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# Optimization of $^{14}C$ liquid scintillation counting of plant and soil lipids to trace short term formation, translocation and degradation of lipids

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**Abstract** Two powerful approaches are frequently used to trace incorporation and degradation of plant derived C in soil: $^{14}C$ labelling/chasing and analysis of lipid composition. In this study, we coupled these approaches in order to trace short term incorporation of plant derived lipids into rhizosphere and non-rhizosphere soil. Methodological optimization was required and implied $^{14}C$ liquid scintillation counting improvement for plant lipid extracts taking into account organic solvents, solvent-to-scintillation cocktail ratio, and amount of lipids. Following method optimization, $^{14}C$ data of fatty acids indicated a notable contribution of root derived lipids to rhizosphere and non-rhizosphere soil. Coupling of $^{14}C$ labelling/chasing with lipid analysis is a powerful and cheap approach for tracing of root derived C in soil allowing for estimation of C budget, for determination of C formation and translocation within plants and from plant to soil, as well as for identification of short term dynamics of specific compound classes within soil.

**Keywords** $^{14}C$ pulse labelling · Lipids · Fatty acids · Loess · Rhizosphere · Liquid scintillation counting

## Introduction

Lipid dynamics in plants and soils have been frequently studied and revealed mean residence times of lipidic compounds in soils of several decades [1, 2]. In addition, long term turnover and age of lipids in soil are commonly studied by radiocarbon dating [3–5], modification of $^{13}C$ signature after $C_3$-/$C_4$-vegetation change [1, 6, 7] or $^{13}C$ signature derived from free air $CO_2$ enrichment experiments using $^{13}C$ depleted $CO_2$ [2]. The methods mentioned above are based on changes of isotopic signature of $\Delta^{14}C$ or $\delta^{13}C$ close to natural abundance and imply slow significant isotopic changes in soil C detectable on a year to decade range. Thus, these approaches are not appropriate to determine short term changes of plant derived lipids in soils on a day to month scale. Some recent studies described short term changes in soil lipid composition combining experiments, which are conducted under controlled conditions, with $^{13}C$ pulse labelling techniques [8, 9]. Additionally, short term incorporation of plant derived lipids in soil was observed to be difficult to follow only by observing changes in abundances of individual substances of fractions, whereby some fatty acids yield potential to trace these changes [9, 10]. However, short term input of other plant derived lipidic components than fatty acids like e.g. alcohols, sterols, or alkanes cannot be followed exclusively by use of lipid distribution patterns, because of a low contribution of plant derived components and slow changes in soil lipid composition in these substance classes [1, 2, 11]. In contrast to analysis of distribution patterns, approaches based on labelling with $^{13}C$ or/and $^{14}C$ allow for tracing of short term processes, i.e. input of plant derived C into soil [12, 13]. Therefore, coupling of lipid distribution patterns with $^{13}C$ or/and $^{14}C$ labelling and chasing techniques might be useful. While continuous labelling techniques might have an effect on synthesis of lipidic components because of artificial environment [11], pulse labelling techniques were described to have no sustainable influence on lipid biosynthesis in plants [8].

$^{14}C$ pulse labelling techniques have been frequently combined with lipid analyses in plant tissues allowing for

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the determination of biosynthesis of individual lipidic compounds [14]. These experiments were found to be useful in order e.g. to determine C incorporation and turnover of C in lipids within plants, to elucidate specific metabolic and enzymatic pathways in plants, and to investigate biosynthesis, transformation, and translocation of individual compounds within plants [15–17]. High sensitivity and simple analysis of $^{14}C$ enabled to elucidate synthesis and transformation mechanisms of fatty acids within plants [18].

In studies concerning rhizosphere processes, $^{14}C$ pulse labelling is frequently applied for the estimation of C input and allocation into soil by plants as well as for partitioning studies [12]. $^{14}C$ labelling has been scarcely applied to determine incorporation and degradation of plant derived lipids in soils [13]. Changes of microbially derived phospholipid fatty acids (PLFA) were obtained by the combination of $^{14}C$ labelling and lipid analyses in soils by Roslev et al. [19]. Therefore, one aim of this study was to check the applicability of the combination of $^{14}C$ pulse labelling with extraction, separation, and subsequent analysis of lipids (other than PLFA) in plants and soils.

The lipid content of soils in temperate climates is about 3–5% of $C_{org}$ [20], while plants can yield typically up to 10 mass-% lipids or sometimes even more [1, 11]. As most of plant lipids are degraded quickly, only part of plant lipids including aliphatic and aromatic hydrocarbons is preserved in soil resulting in mean residence times of several decades [1, 2]. The incorporation of root related lipids has been elucidated seldomly, but is assumed to be a major incorporation pathway of plant derived lipids into soil [1, 13]. Due to large amounts of lipids already present in soil, extraction and analysis of recently synthesized plant derived lipids in soil will be masked. Using natural soil samples for such investigations would result in difficulties to compare the recent lipid input by plants by two methods: total lipid content and $^{14}C$ in lipids. To avoid such uncertainties, soil parent materials together with nutrient solutions may be efficiently used as plant growing substrate, because they provide a nearly natural environment leading to seminatural interactions of plant roots with microorganisms. This is especially important to investigate rhizosphere processes and turnover of organic substances like lipids which will be decomposed and reutilized by microorganisms. Therefore, this study on lipid translocation from plants into the soil was done on loess that is excellent parent material for many soils and offers preferable physico-chemical conditions for roots and microorganisms.

