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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 biopolyesters 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*

al., 2003; Mendez-Millan *et al*., 2011). 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 α,ω-dioic acids, ω-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/), 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 palaeosoil. 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.

148 Samples were air-dried, sieved at 2 mm and ground to pass a 100-µm sieve. The contents of
149 organic carbon (OC) and nitrogen (N), and carbon isotopic signature ($\delta^{13}C$) of the soil
150 samples are given in Table 1.

151 *Plant sampling.* Samples of the three grass species were collected in triplicate in November
152 2011. Shoots and roots were separated, dried at 60 ºC and ground at 100 µm. Roots were
153 taken in the field by manual separation and subsequently washed with deionized water. The
154 contents of organic carbon (OC) and nitrogen (N), and carbon isotopic signature ($\delta^{13}C$) of the
155 shoots and roots are listed in Table 1. The plant inputs to soil under different land use are
156 summarized in Table 2.

157 *Analytical procedures*

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

165 *Saponification and derivatisation.* Saponification was used to release biomarkers of cutins
166 and suberins, as suggested by Mendez-Millan *et al.* (2010b). A lipid-free plant sample of 100
167 mg or of 1 g of soil was refluxed for 18 hours in a solution of water:methanol (MeOH) (1:9,
168 volume:volume) containing 6% of potassium hydroxide (KOH) (Cardoso & Eglinton, 1975).
169 These conditions lead to the depolymerization of cutin and suberin. Hydroxylated fatty acids
170 are released, and epoxy functions only are transformed into methoxy functions. Then, the
171 solution was filtered (GF/A Whatman glass microfibre filters, 1.6 µm) with a Millipore
172 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; CH_2Cl_2). 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 ($C_{19:0}$) 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 ºC. The GC oven temperature was set at 100 ºC for 2 minutes, then from 100 to 150 ºC at 10 ºC $minute^{-1}$, from 150 to 200 ºC at 5 ºC $minute^{-1}$ and finally at a rate of 2 ºC $minute^{-1}$ from 200 to 350ºC and kept for 5 minutes at 350 ºC. Quantification of the monomers was done with a flame ionisation detector (FID) using the internal standard $C_{19:0}$ and an external calibration with 16-hydroxyhexadecanoic acid (ωOH $C_{16:0}$). The chromatographic conditions were the same as for the GC/MS analysis. We obtained a

response factor for the external standard ωOH-$C_{16:0}$ 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 (∑C, ∑S, ∑SC). We calculated the following suberin:cutin ratio adapted from Otto & Simpson (2006):

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

and the sum of suberin and cutin

$$\sum SC = \sum S + \sum C + \sum SC \quad (2)$$

Carbon isotopic signature ($\delta^{13}C$)

We computed the δ^{13}C of each compound class (δ_{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:

$$\delta_{class} = \sum_{i=1}^{N}(\delta_{icomp} \times C_{icomp}) / \sum_{i=1}^{N} C_{icomp}\,, \qquad (3)$$

where C_{icomp} is the concentration of the *i*th compound of the chemical class, δ_{icomp} 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):

$$F = (\delta^{13}C_{maize\text{-}soil} - \delta^{13}C_{grassland\text{-}soil}) / (\delta^{13}C_{maize} - \delta^{13}C_{grasses}). \qquad (4)$$

The $\delta^{13}C_{grasses}$ 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, *α*-hydroxy carboxylic acids, *ω*-hydroxy carboxylic acids, mid-chain hydroxy acids and *α*,*ω*-alkanedioicacids. Because the *n*-carboxylic acids, *n*-alcohols and *α*-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 *ω*-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 *ω*-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 *ω*-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 ω-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 ω-hydroxy carboxylic acids in shoots than in roots (Mendez-Millan *et al*., 2011, for wheat and maize, Figure 2).

We detected three *α,ω*-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, *α,ω-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 α,ω-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 α,ω-

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 α,ω-alkanedioic acids in *Dactylis glomerata* and *Festuca arundinacea* was slightly smaller than for the mid-chain hydroxy

292 acids and ω-hydroxy carboxylic acids, whereas *Lolium perenne* showed no clear differences.
293 Most of the monomers in *Lolium perenne* were more ^{13}C depleted than in the other two
294 grasses. We found the biopolyesters of the grasses investigated to be ^{13}C-depleted compared
295 to the bulk plant tissues (Figure 6). Similar results were obtained by Mendez-Millan *et al.*
296 (2011) and Hamer *et al.* (2012) for several plant species. This ^{13}C depletion is common for
297 lignins and lipids because of isotopic fractionation along transport pathways (Hobbie &
298 Werner, 2004). The same explanation might be applied here to the monomers of cutin and
299 suberin; they have similar biosynthetic pathways to extractable lipids. The ^{13}C of the bulk
300 samples of the grasses agree with those for some C_3 plants (Wiesenberg & Schwark, 2006;
301 Mendez-Millan *et al.*, 2011), such as ryegrass, oats, barley or wheat (from –28.1 to –32.3 ‰
302 on average). The isotopic signature of maize (C_4 plant) reported by the same authors was
303 much larger (12.5 ‰ on average) than for C_3 plants. To the best of our knowledge, the ^{13}C
304 isotopic signature of different cutin and suberin biopolyesters has been determined for a few
305 plant species only: wheat and maize (Mendez-Millan *et al.*, 2010a, 2011), the grass *Setaria*
306 *sphacelata* (Schumach.) and the bracken fern *Pteridium arachnoideum* (Kaulf.) (Hamer *et*
307 *al.*, 2012) and in the grasses investigated here. The differences in ^{13}C content of the aliphatic
308 monomers for different C_3 plants also demonstrates the need to study each plant species
309 independently.

310 *Criteria for selecting cutin and suberin biomarkers in soil*

311 The fact that these aliphatic monomers are considered to be specific for cutin or suberin is not
312 enough to consider them as adequate above- or below-ground biomass markers in soil. The
313 $\omega C_{22:0}$ and $\omega C_{24:0}$ were the predominant ω-hydroxy carboxylic acids (Figure 3) in the soils
314 studied here. Their largest contents were in the 6-year maize crop and the smallest in the 6-
315 year bare soil, with no clear pattern in the other soils. The same was true for the $\omega C_{16:0}$,
316 $\omega C_{18:0}$ and $\omega C_{20:0}$. The $\omega C_{18:1}$ was not detected in the 6-year maize crop. Four alkanedioic

