Florian Wichern

Estimating C and N rhizodeposition of peas and oats

kassel university press

This work has been accepted by the faculty of Organic Agricultural Sciences of the University of Kassel as a thesis for acquiring the academic degree of Doktor der Agrarwissenschaften (Dr. agr.).

Supervisor: Prof. Dr. Rainer Georg Joergensen
Co-Supervisor: Prof. Dr. Torsten Müller

Defense day: 15th December 2006

Bibliographic information published by Deutsche Nationalbibliothek
Die Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at http://dnb.ddb.de

Zugl.: Kassel, Univ., Diss. 2006
ISBN: 978-3-89958-275-8
URN: urn:nbn:de:0002-2759

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www.upress.uni-kassel.de

Printed by: Unidruckerei, University of Kassel
Printed in Germany

To my parents,
my wife Ilka and my daughter Fida
for their love and support.

Preface

The present thesis is submitted to the Faculty of Organic Agricultural Sciences (Fachbereich Ökologische Agrarwissenschaften, FB 11) at the University of Kassel to fulfil the requirements for the degree: Doktor der Agrarwissenschaften (Dr. agr.).

This thesis is based on three papers submitted to internationally refereed journals. The papers are included in chapter 5, 6 and 7. Chapter 1 is a general introduction to the topic of the thesis and chapter 2 gives an overview on relevant literature dealing with rhizodeposition. The objectives of the work are presented in chapter 3 and the methodology used in the present study is shortly described in chapter 4. Chapter 8 gives a synthesis of the results and discussion of chapter 5, 6 and 7 and draws some general conclusions. An outlook is given in chapter 9. Chapter 10 and 11 give a summary in English and German language.

The following papers are enclosed:

Chapter 5

Wichern, F., Mayer, J., Joergensen, R.G., Müller, T. (2007): Evaluation of the wick method for *in situ* application of sugar-urea mixtures into the vascular system of peas (*Pisum sativum* L.) and oats (*Avena sativa* L.). Journal of Plant Nutrition and Soil Science, submitted.

Chapter 6

Wichern, F., Mayer, J., Joergensen, R.G., Müller, T. (2007): Rhizodeposition of C and N in peas and oats after ^{13}C-^{15}N double labelling under field conditions. Soil Biology & Biochemistry, submitted.

Chapter 7

Wichern, F., Mayer, J., Joergensen, R.G., Müller, T. (2007): Availability of rhizodeposition to soil microorganisms differs between peas and oats. Soil Biology & Biochemistry, submitted.

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Glossary: List of plant names and abbreviations

Plant names

Alfalfa	*Medicago sativa* L.
Barley	*Hordeum vulgare* L.
Blue lupin	*Lupinus angustifolius* L.
Carrot	*Daucus carota* L. ssp. sativus [Hoffm.] Schübl. et Mart.
Chickpea	*Cicer arietinum* L.
Cowpea	*Vigna unguiculata* (L.) Walp.
Faba bean	*Vicia faba* L.
Grasspea	*Lathyrus sativus* L.
Groundnut	*Arachis hypogaea* L.
Maize	*Zea mays* L.
Mung bean	*Phaseolus mungo* L.
Oats	*Avena sativa* L.
Pea	*Pisum sativum* L.
Pigeon pea	*Cajanus cajan* L. Huth.
Red clover	*Trifolium pratense* L.
Ryegrass	*Lolium perenne* L.
Serradella	*Ornithopus compressus* L.
Soybean	*Glycine max* (L.) Merr.
Subterranean clover	*Trifolium subterraneum* L.
Wheat	*Triticum aestivum* L.
White clover	*Trifolium repens* L.
White lupin	*Lupinus albus* L.

Abbreviations

AGP	Above-ground plant biomass
AGP-C	Above-ground plant C
AGP-N	Above-ground plant N
asl	Above see level
BGP	Below-ground plant biomass
BGP-C	Below-ground plant derived carbon
BGP-N	Below-ground plant derived nitrogen
C	Carbon
^{11}C	Carbon isotope with the mass 11
^{13}C	Carbon isotope with the mass 13
^{14}C	Carbon isotope with the mass 14
CdfR	Carbon derived from rhizodeposition
^{13}CdfR	^{13}C-carbon derived from rhizodeposition
CH_4	Methane
$CHCl_3$	Chloroform
CO_2	Carbon dioxide
$^{13}CO_2$	^{13}C-carbon dioxide
$^{14}CO_2$	^{14}C-carbon dioxide
$CO(NH_2)_2$	Urea
CV	Coefficient of variance
DC	Decimal code of plant development
E_S	^{15}N enrichment of N in the soil
E_R	^{15}N enrichment of N in roots at time of E_S
df	Derived from

Abbreviations (continued)

dfR	Derived from rhizodeposition
E_C	Organic C extracted from fumigated soils - organic C extracted from non-fumigated soils
E_N	Total N extracted from fumigated soils - total N extracted from non-fumigated soils
H^+	Hydrogen ion
H_2O	Water
HCl	Hydrochloric acid
HCO_3^-	Hydrogen carbonate
HMW	High molecular weight
K	Potassium
K_2SO_4	Potassium sulphate
KCl	Potassium chloride
k_{EC} and k_{EN}	Extractable part of the total amount of carbon (k_{EC}) and nitrogen (k_{EC}) bound in the microbial biomass
LMW	Low molecular weight
MB	Microbial biomass
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
$MB^{15}NdfR$	^{15}N derived from rhizodeposition in the microbial biomass
N	Nitrogen
^{15}N	Nitrogen isotope with the mass 15
N_2	Gaseous nitrogen

Abbreviations (continued)

$^{15}N_2$	Gaseous nitrogen with the nitrogen isotope having the mass 15
N_2O	Nitrous oxide
NaOH	Sodium hydroxide
NdfR	Nitrogen derived from rhizodeposition
^{15}NdfR	^{15}N-Nitrogen derived from rhizodeposition
NH_3	Ammonia
$^{15}NH_3$	^{15}N-ammonia
NH_4^+	Ammonium
$^{15}NH_4^+$	^{15}N-ammonium
$(^{15}NH_4)_2\ SO_4$	^{15}N-ammonium sulphate
NO_3^-	Nitrate
$^{15}NO_3^-$	^{15}N-nitrate
NO_X	Nitrogen oxide (e.g. NO, NO2, N2O)
N_S	N content in the soil or soil fraction
OH^-	Hydroxide
P	Phosphorus
PLSD	Protected least significant difference
S	Sulphur
w/v	Weight per volume

1 Introduction

Environmentally friendly and sustainable production of agricultural commodities gains growing interest, as the potential negative impact of farming systems on the environment becomes obvious. Sustainable agriculture, such as organic farming systems, put a strong focus on a more efficient resource use, minimizing nutrient losses and recycling energy and matter, while optimising yield and crop quality. This aims in contributing to the long term self sustainability of agricultural systems.

Special emphasis has been put on the management of soil organic matter with its positive effect on soil structure, water storage, plant nutrient storage and exchange, and its role as nutrient source and habitat for soil microorganisms. Understanding the processes influencing the mineralisation and immobilisation of organic matter in the soil and therefore the plant nutrient cycling is crucial in agricultural systems with low external input (Paterson, 2003). Sustainable agriculture tends to maintain or even increase soil fertility by using an adapted soil organic matter management system. Simultaneously, plant nutrient efficiency will be maximised to reduce nutrient losses and their negative environmental impact, by coupling plant (primary production) and microbial (secondary production) productivity (Paterson, 2003). In organic farming systems this is aimed at by using crop rotations and mixed cropping.

One of the major future environmental problems and one of growing concern, is the climate change. Agriculture contributes to the increasing concentrations of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) in the atmosphere, and therefore to global warming (Johnson et al., 2005). Beside the oceans and the atmosphere, soil is an important C repository with an estimated annual C release by soil respiration of 7.5×10^{10} t (Nguyen, 2003). This release is strongly influenced by land use, with agriculture contributing to the C release through tillage practices and cropping systems. It is estimated, that organic farming systems have a higher C sequestration potential in comparison with conventional agriculture, at least under northern European conditions (Foereid and Høgh-Jensen, 2004). One reason might be the integration of a high proportion (20 to 40% of the cultivated area) of grain

and fodder legumes, in particular clover-grass leys, into crop rotations, which contribute significantly to the C sequestration (Graham and Vance, 2003). In particular with respect to the increasing atmospheric CO_2 level, it is crucial to know how much of the photosynthetically fixed C of annual crop plants, particularly legumes, is entering the organic matter pool and contributing to the C sequestration potential of the soil (Nguyen, 2003).

Furthermore, legumes serve as an important tool for improved management of phosphorus, nitrogen and other plant nutrients (Vance, 2001), but have not been improved in terms of yield increase compared to *Gramineae* (Graham and Vance, 2003). In particular in developing countries and in organic farming they play a crucial role in the provision of nitrogen to the consecutive crop due to their ability of symbiotic N_2-fixation (Graham and Vance, 2003) and therefore often being the major primary N source. The higher N availability after legumes resulted in higher yields and N uptake of cereals (Soon et al., 2004). Since the 1970s, management of N inputs into agricultural systems has become of increasing interest, as its excessive use in intensive agriculture in humid temperate climate caused NO_3^--leaching into surface and groundwater resulting in eutrophication and loss of nitrogen (Vance, 2001). It also caused gaseous loss as NH_3, NO_x and N_2O in various temperate and tropical farming systems also contributing to global warming (Vance, 2001). However, processes influencing N availability especially in low-input farming systems, have been poorly understood. Minimizing the negative environmental impact of N fertilisation by simultaneously maximizing crop yield and quality, therefore requires a sophisticated management (Vance, 2001) based on a better understanding of C and N turnover processes in the soil. This is particularly important for organic farming systems, which aim in balancing nutrient budgets. For N, however, the balances are often positive, with a high surplus especially in horticultural systems, whereas especially for P, budgets are often negative particularly in mixed and arable systems (Watson et al., 2002).

Hence, it is crucial to understand the interactions of plants and soil influencing C sequestration and turnover of N and other plant nutrients. As plants interact with the soil to acquire and mobilise nutrients, their roots alter the soil biologically, chemi-

cally and physically over variable distances from its surface, defining the extent of the rhizosphere by the respective processes considered in space and time (Paterson, 2003; Gregory, 2006). Nitrate for example, is transported over large distances to the root surface by mass flow and diffusion, therefore inducing a large rhizosphere for N-acquisition. In contrast, the rhizosphere for P-acquisition is very narrow only. It has also been shown, that plant roots increased the decomposition of soil organic matter by stimulating soil microorganisms (Helal and Sauerbeck, 1986), and therefore, especially in low fertility soils, influence plant nutrient availability (Dakora and Phillips, 2002; Paterson, 2003).

The development of roots is influenced by a wide range of abiotic and biotic factors, and the rooting depth and root distribution is influenced by genetic and environmental factors (Weaver, 1926; Gregory, 2006). Additionally, plant roots grow and die continuously during plant development and are thus subject to continuous microbial turnover, with most of the plant roots being already dead at maturity (Steingrobe et al., 2001). Therefore the amount of plant derived below-ground plant biomass N (BGP-N) especially of legumes is underestimated when only taking the N in recoverable roots into account (McNeill et al., 1997). The same holds true for below-ground plant biomass C (BGP-C). Apart from the uptake of mostly inorganic ions, plant roots also release inorganic and organic compounds by exudation and due to the turnover of root tissue into the rhizosphere. This loss is termed rhizodeposition (chapter 2). It is up to 15% of the total assimilated plant C in cereals (Kuzyakov and Schneckenberger, 2004). One advantage of this tremendous loss of C and thus energy from plant roots, lies in an increased nutrient acquisition by exploiting a larger soil volume when availability is low (Steingrobe et al., 2001). It is expected that there is a great potential to use different plant species and genotypes with their diverse root systems and their exuded chemicals, to influence the processes in the rhizosphere for the benefit of crop production (Watt et al., 2006), with the rhizodeposition being a key factor.

Some reviews published in the last years, investigated the effect of root exudates on nutrient availability (Jones and Darrah, 1994; Dakora and Phillips, 2002). Furthermore, reviews on rhizodeposition focussed on the amount released and the methods

for its estimation (Kuzyakov and Domanski, 2000; Kuzyakov and Schneckenberger, 2004), the mechanisms of release and influencing factors (Nguyen, 2003; Jones et al., 2004) and on the impact of rhizodeposition on nutrient mobilisation (Paterson, 2003). These reviews where only investigating the release of rhizodeposition in terms of C. However, there is increasing evidence, that plant roots release significant quantities of N. The detailed composition of the compounds released into the soil under *in situ* conditions and their ecological function are still not known. The following chapter therefore gives an overview of different aspects of rhizodeposition with a focus on methods to estimate N rhizodeposition and the quantities of N rhizodeposition of different annual plant species. Additionally, knowledge gaps, controversial results and unsolved research questions are highlighted.

2 Rhizodeposition

2.1 Definitions and composition of rhizodeposition

The below-ground plant biomass (BGP) can be subdivided into visible fibrous roots and rhizodeposition (Høgh-Jensen and Schjoerring, 2001). At maturity rhizodeposition can be twice as much as roots and represents a substantial energy transfer from plants to soil microorganisms (Gregory, 2006). Rhizodeposition is defined as organic substances released from living roots into the soil during plant growth (Whipps, 1990a; Nguyen, 2003) and contains a wide range of organic compounds (Rovira, 1956; Gregory, 2006). Inorganic ions might also be included in this term (Uren, 2001). Rhizodeposits can be subdivided by their origin into root cap cells and root tissue, such as sloughed root hairs and epidermal cells (Rovira, 1956), which separate from plant roots as these move through the soil (Hawes, 1990), mucilage, and passive or controlled diffused root exudates (Nguyen, 2003). Root border cells are easily separated from the root, are often still viable and beside secretory cells, contribute a high amount to the root exudates (Hawes, 1990) and thus rhizodeposition. The rhizodeposition can furthermore be divided by solubility or extractability into water soluble exudates (e.g. sugars, amino acids, organic acids, hormones, and vitamins), and water insoluble materials, such as decaying fine-

roots, root hairs, cell walls, sloughed cells, and mucilage (Jensen, 1996b; Merbach et al., 1999). Close to the root, the water soluble root-borne compounds are dominated by carbohydrates and organic acids, with only small amounts of amino acids (Merbach et al., 1999).

Rhizodeposition is often quantified as C released by plant roots (CdfR = C derived from rhizodeposition) (Marschner, 1995; Nguyen, 2003). Meharg (1994) subdivides this C lost from roots into four categories: (1) **root exudates**, which are low molecular weight (LMW) substances and some more complex compounds (vitamins, proteins, hormones, enzymes, pigments) *passively lost* from growing roots; (2) **secretions** which are polymeric carbohydrates and enzymes, *actively released* from plant roots; (3) **lysates**, which are substances released from *cell autolysis* including cell walls and whole roots; (4) **gaseous compounds**, mainly CO_2 released by *rhizorespiration* but might also include termenes, ethene and more. Most of the root exudates and some of the secretions and lysates are soluble, whereas mostly rootlets and root-fragments account for the insoluble fraction of the rhizodeposition.

Root exudates are a complex mixture with a wide range of substances (Rovira, 1956) and can be divided into low-molecular-weight (LMW) and high-molecular-weight (HMW) substances (Marschner, 1995). LMW are usually dominated by simple sugars, but also constitute of organic acids, amino sugars, phenolics, and inorganic compounds, whereas high-molecular-weight (HMW) compounds comprise ectoenzymes and root border cells (Marschner, 1995; Dakora and Phillips, 2002; Nguyen, 2003; Paterson, 2003). Root exudates are all LMW organic compounds released from roots irrespective of the process, even though the original definition was related to those LMW compounds passively diffusing into the soil solution (Nguyen, 2003). However, it is difficult to separate true exudates and lysates (Rovira, 1956), in particular in soil systems.

According to Kuzyakov and Domanski (2000) exudates, secretes, root hairs, and rootlets make up only 1-2% of total assimilated C. Other estimates conclude that soluble exudates account for only 1-10% of the total C released by plant roots (Paterson, 2003), leaving most of the rhizodeposits (90-99%) being HMW substances. This is in line with the estimate that 90% of the rhizodeposition is root de-

bris (Uren, 2001). However, in contradiction to that, Nguyen (2003) calculated the amount deposited by root exudation, sloughing of border cells, and mucilage, and assumed that through exudation 10-100 times more C is released from roots than contributed by border cells and mucilage. Mucilage is mostly composed of polymerised sugars and of only up to 6% proteins (Nguyen, 2003) (e.g. in maize (*Zea mays* L.) mucilage had a C-to-N ratio of 64 with 39% C (Mary et al., 1993)). However, the C-to-N ratio of soluble exudates in some grasses was estimated to be 2.0-2.7 (Klein et al., 1988). Taking this into account, root exudates would contain large quantities of N. This is in contradiction to the observation, that root exudates are predominantly sugars (Deubel et al., 2000; Paterson, 2003) and that the release of N from roots is much lower than that of C (Merbach et al., 1999).

Apart from the release of substances containing C, plant roots also deposit various N-compounds, such as NO_3^- (Wacquant et al., 1989), NH_4^+ (Brophy and Heichel, 1989), and amino acids (Rovira, 1956). Cell lysates, sloughed roots, root hairs and root-derived debris also contain N-compounds (Jensen, 1996a). Especially small root fragments, which methodologically can not be easily separated, may represent a significant proportion of the N released from plant roots (NdfR = N derived from rhizodeposition) (Janzen and Bruinsma, 1993). For various plants the dominating N-compounds exuded are NH_4^+ and the amino acids serine and glycine (Brophy and Heichel, 1989; Ofosu-Budu et al., 1990; Paynel et al., 2001). In particular root exudates of legumes contain NH_4^+ (Brophy and Heichel, 1989) and amino acids (Rovira, 1956) and more amino-N than the root exudates of non-legumes (Hale et al., 1978). Amino acids were between 6 and 31% of the total water-soluble exudates in different plant species (Merbach et al., 1999; Hütsch et al., 2002). In sand culture peas exuded large quantities of amino acids (25 mg N $plant^{-1}$) (Virtanen and Miettinen, 1963). Older pea and oat (*Avena sativa* L.) plants excreted material richer in amino compounds with single amino acids (such as alanine in peas) increasing, indicating that the amino acid composition also changes with plant age (Rovira, 1956).

Leachates of alfalfa (*Medicago sativa* L.) in sterile sand culture contained amino acid-N which made up only a small proportion of the total N lost from roots, with

NH_4^+-N released being 50 times higher (Brophy and Heichel, 1989). Most of the N released from soybean (*Glycine max* (L.) Merr.) roots (98.2%) were other forms than amino-N and NH_4^+-N (probably proteins, peptides, and cell debris) (Brophy and Heichel, 1989). The composition of NdfR changed after water stress with more amino-N released (Brophy and Heichel, 1989). In contrast, most of the total plant derived ^{15}N in the soil released from wheat (*Triticum aestivum* L.) plants after labelling with gaseous $^{15}NH_3$, was hydrolysable (HCl) organic N with a large proportion of amino acids and NH_4^+-N (Merbach et al., 1999). About 3 to 5% of the total plant derived ^{15}N in the soil was extractable by KCl and was mostly inorganic N (1/3 NO_3^--N, 2/3 NH_4^+-N) (Merbach et al., 1999). These results show, that knowledge of the quality of N rhizodeposition is still fragmented and inconsistent. One reason for this is the use of different methodological approaches ranging from solution culture and sterile sand culture to the use of soil. The i*n situ* determination of rhizodeposit quality released from roots into the soil, is of great importance to understand turnover processes in the rhizosphere and should be a focus of future research.

2.2 Factors affecting rhizodeposition

2.2.1 Abiotic factors

Rhizodeposition is a highly variable process and quantity and quality are influenced by various abiotic factors such as drought, mechanical impedance, soil texture, anaerobic conditions, light intensity, day length, atmospheric CO_2 concentration, toxicity and nutrient deficiency (Rovira, 1956; Merckx et al., 1985; Janzen, 1990; Lynch and Whipps, 1990; Whipps, 1990a, 1990b; Janzen and Bruinsma, 1993; Marschner, 1995; Nguyen, 2003). Soil texture and density influences rhizodeposition by altering the friction which influences the sloughing and thus the production of border cells which are released into the soil (Nguyen, 2003). Water deficit did not alter the quantity of stem injected ^{15}N recovered in roots but did alter its transfer into leaves and stem (Götz and Herzog, 2000). It was observed, that water stress had no major direct influence on deposition of N, but indirectly influenced rhizodeposition due to reduced plant growth and nutrition (Janzen & Bruinsma, 1993). In

contrast, osmotic stress like water deficit resulted in a difference in quality of alfalfa root leachates under sterile conditions with more amino-N (17.7% of N lost by roots) being released (Brophy & Heichel, 1989). Rhizodeposition of C increased with increasing water stress (Martin, 1977), which can be attributed to an increase in the deposition of mucilage, which has a high polysaccharide and therefore C content and a low N content (Janzen and Bruinsma, 1993).

Nutrient availability influences root growth of plants and therefore rhizodeposition. The mineral nutrients potassium and magnesium are crucial for the shoot to root transfer of photosynthates (Ericsson, 1995; Marschner et al., 1996). Additionally, it was observed, that total root production of winter barley was increased under P deficiency, probably aiming to increase the soil volume for a better P acquisition (Steingrobe et al., 2001). In another study, the net NdfR in non-extractable organic form was on a similar level in the low and high fertility treatments but extractable NdfR was only measurable in the high fertility treatment (Janzen, 1990). Consequently, most of the N released in a low fertility treatment was in organic form, whereas in the high fertility treatment almost half of the NdfR was present in inorganic form (Janzen, 1990). It was therefore concluded, that in a low fertility environment, part of the NdfR was reabsorbed. Janzen (1990) observed a higher amount of NdfR at a high fertility treatment and attributed this to greater translocation of N into the root system, due to excessive accumulation in the shoot, and to reduced reabsorption of NdfR by the plant. In another study, the increase in soil N also resulted in higher quantities of NdfR, probably because increasing N status of the plant results in an increasing proportional translocation of N into the roots (Janzen and Bruinsma, 1989). The opposite effect was observed for C translocation with the portion of BGP-C decreasing with increasing N fertilisation, which however results in a higher total C assimilation and thus a higher amount of C translocated to the soil (Kuzyakov and Domanski, 2000). In response to environmental conditions plants are able to control and alter the amount and quality of exudates released and try to cope with unfavourable conditions in the rhizosphere, such as nutrient deficiency, toxicities or presence of plant pathogens (Nguyen, 2003), and therefore ‘actively’ alter amount and quality of rhizodeposition.

2.2.2 Biotic factors

Biotic factors, such as plant species and variety, their physiological status, competition between individual plants, pathogen infection, soil microorganisms, symbiosis with rhizobia or mycorrhiza and N_2-fixation in legumes, has also been observed to influence rhizodeposition (Van der Krift et al., 2001; Van Hecke et al., 2005). It has also been reported that rhizodeposition of C in dicotyledonous plants was higher than in monocotyledonous plants (Whipps, 1987). Plants with symbiotic N_2-fixation have a higher energy demand resulting in increasing CO_2-assimilation and root respiration (Merbach et al., 1999) and therefore contribute to a higher C rhizodeposition. In red and white clover (*Trifolium pratense* L. and *Trifolium repens* L.) 92 and 95% of plant N was derived from atmospheric N_2-fixation, resulting in the NdfR fraction being mostly N from atmospheric N_2-fixation (Høgh-Jensen and Schjoerring, 2001), which ultimately influence N rhizodeposition.

Rhizodeposition also differed between plant species and variety because the production of border cells which contribute to rhizodeposition, is to a certain extent genetically influenced (Nguyen, 2003). Furthermore, NdfR varied with plant community structure (Høgh-Jensen and Schjoerring, 2001). Pure stands of red or white clover, for example, released a higher percentage of NdfR than clover-grass mixtures (Høgh-Jensen and Schjoerring, 2001). On the other hand, increased associated growth of peas with cereals the N rhizodeposition of peas (Virtanen and Miettinen, 1963). However, Kuzyakov and Domanski (2000) in their review on cereals, assumed that C translocation varies between growth stages rather than between plant species, with the proportion of assimilated C transferred below-ground decreasing during plant growth. One reason for this is the decrease of the root-to-shoot ratio during the phase of fast vegetative growth (Gedroc et al., 1996; Yevdokimov et al., 2006). Furthermore, the release of border cells influences the amount of rhizodeposition. It is influenced by the amount, radius and branching of roots, and the amount of senescence of the epidermis and therefore alters the amount of rhizodeposition during plant growth (Nguyen, 2003). Nguyen (2003) provides a detailed description of the processes involved in the sloughing of root border cells, the secretion of mucilage, and root exudation.

It was also observed, that rhizodeposition of N as a percentage of the BGP-N increased during later plant development stages, because N is translocated from roots to the above-ground plant parts leaving a relative higher proportion of NdfR in the soil (Sawatsky and Soper, 1991). However, other research assumes, that relocation of N from roots is less important than the relocation from leaves and stem tissue during seed filling (Salon et al., 2001). Accessorily, N from rootlets is probably not relocated in the plant, providing a significant N input when roots die to a greater extent, as in the reproductive growth phase (Gordon and Jackson, 2000; Salon et al., 2001).

The quality of rhizodeposits changed with plant age and became more recalcitrant (Jensen, 1996a). This is in particular true for cereals (barley and wheat) where at an early stage of plant development half of the rhizodeposit N was mineralised after 15 weeks, but only 23% at maturity (Jensen, 1996a). The same is true for pea rhizodeposits which, however, were more labile with a higher proportion being mineralisable (Jensen, 1996a). The proportion of NdfR as a percentage of total plant N increased with plant age (Sawatsky and Soper, 1991; Jensen, 1996a). In a high fertility treatment extractable NdfR increased from 8 to 38% with plant age (Janzen, 1990). Furthermore, N-transfer from pea to barley increased with time (Jensen, 1996b). At maturity 46-48% of the BGP-N was present as rhizodeposits (Sawatsky and Soper, 1991; Jensen, 1996a). This increase can be attributed to the decay of roots which are not included in the rhizodeposition at earlier growth stages. However, increasing N fertilisation resulted in a decreasing C translocation into the soil (Kuzyakov and Domanski, 2000). The amount and composition of rhizodeposition, but in particular of the root exudates is also altered in relation to the biomass, activity, and composition of the soil microbial community (Deubel et al., 2000).

Additionally rhizodeposition varies with distance from the root and therefore shaping the extent of the rhizosphere. In pot experiments investigating the presence of root-derived compounds in different proximity to the root surface, it was shown that quantities of C and N were concentrated close to the roots and decreased steeply with increasing distance from the roots (Helal and Sauerbeck, 1986; Merbach et al., 1999). The amount of N derived from rhizodeposition also decreased substantially

with soil depth under different stands of clover, grass, and under clover-grass mixtures and were highest at 0-10 cm depth but very small below 20 cm depth (Høgh-Jensen and Schjoerring, 2001).

2.3 Estimating rhizodeposition

2.3.1 General consideration

Most research on rhizodeposition has focussed on the quantification of CdfR, with a number of reviews been published in the last years (Kuzyakov and Domanski, 2000; Nguyen, 2003; Paterson, 2003; Kuzyakov and Schneckenberger, 2004). Rhizodeposition of N has been quantified only in a smaller number of investigations, and a simultaneous investigation of CdfR and NdfR has been reported only once (Merbach et al., 1999). Therefore the following section deals predominantly with the estimation of NdfR. As under soil conditions a higher N assimilation was observed in comparison with hydroponic conditions (Janzen and Bruinsma, 1989), realistic estimations of rhizodeposition can only be obtained from *in situ* investigations using soil substrate, which is therefore the focus of this section.

Rhizodeposition of C and its fate in the soil is estimated by labelling plants either with $^{14}CO_2$ or $^{13}CO_2$ and tracing the isotope into the various soil pools. For the investigation of NdfR and its dynamics in the soil, plants and in particular BGP is labelled with the stable nitrogen isotope ^{15}N. Isotope tracer techniques give a more accurate estimation of below-ground plant-C (BGP-C) and N (BGP-N) than physical recovery of roots (Sawatsky and Soper, 1991; Khan et al., 2002a; Yasmin et al., 2006). One reason is that approximately only 30% of the BGP is recovered as intact roots (Khan et al., 2002a). The estimation of BGP-N by physical collection of all roots and root fragments, e.g. by washing, underestimates the actual BGP-N and only counts for 20-30% of the BGP-N estimated with isotope methods (Khan et al., 2002a). To estimate NdfR the plant has to be labelled with ^{15}N and the ^{15}N-flux is traced into the roots and soil (Janzen and Bruinsma, 1989). As rhizodeposits are subject to uptake by plants and microbes, adsorption on soil particles, and loss from the system, results are always giving the net outcome from rhizodeposition and the

related processes (Høgh-Jensen and Schjoerring, 2001). A high enrichment of the plant with the stable isotope limits the fractionation and discrimination of the heavy isotope.

2.3.2 Continuous versus pulse labelling

There have been different attempts to label plants with ^{14}C, ^{13}C, or ^{15}N, and quantify their C and N rhizodeposition, mostly in laboratory or pot experiments (Helal and Sauerbeck, 1984, 1986, 1989; Whipps, 1987; Janzen and Bruinsma, 1989, 1993; Janzen, 1990; Reining et al. 1995; Jensen, 1996b; Merbach et al., 2000; Rroço and Mengel, 2000; Kuzyakov et al., 2001; Mayer et al., 2003) and rarely in field studies (Swinnen, 1994; Swinnen et al., 1995), especially for ^{15}N (Høgh-Jensen and Schjoerring, 2001). For a more detailed discussion regarding labelling plants with C see Meharg (1994) or Kuzyakov and Domanski (2000).

To label plants, either continuous labelling or pulse labelling, with single or multiple pulses is used. Often, pulse labelling is easier to handle and allows the investigation of specific growth stages of the plant (Meharg, 1994). An advantage of pulse labelling is that short time periods and shifts can be investigated more precisely (Meharg, 1994). Besides, pulse labelling was often the preferential technique used under field conditions, because continuous labelling requires expensive and cumbersome equipment, which can often not be applied to field conditions (Meharg, 1994). However, the distribution of the isotopic label does not necessarily correspond with the distribution of total plant C or N in the various plant parts. Pulse labelling results in new photosynthates being higher enriched than older ones, with the consequence that the label of root-derived compounds may not be representative for the whole growing period. Labile pools will be preferentially enriched and structural components will be labelled to a lower extend, with no pool being labelled homogenously (Meharg, 1994). Therefore results rather represent the transfer of newly derived compounds obtained from a specific growth phase, and can not be transferred to the whole growing period (Kuzyakov and Domanski, 2000). Quantitative estimates using pulse labelling have to be interpreted with caution. Continuous labelling on the other hand enables a more homogenous and uniform labelling of all

pools giving a more representative estimate of the quantity of root-derived compounds (Jensen, 1996b, 1996a). Using this approach, temporal changes in the enrichment of root exudates and rhizodeposits can be reduced (Janzen and Bruinsma, 1989). Even though continuous labelling enables the determination of the plant derived total C or N transfer into the soil more accurately in comparison with pulse labelling, it gives only cumulative data (Meharg, 1994). In this case no information is gained about the dynamics and pools involved (Meharg, 1994).

A compromise between pulse and continuous labelling provides the use of multiple pulses (Yasmin et al., 2006). Multiple pulses diminish the effect of compartmentalisation associated with a single pulse (Janzen and Bruinsma, 1989). Some of the error associated with pulse labelling can additionally be counteracted by using a higher enrichment and allowing multiple pulse labelling proportional to the biomass (Janzen and Bruinsma, 1989) by frequently harvesting plants growing alongside (Janzen, 1990). The ^{15}N label then represents a constant proportion of the total plant N (Jensen, 1996a).

2.3.3 Methods for labelling plants with ^{15}N

Rhizodeposition of N has been estimated using different approaches (Table 1). Either plants are previously labelled and transplanted (Merbach et al., 2000; Rroço and Mengel, 2000), or plants are labelled with ^{15}N via roots, by labelling the shoot and/or leaves selectively with liquid ^{15}N or with gaseous ^{15}N. In recent years there have been various attempts to compare different ^{15}N labelling techniques (Merbach et al., 2000; Chalk et al., 2002; Khan et al., 2002a; Hertenberger and Wanek, 2004; Yasmin et al., 2006).

The split-root technique

Following the natural uptake of N in the plant, the split-root technique was used in a series of experiments (Sawatsky and Soper, 1991; Jensen, 1996b, 1996a; Merbach et al., 2000; Schmidtke, 2005). For this method, roots are equally split between a compartment with vermiculite or soil containing the ^{15}N-tracer (e.g. $^{15}NO_3^-$, $^{15}NH_4^+$)

and a soil compartment to measure the NdfR (Reining et al., 1995; Jensen, 1996a, 1996b). The split-root technique allows a continuous ^{15}N labelling of various plant

Table 1 Methods for labelling plants with ^{15}N.

Labelling method	Technique	Subtype	References
Shoot labelling	Leaf feeding	Leaf-flap feeding	Zebarth et al., 1991; McNeill et al., 1997, 1998; Høgh-Jensen & Schjoerring, 2001; Khan et al., 2002b; Hertenberger & Wanek, 2004; Yasmin et al., 2006
		Leaf-tip immersion	
		Leaf spraying	
	Petiole feeding		Khan et al., 2002b
	Stem feeding	Cotton wick method	Russell & Fillery, 1996b, 1996a; Mayer et al., 2003; Hertenberger & Wanek, 2004; Yasmin et al., 2006
		Stem injection	Götz & Herzog, 2000
Atmospheric labelling	$^{15}N_2$		Warembourg et al., 1982
	$^{15}NH_3$		Janzen & Bruinsma, 1989, 1993; Janzen, 1993; Merbach et al., 1999
Root labelling	Pre-treatment of plants in ^{15}N-solution		Merbach et al., 2000; Rroço & Mengel, 2000
	Split-root technique		Sawatsky & Soper, 1991; Jensen, 1996b, 1996a; Merbach et al., 2000; Schmidtke, 2005

species (Sawatsky and Soper, 1991; Jensen, 1996a), providing a tool for a continuous labelling of plants using the natural pathway of N assimilation. This guarantees the incorporation of the ^{15}N label in all N pools of the plant (Jensen, 1996b). The

method can also be used to estimate the N-transfer of rhizodeposit-N into associated plants (e.g. from legumes to grasses) (Jensen, 1996b). However, the ^{15}N fertiliser is often a significant proportion of total plant N (Merbach et al., 2000) and therefore influences rhizodeposition patterns. The application of nutrients containing the tracer altered plant development in comparison to an undisturbed control (Jensen, 1996b). Furthermore, the root system is substantially disturbed by the method (McNeill et al., 1997; Khan et al., 2002b), and quantitative estimation of the NdfR accounts only for a part of the root system (Rroço and Mengel, 2000) making a complete ^{15}N-balance difficult (Merbach et al., 2000). The method is not suitable for *in situ* and field investigations and can not be used for plants with tap roots without substantially influencing the rooting system of the plant.

