



Use of molecular ratios to identify changes in fatty acid composition of *Miscanthus × giganteus* (Greef et Deu.) plant tissue, rhizosphere and root-free soil during a laboratory experiment

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ABSTRACT

Fatty acids (FAs) are abundant lipids in plants, microorganisms and soil. Depending on chain length they provide potential for evaluating different sources of C in soil: shoots, roots and microorganisms. This, together with their fast turnover and transformation in living and decaying plant tissues, suggests the use of FA molecular ratios as source indicators in soil. To evaluate the applicability of FAs as source indicators, their dynamics in plant tissue and soil were traced during a laboratory experiment using the highly productive perennial C₄ energy grass *Miscanthus × giganteus* (Greef et Deu.). For the comprehensive use of FAs as source indicators various ratios were calculated: fatty acid ratio (originally defined as carboxylic acid ratio: CAR), carbon preference index (CPI), average chain length (ACL) and unsaturated vs. saturated C₁₈ acids. The FA composition was specific for individual plant tissues as indicated by the CAR, with high values in roots and lower ones in the above ground plant tissue. Based on ACL values of rhizosphere, soil and roots, an enrichment in root derived FAs vs. root-free soil could be estimated. The rhizosphere contained 35–70% more plant derived FAs than root-free soil. The ACL showed potential for estimating root derived carbon in the rhizosphere. The study documents for the first time very fast spatial processes in soil related to plant growth, thereby strongly influencing the FA composition of soil.

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1. Introduction

Soil organic matter (SOM) as a main part of the global C cycle, has frequently been investigated for its stability and sources using chemical and physical fractionation procedures (e.g. Marschner et al., 2008). However, it remains difficult to differentiate individual sources of SOM at a molecular level, including root and shoot derived C. Some molecular proxies derived from cutin and suberin have been suggested as root-specific (α,ω -alkane dioic acids) or shoot-specific (mid-chain hydroxy acids) markers (Feng et al., 2010; Mendez-Millan et al., 2010). Whereas such molecular proxies allow assessment of molecular dynamics in soil over decades, it is well known for other plant derived components that their incorporation into soil occurs on a range of days to months, followed by mineralization of large proportions of the incorporated SOM. Such components include fatty acids (FAs) as ubiquitous components in plant and microbial tissue and SOM (Gregorich et al., 1996). The most abundant short chain saturated FAs containing 16 and 18 carbons are non-specific and occur in the cell membranes of microor-

ganisms and plant cuticles or waxes (Kolattukudy et al., 1976; Harwood and Russell, 1984). Numerous short chain unsaturated or branched FAs from cellular phospholipid FAs (PLFAs) facilitate differentiation of plant and microorganism derived FAs in soil (Kolattukudy et al., 1976; Weete, 1976; Harwood and Russell, 1984; Zelles, 1997; Boeschker and Middelburg, 2002). However, some authors have stated that in soil most short chain (<C₂₀) acids are of microbial origin (Amblès et al., 1993; Naafs et al., 2004).

Plant derived short chain acids have the potential for assessing turnover times of several years to decades in grassland soil (Wiesenberg et al., 2008). In comparison with short chain FAs, long chain FAs in plant tissue are of chemotaxonomic significance (Wiesenberg and Schwark, 2006), comparable to long chain alcohols (Rommerskirchen et al., 2006), β -diketones, hydroxy β -diketones, alkan-2-ol esters, or mid-chain functionalized ketones and secondary alcohols (Bianchi and Bianchi, 1990). These parameters allow molecular differentiation of C₃ and C₄ plants. Hence, the fatty acid ratio [originally defined as carboxylic acid ratio: CAR = $n\text{-C}_{24}/(n\text{-C}_{22} + n\text{-C}_{26})$ fatty acids] was found to be diagnostic for the differentiation of grasses following the C₃ and the C₄ photosynthetic pathway, with CAR of C₄ plants >0.67 and for C₃ plants <0.67 (Wiesenberg and Schwark, 2006). In soil, a change in CAR values occurs as a result

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of decomposition of plant derived acids, resulting in higher CAR values in C₃ soil than in C₃ biomass (Wiesenberg, 2004). Other studies determined a relative enrichment of very long chain acids (>C₂₆) in some C₄ plant leaves (Avato et al., 1990; Hwang et al., 2002; Wiesenberg et al., 2004a; Jandl et al., 2007). This was found to be of low specificity, because enrichment was similarly noted for other plant tissues, including coffee (Stocker and Wanner, 1975) and clover (Body, 1974). Despite the chemotaxonomic significance of long chain FAs, the composition of plant wax lipids including FAs varies slightly during the growing season (Hamilton and Power, 1969; Stocker and Wanner, 1975; Maier and Post-Beittenmiller, 1998; Jenks et al., 2001; Dewhurst et al., 2002; Elgersma et al., 2003). In addition, lipid composition is different for distinct plant tissues, as determined for several grasses (Avato et al., 1990; Dove et al., 1996; Wiesenberg et al., 2004a; Otto and Simpson, 2005).

The input of plant derived lipids to soil follows several pathways, including litter fall, atmospheric deposition of abraded wax (Conte et al., 2003) and rhizodeposition (Wiesenberg et al., 2010). However, knowledge is limited about the degree to which roots contribute to soil FAs and how their spatial and temporal variability is within the short term, i.e. during several weeks of plant growth. Hence, it is not clear if and how incorporation of plant derived lipids into soil occurs predominantly and if the contribution of above ground plant biomass might be negligible vs. the contribution of root derived components. Current crop management practices often involve the complete removal of above ground biomass from the field, leaving the root biomass and shoot stubbles on the field. Thus, the input of above ground plant tissue to soil is limited and roots have the main potential to incorporate plant derived lipids in intensively cropped soil. Additionally, the molecular differentiation of FAs between rhizosphere, i.e. soil adjacent to roots, and soil distant from roots has scarcely been investigated, whereas recent studies clearly demonstrate short term and long term effects of C incorporation and stabilization of lipids nearby the root system (Gocke et al., 2010; Wiesenberg et al., 2010). Nevertheless the different dynamics of FAs in the rhizosphere and root-free soil remain unknown.

Some recent studies observed changes in lipid distribution patterns in soil between summer and winter (Naafs et al., 2004), but knowledge is limited concerning lipid compositional changes during the growing season, i.e. on a time scale of days, weeks or months. In Europe, the C₄ crop, *Miscanthus × giganteus* (Greef et Deu.) is used as a bio-energy crop with high above ground biomass yield potential (e.g. Beuch et al., 2000). Based on the results of Hansen et al. (2004) and Garten and Wullschleger (2000), but especially considering the physiological properties and growth of *Miscanthus*, the C input to soil under it is expected to be high when compared with other agricultural and even energy crops. *Miscanthus* was found to significantly increase and modify the lipid content in a previously C₃ cropped soil (Kahle et al., 2001), like other C₄ plants used in long term C₃/C₄ crop change experiments, including e.g. maize as a C₄ crop (Wiesenberg et al., 2004a).

The objectives of this study were to determine the temporal variability in the FA composition of the fast growing grass *Miscanthus* in different plant tissues (root, stems and leaves) during 1.5 months of growth. In addition, the temporal variability in plant derived FAs was investigated within the rhizosphere and root-free soil to determine, if and to what degree root derived FAs contribute to overall plant derived FAs in soil on a day to month scale.

