Fachgebiet Bodenbiologie und Pflanzenernährung
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Bedeutung der C- und N-Rhizodeposition für das mikrobielle Wachstum und den mikrobiellen Umsatz von C und N in der Rhizosphäre in Abhängigkeit von der Entfernung zur Wurzel

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Mario Freiherr Schenck zu Schweinsberg – Mickan (M. sc. agr.)

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1. Gutachter: Prof. Dr. Rainer Georg Jörgensen

2. Gutachter: Prof. Dr. Torsten Müller

1. Prüfer: Prof. Dr. Bernhard Ludwig

2. Prüfer: Prof. Dr. Michael Wachendorf

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IV Abkürzungen und verwendete Indizes

$^{13}C/^{12}C$	Verhältnis der Kohlenstoffisotope ^{13}C und ^{12}C
$^{15}N/^{14}N$	Verhältnis der Stickstoffisotope ^{15}N und ^{14}N
C	*chem.* Carbon: Kohlenstoff
C_{dfr}	*engl.* Carbon derived from rhizodeposition: Kohlenstoff mit Herkunft aus der Rhizodeposition
CFE	Chloroform-Fumigation-Extraktions Methode
CO_2	*chem.* Carbondioxide: Kohlenstoffdioxid
CO_2-C	Kohlenstoffanteil im Kohlenstoffdioxid
EXT	extrahierbare Fraktion des Bodens
EXT-C	extrahierbarer Kohlenstoff
EXT-N	extrahierbarer Stickstoff
K_2SO_4	*chem.* Kaliumsulfat
MB	*engl.* microbial biomass: mikrobielle Biomasse des Bodens
MBC	Kohlenstoff in der mikrobiellen Biomasse
MBN	Stickstoff in der mikrobiellen Biomasse
MCC	*engl.* Multiple comparisons with a control: Multipler Mittelwertvergleich mit einer Kontrolle
N	*chem.* Nitrogen: Stickstoff
N_{dfr}	*engl.* Nitrogen derived from rhizodeposition: Stickstoff mit Herkunft aus der Rhizodeposition
SOM	*engl.* Soil organic matter: organische Substanz des Bodens
SOM-C	Kohlenstoff in der organischen Bodensubstanz
SOM-N	Stickstoff in der organischen Bodensubstanz
SOM_{next}	nicht extrahierbare organische Substanz des Bodens
TC	*engl.* total carbon: Gesamtkohlenstoff
TM	Trockenmasse
TN	*engl.* total nitrogen: Gesamtstickstoff
WHK_{max}	maximale Wasserhaltekapazität des Bodens
$\delta^{13}C$	Delta Signatur des Kohlenstoffisotops ^{13}C
$\delta^{15}N$	Delta Signatur des Stickstoffisotops ^{15}N

1 Einleitung

Die Pflanze durchdringt mit ihren Wurzeln die *scheinbare* Homogenität des Mikrokosmos Boden in besonderer Weise. BERTIN et al. (2003) beschreiben die Verbindung oder die Schnittstelle zwischen der Pflanze und dem Boden als Ort der höchsten Aktivität innerhalb der Bodenmatrix. Diese auf nur wenige Millimeter um die Wurzel begrenzte Zone wurde von HILTNER 1904 mit dem Begriff der *Rhizosphäre* belegt und hebt so die Besonderheit dieser Zone innerhalb der Bodenmatrix hervor (HARTMANN et al., 2008).

Die komplexen chemischen, biochemischen, physikalischen und mikrobiellen Zusammenhänge in der Rhizosphäre werden durch pflanzeninduzierte Prozesse, die den wurzelnahen Boden beeinflussen, geprägt. Neben dem Nährstoff- und Wasserentzug aus der durchwurzelten Bodenmatrix haben Wurzeln prägenden Einfluss auf die Bodenstruktur, die Durchlüftung, das Bodenleben und auch auf den Status der organischen Bodensubstanz und deren mikrobiellen Auf-, Um- und Abbau. Die Prozesse in der Rhizosphäre sind daher intensives Thema auf den Gebieten bodenbiologischer und pflanzenphysiologischer Forschung, um genauere Kenntnisse über diese Wechselwirkungen zwischen Pflanzen und Boden zu erlangen.

In Bezug auf die mikrobiellen Prozesse in der Rhizosphäre stellen von der Wurzel an den umliegenden Boden ausgeschiedene organische und anorganische Substrate (WHIPPS, 1990; BERTIN et al., 2003; NGUYEN, 2003; UREN, 2007) eine bedeutende Quelle für das mikrobielle Wachstum und den Stoffumsatz in der Rhizosphäre dar (LYNCH und WHIPPS, 1990; MERBACH et al., 1999; HÜTSCH et al., 2002). Diese zusammenfassend als Rhizodeposition beschriebenen Stoffeinträge in den wurzelnahen Boden induzieren zudem qualitative und quantitative Änderungen der mikrobiellen Gemeinschaft in der Rhizosphäre im Vergleich zu unbepflanztem Boden (MARSCHNER et al., 2001; KANDELER et al., 2002; PATERSON et al., 2007). In früheren Untersuchungen wurde als Rhizodeposition im Wesentlichen die Kohlenstoff-Deposition von Wurzeln in den Boden betrachtet (TOAL et al., 2000; KUZYAKOV und DOMANSKI, 2000; KUZYAKOV, 2002; NGUYEN, 2003; KUZYAKOV und SCHNECKENBERGER, 2004). Im Gegensatz dazu fand die Stickstoff-Deposition von Wurzeln in den wurzelnahen Boden bisher nur wenig Beachtung (WICHERN et al., 2008).

Zur Messung der Rhizodeposition wurden in den letzten Jahrzehnten verschiedene ^{13}C-, ^{14}C- und ^{15}N-Tracer-Isotopen-Techniken entwickelt, die es ermöglichen die pflanzenbürtigen Kohlenstoff- und Stickstoffflüsse in den Boden zu untersuchen (JANZEN und BRUINSMA, 1989; MEHARG, 1994; MERBACH et al., 2000; KUZYAKOV und SCHNECKENBERGER, 2004). Zudem stehen Methoden zur Messung von ^{13}C, ^{14}C und ^{15}N in verschiedenen Kohlenstoff- und Stickstoffpools im Boden, wie der organischen Bodensubstanz, den extrahierbaren Fraktionen und der mikrobiellen Biomasse, zur Verfügung (POTTHOFF et al., 2003).

Die Untersuchungen von Prozessen in der Rhizosphäre an der Grenzfläche zwischen Wurzeloberfläche und Boden stoßen dabei jedoch aufgrund der natürlichen Wurzelmorphologie und Beschaffenheit von Böden auf methodische Einschränkungen. HELAL und SAUERBECK (1981) und KUCHENBUCH und JUNGK (1982) haben dazu künstliche Pflanze-Wurzel-Boden-Systeme entwickelt, um Prozesse an der Grenzfläche zwischen Wurzeloberfläche und Boden zu untersuchen. In diesen sogenannten Wurzelgefäß- oder Rhizosphärengefäßsystemen, bei denen die Wurzeln der Versuchspflanzen durch ein feines Gewebe vom angrenzenden Rhizosphärenboden getrennt sind, können Bodenzonen in unterschiedlicher Entfernung von der Wurzeloberfläche (Rhizoplane) untersucht werden. In den folgenden Jahren wurden diese Methoden von YOUSSEF und CHINO (1988) und GAHOONIA und NIELSEN (1991) für weiter reichende Untersuchungen in der Rhizosphäre modifiziert und verbessert.

Diese und ähnliche Systeme wurden inzwischen in einer Vielzahl unterschiedlichster Untersuchungen zur chemischen-, biochemischen- oder physikalischen Charakteristik wurzelbeeinflussten Bodens (z. B.: HELAL und SAUERBECK, 1983; GAHOONIA und NIELSEN, 1991; GAHOONIA und NIELSEN, 1992; MORITSUKA et al. 2000a; MORITSUKA et al. 2000b; WENZEL et al., 2001) und mit Fragestellungen im Bereich der Phytoremediation (z. B.: CORGIÉ et al., 2003; YOSHITOMI und SHANN, 2001; HE et al., 2005) in Abhängigkeit vom Abstand zur Wurzeloberfläche eingesetzt. In nur wenigen Untersuchungen tritt dagegen die Bedeutung und die Verteilung der mikrobiellen Biomasse (HELAL and SAUERBECK, 1986; YOUSSEF et al., 1989; DE NEERGAARD and MAGID, 2001) oder die mikrobielle Zusammensetzung und Diversität in der Rhizosphäre in den Vordergrund (KANDELER et al., 2002; HE et al., 2007).

Das Wissen über die mikrobiellen Prozesse in der Rhizosphäre und über die Bedeutung der von den wachsenden Pflanzen über die Wurzel an den umliegenden Boden, der Rhizosphäre, abgegebenen C- und N-Rhizodeposition sowie deren Schicksal im Boden ist noch immer weitgehend begrenzt (UREN, 2007).