One challenge of combining lipid analyses with $^{14}C$ liquid scintillation counting (LSC) consisted in the optimization for small amount of lipids sufficient for $^{14}C$ analysis. This was connected with the fact that only a small portion of $^{14}C$ assimilated during $^{14}CO_2$ pulses is incorporated into lipids compared to the other substances in plant tissues. So, the $^{14}C$ activity of the lipid fractions is expected to be low, which might result in problems to distinguish $^{14}C$ incorporation from the background of natural radioactivity. At the same time, the largest part of the lipid extract from plant and/or soil should be available for analysis of individual lipidic fractions by gas chromatography. Another task was to select an appropriate solvent for LSC in lipid extracts which (1) allows complete dissolution of extracted lipids, and (2) yields high efficiency of $^{14}C$ analysis by LSC.

The variety of solvents used to dissolve lipidic components and perform $^{14}C$ liquid scintillation counting is very diverse and ranges from toluene [18] over methanol [21], chloroform, aqueous phases [14], to acetone or combinations of these [17]. Our experience shows that not all organic solvents allow for a reliable liquid scintillation counting without quenching, especially for low activity $^{14}C$ labels or without a special calibration. As incorporation of selected lipid fractions into soil can be expected to be low, we tested the optimum solvents for lipid analyses in soils even for trace analyses of $^{14}C$ activity. As numerous different organic solvents are used to extract soil lipids, we first analysed organic solvents yielding lowest background radioactivity signals in order to determine even low levels of $^{14}C$ labelled lipids in soil. Second, we followed the incorporation of $^{14}C$ into extracts of total lipids and fatty acid fractions within plant biomass and the incorporation of plant derived $^{14}C$ into these lipids into a soil like substrate (loess).

## Experimental

### $^{14}C$ labelled plant material and growing substrate

$^{14}C$ labelled samples from ryegrass (*Lolium perenne* [L.]) and associated loess (initial $C_{org}$ content 0.3 mg $g^{-1}$) were collected from a $^{14}C$ multiple pulse labelling experiment. The plants were grown under controlled conditions on loess from Nussloch, SW Germany and were fertilized with a modified Hoagland nutrient solution [22]. Polycarbonate filtration devices were used as plant pots with three inlets in the lid and one main opening for growth of the plant shoots (CombiSart, Sartorius AG, Germany). One day before the first labelling, the plants were sealed around the shoots by silicone rubber (NG 3170, Thauer & Co., Germany). Hence, the aboveground biomass could not enter the loess. Starting from the age of 59 days, the plants received five $^{14}C$ isotopic pulses à 407 kBq by labelling in $^{14}CO_2$ atmosphere (for a detailed method description see Kuzyakov, Shevtzova, and Pustovoytov [23]). Labelling was repeated five times in intervals of 5 days in order to receive

a high $^{14}C$ signal in plant derived components in loess. This led to a total $^{14}C$ activity of $61 \times 10^6$ dpm for shoots ($19 \times 10^6$ dpm $g^{-1}$ biomass) and $15 \times 10^6$ dpm for roots ($4 \times 10^6$ dpm $g^{-1}$ biomass). Between the $^{14}C$ pulses the plants continued growth under normal conditions.

Five days after the last labelling, shoots were cut and roots were separated from loess with tweezers. While the loess almost free of roots was named non-rhizosphere loess, the loess adjacent to roots was separated from roots by washing and was called rhizosphere loess. The water was filtrated using a stainless steel pressure filter holder (SM 16249, Sartorius, Germany) fitted with a mixed cellulose ester membrane filter (0.2 µm, Whatman, UK). All samples were dried at 60 °C and ground in a ball mill (MM200, Retsch, Germany).

## Lipid extraction from loess and plant biomass and fatty acid separation

Complete biomass of plant leaves and roots (between 0.6 and 2.4 g), and part of rhizosphere (10–20 g) and loess samples (app. 100 g) were extracted for free extractable lipids by Soxhlet extraction for at least 40 h using a solvent mixture of dichloromethane/methanol (93:7; v:v [13]). After extraction the solvent mixture was reduced by rotary evaporation. After complete removal of the solvent, lipid extracts were re-dissolved with dichloromethane and aliquots were transferred to scintillation vials for $^{14}C$ liquid scintillation counting. Fatty acids were separated from the remaining lipid extract on a KOH-coated silica gel column [8]. Neutral lipids were eluted first with dichloromethane, followed by fatty acids as a second fraction using dichloromethane/formic acid (99:1; v:v). The solvent was completely removed after elution. After re-dissolving fatty acids with dichloromethane, an aliquot of the fatty acid fraction was transferred to a scintillation vial.

## $^{14}C$ analysis of plant biomass and loess organic matter

To analyse $^{14}C$ incorporated into plant biomass, material from shoots and roots was combusted in an oven (Feststoffmodul 1300, AnalytikJena, Germany) at 800 °C. The $CO_2$ released by combustion was trapped in NaOH. $^{14}C$ activity of the NaOH was measured in 1 mL aliquots mixed with 2 mL of scintillation cocktail (Rotiszint EcoPlus, Carl Roth, Germany) after decay of chemiluminescence. Main components of Rotiszint are according to the safety data sheet [24] diisopropylnaphthalene (50–60%), ethylene oxide nonylphenol polymer (20–30%), phenolethoxylate phosphate ester, and 2-(2-butoxyethoxy)ethanol (5–10% each). In trace amounts occur 2,2'-iminodiethanol, sodium tetrahyborate, 1,4-bis(4-methyl-alpha-steryl)benzene, and 2,5-diphenyloxazole (0.1% each). The $^{14}C$ activity was measured by a 1450 LSC & Luminescence Counter (MicroBeta TriLux, Perkin Elmer Inc., USA). The $^{14}C$ counting efficiency reached at least 70%. The measurement error did not exceed 3.5%. The absolute $^{14}C$ activity was standardized by SQP(I) by addition of increasing amounts of NaOH as a quencher.