2. Materials and methods

2.1. Experimental design

Miscanthus was planted under controlled conditions for 83 days to investigate the dynamics of lipids within the plant tissues and

plant derived lipids in the soil. Rhizome pieces with buds there from were washed and cut into pieces of ca. 0.05 m × 0.02 m before *Miscanthus* planting (Schneckenberger and Kuzyakov, 2007). For each sampling date, three replicate pots with a volume of 4200 ml were planted with four rhizomes. Immediately before planting, the pots were filled with homogenized air-dried soil from the A_p horizon of a Haplic Luvisol developed from loess. The soil was sampled from a green fallow plot on the experimental station 'Heidfeldhof' at the University of Hohenheim (Germany) in 2004 and was sieved before planting (<2 mm). Big roots and most of the small roots were removed with tweezers before the experiment. The previously C₃ plant cropped soil was chosen due to a different molecular composition of the soil vs. the plant biomass used here in order to determine the plant driven changes on a molecular level.

The required soil moisture was held at 60% of water holding capacity by adding a combination of a nutrient solution (Wiesenberg et al., 2009) and water. It was re-adjusted with water in decreasing time intervals to provide the plants with an adequate water supply during the whole plant growth. One week after planting, the weakest plants were removed, leaving three within each pot for the experiment. Plants were illuminated 14 h/day and kept under controlled conditions (800 mol⁻⁶ photons m⁻² s⁻¹ PAR at canopy height, 25/20 °C day/night temperature).

During the first weeks of growth the incorporation of plant lipids into soil could be expected to be low as a result of low rooting and fast growth of above ground biomass of the young plants. Thus, samples were taken 43–83 days after planting on eight different days in order to follow plant evolution and determine the contribution of plant derived lipids to soil. Each result presented refers to three replicate pots containing three plants, which were analyzed as composite samples. Combination of biomass was required due to the low amount of material in some samples. Replicate analyses of composite samples were available for days 47, 61, 75 and for roots from day 70. At the same dates (days 47, 61, 75) aliquots of unplanted soil were collected from control pots treated exactly like the planted pots.

Initial sampling was conducted at a plant age of 43 days. Subsequent destructive sampling was performed every 2, 4 or 8 days until a plant age of 83 days. Different sampling frequency was chosen to investigate the lipid development with increasing plant growth and in different time ranges, without using an extremely high sampling frequency and thus sample numbers.

2.2. Soil and plant samples

Shoot biomass was divided into leaves and stem. For the last two sampling dates (days 75 and 83), leaf sheaths were separated from stems and analyzed separately to determine differences in lipid composition vs. leaves and stems. For all other samples, leaf sheaths were analyzed together with stems. Soil was separated from roots and root adjacent soil by way of careful manual separation. Soil adhering to roots after removal of roots from soil was separated by washing with de-ionized water and was termed 'rhizosphere'. This is the almost root-free soil, directly adhering to the roots, where root-related processes take place. Root fragments were carefully removed from soil and rhizosphere samples by manual picking using tweezers. For days 55 and 57 no root material was available for lipid analysis, due to problems with sample preparation. Plant material and soil material were dried to constant weight at 60 °C in an oven. All samples were milled using a ball mill. For each sampling date three replicate pots were harvested, whereas replicate plant and soil material from the same sample type (leaves, stems, roots, rhizosphere and root-free soil, respectively) was combined and mixed. Duplicate analyses were carried out for three dates (47, 61 and 75 days), whereas for other

sampling dates low biomass did not allow replicate analyses of samples from one sampling date.

2.3. Lipid extraction and separation

Lipids were extracted using accelerated solvent extraction (Dionex ASE 200) with $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (93/7; v/v). Plant material (5 g, where available) or 30 g soil and rhizosphere material, respectively, were filled into stainless steel extraction vessels and extracted for 20 min in two steps at 5×10^6 Pa and 75 °C and 140 °C, respectively (Wiesenberg et al., 2004b). Lipids were separated into FAs and low polarity lipids using solid phase extraction by dissolving extracts in CH_2Cl_2 and applying to KOH coated (5%) silica gel columns (Wiesenberg et al., 2009). Lipids of neutral and intermediate polarity were eluted with CH_2Cl_2 . Thereafter, FAs were recovered by flushing the columns with $\text{CH}_2\text{Cl}_2/\text{CHOOH}$ (99/1; v/v). Volume reduction was performed via a turbo vaporizer (Zymark) or rotary evaporation. Afterwards, extracts were transferred to pre-weighed collection vials and dried under a gentle N_2 stream to constant weight. The amount of fractions was determined by weighing, thus enabling the calculation of FAs accessible for gas chromatographic analysis.

2.4. Gas chromatography–mass spectrometry (GC–MS)

FA fractions were dissolved in 1 ml CH_2Cl_2 and aliquots of the dissolved fractions (containing 1 mg FAs) were transferred to reaction vials, where 5 µg deuterated standard (d_{39} - n - C_{20} FA) was added for quantification. For GC analysis the acids were methylated with CH_2N_2 (Wiesenberg et al., 2004a). Compound identification was performed with a HP 5890 Series II gas chromatograph coupled to a HP 5989A mass spectrometer using a reference mixture of FA standards and comparison with the Wiley mass spectra library. All peak area counts for the individual FAs were determined from the total ion current and corrected using previously determined response factors. To present the results in a more comparable format and to avoid differences within one or more orders of magnitude in the total abundances of individual compounds during plant growth, all results were normalized to the content of unsaturated $\text{C}_{18:1+18:2}$ FAs.

3. Results

3.1. Lipid content

The amount of total extractable lipids was highest for leaves and decreased in the order: leaves > leaf sheaths (avg. 89.1 ± 3.5 mg lipid g^{-1} total organic carbon for the last two sampling dates) > stems > roots > rhizosphere > soil > unplanted control soil (Fig. 1). With plant age lipid content became higher for leaves and stems until day 57 and for rhizosphere until day 55. Thereafter, leaf lipid content remained constant and was slightly lower at the end of the experiment, whereby lipid content of stem biomass and rhizosphere became lower, especially between days 57 and 70. Root lipid content was lower after day 60 vs. previous sampling dates and was similar to that of stems at the end of the experiment. Lipid content of planted and control soils remained almost constant during the whole experimental period. The lipid content for control soil was permanently lower by 15% than lipid content of the planted soil. From day 61, lipid content in the rhizosphere remained constant and was 20–30% higher than that of root-free soil.

3.2. FA composition

Saturated C_{16} or unsaturated $\text{C}_{18:1+2}$ FAs dominated the FA distribution (Fig. 2A–C) in plants. While leaves were characterized by

the highest amount of unsaturated C_{18} acids, the relative contribution of acids with 18 carbons decreased in the direction leaves > stems > roots. Long chain (C_{20-26}) and very long chain ($>\text{C}_{26}$) acids had the lowest abundance in leaves and high abundance in roots and stems. For leaves and stems a relative increase in unsaturated C_{18} acids with plant growth was induced by a relative depletion in most other acids throughout the experiment. Roots showed no change in short chain acid proportion and a relative enrichment in long chain acids with 20–25 carbons with plant growth. While long chain acids from above ground biomass were dominated by n - C_{28} and n - C_{30} , these compounds were less abundant in roots, which had a higher proportion of n - C_{20} to n - C_{24} FAs. Leaf sheaths had a pattern similar to leaves and stems, except for a slightly higher relative abundance of n - C_{28} FA in leaf sheaths than in stem biomass (Figs. 2A, B, and 3A).