Thema dieser Arbeit war es daher, die Bedeutung der C- und N-Rhizodeposition im wurzelnahen Boden auf das Wachstum der mikrobielle Biomasse und die mikrobiellen Umsatzprozesse von C und N in unterschiedlichem Abstand zur Wurzeloberfläche zu untersuchen.

2 Problemstellung und Zielsetzung

Rhizodeposite stellen eine bedeutende Quelle für das mikrobielle Wachstum und den Stoffumsatz in der Rhizosphäre dar. Über den Umsatz von Kohlenstoff und Stickstoff mit Herkunft aus der Rhizodeposition in der Rhizosphäre in Abhängigkeit vom Abstand zur Wurzeloberfläche (Rhizoplane) ist bisher jedoch wenig bekannt.

Ziel der Untersuchungen war es daher, die wurzelbürtigen C- und N-Flüsse (Rhizodeposite) in den Boden zu quantifizieren und deren Einbau in verschiedene Nährstoffpools im Boden in Abhängigkeit von der Entfernung zur Wurzeloberfläche zu verfolgen. Im Mittelpunkt der Betrachtung standen dabei der Einbau des wurzelbürtigen C und N in die mikrobielle Biomasse, der mikrobielle Umsatz des wurzelbürtigen C und N sowie die Bedeutung der extrahierbaren Fraktion und der nicht extrahierbaren organischen Bodensubstanz als Speicher für C und N in definierter Entfernung zur Wurzeloberfläche.

Die Ziele der vorliegenden Arbeit erstreckten sich auf folgende Schwerpunkte:

- Entwicklung der methodischen Voraussetzungen zur simultanen ^{13}C- und ^{15}N-Doppelmarkierung von Pflanzen zur Verfolgung von ^{13}C- und ^{15}N-Rhizodepositen im Boden in definiertem Abstand zur Rhizoplane verschiedener Pflanzen.

- Messung des Konzentrationsgradienten des Rhizodepositions-C und -N im Boden (total-C und total-N im Boden mit Herkunft aus der Rhizodeposition) in definiertem Abstand zur Rhizoplane verschiedener Pflanzen.

- Quantifizierung des Transfers von Rhizodepositions-C und -N in die mikrobielle Biomasse (SMB-C und SMB-N), in die nicht extrahierbare organische Bodensubstanz (SOM_{next}) und in die extrahierbaren C und N Fraktionen (EXT-C und EXT-N) im Boden in Abhängigkeit vom Abstand zur Rhizoplane verschiedener Pflanzen.

- Quantifizierung der Mineralisation und Immobilisation des bodenbürtigen und des wurzelbürtigen C und N im Boden in Abhängigkeit vom Abstand zur Rhizoplane verschiedener Pflanzen.

Als methodische Voraussetzung wurde dazu ein Rhizosphärensystem nach dem Vorbild des von GAHOONIA und NIELSEN (1991) entwickelten Rhizosphärensystems konzipiert und angefertigt, welches sich für den Einsatz in ^{13}C- und ^{15}N-Doppelmarkierungsexperimenten eignet. Das System wurde zudem an eine Bodenbeprobungstechnik nach einem Vorbild von FITZ et al. (2003) angepasst, die es ermöglicht, frische Bodenproben in definiertem Abstand zur Rhizoplane zu gewinnen, ohne, wie bei bisherigen Untersuchungen gebräuchlich, aufwendige und die Bodeneigenschaften beeinflussende Gefriermikrotomtechniken einzusetzen (vgl.: GAHOONIA und NIELSEN, 1991; DE NEERGAARD and MAGID, 2001; KANDELER et al., 2002).

(siehe Kapitel 3: Generelle Methodik)

Im ersten Versuch wurde das Rhizosphärensystem in einem ^{13}C- und ^{15}N-Doppelmarkierungsexperiment zur Untersuchung der Verteilung und der Bedeutung des Rhizodeposition-C und -N in der Rhizosphäre von *Lolium perenne* eingesetzt. Dabei wurde die Eignung der Markierungsmethode und die Verteilung des Rhizodepositions-C und -N im Gesamtboden, der mikrobiellen Biomasse und der extrahierbaren Fraktion in Abhängigkeit von der Entfernung zur Rhizoplane von *Lolium perenne* untersucht.

(siehe Kapitel 4: Fate of ^{13}C- and ^{15}N-labelled rhizodeposition of *Lolium perenne* as function of the distance to the root surface)

In zwei weiteren Versuchen wurde das Rhizosphärensystem in ^{13}C- und ^{15}N-Doppelmarkierungsexperimenten zur Untersuchung der Verteilung und der Bedeutung des Rhizodepositions-C und -N im Vergleich von Hafer (*Avena sativa*) und Weizen (*Triticum aestivum*) eingesetzt. Bei diesen Untersuchungen wurde der Transfer von Rhizodepositions-C und -N in die mikrobielle Biomasse, in die nicht extrahierbare organische Bodensubstanz und in die extrahierbaren C und N Fraktionen im Boden in Abhängigkeit vom Abstand zum Wurzelraum von Hafer und Weizen untersucht. Dazu wurden die C- und N-Umsatzprozesse in Rhizosphärenbodenproben mit zunehmenden Abstand zur Wurzel in Inkubationsexperimenten (42 Tage, 15°C) untersucht. Zur Messung des C-Umsatzes aus der Rhizodeposition und mit Herkunft aus der nativen organischen Bodensubstanz wurde

die mikrobielle CO_2-C und $^{13}CO_2$-C Produktion in den Rhizosphärenbodenproben mit zunehmenden Abständen zur Rhizoplane gemessen. Dazu wurde eine Methode konzipiert, die es ermöglicht die mikrobielle CO_2-C Produktion im Boden und das CO_2-C Isotopenverhältnis ($^{12}C/^{13}C$) in den zur Verfügung stehenden geringen Mengen an Rhizosphärenboden in Inkubationsexperimenten zu messen.

(siehe Kapitel 5: C and N derived from rhizodeposition: Relation to microbial growth and C and N turnover as a function of their spatial distribution within the rhizosphere)

3 Generelle Methodik

3.1 Einführung

Die Untersuchung von Prozessen in der Rhizosphäre an der Grenzfläche zwischen Wurzeloberfläche und Boden stößt aufgrund der natürlichen Wurzelmorphologie und Beschaffenheit von Böden auf methodische Einschränkungen. In zahlreichen Untersuchungen an der Grenzfläche zwischen Wurzeloberfläche und Boden wurden daher künstliche Pflanze-Wurzel-Boden Systeme eingesetzt, um die Prozesse im wurzelnahen Boden, der Rhizosphäre, zu untersuchen (HELAL und SAUERBECK, 1981; HELAL und SAUERBECK, 1986; YOUSSEF et al., 1989; GAHOONIA und NIELSEN, 1991; GAHOONIA und NIELSEN, 1992; MORITSUKA et al. 2000a; MORITSUKA et al. 2000b; DE NEERGAARD und MAGID, 2001; YOSHITOMI und SHANN, 2001; WENZEL et al., 2001; KANDELER et al., 2002; CORGIÉ et al., 2003; HE et al., 2005; HE et al., 2007). In den von HELAL und SAUERBECK (1981) und KUCHENBUCH und JUNGK (1982) entwickelten Methoden werden die Wurzeln der Versuchspflanzen durch ein feines Gewebe (Gaze) vom angrenzenden Rhizosphärenboden getrennt und ermöglichen so die Untersuchung des wurzelbeeinflussten Rhizosphärenbodens ohne eine direkte Durchwurzelung des zu untersuchenden Bodens. In den folgenden Jahren wurden diese Methoden von YOUSSEF und CHINO (1988) und GAHOONIA und NIELSEN (1991) für weiter reichende Untersuchungen in der Rhizosphäre modifiziert und verbessert. Verschiedene Separations- und Beprobungstechniken erlauben zudem die Untersuchung des Rhizosphärenbodens in definiertem Abstand zur künstlichen Wurzeloberfläche (Rhizoplane) (KUCHENBUCH und JUNGK, 1982; YOUSSEF und CHINO, 1988).

Mit verschiedenen Mikrotom-Gefrierschnitt-Techniken wird dabei eine hohe räumliche Auflösung in definiertem Abstand zur Rhizoplane in verschiedenen Untersuchungen angestrebt (KUCHENBUCH und JUNGK, 1982; GAHOONIA und NIELSEN, 1991; WENZEL et al., 2001; KANDELER et al., 2002; KUZYAKOV et al., 2003; SAUER et al., 2006). Nur in wenigen Untersuchungen wurden dagegen Schnitttechniken eingesetzt, die es erlauben, den Rhizosphärenboden in ungefrorenen Zustand zu schneiden (GAILLARD et al., 1999; NURUZZAMAN et al. 2006). Die Probenahme von gefrorenen Bodenproben mittels Mikrotom-Gefrierschnitt-Techniken kann jedoch Auswirkungen auf die mikrobielle Biomasse im Boden aufgrund des Gefrierverfahrens selbst (DE NEERGAARD und MAGID, 2001; FRIEDEL et al., 2003) und kann zudem Auswirkungen auf verschiedene chemische und physikali-

sche Bodeneigenschaften wie die Absorptionskapazität aufgrund der Veränderung der spezifischen Oberfläche der Bodenpartikel nach dem Schneiden haben (FITZ et al., 2003).