Shoot labelling techniques

Several shoot labelling techniques have been used to apply a ^{15}N-carrier following not the natural pathway of N-assimilation. Plants are labelled with a single pulse or multiple pulses either by stem, petiole, or leaf and leaf-flap feeding (Ledgard et al., 1985; Janzen and Bruinsma, 1989; Janzen, 1990; Russell and Fillery, 1996a, 1996b; McNeill et al., 1997; Merbach et al., 1999; Götz and Herzog, 2000; Høgh-Jensen and Schjoerring, 2001; Khan et al., 2002a; Khan et al., 2002b; Mayer et al., 2003; Hertenberger and Wanek, 2004; Yasmin et al., 2006). Common ^{15}N-carriers applied are highly enriched ^{15}N-urea, $^{15}NH_4^+$ and $^{15}NO_3^-$.

Leaf feeding is done by leaf spraying or by leaf, leaf-flap and leaf-tip immersion. For the latter, leaves are either remaining intact (Høgh-Jensen and Schjoerring, 2001), cut at the tip, or cut in half (Yasmin et al., 2006) and are immersed in a solution containing ^{15}N-urea (Yasmin et al., 2006), $^{15}NO_3^-$ or $^{15}NH_4^+$ solution (Hertenberger and Wanek, 2004). Leaf pulse labelling of plants resulted in a similar ^{15}N enrichment of the stem as with a continuous split-root technique, but ^{15}N enrichment in the roots was much lower in the leaf labelling (Jensen, 1996b).

When labelling plants using leaf feeding with urea (spray or mist application) (Zebarth et al., 1991), concentrations have to be considerably low (0.2%-0.7%) to pre-

vent leaf damage (Schmidt and Scrimgeour, 2001). Urea concentrations used were often below 1.0% (e.g. 0.25 and 0.4 % (McNeill et al., 1997, 1998) or 0.5% (Høgh-Jensen and Schjoerring, 2001; Khan et al., 2002b)). Khan et al. (2002b) used highly enriched (98atom%) ^{15}N-urea for labelling faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), mung bean (*Phaseolus mungo* L.), and pigeon pea (*Cajanus cajan* L. Huth.). Also clover and grasses were labelled in multiple pulses using highly enriched (99atom%) urea by inserting leaves into a solution (1.0 ml of 0.5% (v/v)) (Høgh-Jensen and Schjoerring, 2001). Application of a ^{15}N-solution using leaf feeding holds the potential risk of soil contamination by run-off from foliage (McNeill et al., 1997; Khan et al., 2002b) even though precautions can be applied (Zebarth et al., 1991).

The same holds true for **petiole feeding**, where the highly enriched petioles might be detached and contaminating the soil (Khan et al., 2002b). One solution to this problem is to label single leaves in a vial containing the ^{15}N-solution (Ledgard et al., 1985; McNeill et al., 1997). The tip of a single leaf, which was cut under water, was placed into a 2-ml vial containing 1 ml of a 0.25% ^{15}N-labelled (99.6 atom%) urea solution and sealed to prevent solution loss by evaporation (McNeill et al., 1997). Plants took up less solution (deionised water) when instead of leaves petioles were used, and solution uptake varied depending on position of the leaf and the environmental conditions, which are influencing the transpiration stream of plants (McNeill et al., 1997). When comparing leaf-flap feeding and petiole feeding, leaf-flap feeding resulted in more consistent levels of root enrichment in mung bean and in higher enrichment for roots of pigeon pea (Khan et al., 2002b).

One problem associated with leaf feeding methods, is that a preferential partitioning of the labelled assimilates either below-ground or to develop shoot or fruit tissue occurs, depending on the position of the leaf used for labelling (Khan et al., 2002b). Another problem associated with these methods is the substantial difference in the absorption of urea-N, which might be due to differences in the leaf area and the anatomical characteristics of the plant (Zebarth et al., 1991). However, the method of leaf feeding is relatively simple and can be applied under field conditions (McNeill et al., 1997). Solution uptake and labelling efficiency depends on climatic condi-

tions and growth stage of the plant, as solution uptake is driven by the transpiration stream and the actual N accumulation (Høgh-Jensen and Schjoerring, 2001). Even though ^{15}N-urea solution was mostly taken up within 2-3 h when using leaf or petiole feeding, some of the solution remained in the vials when concentrations were higher (Khan et al., 2002b). This explained some of the high variability in enrichment of roots with ^{15}N when using urea solutions at high concentrations, which was due to lower solution uptake where plants were damaged (Khan et al., 2002b). In general, root ^{15}N increased with increasing concentration of the ^{15}N-urea solution and with a second feeding event (Khan et al., 2002b). In chickpea the roots were more enriched when the leaves at the base of the stem were fed (Khan et al., 2002b).

Schmidt and Scrimgeour (2001) described a method for the production of ^{13}C and ^{15}N labelled plant material by daily drizzling of maize leaves with a ^{13}C and ^{15}N enriched urea solution. There is evidence that urea-C is used by plants in a similar way as atmospheric CO_2 (Schmidt and Scrimgeour, 2001). Daily leaf feeding with urea allows continuous and uniform labelling of the plant and thus might provide a tool for accurate quantitative estimation of CdfR and NdfR. However, when using urea as a ^{13}C and ^{15}N-carrier to achieve a detectable ^{13}C signal in the soil, oversupply causing altered carbohydrate accumulation might be the result (Schmidt and Scrimgeour, 2001), making a high enrichment of plant material with ^{13}C using this method difficult. This is especially true, as below-ground enrichment with ^{13}C and ^{15}N was lower in comparison with above-ground plant parts when labelled with a leaf feeding method (Schmidt and Scrimgeour, 2001).

Nevertheless, the common method of labelling plants with ^{13}C, follows the physiological pathway of C assimilation by using $^{13}CO_2$. Uptake of the more heavy $^{13}CO_2$ is commonly partly discriminated and $^{12}CO_2$ is preferentially taken up by plants, in particular under water stress (Dawson et al., 2002). Using a highly enriched (e.g. 99atom%) C source would reduce the discrimination effect. For $^{13}CO_2$ this high enrichment is often not achieved, as a high loss of the expensive $^{13}CO_2$ is common due to relatively low quantities of CO_2 taken up by the plants in relation to the amount in the air.

One of the **stem feeding** techniques used for shoot labelling plants with ^{15}N is the wick method first published by (Russell and Fillery, 1996a). The wick method was developed for labelling plant material and BGP of woody legumes with ^{15}N *in situ*. The wick method might also provide a tool for double labelling plants with ^{13}C and ^{15}N *in situ*, by applying a highly enriched solution with a defined amount of ^{13}C and ^{15}N into the plant. *In situ* methods hold the advantage that the characteristic spatial distribution of roots and root-derived material remains intact (Zebarth et al., 1991) and that the contact between soil and root material with the associated faster turn-over is provided. For the wick method a hole is drilled through the stem of the plant. Then a cotton wick is passed through the hole and covered with a silicone tube. The ends of the wick are passed into a vial containing the labelling solution (e.g. ^{15}N-urea). All connections where solution loss can occur are sealed with a plasticine to prevent solution loss by transpiration.

A comparably uniform labelling of the plant material and a high recovery of the applied ^{15}N was achieved when using this method (Russell and Fillery, 1996a; Mayer et al., 2003). On the other hand Yasmin et al. (2006) observed a non-uniform enrichment of below-ground and above-ground plant parts when using the wick method, indicating inhomogeneous utilisation of the applied N-carrier in the various plant parts. Recently, Hertenberger and Wanek (2004) observed a high ^{15}N-enrichment after labelling plants using the same shoot labelling technique. Yasmin et al. (2006) documented a higher solution uptake using a leaf feeding method in comparison with stem and petiole feeding. Like others (Russell and Fillery, 1996b, 1996a; Mayer et al., 2003), they also observed a reduced uptake in the stem feeding procedure after the second application of the solution, and concluded that it was due to blockage of the drilled hole, probably by development of callus tissue (Russell and Fillery, 1996a). The growth stage at labelling and time of sampling, on the other hand, had no marked effect on the distribution of the ^{15}N in BGP and plant tops (Russell and Fillery, 1996a).

Atmospheric labelling

The technique of atmospheric labelling is technically more demanding than shoot labelling (Khan et al., 2002b). It requires expensive enclosure equipment which limits its application in the field (McNeill et al., 1997). Using this method, legumes can be labelled by applying $^{15}N_2$ which is assimilated symbiotically (Warembourg et al., 1982). Such an approach run at risk of non-symbiotic $^{15}N_2$-fixation influencing the estimates of legume derived BGP-N (McNeill et al., 1997). Plant shoots and leaves can also be labelled by leaf assimilation of $^{15}NH_3$ when plants are exposed to gaseous NH_3 in the atmosphere (Janzen and Bruinsma, 1989, 1993; Janzen, 1993; Merbach et al., 1999). The $^{15}NH_3$ is released from $(^{15}NH_4)_2SO_4$ after placement into NaOH (Merbach et al., 1999). Labelling can be done using multiple pulses (Janzen and Bruinsma, 1989) or a continuous labelling. Short pulses have the advantage that plants are exposed to NH_3 only for a short period of time, making regulation of the atmospheric composition unnecessary (Janzen and Bruinsma, 1989). Atmospheric labelling with $^{15}NH_3$ requires separation of soil and plant by sealing the soil surface during exposure to gas. A further disadvantage is, that quantification of the uptake is difficult, particularly under field conditions. Moreover, the N application does not follow the physiological pathway of N assimilation. After NH_3-exposure, an increased total N uptake but no total dry matter increase was observed, and an increase of shoot yield relative to root yield (dry matter and N) was documented with increasing frequency of NH_3-application (Janzen and Bruinsma, 1989).

Using this method, reasonable uniformity of above-ground plant parts was achieved with the assimilated NH_3 and its derived N-compounds effectively translocated in the various plant parts (Janzen and Bruinsma, 1989). However, ^{15}N enrichment of roots was lower than enrichment of shoots (Janzen and Bruinsma, 1989). The application of $^{15}NH_3$ permits a sequential application of ^{15}N at rates proportional to plant N uptake during growth (Janzen and Bruinsma, 1989). Nevertheless, it was also observed, that only a small amount of ^{15}N is taken up by the plant, making longer investigation periods difficult (Rroço and Mengel, 2000), and do not allow the investigation of the transfer of ^{15}N into small soil pools such as the microbial biomass.

2.3.4 Uniform labelling

The main assumption for the calculation of the rhizodeposition is that the sampled roots have the same isotopic enrichment as their deposits (Janzen and Bruinsma, 1989; Janzen, 1990; Jensen, 1996b; Mayer et al., 2003). Therefore, a requirement for quantifying N rhizodeposition is a relative uniform enrichment of the plant with ^{15}N (Khan et al., 2002b). However, Russell and Fillery (1996b) concluded that, even when plant material is not uniformly labelled between above-ground and below-ground parts, the estimation of BGP-N is adequate.

The accumulation of N in the various plant parts depends on the kind and numbers of sinks present during labelling (Götz and Herzog, 2000), which is influenced by the nutritional status of the plant and the growth stage. Especially when using shoot labelling, the applied ^{15}N is predominantly transferred into stem and leaves (Götz and Herzog, 2000). In an experiment on cowpeas (*Vigna unguiculata* (L.) Walp.), which were labelled by stem injection with a syringe, about 62% of the recovered ^{15}N was accumulated in the grain and 35% in the leaf and stem fraction during pod filling (Götz and Herzog, 2000). When using a leaf or petiole feeding technique on four legume species, fruit enrichment with ^{15}N was almost always lower in comparison with leaves and stem (Khan et al., 2002b). Non-uniform ^{15}N enrichment of plant parts might therefore make these methods impractical for some studies (Zebarth et al., 1991).

Uniformity of ^{15}N enrichment of legume roots is also often not achieved due to dilution in the nodules (Jensen, 1996b). Nodulation in legumes results in lower ^{15}N-enrichment of the nodules in comparison with the roots because the label is diluted by the N_2 fixed from air (Russell and Fillery, 1996a; Khan et al., 2002b). There were also substantial differences in the ^{15}N-enrichment between nodulated and non-nodulated root parts (Khan et al., 2002b). Additionally, the enrichment of roots with ^{15}N increased with increasing depth in serradella (*Ornithopus compressus* L.) but not in subterranean clover (*Trifolium subterraneum* L.) (McNeill et al., 1997). In serradella, this was attributed to the presence of more of the less enriched nodules in the upper part of the root system (McNeill et al., 1997). These examples show, that one of the prerequisites for the estimation of NdfR in legumes, namely that the ^{15}N

enrichment of recovered roots is similar to the enrichment of the root-derived N, does not hold true in all cases, and would lead to an overestimation of the NdfR (Khan et al., 2002b).

2.3.5 Calculation of N rhizodeposition

The percentage of N derived from rhizodeposition (NdfR) is calculated according to Janzen and Bruinsma (1989) as follows: %NdfR = $= \frac{E_S}{E_R} \times 100$, where E_S = ^{15}N enrichment of N in the soil and E_R = ^{15}N enrichment of N in roots at time of E_S. To minimise the error of ^{15}N discrimination, atom% ^{15}Nexcess values should be used. The atom% ^{15}Nexcess of a soil or plant fraction respectively, is the difference of the atom% ^{15}N value of a soil or plant fraction from the labelled sample and an unlabelled control. Consequently, the percentage of N derived from rhizodeposition is: $\%\text{NdfR} = \frac{atom\%\ ^{15}N\ excess_{\ soil}}{atom\%\ ^{15}N\ excess_{\ root}} \times 100$. The amount of rhizodeposition-N is estimated as NdfR or RDN (root-derived N) $= N_S \times \frac{\%NdfR}{100}$, where N_S = N content in the soil or soil fraction.

2.4 Quantities of N rhizodeposition

Below-ground plant biomass C (BGP-C) is estimated to be on average 20-30% of the total assimilated C in cereals (wheat and barley) (Kuzyakov and Domanski, 2000; Kuzyakov and Schneckenberger, 2004) but can also be higher (44%) (Helal and Sauerbeck, 1984). For pasture plants, the percentage of BGP-C is slightly higher (30-50%) in comparison with cereals (Kuzyakov and Domanski, 2000). Half of the BGP-C is found in roots, one third evolves as CO_2 by root and microbial respiration, and 3-9% of the assimilated C is incorporated into the soil microbial biomass and other soil organic matter pools (Kuzyakov and Domanski, 2000; Kuzyakov and Schneckenberger, 2004). Corresponding to these results, Merbach et al. (1999) found 13-32% of the ^{14}C incorporated into wheat plants as primary root-derived C, with 43-86% of it being respired. A rough estimate of the amount of C

transferred below-ground leaves about 1.5-2.0 Mg (megagram) C ha^{-1} remaining in the soil and 0.9-1.2 Mg C ha^{-1} released as CO_2 by rhizo-respiration (Kuzyakov and Domanski, 2000). In a review of 43 published articles, Nguyen (2003) found that on average 17% of the net photosynthetically fixed C is released by roots as rhizodeposition, with most of it (12%) being respired by roots and microorganisms, and only 5% being recovered as residues. The C rhizodeposition thus accounts for 50% of the BGP-C (Nguyen, 2003). It however can also be smaller accounting for only 13% of total plant C (Helal and Sauerbeck, 1984). The average CdfR (CO_2 and incorporated rhizodeposits) of wheat and barley is therefore 10-20% of total assimilated C. The amount of CdfR is highly variable (Marschner, 1995) and often higher for pasture plants than for cereals (Kuzyakov and Schneckenberger, 2004). Merbach et al. (1999) supposed, that the net N release by plants is much lower than net C release but when the rapidly respired C is subtracted, the relative amount is similar.

As legumes have the ability of N_2-fixation in symbiosis with rhizobia, their below-ground transfer of N and rhizodeposition of N is expected to be higher in comparison with non-legumes (e.g. cereals). This holds true in total quantities, due to a higher total N-assimilation of legumes, but not for the below-ground transfer of N as a percentage of total plant N, which varies widely (Table 2a, 2b and 3). In one investigation, BGP-N in peas, faba bean and white lupin (*Lupinus albus* L.) was in the same range, accounting for 15.2, 14.6 and 17.2% of total plant N (Mayer et al., 2003). The quantities, however, were markedly different with faba bean releasing almost four times more N than peas, due to a higher total N-assimilation (Mayer et al., 2003). But also the proportion of NdfR varies markedly. In another study with different legumes, BGP-N represented a higher proportion of total plant N, with 39% in faba bean, 53% in chickpea, 20% in mung bean, and 47% in pigeon pea (Khan et al., 2002a). In blue lupin (*Lupinus angustifolius* L.), BGP-N was 28% of total plant N (Russell and Fillery, 1996b, 1996a), in subterranean clover it was 40-42%, and in serradella 37-47% (McNeill et al., 1997, 1998). The values of the BGP-N in cereals, especially wheat, vary strongly (Table 3) and are between 16% and

Table 2a Quantities of N rhizodeposition of peas (*Pisum sativum* L.) from experiments under controlled conditions.

Plant species	Growth stage or treatment	BGP-N	NdfR	NdfR in % of BGP-N	MBNdfR in % of MBN	MB^{15}NdfR in % of ^{15}NdfR	BGP-N	Total N	Comment	References
		in % of total plant-N					(mg plant^{-1})			
Pea			8.7-12	32					Split-root	Sawatsky & Soper, 1991
		16	10.5	65			64	397	Split-root	Schmidtke, 2005
		15	12.6	82	8	18	56	360	Pot experiment[#]	Mayer et al., 2003
	until flowering	27	4	15			44	165	Split-root	Jensen, 1996a
	until maturity	14	7	48			40	277	Split-root	Jensen, 1996a
Range		**14-27**[‡]	**4**[‡]**-12.6**	**15**[‡]**-82**			**40-64**	**165**[‡]**-397**		
Median		**15**	**9.6**	**48**			**50**	**319**		
Mean		**18**	**9.2**	**48**			**51**	**300**		

[‡] Only vegetative growth stage until late flowering; [#] Outdoor-conditions with exclusion of rain

Table 2b Quantities of N rhizodeposition of various legumes, including peas, from experiments under controlled conditions.

Plant species	Growth stage or treatment	BGP-N (in % of total plant-N)	NdfR (in % of total plant-N)	NdfR in % of BGP-N	MBNdfR in % MBN	MB^{15}NdfR in % of ^{15}NdfR	BGP-N (mg plant^{-1})	Total N (mg plant^{-1})	Comment	References
Peas	see 2.2a									
Faba bean		39	23.5	61			291	745	Pot experiment	Khan et al., 2002a
Faba bean		14	13.4	78	22	14	215	1250	Pot experiment#	Mayer et al., 2003
White lupin		17	15.8	85	20	14	134	720	Pot experiment#	Mayer et al., 2003
Blue lupin		28	18.5	65			213	760	Soil columns#	Russell & Fillery, 1996b
Alfalfa			4.5					151	Sterile cond.	Brophy & Heichel, 1989
Soybean			10.4					123-233	Sterile cond.	Brophy & Heichel, 1989
Chickpea		53	44	88			374	705	Pot experiment	Khan et al., 2002a
Mung bean		20	17	85			89	443	Pot experiment	Khan et al., 2002a
Pigeon pea		47	37	78			446	948	Pot experiment	Khan et al., 2002a
Grasspea		18	9.2	50			51	278	Split-root	Schmidtke, 2005
Serradella		37-47	20	28-70					Soil columns	McNeill et al., 1997
Subterranean clover		40-42	10	24-25					Soil columns	McNeill et al., 1997
Range (including peas)		**14-53**	**4-44**	**15-88**	**8-22***	**14-18***	**40-446**	**123-1250**		
Median (including peas)		**28**	**12.4**	**65**	**20***	**14***	**112**	**397**		
Mean (including peas)		**30**	**15.5**	**58**	**17***	**15***	**168**	**504**		

* Only from one experiment (Mayer et al., 2003); # Outdoor-conditions with exclusion of rain

Table 3 Quantities of N rhizodeposition of cereals (wheat and barley) from experiments under controlled conditions.

Plant species	Growth stage or treatment	BGP-N	NdfR	NdfR in % of BGP-N	MBNdfR in % MBN	MB^{15}NdfR in % of ^{15}NdfR	BGP-N	Total N	Comment	References
		in % of total plant-N					(mg plant^{-1})			
Wheat		60	56	93			25	42	Pot experiment	Khan et al., 2002a
	maturity	16-24	11-13	47-82			10-17	64-71	Pot experiment	Rroço & Mengel, 2000
			5.1-6.1						Pot experiment	Merbach et al., 1999
			4.3-5.6						Pot experiment and split-root	Merbach et al., 2000
	unfertilised	44	25	> 50					Pot experiment	Janzen & Bruinsma, 1989
	N fertilised	53	32	> 50					Pot experiment	Janzen & Bruinsma, 1989
	low N fertility	34	16	47			21	61	Pot experiment	Janzen & Bruinsma, 1993
	high N fertility	30	13	43			45	148	Pot experiment	Janzen & Bruinsma, 1993
	low N fertility	27	18	67			13	48	Pot experiment	Janzen, 1990
	high N fertility	49	33	68			37	76	Pot experiment	Janzen, 1990
	water stress		12-19						Pot experiment	Janzen & Bruinsma, 1993
Range (wheat)		16-60	4.3-56.0	47-93			10-45	42-148		
Median		34	13.0	67			21	64		
Mean		37	17.9	64			24	73		
Barley	ear emergence	25	8	32			19	75	Split-root	Jensen, 1996a
	maturity	24	20	71			23	83		Jensen, 1996a
Range (wheat and barley)		**16-60**	**4.3-56.0**	**32‡-93**			**10-45**	**42-148**		
Median (wheat and barley)		**30**	**13.0**	**67**			**21**	**71**		
Mean (wheat and barley)		**35**	**17.5**	**61**			**23**	**74**		

‡ Only vegetative growth stage until ear emergence

60% of total plant N (Rroço and Mengel, 2000; Khan et al., 2002a). The proportion of BGP-N as a percentage of the assimilated total N, mostly increase with increasing soil fertility (Janzen and Bruinsma, 1989; Janzen, 1990). In barley BGP-N was 24 and 25% of total plant N (Jensen, 1996a), at maturity and ear emergence, respectively.

The amount of N lost from roots of peas has been investigated repeatedly (Sawatsky and Soper, 1991; Jensen, 1996a; Mayer et al., 2003; Schmidtke, 2005), with estimates of NdfR at maturity varying between 7 and 12.6% of total plant N (Table 2a). The mean and median are 9.2 and 9.6%. Applying ^{15}N in multiple pulses equivalent to plant N status using the wick method, NdfR accounted for 12.8% of total plant N in peas representing 82% of BGP-N (Mayer et al., 2003). In a pot experiment with peas, NdfR was 8.7-12% of the total plant N, being 22-46% of the BGP-N and increased as the plant matured (Sawatsky and Soper, 1991). Using continuous split-root techniques NdfR of peas was 10.5% (Schmidtke, 2005) and 7% of total plant N at maturity, representing 48% of the BGP-N (Jensen, 1996a). Until late flowering NdfR accounted for 4% of total plant N of peas, representing 15% of BGP-N (Jensen, 1996a) also indicating that rhizodeposition increases as plants mature.

The proportion of NdfR of the investigated legumes (except for clover) ranged widely between 4 and 44% of total plant N, with a mean of 15.5% and a median of 12.4% (Table 2b). In white lupin 15.8% of total plant N were released by roots representing 85% of the BGP-N (Mayer et al., 2003). In blue lupin NdfR was 18.5% of total plant N and slightly higher, representing 65% of the BGP-N (Russell and Fillery, 1996b). Under sterile conditions NdfR was 4.5% of total plant N at harvest for alfalfa and 10.4% for soybean (Brophy and Heichel, 1989). For subterranean clover NdfR was 10% of total plant N and for serradella it was 20%, representing 17-24% and 34-37% of BGP-N, respectively (McNeill et al., 1997). The visible roots contained 30-62% of BGP-N (McNeill et al., 1997). An investigation under field conditions showed, that rhizodeposition of ryegrass (*Lolium perenne* L.), red clover, white clover and clover-grass mixtures, constituted 80-87% of the BGP-N, and being 56-91% of total plant N (Høgh-Jensen and Schjoerring, 2001). Therefore, rhizodeposition of N contributes a significant amount to the N input in grasslands

(with clover) and may substantially exceed the amount of N removed by harvest and the N retained in roots and stubble (Høgh-Jensen and Schjoerring, 2001).

Estimates of rhizodeposition in wheat vary between 4.3% (Merbach et al., 2000) and 56.0% (Khan et al., 2002a) of total plant N and was 32-93% of the BGP-N (Table 3). The mean was 17.9% and the median was 13.0%. In two experiments (Janzen and Bruinsma, 1989; Janzen, 1990) NdfR of a low fertility and an unfertilised treatment was 18 and 25%, respectively, and therefore a lower proportion than in the high fertility and fertilised treatments (33 and 32% of total plant N). With a plant population of 200 plants m^{-2}, NdfR amounted to approximately 20 kg N ha^{-1} (Janzen, 1990). Contrary to these findings, NdfR of wheat was 16% of total plant N in a low fertility treatment but only 13% in a high fertility treatment (Janzen and Bruinsma, 1993). In another experiment wheat plants released 12% of their assimilated N as rhizodeposition (Rroço and Mengel, 2000). The proportion of NdfR in barley was 8% of total plant N at ear emergence and 20% at maturity, representing 32 and 71% of BGP-N (Jensen, 1996a). The mean NdfR as a percentage of total plant N of all cereals was 17.5%.

These results show, that NdfR as a percentage of total plant N and the amount of NdfR, varies widely, depending on the factors mentioned in chapter 2.2. The variation in the estimation of NdfR of one plant species found in literature, might partly be attributed to methodological differences in root recovery and to the fact that many of the investigations took place using different methods and differing controlled conditions. A source of error in field experiments (Høgh-Jensen and Schjoerring, 2001), is the leaching of ^{15}N from leaves and stems as rainfall might have caused transfer of the tracer into the soil resulting in an overestimating of N rhizodeposition (Rroço and Mengel, 2000).

2.5 Function and fate of rhizodeposits

Rhizodeposition, a highly diverse mixture of chemical compounds released by plant roots, has several effects on the chemical, physical and biological properties of the soil (Dakora and Phillips, 2002; Nguyen, 2003; Fageria and Stone, 2006; Gregory, 2006). It has been shown that roots altered bulk density, which was higher at the

root-soil interface in comparison with the soil further away, that rhizodeposition influenced the mineralogical transformation and soil aggregation, and that mucilage as part of the rhizodeposition, might change the water relations in the rhizosphere (Hawes, 1990; Gregory, 2006).

As a form of chemotaxis (Hawes, 1990), rhizodeposition contains messenger molecules (e.g. root border cells) functioning in sending, perception and disruption of signals for the various communication patterns between plants and soil microorganisms (Bais et al., 2006; Gregory, 2006). A well known example of this, is the release of flavonoids and isoflavonoids from roots of legumes, which induce transcription of nodulation genes in compatible rhizobacteria, which in return form lipochitooligosaccharides as a signal to the host plant for development of nodule tissue (Graham and Vance, 2003). Hence, rhizodeposits stimulate the heterotrophic soil microorganisms, induce rhizobial and mycorrhizal colonization of roots, or influence the infection of roots by soil borne plant pathogens (Rovira, 1956; Meharg, 1994; Nguyen, 2003). As a consequence, the structure of the soil microbial community is altered by the rhizodeposits (Benizri et al., 2002). Differences in exudate or root border cell composition of different plant species and families, account for differences in the microbial rhizosphere community (Rovira, 1956; Hawes, 1990). In addition to the nature of the root exudates, also the location of release and the soil type (Gregory, 2006) influences the structure of the bacterial community in the rhizosphere. It is worth noting, that the root tip is often not as strongly colonised by soil microorganisms as other parts of the root (Hawes, 1990), probably because it is a hot-spot of cell loss. These changes of the microbial community in the rhizosphere in return, affect the rate of soil organic matter turnover and the release of nutrients, compared to bulk soil (Gregory, 2006). As plants develop, sloughed cells are probably becoming quantitatively more important than root exudates, in shaping the microbial community in the rhizosphere, which might change from one predominantly utilizing amino acids and other exudates to one using more complex substances (Rovira, 1956). Other results suggest that root border cells as functional compounds play a crucial role in the regulation of the microbial rhizosphere community, especially in suppressing soil borne plant pathogen populations (Hawes,

1990). For a detailed review on the influence of root border cells on soil microbial communities see Hawes (1990).

The rhizodeposition plays a crucial role for the mobilisation of plant nutrients and serves a complex mixture of ecological functions in the soil (Marschner, 1995; Dakora and Phillips, 2002; Nguyen, 2003; Paterson, 2003; Fageria and Stone, 2006). Table 4 shows the various chemical compounds released by a wide range of plant species and their possible ecological function in nutrient mobilisation. The high diversity of LMW root exudates presented is due to the differences in substances released by different plant species, the variation of exudates in relation to abiotic factors such as nutrient or water availability, and the variation of exudates in reaction to biotic stress. Plant roots therefore actively influence plant nutrient availability, either directly or indirectly (Figure 1). The term 'active' used here, do not refer to the process of root exudate release (secretions, excretions, diffusates), but rather to

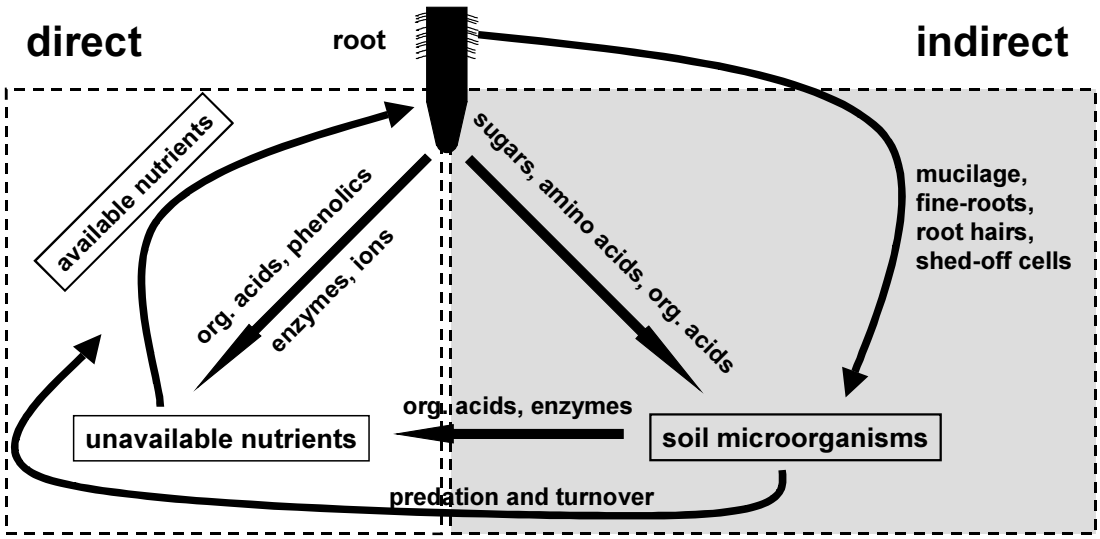

Figure 1 Active nutrient mobilisation by plant roots: Direct and indirect effects of different components of the rhizodeposition on nutrient mobilisation.

the process of nutrient mobilisation which the plant is able to actively influence by root morphological and physiological reactions as well as evolutionary adaptations.

This *active nutrient mobilisation* occurs directly as some of the released root exudates, such as protons, organic acids, phenolics, amino acids including phytosiderophores and enzymes, have a direct effect on nutrient availability, by chemi-

cally solubilising mineral nutrients (Dakora and Phillips, 2002). Special emphasis has been put on the influence of roots on P mobilisation, due to the fact that P availability is one of the major constraints to plant growth (Ryan et al., 2001; Steingrobe et al., 2001; Vance, 2001; Dakora and Phillips, 2002). It was observed that the mobilisation of P from less available fractions and the turnover of organic P increased near the roots, obviously influenced by the rhizodeposition (Helal and Sauerbeck, 1984). Plants excrete more protons and organic acids under nutrient deficiency (Merbach et al., 1999), and therefore counteract low availability in the soil. Additionally, the acid phosphatase activity was increased in the roots and exudates of wheat under phosphorus deficiency (Merbach et al., 1999). Exudation of protons can lead to an acidification of the rhizosphere, which ultimately results in solubilisation of nutrients from soil minerals (Ryan et al., 2001). Organic anions, such as malic acid and citric acid, compete with phosphate groups for binding places in the soil and form stronger complexes than phosphate, therefore increasing the P availability (Marschner, 1995; Ryan et al., 2001). Already fifty years ago it has been observed, that phosphate from glyco-phosphates and nucleic acids was released after liberation of exoenzymes from shed root cap cells (Rovira, 1956), indicating that most of the rhizodeposition components serve nutrient mobilisation means.

Uren (2001) distinguishes the root exudates by their function and considers excretions to ease internal metabolism in reaction to nutrient uptake or respiration, whereas secretions are considered to promote external processes such as chemotaxis or nutrient acquisition. However, some root exudates may also act as both excretion and secretion (Uren, 2001). Beside functional root exudates, Uren (2001) furthermore distinguished non-functional diffusates and root debris, which account for 90% of the rhizodeposition. The latter, however, is a significant energy and nutrient source for soil microorganisms and therefore effects their size and activity (Rovira, 1956; Helal and Sauerbeck, 1984, 1986; Meharg, 1994), which influences the turnover of C and plant nutrients, such as N and P in the soil. In return, it contributes to the indirect active nutrient mobilisation, where soil microorganisms use either root exudates released from plant roots, such as sugars, organic acids, and amino acids,

Table 4 Component, type and compound of rhizodeposits and some of their possible ecological function in the plant-soil interaction for nutrient acquisition (Marschner, 1995; Dakora and Phillips, 2002; Nguyen, 2003; Paterson, 2003).

Component of the rhizodeposition	Type of rhizodeposit	Chemical compounds released (examples)	Ecological function
Sloughed-off cells and root tissue/ debris	lysates, root cap cells, root border cells		nutrient source and chemoattractant for soil micro- and macroorganisms; chelators of poorly soluble mineral nutrients
Mucilage	polymerised sugars, proteins	arabinose, galactose, fucose, glucose, xylose	nutrient source for soil microorganisms;
Low molecular weight root exudates	amino acids and phytosiderophores	α-alanine, β-alanine, arginine, asparagine, cysteine, cystine, glutamine, glycine, homoserine, leucine, lysine, methionine, mugineic, phenylalanine, proline, threonine, tryptophane	nutrient source for soil microorganisms; chelators of poorly soluble mineral plant nutrients; chemoattractant signals for microorganisms
	organic acids	acetic, aconitic, ascorbic, benzoic, butyric, caffeic, citric, isocitric, ferulic, glutaric, glycolic, glyoxilic, maleic, malonic, oxalic, oxalacetic, pyruvic, propionic, succinic, tartaric, vanillic	nutrient source for soil microorganisms; chelators of poorly soluble mineral plant nutrients; chemoattractant for microorganisms
	sugars and polysaccharides	arabinose, desoxyribose, fructose, galactose, glucose, maltose, mannose, oligosaccharides, raffinose, ribose, sucrose	nutrient source for soil microorganisms
	vitamins	Biotin, niacin, pantothenate, pyridoxine (B6), thiamine (B1), riboflavin (B2)	nutrient source for soil microorganisms; promoters of microbial and plant growth
	purines	Adenine, cytidine, guanine, uridine	nutrient source for soil microorganisms
	enzymes	amylase, invertase, peroxidase, phenolase, phosphatase, polygalacturonase, protease, phytase	biocatalysts for organic matter transformation and nutrient release from organic molecules
	phenolics	anthocyanin, flavanol, falvanone, flavone, isoflavonoids	nutrient source for soil microorganisms; chelators of poorly soluble mineral plant nutrients; chemoattractant signals for microorganisms; microbial growth promoters; nod gene inducers and inhibitors in rhizobia; phytoalexins against pathogens
	miscellaneous	auxins, sterols, glycosides, unidentified ninhydrin-positive compounds, unidentified soluble proteins, reducing compounds, ethanol, inositol, dihydroquinone, fatty acids	nutrient source for soil microorganisms; chemoattractant signals for microorganisms; phytohormones
Gaseous compounds		CO_2,O_2,H_2, ethylene	
Ions		H^+, OH^-, HCO_3^-, NH_4^+, NO_3^-	alteration of pH; nutrient acquisition

or substances deposited by roots, such as mucilage, root hairs, rootlets and shed-off cells. Unavailable mineral nutrients are mobilised from soil minerals or organic matter by microbial metabolites and the predation and turnover of the soil microbial biomass. The celerity of active nutrient mobilisation from rhizodeposits might decrease in the order: directly acting root exudates > indirectly acting root exudates > indirectly acting root cells.

