Plant lipid composition is not affected by short-term isotopic (^{13}C) pulse-labelling experiments

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Abstract

Pulse-labelling of plants in a $^{14}CO_2$ or $^{13}CO_2$ atmosphere is a useful tool for investigating C allocation in plants and soils, revelation of C sources in soil organisms, as well as for CO_2 partitioning studies. Recently, these labelling experiments have been used to produce isotopically labelled biomass of plants or microorganisms for the investigation of C dynamics in these organisms or in soil. However, it remains unknown whether these labelling approaches may affect the composition of plant constituents that react to modifications of environmental conditions during biosynthesis. Lipids as primary biosynthesis products and main components of plant waxes are well known to react fast to environmental changes resulting in a modified lipid composition. In this study, we demonstrate that lipid composition may be only slightly affected by the labelling procedure, especially, when only short pulses (only a few hours) are applied and when the sampling does not occur immediately after the labelling. While the differences of plant lipid compositions are obviously modified not as a result of isotopic pulses, the environmental conditions of plants grown under controlled laboratory conditions have a significant effect leading to a shift of the distribution pattern of plant lipids compared to the lipid composition of plants grown under field conditions.

Key words: alkanes / carboxylic acids / distribution patterns / extract yields / $^{13}CO_2$

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1 Introduction

Lipids are main components of plants and soils (*Gregorich* et al., 1996) and are used as diagnostic markers in plants (*e.g., Rommerskirchen* et al., 2006; *Wiesenberg* and *Schwark,* 2006) as well as in sedimentary environments and soils (*e.g., Dinel* et al., 1990). It is documented that they may be useful for turnover estimation of plant-derived lipids in soils (*Cayet* and *Lichtfouse,* 2001; *Wiesenberg* et al., 2004a, 2008a; *Quénéa* et al., 2006). However, it is well known that primary plant lipids like carboxylic acids show a fast reaction on environmental changes (*Jenks* et al., 2001; *Conte* et al., 2003; *Wiesenberg* et al., 2007b) reflected by a modified distribution pattern of cuticular wax components, whereas degradation products of primary plant lipids such as alkanes are less amenable to environmental changes (*Wiesenberg* et al., 2008b).

Pulse-labelling of plants in a $^{14}CO_2$ or $^{13}CO_2$ atmosphere is a useful tool for investigating C allocation in plants (reviewed by *Kuzyakov* and *Domanski*, 2000) and soils, as well as for CO_2 partitioning studies (*Kuzyakov*, 2006). During the last decades, numerous studies focused on C dynamics in plants and soils using pulse-labelling as a powerful tool to analyze C fluxes, to estimate turnover of soil organic matter, to partition fluxes and pools, to evaluate C fluxes through food-webs, and to estimate model parameters. However, to the best of our knowledge there are no studies available demonstrating whether labelling procedures have an effect on the molecular composition of plant tissues. This is one important prerequisite for the application of the pulse-labelling approach and for the transferability of results from laboratory to field conditions. Up to now, it is tacitly accepted that pulse and even continuous isotopic labelling experiments do not change the chemical composition of plant tissues or cuticular waxes. The commonly used comparison in total weight of plant organs between labelled and unlabelled plants as well as their total C and N content is surely insufficient in terms of a rigorous examination of the transferability of the results.

As lipid composition in plant organs is very sensitive to environmental changes, we evaluated lipid distribution patterns as affected by individual or replicate pulse-labelling in a $^{13}CO_2$ atmosphere. We hypothesised that the composition of primary plant lipids is not obviously affected by the $^{13}CO_2$ pulse-labelling procedure due to the short time interval of the modified atmospheric conditions. If the hypotheses were confirmed, the pulse-labelling approach would not affect plant–soil–microorganism interactions and would have a high potential for studies on lipid dynamics in plant, soil, and microbial biomass.

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Therefore, this study focused on the development of lipid distribution patterns of several grasses, which were pulse-labelled in a $^{13}CO_2$ atmosphere and were harvested several days thereafter. The lipid distribution patterns of plants from the labelling experiment were compared with tissues from plants of the same age, which were treated identically, but received no isotopic label.

2 Materials and methods

2.1 Experimental design

To consider differences in lipid composition due to different photosynthetic pathways, the two C_3 grass species wheat and rye and the two C_4 grass species maize and *Miscanthus* were investigated. Seeds of maize (*Zea mays* L. cv. Tassilo), wheat (*Triticum aestivum* L. cv. Thasos), and rye (*Secale cereale* L. cv. Haccada) were pre-germinated in water for 3–4 days. *Miscanthus × giganteus* (Greef et Deu.) rhizome segments with buds were washed and cut to pieces of approximately 0.05 m × 0.02 m before planting (*Schneckenberger* and *Kuzyakov*, 2007).