Zur Messung der Rhizodeposition wurden in den letzten Jahrzehnten verschiedene ^{13}C-, ^{14}C- und ^{15}N-Tracer-Isotopen-Techniken entwickelt, die es ermöglichen die pflanzenbürtigen Kohlenstoff- und Stickstoffflüsse in den Boden zu untersuchen (JANZEN und BRUINSMA, 1989; MEHARG, 1994; MERBACH et al., 1999; KUZYAKOV und SCHNECKENBERGER, 2004). Zudem stehen Methoden zur Messung von ^{13}C, ^{14}C und ^{15}N in verschiedenen Kohlenstoff- und Stickstoffpools im Boden, wie der organischen Bodensubstanz, den extrahierbaren Fraktionen und der mikrobiellen Biomasse, zur Verfügung (POTTHOFF et al., 2003). In bisherigen Untersuchungen wurden Tracer-Isotopen-Techniken zur simultanen Verfolgung der C- und N-Rhizodeposition in den verschiedenen C- und N-Nährstoffpools und in den mikrobiellen Umsatzprozessen in der Rhizosphäre in Abhängigkeit vom Abstand zur Wurzel nicht eingesetzt.

Die Untersuchungen von mikrobiellen Prozessen in Wechselwirkung mit den Prozessen der C- und N-Rhizodeposition von Pflanzen in verschiedenen Nährstoffpools in der Rhizosphäre stoßen dabei an bestimmte methodische Anforderungen mit folgenden Problemen:

1. Der Einsatz von Tracer-Isotopen-Techniken zur Isotopenmarkierung der C- und N-Rhizodeposition muss über Doppelmarkierung (C und N) erfolgen, ohne den Rhizosphärenboden während der Pflanzenmarkierungsphase mit Isotopen zu verunreinigen.

2. Es muss genügend Rhizosphärenboden zur Verfügung stehen, um die C- und N-Flüsse in den verschiedenen Bodenpools untersuchen zu können.

3. Die Beprobung des Rhizosphärenbodens muss eine ausreichende räumliche Auflösung im mm-Bereich oder weniger gewährleisen, die es ermöglicht repräsentative räumliche Gradienten von der Wurzeloberfläche bis in den nicht wurzelbeeinflussten Boden zu untersuchen.

4. Die Beprobungstechnik des Rhizosphärenbodens darf keinen direkten Einfluss auf die zu untersuchenden Kenngrößen wie die mikrobielle Biomasse und andere organische Verbindungen im Rhizosphärenboden haben.

Den Anforderungen entsprechend wurde ein Rhizosphärensystem konstruiert, in dem die Pflanzen mittels Nährlösung ^{15}N-markiert und in Verbindung mit einer Markierungskammer simultan $^{13}CO_2$-markiert werden können. Angepasst an dieses Rhizosphärensystem, wurde eine Schneidapparatur zur Beprobung von frischem Rhizosphärenboden in definiertem Abstand zur Rhizoplane nach einem Vorbild von FITZ et al. (2003) konstruiert.

3.2 Pflanzenanzucht und -markierung in den Rhizosphärensystemen

Zur Pflanzenanzucht und Markierung wurde das von GAHOONIA und NIELSEN (1991) entwickelte Rhizosphärensystem den Versuchsanforderungen entsprechend modifiziert. Das modifizierte System besteht aus einer Anzuchtbox (H x B x T, 10 x 21 x 6 cm) mit einem Dochtsystem zur Bewässerung und Nährstoffversorgung der Versuchspflanzen und einer zweiteiligem Rhizosphärenbodenbox, welche aus einem Wurzelgefäßteil (1,2 x 21 x 6 cm) und einem Rhizosphärenbodenteil (3 x 21 x 6 cm) besteht (Abb. 3-1).

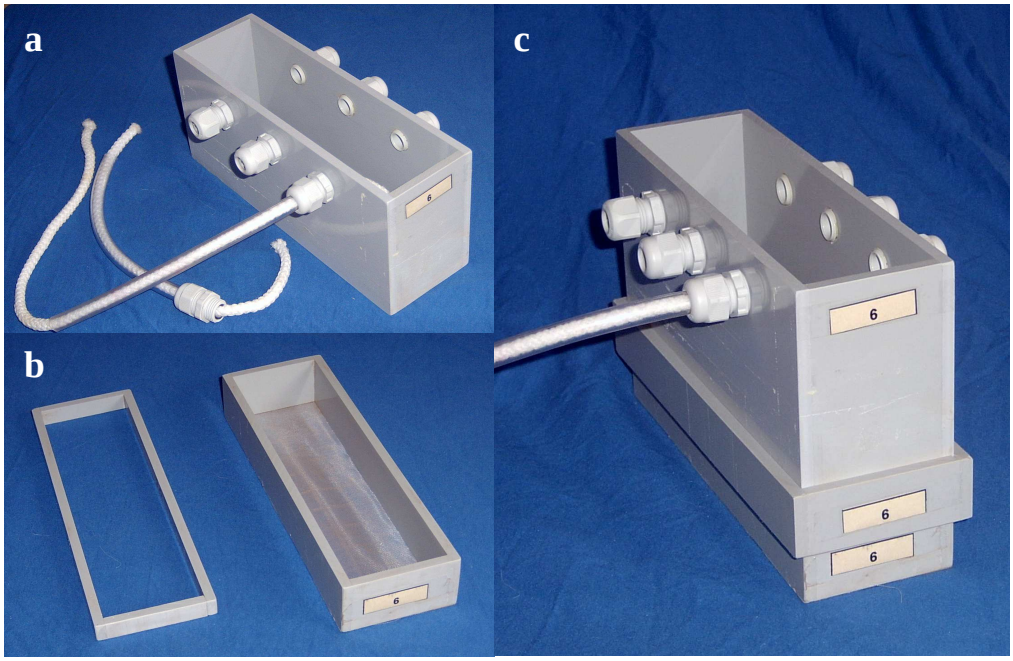

Abb. 3-1: Modifiziertes Rhizosphärensystem nach GAHOONIA und NIELSEN (1991) bestehend aus Anzuchtbox mit Dochtsystem (a) und zweiteiliger Rhizosphärenbodenbox (b: links: Wurzelgefäßteil und rechts: Rhizosphärenbodenteil). Mit Fixierrahmen zusammengesetztes Rhizosphärensystem (c).

Die Unterseite des Rhizosphärenbodenteils wird durch ein Nylongewebe (200µm) verschlossen. Die einzelnen Teile des Systems sind über einen verschiebbaren Fixierrahmen horizontal miteinander zu verbinden (Abb. 3-1). Die Pflanzenanzucht in den Anzuchtboxen erfolgte entweder in einem Gemisch aus Sand und Kies oder Sand und Perlite. Die Gewichtsverhältnisse der Gemische betrug jeweils 2:1. Zur Pflanzenanzucht wurden die Anzuchtboxen an der Unterseite mit einer 500 µm Gaze verschlossen und mit 1600 g des Anzuchtsubstrates befüllt. Die Versuchspflanzen wurden anschließend direkt in das Anzuchtsubstrat ausgesät und kultiviert, bis die Gefäße vollständig durchwurzelt waren (ca. 2-4 Wochen). In dieser Zeit erfolgte die Bewässerung und Nährstoffversorgung der Pflanzen über das Dochtsystem (Abb. 3-2) mit Hoagland's Nährlösung (SCHALLER, 2001). Während dieser Periode wurden die Versuchspflanzen ohne Kontakt zum späteren Probenboden ^{13}C- und ^{15}N-markiert. Die ^{15}N-Markierung der Pflanzen erfolgte mit modifizierter Hoagland's Nährlösung (Tab. 3-1).

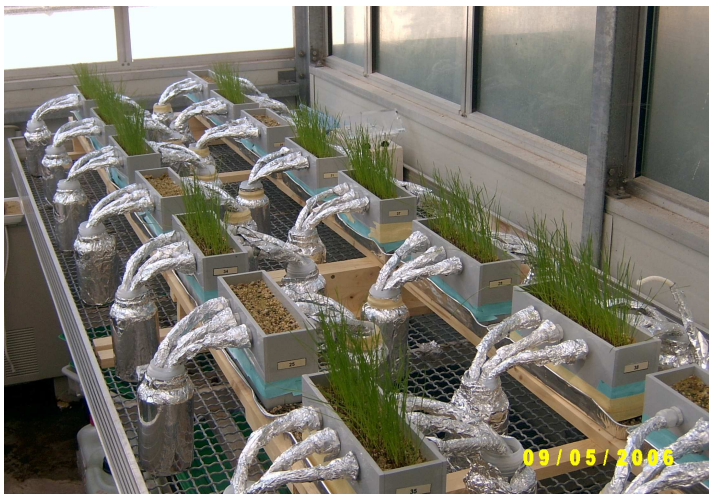

Abb. 3-2: Anzucht von *Lolium perenne* in den Anzuchtboxen des Rhizosphärensystems mit Dochtsystem zur Bewässerung und Nährstoffversorgung der Versuchspflanzen.

