Rhizodeposition
and its effects on C fluxes in the soil

Dissertation

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To Hannes and Xaver

Summary

Plants modify the chemical, physical and biological properties of the soil environment surrounding their roots. Organic compounds released from living roots (rhizodeposits) are easily available sources of energy for microorganisms strongly affecting soil organic matter (SOM) dynamics. Although, rhizodeposition is a key driver of microbially mediated processes in the soils, it still remains the most uncertain component of the terrestrial carbon (C) cycle.

The general aim of the thesis was to gain a more comprehensive understanding of rhizodeposition and its effects on C fluxes in soil by assessing biotic and abiotic factors influencing the rhizodeposition and by providing estimates on the total amounts of root-released C inputs into an agroecosystem with maize.

The input of C into the soil through rhizodeposition occurs in temporal and spatial hotspots. The objective of the first Study was to determine the dynamics of hotspots of recently assimilated C in roots of ryegrass. Shoots were pulse labeled with $^{14}CO_2$ and the allocation patterns at increasing time intervals were visualized by ^{14}C phosphor imaging. We could show a very quick translocation of assimilated C to the roots. Strong ^{14}C hotspots were detected at the root tips already 6 hours after the labeling. The hotspots remained active for at least 2 days. However, 11 days after assimilation the hotspots at the root tips had disappeared, and the ^{14}C distribution was much more even than after 6 hours and 2 days.

Through the availability of rhizodeposits, hotspots create preferred habitats for microbes. Rhizodeposits are an important primary source of carbon and energy for soil microorganisms stimulating their growth and activity. Thereby, roots of living plants can influence the rate of native SOM decomposition in the rhizosphere. This rhizosphere priming effect (RPE) was reported to be plant-species specific. Therefore, we hypothesized that also plant inter-species interactions, like the competition for nutrients and water, affect the RPE.

In Study 2, we used continuous $^{13}CO_2$ labeling to investigate the RPE of monocultures and mixtures of typical agricultural crops. The RPE was consistently positive for all

monocultures and mixed cultures with an increase of 43% to 136% above the unplanted soil. Of particular interest was the result that plant inter-species interactions between sunflower and wheat significantly reduced the RPE in contrast to the other mixtures which included soybean as a legume. It was suggested that priming in the rhizosphere of the sunflower-wheat mixture was reduced through a more severe competition for nitrogen (N), whereas, due to the N-rich rhizodeposits of the legume and its lower demand for soil mineral N the RPE of the legume containing mixtures remained unaffected.

Besides potential differences in the quality and quantity of rhizodeposits, the C allocation pattern in plant and soil pools may also differ between non-legumes and legumes due to high root respiration tied to N_2-fixation. However, not only the plant species may affect rhizodeposition, also photosynthesis could largely control root exudation because of the fast transport of recently assimilated C to belowground processes. Taking both factors into account, in Studies 3 and 4 the effect of limited photosynthesis on the distribution of recently assimilated C, of stored C and of N was investigated. Based on ^{13}C, ^{14}C and ^{15}N labeling of a legume and a non-legume we could demonstrate that high C and N demands of regrowing shoots after clipping led to a remobilization of stored C and N to the shoots for both plant species. Additionally, recently assimilated C was retained in the regrowing shoots. Particularly, in agricultural pasture systems limited photosynthesis after defoliation by grazing may significantly affect belowground C allocation.

Shading, on the other hand, did not induce a remobilization of stored C, since recently assimilated C obviously covered the demand of the shoots with lower growth rates. For both treatments lower amounts of recently assimilated C were observed in the belowground pools emphasizing the importance of the tight coupling of assimilation and belowground processes. Furthermore, different responses of clipping and shading of the legume and the non-legume species could be detected for root-derived CO_2.

In Studies 1-4 we demonstrated the importance of various factors on the rhizodeposition of different agricultural crops under controlled conditions. Under field conditions, however, there is still a great degree of uncertainty about the total quantity of rhizodeposition, primarily because of high and rapid losses of released rhizodeposits through microbial decomposition. Therefore, the quantitative importance of rhizodeposition at field scale was determined in Study 5. We proposed a new approach for an improved quanti-

fication of rhizodeposition under field conditions taking into account the decomposed fraction of rhizodeposits. Based on a $^{14}CO_2$ pulse labeling experiment under controlled conditions a rhizodeposition-to-root ratio was calculated and was applied to the root biomass of the field. The root biomass C of maize, sampled in July 2009, was 298±64 kg C ha^{-1}. Gross rhizodeposition was found to amount to 166±53 kg C ha^{-1}.

With aging of SOM, the availability of C for microbial decomposition declines. In Study 6 the availability of younger relative to older C sources was assessed. The natural isotope abundances of ^{13}C and ^{12}C of SOM and CO_2 were analyzed after a C_3 to C_4 vegetation change. The contribution of younger C, originating from the belowground C input by maize in the previous year, and that of older C sources, derived from the former C_3 vegetation, to SOM and CO_2 was determined. Comparing the proportions of younger and older C in SOM with that in CO_2, we found that the younger C of maize was seven times more available for microbial decomposition than older C pools.

In summary, this thesis extends the understanding of factors affecting rhizodeposition and of processes occurring at the soil-root interface. Furthermore, it presents a new method to quantify gross rhizodeposition at field scale. Although, we could gain insight in temporal changes of the availability of C pools for microbes, the ecological importance of C fluxes in the rhizosphere requires future research on this topic with regard to spatial and temporal predictions.

Zusammenfassung

Pflanzen verändern die chemischen, physikalischen und biologischen Eigenschaften der Bodenumgebung ihrer Wurzeln. Organische Verbindungen, die von lebenden Wurzeln abgegeben werden (Rhizodeposite), sind eine leicht verfügbare Energiequelle für Mikroorganismen und können damit die Dynamik der organischen Bodensubstanz (OBS) stark beeinflussen. Obwohl die Rhizodeposition eine der treibenden Kräfte für die mikrobiellen Prozesse im Boden darstellt, ist sie doch einer der am wenigsten erforschten Faktoren im terrestrischen Kohlenstoffkreislauf.

Das Hauptziel der vorliegenden Arbeit war es, ein umfassenderes Verständnis der Rhizodeposition und ihrer Effekte auf Kohlenstoffflüsse im Boden zu gewinnen. Dabei sollten biotische und abiotische Faktoren, die die Rhizodeposition beeinflussen, bewertet und darüber hinaus die Mengen an Kohlenstoff (C), die über Maiswurzeln in ein Agrarökosystem gelangten, quantifiziert werden.

Der Eintrag von C in den Boden über Rhizodeposition erfolgt in zeitlich und räumlich variierenden Hotspots. In der ersten Studie sollte die Dynamik der Hotspots von neu assimiliertem C in den Wurzeln von Weidelgras untersucht werden. Nach einer $^{14}CO_2$-Pulsmarkierung des Sprosses wurden die Verteilungsmuster in der Pflanze in größer werdenden Zeitabständen mit Hilfe der ^{14}C-Phosphor-Imaging-Methode sichtbar gemacht. Wir konnten eine sehr schnelle Verlagerung des assimilierten C in die Wurzeln zeigen. Starke ^{14}C Hotspots konnten bereits 6 Stunden nach der Isotopen-Markierung an den Wurzelspitzen nachgewiesen werden. Diese Hotspots blieben für mindestens 2 Tage aktiv. Elf Tage nach der Assimilation waren die Hotspots an den Wurzelspitzen verschwunden, und es zeigte sich eine gleichmäßigere ^{14}C-Verteilung als nach 6 Stunden bzw. 2 Tagen.

Durch die verfügbaren Rhizodeposite bilden Hotspots bevorzugte Habitate von Mikroorganismen. Rhizodeposite sind eine wichtige C- und Energiequelle für Bodenmikroorganismen und wirken sich stimulierend auf ihr Wachstum und ihre Aktivität aus. Die Wurzeln lebender Pflanzen können dadurch die Intensität des Abbaus der OBS in der Rhizosphäre beeinflussen. Es ist bekannt, dass dieser Rhizosphäre-Priming-Effekt (RPE) von der Pflanzenart abhängt. Deshalb stellten wir die Hypothese auf, dass sich

auch Interaktionen zwischen verschiedenen Pflanzenarten, wie z.B. die Konkurrenz um Nährstoffe und Wasser, auf den RPE auswirken.

In der zweiten Studie wurde mit Hilfe kontinuierlicher $^{13}CO_2$-Markierung der RPE von Mono- und Mischkulturen typischer landwirtschaftlicher Nutzpflanzen untersucht. Sowohl für die Monokulturen als auch für die gemischten Kulturen waren die RPE mit 43% bis 136% höheren Werten in Bezug auf den unbepflanzten Boden durchwegs positiv. Besonders interessant war das Ergebnis, dass bei der Kultur aus Sonnenblume und Weizen im Gegensatz zu den anderen Mischkulturen, die jeweils die Leguminose Sojabohne enthielten, der RPE durch Interaktionen zwischen den Pflanzenarten signifikant reduziert war. Es wird angenommen, dass eine stärkere Stickstoffkonkurrenz in der Rhizosphäre der Sonnenblume-Weizen-Kultur für den niedrigeren RPE verantwortlich ist, während die stickstoffreichen Rhizodeposite der Leguminose und deren niedrigerer Bedarf an mineralischem Stickstoff (N) aus dem Boden den RPE der anderen Mischkulturen im Vergleich dazu nicht beeinflussten.

Neben potenziellen Unterschieden in Qualität und Quantität der Rhizodeposite können sich Leguminosen und Nicht-Leguminosen, aufgrund der hohen Wurzelatmung verbunden mit der N_2-Fixierung, auch in ihrem C-Verteilungsmuster zwischen den Pflanzen- und Bodenpools unterscheiden. Jedoch nicht nur die Pflanzenart kann sich auf die Rhizodeposition auswirken. Durch den schnellen Transport von neu assimiliertem C in den Boden kann auch die Photosynthese die Wurzelexsudation stark beeinflussen. Unter Berücksichtigung dieser beiden Faktoren wurde in Studie 3 und in Studie 4 der Effekt einer limitierten Photosynthese durch Abschneiden und Beschattung der Pflanzen auf die Verteilung von neu assimiliertem C, von gespeichertem C und von N untersucht. Durch ^{13}C-, ^{14}C- und ^{15}N-Markierung einer Leguminose und einer Nicht-Leguminose konnten wir zeigen, dass der hohe C- und N-Bedarf des nachwachsenden Sprosses nach dem Schneiden in beiden Pflanzenarten zu einer Remobilisierung von gespeichertem C und von N in den Spross führte. Zusätzlich wurde neu assimilierter C im Spross zurückgehalten. Besonders bei landwirtschaftlichen Weideflächen könnte eine limitierte Photosynthese durch Beweidung zu signifikanten Effekten bei der C-Allokation im Boden führen.

Durch die Beschattung wiederum kam es zu keiner Remobilisierung von gespeichertem C. Offensichtlich konnte der neu assimilierte C aufgrund der verringerten

Wachstumsrate des Sprosses dessen Bedarf decken. Sowohl beim Schneiden als auch bei Beschattung wurden geringere Mengen von neu assimiliertem C in den unterirdischen Pools beobachtet, was die Bedeutung der engen Kopplung von Assimilation und Bodenprozessen unterstreicht. Darüber hinaus konnten für wurzelbürtiges CO_2 unterschiedliche Reaktionen auf Schneiden und Beschattung von Leguminose und Nicht-Leguminose festgestellt werden.

In den Studien 1-4 wurde die Bedeutung einzelner Faktoren für die Rhizodeposition verschiedener landwirtschaftlicher Nutzpflanzen unter kontrollierten Bedingungen aufgezeigt. Betrachtet man die Rhizodeposition im Feld, so herrscht jedoch über deren Gesamtmenge aufgrund der hohen und schnellen Verluste der Rhizodeposite durch mikrobiellen Abbau weitestgehend Unklarheit. Deshalb wurde in Studie 5 die quantitative Bedeutung der Rhizodeposition unter Feldbedingungen ermittelt. Wir entwickelten einen neuen Ansatz für eine verbesserte Quantifizierung der Rhizodeposition unter Feldbedingungen, der den Anteil der mikrobiell abgebauten Rhizodeposite mit berücksichtigt. Basierend auf einer $^{14}CO_2$-Pulsmarkierung unter kontrollierten Bedingungen wurde ein Rhizodepositions-Wurzel-Quotient berechnet und auf die Wurzelbiomasse im Feld übertragen. Die Wurzelbiomasse von Mais, die im Juni 2009 beprobt wurde, hatte einen C-Gehalt von 298±64 kg C ha^{-1}. Die Gesamt-Rhizodeposition betrug 166±53 kg C ha^{-1}.

Mit zunehmendem Alter der OBS nimmt die Verfügbarkeit von C für den mikrobiellen Abbau ab. In Studie 6 wurde die Verfügbarkeit von jüngeren im Vergleich zu älteren C-Quellen untersucht. Hierzu wurde die natürliche Häufigkeit von ^{13}C und ^{12}C nach einem C_3/C_4-Nutzungswechsel analysiert. Es wurde der Beitrag des jüngeren C, der im Vorjahr durch Mais unterirdisch eingetragen wurde, sowie des älteren C, der aus der vorherigen C_3-Vegetation stammte, zur OBS und zum CO_2 bestimmt. Beim Vergleich der Anteile des jüngeren und des älteren C in der OBS und im CO_2 stellten wir fest, dass der jüngere C aus dem Mais sieben Mal mehr verfügbar war als C aus den älteren Pools.

Mit der vorliegenden Arbeit konnte das Verständnis wichtiger Faktoren, die die Rhizodeposition beeinflussen, sowie der Prozesse in der Rhizosphäre erweitert werden. Darüber hinaus wurde eine neue Methode zur Quantifizierung der Gesamt-Rhizodeposition auf Feldebene vorgestellt. Obwohl wir Einblick gewinnen konnten, wie sich die Verfügbarkeit von C-Pools für Mikroorganismen mit der Zeit ändert, sind aufgrund der

ökologischen Bedeutung der C-Flüsse in der Rhizosphäre weitere Untersuchungen erforderlich, die räumliche und zeitliche Voraussagen zulassen.

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Abbreviations

ANOVA	Analysis of variance
ANU	Australian National University sucrose
AWC	Available water capacity
BS	Base saturation
^{13}C	Stable carbon isotope with atomic mass 13
^{14}C	Radioactive carbon isotope with atomic mass 14
C_3	C_3 photosynthetic metabolisms
C_4	C_4 photosynthetic metabolisms
CEC	Cation exchange capacity
CL	Continuous labeling
C_{org}	Organic carbon
C_t	Total carbon
cv.	Cultivar
DAG	Days after germination
DAL	Days after labeling
DAP	Days after planting
DLU	Digital light units
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
DW	Dry weight
EA	Elemental analyzer
FC	Field capacity
GC	Gas chromatograph
HAL	Hours after labeling
IRGA	Infrared gas analyzer
IRMS	Isotope ratio mass spectrometry
L.	Carl Linnaeus

LSC	Liquid scintillation counter
LSD	Least significant difference
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
^{15}N	Stable nitrogen isotope with atomic mass 15
N_{min}	Mineral nitrogen
N_{tot}	Total nitrogen
OM	Organic matter
PAR	Photosynthetic active radiation
PE	Priming effect
PL	Pulse labeling
RB	Root biomass
RMR	Rhizomicrobial respiration
RPE	Rhizosphere priming effect
RR	Root respiration
SEM	Standard error of means
SOC	Soil organic carbon
SOM	Soil organic matter
ssp.	Subspecies
TOC	Total organic carbon
WHC	Water holding capacity
PU	Polyurethane
V-PDB	Vienna Pee Dee Belemnite
WRB	World Reference Base for Soil Resources

I Extended Summary

1 Introduction

1.1 Carbon fluxes into and out of the soil

Soils are the largest reservoir of organic carbon (C) in terrestrial ecosystems (Amundson, 2001). It is estimated that soils globally contain about 1,500 Gt of C, which is approximately double the amount of the atmospheric pool and almost three times more than stored in the terrestrial biosphere (Schlesinger, 1997). Annually, land plants assimilate about 120 Gt of C from the atmospheric CO_2 through photosynthesis (Schlesinger, 1997; Lal, 2008). Half of it is transferred from the biosphere into the soil, either as root and shoot litter after plant death or as C released from living roots (Paterson et al., 2009). Again about 60 Gt of C are annually returned back as CO_2 from the soil to the atmosphere (Schlesinger, 1997; Lal, 2008). The soil CO_2 efflux is one of the largest fluxes in the global C cycle (Schlesinger and Andrews, 2000; Amundson, 2001). As such, it is increasingly becoming a focus of scientific interest in the context of climate change, since small alterations in the efflux rate may lead to detectable changes in the atmospheric CO_2 level (Schlesinger and Andrews, 2000; Amundson, 2001).

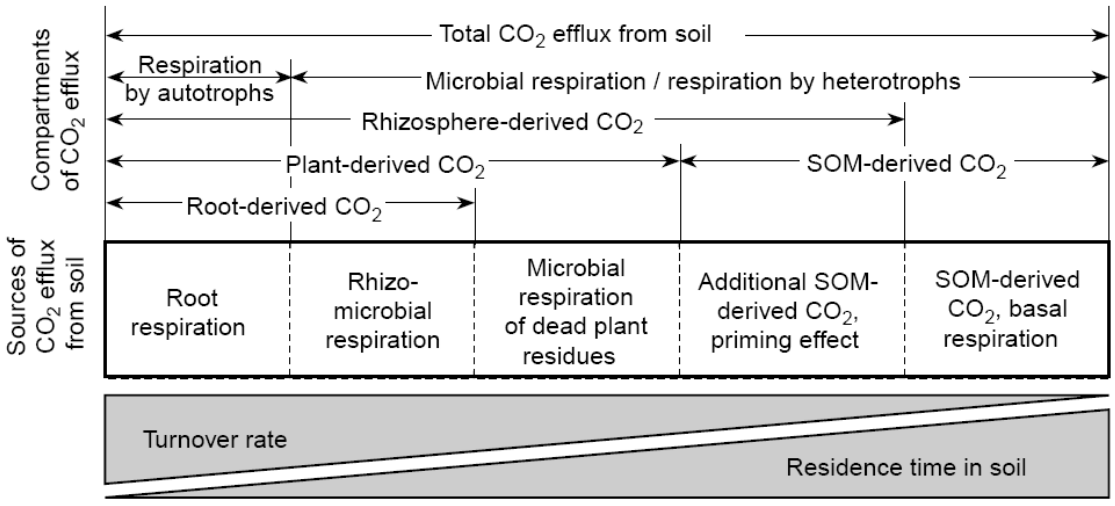

Figure I.1/1: Sources of biogenic CO_2 efflux from soil, ordered by the turnover rate and mean residence time of C in soil (after Kuzyakov, 2006).

The two main sources contributing to the CO_2 efflux from the soil are plant-derived CO_2 and CO_2 from the decomposition of soil organic matter (SOM) (Fig. I.1/1). Plant-derived CO_2 includes mineralization of dead plant residues as well as root-derived CO_2, i.e. root respiration (RR) and CO_2 from microbial decomposition of root-released substances (rhizomicrobial respiration; RMR) (Kuzyakov, 2006). The contribution of the different sources is highly variable for different ecosystems depending on a wide range of plant, soil and environmental factors. Reported contributions of root-derived CO_2 range, for instance, from 10%-90% depending on the type of study and the respective ecosystem (Hanson et al., 2000; Kuzyakov and Gavrichkova, 2010).

Agroecosystems, in particular, are characterized by short-term and long-term changes in the soil CO_2 efflux through alterations in root-derived CO_2 and in SOM-derived CO_2, respectively, based on agricultural management practices (Gavrichkova, 2009). Since cropland and grassland cover 30% of the earth´s land surface (Buyanovsky and Wagner, 1998) our knowledge of the soil CO_2 efflux from these ecosystems is central for understanding the global C exchange between soil and atmosphere.

Due to the high turnover rate of root-released substances, rhizomicrobial respiration is of key importance for the soil CO_2 efflux (Fig. I.1/1). In contrast to C inputs by plant litter, C released from living roots remain, however, a large factor of uncertainty in the C cycle.

The present thesis, therefore, focuses on C inputs into the soil by living roots of crop and grassland species.

1.2 Rhizodeposition

The rhizosphere, a term introduced by Lorenz Hiltner in 1904, is defined as the volume of soil affected by the presence of living roots (Darrah, 1993; Uren, 2007). It describes a complex, unique habitat with chemical, physical and biological conditions different from those of the bulk soil. As plant roots grow, longitudinal and radial gradients between the root surface and the bulk soil are created (Uren, 2007) mainly as a result of nutrient and water uptake and of rhizodeposition, i.e. the release of organic substances by living roots (Nguyen, 2003). Rhizodeposits originate from root exudates of intact cells, which can either be released passively by diffusion or actively by secretion, from

lysates of sloughed-off cells and dead tissues, and from mucilage (Fig. I.1/2; Neumann and Römheld, 2007; Dennis et al., 2010).

Plants translocate about half of the assimilated C to belowground pools (Lynch and Whipps, 1990; Kuzyakov and Domanski, 2000) and up to 40% of the fixed C can be lost through rhizodeposition (Lynch and Whipps, 1990). Root exudates, dominated by low molecular weight solutes such as sugars, carboxylic acids and amino acids, serve as a source of easily available energy for microorganisms in the rhizosphere, stimulating their growth and activity (Merbach et al., 1999; Nguyen, 2003; Jones et al., 2004). In turn, microbes decompose SOM and thereby release plant available nutrients (Paterson, 2003). Rhizodeposition is thus the most important link between plant growth and microbial mediated processes in soils.

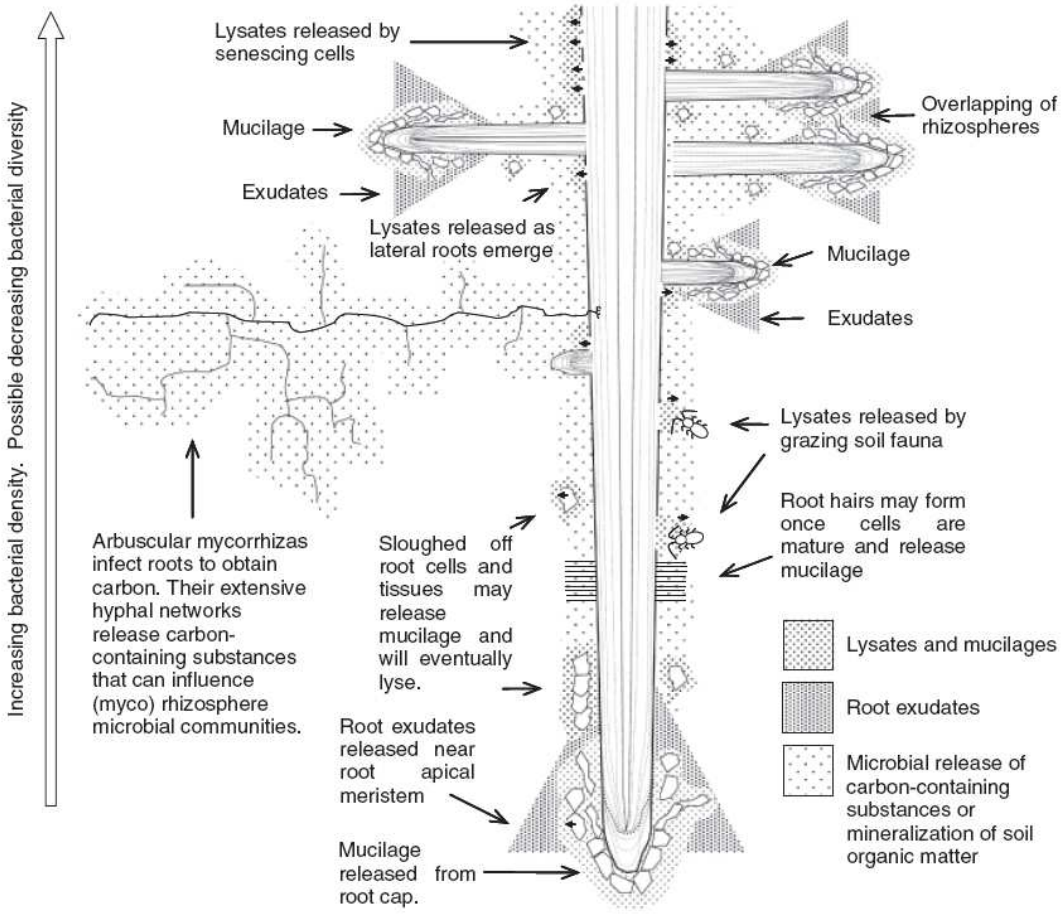

Figure I.1/2: Origin of different types of rhizodeposits (after Dennis et al., 2010)

Rhizodeposition is not homogeneously distributed along the root segments (McDougall and Rovira, 1970; Nguyen, 2003; Dennis et al., 2010). Organic substances are rather released in distinct areas. These hotspots are characterized as small soil volumes rich in easily available organic substrate for microorganisms. Fig. I.1/2 summarizes the origin of different types of rhizodeposits and shows that hotspots mainly occur at root tips (Dennis et al., 2010).

Although it is known that root exudation is mainly supplied from recently assimilated C allocated belowground (Craine et al., 1999; Kuzyakov and Cheng, 2001), the dynamics of hotspots in the rhizosphere remain poorly understood.

Study 1 – *Photoassimilate allocation and dynamics of hotspots in roots visualized by ^{14}C phosphor imaging* – aimed to identify recently assimilated C in roots of *Lolium perenne* presuming that its distribution and dynamics reflect the hotspots in the rhizosphere, since it is the recently assimilated C that mainly fuels rhizosphere processes (Kuzyakov and Cheng, 2001). It was hypothesized that ^{14}C hotspots in the roots occur shortly after the assimilation and are highly dynamic.

1.3 The rhizosphere priming effect

Providing root exudates as energy source for microorganisms, plants influence microbial activity and consequently affect the SOM turnover in the rhizosphere. Changes in the rate of SOM turnover in the presence of living roots are referred to as 'rhizosphere priming effects' (RPE; Kuzyakov, 2002; Cheng and Kuzyakov, 2005). It was found that already small amounts of easily decomposable root exudates can provoke a SOM turnover much higher than would be predicted from the amounts of material added (Kuzyakov, 2002; Paterson, 2003; Cheng and Kuzyakov, 2005). On the other hand, N shortages in the rhizosphere may intensify the competition between plant roots and microorganisms which could inhibit microbial growth and activity and therefore suppress SOM decomposition (Schimel et al., 1989; Van Veen et al., 1989; Wang and Bakken, 1997; Bottner et al., 1999). The rhizosphere priming effect can, hence, be either positive or negative (Kuzyakov, 2002; Cheng and Kuzyakov, 2005).

The RPE was shown to be plant-species specific, since species differ in the quality and quantity of root-released organic compounds (Fu and Cheng, 2002; Cheng et al., 2003).

Also plant diversity may have an impact on the RPE. Higher plant diversity leads to higher diversity of litter quality and quantity entering the soil (Stephan et al., 2000). This could stimulate microbial-mediated processes and consequently increase the potential for positive priming. In contrast, with higher plant richness belowground resources are complementarily and thus more completely consumed. A higher competition for N between microbes and plants may result in a reduced RPE.

Therefore, and because of plant inter-species competition for nutrients and water, it is likely that the RPE is affected by inter-species interactions.

Study 2 – *Plant inter-specific effects on rhizosphere priming of soil organic matter decomposition* – was based on the hypothesis that plant inter-species interactions modulate the RPE, and that the modulation is specific to the plant species composition.

1.4 Factors affecting rhizodeposition

Rhizodeposition is influenced by various biotic and abiotic factors in the plant-soil system (Jones et al., 2004). The soil environment can affect rhizodeposition and especially root exudation through physical aspects (e.g. water availability, temperature, soil texture) and chemical conditions (e.g. pH, availability of nutrient ions), as well as through the activity and diversity of microbial populations (Lynch et al., 2002). One of the most important plant-mediated factors is the plant species (see above) (Vancura, 1964; Van der Krift et al., 2001; Kuzyakov, 2002; Cheng et al., 2003).

Depending on the plant species the amount and composition of rhizodeposits can vary. Legumes, for instance, showed higher N concentrations in the root tissue than non-legume species, which can potentially be released into the soil (Uselman et al., 1999; Paynel et al., 2001). Also the allocation pattern of recently assimilated C differs between legumes and non-legumes, since legumes use a higher proportion of assimilated C for root respiration due to the high energy requirements for N_2 fixation (Warembourg et al., 2003).

Not only the plant species but also environmental factors may have an effect on the quality and quantity of root exudates (e.g. Rovira, 1959; Graystone et al., 1998; Paterson et al., 1996). The C supply to rhizosphere processes via exudation depends to a large extent on the belowground allocation of recently assimilated C and thus on the

intensity of photosynthesis. High transport rates of assimilates from the leaves into the roots and into root exudates has been reported (Gregory and Atwell, 1991; Cheng et al., 1993; Kuzyakov et al., 1999). Thus, belowground C allocation, the release of exudates and the CO_2 efflux from the soil are largely governed by photosynthesis (Craine et al., 1999; Kuzyakov and Cheng, 2001; Kuzyakov, 2002).

In pasture ecosystems periodical partial defoliation occur through grazing which may cause changes in the C fluxes belowground. Results of isotopic labeling experiments under controlled conditions are, however, contradicting and show positive, neutral or negative effects of clipping (simulating grazing or defoliation) on C fluxes in the plant-soil system (Paterson et al., 2005). Besides clipping, also shading may provoke effects on C fluxes belowground. It could, for instance, be demonstrated that shading reduced root-derived respiration (Kuzyakov and Cheng, 2001).

Due to the high C and N demand of regrowing shoots after clipping, we hypothesized that clipping and shading provoke different responses with respect to the remobilization of stored C, the allocation of recently assimilated C, and the uptake and remobilization of N.

Study 3 – *Effect of clipping and shading on C allocation and fluxes in soil under ryegrass and alfalfa estimated by ^{14}C labeling* – was conducted to investigate how a reduction of the photosynthetic activity, either by clipping (grazing) or shading, affects the allocation of stored C and the CO_2 efflux. We hypothesized that (1) clipping and shading show different responses with respect to the redistribution of stored C in plant and soil pools, and that (2) legumes respond differently to limited photosynthesis than non-legume species.

In Study 4 – *C and N allocation in soil under ryegrass and alfalfa estimated by ^{13}C and ^{15}N labeling* – the allocation of recently assimilated C, the N uptake by plants and the remobilization of plant-stored N after reduced photosynthesis was investigated. The hypothesis was that a limitation of photosynthesis alters the distribution of recently assimilated C but also the N distribution in the plant and soil system, and that the magnitude of alteration depends to a large extend on the plant species and on the approach of photosynthetic restriction (clipping or shading).

1.5 Belowground C input at field scale and its microbial availability

As shown in the previous chapters, rhizodeposition is a process of major importance for carbon and nutrient cycling in the soil. However, it is challenging to reliably quantify rhizodeposition, mainly due to the fast decomposition of rhizodeposits (Fig. I.1/1). There is a lack of suitable methods to estimate rhizodeposition at field scale taking into account the fraction of C released from roots that is mineralized (Nguyen, 2003).

The methodological objective of Study 5 – *Estimation of rhizodeposition at field scale: extrapolation of a ^{14}C labeling study* – was to provide a new approach for the estimation of rhizodeposition in the field. This approach is based on the determination of a rhizodeposition-to-root ratio through a $^{14}CO_2$ pulse labeling experiment under controlled conditions and the subsequent application of this ratio to field root biomass data. Maize was planted on an arable field and the root biomass C was determined to estimate rhizodeposition.

It is well established that C that entered the soil recently is more easily decomposable by microorganisms than older, more recalcitrant C pools (e.g. Von Lützow et al., 2006; Jastrow et al., 2007). However, only very few approaches allow for a quantitative estimation of the availability of C in relation to the time it entered the soil.

Study 6 – *Soil organic carbon decomposition from recently added and older sources estimated by $\delta^{13}C$ values of CO_2 and organic matter* – aimed to determine the microbial availability of younger relative to older C sources. We hypothesized, that the $\delta^{13}C$ values of soil CO_2 and the $\delta^{13}C$ values of SOM after a change from C_3 (wheat) to C_4 (maize) vegetation can be used to estimate the relative availability of C sources of different ages.

1.6 Objectives

In summary, the main objectives of the present work were to

(1) identify hotspots of recently assimilated C in roots of *L. perenne* and to determine their dynamics (Study 1),

(2) investigate plant inter-species effects on rhizosphere priming of soil organic matter decomposition (Study 2),

(3) assess the influence of limited photosynthesis on the redistribution of stored C within the plant-soil systems of a legume and a non-legume (Study 3),

(4) assess the influence of limited photosynthesis on the redistribution of recently assimilated C and mineral N within the plant-soil systems of a legume and a non-legume (Study 4),

(5) suggest an approach for the determination of rhizodeposition at a field scale and quantify rhizodeposition of maize (Study 5),

(6) estimate the relative availability of younger C compared to older C for microbial decomposition (Study 6).

2 Materials and Methods

2.1 Isotope approaches

The main challenges for the investigation of rhizodeposition are its occurrence only within a narrow zone around the roots, the fast decomposition of root-released organic C by soil microorganisms and its much lower content compared to other organic substances in the soil (Kuzyakov and Domanski, 2002). To gain a better understanding of rhizosphere processes, stable and radioactive isotope based labeling techniques are applied to differentiate between plant-derived organic C and native SOM. The most common methods are (1) pulse labeling, (2) continuous labeling, and (3) the utilization of natural differences in the isotopic signature between plant C and SOM (Paterson et al., 2009).

For pulse labeling used in Studies 1, 3, 4, 5 the plants were exposed to the isotope-tracer ($^{14}CO_2$ and/or $^{13}CO_2$) in a Plexiglas chamber for a short period of time (several hours), whereas for continuous labeling used in Study 2 the plants were grown in a greenhouse in a tracer atmosphere from the emergence of the first leaf till harvest. Fig. I.2/1 shows the two different experimental set-ups, technical details are provided in the respective studies (Chapter II).

The third method is based on a vegetation change of plants with different photosynthetic pathways, introducing a distinct isotopic signature into the soil (Balesdent and Mariotti, 1987). Depending on the photosynthetic pathways plants differ in their ^{13}C discrimination during photosynthesis, resulting in a higher ^{13}C depletion of C_3 plants compared to C_4 plants (Farquhar et al., 1989). Therefore, when growing C_4 plants on soil originally formed in areas of C_3 vegetation (or vice versa), root- and soil-derived C can be differentiated based on their isotopic differences (Balesdent and Mariotti, 1987). This approach was used in a field experiment in Study 6 (Chapter 2.2).

The three methods fundamentally differ from each other in the resulting distribution of the tracer within the plant and soil (Meharg, 1994), and hence, the choice of the respective method depends on the research question. Pulse labeling provides information on

the relative allocation of recently assimilated C to different C pools and allows studying the dynamics of C translocation between pools (Rattray et al., 1995). The allocation pattern, however, strongly depends on the stage of plant growth, and the results obtained by pulse labeling cannot directly be transferred to the whole growing period (Kuzyakov and Domanski, 2000). Since the tracer is not evenly distributed within the plant after pulse labeling, C fluxes and pools can only roughly be quantified (Lynch and Whipps, 1990; Paterson et al., 2009). Continuous labeling, on the other hand, produces uniformly labeled plant material. The advantage of continuous labeling is that it enables to quantitatively separate root-derived C from native SOM-derived C in soil as well as in soil CO_2 (Lynch and Whipps, 1990; Meharg, 1994). However, the special equipment required for continuous labeling is expensive and the complex experimental set-up cannot easily be adapted to field experiments. Therefore, a vegetation change from C_3 to C_4 plants (or vice versa) has commonly been applied to continuously introduce a natural tracer into a system (Balesdent and Mariotti, 1987). Shortcomings of this method are that different isotopic fractionations may occur between C_3 and C_4 plants which may bias the picture of plant-derived C incorporated into a certain C pool (Hobbie and Werner, 2004; Zhu and Cheng, 2011), and that experiments using the vegetation change are restricted to places where environmental conditions allow to grow C_4 plants on C_3 soil (or vice versa) (Kuzyakov and Domanski, 2000).

In addition to the C isotope labeling approaches, in one study (Study 4) a ^{15}N labeling was performed by adding dissolved $K^{15}NO_3$ to the soil surface shortly before the $^{13}CO_2$ labeling.

Table I.2/1 gives an overview of the different isotope labeling methods and of the treatments applied to the plant cultures. Detailed descriptions are provided in the respective studies (Chapter II).

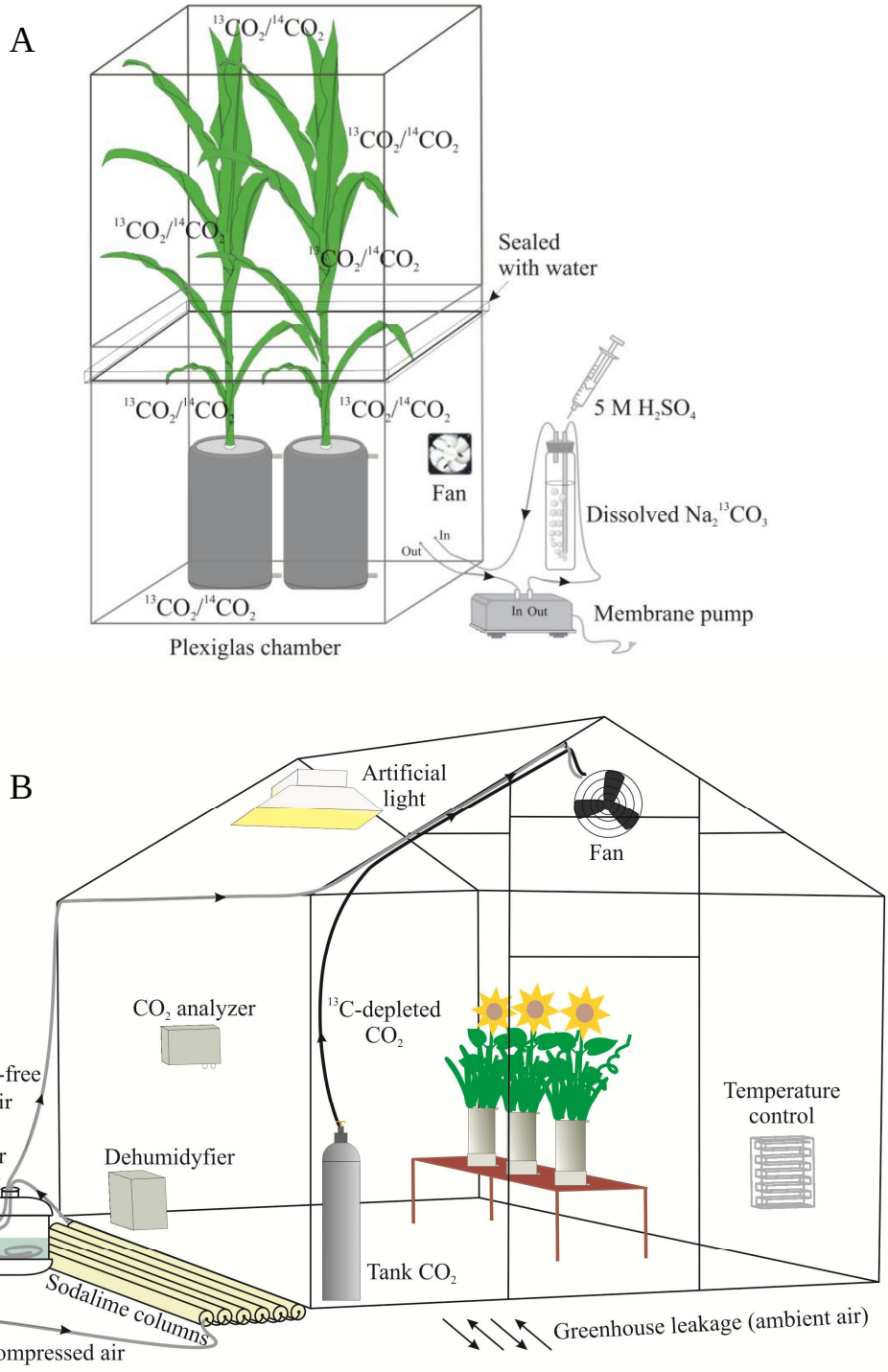

Figure I.2/1: Experimental set-up of labeling approaches. A: Pulse labeling of plants in a $^{13}CO_2$ or $^{14}CO_2$ atmosphere. B: Greenhouse for continuous ^{13}C labeling after the approach developed by Cheng and Dijkstra (2007).

2.2 Experimental site for field studies

The investigations of rhizodeposition at field scale (Study 5) and of the microbial availability of younger and older C sources (Study 6) were carried out on an arable field near Göttingen, Germany (51°33´36.8´´N, 9°53´46.9´´E). The field has been under agricultural use at least since the late 18th century with long-term C_3 vegetation. The soil was classified as a haplic Luvisol (IUSS, 2007). The main soil properties are presented in detail by Kramer et al. (2012).

Before starting the experiment in April 2009, a soil survey was conducted to determine the variability of the isotopic composition and organic C (C_{org}) content of SOM and to identify optimal locations with comparable conditions for establishing the experimental plots. Samples were taken from the upper 10 cm. The field site had a mean C_{org} content of 11.63±0.2 mg C g_{soil}^{-1} and showed $\delta^{13}C$ values typical for C_3 vegetation with a variability of less than 2.2 ‰ (Fig. I.2/2). Overall, the soil survey showed that all plots had comparable isotopic compositions at the start of the experiment.

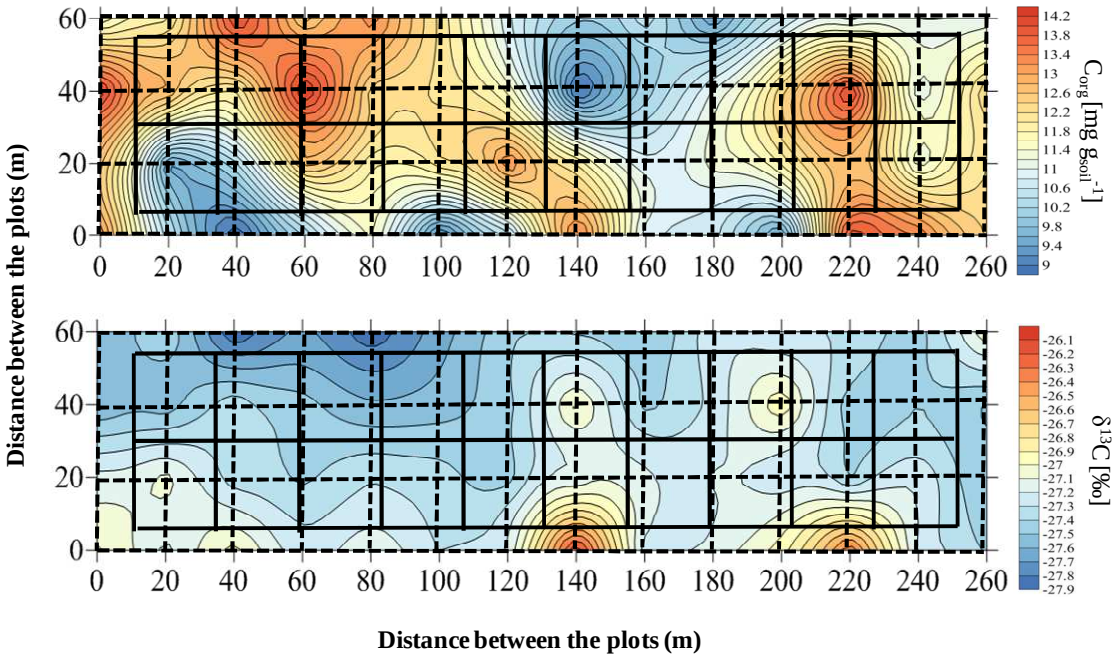

Figure I.2/2: Total organic C content and its $\delta^{13}C$ signature in 0-10 cm depth. Dashed lines: grid established for the soil survey; solid lines: experimental plots.

The experimental plots were established in two rows of 10 plots (24 x 24 m) each (Fig. I.2/2; Kramer et al., 2012). In 2009, one row of plots was planted with the C_4 plant ma-

ize (*Zea mays* L. cv. Ronaldinio) and the second row was used as reference with the C_3 plant winter wheat (*Triticum aestivum* L. cv. Julius). The wheat was harvested in August and the maize in early November. In 2010, in the second year of the study, maize (*Zea mays* L. cv. Fernandez) and summer wheat (*Triticum aestivum* L. cv. Melon) were replanted on the respective plots and on one former maize plot a bare fallow was established. In July 2009 root biomass samples of maize were taken for Study 5 and in 2010 soil CO_2 and soil were sampled during May and November for Study 6.

2.3 Sampling and analyses

Sampling was conducted at defined time intervals after labeling (Table I.2/1).

In the greenhouse and chamber experiments with continuous and pulse labeling (Fig. I.2/1) soil CO_2 was trapped from sealed pots by circulating the soil air through NaOH solution (Studies 2, 3, 4, 5). The total C concentration of the NaOH samples was measured either by titration with HCl against phenolphthalein (Study 3, 4) or by means of a TOC analyzer (Study 2, 5). To measure the ^{14}C activity of CO_2, aliquots of NaOH samples were mixed with a scintillation cocktail and analyzed by a liquid scintillation counter (LSC; Studies 3, 5). In order to determine the $\delta^{13}C$ value, CO_2 trapped in NaOH was precipitated as $SrCO_3$ after the addition of $SrCl_2$ solution (Harris et al., 1997), and analyzed by coupling an elemental analyzer to an isotope ratio mass spectrometer (EA-IRMS; Studies 2, 4).

In the field experiment (Study 6) the method for soil CO_2 sampling was based on the exchange of soil air with the air inside a silicon tube by diffusion (Kammann et al., 2001; Knorr et al., 2008). The tube was buried in the soil at various depths and was gas-tight fitted to a stopcock with a cannula to allow for aboveground air sampling. The $\delta^{13}C$ value and concentration of CO_2 was determined using a gas chromatograph coupled to an isotope ratio mass spectrometer (GC-IRMS).

To determine the ^{14}C activity of plants (roots and shoots) and soils (Studies 3, 5), the samples were combusted in a combustion unit and the released CO_2 was trapped in NaOH. The ^{14}C activity was measured with a LSC and the total C content with a TOC analyzer. To analyze the distribution pattern of ^{14}C (Study 1), shoots and roots were prepared as herbariums and the ^{14}C activity was visualized by phosphor imaging. To

obtain the $\delta^{13}C$ and $\delta^{15}N$ values and total C and N contents, plant and soil materials were analyzed by means of EA-IRMS (Studies 2, 4, 6).

The microbial biomass C (MBC) and the microbial biomass N (MBN) were determined by the chloroform fumigation extraction method using the approach of Vance et al. (1987) with modifications. Soil samples were either directly extracted with K_2SO_4 solution or fumigated with $CHCl_3$ before extraction. The difference between the C or N concentrations of extracts of fumigated and unfumigated samples gives the amount of MBC and MBN. As a measure for the fraction of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) the C and N concentrations of the unfumigated samples were used. To determine the ^{14}C activity of MBC and DOC, the extracts were directly measured by means of a LSC (Studies 3, 5). The $\delta^{13}C$ and $\delta^{15}N$ values of MBC/MBN and DOC/DON were determined in dried extracts using an elemental analyzer coupled to an IRMS (Study 4).

Soil mineral N (N_{min}; NO_3^- + NH_4^+) was extracted with KCl solution and the extracts were analyzed for NO_3^- and NH_4^+ by a flow injection analyzer (Study 2).

Table I.2/1: Overview of approaches, plant species and applied treatments used in Studies 1-6.

Study	Species	Treatment	Time of sampling[1]	Approach[4]	Type of study
1	*Lolium perenne*	-	6 HAL 2 DAL 11 DAL	^{14}C PL	Growth chamber
2	*Triticum aestivum* *Glycine max* *Helianthus annuus*	Monocultures 2-species mixture 3-species mixture Unplanted soil	30 DAP 56 DAP	^{13}C CL	Greenhouse + additional lighting
3	*Lolium perenne* *Medicago sativa*	Clipping Shading	1 DAL[2] 3 DAL[2] 5 DAL[2]	^{14}C repeated PL	Growth chamber
4	*Lolium perenne* *Medicago sativa*	Clipping Shading	1 DAL[2] 3 DAL[2] 5 DAL[2] 6 DAL[3] 8 DAL[3] 11 DAL[3] 13 DAL[3] 15 DAL[3]	^{13}C repeated PL ^{15}N PL	Growth chamber
5	*Zea mays*	-	2 DAL 5 DAL 10 DAL 16 DAL July 2009	^{14}C PL Root biomass sampling	Growth chamber Field
6	*Zea mays* *Triticum aestivum*	C_4/C_4: 2 years maize C_4/fallow: bare fallow after 1 year maize C_3 reference: Long-term wheat	Growing period 2010	δ^{13}C after C_3/C_4 vegetation change	Field

[1] HAL: hours after labeling; DAL: days after labeling; DAP: days after planting.

[2] After each pulse.

[3] After the last pulse.

[4] PL: pulse labeling; CL: continuous labeling.

3 Results and discussion

The methodological innovations and main results of Studies 1-6 are shown in Table I.3/1. The results of the individual studies are summarized in the following chapters.

Table I.3/1: Synthesis of methodological innovations and main innovative results of Studies 1-6.

Study	Aim	Type of study	Methodological innovations	Main innovative results[1]
1	Identification of hotspots of recently assimilated C in roots and determination of their lifetime	Growth chamber	Statistical analysis of digitalized ^{14}C images	Hotspots occurred already 6 HAL and disappeared 11 DAL
2	Effects of plant inter-species interactions on RPE	Greenhouse + additional lighting	Separation of root systems of individual plants grown in mixtures and consideration of ^{13}C fractionations between roots and root-derived CO_2	RPE is affected by plant inter-species interactions
3 / 4	Effects of limited photosynthesis on allocation of stored C, recently assimilated C, N of a legume and a non-legume	Growth chamber	Pulse labeling with 3 isotopes ^{14}C, ^{13}C, ^{15}N	Allocation depends on the approach of photosynthetic restriction
5	Estimation of rhizodeposition at field scale	Growth chamber + Field	Coupling of $^{14}CO_2$ pulse labeling in a growth chamber with root biomass sampled in field	Rhizodeposition of maize in the field is 166±53 kg C ha^{-1}
6	Determination of the availability of younger and older C sources for microbial decomposition	Field	Application of Miller/Tans models to purify soil CO_2 from the atmospheric admixture Application of a new approach for mixing models which considers isotopic fractionation	Younger C is seven times more available than older C sources for microbial decomposition

[1] HAL: hours after labeling; DAL: days after labeling.

3.1 Hotspots of rhizodeposition along the root segments (Study 1)

Hotspots of rhizodeposition were visualized by ^{14}C phosphor imaging, which proofed to be a promising tool for the investigation of processes at the root-soil interface. The results of Study 1 showed that ^{14}C assimilates are allocated to the root tips of *Lolium perenne* which is in accordance with former findings that the majority of exudates are released at the most active root parts, near the root cap, at the meristematic root region (Marschner, 1995; Dennis et al., 2010). Exudation is supplied with recently assimilated C through a fast translocation of assimilates from the leaves to the roots (Gregory and Atwell, 1991; Cheng et al., 1993; Kuzyakov et al., 1999). In the current study hotspots at the roots tips occurred already within 6 hours after $^{14}CO_2$ labeling of the shoots (Fig. I.3/1). Similar results were reported for *Brassica napus* (Dennis and Jones, 2006).

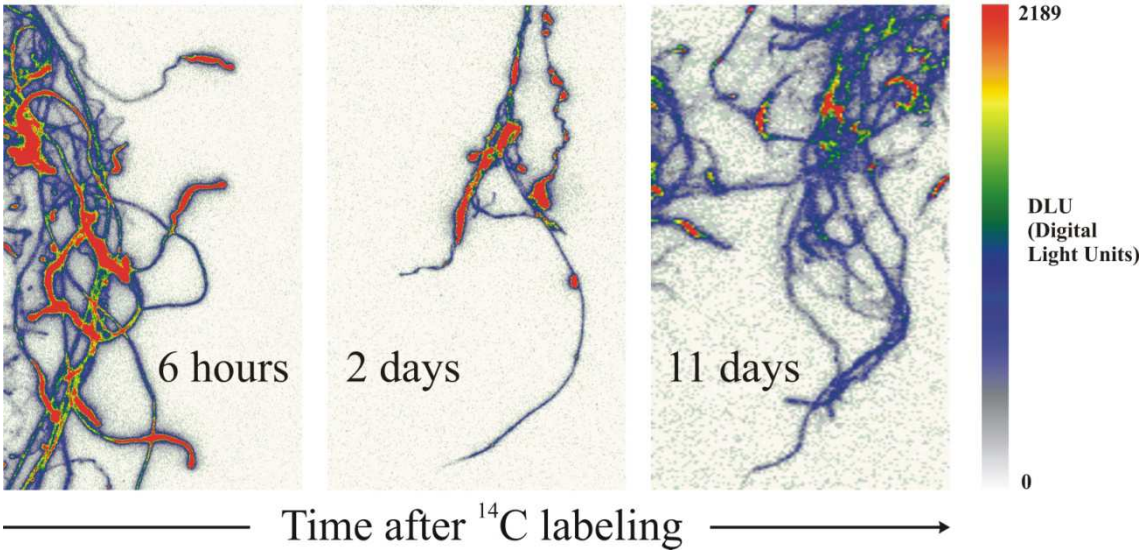

Figure I.3/1: ^{14}C images of roots 6 hours, 2 days and 11 days after labeling. Hotspots of ^{14}C activity are visible at the root tips 6 hours after labeling and the ^{14}C activity at the tips decreased with time. The red color indicates the highest activity.