The release of C-compounds into the rhizosphere has thus important implications on microbial activity, nutrient and pollutant cycling, and soil organic matter transformations (Janzen and Bruinsma, 1989; Janzen, 1990; Nguyen, 2003). Rhizodeposition therefore influences the availability of plant nutrients in the root zone (Høgh-Jensen and Schjoerring, 2001). Living wheat roots, for example, increased the turnover of plant residues and native soil organic matter (Bottner et al., 1999). Helal and Sauerbeck (1986) also observed that maize roots increased the decomposition of soil organic matter by stimulating the soil microorganisms, resulting in a priming effect in the presence of roots (Kuzyakov, 2002). This is important, as especially in low fertility soils, substrate turnover strongly influences plant nutrient availability (Dakora and Phillips, 2002; Paterson, 2003). Under the influence of microorganisms plant roots release more compounds and plants perform better, but the specific carbon use of the microbes decreases (Merbach et al., 1999).

As soil microorganisms are strongly influenced by the C compounds released from plant roots, categorizing rhizodeposits according to their utilization by soil microorganisms might be more helpful when investigating soil organic matter dynamics under the influence of plants (Meharg, 1994). LMW can be easily assimilated by soil microbes, polymeric and more complex compounds (HMW) (polysaccharides, polypeptides, nucleic acids, pigments, etc.) require extracellular enzymatic activity for breakdown, and structural carbon compounds from sources such as cell walls, require saprothrophic degradation before assimilation by the majority of the soil microbes (Meharg, 1994). The decomposition kinetics of different root-derived compounds, therefore vary greatly (Meharg, 1994). Many of the rhizodeposits are highly labile (Janzen, 1990) and will thus be rapidly incorporated into the microbial biomass (McNeill et al., 1997) and its metabolites by processes such as mineralisa-

tion, immobilisation, nitrification and denitrification. It is assumed that rhizodeposits are rapidly utilised by soil microorganisms with photosynthates having a half-life time of 3-6 h in the rhizosphere (Gregory, 2006). It was observed, that about 43-86% of the C rhizodeposition was rapidly respired (Merbach et al., 1999).

Soil microorganisms use the C released from plant roots and transform organic matter, consequently immobilising mineral nutrients such as N and P. These nutrients become available to the plant after consumption of the microorganisms by the soil microfauna ('microbial loop') (Paterson, 2003). The release of C from plant roots obviously bear advantages for the nutrition of the plant but might also have disadvantages. However, it is less clear why the often limiting nutrient N is released by plants which are in this regard in a competitive situation with soil microorganisms. It was observed, that wheat roots continuously, even until maturation, release N, which has been assimilated during early plant development (Rroço and Mengel, 2000). Some of the plant available N-compounds and mineralised organic rhizodeposits might be reabsorbed by the plant, especially under low-N conditions (Janzen, 1990), or lost by denitrification (Jensen, 1996a). The gaseous N-loss by denitrification or as NH_3 released by plants will probably be lower than the respired C but is difficult to quantify (Merbach et al., 1999). The reabsorption of rhizodeposit-N depends on competition of the plant with neighbouring plants for available soil N, capacity of the plant for N uptake, and the availability of other N sources (Jensen, 1996b). Nevertheless, a significant amount of NdfR will be transferred into different soil pools.

In an incubation experiment, Janzen (1990) found that 36% of the root-derived organic N being mineralised after 76 days at 30 °C. In total, inorganic NdfR accounted for 32% of the inorganic N. Janzen and Bruinsma (1993) calculated that 35% of the mineralisable N pool was derived from rhizodeposition. The labile nature of NdfR and the resulting high turnover, is thus an important source of plant-available-N, with the large quantities of NdfR contributing to soil N fertility (Janzen, 1990).

After 7 weeks of plant growth, N rhizodeposits were highly labile, with 79% of NdfR of pea and 48% of NdfR from barley were mineralisable in an incubation ex-

periment (Jensen, 1996a). Root-derived N at maturity, however, was less labile. Only 30% (peas) and 23% (barley) of the NdfR were mineralised upon incubation (Jensen, 1996a). The N-mineralisation in soils was higher after peas in comparison with barley, resulting in a 36% larger inorganic N pool after pea (Jensen, 1996a). This could be attributed to the roots and rhizodeposits of the crop, with NdfR from pea contributing 35% of the N mineralised after three months and NdfR from barley contributing only 12% of the mineralised N, which corresponds to 39 and 18% of the N present in roots and rhizodeposition at maturity (Jensen, 1996a). For peas, roots contributed 69% to the mineralised N, whereas barley roots contributed only 22%. Additionally, N rhizodeposition of legumes may contribute to the N-nutrition of an associated intercropped non-legume (Jensen, 1996b; Xiao et al., 2004). Transfer of N from field pea (donor) to spring barley (receiver) was observed, but no N-transfer from barley to pea occurred (Jensen, 1996b). The N-transfer from pea to associated barley increased with time, indicating increased turnover of rhizodeposition and accelerated turnover of immobilised NdfR already during plant growth (Jensen, 1996b). The N-transfer increased from less than 5% of pea derived total plant N in barley 45 days after planting, to 19% of total plant N in barley 70 days after planting (Jensen, 1996b). This documents, that N rhizodeposition of legumes is re-mineralised by the microbial biomass during plant growth, providing N for an associated crop in mixed cropping (Jensen, 1996a). These results show that the continuous turnover of the more labile pea roots contribute to the positive effect on N availability in mixed cropping. The findings also indicate the importance of legume roots and root-derived compounds for the N nutrition of subsequent crops in crop rotations.

Alike C rhizodeposits, N rhizodeposition quickly becomes immobilised by the soil microorganisms in the rhizosphere. Mineralisation of NdfR and remineralisation of the immobilised N, depends on the amount of rhizodeposition, the quality of the rhizodeposits and the activity of the saprotrophic microorganisms. The mineralisation and immobilisation of N and other mineral nutrients, is driven by the microbial community and its activity. However, these processes are fuelled by the availability of C, e.g. from rhizodeposition. This highlights the interdependence of the C and N

dynamics at the soil-plant interface and the ability of plants to actively influence and control these processes through the release of C compounds (Knops et al., 2002). Quantitative evidence of this process is still scarce, in particular *in situ* estimates under field conditions are lacking.

3 Objectives

As shown above, C rhizodeposition is an easily available energy and nutrient source for soil microorganisms, which influences the turnover processes of plant residues and soil organic matter in the rhizosphere. It is assumed, that also N rhizodeposition is an important easily available nutrient source for soil microorganisms, which is rapidly incorporated into the microbial biomass and its residues. Parts of the C rhizodeposition and N are already immobilised and partly mineralised during plant growth, particularly under field conditions. However, much of the current knowledge is derived from lab- and pot-experiments only, and it is very likely, that the results were biased under artificial conditions.

The present study was therefore conducted to estimate the quantity of CdfR and NdfR of peas and oats simultaneously *in situ* under field conditions and its fate and transfer into different soil compartments during plant growth.

Specific objectives were,

(i) to evaluate the cotton wick method for transferring sugar-urea mixtures as possible ^{13}C and ^{15}N-carriers into the vascular system of peas and oats,
(ii) to examine the suitability of the cotton wick method for labelling oat plants,
(iii) to estimate the quantity of CdfR and NdfR of peas and oats in the reproductive growth phase under field conditions,
(iv) to quantify the amount of CdfR and NdfR of peas and oats at different growth stages under outdoor conditions,
(v) to investigate the fate of CdfR and NdfR and its transfer into different soil pools, such as the microbial biomass and inorganic N,
(vi) to examine the turnover of CdfR and NdfR during plant growth.

4 Methodology

4.1 Overview

Two greenhouse pot experiments (experiments I and II, chapter 5) and two soil column experiments under field (experiment III, chapter 6) and outdoor conditions (experiment IV, chapter 7) were conducted. The greenhouse experiments were conducted to evaluate the potential of the cotton wick method (Figure 2a,b, see also chapter 5.2 for a method description) for double-labelling plants with a ^{13}C-sugar-^{15}N-urea mixture. Based on these experiments, a column experiment under field conditions was accomplished to estimate the net rhizodeposition of C and N under field conditions during the reproductive growth phase of peas and oats (Figure 3). Using the same approach, another column experiment was executed in an elevated bed to estimate the rhizodeposition of C and N at different growth phases of pea and oat plants under outdoor conditions (Figure 3). Columns were 20 cm in diameter and 55 cm in length. This corresponds to a soil depth of 50 cm, which was chosen because of a compaction layer below. Moreover, the chosen soil depth was in the lower range of the rooting depth of different pea genotypes which was between 50 and 75 cm (Thorup-Kristensen, 1998). The soil in the columns was not spatially separated into bulk and rhizosphere soil at harvest time, as at least the first 10 cm of the soil profile is influenced by plant roots (Gregory, 2006), especially in peas which cover a significant soil volume already during early growth (Weaver and Bruner, 1927). In closed soil columns roots might even influence a greater part, especially at 30 cm soil depth, as on average about 80-90% of the root mass is situated in 0 to 30 cm soil depth (Jackson et al., 1996).

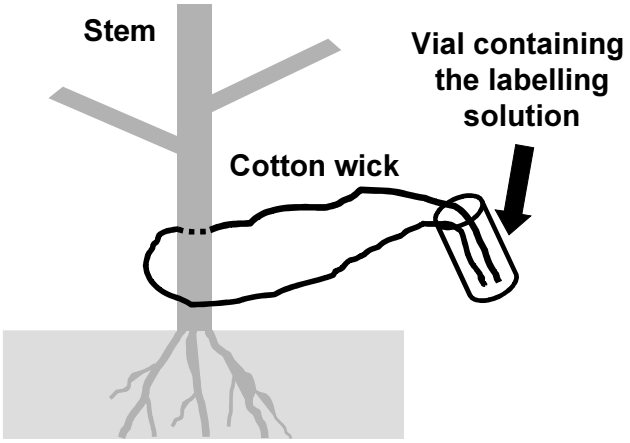

Figure 2a Schematic representation of the cotton wick method for labelling plants with ^{13}C and ^{15}N.

Figure 2b Use of the cotton wick method on peas and oats.

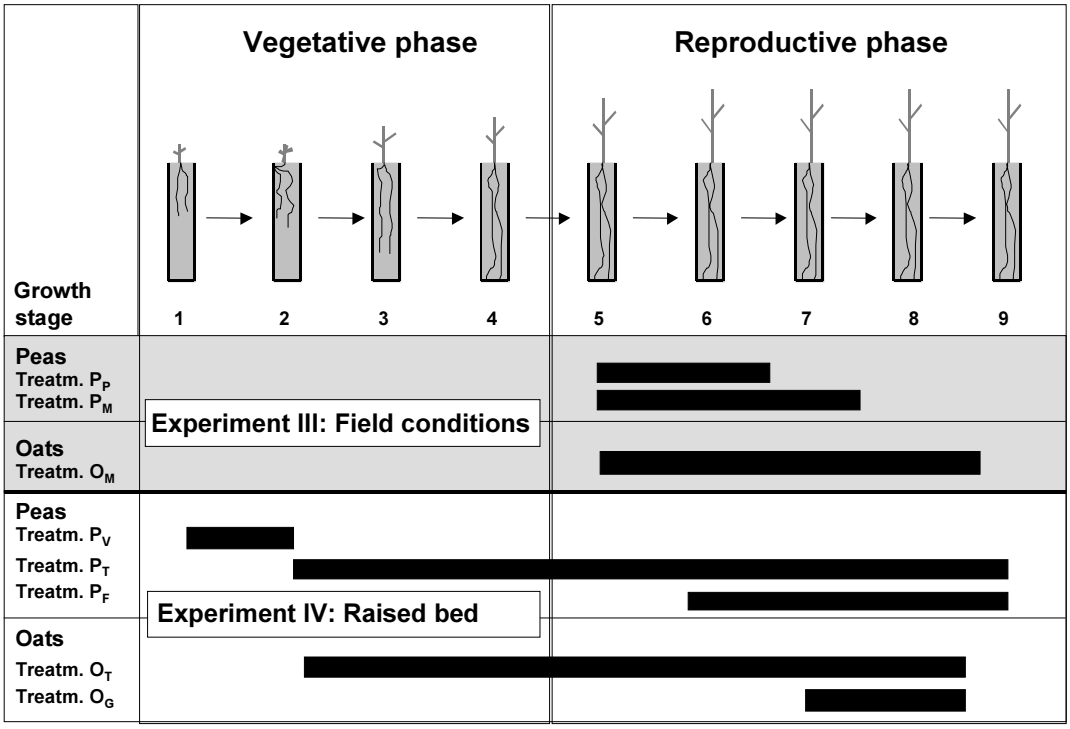

Figure 3 Plant growth phases of peas and oats investigated in the two column experiments under outdoor conditions in the field and in a raised bed.

4.2 Study site, soil and plant material

The greenhouse pot experiments took place in the greenhouse of the University of Kassel, in Witzenhausen, Northern Hesse, Germany. The soil used in the experiment was collected from the research station of the University of Kassel, which is situated at Frankenhausen, Northern Hesse, Germany (51° 24' N, 9° 25' W, 230 m asl) and in Hebenshausen, Northern Hesse, Germany (51° 21' N, 9° 52' W, 230 m asl). The first column experiment was conducted at the research station of the University of Kassel in Frankenhausen. The second column experiment took place in a raised bed at the University of Kassel, Witzenhausen, Germany (51° 34' N, 9° 85' W, 200 m asl) using soil from the research station in Frankenhausen. The chosen fields have been cultivated according to organic farming practices for 6 years. The silty loam soils were Haplic Luvisols developed on loess. Detailed characteristics of the soils used in the experiments are given in chapter 5.2, 6.2 and 7.2 .

The plants used in all experiments were oats (*Avena sativum* L., cv. Freddy) and peas (*Pisum sativum* L., cv. Santana). Oats are a common crop in organic farming systems in north-western Europe and are increasingly planted in mixed cropping systems with peas. Oats are furthermore said to be a 'health crop' reducing weed infestation and the risk of soil borne pathogen infections in preceding cereals (Katonoguchi et al., 1994; Lemanczyk and Sadowski, 2002). Furthermore, oats have a large root system, making the plant suitable for investigation of rhizodeposition. Peas were chosen because they are one of the most important grain legume species in organic cropping systems in north-western Europe and are increasingly planted in mixed cropping with cereals such as oats and barley. Furthermore, there are a number of publications on N rhizodeposition of peas, which allows comparison of the obtained results.

4.3 Experiment I and II: Greenhouse pot experiments

In accordance with objective (i), chapter 3, the aim of the pot experiments was to evaluate the suitability of the cotton wick method for simultaneous *in situ* application of sugar-urea mixtures as possible ^{13}C and ^{15}N-carriers into the vascular system

of peas and oats. Furthermore, the potential use of the wick method on cereals was addressed for oats (objective (ii), chapter 3).

Therefore two pot experiments were conducted in a greenhouse. In a first experiment urea, glucose and cane sugar solutions were applied at different concentrations to pea and oat plants using the cotton wick technique, which allows solution uptake through the vascular system of the plant. In the second experiment glucose-urea and cane sugar-urea mixtures were applied to pea and oat plants with the sugar component at different concentrations using the same approach.

Plant development and solution uptake was monitored throughout the experiment and at maturity plants were harvested to determine plant dry matter. All visible roots and root fragments were collected manually. In the root free soil of the second experiment the microbial biomass C and N was determined by chloroform-fumigation-extraction.

A detailed description of the methodology and analyses is given in chapter 5.2.

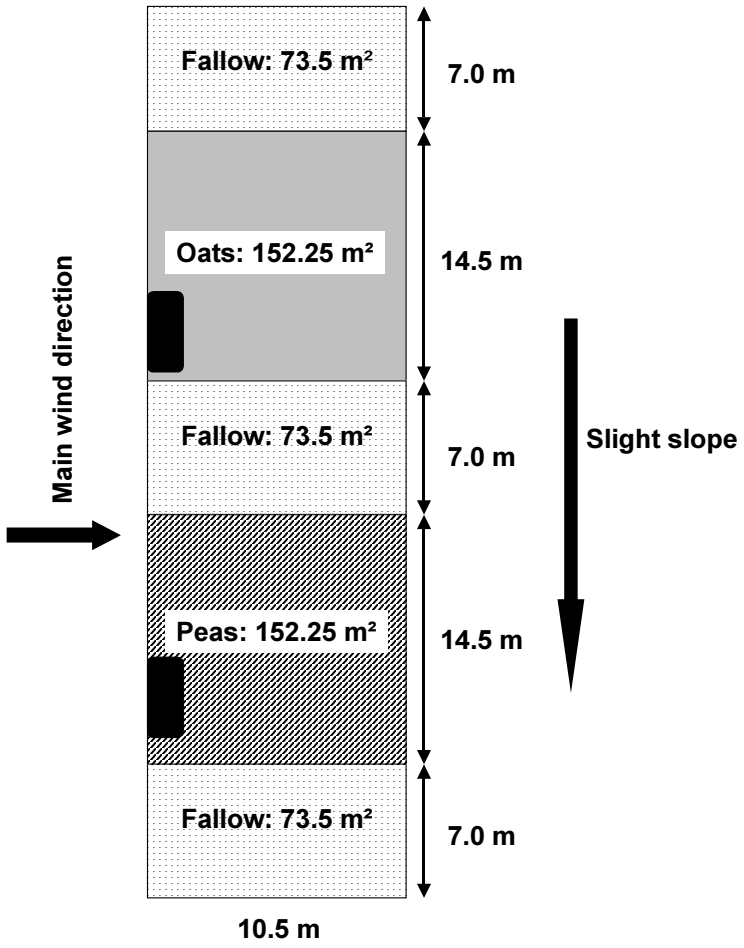

Figure 4 Experimental plots of the field experiment. The black areas indicate where the soil columns were taken for subsequent random transfer into the experimental plots. Fallow plots were established to prevent isotope drift between the experimental plots and for additional investigations.

4.4 Experiment III: Soil column experiment under field conditions

The aim of the experiment was to estimate the amount and fate of C and N rhizodeposition simultaneously during the reproductive growth phase of pea and oat plants under field conditions, according to objective (iii), (v) and (vi), chapter 3.

The experiment was conducted as a column experiment (5 replicates) under field conditions, using undisturbed soil columns (50 cm soil depth, 20 cm diameter) placed randomly blocked into oats and pea plots (Figure 4). Plants were *in situ* pulse labelled at the beginning of the reproductive growth phase with a ^{13}C-glucose-^{15}N-urea mixture using a cotton wick technique, allowing solution uptake through the vascular system of the plant and transfer of the ^{13}C and ^{15}N into the various plant parts.

Plants were harvested at maturity and all visible roots and root fragments were manually collected from the soil. The remaining plant derived C and N in the soil was defined as C and N rhizodeposition. The distribution of the ^{13}C and ^{15}N label in different plant parts (grain, stem, roots) and in various soil pools, such as unrecovered rootlets, microbial biomass and mineral N pool was determined.

Details of the methodology as well as biological and chemical analyses are given in chapter 6.2.

4.5 Experiment IV: Soil column experiment in a raised bed

This experiment was conducted to explore the time course of the amount and fate of C and N rhizodeposition in peas and oats during plant development, according to the objective (iv), (v) and (vi), chapter 3.

For this purpose undisturbed soil columns (50 cm soil depth, 20 cm diameter) were placed into a raised bed filled with soil under outdoor conditions (Figure 5). These experimental units were planted either with peas or with oats, leaving four plants in every column (7 replicates). During the 80 day experiment, the rhizodeposition was investigated for two growth intervals in oats and for three growth intervals in peas. Plants were *in situ* pulse labelled with a ^{13}C-glucose-^{15}N-urea mixture using a cotton wick technique.

Oat plants were harvested at physiological maturity and pea plants at 5 leaves unfolded and maturity, respectively. All visible roots and root fragments were separated from the soil and the remaining plant derived C and N in the soil was defined as C and N rhizodeposition. Distribution of the ^{13}C and ^{15}N label in various plant parts (grain, stem, leaves, roots) and in various soil pools, such as unrecovered rootlets, microbial biomass and mineral N pool was determined in every treatment.

A detailed description of the methodology and biological and chemical analyses is given in chapter 7.2.

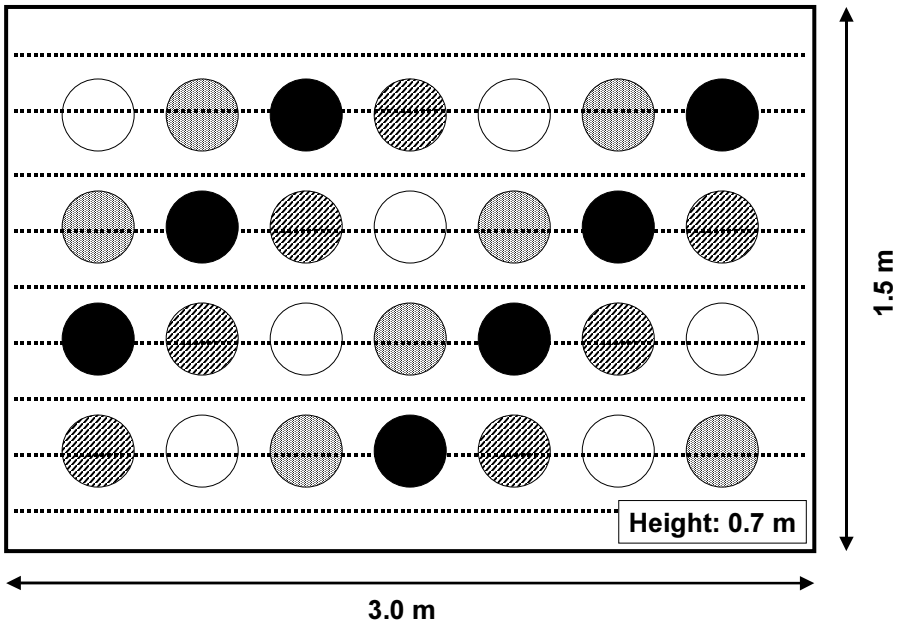

Figure 5 One of the experimental plots of the raised bed experiment under outdoor conditions. Different hatchings show the different treatments (for details see chapter 7.2). The dotted line indicate the location of the plant rows. The wodden box is, 1.5 m wide, 3.0 m long and 0.7 m high.

5 Evaluation of the wick method for in situ application of sugar-urea mixtures into the vascular system of peas (*Pisum sativum* L.) and oats (*Avena sativa* L.)

Florian Wichern [1*], Jochen Mayer [2], Rainer Georg Joergensen [1], and Torsten Müller [3]

Journal of Plant Nutrition and Soil Science, submitted (2007)

[1] *Department of Soil Biology and Plant Nutrition, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany*

[2] *Swiss Federal Research Station for Agroecology and Agriculture, Zürich, Switzerland*

[3] *Institute of Plant Nutrition, University of Hohenheim (330), Germany*

Abstract

The present study was conducted to evaluate the cotton wick method for its suitability for transferring sugar-urea mixtures *in situ* into the stem of plants. Two greenhouse experiments were carried out to investigate the effect of the wick method, including different urea and sugar solutions (cane sugar and glucose) and mixtures thereof on plant development, plant dry matter, and soil microbial biomass C and N. Solution uptake decreased with increasing sugar concentrations. Plant development, below-ground, above-ground and total plant dry matter were not significantly affected by the wick method and the applied solutions. The content of soil microbial biomass C and N was not influenced by the method or the applied solution but by the plant species. This indicates that the wick method is suitable for *in situ* investigation of soil C and N dynamics under the influence of plants. This paper shows that transfer of sugar-urea mixtures into the stem of plants using the wick method is possible. Mixtures of glucose at 2 and 4% with urea at 1% promise to be useful as

[*] Corresponding author: phone +49-5542-981664, fax +49-5542-981596, e-mail fwichern@uni-kassel.de

carriers of ^{13}C and ^{15}N, making *in situ* field investigations of soil C and N dynamics easier. These results should be verified in tracer studies.

Key words: below-ground carbon / below-ground nitrogen / labelling techniques / stem feeding method

5.1 Introduction

Field investigations with *in situ* estimation of amounts, turnover, and fate of plant-derived carbon (C) and nitrogen (N) compounds are crucial in understanding and optimising sustainable farming systems (Paterson, 2003). Research on this issue has flourished since stable isotope (^{13}C, ^{15}N) analytics improved. Isotope tracer techniques provide a tool for differentiating between plant-derived and soil-borne compounds, even in small and labile pools such as the microbial biomass (Mayer et al., 2003; Potthoff et al., 2003).

Three tracer methods can be distinguished: pulse labelling, continuous labelling, or the natural abundance of ^{13}C (Kuzyakov and Domanski, 2000), and more recently of ^{15}N. Quantification of the C input by plants into the soil is usually done by exposure of the plants to CO_2 enriched in ^{13}C, ^{14}C, or ^{11}C (Kuzyakov and Domanski, 2000), following the plants' natural pathway of C assimilation. For investigation of N dynamics, different methods have been developed to enrich plants with ^{15}N and following its transfer into soil pools. They either focus on labelling the plants using the natural way via the root system (e.g. split root technique, transplanting of labelled plants), or labelling above-ground plant parts *in situ* with the root system remaining undisturbed. Investigations using undisturbed plant roots are important, because undisturbed roots decompose faster than disturbed roots (Martin, 1989). Labelling was done by exposure of the plants to gaseous $^{15}NH_3$, or by shoot labelling (e.g. leaf feeding, stem injection, petiole feeding) with $^{15}NH_4^+$, $^{15}NO_3^-$, or urea ($CO(NH_2)_2$). A more detailed overview of the different ^{15}N-labelling methods is provided by Russell and Fillery (1996a) and Hertenberger and Wanek (2004). Most

investigations quantifying plant-derived nitrogenous compounds have been carried out under controlled conditions, probably because of methodological problems and the effort involved with *in situ* labelling (Mayer et al., 2003).

One of the methods used for shoot labelling is the wick method, which allows uniform labelling of plant material and a high recovery of the applied ^{15}N in previous studies (Russell and Fillery, 1996a; Mayer et al., 2003). Enrichment of the roots was as good as using a root feeding method (Hertenberger and Wanek, 2004), indicating that when using stem infiltration the applied solution is metabolised and its metabolites are transferred below-ground. The wick method was developed for labelling plant material and below-ground plant biomass of woody legumes with ^{15}N *in situ*, where the solution uptake through the cotton wick was driven by the transpiration stream in the vascular system (Russell and Fillery, 1996a; Mayer et al., 2003). Russell and Fillery (1996b) were able to transfer urea solutions up to a concentration of 10% into plants. However, solutions at such a concentration severely damaged the plants, as indicated by necroses. Urea was used as the N-carrier because it is non-polar, undissociated, and easily metabolised by the plant. In the vascular system urea is metabolised by the ubiquitous urease, resulting in the release of CO_2 and NH_3 (Hertenberger and Wanek, 2004).

In previous research, a major focus has been put on C deposition by annual plants and its fate in the soil (for a review, see Paterson 2003). Soil dynamics of N deposited by annual plants have been investigated less intensively and mostly under controlled conditions (Mayer et al., 2003), and little data is available on simultaneous investigation of C and N (Merbach et al., 1999). This might be the result of the difficulties involved in field investigations of the deposition and turnover of both, C- and N-derived plant compounds. For labelling plants, either ^{13}C- and ^{15}N-carriers are applied using two different methods (e.g. gaseous $^{13}CO_2$ and foliar application of ^{15}N-urea), or ^{13}C and ^{15}N are applied to the plants as gaseous compounds (e.g. $^{13}CO_2$ and gaseous $^{15}NH_3$), making this procedure very laborious, costly, and time consuming (Kuzyakov and Domanski, 2000). Applying a ^{13}C and ^{15}N enriched urea solution by foliar feeding does not allow a detailed investigation of the below-ground fate of ^{13}C, as urea concentrations have to be low to prevent leaf damage

(Schmidt and Scrimgeour, 2001). As, in the wick method, solution uptake is driven by the transpiration stream, we assume that it should be possible to apply a sugar solution into the stem of plants using this method. The wick method might provide a suitable tool for *in situ* labelling of plants in the field by applying a ^{13}C and ^{15}N labelled solution of sugar and urea. The main sugar in the vascular system of plants is sucrose (Marschner, 1995), which is a non-reducing sugar. However, homogeneously enriched ^{13}C-sucrose is very costly. Glucose enriched with ^{13}C might be a cheaper option for future labelling experiments.

Therefore, we conducted two experiments to evaluate the potential of the wick method for *in situ* transfer of sugar-urea mixtures as future ^{13}C and ^{15}N-carriers into pea and oat plants.

Our hypotheses were:

(1) The wick method provides a tool for *in situ* transfer of sugar-urea mixtures into the stem of plants, and can be used for pea and oat plants alike.

(2) The method and the applied solutions have no measurable effects on plant development, yield, and soil microbial biomass C and N.

5.2 Materials and methods

Experimental design

Two pot experiments were conducted in the greenhouse of the University of Kassel, in Witzenhausen, Northern Hesse, Germany. In a first experiment different concentrations of solutions containing either sugar or urea were applied, whereas in a second experiment different mixtures of sugar and urea were applied in the same manner. The mean temperature during the experiments was 19 °C with on average 110 klxh light per day. The soil used in the experiment was collected from the research station of the University of Kassel, which is situated at the state domain Frankenhausen, Northern Hesse, Germany (51° 24’ N, 9° 25’ W, 230 m asl) and in Hebenshausen, Northern Hesse, Germany (51° 21’ N, 9° 52’ W, 230 m asl). The silty

loam soils were Haplic Luvisols developed on loess and had 1.1% and 1.2% organic C, respectively, 0.12% total N, and a pH (H_2O) of 7.3. Bulk soil was collected in January 2004 and October 2005 and sieved through a 10 mm mesh size sieve.

For the **first experiment** pre-germinated peas (*Pisum sativum* L., cv. Santana) and oats (*Avena sativa* L., cv. Freddy) were planted into 3.5 l pots filled with a soil quartz mixture (3:1) in February 2004. Pots were situated in a randomised block design. After two weeks, pea plants were individualised, leaving two plants per pot. Oat plants were individualised, leaving one plant per pot. Plants were watered with deionised water according to their demand, keeping the soil sufficiently moist. The oats were fertilised with N-P-K (1 ml "Eluxal Super 8-8-6" pot^{-1}) in April and in June. Five weeks after planting, the cotton wicks were installed on the pea plants and sugar and urea solutions were applied (see below). On the oat plants wicks were installed and sugar and urea solutions applied fifteen weeks after planting, because plant development was slow. Pea plants were harvested after three months at 18 leaves unfolded (27th of April, DC 71). Oat plants were harvested after five months at maturity (21st of June, DC 89).

For the **second experiment** peas (*Pisum sativum* L., cv. Santana) and oats (*Avena sativa* L., cv. Freddy) were planted into 3.5 l pots filled with approximately 2000 g (dry weight) of soil on the 16th of January 2006. Pots were situated in a randomised block design with eight treatments in a row and the replicates for each treatment in seven blocks. After two weeks, plants were individualised, leaving two plants per pot which were further reduced to one plant at the time of labelling. Plants were watered with deionised water according to their demand, keeping the soil sufficiently moist. All plants were fertilised with N-P-K (0.5 ml "Eluxal Super 8-8-6" pot^{-1}) in March. At the beginning of March, 1 ml of the sugar-urea mixtures was applied to the pea plants with 8 leaves unfolded (02nd of March, DC 18) using the cotton wick method (see below). An additional 1 ml of each sugar-urea mixture was applied two weeks later at the beginning of flower setting (17th of March, DC 51). Pea plants were then harvested approximately four weeks after the first application of the sugar-urea mixtures at the end of fruit development (03rd of April, DC 78). On the 27th of March, 1 ml of the sugar-urea mixtures was applied to the oat plants

(DC 32). These were harvested seven weeks thereafter at maturity (15th of May, DC 75).

Application of solutions

For the treatments, a cotton wick was passed through a hole in the stem, which was drilled with a 0.5 mm drill approximately 3 cm above the soil surface. The ends of the wick were passed through a silicone tube and placed in a 2 ml vial with lid containing the solutions (Mayer et al., 2003; Russell and Fillery, 1996a). Connections on the plant stem and on the lid were sealed with plasticine (Teroson, Henkel, Düsseldorf, Germany) to prevent transpiration losses. In the **first experiment,** pea plants were treated separately with urea, cane sugar, or glucose solutions whereas only glucose solution was applied to oat plants. Urea was applied to peas at concentrations of 0.5, 1, 2, 4, 6% and to oats at concentrations of 0.5, 2, 6%. Cane sugar concentrations were 5, 10, 16, 22, and 28% for peas and 5, 16, and 28% for oats. Glucose was applied to oats at concentrations of 2, 4, and 10%. Cane sugar concentrations were much higher than those of glucose, because sucrose is the prevalent sugar in the vascular system of plants, whereas glucose is a reducing sugar, which is absent in the phloem and in the plant and is solely present in the mitochondria (Marschner, 1995). Due to the fact that highly enriched ^{13}C-sucrose is expensive, solutions with high concentrations of cane sugar (sucrose) but with low enrichment would therefore be a good option for future investigations.

In the **second experiment** a volume of 1 or 2 ml of mixtures of glucose and urea, or cane sugar and urea were applied to peas and oats, respectively. The concentration of urea in each treatment was 1%. Concentrations of cane sugar in the solutions were 16, 22, and 28% for peas and oats. Glucose-urea solution was applied with glucose at concentrations of 2, 4, and 8%. In both experiments two control treatments were included, one with deionised water applied through the wick and one without using the wick method at all. Solution uptake was monitored throughout the experiment at 24 h intervals by using the scale of the vials. Total solution uptake was calculated as the amount that was left in the vials at harvest subtracted from the amount supplied to the plants (Høgh-Jensen and Schjoerring, 2001).

Sampling and analyses

At harvest, vials and tubes were carefully removed and plants were directly cut right above the soil surface. Plant dry matter was calculated after drying at 60 °C for at least 72 hours. All visible roots were removed from the soil and washed with distilled water, dried at 60 °C for at least 72 hours to determine dry weight. The soil was sieved (< 2 mm) for further processing. Furthermore, a 100 g sub-sample of the sieved soil was wet sieved using a 200 µm sieve to determine the amount of rootlets remaining in the sieve. From this sub-sample, the total amount of rootlets was calculated. In the second experiment soil samples were additionally analysed for soil microbial biomass C and N.