Tab. 3-1: Zusammensetzung der ^{15}N-Nährlösung (modifiziert nach Hoagland's).

Hauptnährstoffe	**Konzentration in der Nährlösung [mg l^{-1}]**
KH_2PO_4	136
NH_4NO_3 (10.26atom% ^{15}N)	160
K_2SO_4	174
KNO_3	303
$MgSO_4$ x 7 H_2O	492
$Ca(NO_3)_2$ x 4 H_2O	944
Fe(III)-Na-EDTA	110
Mikronährstoffe	
H_3BO_4	0,062
$MnSO_4$	0,085
$ZnSO_4$	0,144
$CuSO_4$	0,050
$(NH_4)_6Mo_7O_{24}$	0,012

Die ^{13}C-Markierung der Pflanzen erfolgte in einer gasdichten Markierungskammer aus Plexiglas (120 x 140 x 80 cm) mit einer Kapazität von bis zu 30 Rhizosphärensystemen (Abb. 3-3). Dazu wurden die Gefäße in die Kammer überführt und in Abhängigkeit zur angestrebten ^{13}C-Markierung innerhalb der geschlossenen Kammer eine definierte Menge an $NaHCO_3$ mit einem Gehalt von 99atom% ^{13}C in einem 25 ml Becherglas mit 12 ml 7.5 M Milchsäure versetzt. Die Pflanzen verblieben anschließend je nach Versuchsfrage mehrere Tage in der geschlossenen Kammer. Ein Ventilationssystem gewährleistete eine kontinuierliche Luftbewegung in der Kammer. Je nach angestrebter Markierungshöhe der Versuchspflanzen wurde die Prozedur mehrmals wiederholt. Zwischen den einzelnen Markierungsperioden wurde die Kammer geöffnet und die Gefäße systematisch umgestellt. Während der ^{13}C-Markierung in der Kammer wurden die Versuchspflanzen kontinuierlich mit Nährlösung versorgt (Abb. 3-3).

Abb. 3-3: Anzuchtboxen des Rhizosphärensystems mit Haferpflanzen in geschlossener Markierungskammer (links) während einer $^{13}CO_2$-Markierungsperiode. Innerhalb der Kammer werden die Pflanzen über ein Dochtsystem kontinuierlich mit modifizierter ^{15}N-Hoagland's Nährlösung versorgt (rechts).

Im Anschluss an die Markierungsperiode wurden die Dochte aus den Gefäßen entfernt und überschüssige Nährlösung mit mindestens 4 l H_2O Gefäß$^{-1}$ aus dem Anzuchtsubstrat und von den Wurzeln gespült. Nach dem vollständigem Abtropfen der Gefäße wurden die Anzuchtboxen auf die mit dem Fixierrahmen horizontal verbundenen bodengefüllten Rhizosphärenbodenboxen gesetzt. Dazu wurde der Testboden zuvor nach vollständiger Entfernung aller sichtbaren organischen Bestandteile luftgetrocknet und anschließend gesiebt (1 mm). Die Rhizosphärenbodenteile der Rhizosphärenbodenboxen wurden mit jeweils 600 g des Testbodens befüllt. Während des Befüllens wurde der Boden mit Hilfe eines Spatels verdichtet, um eine gleichmäßige Füllung und eine ebene Oberfläche des Bodens in den Gefäßen zu erreichen. Die Dichte des Testbodens betrug 1,3 g/cm^3. Anschließend wurden die Rhizosphärenbodenteile mit einer feinen Nylongaze (30 µm) verschlossen und die Wurzelgefäßteile mit dem Fixierrahmen auf dem unteren Gefäßteil fixiert. Die Wurzelgefäßteile wurden mit jeweils 210 g des Testbodens befüllt. Die gefüllten Rhizosphärenbodenboxen wurden auf poröse Mineralmatten (Fa. AGRIMEDIA, Eisenberg, Deutschland) in wassergefüllte Schalen (3,5 x 24 x 7,5 cm) gestellt. Die Bewässerung des Testbodens erfolgte so durch das in der porösen Mineralmatte kapillar aufsteigende Wasser. Die Höhe der Mineralmatte (2-5 cm) und der Wasserstand in den Schalen wurden so gewählt, dass durch aufsteigendes Wasser der Wassergehalt des Testbodens bei 50-60% der max. Wasserhaltekapazität des Bodens gehalten wurde. Eine Bewässerung von oben in die Gefäße

erfolgte nicht. Dadurch sollte gewährleistet werden, dass keine Auswaschung von Rhizodepositen aus dem Wurzelgefäßteil in das Rhizosphärenbodenteil stattfindet.

In Abhängigkeit von der Pflanzenart, bildeten die aus den Anzuchtgefäßen in die Wurzelgefäßteile wachsenden Wurzeln auf der Gaze nach ca. 2-4 Wochen eine geschlossene Wurzelmatte (Rhizoplane) aus (Abb. 3-4). Aufgrund der gewählten Maschenweite der Gaze konnten nur Wurzelhaare nicht jedoch Wurzeln die Gaze durchdringen. Zur Beprobung des Rhizosphärenbodens wurden die Gefäße vorsichtig auseinandergebaut und die Wurzelmatte mit der Gaze vom Rhizosphärenboden abgehoben.

Abb. 3-4: Geschlossene Wurzelmatte von *Lolium perenne* nach 21 Tagen

3.3 Probenahme des Rhizosphärenbodens

Der Rhizosphärenboden wurde in definierten Abständen zur künstlichen Rhizoplane (Gaze) untersucht. Dazu wurden mit einer dem Rhizosphärensystem angepassten Schneidapparatur Bodenschichten in definiertem Abstand von der künstlichen Rhizoplane geschnitten (Abb. 3-5). Als Vorbild für diese Apparatur diente ein vom Institut für Bodenkunde in Wien entwickeltes Schneidsystem (FITZ et al., 2003). Die Schneidapparatur ermöglicht die Probenahme von frischen Bodenproben, was einen deutlichen Vorteil bei bodenmikrobiologischen Fragestellungen gegenüber der herkömmlichen Beprobungstechnik mittels Gefriermikrotomschneidtechnik hat (STENBERG et al., 1998; WINTER et al., 1994; PESARO et al., 2003). Das Einfrieren und auch das Auftauen von Boden ist ein gravierender Eingriff in die physikalische und biologische Beschaffenheit des Bodens (WINTER et al., 1994). Untersuchungen von FRIEDEL et al. (2003) und FITZ et al. (2003) zeigten zudem einen deutlichen Einfluss von Gefrier- und Schneidtechniken auf bodenmikrobielle Kenngrößen.

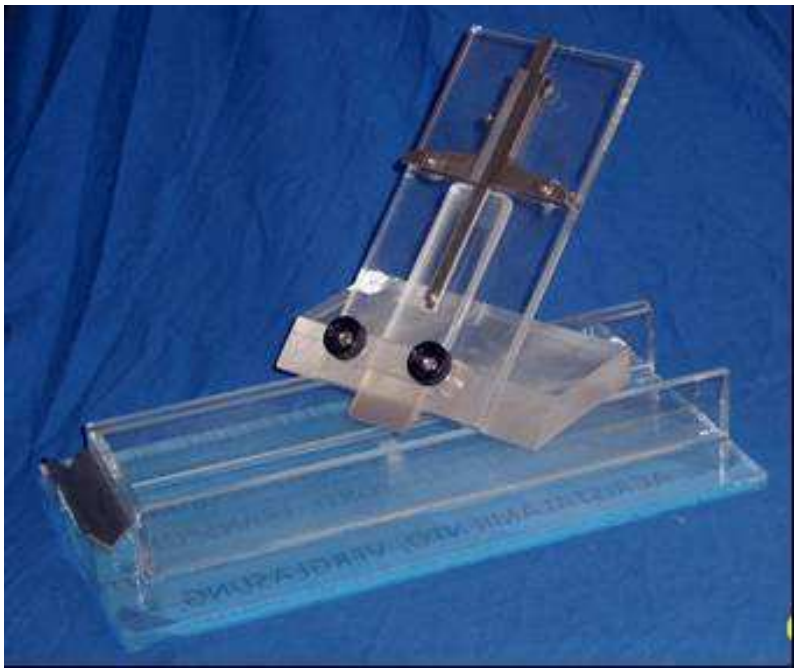

Abb. 3-5: Schneidapparatur zur Probenahme von Rhizosphärenboden in definiertem Abstand zur künstlichen Rhizoplane nach einem Vorbild von FITZ et al. (2003).