For each plant plant species, three replicate pots with a volume of 250 mL for maize, wheat, and rye, or 4200 mL for *Miscanthus*, respectively, were set up with one (maize) or four (rye and wheat) seedlings or rhizomes (*Miscanthus*). Maize, wheat, and rye plants were labelled only once. Detailed studies on *Miscanthus* comprising an application of three pulse-labels required additional pots, so that for each pulse-labelling three replicate pots and, correspondingly, three replicate control pots without labelling were available. For each individual plant species, three pots were treated like the pulse-labelled pots except for receiving the ^{13}C label. Immediately before planting, the pots were filled with homogenised 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" from the University of Hohenheim, Stuttgart, in 2004. Shortly before the experiments were started, the soil was sieved (2 mm). Big roots and most of the small roots were removed with tweezers and the electrostatic method used by *Kuzyakov* and *Siniakina* (2001).

The required water holding capacity of the soil of (60% of maximum water holding capacity) was attained by adding 75 mL deionised water and 25 mL nutrient solution [153.4 mg K_2SO_4 L^{-1}, 7.5 mg KCl L^{-1}, 472.3 mg $Ca(NO_3)_2 \cdot 4H_2O$ L^{-1}, 246.5 mg $MgSO_4 \cdot 7H_2O$ L^{-1}, 34.0 mg KH_2PO_4 L^{-1}, 303.3 mg KNO_3 L^{-1}, 0.0618 mg H_3BO_3 L^{-1}, 0.099 mg $MnCl_2 \cdot 4H_2O$ L^{-1}, 0.0499 mg $CuSO_4 \cdot 5H_2O$ L^{-1}, 0.0247 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ L^{-1}, 0.2875 mg $ZnSO_4 \cdot 7H_2O$ L^{-1}) to each pot. Solutions were added to the pots of the *Miscanthus* experiment in the same relations, but to a higher amount. The nutrient solution was applied weekly. Water content was re-adjusted to 60% water holding capacity in decreasing time intervals to provide the plants with an adequate water supply during the whole experiment. One week after planting, the weakest plant of the four wheat, rye, and *Miscanthus* plants per pot was removed, resulting in three plants remaining for labelling. Results refer to pots containing one maize and three wheat, rye, or *Miscanthus* plants. Plants were illuminated for 14 h daily and kept under controlled conditions (800 mmol photons m^{-2} s^{-1} PAR at canopy height, 25°C/20°C day/night temperature).

The age of the plants at labelling was 37 d (rye), 41 d (*Miscanthus*), or 42 d (wheat, maize). During the day prior to the application of the pulse-label, the pots were sealed with non-phytotoxic silicone rubber (Tacosil 145, Thaurer & Co, Dresden, Germany) between the plant stems and the lid of the pots. Larger holes in the pots of the *Miscanthus* experiment were sealed with paraffin wax, which resulted in a contamination of the aliphatic hydrocarbon fraction of the triple-pulse-labelling experiment. Two holes were reversibly sealed with plastic bucklers during labelling.

Each plant species was labelled independently. For labelling, the plants were placed into an acrylic glass chamber (polymethyl methacrylate, PMMA; 0.5 m × 0.5 m × 0.6 m). Unlabelled plants were used as reference for natural abundance of ^{13}C. The labelling procedure was described in detail by *Kuzyakov* and *Cheng* (2001). Briefly, a glass flask containing 0.11 g (maize, wheat) or 1 g (*Miscanthus*) solid $Na_2{}^{13}CO_3$ (99 atom-%) (Aldrich Chem., Milwaukee, Wisconsin, USA) was connected to the acrylic glass chamber. The amounts of applied ^{13}C varied between the experiments from 12.34 mg ^{13}C (maize and wheat) over 34.78 mg ^{13}C (rye) to 112.2 mg ^{13}C (*Miscanthus*). Five milliliters 5 M H_2SO_4 were added to the sodium carbonate to produce ^{13}C-labelled CO_2. The released $^{13}CO_2$ was disposed into the chamber with a membrane pump (Type 115, Rietschle Thomas, Schopfheim, Germany). The plants were labelled during 5 h (*Miscanthus*) or 8 h (other plants). Afterwards, the labelled air in the chamber was exchanged with a membrane pump for 1.5 h, whereas the remaining CO_2 of the chamber was trapped in 20 mL 0.5 M NaOH. For some of the replicates of *Miscanthus*, the labelling was repeated after 14 d and additionally for the remaining replicates 28 d after the first pulse. All pots (labelled and corresponding control) were harvested 6 d after labelling or 6 d after the second and third labelling, respectively. The *Miscanthus* pots were additionally sampled 2 and 14 d after the individual pulse-labelling. During the time intervals between labelling and sampling, soil air was exchanged as described by *Werth* and *Kuzyakov* (2006).

2.2 Samples

Aboveground plant parts were divided into leaves and stems (*Miscanthus*, wheat, and rye), or flower, leaves, leaf nodes, and culms (maize). Roots were carefully separated from the soil and washed. After weighing, plant material was dried at 60°C to constant weight. Plant material was milled using a ball mill. Individual plant materials from the same treatment and the same plant part were combined. Labelled *Miscanthus* plants from the three different sampling times after each pulse were analyzed separately and the variability was characterised as mean and standard deviation of the mean. Hence, three replicate analyses were carried out for labelled *Miscanthus* plants, while for other samples a combination of replicates was required previous to lipid analyses, because of low amounts of available biomass.