Hotspots along the roots varied in time and space, existing at least for 2 days after labeling (Fig. I.3/1). 11 days after labeling the former hotspot at the tips had disappeared. With continuing root growth new spots are likely to occur at the tips but are no longer visible through ^{14}C imaging since they were supplied with unlabeled assimilates.

Hotspots within the root tips are assumed to correspond to that of exuded C in the rhizosphere. Evidence for this assumption was given by Dennis and Jones (2006) who

compared spots in the roots with that of root exudation captured by filter paper which was exposed to the imaging plate afterwards. Therefore, the fast availability of recently assimilated C at the root tips, as shown in this study, may be decisive for the rhizosphere priming effect.

3.2 Plant inter-species effects on rhizosphere priming (Study 2)

The rhizosphere priming effect was investigated by continuous ^{13}C labeling of sunflower, soybean and wheat grown as monocultures or as mixtures. A consistently positive RPE was found for all monocultures and for all mixtures, the increase above the unplanted soil ranged from 43%-136%. This is explained by the stimulation of microbial growth and activity through rhizodeposition. The higher microbial biomass carbon (MBC) for the two-species mixtures compared to the unplanted soil and to the monocultures suggests that microorganisms were additionally stimulated by the heterogeneity of rhizodeposits originating from different plant species. The MBC of the mixed cultures was, however, lower than would have been expected from the value calculated based on the sum of the MBC of the individual monocultures weighted by their root biomass.

To evaluate plant inter-species effects on rhizosphere priming, an expected RPE was calculated for the mixtures based on the RPE of the monocultures weighted by root biomass and compared to the RPE measured. If the observed value is lower than the expected value, RPE was negatively influenced by inter-species interactions

All mixed cultures showed a tendency towards a reduced RPE through plant inter-species interactions (Fig. I.3/2). However, a significant effect was only observed for the sunflower-wheat mixture. This could be explained by the competition between plant roots and microorganisms for nutrients, especially for N. The competition may be higher in mixed cultures, since plants grown together are able to utilize limited resources more completely (Tilman et al., 1996; Hopper and Vitousek, 1997). A lower mineral N content in the soil of the sunflower-wheat mixture compared to the expectations from the monocultures supports this assumption. With an enhanced competition microbial activity in the rhizosphere may decrease resulting in a reduced RPE (Dijkstra et al., 2010).

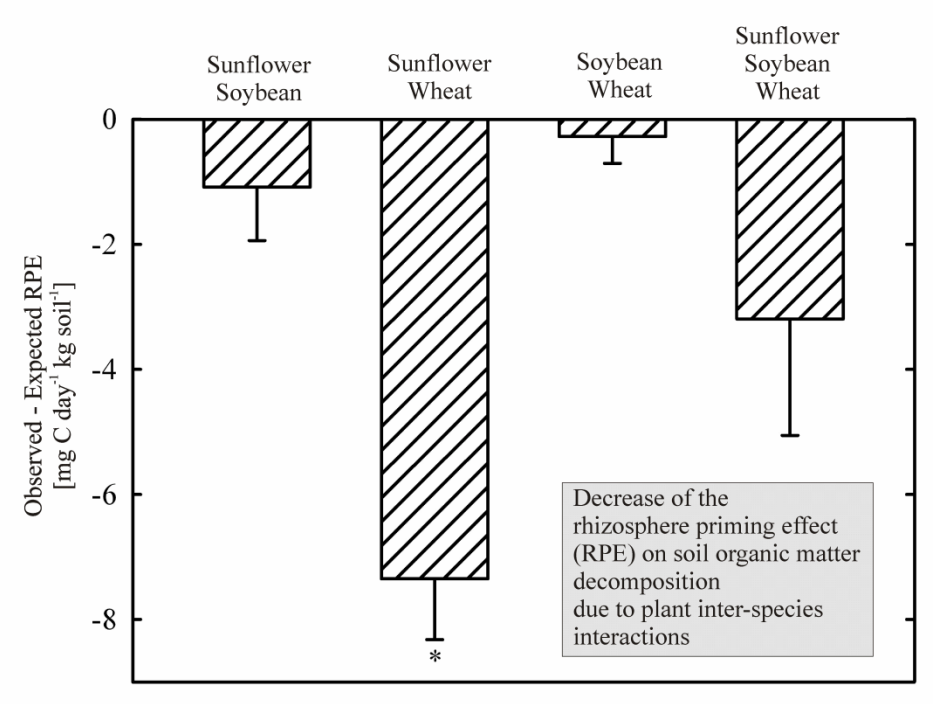

Figure I.3/2: Observed minus expected rhizosphere priming effect. * indicates significant differences from zero.

Interestingly, the RPE was not significantly affected in mixtures with soybean, a N_2-fixing plant. We suggest lower competition for mineral N in these cultures because of the relatively N-rich rhizodeposition of soybeans (Cheng et al., 2003) and because of N sparing, i.e. mineral N not consumed by the legumes (Temperton et al., 2007).

In summary, plant inter-species interactions modulate the rhizosphere priming, and our data provided clear evidence that modulation depends on the species composition.

3.3 Response to limited photosynthesis (Studies 3, 4)

Any change in the photosynthetic activity influences the C allocation in the plant-soil system (Kuzyakov and Cheng, 2001). Aiming to investigate the effect of limited photosynthesis on stored C, a legume and a non-legume were labeled with $^{14}CO_2$. After the ^{14}C distribution between plant and soil pools was mostly completed, the plants were subjected to clipping and shading and the redistribution of stored ^{14}C was analyzed (Study 3). In contrast, Study 4 focused on recently assimilated C. Plants were labeled with $^{13}CO_2$ after clipping and shading to assess the effects of limited photosynthesis on

the allocation of recently assimilated C. Furthermore, Study 4 aimed to investigate the effect of a reduced photosynthetic activity on the N distribution in plant and soil pools after ^{15}N labeling.

It was found that the cumulative CO_2 efflux from soil was higher under the legume *M. sativa* compared to *L. perenne* (Fig. I.3/3), which is explained by the high energy requirement of legumes for N_2 fixation. As reported, 6 mg C are necessary to fix 1 mg N (Vance and Heichel, 1991). The CO_2 efflux decreased after clipping and shading for both species compared to the control due to the lower assimilate supply (Fig. I.3/3). An increase to the level of the control could, however, be detected for clipped *M. sativa* at the end of the experiment, likely due to an enhancement of the nodule respiration to restore the N_2 fixation (Ta et al., 1990).

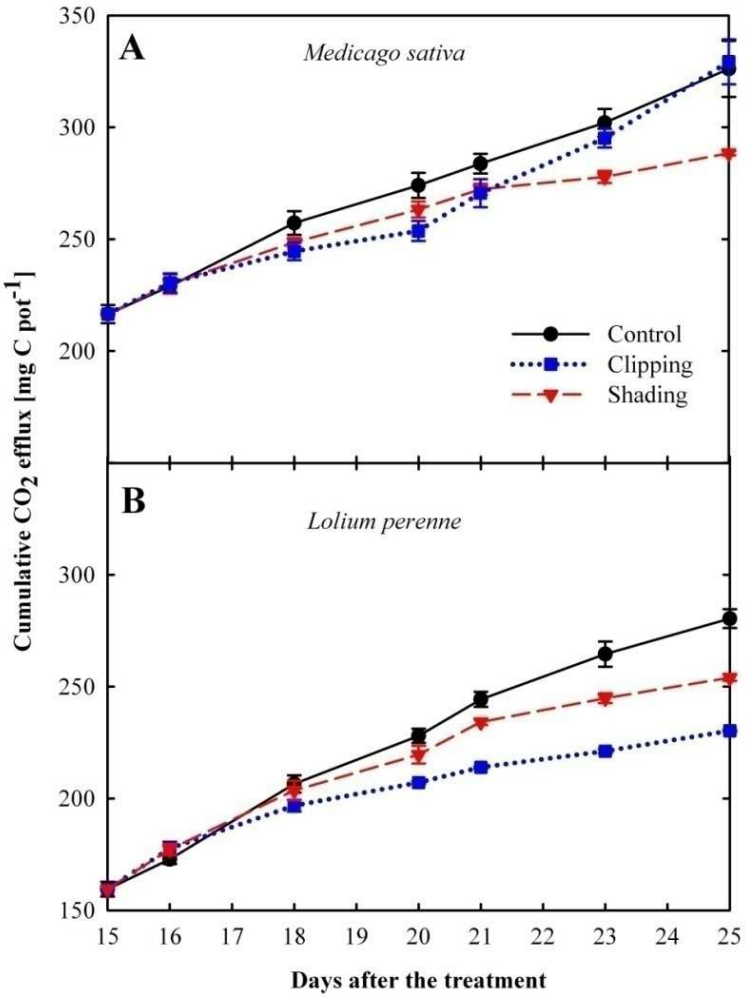

Figure I.3/3: Cumulative CO_2 efflux from soil (±SEM) under *Medicago sativa* (A) and under *Lolium perenne* (B) from the start of clipping and shading.

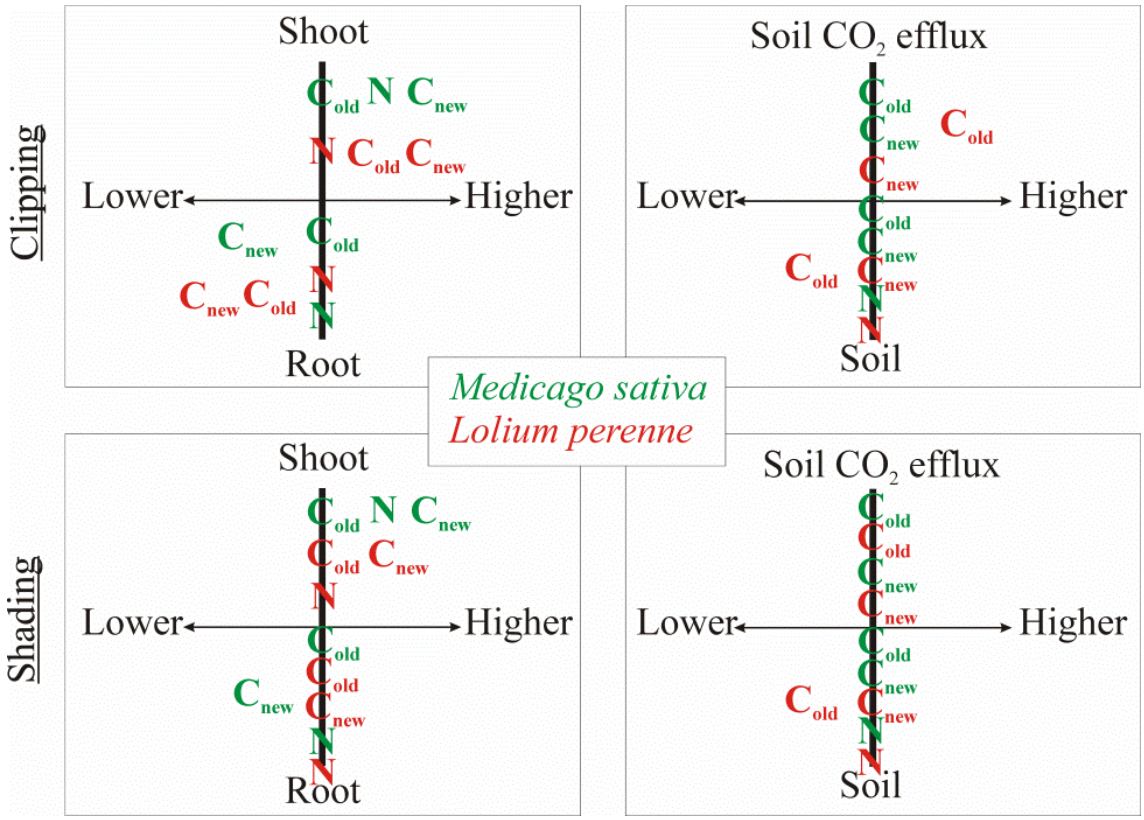

Figure I.3/4: Significant differences of the ^{14}C recovery (C_{old}) (Study 3), the ^{13}C recovery (C_{new}) and ^{15}N recovery (N) (Study 4) in plant and soil pools in comparison to the control (vertical axis). The position of the respective symbols expresses a higher recovery, a lower recovery or no changes (vertical axis) compared to the control. The recoveries under *Medicago sativa* are shown in green color; that under *Lolium perenne* are red colored.

After clipping, the distribution of stored ^{14}C, recently assimilated ^{13}C and ^{15}N was driven by the high C and N demand of the regrowing shoots. In particular, 5% and 8% of stored ^{14}C was remobilized and allocated to regrowing shoots of *L. perenne* and *M. sativa*, respectively. Moreover, clipping provoked a reduced allocation of recently assimilated ^{13}C belowground, since it was retained in the regrowing shoots (Fig. I.3/4).

For *L. perenne* the contribution of recently assimilated ^{13}C to CO_2 was not affected by clipping (Study 3), whereas stored ^{14}C contributes more to CO_2 compared to the control (Fig. I.3/4; Study 4). This demonstrates that *L. perenne* compensates a low assimilate supply by a higher utilization of stored C for maintenance respiration. The ^{13}C and ^{14}C recovery of CO_2 under *M. sativa* was not affected by clipping.

After shading, there is no need for remobilization of stored C, hence, the ^{14}C recovery of the shoots and roots did not differ significantly from that of the control (Fig. I.3/4; Study 3). The plant growth under reduced light is restricted by the low supply of recent assimilates. A reduced growth leads to a lower C and N demand of the shoots. Recently assimilated ^{13}C was preferentially retained in the shoots and is sufficient to cover the low C demand of the shaded plants (Fig. I.3/4; Study 4).

The findings of these studies (Studies 3 and 4) led to the conclusion that the distribution of stored C, recently assimilated C and N after limited photosynthesis depends on the plant species but even more on the approach of photosynthetic restriction (clipping and shading).

3.4 Quantifying rhizodeposition at field scale (Study 5)

Despite the high number of studies in the last years that have been focusing on rhizodeposition, there is still considerable uncertainty regarding the amount of organic C released by living roots especially under field conditions (Nguyen, 2003). The main reason for this uncertainty is that a significant portion of rhizodeposits is rapidly lost (within hours or even minutes) through rhizomicrobial respiration (Jones et al., 2009), and thus hardly measurable in field studies. To overcome this shortcoming, a new approach for an improved estimation of rhizodeposition in the field based on laboratory and field data was developed in Study 5.

A ^{14}C pulse labeling experiment with maize (*Zea mays* cv. Ronaldino) was conducted under controlled conditions, the $^{14}CO_2$ efflux from the soil was measured and the data entered into a simulation model (Kuzyakov et al. 1999; 2001; Kuzyakov and Domanski, 2002). The model allows to differentiate rhizomicrobial and root respiration. The partitioning of root-derived CO_2 into root and rhizomicrobial respiration is essential for research focusing on the impact of rhizodeposition on microbial mediated processes in the soil, since SOM turnover is only affected by rhizomicrobial C, while root respiration biases the picture of SOM turnover (Kuzyakov et al., 1999; Nguyen, 2003).

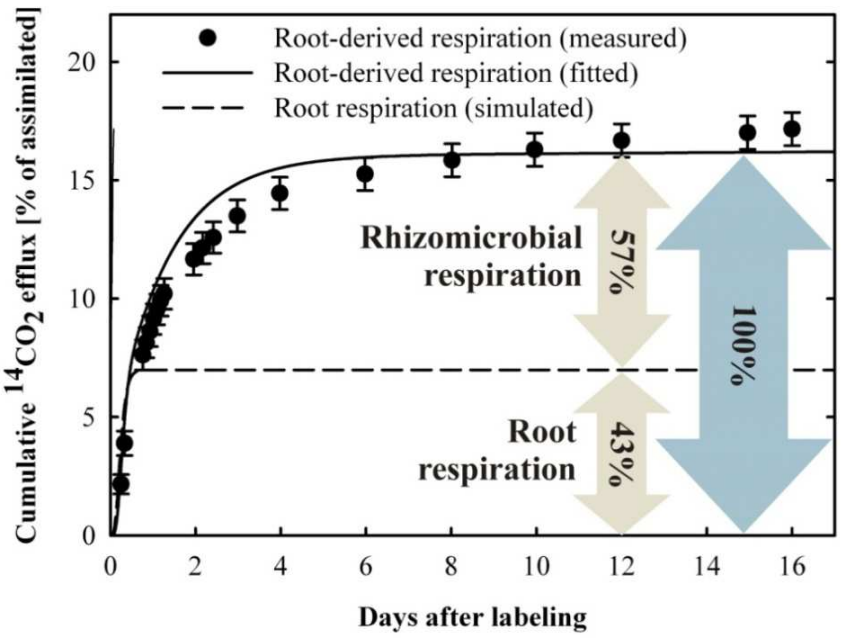

Figure I.3/5: Measured (circles; ±SEM) and fitted (solid line) cumulative $^{14}CO_2$ efflux from the soil and simulated separation of the total $^{14}CO_2$ efflux in root respiration and rhizomicrobial respiration.

It was shown that rhizomicrobial respiration and root respiration contributes 57% and 43% to root-derived CO_2, respectively (Fig. I.3/5).

The ^{14}C activity of total rhizodeposition was then calculated as the sum of the ^{14}C activity of rhizomicrobial respiration, i.e. of the rhizodeposits decomposed, and that of the rhizodeposits remaining in the soil. A rhizodeposition-to-root ratio of 0.56±0.2 was determined by relating the ^{14}C activity of total rhizodeposition to the ^{14}C activity of the roots.

Maize (*Zea mays* cv. Ronaldinio) was cultivated in a field study (Chapter 2.2) and the root biomass was sampled in July 2009. To estimate the total rhizodeposition at a field scale a new approach was used based on the rhizodeposition-to-root ratio from the laboratory experiment which was applied to the root biomass determined in the field.

Maize (25,000 plants ha^{-1}) allocated about 464 kg C ha^{-1} to belowground pools. A total amount of 298±64 kg C ha^{-1} was determined in the roots at harvest. It was found that total rhizodeposition in the field accounted for 166±53 kg C ha^{-1}(Fig. I.3/6).

In addition, the fate of rhizodeposits in the soil was investigated in the current ^{14}C labeling study. 30.6±1.5% of the rhizodeposits remained in the soil, 7.3±1.1% were incorpo-

rated into microbial biomass (MBC) and 0.3±0.1% were detected in dissolved organic carbon (DOC). The main portion of rhizodeposits (61.8±2.1%), however, was decomposed by microorganisms within 16 days after labeling.

The results underline the necessity to consider the portion of rhizodeposits which are quickly mineralized in order to reliably estimate total rhizodeposition. Compared to previous studies, reporting only on the portion of rhizodeposits which remained in the soil (net rhizodeposition), the present study provides an improved approach that offers the possibility to estimate gross rhizodeposition at field scale.

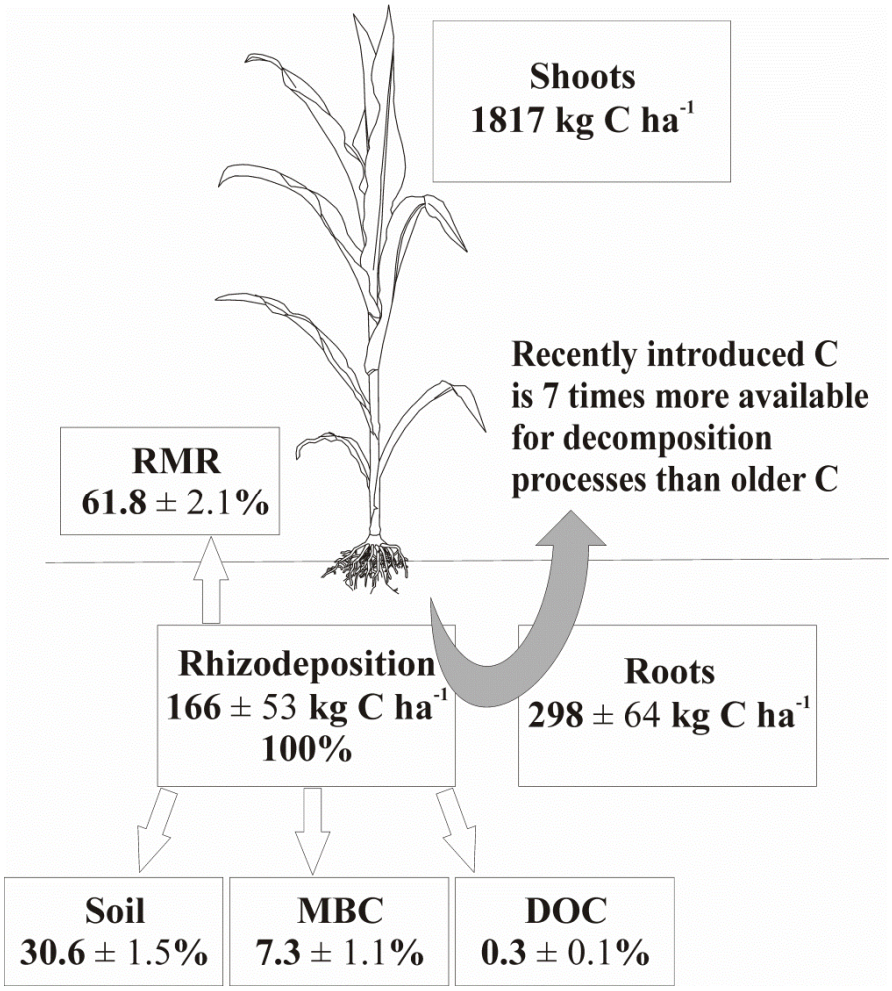

Figure I.3/6: C allocation and rhizodeposition of maize in the field. The amounts of root biomass C and C released by roots via rhizodeposition correspond to a soil depth of 50 cm, a plant population of 25,000 and a growing period from April to July 2009. The fate of rhizodeposits in the soil 16 days after $^{14}CO_2$ pulse labeling under controlled conditions is shown as percentage of total rhizodeposition. The availability of recently introduced C relative to older C pools, estimated in Study 6, is presented.

3.5 Microbial availability of younger and older C sources (Study 6)

The previous results showed a belowground allocation of 464 kg C ha^{-1} for maize (Chapter 3.4), 36% of which was released by living roots. The main part of rhizodeposits could be detected in the CO_2 efflux shortly after their input into the soil (Chapter 3.4). The turnover rate of young C pools is much higher compared to older pools, and decreased with time after the C entered the soil (Von Lützow et al., 2006).

Study 6 aimed to estimate the microbial availability of C introduced during one growing season of maize relative to older C sources. After a change from C_3 to C_4 vegetation, the isotopic composition of soil CO_2 and SOM was determined for three treatments: C_3 reference (long-term C_3 vegetation), C_4/C_4 (two years of maize cropping), and C_4/fallow (bare fallow after one year of maize cropping).

A main problem we had to deal with was the admixture of atmospheric CO_2 to soil CO_2, resulting in a biased isotopic composition. The Miller/Tans model (Miller and Tans, 2003) was successfully applied to obtain an annually integrated isotopic composition of soil CO_2, purified from atmospheric CO_2 (Fig. I.3/7).

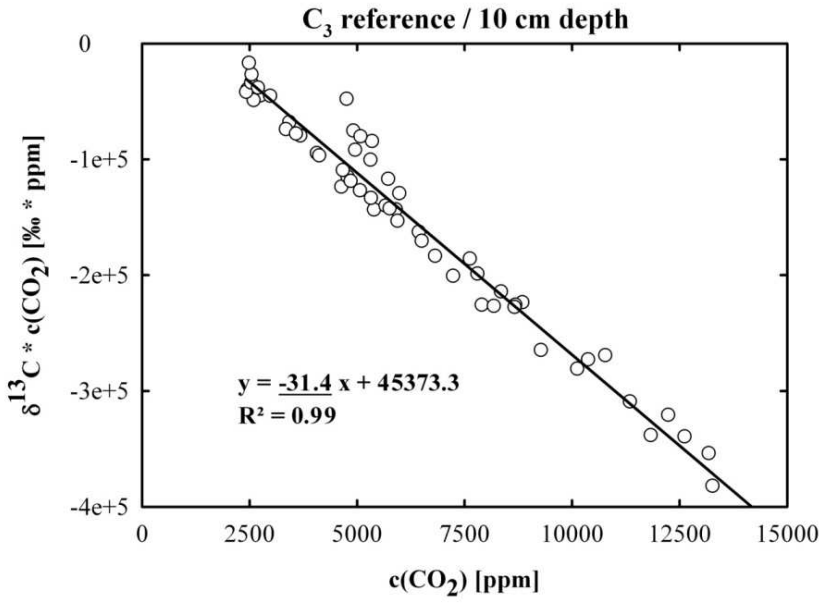

Figure I.3/7: The Miller/Tans model is based on the linear regression between the product of CO_2 concentration and its $\delta^{13}C$ value plotted against the CO_2 concentration. The isotopic composition of pure CO_2 from the soil is equivalent to the slope of a regression line. The Miller/Tans model is exemplarily shown for the C_3 reference at 10 cm soil depth.

Soil CO_2 and SOM were partitioned for younger (C_4) and older (C_3) C sources based on their isotopic composition by applying linear two source isotopic mixing models.

These models revealed that young (C_4) C contributes 27% and 49% to soil CO_2 at the 10 cm depth of the C_4/fallow and the C_4/C_4 treatment, respectively (Fig. I.3/8). The C_4/C_4 treatment showed a higher contribution of younger C compared to the C_4/fallow treatment since root-derived CO_2 contributes to total soil CO_2. Even at a depth of 60-70 cm the contribution of young C to soil CO_2 was high for both treatments with 16% for the C_4/fallow and 43% for the C_4/C_4 treatment. Due to the lower content and slower turnover of SOM at this depth compared to the upper horizons, small amounts of young C highly contributed to CO_2. Despite the high contribution of young C to soil CO_2, its contribution to SOM did not exceed 5%.

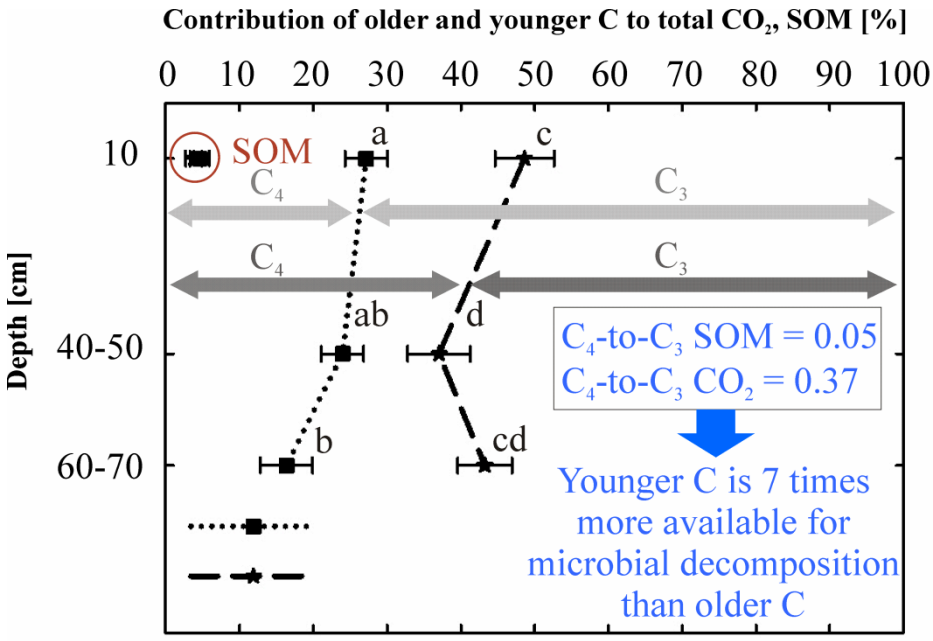

Figure I.3/8: Contribution of younger and older C sources to total CO_2 and SOM at the C_4/fallow and C_4/C_4 treatment and the C_4-to-C_3 ratios of SOM and CO_2 for the 10 cm depth of the C_4/fallow treatment. Significant differences between the depths and treatments are marked by different letters ($P<0.05$).

Estimates of the relative availability of younger and older C pools for microbial decomposition were gained by comparing the C_4-to-C_3 ratio of CO_2 (0.37) to that of SOM (0.05) at the 10 cm depth of the C_4/fallow treatment. The results of Study 6 indicate that younger C that entered the soil during one vegetation period is seven times more available for microbial decomposition relative to older C pools (Fig. I.3/8).

4 Rhizodeposition: Conclusion and outlook

The present thesis leads to the following conclusions:

(1) Hotspots of recently assimilated C quickly appear at the root tips within several hours after the assimilation. These hotspots already disappear within a few days after the assimilation.

(2) Plant inter-species competition reduces rhizosphere priming effects.

(3) Photosynthesis is a key driver for rhizodeposition. The allocation pattern of C and N within the plant-soil system strongly depends on the approach of photosynthetic restriction and on the plant species.

(4) The comparison of $\delta^{13}C$ values of CO_2 and of SOM after a C_3 to C_4 vegetation change allows for a quantitative estimation of the relative availability of younger and older C sources.

(5) The quantity of gross rhizodeposition at field scale can be estimated by applying a rhizodeposition-to-root ratio determined under controlled conditions to the root biomass sampled in the field.

These conclusions are of particular relevance for future investigations, for the following reasons:

(1) Our study of hotspots of recently assimilated C in roots demonstrates the necessity to link rhizodeposition to root morphology. Root branching, the number of root tips and the root diameter are expected to largely impact rhizodeposition. With root growth the tips are moving through the soil and new regions become hotspots of recently assimilated C. There is a need to analyze microbial activity in rhizosphere hotspots. SOM turnover in the hotspots may, for instance, even last longer than available C is present since parts of microorganisms may remain active beyond the period of exudation (Kuzyakov, 2002).

(2) Hotspots of rhizodeposition and rhizosphere priming effects will gain increasing importance in the context of climate change in the future, because it was shown that elevated CO_2 concentrations in the atmosphere stimulate root growth and enhance

root exudation (Dorodnikov et al., 2009). A higher input of C through exudation occurring in hotspots is expected to lead to accelerated SOM turnover through positive priming effects. The higher input can thus be counterbalanced by increasing CO_2 emissions from soils. This calls for further investigations on the response of rhizosphere processes to elevated CO_2.

(3) Rhizosphere priming effects will play an important role in the context of sustainable agriculture and organic farming. The shift towards systems with a low external input of fertilizers increases the dependence of plants on nutrient release from SOM due to RPE.

(4) Substrate availability is a major limiting factor for microbial activity and thus for the CO_2 efflux from soils. Through the tight coupling of assimilation and exudation, photosynthesis is of key importance for microbially mediated processes in the rhizosphere. As we could show, restriction of photosynthesis by clipping does not only evoke a remobilization of stored C in the plant, but also a retention of recently assimilated C in the shoot. Clipping experiments, used to simulate grazing, are valuable for predictions of C fluxes in the soil of agricultural pasture ecosystems and for the assessment of management practices.

(5) There is a critical need for new methods allowing to estimate rhizodeposition under field conditions. Most of the previous methods greatly underestimated rhizodeposition since they do not account for the fraction of rhizodeposits which is fast decomposed by microorganisms. The new approach provided in this thesis is based on the rhizodeposition-to-root ratio determined under controlled conditions. If the ratio is known for particular plants, this approach offers a promising method to estimate rhizodeposition on ecosystem scale as a huge data base of root biomass distributions already exists in the literature.

5 Contributions to the included manuscripts

This Ph.D. thesis comprises five published and one submitted manuscripts which were elaborated in cooperation with various co-authors. The co-authors listed on the manuscripts contributed as follows:

Study 1: Photoassimilate allocation and dynamics of hotspots in roots visualized by ^{14}C phosphor imaging

Status: Published in Journal of Plant Nutrition and Soil Science, 2011, Vol. 174, Page 12-19

J. Pausch: 70% (experimental design, accomplishment of experiment, laboratory analyses, data preparation, manuscript preparation)

Y. Kuzyakov: 30% (discussion of experimental design and results, comments to improve the manuscript)

Study 2: Plant inter-species effects on rhizosphere priming of soil organic matter decomposition

Status: Published in Soil Biology and Biochemistry, 2013, Vol. 57, Page 91-99

J. Pausch: 60% (experimental design, accomplishment of experiment, laboratory analyses, data preparation, manuscript preparation)

B. Zhu: 20% (discussion of experimental design and results, comments to improve the manuscript)

Y. Kuzyakov: 10% (discussion of experimental design and results, comments to improve the manuscript)

W. Cheng: 10% (discussion of experimental design and results, comments to improve the manuscript)

Study 3: Effect of clipping and shading on C allocation and fluxes in soil under ryegrass and alfalfa estimated by ^{14}C labeling

Status: Submitted to Applied Soil Ecology; date: November 11, 2011, resubmitted: February 19, 2012

A. Schmitt: 60% (experimental design, accomplishment of experiment, laboratory analyses, data preparation, manuscript preparation)

J. Pausch: 25% (discussion of experimental design, analyses and results, comments to improve the manuscript)

Y. Kuzyakov: 15% (discussion of experimental design and results, comments to improve the manuscript)

Study 4: C and N allocation in soil under ryegrass and alfalfa estimated by ^{13}C and ^{15}N labeling

Status: Published in Plant and Soil, 2012, DOI: 10.1007/s11104-012-1536-5

A. Schmitt: 60% (experimental design, accomplishment of experiment, laboratory analyses, data preparation, manuscript preparation)

J. Pausch: 25% (discussion of experimental design, analyses and results, comments to improve the manuscript)

Y. Kuzyakov: 15% (discussion of experimental design and results, comments to improve the manuscript)

Study 5: Estimation of rhizodeposition at field scale: upscaling of a ^{14}C labeling study

Status: Published in Plant and Soil, 2012, DOI: 10.1007/s11104-012-1363-8

J. Pausch: 70% (experimental design, accomplishment of experiment, laboratory analyses, data preparation, manuscript preparation)

J. Tian: 10% (laboratory analyses, comments to improve the manuscript)

M. Riederer: 10% (laboratory and field work, comments to improve the manuscript)

Y. Kuzyakov: 10% (discussion of experimental design and results, comments to improve the manuscript)

Study 6: Soil organic carbon decomposition from recently added and older sources estimated by $\delta^{13}C$ values of CO_2 and organic matter

Status: Published in Soil Biology and Biochemistry, 2012, Vol. 55, Page 40-44

J. Pausch: 80% (experimental design, accomplishment of experiment, laboratory analyses, data preparation, manuscript preparation)

Y. Kuzyakov: 20% (discussion of experimental design and results, comments to improve the manuscript)

References

Amundson, R., 2001. The carbon budget in soils. Annual Review of Earth and Planetary Sciences 29, 535-562.

Balesdent, J., Mariotti, A., 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. Soil Biology and Biochemistry 19 (1), 25-30.

Buyanovsky, G.A., Wagner, G.H., 1998. Changing role of cultivated land in the global carbon cycle. Biology and Fertility of Soils 27, 242-245.

Bottner, P., Pansu, M., Sallth, Z., 1999. Modelling the effect of active roots on soil organic matter turnover. Plant and Soil 216, 15-25.

Cheng, W., Coleman, D.C., Carroll, C.R., Hoffman, C.A, 1993. In situ measurement of root respiration and soluble carbon concentrations in the rhizosphere. Soil Biology and Biochemistry 25, 1189-1196.

Cheng, W., Dijkstra, F.A., 2007. Theoretical proof and empirical confirmation of a continuous labeling method using naturally ^{13}C-depleted carbon dioxide. Journal of Integrative Plant Biology 49 (3), 401-407.

Cheng, W., Johnson, D.W., Fu, S., 2003. Rhizosphere effects on decomposition: Controls of plant species, phenology, and fertilization. Soil Science Society of America Journal 67, 1418-1427.

Cheng, W., Kuzyakov, Y., 2005. Root effects on soil organic matter decomposition, in: Zobel, R.W., Wright, S.F. (Eds.), Roots and Soil Management: Interactions between Roots and the Soil. ASA-SSSA, Madison, Wisconsin, pp. 119-143.

Craine, J.M., Wedin, D.A., Chapin III., F.S., 1999. Predominance of ecophysiological controls on soil CO_2 flux in a Minnesota grassland. Plant and Soil 207, 77-86.

Darrah, P.R., 1993. The rhizosphere and plant nutrition: a quantitative approach. Plant and Soil 155/156, 1-20.

Dennis, P.G., Jones, D.L., 2006. ^{14}C pulse labelling and allocation imaging, in: Luster, J., Finlay, R. (Eds.) Handbook of Methods Used in Rhizosphere Research, Swiss Federal Research Institure WSL, Birmensdorf, Switzerland.

Dennis, P.G., Miller, A.J., Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbial Ecology 72, 313-327.

Dijkstra, F.A., Morgan, J.A., Blumenthal, D., Follett, R.F., 2010. Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. Soil Biology and Biochemistry 42, 1073-1082.

Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Marhan, S., Fangmeier, A., Kuzyakov, Y., 2009. Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on soil aggregate size. Global Change Biology 15, 1603-1614.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40, 503-537.

Fu, S., Cheng, W., 2002. Rhizosphere priming effects on the decomposition of soil organic matter in C_4 and C_3 grassland soils. Plant and Soil 238, 289-294.

Gavrichkova, O., 2009. Drivers of soil respiration of root and microbial origin in grasslands. Dissertation.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30 (3), 369-378.

Gregory, P.J., Atwell, B.J., 1991. The fate of carbon in pulse labelled crops of barley and wheat. Plant Soil 136, 205-213.

Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48, 115-146.

Harris, D., Porter, L.K., Paul, E.A., 1997. Continuous flow isotope ratio mass spectrometry of carbon dioxide trapped as strontium carbonate. Communications in Soil Science and Plant Analysis 28, 747-757.

Hiltner, L., 1904. Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. Arbeiten der Deutschen Landwirtschaftlichen Gesellschaft 98, 59-78.

Hobbie, E.A., Werner, R.A., 2004. Intramolecular, compound-specific, and bulk carbon isotope patterns in C_3 and C_4 plants: a review and synthesis. New Phytologist 161, 371-385.

Hooper, D.U., Vitousek, P.M., 1997. The effects of plant composition and diversity on ecosystem processes. Science 277, 1302-1305.

IUSS Working Group WRB, 2007. World Reference Base for Soil Resources, 2006, first update 2007. World Soil Resource Reports No. 103. FAO, Rome.

Jastrow, J.D., Amonette, J.E., Bailey, V.L., 2007. Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Climatic Change 80, 5-23.

Jones, D.L., Hodge, A., Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163, 459-480.

Jones, D.L., Nguyen, C., Finlay, R.D., 2009. Carbon floe in the rhizosphere: carbon trading at the soil-root interface. Plant and Soil 321, 5-33.

Kammann, C., Grünhage, L., Jäger, H.-J., 2001. A new sampling technique to monitor concentrations of CH_4, N_2O and CO_2 in air at well-defined depths in soils with varied water potential. European Journal of Soil Science 52, 297-303.

Knorr, K.-H., Oosterwoud M.R., Blodau C., 2008. Experimental drought alters rates of soil respiration and methanogenesis but not carbon exchange in soil of a temperate fen. Soil Biology and Biochemistry 40, 1781-1791.

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Schoene, J., Scheunemann, N., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E., 2012. Carbon flow into microbial and fungal biomass as a basis to understand the belowground food web in an agroecosystem. Pedobiologia 55, 111-119.

Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 165, 382-396.

Kuzyakov, Y., 2006. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biology and Biochemistry 38, 425-448.

Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology and Biochemistry 33, 1915-1925.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421-431.

Kuzyakov, Y., Domanski, G., 2002. Model for rhizodeposition and CO_2 efflux from planted soil and its validation by ^{14}C pulse labelling of ryegrass. Plant and Soil 239, 87-102.

Kuzyakov, Y., Ehrensberger, H., Stahr, K., 2001. Carbon partitioning and below-ground translocation by *Lolium perenne*. Soil Biology and Biochemistry 33, 61-74.

Kuzyakov, Y., Gavrichkova, O., 2010. Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Global Change Biology 16, 3386-3406.

Kuzyakov, Y, Kretzschmar, A, Stahr, K, 1999. Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. Plant and Soil 213, 127-136.

Lal, R., 2008. Sequestration of atmospheric CO_2 in global carbon pools. Energy and Environmental Science 1, 86-100.

Lynch, J.M., Whipps, J.M., 1990. Substrate flow in the rhizosphere. Plant and Soil 129: 1-10.

Lynch, J.M., Brimecombe, M.J., de Leij, F.A.A.M., 2002. Rhizosphere, in: John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0000403.pub2.

Marschner, H, 1995. Mineral nutrition of higher plants. 2nd edn. Academic Press, London.

McDougall, B.M., Rovira, A.D., 1970. Sites of exudation of ^{14}C-labelled compounds from wheat roots. New Phytologist 69, 999-1003.

Merbach, W., Mirus, E., Knof, G., Remus, R., Ruppel, S., Russow, R., Gransee, A., Schulze, J., 1999. Release of carbon and nitrogen compounds by plant roots and their possible ecological importance. Journal of Plant Nutrition and Soil Science 162, 373-383.

Merharg, A.A., 1994. A critical review of labelling techniques used to quantify rhizosphere carbon-flow. Plant and Soil 166, 55-62.

Miller, J.B., Tans, P.P., 2003. Calculating isotopic fractionation from atmospheric measurements at various scales. Tellus 55B, 207-214.

Neumann, G., Römheld, V., 2007. The release of root exudates as affected by the plant physiological status, in: Pinton, R., Varanini, Z., Nannipieri, P. (Eds.), The Rhizosphere. Biochemistry and organic substances at the soil-plant interface. CRC press, Boca Raton, pp 23-72.

Nguyen, C., 2003. Rhizodeposition of organic C by plant: mechanisms and controls. Agronomie 23, 375-396.

Paterson, E., 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. European Journal of Soil Science 54, 741-750.

Paterson, E., Rattray, E.A.S., Killham, K., 1996. Effect of elevated atmospheric CO_2 concentration on C-partitioning and rhizosphere C-flow for three plant species. Soil Biology and Biochemistry 28 (2), 195-201.

Paterson, E., Midwood, A.J., Millard, P., 2009. Through the eye of the needle: a review of isotope approaches to quantify microbial processes mediating soil carbon balance. New Phytologist 184, 19-33.

Paterson, E., Thornton, B., Midwood, A.J., Sim, A., 2005. Defoliation alters the relative contributions of recent and non-recent assimilate to root exudation from *Festuca rubra*. Plant, Cell and Environment 28, 1525-1533.

Paynel, F., Murray, P.J., Cliquet, J.B., 2001. Root exudates: a pathway for short-term N transfer from clover and ryegrass. Plant and Soil 229, 235-243.

Rattray, E.A.S., Paterson, E., Killham, K., 1995. Characterisation of the dynamics of C-partitioning within *Lolium perenne* and to the rhizosphere microbial biomass using ^{14}C pulse chase. Biology and Fertility of Soils 19, 280-286.

Rovira, A.D., 1959. Root excretions in relation to the rhizosphere effect. IV. Influence of plant species, age of plant, light, temperature, and calcium nutrition on exudation. Plant and Soil 11 (1), 53-64.

Schimel, J.P., Jackson, L.E., Firestone, M.K., 1989. Spatial and temporal effects on plant-microbial competition for inorganic nitrogen in a California annual grassland. Soil Biology and Biochemistry 21 (8), 1059-1066.

Schlesinger, W., 1997. The global carbon cycle, in: Schlesinger, W., (Ed.), Biogeochemistry: An analysis of global change. Academic press, San Diego, pp. 358-382.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7-20.

Stephan, A., Meyer, A.H., Schmid, B., 2000. Plant diversity affects culturable soil bacteria in experimental grassland communities. Journal of Ecology 88, 988-998.

Ta, T.C., MacDowell, F.D.H., Faris, M.A. 1990. Utilization of carbon and nitrogen reserves of alfalfa roots in supporting N_2-fixation and shoot regrowth. Plant and Soil 127, 231-236.

Temperton, V.M., Mwangi, P.N., Scherer-Lorenzen, M., Schmid, B., Buchmann, N., 2007. Positive interactions between nitrogen-fixing legumes and four different neighboring species in a biodiversity experiment. Oecologia 151, 190-205.

Tilman, D., Wedin, D., Knops, J., 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379, 718-720.

Uren, N.C., 2007. Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants, in: Pinton, R., Varanini, Z., Nannipieri, P. (Eds.), The Rhizosphere. Biochemistry and organic substances at the soil-plant interface. CRC press, Boca Raton, pp. 1-21.

Uselman, S.M., Qualls, R.G., Thomas, R.B., 1999. A test of a potential short cut in the nitrogen cycle: The role of exudation of symbiotically fixed nitrogen from roots of a N-fixing tree and the effects of increased atmospheric CO_2 and temperature. Plant and Soil 210, 21-32.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19 (9) 703-707.

Vance, C.P., Heichel, G.H., 1991. Carbon in N_2 fixation: Limitation or exquisite adaption Ann Rev. Plant Physiology 42, 373-392.

Vancura, V., 1964. Root exudates of plants. I. Analysis of root exudates of barley and wheat in their initial phases of growth. Plant and Soil 21, 231-248.

Van der Krift, T., Kuikman, P.J., Möller, F., Berendse, F., 2001. Plant species and nutritional-mediated control over rhizodeposition and root decomposition. Plant and Soil 228, 191-200.

Van Veen, J.A., Merckx, R., Van De Geijn, S.C., 1989. Plant- and soil related controls of the flow of carbon from root through the soil microbial biomass. Plant and Soil 115, 179-188.

Von Lützow, M., Kogel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. European Journal of Soil Science 57, 426-445.

Wang, J.G., Bakken, L.R., 1997. Competition for nitrogen during decomposition of plant residues in soil: Effect of spatial placement of N-rich and N-poor plant residues. Soil Biology and Biochemistry 29, 153-162.

Warembourg, F.R., Roumet, C., Lafont, F., 2003. Differences in rhizosphere carbon-partitioning among plant species of different families. Plant and Soil 256, 347-357.

Zhu, B., Cheng, W., 2011. ^{13}C isotope fractionation during rhizosphere respiration of C_3 and C_4 plants. Plant and Soil 342, 277–287.

II Manuscripts

1 Photoassimilate allocation and dynamics of hotspots in roots visualized by ^{14}C phosphor imaging

Johanna Pausch[1], Yakov Kuzyakov[1*]

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Abstract

Understanding photoassimilate allocation into the roots and the release of organic substances from the roots into the rhizosphere is an important prerequisite for characterizing the belowground C input, the spatial and temporal distribution of carbon, and the interactions between plants and soil microorganisms. Based on ^{14}C phosphor imaging, we visualized the allocation of assimilates into *Lolium perenne* roots and estimated the life time of hotspots at the root tips.

Lolium shoots were labeled in a $^{14}CO_2$ atmosphere, and herbariums of roots and shoots were prepared 6 h, 2 d and 11 d after the ^{14}C pulse. The ^{14}C distribution in roots and leaves revealed that pulse labeling does not yield homogeneously labeled plant material. The spatial distribution of assimilate allocation was evaluated based on the ^{14}C specific activity expressed as digital light units (DLU mm^{-2}) of the imaging plates. Areas with high relative ^{14}C specific activity were classified as hotspots. Strong ^{14}C hotspots were detected mainly at the root tips already 6 h after the ^{14}C assimilation and they remained active for at least 2 d. Eleven days after the ^{14}C assimilation the hotspots at the root tips disappeared and the ^{14}C distribution was much more even than after 6 h or after 2 d.

^{14}C phosphor imaging proved to be a promising tool to visualize the allocation of photoassimilates into the roots and the rhizosphere and can be used to identify hotspots and their dynamics.

Keywords: Rhizodeposition, ^{14}C labeling, hotspots, autoradiography, *Lolium perenne*, rhizosphere.

1.1 Introduction

Although the volume of the rhizosphere is typically < 1% of the total soil volume, the importance of the rhizosphere within the global C cycle is enormous (Hinsinger et al., 2005). The rhizosphere is a complex habitat in which conditions differ from those in the bulk soil. Root-derived C forms a localized source of easily available energy for microbial activity. This leads to a unique biological niche within the soil environment characterized by a high abundance, activity, and diversity of microorganisms. Understanding the allocation of photoassimilates into the roots and the rhizosphere is crucial to provide insight into the complex interactions between soil, microorganisms, and plants. However, investigating root-derived C including rhizodeposition is complicated because roots release organic compounds similar to those already present in the soil and because rhizosphere microorganisms rapidly decompose rhizodeposits. To overcome these problems, most methods for analyzing the distribution of C released by roots in various soil pools are based on applying the C isotopes ^{14}C and ^{13}C and on quantifying the total ^{14}C radioactivity or ^{13}C content in the soil surrounding the roots. This enables distinguishing the root-derived C from the native soil organic compounds (Whipps, 1990; Buyanovsky et al., 1994). A very few studies also applied the short lived ^{11}C to show the translocation of photoassimilates (Farrar et al., 1994; Keutgen et al., 1995). For isotope applications, continuous labeling (e.g. Johnen and Sauerbeck, 1977; Whipps, 1987; Meharg, 1994) or pulse labeling (e.g. Warembourg and Billes, 1979; Meharg and Killham, 1990; Cheng et al., 1993; Swinnen et al., 1994; Nguyen et al., 1999; Kuzyakov et al., 1999; 2001) was used. The advantages and disadvantages of these labeling approaches were reviewed in several publications (Whipps, 1990; Kuzyakov and Domanski, 2000; Nguyen, 2003; Kuzyakov and Schneckenberger, 2004; Werth and Kuzyakov, 2008).

Based on these labeling techniques, total rhizodeposition was estimated for various plants, although most studies focused on agricultural cereals. Therefore, much less is known about pasture plants. The absolute C input is similar for pasture plants and for agricultural cereals (1,500 kg C ha^{-1} y^{-1}) when the same growth period is considered (Jensen, 1993; Swinnen et al., 1995b). Nevertheless, the relative C translocation into soil is higher for pasture plants than for agricultural cereals. In order to determine the allocation of assimilates, ^{14}C or ^{13}C pulse labeling of *Lolium perenne* was used (Meharg,

1994; Swinnen et al., 1994; Tinker et al., 1994; Meharg, 1994). Studies based on the application of C isotopes can help evaluate: 1) the total belowground budget of assimilated C, 2) the dynamics of assimilated C in various belowground pools, and 3) the localization of C allocation. Most of the previous studies focused on the first two items – budget and dynamics – while the present study concentrates on the allocation of ^{14}C assimilates and their dynamics within the root system.

^{14}C can be detected using autoradiography. In recent years, the traditional autoradiographic technique using X-ray films has been replaced by the so-called phosphor imaging approach, which is based on photoinduced chemiluminescence. Compared to traditional autoradiography, phosphor imaging is about two orders more sensitive to β- and γ-rays, it has a wider radioactivity range for imaging, a wider linear dynamic range between the intensity of the image and the activity of the isotope, it avoids handling chemicals necessary for film development, and it reduces the exposure time (Hamaoka, 1990).

The present study was designed to prove the suitability of phosphor imaging for visualizing the allocation of ^{14}C labeled assimilates. As a first step towards more detailed information about the complex interactions between plants and soil, we investigated the allocation of C assimilates in roots of ryegrass (*Lolium perenne* L., ssp. Gremie) and identified the ^{14}C hotspots in these roots.

1.2 Materials and methods

We studied the distribution pattern of the radiotracer ^{14}C in ryegrass at three different time intervals: 6 h, 2 d and 11 d after start of assimilation of $^{14}CO_2$ via the shoots of the plants. The ^{14}C distribution pattern was visualized using phosphor imaging of leaves and roots. The main focus of this study was on the ^{14}C distribution in roots.

1.2.1 Soil properties and plant growth conditions

The experiments were conducted with *Lolium perenne* grown on a fine loamy gleyic Cambisol. The soil samples were taken from the Ah horizon (top 10 cm) of a long-term pasture in Allgäu (S Germany). Basic characteristics of the soil are shown in Table II.1/1.

Table II.1/1: Basic characteristics of the soil sampled from the Ah horizon of a fine loamy gleyic Cambisol from a pasture in the Allgäu (S Germany) (FC, field capacity (pF=1.8); AWC, available water capacity (pF 1.8-4.2)) (Kleber, 1997).