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4 Fate of ^{13}C- and ^{15}N-labelled rhizodeposition of *Lolium perenne* as function of the distance to the root surface

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Mario Schenck zu Schweinsberg-Mickan [1)2)]*, Rainer Georg Joergensen [1)],

Torsten Müller [2)]

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

[2)] Department of Plant Nutrition, Universität Hohenheim, Fruwirthstraße 20, 70593 Stuttgart; Germany

* Corresponding author; Tel +49-5542 98 1574; E-mail: mschenck@uni-kassel.de

4.1 Abstract

A greenhouse rhizobox experiment was carried out to quantify the incorporation of ^{13}C- and ^{15}N-labelled rhizodeposits into different soil pools, especially into the rhizosphere microbial biomass, with increasing distances to the root surface of *Lolium perenne*. Five layers were analysed over 0-4.2 mm distance to an artificial root surface. C and N derived from rhizodeposition were 4.2% of total C and 2.8% of total N in soil at 0-1.0 mm distance and decreased rapidly with increasing distance. Microbial biomass C and N increased significantly towards the roots. At 0-1.0 mm distance microbial biomass C and N accounted for 66% and 29% of C and N derived from rhizodeposition, respectively. These percentages declined with increasing distance to the roots, but were still traceable up to 4.2 mm distance. Only small amounts of root released C and N were found in the 0.05 M K_2SO_4-extractable fraction. Extractable C and N derived from rhizodeposition varied around means of 4% of total C and N derived from rhizodeposition and increased only marginally with increasing distance to the roots. C derived from rhizodeposition in the non-extractable soil organic matter increased from 65 to 89% of total C derived from rhizodeposition at 0-3.4 mm distance. Conversely, microbial biomass C derived from rhizodeposition decreased from 33 to 4%. N derived from rhizodeposition in the non-extractable soil organic matter increased from 61 to 79% of total N derived from rhizodeposition at 0-2.6 mm distance, followed by a decline to roughly 55% in the two outer layers. Microbial biomass N decreased from 37 to 16% at 0-2.6 mm distance, followed by an increase to roughly 41% in the two outer layers. The C/N ratio of total C and N derived from rhizodeposition as well as that of extractable C and N derived from rhizodeposition increased with increasing distance to the roots to values above 30. In contrast, the C/N ratio of incorporated rhizodeposition C and N into the microbial biomass decreased to values less than 5 at 2.6-4.2 mm distance. The data indicate differential microbial response to C and N derived from rhizodeposition at a high spatial resolution from the root surface. The turnover of C and N derived from rhizodeposition in the rhizosphere as a function of the distance to the root surface is discussed.

Keywords: ^{13}C; ^{15}N; Rhizodeposition; Rhizodeposits; Soil microbial biomass; Rhizobox

4.2 Introduction

In the rhizosphere, i.e. the soil surrounding plant roots, microbial growth and turnover processes are fuelled by root-derived substrates, so-called rhizodeposits (LYNCH and WHIPPS, 1990; HÜTSCH et al., 2002; WICHERN et al., 2008). This term comprises a highly diverse variety of root exudates (diffusates, secretions and excretions) as well as lysates and dead root cell material (PATERSON, 2003; UREN, 2007). Earlier investigations were mainly focused on C rhizodeposition (TOAL et al., 2000; KUZYAKOV and DOMANSKI, 2000; KUZYAKOV, 2002; NGUYEN, 2003; KUZYAKOV and SCHNECKENBERGER, 2004) and less often on N rhizodeposition (WICHERN et al., 2008). However, significant amounts of rhizodeposition N, including inorganic N (NO_3, NH_4) and organic N-compounds (amino acids, different phytohormones, vitamins and allelochemicals), released into the rhizosphere by legumes, cereals and grasses were found in several studies (JANZEN, 1990; TOUSSAINT et al., 1995; JENSEN, 1996; MAYER et al., 2003; WICHERN et al., 2007). The amounts of rhizodeposition N are still poorly quantified and estimations vary widely from 4% up to 71% of the total assimilated plant N, depending on plant species, plant physiological status and varying conditions (WICHERN et al., 2008).

Significant amounts of the rhizodeposition are transferred into the organic matter pool during plant growth (SCHMIDTKE, 2005; WICHERN et al., 2007). The fate of rhizodeposits within the rhizosphere is determined by their availability for microbial breakdown and mobilisation/immobilisation patterns, depending on substrate quality. The extension of these effects induced by substrate input from rhizodeposition (i.e. the spatial distribution) in the rhizosphere might be explained as a function of the interactions between substrate solubility, mobility and availability for microbial breakdown. On the one hand, the water-soluble fraction underlies rapid microbial decomposition and immobilisation close to the root surface or directly on the rhizoplane (NEUMANN and RÖMHELD, 2007). On the other hand, more recalcitrant components with a larger C/N ratio diffuse into the more remote soil (UREN, 2007).

Rhizodeposition is, thus, identified as a strong determinant for microbial community development in planted soils (PATERSON et al., 2007). In accordance with this view, KANDELER et al. (2002) detected clear gradients of bacterial community composition between 0 to 2.2 mm and 2.2 to 5.0 mm distance from the root surface of maize, which were also significant in comparison to an unplanted soil. Changes in the functional diversity of

the bacterial communities extend up to several millimetres from roots (KANDELER et al., 2002). However, these changes might be explained as a response of the microbial community to rhizodeposit input, whereas the composition of the substrate presumably also had a decisive impact on the microbial changes within the rhizosphere soil. Less is known about the interdependency of the substrate C and N input by rhizodeposition and the impact on microbial growth and turnover within the rhizosphere. Therefore, simultaneous investigations on the fate of rhizodeposition C and N are necessary for a better understanding of turnover processes occurring within the rhizosphere.

For estimating rhizodeposition C and N, ^{13}C, ^{14}C and ^{15}N isotope-tracing techniques were developed over the past decades for different soil pools such as total and extractable soil organic matter or microbial biomass (POTTHOFF et al., 2003). Due to methodological restrictions, investigations on the spatial distribution of processes at the root-soil interface mostly focus on artificial plant-root-soil systems called rhizobox or rhizosphere systems. HELAL and SAUERBECK (1981) and KUCHENBUCH and JUNGK (1982) introduced these methods, in which roots are separated from soil by a fine mesh for studying rhizosphere processes in soil layers at different proximities to roots. These separation techniques were improved for a wide range of investigations on plant-soil interactions (YOUSSEF and CHINO, 1988; GAHOONIA and NIELSEN, 1991; WENZEL et al., 2001). Only a few investigations have studied the distribution of microbial biomass in the rhizosphere as a function of the distance to the root surface, due to severe methodological problems (HELAL and SAUERBECK, 1986; YOUSSEF et al., 1989; DE NEERGAARD and MAGID, 2001). Sampling of soil layers at high spatial resolution is mainly based on slicing of frozen rhizosphere soil by microtome sectioning (KUCHENBUCH and JUNGK, 1982; GAHOONIA and NIELSEN, 1991; WENZEL et al., 2001; KANDELER et al., 2002; KUZYAKOV et al., 2003; SAUER et al., 2006). However, this type of sampling leads to erroneous reductions in microbial biomass C and microbial biomass N (DE NEERGAARD and MAGID, 2001; FRIEDEL et al., 2003) and also causes artefacts in different soil chemical and physical properties (FITZ et al., 2003). Only very few studies have used cutting devices for slicing of non-frozen soil (BADALUCCO et al., 1996; NURUZZAMAN et al., 2006). There are no reports of a rhizobox system being used in combination with ^{13}C and ^{15}N plant labelling and slicing of non-frozen soil.

The basic aim of the present experiment was to quantify the incorporation of ^{13}C- and ^{15}N-labelled rhizodeposits into different soil pools, especially into the rhizosphere microbial

biomass. The hypothesis was that the spatial distribution and dynamics of rhizodeposits in the different pools differ between the ^{13}C- and ^{15}N-labelled fractions as a function of the distance to the root surface. For investigating this hypothesis, the following four problems must be solved: (1) Rhizodeposition has to be labelled via double labelling (C and N) of a plant without contaminating the rhizosphere soil during the plant-labelling period. (2) Sufficient rhizosphere soil is required for investigations on C and N in different soil pools. (3) The sampling method of rhizosphere soil must have an adequate spatial resolution within the millimetre scale to investigate gradients from the root surface to the bulk soil, and (4) the direct effect of the sampling method of rhizosphere soil on microbial biomass and organic compounds in soil has to be minimised. Consequently, the rhizobox system of GAHOONIA and NIELSEN (1991) was modified for double labelling of plants by application of ^{15}N-nutrient and $^{13}CO_2$ labelling in a closed chamber and combined with slicing of fresh soil with increasing distance to an artificial root surface as proposed by FITZ et al. (2003).