2.3 Bulk analysis

All samples were analyzed for their total organic carbon content by elemental analysis (Leco CS 225). Thereafter, stable carbon isotopic composition was determined using a continuous flow Heraeus CHN-O-Rapid Elemental Analyser coupled to a Finnigan MAT Delta-S mass spectrometer. Carbon isotopic values were expressed in per mil relative to the Vienna Pee Dee Belemnite (V-PDB) standard:

$$\delta^{13}C = [(\{^{13}C/^{12}C_{sample}\} / \{^{13}C/^{12}C_{std}\}) - 1] \times 10^3,$$

where $^{13}C/^{12}C_{std} = 0.0112372$.

2.4 Lipid extraction and separation

Free plant lipids were extracted using accelerated solvent extraction (Dionex ASE 200) with CH_2Cl_2/CH_3OH (93/7; v/v). Five grams of individual dried plant material (when available) were filled into stainless steel extraction vessels and sequentially extracted for 20 min in two steps at 5×10^6 Pa at temperatures of 75°C and 140°C. Extracts of the sequential extraction were combined thereafter. The detailed extraction scheme has been described (*Wiesenberg* et al., 2004b). Lipids were sequentially separated into carboxylic acids and low-polar lipids using solid phase extraction within a manual, simplified step equal to the automated separation of carboxylic acids and neutral/intermediate polar lipids (*Wiesenberg* et al., 2004b). Therefore, dried extracts were redissolved in CH_2Cl_2 and applied to KOH-coated (5%) silica gel columns. Lipids of neutral and intermediate polarity were eluted with CH_2Cl_2 and dried thereafter. Elution of carboxylic acids was performed by flushing with CH_2Cl_2/CHOOH (99/1; v/v). The fraction containing neutral and intermediate-polarity lipids was separated into aliphatic hydrocarbons, aromatic hydrocarbons, and low-polar lipids (mainly alcohols), using automated medium pressure liquid chromatography (MPLC) separation (*Radke* et al., 1980). Volume reduction was performed *via* a turbo vaporization (Zymark) or rotary evaporation. The aliphatic hydrocarbon and the carboxylic acid fractions were studied in detail.

2.5 Gas chromatography/mass spectrometry (GC/MS)

Deuteriated standards (d_{39}-*n*-C_{20} carboxylic acid, d_{50}-*n*-C_{24} alkane) were added to the carboxylic acid and aliphatic hydrocarbon fractions, respectively. Compound identification was performed with a HP 5890 Series II gas chromatograph coupled to a HP 5989A mass spectrometer. Carboxylic acids were methylated with CH_2N_2, while aliphatic hydrocarbons were directly amenable to GC.

2.6 Statistical analysis

The design for the single-pulse experiment implied three replicate pots for control and labelled plants, each. For the *Miscanthus* experiment, three replicates were used for each sampling time. Unfortunately, root dry mass and some other plant parts yielded amounts in a very low range that was not sufficient for lipid analyses. Hence, carbon isotopic values were determined on three replicates for each experiment, while replicates were combined and extracted together for lipid analyses. The three sampling intervals of the *Miscanthus* experiment taken after each pulse were analyzed separately and due to low differences in distribution patterns between these three samples they were expressed as three replicates in this study. To determine significant differences, t-tests for dependant samples were conducted for plant lipid composition using StatSoft STATISTICA 7.0.

3 Results and discussion

3.1 Bulk isotopic values ($\delta^{13}C$)

Isotopic values within control rye plants decreased from –32.9‰ V-PDB in roots to –35.4‰ in leaves (Fig. 1). These values were about 2‰ lower as compared to data typically found in the literature for plants grown under field conditions (*Wiesenberg* and *Schwark*, 2006). This may be due to the early growth stage of the investigated plants in comparison to reported data for mature plants, because increasing isotopic values have been described, *e.g.*, for rye and wheat plants during the growing season (*Yoneyama* et al., 1997; *Wiesenberg* and *Schwark*, 2006). Isotopic values of wheat were highest in roots and lowest in stems while leaves showed intermediate results. Isotopic values for maize plants were almost identical within the whole plant with a low internal variation, which is common (*Wiesenberg* and *Schwark*, 2006). As discussed with respect to isotopic values of rye, those of maize plants were relatively low when compared to published data (*Marino* and *McElroy*, 1991; *Wiesenberg* and *Schwark*, 2006). The low isotopic values of wheat, rye, and maize plants grown under laboratory conditions in comparison to literature results from field studies may also be related to the CO_2 concentration and isotopic composition of the laboratory air as described by *Widory* and *Javoy* (2003). Hence, an enrichment with CO_2 in the laboratory air due to respiration and/or contamination may be associated with a depletion of the isotopic values by 1‰–2‰, which results in an identical isotopic shift in C_4 grasses compared to the air (*Marino* and *McElroy,* 1991) and a larger isotopic depletion in C_3 grasses as discussed by *Zhao* et al. (2001).