Parameter	Value
pH ($CaCl_2$)	5.2
Corg %	4.7
Nt %	0.46
C/N	10.0
Clay (<2 μm) %	28.4
Silt (2-<63 μm) %	47.1
Sand (63-2000 μm) %	24.5
FC %	50.0
AWC %	23.0
$CaCO_3$ %	0.0

The wet soil samples were air dried, homogenized, and passed through a 2 mm sieve to separate large roots and stones. An amount of 1.6 kg of dried soil with a final density of 1.2 g cm^{-3} was filled into each pot (height 10 cm, inner diameter 14 cm). One pre-vernalized seedling of ryegrass was grown per pot. The plants were grown at temperatures of 26°C-28°C (day) and at 22°C-23°C (night) with a day length of 14 h and light

intensity of ≈ 800 μmol m^{-2} s^{-1}. The soil water content of each pot was measured gravimetrically and was adjusted daily to ≈ 60% of the available field capacity.

1.2.2 Labeling of plants in a $^{14}CO_2$ atmosphere

For ^{14}C labeling a perspex chamber previously described by Kuzyakov et al. (1999) was used. The airtight chamber consisted of two compartments. The lower compartment (height 200 mm, inner diameter 138 mm) contained the soil, and the upper compartment (height 300 mm, inner diameter 138 mm) was used for the tracer application to the leaves. Both compartments were separated from each other by a perspex lid with drilled holes (inner diameter 8 mm) for the plants. The day before labeling, the holes were sealed at the base using silicone paste (NG 3170, Thauer & Co., Dresden) (Gregory et al., 1991; Swinnen et al., 1995 a). The seals were tested for air leaks. All plants were labeled simultaneously. 381 kBq of ^{14}C as $Na_2{}^{14}CO_3$ solution were put in a 2 cm^3 Eppendorf micro test tube placed in the upper compartment of the chamber. Then the chamber was closed and 1 ml of 5 M H_2SO_4 was added to the solution through a pipe. Assimilation took place within 2 h after the $^{14}CO_2$ pulsing, but most of the $^{14}CO_2$ was assimilated within the first 30 min. After labeling, the CO_2 from the upper compartment was trapped to remove the remaining non-assimilated $^{14}CO_2$. Finally, the top of the chamber was removed and the plants were grown under normal conditions.

After opening the labeling chamber, the CO_2 evolving from the lower compartment was trapped in a 20 mL solution of 0.5 M NaOH by continuous pumping (100 cm^3 min^{-1}) with a membrane pump. This removes the $^{14}CO_2$ respired by roots and microorganisms and avoids possible re-uptake of ^{14}C from the soil solution by roots.

The plants were harvested at three different times after start of labeling: 6 h, 2 d and 11 d. This was done by cutting the plants at the base and opening the bottom compartment of the chamber. Finally, the soil was pulled out. Roots were carefully separated from the soil by handpicking. All picked roots were gently washed in 400 mL of deionized water to remove the soil adhering to the roots. The leaf material and the roots were distributed on a white paper, prepared as a herbarium and dried at 60°C.

1.2.3 Tracer detection by phosphor imaging

The distribution pattern of the ^{14}C within leaves and roots was determined by Cyclone-Plus Storage Phosphor System (Perkin Elmer, Germany). Each herbarium (6 h, 2 d, 11 d) with roots or shoots was exposed to a sensitive imaging plate in the dark for 1 or 3 weeks. The plate was then scanned by CyclonePlus (Perkin Elmer, Germany) and digitalized by OptiQuant software (Perkin Elmer, Germany). We used two approaches to demonstrate the distribution pattern of assimilated ^{14}C within the roots: 1) evaluation of the evenness of the ^{14}C distribution within the roots and identification of ^{14}C hotspots and 2) visualization of the longitudinal allocation in individual roots.

1.2.3.1 Evenness of the ^{14}C distribution within the roots and identification of ^{14}C hotspots

To verify the visual findings of the image-plate pictures, the evenness of the ^{14}C distribution within the roots was calculated and hotspots were identified by applying a grid to each image (241 columns, 122 rows, square width 1 mm, square length 1 mm, center to center spacing: columns 1 mm, rows 1 mm). The ^{14}C activities of the single squares were added up and set as the total activity of the grid. The activity per square expressed as digital light units (DLU) per mm² was then put in reference to the total activity. The resulting relative activities were categorized into 39 size ranges with the statistical package Statistica7 for Windows. The smallest range with a relative activity of ≤ 0.006 (> 82% of the image area) was set up as background and excluded from the evaluation.

1.2.3.2 Longitudinal ^{14}C allocation in individual roots

Within each image, 10 roots were selected and squares (1 mm x 1 mm) were applied to the individual roots in longitudinal direction up to 19 mm. The squares along each root were numbered starting at the root tip. Subsequently, the mean out of all squares of #1, #2, etc. per image was calculated and the data were normalized with reference to the square with the maximum DLU value out of all images. In this approach we used another reference to normalize the data because, despite the same root length, the area around the roots differs and thus cannot be normalized as described under section 1.2.3.1.

1.3 Results

1.3.1 Distribution of ^{14}C in the shoots

The evenness of the ^{14}C distribution within the shoots (and roots) was estimated based on the number of DLU per area of the phosphor image, with the red color indicating the highest ^{14}C activity (Fig. II.1/1). Hotspots of ^{14}C activity within the image of the shoot 6 h after labeling (Fig. II.1/1A) are located at the growing parts at the leaf base and in the highly photosynthetically active tissue at the tips. Two days and eleven days after labeling, the redistribution of ^{14}C within the shoots was weak (images not presented) and changes in the distribution were minimal. We therefore did not further evaluate the ^{14}C distribution in shoots. Note that 6 h after labeling, the total ^{14}C activity in the shoots was more than 10 times higher than that in the roots (compare DLU scale for shoots and roots).

1.3.2 Evenness of the ^{14}C distribution within the roots and identification of ^{14}C hotspots

The root image-plate pictures of ryegrass harvested 6 h, 2 d and 11 d after labeling (Fig. II.1/1B, C, D) showed a shift of the ^{14}C spots with increasing time after the ^{14}C pulse. Six hours after labeling (Fig. II.1/1B), substantial amounts of ^{14}C were allocated to the youngest parts, i.e., to the root tips. In contrast, 11 d after labeling (Fig. II.1/1D) tracer accumulations were also found in the adventitious roots (black arrows in Fig. II.1/1D).

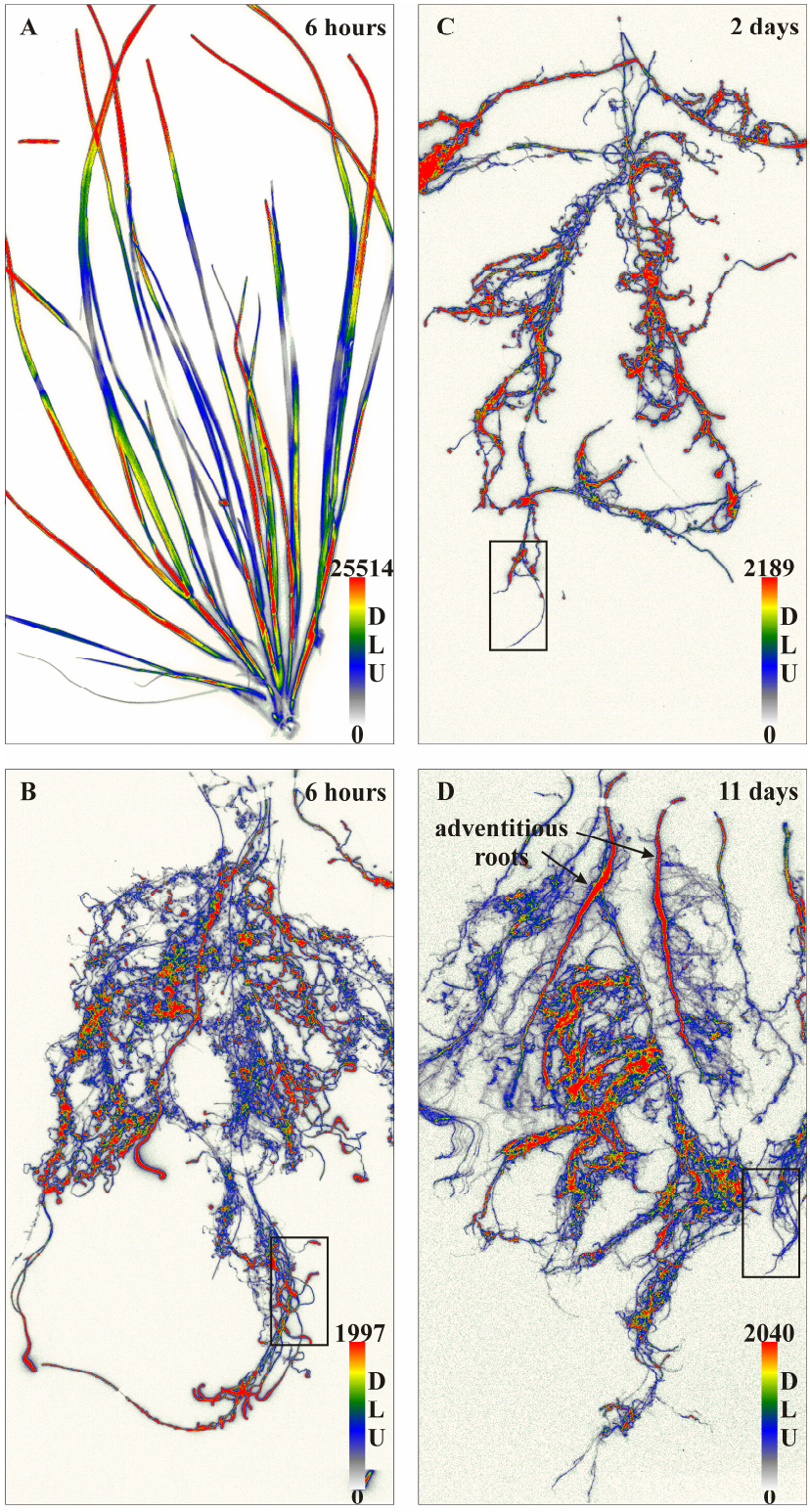

Figure II.1/1: Phosphor images of a shoot 6 h (A) and of roots 6 h (B), 2 d (C) and 11 d (D) after labeling in $^{14}CO_2$ atmosphere. ^{14}C activity expressed as digital light units (DLU). The insets for Fig. II.1/2 are marked by a black rectangle.

To verify the visual findings of Figure II.1/1, the evenness of the ^{14}C distribution in the roots was statistically evaluated (Fig. II.1/2). If ^{14}C was evenly distributed, Figure II.1/2 would show a constant line parallel to the x-axis. However, the ^{14}C distribution obtained consisted of many points with low or very low relative ^{14}C activity and few points with high activity. Up to the threshold of 0.126, the frequency strongly decreased with increasing relative ^{14}C activity (Fig. II.1/2). We defined hotspots as regions with low frequency and high relative ^{14}C activity (> 0.126). The threshold can clearly be identified through the change of the slope of the relative frequency line. Although the number of ^{14}C hotspots was generally low, their frequency after 11 d was higher than after 6 h or after 2 d. Nevertheless, the number of hotspots at the root tips 11 d after ^{14}C photoassimilation clearly decreased.

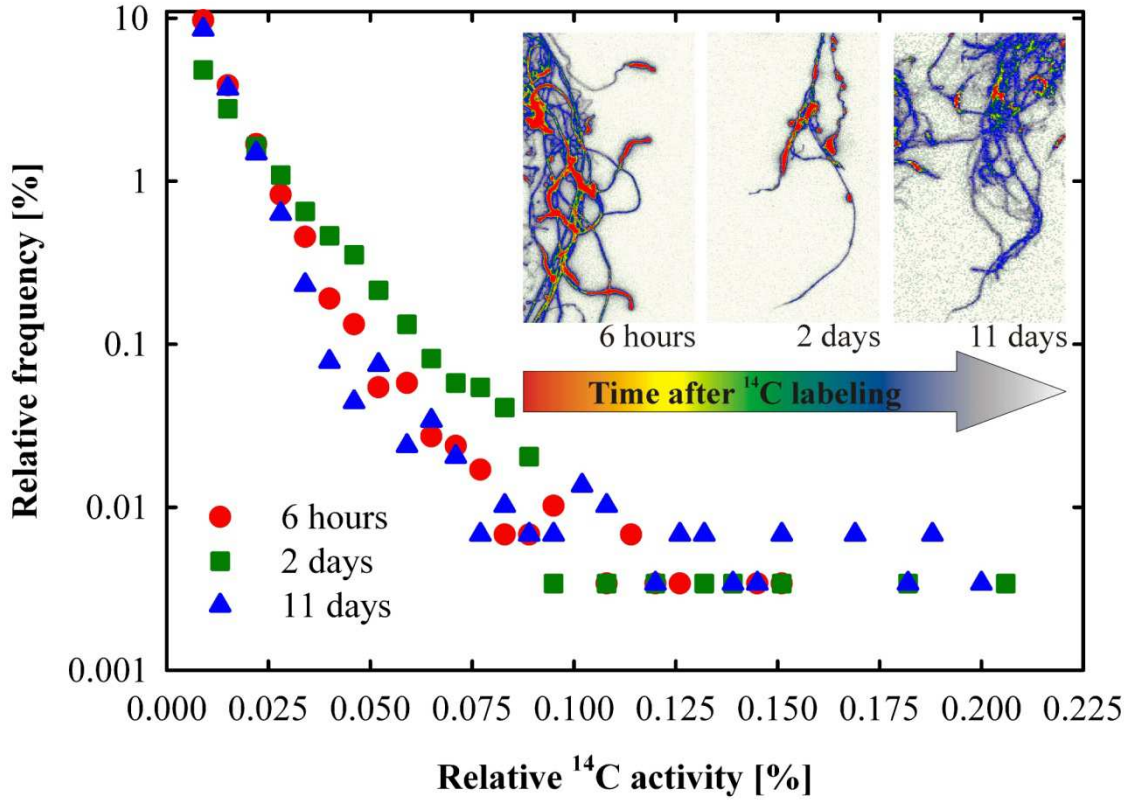

Figure II.1/2: Relationship between the relative frequency of 1 mm x 1 mm squares and their ^{14}C activity at increasing time after tracer application: 6 h, 2 d and 11 d. Note the logarithmic scale of relative frequency. The insets from Fig. II.1/1 show enlarged hotspots at the root tips 6 h after labeling and decreasing ^{14}C activity (DLU) at the tips with time.

1.3.3 Longitudinal ^{14}C allocation in individual roots

In order to better understand the heterogeneity and dynamics of the ^{14}C activity along the roots, individual parts of ryegrass roots prepared 6 h, 2 d and 11 d after labeling were analyzed for ^{14}C allocation. Due to root growth, the tracer accumulation zone changed continuously over the 11 d after the ^{14}C pulse. At the first harvest, ^{14}C activity was highest mainly in the first millimeters from the root tips, and it decreased with increasing distance from the tips (Fig. II.1/3). Two days later, the maximum of ^{14}C enriched tissue still occurred within the first 2 mm from the root tips. The maximum peak shifted 11 d after labeling. The highest activity was now located ≈ 10 mm from the tips. After ^{14}C application, the apical meristem grew mainly with unlabeled C. This resulted in unlabeled tissue at the root tip and explains the shift.

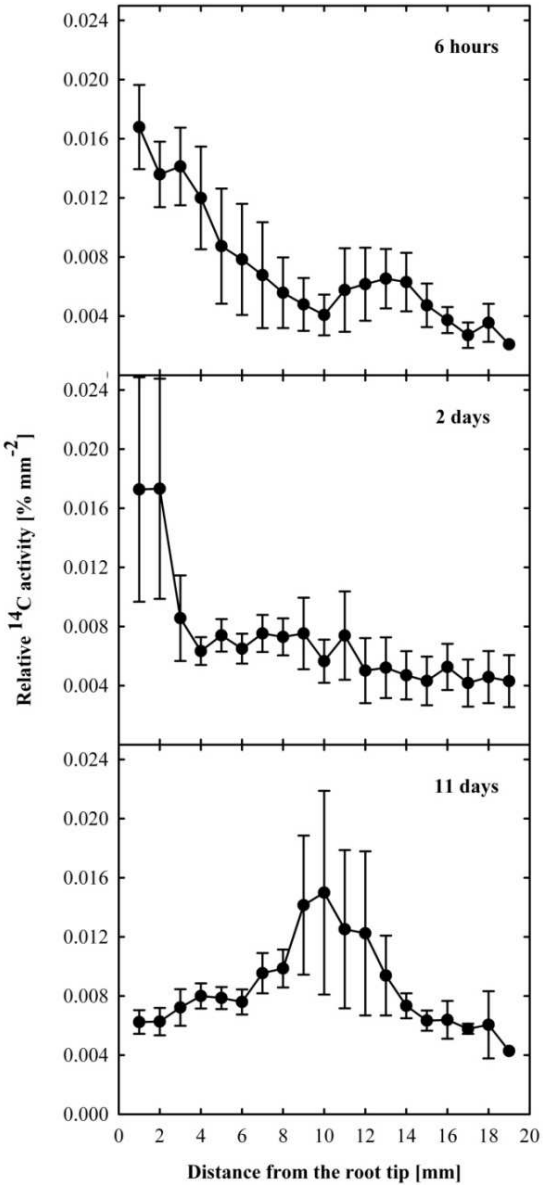

Figure II.1/3: Relationship between the mean relative ^{14}C activities per mm² (%) and the distance from the root tip (mm).

1.4 Discussion

1.4.1 Applicability of ^{14}C phosphor imaging for visualizing and tracing belowground C allocation

Significant amounts of the C allocated belowground are released as organic C *via* rhizodeposition into the rhizosphere (Marschner, 1995). Previously, Meharg and Killham (1990) found that the net photosynthetic C allocated to the roots of *Lolium perenne* ranges from 14%-67%. Recent studies showed an allocation of ≈ 48% of the total assimilated ^{14}C (Domanski et al., 2001). An amount of 10%-15% of the total fixed C is respired by roots, and a further 15%-25% is released *via* exudation (Domanski et al., 2001). In comparison, Farrar et al. (2003) reported that the exudation rate of soil-grown plants, detected in ^{14}C labeling investigations, ranges between 5% and 10% of the net assimilated C. Depending on the level of nutrient supply, Werth and Kuzyakov (2006) recovered 0,4%-0,8% ^{14}C in exudates and 15,1%-16,9% in respired CO_2. It was shown that plants growing in nutrient solution lost only 0.5%-1.5% of the fixed C. Under field conditions, plants import more C belowground than laboratory-grown plants (Meharg and Killham, 1990). Our investigations were carried out with ryegrass grown on a Cambisol, which is closer to field conditions than investigations with plants grown in nutrition solution.

^{14}C can either be released as exudates or CO_2 into the rhizosphere, or can be stored as a relatively stable fraction in growing tissues at the root tips and the lateral root emergence (Henry et al., 2005). The C translocated belowground is not released evenly by the roots, but in distinct areas, thus creating a gradient of rhizodeposits along each root axis. The ^{14}C labeling technique allows distinguishing between root-borne C and C components that are already present in the soil. To date, one approach – the two-compartment rhizobox – was known to identify the gradient of rhizodeposition and thus the active microbial zones. In this rhizobox, the root-containing soil is separated from the root-free soil by gauzes with different hole sizes (0…30 µm). After labeling the shoots in a $^{14}CO_2$ atmosphere, the soil compartment without roots is, after a certain period of time, cut into thin slices by a microtome, and the ^{14}C activity in the different slices is determined (Sauer et al., 2006). Sauer et al. (2006) found maximum ^{14}C accu-

mulation within 2 mm of the root mat at the gauze. This was explained by the 0.08 to 2 mm long root hairs (Esau, 1965; Zhu et al., 2005) penetrating through the 30 µm gauze into the root-free soil. However, in the model of Jones et al. (1996), based mainly on diffusion of organic acids, the maximum distance for the recovery of organic acids was < 1 mm from the root surface.

The main shortcoming of this approach is the creation of a root mat at the gauze, followed by an overestimation of root effects due to specific conditions at the mat, undefined numbers of root layers, as well as variation in age and physiological development. Moreover, root effects may be underestimated due to solute mixing, sorption, and microbial metabolism (Wenzel et al., 2001). To avoid these problems, Wenzel et al. (2001) tested a novel rhizobox design: Plants were grown in a soil plant compartment with roots penetrating through a slit into a soil-free compartment consisting of an acrylic window that moves horizontally when root growth pressure is exerted. This allows root growth, distribution, and morphology to be monitored. A membrane separates the soil-free compartment from the root-free soil compartment in which the rhizodeposition gradient can be determined afterwards. An important advantage of this design is the possibility to measure the exact root biomass. This approach enables the diffusion of exudates from the root compartment to be traced, but provides no information on the allocation of assimilated C along and within individual roots. To overcome this problem and to visualize the allocation of photoassimilates in individual roots, another approach based on ^{14}C phosphor imaging can be applied.

Phosphor imaging had already been used to visualize the allocation and the movement of labeled P (^{32}P and ^{33}P) in leaves of *Vicia faba* (Hüve et al., 2007). At the same time, ^{14}C pulse labeling coupled with phosphor imaging was used to investigate the C flux of assimilated ^{14}C from *Pinus sylvestris* seedlings to the ectomycorrhizal mycelium (Leake et al., 2001) and between the seedlings of *Pinus densiflora* via ectomycorrhizal mycelia (Wu et al., 2001). This approach helps to visualize and quantify the spatial and temporal patterns of the allocation of ^{14}C assimilates.

In the present study, the ^{14}C phosphor imaging approach enabled us to use the image-plate pictures to statistically evaluate the ^{14}C distribution. The methodology we used in preparing the herbariums did not include measuring rhizodeposition and CO_2 but focused on the ^{14}C allocation within the roots. The limitations of this approach are mainly

linked to the preparation of the root herbariums. Firstly, after carefully extracting the roots, their separation from mineral soil material and washing may lead to a significant loss of fine roots. Secondly, root exudates and rhizodeposits are completely lost during the washing procedure. Therefore, very fine roots, rhizodeposits, and exudates cannot be determined with this approach. Thirdly, the preparation of root (and shoot) herbariums may cause some parts of the roots to overlap (Fig. II.1/1B, C, D). Such overlapping of 2-3 roots could locally increase the ^{14}C specific activity of the area (DLU mm^{-2}), thus overestimating hotspots. The energy of β^- particles released by ^{14}C decay, however, is very low (E_{max} = 0.156 MeV), and the probability that they will penetrate the overlapped roots is very small. We therefore assume that the number of hotspots is not overestimated by the image of ^{14}C on the imaging plates.

The three shortcomings described above (loss of fine roots, loss of exudates, and overlapping) could be overcome using a specially constructed, thin rhizobox with a removable front wall. Placing the boxes in tilted position (20°-30° vertical) causes roots to grow along this wall. The front wall can be removed after labeling. Then, after drying or freezing the roots and the soil in the rhizobox, the phosphor imaging plate can be placed instead of the wall to prepare the root image. After developing the image, the roots can be carefully removed, and the phosphor imaging plate can be placed again to the soil. This yields an image of rhizodeposition without roots.

1.4.2 Relative carbon allocation in shoots and roots

We compared the allocation of assimilated C in shoots with the allocation in roots based on the intensity of DLU mm^{-2}. The amount of assimilates in the shoots 6 h after labeling was ≈ 10 times higher than in the roots. Surprisingly, this ratio did not strongly decrease within 2 or 11 d after labeling. Other authors have also reported this interesting phenomenon (Hill et al., 2007). Similar results were frequently obtained for ^{13}C pulse-labeling experiments based on higher ^{13}C enrichment in shoots versus roots (Kastovska and Santruckova, 2007). The difference cannot be adequately explained by less total assimilates allocated to roots compared to shoots: considering the ratio of ^{14}C shoot to ^{14}C roots of ≈ 10 (Fig. II.1/1A, B), the total shoot mass should also be ≈ 10 times higher. This phenomenon can be explained either by label storage in pools of the shoots with a long turnover time, or by a much higher turnover of roots compared with shoots and

thus a much higher replacement of ^{14}C (or ^{13}C) from the pulse by subsequent unlabeled C.

Another surprising result clearly visualized by the ^{14}C distribution was the high inhomogeneity of the C allocation within the shoots. Even within a single leaf, the ^{14}C activity can differ by 5-7 times (compare parts of individual leaves in Fig. II.1/1A). This shows that it is impossible to produce homogeneously labeled plant biomass by pulse labeling (^{14}C or ^{13}C). Probably, repeated pulse labeling will also be insufficient to produce plant biomass with a homogeneous distribution of the label.

1.5 Conclusions

^{14}C labeling, coupled with phosphor imaging, can provide detailed insights into the C flows into and through plant roots. The spatial distribution pattern of allocated ^{14}C was already visible 6 h after assimilation. The spatial distribution of the C allocation in the roots was uneven: up to 2 d after assimilation, it was associated with the root tips, whereas 11 d after assimilation the ^{14}C allocation to the hotspots at the root tips disappeared. Moreover, it was impossible to produce homogeneously labeled shoot or root biomass by pulse labeling.

^{14}C phosphor imaging is a promising tool for visualizing C translocation in both plants and rhizosphere. This technique will yield further progress in describing and interpreting soil-root interactions.

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References

Buyanovky, G.A., Aslam, M., Wagner, G., 1994. Carbon turnover in soil physical fractions. Soil Science Society of America Journal 58, 1167-1173.

Cheng, W., Coleman, D.C., Carroll, C.R., Hoffman, C.A., 1993. In situ measurements of root respiration and soluble C concentrations in the rhizosphere. Soil Biology and Biochemistry 25, 1189-1196.

Domanski, G., Kuzyakov, Y., Siniakina, S.V., Stahr, K., 2001. Carbon flow in the rhizosphere of ryegrass (*Lolium perenne*). Journal of Plant Nutrition and Soil Science 164, 381.387.

Esau, K., 1965. Plant Anatomy. John Wiley & Sons, New York.

Farrar, J.F., Minchin, P.E.H., Thorpe, M.R., 1994. Carbon import into barley roots- stimulation by galactose. Journal of Experimental Botany 47, 17-22.

Farrar, J., Hawes, M., Jones, D., Lindow, S., 2003. How roots control the flux of carbon to the rhizosphere. Ecology 84, 827-837.

Gregory, P.J., Atwell, B.J., 1991. The fate of carbon in pulse labelled crops of barley and wheat. Plant and Soil 136, 205-213.

Hamaoka, T., 1990. Autoradiography of a new era replacing traditional X-ray film. Cell Technology 9, 456-462.

Henry, F., Nguyen, C., Paterson, E., Robin, A.S., Robin, C., 2005. How does nitrogen availability alter rhizodeposition in Lolium multiflorum Lam. during vegetative growth? Plant and Soil 269, 181–191.

Hill, P.W., Marshall, C., Williams, G.G., Blum, H., Harmens, H., Jones, D.L., Farrar, J.F., 2007. The fate of photosynthetically-fixed carbon in *Lolium perenne* grassland as modified by elevated CO_2 and sward management. New Phytologist 173, 766-777.

Hinsinger, P., Gobran, G.R., Gregory, P.J., Wenzel, W., 2005. Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytologist 168, 293-303.

Hüve, K., Merbach, W., Remus, R., Lüttschwager, D., Wittenmayer, L., Hertel, K., Schurr, U., 2007. Transport of phosphorus in leaf of *Vicia faba* L. Journal of Plant Nutrition and Soil Science 170, 14-23.

Jensen, B., 1993. Rhizodeposition by $^{14}CO_2$-pulse-labelled spring barley grown in small field pots on sandy loam. Soil Biology and Biochemistry 25, 1553-1559.

Johnen, B.G., Sauerbeck, D., 1977. A tracer technique for measuring growth, mass and microbial breakdown of plant roots during vegetation, in Lohm, U., Persson, T.: Soil Organisms as Components of Ecosystems. Ecological Bulletins, Stockholm 25, pp. 366-373.

Jones, D.L., Darrah, P.R., Kochian, L.V., 1996. Critical evaluation of organic acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. Plant and Soil 180, 57–66.

Kastovska, E., Santruckova, H., 2007. Fate and dynamics of recently fixed C in pasture plant-soil system under field conditions. Plant and Soil 300, 61-69.

Keutgen, N., Roeb, G.W., Minchin, P.E.H., Fuhr, F., 1995. Use of transfer function and compartmental analysis to quantify ^{11}C-labelled photoassimilate export from wheat leaves. Journal of Experimental Botany 46, 489-496.

Kleber, M., 1997. Carbon exchange in humid grassland soils. Hohenheimer Bodenkundliche Hefte, 41.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421-431.

Kuzyakov, Y., Ehrensberger, H., Stahr, K., 2001. Carbon partitioning and below-ground translocation by *Lolium perenne*. Biology and Biochemistry 33, 61-74.

Kuzyakov, Y., Kretzschmar, A., Stahr, K., 1999. Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. Plant and Soil 213, 127-136.

Kuzyakov, Y., Schneckenberger, K., 2004. Review of estimation of plant rhizodeposition and their contribution to soil organic matter formation. Archives of Agronomy and Soil Science 50, 115-132.

Leake, J.R., Donnelly, D.P., Saunders, E.M., Boddy, L., Read, D.J., 2001. Rates and quantities of carbon flux to ectomycorrhizal mycelium following ^{14}C pulse labeling

of *Pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus. Tree Physiology 21, 71-82.

Marschner, H., 1995. Mineral nutrition of higher plants. Academic Press, London.

Meharg, A.A., 1994. A critical review of labelling techniques used to quantify rhizosphere carbon flow. Plant and Soil 166, 55-62.

Meharg, A.A., Killham, K., 1990. Carbon distribution within the plant and rhizosphere in laboratory and field grown *Lolium perenne* at different stages of development. Biology and Biochemistry 22, 471-477.

Nguyen, C., 2003. Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23, 375-396.

Nguyen, C., Todorovic, C., Robin, C., Christophe, A., Guckert, A., 1999. Continuous monitoring of rhizosphere respiration after labelling of plant shoots with $^{14}CO_2$. Plant and Soil 212, 191-201.

Sauer, D., Kuzyakov, Y., Stahr, K., 2006. Short Communication Special distribution of root exudates of five plant species as assessed by ^{14}C labeling. Journal of Plant Nutrition and Soil Science 169, 360-362.

Swinnen, J., Van Veen, J.A., Merckx, R., 1994. ^{14}C pulse-labelling of field-grown spring wheat: an evaluation of its use in rhizosphere carbon budget estimations. Soil Biology and Biochemistry 26, 171-182.

Swinnen, J., Van Veen, J.A., Merckx, R., 1995 a. Carbon fluxes in the rhizosphere of winter wheat and spring barley with conventional vs. integrated farming. Soil Biology and Biochemistry 27, 811-820.

Swinnen, J., Van Veen, J.A., Merckx, R., 1995 b. Root decay and turnover of rhizodeposits in field-grown winter wheat and spring barley estimated by ^{14}C pulse-labelling. Soil Biology and Biochemistry 27, 211-217.

Tinker, P.B., Durrall, D.M., Jones, M.D., 1994. Carbon use efficiency in mycorrhizas: theory and sample calculations. New Phytologist 128, 115-122.

Warembourg, F.R., Billes, G., 1979. Estimation carbon transfers in the plant rhizosphere, in Harley, J.L., Russell, R.S.: The Soil-Root Interface. Academic Press, London, pp. 183-196.

Wenzel, W.W., Wieshammer, G., Fitz, W.J., Puschenreiter, M., 2001. Novel rhizobox design to assess rhizosphere characteristics at high spatial resolution. Plant and Soil 237, 37-45.

Werth, M., Kuzyakov, Y., 2006. Assimilate partitioning affects ^{13}C fractionation of recently assimilated carbon in maize. Plant and Soil 284, 319-333.

Werth, M., Kuzyakov, Y., 2008. Root-derived carbon in soil respiration and microbial biomass determination by ^{14}C and ^{13}C. Soil Biology and Biochemistry 40, 625-637.

Whipps, J.M., 1987. Carbon loss from the roots of tomato and pea seedlings grown in soil. Plant and Soil 103, 95-100.

Whipps, J.M., 1990. Carbon Economy, in Lynch, J.M.: The Rhizosphere. Wiley, Chichester, pp. 59-97.

Wu, B., Nara, K., Hogetsu, T., 2001. Can ^{14}C-labeled photosynthetic products move between Pinus densiflora seedlings linked by ectomycorrhizal mycelia? New Phytologist 149 (1), 137-146.

Zhu, J.M., Kaeppler, S.M., Lynch, J.P., 2005. Mapping of QTL controlling root hair length in maize (*Zea mays* L.) under phosphorus deficiency. Plant and Soil 270, 299-310.

2 Plant inter-species effects on rhizosphere priming of soil organic matter decomposition

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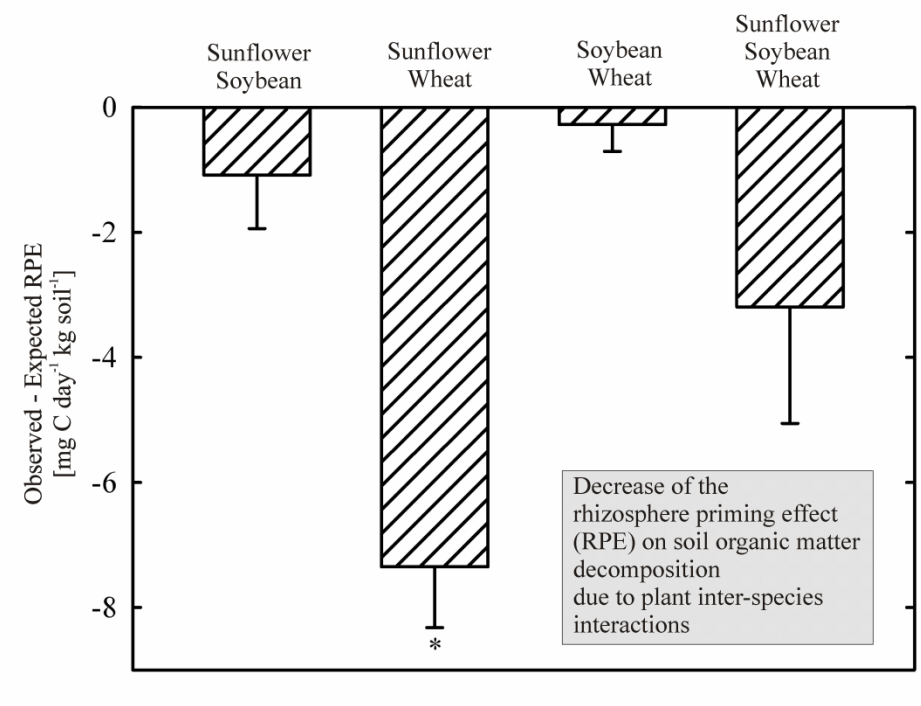
Research Highlights

- We studied effects of plant-plant interactions on rhizosphere priming effects (RPE).
- Sunflower, soybean and wheat were grown as monocultures or as mixtures.
- RPE was studied by continuous ^{13}C labeling of the monocultures and of the mixtures.
- All planted treatments induced positive RPE (43%-136% of the unplanted control).
- Inter-species interactions can reduce the intensity of priming on SOM decomposition.

Graphical Abstract



Abstract

Living roots and their rhizodeposits can stimulate microbial activity and soil organic matter (SOM) decomposition up to several folds. This so-called rhizosphere priming effect (RPE) varies widely among plant species possibly due to species-specific differences in the quality and quantity of rhizodeposits and other root functions. However, whether the RPE is influenced by plant inter-species interactions remains largely unexplored, even though these interactions can fundamentally shape plant functions such as carbon allocation and nutrient uptake.

In a 60-day greenhouse experiment, we continuously labeled monocultures and mixtures of sunflower, soybean and wheat with ^{13}C-depleted CO_2 and partitioned total CO_2 efflux released from soil at two stages of plant development for SOM- and root-derived CO_2. The RPE was calculated as the difference in SOM-derived CO_2 between the planted and the unplanted soil, and was compared among the monocultures and mixtures.

We found that the RPE was positive under all plants, ranging from 43% to 136% increase above the unplanted control. There were no significant differences in RPE at the vegetative stage. At the flowering stage however, the RPE in the soybean-wheat mixture was significantly higher than those in the sunflower monoculture, the sunflower-wheat mixture, and the sunflower-soybean mixture. These results indicated that the influence of plant inter-specific interactions on the RPE is case-specific and phenology-dependent. To evaluate the intensity of inter-specific effects on priming, we calculated an expected RPE for the mixtures based on the RPE of the monocultures weighted by their root biomass and compared it to the measured RPE under mixtures. At flowering, the measured RPE was significantly lower for the sunflower-wheat mixture than what can be expected from their monocultures, suggesting that RPE was significantly reduced by the inter-species effects of sunflower and wheat. In summary, our results clearly demonstrated that inter-species interactions can significantly modify rhizosphere priming on SOM decomposition.

Keywords: Priming effect, ^{13}C-continuous labeling, soil respiration, microbial biomass, nitrogen competition, biodiversity, plant-microbial competition.

2.1 Introduction

Soil organic carbon (C) functions as both an important source and a sink of atmospheric CO_2. Many uncertain parameters of the global C cycle are associated with complex soil processes and biological communities, which are both difficult to measure and highly sensitive to disturbance (Moyes et al., 2010). Soil CO_2 mainly consists of (1) root-derived CO_2, including root respiration and microbial decomposition of rhizodeposits from living roots (rhizomicrobial respiration), and (2) CO_2 derived from microbial decomposition of soil organic matter (SOM) (Kuzyakov, 2006). Both sources are linked through the rhizosphere priming effect (RPE) which describes changes in the rate of SOM decomposition in the presence of living roots (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Plant roots can alter microbial activities by providing organic substances (rhizodeposits) (Paterson, 2003), by competing with microorganisms for mineral nutrients (Schimel et al., 1989; Wang and Bakken, 1997), and by changing the physical and chemical conditions in the rhizosphere (e.g. water, pH) (Shields and Paul, 1973; Jenkinson, 1977). These alterations can lead to either stimulation (positive RPE) or retardation (negative RPE) of SOM decomposition with rates ranging from 70% reduction to as high as 330% increase compared to an unplanted control (Cheng and Kuzyakov, 2005). The direction and magnitude of RPE on SOM decomposition depend on both the plant and the soil. The amount of decomposable organic C and the mineral nitrogen (N_{min}) content of the soil have been identified as two of the main soil factors that significantly influence RPE (Liljeroth et al., 1994; Cheng and Johnson, 1998; Bottner et al., 1999; Kuzyakov, 2002). On the other hand, plant species and their developmental stages (Fu and Cheng, 2002; Cheng et al., 2003) also strongly influence the RPE, possibly through differences in the quality and quantity of rhizodeposits (Van der Krift et al., 2001; Nguyen, 2003; Jones et al., 2004).

Since the RPE is plant-species specific (Fu and Cheng, 2002; Cheng et al., 2003), it may also vary with plant inter-species interactions. It was proposed that with higher plant diversity the diversity of root exudates may also increase (Lavelle et al., 1995; Hooper et al., 2000). This wider spectrum of root exudates from mixed plant species may support a higher microbial biomass and activity in the rhizosphere (Hooper et al., 2000; Spehn et al., 2000; Stephan et al., 2000) whereby, the production of extracellular en-

zymes can be enhanced. Consequently, the potential for a positive RPE may increase (Fontaine et al., 2003). In contrast, Dijkstra et al. (2010) suggested a plant-diversity-induced decrease in SOM decomposition for systems with low water availability. With higher plant richness, belowground resources, especially N, are being complementarily used (Hooper and Vitousek, 1997; von Felten et al., 2009). This may result in a lower availability of belowground resources and thus, in a decline in the decomposition of SOM (Dijkstra et al., 2010).

In this study monocultures and mixtures of sunflower, soybean and wheat were continuously exposed to ^{13}C-depleted CO_2. The soil CO_2 efflux was measured at an early stage of plant development (day 29-30 after planting), and at flowering of sunflower and soybean (day 55-56 after planting). Based on its $\delta^{13}C$ values the total soil CO_2 efflux was separated into root- and SOM-derived CO_2. The RPE was calculated as the difference in SOM-derived CO_2 between planted and unplanted treatments. To our knowledge, up to now the experimental work of Dijkstra et al. (2010) is the only study investigating the effect of plant diversity on the RPE, although inter-specific effects on carbon allocation belowground (Sanaullah et al., 2012) and on the activity of microorganisms (Sanaullah et al., 2011) are known. Although the results of Dijkstra et al. (2010) provide evidence that plant-plant interactions modify RPE, no firm conclusions on general patterns could be drawn, which necessitates future research on this topic.

Therefore, the aim of this study was to gain a more comprehensive understanding of modified RPE due to plant inter-species interactions. We hypothesized that the modulation of RPE by inter-species interactions is specific to the plant species composition and dependent on plant developmental stage.

2.2 Materials and methods

2.2.1 Experimental set-up

Monocultures of sunflower (Sun) (*Helianthus annuus* L.), soybean (Soy) (*Glycine max* L. Merr.) and spring wheat (Wh) (*Triticum aestivum* L.) and mixed cultures of sunflower/soybean (Sun/Soy), sunflower/wheat (Sun/Wh), soybean/wheat (Soy/Wh) and sunflower/soybean/wheat (Sun/Soy/Wh) were grown in PVC pots (15 cm diameter, 40 cm height, equipped with an inlet tube at the bottom for aeration and soil CO_2 trapping) with four replicates of the monocultures and six replicates of the mixed cultures. Including two-species mixtures in our study allowed investigation of possible patterns of individual species' influence on RPE when they were grown in mixtures. In addition four unplanted pots were prepared. A nylon bag filled with 1500 g sand was placed at the bottom of each PVC pot to improve air circulation. The pots were then filled with 7.9 kg of air-dried, sieved (<4mm) soil taken from the plough horizon (top 30 cm) of a sandy loam from a farm on the campus reserves of the University of California, Santa Cruz. Air drying and sieving allowed us to achieve a high degree of soil homogeneity and reduced the variability among the treatments and replicates. The soil contained 1.1% organic C and 0.1% N, had a $\delta^{13}C$ value of -26.0‰ and a pH value of 5.8. All filled pots were wetted to 20% gravimetric soil moisture content (equivalent of 80% of the water holding capacity) with deionized water.

Seeds were presoaked over night in deionized water before planting. For the mixed cultures we used one individual plant of each species. For all monocultures we used two individual plants per pot to establish comparable growing conditions for all treatments. To get one individual plant, six seeds of wheat, two of sunflower and three of soybean were planted and thinned to one after seedling emergence. The soil moisture content was measured gravimetrically and adjusted daily to 80% of the water holding capacity. To maintain homogeneous soil moisture and good soil structure, water was added through perforated tubes (inner diameter 0.32 cm, total length 180 cm, buried length approximately 140 cm) as described by Dijkstra and Cheng (2007). The location of the pots in the greenhouse was changed weekly by mixing them randomly to guarantee similar growing conditions for the plants.

The experiment was conducted from January to March 2011 in a greenhouse equipped with continuous labeling by ^{13}C-depleted CO_2 at the University of California, Santa Cruz. Plants were continuously, i.e. from the emergence of the first leaf till harvest, exposed to ^{13}C-depleted CO_2 (-15‰). The continuous labeling technique allows us to quantitatively differentiate root-derived CO_2 from native SOM-derived CO_2 since both C pools differ in their isotopic composition after labeling (Table II.2/1). During plant growth the day time air temperature inside the greenhouse was maintained at 28°C by two AC units. The night time temperature was kept above 18°C. Artificial lighting (1100W lights, P.L. Light Systems, Beamsville, ON) was used when the natural light intensity was less than 900 W m^{-2}. The photoperiod was set from 6AM to 6PM. The relative air humidity was kept at 45% by a dehumidifier (Kenmore Elite 70 pint, Sears, Chicago, IL, USA). We continuously labeled the plants with naturally ^{13}C-depleted CO_2 using the method described in detail by Cheng and Dijkstra (2007). Briefly, a constant CO_2 concentration of 400±5 ppm and a constant $\delta^{13}C$ value (see below) was maintained inside the greenhouse by regulating the flow of pure, ^{13}C-depleted CO_2 (99.9% CO_2, -35‰) from a tank and setting CO_2-free air flow rate proportional to the leakage rate of the greenhouse (Zhu and Cheng, 2012). The CO_2-free air was produced from compressed air passed through six soda lime columns (20 cm diameter, 200 cm length) filled with approximately 40 kg soda lime each. The CO_2-free air flow was set at 120 L/min. The leakage rate of the greenhouse (300 L/min) was determined without plants shortly before the start of the experiment. This was done, after closing all inputs (CO_2-free air, tank CO_2), by raising the CO_2 concentration inside the greenhouse to a certain level and monitoring the decrease of the concentration which is proportional to the leakage rate. The CO_2 concentration inside the greenhouse was continuously monitored by an infra red gas analyzer (Model LI-820, Li-COR, Lincoln, NE, USA) and stabilized at 400 ± 5 ppm by computer controlled CO_2 injection from the tank. A fan was used to ensure a uniform distribution of the CO_2 inside the greenhouse. For the duration of the experiment the $\delta^{13}C$ value of the greenhouse air was measured every three days during the light period by pumping air through a glass airstone immersed in 50 mL of 0.5 M NaOH solution. The CO_2 trapping efficiency was nearly 100% as checked by an infra red gas analyzer (Model LI-6262, Li-COR, Lincoln, NE, USA). An aliquot of the sample was precipitated with $SrCl_2$ as $SrCO_3$ using the method described by Harris et al. (1997) and analyzed for $\delta^{13}C$ (relative to PDB standard) using a PDZ Europa ANCA-

GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. The mean $\delta^{13}C$ value of the greenhouse air was -15.2‰ with a day-to-day variability of <0.7‰.

The inlet tube at the bottom of each pot was connected to an aquarium pump (Elite 802, Hagen Corp., Mansfield, MA, USA) to aerate the pots. This was done two times during the dark period (from 6:30PM to 7PM and from 12AM to 1AM) to avoid contamination of the greenhouse $\delta^{13}C$ signal with that of soil-derived CO_2 during the assimilation period. Before the start of each photoperiod the isotopic composition of the greenhouse CO_2 returned to equilibrium.

2.2.2 Measurements

Soil CO_2 efflux was measured on day 29-30 after planting (T1) when the plants were still at vegetative stage and on day 55-56 after planting (T2) during the flowering of sunflower and soybean by means of a closed-circulation CO_2 trapping system (Cheng et al. 2003). Briefly, a Plexiglas lid with holes for the shoots was placed direct on the soil surface. A plastic tube was attached to the lid for CO_2 trapping. The holes around the shoots and between the lid and the rim of the pot were sealed with non-toxic silicone rubber (GI-1000, Silicones Inc., NC, USA). The pots were checked for airtightness. The CO_2 inside the pots was removed by circulating the isolated air through a soda lime column (3 cm diameter, 50 cm length) for 40 min. Then CO_2 produced in the sealed pot was trapped for 24 h in 400 ml of 0.5 M NaOH solution. Four blanks were included to correct the total inorganic C content for possible contamination from carbonate in the NaOH stock solution and from sample handling. An aliquot of each NaOH solution was analyzed for total inorganic carbon using a Shimadzu TOC-5050A Total Organic Carbon Analyzer. Another aliquot was precipitated as $SrCO_3$ (Harris et al., 1997) and analyzed for $\delta^{13}C$ by means of a continuous flow isotopic ratio mass spectrometer (see description above).

At the end of the experiment the pots were destructively sampled. The shoots were cut at the base, dried at 60°C and weighed. Three out of the six replicates of the mixtures were used to determine the root dry weight of each individual plant. All the soil in each pot was pulled out and soaked in deionized water for 24 h. All soil was washed away and the roots of each single plant were carefully separated in a water bath with tweezers,

dried at 60°C and weighed. The shoot-to-root ratio was determined and used to calculate the root biomass of each individual plant for the three remaining replicates based on their shoot biomass. Careful and accurate separation of roots of individual plant species in the mixtures was required to compare RPE of the mixtures with expected RPE calculated for the respective monocultures in a reliable manner (see below). Furthermore, to obtain root-free soil the roots of the three remaining replicates of each mixed treatment as well as the roots of all monocultures were separated from soil by hand-picking. The monoculture roots were rinsed with deionized water, dried as described above and weighed. A part of the soil remaining after root-picking of all treatments as well as of the soil from the unplanted pots was dried at 60°C for three days. All dried samples were ground in a ball mill and analyzed for C%, N% and $\delta^{13}C$ using a Carlo Elba 1108 elemental analyzer interfaced to a ThermoFinningan Delta Plus XP isotope ratio mass spectrometer.

Soil microbial biomass C (MBC) was determined on all remaining soil samples by the chloroform fumigation extraction method described by Vance et al. (1987) with the modification that fumigated and unfumigated soil samples (30 g fresh soil) were extracted for 2 hours with 60 mL of 0.5 M K_2SO_4 solution. The samples were filtered and the extracts analyzed for total organic carbon by means of a Shimadzu TOC-5050A Total Organic Carbon Analyzer. The difference between the extracts of fumigated and unfumigated samples corrected for a k_{EC} factor of 0.45 (Wu et al., 1990) gives the total amount of microbial biomass C.

Furthermore, soil mineral N (N_{min}; NO_3^- + NH_4^+) was extracted from 30 g fresh soil with 60 ml of 2 M KCl solution. Samples were shaken for 2 hours, filtered and the extracts were analyzed for NO_3^- and NH_4^+ by a flow injection analyzer (Lachat QuikChem 8000, Milwaukee, WI).

2.2.3 Calculations

The contribution of CO_2 derived from SOM decomposition ($C_{SOM-DERIVED}$, mg C day^{-1} $kg\ soil^{-1}$) to total soil respiration was calculated using a linear two-source isotopic mixing model:

$$C_{SOM-DERIVED} = C_{TOTAL} \cdot \frac{\delta^{13}C_{TOTAL} - \delta^{13}C_{ROOT-DERIVED}}{\delta^{13}C_{SOM-DERIVED} - \delta^{13}C_{ROOT-DERIVED}} \quad (1)$$

$$C_{ROOT-DERIVED} = C_{TOTAL} - C_{SOM-DERIVED} \quad (2)$$

where C_{TOTAL} is the total CO_2 efflux of the planted treatment (mg C day^{-1} kg $soil^{-1}$) and $\delta^{13}C_{TOTAL}$ the corresponding $\delta^{13}C$ value (‰). $\delta^{13}C_{SOM-DERIVED}$ is the $\delta^{13}C$ value of CO_2 from SOM decomposition measured in the unplanted pots (‰). $C_{ROOT-DERIVED}$ is the root-derived CO_2 in the planted pot (mg C day^{-1} kg $soil^{-1}$) with $\delta^{13}C_{ROOT-DERIVED}$ as the corresponding $\delta^{13}C$ value (‰).

The separation of root- and SOM-derived CO_2, as a prerequisite to calculate RPE, often involves the assumption that the net isotopic fractionation during respiration processes is negligible (e.g. Buchmann and Ehleringer, 1998; Rochette et al., 1999; Søe et al., 2004). A recent study by Zhu and Cheng (2011a), however, reported a ^{13}C-depletion of rhizosphere respiration compared to the isotopic composition of roots or shoots. Furthermore, a review of ^{13}C fractionation at the root-microorganisms-soil interface showed that the mean difference between $\delta^{13}C$ of root-derived CO_2 and that of roots was –2.3‰ for C_3 plants and –1.3‰ for C_4 plants, by variation of more than ±2.0‰ (Werth and Kuzyakov, 2010). Moreover, it has been discussed by Dijkstra et al. (2010) that a 1‰ deviation in the isotopic composition of the plant tissue may result in variations of up to 40% in the RPE in their particular experimental configuration.

In order to minimize the influence of isotopic fractionation on the RPE results, we investigated target species for which the fractionation between roots and root-derived CO_2 (f) was determined recently. Accounting for ^{13}C fractionations associated with rhizosphere respiration by using directly-measured species-specific data in our calculations of SOM-derived CO_2 and root-derived CO_2 eliminates a major uncertainty or possible erroneous conclusions. The f factors were taken from an earlier study by Zhu and Cheng (2011a) and are -1.01‰ for sunflower, -1.71‰ for soybean and -0.87‰ for wheat. We kept the conditions as similar as possible by using the same seeds, soil, and equipment with similar growing conditions (Zhu and Cheng, 2011a). By doing so we accounted for the effect of isotopic fractionation on the RPE results.

$\delta^{13}C_{ROOT-DERIVED}$ was differently calculated for monocultures and mixed cultures.

$\delta^{13}C_{ROOT-DERIVED}$ of the monocultures was calculated by correcting the $\delta^{13}C$ value of the root ($\delta^{13}C_{ROOT}$) by a fractionation factor (f):

$$\delta^{13}C_{ROOT-DERIVED} = \delta^{13}C_{ROOT} + f \quad (3)$$

For mixed cultures we calculated a root biomass weighted $\delta^{13}C_{ROOT-DERIVED}$ value:

$$\delta^{13}C_{ROOT-DERIVED} = \sum(\delta^{13}C_{ROOT,i} + f_i) \cdot a_i \quad (4)$$

where a_i is the percentage of root dry weight of species i on total root dry weight per pot.