317 acids ($C_{16:0}$, $C_{18:1}$, $C_{20:0}$, $C_{22:0}$) were identified in soil under grass and crops (Figure 4). In the
318 6-year bare soil $C_{22:0}$ only was detected. The largest values for the $C_{16:0}$ diacid were in the 6-
319 year maize crop. This monomer was not detected in the grasses but it is present in the roots of
320 maize and wheat (Mendez-Millan *et al.*, 2010a, 2011), which are the likely sources in our
321 study. The $C_{18:1}$ diacid, which was predominant among the alkanedioic acids of the grasses,
322 had a much smaller relative abundance in soil. The total concentration of mid-chain hydroxy
323 acids was much smaller in the 6-year bare soil than in the other soils. The 8(9)(10),16-diOH
324 $C_{16:0}$ diacid and the 9-OH $C_{16:0}$ diacid were the most abundant compounds (Figure 5).
325 Moreover, in our study the relative contribution of the different compounds to the total of
326 released monomers often varied considerably from plant to soil. These changes were almost
327 exclusively the result of a drastic decrease in the relative abundance of a few monomers that
328 contained either epoxy functions or double bonds: $\omega C_{18:1}$, $C_{18:1}$ diacid, 11,18-diOH $C_{18:1}$,
329 9(10),18-diOH $C_{18:1}$ and 9,10-epoxy, 18-OH $C_{18:0}$. In contrast, the 8(9)(10),16-diOH $C_{16:0}$ and
330 the 9-OH $C_{16:0}$ diacid showed a larger relative distribution in soil (4.9–10.5 % and 2.7–4.2 %,
331 respectively) than in the plant tissues (5.0–6.9 % and 0.7–1.1 %). The decrease in the double
332 bond functions from plant to soil might be explained by their preferential degradation with
333 respect to the saturated building blocks (Nierop, 2001; Nierop *et al.*, 2003) because the epoxy
334 groups are considered as first intermediates in the oxidation of double bonds (Watkinson &
335 Morgan, 1990). There is more debate about the preservation of cutin and suberin monomers
336 in soil. Some authors have reported a similar rate of decomposition irrespective of their
337 chemical composition (e.g. Riederer *et al.* (1993) in an *in vitro* decomposition experiment of
338 cutin in *Fagus sylvatica* (L.) leaves; Nierop *et al.* (2003), for suberin monomers in an oak
339 forest), whereas other authors have proposed different mechanisms for preservation of these
340 monomers in soil. Mendez-Millan *et al.* (2011) suggested chemical recalcitrance for some
341 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-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-OH $C_{16:0}$ diacid and 8(9)(10),16-diOH $C_{16:0}$ were the most appropriate aboveground biomarkers for grassland species. On the other hand, the $C_{22:0}$ diacid, the $\omega C_{22:0}$, the $\omega C_{24:0}$ and to a lesser extent the $\omega C_{20:0}$ and $\omega C_{26:0}$, 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 ω-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.

392 The average isotopic signature of the hydroxy carboxylic acids was largest in the 6-year
393 maize crop and tended to be slightly smaller in the 6-year bare soil and the 6-year continuous
394 grassland soil (Table 4, Figure 6). The ^{13}C content varied according to the type of monomer.
395 For the 6-year maize crop, the largest enrichment in ^{13}C compared to the grassland soil was in
396 ω-$C_{22:0}$, followed by ω-$C_{24:0}$. The ^{13}C was more depleted in ω-$C_{20:0}$, ω-$C_{24:0}$ and ω-$C_{18:1}$ for
397 the 6-year bare soil than for the cropland soil. The largest average content of ^{13}C in the α,ω-
398 alkanedioic acids was for the 6-year bare soil (Table 4, Figure 6). The 6-year maize had
399 intermediate values and the smallest average content was for the 6-year continuous grassland
400 soil. The $C_{16:0}$ and $C_{22:0}$ diacids were ^{13}C-enriched more in the 6-year maize crop than in the
401 grassland soil. The $C_{22:0}$ diacid was the only α,ω-alkanedioic acid detected in the 6-year bare
402 soil and it had the largest ^{13}C content. Lastly, the $C_{20:0}$ diacid had a slightly larger isotopic
403 signature in the 6-year continuous grassland than for the 6-year maize crop. The average ^{13}C
404 content of the mid-chain hydroxy acids was similar under the 6-year maize crop and 6-year
405 continuous grassland soil, with a slightly smaller value in the 6-year bare soil (Table 4,
406 Figure 6). The isotopic signature of each monomer was somewhat irregular: the 8(9)(10),16-
407 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
408 more in the soil of the 6-year maize crop than for the 6-year continuous grassland, whereas
409 9,10,18-triOH $C_{18:0}$ was strongly ^{13}C-enriched (Table 4, Figure 6). These results should be
410 treated with caution because we do not know the effect of the methylated hydroxy group on
411 the epoxy functions. In all cases, the smallest ^{13}C contents were for the 6-year bare soil.
412 We calculated the proportion of cutin and suberin markers with Equation (4) that are maize-
413 derived 6 years after maize was introduced on land that had been cultivated previously with
414 C_3 plants. This enabled us to estimate the rate of turnover of shoot- and root-derived OM in
415 soil. The rate was large, about 29 %, for the α,ω-alkanedioic acids, whereas it was much less
416 for the ω-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 C_4-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.

442 The concentrations of cutin and suberin in soil were related to the amount of organic inputs.
443 We found no differences in the amounts of cutin and suberin in soil under continuous and
444 temporary grassland, which might indicate that the disturbance caused by conversion from
445 grassland to cropland was transitory only. For bare soil, suberin decreased by 40–64 % and
446 cutin by 24–40 % during a 6-year fallow, which indicated that root markers were more
447 sensitive to degradation than shoot markers.
448 The changes detected in the ^{13}C isotopic signatures of specific biomarkers after 6 years of
449 maize cropping showed that incorporation into SOM was greater for roots than for shoot
450 markers, in spite of the much smaller root inputs from maize than from grasses. Roots
451 contributed more to SOM accumulation, but had a shorter residence time in soil than shoots.
452 The sequestration of suberin in soil was more rapid, but less durable than that of cutin.
453 To specify the mechanisms and processes that lead to the turnover of cutin and suberin
454 monomers with land-use changes, future studies should focus on the organo-mineral
455 associations to protect these macromolecules in soil.
456

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464

465 **Figure captions**

466 **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.

