory and one year only after conversion the chemical characteristics of SOM returned to their initial status. Our results corroborate this finding for cutin and suberin because we found no difference in their concentrations between the continuous and temporary crop rotation and grasslands. The most prominent change in the concentration of monomers was in the 6-year bare soil where they decreased from 40 to 64 % for suberin and from 24 to 40 % for cutin. There was also a smaller suberin/cutin ratio (1.01) than for the other soil uses (1.12–1.54). Suberin is considered to be more resistant to degradation than cutin because of the larger concentration of aromatic compounds from its polyphenolic domain (Riederer et al., 1993; Nierop et al., 2003; Otto & Simpson, 2006). The residence time of organic compounds in soil, however, depends more on their susceptibility to physicochemical stabilization through incorporation into soil aggregates or chemical interactions with the mineral phase or both than on their chemical composition (Marschner et al., 2008). Cutin and suberin compounds sorb strongly to clay mineral surfaces (Feng et al., 2005; Simpson et al., 2006). In a long-term bare-fallow experiment (Closeaux experiment, from 1928, in Versailles, France), the clay–SOM association in macroaggregates was the most important sink for stabilized organic C (Balabane & Plante, 2004). In our study, cutins seem either to be more protected from biodegradation than suberins in the 6-year bare soil, or to have more effective mechanisms of stabilization than those for suberins. Thus, root markers seem to be more sensitive than shoot markers to microbial degradation.

**Turnover rate of shoot and root-derived organic matter in soils after land-use change**

The $^{13}$C concentrations of the monomers of cutins and suberins in soil was measured only in the 6-year continuous grassland, 6-year maize crop and 6-year bare soil. In the rotation with crops, the $^{13}$C signal of biomass input changes every year because both C$_3$ and C$_4$ plants are present, which prevents the fate of these plant biomarkers in soil from being followed.
The average isotopic signature of the hydroxy carboxylic acids was largest in the 6-year maize crop and tended to be slightly smaller in the 6-year bare soil and the 6-year continuous grassland soil (Table 4, Figure 6). The $^{13}$C content varied according to the type of monomer. For the 6-year maize crop, the largest enrichment in $^{13}$C compared to the grassland soil was in $\omega$-C$_{22:0}$, followed by $\omega$-C$_{24:0}$. The $^{13}$C was more depleted in $\omega$-C$_{20:0}$, $\omega$-C$_{24:0}$ and $\omega$-C$_{18:1}$ for the 6-year bare soil than for the cropland soil. The largest average content of $^{13}$C in the $\alpha,\omega$-alkanedioic acids was for the 6-year bare soil (Table 4, Figure 6). The 6-year maize had intermediate values and the smallest average content was for the 6-year continuous grassland soil. The C$_{16:0}$ and C$_{22:0}$ diacids were $^{13}$C-enriched more in the 6-year maize crop than in the grassland soil. The C$_{22:0}$ diacid was the only $\alpha,\omega$-alkanedioic acid detected in the 6-year bare soil and it had the largest $^{13}$C content. Lastly, the C$_{20:0}$ diacid had a slightly larger isotopic signature in the 6-year continuous grassland than for the 6-year maize crop. The average $^{13}$C content of the mid-chain hydroxy acids was similar under the 6-year maize crop and 6-year continuous grassland soil, with a slightly smaller value in the 6-year bare soil (Table 4, Figure 6). The isotopic signature of each monomer was somewhat irregular: the 8(9)(10),16-diOH C$_{16:0}$, 9-OH C$_{16:0}$ diacid and 9,10-epoxy, 18-OH C$_{18:0}$ were $^{13}$C-enriched only slightly more in the soil of the 6-year maize crop than for the 6-year continuous grassland, whereas 9,10,18-triOH C$_{18:0}$ was strongly $^{13}$C-enriched (Table 4, Figure 6). These results should be treated with caution because we do not know the effect of the methylated hydroxy group on the epoxy functions. In all cases, the smallest $^{13}$C contents were for the 6-year bare soil.