In comparison to plant biomass, all soil and rhizosphere samples were characterized by a higher content of long chain acids. Control soil in particular had a large amount of long chain homologs and a low amount of short chain FAs (Fig. 3B) with a low variation over time. A similar distribution pattern of FAs was observed in the rhizosphere and root-free soil under *Miscanthus* (Fig. 2D–E). The proportion of unsaturated C_{18} acids became higher over 1.5 months in soil samples, accompanied by a depletion in the proportion of most other acids. The FA distribution for the rhizosphere was characterized by a higher proportion of $\text{C}_{16:0}$ and unsaturated C_{18} acids, being intermediate between planted soil and roots. The distribution of acids in the rhizosphere showed some variation with time. $\text{C}_{19:1}$ was missing from all plant samples, whereas it was abundant in most soil and rhizosphere samples and was higher at the end vs. the start of the investigated period.

3.3. Molecular proxies for determination of plant derived FAs in soil

To determine the dynamics of lipid composition of plants and follow the contribution of plant derived lipids to soil lipids, several molecular ratios were evaluated (Fig. 4).

The diagnostic fatty acid ratio (originally defined as carboxylic acid ratio: CAR), which separates C_4 crops ($\text{CAR} > 0.67$) from C_3 crops ($\text{CAR} < 0.67$) (Wiesenberg and Schwark, 2006), was highest for roots and lower for leaves and stems (Fig. 4A). During the experiment CAR values became higher for roots and leaves, while they remained almost constant for stems. In the control soil the CAR was similar to the CAR of above ground biomass and revealed a low temporal variability. Planted soil and rhizosphere had low CAR values during days 55–62, whereas they were higher at the start and the end of the investigated period. The difference was stronger for rhizosphere than for root-free soil. During the whole experiment, rhizosphere soil and root-free soil under *Miscanthus* were characterized by slightly lower CAR values than the unplanted control soil.

The ratio of unsaturated vs. saturated C_{18} acids [$(\text{C}_{18:1} + \text{C}_{18:2}) \times n$ - C_{18}^{-1}] revealed high values (>15) for leaves and lower values for stems and roots (Fig. 4B), whereas all plant values were >5 . While for the above ground biomass (leaves and stems) the ratio was higher at the end of the experiment than at the beginning and it remained essentially constant for roots throughout the period. It became higher with time for all soil samples, whereas for rhizosphere exceptionally high values were observed within the first 2 weeks. After day 61 the ratio showed virtually identical results for all soil samples, except for slightly higher values within the rhizosphere.

The even/odd predominance or the carbon preference index (CPI; Table 1) for C_{20-30} FAs showed high values for plant tissues (Fig. 4C). Within plants values were highest for stems lower for leaves and lowest for roots. While values for above ground biomass became slightly higher with time, values for roots remained

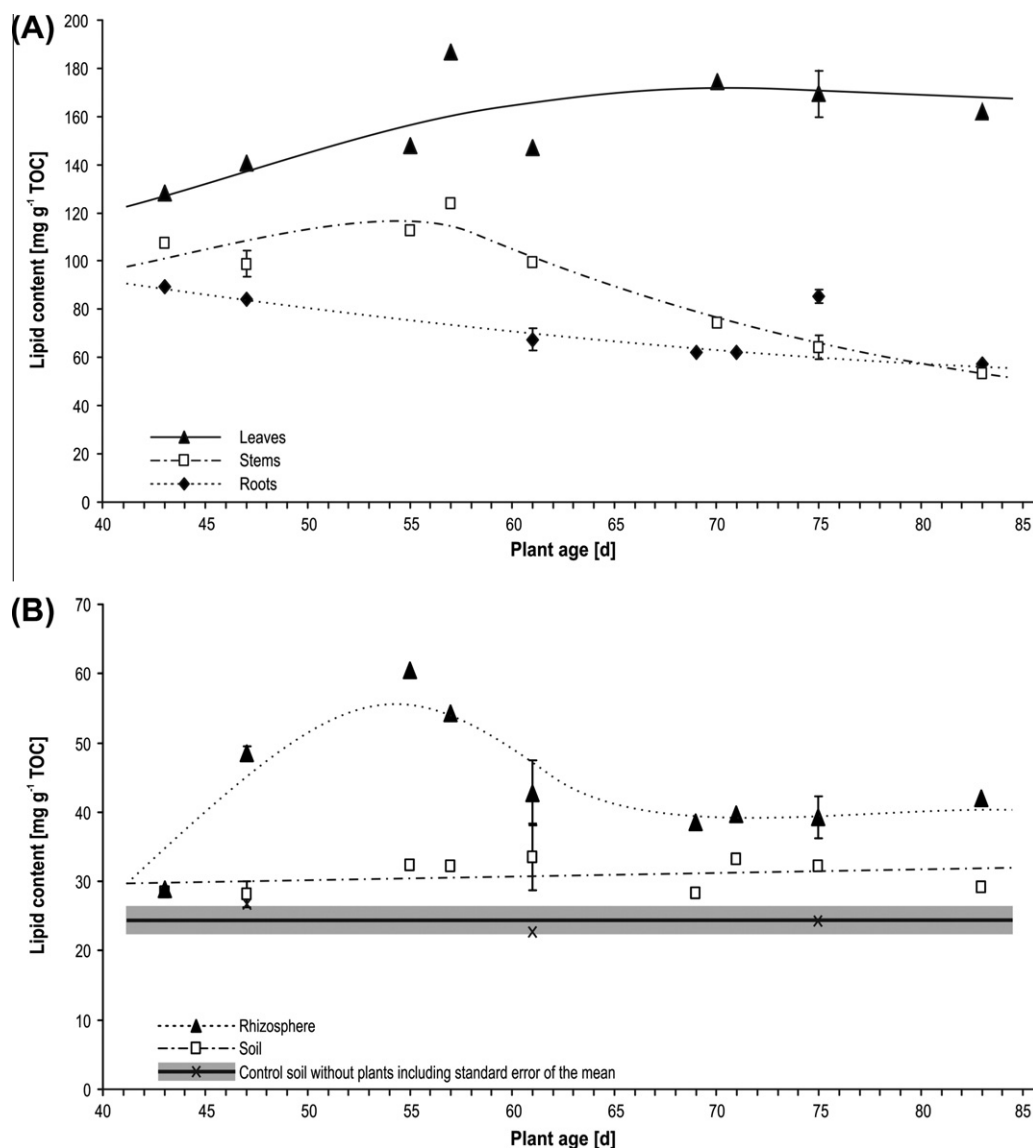


Fig. 1. Dynamics of lipid content normalized to TOC content: (A) *Miscanthus* plant parts and (B) soil and rhizosphere under *Miscanthus* as well as unplanted soil.

constant throughout the experiment. The low values for stems at the last two sampling dates were due to the separation of leaf sheaths from stem biomass for these samples. Without separation of leaf sheaths from stems, the ratio would have remained constant. For control and planted soil, CPI values were in the range of root biomass. At the end of the experiment slightly higher values were observed for soil vs. the beginning of the monitoring period. Rhizosphere revealed lowering of the values within the first 2 weeks and higher values thereafter, but never reached the values for root or soil CPI.