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Table 1 Carbon (C) and nitrogen (N) contents and carbon isotopic signature ($\delta^{13}C$) of grassland plants and soil at the SOERE-ACBB site in Lusignan (France)

		C /mg g^{-1}	N /mg g^{-1}	C/N	$\delta^{13}C$ /‰
Plants					
Dactylis glomerata	Shoot	384 (29)	22.8 (1.4)	16.9 (0.8)	–26.7 (0.1)
	Root	350 (34)	11.5 (1.5)	27.8 (0.8)	–26.2 (0.3)
Festuca arundinacea	Shoot	389 (17)	16.3 (0.3)	23.7 (1.8)	–26.1 (0.2)
	Root	375 (8)	10.6 (0.7)	35.5 (2.4)	–26.3 (0.4)
Lolium perenne	Shoot	385 (27)	27.9 (6.4)	15.5 (0.5)	–28.5 (0.4)
	Root	332 (18)	13.2 (0.8)	25.2 (0.2)	–28.4 (0.1)
*Zea mays**	Bulk plant	425	na	na	–12.5
Soil (0–30 cm)					
6-year continuous grassland		11.2 (0.6)	1.20 (0.0)	9.35 (0.2)	–25.7 (0.2)
6-year ley grassland		11.3 (1.3)	1.24 (0.1)	9.10 (0.5)	–25.8 (0.2)
6-year crop rotation		10.1 (1.3)	1.11 (0.1)	9.06 (0.2)	–24.9 (0.2)
6-year maize crop		11.0 (0.4)	1.23 (0.1)	9.59 (0.1)	–25.4 (0.3)
3-year ley grassland		11.0 (0.7)	1.08 (0.1)	9.47 (0.4)	–25.2 (0.0)
6-year bare soil		8.29 (0.5)	0.96 (0.1)	8.59 (0.2)	–25.1 (0.2)

*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, $n = 4$)

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

*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

	[a]∑C Cutin	[b]∑S Suberin	[c]∑CS Cutin+suberin	[e]Suberin + cutin ∑SC	[d]Suberin / cutin
Plants	C /µg g^{-1}	C /µg g^{-1}	C /µg g^{-1}	C /µg g^{-1}	
Dactylis glomerata					
Shoots	9574 (446)	911 (42)	3877 (159)	14 361 (551)	0.25 (0.01)
Roots	1239 (150)	6394 279)	3950 (375)	11 583 (765)	2.61 (0.13)
Festuca arundinacea					
Shoots	6940 (536)	721 (101)	3950 (375)	11 620 (662)	0.30 (0.03)
Roots	1301 (73)	5411 (763)	5282 (394)	11 994 (862)	2.05 (0.22)
Lolium perenne					
Shoots	9341 (632)	891 (113)	3796 (374)	14 028 (930)	0.25 (0.01)
Roots	1186 (173)	7305 (763)	3835 (90)	12 325 (624)	2.99 (0.38)
Soil					
6-year continuous grassland	2258 (279)	3209 (276)	1128 (200)	6595 (259)	1.27 (0.00)
6-year ley grassland	2152 (257)	2451 (294)	1093 (39)	5696 (77)	1.12 (0.21)
6-year crop rotation	1920 (72)	2463 (198)	925 (61)	5308 (209)	1.23 (0.05)
6-year maize crop	2409 (214)	4061 (168)	1338 (110)	7808 (260)	1.54 (0.15)
3-year ley grassland	2340 (77)	3108 (65)	1111 (163)	6560 (22)	1.27 (0.00)
6-year bare soil	1457 (129)	1461 (71)	707 (79)	3625 (137)	1.01 (0.11)

[a]Cutin ∑C (µg g^{-1} C) = 8(9)(10),16-diOH $C_{16:0}$ + 9-OH $C_{16:0}$ diacid + 11,18-diOH $C_{18:1}$ + 9,10-epoxy, 18-OH $C_{18:0}$

[b]Suberin ∑S (µg g^{-1} C) = $\omega C_{20:0}$ + $\omega C_{22:0}$ + $\omega C_{24:0}$ + $\omega C_{26:0}$ + $C_{16:0}$ diacid + $C_{18:1}$ diacid + $C_{20:0}$ diacid + $C_{22:0}$ diacid

[c]Suberin or cutin ∑SC (µg g^{-1} C) = $\omega C_{16:0}$ + $\omega C_{18:0}$ + $\omega C_{18:1}$ + 9(10),18-diOH $C_{18:1}$ + 9,10,18-triOH $C_{18:0}$

[d]Suberin/cutin ratio = (∑S + ∑SC/2) / (∑C + ∑SC/2)

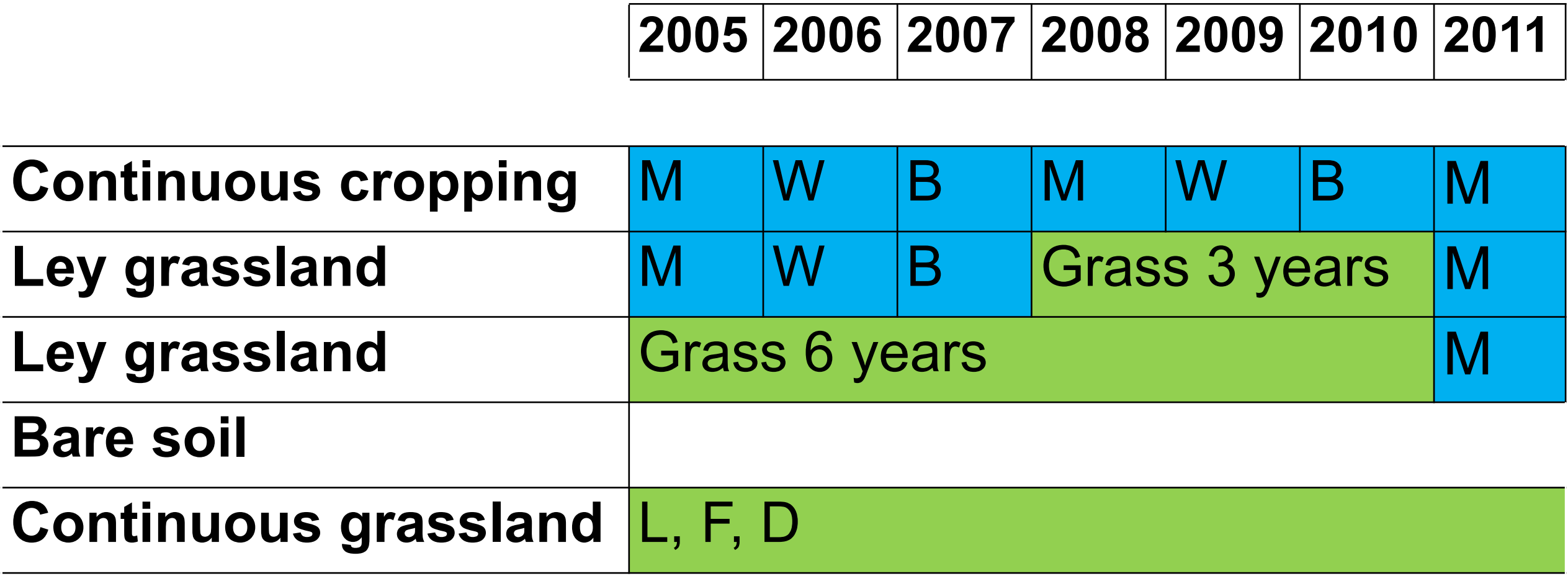
[e]Sum of suberin and cutin ∑SC (µg g^{-1} C)= ∑S + ∑C + ∑SC