Determination of the microbial biomass

Microbial biomass C and biomass N in the soil were estimated by fumigation-extraction (Brookes et al., 1985; Vance et al., 1987), including a pre-extraction step to remove living roots (Mueller et al., 1992; Mayer et al., 2003). Two portions of 25 g soil (on an oven-dry basis) were extracted with 100 ml 0.05 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1}. The soil suspension was completely transferred into a 600 ml beaker with an additional 25 ml 0.05 M K_2SO_4 where all visible roots were taken out. After that, the soil suspension was transferred into a filter with another 25 ml 0.05 M K_2SO_4. Then, one of the two soil samples of the extracted soil including the filter, was immediately fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$. Following fumigant removal, the sample was extracted with 100 ml 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1}. The soil suspension was filtered and the supernatant collected for determination of organic C and total N. The non-fumigated portion was extracted similarly at the time when fumigation commenced. Organic C in the extracts was measured as CO_2 by infrared absorption after combustion at 850 °C using a Dimatoc 100 automatic analyser (Dimatec, Essen, Germany). Microbial biomass C was calculated as E_C / k_{EC}, where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al., 1990). Total N in the extracts was measured by

chemoluminescence detection after combustion at 850 °C, using a Dima-N (Dimatec, Essen, Germany) chemoluminescence detector. Microbial biomass N was calculated as E_N / k_{EC}, where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996).

Calculations and statistical analysis

Results presented in the tables and figures are calculated on an oven-dry basis (about 24 h at 105 °C). The significance of differences between the treatments in the first experiment was tested by one-way analysis of variance and the Fisher's PLSD test was computed to separate means. In the second experiment two-way analysis of variance was carried out and the more conservative Tukey/Kramer post-hoc test was computed to separate means. All statistical analyses were done using StatView 5.0 (SAS Inst. Inc., Cary, NC, USA).

5.3 Results

Pea and oat plants took up urea, cane sugar, and glucose solutions, as well as mixtures thereof. Solution uptake rate was reduced when a second application took place using the same wick and took longer for oats than for pea plants (data not shown). In the second experiment with oats, visible fungal hyphae were detected in the vials of five samples where glucose-urea solution was applied and in four samples where cane sugar-urea solution was applied. Uptake of N for urea (Figure 6) and C for cane sugar and glucose (Figure 7 and 8) was related to the concentration of the solution and increased with increasing concentration. However, the solution uptake varied strongly between individual plants, resulting in increasing variation of the C- and N uptake with increasing concentration of the solution (Figures 6 to 8). When sugar-urea mixtures were applied, total solution uptake decreased with increasing sugar concentration, resulting in a reduced N uptake, as the concentration of urea was 1% in all treatments (Table 5). Pea and oat plants took up almost the same amount of glucose-urea solution with glucose concentrations of 2 and 4% as

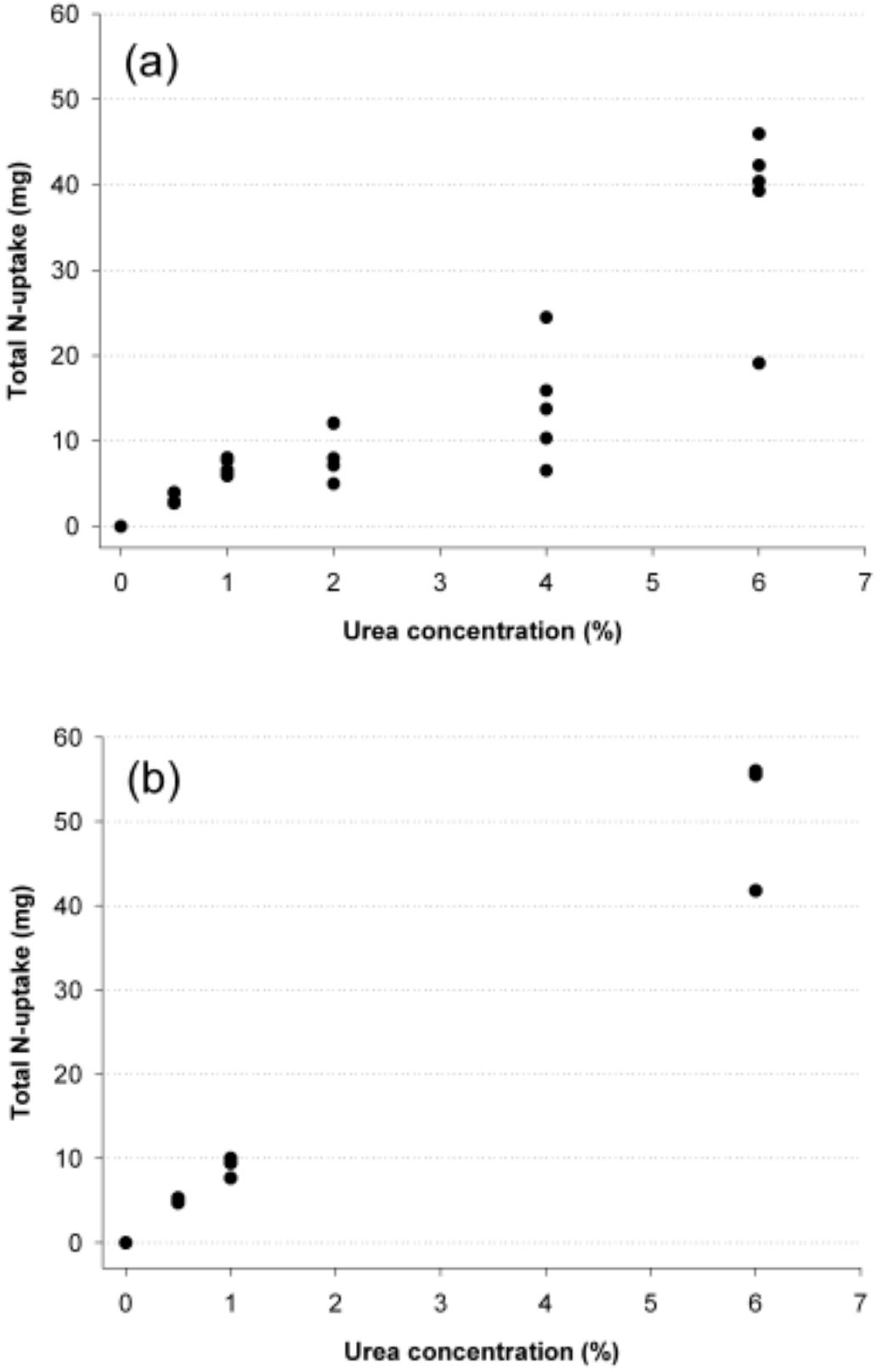

Figure 6 N uptake in relation to the concentration of urea solutions applied to pea (Fig. 6a) and oat (Fig. 6b) plants using the wick method.

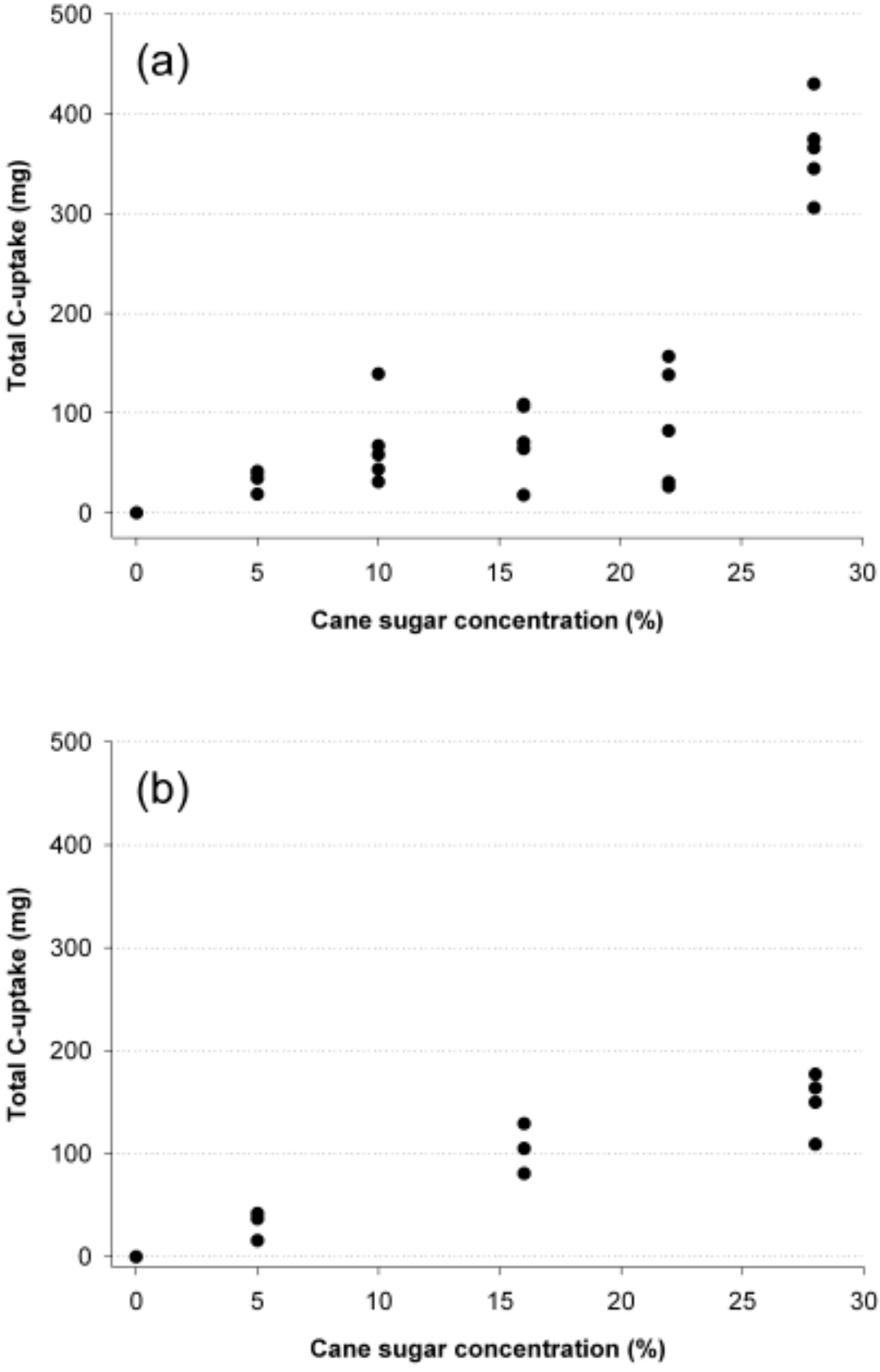

Figure 7 C uptake in relation to the concentration of cane sugar solutions applied to pea (Fig. 7a) and oat (Fig. 7b) plants using the wick method.

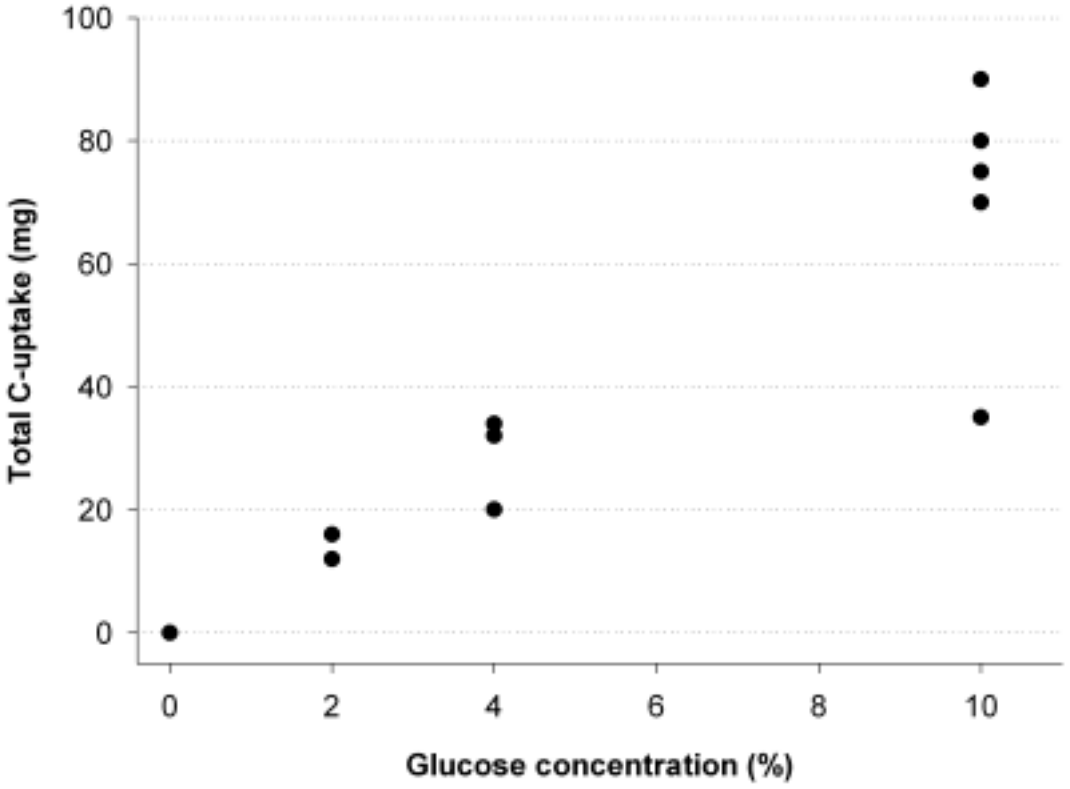

Figure 8 C uptake in relation to the concentration of glucose solutions applied to oat plants using the wick method.

they took up plain distilled deionised water. In these treatments the highest N uptake but the lowest C uptake could be achieved. The highest C uptake was realised when high concentrations of cane sugar in cane sugar-urea solutions were applied. Nevertheless, only 20 to 50% of the applied solutions containing cane sugar was taken up by the plants, resulting in a very low uptake of N. Pea plants took up urea solutions to a concentration of 4% without having any visible damage or being hampered in their development, confirmed by a lack of significant differences in plant dry matter between the different urea concentrations (Table 6). Where the urea solution of 6% was applied to the peas, some individual plants showed necroses. The lower above-ground dry matter of these plants was not significantly different from the other treatments, due to strong variation of individual plants in all treatments. Cane sugar solution was taken up similarly without any visible plant damage either of peas or of oats, even at high concentrations (Table 6). The same holds true

Table 5 Total solution, C, and N uptake of pea and oat plants after application of glucose-urea and cane sugar-urea solutions at different concentrations. Values show means. Different letters within a crop specific column indicate significant (Tukey/Kramer, $p < 0.05$) differences between the means. The lower part of the table shows F-values from the analysis of variance; degrees of freedom: crop (1), treatment (6).

Crop	Solution type	Conc. (%)		Solution uptake	C uptake	N uptake
		sugar	urea	(ml)	(mg)	(mg)
Peas	Glucose-urea	2	1	1.8 a	18 ab	8 a
	Glucose-urea	4	1	1.6 ac	30 ab	8 ad
	Glucose-urea	8	1	1.1 bc	37 b	5 bd
	Cane sugar-urea	16	1	0.7 b	52 bc	3 b
	Cane sugar-urea	22	1	0.5 b	45 bc	2 bc
	Cane sugar-urea	28	1	0.6 b	74 c	3 bc
	Water	0	0	2.0 a	0 a	0 c
Oats	Glucose-urea	2	1	0.8 ac	8 a	4 ac
	Glucose-urea	4	1	0.9 a	16 ac	4 a
	Glucose-urea	8	1	0.8 ac	29 bc	4 ac
	Cane sugar-urea	16	1	0.5 bc	33 bc	2 cd
	Cane sugar-urea	22	1	0.4 b	35 b	2 bd
	Cane sugar-urea	28	1	0.2 b	30 bc	1 bd
	Water	0	0	1.0 a	0 a	0 b
Analysis of variance						
Crop				70.7 ***	18.7 ***	37.4 ***
Treatment				29.5 ***	15.4 ***	33.4 ***
C x **T**				5.1 ***	2.4 ***	4.5 ***
CV (%)				70	90	80
n				7	7	7

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; CV = Coefficient of variance

Table 6 Total, above-ground, and below-ground dry matter of pea and oat plants after application of urea, cane sugar, and glucose solutions at different concentrations. Values show means. Different letters within a column indicate significant (Fisher's, $p < 0.05$) differences between the means.

Crop	Solution		Plant dry matter (g)			
	type	conc. (%)	total	above-ground	below-ground	n
Peas	Urea	0.5	6.5 a	3.2 a	3.4 a	5
	Urea	1	5.5 a	2.6 a	2.9 a	5
	Urea	2	5.9 a	2.6 a	2.4 a	5
	Urea	4	4.7 a	2.9 a	1.8 a	5
	Urea	6	4.9 a	1.9 a	3.0 a	5
	Water		7.6 a	3.1 a	4.5 a	5
	No wick		6.9 a	2.8 a	4.0 a	5
	Cane sugar	5	5.8 a	3.0 a	2.8 a	5
	Cane sugar	10	4.6 a	2.7 a	1.8 a	5
	Cane sugar	16	5.5 a	3.2 a	2.2 a	5
	Cane sugar	22	6.1 a	3.0 a	3.1 a	5
	Cane sugar	28	5.3 a	2.7 a	2.6 a	5
	Water		5.2 a	2.8 a	2.4 a	5
	No wick		5.0 a	2.6 a	2.3 a	5
Oats	Urea	0.5	10.3 b	4.5 b	5.8 b	5
	Urea	1	12.5 b	4.1 b	8.4 b	5
	Urea	6	10.2 b	3.4 b	6.8 b	4
	Cane sugar	5	10.7 b	4.6 b	6.1 b	4
	Cane sugar	16	14.4 b	4.2 b	10.2 b	5
	Cane sugar	28	11.2 b	4.2 b	7.0 b	5
	Glucose	2	10.4 b	4.1 b	6.3 b	5
	Glucose	4	10.0 b	4.1 b	5.9 b	5
	Glucose	10	10.1 b	5.1 b	5.1 b	5
	Water		12.9 b	4.0 b	8.9 b	5
	No wick		11.1 b	4.4 b	6.8 b	8

Table 7 Total, above-ground, and below-ground dry matter of pea and oat plants after application of glucose-urea and cane sugar-urea solutions at different concentrations. Values show means. The lower part of the table shows *F*-values from the analysis of variance; degrees of freedom: crop (1), treatment (7).

Crop	Solution type	Conc. (%)		Plant dry matter (g)		
		sugar	urea	total	above-ground	below-ground
Peas	Glucose-urea	2	1	6.3	5.8	0.5
	Glucose-urea	4	1	5.8	5.4	0.4
	Glucose-urea	8	1	6.1	5.7	0.4
	Cane sugar-urea	16	1	6.2	5.9	0.4
	Cane sugar-urea	22	1	5.9	5.4	0.4
	Cane sugar-urea	28	1	6.1	5.6	0.4
	Water	0	0	5.9	5.5	0.4
	No wick			5.9	5.5	0.4
Oats	Glucose-urea	2	1	8.6	7.4	1.1
	Glucose-urea	4	1	7.5	6.6	0.9
	Glucose-urea	8	1	8.5	7.2	1.2
	Cane sugar-urea	16	1	7.7	6.6	1.2
	Cane sugar-urea	22	1	7.7	6.8	1.0
	Cane sugar-urea	28	1	7.5	6.5	1.0
	Water	0	0	7.9	6.9	1.0
	No wick			8.1	7.0	1.1
Analysis of variance						
Crop				49.9 ***	29.4 ***	173.3 ***
Treatment				0.4	0.4	0.5
C x **T**				0.2	0.3	0.5
CV (%)				20	20	60
n				7	7	7

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; CV = Coefficient of variance

for glucose solution applied to oat plants (Table 6). The results of the second experiment support this observation (Table 7). No visible plant damage was observed during plant growth and no treatment-specific difference in below-ground, above-ground, or total plant dry matter could be detected (Table 7). However, plant dry matter of oats was on average 7.9 g $plant^{-1}$ and therefore 30% higher than the dry matter of pea plants, which was 6.0 g $plant^{-1}$.

The soil microbial biomass C and N content was not significantly different between the investigated treatments in the second experiment (Table 8). Under oats, the microbial biomass C content was on average 308 μg g^{-1} soil and lower in comparison with peas (379 μg g^{-1} soil). The microbial N content, however, was on a similar level (46 and 48 μg g^{-1} soil for oats and peas, respectively) in both soils, resulting in a lower C-to-N ratio of the microbial biomass under oats (6.8) in comparison with peas (7.9).

5.4 Discussion

In previous investigations, the wick method was used for labelling legumes with thick stems (Russell and Fillery, 1996a; Mayer et al., 2003; Hertenberger and Wanek, 2004; Yasmin et al., 2006). We were able to use the wick method on oat plants at elongation growth without damaging the plant as supposed by Yasmin et al. (2006). This stage of growth was chosen for labelling because the wick method can be used on oats only after the appearance of the first node to prevent solution loss by capillary transport between the leaves. Therefore, the conclusion of Hertenberger and Wanek (2004) that plant labelling using a stem feeding method is not appropriate for grasses, holds true only when an early labelling is striven for.

The wick method had no effect on total and below-ground plant dry matter in comparison to a control. Furthermore, the type of the solution applied via wick did not significantly influence plant development or dry matter production in our experiments. Yasmin et al. (2006) also found no significant effect on plant dry matter when comparing different labelling methods. Urea can be used as the N-carrier up to a concentration of 4% without damaging the plant. Only where a urea solution of

Table 8 Soil microbial biomass C and N, and its ratio after treatment of pea and oat plants with glucose-urea and cane sugar-urea solutions at different concentrations. Values show means. The lower part of the table shows F-values from the analysis of variance; degrees of freedom: crop (1), treatment (7).

Crop	Solution type	Concentration (%)		Microbial biomass		
		sugar	urea	C	N	C-to-N
				(µg g^{-1} soil)		
Peas	Glucose-urea	2	1	409	52	7.9
	Glucose-urea	4	1	382	48	8.0
	Glucose-urea	8	1	391	50	7.8
	Cane sugar-urea	16	1	365	44	7.7
	Cane sugar-urea	22	1	356	46	7.8
	Cane sugar-urea	28	1	401	50	7.9
	Water	0	0	373	46	8.1
	No wick			352	46	7.6
Oats	Glucose-urea	2	1	316	51	6.3
	Glucose-urea	4	1	308	47	6.6
	Glucose-urea	8	1	303	44	6.9
	Cane sugar-urea	16	1	315	44	7.1
	Cane sugar-urea	22	1	314	45	7.0
	Cane sugar-urea	28	1	314	47	6.7
	Water	0	0	306	45	6.8
	No wick			290	48	6.8
Analysis of variance						
Crop				50.7 ***	3.3	70.7 ***
Treatment				0.9	1.4	0.7
C x **T**				0.4	0.3	0.9
CV (%)				20	10	10
n				7	7	7

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; CV = Coefficient of variance

6% was applied to the peas did plants have a lower total plant dry matter, which was due to the toxic effect of the solution resulting in damage of the plant tissue. This has also been observed for lupines (Russell and Fillery, 1996a). In our experiments, we did not observe any difference in root biomass production of the treatments having a wick in comparison with an untreated control. This is in contradiction to the findings of Yasmin et al. (2006) who observed stimulated root growth when using the stem feeding technique in comparison with leaf and petiole feeding. Necroses have been reported after labelling with urea when using a leaf feeding technique (Yasmin et al., 2006). In our experiments using the wick method, necrotic tissue was only observed at urea concentrations of 6%, making this technique suitable for high enrichment of above- and below-ground plant biomass without strongly damaging the plant. This is in line with the observation of Mayer et al. (2003) that plants tolerate higher concentrations of urea when using the wick method in comparison with foliar feeding.

The soil microbial biomass as a sensitive indicator of short-term changes in the organic matter pool (Sparling, 1997), was not influenced by the method or the solution applied using the wick method. However, its size and the C-to-N ratio was lower under oats in comparison to peas, even though the below-ground biomass of oats was more than twice as much. Plant species thus have a stronger effect on the soil microbial biomass than the wick method and the applied solutions.

In the second experiment, solution uptake after the second application to pea plants was slower in comparison with the first application. Yasmin et al. (2006) observed a reduced solution uptake after the second feeding event, and concluded that it was due to blockage of the drilled hole. This was previously also observed by others (Russell and Fillery, 1996a; Mayer et al., 2003). It is assumed that tissue had developed around the wick and that air bubbles hampered solution uptake (Russell and Fillery, 1996a). In our second experiment with oats, fungal growth might be one reason for lower solution uptake, especially in the cane sugar treatments. For the application of a sugar-urea mixture using the wick method, sterility of the solution and vessels is thus crucial. Due to this fact and the observation that solution uptake by the oat plants was slow in comparison with pea plants, and that uptake was

slower at the second application event for peas, the wick method should be used as a method for pulse labelling. Multiple pulses might be achieved when small amounts of solutions with high concentrations are applied or when a new wick is installed for every pulse. This allows smaller growth intervals to be investigated and helps to overcome some of the problems described. In their study, Russell and Fillery (1996a) concluded that the wick method should be used for sequential application with proportional rates to plant N content. Due to the reasons described above, we propose that the wick method should be preferentially used for pulse labelling instead of the recommended continuous labelling. However, with a continuous labelling throughout the growth of the plant, a more homogeneous labelling would be achieved (Russell and Fillery, 1996a; Mayer et al., 2003). This would be preferable especially when investigating the turnover of the labelled plant material in further investigations (Mayer et al., 2003).

In our experiments a mixture of glucose and urea was taken up at higher amounts as a cane sugar-urea mixture. One reason might have been the high concentration of cane sugar in the solution making it more viscous. Clearly, applying glucose as the C-carrier using the wick method does not represent the natural way of C assimilation of the plant. Nevertheless, this method provides the opportunity to simultaneously investigate soil C and N dynamics under the influence of vegetation in particular *in situ* and under field conditions, with relatively low costs and without the need of an airtight chamber for CO_2-application. The wick method is a tool for transferring sugar-urea mixtures into the vascular system of pea plants and in addition also to oat plants, corroborating our hypothesis.

5.5 Conclusions

It is possible to transfer sugar solutions and sugar-urea mixtures into the stem of pea and oat plants using the wick method. The method itself has no measurable effect on plant development, yield, or soil microbial biomass. This method may thus be used for pulse double labelling plant material and below-ground plant biomass *in situ* with stable and radioactive C and N isotopes. If sterile conditions in the wick

system are ensured, this method can be a useful tool for field investigations of C and N dynamics.

Acknowledgements

We acknowledge the kind help of Gabi Dormann, Christian Maack, Manuel Renneberg, Sarina Weber, Markus Peters, Birge Ude, Nicole Gauss, and Elmar Eberhardt. We are highly indebted to Rainer Brauckmann who provided technical support using the greenhouse. We further thank Mick Locke for correcting our English. This study was financed by the German Research Foundation (MU1711/3-1 and JO362/6-1).

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6 Rhizodeposition of C and N in peas and oats after ^{13}C-^{15}N double labelling under field conditions

Florian Wichern [1*], Jochen Mayer [2], Rainer Georg Joergensen [1], and Torsten Müller [3]

Soil Biology & Biochemistry, submitted (2006)

[1] *Department of Soil Biology and Plant Nutrition, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany*

[2] *Swiss Federal Research Station for Agroecology and Agriculture, Zürich, Switzerland*

[3] *Institute of Plant Nutrition, University of Hohenheim (330), Germany*

Abstract

Compounds released by plant roots during growth can make up a high proportion of below-ground plant carbon and nitrogen, and therefore influence soil organic matter turnover and plant nutrient availability by stimulating the soil microorganisms. The present study was conducted to examine the amount and fate of C (CdfR) and N rhizodeposition (NdfR), in this study defined as root-derived C or N present in the soil after removal of roots and root fragments, released during reproductive growth. Below-ground plant biomass (BGP) of peas (Pisum sativum L.) and oats (Avena sativa L.) was successfully labelled in situ with a ^{13}C-glucose-^{15}N-urea mixture under field conditions using a stem feeding method. Pea plants were labelled at the beginning of flowering and harvested at pod filling (PP) and maturity (PM). Oat plants were labelled at grain filling and harvested at maturity (OM). CdfR was between 24.2% (PP) and 30.8% (OM) of total recovered plant C. NdfR was between 30.0% (OM) and 36.4% (PM) of total plant N. Amounts of NdfR were four times higher in peas in comparison with oats. These results are higher than results from

[*] Corresponding author: phone +49-5542-981664, fax +49-5542-981596, e-mail fwichern@uni-kassel.de

other studies. Microbial CdfR at 0-30 cm soil depth was 67% and 37% of the microbial biomass C in peas and 23% in oats. Microbial NdfR was 17% and 15% of microbial N in peas and 54% in oats. Furthermore, inorganic NdfR was 32% and 34% in peas and 9% in oats at 0-30 cm. The C-to-N ratio of rhizodeposition was lower under peas (17.3) than under oats (41.9) at maturity. These results show that rhizodeposits of peas provide a more easily available substrate to soil microorganisms, which are incorporated to a greater extent and turned over faster in comparison with oats. Beside the higher amounts of N released from pea roots, this process contributes to the higher N-availability for subsequent crops.

Keywords: ^{13}C; Below-ground carbon; Below-ground nitrogen; Double labelling technique; ^{15}N; Rhizodeposits; Soil microbial biomass; Stem feeding method

6.1 Introduction

Sustainable and organic farming systems attempt to increase soil fertility by introducing legumes and clover grass mixtures into crop rotations, which contribute to a higher N-availability for subsequent non-legumes (Chalk, 1998; Mayer et al., 2003). Beside N_2-fixation, 'N-sparing' (reduced inorganic N-uptake by legumes in comparison with non-legumes), plant residue quality, turnover of parent soil organic matter, and the amount and quality of rhizodeposition released by plant roots are assumed to be critical factors (Chalk, 1998; Mayer et al., 2003). Understanding the processes of C and N turnover in the soil under the influence of living roots is therefore crucial for assessing the contribution of below-ground N of legumes to succeeding crops (Høgh-Jensen and Schjoerring, 2001), and for creating sustainable agricultural systems (Merbach et al., 1999).

Rhizodeposition is the release of all root derived compounds and plant material into the soil during plant growth and comprises root exudates, sloughed-off cells, and tissue, such as rootlets and root hairs (Marschner, 1995). It is mostly estimated as C derived from rhizodeposition (CdfR) and less often in terms of N derived from

rhizodeposition (NdfR) and always estimated as the net release from the roots. At the time of investigation, visible morphologically intact roots and root fragments are separated and not included in this part of the below-ground plant fraction. Later on, these fractions may become part of rhizodeposition as soon as they are subject to microbial decomposition. Composition and amounts of rhizodeposition vary depending on various environmental factors (e.g. nutrient status, soil type, water availability, soil microbial community), plant specific factors (e.g. species or variety, growth stage), and their interactions (Marschner, 1995; Deubel et al., 2000). Rhizodeposition was observed to be highest in the phase of fast vegetative growth (Sauerbeck and Johnen, 1976). In wheat plants N release was highest from ear emergence until the beginning of grain filling (Rroço and Mengel, 2000). With increasing plant development, however, below ground translocation of photosynthates often decreased in cereals (Helal and Sauerbeck, 1986; Meharg, 1994; Yevdokimov et al., 2006). The reproductive growth phase, however, is an important stage of grain filling, where a strong relocation of nitrogen into the sinks of the plant, in particular the grains, can be expected (Rroço and Mengel, 2000). In addition, existing roots die during this phase and therefore contribute to rhizodeposition. It was also observed that especially during pod filling in grain legumes a high quantity of readily mineralisable N-compounds are lost from roots (Ofosu-Budu et al., 1990). The reproductive growth stage is therefore an important phase for investigating rhizodeposition. Information on the amounts of rhizodeposition of C and N under field conditions is still relatively scarce, with a simultaneous assessment of it missing.

On average 20 to 30% of the net fixed C of cereals is translocated below-ground with half of it accounting for rhizodeposition (Kuzyakov and Domanski, 2000; Kuzyakov and Schneckenberger, 2004). Most of the C rhizodeposition is respired as CO_2 by roots and microorganisms in the rhizosphere, leaving only 3 to 9% of the net fixed C as residues in the soil (Kuzyakov and Domanski, 2000; Nguyen, 2003; Kuzyakov and Schneckenberger, 2004). The proportion of net assimilated N transferred below-ground varies between 16 and 60% in cereals (Rroço and Mengel, 2000; Khan et al., 2002a), whereas in legumes 14 to 53% is transferred below-ground (Khan et al., 2002a; Mayer et al., 2003). Nitrogen derived from rhizodeposi-

tion varies widely between 4.3 and 56% of net assimilated N in wheat (Merbach et al., 2000; Khan et al., 2002a) and 4.5 and 44% in legumes (Brophy and Heichel, 1989; Khan et al., 2002a). This high variability of N rhizodeposition found in literature, is due to the use of different methods for labelling plants with ^{15}N and varying experimental conditions.

Most of the different compounds released by plants serve as substrate for soil microorganisms (Paterson, 2003) and are often highly labile (Janzen, 1990). However, the decomposition of different root derived compounds may vary greatly (Meharg, 1994). Low molecular weight substances are metabolised relatively fast, whereas mucilage, sloughed-off cells, and other high molecular weight rhizodeposits are metabolised by microorganisms with a time delay. This is particularly important with respect to N. During plant growth, N rhizodeposits may quickly become immobilized by rhizosphere microorganisms. Its remineralisation is driven by the turnover of the microbial biomass, which depends on the availability of C, e.g. from rhizodeposition. Therefore the rhizodeposition of C and also of N influences the plant nutrient availability, in particular for the succeeding crop.

As root turnover is much faster under undisturbed conditions at the soil-root interface, *in situ* measurement may register higher amounts of rhizodeposition (Meharg, 1994; Mayer et al., 2003). Furthermore, the dynamics of carbon and nitrogen released by plant roots and their turnover can be investigated much more realistically under field conditions than under laboratory conditions, because the constantly changing biotic and abiotic conditions are included (Meharg, 1994). With isotope tracer techniques, a tool is provided which allows the differentiation between plant-derived and soil-borne compounds. There have been different approaches to labelling plants with ^{13}C or ^{15}N individually, but hardly ever have the two tracers been combined to investigate the fate of root derived compounds in the soil (Merbach et al., 1999). For a detailed overview of different ^{13}C and ^{15}N labelling techniques, see e.g. Russell and Fillery (1996a), Hertenberger and Wanek (2004), and Kuzyakov and Schneckenberger (2004). A common method of labelling plants with ^{13}C is using the physiological pathway of C assimilation and applying $^{13}CO_2$ to an airtight chamber. This is laborious and expensive when applied in the field and therefore

impractical. Another option is provided by the wick method (Russell and Fillery, 1996a), originally developed for labelling grain legumes with ^{15}N. It is a tool for applying a highly enriched solution of glucose and urea with a defined amount of ^{13}C and ^{15}N into the plant (Wichern et al., 2007).

Therefore, we conducted an experiment under field conditions where plants were pulse-labelled with ^{13}C and ^{15}N, with the objectives (I) to quantify *in situ* rhizodeposition of C and N in the reproductive growth phase of peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) under field conditions, (II) to investigate the transfer of C and N derived from rhizodeposition into different soil pools, and (III) to examine the interaction of C and N derived from rhizodeposition in the soil.