Note: Because root biomass was analyzed at the end of the experiment only, the calculation (Eq. 4) involves the assumption that a_i does not change between T1 and T2. We examined the sensitivity of this assumption by changing a_i from 0 to 100% and found that variations of $C_{SOM-DERIVED}$ and $C_{ROOT-DERIVED}$ did not exceed 15% and 19%, respectively for the treatments including soybean and were even smaller than 5% for the Sun/Wh treatment.

The isotopic composition of root-derived CO_2 and SOM-derived CO_2 from the unplanted soil used as end members of the linear two-source isotopic mixing models are given in Table II.2/1.

Table II.2/1: End member values (±SEM) used in two-source isotopic mixing models in order to calculate the contribution of SOM-derived CO_2 to total soil CO_2 of the planted treatments.

Treatment	**Root-derived CO_2 [‰]**	**SOM-derived CO_2 of the unplanted soil [‰]**
Sun	-39.6 ± 0.09	**T1**
Soy	-37.0 ± 0.3	-23.9 ± 0.2
Wh	-39.4 ± 0.4	**T2**
Sun/Soy	-39.2 ± 0.1	-23.7 ± 0.1
Sun/Wh	-39.6 ± 0.06	
Soy/Wh	-39.4 ± 0.2	
Sun/Soy/Wh	-39.1 ± 0.07	

The RPE on SOM decomposition (observed RPE) was calculated by subtracting the CO_2-C flux of the unplanted treatment ($C_{SOM-DERIVED}(UP)$) from the SOM-derived CO_2-C flux of the planted treatment ($C_{SOM-DERIVED}(P)$) and was denoted as mg C day^{-1} kg $soil^{-1}$.

$$RPE = C_{SOM-DERIVED}(P) - C_{SOM-DERIVED}(UP) \quad (5)$$

Since changes of root biomass may have occurred when plants were grown in mixed cultures and because the RPE as well as the MBC, N_{min} and the root-derived CO_2 can be positively related to root biomass (Dijkstra et al., 2006), we calculated expected values (EXP) for the mixtures:

$$EXP = \sum RB_{mix,i} \cdot \frac{OBS_{mono,i}}{RB_{mono,i}} \quad (6)$$

$RB_{mix,i}$ is the root dry weight of species *i* in the mixture (g pot^{-1}), $OBS_{mono,i}$ is the observed value of species *i* growing in monoculture, denoted as mg C day^{-1} kg $soil^{-1}$ for the RPE and the root-derived CO_2, as mg C kg $soil^{-1}$ for the MBC and as mg N kg $soil^{-1}$ for N_{min}. $RB_{mono,i}$ is the root dry weight of species *i* in the monoculture (g pot^{-1}).

To estimate the effect of plant inter-species interactions, the expected values were subtracted from the observed values. If the observed value is lower than the expected value, RPE, root-derived CO_2, MBC or N_{min} was negatively influenced by inter-species interactions.

2.2.4 Statistics

The values presented in the figures and tables are given as means ± standard errors of the means (±SEM). Significant differences in total microbial biomass C, shoot and root N content, soil mineral N, shoot and root dry matter and in their isotopic composition between the treatments were obtained by a one-way analysis of variance (ANOVA) in combination with a *post hoc* unequal N HSD test, a modification of the Tukey HSD test. A one-way ANOVA was also conducted to test for significant differences in root-derived and SOM-derived CO_2 and RPE between the treatments by calculating the ANOVA separately for each sampling date. The significance of differences between individual means was obtained by the unequal N HSD test. To test for significant differ-

ences in root- and SOM-derived CO_2 and RPE within each treatment but between T1 and T2 (phenological effects) a dependent (paired) t-test was used.

Before calculating the RPE, as difference in SOM-derived CO_2 between a planted treatment and the unplanted control, we further tested for significant differences in SOM-derived CO_2 between the unplanted control and each single treatment and sampling day using an independent t-test. The unplanted control always showed significant ($P \leq 0.05$) lower values in comparison to the planted treatments (statistics are not presented in the figures).

Moreover, observed minus expected values were tested for significant deviation from zero by a t-test. All statistical analyses were performed with the statistical package STATISTICA for Windows (version 7.0; StatSoft Inc., OK, USA).

2.3 Results

2.3.1 Plant biomass, plant $\delta^{13}C$ and microbial biomass C

Sunflower grown in monoculture produced a significantly higher shoot biomass per pot than soybean and wheat, whereas wheat developed the highest root biomass (data not presented). The root biomass per wheat plant was significantly lower in the monoculture compared to that of wheat grown in the mixtures (Table II.2/2). In contrast, sunflower as well as soybean produced similar root biomass in all treatments independently of the neighboring plants.

The plant biomass of sunflower and wheat was significantly depleted in ^{13}C compared to that of soybean (Table II.2/2). Wheat and sunflower showed similar isotopic compositions of their plant tissue.

Planting increased the microbial biomass C (Table II.2/2). The lowest microbial biomass C was found in the unplanted control with values of about 118 mg C kg soil^{-1}. While the wheat monoculture showed a significant higher microbial biomass C than the control, only a slightly but not significantly higher microbial biomass was detected for the monocultures of sunflower and soybean. Compared to the unplanted control, all mixed croppings had significantly higher microbial biomass C, with values ranging from 188 mg C kg soil^{-1} for the Sun/Soy treatment to 212 mg C kg soil^{-1} for the Sun/Wh treatment. The microbial biomass C of the three-species mixture (172 mg C kg soil^{-1}) was, however, significantly lower than that measured for the Sun/Wh treatment.

Table II.2/2: Plant biomass (±SEM), $\delta^{13}C$ values (±SEM) and microbial biomass C (±SEM) compiled at the end of the experiment. N=4 for the monocultures; N=6 for the mixed cultures except for root $\delta^{13}C$ and microbial biomass C of the mixed cultures for which N=3.

			Plant Biomass			$\delta^{13}C$		Microbial biomass C
Cultures	**Treatment**	**Species**	**Shoot** [g_{DW} $plant^{-1}$]	**Root** [g_{DW} $plant^{-1}$]	**Shoot/Root**	**Shoot** [‰]	**Root** [‰]	[mg $kg\ soil^{-1}$]
	Unplanted soil							117.7±7.9a
Monoculture	**Sun**	*H. annuus*	27.9±3.7a[1]	2.7±0.3abdf[1]	10.5±1.1	-38.9±0.1ab	-38.6±0.1a	136.7±5.9ab
	Soy	*G. max*	17.0±1.7b[1]	2.0±0.2abdf[1]	8.7±1.0	-35.4±0.2b	-35.1±0.3b	140.0±8.8ac
	Wh	*T. aestivum*	10.9±0.7bc[1]	5.0±0.5abg[1]	2.2±0.1	-39.8±0.2acd	-38.6±0.4a	165.7±6.5bcd
Mixed Culture	**Sun/Soy**	*H. annuus*	35.8±1.7a	5.0±0.4a	7.3±1.0	-38.8±0.2ad	-38.9±0.2a	187.5±7.1def
		G. max	8.5±0.9bc	2.0±0.2bdf	4.2±0.7	-36.3±0.6b	-35.8±0.1b	
	Sun/Wh	*H. annuus*	31.2±1.1a	3.4±0.3abdf	9.5±1.4	-39.0±0.2d	-38.8±0.2a	211.5±4.1f
		T. aestivum	8.9±0.4bc	11.5±1.0c	0.8±0.1	-40.1±0.0c	-38.7±0.2a	
	Soy/Wh	*G. max*	10.6±2.6bc	1.3±0.4dfh	8.7±1.5	-35.8±0.1b	-35.9±0.3b	207.0±6.9fg
		T. aestivum	13.3±1.3b	9.6±1.0ce	1.4±0.1	-40.3±0.1c	-38.8±0.2a	
	Sun/Soy/Wh	*H. annuus*	28.9±1.7a	2.9±0.4ah	10.3±1.7	-39.0±0.0ad	-38.8±0.1a	
		G. max	5.0±1.1c	1.2±0.3f	4.1±0.5	-36.1±0.3b	-35.6±0.2b	172.4±5.5bceg
		T. aestivum	7.7±0.4bc	7.8±0.8eg	1.0±0.2	-40.2±0.1ac	-38.3±0.2a	

[1] dry weight per pot divided by two because two individual plants were grown in these pots.

The legume soybean showed consistently higher root N concentrations than the non-legume species (Fig. II.2/1). The N concentration of the wheat roots increased when grown in combination with soybean compared to the wheat monoculture. However, the root N concentration of sunflower was approximately 8 mg N g_{dw}^{-1} regardless of treatments and did not increase with a neighboring soybean.

Because of plant uptake, the mineral N (NH_4^+ + NO_3^-) content in the soil was roughly one order of magnitude lower for all planted treatments compared to the unplanted control at harvest (Fig. II.2/1C). There were no significant differences in soil mineral N content between any planted treatments.

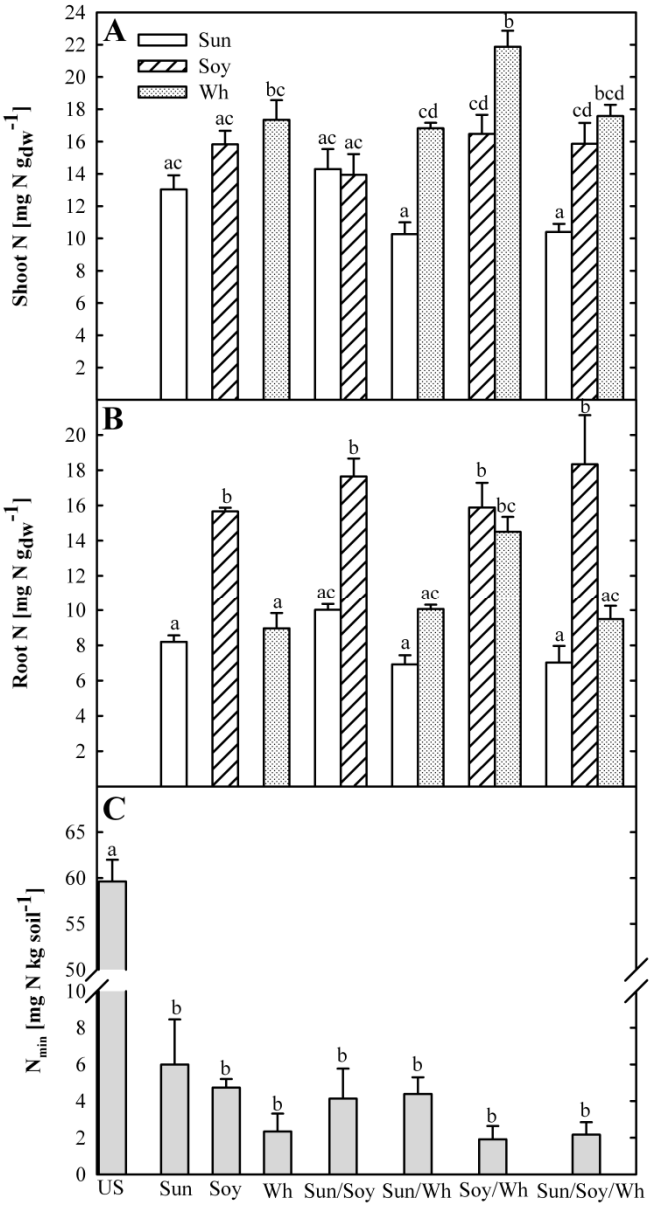


Figure II.2/1: N concentration (±SEM) of A: shoots (N=4 for the monocultures; N=6 for the mixtures) and B: roots (N=4 for the monocultures; N=3 for the mixtures). C: soil mineral N (NH_4^+ + NO_3^-) (N=4 for the monocultures; N=3 for the mixtures). Bars followed by the same lowercase letter are not significantly different at P=0.05.

2.3.2 CO_2 efflux partitioning

The contributions of SOM- and root-derived sources to total soil CO_2 efflux were calculated based on a linear two source isotopic mixing model (Fig. II.2/2). Since the $\delta^{13}C$ values of the roots differed among the species (Table II.2/2), we assumed that the rate of root-derived CO_2 per unit of root dry weight for each species was the same in monoculture and in all mixtures in order to calculate the species-weighed $\delta^{13}C_{ROOT-DERIVED}$ of the mixtures (Dijkstra et al., 2010). A significant positive correlation (R^2=0.86, N=12, P<0.001) between root-derived CO_2 and root biomass of the monocultures measured at T2 actually supported this assumption (data not shown).

Root-derived CO_2 varied between the treatments at T1 as well as at T2, probably due to varying root biomass (as indicated for T2 in Table II.2/2) (Fig. II.2/2A). A species effect was detected with low rates of root-derived CO_2 at T1 for the soybean and high rates for the sunflower monoculture. A combination of soybean and wheat resulted in a low rate of root-derived CO_2. At T2 wheat showed a very high rate of root-derived CO_2 when grown in monoculture, mainly because of its high root biomass (Table II.2/2). The species composition effect was mainly influenced by the presence of wheat, leading to high rates of root-derived CO_2. When comparing T1 with T2, the root-derived CO_2 decreased for the sunflower and soybean monoculture as well as for the mixture of both species (Sun/Soy). In contrast, the root-derived CO_2 significantly increased for the wheat monoculture and the Soy/Wh treatment. The Sun/Wh and Sun/Soy/Wh treatments did no differ significantly between T1 and T2.

SOM-derived CO_2 did not differ between the planted treatments at T1 (Fig. II.2/2B). Likewise, there is no statistically significant difference between the planted treatments in SOM-derived CO_2 at T2 with the exception of the Soy/Wh treatment showing higher values of about 15 mg C day^{-1} kg $soil^{-1}$ compared to Sun/Soy and Sun/Wh mixtures, the sunflower monoculture and the unplanted control. When comparing T1 with T2 SOM-derived CO_2 decreased significantly for the Sun and the Sun/Wh treatment but increased for the Soy/Wh treatment. No statistically significant differences between T1 and T2 could be observed for the other treatments.

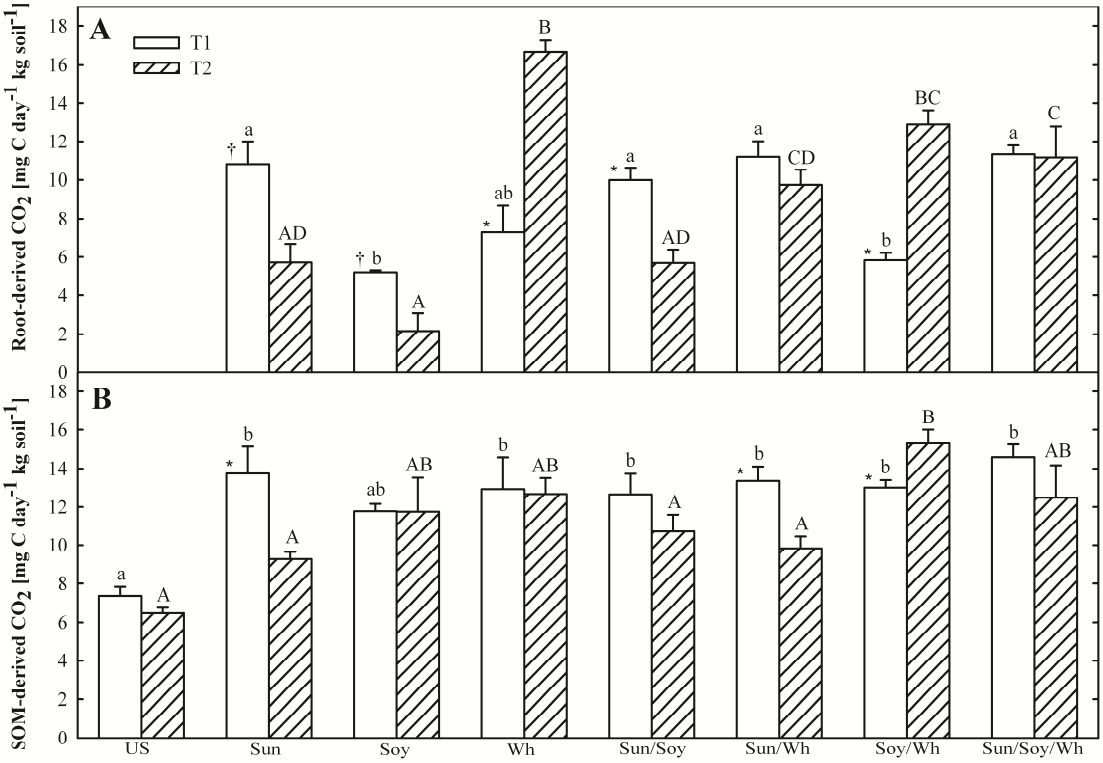


Figure II.2/2: Root-derived (A) and SOM-derived CO_2 (B) (±SEM) at T1 and T2. Significant differences between T1 and T2 within a treatment are presented as †: P<0.1 and *: P<0.05. Bars followed by different lowercase letters indicate significant differences between the treatments at T1 (P<0.05). Significant differences between the treatments at T2 are marked by different uppercase letters (P<0.05). N=4 for the unplanted soil and the monocultures; N=6 for the mixed cultures.

2.3.3 Rhizosphere priming effect

All planted treatments resulted in stimulation of SOM decomposition and hence, we found a consistently positive RPE (Fig. II.2/3). The primed C at T1 ranges from 60% of SOM-derived CO_2 of the unplanted control for the Soy treatment to 98% for the Sun/Soy/Wh treatment. At T2 the values showed a broader range from 43% of SOM-derived CO_2 of the unplanted control for the Sun treatment to 136% for the Soy/Wh mixture. The RPE did not differ significantly between the planted treatments at T1 (Fig. II.2/3). However, at T2 the Soy/Wh treatment showed a significantly higher RPE compared to Sun/Soy and Sun/Wh mixtures and sunflower monoculture. When comparing T1 with T2 no significant difference in the RPE could be detected for most treatments,

except that the Soy/Wh treatment showed a significantly higher RPE at T2 compared to T1, and that the RPE decreased significantly for the sunflower monoculture and the Sun/Wh treatment from T1 to T2.

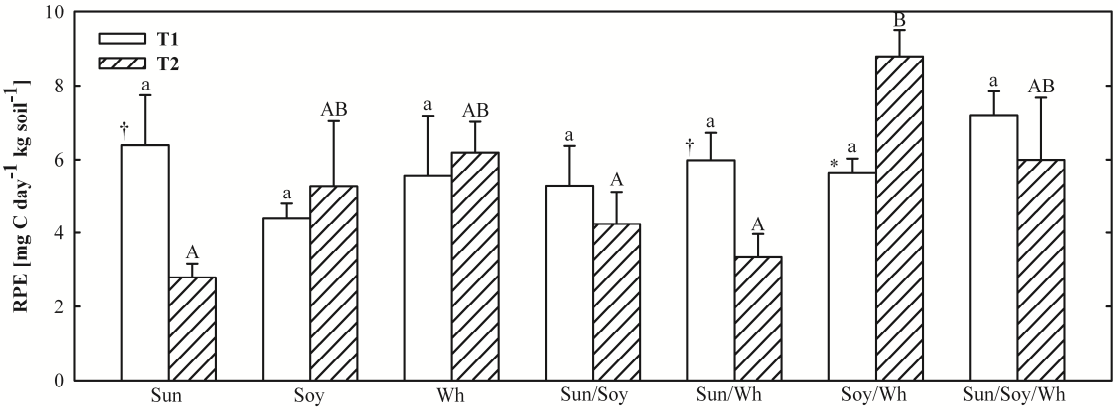


Figure II.2/3: Rhizosphere priming effect (±SEM) calculated for T1 and T2. Significant differences between T1 and T2 within a treatment are presented as †: P<0.1 and *: P<0.05. Bars followed by different lowercase letters indicate significant differences between the treatments at T1 (P<0.05). Significant differences between the treatments at T2 are marked by different uppercase letters (P<0.05). For the monocultures N=4, for the mixed cultures N=6.

2.3.4 Effect of inter-species interactions

The observed RPE was compared to an expected value calculated for the mixtures (Fig. II.2/4). The expected RPE was always slightly higher compared to the observed, but significantly higher only for the Sun/Wh treatment (Table II.2/3). Modulations of RPE by plant inter-species interactions were specific to the plant species composition and tended to inhibit the RPE. However, the replicates of the treatments showed high variations. All combinations that contained the legume soybean did not show a significant effect of inter-species interactions on the RPE suggesting that available N may be an important factor modulating RPE. In contrast to that, the rhizosphere induced decomposition of SOM was significantly inhibited when growing sunflower and wheat together.

Similar to the RPE we estimated the effect of plant inter-species interactions on MBC, N_{min} and root-derived CO_2 by comparing the observed values with the expected values (Table II.2/3). The MBC was negatively affected by mixed-cropping with the exception

of the Soy/Wh treatment where no influence could be detected (Table II.2/3). A significantly negative effect on N_{min} by mixed cropping occurred for the Sun/Soy and the Sun/Wh treatments. Root-derived CO_2 was also significantly and negatively affected by most mixed-cropings (Table II.2/3).

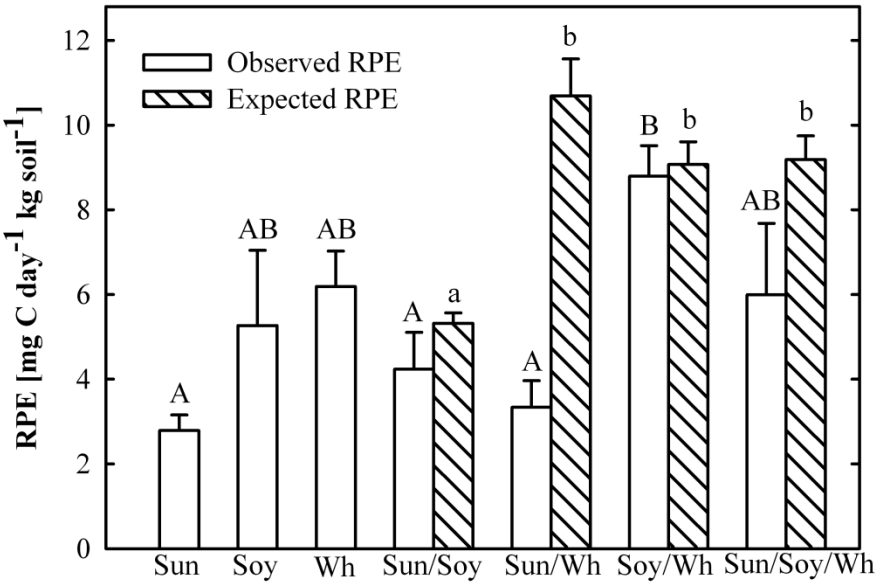


Figure II.2/4: Observed and expected RPE (±SEM). Significant differences between the observed values are marked by different uppercase letters (P<0.05). Bars followed by different lowercase letters indicate significant differences between the expected RPE (P<0.05).

Table II.2/3: Observed minus expected values (±SEM) of RPE at T2 (N=6), MBC (N=3), mineral soil N (N=3), and root-derived CO_2 (N=6).

	Observed minus expected values (±SEM)			
Treatment	**RPE [mg C day^{-1} kg soil^{-1}]**	**MBC [mg C kg soil^{-1}]**	**N_{min} [mg N kg soil^{-1}]**	**Root-derived CO_2 [mg C day^{-1} kg soil^{-1}]**
Sun/Soy	-1.1 ± 0.9	-21.6 ± 10.1†	-3.5 ± 0.3*	-0.9 ± 0.7
Sun/Wh	-7.4 ± 1.0*	-74.7 ± 21.9*	-5.4 ± 0.7*	-14.4 ± 1.8*
Soy/Wh	-0.3 ± 0.4	-4.1 ± 12.6	-1.3 ± 0.7	-4.8 ± 1.2*
Sun/Soy/Wh	-3.2 ± 1.9	-84.3 ± 15.6*	0.5 ± 0.7	-6.6 ± 2.4*

* Significant difference from zero: P<0.05. † Significant difference from zero: P<0.1.

2.4 Discussion

2.4.1 Plant species and plant phenology effects on the RPE

The type of plant species did not significantly affect the RPE on SOM decomposition, as shown by the similar RPEs of the monocultures (Fig. II.2/3). Even after normalization for root dry weight, an equivalent RPE was measured for the monocultures despite slight variations in the root biomass per pot (data not presented). However, the high variability between the replicates of one treatment (sunflower monoculture) might have masked the differences between plant species at T2. The absence of significant plant specific differences seemed contradictory to recent results which revealed a species specific effect on the RPE (Fu and Cheng, 2002; Cheng et al., 2003). Fu and Cheng (2002) reported a stronger priming effect under soybean, a N_2-fixing plant, compared to sunflower. A more pronounced RPE of soybean was also detected compared to wheat (Cheng et al., 2003). However, both studies compared the cumulative primed C over the whole growing period, which was, with more than 100 days, much longer than in our experiment. When considering only the first CO_2-trapping during the vegetative growth stage, the previous studies did not detect any effect of the plant species on RPE either. During the early stages of plant development, exudates, as a source of easily available C, may stimulate the growth and activity of rhizosphere microorganisms resulting in an increased rate of SOM decomposition (‘Microbial activation hypothesis’ Kuzyakov, 2002; Cheng and Kuzyakov, 2005). At later stages of plant development, other mechanisms controlling the RPE may gain increasing significance, such as the competition between roots and microorganisms for mineral N which may explain a negative RPE (‘Competition hypothesis’ Dormaar, 1990; Kuzyakov, 2002; Cheng and Kuzyakov, 2005).

Therefore, the plant age itself governs the amount of primed C in the rhizosphere due to changes of the exudation intensity with the growth stages (Kuzyakov, 2002; Cheng et al., 2003). The stage of plant development controls C translocation belowground, in addition to the type of plant species (Kuzyakov and Domanski, 2000). Young plants translocate more carbon to the roots, whereas older plants preferably allocate the newly assimilated C to the shoots (Keith et al., 1986; Gregory and Atwell, 1991; Palta and

Gregory, 1997) thus, leading to reduced C inputs per root biomass into the soil via exudation at older stages (reviewed by Nguyen, 2003). We found significantly higher ($P \leq 0.1$) root-derived CO_2 of the sunflower and the soybean monocultures at the vegetative stage (T1) compared to the flowering stage (T2; Fig. II.2/2). In contrast, wheat was still at the vegetative stage at the second CO_2 trapping and showed a strong increase of root-derived CO_2 as compared to the first CO_2 trapping (Fig. II.2/2). It has been reported that the rhizodeposition of annual plants increased until the end of tillering because the decrease of the exudation intensity with age is, at this stage of development, slower than the root growth (Kuzyakov, 2002). However, we found no phenological effect of wheat on the amount of primed soil C (Fig. II.2/3), likely because an increased nutrients uptake intensifies the competition between roots and microorganisms. In an experiment where the plants had developed over a longer period, a strong reduction of RPE after flowering of wheat has been reported (Cheng et al., 2003). We detected a phenology effect on RPE only for sunflower (Fig. II.2/3). The priming for sunflower was lower ($P \leq 0.1$) during flowering compared to the vegetative stage, likely due to a higher need of assimilates for flower development and hence, a lower C allocation belowground.

2.4.2 Plant inter-species interactions modify RPE

The RPE was consistently positive for all planted treatments (Fig. II.2/3). The increase in the SOM decomposition rates in the planted treatments was likely induced by inputs of organic substances via rhizodeposition, which often stimulate, as a source of easily available C, the growth of microorganisms in the rhizosphere (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Moreover, it could be assumed that with higher species richness the types of organic compounds released by plants into the soil might have increased. This would further stimulate the microbial biomass and its activity, resulting in a greater diversity of extracellular enzyme production which subsequently contributed to positive priming (Hooper et al., 2000; Spehn et al., 2000; Stephan et al., 2000; Fontaine et al., 2003; Dijkstra et al., 2010). Our results partly support this line of reasoning since all planted treatments generally resulted in higher microbial biomass C than the unplanted control, and the two-species mixtures showed higher MBC values than the monocultures. However, the three-species mixture has lower MBC compared to the Sun/Wh treatment (Table II.2/2).

Our results indicate that plant inter-species interactions can significantly modify the rhizosphere priming effect on SOM decomposition (Table II.2/3) with a tendency of reducing the root-biomass-adjusted RPE than what could be expected from their monocultures. A similar trend was also reported for five semi-arid grassland species when grown in mixture compared to monocultures even though no significant treatment differences could be detected because of their high experimental variability (Dijkstra et al., 2010). Plant species may differ in their nutrient acquisition. More diverse plant communities may better utilize limited resources such as available N (Tilman et al., 1996; Hopper and Vitousek, 1997). Hence, the plant-microbial competition, especially for mineral N, may increase with higher plant diversity leading to partial reduction of microbial activity, which is accompanied with a decrease of the RPE (Dijkstra et al., 2010).

We suggest that the competition hypothesis applies for the mixture containing sunflower and wheat, the only treatment where a significantly lower root-biomass-adjusted RPE was observed than expected (Table II.2/3). This is further supported by the lower N_{min} content of the Sun/Wh treatment than expected (Table II.2/3). An increasing competition for mineral N between roots and microorganisms may also cause the lower observed microbial biomass C compared to the expected (Table II.2/3). Moreover, the decreasing N_{min} content with time was accompanied with the reduced RPE at T2 compared to T1 (Fig. II.2/3), despite the fact that the root-derived CO_2, reflecting exudation intensity, remained constant (Fig. II.2/2). On the other hand, root-derived CO_2 was also significantly influenced by mixed cropping for all treatments containing wheat (Table II.2/3). Therefore, the intensified competition for mineral N and the lower than expected exudation intensity together suppressed the RPE of the Sun/Wh mixture compared to the monocultures. However, the exact mechanisms behind these findings remain unknown.

Our results demonstrated for the first time that mixed cropping of typical agricultural plants may reduce the decomposition of SOM compared to monocultures. Generally, this result indicates that on a longer-term C storage may be reduced through the cultivation of plants in monocultures. However, it has to be considered that the RPE strongly depends on soil properties, mainly on the organic C and mineral N content (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Agricultural soils are characterized by low contents

of decomposable C and high mineral N contents through fertilization. It was hypothesized that microorganisms, not limited in N, can switch from the decomposition of SOM to the decomposition of rhizodeposits which provide easily available energy and C for microbial activity and growth (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Thus, RPE in agricultural soils are largely controlled by this preferential substrate utilization (Kuzyakov, 2002). However, rhizosphere priming will gain increasing importance in the future in the context of sustainable agriculture and organic farming. The shift towards systems with a low external input of fertilizers increases the dependence of plants on nutrient release from SOM due to RPE (Paterson, 2003).

Apart from the agricultural point of view, inter-species interactions have implications on C and N cycling in natural ecosystems with high plant diversity, not only through altered productivity and litter inputs but also through altered RPE. The reduced priming measured in this study may contribute to a long-term increase in SOC in mixed cultures compared to monocultures.

2.5 Conclusions

During the early stage of plant development the RPE was not specific to the plant species and was positive for all planted treatments. The modulation of RPE by plant inter-species interactions was specific to the species composition. The RPE was significantly reduced for the sunflower-wheat mixture compared to the monocultures. Our data provided clear evidence that plant species composition affects the RPE. Future research is needed to identify mechanisms and clarify the role of inter-species interactions, especially among plant functional groups, on RPE.

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References

Bottner, P., Pansu, M., Sallth, Z., 1999. Modelling the effect of active roots on soil organic matter turnover. Plant and Soil 216, 15-25.

Buchmann, N., Ehleringer, J.R., 1998. CO_2 concentration profiles, and carbon and oxygen isotopes in C_3 and C_4 crop canopies. Agricultural and Forest Meteorology 89, 45-58.

Cheng, W., Dijkstra, F.A., 2007. Theoretical proof and empirical confirmation of a continuous labeling method using naturally ^{13}C-depleted carbon dioxide. Journal of Integrative Plant Biology 49 (3), 401-407.

Cheng, W., Johnson, D.W., 1998. Elevated CO_2, rhizosphere processes, and soil organic matter decomposition. Plant and Soil 202 (2), 167-174.

Cheng, W., Johnson, D.W., Fu, S., 2003. Rhizosphere effects on decomposition: Controls of plant species, phenology, and fertilization. Soil Science Society of America Journal 67, 1418-1427.

Cheng, W., Kuzyakov, Y., 2005. Root effects on soil organic matter decomposition, in: Zobel, R.W., Wright, S.F. (Eds.), Roots and Soil Management: Interactions between Roots and the Soil. ASA-SSSA, Madison, Wisconsin, pp. 119-143.

Dijkstra, F.A., Cheng, W., 2007. Moisture modulates rhizosphere effects on C decomposition in two different soil types. Soil Biology and Biochemistry 39, 2264-2274.

Dijkstra, F.A., Cheng, W., Johnson, D.W, 2006. Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. Soil Biology and Biochemistry 38, 2519-2526.

Dijkstra, F.A., Morgan, J.A., Blumenthal, D., Follett, R.F., 2010. Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. Soil Biology and Biochemistry 42, 1073-1082.

Dormaar, J.F., 1990. Effect of active roots on the decomposition of soil organic materials. Biology and Fertility of Soils 10, 121-126.

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology and Biochemistry 35, 837-843.

Fu, S., Cheng, W., 2002. Rhizosphere priming effects on the decomposition of soil organic matter in C_4 and C_3 grassland soils. Plant and Soil 238, 289-294.

Gregory, P.J., Atwell, B.J., 1991. The fate of carbon in pulse-labelled crops of barley and wheat. Plant and Soil 136, 205-213.

Harris, D., Porter, L.K., Paul, E.A., 1997. Continuous flow isotope ratio mass spectrometry of carbon dioxide trapped as strontium carbonate. Communications in Soil Science and Plant Analysis 28, 747-757.

Hooper, D.U., Bignell, D.E., Brown, V.K., Brussard, L., Dangerfield, J.M., Wall, D.H., Wardle, D.A., Coleman, D.C., Giller, K.E., Lavelle, P., Van der Putten, W.H., De Ruiter, P.C., Rusek, J., Silver, W. L., Tiedje, J.M., Wolters, V., 2000. Interactions between aboveground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. BioScience 55 (12), 1049-1061.

Hooper, D.U., Vitousek, P.M., 1997. The effects of plant composition and diversity on ecosystem processes. Science 277, 1302-1305.

Jenkinson, D.S., 1977. Studies on decomposition of plant material in soil. 5: Effects of plant cover and soil type on loss of carbon from C-14-labeled ryegrass decomposing under field conditions. Journal of Soil Science 28, 424-434.

Jones, D.L., Hodge, A., Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163, 459-480.

Keith, H., Oades, J.M., Martin, J.K., 1986. Input of carbon to soil from wheat plants. Soil Biology and Biochemistry 18, 445-449.

Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 165, 382-396.

Kuzyakov , Y., 2006. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biology and Biochemistry 38, 425-448.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421-431.

Lavelle, P., Lattaud, C., Trigo, D., Barois, I., 1995. Mutualism and biodiversity in soils. Plant and Soil 179, 23-33.

Liljeroth, E., Kuikman, P., Van Veen, J.A., 1994. Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels. Plant and Soil 161, 233-240.

Moyes, A.B., Gaines, S.J., Siegwolf, R.T.W., Bowling, D.R., 2010. Diffusive fractionation complicates isotopic partitioning of autotrophic and heterotrophic sources of soil respiration. Plant, Cell and Environment 33, 1804-1819.

Nguyen, C., 2003. Rhizodeposition of organic carbon by plant: Mechanisms and controls. Agronomie 23, 375–396.

Palta, J.A., Gregory, P.J., 1997. Drought affects the fluxes of carbon to roots and soil in ^{13}C pulse-labelled plants of wheat. Soil Biology and Biochemistry 29 (9/10), 1395-1403.

Paterson, E., 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. European journal of Soil Science 54, 741-750.

Rochette, P., Flanagan, L.B., Gregorich, E.G. 1999: Separating soil respiration into plant and soil components using analyses of the natural abundance of carbon-13. Soil Science Society of America Journal 63, 1207-1213.

Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2011. Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depending on plant community composition. Applied Soil Ecology 48, 38-44.

Sanaullah, M., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2012. Carbon allocation in grassland communities under drought stress followed by ^{14}C pulse labelling. Soil Biology and Biochemistry 55, 132-139.

Schimel, J.P., Jackson, L.E., Firestone, M.K., 1989. Spatial and temporal effects on plant-microbial competition for inorganic nitrogen in a California annual grassland. Soil Biology and Biochemistry 21 (8), 1059-1066.

Shields, J.A., Paul, E.A., 1973. Decomposition of ^{14}C-labeled plant material under field conditions. Canadian Journal of Soil Science 53, 297-306.

Søe, A.R.B., Giesemann, A., Anderson, T.-H., Weigel, H.-J., Buchmann, N., 2004. Soil respiration under elevated CO_2 and its partitioning into recently assimilated and older carbon sources. Plant and Soil 262, 85-94.

Stephan, A., Meyer, A.H., Schmid, B., 2000. Plant diversity affects culturable soil bacteria in experimental grassland communities. Journal of Ecology 88, 988-998.

Tilman, D., Wedin, D., Knops, J., 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379, 718-720.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19 (9) 703-707.

Van der Krift, T., Kuikman, P.J., Möller, F., Berendse, F., 2001. Plant species and nutritional-mediated control over rhizodeposition and root decomposition. Plant and Soil 228, 191-200.

Von Felten, S., Hector, A., Buchmann, N., Niklaus, P.A., Schmid, B., Scherer-Lorenzen, M., 2009. Belowground nitrogen partitioning in experimental grassland plant communities of varying species richness. Ecology, 90, 1389-1399.

Wang, J.G., Bakken, L.R., 1997. Competition for nitrogen during decomposition of plant residues in soil: Effect of spatial placement of N-rich and N-poor plant residues. Soil Biology and Biochemistry 29, 153-162.

Werth, M., Kuzyakov, Y., 2010. ^{13}C fractionation at the root–microorganisms–soil interface: a review and outlook for partitioning studies. Soil Biology and Biochemistry 42, 1372-1384.

Wu, J., Jörgensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass-C by fumigation-extraction – an automated procedure. Soil Biology and Biochemistry 22, 1167-1169.

Zhu, B., Cheng, W., 2011. ^{13}C isotope fractionation during rhizosphere respiration of C_3 and C_4 plants. Plant and Soil 342, 277–287.

3 Effect of clipping and shading on C allocation and fluxes in soil under ryegrass and alfalfa estimated by ^{14}C labeling

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Abstract

Photosynthesis of higher plants drives carbon (C) allocation belowground and controls the supply of assimilates to roots and to rhizosphere microorganisms. To investigate the effect of limited photosynthesis on C allocation, redistribution and reutilization in plant and soil microorganisms, perennial grass *Lolium perenne* and legume *Medicago sativa* were clipped or shaded. Plants were labeled with three ^{14}C pulses to trace allocation and reutilization of C assimilated before clipping or shading. Five days after the last ^{14}C pulse, plants were clipped or shaded and the total CO_2 and $^{14}CO_2$ efflux from the soil was measured. ^{14}C in above- and belowground plant biomass and bulk soil, rhizosphere soil and microorganisms was determined 10 days after clipping or shading.

After clipping, 2% of the total assimilated ^{14}C originating mainly from root reserves were detected in the newly grown shoots. This corresponded to a translocation of 5% and 8% of total ^{14}C from reserve organs to new shoots of *L. perenne* and *M. sativa*, respectively. The total CO_2 efflux from soil decreased after shading of both plant species, whereas after clipping, this was only true for *L. perenne*. The $^{14}CO_2$ efflux from soil did not change after clipping of both species. An increased $^{14}CO_2$ efflux from soil after shading for both plants indicated that lower assimilation was compensated by higher utilization of the reserve C for root and rhizomicrobial respiration.

We conclude that C stored in roots is an important factor for plant recovery after limiting photosynthesis. This stored C is important for shoot regrowth after clipping, whereas after shading, it is utilized mainly for maintenance of root respiration. Based on our results as well as on a review of several studies on C reutilization for regrowth after clipping, we conclude that because of the high energy demand for nitrogen fixation, legumes use a higher portion (9% to 10%) of stored C for regrowth compared to grasses (5% to 7%). The effects of limited photosynthesis were of minor importance for the exudation of the reserve C and thus, have no effect on the uptake of this C by microorganisms.

Keywords: Carbon allocation and partitioning, isotope labeling, grazing effects, assimilate reutilization, shoot regrowth, CO_2 sources, photosynthesis reduction, rhizosphere processes.

3.1 Introduction

Belowground translocation of carbon (C) by plants and its turnover in soils are important processes affecting the global C cycle. Thus, in the last decades, many studies have investigated the distribution and dynamics of assimilates in the plant-soil system, their utilization by microorganisms and contribution to carbon dioxide (CO_2) efflux. It has been shown that pasture plants translocate 30% to 50% of assimilated C belowground. Approximately half of this C is incorporated into the root biomass, 12% remains in the soil and microbial biomass, and 36% is respired by roots or microorganisms, whereby about 5% of the fixed C is respired by mycorrhizas (Johnson et al., 2002; Kuzyakov and Domanski, 2000; Leake et al., 2006). Roots contribute 30% to 70% of the soil CO_2 efflux (Schlesinger, 1977; Subke et al., 2006), which is the second largest C flux in terrestrial ecosystems and accounts for 60% to 90% of ecosystem respiration (Goulden et al., 1996; Longdoz et al., 2000). Rhizodeposition is an important driver for many processes in terrestrial ecosystems, such as nutrient availability for plants, activity and turnover of microbes (Blagodatskaya et al., 2010) in addition to turnover of soil organic matter (Merbach et al., 1999).

The belowground translocation of recently assimilated C is a very rapid process. The highest exudation rate of photosynthates by wheat roots is reached 2 to 3 hours after fixation, declining to a third of the maximum after 5 hours (Dilkes et al., 2004). Also for the grass *Nardus stricta* a fast transport of recent assimilates to soil and DOC has been reported (Johnson et al., 2011). In a tree girdling experiment, a large decrease in soil respiration was observed after disrupting assimilate transport to the roots (Högberg et al., 2001). These studies indicate that current photosynthesis and the supply of recent assimilates to roots are the main drivers for rhizodeposition and soil respiration (Kuzyakov and Gavrichkova, 2010). Thus, any alteration in environmental factors affecting photosynthetic activity, and thereby influencing availability of recent assimilates, is assumed to influence fast C pools and fluxes of plant-derived C, such as dissolved organic matter, soil CO_2 or microbial biomass. Defoliation by grazing (Detling et al., 1979) and shading are factors that reduce the photosynthesis rate due to lower leaf surface areas and less available light, respectively. It has been shown that defoliation increases the sink strength of regrowing leaves and, therefore, reduces C allocation be-

lowground (Detling et al., 1979; Mackie-Dawson, 1999). On the contrary, Holland et al. (1996) found a positive relationship between herbivory and belowground C allocation for *Zea mays*. Defoliation by grazing affects plant biomass and soil respiration, depending on the grazing intensity, history and composition of vegetation (Cao et al., 2004; Milchunas and Lauenroth, 1993). Thus, grazing management can play an important role in C economy of grasslands.

Less is known about the effect of shading on the redistribution of C reserves. A rapid reduction of C reserves under low light conditions due to limited C supply has been observed (Merlo et al., 1994). Low light intensity decreased the root-to-shoot ratio (R:S) of *Zea mays* (Lambers and Posthumus, 1980), whereas an increase was observed for *Lolium perenne* (Hodge et al., 1997). To compensate temporary limited photosynthesis by defoliation or shading, plants are able to store C. Although both defoliation and reduced light intensity lead to reduced assimilation, it is assumed that because of the removal of plant biomass caused by defoliation, they have different impacts on the redistribution of stored C and thus on the C input into the soil and the C availability for soil microorganisms.

C allocation in plant and soil is also affected by plant properties. During plant development, the portion of C stored in shoots increases, leading to a decrease in belowground translocation (Gregory and Atwell, 1991; Keith and Oades, 1986; Meharg and Killham, 1990). Furthermore, C allocation patterns differ between plant species. The relative belowground translocation of C of perennial plants is higher compared to annual plants. This indicates a higher C storage in roots of perennial plants, whereas annual plants allocate more C in aboveground parts, especially grains (Kuzyakov and Domanski, 2000). Warembourg et al. (2003) investigated the C input into the rhizosphere of 12 Mediterranean plants. They found significant species-dependent differences in the belowground allocation of assimilated C, with portions ranging from 41% to 76%. Among functional plant groups, legumes use the highest C portion for rhizosphere respiration compared to grasses and especially to non-legume forbs (Warembourg et al., 2003). This is because of the high energy requirement and consequently high C demand for N_2 fixation by symbiotic rhizobia (Philips, 1980). Estimations give evidence that about 6 mg of C are necessary to fix 1 mg of nitrogen (N) (Vance and Heichel, 1991). The respiration losses tied to N_2 fixation can account for up to 70% of total root respiration

(Witty et al., 1983). Thus, because of the high C costs for N_2 fixation, we hypothesized that changing rates of photosynthesis provoked different effects between a legume species (*Medicago sativa* L.) and a non-legume species (*Lolium perenne* L.) regarding the distribution of assimilates.

Using repeated $^{14}CO_2$ labeling of two plant species, *M. sativa* and a *L. perenne*, we investigated how defoliation (simulated grazing) and shading affected C allocation within the plant, belowground C translocation and reutilization of stored C. The specific questions were:

(1) How does clipping and shading affect biomass production and ^{14}C distribution between various pools?

(2) Which plant parts provide C for growth of new shoots after clipping?

(3) How does limited photosynthesis after clipping or shading alter the redistribution of stored C in plant, soil, microorganisms and soil CO_2?

(4) Do clipping and shading induce different responses with respect to the redistribution of stored C in the plant and soil pools?

3.2 Materials and Methods

3.2.1 Soil properties and plant growing conditions

Plants were grown on an arable loamy haplic Luvisol developed on loess. This soil was collected near Göttingen (Germany, 51°33´36.8´´N, 9°53´46.9´´E) from the upper 10 cm of the Ap horizon. The basic characteristics of the soil are shown in Table II.3/1.

Seeds of ryegrass (*Lolium perenne* L.) and alfalfa (*Medicago sativa* L.) were germinated on wet filter paper in Petri dishes. After 5 (*M. sativa*) and 8 days (*L. perenne*), the seedlings were transferred to pots (inner diameter 7 cm, height 20 cm), each filled with 700 g of air-dried, sieved (≤ 2 mm) soil. For *M. sativa*, each pot contained 3 plants and for *L. perenne* 5 plants, because of the lower biomass of *L. perenne*. In total 12 pots per plant species were prepared for the experiment. The pots were closed with a plastic lid with holes for shoots. The plants were grown at 26° to 28°C day temperature and at 22° to 23°C night temperature with a day-length of 14 h and a light intensity of approximately 211 µmol $m^{-2}s^{-1}$. Thus, the cumulative daily radiation was approximately in the range of field conditions. The soil water content was measured gravimetrically and adjusted daily to 70% of the available field capacity.

Parameter	**Value**
N_{tot} (mg g^{-1})	1.200
C_{org} (mg g^{-1})	11.700
C/N	9.76
NO_3^- (mg g^{-1})	0.083
P (mg g^{-1})	0.160
S (mg g^{-1})	0.009
CEC ($mmol_c$ kg^{-1})	108.000
BS (%)	99.700
Texture[1]	
clay/silt/sand (% w/w))	7.0/87.2/5.8
pH (H_2O)	6.600
pH ($CaCl_2$)	6.000

Table II.3/1: Basic characteristics of the soil sampled from the A_p horizon of a haplic Luvisol near Göttingen (Germany) (Kramer et al., 2012). CEC: Cation exchange capacity; BS: Base saturation.

[1]Texture according to the German classification system.

3.2.2 ^{14}C labeling procedure

Repeated ^{14}C pulse labeling was used to evaluate C reutilisation and C input into the soil. All plants of one species (12 pots) were labeled simultaneously in a $^{14}CO_2$ atmosphere on days 35, 40 and 45 after planting. The day before the first labeling, holes in the plastic lids were sealed around the shoots with silicone paste (NG 3170, Thauer & Co., Dresden) and checked for air tightness. For labeling, the plants were placed in an acryl glass chamber. The chamber and the labeling technique are described in detail elsewhere (Werth and Kuzyakov, 2008). Briefly, the chamber was connected by tubing with a flask containing 10 ml of diluted $Na_2{}^{14}CO_3$ (ARC Inc., USA) solution with 1.67 MBq. $^{14}CO_2$ was released into the chamber by adding 3 ml of 5 M H_2SO_4 to the labeling solution. Plants were labeled during 3 hours in the $^{14}CO_2$ atmosphere. Thereafter, the air from the chamber was pumped through 15 ml of 1 M NaOH solution for 2 hours to trap the remaining unassimilated $^{14}CO_2$. Finally, plants were removed from the chamber and grown under normal conditions until the next $^{14}CO_2$ pulse.

3.2.3 Clipping and light reduction

Both plant species were subjected to reduced light or clipping 5 days after the last $^{14}CO_2$ pulse because it was assumed that after this period, the distribution of assimilated C between above- and belowground pools was mostly complete (Domanski et al., 2001). Consequently, the translocated ^{14}C found in the various pools after shading or clipping was considered as remobilized reserve C. This is in agreement with Danckwerts and Gordon (1987) who found that assimilated ^{14}C reached its final destination within 4 to 6 days and termed this ^{14}C as reserve C. For clipping, the shoots were cut 4 cm above the soil surface for *L. perenne* and 8 cm for *M. sativa*. We used 4 replicates for each species. Different clipping heights were applied to achieve a similar stubble biomass of both plant species. Subsequently, plants continued growth under normal conditions. For shading, 4 planted pots of both species were exposed to a reduced light intensity of about 17 $\mu mol\ m^{-2}\ s^{-1}$ for 10 days. In addition, 4 pots per species were kept under normal conditions and used as controls with untreated plants (no reduced light and no clipping). All pots, including the controls, were harvested 10 days after the clipping or the beginning of light reduction.

3.2.4 Sampling

At harvest, aboveground biomass of all treatments was divided into 'shoot' (biomass above the cutting height of 4 cm or 8 cm) and 'stubble' (biomass between cutting height of shoots and soil surface). Furthermore, the shoots of the clipped plants were divided into 'clipped shoots' (the shoots already cut 5 days after labeling) and 'regrown shoot' (the shoots cut at harvest). Roots were separated from the soil by tweezers. To separate rhizosphere soil and bulk soil, the roots were slightly shaken and the remaining soil attached to the roots was accepted as rhizosphere soil.

To determine the impact of clipping and shading on the dynamics of soil CO_2 efflux, the soil air was trapped in 15 ml of 1 M NaOH solution by pumping with a membrane pump. Sampling of CO_2 started directly after the first $^{14}CO_2$ pulse. The NaOH solution was changed 3 times after each labeling (day 1, 3 and 5 after each labeling) and 6 times after clipping or the beginning of the light reduction (day 1, 3, 5, 6, 8 and 10 after the treatments).

3.2.5 Sample analysis

All plant and soil samples were dried at 65°C for 3 days, weighed and ground in a ball mill. Prior to liquid scintillation counting (LSC) for ^{14}C analyses, the solid samples (50 mg of plant material, 500 mg of soil) were combusted in an oxidizer unit (Feststoffmodul 1300, AnalytikJena, Germany) at 900°C. The CO_2 released during combustion was trapped in 10 ml of 1 M NaOH. 2 ml aliquots of the NaOH solution were mixed with 4 ml of the scintillation cocktail Rotiszint Eco Plus (Carl Roth, Germany). After decay of chemiluminescence, the ^{14}C activity was measured by means of LSC (LS 6500 Multi-Purpose Scintillation Counter, 217 Beckman, USA). The ^{14}C activity of $^{14}CO_2$ trapped in NaOH solution during the experiment was measured in 1 ml aliquots added to 2 ml scintillation cocktail Rotiszint Eco Plus (Carl Roth, Germany) after decay of chemiluminescence. The ^{14}C measurements were carried out with an LSC (MicroBeta TriLux, 205 Perkin Elmer Inc., USA). The total C content in trapped CO_2 was determined by titration of the NaOH solution with 0.01 M HCl against Phenolphthalein after addition of 1.5 M $BaCl_2$ solution.

Total C and ^{14}C incorporated into the microbial biomass in the bulk soil and rhizosphere soil during the experiment were analyzed by the chloroform-fumigation extraction method (CFE) (modified after Vance et al., 1987). 5 g of fresh soil were extracted with 20 ml of 0.05 M K_2SO_4 solution. Another 5 g of soil were first fumigated with ethanol-free chloroform for 24 hours and then extracted in the same way. Both extracts were shaken for 1 h at 200 rpm and then centrifuged for 10 min at 3070 rpm. The extracts were frozen until analysis of total C and ^{14}C. The total C content in the extracts of the fumigated and unfumigated soil samples was measured using an N/C analyzer (Multi N/C 2100, AnalytikJena, Germany). The ^{14}C activity of the extracts was measured by means of an LSC (LS 6500 Multi-Purpose Scintillation Counter, 217 Beckman, USA) as described for plant and soil material.