Table 4 Carbon isotopic signature ($\delta^{13}C$) values of the aliphatic monomers of cutins and suberins present in grassland, crop plants and soil at the SOERE-ACBB site in Lusignan (France)

	Plants /‰ $\delta^{13}C$							Soil /‰ $\delta^{13}C$		
	Dactylis glomerata		*Festuca arundinacea*		*Lolium perenne*		*Zea mays*[a]	6-year	6-year	6-year
	Shoot	Root	Shoot	Root	Shoot	Root	Bulk plant	grassland	maize crop	bare soil
ω-Hydroxy carboxylic acids										
16-Hydroxy hexadecanoic acid ($\omega C_{16:0}$)	na	na	na	na	na	na	−17.1 (0.9)	na	na	na
18-Hydroxy octadecanoic acid ($\omega C_{18:0}$)	na	na	na	na	na	na	na	na	na	na
18-Hydroxy octadecenoic acid ($\omega C_{18:1}$)	−35.5	−35.2	−34.3	−34.7	−36.0	−36.2	−17.1 (0.3)	−38.3 (0.5)	na	−39.3 (0.7)
20-Hydroxy eicosanoic acid ($\omega C_{20:0}$)		−36.2		−34.8		−37.8	na	−35.5 (0.6)	−35.3 (1.1)	−37.0 (0.1)
22-Hydroxy docosanoic acid ($\omega C_{22:0}$)	−36.9	−36.8	−32.6	−38.2	−37.8	−37.9	−19.8 (0.7)	−36.8 (0.8)	−35.4 (0.2)	−36.2 (2.7)
24-Hydroxy tetracosanoic acid ($\omega C_{24:0}$)	−36.5	−29.5	−30.3	−36.6	−37.0	−37.8	−21.2 (0.4)	−35.2 (0.2)	−34.6 (0.4)	−36.6 (0.1)
26-Hydroxy hexacosanoic acid ($\omega C_{26:0}$)		−28.0		−33.0		−34.6	−19.1 (0.8)	na	na	na
Weighted average for *ω*-hydroxy carboxylic acids	−35.7	−34.2	−33.7	−35.4	−36.3	−37.1	na	−36.4 (0.5)	−35.1 (0.3)	−36.8 (1.5)
α,*ω*-Alkanedioic acids										
1,16-Hexadecadioic acid ($C_{16:0}$ diacid)	na	na	na		na	na	−15.1 (0.5)	−32.4 (0.1)	−29.0 (0.8)	na
1,18-Octadecendioic acid ($C_{18:1}$ diacid)	na	−35.4	na	−36.3	na	−36.2	−17.0 (2.2)	−36.8 (4.6)	na	na
1,20-Neodecandioic acid ($C_{20:0}$ diacid)	na	−36.6	na	−33.1	na	−31.3	na	−30.6 (2.2)	−31.2 (1.7)	na
1,22-Docosandioic acid ($C_{22:0}$ diacid)	na	−34.9	na	−37.5	na	−38.7	na	−37.4 (0.4)	−34.8 (0.9)	−30.5 (1.1)
Weighted average for *α*,*ω*-alkanedioic acids		−35.4		−36.2		−36.1	na	−33.6 (1.2)	−31.3 (1.0)	−30.5 (1.1)
Mid-chain hydroxy acids										
8(9)(10),16-Dihydroxy hexadecanoic acids (8(9)(10),16-diOH $C_{16:0}$)	−34.6	−32.4	−34.7	−30.8	−32.4	−35.8	−16.4 (0.5)	−34.2 (0.3)	−33.8 (0.1)	−35.0 (0.9)
9-Hydroxy hexadecanedioic acid (9-OH $C_{16:0}$ diacid)	na	na	−34.7	na	−33.9	na	−16.1 (0.5)	−34.5 (0.3)	−34.1 (0.3)	−36.6 (0.4)
11,18-Dihydroxyoctadecenoic acid (11,18-diOH $C_{18:1}$)[b]	−35.9	−37.4	−35.8	−37.5	−37.3	−39.6	−17.1 (0.5)	−37.5 (0.4)	−38.4 (0.1)	−36.9 (0.2)
9(10),18-Dihydroxyoctadecenoic acid (9(10),18-diOH $C_{18:1}$)[b]	−33.6	−29.6	−34.7	−36.7	−36.1	−38.0	−17.7 (0.5)	−31.6 (0.3)	−32.9 (0.5)	na
9,10-Epoxy, 18-hydroxyoctadecanoic acid (9,10-epoxy, 18-OH $C_{18:0}$)[c]	−34.2	−30.4	−34.9	−34.7	−33.6	−37.4	−19.1 (0.5)	−33.8 (0.9)	−33.5 (0.6)	na
9,10,18-Trihydroxyoctadecanoic acid (9,10,18-triOH $C_{18:0}$)	−34.6	−36.8	−39.4	−37.3	−41.8	na	−17.9 (0.5)	−35.3 (0.5)	−31.9 (0.3)	na
Weighted average for mid-chain hydroxy acids	−34.4	−33.1	−35.4	−34.8	−34.3	−37.4	na	−34.4 (0.4)	−34.1 (0.2)	−35.7 (0.6)

[a]Data from Mendez-Millan *et al*. (2011); [b]Position of the double bond not confirmed; [c]Isomeric mixture of 9-methoxy, 10,18-dihydroxyoctadecanoic acid and 9-hydroxy,10-methoxy, 18-hydroxyoctadecanoic acids

(SD in parentheses, $n = 2$, na = not analysed)