Total $^{14}C$ incorporated into loess organic matter (rhizosphere and non-rhizosphere loess) was calculated as the difference between total $^{14}C$ recovered in loess and $^{14}C$ incorporated into loess carbonate by recrystallization with root derived $CO_2$. Total $^{14}C$ activity in loess was analyzed by combustion (see above) at 1200 °C. As we expected lower $^{14}C$ activity in loess than in plant biomass, $^{14}C$ activity in NaOH was measured on 6 mL aliquots added to 12 mL of Rotiszint. The $^{14}C$ counting efficiency of the liquid scintillation counter used (LS 6500 Multi-Purpose Scintillation Counter, Beckman, USA) was at least 90% and the measurement error did not exceed 4%. The absolute $^{14}C$ activity was standardized by the H number method, using a $^{137}Cs$ external standard. To analyse $^{14}C$ activity only in loess carbonate, loess samples were dissolved by $H_3PO_4$, and $CO_2$ released from $CaCO_3$ was trapped in NaOH. $^{14}C$ activity in NaOH was measured as described above.

## Organic solvents and $^{14}C$ sample analysis of lipid extracts and fatty acids

A wide range of solvents is used in lipid analyses depending on the polarity of lipids to be dissolved. Polar lipids are commonly extracted by polar solvents like methanol (MeOH) or MeOH combined with low polarity solvents to extract both, low and high polarity lipids. Before measuring $^{14}C$ activities of lipid extracts and fatty acids dissolved in organic solvent, we performed several tests for method optimization. First, the appropriate ratio of solvent and Rotiszint was determined. Reagent blanks of MeOH were mixed with Rotiszint in ratios of 1:1, 1:2, and 1:3 (v:v), where the volume of methanol was 3, 2, and 2 mL, respectively. Second, different solvents frequently used for a variety of lipid analysis (methanol, dichloromethane, chloroform, hexane, toluene, and acetone) were tested for background signals and possible interference with Rotiszint during scintillation counting due to composition of the solvent. The most suitable solvent was determined after addition of 6 mL Rotiszint to 2 mL of each solvent. Third, the optimum amount of $^{14}C$ labelled lipids for $^{14}C$ analyses was determined, because dissolved polar lipid extracts and lipid fractions are often coloured and thus may cause problems related to colour quenching during liquid scintillation counting. The optimum lipid amount was determined by preparation of 4 different aliquots (5, 10, 50, or 100 µg) of the same ryegrass shoot lipid

extract. The aliquots were filled in scintillation vials and re-dissolved after drying again in 2 mL of MeOH and mixed with 4 mL of Rotiszint. Fourth, possible interference between organic solvent and the lipidic fraction might yield different $^{14}C$ values of one sample, depending on the applied solvent. Therefore, scintillation vials were filled with 10 µg of fatty acids, each, which were obtained from ryegrass shoots. After drying of the lipids, several individual solvents were added to the scintillation cocktail.

$^{14}C$ activities of lipids and fatty acids as well as background of the respective blanks were measured in 3 replicates after decay of chemiluminescence by LS 6500 Multi-Purpose Scintillation Counter. In order to keep counting errors as low as possible, blanks were measured for 120 min while counting times of 60 min were sufficient for $^{14}C$ labelled samples to keep counting errors below 1%.

## Results

### Solvent and sample quantity optimization

The first part of scintillation counting adjustment was pointed on optimization of MeOH to scintillation cocktail ratio. In order to optimize the counting efficiency, different mixtures of MeOH to scintillation cocktail (1:1, 1:2 and 1:3 of MeOH:Rotiszint; v:v) were tested (Fig. 1). A decreasing ratio of MeOH to Rotiszint cocktail increased the counting efficiency. In the same direction the background signal decreased and was almost constant for 1:2 and 1:3 ratios.

The second aim of scintillation counting optimization was to diminish chemiluminescence problems. The Rotiszint scintillation cocktail itself showed a high counting efficiency and a low background signal, while counting error and chemiluminescence were in a usual range (Fig. 2). The Rotiszint scintillation cocktail was designed for high counting efficiency for hydrophilic and lipophilic solutions [24]. Hence, it was not surprising that lowest background signals were obtained for pure Rotiszint. Due to the composition of the scintillation cocktail low polar lipophilic organic compounds were dissolved only by applying ultrasonic energy and high temperature while polar lipidic components and compounds dissolvable in water were easily dissolved. Hence, pure Rotiszint scintillation cocktail was not ideal for the determination of $^{14}C$ in lipid extracts. These lipids require dissolution in organic solvent with medium polarity prior to addition of Rotiszint. To test for background signals, several organic solvents frequently used in lipid analyses were mixed with Rotiszint in a relationship 1:3 (v:v) which had been determined to yield the lowest background signal. Low background signal, counting error, and chemiluminescence as well as a high counting efficiency have to be recommended for pure solvents in order to keep correction low. From the tested solvents, chloroform showed the lowest counting efficiency and the highest background signal, which might be due to a standardization that was not ideal for chloroform, but all other organic solvents. Acetone revealed highest chemiluminescence values. The other solvents like methanol, dichloromethane, hexane and toluene were characterized by low background signal, chemiluminescence, and high counting efficiency (>90%), whereas counting errors are slightly lower for methanol and dichloromethane.