The average chain length (ACL; Table 1) in soil has been used for the differentiation of microorganism and plant derived OM, with higher values typical for plant biomass (Kolattukudy et al., 1976). During degradation of plant biomass long chain acids are selectively preserved in soils and indicate a plant derived origin. ACL values for *Miscanthus* FAs were different for plant tissue and ranged from ca. 17 in roots, over ca. 18.3 for leaves, to ca. 19.5 for stems, and remained almost constant during the experiment (Fig. 4D). As for some other parameters, the separation of leaf sheaths from stem biomass led to reduction of the ACL values for stem samples from days 75 and 83. ACL became higher for the control soil and lower for the planted soil with time. The ACL ratio was

two carbons lower for the rhizosphere than for soil samples and showed some variation with time.

To obtain information about plant biomass input to soil, CPI was plotted vs. ACL (Fig. 5), because specific variation was obtained either for soil or for plant samples. Values for leaves plotted on a mixing line between those of roots, which were characterized by low CPI and ACL values, and most stem values, which showed high values for CPI and ACL. The few stem samples with lower values than leaves were those where leaf sheaths had been analyzed separately. Soil and rhizosphere revealed low CPI values in the range of roots. Rhizosphere FAs plotted on a mixing line between root and soil samples, but sometimes showed lower CPI values than roots. Due to the fact that the ACL of rhizosphere samples plotted on a mixing line between roots and root-free soils, the enrichment in root and microbial biomass in the rhizosphere (E_R) vs. root-free soil could be estimated as follows using the ACL values of the identified samples (rhizosphere, soil and root):

$$E_R[\%] = (\text{ACL}_{\text{rhizosphere}} - \text{ACL}_{\text{soil}}) \times (\text{ACL}_{\text{root}} - \text{ACL}_{\text{soil}})^{-1} \times 100$$

Thus, E_R enabled estimation of the enrichment in fresh biomass to rhizosphere vs. corresponding soil without fresh incorporated biomass during the experiment (Fig. 6). Due to the experimental

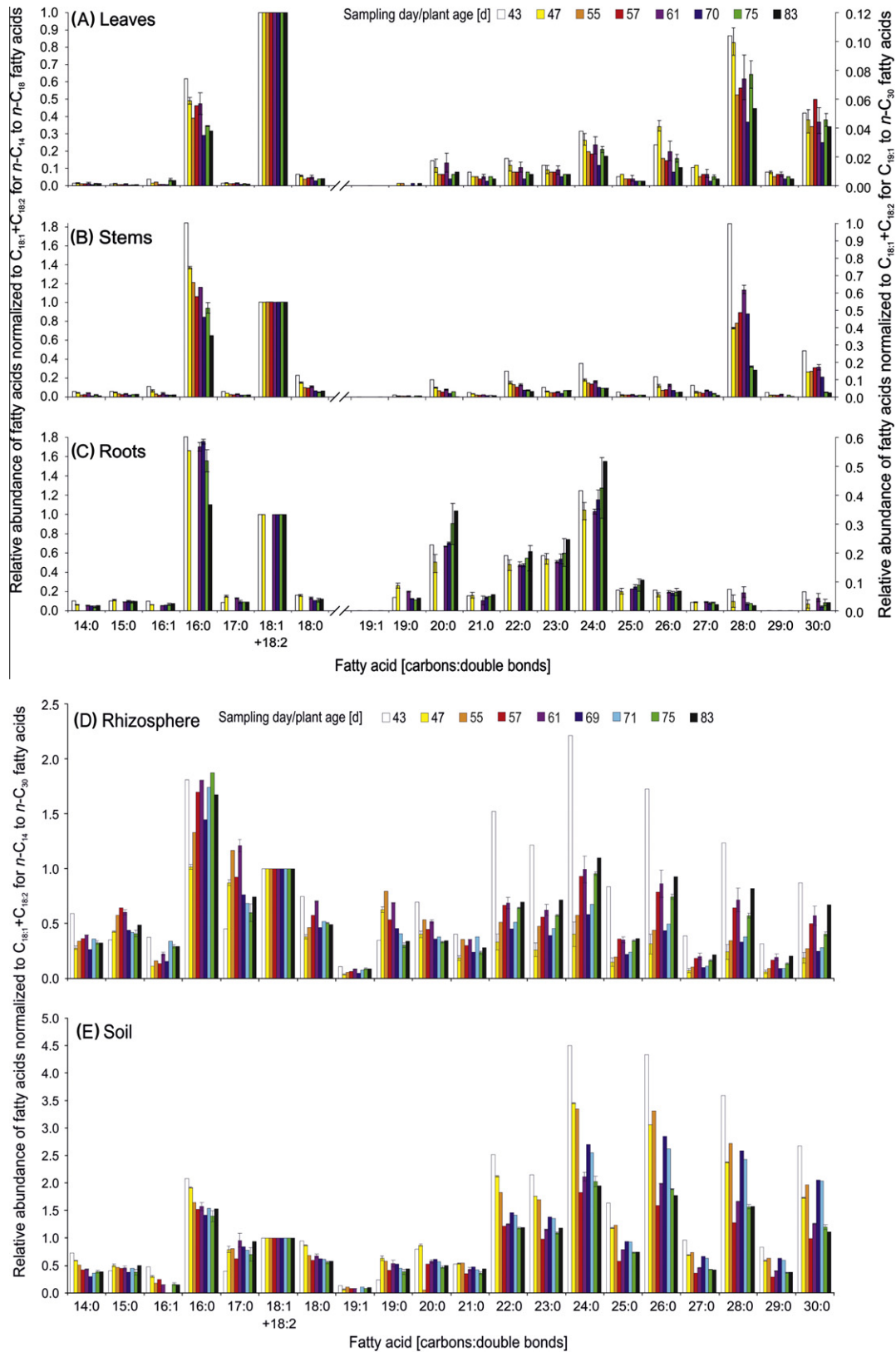


Fig. 2. Dynamics of FA distribution patterns normalized to $C_{18:1}$ and $C_{18:2}$ in *Miscanthus*: (A) leaves, (B) stems and (C) roots, as well as in (D) rhizosphere and (E) root-free soil under *Miscanthus*. For (A–C) the left y-axis refers to the most abundant acids with 14–18 carbons, while right y-axis refers to acids with 19–30 carbons.

set-up, the contribution of fresh above ground biomass was precluded, facilitating the use of the formula for E_R . The rhizosphere was enriched by 35–75% of fresh root and microbial biomass in

comparison to corresponding soil. After high values between days 47 and 55 and low values at the beginning, the enrichment was lower thereafter and reached a second relative maximum after