M= maize, W= wheat, B= barley

L= *Lolium*, F= *Festuca*, D= *Dactylis*

Figure 1

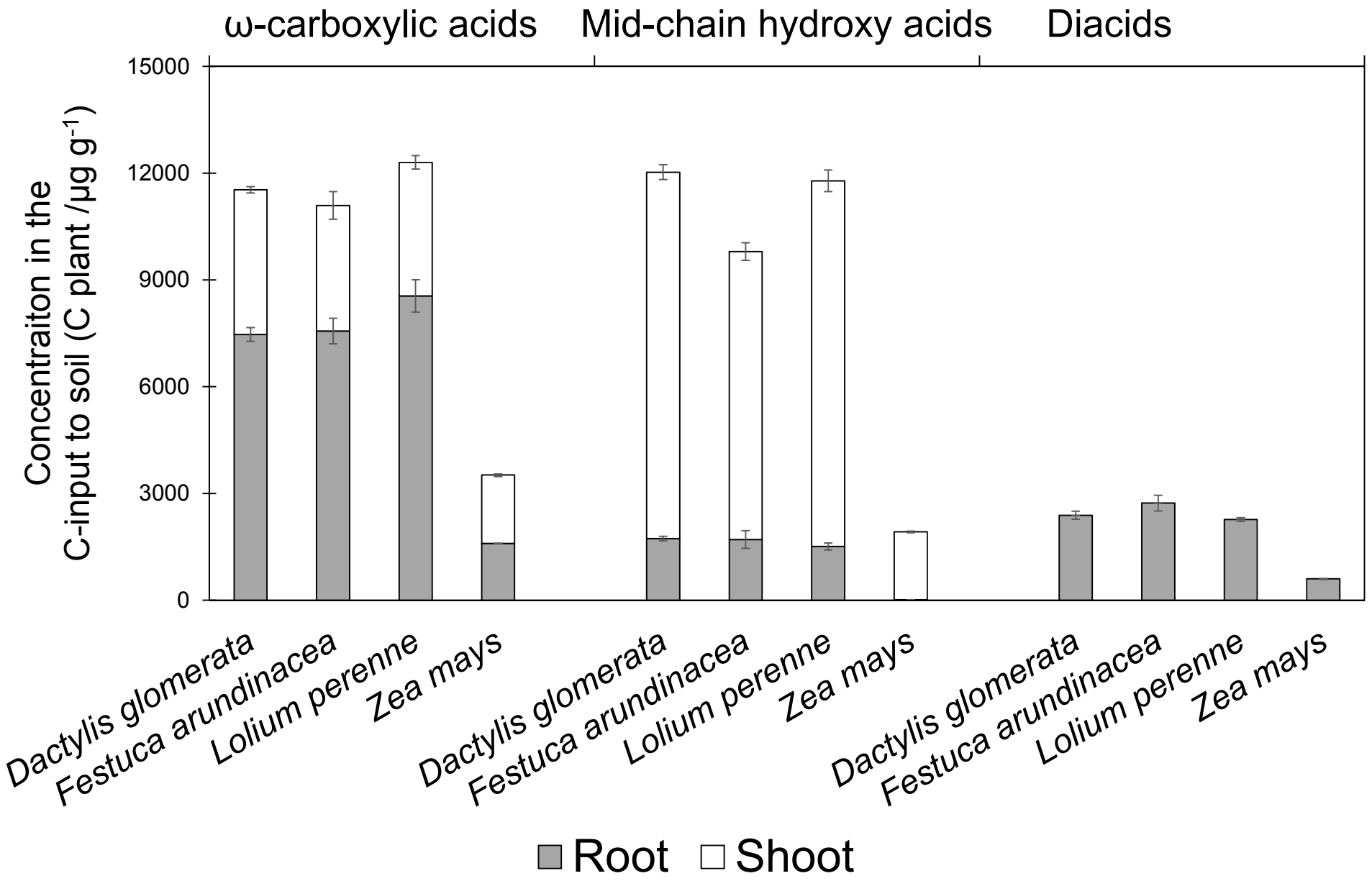

ω-carboxylic acids
Mid-chain hydroxy acids
Diacids
Concentraiton in the C-input to soil (C plant /µg g-1)
15000
12000
9000
6000
3000
0
Dactylis glomerata
Festuca arundinacea
Lolium perenne
Zea mays
Dactylis glomerata
Festuca arundinacea
Lolium perenne
Zea mays
Dactylis glomerata
Festuca arundinacea
Lolium perenne
Zea mays
Root
Shoot