Miscanthus plants showed largest isotopic values for stems and lowest isotopic values for roots or leaves depending on plant age (Fig. 1). In general, isotopic values decreased for *Miscanthus* with plant age in agreement with observations made by *Yoneyama* et al. (1997) and *Wiesenberg* and *Schwark* (2006) for other grasses. Compared to results of *Wiesenberg* and *Schwark* (2006), isotopic values were depleted by at least 2‰ in comparison to isotopic values derived from plant tissues grown under field conditions. Hence, we assume that the laboratory conditions resulted in special environmental conditions for plant growth, which resulted in depleted isotopic signatures of plant tissues and which may have an effect on the lipid composition when compared with plants grown under field conditions. Despite these special environmental conditions in the laboratory, a comparison of control plants and ^{13}C-labelled plants, both grown under iden-

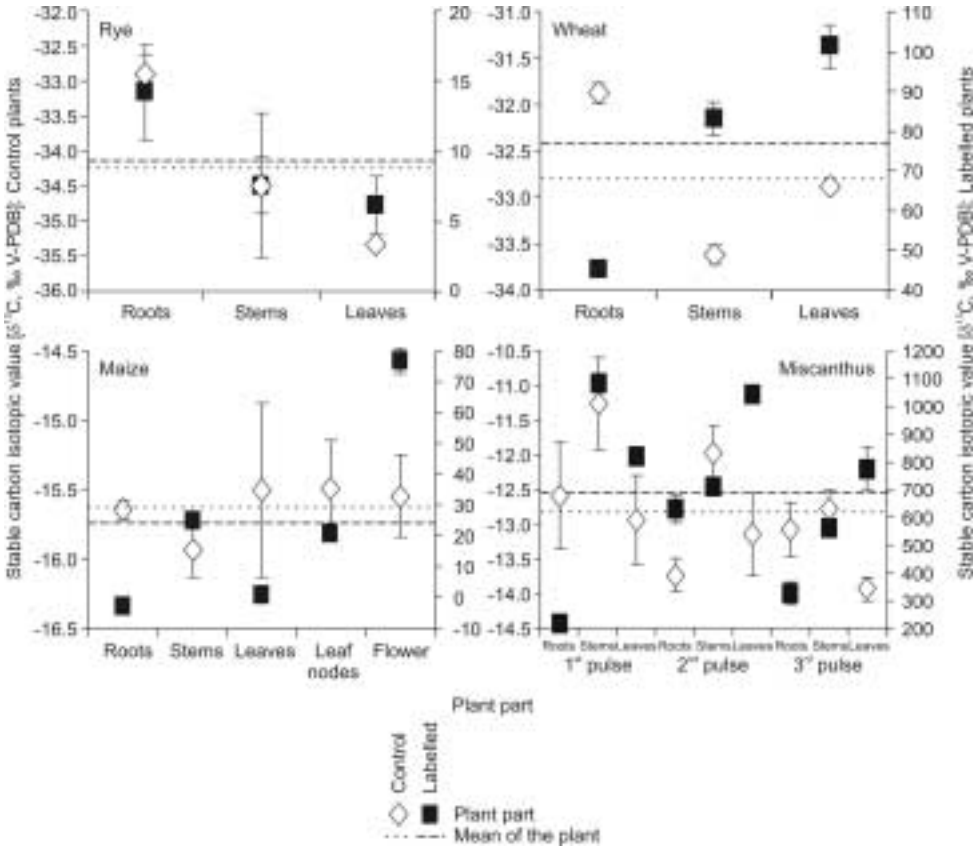

Figure 1: Stable carbon isotopic values ($\delta^{13}C$) of ^{13}C-labelled *versus* untreated control plants. For *Miscanthus* pulse labelling was repeated 14 d after the previous pulse for three times.

tical conditions except for the isotopic pulse-label, gives the opportunity to determine the influence of the isotopic labelling procedure on the plant lipid composition.

Labelled rye plants revealed the lowest isotopic label ranging between 6‰–16‰ V-PDB, whilst for labelling an intermediate amount of ^{13}C was available compared to all other plants. Lowest values in aboveground plant tissues compiled with root biomass indicate a faster replacement of ^{13}C in aboveground tissues than in root biomass. However, the low ^{13}C values may be due to either a limited uptake of CO_2 by rye during labelling or problems during the labelling procedure, *e.g.*, a potential leakage of the labelling chamber or an insufficient dissolution of the labelled substrate. Wheat and maize plants received an identical amount of $Na_2{}^{13}CO_3$ and showed isotopic values in the range of 45‰–102‰ V-PDB for wheat and 0‰–78‰ V-PDB for maize. The lower isotopic label of maize plants was related to a higher amount of biomass and thus a higher dilution effect of the assimilated ^{13}C in the plant biomass. For both plant species, the isotopic values were highest in aboveground tissues and lowest in root biomass due to a reduced translocation towards root biomass. For *Miscanthus*, isotopic values were significantly higher than for the other plant species due to larger amounts of $Na_2{}^{13}CO_3$ dissolved during the labelling procedure. For each sampling interval, isotopic values were highest in aboveground tissues and lowest in root biomass. The low isotopic value of the root biomass after the first labelling must be related to a delayed internal transfer of ^{13}C plant from aboveground biomass towards roots. Except for those roots, isotopic values decreased with plant age despite applying additional isotopic labels with identical amounts of $Na_2{}^{13}CO_3$. This was caused by a dilution effect of the growing biomass and thus a reduction in the isotopic values occurred although additional isotopic labelling procedures were applied.