In the third part of the method optimization, the lipid amount yielding a high relation of $^{14}C$ activity in lipids compared to the $^{14}C$ activity in the solvent background as well as a high counting efficiency was determined. At the same time the required sample amount should be as low as possible in order to keep large amounts for fraction specific gas chromatographic analyses of extracted lipids and to keep the soil or plant sample amounts needed for extraction as low as possible. The latter is important especially in rhizosphere studies, because often available sample amounts are limited and hence large proportions of

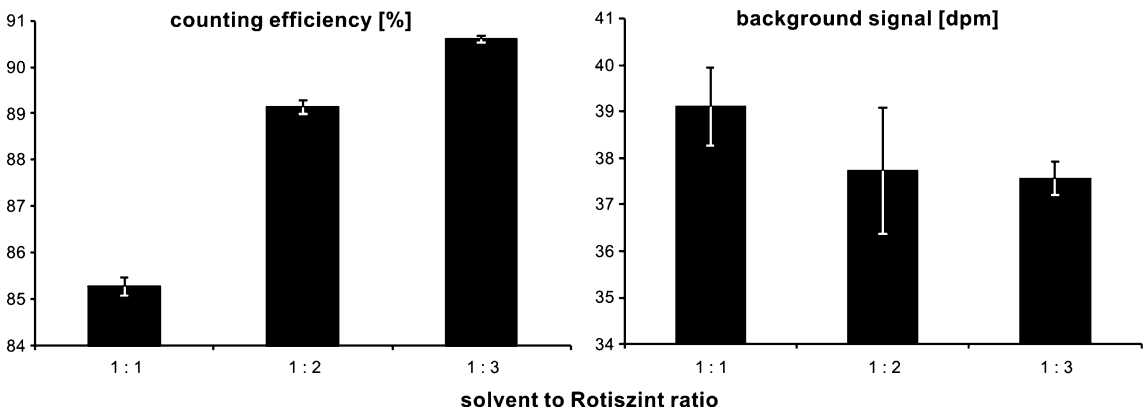


**Fig. 1** Determination of the optimum solvent/Rotiszint mixture for MeOH. Relations are given in one part MeOH compared to one, two, or three parts of Rotiszint scintillation cocktail, respectively. Counting efficiency [%] and background signal [dpm] are shown

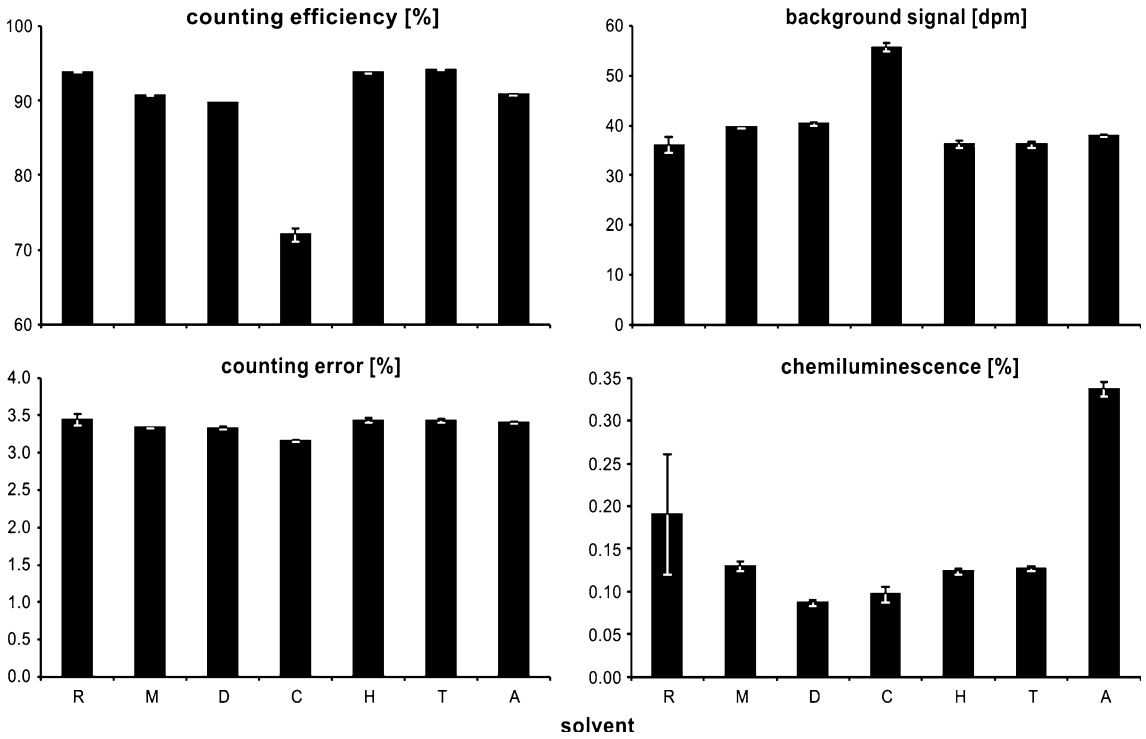


**Fig. 2** Counting efficiency [%], $^{14}C$ background activity [dpm], counting error [%] and chemiluminescence [%] of the following solutions: Pure Rotiszint scintillation cocktail (R); Rotiszint mixed with methanol (M), dichloromethane (D), chloroform (C), hexane (H), toluene (T), acetone (A)

extracted components are required for the determination of lipid composition by gas chromatography. With increasing lipid concentrations the $^{14}C$ activity increased linearly with decreasing variability between the replicates (Fig. 3). At the same time, counting efficiency decreased linearly due to increasing colour quenching (despite dilution by organic solvent and Rotiszint). As high counting efficiency is important for precision of recalculated $^{14}C$ activity, we used an optimum lipid amount of max. 50 µg in order to achieve counting efficiencies >80%. However, as colouring agents in lipids for plant and soil samples differ, this test may be required for each sample set, especially if the $^{14}C$ activity is low and therefore, the amount of lipids for LSC should be increased.