3.2.6 Calculations and statistics

The ^{14}C activity in shoots, stubbles, roots, bulk soil, rhizosphere soil, microbial biomass and in CO_2 efflux are presented as percentage of total recovered ^{14}C. Specific ^{14}C activities are expressed as kBq g^{-1} dry weight for shoots, stubbles, roots and soil samples, and as kBq g^{-1} C for CO_2 and microbial biomass. The total C and ^{14}C in microbial biomass was calculated by dividing the microbial C flush (difference between extractable C from fumigated and unfumigated soil samples) with a k_{EC} factor of 0.45 (Wu et al., 1990).

The experiment was conducted with 4 replicates for all treatments. All results are presented as mean values with standard errors of the mean. If the standard error exceeded the mean by more than 10%, the replicate with the highest deviation was not considered. Significances between the treatment and the plant species were obtained by a two-factor analysis of variance (ANOVA) in combination with a *post hoc* Newman-Keuls test as least significant differences between the means (LSD; $P<0.05$).

3.3 Results

3.3.1 Plant biomass production

Plants of *M. sativa* produced significantly more shoot biomass as well as stubble biomass compared to *L. perenne* (Fig. II.3/1). Only after shading the stubble biomass was the same for both plant species. *M. sativa* had slightly higher root biomass compared to *L. perenne*, resulting in a slightly higher R:S ratio by *L. perenne* (Table II.3/2).

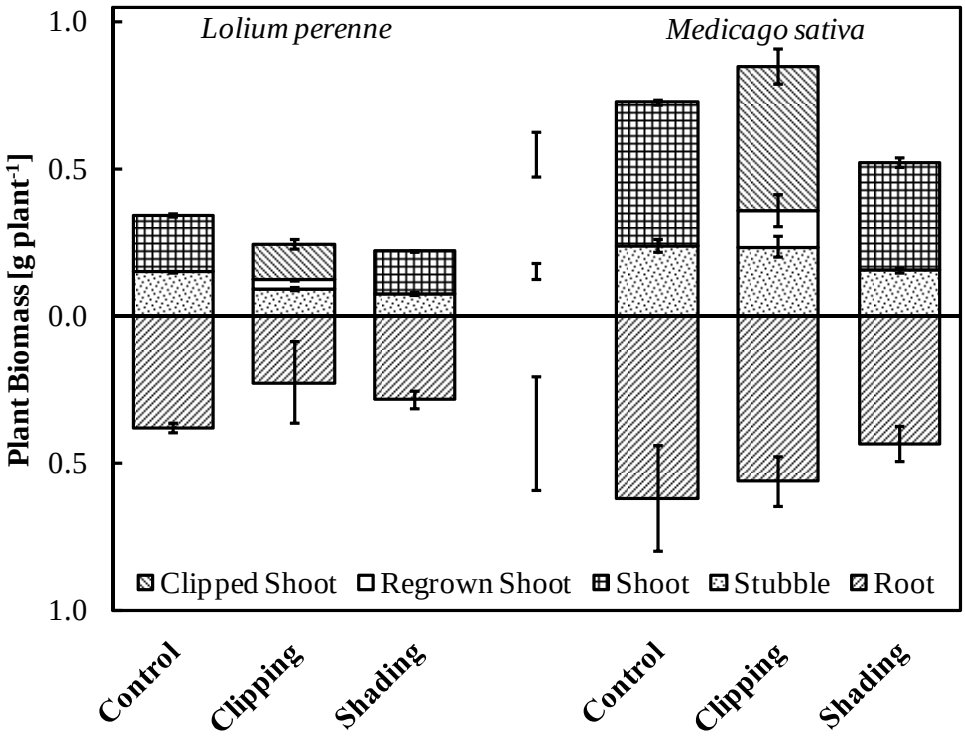

Figure II.3/1: Aboveground and belowground plant dry mass (±SEM) of 60 days old *L. perenne* and *M. sativa* 10 days after clipping or shading. LSD values ($P<0.05$) are presented as whisked segments.

Table II.3/2: Root-to-shoot (R:S) ratio (±SEM) of *L. perenne* and *M. sativa* 10 days after clipping and shading. The statistical analyses showed no significant differences between the results.

	R:S ratio	
	Lolium perenne	***Medicago sativa***
Control	1.12±0.06	0.85±0.25
Clipping	1.00±0.60	0.61±0.10
Shading	1.23±0.14	0.84±0.12

Clipping caused an increase in shoot biomass (including clipped shoots) of *M. sativa* after 10 days of regrowth. These results indicate faster regrowth of *M. sativa* compared to *L. perenne*. For the stubble biomass, a significant decrease after clipping was observed only for *L. perenne*, while there was no change for *M. sativa*. Shading for 10 days reduced the biomass of the stubbles of both plant species (Fig. II.3/1). The amount of root biomass showed no significant differences between the different treatments, and thus, also the R:S ratio was unaffected (Table II.3/2).

3.3.2 Distribution of ^{14}C in plant and soil pools

The amount of C allocated into shoots, stubbles, roots, bulk soil and rhizosphere soil was determined as percentage of total ^{14}C recovery and as ^{14}C specific activity. The ^{14}C specific activity of a pool allowed comparison of C allocation with respect to the pool size, while ^{14}C recovery within this pool showed the allocation of total C after the start of labeling and thus reflected the effect of clipping and shading.

About 50% of the recovered ^{14}C was found in the aboveground biomass for both plant species (Fig. II.3/2). Except for the control plants, where the ^{14}C recovery in the shoots was higher for *M. sativa* than *L. perenne* there was no difference in the shoot ^{14}C recovery between both plant species. The ^{14}C recovery in the roots reached about 20% for *M. sativa* and, depending on the treatment, between 6% and 15% for *L. perenne* (Fig. II.3/2). ^{14}C recovery for the stubbles was nearly identical for both species as well as between the treatments and ranged from about 10% to 15%.

Translocation of reserve C to newly grown shoots after clipping was measured by ^{14}C in the regrown shoots. The reserve C used for shoot regrowth contributed about 2% of total ^{14}C recovery for both plants. After clipping, there was no significant change of ^{14}C recovery and ^{14}C specific activity in the stubbles and in the roots (Fig. II.3/2 and II.3/3). However, a relative ^{14}C decrease in the roots of *L. perenne* was observed, indicating that roots are a probable source of reused C reserves after clipping.

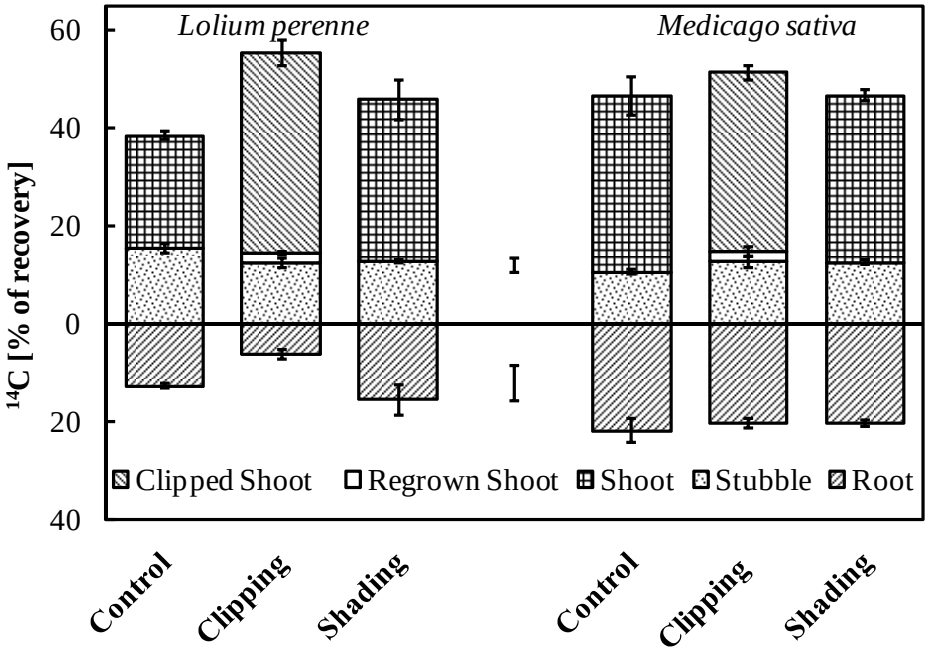

Figure II.3/2: ^{14}C recovery (±SEM) in the above- and belowground plant parts 10 days after clipping or shading of 60 days old *L. perenne* and *M. sativa* presented as portions of ^{14}C recoveries. LSD values ($P<0.05$) are presented as whisked segments.

There was no effect of shading on the ^{14}C recovery as compared to the controls (Fig. II.3/2). However, due to lower amounts of aboveground biomass (Fig. II.3/1) and a lower assimilation of new C compared to plants grown under control conditions, ^{14}C specific activity of the stubble and shoots of *L. perenne* grown under reduced light was higher than under normal light conditions. For *M. sativa*, however, this increase was only observed for the stubbles (Fig. II.3/3). There was no change in the ^{14}C specific activity in roots.

In the control and the shaded plants, higher portions of ^{14}C were recovered in the rhizosphere of *L. perenne* compared to *M. sativa* (Fig. II.3/4A). Clipping and shading showed no significant effects on ^{14}C recovery in the soil pools of both plants compared to their respective control plants (Fig. II.3/4B). ^{14}C recovery and specific activity in the microbial biomass was similar for both plant species and was unaffected by clipping and shading (Fig. II.3/4A).

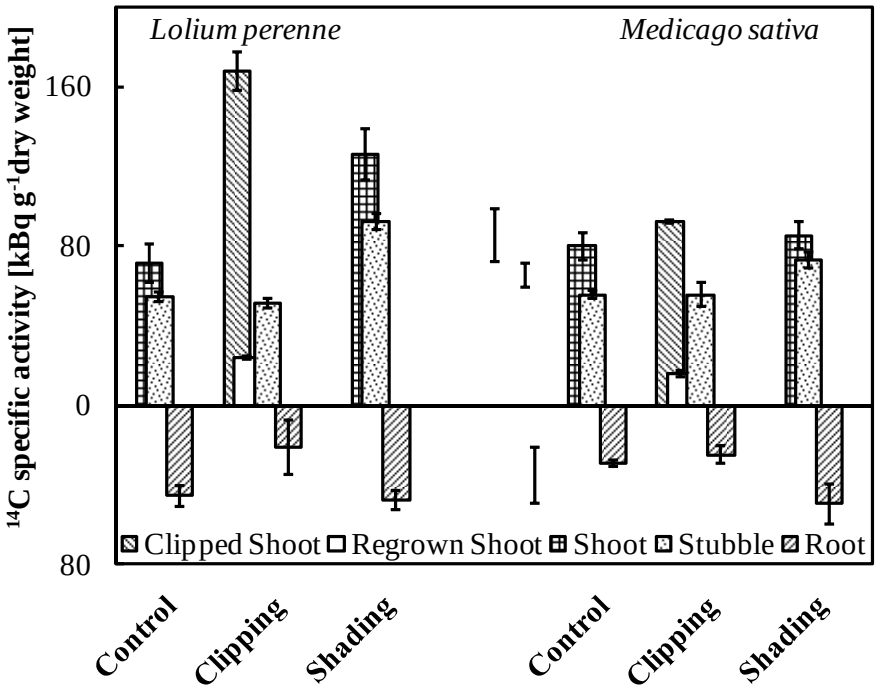

Figure II.3/3: ^{14}C specific activity (±SEM) of aboveground and belowground plant parts for different treatments 10 days after clipping or shading. LSD values ($P<0.05$) are presented as whisked segments.

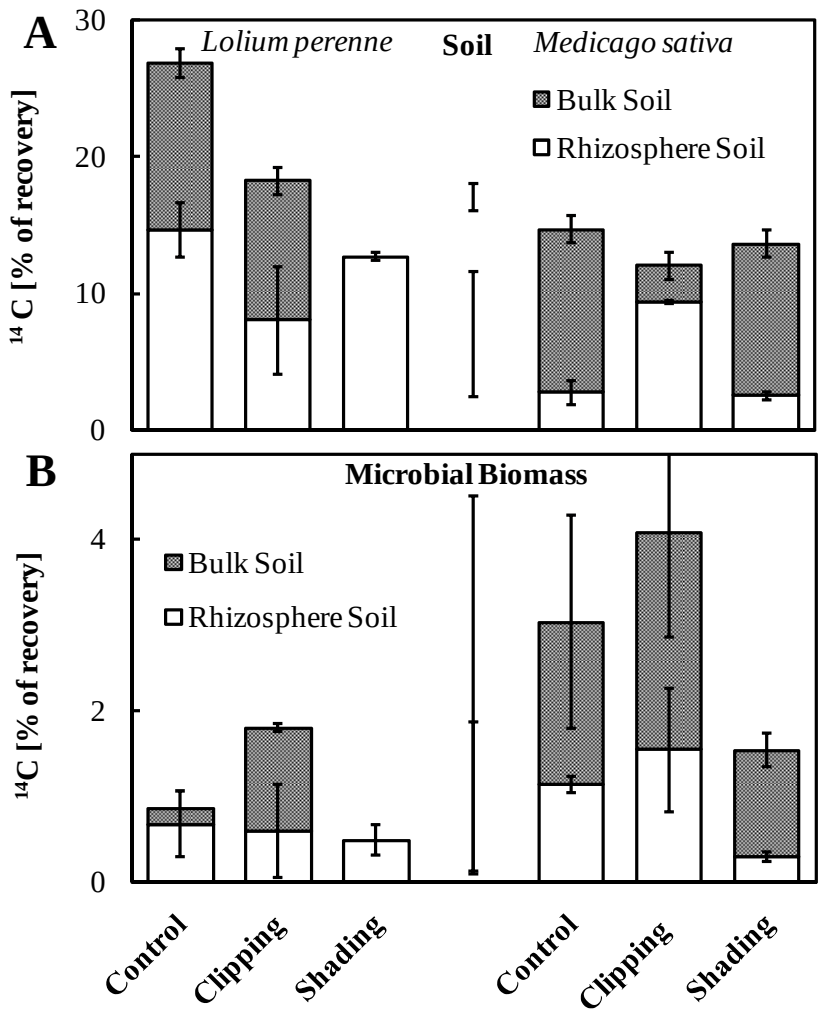

Figure II.3/4: ^{14}C recovery (±SEM) in soil (A) and microbial biomass (B) under *L. perenne* and M. *sativa* 10 days after clipping or shading. LSD values ($P<0.05$) are presented as whisked segments. Soil of shaded *L. perenne* was completely rooted and therefore no data for bulk soil are available.

3.3.3 Total CO_2 and ^{14}C efflux from soil

The cumulative CO_2 efflux from soil under *L. perenne* was highest for the control treatments (Fig. II.3/5A). The reduced availability of assimilates after clipping or shading decreased the CO_2 efflux, with a larger decrease after clipping. For *M. sativa*, soil CO_2 efflux was also reduced after clipping or shading (Fig. II.3/5B). However, after clipping this was only observed for 5 days and after 10 days, it reached the same level as that of control plants. The lowest amounts of soil CO_2 for *M. sativa* were observed

after shading. Comparing both plant species, total soil CO_2 efflux was higher for *M. sativa* than for *L. perenne.*

The percentage of ^{14}C recovery in the CO_2 efflux increased in response to clipping under *L. perenne*, whereas it showed no significant change after reducing light (Fig. II.3/6A). ^{14}C specific activity, calculated as mean of the time between the beginning of treatment and harvest, was higher under *M. sativa* than under *L. perenne* for clipped plants and shaded plants (Fig. II.3/6B). Clipping increased the ^{14}C specific activity of the soil CO_2 efflux under *M. sativa*, whereas there was no effect under *L. perenne*. After shading, an increase in ^{14}C specific activity of CO_2 was observed for both plant species. In contrast to clipping, the remobilization of reserve C may play a more important role in maintaining respiration after shading.

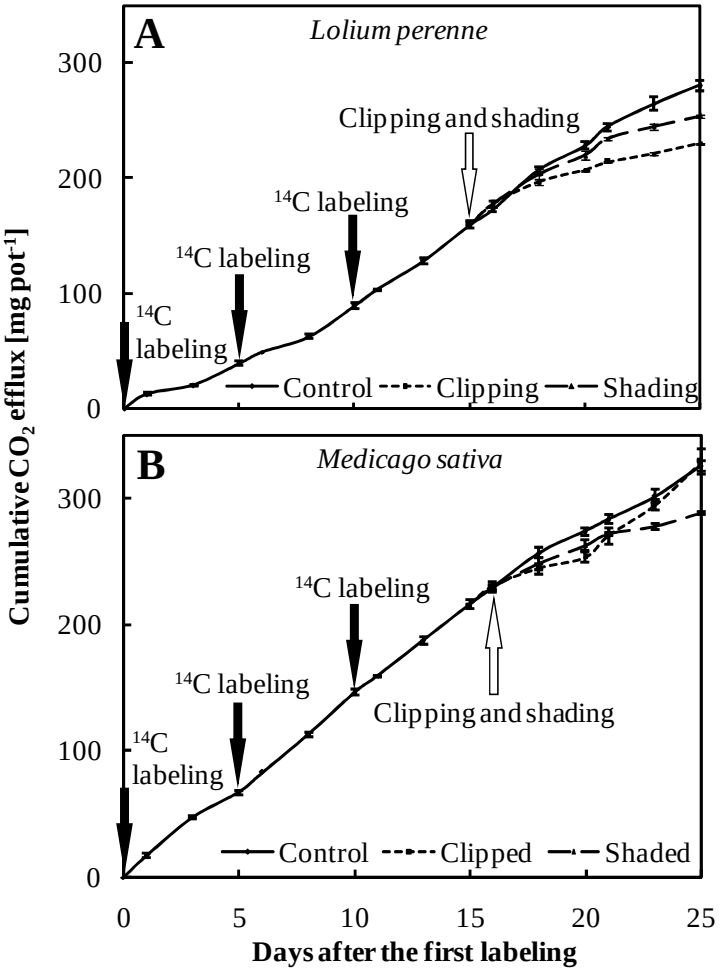

Figure II.3/5: Cumulative C-CO_2 efflux (±SEM) from soil under *L. perenne* (A) and *M. sativa* (B) beginning after the first ^{14}C labeling and the effect of clipping and shading on the CO_2 efflux.

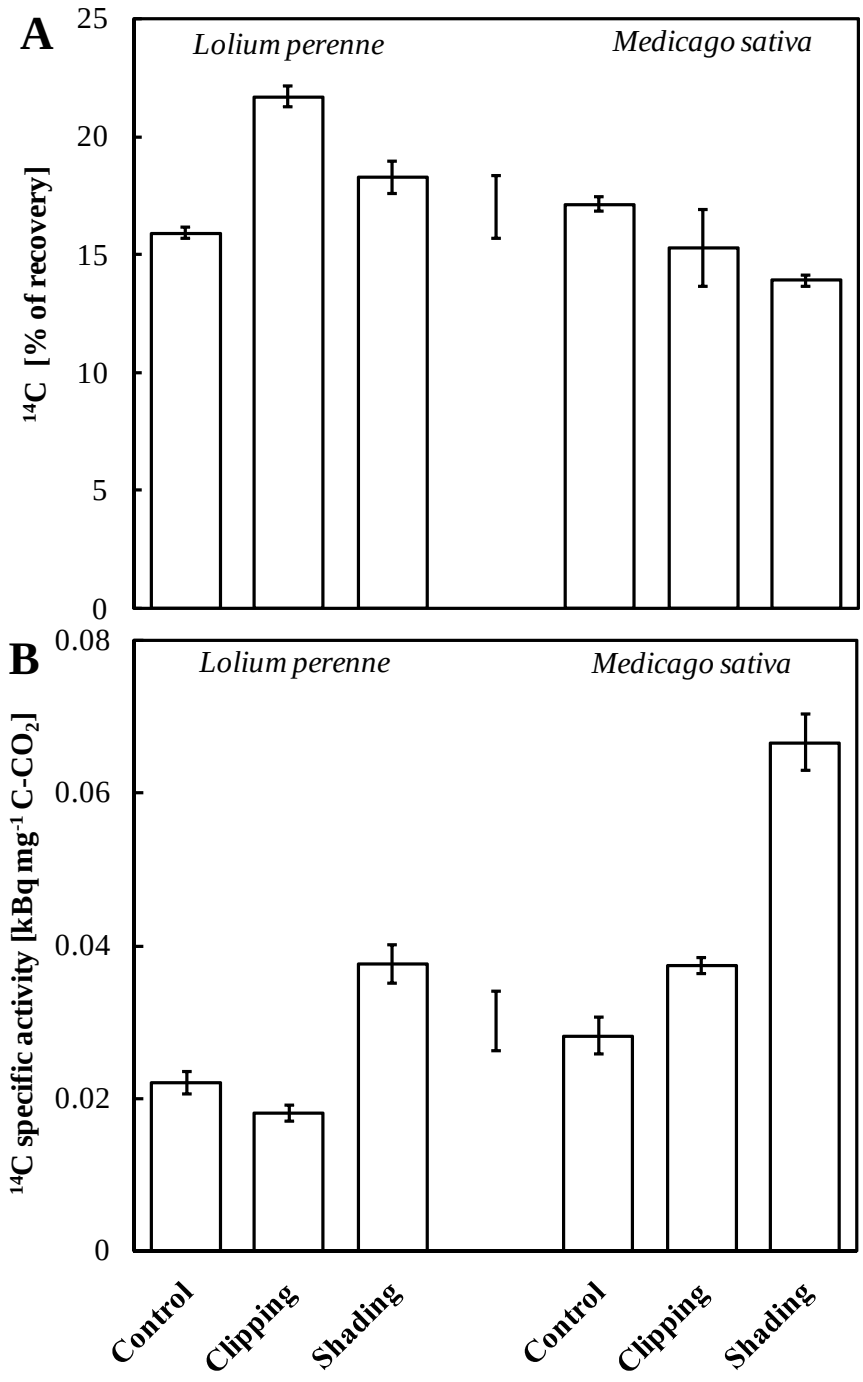

Figure II.3/6: ^{14}C recovery (±SEM) in CO_2 efflux from soil under *L. perenne* and *M. sativa*, calculated from the cumulated ^{14}C efflux (A), and mean value of ^{14}C specific activity (±SEM) of the soil CO_2 under *L. perenne* and *M. sativa* measured from clipping or shading until harvest (B). LSD value ($P<0.05$) is presented as whisked segment.

3.4 Discussion

3.4.1 C allocation by *Lolium perenne* and *Medicago sativa*

The biomass of the aboveground plant parts and roots was higher for *M. sativa* than for *L. perenne* (Fig. II.3/1). These results are in accordance with the higher ^{14}C recovery found in shoots of the control of *M. sativa* compared to *L. perenne* (Fig. II.3/2). The lower R:S ratio of *M. sativa* showed that this legume allocates more C in its aboveground biomass, whereas C allocation in roots is higher for the non-legume *L. perenne*. This is also supported by the higher specific ^{14}C activity of the roots of *L. perenne*. The higher ^{14}C recovery found in the soil under *L. perenne* compared to *M. sativa* (Fig. II.3/4) can be explained by a higher investment of *L. perenne* for rhizodeposition since an enhanced rhizodeposition leads to increased nutrient availability for roots (Kuzyakov, 2002), which is of more importance for non-legumes than for legumes. On the other hand, legumes have higher C costs for N_2 fixation estimated as between 4% and 12% of photosynthesis (Lambers, 1987), resulting in higher root and rhizomicrobial respiration. Thus, the higher soil CO_2 efflux of *M. sativa* compared to the non-legume *L. perenne* (Fig. II.3/5) can be explained by higher root respiration to maintain N_2 fixation.

3.4.2 Redistribution of stored C in plant pools

The results of 28 studies investigating the effect of defoliation on growth of grasses and herbs were reviewed by Ferraro and Oesterheld (2002). Most plant species decrease their biomass production after defoliation, depending on (a) the recovery period after the last defoliation, (b) the time interval between defoliation events and (c) N availability. In our study, the aboveground biomass (including clipped shoots) of *L. perenne* was reduced after clipping, whereas that of *M. sativa* was increased (Fig. II.3/1). A trend of biomass reduction of *L. perenne* roots was observed after clipping because of higher herbivory tolerance of *L. perenne* compared to *M. sativa* (Counce et al., 1984). For herbivory-tolerant grass species, defoliation-induced reduction of root growth was a consequence of allocation of assimilates to support shoot regrowth (Guitian and Bardgett, 2000). The decreased R:S ratio of both plant species indicated assimilate translocation from roots to shoots after clipping (Table II.3/2).^{14}C was found in the newly grown shoots of both species. This is supported by many other studies that have labeled grasses

with ^{14}C or ^{13}C (Johansson, 1993; Kuzyakov et al., 2002; Morvan-Bertrand et al., 1999). The ^{14}C in the shoot must have been translocated from the stubbles or roots left after clipping. The translocation of C is very important for the growth of new tissue since 91% of the C in these plant parts is derived from reserves (Morvan-Bertrand et al., 1999). Five and 8% of ^{14}C in *L. perenne* and *M. sativa*, respectively, were translocated from storage pools to newly grown shoots. The remobilization was, however, too low to cause significant changes in ^{14}C recovery in the stubble or roots. A greater use of stored C by *M. sativa* can be explained by a faster growth of the new shoots compared to *L. perenne*. However, higher ^{14}C specific activity in newly grown shoots of *L. perenne* indicated a higher use of stored C related to biomass increase compared to *M. sativa.* Since *L. perenne* is more herbivory-tolerant, it is better adapted to the removal of biomass by means of a higher ability to use reserve C as compared to *M. sativa.* A trend for reduced portion of recovered ^{14}C was determined in roots of *L. perenne* but not in its stubbles, indicating remobilization of stored C from roots rather than from the stubble. In contrast, no difference in ^{14}C recoveries was observed between clipped and control treatments, neither in roots nor in stubbles of *M. sativa* (Fig. II.3/2 and II.3/3). The results of *M. sativa* were surprising since no source of the ^{14}C in the new shoot could be found. However, a decrease in reserve C in the root by translocation to the shoots could be counterbalanced by a reduced proportion of reserve C in root respiration (discussed below).

We reviewed several studies focusing on the effects of clipping (simulated grazing) on the portion of C translocated to the newly grown shoots of grassland species (Table II.3/3). Legumes use significantly higher portion of C (10%) for support of the new shoots as compared to grasses (7%). However, the reviewed studies did not allow conclusions about the absolute amount of C reutilization since the amount of stored C was not measured nor presented. The source of C reutilized by grasses and legumes for shoot regrowth was mainly roots (Table II.3/3). The relative amount of translocated reserve C in newly grown shoots depends on the period after defoliation (Briske et al., 1996). During the first three days after defoliation, the most important C source for the elongation and maturation zone is stored C (Schnyder and de Visser, 1999). However, when comparing the reviewed studies, plant species and clipping height is more important than the time of regrowth.

Table II.3/3: Review of sources and amounts of C relocated in the newly grown shoots after clipping of grassland species.

Plant species	**Approach**	**Source of retranslocated C**	**Days after clipping**	**Clipping height [cm]**	**C retrans-located [%]**	**Reference**
Medicago sativa	^{13}C pulse	Roots (ta-proots, lateral roots), Stubble stem	30	6	5	Avice et al., 1996
	^{14}C pulse	Roots (stubbles were not measured)	28	5	12	Ta et al., 1990
	^{14}C pulse	np*	28		19	Pearce et al.,1969
	^{14}C continuous	Stubbles and Roots		5	9	Smith and Marten, 1969
	Repeated ^{14}C pulse	Roots	10	8	8	This study
	^{14}C + ^{13}C continuous	Stubbles	23	5		Crawford et al., 2000
Medicago truncatula	^{14}C pulse to a single leaf	At the begin-ning stolons after 5 days roots	10	Removing of all meristems and leaves	11	Danckwerts and Gordon, 1989
Trifolium repens		np		Removing of all meristems and all, except two, leaves	5-6	
Legumes		**Mainly roots**			**9 / 9.9**	**Median / average**

Table II.3/3 continued:

Lolium perenne	^{14}C pulse to a single leaf	Stem bases (stubbles)	10	2		Danckwerts and Gordon, 1987
	Repeated ^{14}C pulse	Predominantly stubbles	15	4	2.4 - 4.7	Kuzyakov et al., 2002
	^{13}C continuous	Elongated leaf bases, sheaths of stubble	28	4	1	Morvan-Bertrand et al., 1999
	Repeated ^{14}C pulse	Roots	10	4	5	This study
Panicum maximum	^{14}C continuous	Crowns and Roots	19	8		Bushby et al., 1992
Festuca pratensis	^{14}C + ^{13}C continuous	Stubbles and Root	15	1.5	21	Johansson, 1993
Agropyron-Koeleria association	^{14}C continuous	Roots (stubbles were not measured)	120		6	Warembourg and Paul, 1977
Grasses		**Mainly roots**			5 / 6.8	**Median / average**

*np data were not presented in the paper.

Shading allows the sole investigation of the effect of limited photosynthesis on the redistribution of reserve C, without the effect of C translocation to support shoot regrowth, as is the case after clipping. This study showed that low light reduced the amount of dry matter in aboveground biomass and roots but had no effect on the R:S ratio of *M. sativa* and *L. perenne* (Fig. II.3/1 and Table II.3/2). This indicates that the C stored in shoots and roots was used for maintenance proportional to the weight of the plant parts. A positive relationship between plant biomass and light intensity has also been observed in many other studies (Lambers and Posthumus, 1980; Zagal, 1994). In comparison to clipped plants, plants grown under low light showed a higher R:S ratio and the ^{14}C recovery in roots was higher after shading for *L. perenne*. Thus, clipped plants rely more

on translocated C for regrowth compared to shaded plants. ^{14}C specific activity in the aboveground biomass of *L. perenne* was higher after shading compared to control plants and clipped plants (Fig. II.3/3). This is because lower photosynthesis after shading led to less dilution of ^{14}C by unlabeled assimilates. For *M. sativa,* biomass production and ^{14}C specific activity were less affected by shading compared to *L. perenne*. This indicate a better strategy of *M. sativa* to cope with low light conditions.

3.4.3 Redistribution of stored C in soil and soil CO_2

Many studies investigated the effect of clipping on root exudation, however, with contradicting results. An increase (Hamilton et al., 2001; Paterson and Sim, 1999), no change (Kuzyakov et al., 2002; Murray et al., 2004; Todorovic et al., 1999) or decrease (Mikola and Kytöviita, 2002) of exudation after defoliation have been noted. These differences depend on plant species and methods used in the studies (Mikola and Kytöviita, 2002). Paterson and Sim (1999) measured the release of total organic C and hypothesized that an increase in exudation after defoliation was a consequence of the remobilization of storage compounds in roots, increasing the concentration of diffusible exudates in the root system. In our study, an increased ^{14}C recovery rate, indicating a remobilization of stored C was only found in the rhizosphere soil under *M. sativa*. This is caused by a higher exudation and/or an increased root senescence. However, this was not found in any of the other investigated soil pools (bulk and rhizosphere soil) under both plants (Fig. II.3/4A). The increase in total root exudation lasts only two days after defoliation (Paterson et al., 2005), which may explain that no effects were detected 10 days after clipping.

Many authors observed an increase in soil microbial biomass after defoliation (Butenschoen et al., 2008; Guitian and Bardgett, 2000). It is assumed that plants are able to stimulate rhizodeposition to enhance nutrient availability by promoting the activity of microbial populations (Blagodatskaya et al., 2010; Hamilton et al., 2001; Lambers et al., 2009). In our study the results of the ^{14}C recovery and the ^{14}C specific activity (data not shown) indicates that there is no effect of clipping on the availability and uptake of plant-stored C by microorganisms (Fig. II.3/4B).

Rhizodeposits are an important driver for soil CO_2 efflux, as their microbial decomposition is an important source for soil CO_2 (Kuzyakov, 2006). After clipping, a decrease in

total CO_2 efflux was observed for *L. perenne*, confirming the results from previous studies (Craine et al., 1999; Detling et al., 1979; Kuzyakov et al., 2002). This decrease is caused by reduced root respiration and microbial respiration after clipping (Gavrichkova et al., 2010) and indicates a strong connection between photosynthesis and soil respiration (Kuzyakov and Gavrichkova, 2010). Lower assimilation after clipping leads to less available C for belowground translocation and thus, reduces soil CO_2 efflux. The unaltered CO_2 efflux under *M. sativa* (Fig. II.3/5B) by clipping was unexpected. Like *L. perenne*, a lower CO_2 efflux from soil was assumed due to a lower photosynthesis after clipping. A high energy demand for N_2 fixation by legumes may lead to an increase in root and rhizomicrobial respiration after clipping, diminishing the effect of limited photosynthesis. The ^{14}C specific activity of soil CO_2 increased after clipping of *M. sativa* (Fig. II.3/6). C stored in nodules plays an important role in supporting N_2 fixation after defoliation of *M. sativa* (Ta et al., 1990). Thus, in contrast to *L. perenne*, *M. sativa* showed increased ^{14}C specific activity of the CO_2 efflux after clipping.

In former studies a limited photosynthesis after reduced light intensity decreases root exudation (Hill et al., 2007). This leads to a reduced incorporation of exuded C into microorganisms and decreased microbial growth (Zagal, 1994). In the present study, no change in the ^{14}C specific activities (data not shown) and ^{14}C recoveries of the soil and microbial biomass were observed (Fig. II.3/4A, B).

Root respiration and rhizomicrobial respiration are very closely linked to the supply of assimilates (Kuzyakov and Gavrichkova, 2010). In grassland, shading reduces the soil CO_2 flux by 40% (Craine et al., 1999). Our results also showed a decrease in the CO_2 efflux after shading (Fig. II.3/5). The higher $^{14}CO_2$ efflux (Fig. II.3/6) seems to contradict the decreasing total CO_2 efflux from soil for *L. perenne* and for *M. sativa*. This effect of low light conditions was also observed for wheat and maize (Kuzyakov and Cheng, 2001; 2004). The authors of these studies explained the effects on the need for recently assimilated C for maintaining respiration, increasing ^{14}C efflux, and also because of the reduced photosynthesis, decreasing the total CO_2 efflux from soil. Our results demonstrate that the respired CO_2 was not only composed of recently assimilated C but also of translocated reserve C (^{14}C). Indeed, respiration of old C was closely related to maintenance, which dominated the respiratory costs when relative growth rate was low, e.g., after shading (Lötscher et al., 2004). Changing respiration regimes (in-

creased maintenance respiration after shading and increased growth respiration after clipping) with their different demands on stored C and newly assimilated C influence the relative amount of reserve C in the root respiration.

3.5 Conclusions

Limited photosynthesis after clipping or shading alters C allocation in grassland plants. Shading reduced the total biomass of both *L. perenne* and *M. sativa*, whereas the response to clipping was different between the two species. While the biomass of *L. perenne* decreased, the biomass of *M. sativa* increased by regrowth after clipping. The redistribution of reserve C after clipping was governed not only by the lower photosynthesis but also by the C demand for the regrowth of new shoots. In particular, clipping induced a higher demand of reserve C for newly growing shoots. In contrast, only the lower photosynthesis, without the regrowth of shoots, determined the redistribution of reserve C after shading. The main effect after shading was a higher utilization of stored C for maintaining respiration. These differences indicate that the removal of biomass after clipping is more important for the translocation of stored C than limited photosynthesis.

The CO_2 efflux from soil declined by *L. perenne* after shading. The decrease of the CO_2 efflux is more pronounced after clipping compared to shading because of a higher C demand for the newly growing shoots. For *M. sativa*, a decrease in soil CO_2 efflux was observed only after shading but not after clipping. This indicates that the non-legume *L. perenne* and the legume *M. sativa* have different mechanisms to cope with clipping. While *L. perenne* uses stored C mainly for shoot regrowth, *M. sativa* has also a high C demand for N_2 fixation compared to the nutrient uptake of non-legumes.

The results show that C storage by plants is a very important mechanism to overcome stress periods like grazing or limited light availability. This C can be useful to recover from the removal of biomass by supporting the regrowth of new shoots or to obtain vital functions like respiration or the N_2 fixation of legumes.

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References

Avice, J.C., Ourry, A., Lemaire, G., Boucaud, J., 1996. Nitrogen and carbon flows estimated by ^{15}N and ^{13}C pulse-chase labeling during regrowth of alfalfa plant. Plant Physiology 112, 281–290.

Blagodatskaya, E., Blagodatsky, S., Dorodnikov, M., Kuzyakov, Y., 2010. Elevated atmospheric CO_2 increases microbial growth rates in soil: results of three CO_2 enrichment experiments. Global Change Biology 16, 836–848.

Briske, D.D., Boutton, T.W., Wang, Z., 1996. Contribution of flexible allocation priorities to herbivory tolerance in C4 perennial grasses: an evaluation with ^{13}C. Oecologia 105, 151-159.

Bushby, H.V.A., Vallis, I., Myers, R.J.K., 1992. Dynamics of C in a pasture grass (*Panicum maximum* var. Trichoglume) - soil system. Soil Biology and Biochemistry 24, 381-187.

Butenschoen, O., Marhan, S., Scheu, S., 2008. Response of soil microorganisms and endogeic earthworms to cutting of grassland plants in a laboratory experiment. Applied Soil Ecology 38, 152-160.

Cao, G., Tang, Y., Mo, W., Wang, Y., Li, Y., Zhao, X., 2004. Grazing intensity alters soil respiration in an alpine meadow on the Tibetan plateau. Soil Biology and Biochemistry 36, 237-243.

Counce, P.A., Bouton, J.H., Brown, R.H., 1984. Screening and characterizing alfalfa for persistence under mowing and continuous grazing. Crop Science 24, 282-285.

Craine, F.M., Wedin, D.A., Chapin III, F.S., 1999. Predominance of ecophysiological controls on soil CO_2 flux in a Minnesota grassland. Plant and Soil 207, 77-86.

Crawford, M.C., Grace, P.R., Oades, J.M., 2000. Allocation of carbon to shoots, roots and rhizosphere respiration by barrel medic (*Medicago truncatula*) before and after defoliation. Plant and Soil 227, 67-75.

Danckwerts, J.E., Gordon, A.J., 1987. Long-term partitioning, storage and remobilization of ^{14}C assimilated by *Lolium perenne* (cv. Melle). Annals of Botany 59, 55-66.

Danckwerts, J.E., Gordon, A.J., 1989. Long-term Partitioning, Storage and Remobilization of ^{14}C Assimilated by *Trifolium repens* (cv. Blanca). Annals of Botany 64, 533-544.

Detling, J.K., Dyer, M.I., Winn, D.T., 1979. Net photosynthesis, root respiration, and regrowth of *Bouteloua gracilis* following simulated grazing. Oecologia 41, 127-134.

Dilkes, N.B., Jones, D.L., Farrar, J., 2004. Temporal dynamics of carbon partitioning and rhizodeposition in wheat. Plant Physiology 134, 706-715.

Domanski, G., Kuzyakov, Y., Siniakina, S.V., Stahr, K., 2001. Carbon flows in the rhizosphere of ryegrass (*Lolium perenne*). Journal of Plant Nutrition and Soil Science. 164, 381–387.

Ferraro, D.O., Oesterheld, M., 2002. Effect of defoliation on grass growth. A quantitative analysis. OIKOS 98, 125-133.

Gavrichkova, O., Moscatelli, M.C., Kuzyakov, Y., Grego, S., Valentini, R., 2010. Influence of defoliation on CO_2 efflux from soil and microbial activity in a Mediterranean grassland. Agriculture, Ecosystems and Environment 136, 87-96.

Goulden, M.L., Munger, J.W., Fan, S.M., Daube, B.C., Wofsy, S.C., 1996. Exchange of carbon dioxide by a deciduous forest: response of interannual climate variability. Science 271, 1576–1578.

Gregory, P.J., Atwell, B.J., 1991. The fate of carbon in pulse labelled crops of barley and wheat. Plant and Soil 136, 205-213.

Guitian, R., Bardgett, R.D., 2000. Plant and soil microbial responses to defoliation in temperate semi-natural grassland. Plant and Soil 220, 271–277.

Hamilton, E.W., Frank, D.A., 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82, 2397-2402.

Hill, P., Kuzyakov, Y., Jones, D., Farrar, J., 2007. Response of root respiration and root exudation to alterations in root C supply and demand in wheat. Plant and Soil 291, 131-141.

Hodge, A., Paterson, E., Thornton, B., Millard, P., Killham, K., 1997. Effects of photon flux density on carbon partitioning and rhizosphere carbon flow of *Lolium perenne.* Journal of Experimental Botany 48, 1797-1805.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001 Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789-79.

Holland, J.N., Cheng, W., Crossley, D.A.J., 1996. Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using ^{14}C. Oecologia 107, 87-94.

Johansson, G., 1993. Carbon distribution in grass (*Festuca pratensis* L.) during re-growth after cutting-utilization of stored and newly assimilated carbon. Plant and Soil 151, 11-20.

Johnson, D., Leake, J.R., Ostle, N., Ineson, P., Read, D.J., 2002. *In situ* $^{13}CO_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. New Phytologist 153, 327-334.

Johnson, D., Vachon, J., Britton A.J., Helliwelli R.C., 2011. Drought alters carbon fluxes in alpine snowbed ecosystems through contrasting impacts on graminoids and forbs. New Phytologist 190, 740-749.

KA5, 2005. Bodenkundliche Kartieranleitung. Ad-Hoc-Arbeitsgruppe Boden:5. verbesserte und erweiterte Auflage, ISBN 978-3-510-95920-4, E.Schweizerbart Science Publishers.

Keith, H., Oades, J.M., 1986. Input of carbon to soil from wheat plants. Soil Biology and Biochemistry 18, 445-449.

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Schoene, J., Scheunemann, N., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E., 2012. Carbon flow into microbial and fungal biomass as a basis to understand the belowground food web in an agroecosystem. Pedobiologia 55, 111-119.

Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 16, 382-396.

Kuzyakov, Y., 2006. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biology and Biochemistry 38, 425-448.

Kuzyakov, Y., Domanski, G., 2000. Carbon inputs into soil. Review. Journal of Plant Nutrition and Soil Science 163, 421-423.

Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology and Biochemistry 33, 1915-1922.

Kuzyakov, Y., Cheng, W., 2004. Photosynthesis controls of CO_2 efflux from maize rhizosphere. Plant and Soil 263, 85-99.

Kuzyakov, Y., Biryukova, O.V., Kuznetzova, T.V., Molter, K., Kandeler, E., Stahr, K., 2002. Carbon partitioning in plant and soil carbon dioxide fluxes and enzyme activities as affected by cutting ryegrass. Biology and Fertility of Soils 35, 348–358.

Kuzyakov, Y., Gavrichkova, O., 2010. Time lag between photosynthesis and carbon dioxide efflux from soil: a review. Global Change Biology 16, 3386-3406.

Lambers, H., 1987. Growth, respiration, exudation and symbiotic associations: The fate of carbon translocated to the roots, in: Gregory P.J., Lake J.K., Rose D.A. (Eds.), Root development and function. DA Cambridge University Press, Cambridge, pp. 125-145.

Lambers, H., Posthumus, L., 1980. The effect of light intensity and relative humidity on growth rate and root respiration of *Plantago lanceolata* and *Zea mays.* Journal of Experimental Botany 31, 1621-1630.

Lambers, H., Mougel, C., Jaillard, B., Hinsinger, P., 2009. Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. Plant and Soil 321, 83-115.

Leake, J.R., Ostle, N., Rangel-Castro, J.I., Johnson, D., 2006. Carbon fluxes from plants through soil organisms determined by field $^{13}CO_2$ pulse-labelling in an upland grassland. Applied Soil Ecology 33, 152–175.

Longdoz, B., Yernaux, M., Aubinet, M., 2000. Soil CO_2 efflux measurements in a mixed forest: impact of chamber distances, spatial variability and seasonal evolution. Global Change Biology 6, 907-917.

Lötscher, M., Klumpp, K., Schnyder, H., 2004. Growth and maintenance respiration for individual plants in hierarchically structured canopies of *Medicago sativa* and *He-*

lianthus annuus: the contribution of current and old assimilates. New Phytologist 164, 305-316.

Mackie-Dawson, L.A., 1999. Nitrogen uptake and root morphological responses of defoliated *Lolium perenne* (L.) to a heterogeneous nitrogen supply. Plant and Soil 209, 111-118.

Meharg, A.A., Killham, K., 1990. Carbon distribution within the plant and rhizosphere in laboratory and field grown *Lolium perenne* at different stages of development. Soil Biology and Biochemistry 22, 471-477.

Merbach, W., Mirus, E., Knof, G., Remus, R., Ruppel, S., Russow, R., Gransee, A., Schulze, J., 1999. Release of carbon and nitrogen compounds and their possible ecological importance. Journal of Plant Nutrition and Soil Science 162, 373-383.

Merlo, L., Ferretti, M., Passera, C., Ghisi, R., 1994. Effect of decreased irradiance on N and C metabolism in leaves and roots of maize. Physiologia Plantarum 90, 72-80.

Mikola, J., Kytöviita, M.M., 2002. Defoliation and the availability of currently assimilated carbon in the *Phleum pratense* rhizosphere. Soil Biology and Biochemistry 34, 1869-1874.

Milchunas, D.G., Lauenroth, W.K., 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. Ecological Monographs 63, 327-366.

Morvan-Bertrand, A., Pavis, J., Boucaud, J., Prud´homme, M.-P., 1999. Partitioning of reserve and newly assimilated carbon in roots and leaf tissues of *Lolium perenne* during regrowth after defoliation: assessment by ^{13}C steady-state labelling and carbohydrate analysis. Plant, Cell and Environment 22, 1097-1108.

Murray, P., Ostle, N., Kenny, C., Grant, H., 2004. Effect of defoliation on patterns of carbon exudation from *Agrostis capillaries.* Journal of Plant Nutrition and Soil Science 167, 487-493.

Paterson, E., Sim, A., 1999. Rhizodeposition and C partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation. Plant and Soil 216, 155-164.

Paterson, E., Thornton, B., Midwood, A.J., Sim, A., 2005. Defoliation alters the relative contributions of recent and non-recent assimilate to root exudation from *Festuca rubra*. Plant, Cell and Environment 28, 1525-1533.

Phillips, D.A., 1980. Efficiency of symbiotic nitrogen fixation in legumes. Ann. Rev. Plant Physiology 31, 29-49.

Pearce, R.B., Fissel, G., Carlson, G.E., 1969. Carbon uptake and distribution before and after defoliation of alfalfa. Crop Science 9, 756-759.

Schlesinger, W.H., 1977. Carbon balance in terrestrial detritus. Annual Review of Ecology and Systematics 8, 51-81.

Smith, L.H., Marten, G.C., 1969. Foliar regrowth of alfalfa utilizing ^{14}C-labeled carbohydrates stored in roots. Crop Science 9, 146-150.

Schnyder, H., De Visser, R., 1999. Fluxes of reserve-derived and currently assimilated carbon and nitrogen in perennial ryegrass recovering from defoliation. The regrowing tiller and its component functionally distinct zones. Plant Physiology 119, 1423-1435.

Subke, J.-A., Inglima, I., Cotrufo, M.F., 2006. Trends and methodological impacts in soil CO_2 efflux partitioning: A metaanalytical review. Global Change Biology 12, 921-943.

Todorovic, C., Nguyen, C., Robin, C., Guckert, A., 1999. ^{14}C-assimilate partitioning within white clover plant–soil system: effects of photoperiod/temperature treatments and defoliation. European Journal of Agronomy 11, 13–21.

Vance, E., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703-707.

Vance, C.P., Heichel, G.H., 1991. Carbon in N_2 fixation: Limitation or exquisite adaption Ann Rev. Plant Physiology 42, 373-392.

Warembourg, F.R., Paul, E.A., 1977. Seasonal transfers of assimilated ^{14}C in grassland: Plant production and turnover, soil and plant respiration. Soil Biology and Biochemistry 9, 295-301.

Warembourg, F.R., Roumet, C., Lafont, F., 2003. Differences in rhizosphere carbon-partitioning among plant species of different plant families. Plant and Soil 256, 347-357.

Werth, M., Kuzyakov, Y., 2008. Determining root-derived carbon in soil respiration and microbial biomass using ^{14}C and ^{13}C. Soil Biology and Biochemistry 40, 625-637.

Witty, J.F., Minchin, F.R., Sheehy, J.E., 1983. Carbon costs of nitrogenase activity in legume root nodules determined using acetylene and oxygen. Journal of Experimental Botany 34, 951-963.

Wu, J., Jörgensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass-C by fumigation-extraction – an automated procedure. Soil Biology and Biochemistry 22, 1167-1169.

Zagal, E., 1994. Influence of light intensity on the distribution of carbon and consequent effects on mineralization of soil nitrogen in a barley *(Hordeum vulgare* L.)-soil system. Plant and Soil 160, 21-31.

Supplementary material

Table II.3/4: Statistical analysis (Newman-Keuls test) of significance of treatment effects (clipping or shading) on ^{14}C specific activity and ^{14}C recovery.

Parameters	**^{14}C specific activity**			**^{14}C recovery**		
	LSD (P<0.05)	Between MS	df	LSD (P<0.05)	Between MS	df
Stubble	12.09	55.064	15	2.98	2.8454	13
Shoot	26.53	265.31	15	7.77	25.67	13
Root	27.78	258.44	13	6.94	14.569	12
Rhizosphere soil	0.04	0.00056	13	9.09	20.652	10
Bulk soil	0.06	0.00042	5	8.73	13.138	7
Microbial biomass (rhizosphere soil)	2.21	1.0289	8	4.41	0.82040	11
Microbial biomass (bulk soil)	2.68	0.96122	5	1.73	3.0506	6
Belowground respiration	4.93	9.1654	15	2.61	2.2859	13

Table II.3/5: Statistical analysis (Newman-Keuls test) of significance of treatment effects (clipping or shading) on plant biomass and the cumulative belowground respiration at the last day of the experiment.

Parameters			
	LSD (P<0.05)	Between MS	df
stubble	0.09	0.00924	14
shoot	0.05	0.5037	17
root	0.45	258.44	13
belowground respiraion	20.30	186.45	18

4 C and N allocation in soil under ryegrass and alfalfa estimated by ^{13}C and ^{15}N labeling

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Abstract

Background and Aims

Belowground translocated carbon (C) released as rhizodeposits is an important driver for microbial mobilization of nitrogen (N) for plants. We investigated how a limited substrate supply due to reduced photoassimilation alters the allocation of recently assimilated C in plant and soil pools under legume and non-legume species.

Methods

A non-legume (*Lolium perenne*) and a legume (*Medicago sativa*) were labeled with ^{15}N before the plants were clipped or shaded, and labeled twice with $^{13}CO_2$ thereafter. Ten days after clipping and shading, the ^{15}N and ^{13}C in shoots, roots, soil, dissolved organic nitrogen (DON) and carbon (DOC) and in microbial biomass, as well as the ^{13}C in soil CO_2 were analyzed.

Results

After clipping, about 50% more ^{13}C was allocated to regrowing shoots, resulting in a lower translocation to roots compared to the unclipped control. Clipping also reduced the total soil CO_2 efflux under both species and the ^{13}C recovery of soil CO_2 under *L. perenne*. The ^{15}N recovery increased in the shoots of *M. sativa* after clipping, because storage compounds were remobilized from the roots and/or the N uptake from the soil increased.

After shading, the assimilated ^{13}C was preferentially retained in the shoots of both species. This caused a decreased ^{13}C recovery in the roots of *M. sativa*. Similarly, the total soil CO_2 efflux under *M. sativa* decreased more than 50% after shading. The ^{15}N recovery in plant and soil pools showed that shading has no effect on the N uptake and N remobilization for *L. perenne*, but, the ^{15}N recovery increased in the shoot of *M. sativa*.

Conclusions

The experiment showed that the dominating effect on C and N allocation after clipping is the need of C and N for shoot regrowth, whereas the dominating effect after shading is the reduced substrate supply for growth and respiration. Only slight differences could

be observed between *L. perenne* and *M. sativa* in the C and N distribution after clipping or shading.

Keywords: Carbon allocation, N allocation, isotope labeling, grazing effects, N_2 fixation.

4.1 Introduction

Belowground translocation of carbon (C) by plants and its turnover are important drivers for ecological processes and functions in soil. These include nutrient availability for plants, microbe activity and turnover, or the turnover of soil organic matter (SOM) (Merbach et al., 1999; Blagodatskaya et al., 2010). The amount of C allocation by plants into the soil is affected by many factors such as plant development (Gregory and Atwell, 1991; Meharg and Killham, 1999), nutrient availability (Merckx et al., 1987) or plant species and plant functional groups (Warembourg et al., 2003). Since symbiotic N_2 fixation requires abundant energy, legumes have a higher demand for the assimilated C for rhizosphere respiration than grasses and non-legume forbs (Philips, 1980; Vance and Heichel, 1991; Warembourg et al., 2003).

For grasses, rhizodeposition is an important process affecting N availability and N uptake (Frank and Groffman, 2009). Rhizodeposits enhance N mobilization by stimulating microbial activity and SOM degradation; this is termed as the 'priming effect' (Kuzyakov, 2002). Thus, we expect that alterations in the amount of C translocated belowground will trigger different responses in the N uptake between legumes and non-legumes.