3.2 Lipid extract yields

Lipid extract yields revealed differences between individual plant parts for all analyzed grasses (Fig. 2). In general, lipid extract yields were highest for leaves, followed by those of stems and roots and varied between 20 and 90 mg g^{-1}. Especially in stems and leaves, lipid concentrations were higher for C_3 plants than for C_4 plants, which has been previously described (*Wiesenberg*, 2004). An increase in lipid concentrations with plant growth was obtained for *Miscanthus* leaves, as previously determined for several crop plants grown under field conditions (*Wiesenberg*, 2004) and tree leaves (*Prasad* and *Gülz*, 1990). Maize plants showed similar total lipid concentrations in flowers, leaf nodes, and stems. Highest proportions of lipids in leaves and lower amounts in the other plant parts resulted from a higher surface to volume ratio in leaves and the high concentration of surface waxes in leaves (*e.g., Bianchi*, 1994).

Commonly, lipid extract yields were similar for labelled and control plants. Especially the mean values of lipid yields in the plants indicate only marginal differences, which are com-

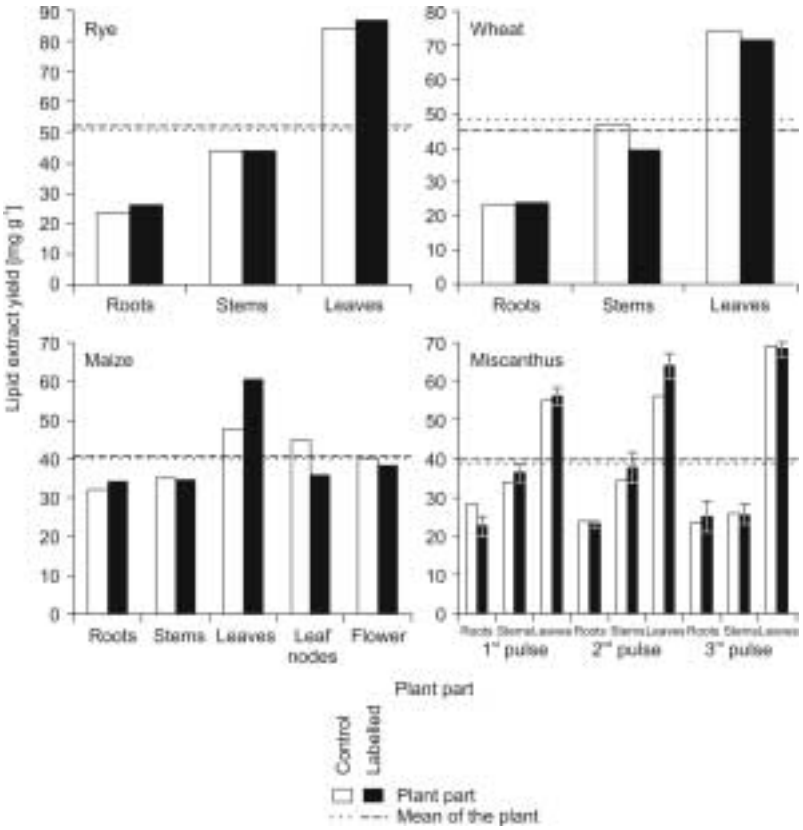

Figure 2: Lipid concentration of plant parts and mean lipid extract yields of ^{13}C-labelled *versus* untreated control plants. For *Miscanthus*, pulse labelling was repeated 14 d after the previous pulse for three times.

mon due to an inhomogeneous growth of individual plants. Differences between labelled and unlabelled plant tissues (*e.g.*, maize leaves or *Miscanthus* roots) are due to uncertainty in the gravimetric determination of the results: (1) root samples were available only in low amounts and due to low extract yields, the lipids were in the range of weighing errors; and (2) some of the extracts especially from plant leaves were very oily leading to difficulties in complete solvent removal without loss of volatile lipidic compounds. Despite those problems during gravimetric determinations of lipid extract yields, only marginal differences (<7%) between labelled and control plants were observed for all plants. This argues for a low effect of isotopic pulse-labelling on the total extractable lipid concentrations.