Figure 2

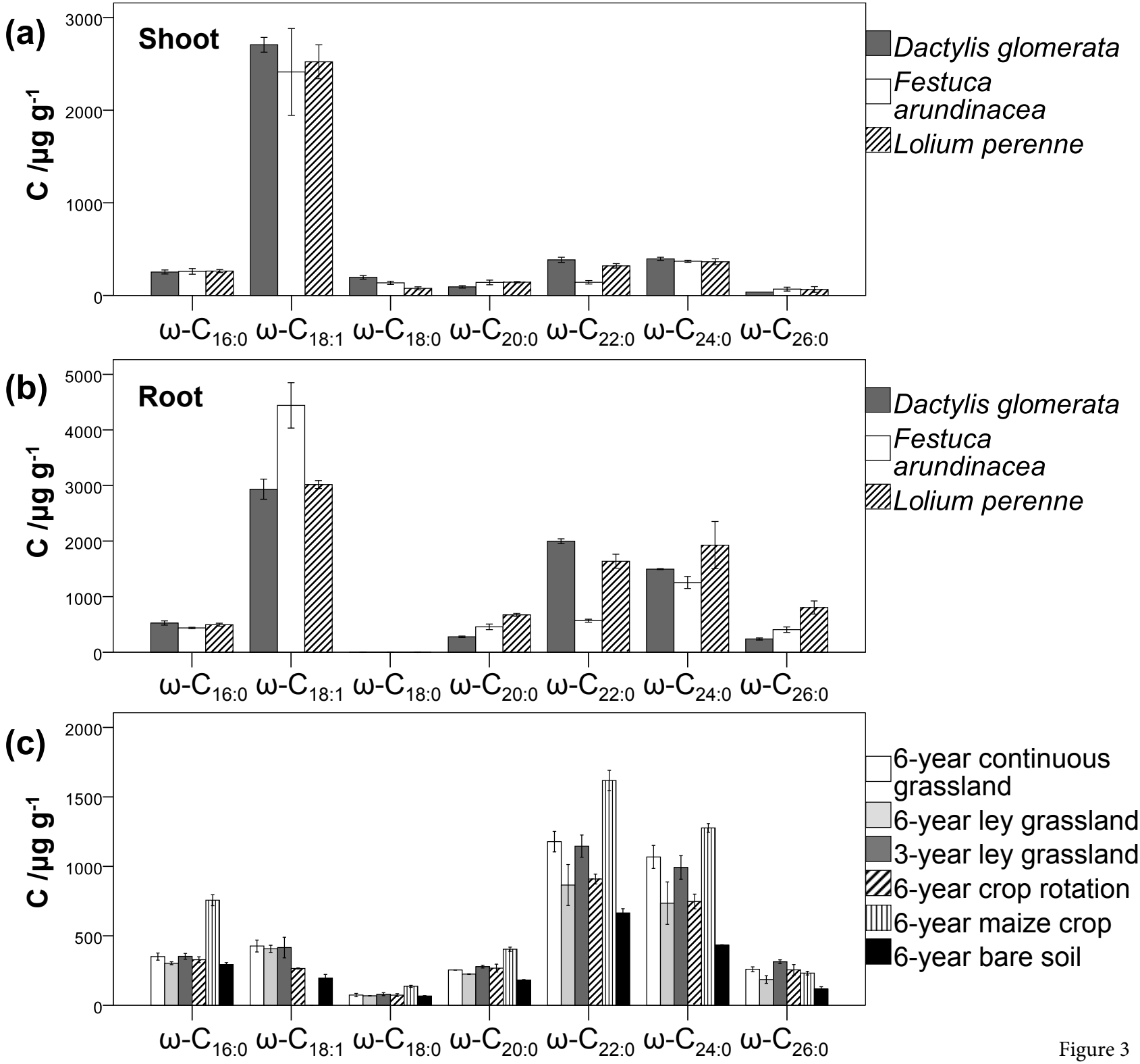

(a)
Shoot
C /µg g-1
3000
2000
1000
0
Dactylis glomerata
Festuca arundinacea
Lolium perenne
ω-C16:0
ω-C18:1
ω-C18:0
ω-C20:0
ω-C22:0
ω-C24:0
ω-C26:0
(b)
Root
C /µg g-1
5000
4000
3000
2000
1000
0
Dactylis glomerata
Festuca arundinacea
Lolium perenne
ω-C16:0
ω-C18:1
ω-C18:0
ω-C20:0
ω-C22:0
ω-C24:0
ω-C26:0
(c)
C /µg g-1
2000
1500
1000
500
0
6-year continuous grassland
6-year ley grassland
3-year ley grassland
6-year crop rotation
6-year maize crop
6-year bare soil
ω-C16:0
ω-C18:1
ω-C18:0
ω-C20:0
ω-C22:0
ω-C24:0
ω-C26:0

Figure 3

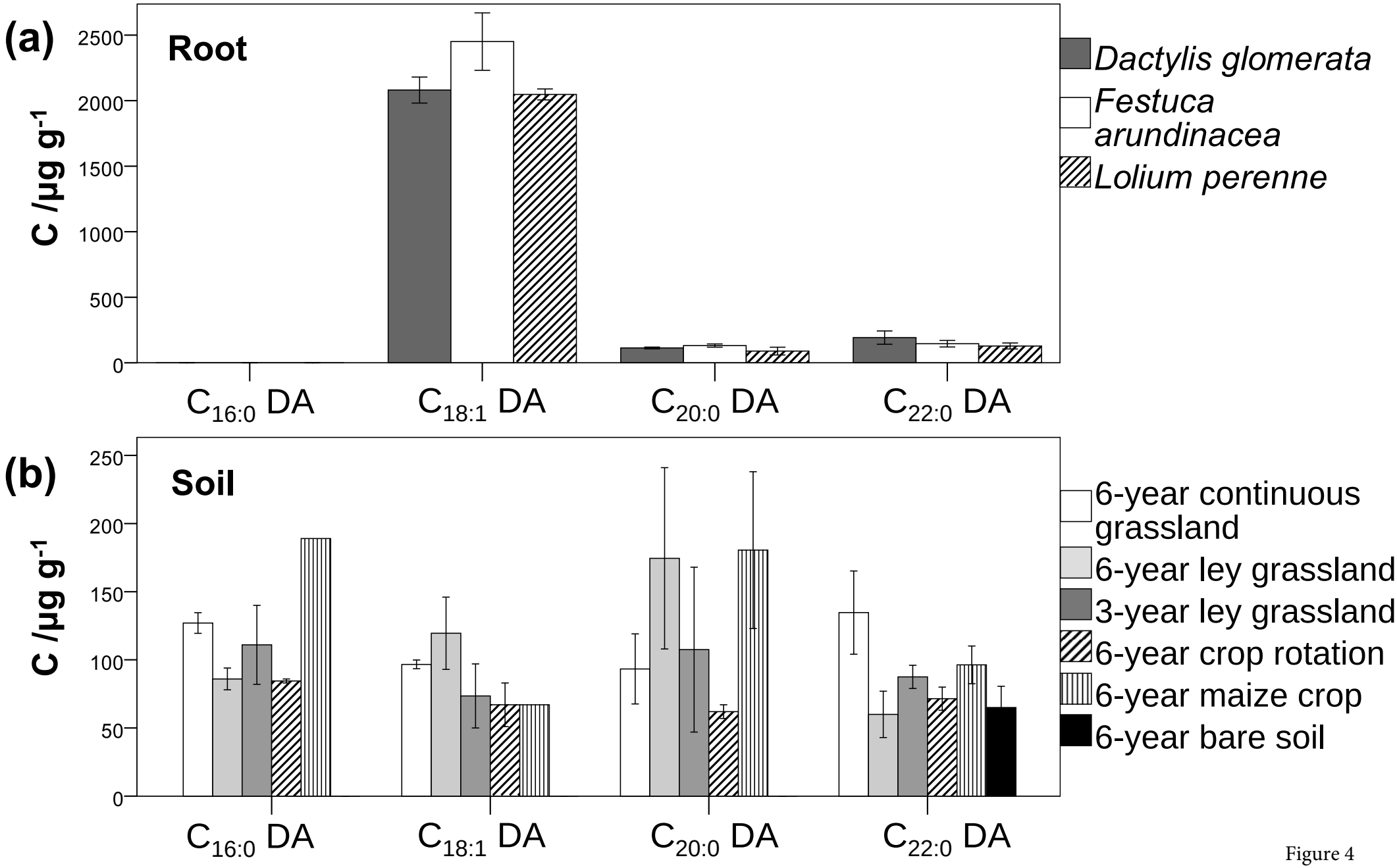

(a)
Root
C /µg g-1
2500
2000
1500
1000
500
0
C16:0 DA
C18:1 DA
C20:0 DA
C22:0 DA
Dactylis glomerata
Festuca arundinacea
Lolium perenne
(b)
Soil
C /µg g-1
250
200
150
100
50
0
C16:0 DA
C18:1 DA
C20:0 DA
C22:0 DA
6-year continuous grassland
6-year ley grassland
3-year ley grassland
6-year crop rotation
6-year maize crop
6-year bare soil