We calculated the proportion of cutin and suberin markers with Equation (4) that are maize-derived 6 years after maize was introduced on land that had been cultivated previously with C$_3$ plants. This enabled us to estimate the rate of turnover of shoot- and root-derived OM in soil. The rate was large, about 29 %, for the $\alpha,\omega$-alkanedioic acids, whereas it was much less for the $\omega$-hydroxy carboxylic acids (8 %) and the mid-chain hydroxy acids (5 %). The small
rate of incorporation of both ω-hydroxy carboxylic acids and mid-chain hydroxy acids into SOM suggests that they are degraded faster than α,ω-alkanedioic acids, in spite of the increase in shoot inputs after conversion from grassland to maize crop (Table 2). Hamer et al. (2012) found that about 75% of newly introduced C₄-ω-hydroxy carboxylic acids was decomposed 15 years after the pasture was abandoned. This relatively small rate of biodegradability was explained by the binding of these compounds to soil minerals (Hamer et al., 2012). The mid-chain hydroxy acids, however, decompose rapidly in soil (Mendez-Millan et al., 2011). The small variation in the isotopic signature after 6 years of maize suggest that at least a fraction of these compounds was stabilized previously in the soil that might have come from the ancient forest vegetation (Mendez-Millan et al., 2011; Hamer et al., 2012). In summary, our finding suggests that root C contributed more to the SOM accumulation. However, root C showed a shorter residence time in soil compared to shoot C. Thus, the sequestration of suberin in soils was less durable than that of cutin.

Conclusions

There were marked differences in monomer composition, abundance and patterns of shoot–root allocation of cutin and suberin in the plant species analysed (Dactylis glomerata, Festuca arundinacea and Lolium perenne) than for other plant species. These results emphasize the need to identify specific cutin and suberin biomarkers for each plant species to study the incorporation of their biomass into SOM.

To be shoot and root markers cutin and suberin monomers should have strong differences in their shoot and root concentrations and measurable concentrations in soil. According to these criteria, 9-hydroxy hexadecanedioic and 8(9)(10),16-dihydroxy hexadecanoic acids can be used as aboveground biomarkers, and 1,22-docosandioic, 22-hydroxy docosanoic and 24-hydroxy tetracosanoic acids for belowground biomarkers for the plants investigated.
The concentrations of cutin and suberin in soil were related to the amount of organic inputs. We found no differences in the amounts of cutin and suberin in soil under continuous and temporary grassland, which might indicate that the disturbance caused by conversion from grassland to cropland was transitory only. For bare soil, suberin decreased by 40–64 % and cutin by 24–40 % during a 6-year fallow, which indicated that root markers were more sensitive to degradation than shoot markers.

The changes detected in the $^{13}$C isotopic signatures of specific biomarkers after 6 years of maize cropping showed that incorporation into SOM was greater for roots than for shoot markers, in spite of the much smaller root inputs from maize than from grasses. Roots contributed more to SOM accumulation, but had a shorter residence time in soil than shoots. The sequestration of suberin in soil was more rapid, but less durable than that of cutin.

To specify the mechanisms and processes that lead to the turnover of cutin and suberin monomers with land-use changes, future studies should focus on the organo-mineral associations to protect these macromolecules in soil.

Acknowledgements

The authors thank the European Commission for funding under the transnational access of the European Project EXPEER (Distributed Infrastructure for EXPERimentation in Ecosystem Research; Grant Agreement Number: 262060). Also, C.M. Armas-Herrera thanks the European Science Foundation for funding under the framework of the MOLTER program. Moreover, we thank the SOERE ACBB for access to the experimental site and for providing data on biomass input and site characteristics.

Figure captions

Figure 1. Sequence of soil cover for the various treatments.
**Figure 2.** Concentrations (C /µg g⁻¹) of plant-specific monomers in shoots and roots of *Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Lolium perenne* L., and *Zea mays* L.

The standard errors were calculated from three replicates.