4.3 Materials and Methods

4.3.1 Soil and growing conditions

Soil (clayey silt soil, pH-H_2O of 6.8, total C 1.01%, total N 0.09%) from the research farm Hessian State Farm Frankenhausen of the University of Kassel was collected in February 2006 from the top 10 cm of arable land. After removing visible plant debris, the soil was incubated for 2 weeks at 15°C, then air-dried and sieved to 1 mm and stored in the dark at 4°C until the experiment started. The experiment was conducted in a conditioned greenhouse cabin (temperature: 17 ± 2°C night, 22 ± 3°C day; light exposure day^{-1}: 40.8 lm m^{-2} s).

4.3.2 Rhizobox system

Modified three-compartment rhizoboxes based on the system developed by GAHOONIA and NIELSEN (1991) were placed in a gas-tight labelling chamber (L × W × H = 130 × 80 × 120 cm) made of Plexiglas with a capacity for 30 labelling compartments (compartment 1: 22 × 6 × 10 cm). Inside this chamber, the plants were $^{13}CO_2$-C labelled and simultaneously labelled via a wick-system with ^{15}N-nutrient solution. In the upper labelling compartment, the plants were pre-cultivated in a sand/grit mixture and labelled without contact to the

later test soil. After two 4-day labelling periods, the labelling compartments were taken out of the chamber. The 500 µm meshes underneath the labelling compartments and the wick system were removed. Then, the labelling compartments were tightly attached to the rooting and rhizosphere compartments, which were filled with unlabelled test soil. The rooting soil compartment (compartment 2: 22 × 6 × 1 cm) and the rhizosphere soil compartment (compartment 3: 22 × 6 × 3 cm) were horizontally separated by a 30 µm nylon gauze. Roots penetrated the 1 cm soil layer of the rooting compartment and formed a closed root mat above the nylon gauze, which could be penetrated only by root hairs. Both compartments were fixed by a removable frame, which also fixed the labelling compartment later transferred onto the rooting/rhizosphere compartment. The soil in the compartments was wetted via capillary action from watered porous mineral mats (thickness: 5 cm), which were placed under the boxes in water filled dishes, where the water was regularly replenished.

4.3.3 Plant cultivation, labelling and sampling

Twenty-eight labelling compartments closed at the bottom by a 500 µm mesh were filled with 1600 g of a sand/grit mixture (ratio 2/1) and wetted to 100% water holding capacity. 0.8 g *Lolium perenne* were sown into each of 16 compartments, 12 compartments were left unplanted. Ten days after complete germination of the seeds, 12 compartments with *L. perenne* and the unplanted compartments were transferred into the labelling chamber and supplied via wicks with a modified Hoaglands nutrient solution including 2.66 atom% ^{15}N (as NH_4NO_3 10.26atom% ^{15}N). Four reference compartments with *L. perenne* were kept outside of the chamber and supplied with unlabelled nutrient solution. Two beakers (25 ml) each containing 1.2 g $NaHCO_3$ with 99 atom% ^{13}C were placed inside the chamber. The chamber was closed for 4 days and 12 ml 7.5 M lactic acid were added through a pipe to one of the beakers. After 24 h, again 12 ml 7.5 M lactic acid were added through a pipe to the second beaker. After this first 4-day labelling period, the compartments were relocated inside the chamber and a second 4-day labelling period was started as described above. Then, the compartments were taken out of the chamber and the sand/grit mixture was carefully rinsed with 4 l H_2O to wash out potential adherent labelled nutrient solution from roots and the substrate. After complete drainage, the substrate labelling compartments were tightly attached to the rooting and rhizosphere compartments, filled with 210 and 900 g air-

dry test soils, respectively. Plants and soil were collected for analyses 25 days after labelling.

Plants were immediately separated into above ground plant material and roots creating the artificial root mat. Roots growing inside the labelling compartment were not analysed. All plant material was dried at 60°C until constant weight for analyses. For determination of soil moisture and soil chemical analyses, aliquots of 5 g of each soil layer were dried at 105°C until constant weight. For each plant and soil analysis, samples of 3 compartments were merged. The root mats including the gauze were carefully removed and the soil was sliced with a modified cutting device as described by FITZ et al. (2003) at 1.0, 1.8, 2.6, 3.4 and 4.2 mm distance to the artificial root surface. According to FITZ et al. (2003), the cutting device allows for slicing fresh soil at a spatial resolution of 250 µm and less.

4.3.4 Analysis and calculations

Soil microbial biomass C and N were estimated by fumigation extraction (BROOKES et al., 1985; VANCE et al., 1987), modified for simultaneous isotopic determination of $^{12}C/^{13}C$ and $^{14}N/^{15}N$ in the extract (POTTHOFF et al., 2003). Immediately after harvest, two portions of 10 g fresh soil of each soil layer were taken from separated samples. One portion was fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Following fumigant removal, the soil was extracted with 40 ml 0.05 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered. The other non-fumigated portion was extracted similarly at the time fumigation commenced. Sub-samples of the extracts were freeze-dried until constant weight.

The delta signatures and the total C and N concentrations in all samples were measured by isotope ratio mass spectroscopy using a Delta plus IRMS (Finnigan Mat, Bremen, Germany) after combustion in combination with an NA 1500 elemental analyser (Carlo Erba, Milan, Italy). The ^{13}C and ^{15}N abundances of the samples were expressed in delta signatures relative to the Vienna Pee Dee Belemnite standard in the case of ^{13}C and relative to atmospheric nitrogen in the case of ^{15}N (SLATER et al., 2001). Soil microbial biomass C (SMB-C) was calculated as follows: SMB-C = 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; JOERGENSEN, 1996). Soil microbial biomass N (SMB-N) was calculated as follows: SMB-N = E_N / k_{EN}, 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 MÜLLER, 1996).

The delta signatures for soil microbial biomass C ($\delta^{13}C_{SMB}$) and N ($\delta^{15}N_{SMB}$) were calculated using the following equations (RYAN and ARAVENA, 1994):

$$\delta^{13}C_{SMB} = \frac{(\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{unfum} \times C_{unfum})}{C_{fum} - C_{unfum}}$$

and

$$\delta^{15}N_{SMB} = \frac{(\delta^{15}N_{fum} \times N_{fum} - \delta^{15}N_{unfum} \times N_{unfum})}{N_{fum} - N_{unfum}},$$

where $\delta^{13}C$ and $\delta^{15}N$ are the isotope signatures of the fumigated (fum) and unfumigated (unfum) soil extracts, respectively. C and N are the corresponding amounts of extractable C and N in the extracts. The proportions of C (C_{dfr}) and N (N_{dfr}) derived from rhizodeposition in total soil, extractable fractions and soil microbial biomass were calculated by using the following equations according to BALESDENT and MARIOTTI (1996):

$$C_{dfr}[\%] = \frac{\delta^{13}C_{sample} - \delta^{13}C_{control}}{\delta^{13}C_{rhizodeposition} - \delta^{13}C_{control}} \times 100$$

and

$$N_{dfr}[\%] = \frac{\delta^{15}N_{sample} - \delta^{15}N_{control}}{\delta^{15}N_{rhizodeposition} - \delta^{15}N_{control}} \times 100,$$

where $\delta^{13}C_{sample}$ and $\delta^{15}N_{sample}$ are the isotope signatures of total C and N, extractable C and N or microbial biomass C and N in the planted soil and $\delta^{13}C_{control}$ and $\delta^{15}N_{control}$ are the isotope signatures of the related pool in the unplanted control soil. $\delta^{13}C_{rhizodeposition}$ and $\delta^{15}N_{rhizodeposition}$ are the isotope signatures of the roots of the labelled plants, assuming that these isotope signatures are also representative for the rhizodeposition. The total amounts of rhizodeposition C and N in the different fractions were calculated on the basis of the total amounts by multiplying by the respective proportions of rhizodeposition C and N (see above).

4.3.5 Statistical analysis

All treatments were carried out in four replicates and a completely randomised design during the labelling procedure inside the chamber. To adjust potential light and assimilation differences inside the chamber, the boxes of the labelled treatments were relocated between the labelling periods. Differences of means between rhizosphere soil and unplanted control soil were tested for significance using the unpaired t-test. Differences between distances to the root mat or gauze within rhizosphere or unplanted control soil were tested using paired t-test for dependent treatments. All delta signatures were tested one-tailed, assuming a potential increase in the case of isotope labelling (rhizosphere vs. unplanted control soil) or an increase with increasing proximity to the labelled root surface. All other tests were evaluated two-tailed. All statistics were analysed using JMP 7.0 statistical software package (SAS Institute, 2007).

4.4 Results

In all rhizoboxes, *L. perenne* developed a dense root mat above the gauze and created an artificial root surface. Underneath, the gauze was densely covered by root hairs penetrating the 30 µm gauze. The gauze including the penetrating root hairs was easily removed from the soil inside the rhizosphere compartment. In two of the unplanted treatments, soil adhered to the nylon gauze. These boxes were discarded. There were no significant differences in leaf (4.9 g ± 0.4 standard deviation) and root mat dry matter (1.1 g ± 0.2) between the labelled and unlabelled plants at harvest. The total C to N ratio of the leaves and root mat were 25.7 ± 1.8 and 32.7 ± 2.4, respectively.