6.2 Materials and methods

Study site

The experiment took place at the research station of the University of Kassel, which is situated in Frankenhausen, Northern Hesse, Germany (51° 34' N, 9° 85' W, 230 m asl). Total annual rainfall in 2004 was 585 mm. During the investigation, precipitation was 250 mm and mean average temperature was 15.8 °C, with a maximum of 33.5 °C and a minimum of –0.5 °C. Fields have been cultivated according to organic farming practices for 6 years. The preceding crop on the experimental field was barley (*Hordeum vulgare* L.). There has been neither clover-rye grass, nor a legume crop on the experimental site for at least six years. The clayey silt soil was a Haplic Luvisol on loess with 2% sand and 22 to 30% clay. The pH (H_2O) was 7.5 and the content of organic C and total N in the soil was 1.12 and 0.12% in 0-30 cm soil depth and 0.58 and 0.06% in 30-50 cm, respectively. In 0-30 cm soil depth phosphorus content (CAL) was 60 µg g^{-1} soil, potassium content (CAL) was 121 µg g^{-1} soil and magnesium ($CaCl_2$) content was 93 µg g^{-1} soil.

Experimental design and labelling

In spring 2004 (15th of April) two plots of 14.5 m to 10.5 m were cultivated either with peas (*Pisum sativum* L., cv. Santana) or with oats (*Avena sativa* L., cv. Freddy). After germination of the pea and oat plants, ten and twenty PVC-columns (20 cm in diameter and 55 cm long) were forced into the soil at the fringe of the plots. Columns were excavated, closed with a lid at the bottom, and transferred to prepared holes in the respective experimental plot at least 2 m from the edge of the plot. For oats, 4 plants were left in the columns and for peas 5 plants. The columns were situated in a randomised block design with five replicates of each treatment.

Plants were labelled with a solution of on average 2% (w/v) ^{13}C enriched (99atom%) glucose and 0.5% (w/v) ^{15}N enriched (99atom%) urea. The wick method was used (Russell and Fillery, 1996a; Mayer et al., 2003) for solution transfer into the plant. Briefly, a cotton wick was passed through a hole in the stem, which was drilled with a 0.5 mm drill approximately 3 cm above the soil surface. The ends of the wick were passed through a silicone tube and inserted into a 2 ml vial with a lid containing the solution. To prevent transpiration losses the connections at the plant stem and at the lid were sealed with plasticine (Teroson, Henkel, Düsseldorf, Germany). All vials and materials used in the system were steam sterilised for 20 min at 121 °C. Solution was produced using sterile deionised water.

For peas, two treatments were labelled ($\mathbf{P_P}$ = **P**eas harvested at **P**od setting, and $\mathbf{P_M}$ = **P**eas harvested at **M**aturity). Treatment P_p was labelled at the beginning of flowering with 12 leaves unfolded (14th of June, EC 58) by applying 2 ml of the solution. Treatment P_M was labelled in two dispensations with 1 ml of the solution applied also at 12 leaves unfolded (14th of June, EC 58) and further 1 ml applied during flowering (23rd of June, EC 59). After the solution was taken up, 1 ml of sterile deionised water was placed in the empty vials (16th of July) to assure uptake of remaining glucose and urea. Harvest of the treatment P_P and the respective control took place at 14 leaves unfolded (20th of July, EC 69) at the beginning of pod setting, and of the treatment P_M and the respective control at maturity (5th of August, EC 81). Oat plants were labelled at the end of elongation growth (30th of June, EC 40) and harvested at maturity (11th of August, EC 86, treatment $\mathbf{O_M}$ = **O**ats har-

vested at **M**aturity). After labelling, a mesh (1 mm) was put around the plants and covering the soil, to prevent any contamination from shed leaves, which were collected regularly. With each labelled treatment an unlabelled control treatment with plants of the same kind was harvested to obtain background values of ^{13}C and ^{15}N enrichment of the various plant parts and soil fractions.

Plant and soil sampling

At harvest, vials and wicks were carefully removed and plants were cut off directly above the soil surface. Plants were separated into stem, pod, and grain. Soil columns were excavated, closed with a plastic bag on top, transferred to the laboratory, and stored at 5 °C until further processing for a maximum of three days. The soil was separated into two soil layers (0-30 cm and 30-50 cm), all visible roots of each layer were collected manually, and the soil was sieved (< 2mm) for further analyses. Roots and root fragments were manually collected during the sieving process and washed with distilled water. All plant material was dried at 60 °C for at least 72 hours to determine dry weight, ground to fine powder using a ball mill, and analysed for total C, total N, and the isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ (see below). Additionally, a 200 g sub-sample of the sieved soil was washed over a 200 µm sieve to determine the amount of remaining rootlets. A sub-sample of the rootlet material was burned to ash at 550 °C to determine the ash-free dry matter and corrected with the ash content of the roots. Sub-samples of dried root free soil material before the pre-extraction step (see below) were homogenised in a ball mill and analysed for total C and total N.

Analyses

Total C and total N in plant and soil samples were determined gas-chromatographically after combustion using a Carlo Vario Max CN analyser (elementar, Hanau). The isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were determined using isotope ratio mass spectrometry (Delta plus IRMS 251, Finnigan Mat, Bremen, Ger-

many) after combustion using a Carlo Erba NA 1500 gas chromatograph (Carlo Erba Instruments, Milano, Italy).

Microbial biomass C and N in the soil were estimated by chloroform-fumigation-extraction (Brookes et al., 1985; Vance et al., 1987), including a pre-extraction step to remove living roots (Mueller et al., 1992; Mayer et al., 2003). Briefly, one portion of 100 g (on an oven-dry basis) soil was extracted with 400 ml 0.05 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1}. The soil suspension was completely transferred into a 600 ml beaker with an additional 50 ml 0.05 M K_2SO_4 and all visible root fragments were taken out manually. After that, the soil suspension was transferred into a centrifuge beaker with another 50 ml 0.05 M K_2SO_4 and centrifuged for 10 min at 500 *g*. An aliquot of the supernatant (pre-extract) was collected for measuring organic C and total N in the extract. Then, one portion of approximately 25 g (on an oven-dry basis) of the extracted soil was immediately fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$. Following fumigant removal, the sample was extracted with 100 ml 0.05 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and centrifuged for 10 min at 500 *g,* the supernatant was collected for determination of organic C and total N. The non-fumigated 25 g portion was extracted similarly at the time when fumigation commenced. Organic C in all extracts was measured as CO_2 by infrared absorption after combustion at 850 °C using a Dimatoc 100 automatic analyser (Dimatec, Essen, Germany). Microbial biomass C was calculated as E_C / k_{EC}, where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al., 1990). Total N in the extracts was measured by chemoluminescence detection after combustion using a Dima-N analyser. Microbial biomass N was calculated as E_N / k_{EC}, where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996). In the K_2SO_4 extracts of all non-fumigated samples and in all pre-extracts, NO_3^--N was determined using segmented flow analysis (Alliance Instruments, Friedrichsdorf).

For measuring the isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in the extracts, a sub-sample of the K_2SO_4-extract was freeze-dried until constant weight. The isotope ratios $^{13}C/^{12}C$

and $^{15}N/^{14}N$ of the dry material was determined using isotope ratio mass spectrometry.

Calculations and statistical analysis

The percentage of rhizodeposition derived total soil C (CdfR), total soil N (NdfR), K_2SO_4 extractable C (extractable CdfR) inorganic N (inorganic NdfR), microbial C (microbial CdfR), and N (microbial NdfR) was calculated using the equation of Janzen and Bruinsma (1989):

$$\%\text{CdfR} = \frac{atom\%\ ^{13}C\ excess\ soil}{atom\%\ ^{13}C\ excess\ root} \times 100 \quad \text{and} \quad \%\text{NdfR} = \frac{atom\%\ ^{15}N\ excess\ soil}{atom\%\ ^{15}N\ excess\ root} \times 100$$

Amounts of CdfR and NdfR in the various pools were obtained by multiplying the total amounts of C and N in the pool by the respective %CdfR and %NdfR values. The enrichment of microbial biomass C and N with ^{13}C and ^{15}N respectively was calculated according to Mayer et al. (2003):

$$\text{atom\% microbial } ^{13}\text{Cexcess} = \frac{(C_{fum} \times \%^{13}\text{Cexcess}_{fum} - C_{nonfum} \times \%^{13}\text{Cexcess}_{nonfum})}{(C_{fum} - C_{nonfum})} \times 100,$$

$$\text{atom\% microbial } ^{15}\text{Nexcess} = \frac{(N_{fum} \times \%^{15}\text{Nexcess}_{fum} - N_{nonfum} \times \%^{15}\text{Nexcess}_{nonfum})}{(N_{fum} - N_{nonfum})} \times 100,$$

where 'fum' is the fumigated soil and 'nonfum' is the non-fumigated soil. The ^{13}C and ^{15}N atom% values of every investigated plant and soil fraction were corrected by subtracting the ^{13}C and ^{15}N enrichment of the non-labelled control treatments containing plants of the same kind. For these calculations, the following assumptions were made: (1) rhizodeposition has the same ^{13}C and ^{15}N enrichment as the recovered roots, (2) rootlets recovered by wet sieving have the same enrichment as roots, and (3) the enrichment of the roots with ^{13}C and ^{15}N was constant during the time of investigation.

Results in the tables are presented on an oven-dry basis (about 24 h at 105°C for soil samples and about 72 h at 60 °C for plant parts). Means of microbial biomass C and N, its ratio (microbial biomass C-to-N), extractable C, inorganic N, and the per-

centage of each fraction derived from rhizodeposition (dfR), were compared between the treatments and depth using a three-way ANOVA, with block as random factor and treatment and depth as fixed factors. The Tukey/Kramer post-hoc test was computed to separate means. All statistical analyses were performed using JMP 5.1 (SAS Institute Inc., Cary, USA).

6.3 Results

Recovery and distribution of ^{13}C and ^{15}N

Enrichment of plants with ^{13}C was highest in the stem and leaf fraction, followed by the grain fraction for pea and the below-ground plant biomass (BGP) fraction for oats, but was generally below 0.5 atom%^{13}Cexcess (Table 9). The enrichment with ^{15}N varied between 1.15 atom%^{15}Nexcess and 7.12 atom%^{15}Nexcess, and was on a similar level in the grain and stem fractions of each treatment and lower in the BGP fraction (Table 10).

The total recovery of the applied ^{13}C in the above and below-ground plant parts varied between 32.4 and 53.2%. These values do not include $^{13}CO_2$ lost from foliage, roots, and microbial respiration, as these fractions cannot be measured in this system. Total recovery of the applied ^{15}N was higher, ranging from 59.2 to 77.0%. More than half of the recovered ^{13}C was detected in the leaves and stem fraction. In oats, additionally about one third of the recovered ^{13}C and twice as much as in peas was detected in the BGP. The same trend was observed for recovered ^{15}N in the BGP as a percentage of total ^{15}N, which was higher in oats. In treatment P_P, most of the ^{15}N was recovered in the stem and leaf fraction, whereas for treatment P_M and treatment O_M most of the applied ^{15}N was detected in the grains. The ^{13}C and ^{15}N recovered in the grain and BGP of peas increased from pod setting to maturity. This was reflected by an increase of plant C and N in these fractions.

The distribution of total plant C and N in treatment O_M was similar to treatment P_M, with the BGP-C fraction of treatment OM being 5% higher, due to a higher contribution from roots. The percentage of BGP-C and of grain-C, was much higher than for ^{13}C in these fractions, showing that the distribution of the recovered ^{13}C did not

Table 9 Enrichment with ^{13}C, recovery and distribution of the ^{13}C label, and distribution of plant C in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.). Peas were labelled at 12 leaves unfolded and harvested at the beginning of pod setting (P_P) and at maturity (P_M). Oats were labelled at the end of elongation growth and harvested at maturity (O_M). Values show means ± standard error of the mean (n=5).

		atom% ^{13}C excess	**Recovery of ^{13}C (%)**	**Distribution of recovered ^{13}C (%)**	**Distribution of total C (%)**
P_P	Grain	0.25 ± 0.03	7.8 ± 0.5	14.8 ± 1.3	17.9 ± 0.5
	Stem and leaves	0.41 ± 0.04	39.2 ± 3.0	73.3 ± 2.7	54.3 ± 2.8
	BGP-C	0.13 ± 0.02	6.2 ± 1.1	11.9 ± 2.4	27.9 ± 3.2
	Roots		*0.8 ± 0.1*	*1.5 ± 0.2*	*3.6 ± 0.2*
	Rhizodeposition		*5.4 ± 1.0*	*10.4 ± 2.3*	*24.2 ± 3.4*
	Total		53.2 ± 2.2	100	100
P_M	Grain	0.29 ± 0.05	8.4 ± 0.9	25.8 ± 2.3	26.3 ± 1.2
	Stem and leaves	0.40 ± 0.03	18.3 ± 1.1	56.6 ± 1.0	40.0 ± 1.7
	BGP-C	0.15 ± 0.02	5.7 ± 0.5	17.6 ± 1.5	33.7 ± 1.3
	Roots		*0.7 ± 0.1*	*2.1 ± 0.2*	*4.1 ± 0.3*
	Rhizodeposition		*5.0 ± 0.5*	*15.4 ± 1.6*	*29.6 ± 1.6*
	Total		32.4 ± 1.7	100	100
O_M	Grain	0.08 ± 0.01	3.2 ± 0.8	7.5 ± 1.3	26.0 ± 3.1
	Stem and leaves	0.43 ± 0.06	23.9 ± 4.6	57.3 ± 7.3	35.3 ± 3.4
	BGP-C	0.25 ± 0.08	13.3 ± 2.6	35.2 ± 8.3	38.7 ± 5.6
	Roots		*2.7 ± 0.4*	*7.1 ± 1.6*	*7.9 ± 0.5*
	Rhizodeposition		*10.6 ± 2.5*	*28.1 ± 7.3*	*30.8 ± 6.0*
	Total		40.4 ± 3.6	100	100

Table 10 Enrichment with ^{15}N, recovery and distribution of the ^{15}N label, and distribution of plant N in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.). Peas were labelled at 12 leaves unfolded and harvested at the beginning of pod setting (P_P) and at maturity (P_M). Oats were labelled at the end of elongation growth and harvested at maturity (O_M). Values show means ± standard error of the mean (n=5).

		atom% ^{15}N excess	**Recovery of ^{15}N (%)**	**Distribution of recovered ^{15}N (%)**	**Distribution of total N (%)**
P_P	Grain	3.72 ± 0.16	28.1 ± 2.8	36.4 ± 1.2	27.9 ± 0.6
	Stem and leaves	3.93 ± 0.25	38.0 ± 3.3	49.3 ± 2.4	35.9 ± 1.3
	BGP-N	1.15 ± 0.18	10.9 ± 1.2	14.3 ± 1.7	36.2 ± 1.7
	Roots		*1.2 ± 0.1*	*1.7 ± 0.3*	*4.2 ± 0.3*
	Rhizodeposition		*9.6 ± 1.2*	*12.6 ± 1.5*	*32.1 ± 2.0*
	Total		77.0 ± 6.0	100	100
P_M	Grain	4.90 ± 0.75	32.2 ± 2.5	54.0 ± 2.6	36.9 ± 1.7
	Stem and leaves	4.19 ± 0.33	17.3 ± 0.9	29.1 ± 1.0	22.3 ± 1.1
	BGP-N	1.32 ± 0.19	9.9 ± 1.4	16.9 ± 2.2	40.8 ± 1.5
	Roots		*1.1 ± 0.2*	*1.8 ± 0.3*	*4.4 ±0.4*
	Rhizodeposition		*8.9 ± 1.2*	*15.1 ± 1.9*	*36.4 ± 1.5*
	Total		59.2 ± 2.7	100	100
O_M	Grain	7.06 ± 0.82	29.1 ± 3.1	49.1 ± 5.2	40.7 ± 5.2
	Stem and leaves	7.12 ± 0.35	14.8 ± 1.6	24.8 ± 2.3	20.6 ± 4.2
	BGP-N	3.20 ± 0.72	15.5 ± 3.1	26.1 ± 5.4	38.7 ± 4.3
	Roots		*2.8 ± 0.5*	*4.7 ± 0.8*	*8.7 ± 0.6*
	Rhizodeposition		*12.7 ± 2.8*	*21.4 ± 4.9*	*30.0 ± 4.6*
	Total		59.5 ± 2.3	100	100

represent the distribution of total plant C. The same holds true for the distribution of total N in the various plant fractions, which was not represented by the distribution of the recovered ^{15}N. In comparison with the distribution of total N, a higher percentage of ^{15}N was recovered in the above-ground plant parts and a lower percentage in BGP-N. About one third of total plant C was recovered as rhizodeposition (CdfR) and in the stem and leaves fraction. One fourth of total plant C was recovered in grains. In treatment P_M and treatment O_M, more than one third of total plant N was found in grains, and 30 to 36% of total pant N was rhizodeposition.

Quantity and partition of C and N rhizodeposition

In peas, C derived from rhizodeposition (CdfR) was 24.2 and 29.6% of total recovered plant C (Table 9). It made up 87 and 88% of the BGP-C. In oats CdfR was in the same range making up 30.8% of total recovered plant C and representing 80% of the BGP-C. Rhizodeposition of N (NdfR) was higher than CdfR, being 32.1 and 36.4% of total plant N in peas, contributing with 88 and 89% to BGP-N, and a lower value in oats, being 30% of total plant N, contributing 78% to BGP-N (Table 10). Beside the proportion of CdfR being alike in peas and oats, the amounts of CdfR were also in the same range and only slightly lower for oats (Table 11). Amounts of NdfR, however, were four times higher for peas in comparison with oats, due to a higher total N uptake. The amounts of grain-C and grain-N in treatment P_P were lower in comparison with treatment P_M, yet the amounts of CdfR, NdfR, BGP-C, BGP-N, and total plant C and N were higher.

Most ^{13}CdfR and ^{15}NdfR was present at 0-30 cm soil depth (data not shown) and a high proportion was recovered in rootlet material or other pools which have not been further differentiated (Table 12 and 13). In oats, almost half of the ^{13}CdfR and one third of ^{15}NdfR was present in rootlets, whereas it was only one third and one fourth, respectively, in peas. In contrast, other pools not further differentiated, contained 45 and 46% of the ^{13}CdfR in peas, and therefore almost 10% more than in oats. A marked difference between the two crops was observed for the microbial

Table 11 Total plant C, grain C, below-ground plant C (BGP-C), C derived from rhizodeposition, total plant N, grain N, below-ground plant N (BGP-N), and N derived from rhizodeposition (NdfR) in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.). Peas were labelled at 12 leaves unfolded and harvested at the beginning of pod setting (P_P) and at maturity (P_M). Oats were labelled at the end of elongation growth and harvested at maturity (O_M). Values show means ± standard error of the mean (n=5).

		Total	Grain	BGP	dfR
		(g plant^{-1})			
C	**P_P**	5.03 ± 0.30	0.89 ± 0.04	1.44 ± 0.26	1.26 ± 0.26
	P_M	3.49 ± 0.20	0.92 ± 0.08	1.18 ± 0.11	1.04 ± 0.11
	O_M	2.33 ± 0.30	0.58 ± 0.06	0.97 ± 0.25	0.79 ± 0.24
		(mg plant^{-1})			
N	**P_P**	204.7 ± 13.0	56.9 ± 2.8	75.0 ± 8.3	66.6 ± 8.4
	P_M	160.9 ± 11.1	59.5 ± 5.7	65.9 ± 5.7	59.0 ± 5.8
	O_M	46.3 ± 4.8	18.0 ± 1.7	18.6 ± 3.8	14.6 ± 3.5

^{13}CdfR and ^{15}NdfR. The microbial biomass under oats contained about one third of it, whereas in peas microbial ^{13}CdfR was 10% lower and ^{15}NdfR was only half of that recovered in oats. The lower recovery of microbial ^{15}NdfR in peas in comparison with oats, was reflected by a more than five times higher recovery in the inorganic N pool. This corresponds to a higher amount of inorganic N and a higher part of it derived from rhizodeposition under peas (Table 14). Furthermore, the amounts under peas were also higher in the upper soil layer at 0-30 cm depth and remained on a similar level from pod filling to maturity, as did the percentage of it derived from rhizodeposition. However, the proportion of inorganic NdfR at 30-50 cm increased from treatment P_P to treatment P_M. In contrast to inorganic N, the content of extractable C was three times higher under oats in comparison with peas and on a similar level in both soil layers. The proportion of it derived from rhizodeposition, however, was only 6% at 0-30 cm and no contribution was detectable in the lower soil layer. In peas, a two to three times higher proportion was derived from rhizodeposition.

Table 12 Total ^{13}C derived from rhizodeposition ($^{13}CdfR$), $^{13}CdfR$ in rootlets, microbial $^{13}CdfR$, extractable $^{13}CdfR$, and $^{13}CdfR$ in other pools in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.). Peas were labelled at 12 leaves unfolded and harvested at the beginning of pod setting (P_P) and at maturity (P_M). Oats were labelled at the end of elongation growth and harvested at maturity (O_M). Different letters within a row indicate significant differences between the means (Tukey/Kramer, $p < 0.05$; n=5).

	Total	**Rootlet**	**Microbial**	**Extractable**	**Other pools**
			µg $^{13}CdfR$ plant^{-1}		
P_P	1551 ± 315	429 ± 61	298 ± 57	37 ± 5	786 ± 246
P_M	1488 ± 143	495 ± 86	288 ± 22	25 ± 2	680 ± 89
O_M	1720 ± 501	644 ± 251	468 ± 177	12 ± 3	704 ± 348
			recovery of $^{13}CdfR$ (%)		
P_P	101	32 ab	20 bc	3 c	46 a
P_M	100	33 ab	20 b	2 c	45 a
O_M	115	46 a	31 a	1 b	37 ab

Table 13 Total ^{15}N derived from rhizodeposition ($^{15}NdfR$), $^{15}NdfR$ in rootlets, microbial $^{15}NdfR$, inorganic $^{15}NdfR$, and $^{15}NdfR$ in other pools in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.). Peas were labelled at 12 leaves unfolded and harvested at the beginning of pod setting (P_P) and at maturity (P_M). Oats were labelled at the end of elongation growth and harvested at maturity (O_M). Values show means ± standard error of the mean. Different letters within a row indicate significant differences between the means (Tukey/Kramer, $p < 0.05$; n=5).

	Total	**Rootlet**	**Microbial**	**Inorganic**	**Other pools**
			µg $^{15}NdfR$ plant^{-1}		
P_P	740 ± 105	182 ± 26	115 ± 11	145 ± 41	297 ± 56
P_M	765 ± 113	219 ± 48	119 ± 13	195 ± 15	233 ± 84
O_M	619 ± 192	189 ± 63	154 ± 35	23 ± 7	318 ± 143
			recovery of $^{15}NdfR$ (%)		
P_P	100	26 ab	16 b	19 b	39 a
P_M	108	28 ab	17 b	27 ab	36 a
O_M	108	36 ab	31 ab	4 b	37 a

Table 14 Microbial biomass C (MBC), microbial C derived from rhizodeposition (dfR), microbial biomass N (MBN), microbial NdfR, inorganic N and its proportion dfR in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.). Peas were labelled at 12 leaves unfolded and harvested at the beginning of pod setting (P_P) and at maturity (P_M). Oats were labelled at the end of elongation growth and harvested at maturity (O_M). Values with different letters within a column indicate significant differences of the means. Different letters within a row indicate significant differences between the means (Tukey/Kramer, $p < 0.05$; n=5).

		MBC		**Extractable C**		**MBN**		**Inorganic N**	
		µg g^{-1} soil	dfR (%)	µg g^{-1} soil	dfR (%)	µg g^{-1} soil	dfR (%)	µg g^{-1} soil	dfR (%)
0-30 cm	**P_P**	155 ab	67 a	55 b	18 ab	24 a	17 a	16.2 a	32 ab
	P_M	193 a	37 b	46 b	12 bc	25 a	15 a	17.3 a	34 ab
	O_M	108 cd	59 e	164 a	6 c	14 b	5 b	2.3 bc	9 b
30-50 cm	**P_P**	69 de	15 b	24 b	19 a	8 cd	12 ab	5.9 b	18 b
	P_M	119 bc	23 b	26 b	13 ab	12 bc	5 b	2.3 bc	63 a
	O_M	59 e	n.d.	136 a	n.d.	6 d	n.d.	0.3 c	n.d.

n.d. = not detectable

The microbial biomass was generally higher at 0-30 cm in comparison to 30-50 cm depth and in peas in comparison with oats (Table 14). Microbial biomass C increased from treatment P_P to treatment P_M in both soil layers under peas, yet microbial biomass N increased only at 30-50 cm depth. At the same time, the proportion of microbial CdfR decreased from 67 to 37% at 0-30 cm depth and increased from 15 to 23% at 30–50 cm depth, and was higher under peas than under oats. The proportion of microbial NdfR remained on a similar level at 0-30 cm and slightly decreased at 30-50 cm in the peas treatments, but was also higher than in treatment O_M.

The C-to-N ratio of the rhizodeposits was neither significantly different between the treatments, nor between the soil layers and was in the same range as the C-to-N ra-

tio of the roots (Table 15). However, like the latter, the C-to-N ratio of the rhizodeposits was more than two times higher under oats and slightly higher at 30-50 cm

Table 15 C-to-N ratio and the ^{13}C-to-^{15}N ratio of roots, rhizodeposition, and the microbial biomass in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.). Peas were labelled at 12 leaves unfolded and harvested at the beginning of pod setting (P_P) and at maturity (P_M). Oats were labelled at the end of elongation growth and harvested at maturity (O_M). Values with different letters within a row indicate significant differences of the means. Different letters within a row indicate significant differences between the means (Tukey/Kramer, $p < 0.05$; n=5).

		Root		Rhizodeposition		Microbial biomass	
		C-to-N	**^{13}C-to-^{15}N**	**C-to-N**	**^{13}C-to-^{15}N**	**C-to-N**	**^{13}C-to-^{15}N**
0-30 cm	**P_P**	21.4 a	2.4 a	18.0 a	2.0 a	6.4 a	2.7 a
	P_M	20.6 a	2.4 a	17.3 a	2.1 a	7.6 ab	2.2 a
	O_M	45.4 b	4.5 b	41.9 a	3.1 a	7.8 abc	2.6 a
30-50 cm	**P_P**	21.4 a	2.4 a	29.9 a	3.3 a	8.5 abc	1.6 a
	P_M	20.6 a	2.4 a	18.7 a	1.8 a	10.2 c	4.8 a
	O_M	45.4 b	4.5 b	62.1 a	5.6 a	9.9 bc	n.d.

n.d. = not detectable

depth. The C-to-N ratio of the microbial biomass varied from 6.4 to 10.2 and was not significantly different between the treatments, but also revealed slightly higher values at 30-50 cm depth. The ratio of ^{13}C and ^{15}N was in the same range in roots, rhizodeposition, and the microbial biomass for peas. In oats, this ratio was slightly higher in roots and rhizodeposition.

6.4 Discussion

Recovery of ^{13}C and ^{15}N

The relatively low recovery of applied ^{13}C of less than 55% in our study can be partly attributed to loss of assimilated ^{13}C as CO_2 by shoot and root respiration. Additionally, root derived ^{13}C is metabolised by the soil microorganisms and partly released as CO_2, which can be one third of the C transferred below-ground (Kuzyakov and Domanski, 2000) or up to 12% of total assimilated plant C (Nguyen, 2003). Recovery of ^{15}N in treatment P_M and treatment O_M was relatively low, in comparison with pot experiments under controlled conditions using the same methodology, and reaching recoveries of above 80% (Russell and Fillery, 1996a, 1996b; Mayer et al., 2003). The loss of ^{13}C and ^{15}N is partly a consequence of the present experimental conditions in the field, which might have caused losses by unrecovered shed leaves and by experimental errors in the mass balance between the recovered ^{13}C and ^{15}N in the various plant parts and the added amounts. In addition to that, minor losses of ^{15}N from shoots and particularly leaves may have occurred after hydrolysis of urea in the plant and subsequent volatilisation of NH_3 (Zebarth et al., 1991), or from the soil as N_2 and N_2O after denitrification of root derived compounds.

Furthermore, enzymatic decomposition of urea and glucose by microorganisms in the wick system, probably contributed to the loss of the applied isotopes. This is an explanation for the lower recovery of applied ^{13}C and ^{15}N in the treatment P_M, where the solution remained in the system for a longer period of time, compared to treatment P_P. The applied mixture of glucose and urea in the present investigation served as energy and nutrient source for microbes, which might have entered the sterile system during handling in the field and through leaks. However, the magnitude of these amounts was not investigated in the present study. As a consequence, short-duration feeding is advantageous to longer-term feeding under field conditions, where the feeding system is more likely be exposed to the weather conditions (Khan et al., 2002b).

Suitability of the wick method for in situ labelling of rhizodeposits

Using the wick method for in situ stem feeding of pea and oat plants with a ^{13}C-glucose-^{15}N-urea solution resulted in a high enrichment of above-ground biomass with ^{13}C and ^{15}N, and was sufficient to trace the label in the soil including the soil microbial biomass and the inorganic N pool (Table 12 and 13). The different plant parts were labelled heterogeneously, with the BGP having a lower enrichment in comparison with stem, leaves, and fruit (Table 10), which has also been observed earlier for ^{15}N labelled annual plants (Russell and Fillery, 1996a, 1996b; Mayer et al., 2003; Yasmin et al., 2006). Nevertheless, a significant proportion of assimilates was transferred below-ground even during the reproductive growth stage, indicated by the increase in ^{13}C and ^{15}N from treatment Pp to treatment PM (Table 9 and 10). The described labelling procedure therefore provides a useful tool for labelling BGP and rhizodeposits in situ simultaneously with ^{13}C and ^{15}N.

One prerequisite for the estimation of the rhizodeposition is homogeneous enrichment of roots and rhizodeposits. Pulse labelling, as in the present study, might result in a preferential enrichment of the labile and young pools and to a low enrichment of structural root components (Meharg, 1994). Labelled rhizodeposits might thus be dominated by recently assimilated compounds such as exudates, detached apical cells, sloughed root caps and their associated polysaccharides (Meharg, 1994). This would ultimately result in an overestimation of the rhizodeposition, when the enrichment of roots is lower than the enrichment of rhizodeposits. The magnitude of this error cannot be estimated in situ yet but can be counteracted by using multiple short pulses or a continuous labelling approach.

C and N derived from rhizodeposition

Rhizodeposition of C as a percentage of total recovered plant C in the present study (Table 9) was higher than results for cereals and grasses derived from pot experiments (Helal and Sauerbeck, 1984; Kuzyakov and Domanski, 2000; Nguyen, 2003; Kuzyakov and Schneckenberger, 2004), and made up a two to four times higher proportion of the BGP-C (Kuzyakov and Domanski, 2000; Nguyen, 2003). Fur-

thermore, the BGP-C was about one third of total plant C, increasing with plant growth in peas, and is therefore slightly higher than the average estimate in cereals of 20 to 30% of total plant C (Kuzyakov and Schneckenberger, 2004). Rhizodeposition of N as a percentage of total plant N in oats (Table 10) was in the upper range of previous results from pot experiments with wheat (Janzen and Bruinsma, 1989; Janzen, 1990). For NdfR of peas, results from pot experiments were much lower than in the present study, ranging from 4% of total plant N at flowering and 7% to more than 10% at maturity (Sawatsky, 1991; Jensen, 1996; Mayer, 2003). Additionally, BGP-N of peas as a percentage of total plant N was two to three times higher than the 15% estimated in a pot experiment (Mayer et al., 2003). Rhizodeposition contributed 78% to BGP-N in oats at maturity and 89% in peas, being in the range of 82% reported for peas in a pot experiment using the same methodology (Mayer et al., 2003) and 71% for barley (Jensen, 1996). These values correspond well to the finding, that only 30% of the BGP is recovered as intact roots (Khan et al., 2002a). Much lower values for NdfR, being 15 to 48% of BGP-N, were also reported for peas from pot experiments (Sawatsky and Soper, 1991; Jensen, 1996), indicating that the experimental conditions and methodological differences strongly influence the estimations.

These results show that rhizodeposition of C and N in the present investigation using soil columns under field conditions was higher than in pot experiments under controlled conditions and contributed more to BGP. One reason is the altered root-to-shoot ratio under field conditions, which is shifted in favour of roots in comparison with pot experiments (Mayer et al., 2003). This higher root-to-shoot ratio contributed to the higher amounts of rhizodeposition estimated in the present study. Another reason might be a faster senescence and decay of roots caused by pronounced changes in temperature and soil moisture during plant growth in the restricted rooting volume of the columns used. A methodological reason for the high percentage of C and N released by roots might be incomplete recovery of root fragments, resulting in an overestimation of CdfR and NdfR. However, this error seems to be small, especially when comparing our results of ^{15}N-recovery (Table 13) in rootlets (20 to 31% of ^{15}NdfR) with the value reported by Mayer et al. (2003)

(31% of ^{15}NdfR), using the same methodology. The main reason for the obtained high values of C and N rhizodeposition might be heterogeneous enrichment of roots and rhizodeposits as discussed above.

Nevertheless, rhizodeposition under field conditions might be higher than previously thought, these estimates give a more realistic picture of the amounts of rhizodeposition than those values from pot experiments under controlled conditions. Future investigations should therefore focus on investigating the influence of various environmental factors, such as soil type or change in temperature and moisture, on the amounts of rhizodeposition in different crops. Consequently, total plant C and N balances have to be reassessed. This is especially true for the assumption that grain legumes contain two thirds of total plant N in the grain and thus leave only a minor part of total plant N in the plant residues. Furthermore, N_2 fixation rates by legumes under field conditions have to be reassessed, taking higher rhizodeposition into account.

Turnover of rhizodeposits

In the present study the soil microbial biomass contained 20% of the ^{13}CdfR and 17% of the ^{15}NdfR in peas (Table 12 and 13). Similar to that, Mayer et al. (2003) recovered 18% of the ^{15}NdfR in the microbial biomass using a similar methodology in a pot experiment. Under oats 31% of the ^{13}CdfR and ^{15}NdfR was recovered in the soil microbial biomass. This supports the assumption that a large part of the rhizodeposits are highly labile (Janzen, 1990) and are therefore rapidly incorporated into the microbial biomass (McNeill et al., 1997) and its metabolites, and are also partly lost as CO_2. Additionally, most of the N derived from decaying roots is mainly amino acids, polypeptides, and proteins, which are highly labile and decompose very rapidly. However, decomposition kinetics of different root derived compounds vary (Meharg, 1994), depending on plant species and growth stage. In the present investigation, the inorganic N content at maturity and the proportion derived from rhizodeposition was much higher under peas in comparison with oats (Table 14). This indicates a faster turnover of pea rhizodeposits in comparison with oats. The average C-to-N ratio of roots and rhizodeposits was two times higher in oats

than in peas (Table 15), reflecting the lower availability of oat rhizodeposits to the soil microorganisms, indicated by a lower microbial biomass and a lower proportion of it derived from rhizodeposition (Table 14).