To test for a potential effect of individual solvents on $^{14}C$ activity of lipidic fractions, 10 µg of fatty acids extracted from ryegrass shoots were analyzed for $^{14}C$ after addition of the same individual organic solvents previously tested for their background value (see above). The optimum results would reveal high $^{14}C$ activity and a high efficiency. For fatty acids dissolved in chloroform significantly lower $^{14}C$ activity and counting efficiency associated by larger counting errors occurred when compared to all other solvents (Fig. 4). Fatty acids dissolved in the other solvents were characterized by higher $^{14}C$ signals ($46 - 48 \times 10^6$ dpm $g^{-1}$) uniform counting errors (0.42–0.45%, Fig. 4) and a high efficiency (>87%, Fig. 4) with highest signals observed for toluene and hexane, followed by dichloromethane, methanol, and acetone.

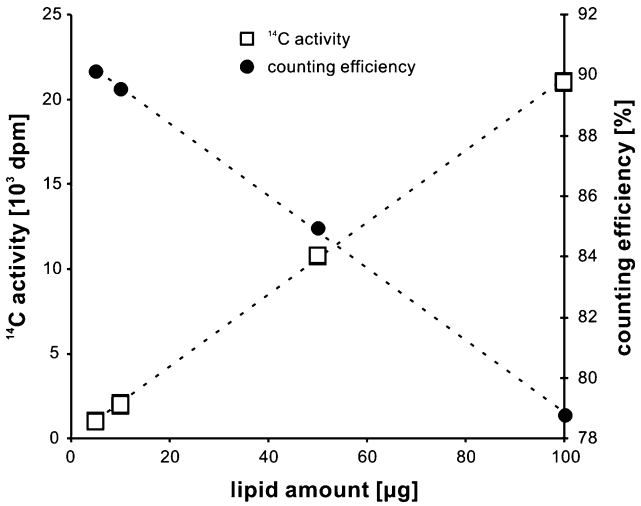


**Fig. 3** Determination of the optimum lipid amount for $^{14}C$ LSC of lipid extracts of *Lolium perenne* shoots derived from a $^{14}C$ labelling experiment

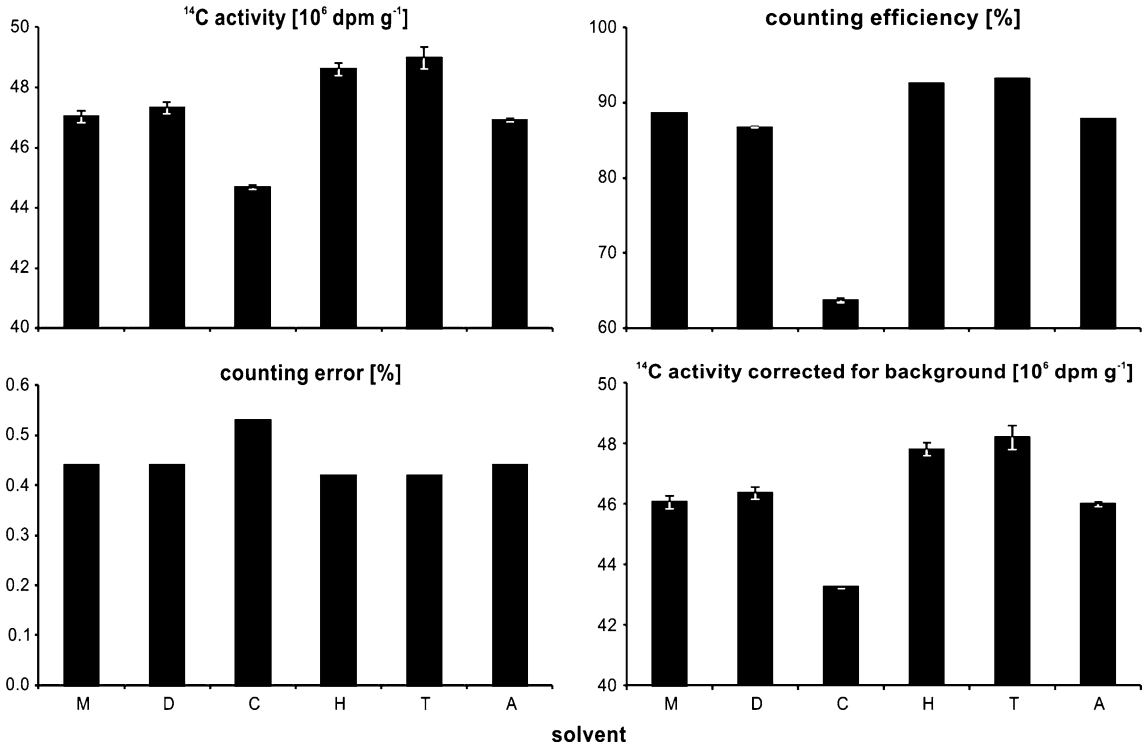


**Fig. 4** $^{14}C$ activity, counting efficiency, counting error, and blank-corrected $^{14}C$ activity within fatty acids of $^{14}C$ labelled shoots using the following solvents: methanol (M), dichloromethane (D), chloroform (C), hexane (H), toluene (T), acetone (A), each mixed with Rotiszint in the relationship 1:3 (v:v)

## Application of LSC to lipid extracts and fatty acid fraction after a $^{14}C$ pulse labelling

After the labelling experiment the specific $^{14}C$ activity related to 1 g of biomass or loess was highest in shoots followed by roots, rhizosphere loess, and non-rhizosphere loess (Fig. 5). The $^{14}C$ activity was approximately 5 times higher in shoots than in roots. The total $^{14}C$ activity was ~260–390 times higher in plant tissues than in rhizosphere and root-free loess.