**Figure 3.** Concentrations (C /µg g⁻¹) of ω-hydroxy carboxylic acids in shoots and roots of *Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Lolium perenne* L., and *Zea mays* L., and in soil at the SOERE-ACBB site in Lusignan (France)

The standard errors were calculated from three replicates.

**Figure 4.** Concentrations (C /µg g⁻¹) of α,ω-alkanedioic acids in shoots and roots of *Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Lolium perenne* L., and *Zea mays* L., and in soil at the SOERE-ACBB site in Lusignan (France)

The standard errors were calculated from three replicates.

**Figure 5.** Concentrations (C /µg g⁻¹) of mid-chain hydroxy acids in shoots and roots of *Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Lolium perenne* L., and *Zea mays* L., and in soil at the SOERE-ACBB site in Lusignan (France)

The standard errors were calculated from three replicates.

**Figure 6.** Bulk and molecular isotopic signatures in the grasses, maize and soil. The values for the compound classes are calculated as the average of the signatures of the monomers weighted by their concentration.
References


<table>
<thead>
<tr>
<th>Plants</th>
<th>C /mg g⁻¹</th>
<th>N /mg g⁻¹</th>
<th>C/N</th>
<th>δ¹³C /‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dactylis glomerata</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>384 (29)</td>
<td>22.8 (1.4)</td>
<td>16.9 (0.8)</td>
<td>−26.7 (0.1)</td>
</tr>
<tr>
<td>Root</td>
<td>350 (34)</td>
<td>11.5 (1.5)</td>
<td>27.8 (0.8)</td>
<td>−26.2 (0.3)</td>
</tr>
<tr>
<td>Festuca arundinacea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>389 (17)</td>
<td>16.3 (0.3)</td>
<td>23.7 (1.8)</td>
<td>−26.1 (0.2)</td>
</tr>
<tr>
<td>Root</td>
<td>375 (8)</td>
<td>10.6 (0.7)</td>
<td>35.5 (2.4)</td>
<td>−26.3 (0.4)</td>
</tr>
<tr>
<td>Lolium perenne</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>385 (27)</td>
<td>27.9 (6.4)</td>
<td>15.5 (0.5)</td>
<td>−28.5 (0.4)</td>
</tr>
<tr>
<td>Root</td>
<td>332 (18)</td>
<td>13.2 (0.8)</td>
<td>25.2 (0.2)</td>
<td>−28.4 (0.1)</td>
</tr>
<tr>
<td>Zea mays*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk plant</td>
<td>425</td>
<td>na</td>
<td>na</td>
<td>−12.5</td>
</tr>
<tr>
<td>Soil (0–30 cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-year continuous grassland</td>
<td>11.2 (0.6)</td>
<td>1.20 (0.0)</td>
<td>9.35 (0.2)</td>
<td>−25.7 (0.2)</td>
</tr>
<tr>
<td>6-year ley grassland</td>
<td>11.3 (1.3)</td>
<td>1.24 (0.1)</td>
<td>9.10 (0.5)</td>
<td>−25.8 (0.2)</td>
</tr>
<tr>
<td>6-year crop rotation</td>
<td>10.1 (1.3)</td>
<td>1.11 (0.1)</td>
<td>9.06 (0.2)</td>
<td>−24.9 (0.2)</td>
</tr>
<tr>
<td>6-year maize crop</td>
<td>11.0 (0.4)</td>
<td>1.23 (0.1)</td>
<td>9.59 (0.1)</td>
<td>−25.4 (0.3)</td>
</tr>
<tr>
<td>3-year ley grassland</td>
<td>11.0 (0.7)</td>
<td>1.08 (0.1)</td>
<td>9.47 (0.4)</td>
<td>−25.2 (0.0)</td>
</tr>
<tr>
<td>6-year bare soil</td>
<td>8.29 (0.5)</td>
<td>0.96 (0.1)</td>
<td>8.59 (0.2)</td>
<td>−25.1 (0.2)</td>
</tr>
</tbody>
</table>