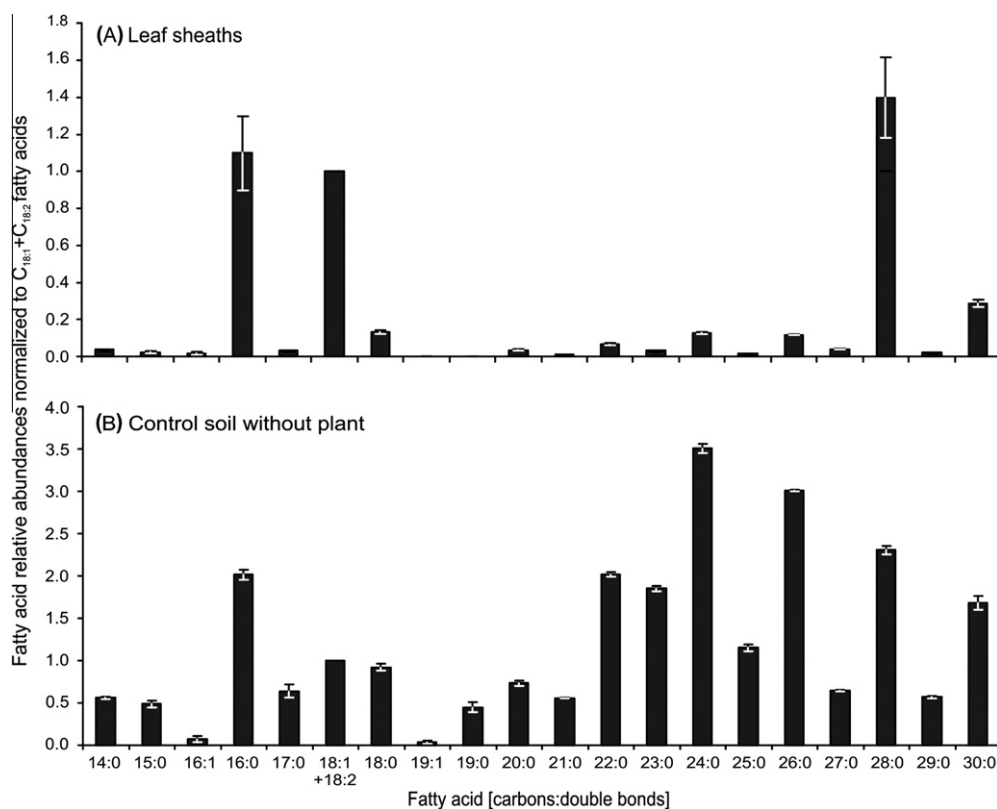


Fig. 3. Relative abundance of FAs normalized to C_{18:1} and C_{18:2} in (A) *Miscanthus* leaf sheaths from days 75 and 83 and (B) unplanted soil taken after 47, 61, and 75 days. Columns represent mean values of all sampling dates and errors indicate standard error of the mean.

70 days. Thereafter, the fresh plant and microbial biomass enrichment decreased in the rhizosphere vs. soil until the end of the experiment.

4. Discussion

4.1. Lipid content

The lipid content of *Miscanthus* tissue (Fig. 1) was in the range for crop plant tissues (e.g. Dove et al., 1996; Wiesenberg et al., 2009). The root-free soil samples correspond to extract yields from other bulk arable soils (Lichtfouse et al., 1995; Wiesenberg et al., 2004a, 2006). The highest lipid content was in leaves and a decrease towards roots resulted from higher surface to volume ratio in leaves than in stems and higher content of surface wax in above ground tissue vs. roots (e.g. Bianchi, 1994). The lower lipid content in stems after day 57 might be associated with plant development and could be related to a growth phase where preferentially other components (like starch) were produced and lipid production decreased at the same time. The high lipid content of the rhizosphere between days 47 and 61 might be due to improved growth of very fine roots, which could not be separated from the rhizosphere soil by manual picking. During later development stages, larger roots could be easily separated from the rhizosphere soil, resulting in uniform lipid content during the remaining weeks of the experiment. The higher lipid content of rhizosphere soil than root-free soil indicate a permanent presence of fine root material or a microbial contribution to the rhizosphere (Wiesenberg et al., 2010), whereas after root death, lipid content might also rapidly decrease again in the rhizosphere (Gocke et al., 2010). Thus, after the death of fine and coarse roots, the total lipid amount of the former rhizosphere might not necessarily indicate the presence of root derived

OM contribution, but must contain molecular remains of roots and microorganisms feeding on roots (Gocke et al., 2010). Higher lipid content in planted than control soil revealed a greater contribution of root derived and/or microorganism derived lipids to the planted soil, in agreement with a long term enrichment of lipids in soils under *Miscanthus* over 9 years in a field experiment (Kahle et al., 2001).

4.2. FA distribution patterns

The function of FAs in individual plant parts is reflected in different distribution patterns. Leaves, with the largest surface to volume ratio, contain more protective epicuticular waxes with 16 and 18 carbons and particularly long chain FAs with more than 20 carbons, typical of plant leaf waxes (Harwood and Russell, 1984; Walton, 1990). The distribution patterns of the FAs in plant tissue remained similar during the whole experiment, indicating the constant climatic conditions in the laboratory. Leaf sheaths and stems, with a lower surface to volume ratio, were characterized by very long chain acids (*n*-C₂₈ and *n*-C₃₀), which are thought to be related to intra- as well as epicuticular waxes. A similar enrichment of very long chain acids in leaves and stems vs. other plant tissues has been described for several C₄ crops like *Sorghum* (Avato et al., 1990; Hwang et al., 2002) or maize (Wiesenberg et al., 2004a; Jandl et al., 2007), but rarely for other plant groups like legumes (Body, 1974) or coffee (Stocker and Wanner, 1975). In agreement with previous studies (e.g. Dewhurst et al., 2002; Elgersma et al., 2003) of another perennial crop, increasing relative amounts of unsaturated C₁₈ FAs in the above ground biomass were observed during the growing season and indicated increasing amounts of primary epicuticular wax. For roots, long chain acids are produced in a different way than in above ground biomass due to potentially different FA chain elongase activity in the roots than in above