A comparatively large portion of $^{14}C$ was incorporated into shoot lipids and shoot fatty acids, while a lower $^{14}C$ activity was found in lipidic components in roots (Fig. 5). Only a low portion of assimilated $^{14}C$ was incorporated into rhizosphere lipids, whereas $^{14}C$ incorporation was higher in non-rhizosphere soil lipids, especially for fatty acids.

In total, 66.7% of the assimilated $^{14}C$ was determined in plant biomass and loess with largest incorporation into shoot biomass and decreasing amounts from shoot over

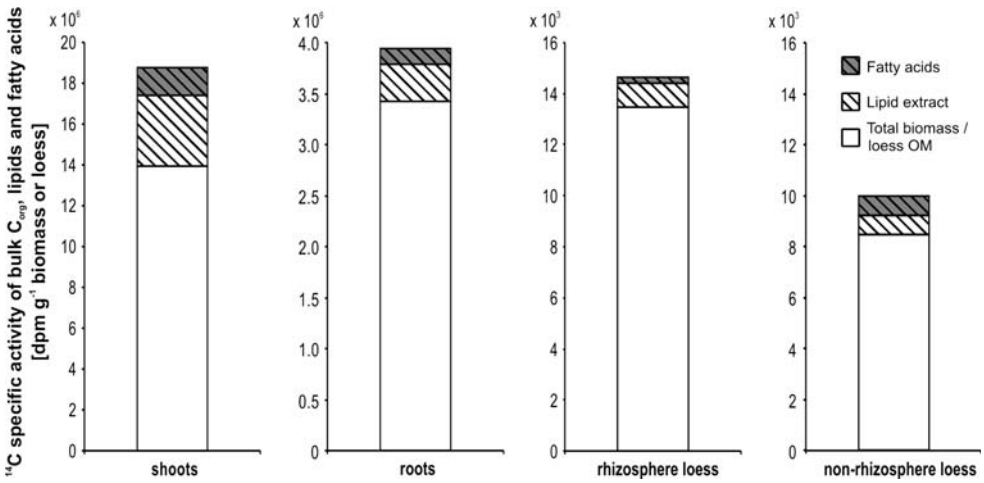


**Fig. 5** $^{14}C$ specific activity of lipid extract and fatty acids in plant compartments, rhizosphere and loess after a laboratory $^{14}C$ pulse labelling experiment with *Lolium perenne*

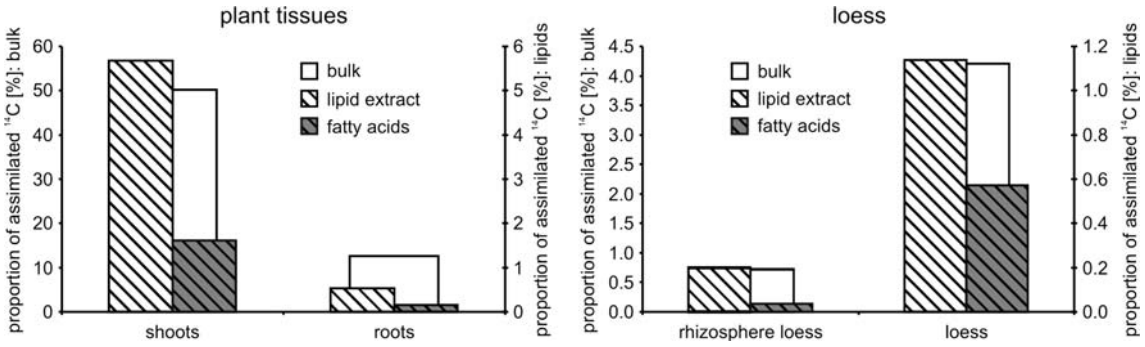


**Fig. 6** Proportion of assimilated $^{14}C$ determined in plant tissues and soil 5 days after the fifth isotopic pulse label. On the left *y*-axis scales of bulk organic carbon are given whereas those of total lipids and fatty acids can be found on the right *y*-axis

root biomass to rhizosphere loess (Fig. 6). From rhizosphere towards root free loess an increase of $^{14}C$ activity was determined due to larger absolute volume (10 times higher than rhizosphere volume) of the latter and due to a progressive translocation of $^{14}C$ towards non-rhizosphere substrate. 7.5% of the assimilated $^{14}C$ were observed in lipids over all compartments. Fatty acids yielded 31.7% of the $^{14}C$ label determined in total lipids and account for 2.4% of the total assimilated $CO_2$.

## Discussion

### Solvent optimization

A solvent/scintillation cocktail relationship of 1:3 (MeOH:Rotiszint; v:v) was found to be the best of the tested mixtures for the liquid scintillation counting of dissolved lipidic components (Fig. 1). This mixture enables a low background accompanied by high counting efficiency. Hence, the background and efficiency correction factor, which has to be applied for lipids dissolved in the solvent, is low for this relationship, leading to low potential interference related to pure solvent and enables the detection of low $^{14}C$ activity.