The fast translocation of assimilates belowground indicates a strong connection between current photosynthesis and root exudation (Gregory and Atwell, 1991; Cheng et al., 1993; Kuzyakov et al., 1999; Jones et al., 2004). Thus, any change in photosynthetic activity will affect the turnover processes in the rhizosphere and thus influence N availability for plants (Kuzyakov, 2002).

In this study we manipulated the photosynthetic activity by clipping or shading. After clipping (simulated grazing), photosynthesis is reduced due to a smaller leaf area (Detling et al., 1979). Clipped plants can meet their C supply for regrowth by remobilizing stored C from roots or from remaining shoot parts (Avice et al., 1996; Johansson, 1993). Despite the demand for C for regrowth, root exudation after clipping was higher in many studies (Paterson and Sim, 1999; 2000), however, also a reduced root exudation was found (Augustine et al., 2011). Some authors suggest that, besides C reserves, the remobilization of organic N compounds stored in roots or stubbles – such as amino ac-

ids or vegetative storage proteins – is also important for regrowth after clipping (Volenec et al., 1996).

In contrast, shading reduced the photosynthesis rate only at a lower light availability, without the removal of shoots. Like after clipping, C is preferentially allocated in aboveground plant parts after shading, as indicated by a decrease of the R:S ratio in *Lolium perenne* (Lambers and Posthumus, 1980). Consequently, shading leads to less rhizodeposition (Hill et al., 2007). Thus, based on the different effects of clipping and shading on rhizodeposition, and based on the high demand of N for regrowth after clipping, we hypothesize that clipping enhances N uptake by plants, whereas shading reduces it.

Using repeated $^{13}CO_2$ labeling of two plant species, a legume (*Medicago sativa*) and a non-legume plant (*Lolium perenne*), we investigated how a limited substrate supply after clipping and shading affected the C allocation within the plant and the belowground C translocation. Labeling with $^{15}NO_3^-$ was carried out to investigate how the altered C allocation after limited assimilate supply affects N remobilization and N uptake by both plant species. The specific questions were:

(1) How does a limited substrate supply affect plant biomass production and alter the distribution of C in plant, soil, microorganisms and CO_2 efflux from soil?

(2) How does a limited assimilate supply affect the remobilization of plant-stored N?

(3) How does the effect of a limited substrate supply affect the N uptake by plants from soil?

(4) Do shading and clipping induce different responses with respect to the distribution of C and N in the plant and soil pools?

4.2 Materials and Methods

4.2.1 Soil properties and plant growing conditions

The soil used in the experiment was an arable loamy haplic Luvisol developed on loess, collected near Göttingen (Germany, 51°33´36.8´´N, 9°53´46.9´´E) from the upper 10 cm of the Ap-horizon. The basic characteristics of the soil are shown in Table II.4/1.

Table II.4/1: Basic characteristics of the soil sampled from the A_p horizon of a haplic Luvisol originated from loess near Göttingen (Germany, Kramer et al., 2012). CEC: Cation exchange capacity; BS: Base saturation.

Parameter	**Value**
N_{tot} (mg g^{-1})	1.200
C_{org} (mg g^{-1})	11.700
C/N	9.76
NO_3^- (mg g^{-1})	0.083
P (mg g^{-1})	0.160
S (mg g^{-1})	0.009
CEC ($mmol_c$ kg^{-1})	108.000
BS (%)	99.700
Texture[1]	
clay/silt/sand (% w/w))	7.0/87.2/5.8
pH (H_2O)	6.600
pH ($CaCl_2$)	6.000

[1] Texture according to the German classification system.

The seedlings of ryegrass (*Lolium perenne* L.) and alfalfa (*Medicago sativa* L.) were first germinated on wet filter paper for 5 (*M. sativa)* and 8 days (*L. perenne*) and thereafter transferred to the plant pots (inner diameter 7 cm, height 20 cm), each of them filled with 700 g of air-dried, sieved (≤ 2 mm) soil. In each pot, 3 seedlings of *M. sativa* or 5 seedlings of *L. perenne* were transferred to achieve a similar biomass for both plant species. The pots were closed with a plastic lid with holes for shoots. The plants were

grown at 26 to 28 °C day temperature and at 22 to 23 °C night temperature. At a day length of 14 h the light intensity was approximately 210 µmol m^{-2} s^{-1}, approximately corresponding to a cumulative daily radiation in the range of field conditions. The soil moisture was maintained at 70% of the available field capacity by daily watering with distilled water.

4.2.2 ^{13}C and ^{15}N labeling

To label the soil of all pots with ^{15}N, 16 mg of $K^{15}NO_3$ (enrichment: 52.7 atom%) were dissolved in water and added to the pots with the watering (28 days after planting).

The ^{13}C labeling was conducted for the first time 50 days after planting (the day of clipping or beginning of shading). One day before ^{13}C labeling, all pots were sealed with silicone paste (NG 3170, Thauer & Co., Dresden). All plants were labeled in a Plexiglas chamber as described by Werth and Kuzyakov (2008). Briefly, $^{13}CO_2$ was introduced to the chamber by circulating air through a flask containing 150 mg of $Na_2{}^{13}CO_3$ (^{13}C enrichment: 99.9 atom%) for labeling of *L. perenne* or 15 mg of the same $Na_2{}^{13}CO_3$ for *M. sativa* solved in 10 ml deionized water. To produce $^{13}CO_2$, an excess of 5 M H_2SO_4 was added to the $Na_2{}^{13}CO_3$ solution. The plants were labeled in the $^{13}CO_2$ enriched atmosphere for 3 h. Before opening the labeling chamber, the chamber air was pumped through 1 M NaOH solution to remove unassimilated $^{13}CO_2$. Since the amount of ^{13}C found in the NaOH solution was negligible, it can be assumed, that all $^{13}CO_2$ was assimilated. Then the chamber was opened and the trapping of CO_2 evolved from the soil started. ^{13}C labeling was repeated on day 55 after planting.

4.2.3 Clipping and shading

Three pots of each plant species was used for the clipping procedure or exposed to shading. Additionally, three pots of each plant species were grown under normal conditions as a control treatment. The plants were clipped or shaded 2 hours before the first $^{13}CO_2$ pulse. *Lolium perenne* shoots were clipped 4 cm above the soil surface, those of *M. sativa* 8 cm above the surface. Due to the different clipping heights, both plant species achieve similar stubble biomass. The clipped plants continued growth under the conditions described above. For shading, the light intensity was reduced to about 17 µmol m^{-2} s^{-1} for 10 days.

4.2.4 Sampling and analysis

Starting after the first labeling, the CO_2 evolved from soil was trapped using a closed-circulating system. The air was pumped through tubes containing 15 ml of 1 M NaOH solution. Because of the circulation there were no losses of CO_2 due to incomplete absorption by NaOH solution. The NaOH solution was changed 1, 3 and 5 days after each labeling. The pots were destructively harvested at day 60 after planting. Roots were separated from soil by handpicking. Plant and soil material was dried at 65 °C for 3 days.

To estimate total CO_2 efflux, the C content of the NaOH solution was determined by titration with 0.01 M HCl against phenolphthalein after adding 1.5 M $BaCl_2$ solution. For ^{13}C measurements the CO_2 trapped in NaOH was precipitated as $SrCO_3$ with an excess of 0.5 M $SrCl_2$ solution. The precipitants were centrifuged at 3000 rev min^{-1}, washed with deionized water until the pH reached neutral conditions and dried at 65 °C.

Microbial biomass C and N was determined by the chloroform fumigation-extraction-method (CFE) (modified after Vance et al. 1987). For this, the soil was separated into two samples with 5 g each. One of these samples was firstly fumigated with chloroform for 24 h. Both samples were extracted with 20 ml of 0.05 M K_2SO_4, shaken for 1 h and, thereafter, centrifuged for 10 min at 3070 rev min^{-1}. Total C and N contents of fumigated and non-fumigated soil extracts were measured using a N/C analyzer (Multi N/C 2100, AnalytikJena, Germany). The extracts of the non-fumigated samples were used to measure dissolved organic carbon (DOC) and dissolved organic nitrogen (DON). For the determination of ^{13}C and ^{15}N in the microbial biomass, DOC and DON the extracts were oven-dried at 60 °C and measured as described below.

The ground plant and soil material (ball mill), the $SrCO_3$ and the dried extracts of the CFE were analyzed for their ^{13}C and ^{15}N isotope ratios. This was done using an elemental analyzer NC 2500 (CE Instruments, Milano, Italy) linked to a delta plus gas-isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) via a ConFlo III (Thermo Fisher Scientific, Bremen, Germany) interface.

4.2.5 Calculations and statistics

The ^{13}C enrichment of a particular C pool ($^{13}C_{excess;p}$; µg g^{-1}) was calculated as follows:

$$^{13}C_{excess;p} = (^{13}C_p - {}^{13}C_{NA;p}) \cdot C_p \quad (1)$$

where $^{13}C_{NA;p}$ is the ^{13}C natural abundance of the respective pool (atom%), $^{13}C_p$ is the amount of ^{13}C of the pool after labeling (atom%), and C_p is the total amount of C in this pool (µg g^{-1}).

The ^{13}C recovery in a particular C pool ($^{13}C_{rec;p}$; %) was calculated by dividing the amount of ^{13}C (mg) of that particular pool (^{13}C enrichment multiplied by the pool mass (mg)) by the sum of the ^{13}C amount (mg) of all pools (shoot, root, soil, DOC, soil microbial biomass and soil CO_2):

$$^{13}C_{rec;p} = \frac{^{13}C_{excess;p} \times mass_p}{\Sigma^{13}C_{excess;p} \times mass_p} \times 100 \quad (2)$$

To determine the $\delta^{13}C$ value of microbial biomass ($\delta^{13}C_{MB}$; ‰) a mass balance equation was used:

$$\delta^{13}C_{MB} = \frac{\delta^{13}C_{fum} \cdot C_{fum} - \delta^{13}C_{nf} \cdot C_{nf}}{C_{fum} - C_{nf}} \quad (3)$$

where $\delta^{13}C_{fum}$(‰) and $\delta^{13}C_{nf}$(‰) are the $\delta^{13}C$ values of the fumigated and unfumigated samples, respectively, and C_{fum} (mg) and C_{nf} (mg) are the amounts of C in the fumigated and unfumigated samples, respectively.

The calculations for ^{15}N correspond to those for ^{13}C.

The experiment was conducted with 3 replicates for all treatments. The values presented in the figures and tables are given as means ± standard errors of the means (±SEM). Significant differences between the treatment and the plant species were obtained by a two-factor analysis of variance (ANOVA) in combination with a post hoc Fisher LSD test.

4.3 Results

4.3.1 Plant biomass production

M. sativa produced significantly more shoot biomass per plant than *L. perenne* during 60 days (Table II.4/2). Clipping has no effects on the shoot and root biomass of *M. sativa* and *L. perenne* when measured after 10 days of regrowth (Table II.4/2). Ten days of shading were also not sufficient to decrease the shoot or root biomass of both species. The R:S ratio decreased after clipping and shading of *L. perenne*, whereas it increased for *M. sativa* after clipping and slightly after shading (Table II.4/2).

Table II.4/2: Plant biomass (±SEM) and root-to-shoot ratio (R:S) (±SEM) of *L. perenne* and *M. sativa* 10 days after clipping or shading. Significant differences are marked by different letters (P<0.05).

		Biomass [g plant^{-1}]				**R:S**
		Shoot	**Clipped Shoot**	**Total Aboveground**	**Root**	
Lolium perenne	Control	0.36±0.02ac		0.36±0.02ad	0.38±0.02ab	1.08±0.09
	Clipping	0.12±0.01a	0.13±0.02	0.25±0.03a	0.23±0.16a	1.04±0.77
	Shading	0.24±0.01a		0.24±0.01a	0.21±0.07a	0.88±0.26
Medicago sativa	Control	0.67±0.10b		0.67±0.10bc	0.59±0.25ab	0.82±0.30
	Clipping	0.43±0.15b	0.45±0.06	0.88±0.21b	0.78±0.18b	1.09±0.46
	Shading	0.52±0.03abc		0.52±0.03ac	0.44±0.07ab	0.85±0.17

4.3.2 Effect of clipping and shading on ^{13}C distribution in plant and soil

In the control treatments of *L. perenne* and *M. sativa*, about 50% of ^{13}C were recovered in shoots; 30% and 20% were found in the roots of *L. perenne* and *M. sativa*, respectively (Fig. II.4/1). The ^{13}C recovery in CO_2 efflux, the soil, microbial biomass and DOC did not differ between both plant species (Fig. II.4/2).

Clipping increased the ^{13}C recovery in the shoot by about 30% and 20% for *L. perenne* and *M. sativa*, respectively. The retention of newly assimilated C (^{13}C) in the shoots resulted in a lower translocation to the roots, and thus, the ^{13}C recovery of the roots of both plant species was lower compared to the respective control (Fig. II.4/1). However, the retention of ^{13}C in the shoots after clipping had no effects on the ^{13}C recovery in the soil (Fig. II.4/2). Also, all other belowground C pools of both plant species were not affected by clipping (Fig. II.4/2).

Shading increased the ^{13}C recovery in the shoots of *L. perenne* and *M. sativa* (Fig. II.4/1). The ^{13}C recovery was reduced only in the roots of *M. sativa* (Fig. II.4/1). Like after clipping, the ^{13}C recovery in the soil, microbial biomass and DOC was not affected by shading (Fig. II.4/2).

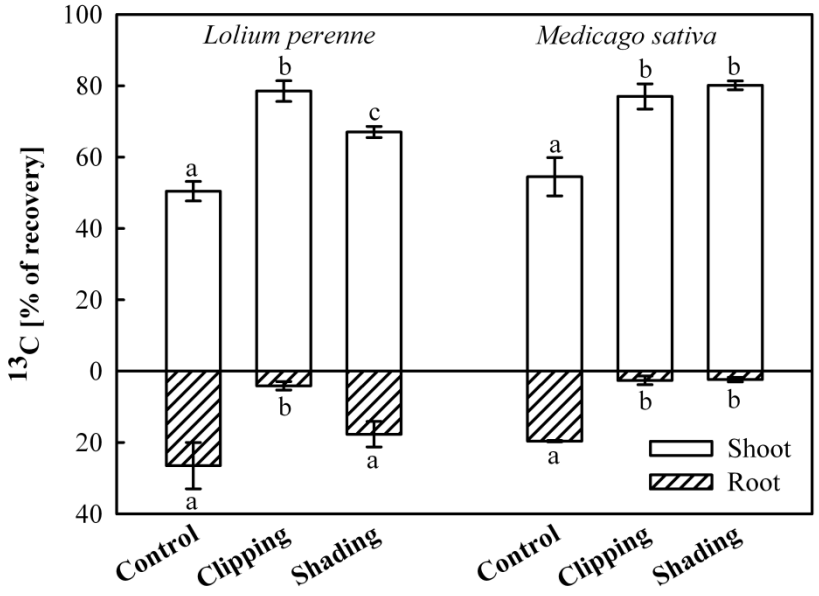

Figure II.4/1: ^{13}C recovery (±SEM) in shoots and roots 10 days after clipping or beginning of shading of 60 days old *L. perenne* and *M. sativa*. Significant differences are marked by different letters ($P<0.05$).

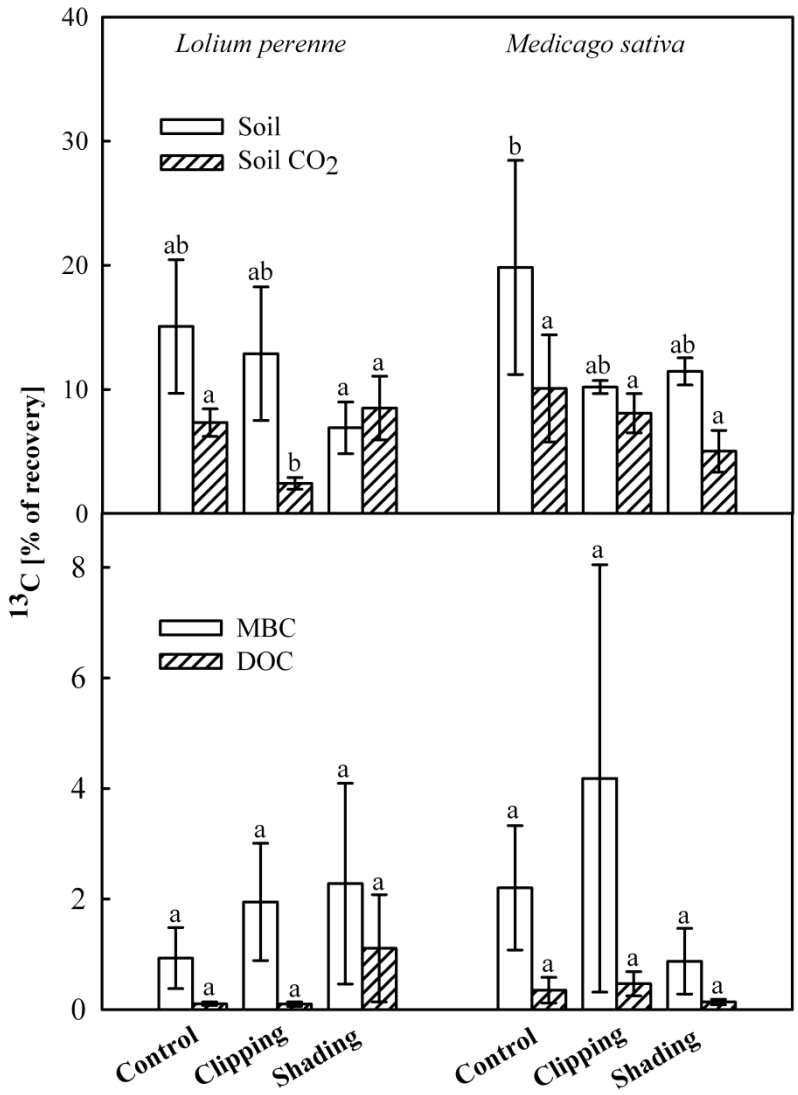

Figure II.4/2: ^{13}C recovery (±SEM) in the soil and in soil CO_2 (top), and in DOC and microbial biomass (bottom) under *L. perenne* and *M. sativa* 10 days after clipping and beginning of shading. Significant differences are marked by different letters (P<0.05).

4.3.3 Effect of clipping and shading on total CO_2 and ^{13}C efflux from soil

The total CO_2 efflux from soil was significantly higher under *M. sativa* than under *L. perenne* (Fig. II.4/3); this indicates the higher C demand in legume roots. Both treatments for reduced C assimilation decreased the CO_2 efflux from soil under *L. perenne*. This reflects the limited substrate availability, whereby the CO_2 reduction was significant only after clipping at the end of the experiment (Fig. II.4/3). Under *M. sativa*, clipping and shading significantly decreased the soil CO_2 efflux (Fig. II.4/3). After clipping,

however, this reduced CO_2 efflux from soil lasted only until day 5. Contrary to *L. perenne*, the soil CO_2 efflux under *M. sativa* was lowest after shading (Fig. II.4/3).

Clipping also significantly reduced the ^{13}C recovery of the soil CO_2 efflux under *L. perenne*; because ^{13}C was used for shoot regrowth (Fig. II.4/2). Shading had no effect on the ^{13}C recovery in CO_2 under *L. perenne*. The ^{13}C recovery of the soil CO_2 efflux under *M. sativa* was not affected by clipping or shading (Fig. II.4/2).

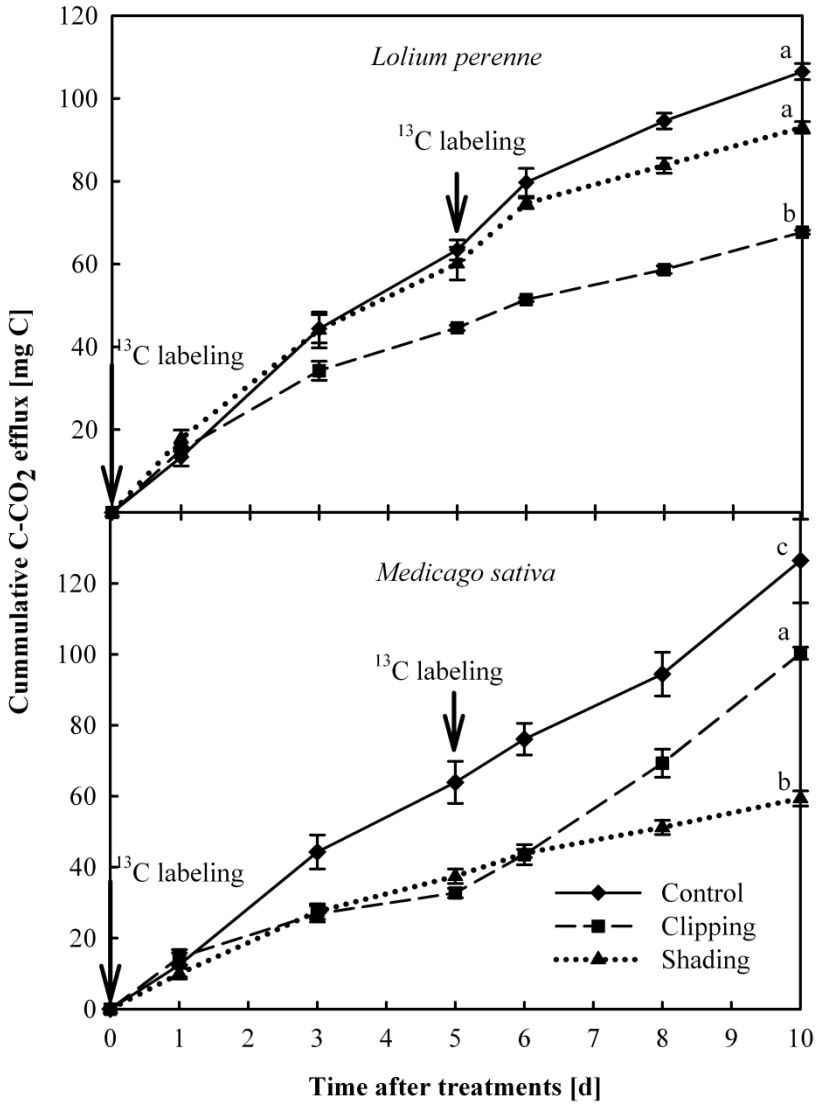

Figure II.4/3: Cumulative CO_2 efflux from soil (±SEM) under *L. perenne* (top) and *M. sativa* (bottom) beginning at clipping or start of shading and the effect of clipping and shading on the CO_2 efflux. Significant differences at the end of the experiment are marked by different letters ($P<0.05$).

4.3.4 Distribution of ^{15}N in plant and soil

Under normal light conditions a higher ^{15}N recovery was detected for the shoots of *L. perenne* compared to *M. sativa* (Fig. II.4/4). In the roots, the ^{15}N recovery showed no significant differences between *M. sativa* and *L. perenne* (Fig. II.4/4).

Clipping increased the ^{15}N recovery only in the shoots of *M. sativa*, but had no effect on the ^{15}N recovery in the roots of both plant species (Fig. II.4/4). Also the ^{15}N recovery in the soil, DON and microbial biomass N was unaffected by clipping (Fig. II.4/5).

The ^{15}N recovery in the shoots and roots of *L. perenne* was not affected by shading, however, it increased in the shoots of *M. sativa* (Fig. II.4/4). In the soil, the DON and the microbial biomass under both plant species, shading showed no influence on the ^{15}N recovery (Fig. II.4/5).

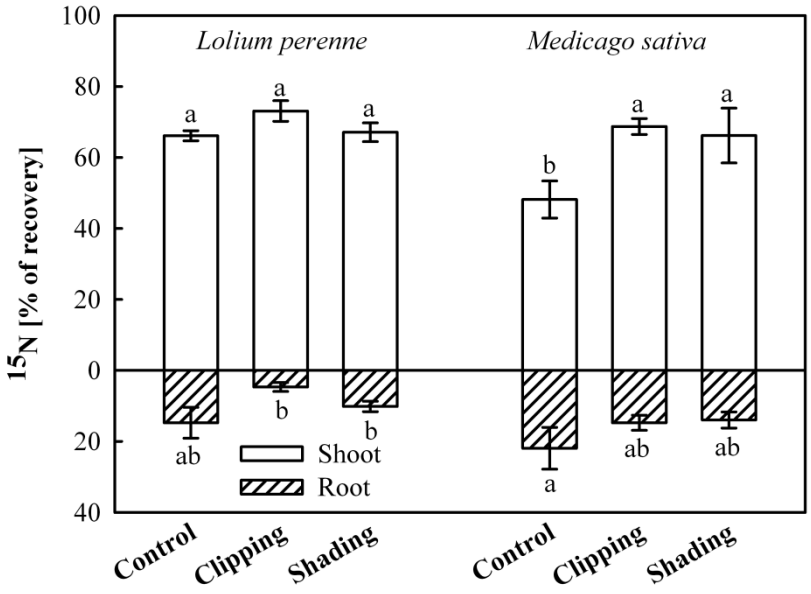

Figure II.4/4: ^{15}N recovery (±SEM) (top) in shoots and roots 10 days after clipping or beginning of shading of 60 days old *L. perenne* and *M. sativa*. Significant differences are marked by different letters ($P<0.05$).

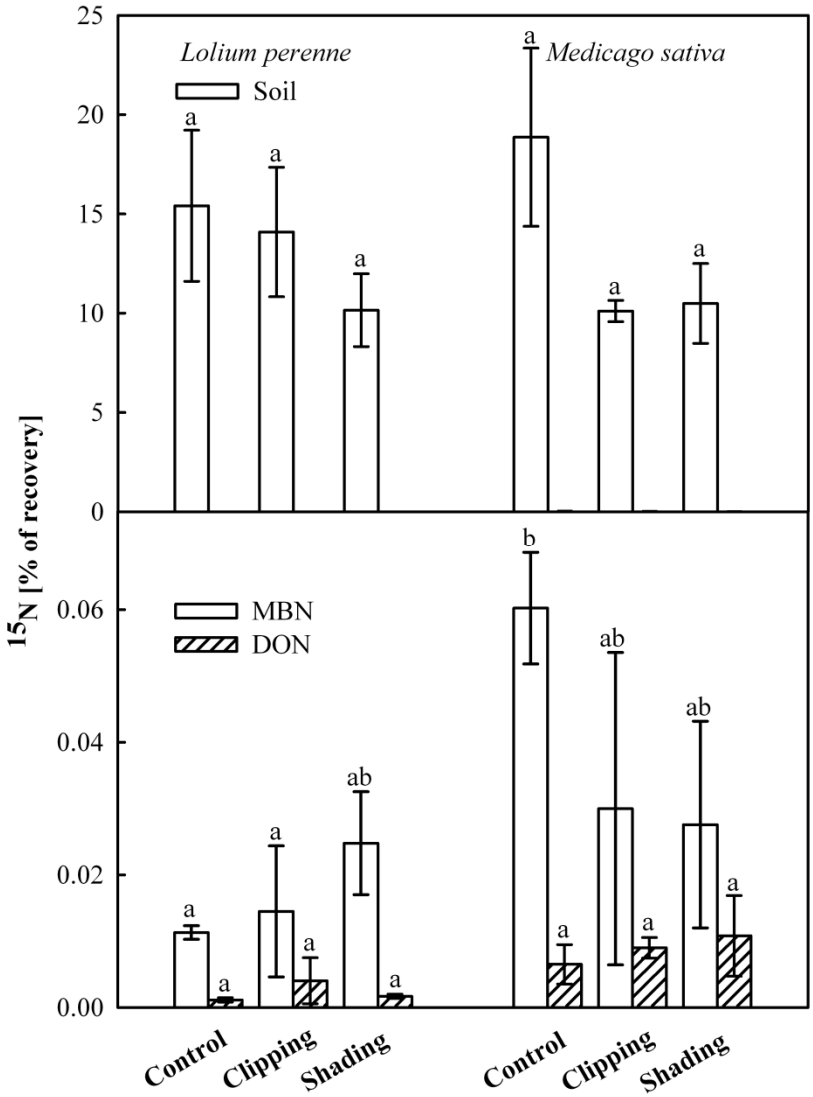

Figure II.4/5: ^{15}N recovery (±SEM) in soil (top), and in DON and microbial biomass (bottom) of *L. perenne* and *M. sativa* 10 days after clipping or beginning of shading. Significant differences are marked by different letters (P<0.05).

4.4 Discussion

4.4.1 Effect of plant species

The distribution of ^{13}C between above- and belowground pools in the control treatment was similar for *L. perenne* and *M. sativa*, with about one half of the labeled assimilates being incorporated in the shoots (Fig. II.4/1). This is in the range of earlier studies, reviewed by Kuzyakov and Domanski (2000). The roots of *L. perenne* recovered more ^{13}C than *M. sativa*, whereas the portion of ^{13}C found in the soil CO_2 was higher under *M. sativa* (Fig. II.4/1 and Fig. II.4/2). A higher incorporation of assimilated C was found in the roots of the legume *Trifolium repens* compared to the roots of *L. perenne* (Neergaard and Gorissen, 2004), however, in our study there was no difference between the legume species and *L. perenne*. A higher total CO_2 efflux from the soil was found under *M. sativa* compared to *L. perenne*, indicating a high energy need for N_2 fixation.

4.4.2 Effect of clipping

After clipping, both species preferentially allocated ^{13}C in the aboveground biomass as shown by an increased ^{13}C recovery in shoots (Fig. II.4/1). Recent studies observed an increased aboveground C allocation after clipping (Kuzyakov et al., 2002; Detling et al., 1979; Mackie-Dawson, 1999). The assumption is that regrowing shoots retain photosynthates and prevent a translocation belowground (Mackie-Dawson, 1999). This agrees with our results of less ^{13}C recovery in the roots of both plants after clipping (Fig. II.4/1).

Especially on the first days after clipping, the remobilization of storage compounds is the major substrate supply for the regrowing shoots, including N compounds (Morvan-Bertrand et al., 1999; Ourry et al., 1988). This is confirmed by the higher post-clipping ^{15}N recovery in the shoots of *M. sativa* in our study (Fig. II.4/4). The re-translocation of root N contributes substantially to the synthesis of amino acids and proteins in the regrowing tissue of *M. sativa* (Avice et al., 1996). In our study there were no indications for a re-translocation of N compounds from roots to shoots of *M. sativa*, since there was no significant decrease of the ^{15}N recovery in the roots. However, the design of our ex-

periment does not allow us to make any predictions about a possible retranslocation of N which is taken up by N_2 -Fixation.

It is likely that the reduced C translocation to roots has implications for root respiration and rhizodeposition, as well as for ^{13}C incorporation in soil and availability for soil microorganisms. However, the unaffected ^{13}C recovery in the soil shows that exudation of newly assimilated C did not change after clipping because of assimilate retention in the shoots. The increased rhizodeposition found in earlier studies (e.g. Bardgett et al., 1998) may reflect remobilization of storage compounds in roots, which would increase the release of stored C in the soil (Paterson and Sim, 1999). Our ^{13}C results, however, provide no information about the total rhizodeposition and the release of stored C. Former studies showed that an increased rhizodeposition has a positive effect on microbial activity, stimulates N cycling and thus enhances N availability for plant roots after defoliation (Guitian and Bardgett, 2000; Hamilton and Frank, 2001). It can be expected that this would lead to a reduced ^{15}N recovery in the soil, however, the high variability of the results of our results makes it impossible to see these effect.

The assimilate supply is a major factor affecting root respiration (Gavrichkova et al., 2010). A reduced soil CO_2 efflux after clipping, as observed for *L. perenne* (Fig. II.4/3), was also found in many other studies (Detling et al., 1979; Craine et al., 1999; Kuzyakov et al., 2002). Since the ^{13}C recovery in microbial biomass and DOC under *L. perenne* did not change after clipping (Fig. II.4/2), it can be concluded that these pools were not affected by clipping. Thus, the decrease in soil CO_2 can be ascribed to a reduced root respiration of current assimilates rather than reduced microbial respiration.

The soil processes under the legume *M. sativa* differed from those under *L. perenne*. The total CO_2 efflux under *M. sativa* decreased until day 5 after clipping and, thereafter, recovered and was approximately at the same level as observed in the control pots (Fig. II.4/3). In the same time the ^{13}C recovery of the CO_2 efflux remained unchanged. Thus, the portion of newly assimilated C in the soil CO_2 is increasing after clipping. This corresponds with findings that newly assimilated C is closely related to growth respiration (Lötscher et al., 2004), which is important after clipping for the biomass production. The increasing CO_2 efflux after 5 days may point to enhanced nodule respiration to restore the N_2 fixation.

We conclude that high C and N demands of regrowing shoots after clipping led to a remobilization of N to the shoots and additionally, recently assimilated C was retained in the regrowing shoots.

4.4.3 Effect of shading

We implemented shading (besides clipping) to evaluate the effect of a limited substrate supply on the distribution of recently assimilated C and the impacts of such a limited supply on the N budget in plant and soil. In contrast to clipping, however, the effect of shading in limiting the substrate supply is not connected with the high demand for reserve C and N for shoot regrowth. The R:S ratio of *L. perenne* was reduced after shading (Table II.4/2). The increased preference for shoot versus root growth is also reflected by the higher recovery of currently assimilated C (^{13}C) in the shoots. After shading, more assimilates are allocated into the terminal meristems to compensate for the reduced photosynthesis rate (Ryle and Powell, 1976). For *M. sativa* the ^{13}C recovery in the shoots was very high after shading and was in the range of the clipped plants. Like after clipping, this took place at the expense of the ^{13}C translocation into the roots, however, this is significant only for *M. sativa*.

Belowground translocation of C is very closely linked to the assimilate supply (Kuzyakov and Gavrichkova, 2010). Reduced soil CO_2 efflux and rhizodeposition have been observed after shading (Craine et al., 1999; Hill et al., 2007). The present study indicates that the shading effect on the CO_2 efflux from soil of currently assimilated C depends on the plant species.

For *M. sativa* the total soil CO_2 efflux decreased, whereas the portion of ^{13}C in CO_2 was not influenced by shading (Fig. II.4/2 and Fig. II.4/3). These apparently contradictory results can be explained by the need for recently assimilated C to maintain respiration (shown by the unchanged ^{13}C efflux) and by the reduced substrate supply (decreasing the total CO_2 efflux from soil) (Kuzyakov and Cheng, 2001; 2004). Contrary, for *L. perenne*, the total CO_2 efflux and the ^{13}C recovery in the CO_2 did not change after shading.

Plants grown under normal light conditions have a higher N demand compared to shaded plants, which can be met by a higher rhizodeposition and the resulting SOM decomposition (Frank and Groffman, 2009). The growth after shading is restricted by

low assimilation rates (Shipley, 2002), which also reduces the demand for N in the shoots. Moreover, under shaded conditions a reduced rhizodeposition causes a decreased turnover of the microbial biomass and SOM and, thus, a lower N mineralization (Zagal, 1994). In our study no change of the ^{13}C recovery in the soil of both plants and no change of the ^{15}N recovery in the shoots of *L. perenne* was observed after shading. Thus, our results show no effect of shading on the rhizodeposition or the N uptake by this species. The unchanging ^{13}C recovery at a concurrent decreasing of the total CO_2 efflux underlines the importance of recently fixed C for the legume *M. sativa*. *M. sativa* uses recently fixed C for nodule respiration and stored C for root respiration (Avice et al., 1996). The decreased CO_2 efflux, however, indicates overall that the nodule respiration and the root respiration were reduced. It was expected that *M. sativa* would remobilize storage N from roots to overcome this limitation of the N supply to shoots, since remobilization requires less energy than N fixation and can thus be an adequate mechanism to meet the N demand in the shoots (Bakken, 1998). The increased ^{15}N recovery in the shoots of shaded *M. sativa* may be due to a reduced uptake of unlabeled N by the N_2 fixation after shading. However, our results cannot clarify if the origin of the increased recovery of ^{15}N in the shoots is the remobilization of N from roots or a higher ^{15}N uptake from soil. Both pools show a decrease of ^{15}N after shading, however for both this decrease was not significant.

We conclude that shading has a pronounced effect on the belowground allocation of currently assimilated C for both plant species; on the other hand shading has effects on the N distribution only for *M. sativa* with a higher allocation of N in the shoots. However the origin of this N remains unclear.

4.5 Conclusion

After clipping, shoot regrowth is an important sink affecting the C distribution of newly assimilated C. To meet the demand of N for regrowth, the legume *M. sativa* increased the N allocation in the shoots. We assume that this is supported by a higher N uptake by the roots. The N pools in *L. perenne* were not affected by clipping. After shading, more C was allocated aboveground compared to normal light conditions leading to reduced translocation of assimilates in the roots of *M. sativa*. An increased need for N after shading was observed for the shoots of *M. sativa*, but the source of this N remains unclear. The results indicate that the allocation of recently assimilated C in plants and its translocation belowground is strongly influenced by the altered substrate supply after clipping and shading. However, the reduced assimilation is of minor importance for the N distribution.

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References

Augustine, D.J., Dijkstra, F.A., Hamilton III., E.W., Morgan, J.A., 2011. Rhizosphere interactions, carbon allocation, and nitrogen acquisition of two perennial North American grasses in response to defoliation and elevated atmospheric CO_2. Oecologia 165, 755-770

Avice, J.C., Ourry, A., Lemaire, G., Boucaud, J., 1996. Nitrogen and carbon flows estimated by ^{15}N and ^{13}C pulse-chase labeling during regrowth of alfalfa plant. Plant Physiology 112, 281–290.

Bakken, A.K, Macduff, J.H., Collison, M., 1998. Dynamics of nitrogen remobilization in defoliated *Phleum pratense* and *Festuca pratensis* under short and long photoperiods. Physiologica Plantarum 103, 426-436.

Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry 30, 1867-1878.

Blagodatskaya, E., Blagodatsky, S., Dorodnikov, M., Kuzyakov, Y., 2010. Elevated atmospheric CO_2 increases microbial growth rates in soil: results of three CO_2 enrichment experiments. Global Change Biology 16, 836–848.

Cheng, W., Coleman, D.C, Carroll, C.R., Hoffman, C.A., 1993. In situ measurement of root respiration and soluble C concentrations in the rhizosphere. Soil Biology and Biochemistry 25, 1189-1196.

Craine, F.M., Wedin, D.A., Chapin III, F.S., 1999. Predominance of ecophysiological controls on soil CO_2 flux in a Minnesota grassland. Plant and Soil 207, 77–86.

Detling, J.K., Dyer, M.I., Winn, D.T., 1979. Net photosynthesis, root respiration, and regrowth of Bouteloua gracilis following simulated grazing. Oecologia 41, 127-134.

Frank, D.A., Groffman, P.M., 2009. Plant rhizospheric N processes: what we don't know and why we should care. Ecology 90, 1512–1519.

Gavrichkova, O., Moscatelli, M.C., Kuzyakov, Y., Grego, S., Valentini, R., 2010. Influence of defoliation on CO_2 efflux from soil and microbial activity in a Mediterranean grassland. Agriculture, Ecosystems and Envionment 136, 87-96.

Gregory, P.J., Atwell, B.J., 1991. The fate of carbon in pulse labelled crops of barley and wheat. Plant and Soil 136, 205-213.

Guitian, R., Bardgett, R.D., 2000. Plant and soil microbial responses to defoliation in temperate semi-natural grassland. Plant and Soil 220, 271-277.

Hill, P., Kuzyakov, Y., Jones, D., Farrar, J., 2007. Response of root respiration and root exudation to alterations in root C supply and demand in wheat. Plant and Soil 291, 131-141.

Johansson, G., 1993. Carbon distribution in grass (*Festuca pratensis* L.) during regrowth after cutting-utilization of stored and newly assimilated carbon. Plant and Soil 151, 11–20.

Jones, D.L., Hodge, A., Kuzyakov, K., 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist163, 459–480.

KA5, 2005. Bodenkundliche Kartieranleitung. Ad-Hoc-Arbeitsgruppe Boden; 5. Auflage. Science Publishers.

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Schoene, J., Scheunemann, N., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E., 2012. Carbon flow into microbial and fungal biomass as a basis to understand the belowground food web in an agroecosystem. Pedobiologia 55, 111-119.

Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 16, 382-396.

Kuzyakov, Y., Kretzschmar, A., Stahr, K., 1999. Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. Plant and Soil 213, 127-136.

Kuzyakov, Y., Domanski, G., 2000. Carbon inputs into soil. Review. Journal of Plant Nutrition and Soil Science 163, 421–423.

Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology and Biochemistry 33, 1915-1922.

Kuzyakov, Y., Cheng, W., 2004. Photosynthesis controls of CO_2 efflux from maize rhizosphere. Plant and Soil 263, 85-99.

Kuzyakov, Y., Biryukova, O.V., Kuznetzova, T.V., Molter, K., Kandeler, E., Stahr, K., 2002. Carbon partitioning in plant and soil carbon dioxide fluxes and enzyme activities as affected by cutting ryegrass. Biology and Fertility of Soils 35, 348–358.

Kuzyakov, Y., Gavrichkova, O., 2010. Time lag between photosynthesis and carbon dioxide efflux from soil: a review. Global Change Biology 16, 3386-3406.

Lambers, H., Posthumus, L., 1980. The effect of light intensity and relative humidity on growth rate and root respiration of *Plantago lanceolata* and *Zea mays*. Journal of Experimental Botany 31, 1621-1630.

Mackie-Dawson, L.A., 1999. Nitrogen uptake and root morphological responses of defoliated *Lolium perenne* (L.) to a heterogeneous nitrogen supply. Plant and Soil 209, 111-118.

Meharg, A.A., Killham, K., 1990. Carbon distribution within the plant and rhizosphere in laboratory and field grown *Lolium perenne* at different stages of development. Soil Biology and Biochemistry 22, 471-477.

Merbach, W., Mirus, E., Knof, G., Remus, R., Ruppel, S., Russow, R., Gransee, A., Schulze, J., 1999. Release of carbon and nitrogen compounds and their possible ecological importance. Journal of Plant Nutrition and Soil Science 162, 373-383.

Merckx, R., Dijkstra, A., Hartog, A., Veen, J. A., 1987. Production of root-derived material and associated microbial growth in soil at different nutrient levels. Biology and Fertility of Soils 5, 126-132.

Morvan-Bertrand, A., Pavis, J., Boucaud ,J., Prud'homme, M.-P., 1999. Partitioning of reserve and newly assimilated carbon in roots and leaf tissues of *Lolium perenne* during regrowth after defoliation: assessment by ^{13}C steady-state labelling and carbohydrate analysis. Plant, Cell and Environment 22, 1097-1108.

Neergaard de, A., Gorissen, A., 2004. Carbon allocation to roots, rhizodeposits and soil after pulse labelling: a comparison of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.). Biology and Fertility of Soils 39, 228-234.

Ourry, A., Boucaud, J., Salette, L., 1988. Nitrogen Mobilization from Stubble and Roots during Re-growth of Defoliated Perennial Ryegrass. Journal of Experimental Botany 39, 803-809.

Paterson, E., Sim, A., 1999. Rhizodeposition and C partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation. Plant and Soil 216, 155–164.

Paterson, E., Sim, A., 2000. Effect of nitrogen supply and defoliation on loss of organic compounds from roots of *Festuca rubra*. Journal of Experimental Botany 51, 1449–1457.

Phillips, D.A., 1980. Efficiency of symbiotic nitrogen fixation in legumes. Annual Review of Plant Physiology 31, 29-49.

Ryle, G.J.A., Powell, C.E., 1976. Effect of rate of photosynthesis on the pattern of assimilate distribution in the graminaceous plant. Journal of Experimental Botany 27, 189-199.

Shipley, B., 2002. Trade-offs between net assimilation rate and specific leaf area in determining relative growth rate: relationship with daily irradiance. Functional Ecology 16, 682-689.

Ta, T.C., Macdowell, F.D.H., Faris, M.A., 1990. Utilization of carbon and nitrogen reserves of alfalfa roots in supporting N_2-fixation and shoot regrowth. Plant and Soil 127, 231-236.

Vance, E., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703-707.

Vance, C.P., Heichel, G.H., 1991. Carbon in N_2 fixation: Limitation or exquisite adaption. Annual Review of Plant Physiology 42, 373-392.

Volenec, J. J., Ourry, A., Joern, B. C., 1996. A role for nitrogen reserves in forage regrowth and stress tolerance. Physiologica Plantarum 97, 185-193.

Warembourg, F.R., Roumet, C., Lafont, F., 2003. Differences in rhizosphere carbon-partitioning among plant species of different plant families. Plant and Soil 256, 347-357.

Werth, M., Kuzyakov, Y., 2008. Determining root-derived carbon in soil respiration and microbial biomass using ^{14}C and ^{13}C. Soil Biology and Biochemistry 40, 625-637.

Xu, X., Ouyang, H., Richter, A., Wanek, W., Cao, G., Kuzyakov, Y., 2011. Spatio-temporal variations determine plant–microbe competition for inorganic nitrogen in an alpine meadow. Journal of Ecology 99, 563–571.

Xu, X., Stange, F., Richter, A., Wanek ,W., Kuzyakov, Y., 2008. Light affects competition for inorganic and organic nitrogen between maize seedlings and soil microorganisms. Plant and Soil 304, 59-72.

Zagal, E., 1994. Influence of light intensity on the distribution of carbon and consequent effects on mineralization of soil nitrogen in a barley *(Hordeum vulgare* L.)-soil system. Plant andSoil 160, 21-31.

5 Estimation of rhizodeposition at field scale: upscaling of a ^{14}C labeling study

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Abstract

Background and Aims

Rhizodeposition of plants is the most uncertain component of the carbon (C) cycle. By existing approaches the amount of rhizodeposition can only roughly be estimated since its persistence in soil is very short compared to other organic C pools. We suggest an approach to quantify rhizodeposition at the field scale by assuming a constant ratio between rhizodeposited-C to root-C.

Methods

Maize plants were pulse-labeled with $^{14}CO_2$ under controlled conditions and the soil $^{14}CO_2$ efflux was separated into root and rhizomicrobial respiration. The latter and the ^{14}C activity remaining in the soil corresponded to total rhizodeposition. By relating rhizodeposited-^{14}C to root-^{14}C a rhizodeposition-to-root ratio of 0.56 was calculated. This ratio was applied to the root biomass C measured in the field to estimate rhizodeposition under field conditions.

Results

Maize allocated 298 kg C ha^{-1} as root-C and 166 kg C ha^{-1} as rhizodeposited-C belowground, 50% of which were recovered in the upper 10 cm. The fate of rhizodeposits was estimated based on the ^{14}C data, which showed that 62% of total rhizodeposition was mineralized within 16 days, 7% and 0.3% was incorporated into microbial biomass and DOC, respectively, and 31% was recovered in the soil.

Conclusions

We conclude that the present approach allows for an improved estimation of total rhizodeposition, since it accounts not only for the fraction of rhizodeposits remaining in soil, but also for that decomposed by microorganisms and released from the soil as CO_2.

Keywords: Isotopic methods, belowground C, root-derived C, modeling $^{14}CO_2$ efflux, microbial biomass, dissolved organic C, CO_2 partitioning, upscaling approach.

5.1 Introduction

Plants modify chemical, physical and biological properties of the soil environment surrounding the roots. Organic compounds released from living roots (rhizodeposits), originating from roots exudates of intact cells, from lysates of sloughed-off cells and dead tissues, and from mucilage (Dennis et al., 2010) represent an important carbon (C) flux into the soil. Especially root exudates are a primary source of energy for microorganisms strongly affecting soil organic matter (SOM) dynamics (Kuzyakov et al., 2007). This ecological importance calls for a better estimation of rhizodeposition, which still remains the most uncertain part of the soil C cycle (Nguyen, 2003). There are several reasons why it is difficult to reliably quantify rhizodeposition. Organic substances released by living roots occur in a much lower content than other organics in soil and are restricted to the narrow zone around the roots (Kuzyakov and Domanski, 2000). Fast decomposition of root-released organics due to their high availability for microorganisms further makes rhizodeposition difficult to assess for analytics (Jones et al., 2005).

To distinguish rhizodeposited-C from native soil organic carbon (SOC), ^{14}C and/or ^{13}C labeling of plants has commonly been applied leading to distinct isotopic differences of root- and SOC-derived C (Werth and Kuzyakov, 2008). The portion of root-released C remaining in soil (net rhizodeposition) can thus be quantified. However, these approaches largely underestimate rhizodeposition since they did not account for the amount of rhizodeposits rapidly decomposed by microorganisms (Amos and Walters, 2006; Werth and Kuzyakov, 2008). The portion of rhizodeposits mineralized to CO_2 (rhizomicrobial respiration; RMR) contributes, together with root respiration (RR), to root-derived CO_2, a main source of the soil CO_2 efflux (Cheng et al., 2003; Kuzyakov, 2006). For further partitioning of root-derived CO_2 into RMR and RR the isotopic labeling approaches reach their limit since both sources of root-derived CO_2 are labeled by the tracer. It is, however, necessary to consider them separately because C input to soil and SOM turnover is only affected by rhizomicrobial C, while root respiration biases the picture of SOM turnover.

C accumulation and consumption in soil are closely coupled with microbial activity and in turn are influenced by rhizodeposition (Kuzyakov et al., 1999). The easily available part of rhizodeposition fuels microbial activity in the rhizosphere and thus represents a

direct link between roots and soil microorganisms. Despite the importance of separating rhizomicrobial from root respiration suitable approaches are rare. After ^{14}C pulse labeling of plants, root-derived $^{14}CO_2$ can be partitioned into CO_2 coming from the decomposition of rhizodeposits and CO_2 from root respiration by means of a simulation model (Kuzyakov and Domanski, 2002; Kuzyakov et al., 1999). The model is based on the assumption that both respiration processes reach their maximum at different times after pulse labeling. While root respiration occurs immediately, rhizomicrobial respiration appears at a later stage after labeling because a chain of successive processes is passed before (exudation, microbial uptake and respiration) (Kuzyakov et al., 2001). Special experiments are necessary to determine the $^{14}CO_2$ efflux dynamics, which are applicable under controlled conditions, but hardly possible under field conditions. Thus, estimation of rhizodeposition under field conditions remains a challenge for quantification of C budget and fluxes.

Under field conditions, root biomass (RB) was measured to estimate the portion of photosynthetically fixed C allocated to belowground pools. Those measurements alone may greatly underestimate the C input by roots into the soil since rhizodeposition is ignored (Amos and Walter, 2006; Johnson et al., 2006). The portion of net photosynthetic C translocated belowground and released by living roots can even be higher as the C retained in the roots (Johnson et al., 2006). However, root biomass contributes more C to SOC than rhizodeposition, because the latter is easily decomposable by microorganisms (Johnson et al., 2006). On average 17% of net assimilated C is released by roots via rhizodeposition, with 12% of which being mineralized to CO_2 (RMR) and only 5% remaining in the soil (Nguyen, 2003). Attempts to include rhizodeposition in estimates of C inputs into the soil by roots often only very roughly assumed that the quantity of rhizodeposited C equals that of root biomass at harvest (Bolinder et al., 1999; Amos and Walter, 2006). However, reliable data on rhizodeposition under field conditions are absent.

In this paper we provide a method for an improved quantification of total rhizodeposition, including C losses by rhizomicrobial respiration, under field conditions. After $^{14}CO_2$ pulse labeling of maize plants under controlled conditions, we measured the root-derived $^{14}CO_2$ and determined the contributions of root and rhizomicrobial respiration based on model calculations. The rhizodeposition-to-root ratio determined under con-

trolled conditions was applied to the maize root biomass measured in the field in order to estimate the rhizodeposition at a field scale.

5.2 Materials and Methods

5.2.1 Determination of rhizodeposition-to-root ratio (R) under controlled conditions

5.2.1.1 Soil and growing conditions

Intact soil cores were collected with a soil corer (inner diameter 12 cm, height 30 cm) from the upper 30 cm on the experimental site and placed in cylindrical Plexiglas pots (inner diameter 13 cm, height 30 cm, covered with dark foil). Maize seeds (*Zea mays* L. cv. Ronaldinio) were germinated on wet filter paper and transferred to the 16 pots 3 days after germination. The pots were closed with a plastic lid with holes for the shoots. The soil water content was measured gravimetrically and adjusted daily to 70% of the water holding capacity (WHC). The plants were grown at 26 to 28°C day temperature and at 22 to 23°C night temperature with a day-length of 14 h and a light intensity of about 400 μmol m^{-2} s^{-1}.

5.2.1.2 ^{14}C pulse labeling

The plants were labeled at the tillering stage, 28 days after germination. The day before labeling, the holes in the plastic lids were sealed around the shoots with silicon paste (NG 3170, Thauer & Co., Germany) and the seals were tested for air leaks. The labeling procedure is described by Kuzyakov et al. (1999). Briefly, eight pots were placed in a Plexiglas chamber (48.1 x 48.1 x 158 cm). The chamber was connected with a flask containing 5 ml of $Na_2{}^{14}CO_3$ (ARC Inc., USA) solution with a ^{14}C activity of 1.2 MBq per pot. $^{14}CO_2$ was released into the chamber by addition of 10 ml of 5 M H_2SO_4 to the labeling solution. The plants were labeled during 4 h in the $^{14}CO_2$ atmosphere. Thereafter, the chamber air was pumped through 15 ml of 1 M NaOH solution to remove unassimilated $^{14}CO_2$ for 2 h. Finally, the chamber was opened and trapping of CO_2 evolved from the soil started. CO_2 produced in four sealed pots was trapped by circulating the air through 15 ml of 1 M NaOH solution. The NaOH solution was changed every two hours after labeling for the first day, then twice daily, then once every 2 days until 16 days after labeling.