Figure 4

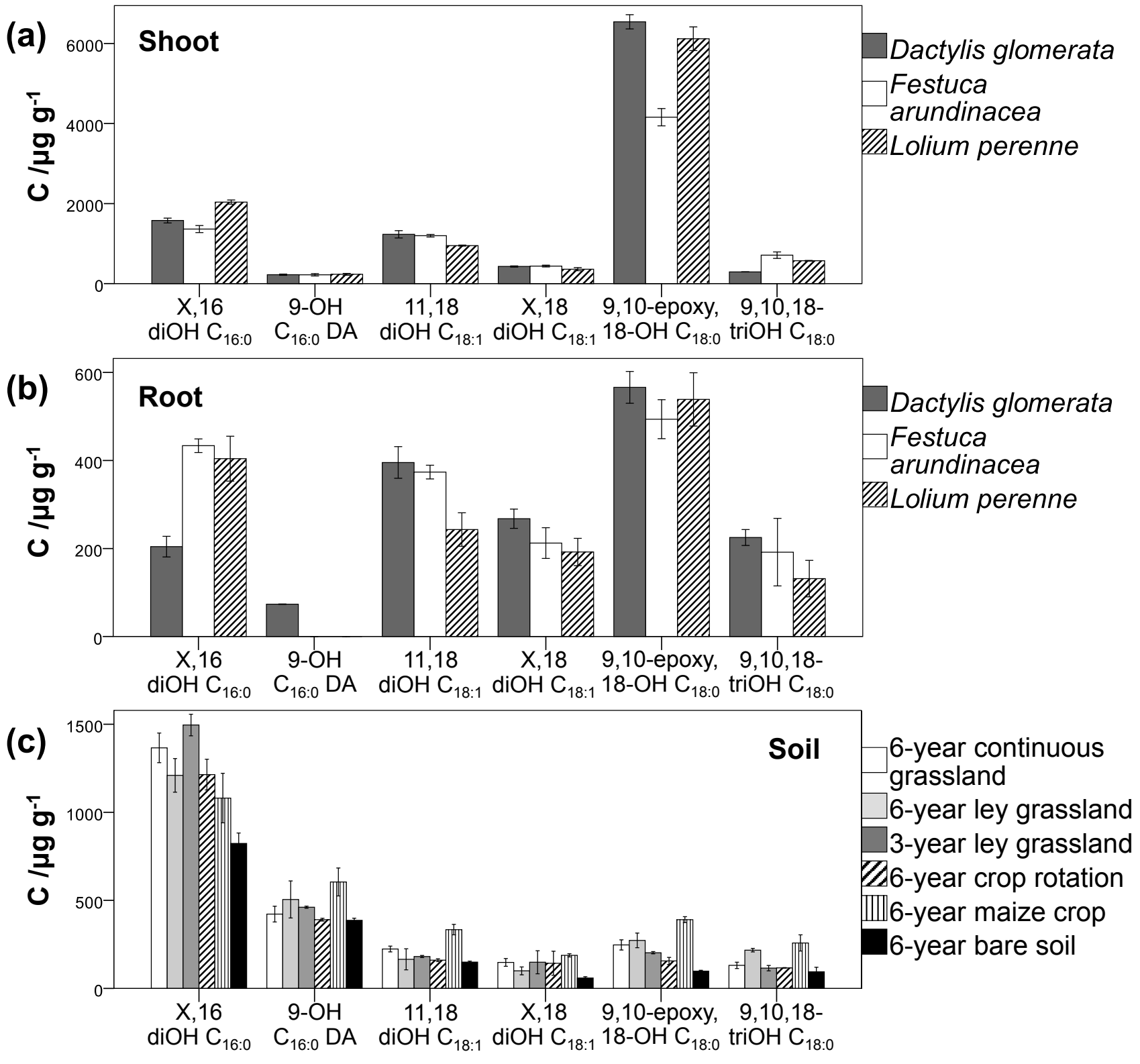
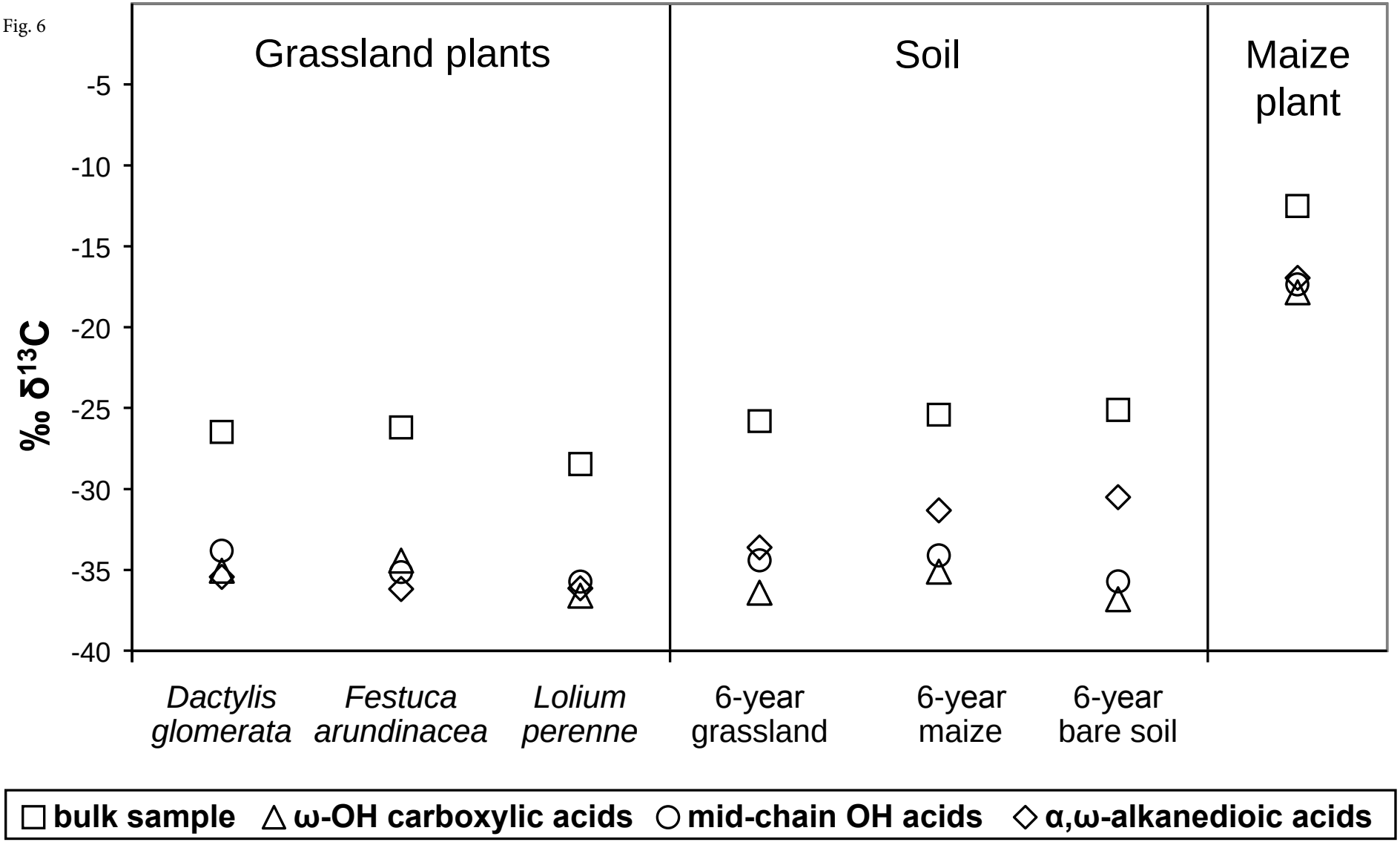
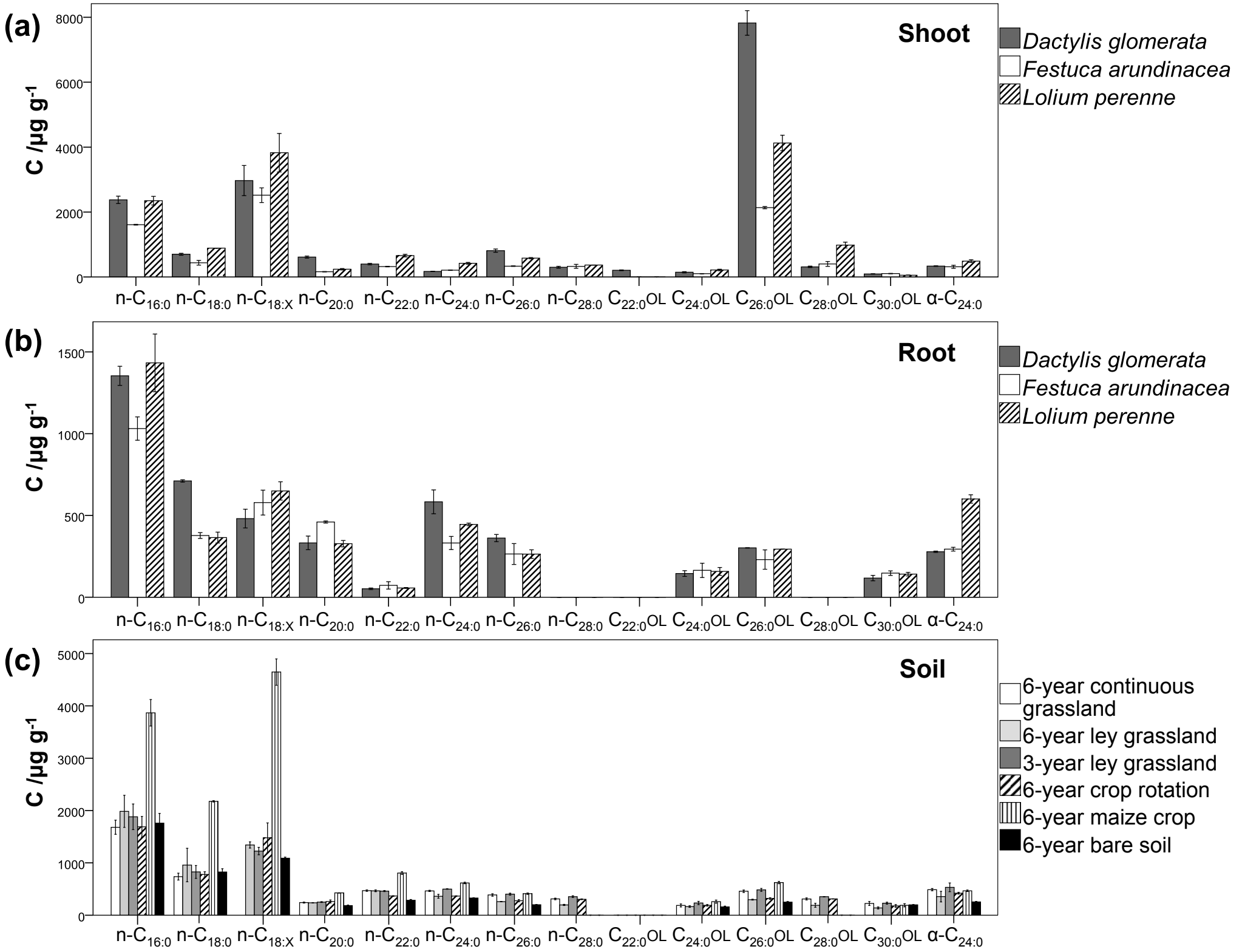