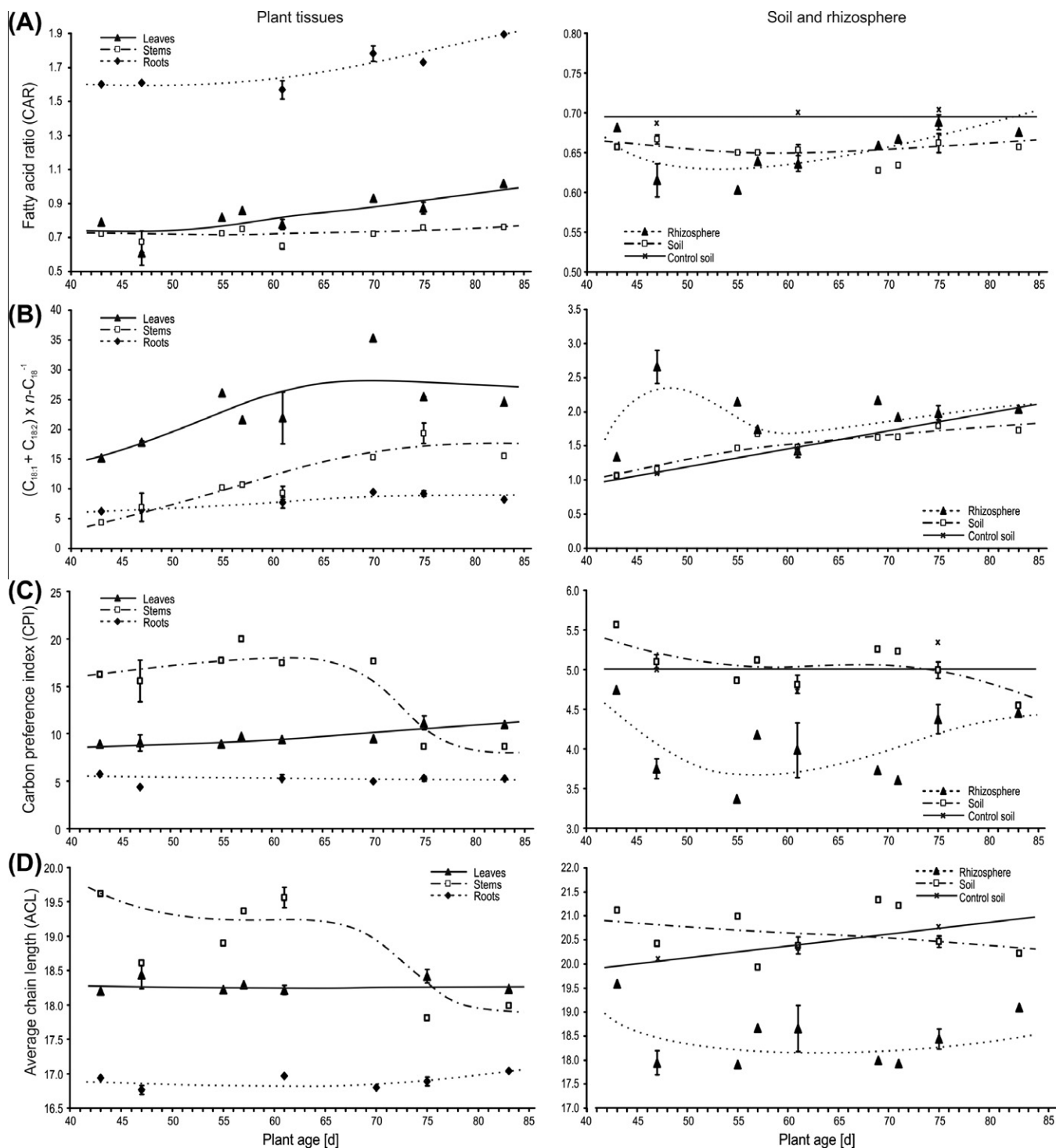


Fig. 4. Dynamics of diagnostic molecular ratios for *Miscanthus* plant tissues, soil, and rhizosphere to differentiate plant compositional changes and input of plant derived lipids into soils. (A) Fatty acid ratio [$n\text{-C}_{24} \times (n\text{-C}_{22} + n\text{-C}_{26})^{-1}$; CAR]. (B) Unsaturated vs. $n\text{-C}_{18}$ acids [$(\text{C}_{18:1} + \text{C}_{18:2}) \times n\text{-C}_{18}^{-1}$]. (C) Carbon preference index [$\{(\sum n\text{-C}_{20+22+24+26+28+30} / \sum n\text{-C}_{19+21+23+25+27+29}) + (\sum n\text{-C}_{20+22+24+26+28+30} / \sum n\text{-C}_{21+23+25+27+29+31})\} / 2$; CPI]. (D) Average chain length [$\sum(a \times A) \times n^{-1}$; ACL] with a as the amount of compound with A carbons and n as the number of acids used in the formula ($n = 20$). Interpolated lines are only for optical guidance.

ground biomass (Kolattukudy et al., 1976). Probably, different enzymes like root elongase (Schreiber et al., 2000) or a different cell wall composition (Schreiber et al., 1999) in roots is responsible for a lower chain elongation metabolism than above ground biomass. Minor amounts of endophytic diazotrophic bacteria as determined for other *Miscanthus* species (Kirchhof et al., 1997) might also result in a slightly different lipid composition of roots than above ground biomass, where these bacteria are absent. However, the lat-

ter cannot explain the major differences between these plant tissues. Additionally, some mycorrhizal fungi are associated and commonly analyzed together with roots, which might have modified the lipid composition to more acids with 16 carbons (Olsson, 1999).

Soil samples with low amounts of short chain acids and large amounts of long chain acids (Figs. 2D, E, and 3B) are typical for arable and grassland soils (e.g. van Bergen et al., 1998; Wiesenberg

Table 1
Mean values for molecular ratios of FAs for individual plant and soil samples. Arrows indicate trends for temporal changes in individual ratios during growth period ^a.

	CAR ^b	$C_{18:1+2} \times C_{18:0}^{-1}$ ^c	CPI (C ₂₀ –C ₃₀) ^d	ACL ^e
Leaves	0.81 ± 0.10 ↗	23.5 ± 6.1 ↗→	9.7 ± 0.9 ↗	18.3 ± 0.1 →
Stems	0.72 ± 0.04 →	11.4 ± 4.9 ↗	15.2 ± 4.3 ↗	18.7 ± 0.7 →
Roots	1.71 ± 0.12 ↗	8.1 ± 1.3 →	5.1 ± 0.4 →	16.9 ± 0.1 →
Rhizosphere	0.65 ± 0.03 ↘↗	1.9 ± 0.4 ↗	4.0 ± 0.4 ↘↗	18.5 ± 0.6 ↘↗
Root-free soil	0.65 ± 0.03 ↘↗	1.5 ± 0.3 ↗	5.1 ± 0.3 ↘	20.7 ± 0.5 ↘
Unplanted soil	0.70 ± 0.01 →	1.4 ± 0.4 ↗	5.0 ± 0.3 →	20.4 ± 0.4 ↗
Microorganisms ^f	ca. 1.0 ^h →	<1.0–100	1.0–2.0	16.7–17.2
Degradation ^g	↘	↘	↘	↗

^a Mean values and standard errors of mean are given; temporal tendencies are shown either in general or, if several trends were observable, are indicated by multiple arrows.

^b Fatty acid ratio: $[n-C_{24} \times (n-C_{22} + n-C_{26}) - 1]$.

^c Ratio of unsaturated vs. saturated C₁₈ acids $[C_{18:1+2} \times C_{18:0}^{-1}]$.

^d CPI for long chain acids $\{[(\Sigma C_{20+22+24+26+28+30}/\Sigma C_{19+21+23+25+27+29}) + (\Sigma C_{20+22+24+26+28+30}/\Sigma C_{21+23+25+27+29+31})]/2\}$.

^e ACL of FAs $[\Sigma(a \times A) \times n^{-1}]$.

^f Estimates based on literature results for microorganisms (Zelles and Bai, 1993; Zelles, 1997).

^g Degradation of plant biomass can be expected to modify the ratios in the way indicated.

^h Ratio can be expected to be in this range, because of very low or non-detectable amounts of long chain acids in microbial communities.