*Data from Mendez-Millan et al. (2011)

n = 3; SD is in parentheses, na = not analysed
Table 2 Mean annual sources of shoot and root into soil under different management for the study period (2005–2011) at the SOERE-ACBB site in Lusignan (France) (SD in parentheses, n = 4)

<table>
<thead>
<tr>
<th></th>
<th>6-year continuous grassland</th>
<th>6-year ley grassland rotation</th>
<th>6-year maize crop</th>
<th>3-year ley grassland</th>
<th>6-year bare soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot /t ha⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grasses</td>
<td>1.93*</td>
<td>1.94*</td>
<td>0</td>
<td>0</td>
<td>2.29 (0.66)*</td>
</tr>
<tr>
<td>Maize</td>
<td>0</td>
<td>0</td>
<td>14.8 (2.74)</td>
<td>14.8 (2.7)</td>
<td>8.02 (0.35)</td>
</tr>
<tr>
<td>Wheat</td>
<td>0</td>
<td>0</td>
<td>1.62 (0.12)</td>
<td>0</td>
<td>1.86 (0.21)</td>
</tr>
<tr>
<td>Barley</td>
<td>0</td>
<td>0</td>
<td>1.19 (0.08)</td>
<td>0</td>
<td>1.56 (0.11)</td>
</tr>
<tr>
<td><strong>Shoot weighted average</strong></td>
<td>1.93</td>
<td>1.94</td>
<td>5.87 (0.98)</td>
<td>14.8 (2.7)</td>
<td>1.14 (0.11)</td>
</tr>
<tr>
<td>Root /t ha⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grasses</td>
<td>7.63 (1.47)</td>
<td>8.46 (2.88)</td>
<td>0</td>
<td>0</td>
<td>9.21 (1.04)</td>
</tr>
<tr>
<td>Maize</td>
<td>0</td>
<td>0</td>
<td>1.09 (0.70)</td>
<td>1.09 (0.70)</td>
<td>4.32 (2.54)</td>
</tr>
<tr>
<td>Wheat</td>
<td>0</td>
<td>0</td>
<td>1.06 (0.32)</td>
<td>0</td>
<td>0.47 (0.05)</td>
</tr>
<tr>
<td>Barley</td>
<td>0</td>
<td>0</td>
<td>0.99 (0.23)</td>
<td>0</td>
<td>0.66 (0.17)</td>
</tr>
<tr>
<td><strong>Root weighted average</strong></td>
<td>7.63 (1.47)</td>
<td>8.46 (2.88)</td>
<td>1.04 (0.41)</td>
<td>1.09 (0.70)</td>
<td>4.72 (1.43)</td>
</tr>
</tbody>
</table>

*These values correspond to 20% of total shoot biomass because of removal by mowing the grasses. Source: Sanaullah et al. (2010), at the same experimental site
Table 3 Cutin and suberin concentrations and calculated ratios for plants and soil