(a)
Shoot
C /µg g-1
6000
4000
2000
0
X,16 diOH C16:0
9-OH C16:0 DA
11,18 diOH C18:1
X,18 diOH C18:1
9,10-epoxy, 18-OH C18:0
9,10,18-triOH C18:0
Dactylis glomerata
Festuca arundinacea
Lolium perenne
(b)
Root
C /µg g-1
600
400
200
0
X,16 diOH C16:0
9-OH C16:0 DA
11,18 diOH C18:1
X,18 diOH C18:1
9,10-epoxy, 18-OH C18:0
9,10,18-triOH C18:0
Dactylis glomerata
Festuca arundinacea
Lolium perenne
(c)
Soil
C /µg g-1
1500
1000
500
0
X,16 diOH C16:0
9-OH C16:0 DA
11,18 diOH C18:1
X,18 diOH C18:1
9,10-epoxy, 18-OH C18:0
9,10,18-triOH C18:0
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 5

Fig. 6


Grassland plants
Soil
Maize plant
‰ δ13C
-5
-10
-15
-20
-25
-30
-35
-40
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


(a)
Shoot
C /µg g-1
8000
6000
4000
2000
0
Dactylis glomerata
Festuca arundinacea
Lolium perenne
n-C16:0 n-C18:0 n-C18:X n-C20:0 n-C22:0 n-C24:0 n-C26:0 n-C28:0 C22:0OL C24:0OL C26:0OL C28:0OL C30:0OL α-C24:0
(b)
Root
C /µg g-1
1500
1000
500
0
Dactylis glomerata
Festuca arundinacea
Lolium perenne
n-C16:0 n-C18:0 n-C18:X n-C20:0 n-C22:0 n-C24:0 n-C26:0 n-C28:0 C22:0OL C24:0OL C26:0OL C28:0OL C30:0OL α-C24:0
(c)
Soil
C /µg g-1
5000
4000
3000
2000
1000
0
6-year continuous grassland
6-year ley grassland
3-year ley grassland
6-year crop rotation
6-year maize crop
6-year bare soil
n-C16:0 n-C18:0 n-C18:X n-C20:0 n-C22:0 n-C24:0 n-C26:0 n-C28:0 C22:0OL C24:0OL C26:0OL C28:0OL C30:0OL α-C24:0