5.2.1.3 Sampling

Plants and soil were sampled 2, 5, 10, and 16 days after labeling with four replicates for each sampling day. At harvest, shoots were cut at the base and roots were separated from the soil of each layer by handpicking. The soil adhering to the roots was shaken gently and termed 'rhizosphere soil'. The roots were washed with 50 ml deionized water to remove the soil still attached to the roots. The soil was sieved (< 2 mm). Shoots, roots, bulk and rhizosphere soil were dried at 60 °C, weighed and pulverized in a ball mill.

5.2.1.4 Sample analysis

The ^{14}C activity of unassimilated $^{14}CO_2$ after labeling, trapped in NaOH, and the remaining ^{14}C activity in the tracer solution was measured in 2 ml aliquots added to 4 ml Rothiscint scintillation cocktail (Roth, Germany) with a Liquid Scintillation Counter (LS 6500 Multi-Purpose Scintillation Counter, 217 Beckman, USA) after the decay of chemiluminescence. The ^{14}C activity of soil CO_2 trapped in the NaOH solution was measured in the same way. The ^{14}C counting efficiency was about 92% and the ^{14}C activity measurement error did not exceed 2%. Total C of soil CO_2 was analyzed by an N/C analyzer (Multi N/C 2100, AnalytikJena, Germany)

50 mg of plant samples (shoots, roots) or 500 mg of soil samples (bulk and rhizosphere soil) were combusted in an oxidizer unit (Feststoffmodul 1300, AnalytikJena, Germany) and released CO_2 was trapped in 10 ml of 1 M NaOH. The radioactivity was measured by means of a Scintillation Counter (LS 6500 Multi-Purpose Scintillation Counter, 217 Beckman, USA) as described above. Total C concentrations for those samples were measured by a N/C analyzer (Multi N/C 2100, AnalytikJena, Germany)

The ^{14}C activity of the soil microbial biomass C (MBC) was determined for the four replicates sampled on day 16 after labeling by the chloroform fumigation extraction method described by Vance et al. (1987). Briefly, 5 g fresh soil were shaken with 20 ml of 0.05 M K_2SO_4 for 1 h at 200 rev min^{-1}, centrifuged at 3000 rev min^{-1} for 10 min, and filtrated. Another 5 g fresh soil were fumigated with chloroform for 24 h and extracted in the same way. The extracts were analyzed for total organic carbon by means of an N/C analyzer (Multi N/C 2100, AnalytikJena, Germany). The ^{14}C activities of the ex-

tracts of unfumigated and fumigated soils were measured using a LS 6500 Multi-Purpose Scintillation Counter, 217 Beckman, USA). Measurements were conducted on 1 mL aliquots added to 6 mL scintillation cocktail Rothiscint (Roth, Germany).

5.2.1.5 Calculation of the ^{14}C budget

A ^{14}C budget was compiled for each sampling day separately. The percentage of ^{14}C recovered in a C pool ($r(^{14}C)_P$, %) was calculated by relating the ^{14}C activity of the respective C pool ($a(^{14}C)_P$, kBq) to the total ^{14}C recovery after each harvest ($a(^{14}C)_T$, kBq), i.e. to the sum of the ^{14}C activity in shoot, root, bulk soil, rhizosphere soil and CO_2:

$$r(^{14}C)_P = \frac{a(^{14}C)_P}{a(^{14}C)_T} \cdot 100 \quad (1)$$

Note, CO_2 measurements started directly after labeling, but only for the pots harvested 16 days after labeling. Therefore, from those pots the cumulative $^{14}CO_2$ efflux after 2, 5 and 10 days of labeling was added to the total ^{14}C recovery on the respective day.

The ^{14}C results obtained from the measurement of the extracts of fumigated and unfumigated soil were converted to the ^{14}C activity in microbial biomass ($^{14}C_{mic}$) using the following equation:

$$^{14}C_{mic} = \frac{^{14}C_{flush}}{0.45} \quad (2)$$

where $^{14}C_{flush}$ is the difference between the ^{14}C activity in fumigated and in unfumigated samples (kBq) and 0.45 is the conversion factor (Wu et al., 1990). As a measure for the fraction of dissolved organic carbon (DOC) we used the ^{14}C activity of the unfumigated soils.

The percentage of ^{14}C recovered in MBC and DOC on day 16 after labeling was calculated using Eq. (1).

5.2.1.6 Model calculations for separating root and rhizomicrobial respiration

In order to estimate the percentage of root respiration and rhizomicrobial respiration on total $^{14}CO_2$ efflux a model approach was applied. The model design is described in detail by Kuzyakov and Domanski (2002). The ^{14}C activity of total CO_2 $a(^{14}C)_{CO_2}$ (kBq) was converted into percentage of total assimilated CO_2 $r(^{14}C)_{CO_2}$ before using it in the model. The amount of total assimilated ^{14}C $a(^{14}C)_{TA}$ (kBq) was assumed to be equal to the ^{14}C activity of the tracer introduced into the chamber $a(^{14}C)_{C}$ (kBq) at the beginning of labeling minus the ^{14}C activity remaining in the chamber $a(^{14}C)_{RC}$ (kBq) and in the tracer solution $a(^{14}C)_{RS}$ (kBq) after labeling (Kuzyakov and Domanski, 2002).

$$r(^{14}C)_{CO_2} = \frac{a(^{14}C)_{CO_2}}{a(^{14}C)_{TA}} \cdot 100 \quad (3)$$

$$a(^{14}C)_{TA} = a(^{14}C)_{C} - a(^{14}C)_{RC} - a(^{14}C)_{RS} \quad (4)$$

The model parameters (Table II.5/1) were adjusted based on 1) the $^{14}CO_2$ efflux rate from soil, expressed in % of assimilated per hour, and based on 2) the cumulative $^{14}CO_2$ efflux, expressed as % of assimilated. Thereby, the cumulative $^{14}CO_2$ efflux allows to adjust parameters responsible for the amount of respired $^{14}CO_2$, while the $^{14}CO_2$ efflux rate was used to adjust parameters responsible for the dynamics of the respiration rates (Kuzyakov and Domanski, 2002). The distribution between above- and belowground C pools was considered by the shoot-to-root ratio. The parameters shoot growth rate, short-term shoot respiration and long-term shoot respiration were not considered here since they did not affect the belowground ^{14}C fluxes. RR and RMR were simulated based on the Model-maker (3) software (ModelKinetix, Oxford, UK; www.modelkinetix.com).

Table II.5/1: Model parameters of belowground C fluxes fitted by experimental data of ^{14}C distribution, total $^{14}CO_2$ efflux and its dynamics.

Parameter	**Value [h^{-1}]**
Assimilation rate	0.617
Ratio: shoot/root	0.815[a]
Exudation rate	0.383
Exudate mineralization	0.05
Exudate stabilization	0.001
Root growth	0.004
Root respiration	0.227
Root mineralization	0.0012[b]
Biomass respiration	0.35
Biomass stabilization	0.016[b]
Biomass exudation	0.2
SOM mineralization	0.0004[b]

[a] unitless: refractive index.

[b] values were taken from earlier model parameterization (Kuzyakov et al., 2001).

5.2.1.7 Rhizodeposition-to-root ratio

The contribution of rhizomicrobial respiration ($r(^{14}C)_{RMR}$, % of assimilated) to total root-derived $^{14}CO_2$, simulated by the $^{14}CO_2$ efflux model, was converted into the ^{14}C activity of rhizomicrobial respiration ($a(^{14}C)_{RMR}$, kBq):

$$a(^{14}C)_{RMR} = \frac{a(^{14}C)_{CumCO_2} \cdot r(^{14}C)_{RMR}}{100} \quad (5)$$

where $(^{14}C)_{CumCO_2}$ (kBq) is the fitted ^{14}C activity of the cumulative $^{14}CO_2$ efflux at day 16 after labeling.

The rhizodeposition-to-root ratio (R) was calculated as follows:

$$R = \frac{a(^{14}C)_{BS} + a(^{14}C)_{RS} + a(^{14}C)_{RMR}}{a(^{14}C)_{Root}} \quad (6)$$

where $a(^{14}C)_{BS}$ and $a(^{14}C)_{RS}$ are the ^{14}C activities in kBq of the bulk and the rhizosphere soil, respectively, and $a(^{14}C)_{Root}$ is the ^{14}C activity in kBq of the root.

5.2.2 Root biomass measurements in the field - experimental design and root sampling

The experimental site was established on an arable field in the north-west of Göttingen, Germany (51°33´36.8´´N, 9°53´46.9´´E) in 2009. The soil type was classified as a haplic Luvisol. Detailed information about soil properties and the experimental site are given by Kramer et al. (2012). Maize (*Zea mays* L. cv. Ronaldinio) was planted on a 24 x 240 m plot in April 2009 after removing wheat seedlings sown in October 2008 with a non-selective herbicide ("Round-up", Monsanto Agrar, Germany). The mean distance between the maize rows was 0.8 m and the mean distance between the plants in row was 0.5 m. Maize plants on the experimental plots were harvested in November 2009.

Root biomass was sampled in July 2009, at the silking stage of the maize plants, 12 weeks after planting. To investigate the spatial distribution of maize roots we sampled direct at the position of the maize plant, 12.5 cm and 25 cm away from the plant in row, 20 cm and 40 cm away in the inter-row and 23.5 cm and 47 cm away from the plant at the diagonal between row and inter-row. Soil samples were taken with an auger (Riverside auger, Eijkelkamp, The Netherlands) at each position up to 50 cm depth in 10 cm layers. This sampling procedure allowed to cover spatial variability of maize roots under the plant, within and between the maize rows.

Each fresh sample was weighed and a subsample of the soil (without roots) was dried for 3 days at 60°C. The water content of the subsample was used to determine the dry weight of the total sample. All roots were carefully washed free from soil using the method described by Smucker et al. (1982). The remaining non-root material was separated from the sample by handpicking. The samples were dried at 60°C for 3 days and weighed. The C content of the roots was determined on five replicates using a multi N/C 2100 S analyzer (Analytik, Jena, Germany). Root biomass was expressed as mg C per g dry soil. Note, in the present study only the portion of the root system below the soil surface was considered as root biomass and thus, the aboveground crown was not included.

5.2.3 Upscaling: Root biomass C and total C from rhizodeposition in the field

The amount of maize root C ($n(C_{Root})_F$, kg C ha^{-1}) was calculated for each 10 cm layer until 50 cm.

$$n(C_{Root})_F = z \cdot \rho \cdot n(C_{Root}) \cdot 100 \quad (7)$$

where z (cm) is the thickness of the respective soil layer (10 cm), ρ (g cm^{-3}) is the bulk density of the layer and $n(C_{Root})$ is the C (mg C g_{soil}^{-1}) content of the roots. Bulk density values were taken from Kramer et al. (2012) and are 1.4±0.0 g cm^{-3} for the Ap1 horizon (0-25 cm), 1.6±0.0 g cm^{-3} for the Ap2 horizon (25-37 cm) and 1.7±0.0 g cm^{-3} for the Btw1 horizon (37-65cm).

The amount ($n(C_{RD})_F$) of root released C in the field during one growing season was estimated by multiplying the amount of maize root C ($n(C_{Root})_F$) with the rhizodeposition-to-root ratio (R) and was expressed as kg C ha^{-1}:

$$n(C_{RD})_F = R \cdot n(C_{Root})_F \quad (8)$$

5.2.4 Statistics

The values presented in the figures are given as means ± standard errors of means (SEM). A one-way analysis of variance (ANOVA) was conducted to test for significant differences in root biomass C between the sampling depths. The significance of differences between the depths at individual sampling positions was obtained by the *post hoc* Tukey HSD test for unequal N, while the significance of differences for the mean root biomass C between depths was calculated by the *post hoc* Tukey HSD test. Significant differences in the ^{14}C recovery between the sampling dates were also obtained by a one-way ANOVA in combination with a *post hoc* Tukey HSD test for unequal N. All statistical analysis were performed with the statistical package STATISTICA for Windows (version 7.0; StatSoft Inc., OK, USA).

5.3 Results

5.3.1 ^{14}C pulse labeling under controlled conditions

5.3.1.1 Budget of assimilated ^{14}C

The precondition for the determination of the rhizodeposition-to-root ratio at the end of the chase period was that the ^{14}C allocation between above- and belowground C pools was mostly completed. To demonstrate this, pots were sampled 2, 5, 10 and 16 days after the labeling and the ^{14}C budgets of the individual sampling dates were checked for statistical differences. We could not find significant differences between ^{14}C budgets of the investigated sampling dates with the exception of the difference in the ^{14}C recovery of the bulk soil between day 2 and 5 (Table II.5/2). Thus, the main part of the tracer was allocated to various pools already in the first two days after the labeling.

At all sampling dates about half of the tracer was incorporated into the shoot biomass and 21%-28% was recovered in the roots (Table II.5/2). While about 7% of ^{14}C retained in the bulk soil, the ^{14}C recovered in the rhizosphere soil was only about 0.1% because of its small volume. The missing portion of ^{14}C in the complete balance is connected with the CO_2 efflux from soil, which was included in the calculations of the ^{14}C recovery.

Table II.5/2: ^{14}C budget calculated as % of ^{14}C recovered in all pools at each sampling date (±SEM). Different letters indicate significant differences between the treatments. Note that the difference to 100% represents the portion of the CO_2 efflux from soil.

	^{14}C [% recovery]			
Pool	**2 d**	**5 d**	**10 d**	**16 d**
Shoot	53.5±3.0	53.9±3.0	53.5±0.4	50.9±3.7
Root	22.8±3.5	21.6±3.8	20.6±4.1	28.0±5.4
Bulk soil	8.2±0.9 a	4.3±1.1 b	8.3±1.9 ab	5.2±0.3 ab
Rhizosphere soil	0.2±0.04	0.1±0.02	0.1±0.01	0.1±0.03

5.3.1.2 Separating root and rhizomicrobial respiration

An earlier developed model of belowground C fluxes was applied to determine the contributions of RR and RMR to total root-derived CO_2.

Cumulative $^{14}CO_2$ efflux and the $^{14}CO_2$ efflux rate measured under controlled conditions were used to fit most of the model parameters (Table II.5/1). The root growth rate (h^{-1}) was measured as root biomass increase between the sampling dates. For parameters which can be varied over a wide range we used the values from the previous model parameterization (Kuzyakov and Domanski, 2002) (Table II.5/1). A good correlation between the measured and the fitted data were obtained, for the cumulative $^{14}CO_2$ efflux (Fig. II.5/1A) as well as for the $^{14}CO_2$ efflux dynamics (Fig. II.5/1B). The model is based on the finding that the ^{14}C activity of the CO_2 efflux after pulse labeling shows two peaks (Warembourg and Billes, 1979; Nguyen et al., 1999; Kuzyakov et al., 1999; 2001; Kuzyakov and Domanski, 2002). Warembourg and Billes (1979) assumed that the second peak of ^{14}C activity can be attributed to the decomposition of rhizodeposits by microorganisms, and is delayed compared to root respiration because of the time necessary for roots to synthesize and release substances which are decomposed later on. We found the highest ^{14}C activity of the CO_2 efflux already 6 hours after the start of the labeling (Fig. II.5/1). Thereafter, the ^{14}C activity strongly decreased within the first 20 hours. The data did not show a distinctive second peak. However, the CO_2 efflux rate remained on a constant level between 20 h and 24 h after the labeling before it gradually declined. The measured kinetics is similar to that reported by Nuygen et al. (1999) and Todorovic et al. (2001). The assumption of different process rates of root and rhizomicrobial respiration allow to separate both based on the simulation model. During the 16 days after labeling about 16.2% of total assimilated ^{14}C was detected in root-derived CO_2 (Fig. II.5/1A). Rhizomicrobial respiration accounted for 9.2% of assimilated $^{14}CO_2$, which equals 56.8% of total root-derived $^{14}CO_2$.

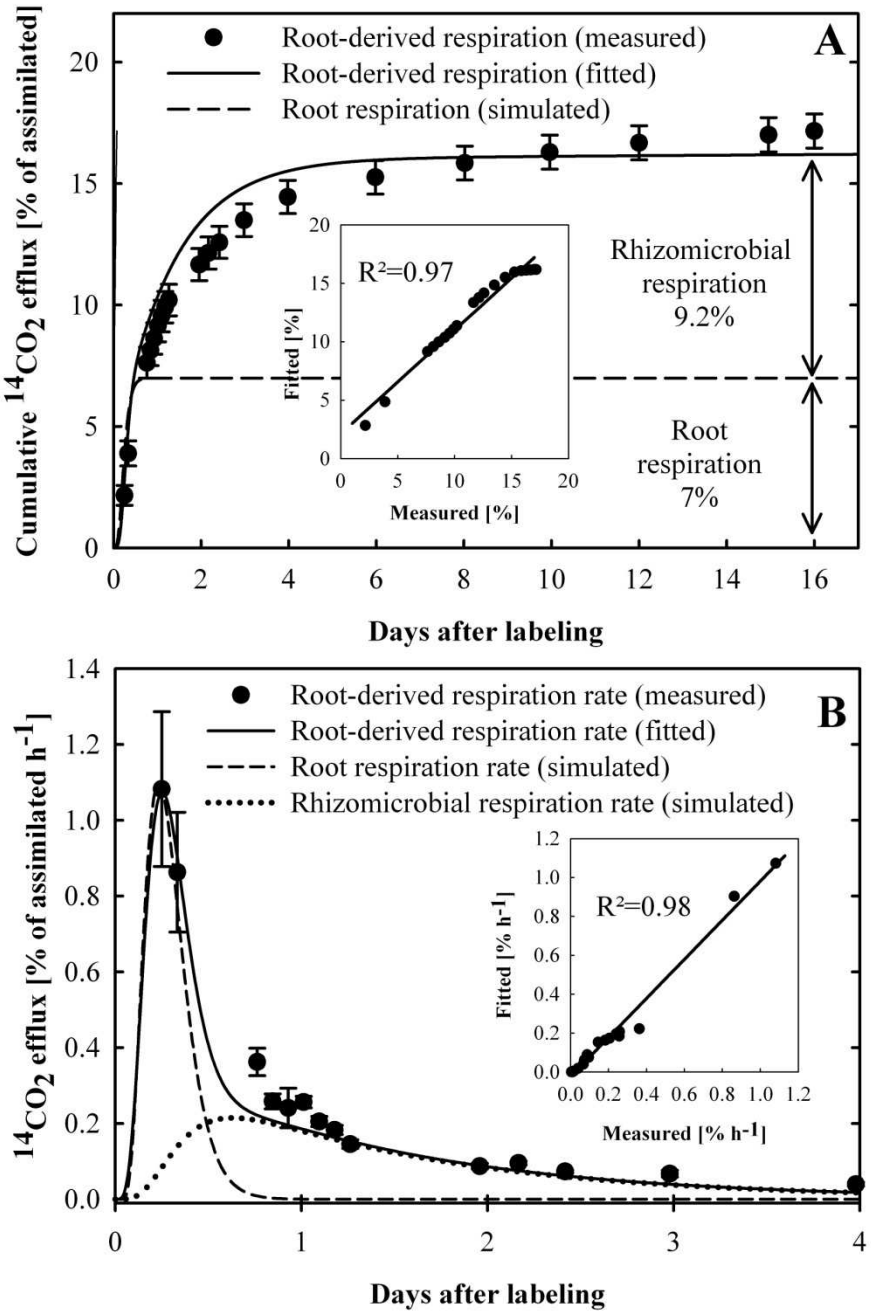

Figure II.5/1: Measured (circles; ±SEM) and fitted (solid line) $^{14}CO_2$ efflux from the soil and simulated separation of the total $^{14}CO_2$ efflux in root respiration and rhizomicrobial respiration. A: Cumulative $^{14}CO_2$ efflux; B: $^{14}CO_2$ efflux dynamics.

5.3.1.3 Rhizodeposition-to-root ratio

The ^{14}C activities of the bulk soil, of the rhizosphere soil and of microbially respired $^{14}CO_2$ were considered as total ^{14}C rhizodeposition and related to the ^{14}C activity of the roots. The respective rhizodeposition-to-root ratio was on average 0.56±0.2 (Fig. II.5/2). To investigate the fate of the rhizodeposits, total rhizodeposition (16 days after labeling) was partitioned into four C fluxes. The largest portion of rhizodeposits was respired by microorganisms and released as CO_2. This portion accounted for 61.8% of total rhizodeposition. About 30.6% of the ^{14}C released by roots retained in the soil longer than 16 days, with further 7.3% being incorporated into the microbial biomass and only 0.3% recovered in DOC.

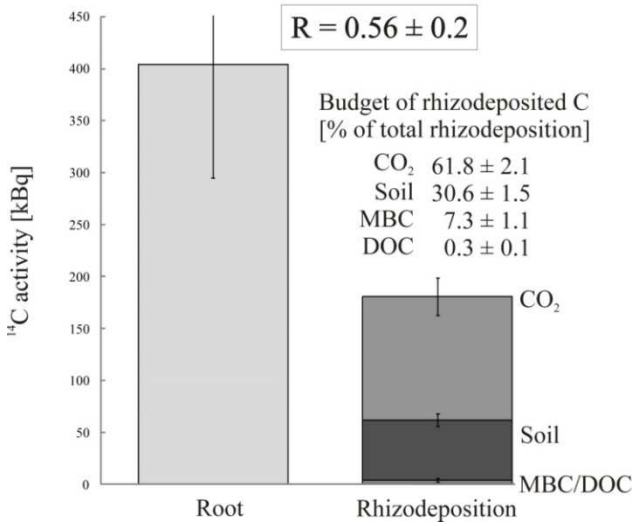

Figure II.5/2: Determination of the rhizodeposition-to-root ratio (R). The ^{14}C activity (±SEM, kBq) of roots and of total rhizodeposition is shown. The allocation of root released C to different pools is presented as percentage of total rhizodeposition

5.3.2 Root biomass in the field

Root biomass sampled directly over the plant showed a decline with depth (Fig. II.5/3). About 50% of the roots were distributed in the upper 10 cm of the Ap horizon. The decline was still present 12.5 cm away from the plant in row and 23.5 cm away on the diagonal between row and inter-row. The RB did not differ significantly at one depth between the seven sampling positions (aboveground crown not included; Fig. II.5/3). The weighted average biomass C also declined with depth, from 104 kg C ha^{-1} at the 0-10 cm to 15 kg C ha^{-1} at the 40-50 cm depth (Fig. II.5/4).

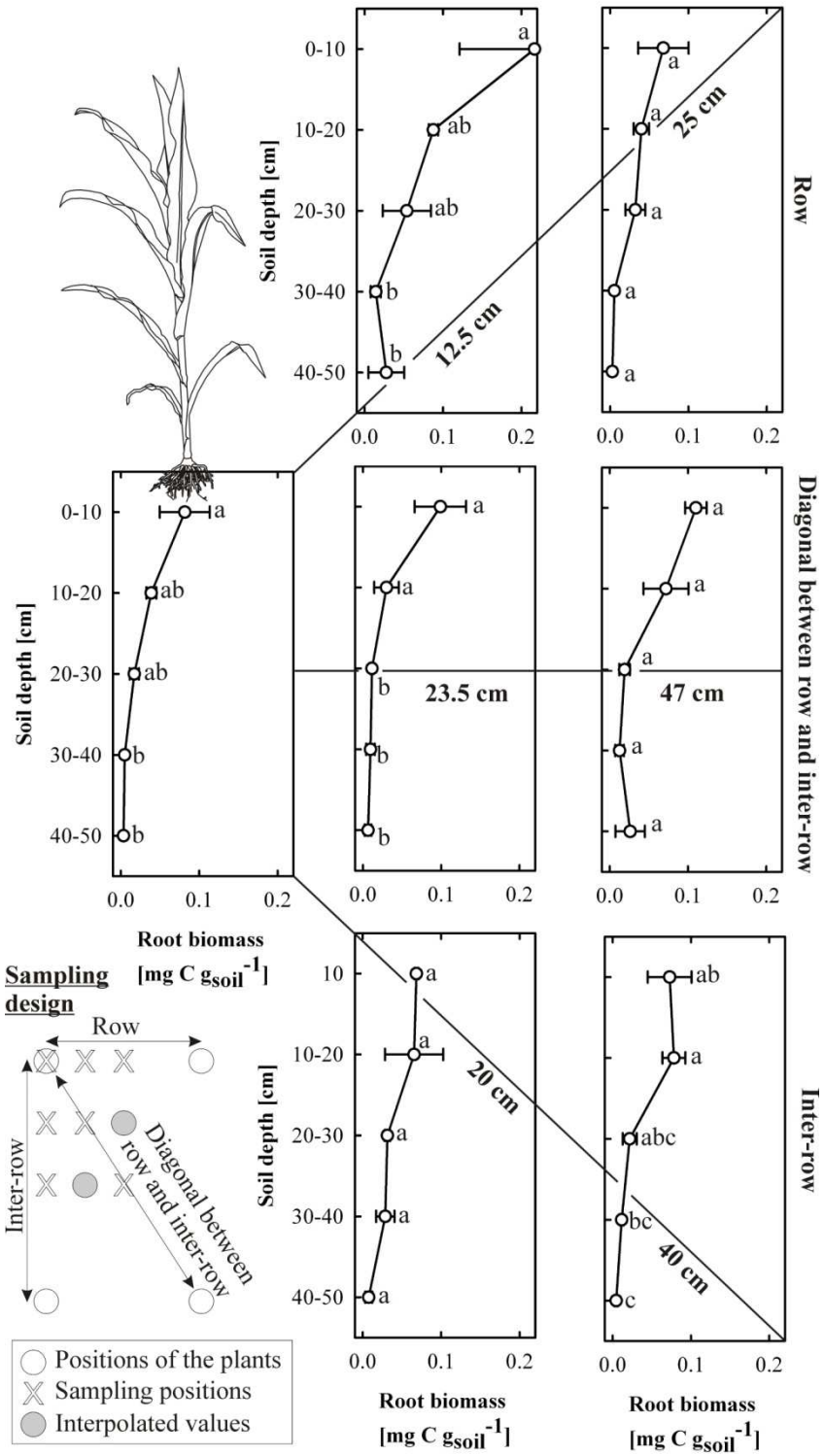

Figure II.5/3: Vertical and horizontal root biomass C distribution (±SEM) of maize sampled in July 2009. Different letters indicate significant differences ($P<0.05$) of root biomass C between the depths (vertical). The samples of a single depth did not differ significantly between different positions (horizontal). The sampling design is shown bottom left

5.3.3 Upscaling: Root biomass C and total C from rhizodeposition in the field

Considering the spatial variability of roots between and within the rows, the measured maize root C in the upper 50 cm was 298±64 kg C ha^{-1} (Fig. II.5/4). By applying the rhizodeposition-to-root ratio of 0.56±0.2 analyzed by ^{14}C labeling under controlled conditions (Fig. II.5/3) to the root C measured in field we estimated that 166±53 kg C ha^{-1} was released from living roots as rhizodeposits between April and July 2009 in the upper 50 cm of the soil. Half of these rhizodeposits was released into the upper 10 cm soil. The portion declined with depth (Fig. II.5/4).

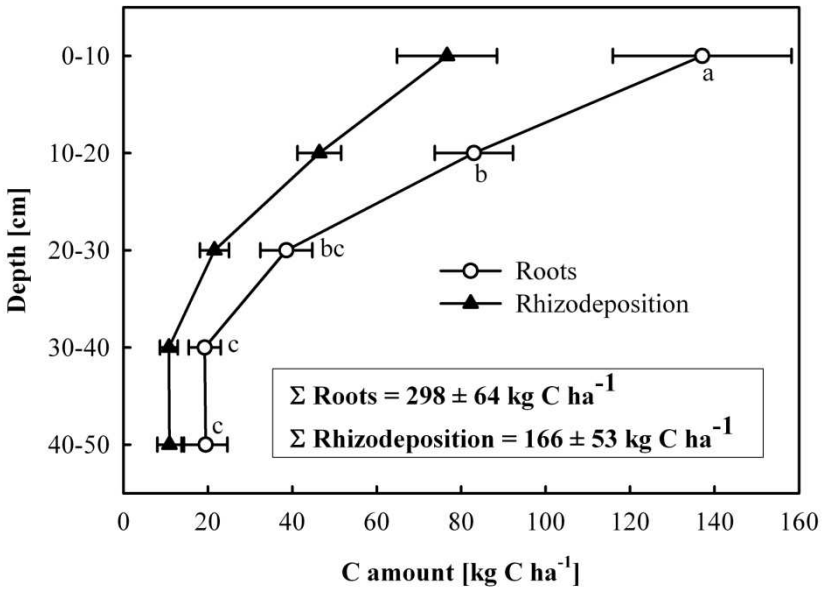

Figure II.5/4: Rhizodeposition at field scale. Weighted maize root biomass C (±SEM, kg C ha^{-1}) measured in July 2009 and C released by roots via rhizodeposition (±SEM, kg C ha^{-1}) during the growing season 2009. The extrapolated values, shown in the frame, correspond to a soil depth of 50 cm, a plant population of 25,000 and a growing period from April to July 2009. Different letters indicate significant differences between the depths

5.4 Discussion

5.4.1 Root biomass determination in the field

A reliable quantification of the maize root biomass in the field, as a precondition to estimate rhizodeposition, strongly depends on 1) the sampling design and time of sampling and on 2) losses during the root washing procedure. The sampling design must cover the spatial variability of the RB to accurately extrapolate to the basis of RB per hectare. It was shown that the maize RB was highest close to the base of the plants, than decreased with distance from the plant and increased again at the mid-row position where adjacent plants contribute to the RB (Gajri et al., 1994). RB sampling at different positions in row, in inter-row and on the diagonal between row and inter-row allows to cover the spatial variability. However, in the present experiment with already developed maize plants, the RB did not differ significantly between the sampling locations (Fig. II.5/3). Furthermore, the rooting depth must be considered. The portion of roots grown deeper than the sampling depth of 50 cm was not considered and therefore, the total RB may be slightly underestimated. However, the majority of roots were allocated directly below the soil surface. The upper 30 cm contain 70%-90% of the RB of maize (reviewed by Amos and Walter, 2006). Therefore, samples were taken up to 50 cm depth included the main part of the RB. Root biomass was sampled on day 84 after planting, at the silking stage of the plant growth. This stage was chosen since it has been reported that the maize root biomass is maximal just after anthesis (Amos and Walters, 2006; Anderson, 1988).

The second source of uncertainty of RB determination may have been the washing procedure. Despite a considerable loss of root hairs and fine roots during the washing procedure (see discussion below), 93% to 96% of total maize RB has been recovered when using a sieve with a mesh size of 0.5 mm (Livesley et al., 1999), as done in the present experiment.

The root-to-shoot ratio (R/S) measured in the current study was compared with literature data. For calculating R/S, the aboveground biomass (without the crown) of nine representative maize plants was measured. The dry weight per plant was on average 173 g plant^{-1}. The average distance between maize rows (inter-row) in the field was 0.8 m and the average distance between plants in row was 0.5 m, resulting in a theoretical

number of 25,000 plants ha^{-1}. Therefore, the shoot biomass accounts for 4325 kg dry weight ha^{-1}. We calculated a spatially weighted mean RB of 960 kg dry weight ha^{-1} with reference to the upper 50 cm of soil. The resulting R/S of 0.22 is in agreement with the R/S ratios reviewed by Amos and Walter (2006). With plant age a decrease in the root-to-shoot ratio has been reported, from 0.68 at emergence to 0.16 at physiological maturity (Amos and Walter, 2006).

5.4.2 Factors affecting root biomass and/or rhizodeposition

The type of study, i.e. field or controlled conditions, may affect root biomass and rhizodeposition through differences in growth conditions. To keep the soil conditions as comparable as possible, intact soil cores from the field site were used for the controlled conditions experiment. To exclude plant genetic influences on the root system and on rhizodeposition the same maize variety as in the field was used.

The root biomass and the quantity of C released by living roots depend on the plant phenology and on environmental factors (Grayston et al., 1996; Hütsch et al., 2002; Nguyen, 2003). Plant phenology may influence root biomass as well as rhizodeposition, mainly through root growth dynamics and differences in the quantity of rhizodeposits (Vancura, 1964; Klein et al., 1988; Van der Krift et al., 2001; Jones et al., 2004). At young age, plants translocate more carbon to the roots, whereas older plants preferably retain newly assimilated C in the shoots (Keith et al., 1986; Gregory and Atwell, 1991; Palta and Gregory, 1997; Gransee and Wittenmayer, 2000) thus, leading to decreased C inputs into the soil due to a decreased assimilates allocation to the roots (reviewed by Nguyen, 2003). Aging of plants decreases the exudation intensity, however, if this decrease is slower than the root growth total rhizodeposition will increase (Kuzyakov, 2002). On the other hand an enhanced die of root material with plant age increased C inputs into the soil. It was shown that rhizodeposition is positively correlated to root biomass (Van der Krift et al., 2001). To adequately estimate the rhizodeposition under field conditions, we sampled root biomass at the maximum development stage of the root system. However, under controlled conditions the size of the pots may restrict the rooting volume and the amount of nutrients available for plants. To overcome these restrictions an earlier stage of plant development were studied under controlled conditions as compared to the field. Here we assumed that changes in the root biomass between

field and controlled conditions are accompanied with the same relative changes in rhizodeposition. This assumption allows to conclude that despite the differences between the root biomass (and rhizodeposition) in field and under controlled conditions, the rhizodeposition-to-root ratio remains nearly constant and its changes are much lower than variations in the both C pools.

Furthermore, not only plant phenology but also environmental factors may alter the root growth pattern and the amount of rhizodeposition (Grayston et al., 1996; Hütsch et al., 2002; Nguyen, 2003). The release of C by living roots is driven by the allocation of recently assimilated C belowground and thus, depends to a large degree on the intensity of photosynthesis (Kuzyakov and Cheng, 2001) and growth rates of individual organs. It was suggested that plants grown under natural sunlight released a higher amount of C compared to plants grown under artificial light, the latter showing highly variable values (Amos and Walter, 2006). On the other hand, consistently lower light conditions may not only reduce the rhizodeposition of maize plants (Kuzyakov and Cheng, 2004), but may also lead to a lower root biomass (Hébert et al., 2001).

The main assumption involved in the current study was, that the ratio of rhizodeposition-to-root is much more stable than changes in the C amount of roots and rhizodeposits between field and controlled conditions.

5.4.3 ^{14}C-Partitioning

Sixteen days after the labeling about 51% of the ^{14}C activity was recovered in the maize shoot, 28% in the roots, 5% in the soil and 16% in the CO_2 efflux. The ^{14}C recovery in the CO_2 efflux was within the range of 21% reported by Werth and Kuzyakov (2008) and of 14% measured by Trodorovic et al. (2001). Based on a $^{14}CO_2$ efflux model we found that root respiration accounts for 7% of total assimilated ^{14}C and about 9.2% of assimilated ^{14}C was released as CO_2 from rhizomicrobial respiration. These values are close to the results obtained for *Lolium perenne*, ranging 1.4%-7.6% and 0.9%-8% of assimilated ^{14}C for RR and RMR, respectively (Kuzyakov et al., 1999; 2001; Kuzyakov and Domanski, 2002). In a further study, conducted with wheat, Cheng et al. (1993) used the isotope dilution method to separate root and rhizomicrobial respiration and found that 59% of root-derived C is coming from rhizomicrobial respiration. This is in accordance to our value of 57% rhizomicrobial respiration on total root-derived CO_2.

5.4.4 Rhizodeposition at field scale

The main obstacle to quantify total rhizodeposition is the separation of root-derived CO_2 into root and rhizomicrobial respiration. Limitations and advantages of methods used to separate the sources of root-derived CO_2 were reviewed earlier (Hanson et al., 2000; Kuzyakov and Larinova, 2005; Sapronov and Kuzyakov, 2007). Due to methodological difficulties and various assumptions involved in the separation methods, most studies, aiming to quantify the amount of C released from living roots, are focusing on the net rhizodeposition, i.e. on the amount of rhizodeposits that remained in the soil at harvest. In order to compare our data from the ^{14}C labeling experiment with data from the literature we calculated a net rhizodeposition-to-root ratio based on the results of eight studies conducted with maize. CO_2 from rhizomicrobial respiration was not included (Table II.5/3). Thus, net rhizodeposition is equal to the portion of ^{14}C measured in the soil at harvest.

The net rhizodeposition-to-root ratio ranged from 0.04 to 0.84 (Table II.5/3), with a mean value of 0.34 and a median of 0.35. In our study the net rhizodeposition-to-root ratio (decomposition to CO_2 is not included) was on average 0.29. However, when including the CO_2 from RMR the ratio was almost twice as high since about 62% of released rhizodeposits were decomposed within 16 days (Fig. II.5/2).

The amounts of rhizodeposition and root biomass C are influenced by various biotic and abiotic factors in the plant-soil system (Jones et al., 2004; Amos and Walter, 2006). The soil environment can affect rhizodeposition and root biomass through physical aspects (e.g. water availability, temperature, soil texture) and chemical conditions (e.g. pH, availability of nutrient ions), as well as through the activity and diversity of microbial populations (Lynch et al., 2002). Moreover, plant-mediated factors, like the maize variety and the plant phenological stage, are influencing the root biomass and the rhizodeposition. The mentioned factors may alter the rhizodeposition-to-root ratio and thus, may provide the explanation for the variability in the literature data (Table II.5/3). This variability underlines the necessity for future experiments assessing the effects of various factors influencing rhizodeposition and/or root biomass on the robustness of the rhizodeposition-to-root ratio. In our study, however, the same plant species and variety and

the same soil as in the field was used, thus, we assumed a constant rhizodeposition-to-root ratio for controlled and field conditions.

By applying the rhizodeposition-to-root ratio of 0.56 to the root biomass determined in the field, we estimated that about 166 kg C ha^{-1} was released by living roots into the soil in the time from planting to sampling (April to July 2009) for a theoretical plant population of 25,000 plants ha^{-1}. Amos and Walter (2006) concluded in their review, that net belowground C accounts for about 29±13% of shoot biomass C of maize when assuming similar C contents of roots and shoots. By doing so, we found 34.6% of the shoot biomass C allocated belowground (roots + rhizodeposition). Root respiration was not included. This value, however, includes RMR and may thus slightly be higher than the reported average.

Table II.5/3: Net rhizodeposition-to-root ratio calculated based on ^{14}C labeling studies with maize (recalculated and modified after Amos and Walter, 2006).

Net rhizo-deposition/ Root	**Time of sampling[a]**	**Approach[c]**	**Reference**
0.11	30 DAG	^{14}C continuous labeling	Helal and Sauerbeck, 1986
0.16	21 DAG	^{14}C continuous labeling	Helal and Sauerbeck, 1989
0.84 [b]	3 DAL	^{14}C pulse labeling (field)	Kisselle et al., 2001
0.73 [b]	13 DAL		
0.61 [b]	55 DAL		
0.37	46 DAP/ 48 DAG	^{14}C continuous labeling	Martens, 1990
0.40	76 DAP/ 78 DAG		
0.54	111 DAP/ 113 DAG		
0.09	21 DAG	^{14}C continuous labeling	Merckx et al., 1986
0.17	28 DAG		
0.06	35 DAG		
0.04	42 DAG		
0.54	24 DAG	^{14}C pulse labeling	Todorovic et al., 2001
0.54	21 DAP/ 24 DAG	^{14}C pulse labeling	Tubeileh et al., 2003
0.36	35 DAP/ 38 DAG		
0.75	42 DAP/ 45 DAG		
0.34	9 DAG	^{14}C pulse labeling	Werth and Kuzyakov, 2008
0.19	22 DAG	Repeated ^{14}C pulse labeling	
0.15	28 DAG		
0.24	34 DAG		
0.27	40 DAG		
0.37	2 DAL/ 30 DAG	^{14}C pulse labeling	This study
0.20	5 DAL/ 33 DAG		
0.41	10 DAL/ 38 DAG		
0.19	16 DAL/ 44 DAG		
Median / Mean 0.34 / 0.35			

[a] DAG, days after germination; DAP, days after planting; DAL, days after labeling.

[b] Average across treatments.

[c] All studies were conducted under light intensities between 180 and 400 µmol m^{-2} s^{-1}.

5.5 Conclusion

We showed that the combination of root biomass data from the field with the rhizodeposition-to-root ratio determined under controlled condition allow to quantify rhizodeposition at a field scale. The advantage of the present approach compared to recent estimates is that the portion of rhizodeposits, which are quickly mineralized by microorganisms (rhizomicrobial respiration), is considered. Thus, in contrast to previous studies estimating net rhizodeposition, here the gross rhizodeposition was measured and upscaled to the field. The portion of rhizodeposits decomposed to CO_2 within 16 days accounts for about 57% of total root-derived CO_2. Therefore, including RMR led to an improved estimation of the total rhizodeposition under field conditions. Our data showed a total rhizodeposition by maize of 166±53 kg C ha^{-1}. If the rhizodeposition-to-root ratio is known for particular plants, the new approach offers a promising estimation of rhizodeposition at field scale as a huge data base of root biomass distributions already exists in the literature.

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References

Amos, B., Walters, D.T., 2006. Maize root biomass and net rhizodeposited carbon: An analysis of the literature. Soil Science Society of America Journal 70, 1489-1503.

Anderson, E.L., 1988. Tillage and N fertilization effects on maize root growth and root:shoot ratio. Plant and Soil 108, 245-251.

Bolinder, M.A., Angers, D.A., Giroux, M., Laverdiere, M.R., 1999. Estimating C inputs retained as soil organic matter from corn (*Zea Mays* L.). Plant and Soil 215, 85-91.

Cheng, W., Coleman, D.C., Carroll, C.R., Hoffman, C.A., 1993. In situ measurement of root respiration and soluble carbon concentrations in the rhizosphere. Soil Biology and Biochemistry 25, 1189-1196.

Cheng, W., Johnson, D.W., Fu, S., 2003. Rhizosphere effects on decomposition: controls of plant species, phenology, and fertilization. Soil Science Society of America 67, 1418-1427.

Dennis, P.G., Miller, A.J., Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiology Ecology 72, 313-327.

Gajri, P.R., Arora, V.K., Kumar, K., 1994. A procedure for determining average root length density in row crops by single-site augering. Plant and Soil 160, 41-47.

Gransee, A., Wittenmayer, L., 2000. Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. Journal of Plant Nutrition and Soil Science 163, 381-385.

Gregory, P.J., Atwell, B.J., 1991. The fate of carbon in pulse-labelled crops of barley and wheat. Plant and Soil 136, 205-213.

Grayston, S.J., Vaughan, D., Jones, D., 1996. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5, 29-56.

Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48, 115-146.

Hébert, Y., Guingo, E., Loudet, O., 2001. The response of root/shoot partitioning and root morphology to light reduction in maize genotypes. Crop Science 41, 363-371.

Helal, H.M., Sauerbeck, D., 1986. Effect of plant roots on carbon metabolism of soil microbial biomass. Zeitschrift für Pflanzenernährung und Bodenkunde 149, 181-188.

Helal, H.M., Sauerbeck, D., 1989. Carbon turnover in the rhizosphere. Zeitschrift für Pflanzenernährung und Bodenkunde 152, 211-216.

Hütsch, B.W., Augustin, J., Merbach, W., 2002. Plant rhizodeposition - an important source for carbon turnover in soils. Journal of Plant Nutrition and Soil Science 165, 397-407.

Johnson, J.M.-F., Allmaras, R.R., Reicosky, D.C., 2006. Estimating source carbon from crop residues, roots and rhizodeposits using the national grain-yield database. Agronomy Journal 98, 622-636.

Jones, D.L., Hodge, A., Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163, 459-480.

Jones, D.L., Kemmitt, S.J., Wright, D., Cuttle, S.P., Bol, R., Edwards, A.C., 2005. Rapid intrinsic rates of amino acid biodegradation in soils are unaffected by agricultural management strategy. Soil Biology and Biochemistry 37, 1267–1275.

Keith, H., Oades, J.M., Martin, J.K., 1986. Input of carbon to soil from wheat plants. Soil Biology and Biochemistry 18, 445-449.

Kisselle, K.W., Garrett, C.J., Fu, S., Hendrix, P.F., Crossley Jr., D.A., Coleman, D.C., Potter, R.L., 2001. Budgets for root-derived C and litter-derived C: comparison between conventional tillage and no tillage soils. Soil Biology and Biochemistry 33, 1067-1075.

Klein, D.A., Frederick, B.A., Biondini, M., Trlica, M.J., 1988. Rhizosphere microorganisms effects on soluble amino acids, sugars and organic acids in the root zone of *Agropyron cristatum*, *A. smithii* and *Bouteloua gracilis*. Plant and Soil 110, 19-25.

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Schoene, J., Scheunemann, N., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E., 2012. Carbon flow into microbial and fungal biomass as a basis to understand the belowground food web in an agroecosystem. Pedobiologia 55, 111-119.

Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 165, 382-396.

Kuzyakov, Y., 2006. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biology and Biochemistry 38 (3), 425-448.

Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology and Biochemistry 33 (14), 1915-1925.

Kuzyakov, Y., Cheng, W., 2004. Photosynthesis controls of $_{CO2}$ efflux from maize rhizosphere. Plant and Soil 263, 85-99.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421-431.

Kuzyakov, Y., Domanski, G., 2002. Model for rhizodeposition and CO_2 efflux from planted soil and its validation by ^{14}C pulse labelling of ryegrass. Plant and Soil 239, 87-102.

Kuzyakov, Y., Ehrensberger, H., Stahr, K., 2001. Carbon partitioning and below-ground translocation by *Lolium perenne*. Soil Biology and Biochemistry 33, 61-74.

Kuzyakov, Y., Hill, P.W., Jones, D.L., 2007. Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature. Plant and Soil 290, 293-305.

Kuzyakov, Y., Kretzschmar, A., Stahr, K., 1999. Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. Plant and Soil 213, 127-136.

Kuzyakov, Y., Larionova, A.A., 2005. Root and rhizomicrobial respiration: A review of approaches to estimate respiration of autotrophic and heterotrophic organisms in soil. Journal of Plant Nutrition and Soil Science 168 (4), 503-520.

Livesley, S.J., Stancey, C.L., Gregory, P.J., Buresh, R.J., 1999. Sieve size effects on root length and biomass measurements of maize (*Zea mays*) and *Grevillea robusta*. Plant and Soil 207, 183-193.

Lynch, J.M., Brimecombe, M., De Leij, F.A.A.M., 2002. Rhizosphere, in: John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0000403.pub2.

Martens, R., 1990. Contribution of rhizodeposits to the maintenance and growth of soil microbial biomass. Soil Biology and Biochemistry 22, 141-147.

Merckx, R., Van Ginkel, J.H., Sinnaeve, J., Cremers, A., 1986. Plant-induced changes in the rhizosphere of maize and wheat. Plant and Soil 96, 85-93.

Nguyen, C., Todorovic, C., Robin, C., Christophe, A., Guckert, A., 1999. Continuous monitoring of rhizosphere respiration after labelling of plant shoots with $^{14}CO_2$. Plant and Soil 212, 191-201.

Nguyen, C., 2003. Rhizodeposition of organic C by plant: Mechanisms and controls. Agronomie 23, 375-396.

Palta, J.A., Gregory, P.J., 1997. Drought affects the fluxes of carbon to roots and soil in ^{13}C pulse-labelled plants of wheat. Soil Biology and Biochemistry 29 (9/10), 1395-1403.

Sapronov, D.V., Kuzyakov, Y., 2007. Separation of root and microbial respiration: Comparison of three methods. Eurasian Soil Science 40 (7), 775-784.

Smucker, A.J.M., McBurney, S.L., Srivastava, A.K., 1982. Quantitative separation of roots from compacted soil profiles by the Hydropneumatic Elutriation System. Agronomy Journal 74, 500-503.

Todorovic, C., Nguyen, C., Robi, C., Guckert, A., 2001. Root and microbial involvement in the kinetics of ^{14}C-partitioning to rhizosphere respiration after a pulse labelling of maize assimilates. Plant and Soil 228, 179-189.

Tubeileh, A., Groleau-Renaud, V., Plantureux, S., Guckert, A., 2003. Effect of soil compaction on photosynthesis and carbon partitioning within a maize-soil system. Soil and Tillage Research 71, 151-161.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19 (9), 703-707.

Vancura, V., 1964. Root exudates of plants. I. Analysis of root exudates of barley and wheat in their initial phases of growth. Plant and Soil 21 (2), 231-248.

Van der Krift, T., Kuikman, P.J., Möller, F., Berendse, F., 2001. Plant species and nutritional-mediated control over rhizodeposition and root decomposition. Plant and Soil 228, 191-200.

Warembourg, F.R., Billes, G., 1979. Estimating carbon transfers in the plant rhizosphere, in: Harley, J.L., Russel, R.S. (Eds) The soil-root interface. Academic Press, London, pp 183-196.

Werth, M., Kuzyakov, Y., 2008. Root-derived carbon in soil respiration and microbial biomass determination by ^{14}C and ^{13}C. Soil Biology and Biochemistry 40, 625-637.

Wu, J., Jörgensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass-C by fumigation-extraction – an automated procedure. Soil Biology and Biochemistry 22, 1167-1169.

6 Soil organic carbon decomposition from recently added and older sources estimated by $\delta^{13}C$ values of CO_2 and organic matter

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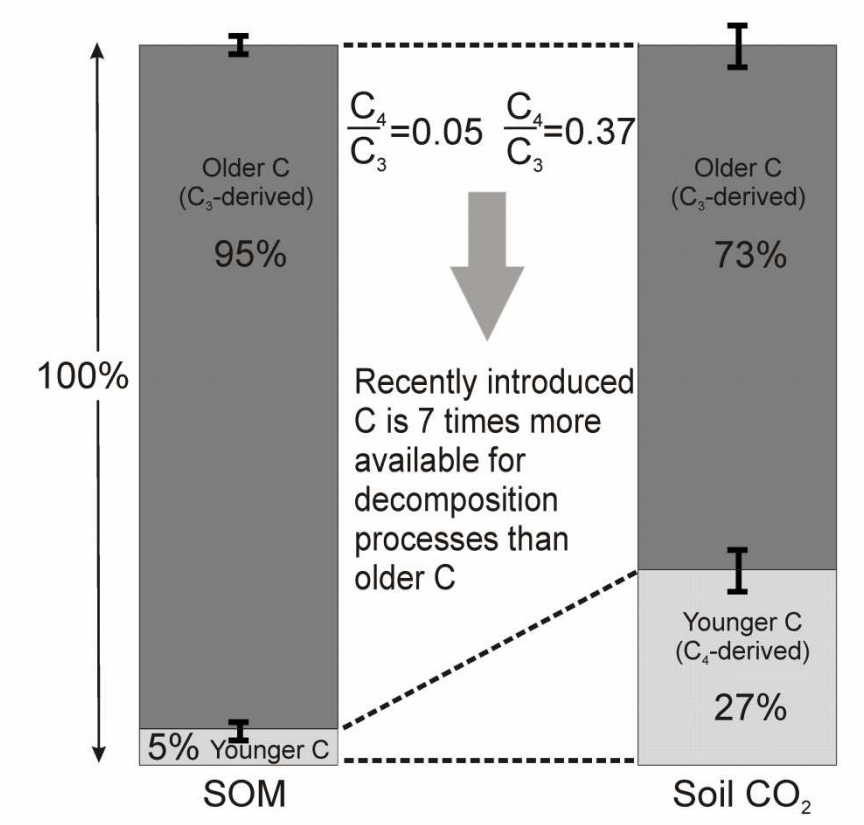
Research Highlights

- The $\delta^{13}C$ signature of soil CO_2 reflects that of organics decomposed.
- The admixture of atmospheric CO_2 to soil CO_2 was removed by Miller/Tans models.
- The contribution of younger and older C to CO_2 and to SOM was compared.
- This allows to estimate microbial availability of younger relative to older C sources.
- Younger C (<2 years) is seven times more available for microorganisms than older C.

Graphical Abstract



Abstract

The production of CO_2 in soil strongly depends on the availability of organic carbon (C) for microorganisms. It is obvious, that C that entered the soil recently is more easily available for microorganisms in comparison to older C. However, only very few approaches allow for a quantitative estimation of the availability of C in relation to the time it is entering the soil. We hypothesized that $\delta^{13}C$ values of CO_2 and of soil organic matter (SOM) after a C_3 to C_4 vegetation change will enable to calculate the relative availability of younger (C_4-derived) and older C (C_3-derived) sources for microorganisms. Soil CO_2 was sampled over one vegetation period at depths of 10, 40-50 and 60-70 cm at three treatments: a C_3 reference (wheat), a C_4/fallow (fallow after one year of maize cropping), and a C_4/C_4 (two years of maize cropping). Based on the $\delta^{13}C$ of CO_2 purified from the admixture of atmospheric CO_2 by the Miller/Tans model and on the $\delta^{13}C$ values of SOM, the contributions of younger and older C sources to CO_2 and SOM were assessed. Depending on the soil depth and the presence of living roots, the contribution of younger C to soil CO_2 ranged from 16 to 50%, but that to SOM was less than 5%. By comparing the contributions of older and younger C to CO_2 and SOM, we found that the relative availability of organics recently introduced into the soil (C_4-derived) was about 7 times higher than the availability of C stabilized in soil for longer than one year (C_3-derived). We concluded that simultaneous analysis of the $\delta^{13}C$ values of both SOM and of CO_2 allows not only for the quantification of the CO_2 sources, but also for the estimation of the availability of soil C pools of different age for microorganisms.