The ^{13}C and ^{15}N double labelling led to significant enrichment for ^{13}C and ^{15}N in leaves and roots of the labelled plants in comparison with the unlabelled control plants: -29.89‰ ± 0.96 δ^{13}C and 3.50‰ ± 1.10 δ^{15}N. At harvest, the δ^{13}C values in leaves and in roots were 530‰ ± 98 and 37.90‰ ± 10.32, respectively, and the δ^{15}N values in leaves and in roots were 1312‰ ± 171 and 960‰ ± 95, respectively. ^{13}C and ^{15}N in the shoots were correlated with ^{13}C and ^{15}N in the roots by $R^2 = 0.76$ and $R^2 = 0.72$, respectively.

Soil moisture was significantly higher in the unplanted (24.2% dry weight ± 2.6) than in the planted soil (21.6% dry weight ± 1.5). There was no significant change in soil moisture

depending on the distance to the gauze in the planted and unplanted treatments. The contents of total C and N in the planted and unplanted soil did not differ between the treatments and between different distances to the artificial root surface (total C: 1.02% ± 0.08; total N: 0.09% ± 0.01).

The contents of 0.05 M K_2SO_4-extractable C and N were decreased on average by 12 µg and 10 µg g^{-1} soil, respectively, in the planted soil in comparison with the unplanted soil (Fig. 4-1). Within the rhizosphere in the planted soil, extractable C and N did not show any clear gradient. The differences between the planted and unplanted soil were significant in most of the five distance layers, with the exception of the extractable C content at 0-1.0 mm distance to the roots. Total microbial biomass C was significantly increased by 63 µg g^{-1} soil at 0-1.0 mm distance and total microbial biomass N was nearly doubled, with an increase by 13 µg g^{-1} soil at 0-1.0 mm distance in comparison with the unplanted soil. The increase of microbial biomass N was still significant up to 1.8 mm distance.

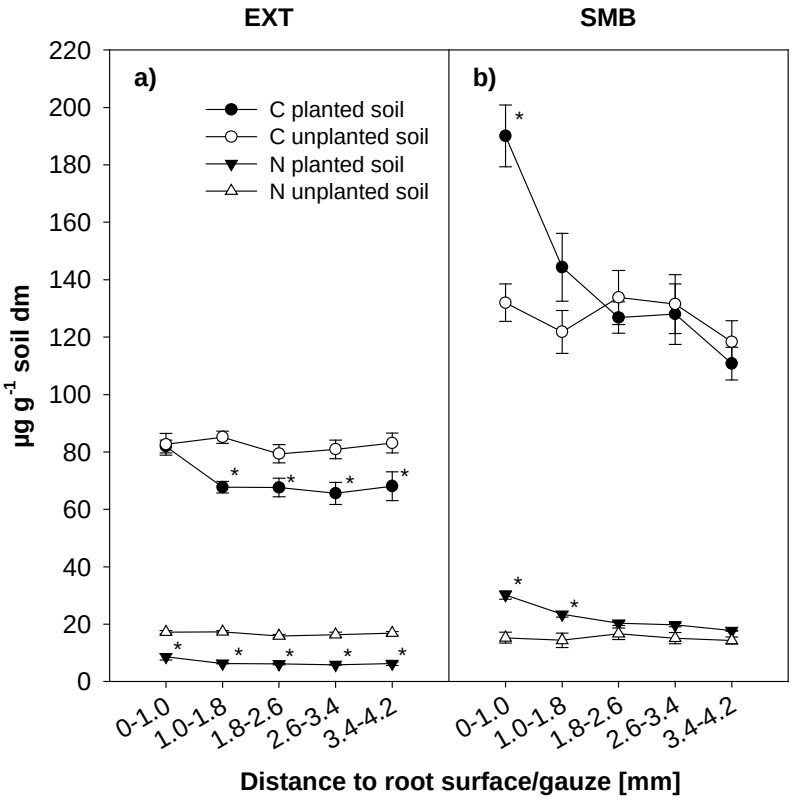

Fig. 4-1: Means of 0.05 M K_2SO_4-extractable C and N (a) and soil microbial biomass C and N (b) [µg g^{-1} soil dm] in the rhizosphere of ^{13}C- and ^{15}N-labelled *Lolium perenne* (planted soil) at different distances to an artificial root surface and a nylon gauze in an unplanted control soil (unplanted soil). The * indicate significant differences in the rhizosphere soil related to the unplanted control soil (two-tailed, unpaired t-test); I = standard error of mean (n = 4).

The δ^{13}C and δ^{15}N values of all three fractions (total soil, extractable C and N and microbial biomass) increased with increasing proximity to the artificial root surface in the planted soil (Fig. 4-2). At 0-1.8 mm distance in the planted soil, the δ^{13}C and δ^{15}N values of all three fractions were significantly increased in comparison with the unplanted soil. The δ^{13}C values of total C and microbial biomass C were significantly higher up to 2.6 mm and the δ^{13}C values of the extractable fraction and the δ^{15}N values of total N and microbial biomass N up to 4.2 mm distance.

In 0-1.0 mm distance, C and N derived from rhizodeposition were 4.2% of total C and 2.8% of total N in soil at 0-1.0 mm distance. Microbial biomass C and N accounted for 66% and 29% of C and N derived from rhizodeposition, respectively. Also 9.9% of extractable C and 6.5% of extractable N were derived from rhizodeposition in this soil layer close to the root surface. The contents of all three C and N fractions derived from rhizodeposition decreased with increasing distance to the roots (Tab. 4-1). However, all fractions were still traceable up to 4.2 mm distance, except the fraction of total C derived from rhizodeposition. The fraction of C derived from rhizodeposition transferred into the non-extractable soil organic matter as calculated by the difference of total soil C and the amounts of extractable and microbial biomass C increased from 65 to 89% of total C derived from rhizodeposition at 2.6-3.4 mm distance (Fig. 4-3). Conversely, C derived from rhizodeposition incorporated into the microbial biomass pool decreased from 33 to 4% along the rhizosphere gradient. The fraction of N derived from rhizodeposition transferred into the soil organic matter increased from 61 to 79% of total N derived from rhizodeposition at 1.8-2.6 mm distance, followed by a decline to roughly 55% in the two outer distances. Microbial biomass N derived from rhizodeposition decreased from 37 to 16% at 1.8-2.6 mm distance, followed by an increase to roughly 41% in the two outer distances. Extractable C and N derived from rhizodeposition varied widely around means of 4% of total C and N derived from rhizodeposition within the entire investigated distance.