<table>
<thead>
<tr>
<th></th>
<th>aΣC Cutin C /µg g⁻¹</th>
<th>bΣS Suberin C /µg g⁻¹</th>
<th>cΣCS Cutin+suberin C /µg g⁻¹</th>
<th>dSuberin + cutin ΣSC C /µg g⁻¹</th>
<th>eSuberin / cutin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dactylis glomerata</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoots</td>
<td>9574 (446)</td>
<td>911 (42)</td>
<td>3877 (159)</td>
<td>14,361 (551)</td>
<td>0.25 (0.01)</td>
</tr>
<tr>
<td>Roots</td>
<td>1239 (150)</td>
<td>639 (279)</td>
<td>3950 (375)</td>
<td>11,583 (765)</td>
<td>2.61 (0.13)</td>
</tr>
<tr>
<td><em>Festuca arundinacea</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoots</td>
<td>6940 (536)</td>
<td>721 (101)</td>
<td>3950 (375)</td>
<td>11,620 (662)</td>
<td>0.30 (0.03)</td>
</tr>
<tr>
<td>Roots</td>
<td>1301 (73)</td>
<td>541 (763)</td>
<td>5282 (394)</td>
<td>11,994 (862)</td>
<td>2.05 (0.22)</td>
</tr>
<tr>
<td><em>Lolium perenne</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoots</td>
<td>9341 (632)</td>
<td>891 (113)</td>
<td>3796 (374)</td>
<td>14,028 (930)</td>
<td>0.25 (0.01)</td>
</tr>
<tr>
<td>Roots</td>
<td>1186 (173)</td>
<td>7305 (763)</td>
<td>3835 (90)</td>
<td>12,325 (624)</td>
<td>2.99 (0.38)</td>
</tr>
<tr>
<td><strong>Soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-year continuous grassland</td>
<td>2258 (279)</td>
<td>3209 (276)</td>
<td>1128 (200)</td>
<td>6595 (259)</td>
<td>1.27 (0.00)</td>
</tr>
<tr>
<td>6-year ley grassland</td>
<td>2152 (257)</td>
<td>2451 (294)</td>
<td>1093 (39)</td>
<td>5696 (77)</td>
<td>1.12 (0.21)</td>
</tr>
<tr>
<td>6-year crop rotation</td>
<td>1920 (72)</td>
<td>2463 (198)</td>
<td>925 (61)</td>
<td>5308 (209)</td>
<td>1.23 (0.05)</td>
</tr>
<tr>
<td>6-year maize crop</td>
<td>2409 (214)</td>
<td>4061 (168)</td>
<td>1338 (110)</td>
<td>7808 (260)</td>
<td>1.54 (0.15)</td>
</tr>
<tr>
<td>3-year ley grassland</td>
<td>2340 (77)</td>
<td>3108 (65)</td>
<td>1111 (163)</td>
<td>6560 (22)</td>
<td>1.27 (0.00)</td>
</tr>
<tr>
<td>6-year bare soil</td>
<td>1457 (129)</td>
<td>1461 (71)</td>
<td>707 (79)</td>
<td>3625 (137)</td>
<td>1.01 (0.11)</td>
</tr>
</tbody>
</table>

a) Cutin ΣC (µg g⁻¹ C) = 8(9)(10),16-dioH C₁₆:₀ + 9-OH C₁₆:₀ diacid + 11,18-dioH C₁₈:₁ + 9,10-epoxy, 18-OH C₁₈:₀  
b) Suberin ΣS (µg g⁻¹ C) = ωC₂₀:₀ + ωC₂₂:₀ + ωC₂₄:₀ + ωC₂₆:₀ + C₁₆:₀ diacid + C₁₈:₁ diacid + C₂₀:₈ diacid + C₂₂:₀ diacid  
c) Suberin or cutin ΣSC (µg g⁻¹ C) = ωC₁₆:₀ + ωC₁₈:₀ + ωC₁₈:₁ + 9(10),18-dioH C₁₈:₁ + 9,10,18-trioH C₁₈:₀  
d) Suberin/cutin ratio = (ΣS + ΣSC/2) / (ΣC + ΣSC/2)  
e) Sum of suberin and cutin ΣSC (µg g⁻¹ C) = ΣS + ΣC + ΣSC
Table 4 Carbon isotopic signature (δ¹³C) values of the aliphatic monomers of cutins and suberins present in grassland, crop plants and soil at the SOERE-ACBB site in Lusignan (France)