Between pod setting and maturity of peas, neither the amount of inorganic N at 0-30 cm soil depth, nor the percentage of it derived from rhizodeposition significantly increased. However, at 30-50 cm soil depth, where amounts of inorganic N were much lower, the percentage of inorganic N derived from rhizodeposition increased significantly during this time. This might reflect an increasing turnover of rhizodeposits and decaying roots in the lower soil layer with maturation of plants, which is supported by a significant increase of the soil microbial biomass and a slight increase of the proportion of the microbial CdfR (Table 14). At 0-30 cm soil depth, on the other hand, the proportion of the microbial CdfR decreased between pod setting and maturity in peas, even though the microbial biomass C content increased. The lower proportion of microbial CdfR at maturity might reflect a decreasing availability of rhizodeposits with plant age. As the plant matures, the senescence of roots increases, and structural root components contribute more strongly to the rhizodeposition as root exudates. This would ultimately lead to the rhizodeposition having a lower availability to soil microorganisms when the plant matures. This, however, was not reflected by a change in the C-to-N ratio of the rhizodeposition, which was higher at 30-50 cm soil depth but did not significantly change from pod setting to maturity (Table 15).

6.5 Conclusions

Peas and oats can be sufficiently labelled with ^{13}C and ^{15}N *in situ* under field conditions by applying glucose-urea mixtures using the wick method. The label can be traced in the soil and in different soil pools such as the soil microbial biomass.

The findings of the present study under field conditions, show that high inputs of N through rhizodeposition and the continuous turnover of pea roots and rhizodeposits, which are an easily available substrate for soil microorganisms, contribute to a great extent to the positive crop rotation effect of this legume on N-availability for subse-

quent crops. This process leaves high amounts of inorganic N in the soil. Rhizodeposition of oats on the other hand, has a lower availability to soil microorganisms, as indicated by a higher C-to-N ratio and a lower proportion of the microbial biomass derived from it.

Acknowledgements

We acknowledge the kind help of Gabi Dormann, Christian Maack, Manuel Renneberg, Sarina Weber, Judith Isele, Markus Peters, and Eberhardt Kölsch. We further thank Mick Locke for correcting our English. This study was financed by the German Research Foundation (MU1711/3-1 and JO362/6-1).

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7 Availability of rhizodeposition to soil microorganisms differs between peas and oats

Florian Wichern [1*], Jochen Mayer [2], Rainer Georg Joergensen [1], and Torsten Müller [3]

Soil Biology & Biochemistry, submitted (2007)

[1] *Department of Soil Biology and Plant Nutrition, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany*

[2] *Swiss Federal Research Station for Agroecology and Agriculture, Zürich, Switzerland*

[3] *Institute of Plant Nutrition, University of Hohenheim (330), Germany*

Abstract

Nutrient mobilisation in the rhizosphere is driven by soil microorganisms and controlled by the release of available C compounds from roots. It is not known how the quality of release influences this process in situ. Therefore, the present study was conducted to investigate the amount and turnover of rhizodeposition of C and N in peas (Pisum sativum L.) and oats (Avena sativa L.) at different growth stages. Plants were grown in soil columns placed in a raised bed under outdoor conditions and simultaneously pulse labelled in situ with a 13C-glucose-15N-urea solution using a stem feeding method. After harvest, 13C and 15N was recovered in plant parts and soil pools, including the microbial biomass. Net rhizodeposition of C and N as a percentage of total plant C and N, was higher in peas compared to oats. Moreover, the C-to-N ratio of the rhizodeposits was lower in peas and a higher proportion of the microbial biomass and inorganic N derived from rhizodeposition. Evidence suggests a positive plant-soil feedback shaping nutrient mobilisation. This process

[*] Corresponding author: phone +49-5542-981664, fax +49-5542-981596, e-mail fwichern@uni-kassel.de

is driven by the C and N supply of roots which has a higher availability in peas in comparison with oats.

Keywords: ^{13}C; Below-ground plant biomass; Feedback; ^{15}N; N cycling; Rhizodeposits; Soil microbial biomass; Stem feeding method

7.1 Introduction

In addition to mineral nutrient uptake, plant roots release a variety of different chemical compounds, often referred to as rhizodeposition, which consist of low and high molecular weight root exudates, mucilage, sloughed cells and tissue, cell lysates, root debris and decomposed root material (Marschner, 1995; Uren, 2001; Nguyen, 2003; Gregory, 2006). The composition of root exudates varies between plant species, variety and age, as well as the abiotic and biotic factors influencing root development (Dakora and Phillips, 2002). However, it was estimated, that soluble root exudates account for only 1 to 10% of C derived from rhizodeposition (CdfR) with the majority of it being simple sugars (Paterson, 2003; Jones et al., 2004). Only a small proportion of the root exudates directly mobilises nutrients, whereas other exudates, such as sugars, act as energy source for microorganisms and therefore indirectly influence plant nutrient availability, in particular in low fertility soils (Paterson, 2003; Jones et al., 2004). Besides the released exudates, the remaining 90 to 99% of the CdfR also act as substrate for soil microorganisms. Thereby, rhizodeposition fuels the turnover processes in the rhizosphere and strongly influences nutrient availability by direct and indirect means already during plant growth. This concept suggests a positive plant-soil feedback as proposed by Chapman et al. (2006). The indirect nutrient mobilisation effects are probably more important than the direct ones (Merbach et al., 1999). Hence, the quantification of rhizodeposition and its turnover, especially of C and N, is crucial in particular for plant nutrition in low input systems and in mixed cropping systems, which are gaining importance for biomass and biofuel production.

Several authors observed, that amounts of CdfR were highest at fast and early vegetative growth (Sauerbeck and Johnen, 1976; Yevdokimov et al., 2006) and that nitrogen derived from rhizodeposition (NdfR) was highest between ear emergence and grain filling in wheat (*Triticum aestivum* L.) (Rroço and Mengel, 2000). In the reproductive growth phase, nitrogen is taken up and relocated into sinks, particularly into grains (Götz and Herzog, 2000; Rroço and Mengel, 2000). However, it has been observed, that in this phase increasing amounts of readily mineralisable N-compounds were emitted (Ofosu-Budu et al., 1990). The species-specific differences of root-derived compounds was highlighted by Rovira (1956), who showed that amounts of root exudates in peas were higher than in oats. At the same time, cell debris of peas, such as sloughed root cells, was twice the amount of oats and increased stronger during plant growth than the dry weight of the root exudates. Additionally, peas exuded a higher number of amino acids (22) in comparison with oats (14), with glycine only found in oats. The amounts and composition of sugars exuded by peas and oats on the other hand, were similar and dominated by glucose and fructose (Rovira, 1956).

As nitrogen is often a limiting factor for plant and microbial nutrition in the rhizosphere (Jones et al., 2004), it can be assumed that nitrogen rhizodeposits quickly become immobilized by rhizosphere microorganisms (Ehrenfeld et al., 2005). The subsequent remineralisation of this immobilised N depends on the quality of the rhizodeposits and on the microbial activity, which is driven by the availability of C, e.g. from rhizodeposition (Knops, et al., 2002; Paterson, 2003). Thus, CdfR and NdfR have to be investigated simultaneously to explore their interactions and turnover during plant growth. However, until now only little is known about the quantities of C, and in particular N, released from intact plant roots into soil *in situ* at different growth stages, especially under outdoor conditions. In addition, a simultaneous investigation of CdfR and NdfR is lacking.

Isotope tracer techniques allow the quantification of root-derived C and N in the soil. The wick method was developed for ^{15}N-labelling of grain legumes and resulted in homogenously labelled plant material with a high recovery of the applied ^{15}N (Russell and Fillery, 1996a; Mayer et al., 2003). Furthermore, plants showed a

similar labelling pattern in different plant parts, as was the case for the split-root technique (Hertenberger and Wanek, 2004), where ^{15}N-uptake took place via the roots. Enrichment of plant material with ^{13}C is typically done using an airtight chamber and applying $^{13}CO_2$. This method is laborious and expensive when applied under outdoor conditions. However, it is expected, that rhizodeposition under outdoor conditions is higher than in pot experiments, as in the latter the root-to-shoot ratio is shifted in favour of shoot biomass (Mayer et al., 2003). This led us to the assumption that the wick method provides a suitable tool to pulse label plants with ^{13}C and ^{15}N without a strong disturbance of the plants physiology. Meharg (1994) stated, that the investigation of rhizodeposition at different plant development stages, such as the reproductive growth phase, might more suitably be addressed by using a pulse labelling approach.

Therefore, we conducted an experiment under outdoor conditions in a raised bed, where pea (*Pisum sativum* L.) and oat (*Avena sativa* L.) plants were pulse labelled *in situ* with ^{13}C and ^{15}N simultaneously, to investigate (I) the amount of C and N rhizodeposition at different growth stages, (II) the species-specific transfer of C and N derived from rhizodeposition into different labile soil pools, and (III) the turnover of C and N rhizodeposits.

7.2 Materials and methods

Study site

The 80 day experiment took place in a raised bed at the University of Kassel, Witzenhausen, Germany (51° 34’ N, 9° 85’ W, 200 m asl). Total rainfall during the investigation period was 74.5 mm with a daily maximum of 11.2 mm. Air temperature during the investigation ranged from 2.8 to 33.2 °C with an average temperature of 16.6 °C. Soil temperature ranged from 7.2 to 26.6 °C with the mean being 16.3 °C and the median 16.7 °C. Soil temperature at the edge of the raised bed was on average approximately 0.5 °C higher in comparison with the middle. On very hot days the soil at the edge of the raised bed was slightly watered to reduce strong temperature increase.

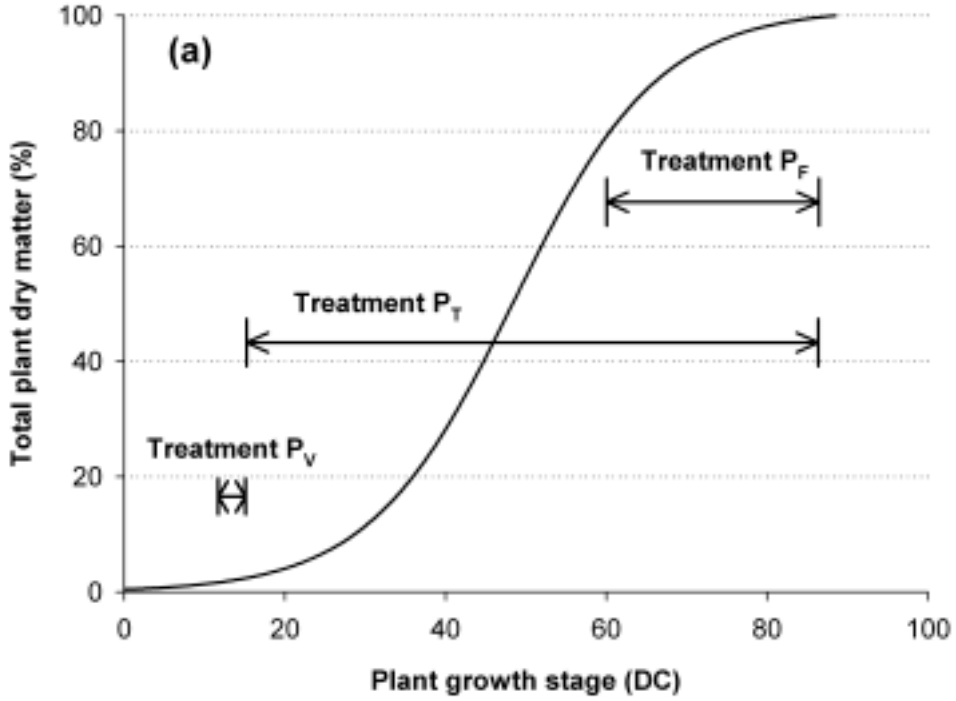
The soil used in the experiment was collected in April 2005 from the research station of the University of Kassel, which is situated at Frankenhausen, Northern Hesse, Germany (51° 24' N, 9° 25' W, 230 m asl). The long term average annual rainfall here was 698 mm. Mean annual temperature at the site was 8.5 °C. The fields have been cultivated according to organic farming practices for 6 years. The preceding crop on the experimental field was carrot (*Daucus carota* L. ssp. sativus [Hoffm.] Schübl. et Mart.), and there were no legumes on the experimental site for two years. The clayey silt soil (16-23% clay, 75-82% silt, 2% sand) was classified as Haplic Luvisol on loess, with a bulk density of 1.3 g cm^{-3}, and a pH (H_2O) of 7.3 in 0-30 cm and 7.6 in 30-50 cm soil depth. The contents of organic C and total N in the soil were 1.22 and 0.12% at 0-30 cm soil depth and 0.69 and 0.07% at 30-50 cm, respectively. At 0-30 cm soil depth phosphorus content (CAL) was 95 µg g^{-1} soil, potassium content (CAL) was 136 µg g^{-1} soil and magnesium ($CaCl_2$) content was 83 µg g^{-1} soil. Soil microbial biomass C was 270 µg g^{-1} soil and microbial N was 50 µg g^{-1} soil at 0-30 cm soil depth. For soil collection 56 columns (PVC-tubes: 20 cm in diameter and 55 cm long) were forced into the soil, excavated, closed with plastic bags on top, and with a lid at the bottom, and transferred to Witzenhausen for further processing.

Experimental design and labelling

The collected columns were placed into a box of 1.5 x 3.0 x 0.7 m (width x length x height) in a randomised block design with 7 replicates of 4 treatments for peas (*Pisum sativum* L., cv. Santana) and 3 treatments for oats (*Avena sativa* L., cv. Freddy). The space between the columns was filled with soil and compacted, creating the raised bed. On the 29th of April, peas and oats were sown into the box and into the columns in rows, with one row crossing the column and one row between each two columns. After germination, plants in the columns were thinned to 4 plants per column (equivalent to approximately 100 plants m^{-2}). At the edge of the raised bed containing oat plants, plant density was higher for wind protection purposes.

Plants were labelled with a solution of ^{13}C enriched (99atom%) glucose and ^{15}N enriched (99atom%) urea. Concentrations ranged from 0.6 to 8.1% (w/v) for glucose and 0.04 to 0.89% (w/v) for urea, depending on the estimated dry matter increase. For solution transfer into the plant, the wick method was used (Russell and Fillery, 1996a; Mayer et al., 2003). Briefly, a cotton wick was passed through a hole in the stem, which was drilled with a 0.5 mm drill approximately 3 cm above the soil surface. The ends of the wick were passed through a silicone tube and reached into a 2 ml vial with lid, containing the solution. To prevent transpiration losses, the connections at the plant stem and at the lid were sealed with plasticine (Teroson, Henkel, Düsseldorf, Germany). All vials and materials used in the system were steam sterilised for 20 min at 121 °C. Solution was produced using sterile deionised water and filtrated (< 0.2µm) before application.

For peas, three treatments were labelled using the wick method and one treatment was the respective unlabelled control treatment. Treatment $\mathbf{P_V}$ (**P**eas labelled at early **V**egetative growth) focussed on the very early growth phase of peas. For treatment $\mathbf{P_T}$ (**P**eas labelled **T**wice during plant growth) the same growth period was chosen as previously investigated by Mayer et al. (2003) in a pot experiment. Treatment $\mathbf{P_F}$ (**P**eas labelled at **F**lowering) looked at the reproductive growth phase. Plants of treatment P_V were labelled (Figure 9a) at 3 leaves unfolded (23rd of May, DC 13; DC = decimal code of plant development) by applying 1 ml of a glucose-urea solution. After the solution has been taken up, 1 ml of sterile deionised water was applied to the empty vials (27th of May) to assure uptake of the remaining glucose and urea. Treatment P_T was labelled in two dispensations with 1 ml of the solution applied at 5 leaves unfolded (1st of June, DC 15) and 1 ml at 8 leaves unfolded (13th of June, DC 18). The treatment P_F was labelled at the beginning of flowering with 12 leaves unfolded (1st of July, DC 59). After solution uptake by the plants, 0.5 ml of sterile distilled deionised water was applied to the empty vials (4th of July). At 5 leaves unfolded (31st of May, DC 15), treatment P_V and three columns of the control treatment were harvested. Harvest of the treatments P_T and P_F with the remaining 4 control columns took place at maturity (18th of July, DC 85). For oats, two



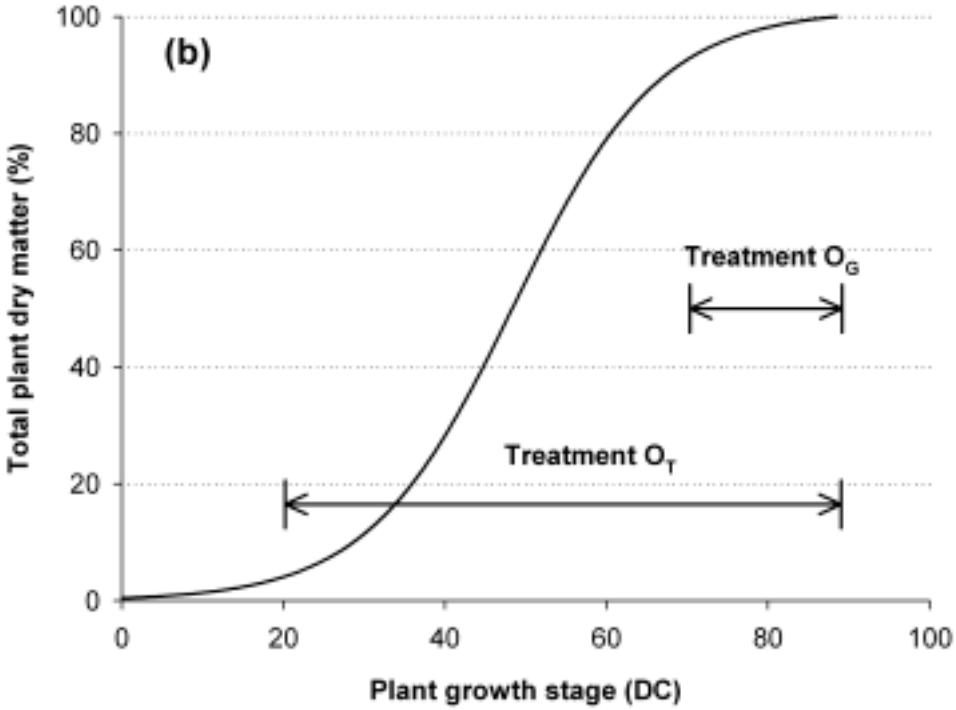


Figure 9 Periods of plant growth investigated in peas (*Pisum sativum* L.) (Fig. 9a) and in oats (*Avena sativa* L.) (Fig. 9b). The growth curves show the typical development of plant dry matter and C and N uptake during plant growth which has been determined experimentally (data not shown).

treatments were labelled (**O_T** = **O**ats labelled **T**wice during plant growth and **O_G** = **O**ats labelled at **G**rain development) (Figure 9b) and one left as unlabelled control. In treatment O_T, the growth period from tillering until maturity was investigated, with plants labelled at the beginning of tillering (15th of June, DC 21). Treatment O_G was labelled at grain development (5th of July, DC 70) to focus on the reproductive growth phase. After solution uptake by the plants, 0.5 ml of sterile distilled deionised water was applied to the empty vials (4th and 8th of July). All oats treatments (O_T, O_G and CON_O) were harvested at approximate physiological maturity (28th of July, DC 86). After labelling, a mesh (1 mm) was put around the plants to prevent soil contamination from shed leaves which were collected regularly. To prevent isotope drift the columns with the labelled plants were protected with a plastic bag covering the plant to a height of approximately 2/3 of the plant. After harvesting the plants, wicks and vials were extracted with 200 ml 0.05 M K_2SO_4 to determine the remaining amounts of organic C and total N and their isotopic composition, as described for the soil extracts (see below).

Plant and soil sampling

At harvest, vials and wicks were carefully removed and plants were cut off directly above the soil surface. After harvest, plants were immediately separated into stem, leaves, pods, and grain and dried at 60 °C for at least 72 hours. The dry plant material was ground using a ball mill and analysed for total C, total N, and the isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ for every fraction.

Soil columns were excavated, closed with a plastic bag on top, transferred to the laboratory, and stored at 5 °C until further processing. The soil was then separated into two soil layers (0-30 cm and 30-50 cm). All visible roots in each layer were collected by hand, and the soil was sieved (< 2 mm) for further analyses. Collected roots were washed with distilled water, dried at 60 °C for at least 72 hours to determine dry weight, ground to a fine powder using a ball mill, and analysed for total C, total N, and the isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$. Additionally, a 200 g sub-sample of the sieved soil was washed over a 200 µm sieve to determine the amount of remaining rootlets. A sub-sample of the root and rootlet material was burned to ash at

550 °C. The dry matter of the rootlets was corrected with the ash content of the roots. Sub-samples of dried root free soil material were homogenised in a ball mill and analysed for total C and total N.

Analyses

Total C and total N in plant and soil samples were determined gas-chromatographically after combustion using a Carlo Vario Max CN analyser (elementar, Hanau, Germany). The isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were determined using isotope ratio mass spectrometry (Delta plus IRMS 251, Finnigan Mat, Bremen, Germany) after combustion using a Carlo Erba NA 1500 gas chromatograph (Carlo Erba Instruments, Milano, Italy). Microbial biomass C and N in the soil were estimated by chloroform-fumigation-extraction (Brookes et al., 1985; Vance et al., 1987), including a pre-extraction step to remove living roots (Mueller et al., 1992; Mayer et al., 2003).

Briefly, one portion of 100 g (on an oven-dry basis) soil was extracted with 400 ml 0.05 M K_2SO_4 by shaking it on a horizontal shaker at 200 rev min^{-1} for 30 min. The soil suspension was completely transferred into a 600 ml beaker with an additional 50 ml 0.05 M K_2SO_4, and all visible root fragments were taken out manually. The soil suspension was transferred into a centrifuge beaker with another 50 ml 0.05 M K_2SO_4 and centrifuged for 10 min at 500 *g*. An aliquot of the supernatant (pre-extract) was collected for measuring organic C and total N in the extract. One portion of approximately 25 g (on an oven-dry basis) of the pre-extracted wet soil was immediately fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$. Following fumigant removal, the sample was extracted with 100 ml 0.05 M K_2SO_4 by shaking for 30 min on a horizontal shaker at 200 rev min^{-1} and centrifuged for 10 min at 500 *g*. The supernatant was collected for determination of organic C and total N. The non-fumigated 25-g portion was extracted similarly at the time when fumigation commenced.

Organic C in all extracts was measured as CO_2 by infrared absorption after combustion at 850 °C using a Dimatoc 100 automatic analyser (Dimatec, Essen, Germany).

Microbial biomass C was calculated as E_C / k_{EC}, where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al., 1990). Total N in the extracts was measured by chemoluminescence detection after combustion using a Dima-N (Dimatec, Essen, Germany) detector. Microbial biomass N was calculated as E_N / k_{EC}, where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996). In the K_2SO_4 extracts of all non-fumigated samples and in all pre-extracts, NO_3^--N was determined using segmented flow analysis (Alliance Instruments, Friedrichsdorf, Germany). For measuring the isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in the extracts, a sub-sample of the K_2SO_4-extract was freeze-dried until constant weight. The isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ of the dry material were determined using isotope ratio mass spectrometry.

Calculations and statistical analysis

The percentage of rhizodeposition-derived total soil C (CdfR), total soil N (NdfR), K_2SO_4 extractable C (extractable CdfR) inorganic N (inorganic NdfR), microbial C (microbial CdfR), and N (microbial NdfR) was calculated using the equation of Janzen and Bruinsma (1989):

$$\%\text{CdfR} = \frac{atom\%\ ^{13}C\ excess_{\ soil}}{atom\%\ ^{13}C\ excess_{\ root}} \times 100 \quad \text{and} \quad \%\text{NdfR} = \frac{atom\%\ ^{15}N\ excess_{\ soil}}{atom\%\ ^{15}N\ excess_{\ root}} \times 100$$

Amounts of CdfR and NdfR in the various pools were obtained by multiplying the total amounts of C and N in the pool by the respective %CdfR and %NdfR values. The enrichment of microbial biomass C and N with ^{13}C and ^{15}N respectively was calculated according to Mayer et al. (2003):

$$\text{atom\% microbial } ^{13}\text{Cexcess} = \frac{(C_{fum} \times \%^{13}\text{Cexcess}_{fum} - C_{nonfum} \times \%^{13}\text{Cexcess}_{nonfum})}{(C_{fum} - C_{nonfum})} \times 100,$$

$$\text{atom\% microbial } ^{15}\text{Nexcess} = \frac{(N_{fum} \times \%^{15}\text{Nexcess}_{fum} - N_{nonfum} \times \%^{15}\text{Nexcess}_{nonfum})}{(N_{fum} - N_{nonfum})} \times 100,$$

where 'fum' is the fumigated soil and 'nonfum' is the non-fumigated soil. The ^{13}C and ^{15}N atom%excess values were calculated by subtracting the ^{13}C and ^{15}N atom% values of the non-labelled control treatments from measured atom% value in the labelled treatments. This was done separately for every investigated plant and soil fraction. For these calculations, the following assumptions were made: (i) rhizodeposition has the same ^{13}C and ^{15}N enrichment as the recovered roots and (ii) rootlets recovered by wet sieving have the same enrichment as roots.

Results in the tables and figures are presented on an oven-dry basis (about 24 h at 105 °C for soil samples and about 72 h at 60 °C for plant parts). Means of microbial biomass C and N, its ratio (microbial biomass C-to-N), extractable C, inorganic N, and the percentage of each fraction derived from rhizodeposition (dfR), were compared between each treatment and depth using a one-way analysis of variance (ANOVA). Student's t-test was computed to separate means by pairwise comparison. All statistical analyses were performed using JMP 5.1 (SAS Institute Inc., Cary, USA).

7.3 Results

Isotope recovery and distribution

About 38 to 74% of the applied ^{13}C was recovered from soil and plant material (Table 16). These values do not include $^{13}CO_2$ lost from plant and soil, by foliage, root and microbial respiration, which cannot be measured separately in the system used here. Where pea and oat plants were labelled twice and for a long growth period (P_T, O_T), recovery from soil and plant material and total recovered ^{13}C was lowest for each plant species. Total recovery from plant, soil and the wick system was very high for treatment P_V (81%) and the oats treatments (82 and 98%), with 23 and 26% of the applied ^{13}C recovered in the wick system. Generally, loss of ^{13}C was higher in the pea treatments than in oats treatments. Recovery of ^{15}N from plant and soil material was very high, ranging from 73% in treatment O_T to 99% in the treatment P_V. In the pea treatments, only 2 to 9% of the applied ^{15}N were extractable in the wick system, compared to 23 and 29% in the oats treatments. Total ^{15}N recovery was 87-

108%. The highest loss of ^{15}N occurred in the treatment P_T, where plants were labelled twice during the investigation period.

Table 16 Recovery of ^{13}C and ^{15}N (in % of applied) in the plant-soil system, the wick system, in total and loss in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.).

		Isotope recovery (% of applied)			
		in plant and soil	**in the wick system**	**In total**	**loss**
^{13}C	**P_V**	58 ± 4	23 ± 8	81 ± 7	19 ± 7
	P_T	38 ± 3	2 ± 1	41 ± 3	59 ± 3
	O_T	56 ± 10	26 ± 5	82 ± 12	18 ± 12
	P_F	53 ± 4	3 ± 1	56 ± 3	44 ± 3
	O_G	74 ± 7	23 ± 6	98 ± 12	2 ± 12
^{15}N	**P_V**	99 ± 5	9 ± 3	108 ± 6	-8 ± 6
	P_T	84 ± 7	2 ± 1	87 ± 7	13 ± 7
	O_T	73 ± 9	29 ± 8	102 ± 4	-2 ± 4
	P_F	91 ± 6	2 ± 2	94 ± 6	6 ± 6
	O_G	82 ± 9	23 ± 12	105 ± 4	-5 ± 4

P stands for peas, O means oats. Plants were labelled once at early vegetative growth (subscript V), twice during plant growth (subscript T), or once at flowering (subscript F) or grain filling (subscript G). Values show means ± standard error of the mean (n = 7).

Enrichment of the different plant parts with ^{13}C varied between 0.01 atom%excess in the grain of the treatment O_T and 0.99 atom%excess in the stem of treatment P_V (Table 17). Generally, stem material showed the highest enrichment. Roots usually had the lowest enrichment with ^{13}C and ^{15}N. In the treatments P_V, P_T and O_T, ^{15}N enrichment was highest in the stem, whereas treatments P_F and O_G showed the highest enrichment with ^{15}N in the grain.

Most of the ^{13}C and ^{15}N applied to peas was recovered in the above-ground plant material (Table 18). Between 15.4 and 29.1% of the recovered ^{13}C and 13.4 and 22.8% of the recovered ^{15}N was detected in below-ground plant biomass (BGP) of peas. In treatment P_V, the highest recovery of ^{13}C and ^{15}N was found in the leaves, whereas in treatments P_T and P_F most of the applied ^{13}C was detected in the stem, and most of the applied ^{15}N was recovered from the grain. The ^{13}CdfR represented 81 to 91% of the BGP-^{13}C in peas. A high proportion of it (71 to 87% of the ^{13}CdfR) was present in soil at 0-30 cm depth, whereas only 13 to 29% were present

Table 17 Enrichment of the plant parts with ^{13}C and ^{15}N (in atom % excess) in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.).

		Grain	Pods	Leaves	Stem	Roots
		enrichment in atom%excess				
^{13}C	**P_V**	-	-	0.43 ± 0.04	0.99 ± 0.08	0.12 ± 0.01
	P_T	0.11 ± 0.02	0.11 ± 0.03	0.25 ± 0.05	0.34 ± 0.04	0.19 ± 0.03
	O_T	0.01 ± <0.01	-	-	0.19 ± 0.03	0.07 ± 0.02
	P_F	0.31 ± 0.05	0.58 ± 0.13	0.19 ± 0.03	0.38 ± 0.05	0.14 ± 0.02
	O_G	0.24 ± 0.04	-	-	0.36 ± 0.04	0.16 ± 0.02
^{15}N	**P_V**	-	-	0.66 ± 0.11	0.95 ± 0.20	0.12 ± 0.04
	P_T	1.25 ± 0.31	1.25 ± 0.36	1.29 ± 0.21	1.77 ± 0.39	0.60 ± 0.18
	O_T	1.21 ± 0.46	-	-	1.34 ± 0.56	0.36 ± 0.15
	P_F	1.56 ± 0.34	1.51 ± 0.61	1.12 ± 0.65	1.54 ± 0.68	0.45 ± 0.24
	O_G	2.72 ± 0.75	-	-	2.52 ± 0.46	0.79 ± 0.30

P stands for peas, O means oats. Plants were labelled once at early vegetative growth (subscript V), twice during plant growth (subscript T), or once at flowering (subscript F) or grain filling (subscript G). Values show means ± standard error of the mean (n = 7).

Table 18 Distribution of ^{13}C and ^{15}N recovered in plant and soil, in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.).

		Grain	**Pods**	**Leaves**	**Stem**	**BGP**	***Roots***	***Rhizodeposition***
		% of recovered						
^{13}C	**P_V**	-	-	43.9 ± 2.6	27.0 ± 2.3	29.2 ± 3.4	*2.7 ± 0.3*	*26.5 ± 3.3*
	P_T	10.9 ± 1.9	2.2 ± 0.5	6.5 ± 1.0	56.8 ± 3.4	23.2 ± 5.7	*3.5 ± 0.6*	*19.7 ± 5.7*
	O_T	2.2 ± 0.4	-	-	45.7 ± 4.0	52.2 ± 4.1	*40.6 ± 4.6*	*11.6 ± 3.5*
	P_F	22.1 ± 3.9	8.6 ± 1.8	4.6 ± 0.6	49.3 ± 4.3	15.4 ± 4.2	*2.9 ± 0.5*	*12.5 ± 4.0*
	O_G	21.7 ± 2.2	-	-	34.9 ± 2.9	43.4 ± 3.0	*37.6 ± 2.1*	*5.8 ± 1.5*
^{15}N	**P_V**	-	-	59.4 ± 3.8	17.8 ± 3.2	22.8 ± 1.7	*1.6 ± 0.2*	*21.2 ± 1.8*
	P_T	42.4 ± 4.1	3.2 ± 0.3	6.8 ± 0.7	30.0 ± 2.7	17.6 ± 2.4	*2.3 ± 0.4*	*15.3 ± 2.7*
	O_T	48.6 ± 3.1	-	-	10.7 ± 1.3	40.7 ± 3.6	*28.7 ± 4.1*	*12.0 ± 3.8*
	P_F	46.3 ± 6.8	3.2 ± 0.7	7.1 ± 1.8	30.0 ± 5.2	13.4 ± 3.5	*2.5 ± 0.4*	*10.9 ± 3.5*
	O_G	60.1 ± 2.3	-	-	14.1 ± 1.6	25.5 ± 2.8	*23.5 ± 2.8*	*2.0 ± 0.4*

P stands for peas, O means oats. Plants were labelled once at early vegetative growth (subscript V), twice during plant growth (subscript T), or once at flowering (subscript F) or grain filling (subscript G). Values show means ± standard error of the mean (n = 7).

Table 19 Distribution of total plant C and N recovered in plant and soil, in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.).

		Grain	**Pods**	**Leaves**	**Stem**	**BGP**	***Roots***	***Rhizodeposition***
		% of recovered						
C	**P_V**	-	-	28.3 ± 4.4	7.5 ± 1.2	64.1 ± 5.6	*6.0 ± 0.5*	*58.2 ± 5.6*
	P_T	22.7 ± 3.3	4.9 ± 0.5	6.0 ± 0.7	37.9 ± 3.9	28.5 ± 7.6	*4.0 ± 0.5*	*24.5 ± 7.8*
	O_T	17.3 ± 1.1	-	-	18.7 ± 1.2	63.9 ± 2.3	*48.7 ± 4.1*	*15.2 ± 3.9*
	P_F	20.5 ± 1.9	4.6 ± 0.3	7.2 ± 0.7	38.9 ± 3.1	28.7 ± 4.4	*6.1 ± 0.7*	*22.6 ± 4.9*
	O_G	19.9 ± 1.6	-	-	20.9 ± 2.3	59.2 ± 3.4	*51.4 ± 2.4*	*7.9 ± 2.0*
N	**P_V**	-	-	21.5 ± 2.8	4.2 ± 0.4	74.3 ± 2.7	*3.2 ± 0.6*	*71.1 ± 3.0*
	P_T	39.0 ± 4.8	2.9 ± 0.3	5.9 ± 0.7	18.7 ± 1.6	33.4 ± 5.3	*4.3 ± 0.6*	*29.2 ± 5.7*
	O_T	25.4 ± 2.5	-	-	5.1 ± 0.8	69.5 ± 3.1	*49.2 ± 5.9*	*20.3 ± 6.1*
	P_F	34.5 ± 3.9	2.5 ± 0.4	7.5 ± 0.8	22.9 ± 2.9	32.5 ± 4.6	*7.1 ± 0.9*	*22.9 ± 2.9*
	O_G	35.8 ± 2.8	-	-	9.1 ± 1.4	55.1 ± 4.0	*50.6 ± 4.0*	*4.5 ± 1.1*

P stands for peas, O means oats. Plants were labelled once at early vegetative growth (subscript V), twice during plant growth (subscript T), or once at flowering (subscript F) or grain filling (subscript G). Values show means ± standard error of the mean (n = 7).

Table 20 Proportion of total ^{13}C and ^{15}N derived from rhizodeposition ($^{13}CdfR$, $^{15}NdfR$) recovered as microbial $^{13}CdfR$ and $^{15}NdfR$, extractable $^{13}CdfR$, inorganic $^{15}NdfR$ and $^{13}CdfR$ and $^{15}NdfR$ in other soil pools including rootlets, in peas (*Pisum sativum* L.) and oats (*Avena sativa* L.).