Rotiszint scintillation cocktail itself was unsuitable to completely dissolve the lipid extract. Therefore, a mixture with solvents depending on the polarity and solubility of lipidic compounds, which should be amended to $^{14}C$ liquid scintillation counting, is necessary. Most tested solvents showed background signals in the range of Rotiszint and are useful for LSC (Fig. 2). Especially methanol and dichloromethane can be recommended due to low background signals and comparatively high counting efficiencies. This is partly connected with the fact that ethanol is used in this cocktail to increase the solubility of aqueous solutions. Solvents like hexane and toluene are suitable as well, but a slightly higher counting error has to be taken into account. Due to high background signal and low counting efficiency connected with high quenching, chloroform cannot be recommended as optimum solvent for LSC of organic substances without further optimization. Especially for samples with low $^{14}C$ activity the potential errors caused by a large background and low counting efficiency might be problematic. Therefore, methanol and dichloromethane should be used, while hexane and toluene are still suitable and further adjustments are required for different solvents like chloroform.

For plant lipid samples, a quantity of 50 µg revealed the lowest standard errors for replicate analyses of $^{14}C$ activity associated by a comparatively high counting efficiency (Fig. 3). Higher lipid concentrations (multiples of 100 µg) resulted in an intensive colour and thus a high colour quench. Due to the colour quenching, a very low counting efficiency (<45%, not shown) was present. For some replicates, the value was outside the quench curve leading to high error of DPM counting, which is a common fact for intensively coloured liquids that are measured by LSC [25, 26]. Therefore, the amount of 50 µg lipids was chosen as a maximum in order to avoid variability between the replicates (as was the case for smaller aliquots) on the one hand, and an extraordinary low counting efficiency due to colour quench on the other hand. With respect to the intensity of the colour present in individual lipid fractions, adjustments might be required in different sample sets. Hence, typically non coloured fractions like aliphatic hydrocarbons can be measured in higher concentrations, while more intensive coloured fractions like acids, alcohols, or wax esters, might require a further dilution.

The test on fatty acids in combination with different solvents showed that chloroform cannot be recommended for LSC due to strong quenching, associated by larger counting errors and lower $^{14}C$ values compared to all other solvents (Fig. 3). Even after background correction these differences are abundant and should not be ignored by use of chloroform. Most likely, a specific standardization of the liquid scintillation counter for chloroform or the use of a different scintillation cocktail would give improved results,

which was not the intention of this study. However, the aim of this study was to use one method and standardization for a variety of applications in order to avoid specific adjustments for each single method and sample series. In contrast to chloroform, the other solvents in general revealed uniform results especially with respect to counting efficiency and error as well as background signal. This supports the general applicability of the standardization and scintillation cocktail for a broad variety of hydrophilic and lipophilic solutions [24]. Fatty acids were not completely dissolved in hexane, while these are completely dissolvable in all other used solvents. Hence hexane might be a choice for low polarity lipid fractions, while for polar lipid fractions this solvent is not suitable. However, the test did not show this potential problem related to the limited solubility, but it is well-established that solvents should match with the polarity of the substances to be dissolved. Differences between most solvents except for chloroform are within the general error which has to be taken into account during labelling experiments (<5%). Except for chloroform all tested solvents can be recommended for the determination of $^{14}C$ labels in lipid extracts and fractions.

## Plant and loess lipids and fatty acids

Largest portion of assimilated $^{14}C$ was found in shoots and lipidic components in plants, because they directly received the isotopic pulse label as previously described elsewhere [27]. This is in accordance with literature results, which describe a fast incorporation of the $^{14}C$ signal into lipids in leaves with a low lag of 3 min after application of a short term (5 min) isotopic pulse label due to direct biosynthesis [15]. The lower $^{14}C$ signal of bulk carbon in roots when compared to shoots was due to a limited plant internal transfer of assimilated carbon towards root biomass, which has been frequently described in previous experiments [27]. In comparison to the study of Hill et al. [27] we observed a higher proportion of $^{14}C$ in roots when compared to shoots (root $^{14}C$:shoot $^{14}C$ = 1:4 this study and 1:~10 reported by Hill et al. [27]) due to the experimental setup. While plants in our experiment received five $^{14}C$ pulses in intervals of 5 days, e.g. Hill et al. [27] obtained their results after a single pulse. Therefore, larger $^{14}C$ activity remained in root tissues from previous isotopic pulses, while shoots grew more intensively and individual leaves received one to five isotopic pulses, which results in a modification of this ratio. In comparison to lipids of aboveground biomass root lipids received a low $^{14}C$ label as a result of the limited transfer of biosynthetic precursor components towards root biomass. Hence a reduced formation of $^{14}C$ labelled lipids occurs in situ within roots, whereas amounts of extractable lipids in roots are significantly lower than those in aboveground biomass [8, 9]. Due to a low transfer of $^{14}C$ towards root biomass and especially rhizosphere a further depletion of the specific $^{14}C$ activity in fatty acids compared to other lipids is to be expected as observed. Even within non-rhizosphere loess distant to roots as well as rhizosphere lipids and fatty acids show a high $^{14}C$ activity, which is evidence for short term input of lipidic compounds in non-rhizosphere substrates. This effect of incorporation of root derived lipids via exudation has been seldomly described [9, 13, 28] and was mostly limited to short chain acids (<$C_{16}$) which have a higher affinity to dissolution in root exudates than long chain and thus more hydrophobic aliphatic compounds. During degradation of plant litter the portion of fatty acids vs. lipids should decrease due to different chemical reactions like defunctionalisation, oxidation and/or reduction of fatty acids. The opposite trend is regarded from root tissue towards non-rhizosphere soil. Hence, incorporation of plant derived fatty acids into non-rhizosphere tissues via exudation seems to be possible.