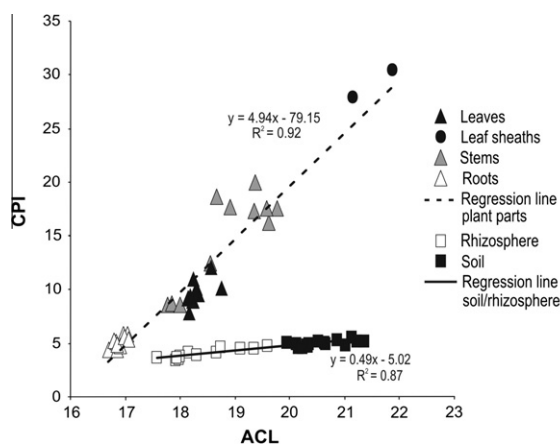


Fig. 5. CPI vs. ACL for source discrimination and apportionment.

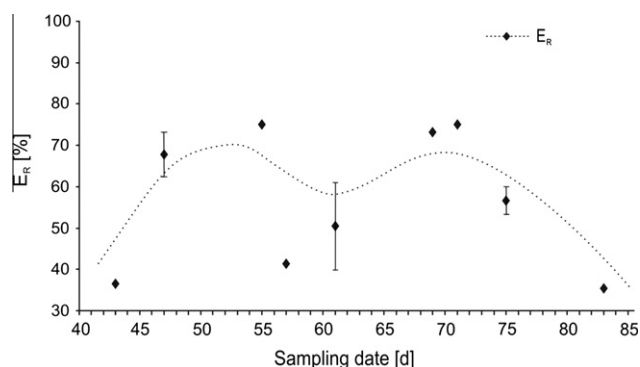


Fig. 6. Enrichment in fresh biomass (E_R) in rhizosphere when compared with soil distant from roots (root-free soil) based on FA ACL.

et al., 2004a; Quénéa et al., 2006). Whereas the overall variability in the FA patterns in root-free soil was low, the variation in the rhizosphere was most likely due to varying contributions of fine root and mycorrhizal hyphae in individual rhizosphere samples. Soil samples did not reveal a major contribution from fresh root biomass, which showed a high content of short-chain FAs and a low content of long-chain FAs. The changing amount of unsaturated C₁₈ acids during the experiment might indicate a subsequent marginal enhancement in the contribution of root derived FAs in soil.

Unsaturated C₁₉ acids were exclusively found in soil material and were absent from plant material. Therefore, in soil this FA must derive from a different source, thought to be the microbial biomass. Microorganisms do not comprise the sole source of short chain acids (<C₂₀) in soils as proposed by several authors (e.g. Amblès et al., 1993; Naafs et al., 2004) due to a similar FA composition of roots. Some unsaturated FAs in soils and rhizosphere are only present in low amount (C_{16:1}) potentially due to symbiotic mycorrhiza (Olsson, 1999) or absent (C_{19:1}) from roots, indicating a direct microbial origin.

4.3. Diagnostic ratios for determination of plant derived FAs in soil

The fatty acid ratio (CAR) of plants varied between 1.71 ± 0.12 for roots, 0.72 ± 0.04 for stems and 0.81 ± 0.10 for leaves (Fig. 4A). The high values for roots, followed by low values of stems and leaves, confirmed typical CAR values (>0.67) for C₄ plants (Wiesenberg and Schwark, 2006). The change in values for plant tissue with time was not previously observed for C₄ plants and can be related to plant growth and the successive alteration of the lipid composition with plant age. The CAR of the control soil varied slightly around 0.7 and thus was similar to values for the above ground biomass of *Miscanthus*. However, this cannot be described as influenced by the C₄ plant, because the soil was unplanted. For control and even for the planted pots almost no above ground litter could enter the soil as the soil was completely separated from shoots throughout the experiment. As previously described, C₃ cropped soils have CAR < 0.8 and therefore plot on a mixing line between C₃ and C₄ plant biomass as a result of degradation of plant biomass (Wiesenberg, 2004). The degradation of primary plant biomass leads to an increase in CAR values in C₃ cropped soils vs. C₃ biomass due to approaching an equilibration of C₂₂, C₂₄ and C₂₆ FAs during degradation. Thus, the lipids in the control soil originated from degraded C₃ plant biomass without measurable contribution from C₄ plant material, which would reveal higher CAR values vs. the control soil. Surprisingly, CAR values became lower for root-free soil and rhizosphere in spite of cropping with *Miscanthus*, which showed very high CAR values in root material. This implies that even in the rhizosphere the potential fine root material incorporated by *Miscanthus* did not obviously contribute to soil FAs. Therefore, it is more likely that higher microbial activity in the rhizosphere led to improved selective degradation of the most abundant plant derived lipids and resulted in decreasing CAR values. Thereafter, rooting and rhizodeposition were more pronounced throughout the experiment as indicated

by greater root biomass (Schneckenberger et al., unpublished data), resulting in increasing CAR values within the rhizosphere and root-free soil under *Miscanthus* over the last month investigated. This obvious change to higher values suggests that increasing contribution of root derived FAs can be expected in the long term and that this is associated with high productivity of root tissues at specific growth stages. This hypothesis must be proved by monitoring the lipid composition in *Miscanthus* plants and soils for more than 1.5 months in further experiments.

The ratio of unsaturated to saturated C_{18} acids documents the temporal variability in epicuticular wax in above ground biomass, which revealed an enrichment in unsaturated acids until a plant age of 70 days and remained constant until the end of the experiment (Fig. 4B). This was associated with preferential growth of above ground biomass during the experiment until day 70, followed by a preferential growth of root biomass as indicated by elevated root:shoot values (Schneckenberger et al., unpublished data). This is in agreement with improved root production after several weeks of plant growth as reported for *Miscanthus* and some other C_4 plants (Jin et al., 1997; Neukirchen et al., 1999). The high amounts of unsaturated C_{18} acids within the rhizosphere during the first 2 weeks seem to be related to enhanced microbial activity during this period, because these compounds are abundant in both plant and microbial tissues (Harwood and Russell, 1984). An increased contribution of plant biomass (i.e. roots) was not determined during monitoring of the plant biomass. The stronger microbial contribution is in agreement with the low CAR values in the rhizosphere at the same time. Thus, higher values of unsaturated vs. saturated C_{18} acids support the assumption of higher microbial activity and contribution of root biomass in the rhizosphere in comparison to root-free soil, which is a common feature of the rhizosphere (e.g. Singh et al., 2004).

The higher CPI values of the above ground biomass at the end of the experiment vs. the beginning indicates a further enrichment of predominant even FA homologs during plant growth and not e.g. partial microbial degradation of the litter (Fig. 4C) as proposed by some authors (Zech et al., 2009; Buggle et al., 2010). The high CPI values of stems are related to the leaf sheaths, which were analyzed together except for the last sampling dates. As expected, the CPI was lower for root tissues due to a lower content of primary epicuticular waxes. The constant CPI of soil indicates that no additional contribution of above ground biomass occurred during the experiment. Whether root biomass might have entered the root-free soil or not cannot be determined via this ratio due to identical CPI for roots and soil. Higher CPI values in the rhizosphere at the end than in the beginning of the investigated period documents a stronger degradation of primary plant biomass (Zech et al., 2009; Buggle et al., 2010) and/or FAs in rhizosphere than in root-free soil, which may occur only under increased microbial activity. Like other parameters (CAR, unsaturated vs. saturated C_{18} acids), the CPI was lowest within the first 2 weeks, indicating high microbial activity at this stage, which must have been stronger in the rhizosphere than in the root-free soil (Singh et al., 2004). Thereafter, the CPI could only increase very slowly due to the low input of primary plant lipids via rhizodeposition and root exudates. The enhancement of CPI values supports the findings based on the CAR that the input of root derived plant biomass occurred predominantly during the experiment within a couple of weeks.