		Percentage of total $^{13}CdfR$ and $^{15}NdfR$ recovered in			
total=100%		**microbial biomass**	**extractable**	**inorganic**	**other pools and rootlets**
$^{13}CdfR$	**P_V**	11	1		88
	P_T	21	2		77
	O_T	14	2		84
	P_F	14	2		72
	O_G	14	3		83
$^{15}NdfR$	**P_V**	1		7	92
	P_T	23		38	39
	O_T	11		27	62
	P_F	17		38	45
	O_G	17		40	43

P stands for peas, O means oats. Plants were labelled once at early vegetative growth (subscript V), twice during plant growth (subscript T), or once at flowering (subscript F) or grain filling (subscript G). Values show means ± standard error of the mean (n = 7).

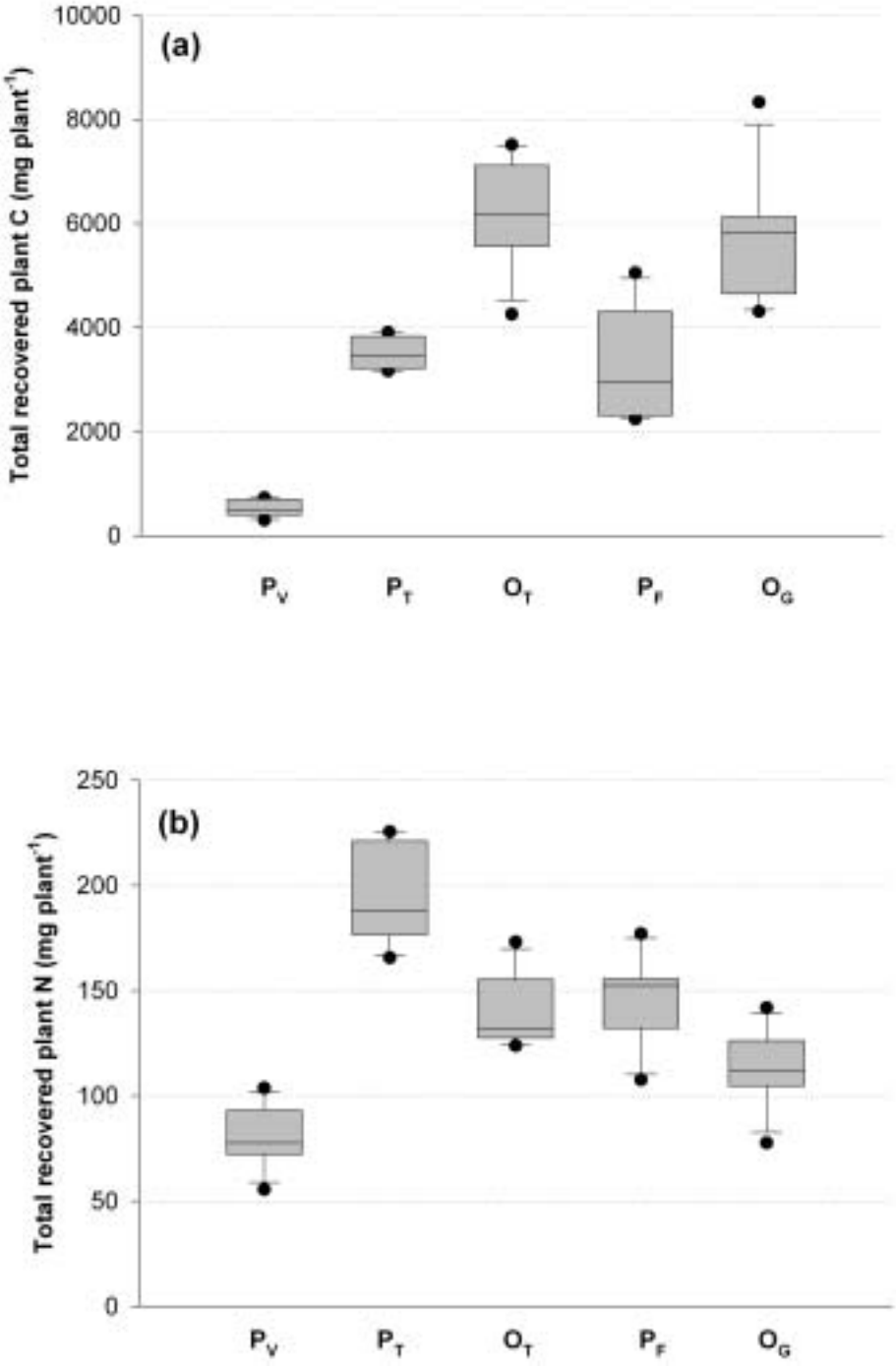


Figure 10 Amounts of total recovered plant C (Fig. 10a) and plant N (Fig. 10b) after harvest of peas (*Pisum sativum* L.) and oats (*Avena sativa* L.). Peas were labelled at early vegetative growth (P_V), twice during plant growth (P_T), and at flowering (P_F). Oats were labelled twice during plant growth (O_T) and once at grain development (O_G). Different letters indicate significant differences between the means (Student's t-test, pairwise comparison, $p < 0.05$, n=7, except P_T and total N in P_F, where n=6).

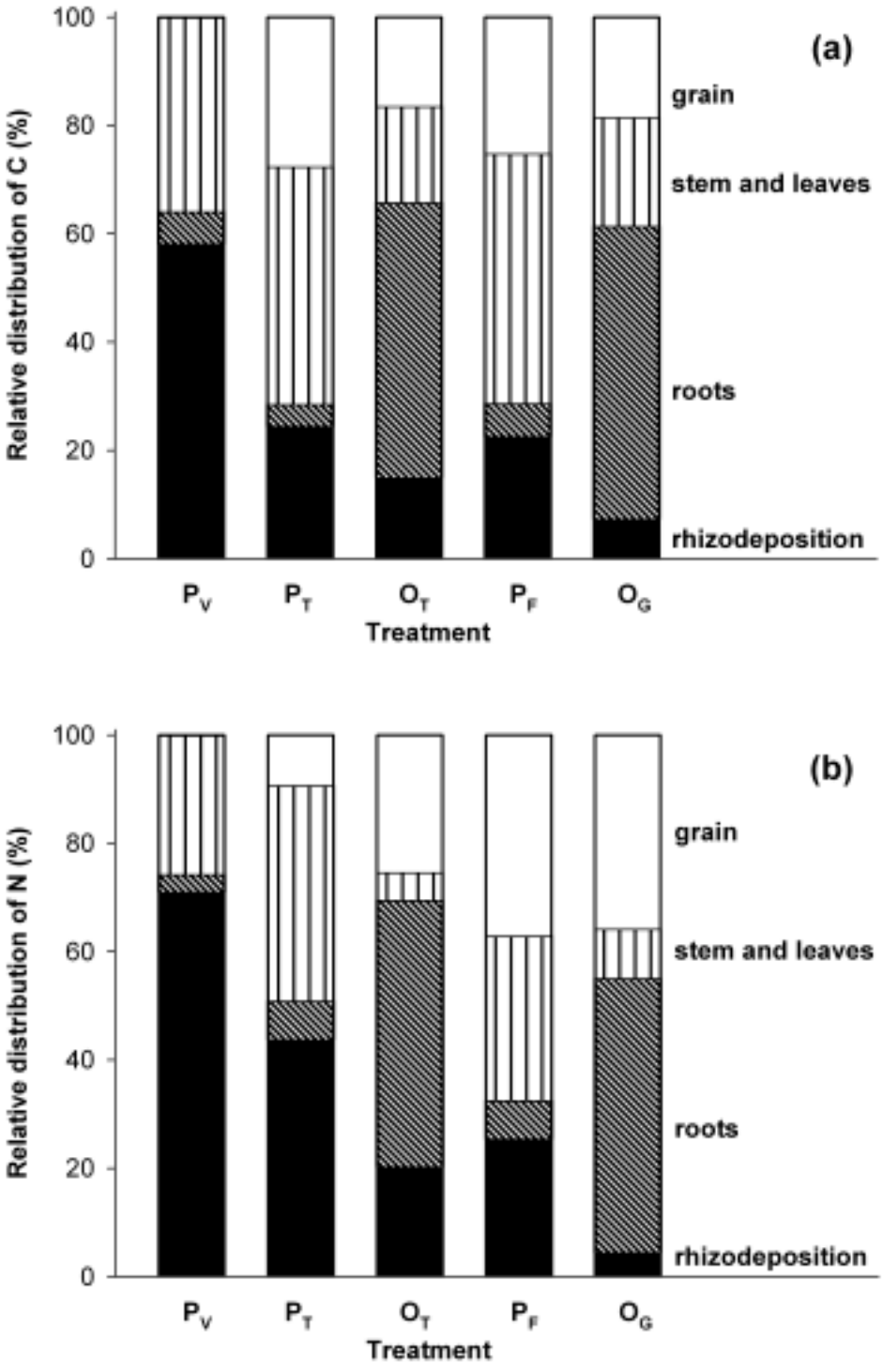


Figure 11 Relative mean distribution of plant C (Fig. 11a) and N (Fig. 11b) in grain, stem and leaves, roots and rhizodeposition after harvest of peas (*Pisum sativum* L.) and oats (*Avena sativa* L.). Peas were labelled at early vegetative growth (P_V), twice during plant growth (P_T), and at flowering (P_F). Oats were labelled twice during plant growth (O_T) and once at grain development (O_G).

in soil at 30-50 cm depth. Amounts of ^{15}N released from pea roots ($^{15}NdfR$) represented 81 to 93% of the BGP-^{15}N. At 0-30 cm soil depth 58, 83, and 79% of the $^{15}NdfR$ was present in the treatments P_V, P_T, and P_F, leaving 42, 17, and 21% of the $^{15}NdfR$ at 30-50 cm soil depth, respectively. In the oats treatments, recovery of ^{15}N was highest in grains (Table 18). However, recovery of ^{13}C was highest in the BGP. Additionally, most of the ^{13}C and ^{15}N recovered in the BGP was present in root material, representing more than 70% of the ^{13}C and ^{15}N in BGP. Similarly to peas, 71 and 75% of the $^{13}CdfR$ and 89 and 69% of the $^{15}NdfR$ in treatment O_T and O_G were detected at 0-30 cm soil depth. The percentages of $^{13}CdfR$ and $^{15}NdfR$ were higher in the treatments P_T and P_F than in treatments O_T and O_G.

Plant uptake and release of C and N

Amounts of total recovered C were higher in oats than in peas (Figure 10a), whereas the amount of total recovered N was highest in treatment P_T (Figure 10b). At early vegetative growth of peas, C and N was predominantly transferred below-ground (Table 19, Figure 11), resulting in an above-ground plant-C to below-ground plant-C ratio (AGP-C to BGP-C) of 0.6 and an AGP-N to BGP-N ratio of 0.3 in treatment P_V. The recovered C did not include CO_2 lost by respiration of roots, microorganisms and leaves. At maturity, however, most of the plant C and N recovered from treatments P_T and P_F was present above-ground, resulting in an AGP-C to BGP-C ratio of 2.5 and an AGP-N to BGP-N ratio of 2.0. Contrary to peas, most plant C and N in the oats treatments was present below-ground, with a ratio of AGP-C-to-BGP-C between 0.6 and 0.7, and a ratio of AGP-N to BGP-N of 0.4 and 0.8. Additionally, most BGP-C and BGP-N in oats was present in roots, with only 24 and 13% of the BGP-C and 29 and 8% of the BGP-N recovered as rhizodeposition in the treatments O_T and O_G. On the other hand, BGP-C and BGP-N in peas was dominated by CdfR and NdfR, which was 70 to 96% of the BGP. In treatment P_V, NdfR was very high at 71.1% of recovered N. In treatment P_T and P_F, NdfR was 29.2 and 25.5% of the recovered N, representing 87 and 78% of the BGP-N. The proportion of rhizodeposition C and N at 0-30 cm and 30-50 cm soil

depth were similar to the proportion of ^{13}CdfR and ^{15}NdfR in the respective soil layer (see above).

Distribution of CdfR and NdfR in the soil

Most of the ^{13}CdfR and ^{15}NdfR was present in other soil pools, which were not differentiated and including the rootlets of the plants (Table 20). Extractable C contained only negligible amounts of ^{13}CdfR, whereas inorganic ^{15}N was 38% of ^{15}NdfR in treatment P_T and P_F, and 27 and 40% in treatment O_T and O_G, respectively. Between 11 and 21% of the ^{13}CdfR and 1 to 23% of the ^{15}NdfR were recovered in the microbial biomass, with a lower proportion in treatment O_T, in comparison with treatment P_T. In treatments P_F and O_G, proportions of ^{13}CdfR and ^{15}NdfR present in microbial biomass were similar. Additionally, microbial biomass C and N were in the same range in the pea and oats treatments at 0-30 cm and at 30-50 cm soil depth (Table 21). However, the proportion of the microbial biomass derived from rhizodeposition was higher in peas than in oats, which was significant for treatment P_T at 0-30 cm soil depth. In treatment P_V, the proportion of microbial biomass C and N derived from rhizodeposition was much smaller than in the other pea treatments, even though amounts of microbial biomass C and N were similar.

Amounts of extractable C and amounts of inorganic N were significantly higher in the treatments P_T and P_F in comparison with the treatments O_T and O_G (Table 21). In treatment P_V, however, the content of extractable C was lower and inorganic N was higher in comparison with all the other treatments. The proportion of extractable CdfR was generally very low, and at 0-30 cm soil depth it was slightly higher in the pea treatments than in the oats treatments. Inorganic NdfR was 12 to 46% of inorganic N and higher in the peas than in the respective oats treatments. However, the lowest values were obtained for treatment P_V, where inorganic NdfR was only 13% at 0-30 cm and 12% at 30-50 cm soil depth. However, when calculating the amounts of inorganic NdfR in the pea treatments, values were higher in P_V (2.1 µg g^{-1} soil at 0-30 cm) than in P_T and P_F (1.1 and 0.4 µg g^{-1} soil).

Table 21 Microbial biomass C (MBC) and N (MBN), extractable C, and inorganic N in µg g^{-1} soil and percentage of it derived from rhizodeposition (%dfR), after harvest of peas (*Pisum sativum* L.) and oats (*Avena sativa* L.).

		MBC		Extractable C		MBN		Inorganic N	
		µg g^{-1} soil	dfR (%)	µg g^{-1} soil	dfR (%)	µg g^{-1} soil	dfR (%)	µg g^{-1} soil	dfR (%)
0-30 cm	$\mathbf{P_V}$	189 a	4 b	31 c	1.0 cde	24 a	2 b	16.2 a	13 cd
	$\mathbf{P_T}$	196 a	52 a	52 a	3.5 ab	25 a	18 a	2.3 c	46 a
	$\mathbf{O_T}$	172 a	19 b	41 b	1.7 cd	23 a	5 b	1.0 cde	36 ab
	$\mathbf{P_F}$	195 a	27 ab	52 a	3.7 a	25 a	8 b	2.1 cd	21 bcd
	$\mathbf{O_G}$	167 a	9 b	39 b	1.7 cde	22 a	1 b	0.9 cde	16 bcd
30-50 cm	$\mathbf{P_V}$	62 b	n.d.	28 c	0.3 e	8 b	n.d.	5.4 b	12 d
	$\mathbf{P_T}$	62 b	13 b	29 c	0.7 de	6 b	8 b	0.4 d	31 abc
	$\mathbf{O_T}$	38 b	2 b	25 c	1.8 cd	4 b	n.d.	0.1 e	n.d.
	$\mathbf{P_F}$	39 b	9 b	39 b	2.2 bc	4 b	5 b	0.5 de	45 a
	$\mathbf{O_G}$	33 b	12 b	27 c	1.2 cde	4 b	n.d.	0.1 e	n.d.

P stands for peas, O means oats. Plants were labelled once at early vegetative growth (subscript V), twice during plant growth (subscript T), or once at flowering (subscript F) or grain filling (subscript G). Values with different letters within a column indicate significant differences between the means (Student's t-test, pairwise comparison, $p < 0.05$, n=7; n.d. = not detectable).

The C-to-N ratio of the rhizodeposition estimated from the total amounts released was higher in oats than in peas, corresponding to a higher C-to-N and ^{13}C-to-^{15}N ratio in the roots (Table 22). Additionally, the ^{13}C-to-^{15}N ratio in the soil and in the microbial biomass was higher in treatment O_T at 30-50 cm soil depth and in treatment O_G at both soil depths, in comparison with the respective pea treatments.

Table 22 The C-to-N ratio and the ^{13}Cexcess-to-^{15}Nexcess ratio in roots, rhizodeposition, soil, and the microbial biomass, after harvest of peas (*Pisum sativum* L.) and oats (*Avena sativa* L.).

		Root		Rhizodeposition		Soil	Microbial biomass	
		C-to-N	**^{13}C-to-^{15}N**	**C-to-N**	**^{13}C-to-^{15}N**	**C-to-N**	**^{13}C-to-^{15}N**
0-30 cm	**P_V**	13.2 b	12.7 a	8.0 c	7.6 b	7.8 b	8 b
	P_T	17.1 b	5.3 d	16.8 c	4.9 b	7.7 b	5 bc
	O_T	46.1 a	8.9 bc	33.3 bc	6.3 b	7.5 b	4 bc
	P_F	16.7 b	5.5 cd	25.0 c	7.1 b	7.8 b	6 bc
	O_G	55.5 a	11.6 ab	142.5 a	30.7 a	7.6 b	14 a
30-50 cm	**P_V**	13.2 b	12.7 a	4.5 c	4.3 b	8.3 b	n.d.
	P_T	17.1 b	5.3 d	16.2 c	4.8 b	9.8 a	3c
	O_T	46.1 a	8.9 bc	97.3 ab	17.8 ab	9.6 a	n.d.
	P_F	16.7 b	95.5 cd	17.2 c	5.3 b	9.5 a	4 bc
	O_G	55.5 a	11.6 ab	142.6 a	32.1 a	7.6 b	n.d.

P stands for peas, O means oats. Plants were labelled once at early vegetative growth (subscript V), twice during plant growth (subscript T), or once at flowering (subscript F) or grain filling (subscript G). Values with different letters within a column indicate significant differences between the means (Student's t-test, pairwise comparison, $p < 0.05$; n=7, except for ^{13}C-to-^{15}N in the microbial biomass of treatment P_V n=5 and O_T n=6; n.d. = not detectable).

7.4 Discussion

Simultaneous ^{13}C-^{15}N double labelling of plants *in situ*

Total recovery of applied ^{13}C and ^{15}N (Table 16) was very high and close to 100% for ^{15}N, indicating that only minor losses occurred when working with sterile glucose and urea solutions using multiple pulses for labelling. Recovery of ^{15}N from plants and soil was in the range previously reported by others using the wick method for application of ^{15}N-urea (Russell and Fillery, 1996a; 1996b; Mayer et al., 2003). Where solution remained in the wick system for a longer period of time (treatment P_T), loss of ^{15}N was on average 13%. This might be due to immobilisation of ^{15}N in the wick system by microorganisms, which entered the system during handling. Additionally, gaseous loss of ^{15}N from plants and soil might have occurred, but would only account for a small proportion of total losses. The ^{13}C not recovered from plants and soil or the wick system, can be attributed to respiration of roots and soil microorganisms and partly to shoot respiration. Moreover, $^{13}CO_2$ might have evolved from the wick system after assimilation of the glucose-urea mixture by microorganisms, which might have entered the system and immobilised ^{13}C and ^{15}N. In the experimental system used here, $^{13}CO_2$ evolution cannot be detected. The extent of reassimilation of root-derived $^{13}CO_2$ by the plant therefore remains unknown.

Nevertheless, the present results show that the wick method can be used for simultaneous double labelling of plants *in situ* with both ^{13}C and ^{15}N simultaneously. Using glucose as a ^{13}C carrier contributes to the influx of the solution into the cells at the wick-stem interface, due to its osmotic potential (Amberger, 1996). Applying glucose and urea as carriers for ^{13}C and ^{15}N might have no strong disadvantage for the plant, as urea is decomposed enzymatically into CO_2 and NH_3 by urease (Hertenberger and Wanek, 2004). Glucose is known to be a hormone-like regulator of growth and metabolism including vegetative growth, reproduction and senescence (Yanagisawa et al., 2003), and is probably metabolised or stored in the cells for further relocation and metabolic use. A previous study, showed that application of glucose using the wick method had no effect on plant development and the investigated soil properties (Wichern et al., 2007).

The distribution of ^{13}C and ^{15}N in the various plant parts did not follow the distribution of C and N in the plant. Enrichment of the BGP with ^{13}C was in many cases lower than the above-ground plant biomass (Table 17). Stems of peas and oats were preferentially enriched with ^{13}C and, together with leaves, contained a high proportion of the applied ^{13}C (Table 18). The ^{15}N enrichment was similar among the above-ground plant parts, and lower for the BGP-N. Many authors reported for shoot feeding techniques, that above-ground plant biomass (AGP) is preferentially enriched with ^{15}N (Janzen and Bruinsma, 1993; Russell and Fillery, 1996a; Schmidt and Scrimgeour, 2001; Yasmin et al., 2006). This is probably due to preferential use of the compounds at the site of application. Nevertheless, BGP was significantly enriched with ^{13}C and ^{15}N allowing isotope tracing in the soil and estimation of rhizodeposition and the related turnover in the soil.

Most of the recovered ^{13}CdfR and ^{15}NdfR was detected at 0-30 cm soil depth, as observed previously (Høgh-Jensen and Schjoerring, 2001). This indicates that rhizodeposition occurs where most of the roots are present and that amounts depend on root density. In pea treatments, 79 to 85% and in oats treatments 63 to 64% of the recovered roots were detected at 0-30 cm (data not shown), corresponding to the distribution of ^{13}CdfR and ^{15}NdfR in the two soil layers in most of the treatments. An exception is treatment P_V, where the proportion of roots in 30-50 cm soil depth was much lower than the proportion of ^{13}CdfR and ^{15}NdfR. In this case, transfer of soluble rhizodeposits, especially N, into the deeper soil layer might have happened on occasions of downward water movement. This is common in early vegetative growth stages when transpiration and therefore water uptake is low under temperate humid conditions.

Transfer of plant C and N into the soil

At early vegetative growth of peas (treatment P_V), C and N was predominantly present in below-ground parts, whereas at maturity (treatments P_T and P_F) most C and N was present in above-ground plant parts. A similar pattern was also observed for other plant species (Gedroc et al., 1996; Mokany et al., 2006; Yevdokimov et al., 2006). Ericsson (1995) proposed that the internal relation of labile N and C in root

and shoot tissue affects dry matter partitioning of plant parts. Additionally, this relation is influenced by the availability of mineral nutrients, with low availability of N, P or S favouring root growth. Low availability of nutrients might therefore be one reason for increased below-ground transfer of C and N during the early growth phase. However, N might not have been deficient, as high amounts of N were released from roots, and inorganic N content was sufficiently high in treatment P_V. Whether other plant nutrients were deficient causing the initial root growth remains unclear.

At maturity, most of the N recovered from peas was found in grains and rhizodeposition. In treatment P_F, where plants were only labelled during the reproductive growth phase, 10.9% of the applied ^{15}N was transferred below-ground as rhizodeposition and 46.3% was detected in grain tissue. These results clearly show the transfer of N into the grain as the dominating above-ground sink of the plant during the reproductive growth phase (Götz and Herzog, 2000; Rroço and Mengel, 2000). However, a significant proportion of N was also transferred below-ground.

Higher amounts of total plant C and BGP-C, dominated by root C, were recovered from oats than from peas. These values, however, did not include C lost as CO_2. In peas, more assimilated C might be lost by rhizo-respiration, due to the fact that legumes have a higher assimilate demand for symbiotic N_2-fixation (Merbach et al., 1999), as observed for alfalfa (*Medicago sativa* L.) and wheat (Hütsch et al., 2002; Wichern et al., 2004). This is supported by the higher proportion of unaccounted-for loss of ^{13}C in the pea treatments (Table 16). A higher respiration would result in a stronger below-ground transfer of C and in a reduced contribution of recovered CdfR to the BGP-C and total plant-C. Furthermore, the turnover of pea roots, might be higher than that of oats, contributing to the loss of plant C as CO_2. The stronger decomposition of pea roots is indicated by a higher contribution of CdfR to BGP-C than roots compared with oats.

Additionally, BGP-N was dominated by NdfR in peas, whereas in oats mostly roots accounted for the BGP-N. Total amounts of plant N, however, were higher in peas than in oats, which must be expected from a comparison of legumes and oats. In the treatments P_F and O_G labelled at the reproductive growth phase, total amounts of N

were lower than in treatments P_T and O_T, which investigated a longer growth period. The reason for this might be a lower recovery of NdfR evolved during the vegetative growth phase before labelling and still remaining in the soil in the treatments P_F and O_G. Amounts of net rhizodeposition seem to increase during plant growth, while the proportion of total plant C that is deposited in the root zone decreases (Yevdokimov et al., 2006). The observed differences between peas and oats in the contribution of rhizodeposition to the BGP-C and N indicate that peas release higher amounts of rhizodeposition and that roots of peas are subject to faster turnover.

In peas, one to two-third and in oats two-third of the recovered C was transferred below the soil surface. These results are higher than BGP-C values in the range of 20-30% for cereals obtained from experiments where CO_2 was included in the balances (Nguyen, 2003; Kuzyakov and Schneckenberger, 2004). Taking the loss of C by rhizo-respiration into account, the proportion of assimilated C transferred below ground level would even be higher. In addition, values obtained for CdfR as a percentage of total plant C would only be slightly reduced and still be higher than most of the results reported in the literature. The proportion of NdfR in treatment O_T, was in the range of reports from pot and split-root experiments with barley (*Hordeum vulgare* L.) (Jensen, 1996) and low fertility treatments of wheat (Janzen, 1990; Janzen and Bruinsma, 1989; 1993). However, the contribution of NdfR to the BGP-N was only 8 to 29% and much lower in the present investigation than in the other studies. On the other hand, BGP-N as a percentage of total plant N was higher than in previous studies. In contrast, NdfR contributed more than 70% to the BGP-N in peas, which was higher than most previous results (Sawatsky and Soper, 1991; Jensen, 1996), but in the range of the values reported by Mayer et al. (2003).

Our results show that rhizodeposition is higher under outdoor conditions than in pot experiments under controlled conditions. This is probably due to a higher root-to-shoot ratio and a higher root turnover due to the changing water and temperature regime (Mayer et al., 2003). In addition, reuptake of mineralised NdfR might have occurred, especially during the reproductive growth phase. This would ultimately result in a much higher gross rhizodeposition of N. The question remains, why such

high amounts of N are released by plant roots, and in which way a positive plant-soil feedback occurs. Most N released is probably passively lost from decaying roots through autolysis. Gordon and Jackson (2000) observed, that N from fine roots is only relocated to above-ground plant parts to a small extent and therefore remains in the soil contributing to rhizodeposition. They assumed that parts of this below-ground litter provide an immediate source of plant available N (Chapman et al., 2006).

Turnover of root-derived C and N during plant growth

In treatment P_T, 21% of the ^{13}CdfR and 23% of the ^{15}NdfR was detected in the microbial biomass at maturity (Table 20). This was in the range of the results obtained by Mayer et al. (2003) for ^{15}N, using a similar methodology and investigating the same growth period. The proportion of microbial ^{13}C and ^{15}NdfR in oats, was somewhat lower, and for ^{13}CdfR in the same range as reported from a greenhouse experiment with oats (Yevdokimov et al., 2006). These results indicate, that rhizodeposition of peas and oats, is highly labile and rapidly incorporated into the soil microbial biomass (Janzen, 1990; McNeill et al., 1997), but to a varying degree. However, only a small proportion of the extractable C was derived from rhizodeposition, reflecting either rapid utilization of CdfR by soil microbes and loss as CO_2 (Merbach et al., 1999; Yevdokimov et al., 2006), or indicating that the extractable C pool is not strongly related to substrate availability (Cookson and Murphy, 2004).

The quality of rhizodeposits varies with plant species, resulting in a lower availability of oat rhizodeposits for soil microorganisms in comparison with peas. This is suggested by the observation that in peas a higher proportion of the microbial biomass C and N originated from rhizodeposition. (Table 21). On the other hand, the microbial biomass and its C-to-N ratio were similar in peas and oats, indicating that rhizodeposits of peas increase microbial activity and turnover in comparison with oats, while leaving the size of the microbial biomass unaffected. A high turnover of the microbial biomass consequently results in NdfR being immobilised in microbial

residues (Knops et al., 2002; Mayer et al., 2003). However, almost half of the inorganic N in peas (P_T) and more than one third in oats (O_T) was derived from rhizodeposition (Table 21). Moreover, 27 and 38% of the ^{15}NdfR was found in the inorganic soil N pool of these treatments (Table 20), indicating that a large part of the NdfR was already mineralised, either by microbial turnover or by direct leaching from living and decaying roots (Chapman et al., 2006).

The rate of N mineralisation and immobilisation is affected by the chemical composition of the compounds released from plant roots (Ehrenfeld et al., 2005; Chapman et al., 2006). In the present study the C-to-N ratio of the roots and of the rhizodeposition were lower in peas than in oats, reflecting that availability of rhizodeposits to soil microorganisms is higher. However, in future investigations, other quality indices should be taken into consideration to explore the mechanisms driving the interacting C and N mineralisation and immobilisation processes of root derived compounds. The slower turnover of oat rhizodeposition can partly be attributed to a higher content of rootlets in comparison with peas. It is assumed that rootlets decompose more slowly and therefore contribute to soil organic matter accumulation (Pelz et al., 2005). Again, the quality of the rootlets and root fragments strongly influence their decomposition kinetics.

The availability of rhizodeposits changes as the plant develops. Especially during the reproductive growth phase, where root decay increases, structural compounds of lower availability are released from the roots. In treatment O_G, the elevated C-to-N ratio of rhizodeposition during reproductive growth, in particular at 0-30 cm soil depth, reflected this change. However, at the same time 40% of the ^{15}NdfR was recovered as inorganic N, and even though the concentration of inorganic N was low, 16% of it derived from rhizodeposition. This plant derived inorganic N might have originated directly from dying plant roots (Chapman et al., 2006), as the contribution of plant-derived N to the microbial biomass was only 1%.

In peas, the proportion of inorganic N derived from rhizodeposition was smaller at early vegetative growth, but amounts were higher than during later growth stages. This was most pronounced in comparison with the reproductive growth phase (Table 22). This indicates, that during early development pea plants release N-

compounds that are more rapidly mineralised than those released during a later development stage. It also indicates that re-uptake of inorganic NdfR occurs during plant growth, in particular during grain filling.

7.5 Conclusions

Amounts of rhizodeposition C and N can be estimated simultaneously *in situ* using the wick method for double labelling plants. Our results are the first describing C and N rhizodeposition and its turnover *in situ* under outdoor conditions. From the presented results, it can be concluded, that plant species differ in amount and quality of compounds released by roots. These differences strongly influence the activity of the microbial community. Consequently, highly available rhizodeposits fuel the turnover of the microbial biomass, as shown for peas in comparison with oats. This results in strong immobilisation and subsequent remineralisation of mineral nutrients already during plant growth. It therefore might serve the indirect nutrient mobilisation, actively controlled by plant physiology and morphology, which gives evidence for a stronger positive plant-soil feedback in peas than in oats.

Acknowledgements

We acknowledge the technical assistance and kind help of Gabi Dormann, Christian Maack, Manuel Renneberg, Sarina Weber, Elmar Eberhardt, Julia Hartkemeyer, and Rainer Brauckmann. We also thank Reinhard Langel and Dr. Jens Dyckmans for isotope analyses and valuable discussions. This study was financed by the German Research Foundation (MU1711/3-1 and JO362/6-1).

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8 Synthesis

8.1 Plants can be *in situ* labelled simultaneously with ^{13}C and ^{15}N

Labelling plants with ^{13}C and ^{15}N allows the quantification of plant C and N in the soil, facilitates tracing of their transfer into different soil pools and enables estimation of the related turnover processes. One technique developed for pulse labelling grain legumes with ^{15}N, is the cotton wick method. Using this method showed a similar distribution pattern of applied ^{15}N as a split-root feeding technique (Hertenberger and Wanek, 2004). In contrast, this was not the case for a leaf-feeding method (Hertenberger and Wanek, 2004). This indicates that the applied ^{15}N is metabolised in a similar way when using the wick method or a natural N uptake pathway for labelling. Merbach et al. (2000) argued that all the methods they compared have some disadvantages but are also all suitable for estimation of N rhizodeposition.

The amount of N applied with the highly enriched ^{15}N-urea solution by the wick method, is only a tiny proportion of total plant N. It therefore has no fertilisation effect. This is in contrast to other methods, such as gaseous NH_3 application, precultivation of plants in ^{15}N-labelled quartz sand and the split-root technique, where a significant amount of N is applied to the plant (Merbach et al., 2000). Furthermore, in contrast to the split-root technique (Merbach et al., 2000), the wick method allows a complete balance of ^{15}N in the plant-soil system (Mayer et al., 2003), can be used *in situ* with undisturbed soil and under field conditions, and has been successfully used on oats (Objective (ii), chapter 3), after the emergence of the first knot (Chapter 5). Additionally, it allows simultaneous double labelling of plants with ^{13}C and ^{15}N *in situ* and under field conditions using a ^{13}C-glucose-^{15}N-urea mixture (Objective (i), chapter 3), as shown in the present study (Chapter 6). For this approach, conditions in the wick system have to be kept as sterile as possible, to achieve a high recovery of the applied isotopes and a sufficient enrichment of the below-ground plant biomass as shown in chapter 7. The cotton wick method used in the present study provides a useful tool for *in situ* quantification of rhizodeposition

and the investigation of processes involved in the mobilisation and immobilisation of mineral plant nutrients at the soil-plant-atmosphere interface.

As presented here shortly, the wick method has several advantages in comparison with most of the other methods for labelling plants with ^{13}C and ^{15}N. However, homogeneous labelling of the different plant parts and homogeneous enrichment of soil C and N pools among the replicates was not always achieved, which requires further improvement of the methodology. This limits the use of the method and requires further improvement to guarantee homogeneous enrichment of root and soil C and N. Therefore, the documentation of rhizodeposition as a percentage of recovered C or N seems to be more reliable than giving the total quantities released. A high enrichment of roots and rhizodeposits might counteract the uncertainty in quantitative detection of CdfR and NdfR and can be achieved by applying a small amount of highly enriched solutions in multiple pulses. Moreover, using homogenised soil material and therefore disturbed soil conditions, is another option to reduce the small-scale variability between the soil samples. A higher number of replicates might be the best option to gain more reliable average quantities of CdfR and NdfR but holds the disadvantage of the increasing amount of work and expenses for isotope analyses in the various soil and plant pools.

8.2 The determination of ^{13}C and ^{15}N in the soil microbial biomass has to be improved

In the column experiments (Experiments III and IV) the transfer of ^{13}C and ^{15}N into the microbial biomass was determined by estimating the isotopic enrichment of the microbial biomass. Therefore, the microbial biomass C and N content in the soil was estimated using the chloroform-fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987) including a pre-extraction step (Mueller et al., 1992; Mayer et al., 2003) to remove living root fragments. Instead of the common 0.5 M K_2SO_4 solution, similarly to the pre-extraction a 0.05 M K_2SO_4 solution was used for soil extraction before and after fumigation. It was necessary to reduce the salt con content in the extracts, and therefore the salt freight in the isotope analysis, as it

Table 23 Microbial C, microbial N, extractable C, and extractable N in the soil used for the first column experiment in the field (soil A) and the second experiment in the raised bed (soil B), after extraction with 0.05 and 0.5 M K_2SO_4. Values show means ± standard deviation. Values with different letters within a soil depth specific treatment pair, show means with significant differences between the extraction treatments ($p < 0.05$; Fisher's PLSD-test). The lower part of the table shows F-values from the analysis of variance; degrees of freedom: 1.