3.3 Alkanes

Distribution patterns of *n*-alkanes (Fig. 3) were similar for all plants maximising at C_{27}, C_{29}, or C_{31}, which is typical for *Poaceae* and other terrestrial plants (*e.g., Eglinton* et al., 1962; *Maffei,* 1996a, b). The distribution patterns of plants grown under controlled conditions in this study were similar to patterns for grasses grown under field conditions (*e.g., Bianchi* and *Corbellini,* 1977; *Wiesenberg* et al., 2004a). For all grasses, an increase in alkane chain length can be observed from root over culm towards leaf biomass. This tendency has been rarely described for plants grown under field conditions (*Dove* et al., 1996; *Wiesenberg,* 2004; *Wiesenberg* et al., 2004a) and may be attributed to the early growth stage of the plants and to different requirements concerning the regulation of evapotranspiration in each plant compartment. Root materials comprised the largest differences between labelled and control plants, which can be related to the lowest amount of biomass available for extraction and possible contamination by *n*-alkanes derived from root-adhering soil particles. The differences between alkanes of labelled and control plants were not significant (p = 5%) except for root tissues showing significant differences. These differences in root tissues can be attributed to low amounts of available biomass. Additionally, small differences of lipid distribution patterns between individual plants may be related to differences in nutrient supply and plant growth occurring in individual pots. In field experiments, it was observed that these effects may be related to numerous environmental factors, *e.g.*, exposition of plant tissues and sunlight availability (*Lockheart* et al., 1997) or nutrient and water availability (*Jenks* et al., 2001). Despite controlled growth conditions in the laboratory in our experiments, minor differences in plant growth occurred and may be responsible for these variations. Alkane distribution patterns for *Miscanthus* are not available here because of contamination by non-plant-derived alkanes during the experiment, as described in the section 2.

3.4 Carboxylic acids

Carboxylic acid distribution patterns (Fig. 4) were predominated by saturated C_{16} acids or unsaturated C_{18} acids, as a result of large abundances of these compounds in plant cuticles and waxes. Due to significantly different compositions (p < 5%) of various plant tissues, root biomass was

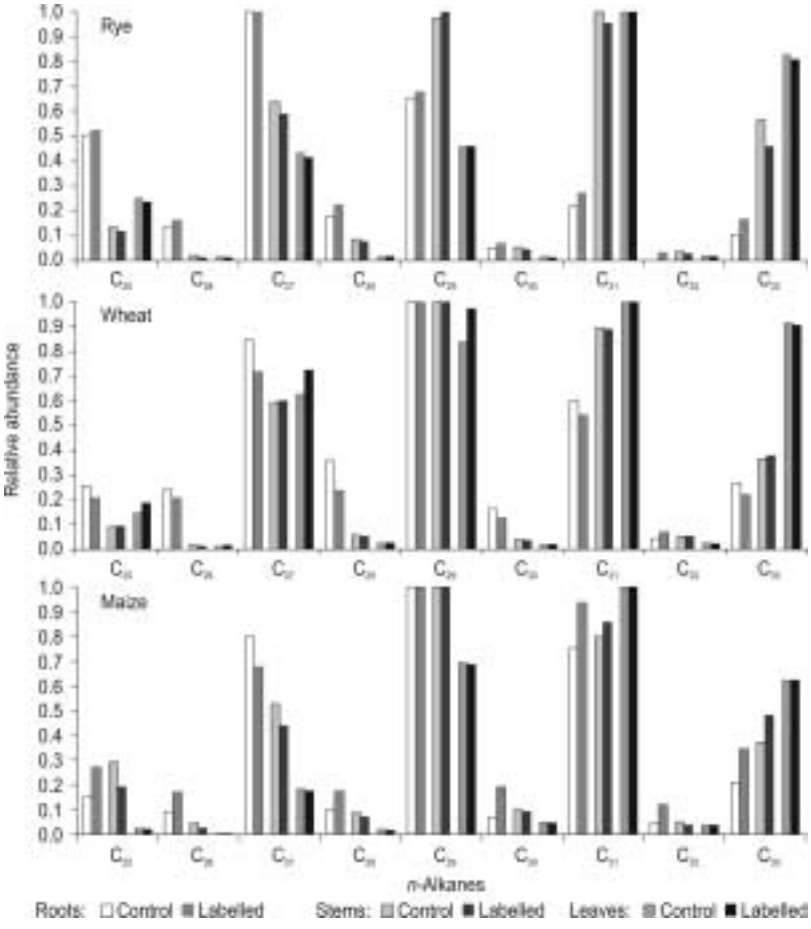

Figure 3: Relative abundances of *n*-alkanes normalised to the most abundant alkane of labelled and control plant parts for rye, wheat, and maize.

often characterised by large amounts of $C_{16:0}$, while aboveground biomass commonly contained lower amounts of $C_{16:0}$ in comparison to the most abundant unsaturated C_{18} acids. This is contrary to similar plants taken from field experiments (*Wiesenberg*, 2004; *Wiesenberg* et al., 2004a), where commonly unsaturated acids with 18 carbon atoms were most abundant in all plant tissues. Most likely these exceptional distribution patterns are related to the growth conditions in the laboratory, where a specific microclimate without rainfall and increased temperatures and presumably higher CO_2 concentrations, as compared to field conditions, may be responsible for the composition of primarily epicuticular lipids. Exceptionally low amounts of $C_{16:0}$ in control maize roots are difficult to interpret, but are similar to those previously determined under field conditions (*Wiesenberg*, 2004; *Wiesenberg* et al., 2004a). Even long-chain carboxylic acids between C_{22} and C_{30} were slightly enriched and revealed a specific pattern for each plant. In agreement with *Wiesenberg* and *Schwark* (2006), C_3 and C_4 plants can be differentiated by higher relative abundances of C_{24} in C_4 plants in comparison to higher amounts of C_{22} and C_{26} in C_3 plants.