Keywords: CO_2 partitioning, Miller/Tans model, C_3/C_4 vegetation change, isotopic approaches.

6.1 Introduction

The availability of soil organic C for microbial decomposition is crucial for many processes within the C cycle since it controls the rate of CO_2 flux to the atmosphere, determines the sources contributing to soil CO_2, affects microbial activity and composition, and reflects C sequestration. Soil organic C consists of various heterogeneous pools which differ in their stability and availability and are characterized by particular turnover rates (Von Lützow et al., 2007; Blagodatskaya et al., 2011). Older, more recalcitrant C pools are less decomposable by microorganisms in comparison to younger C pools (Von Lützow et al. 2006; Jastrow et al. 2007). According to their turnover time various C pools contribute differently to soil CO_2 as the major product of microbial decomposition.

To distinguish between C pools and to determine their contribution to soil CO_2, isotopic tracer techniques have been applied. A vegetation change from C_3 to C_4 plants results in different isotopic composition of young and old C pools, which allows for their separation (Balesdent and Mariotti, 1987). Depending on the photosynthetic pathways, different isotopic ^{13}C fractionations occur during CO_2 assimilation, leading to a distinct isotopic composition of C_3 and C_4 plants (Farquhar et al., 1989). Therefore, when growing C_4 plants on soil originally formed in areas of C_3 vegetation (or vice versa), older (C_3-derived) and younger (C_4-derived) C can be differentiated based on their isotopic differences (Balesdent and Mariotti, 1987).

The first aim of this study was the partitioning of soil CO_2 and SOM into their C sources after the application of a C_3 to C_4 vegetation change. Over one vegetation period soil CO_2 was sampled at depths of 10, 40-50 and 60-70 cm at three treatments: 1) C_3 reference with long-term C_3 plant cropping; 2) C_4/fallow with maize in the first year of the experiment, and a bare fallow in the second year; and 3) C_4/C_4 with two years' maize cropping.

A major problem for the evaluation of the isotopic composition of soil CO_2 and the subsequent determination of the contribution of various C sources, is the admixture of atmospheric CO_2 to soil air resulting in the modification of its $\delta^{13}C$ value. This strongly limits the application of $\delta^{13}C$ values of CO_2 for the estimation of CO_2 sources and for

the evaluation of their availability for microorganisms (Søe et al., 2004). Removal of atmospheric CO_2 is therefore a prerequisite for analyzing the soil CO_2. For this purpose, Keeling (1958; 1961) and Miller and Tans (2003) suggested different approaches based on two component isotopic mixing lines. When applying the commonly used Keeling plot approach, the isotopic composition of sampled air versus the inverse of the respective CO_2 concentration is used to estimate the y-axis intercept equivalent to the $\delta^{13}C$ value of pure soil CO_2. The Miller/Tans model is based on the linear regression between the product of CO_2 concentration and its $\delta^{13}C$ value plotted against the CO_2 concentration. The isotopic composition of pure CO_2 from the soil is then determined as the slope of the regression line. Although the Miller/Tans model has seldom been used before, its important advantage is that the calculated $\delta^{13}C$ values of soil CO_2 are less variable in comparison to the Keeling plot approach, especially if the measured CO_2 concentration varies across a broad range. As soil CO_2 concentrations usually do vary over a broad range (more than two orders of magnitude), we used the Miller/Tans model to remove the admixture of atmospheric CO_2 before calculating the contribution of older (C_3-derived) and younger (C_4-derived) sources to CO_2.

The C_3 to C_4 vegetation change approach has commonly been applied to determine either the sources of CO_2 or of SOM, but has very seldom been used to relate isotopic compositions of CO_2 to that of SOM in a way that allows for a quantitative estimation of the availability of C pools for microorganisms (Flessa et al., 2000; Kuzyakov, 2011). We therefore hypothesized that the purified $\delta^{13}C$ values of CO_2 and the $\delta^{13}C$ values of SOM after a C_3 to C_4 vegetation change can be used to estimate the relative availability of younger and older C sources for soil microorganisms.

6.2 Materials and Methods

6.2.1 Experimental design

The experimental site was established on an arable field in the north-west of Göttingen, Germany (51°33´36.8´´N, 9°53´46.9´´E). The soil is a haplic Luvisol the organic carbon of which originates from permanent C_3 vegetation. The main soil properties are presented in Table II.6/1.

A vegetation change in 2009 from C_3 to C_4 crops was used to introduce a distinct ^{13}C signal into the soil. The experimental site and design is described in detail by Kramer et al. (2012). In the first year of the study, plots (24 x 24 m) with maize (*Zea mays* L. cv. Ronaldinio) and reference plots (24 x 24 m) with winter wheat (*Triticum aestivum* L. cv. Julius) were established. Wheat seedlings, sown in October 2008, had been removed from the maize plots with a non-selective herbicide (“Roundup”, Monsanto Agrar, Düsseldorf, Germany) before sowing maize in April 2009. Wheat was harvested in August and maize in early November 2009 and the straw was removed from the plots. In the second year of the study, in April 2010, maize (*Zea mays* L. cv. Fernandez) and summer wheat (*Triticum aestivum* L. cv. Melon) were replanted. A bare fallow area (2 x 5 m) was established within one of the maize plots. This area was manually kept free from vegetation during the growing season and roots from the neighboring plants were severed with a spade up to 20 cm depth every two weeks in order to eliminate lateral root ingrowth. Maize and wheat plants were harvested in November 2010. In this paper, the treatments will be referred to as 'C_3 reference' for the plots with continuous wheat cropping, 'C_4/C_4' for the plots with maize cropping in the first and in the second year of the experiment, and 'C_4/fallow' for the plots with maize cropping in the first year and bare fallow in the second year.

Five soil moisture sensors (EC-5, Decagon Devices, Pullman, USA) were installed at 48 cm depth at the C_3 reference. The water content of the soil was measured every 30 minutes and displayed as daily average.

Table II.6/1: Selected properties (±SEM) of the haplic Luvisol determined before the start of the experiment (Kramer et al., 2012, modified). Significant differences between the horizons are indicated by different letters (P<0.05).

Horizon	Depth [cm]	pH ($CaCl_2$)	Bulk density [g cm^{-3}]	C_{org} [g kg^{-1}]	Total N [g kg^{-1}]	C/N	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]
Ap1	0-25	6.0±0.1 a	1.4±0.0 a	12.4±0.4 a	1.3±0.0 a	9.8 a	8.0±0.2 a	-27.4±0.1 a
Ap2	25-37	6.2±0.1 a	1.6±0.0 b	6.9±1.2 b	0.8±0.1 b	9.2 a	7.9±0.5 ab	-26.5±0.2 b
Btw1	37-65	6.6±0.1 b	1.7±0.0 c	3.3±0.5 c	0.4±0.0 c	8.9 ab	6.4±0.3 bc	-26.1±0.1 bc
Btw2	>65	7.0±0.1 c	1.6±0.0 b	1.8±0.4 c*	0.3±0.0 c	6.9 b	5.6±0.6 c	-25.5±0.3 c

* Small quantities of $CaCO_3$ may occur below 65 cm.

6.2.2 Soil air samplers

To sample all three treatments during the same growing season, sampling was done in 2010, in the second year of maize cropping, after establishing the bare fallow (C_4/fallow). The soil air samplers were installed in April 2010 two weeks before the first sampling, in order to reduce disturbance. The air samplers were constructed according to the principle described by Kammann et al. (2001) with the difference that we did not roll them up to a spiral. The sampler, consisting of a silicone tube (length 14.5 cm, inner diameter 10 mm, wall thickness 3 mm), was sealed at one end with a silicone stopper (length 2 cm). Teflon rings (height 5 mm, inner diameter 8 mm) were placed inside the tube to prevent compression by the overlying soil. The other end of the sampler was connected to a non-permeable polyurethane- (PU) tube (consisting of a 5 cm tube with an inner diameter of 4 mm and a tube with ≤ 80 cm, depending on the sampling depth, with an inner diameter of 1.8 mm) fitted with a three-way stopcock with a cannula to allow for above soil sampling (Knorr et al., 2008).

The air samplers were installed with four replicates at randomly selected positions within all three treatments (C_3 reference, C_4/C_4, and C_4/fallow), at three depths of 10 cm, 40-50 cm and 60-70 cm. The samplers at 40-50 cm and 60-70 cm were vertically installed into a hole made by a Pürckhauer sampler (groove width 18 mm, ecoTech, Bonn, Germany). The hole was refilled with the soil core after installation and sealed with mud at

the surface. Due to a steeper CO_2 concentration gradient in the upper soil depths, the 10 cm samplers were horizontally inserted after excavating a soil core (0.008 m^3). The soil core was placed back and the soil surface was slightly compressed.

6.2.3 Sampling and analysis

Soil air samples were taken twice per month throughout the maize growing season (from May to October 2010) and once after the maize harvest in November 2010. We sampled soil air by plugging evacuated 5 ml vials into the cannula. The stopcock was carefully opened and soil air was soaked into the vial.

The relative ^{13}C abundances and total C concentrations of the CO_2 in the soil air samples were measured by a gas chromatograph 5890 Series II (Hewlett-Packard, Wilmington, USA) coupled to a Delta V Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, Bremen, Germany) via a Combustion Interface II (Thermo Fisher Scientific, Bremen, Germany).

Soil from each treatment was sampled four times during the growing season (May, June, July and August 2010) from depths of 0-10 cm, 40-50 cm and 60-70 cm using a Riverside auger (inner diameter 5 cm, Eijkelkamp, Giesbeek, The Netherlands). Roots from the 0-10 cm soil depth were separated from the soil and rinsed with deionized water. Soil and root material was dried at 60 °C for three days and homogenized in a ball mill. Relative C isotope abundances and total C contents of root and soil samples were measured using an elemental analyzer (Carlo Erba 1108, Milano, Italy) coupled to a Delta S Gas-isotope Ratio Mass Spectrometer (Finnigan MAT, Bremen, Germany) through a ConFlo III interface (Thermo Fisher Scientific, Bremen, Germany). The standard gases (Australian National University sucrose (ANU) and NBS 19) were calibrated with reference to the international standard (Vienna Pee Dee Belemnite (V-PDB)).

6.2.4 Calculations and statistics

6.2.4.1 Miller/Tans model

The $\delta^{13}C$ value of pure soil CO_2 was determined by correcting the measured $\delta^{13}C$ value for the admixture of atmospheric CO_2 based on the Miller/Tans model (Miller and Tans, 2003), using a geometric mean regression (GMR) as suggested for soil CO_2 (Kayler et

al., 2010). A GMR through the individual data points of all samplings was calculated for each depth separately for the C_3 reference, C_4/fallow and C_4/C_4 treatments. The slope of the GMR is equivalent to a seasonally integrated $\delta^{13}C$ value of pure soil CO_2. Standard errors for the slope of the GMR were calculated from the respective ordinary least square regression (Sokal and Rohlf, 1995). These standard errors may not completely characterize the uncertainty (Zobitz et al., 2006).

6.2.4.2 Contribution of recently added C to total soil CO_2 and total SOM

The contributions of older (C_3-derived) and younger (C_4-derived) sources to total soil CO_2 or total SOM were calculated using linear two source isotopic mixing models.

$$\delta^{13}C_t \cdot C_t = \delta^{13}C_{C_3} \cdot C_{C_3} + \delta^{13}C_{C_4} \cdot C_{C_4} \quad (1)$$

$$C_t = C_{C_3} + C_{C_4} \quad (2)$$

$$f_{C_4} = \frac{\delta^{13}C_t - \delta^{13}C_{C_3}}{\delta^{13}C_{C_4} - \delta^{13}C_{C_3}} \quad (3)$$

$$f_{C_3} = 1 - f_{C_4} \quad (4)$$

where $\delta^{13}C_t$ is the isotopic composition of either total CO_2 or total SOM and C_t is the total CO_2 concentration or total C content of SOM. $\delta^{13}C_{C_3}$ and $\delta^{13}C_{C_4}$ are the isotopic compostions of the C_3 and C_4 sources, respectively. C_{C_3} and C_{C_4} are the CO_2 concentrations or C contents of SOM of the C_3 and C_4 sources. f_{C_3} and f_{C_4} are the proportional contributions of the C_3 and the C_4 source to total CO_2 or SOM.

The C_3 source ($\delta^{13}C_{C_3}$) was either defined by the calculated Miller/Tans $\delta^{13}C$ value of the C_3 reference soil when partitioning total CO_2, or by the $\delta^{13}C$ value of SOM of the C_3 reference soil when partitioning total SOM. For partitioning total CO_2 we used the isotopic composition of the maize roots as C_4 source ($\delta^{13}C_{C_4}$). Apparent ^{13}C fractionations between roots and SOM (F_{SOM}) from the pure C_3 system were assumed to be the same in a pure C_4 system and were therefore applied to maize roots to calculate the isotopic composition of the C_4 source ($\delta^{13}C_{C_4}$) when partitioning SOM:

$$F_{SOM} = \delta^{13}C_{C_3} - \delta^{13}C_{C_3-Root} \quad (‰) \quad (5)$$

$$\delta^{13}C_{C_4} = \delta^{13}C_{C_4-Root} + F_{SOM} \quad (‰) \quad (6)$$

where $\delta^{13}C_{C_3-Root}$ and $\delta^{13}C_{C_4-Root}$ are the $\delta^{13}C$ values of the wheat and the maize root, respectively.

Standard errors of f_{C_4} and f_{C_3} were calculated as described by Phillips and Gregg (2001).

6.2.4.3 Relative availability of older and younger C

The relative availability of SOM for soil microorganisms was estimated based on its $\delta^{13}C$ values and on the $\delta^{13}C$ of soil CO_2. In order to evaluate the availability of organics that entered the soil after the start of maize cropping (younger C) and to compare it with the availability of organics that had entered the soil before maize cropping (older C), the ratios of C_4- to C_3-derived C in SOM were related to that in CO_2. The respective C_4/C_3 ratios in SOM and in CO_2 were calculated using linear two source isotopic mixing models (Phillips and Gregg, 2001).

6.2.4.4 Statistics

The values presented in the figures and tables are given as means ± standard errors of means (±SEM). Significant differences of the soil properties (Table II.6/1) between the horizons were obtained by a one-way analysis of variance (ANOVA) in combination with a *post hoc* unequal N HSD test. The slopes of the regression lines calculated by the Miller/Tans model showed P-values always lower than 0.001. A Fisher's z-transformation of the correlation coefficient (r) of the Miller/Tans models showed that r was always highly significant ($P \leq 0.001$). T-tests were used to evaluate differences between depths and treatments in the isotopic composition of CO_2 as well as of SOM and in the contribution of C_4-derived C to total soil CO_2 and SOM, respectively. Statistical analyses were performed with the statistical package STATISTICA for Windows (version 7.0; StatSoft Inc., OK, USA).

6.3 Results

6.3.1 Isotopic composition of soil CO_2 and removal of atmospheric CO_2 admixture

The raw data shows that the CO_2 concentrations of the three treatments C_3 reference, C_4/fallow and C_4/C_4 are in a similar range and display similar dynamics, i.e. the treatment itself had no impact (Fig. II.6/1). Consistently lower CO_2 concentrations were detected at the 10 cm soil depth for all treatments, in comparison to the concentrations at the 40-50 cm and at the 60-70 cm depth, where the concentrations were twice as high. In August, the CO_2 concentration increased at all depths and treatments. This increase can be explained by an increasing soil water content as shown for the C_3 reference at a depth of 48 cm (Fig. II.6/1).

The dynamics of the isotopic composition of CO_2 differed between the treatments (Fig. II.6/1). Whereas the $\delta^{13}C$ values of the C_3 reference at the 40-50 cm and at the 60-70 cm soil depths, ranging from -30‰ to -24‰, did not show a trend over time, the $\delta^{13}C$ values of the C_4/C_4 treatment increased at mid-June and reached values of up to -16‰, and decreased again in August at all depths. The $\delta^{13}C$ of CO_2 in the C_4/fallow treatment decreased over the season due to the absence of new C_4 inputs in the second year, and due to the decomposition of the C_4 organics which remained after the first year of maize cropping. At the end of the vegetation period, $\delta^{13}C$ values of -27‰ were detected, which were within the $\delta^{13}C$ range of the C_3 reference. During the vegetation period the $\delta^{13}C$ values of CO_2 of the C_3 reference soil at the depth of 40-50 cm and 60-70 cm were lower compared to the values at the C_4/fallow and the C_4/C_4 treatments. The different dynamics of the $\delta^{13}C$ values indicate changes in the contribution of C_3 and C_4 sources to total CO_2.

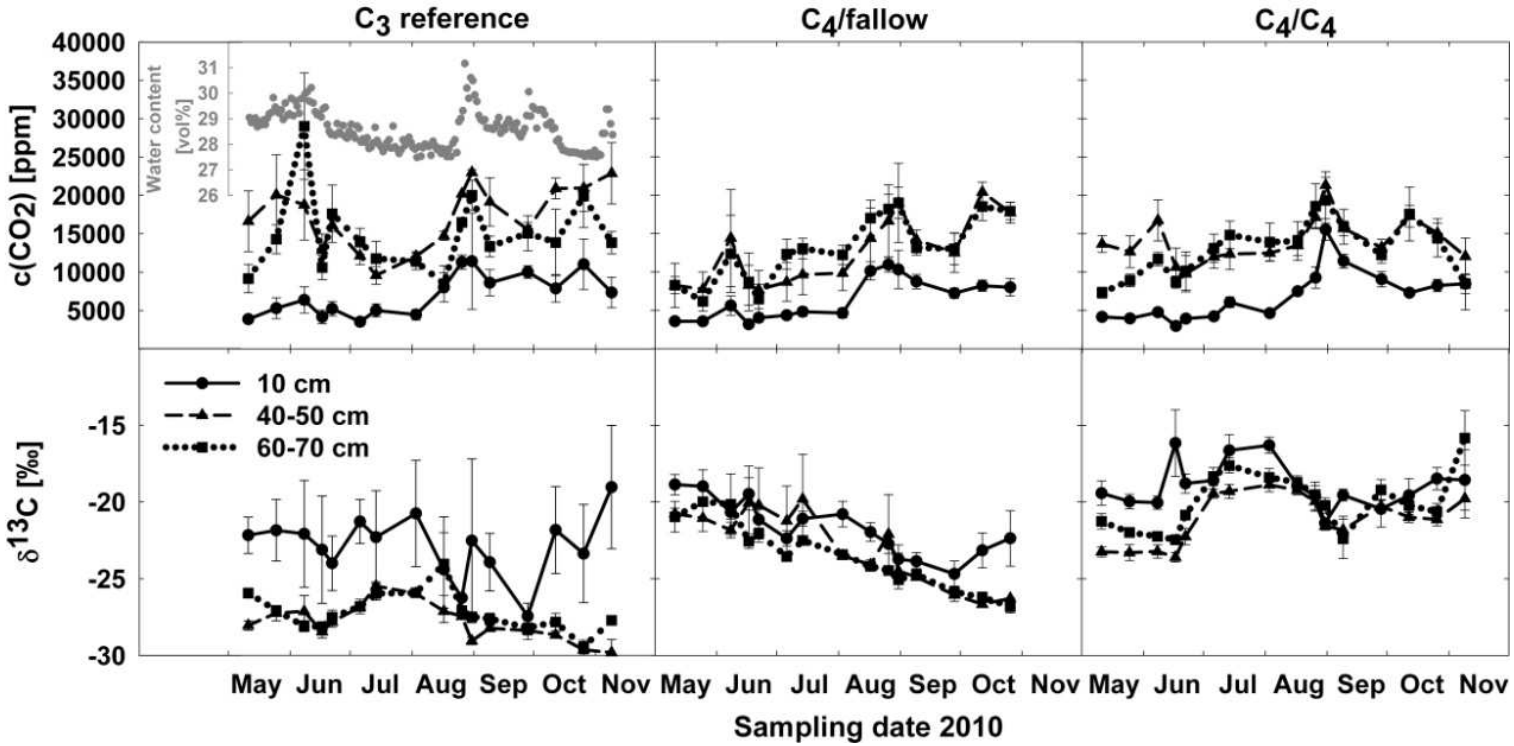


Figure II.6/1: Top: CO_2 concentration (mean ±SEM, N=4) and bottom: $\delta^{13}C$ values (mean ±SEM, N=4) of total soil CO_2 during the growing season 2010 at 10 cm, 40-50 cm and 60-70 cm depth at the C_3 reference (left), the C_4/fallow (middle) and the C_4/C_4 (right) treatment. Raw data without purification from the admixture of atmospheric CO_2 are presented. The soil water content at 48 cm depth under the C_3 reference is shown in gray color.

For all three treatments, the highest $\delta^{13}C$ values were found at the 10 cm depth, but with the highest variation for the C_3 reference soil. High variation of CO_2 concentrations and especially of $\delta^{13}C$ values, due to the admixture of atmospheric CO_2, confirmed that a correction for the admixture was required to determine the $\delta^{13}C$ of pure soil CO_2. Using the Miller/Tans model the single CO_2 concentration data (shown as means in Fig. II.6/1) was plotted against the $\delta^{13}C$ value multiplied by the CO_2 concentration (Miller and Tans, 2003) (Fig. II.6/2). The best correlations were found for samples taken from the C_3 reference soil because of the highest difference of $\delta^{13}C$ values of organics utilized by microorganisms and atmospheric CO_2. The slope of the regression line equals the integrated $\delta^{13}C$ value of pure soil CO_2 without atmospheric CO_2 (Fig. II.6/2, II.6/3A).

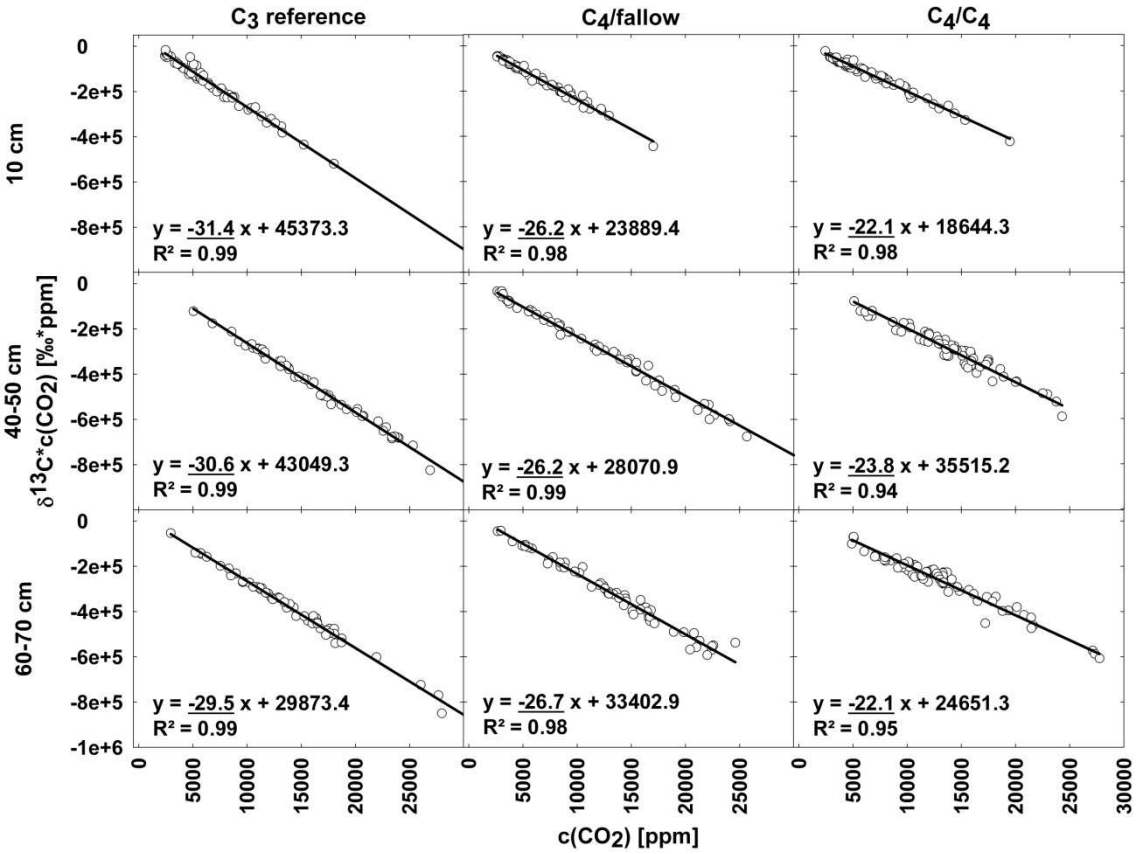


Figure II.6/2: Miller/Tans models for the C_3 reference, the C_4/fallow and the C_4/C_4 treatment at 10 cm, 40-50 cm and 60-70 cm soil depth. The slope of the regression line is equivalent to the $\delta^{13}C$ of pure soil CO_2 without admixture of atmospheric CO_2. Since the samples were taken over one growing period (2010), the slope represents a seasonally integrated $\delta^{13}C$ value. Note: for a better visualization (not for the calculations), the x-axis was truncated at 30,000 ppm, as only 5 points were higher.

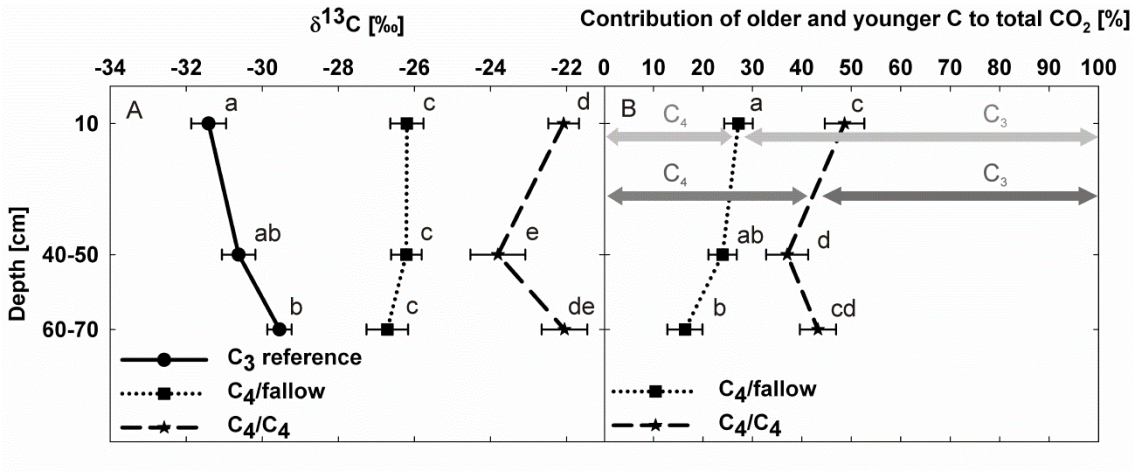


Figure II.6/3: A: $\delta^{13}C$ values (±SEM) of soil CO_2 calculated by Miller/Tans models for the C_3 reference, the C_4/fallow and the C_4/C_4 treatment at 10 cm, 40-50 cm and 60-70 cm depth, and B: contribution of younger and older C sources to total CO_2 at the C_4/fallow and C_4/C_4 treatment at 10 cm, 40-50 cm and 60-70 cm soil depth. Significant differences between the depths and treatments are marked by different letters ($P<0.05$).

The purified $\delta^{13}C$ values of soil CO_2 consistently and strongly differed between the three treatments for all depths investigated (Fig. II.6/3A). The lowest $\delta^{13}C$ values were calculated for CO_2 produced at the C_3 reference site. The C_4/fallow treatment represented an intermediate stage and the highest $\delta^{13}C$ values were detected for the C_4/C_4 treatment. Correspondingly to the $\delta^{13}C$ of SOM (Table II.6/2) the $\delta^{13}C$ of CO_2 at the C_3 reference increased slightly with depth, from about -31‰ at the 10 cm depth to -30‰ at the 60-70 cm depth (Fig. II.6/3A). However, there were no depth gradients for the C_4/fallow and the C_4/C_4 treatments with average $\delta^{13}C$ values of approximately -26‰ and -23‰, respectively.

6.3.2 Contribution of recently added C to total soil CO_2 and SOM and relative availability of younger and older C

Based on two component isotopic mixing lines, the contribution of younger C (C_4-derived) to soil CO_2, after the removal of the admixture of atmospheric air (Fig. II.6/3B) and its contribution to SOM (Table II.6/2) were calculated. The contribution of recently added C to CO_2 at the C_4/C_4 treatment site was approximately 50% at the 10 cm depth, which was twice as high compared to the C_4/fallow treatment. This can be explained by

root-derived CO_2 (root and rhizomicrobial respiration) in the presence of living roots in the C_4/C_4 soil.

The contribution of younger C to soil CO_2 decreased with depth for both C_4 treatments. At the 40-50 cm and the 60-70 cm depth of the C_4/C_4 treatment, younger C contributed about 43% to soil CO_2, while at the C_4/fallow treatment only 16% C_4-derived C was detected at the 60-70 cm depth. Despite the high contribution of recently added C to soil CO_2, even at the 60-70 cm soil depth of the C_4/fallow treatment, the bulk SOM was only slightly (< 5%) enriched by the C_4 signal after one year of maize cropping (Table II.6/2).

Table II.6/2: $\delta^{13}C$ values of SOM (±SEM) of the C_3 reference, the C_4/fallow and the C_4/C_4 treatments and the contribution of C_4-derived C to total SOM (±SEM) on C_4/fallow and C_4/C_4 treatments at 10 cm, 40-50 cm and 60-70 cm depths. The $\delta^{13}C$ values of pure C_4 soil were: -11.4±0.2‰ for 10 cm; -10.5±0.2‰ for 40-50 cm; -10.6±0.2‰ for 60-70 cm. Significant differences between the depths within a treatment are marked by different lowercase letters (P<0.05). Values followed by different uppercase letters indicate statistical differences between the treatments at a certain soil depth (P<0.05).

	$\delta^{13}C$ [‰]			**Contribution of recently added C to total SOM [%]**	
Depth [cm]	**C_3 reference**	**C_4/fallow**	**C_4/C_4**	**C_4/fallow**	**C_4/C_4**
10	-27.0±0.2 aA	-26.3±0.1 aB	-26.4±0.2 aAB	4.6±1.3 aA	3.8±1.1 aA
40-50	-26.2±0.1 bA	-26.0±0.2 abA	-26.2±0.1 aA	1.4±1.7 aA	0±1.2 bA
60-70	-26.3±0.1 bA	-25.7±0.1 bB	-26.2±0.1 aA	3.6±1.1 aA	0.6±1.0 bA

By relating the contribution of recently added C to SOM to its contribution to CO_2, the availability of younger C relative to older C was estimated. This was done only for the 10 cm depth of the C_4/fallow treatment, because of the absence of significant differences in $\delta^{13}C$ of SOM below the plough horizon at 40-50 cm between the C_3 reference and the C_4/fallow treatment (Table II.6/2) and because we cannot completely exclude the influence of carbonates at the 60-70 cm depth (see Table II.6/1). The younger C, introduced into the soil at a depth of 10 cm in the first year of maize cropping, was about 7 times more available for microbial decomposition in comparison to more recalcitrant C which was older than two years.

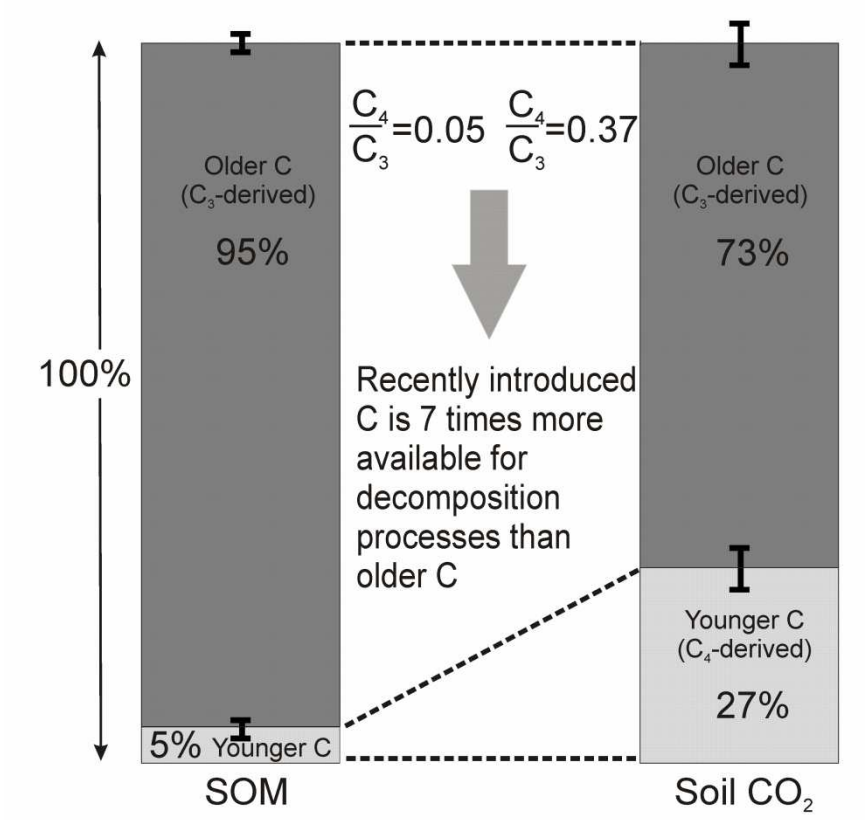


Figure II.6/4: Contribution of older and younger C to SOM and soil CO_2 at the 10 cm depth of the C_4/fallow treatment, and calculation of relative availability of recently added and older C. The seasonally integrated $\delta^{13}C$ value of CO_2 at this treatment and depth was -26.2±0.4‰. The $\delta^{13}C$ value of SOM was -26.3±0.1‰. The contribution of C_4-derived C to total CO_2 or SOM was calculated based on the following isotopic composition of the C_3 and C_4 sources: -27.0±0.2‰ and -11.4±0.2‰ for SOM in a pure C_3 system and a pure C_4 system, respectively; -31.4±0.5‰ and -12.2±0.2‰ for CO_2 in a pure C_3 system and maize roots, respectively.

6.4 Discussion

6.4.1 Dynamics of isotopic composition of soil CO_2

The $\delta^{13}C$ values of the measured soil CO_2 of the C_4/C_4 treatment showed a clear pattern over the growing season with increasing values at mid-June (Fig. II.6/1). Similar patterns had been observed in earlier studies (Rochette and Flanagan, 1997; Rochette et al., 1999) and were explained by a higher contribution of root-derived respiration to total soil CO_2 with increasing root biomass during the growing season. At the end of the growing season, the $\delta^{13}C$ values of CO_2 declined again to the initial level due to reduced root-derived respiration.

The $\delta^{13}C$ of CO_2 in the C_4/fallow soil decreased consistently with time, and converged to the range of the $\delta^{13}C$ of CO_2 measured at the C_3 reference plot. The relative contribution of younger C to soil CO_2 at the C_4/fallow treatment decreased in the absence of new C_4 inputs, because younger (C_4-derived) organics remaining in the soil after the first year of maize cropping had higher availability and, consequently, faster decomposition rates compared to older (C_3-derived) organics. CO_2 produced at the beginning of the vegetation season mainly originated from the compounds with fast decomposition rates, while compounds with lower decomposition rates gained an increasing influence on the CO_2 with time (Werth and Kuzyakov, 2008). This led to a decrease of $\delta^{13}C$ values of soil CO_2 within the C_4/fallow treatment.

The CO_2 measured at the 10 cm depth of the C_3 reference soil was characterized by highly variable $\delta^{13}C$ values ranging from -27‰ to -19‰ (Fig. II.6/1). This high spatial (indicated by large standard errors) and temporal variability can be explained by a higher variability in soil moisture and temperature at the wheat site compared to the C_4/C_4 and to the C_4/fallow treatment, which might have led to bigger differences in microbial activity and hence in C decomposition on the wheat site. Furthermore, soil moisture is a key factor controlling the intensity of atmospheric air mixing on all three sites, because it alters the rate of gaseous diffusion (Susfalk et al., 2002). The magnitude of the atmospheric air admixture mainly depends on the decomposition rates of SOM and on the rate of gaseous diffusion within the soil (Amundson et al., 1998; Cerling, 1984; Dudziak and Halas, 1996; Susfalk et al., 2002). The $\delta^{13}C$ values at the 10 cm depth of the C_3 refer-

ence increased at the end of the vegetation period due to a higher admixture of atmospheric CO_2 as a result of a decreasing water content (Fig. II.6/1). The spatial and temporal variability of soil respiration, as well as of the effective soil porosity, led to a variation in the contribution of atmospheric air to total soil CO_2. A contribution of up to 35% of atmospheric air to near-surface soil gas has been reported (Susfalk et al., 2002). For grassland, Millard et al. (2008) calculated contributions of even 61%.

In this study, it was not possible to determine the percentage of atmospheric air mixed to soil CO_2 because the isotopic composition of atmospheric CO_2 in the vicinity of the soil surface was not measured. The use of the atmospheric $\delta^{13}C$ value of -8‰ and a CO_2 concentration of about 390 ppm, as an average for the bulk atmosphere, is inappropriate because the air close to the surface is strongly affected by CO_2 from the soil and by gaseous exchange with the vegetation.

6.4.2 Application of the Miller/Tans model to determine the isotopic composition of soil CO_2

The isotopic signature of pure soil CO_2, without admixing of atmospheric CO_2, can be determined using two component isotopic mixing lines (Fig. II.6/2). The application of the various mixing models in combination with different regression approaches, such as ordinary least squares or GMR, was discussed in detail by Zobitz et al. (2006) and Kayler et al. (2010). For systems with broad CO_2 concentration ranges, such as soil CO_2, the application of the Miller/Tans model used with a GMR was recommended, since it provides the most accurate and precise estimate of the $\delta^{13}C$ value of purely soil-respired CO_2 (Kayler et al., 2010).

The basis of the mixing model is a mass balance equation which relies on the assumption of a simple mixing of only two gas components, soil CO_2 and atmospheric CO_2 (Pataki et al., 2003). During the sampling period, the contribution of the two components may change, but the isotope composition of the single components does not. In our experiment, besides the $\delta^{13}C$ value of the former C_3 vegetation and that of the atmospheric CO_2 mixed into the soil, a third source, with a C_4 isotopic signal, was added to soil by maize cropping. Since the contribution of the C_4 signal varied spatially and temporally, e.g. with changing root biomass and soil moisture (Fig. II.6/1), the $\delta^{13}C$ of soil CO_2 did not remain constant. Nevertheless, both C_3- and C_4-derived CO_2 were ac-

companied by high CO_2 concentrations, whereas atmospheric CO_2, strongly enriched in ^{13}C compared to C_3-CO_2, had constantly lower concentrations. This enabled us to distinguish between soil CO_2 and atmospheric air mixed into the soil.

Additionally, the second component of the mixing model, atmospheric CO_2, may be a source of uncertainty since its $\delta^{13}C$ values can also differ spatially and temporally. The atmospheric CO_2 in the vicinity of the soil surface did not reflect the isotopic composition of the bulk atmospheric CO_2 of -8‰ but is, as a function of the distance from the soil surface, influenced by respiration and assimilation processes and the intensity of air mixing from higher atmospheric layers. Temporal variations in the isotopic composition of atmospheric CO_2 have also been reported, with increasing $\delta^{13}C$ values during periods of high photosynthetic activity (spring and summer), and declining $\delta^{13}C$ values during periods dominated by soil respiration (fall and winter) (Amundson et al., 1998). We concluded, that despite the mixing model requirement of constant $\delta^{13}C$ values for both components being violated, in particular for treatments with a C_4 source, reasonable results can nonetheless be obtained by applying the Miller/Tans model, mainly because of the broad CO_2 concentration range and the vicinity of our data to the $\delta^{13}C$ value and concentration of CO_2 from soil CO_2. Spatial and temporal fluctuations in the $\delta^{13}C$ values of both components of the mixing model may explain variations from the defined mixing line.

6.4.3 Contribution of recently added C to total soil CO_2

The application of a two source isotopic mixing model for partitioning total soil CO_2 into younger (C_4-derived) and older (C_3-derived) sources required the $\delta^{13}C$ values of both endmembers, i.e. (1) of the CO_2 from the decomposition of SOM from a C_3 bare fallow (C_3-derived), and (2) of pure root and/or rhizosphere respiration (C_4-derived). As a C_3 endmember for the mixing models (Eq. 1) the isotopic composition of CO_2 from the C_3-reference site, obtained from the Miller/Tans models was used. This allowed accounting for ^{13}C fractionation between roots and soil CO_2. However, the ^{13}C fractionation between wheat roots and soil CO_2 under wheat not only comprised ^{13}C fractionation between SOM and SOM-derived CO_2, but also ^{13}C fractionation by root-derived respiration, i.e. between $\delta^{13}C$ of roots and that of root-derived CO_2. The latter may have lead to a slightly biased contribution of younger and older C to total CO_2 since ^{13}C frac-

tionation by root-derived respiration should not have been accounted for in the C_3 endmember because of the absence of wheat roots at the C_4/fallow and at the C_4/C_4 treatment. To overcome this problem, the $\delta^{13}C$ of CO_2 from a bare fallow with long-term C_3 history can be used as the C_3 endmember of the mixing model.

As done in most recent studies, we assumed that the net ^{13}C fractionation during respiration is negligible and, hence, the bulk isotopic composition of roots was used as the $\delta^{13}C$ value of the C_4 endmember when partitioning CO_2 (e.g. Buchmann and Ehleringer, 1998; Rochette et al., 1999, Rochette and Flanagan, 1997). Thus, this assumption may result in a slightly biased contribution of younger and older C to total CO_2 since ^{13}C fractionation in the C_4 endmember (maize roots) was not accounted for. To overcome this problem root-derived CO_2 measured in hydrocultures can be used as C_4 endmember. However, this was not applicable in the present experiment.

An estimated 50% of CO_2 released at the 10 cm depth of the C_4/C_4 soil was derived from recently added C (C_4-derived). This percentage decreased with depth, because there were fewer roots in deeper soil. Furthermore, the content of SOM decreased with depth (Table II.6/1) and its turnover in deeper soil horizons is slower compared to upper horizons. Thus, the relative contribution of the roots to the CO_2 signature increased. Maize roots, and consequently rhizodeposition, are concentrated near the soil surface (Amos and Walters, 2006). The contribution of root-derived CO_2 under maize can account for up to 45% of total soil CO_2 (Rochette et al., 1999; Werth and Kuzyakov, 2009). Similar values have been reported for wheat (Kuzyakov and Cheng, 2001). Root-derived respiration explained the higher percentage of C_4-derived CO_2 for the C_4/C_4 treatment in comparison to the C_4/fallow treatment.

6.4.4 Relative availability of younger and older C

A high contribution of younger C to CO_2 was detected for the C_4/fallow as well as for the C_4/C_4 treatment at each sampling depth, whereas there was a much lower impact of younger C on SOM. This finding was confirmed by other studies. Flessa et al. (2000) reported that even after 37 years of maize cropping, the contribution of maize-derived C to SOM accounted for 15% of total C, but for about 58% of CO_2. A discussion by Kuzyakov (2011) showed that the C_4-to-C_3 ratio of SOM slowly increases and reaches saturation while it rises exponentially in CO_2 (outlined in Fig. 5 by Kuzyakov, 2011).

Therefore, even a few decades after the C_3/C_4 vegetation change there will still be a high proportion of C_3-derived C in SOM, but a low contribution to the CO_2 efflux (Kuzyakov, 2011). This reflects the availability of recent and old SOM pools and allows to calculate the relative availability of C_4- and C_3-derived C by relating the C_4-to-C_3 ratio of CO_2 to that of SOM. Since root-derived CO_2 played a crucial role for the C_4/C_4 treatment, we only calculated the relative availability for the 10 cm depth of the C_4/fallow treatment (Fig. II.6/4). While the ratio of C_4-to-C_3 at the 10 cm soil depth was 0.05 in SOM, it was about 0.5 in CO_2 (Fig. II.6/4). Thus, the younger C, introduced into the soil in the previous year, was 10 times more available for microorganisms than the older C. The availability of soil C decreased with time as shown by the high proportion of younger C in CO_2 compared to older C and so indicated C stabilization.

6.5 Conclusions

In order to evaluate the availability of younger relative to older C for soil microorganisms a number of calculation steps were performed. In the first step – the removal of the admixture of atmospheric CO_2 and the estimation of pure soil CO_2 – the Miller/Tans model was successfully applied and provided a clear $\delta^{13}C$ signature of the soil CO_2. In the second step we estimated the contributions of younger (C_4-derived) and older (C_3-derived) carbon to CO_2 and to SOM. In the last step, we compared the contributions of older and younger sources to CO_2 and to SOM, and calculated the relative availability of recent and of old C. We showed that, despite the fact that the contribution of recent C to SOM was less than 5%, the contribution of recent C to produced CO_2 was about 27% at a soil depth of 10 cm within the C_4/fallow treatment. This indicates that one year after the C input into the soil, its availability for microorganisms was about 7 times higher than the availability of C sources older than one year.

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References

Amos, B., Walters, D.T., 2006. Maize root biomass and net rhizodeposited Carbon: An analysis of the literature. Soil Science Society of America Journal 70, 1489-1503.

Amundson, R., Stern, L., Baisden, T., Wang, Y., 1998. The isotopic composition of soil and soil-respired CO_2. Geoderma 82, 83-114.

Balesdent, J., Mariotti, A., 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. Soil Biology and Biochemistry 19 (1), 25-30.

Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., Kuzyakov, Y., 2011. Turnover of soil organic matter and microbial biomass under C_3 - C_4 vegetation change: Consideration of ^{13}C fractionation and preferential substrate utilization. Soil Biology and Biochemistry 43, 159-166.

Buchmann, N., Ehleringer, J.R., 1998. CO_2 concentration profiles, and carbon and oxygen isotopes in C_3 and C_4 crop canopies. Agricultural and Forest Meteorology 89, 45-58.

Cerling, T.E., 1984. The stable isotopic composition of modern soil carbonate and its relationship to climate. Earth and Planetary Science Letters 71, 229-240.

Dudziak, A., Halas, S., 1996. Influence of freezing and thawing on the carbon isotope composition in soil CO_2. Geoderma 69, 209-216.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40, 503-537.

Flessa, H., Ludwig, B., Heil, B., Merbach, W., 2000. The origin of soil organic C, dissolved organic C and respiration in a long-term maize experiment in Halle, Germany, determined by ^{13}C natural abundance. Journal of Plant Nutrition and Soil Science 163, 157-163.

Jastrow, J.D., Amonette, J.E., Bailey, V.L., 2007. Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Climatic Change 80, 5-23.

Kammann, C., Grünhage, L., Jäger, H.-J., 2001. A new sampling technique to monitor concentrations of CH_4, N_2O and CO_2 in air at well-defined depths in soils with varied water potential. European Journal of Soil Science 52, 297-303.

Kayler, Z.E., Ganio, L., Hauck, M., Pypker, T.G., Sulzman, E.W., Mix, A.C., Bond, B.J., 2010. Bias and uncertainty of $\delta^{13}CO_2$ isotopic mixing models. Oecologia 163, 227-234.

Keeling, C.D., 1958. The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochimica et Cosmochimica Acta 13, 322-334.

Keeling, C.D., 1961. The concentration and isotopic abundances of carbon dioxide in rural and marine air. Geochimica et Cosmochimica Acta 24, 277-298.

Knorr, K.-H., Oosterwoud M.R., Blodau C., 2008. Experimental drought alters rates of soil respiration and methanogenesis but not carbon exchange in soil of a temperate fen. Soil Biology and Biochemistry 40, 1781-1791.

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Schoene, J., Scheunemann, N., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E., 2012. Carbon flow into microbial and fungal biomass as a basis to understand the belowground food web in an agroecosystem. Pedobiologia 55, 111-119.

Kuzyakov, Y., 2011. How to link soil C pools with CO_2 fluxes? Biogeosciences 8, 1523-1537.

Kuzyakov, Y., Cheng W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology and Biochemistry 33, 1915-1925.

Millard, P., Midwood, A.J., Hunt, J.E., Whitehead, D., Boutton, T.W., 2008. Partitioning soil surface CO_2 efflux into autotrophic and heterotrophic components, using natural gradients in soil $\delta^{13}C$ in an undisturbed savannah soil. Soil Biology and Biochemistry 40, 1575-1582.

Miller, J.B., Tans, P.P., 2003. Calculating isotopic fractionation from atmospheric measurements at various scales. Tellus 55B, 207-214.

Pataki, D.E., Ehleringer, J.R., Flanagan, L.B., Yakir, D., Bowling, D.R., Still, C.J., Buchmann, N., Kaplan, J.O., Berry, J.A., 2003. The application and interpretation

of Keeling plots in terrestrial carbon cycle research. Global Biogeochemical Cycles 17, 1022, doi:10.1029/2001GB001850.

Phillips, D.L., Gregg, J.W., 2001. Uncertainty in source partitioning using stable isotopes. Oecologia 127, 171-179.

Rochette, P., Flanagan, L.B., 1997. Quantifying rhizosphere respiration on a corn crop under field conditions. Soil Science Society of America Journal 61, 466-474.

Rochette, P., Flanagan, L.B., Gregorich, E.G., 1999. Separating soil respiration into plant and soil components using analyses of the natural abundance of carbon-13. Soil Science Society of America Journal 63, 1207-1213.

Søe, A.R.B., Giesemann, A., Anderson, T.-H., Weigel, H.-J., Buchmann, N., 2004. Soil respiration under elevated CO_2 and its partitioning into recently assimilated and older carbon sources. Plant and Soil 262, 85-94.

Sokal, R.R., Rohlf, F.J., 1995. Biometry. W.H. Freeman, New York.

Susfalk, R.B., Cheng, W.X., Johnson, D.W., Walker, R.F., Verburg, P., Fu, S., 2002. Lateral diffusion and atmospheric CO2 mixing comprise estimates of rhizosphere respiration in a forest soil. Canadian Journal of Forest Research 32, 1005-1015.

Von Lützow, M., Kogel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. European Journal of Soil Science 57, 426-445.

Von Lützow, M., Kogel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry 39, 2183-2207.

Werth, M., Kuzyakov, Y., 2008. Root-derived carbon in soil respiration and microbial biomass determined by ^{14}C and ^{13}C. Soil Biology and Biochemistry 40, 625-637.

Werth, M., Kuzyakov, Y., 2009. Three-source partitioning of CO_2 efflux from maize field soil by ^{13}C natural abundance. Journal of Plant Nutrition and Soil Science 172, 487-499.

Zobitz, J.M., Keener, J.P., Schnyder, H., Bowling, D.R., 2006. Sensitivity analysis and quantification of uncertainty for isotopic mixing relationships in carbon cycle research. Agricultural and Forest Meteorology 13, 56-75.

Previous own publications

First author publication:

Sommer, J., Pausch, J., Brundrett, M.C., Dixon, K.W., Bidartondo, M.I., Gebauer, G. Limited carbon and mineral nutrient gain from mycorrhizal fungi by adult Australian orchids. Published in American Journal of Botany 2012, Vol. 99, Page 1133-1145.

Co-author publications:

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Schoene, J., Scheunemann, N., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E. Carbon flow into microbial and fungal biomass as a basis to understand the belowground food web in an agroecosystem. Published in Pedobiologia 2012, Vol. 55, Page 111-119.

Tian, J., Pausch, J., Fan, M., Li, X., Tang, Q., Kuzyakov, Y. Effect of water management on allocation and dynamics of assimilated carbon in rice-soil system. Accepted for publication in Plant and Soil, 2012, DOI: 10.1007/s11104-012-1327-z.

Awad, Y.M., Pausch, J., Ok, Y.S., Kuzyakov, Y. Effects of biochar and polyacrylamide on decomposition of soil organic carbon and ^{14}C labeled maize rhizodeposits as evaluated by metabolic quotient and microbial biomass. To be submitted to European Journal of Soil Science.

Riederer, M., Pausch, J., Kuzyakov, Y., Foken, T. Partitioning of atmospheric C fluxes into diverse ecosystem C pools by coupling stable isotope labeling and eddy covariance technique. Submitted to Agricultural and Forest Meteorology; date: September 25, 2012.

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I hereby declare, to the best of my knowledge and belief, that this thesis contains no material previously published or written by another person, except where due reference has been made in the text of the thesis.

This thesis contains no material which has been accepted or definitely rejected for the award of any other doctoral degree at any university.

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Hiermit erkläre ich, die vorliegende Arbeit selbst verfasst, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt sowie alle wörtlich und sinngemäß übernommenen Stellen in der Arbeit gekennzeichnet zu haben.

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Bayreuth, den 9. Mai 2012

Johanna Pausch