<table>
<thead>
<tr>
<th></th>
<th>Dactylis glomerata</th>
<th>Festuca arundinacea</th>
<th>Lolium perenne</th>
<th>Zea maysa</th>
<th>Soil %δ ¹³C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td>Bulk plant</td>
</tr>
<tr>
<td>o-Hydroxy carboxylic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-Hydroxy hexadecanoic acid (oC₁₆:0)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>−17.1 (0.9)</td>
</tr>
<tr>
<td>18-Hydroxy octadecanoic acid (oC₁₈:0)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>−36.0 (0.3)</td>
</tr>
<tr>
<td>18-Hydroxy octadecenoic acid (oC₁₈:1ω)</td>
<td>−35.5</td>
<td>−35.2</td>
<td>−34.3</td>
<td>−34.7</td>
<td>−36.2</td>
</tr>
<tr>
<td>20-Hydroxy eicosanoic acid (oC₂₀:0)</td>
<td>−36.2</td>
<td>−34.8</td>
<td>−37.8</td>
<td>na</td>
<td>−35.5 (0.6)</td>
</tr>
<tr>
<td>22-Hydroxy docosanoic acid (oC₂₂:0)</td>
<td>−36.9</td>
<td>−36.8</td>
<td>−32.6</td>
<td>−38.2</td>
<td>−37.9</td>
</tr>
<tr>
<td>24-Hydroxy tetracosanoic acid (oC₂₄:0)</td>
<td>−36.5</td>
<td>−29.5</td>
<td>−30.3</td>
<td>−36.6</td>
<td>−37.8</td>
</tr>
<tr>
<td>26-Hydroxy hexacosanoic acid (oC₂₆:0)</td>
<td>−28.0</td>
<td>−33.0</td>
<td>−34.6</td>
<td>−19.1 (0.8)</td>
<td>na</td>
</tr>
<tr>
<td>Weighted average for o-Hydroxy carboxylic acids</td>
<td>−35.7</td>
<td>−34.2</td>
<td>−33.7</td>
<td>−35.4</td>
<td>−36.3</td>
</tr>
</tbody>
</table>

a,ω-Alkanedioic acids

<table>
<thead>
<tr>
<th></th>
<th>Dactylis glomerata</th>
<th>Festuca arundinacea</th>
<th>Lolium perenne</th>
<th>Zea maysa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,16-Hexadecadioic acid (C₁₆:₀ diacid)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>−15.1 (0.5)</td>
</tr>
<tr>
<td>1,18-Octadecadiioic acid (C₁₈:₁ diacid)</td>
<td>−35.4</td>
<td>na</td>
<td>−36.3</td>
<td>−36.2</td>
</tr>
<tr>
<td>1,20-Neodecanedioic acid (C₂₀:₀ diacid)</td>
<td>−36.6</td>
<td>−33.1</td>
<td>−31.3</td>
<td>na</td>
</tr>
<tr>
<td>1,22-Docosadiioic acid (C₂₂:₀ diacid)</td>
<td>−34.9</td>
<td>−37.5</td>
<td>−38.7</td>
<td>na</td>
</tr>
<tr>
<td>Weighted average for a,ω-alkanedioic acids</td>
<td>−35.4</td>
<td>−36.2</td>
<td>−36.1</td>
<td>na</td>
</tr>
</tbody>
</table>

Mid-chain hydroxy acids

<table>
<thead>
<tr>
<th></th>
<th>Dactylis glomerata</th>
<th>Festuca arundinacea</th>
<th>Lolium perenne</th>
<th>Zea maysa</th>
</tr>
</thead>
<tbody>
<tr>
<td>8(9)/10,16-Dihydroxy hexadecanoic acid (8/9(10)16-oh C₁₆:₀)</td>
<td>−34.6</td>
<td>−32.4</td>
<td>−34.7</td>
<td>−30.8</td>
</tr>
<tr>
<td>9-Hydroxy hexadecanedioic acid (9-OH C₁₆:₀ diacid)</td>
<td>na</td>
<td>na</td>
<td>−34.7</td>
<td>−33.9</td>
</tr>
<tr>
<td>11,18-Dihydroxyoctadecenoic acid (11,18-diOH C₁₈:₁)</td>
<td>−35.9</td>
<td>−37.4</td>
<td>−35.8</td>
<td>−37.5</td>
</tr>
<tr>
<td>9(10),18-Dihydroxyoctadecenoic acid (9(10),18-diOH C₁₈:₁)</td>
<td>−33.6</td>
<td>−29.6</td>
<td>−34.7</td>
<td>−36.7</td>
</tr>
<tr>
<td>9,10-Epoxy, 18-hydroxyoctadecanoic acid (9,10-epoxy, 18-OH C₁₈:₀)</td>
<td>−34.2</td>
<td>−30.4</td>
<td>−34.9</td>
<td>−34.7</td>
</tr>
<tr>
<td>9,10,18-Trihydroxyoctadecanoic acid (9,10,18-triOH C₁₈:₀)</td>
<td>−34.6</td>
<td>−36.8</td>
<td>−39.4</td>
<td>−37.3</td>
</tr>
<tr>
<td>Weighted average for mid-chain hydroxy acids</td>
<td>−34.4</td>
<td>−33.1</td>
<td>−35.4</td>
<td>−34.8</td>
</tr>
</tbody>
</table>