Distribution patterns of carboxylic acids changed ($p < 5\%$) during the multiple pulse-labelling of *Miscanthus* (Fig. 4), which is related to different growth stages and thus an ongoing change in biosynthetic activity. Means of plant tissues taken 2, 6, and 14 d after the individual pulse-labelling showed a slight variation for most compounds, indicated by standard errors of the mean. Largest variations occurred in root biomass ($p < 0.5\%$) and may be related to inhomogeneities in root tissues and a varying composition of the microbial communities on the roots with time. In general, differences between carboxylic acid distribution patterns of control and means of labelled plants were small and not significant in most cases ($p = 5\%$). However, there were some significant ($p < 5\%$) differences between labelled and control plant tissues, *e.g.*, wheat leaves and *Miscanthus* roots after the first and third pulse-labelling as well as *Miscanthus* leaves after the second labelling. These may be related to plant growth conditions at that stage of the experiment or microbial communities analyzed on the root tissues and reflecting their carboxylic acid distribution patterns.

3.5 Comparison of alkanes and carboxylic acid contents

In order to test the comparability of the relative abundances, lipids in samples derived from labelled plants were plotted *versus* control lipid concentrations (Fig. 5). Alkanes showed an excellent correlation ($r^2 = 0.98$) of relative abundances of labelled and control lipids with all individual components close to the 1:1 line. The correlation for carboxylic acid