The ACL was greatest for stems and lower for leaf and root tissues due to the largest amount of very long chain acids in stems followed by the other tissues (Fig. 4D). The varying ACL values for unplanted soil with time results from a preferential degradation of short chain and mid-chain acids, as shown by other parameters, hence leading successively to higher values. Lower ACL values with time for planted soil can be related to a stronger contribution from microorganism and root derived short chain acids

with time. Low ACL values for the rhizosphere, intermediate between soil and root ACL values, correspond to a large contribution of root and microorganism derived FAs to the rhizosphere. Hence, the ACL clearly indicates a contribution of microorganism derived OM and degradation effects, where the latter are associated with the presence of the microorganisms.

The higher CPI and ACL values in above ground biomass than in roots (Table 1 and Fig. 5) were due to the specific composition of the plant tissues, with high amounts of very long chain acids ($n-C_{28}$ and $n-C_{30}$), while roots were characterized by larger amounts of long chain acids (maximizing at $n-C_{24}$). During degradation of plant derived biomass long chain and very long chain acids are relatively enriched in soils, while short chain acids are rapidly degraded and the preference of even homologs is reduced, which is common during degradation (Otto and Simpson, 2005; Zech et al., 2009; Buggle et al., 2010). The composition of long chain acids in planted and control soils was predominantly due to 'old' plant biomass, which was incorporated into soil prior to the experiment. While the general composition was related to old biomass, variation in soil FAs during the experiment was due to ongoing changes in the input of fresh biomass. Especially the rhizosphere was enriched in root and microbial biomass due to a large contribution of fresh plant derived C. For some rhizosphere samples the CPI was lower than that of root biomass, indicating a high contribution of microorganism derived compounds, where microorganisms were responsible for degradation of fresh and old FAs, therefore causing the lower CPI values in rhizosphere vs. root biomass.

As a result of microbial decomposition of plant biomass, most of the ratios (CAR, ratio of unsaturated vs. saturated acids, CPI) showed lower values with time, whereas others [long chain/short chain (L/S) acids, ACL] are supposed to reveal the opposite (Table 1). This leads to a typical FA distribution pattern found in agricultural soils with a predominance of long chain and very long chain FAs and very low amounts of short chain and unsaturated FAs (van Bergen et al., 1998; Wiesenberg et al., 2004a; Quénéa et al., 2006). Taking this into account, CAR and CPI in particular indicate decomposition of plant derived biomass in soil and/or rhizosphere between 43 and 61 days. Ratios like ACL and unsaturated vs. saturated C_{18} acids suggest an incorporation of FAs derived from microorganisms into the planted soil in the same period. In the control soil the ratios indicated a successive degradation of plant derived old biomass with time. Also, some ratios showed variable trends during the monitoring time. From days 57 or 70, respectively, a lower tendency to decomposition than for days 43–57 was indicated. Moreover, successive incorporation of new root derived C into the planted soil and strong incorporation into the rhizosphere occurred, as indicated by CAR and CPI ratios.

The enrichment of fresh microbial and root biomass in the rhizosphere based on ACL indicates a high microbial contribution to the rhizosphere within the first 2 weeks (Fig. 6). Thereafter, the constant low contribution of fresh plant derived biomass resulted in a lower nutrient supply for microorganisms within the rhizosphere and led to a decrease in microbial activity and contribution of molecular microorganism remains. Afterwards, the enhanced root growth resulted in a stronger contribution in fresh biomass again until day 71, as indicated by the enrichment factor E_R . The varying enrichment factor with time and the decrease until the end of the experiment can be related to three major factors. First, samples of the laboratory experiment were taken from different pots and represent soil under different plants and therefore can show different amounts of plant derived C in soil related to specific growth of plant individuals. Second, separation of root-free soil and rhizosphere could be insufficient for individual pots, which is not likely due to several parameters showing almost no variability. Third, the development of lipid composition is a reflection of the

on-going molecular changes in the soil. The first point cannot be completely neglected but, due to a combination of several replicates for analysis, the differences can be expected to be low. The second point can be also neglected due to different lipid content and composition for rhizosphere and related root-free soil samples. Hence, the molecular trends reflect the development of a root derived C contribution and subsequent microbial influence within soil. Taking natural variation between plant individuals into account, E_R and the other ratios indicate that an input of root derived lipids occurs within months and is faster in the rhizosphere than in root-free soil, which confirms recent studies using ^{14}C labelling techniques (Wiesenberg et al., 2010). However, the current study is the first showing molecular changes in soil on a scale of weeks to months. Due to the strong enrichment of plant and microorganism related FAs in the rhizosphere vs. root-free soil, the contribution of root derived lipids to soil lipids is an important incorporation pathway and should be mentioned in future studies focussing on soil lipids. However, further studies are needed to differentiate the amount of the contribution from litter and root derived C to soil lipids and the degradation of C on a molecular level during periods with no or limited plant growth.

5. Conclusions

The lipid content and FA composition was followed during a laboratory experiment on *Miscanthus x giganteus* for 40 days to assess the short term temporal and spatial variability in the molecular composition in the plant–soil system. The individual plant tissues—leaves, stems and roots—were characterized by a different lipid composition as shown by FA distribution patterns and several molecular ratios, including e.g. the fatty acid ratio (CAR), carbon preference index (CPI) and average chain length (ACL). While CPI and ACL increased, CAR decreased from roots over leaves to stems. The plant lipid distribution of different plant tissues changed with time to more very long chain ($>\text{C}_{26}$) and more unsaturated C_{18} acids for above ground biomass and decreasing amounts of very long chain acids and increasing CAR for roots. Incorporation of root derived FAs into the soil distant from roots was low. Even in the rhizosphere, rhizodeposition was masked by microorganism derived FAs, especially during early plant growth. Only at the end of the experiment the contribution of root derived carbon increased, especially in the rhizosphere and marginally in soil distant from roots, as documented in the CAR. Root and microorganism derived FAs were enriched in the rhizosphere by 30–75% when compared to root-free soil, which was determined by the ACL based enrichment factor E_R . The study clearly showed that molecular changes in plant tissues and input of root derived lipids to soil occur on a day to month scale. This must be taken into account when choosing a sampling date for samples deriving from natural soils as, like in the laboratory experiment, a strong temporal and spatial variability in the molecular composition can be expected. The input of root lipids might be completely different from input of above ground biomass as derived from various examples from short chain to very long chain FAs for *Miscanthus* plants. Thus, a differentiation of root and above ground plant derived FAs in soil is possible, especially when long chain and very long chain FAs are investigated. However, further research is required regarding spatial and temporal variability of molecular patterns under field conditions.

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