Soil depth (cm)		Molarity of K_2SO_4	Microbial		Extractable		n
			C	N	C	N	
			($\mu g\ g^{-1}$ soil)				
Soil A	0-30	0.05	153 ± 9 a	36.1 ± 5.9 a	15.3 ± 6.8 a	6.0 ± 3.2 a	3
		0.5	158 ± 8 a	45.5 ± 6.6 a	45.9 ± 3.1 b	7.2 ± 3.1 a	4
	30-50	0.05	56 ± 12 a	10.5 ± 3.6 a	14.9 ± 6.4 a	0.5 ± 0.5 a	3
		0.5	41 ± 9 a	13.3 ± 4.5 a	39.4 ± 4.3 b	1.1 ± 0.2 a	3
Soil B	0-30	0.05	151 ± 23 a	24.3 ± 3.3 a	29.9 ± 4.9 a	1.5 ± 0.8 a	4
		0.5	131 ± 6 a	26.9 ± 2.3 a	73.8 ± 7.8 b	3.0 ± 0.6 b	4
	30-50	0.05	29 ± 17 a	6.4 ± 0.4 a	23.9 ± 5.7 a	0.6 ± 0.2 a	4
		0.5	22 ± 8 a	10.8 ± 0.4 b	52.8 ± 4.6 b	1.0 ± 0.2 a	4
Analysis of variance							
Molarity			4.1	10.8 **	232.3 ***	2.6	
Soil			15.0 ***	40.2 ***	60.1 ***	13.4 **	
Depth			536.4 ***	249.2 ***	16.4 ***	37.7 ***	
M x **S**			0.9	0.8	4.4 *	< 0.1	
M x **D**			0.2	0.7	6.3 *	0.5	
S x **D**			0.8	16.7 ***	5.7 *	13.6 **	
M x **S** x **D**			3.0	2.1	1.1	0.1	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

affected the flash combustion in the elementar analyser (Potthoff et al., 2003). The ^{13}C and ^{15}N enrichment of the soil extracts was determined after freeze-drying a sub-sample of the extract (15 ml) and measuring the isotope ratio $^{13}C/^{12}C$ and

$^{15}N/^{14}N$ in the dry salt residues using isotope ratio mass spectrometry (Delta plus IRMS 251, Finnigan Mat, Bremen, Germany) after combustion using a Carlo Erba NA 1500 gas chromatograph (Carlo Erba Instruments, Milano, Italy). Determination of the microbial biomass C can be done using a 0.05 M K_2SO_4 solution, with no difference in extractable chloroform labile organic C, as long as flocculation is ensured (Potthoff et al., 2003). This observation was confirmed by our results obtained from a comparison of 0.5 and 0.05 M K_2SO_4 solution for fumigation-extraction on the two soils used in the experiments (Table 23). We were not able to detect any significant difference in the microbial biomass C between the two molarity treatments. However, extractable C was highly significantly affected by the molarity and was higher where soils were extracted with 0.5 M K_2SO_4 solution, as observed before (Potthoff et al., 2003). Furthermore, extractable N was also slightly higher in the treatment with 0.5 M K_2SO_4 solution, but this was not significant. Microbial N was also always higher after extraction with 0.5 M K_2SO_4 solution. This effect was significant, however, the values of the two molarity treatments was not significantly different for each soil depth. These results show, that the methodology of simultaneous determination of ^{13}C and ^{15}N in the microbial biomass, has to be improved further. Soil extraction with the commonly used 0.5 M K_2SO_4 solution, would require a high amount of N in the solution. When investigating rhizodeposition, a pre-extraction step has to be included to remove living roots and root fragments, which ultimately reduces the N concentration in the extract. This dilemma might be overcome by developing or improving methods for online determination of the $\delta^{13}C$ and $\delta^{15}N$ values in the 0.5 M K_2SO_4 soil extract. However, whether the obtained values are more reliable has to be proven. Using the common 0.5 M K_2SO_4 solution, additionally holds the advantage of preventing microbial decomposition of easily available chloroform labile organic material in the extract (Potthoff et al., 2003).

8.3 Rhizodeposition of C and N in outdoor experiments is higher than in pot experiments

One of the objectives of the present work was to quantify rhizodeposition of C (CdfR) and N (NdfR) during different growth phases of peas and oats (objective (iv), chapter 3) in particular in the reproductive growth phase (objective (iii), chapter 3). The results of the present study show, that CdfR and NdfR estimated under outdoor conditions contribute a higher proportion to total plant C and N as previously estimated in pot experiments. However, values from the two studies under outdoor conditions presented here, are varying considerably in particular for oats. In general, CdfR was between one fourth and one third of total recovered plant C. Only in the early vegetative growth phase of peas it was remarkably higher, representing 58.2% of total recovered plant C. The same holds true for NdfR, which was 71.1% of total plant N at an early vegetative growth phase of peas. For the reproductive and the whole growth period, it was one fifth to more than one third in peas. In oats, it varied between 4.5 and 30% of plant N with the lowest value obtained when investigating the period of grain filling. As pea plants take up more N, the amount of NdfR is higher in comparison with oats. During a whole vegetation period, peas (Treatment P_T, Chapter 7) released 24.5% of total recovered plant C as CdfR and 29.3% of total plant N as NdfR. In oats (Treatment O_T, Chapter 7), CdfR was 15.2% of total recovered plant C and NdfR was 20.3% of total plant N.

For a rough estimation of the C and N input by oats and peas through roots and rhizodeposition under field conditions, a crop grain yield of 6 Mg (megagram) ha^{-1} was assumed. The amount of CdfR in oats was calculated to be 2.2 Mg ha^{-1} and BGP-C was estimated to be 9.3 Mg ha^{-1}. This is much higher than the amount reported by Kuzyakov and Schneckenberger for wheat and barley. For peas, CdfR in our study was 2.7 Mg ha^{-1} with a BGP-C of 3.2 Mg ha^{-1} and therefore much lower than for oats. Oats might therefore contribute stronger to C sequestration in the soil than peas. It was surprising, that the amount of BGP-N was higher for oats with 0.28 Mg ha^{-1} compared to 0.21 Mg ha^{-1} for peas. However, the amount of NdfR was only 0.02 Mg ha^{-1} in oats, whereas it was ten times higher in peas (0.18 Mg ha^{-1}). These results show, that pot experiments under controlled conditions underestimate

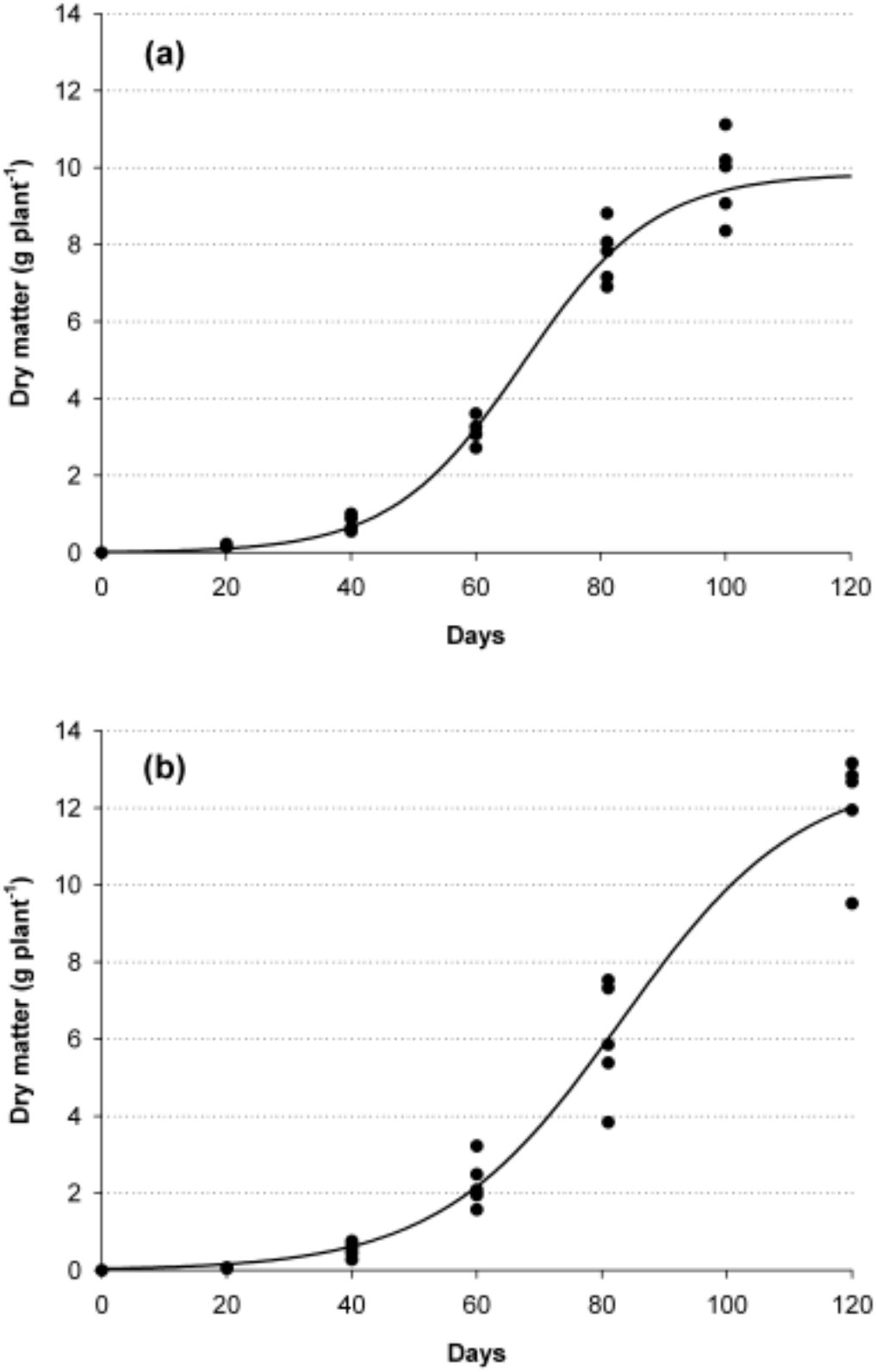

Figure 12 Above-ground dry matter development in peas (*Pisum sativum* L.) (Fig. 12a) and oats (*Avena sativa* L.) (Fig. 12b). Results are obtained from a pot experiment in the greenhouse using soil as growth medium (n=5).

the amount of CdfR and NdfR. Reasons for this are a higher root-to-shoot ratio under field conditions in comparison with pot experiments (Mayer et al., 2003) and probably a higher and continuous turnover of roots due to a changing moisture and temperature regime.

Table 24 Change of the root-to-shoot ratio and total plant dry matter of peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) during plant growth. Results are obtained from a pot experiment in the greenhouse using soil as growth medium. Values with different letters within a column indicate significant differences between the means (Tukey/Kramer, $p < 0.05$; n=5).

Time after sowing	Root-to-shoot ratio		Total plant dry matter (g plant^{-1})	
(d)	peas	oats	peas	oats
20	5.3 a	10.5 a	1.0 d	0.6 c
40	0.4 b	0.4 b	1.1 d	0.7 c
60	0.1 bc	0.3 b	3.5 c	2.9 c
81	0.1 b	0.3 b	8.7 b	7.9 b
100	0.1 b		10.4 a	
120		0.2 b		14.4 a

In addition to the rhizodeposition of the whole growth period, the vegetative and generative growth phases were investigated and the release of root-derived compounds was estimated. The dry matter development of above-ground plant material in pea (Figure 12a) and oat plants (Figure 12b) fitted a typical sigmoidal growth curve. The development of below-ground plant biomass, however, showed a different dynamic. Early vegetative development of plants is associated with stronger root growth, resulting in higher root-to-shoot ratios (Table 24) (Gedroc et al., 1996; Yevdokimov et al., 2006). Therefore, a high proportion of plant assimilates is transferred below-ground resulting in the rhizodeposition accounting for a high proportion of the total plant C and N as shown in chapter 7.3. CdfR (% of total recovered plant C) was lower in plants labelled during the reproductive growth phase in com-

parison with plants labelled twice during vegetative growth. This indicates reduced transfer of assimilates below-ground during the phase of grain development and grain filling. Pea plants labelled at a very early vegetative growth phase (3 leaves unfolded) on the other hand, showed a strong transfer of C and N below-ground.

There is a methodological uncertainty of root recovery, with only a certain amount of the roots detected, due to their soft and fragile nature at early growth. This ultimately would result in an overestimation of rhizodeposition. Nevertheless, the presented data (Chapter 7.3) give evidence, that soluble compounds are released from plant roots of peas at an early vegetative growth stage. This was supported by the observation, that even at 30-50 cm soil depth, where no roots were detected, ^{15}N enrichment of the soil N pool could be detected, leading to the conclusion, that soluble exudates or mineralised rhizodeposits were transferred into the deeper soil layer after heavy rain events.

Consequently, rhizodeposition of C and N as a percentage of total plant C and N, is higher during vegetative growth and decreases during plant growth in particular during the reproductive growth phase. The total quantities of C and N released from roots are probably increasing. However, as respiration of C and re-uptake of N occurs throughout the growing season, the net amount might be more similar in the different growth phases, whereas composition of the rhizodeposition changes. Rhizodeposition constitutes of soluble low and high molecular weight root exudates, mucilage, sloughed cells and tissue, cell lysates and decomposing rootlets, and during the reproductive growth phase of decaying roots. Root death therefore strongly contributes to the rhizodeposition of C and N during the reproductive growth phase in peas and oats. Most roots in peas were present approximately 10 days after the onset of flowering (Thorup-Kristensen, 1998), which indicates death and turnover of roots thereafter. Besides, the reallocation of N from rootlets into above-ground plant biomass was estimated to be small or nonexistent (Gordon and Jackson, 2000; Salon et al., 2001). Therefore rootlets and root fragments contribute to a great extent to the rhizodeposition of N, especially during the reproductive growth phase, where many roots are dying. However, peas labelled at flowering and oats labelled at grain filling released significant quantities of C and N derived from

new photosynthates, indicating, that during the reproductive growth phase also soluble compounds, such as root exudates are released.

8.4 The turnover of rhizodeposits is influenced by its availability to microorganisms

The ecological function of the different compounds released by plant roots is manifold. However, the reason for the release of N is still unclear. Many of the rhizodeposits contribute to nutrient mobilisation, with the bulk of it having indirect, rather than direct effects on the solubilisation of mineral nutrients. These mobilisation processes are largely driven by the turnover of rhizodeposits through the microbial biomass, depending on the availability of the compounds released. Moreover, it is assumed, that quantity and quality of rhizodeposition influence the microbial community structure (Paterson, 2003), which explains some of the plant species-specific differences in microbial properties observed in roots and rhizosphere soil of different plant species (Appuhn and Joergensen, 2006).

Rhizodeposits are an easily available nutrient source for soil microorganisms. Our results give evidence that rhizodeposits of peas are more easily available to soil microorganisms in comparison with oats. In the column experiment under field conditions (Chapter 6), a higher proportion of microbial biomass C and N and inorganic N derived from rhizodeposition in peas in comparison with oats. This indicates immobilisation and mineralisation of pea rhizodeposits during the investigated reproductive growth phase. Additionally, this was supported by the observation that the proportion of ^{15}NdfR in the inorganic N pool was higher under peas in comparison with oats. The difference was not as pronounced for the proportion of inorganic NdfR and inorganic ^{15}NdfR in the raised bed column experiment (Chapter 7). However, the microbial biomass C and N derived from rhizodeposition in the treatments investigating the whole growth period (treatments P_T and O_T), was also much higher in peas in comparison with oats. Microbial biomass CdfR was 52% in peas and 19% in oats in this experiment and microbial biomass NdfR was 18% in peas and 5% in oats. The treatments investigating the reproductive growth phase (treatment P_F and

O_G) showed the same difference but less pronounced. Furthermore, the percentage of the microbial biomass derived from rhizodeposition in these treatments was lower in comparison with the treatments where the whole growth period was investigated (treatment P_T and O_T). These results indicate that rhizodeposits released during the reproductive growth phase are less available to soil microorganisms, which is supported by the finding that the C-to-N ratio of the rhizodeposition in the reproductive growth phase was higher. This difference was more pronounced for oats.

Generally, the C-to-N ratio of the roots and of the rhizodeposition was in the same range in most treatments, except for the treatment P_V labelled at the very early vegetative growth phase in the raised bed column experiment. In general, both ratios were lower in peas in comparison with oats. A lower C-to-N ratio of the rhizodeposition indicates higher availability of pea rhizodeposits. The C-to-N ratio of the roots of peas and oats can therefore be used as an indicator for rhizodeposit availability to soil microorganisms, at least for the whole growth period and in comparison between the two species. In addition to the difference of the C-to-N ratio in roots, a higher proportion of ^{13}C and ^{15}N was found in the recovered rootlets of oats in comparison with peas in the field experiment. Rootlets are assumed to decompose slower than other rhizodeposits (Pelz et al., 2005). However, the decomposability strongly depends on their chemical composition. The content of other root compounds, such as lignin, cellulose, or polyphenolic compounds are probably strongly influencing the degradability of rhizodeposits, which should be taken into consideration in future investigations.

Recently it was shown, that roots contribute to a greater extent to C sequestration as previously thought (Mokany et al., 2006). The data obtained in the present study support this observation, with a high amount of C released from roots especially of oats. However, the study of Mokany et al. (2006) did not include rhizodeposition in their appraisal and therefore underestimated the contribution of root biomass to C sequestration. Admittedly, the quality of roots and rhizodeposition strongly influence C sequestration. Pea roots and rhizodeposits decompose faster than those of cereals and therefore might contribute only to a smaller extent to C accumulation. Due to higher quantities of N released and due to a higher decomposition and turn-

over of roots and rhizodeposits during plant growth and after harvest, peas contribute to a higher N availability for the subsequent crop. They therefore might also contribute to their own nutrition and that of associated plants with respect to other mineral nutrients.

In conclusion, the results of the present study show the interdependence of C and N turnover at the soil-plant interface. The mineralisation of N and other plant nutrients, which is controlled by the soil microorganisms and their turnover through the 'microbial loop', is driven by the input of easily available C by plant roots (Knops et al., 2002; Chapman et al., 2006). During plant growth the composition of the compounds released by plant roots changes. At maturity, a high proportion of these rhizodeposits are probably sloughed cells, root debris, rootlets, mucilage and cell lysates, with different availability to soil microorganisms. Additionally, the quality of the rhizodeposition differs between plant species influencing microbial activity and turnover. The lower C-to-N ratio of pea rhizodeposition in comparison with oats, indicates a higher availability to soil microorganisms. An increased immobilisation and subsequent mineralisation of the rhizodeposits in peas, aid the nutrition of the plant itself and associated or subsequent plants.

9 Outlook

Quantitative appraisal of rhizodeposition

Most of the research conducted on quantitative appraisal of rhizodeposition has been done under controlled conditions in the laboratory or greenhouse. Like the present study shows, results obtained from experiments under outdoor conditions are higher. Future investigations on rhizodeposition should therefore focus on estimating the net rhizodeposition of C and N for various plant species and crop varieties under field conditions, with and without separation of soil space by columns. Moreover, the various abiotic and biotic factors influencing rhizodeposition have to be taken into consideration. This would effectively be done in pot and laboratory experiments under controlled conditions to guarantee a clear treatment effect. The main abiotic factors to be examined are soil type, soil nutrient status, soil moisture, soil and air temperature, whereas biotic factors, such as root nodulation, mycorrhiza, pests and pathogens should be a focus of quantitative research in the first place. Furthermore, laboratory and pot experiments using the wick method can be designed to estimate the gross rhizodeposition of C and N, especially in vicinity

Investigating the active nutrient mobilisation of different plants

The transfer of N rhizodeposition to associated plants of the same species or another kind, and into subsequent crops is of valuable interest, especially for agronomic management of sustainable cropping systems. These mixed cropping systems with two or more crops grown together, gain interest for the production of food, fibre, biomass or biomass fuel. Especially where nutrient availability is low, the interactions of plants and their ability of nutrient acquisition in such systems, might be suitably addressed by a similar methodology as used in the present study. For this field of research, pot experiments should be conducted to estimate the influence of rhizodeposition on the active nutrient mobilisation of different crop species, in particular legumes. A good model plant for the influence of rhizodeposition on phosphorus availability is the groundnut (*Arachis hypogaea* L.). It is well known, that due to the ‘contact reaction’, where rhizodermis cells are continuously renewed,

higher quantities of Al^{3+} and Fe^{3+} are chelated and phosphorus is mobilised. These quantities might be quantitatively estimated using a similar methodological approach as in the present study. Furthermore, research on remediation plants, that solubilise and store heavy metals might profit from the use of the present methodology to evaluate the potential of different plant species.

Another focus has to be put on investigating the chemical composition of rhizodeposition, its change with plant growth, and its fate in the soil. Experiments focussing on the processes involved in rhizodeposition and plant physiological investigations, such as nutrient acquisition and relocation studies, the application of other labelled compounds using the wick methods might be useful. Application of sugars or amino acids with selectively labelled C-atoms or labelled N in the amino group, offer a possibility to investigate the fate of single C and N-atoms in the plant and at the plant-soil interface. The application of a mixture of tracers or isotopes, such as ^{14}C in combination with ^{13}C and/or ^{12}C, might also give further insight into the processes involved in rhizodeposition and nutrient acquisition and relocation.

Linking microbial community structure with function

With increasing accuracy of isotopic analysis, the use of compound specific isotope analysis in soil science will be valuable for a more detailed characterisation of the microbial community structure as influenced by rhizodeposition. The existing techniques to determine ^{13}C in PLFA, DNA, RNA and microbial biomass or ^{15}N in DNA, RNA and the microbial biomass, or still to be developed techniques, such as e.g. ^{15}N in amino sugars, may contribute in linking function and community structure of the soil microorganisms. Combining these methods with the use of the wick method can contribute to a better understanding of the nutrient, especially C and N dynamics in the rhizoplane, the rhizosphere and the bulk soil.

However, there is a strong need to integrate research in plant physiology and molecular biology with soil science disciplines, such as soil chemistry, physics and soil microbial and mesofaunal ecology. This integration can contribute to bigger advances in rhizosphere research.

10 Summary

The objective of the present work was to quantify the rhizodeposition of C and N simultaneously *in situ* under field conditions and to estimate its turnover and transfer into different soil compartments. For this purpose two greenhouse pot experiments and two column experiments under outdoor conditions were conducted using peas (*Pisum sativum* L.) and oats (*Avena sativa* L.).

Experiments I and II aimed at evaluating the cotton wick method for its suitability of simultaneous *in situ* application of sugar-urea mixtures as possible ^{13}C and ^{15}N-carriers into the stem of the plants. Two greenhouse experiments were conducted using soil as substrate. Different concentrations of cane sugar, glucose and urea solutions and mixtures thereof were applied to the plants. Solution uptake and plant development was monitored throughout the experiments. At harvest, dry matter of above-ground plant parts and roots was determined and the soil microbial biomass was estimated. It was examined whether the wick method or the applied solutions had any effect on plants and the soil microbial biomass.

In experiment III, rhizodeposition C (CdfR=C derived from rhizodeposition) and N (NdfR), defined as root-derived C or N present in the soil after removal of roots and root fragments, was quantified in a column experiment under field conditions. In the reproductive growth phase of peas and oats, plants were *in situ* labelled with a ^{13}C-glucose-^{15}N-urea mixture using the cotton wick method. The fate of CdfR and NdfR in the soil microbial biomass, the soil inorganic N pool and the remaining rootlets was examined, to estimate the turnover of CdfR and NdfR.

In experiment IV, quantities and fate of CdfR and NdfR was estimated at different growth phases. Plants were grown in soil columns placed in a raised bed under outdoor conditions and successfully *in situ* labelled with a ^{13}C-glucose-^{15}N-urea mixture simultaneously using the cotton wick method. After harvest, ^{13}C and ^{15}N was recovered in plant parts and soil pools, including the microbial biomass to estimate the turnover of rhizodeposits at different growth stages.

Sugar-urea mixtures were successful transferred into the plant. The solution uptake was driven by the transpiration stream. Plant development, below-ground, above-

ground and total plant dry matter, as well as the soil microbial biomass were not affected by the method or the solution applied. Therefore, glucose-urea mixtures can be used as ^{13}C and ^{15}N-carriers, making *in situ* field investigation of C and N dynamics easier. In the column experiments under outdoor conditions, below-ground plant biomass was successfully labelled using ^{13}C-glucose-^{15}N-urea mixtures. Moreover, a high isotope recovery was achieved. To guarantee enrichment of the labile soil C and N pools, such as the microbial biomass, glucose and urea have to be highly enriched.

The estimated amount of net CdfR and NdfR under outdoor conditions, was higher than previous reports with results mostly obtained from pot experiments. However, values varied strongly between growth stages and between the two conducted experiments. The CdfR ranged from 22.6 to 58.2% of the recovered plant C in peas and from 7.9 to 30.8% in oats. In addition, NdfR was 22.9 to 71.1% of total plant N in peas and 4.5 to 30.0% in oats. The proportion of total plant C and N derived from rhizodeposition was highest during early plant growth and probably decreased during plant growth. Alike the majority of roots, most of the rhizodeposition was present at 0-30 cm soil depth.

Depending on the growth phase, 1 to 31% of CdfR and NdfR was recovered in the microbial biomass and 4 to 40% of the NdfR inorganic N pool. Most of the recovered CdfR and NdfR, was present in rootlets and in other pools not further differentiated. Hence, a major part of the unrecovered C and N was probably immobilised in microbial residues. A higher proportion of the microbial biomass was derived from rhizodeposition in peas in comparison with oats. Additionally, the proportion of inorganic N derived from rhizodeposition was slightly higher in peas. Furthermore, the C-to-N ratio of the rhizodeposition and the roots was higher in oats in comparison with peas, indicating that rhizodeposits of peas were more easily available to soil microorganisms than those of oats.

Rhizodeposition of peas and oats represented a significant easily available C input into the soil which fuelled the turnover processes in the rhizosphere and therefore influence nutrient availability. Rhizodeposition N as a percentage of total plant N in oats and peas were in the same range, but quantities of N taken up by peas were

higher and therefore also the amount of NdfR. Hence, rhizodeposition N represents a significant N pool contributing to the higher N availability after peas and has to be taken into consideration for N balances and estimations of N_2-fixation. Moreover, differences in the turnover of NdfR and roots determine the N availability after the two crops.

11 Zusammenfassung

Das Ziel der vorliegenden Arbeit war es, die Mengen der Rhizodeposition von C und N sowie deren Umsatz *in situ* und unter Freilandbedingungen abzuschätzen. Zu diesem Zweck wurden zwei Gefäßversuche im Gewächshaus und zwei Versuche mit ungestörten Bodensäulen unter Freilandbedingungen durchgeführt. Als Pflanzen wurden Erbse (*Pisum sativum* L.) und Hafer (*Avena sativa* L.) verwendet.

Die Versuche I und II dienten der Evaluation der Dochtmethode und ihrer Eignung zur simultanen Applikation von Zucker-Harnstoff-Gemischen als potenzielle ^{13}C- und ^{15}N-Träger bei Körnerleguminosen (Erbse) und bei Getreide (Hafer). Die Pflanzen wurden in Gefäßen mit Boden im Gewächshaus angezogen und mittels Dochtmethode mit verschiedenen Rohrzucker-, Glukose- und Harnstofflösungen sowie deren Gemischen, in verschiedenen Konzentrationen versehen. Lösungsaufnahme und Pflanzenentwicklung wurden regelmäßig notiert. Nach der Ernte wurde die Trockenmasse der ober- und unterirdischen Pflanzenteile separat bestimmt sowie die mikrobielle Biomasse gemessen. Es wurde untersucht, ob die Methode oder die applizierten Lösungen einen Effekt auf die Pflanzenentwicklung und die mikrobielle Biomasse haben.

Im Säulenversuch unter Feldbedingungen (Versuch III), wurde die Rhizodeposition von C und N, hier definiert als wurzelbürtiger C und N im Boden nach Entfernung aller sichtbaren Wurzeln und Wurzelfragmente, gemessen und ihr Verbleib in verschiedenen Bodenkompartimenten ermittelt. In der generativen Phase wurden die Pflanzen *in situ* mittels Applikation einer ^{13}C-Glukose-^{15}N-Harnstofflösung mit der Dochtmethode markiert. Um den Umsatz der Rhizodeposition abzuschätzen, wurden die Mengen an wurzelbürtigem ^{13}C und ^{15}N im Boden sowie in der mikrobiel-

len Biomasse, im extrahierbaren C, im mineralischen N und in den Feinwurzeln bestimmt.

Der Hochbeet-Säulenversuch im Freiland (Versuch IV), diente der Abschätzung der Mengen an Rhizodeposition C und N sowie deren Transfer in verschiedene Bodenkompartimente in verschiedenen Wachstumsphasen der Pflanzen. Die Pflanzen wurden in ungestörten Bodensäulen angezogen die in einem Hochbeet positioniert wurden, und mittels Dochtmethode mit einer ^{13}C-Glukose-^{15}N-Harnstofflösung *in situ* markiert. Auch in diesem Versuch wurden die Mengen an wurzelbürtigem ^{13}C und ^{15}N im Boden, in der mikrobiellen Biomasse, im extrahierbaren C, im mineralischen N und in den Feinwurzeln ermittelt. Aus diesen Ergebnissen sollte der Umsatz der Rhizodeposition in verschiedenen Wachstumsphasen abgeschätzt werden.

Die Ergebnisse aus den Gefäßversuchen zeigen, dass es möglich ist, Pflanzen *in situ* mittels Dochtmethode Zucker-Harnstofflösungen zu applizieren. Die Lösungen wurden durch die Transpiration der Pflanze in den Spross gesogen. Die Pflanzenentwicklung, unter- und oberirdische Trockenmasse sowie die mikrobielle Biomasse wurden weder von der Methode noch von den applizierten Lösungen messbar beeinflusst. Glukose-Harnstofflösungsgemische eignen sich zur simultanen Markierung sowohl von Erbse wie auch von Hafer mit ^{13}C und ^{15}N und erleichtern daher die *in situ* Untersuchung der C- und N-Umsatzprozesse unter Freiland- und Feldbedingungen. In den Säulenversuchen konnten die ober- und unterirdischen Pflanzenteile sowie die labilen Bodenkompartimente mit ^{13}C und ^{15}N angereichert werden. Zudem wurde eine hohe Wiederfindung der Isotope realisiert. Zur Untersuchung des Transfers von wurzelbürtigen Verbindungen in die labilen Bodenkompartimente, muss für die Glukose und den Harnstoff eine hohe Anreicherung gewählt werden.

Die Netto-Rhizodeposition von C und N unter Freilandbedingungen war höher, als die Ergebnisse bisheriger Studien aus Gefäßversuchen unter kontrollierten Bedingungen zeigten. Die Werte aus den beiden Versuchen schwankten jedoch in Abhängigkeit von untersuchter Wachstumsphase und zwischen den Versuchen. Die C-Rhizodeposition war 22.6 bis 58.2 % des Pflanzen-C zum Zeitpunkt der Ernte bei Erbse und 7.9 bis 30.8 % bei Hafer. Die N-Rhizodeposition war 22.9 bis 71.1 % des

Pflanzen-N bei Erbse und 4.5 bis 30.0 % bei Hafer. Der Anteil der Rhizodeposition am Pflanzen-C und -N, war am höchsten in der frühen vegetativen Wachstumsphase bei Erbse und scheint im Laufe der Vegetation abzunehmen. Ebenso wie die Wurzelmasse, war auch der größte Teil der Rhizodeposition in 0-30 cm Bodentiefe.

In Abhängigkeit von der Wachstumsphase wurden 1 bis 31 % des Netto-C und -N der Rhizodeposition in die mikrobielle Biomasse inkorporiert. Zwischen 4 und 40 % der N-Rhizodeposition wurde im mineralischen N wiedergefunden. Die größte Menge C- und N-Rhizodeposition lag allerdings in Feinwurzeln und anderen nicht detailliert untersuchten Bodenkompartimenten vor. Dieses lässt vermuten, dass schon während des Pflanzenwachstums ein Großteil der Rhizodeposition in mikrobiellen Residualprodukten immobilisiert wurde. Unter Erbse stammt ein größerer Anteil der mikrobiellen Biomasse aus der Rhizodeposition als unter Hafer. Auch der Anteil des mineralischen Stickstoffs aus der Rhizodeposition ist unter Erbse höher als unter Hafer. Zusätzlich ist das C/N-Verhältnis der Rhizodeposition und der Grobwurzeln bei Hafer größer als bei Erbse. Dieses lässt daher vermuten, dass die Rhizodeposition von Erbse ein leichter verfügbares Substrat für die Bodenmikroorganismen darstellt als die Rhizodeposition von Hafer.

Rhizodeposition von Erbse und Hafer stellt einen hohen Eintrag an leicht verfügbarem C dar, der die Umsatzprozesse im Boden antreibt und somit die Nährstoffverfügbarkeit entscheidend beeinflusst. Obwohl der Anteil der Rhizodeposition am Pflanzen-N von Erbse und Hafer in einem ähnlichen Bereich liegt, ist aufgrund der höheren N-Aufnahme von Erbse die Menge der N-Rhizodeposition höher. Die Menge an N-Rhizodeposition und der vermehrte Umsatz der Rhizodeposition trägt stark zu der höheren N-Verfügbarkeit nach Erbse im Vergleich zu Hafer bei. Dieses muss zukünftig für die Ermittlung von N_2-Fixierungsleistungen von Leguminosen und N-Bilanzen stärker berücksichtigt werden.

12 Acknowledgements

This thesis would have not been possible without the support of many people. First of all, I would like to thank my supervisors Prof. Dr. Torsten Müller and Prof. Dr. Rainer Georg Jörgensen for providing the opportunity to start this project and for the trust they have put in me during the last three years. They were always receptive to discussions, whenever I asked for it.

I am highly indebted to Gabi Dormann. Without her technical support and the shard coffee breaks, this thesis would have not succeeded. I would like to acknowledge the technical assistance of Christian Maack, Manuel Renneberg, Sarina Weber, Nicole Gauss, Susanne Beck, Judith Isele, Markus Peters, Marie-Catherine Apolot, Luis Eliezer Cruz Bacab, Birge Ude, Julia Hartkemeyer, Elmar Eberhardt, Peer Urbatzka, Eberhardt Kölsch and Rainer Brauckmann. Additionally, I am grateful to my Diploma students Isabel Winter and Elmar Eberhardt for all the useful information, ideas and discussions on the topic of nutrient mobilisation by plants. Furthermore, I thank Jochen Mayer for valuable comments and discussions and Heinz Bruns, Beate Formowitz and Eike Lüdeling for valuable comments on the present thesis and the linguistic help. I would like to thank Thomas Terhoeven-Urselmans for companionship especially during the weeks before submission of our theses. I am indebted to Reinhard Langel and Dr. Jens Dyckmans for the isotope analyses. I further thank Mick Locke for correcting my English in the chapter 5 and 6.

However, the work would have not proceeded that fast without the support of Heinz and Ines, Susi and Markus, and especially the warmth and comfort of my beloved wife Ilka.

This study was financed by the German Research Foundation (MU1711/3-1 and JO362/6-1).

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