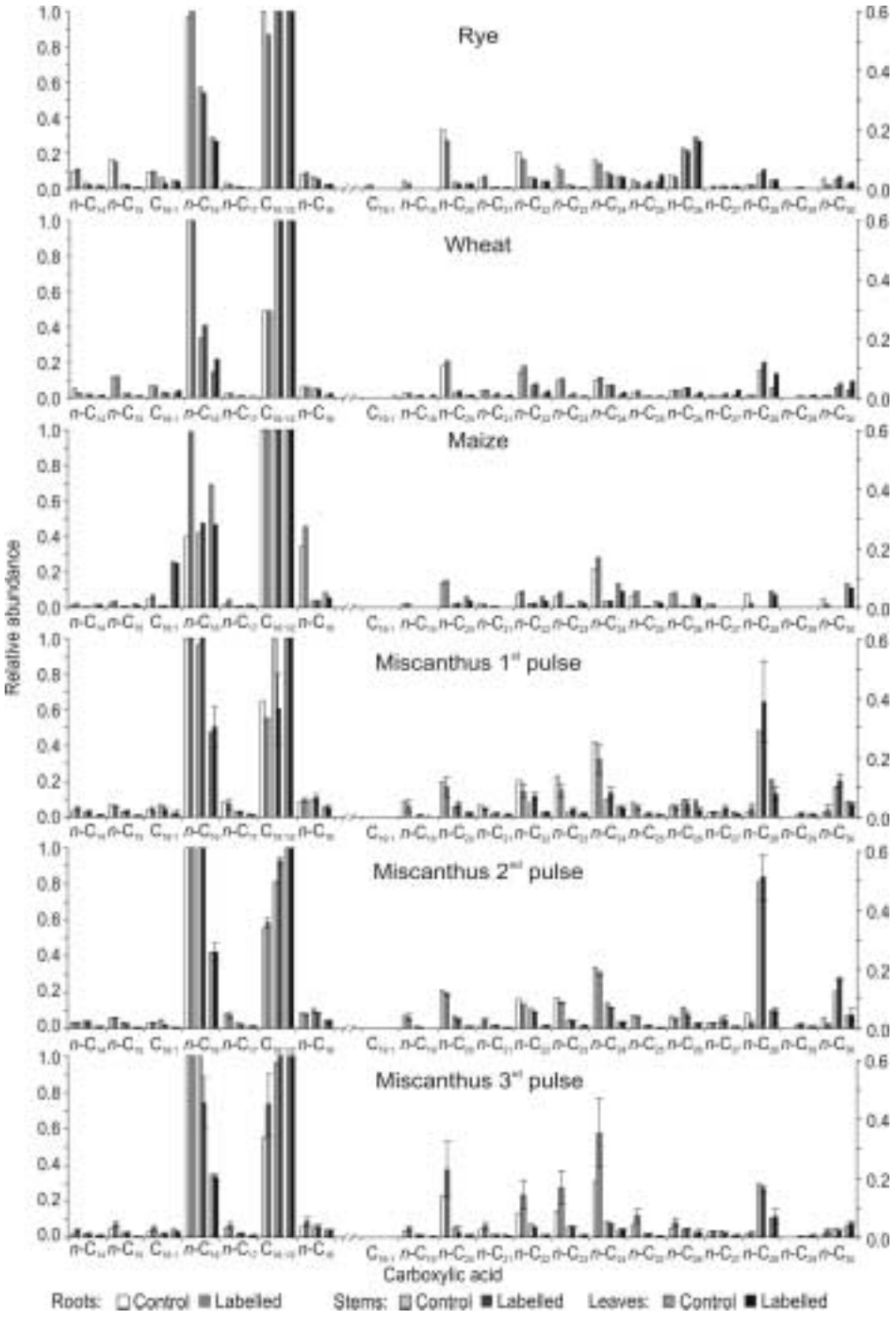

Figure 4: Relative abundances of carboxylic acids normalised to the most abundant carboxylic acids of individual labelled and control plant parts for rye, wheat, maize, and three pulses for *Miscanthus.* For *Miscanthus* mean values of samples taken 2, 6, and 14 d after the individual pulse labelling are given and standard errors of the mean are indicated by error bars for three replicates of labelled plants.

relative concentrations (r^2 = 0.95) was similar to that for *n*-alkanes, except for two data points as explained above. For alkanes and carboxylic acids, both, consistent slopes and intercepts of the regression line close to the origin indicate the low variation of individual lipids from labelled and control plants. Only the carboxylic acids from the repeated pulse-labelling of *Miscanthus* seem to reveal elevated proportions of some acids in the labelled plants. Hence, after several pulses a moderately lasting influence on plant lipid distribution pattern may occur.

4 Conclusions

Lipid composition (extract yield and distribution patterns of alkanes and carboxylic acids) of plants is not modified if only one short ^{13}C pulse is applied during labelling in $^{13}CO_2$ atmosphere and if sampling occurs several days after the ^{13}C pulse. When several ^{13}C pulses are applied, minor variation of plant lipid composition is possible. Isotopic labelling of plant materials offers a powerful tool to investigate biosynthesis and metabolic pathways of individual plant components

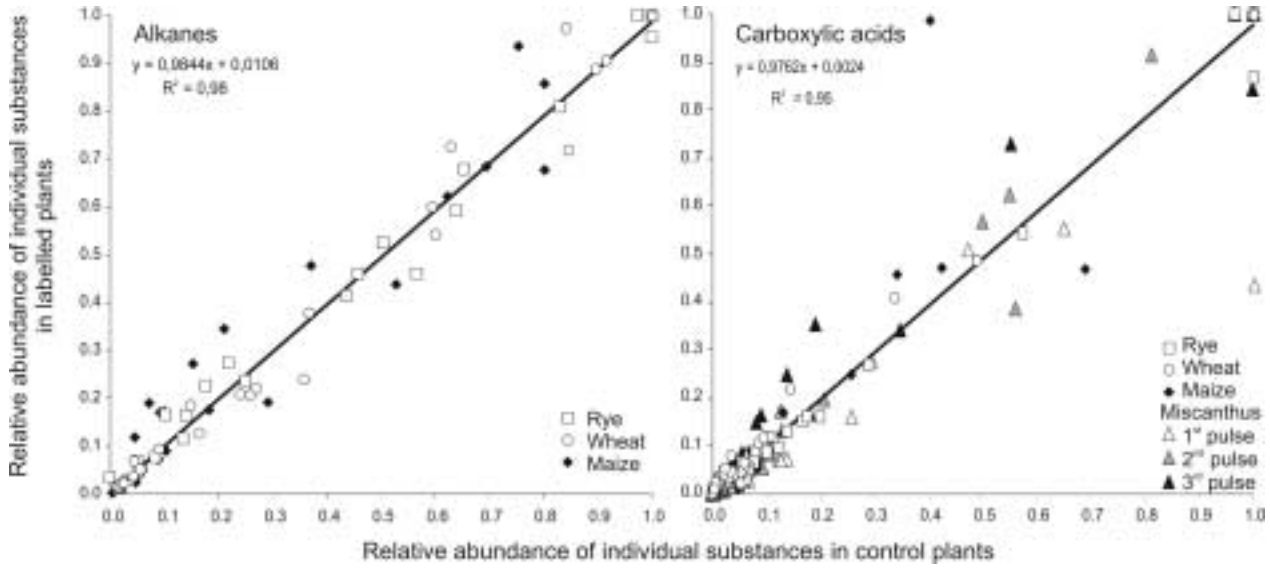

Figure 5: Correlation of labelled and control plant alkanes and carboxylic acids for rye, wheat, maize, and for triplicate pulses of *Miscanthus* (carboxylic acids only).

without affecting the lipid composition of the plant tissue itself. Plants grown under laboratory conditions reveal a different lipid distribution pattern and $\delta^{13}C$ isotopic values, when compared to plants grown under field conditions.

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