Regarding the budgeting of total C and $^{14}C$, lipids always showed higher $^{14}C$ activity than their total content could account for (Table 1). This indicates an improved storage capacity of lipidic compounds within plants once they are synthesized compared to other plant components, which are characterized by a faster turnover than lipids like

**Table 1** Relationship between lipids, fatty acids, and bulk samples determined for $^{14}C$ specific activities and masses

| | | Shoots | Roots | Rhizosphere loess | Non-rhizosphere loess |
|---|---|---|---|---|---|
| Lipids vs. biomass/loess | Lipids vs. biomass/loess [mg $g^{-1}$] | 98.0 | 44.2 | 0.7 | 0.2 |
| | $^{14}C$ activity in lipids vs. $^{14}C$ biomass/loess [(dpm $mg^{-1}$) (dpm $g^{-1}$)$^{-1}$] | 256.8 | 129.8 | 81.0 | 150.2 |
| Fatty acids vs. biomass/loess | Fatty acids vs. biomass/loess [mg $g^{-1}$] | 13.8 | 3.4 | 0.027 | 0.041 |
| | $^{14}C$ activity in fatty acids vs. $^{14}C$ biomass/loess [(dpm $mg^{-1}$) (dpm $g^{-1}$)$^{-1}$] | 73.2 | 39.2 | 15.0 | 75.6 |
| Fatty acids vs. lipids | Fatty acids vs. biomass/loess [mg $g^{-1}$] | 140.7 | 77.2 | 38.1 | 212.2 |
| | $^{14}C$ activity in lipids vs. $^{14}C$ biomass/loess [(dpm $mg^{-1}$) (dpm $g^{-1}$)$^{-1}$] | 285.1 | 301.7 | 185.6 | 503.2 |

e.g. sugars. Similarly, fatty acids accounted for a higher $^{14}C$ activity with respect to total lipids when compared to corresponding mass relationship. This confirms the fast synthesis of fatty acids in plants and a slower reutilzation of these components thereafter [15]. Other lipids like alkanes and alcohols are less reactive than fatty acids plant internally and in soils as previously described [1, 2]. Especially in rhizosphere, fatty acids were characterized by the lowest $^{14}C$ activity when compared to total lipids, which must be due to an intensive throughput of plant derived exudates accelerated by an increased microbial activity adjacent to roots [29]. Contrary, in the non-rhizosphere loess $^{14}C$ activity of fatty acids accounts for approximately one half of the $^{14}C$ signal of total lipids, which indicates the preferential incorporation of fatty acid containing root exudates, while other hydrophobic lipids were not able to enter this area distant from roots. Additionally, a part of fatty acids can be derived from microorganisms feeding on $^{14}C$ containing substrates incorporated as dissolved organic components from the rhizosphere. However, gas chromatographic analyses [9] did not show significant amounts of microbial derived components in the fatty acid fraction (including $C_{16:1}$ and $C_{19:1}$ fatty acids), while some plant derived components like polyunsaturated $C_{18}$ fatty acids ($C_{18:2}$ and $C_{18:3}$) could be observed in the non-rhizosphere loess after the experiment (data not shown here). Thus, both microbial and plant derived lipids were incorporated in non-rhizosphere loess within this short term experiment.

Hence, the $^{14}C$ labelling and lipid determination even in roots is very useful to determine biosynthetic pathways. The low transfer of the $^{14}C$ activity towards rhizosphere and non-rhizosphere loess is indicated by the differences in several orders of magnitude of the signal. Especially in the rhizosphere, extremely low $^{14}C$ activities were determined in lipids and fatty acids when compared to the bulk $^{14}C_{org}$ activity. This is partly connected with microbial decomposition of the $^{14}C$ labelled substances by microorganisms, of which the biological activity in rhizosphere is much higher than in non-rhizosphere loess [29]. This exceptional low $^{14}C$ activity might be due to a fast turnover and/or translocation of $^{14}C$ labelled substances from roots towards non-rhizosphere loess within 5 days after the last isotopic pulse. Lipids in non-rhizosphere loess received a stronger $^{14}C$ label than in rhizosphere due to present residues of dead plant materials, which could not be sufficiently separated from loess, and especially lipids derived from root exudates. Fatty acids in non-rhizosphere loess were predominantly labelled within the lipids (app. 50% of $^{14}C$ of bulk lipids, Table 1), which might be related to rhizodeposition in non-rhizosphere soil. Hence, during the $^{14}C$ pulse-labelling lipids in root-free substrates received a $^{14}C$ isotopic label indicating the incorporation of fresh lipidic components, especially related to fatty acids. The combination of lipid analyses and LSC is a useful tool in order to investigate biosynthesis of lipidic fractions or even individual components within the whole plant biomass and is not limited to aboveground biomass. Additionally, soil lipid incorporation and transformation can be easily determined in substrates adjacent and distant to roots. This enables the determination of short-term dynamics of lipids in soils, which have not been subjected to investigations so far.

The $^{14}C$ labelling technique has been shown to be a very effective tool to follow biosynthesis in plants and incorporation of plant derived components on a molecular level with the example of lipids and fatty acids as a lipidic fraction. As lipid composition varies within plants and time, biosynthesis and degradation rates cannot be exactly described using lipid composition only [9]. $^{14}C$ LSC combined with lipid analyses provide the unique opportunity to receive detailed information on lipid fluxes and dynamics within plants and incorporation of lipidic components into soils using the cheap LSC technique, which requires low amount of lipidic material in a ng-µg range depending on the $^{14}C$ activity the substances or fractions received. A further application of LSC on PLFA and other lipid fractions was successful, too (not shown here) and offers a wide range of potential applications in biochemical and geochemical sciences. Not only on a fraction- but even on a compound-specific level the application of the technique is supposed to yield high potential for future studies in plant and soil science.

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