aData from Mendez-Millan et al. (2011); bPosition of the double bond not confirmed; cIsomeric mixture of 9-methoxy, 10,18-dihydroxyoctadecanoic acid and 9-hydroxy, 10-methoxy, 18-hydroxyoctadecanoic acids

(SD in parentheses, n = 2, na = not analysed)
<table>
<thead>
<tr>
<th>Crop Type</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous cropping</td>
<td>M</td>
<td>W</td>
<td>B</td>
<td>M</td>
<td>W</td>
<td>B</td>
<td>M</td>
</tr>
<tr>
<td>Ley grassland</td>
<td>M</td>
<td>W</td>
<td>B</td>
<td>Grass 3 years</td>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ley grassland</td>
<td>Grass 6 years</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bare soil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous grassland</td>
<td>L, F, D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M = maize, W = wheat, B = barley
L = Lolium, F = Festuca, D = Dactylis

Figure 1
ω-carboxylic acids  Mid-chain hydroxy acids  Diacids

Concentration in the C-input to soil (C plant/μg g⁻¹)

Dactylis glomerata  Festuca arundinacea  Lolium perenne  Zea mays
Dactylis glomerata  Festuca arundinacea  Lolium perenne  Zea mays
Dactylis glomerata  Festuca arundinacea  Lolium perenne  Zea mays
Dactylis glomerata  Festuca arundinacea  Lolium perenne  Zea mays

Root  Shoot

Figure 2
Figure 3

(a) Shoot

-ω-C16:0
-ω-C18:1
-ω-C18:0
-ω-C20:0
-ω-C22:0
-ω-C24:0
-ω-C26:0

(b) Root

-ω-C16:0
-ω-C18:1
-ω-C18:0
-ω-C20:0
-ω-C22:0
-ω-C24:0
-ω-C26:0

(c) Soil

-6-year continuous grassland
-6-year ley grassland
-3-year ley grassland
-6-year crop rotation
-6-year maize crop
-6-year bare soil
Figure 4

(a) Root

- **C / μg g⁻¹**
- **Y-axis**: 0 to 2500
- **X-axis**: C₁₆:0 DA, C₁₈:1 DA, C₂₀:0 DA, C₂₂:0 DA

(b) Soil

- **C / μg g⁻¹**
- **Y-axis**: 0 to 250
- **X-axis**: C₁₆:0 DA, C₁₈:1 DA, C₂₀:0 DA, C₂₂:0 DA

Legend:
- Dactylis glomerata
- Festuca arundinacea
- Lolium perenne

- 6-year continuous grassland
- 6-year ley grassland
- 3-year ley grassland
- 6-year crop rotation
- 6-year maize crop
- 6-year bare soil
(a) Shoot

(b) Root

(c) Soil

Figure 5
Fig. 6

Grassland plants

Soil

Maize plant

% δ¹³C

Dactylis glomerata
Festuca arundinacea
Lolium perenne

6-year grassland
6-year maize
6-year bare soil

 bulk sample
ω-OH carboxylic acids
mid-chain OH acids
α,ω-alkanedioic acids