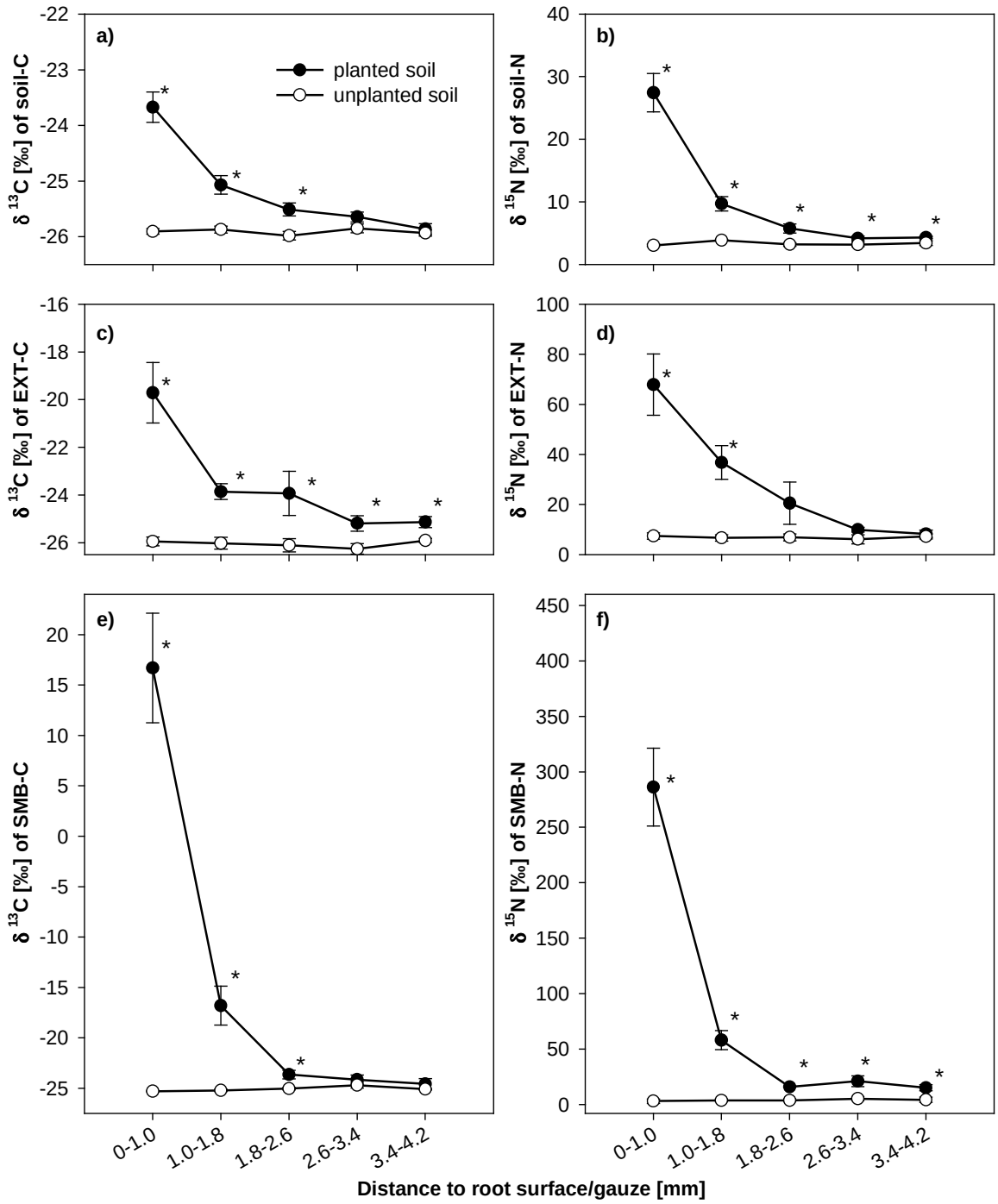

Fig. 4-2: Means of δ values of total C (a), total N (b), 0.05 M K_2SO_4-extractable C (c), 0.05 M K_2SO_4-extractable N (d), soil microbial biomass C (e) and soil microbial biomass N (f) in the rhizosphere of ^{13}C- and ^{15}N-labelled *Lolium perenne* at different distances to an artificial root surface (planted soil) and a nylon gauze in an unplanted control soil (unplanted soil). The * indicate significant differences of soil, EXT and SMB in the rhizosphere soil related to the unplanted control soil (one-tailed, unpaired t-test). I = standard error of mean; n = 8 for total soil δ values; n = 4 for EXT and SMB δ values.

Tab. 4-1: C and N derived from rhizodeposition (Cdfr, Ndfr) in total soil (Total), 0.05 M K_2SO_4 extractable fraction (EXT) and soil microbial biomass (SMB) in the rhizosphere of ^{13}C- and ^{15}N-labelled *Lolium perenne* at different distances to an artificial root surface.

Distance to gauze (mm)	**Total C_{dfr}**		**EXT-C_{dfr}**		**SMB-C_{dfr}**	
	% of total soil C	**µg g^{-1} soil**	**% of EXT-C**	**µg g^{-1} soil**	**% of SMB-C**	**µg g^{-1} soil**
0-1.0	4.2 ± 1.4	389 ± 104	9.9 ± 4.0	8.1 ± 3.0	66.0 ± 9.8	126 ± 25
1.0-1.8	1.3 ± 0.2	122 ± 23	3.4 ± 1.0	2.3 ± 0.8	13.0 ± 6.2	19.2 ± 11
1.8-2.6	0.6 ± 0.4	53 ± 39	3.3 ± 2.1	2.2 ± 1.4	2.3 ± 1.3	2.8 ± 1.6
2.6-3.4	0.3 ± 0.2	33 ± 22	1.3 ± 1.0	0.9 ± 0.7	1.4 ± 1.5	1.8 ± 2.2
3.4-4.2	n.t.	n.t.	1.4 ± 0.7	1.0 ± 0.5	0.6 ± 1.6	0.6 ± 1.8
Distance to gauze (mm)	**Total N_{dfr}**		**EXT-N_{dfr}**		**SMB-N_{dfr}**	
	% of total soil N	**µg g^{-1} soil**	**% of EXT-N**	**µg g^{-1} soil**	**% of SMB-N**	**µg g^{-1} soil**
0-1.0	2.8 ± 0.6	25.0 ± 5.0	6.5 ± 1.8	0.53 ± 0.04	29.3 ± 5.1	8.8 ± 1.9
1.0-1.8	0.6 ± 0.4	5.2 ± 3.5	3.3 ± 1.4	0.21 ± 0.10	5.9 ± 2.6	1.4 ± 0.7
1.8-2.6	0.2 ± 0.03	1.7 ± 0.3	1.6 ± 1.8	0.10 ± 0.11	1.3 ± 0.1	0.3 ± 0.03
2.6-3.4	0.1 ± 0.07	0.8 ± 0.6	0.5 ± 0.2	0.03 ± 0.01	1.9 ± 1.3	0.4 ± 0.3
3.4-4.2	0.1 ± 0.02	0.6 ± 0.1	0.3 ± 0.3	0.02 ± 0.02	0.2 ± 0.09	1.2 ± 0.5

n.t.: not traceable, mean ± standard deviation (n = 4)

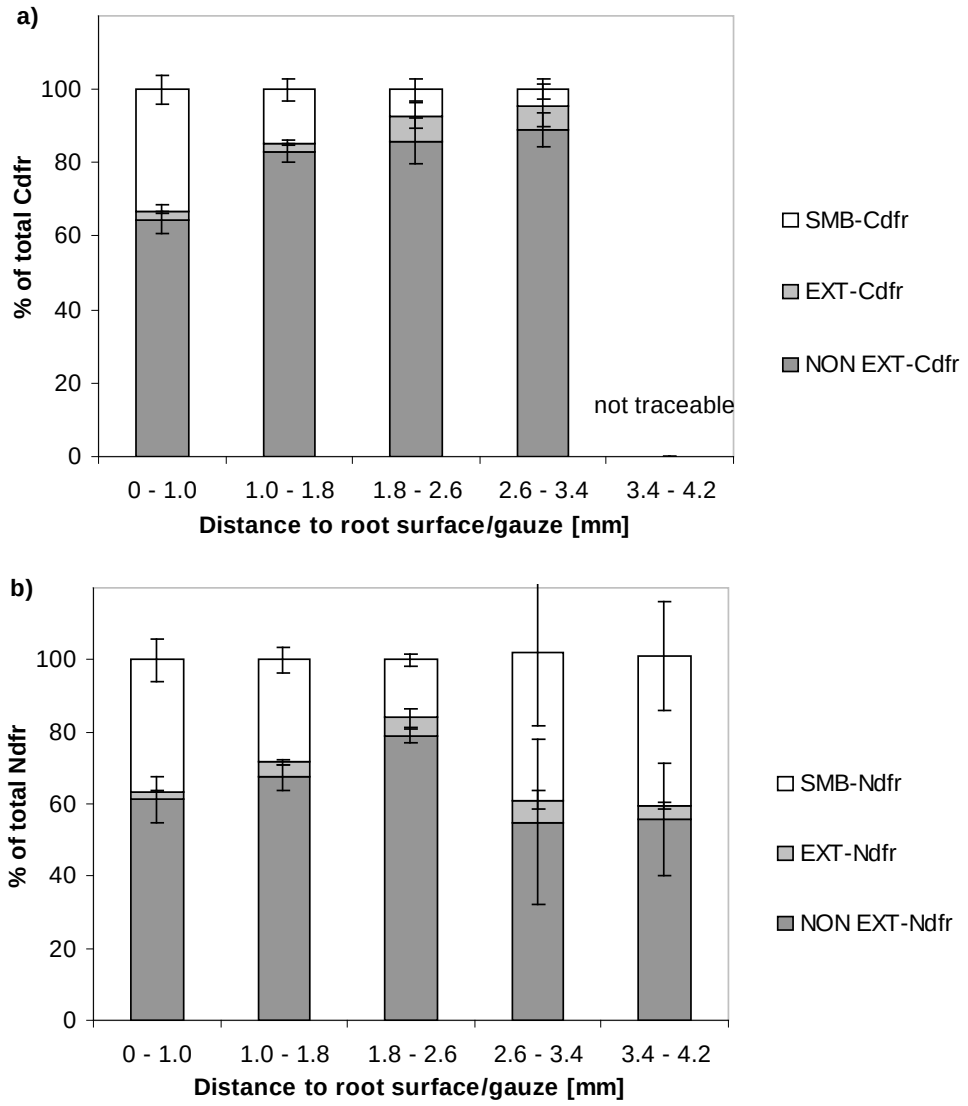

Fig. 4-3: Partitioning of total C (a) and N (b) derived from rhizodeposition in the non extractable soil organic matter fraction (NON EXT), 0.05 M K_2SO_4-extractable fraction (EXT) and in soil microbial biomass (SMB) in percent of total derived C and N from rhizodeposition in soil at different distances to an artificial root surface of ^{13}C- and ^{15}N-labelled *Lolium perenne*. I = standard error of mean; n = 4.

The C/N ratio of C and N derived from rhizodeposition in soil at 0-1.0 mm distance to the roots varied only marginally in all fractions (C/N: 15 ± 3) (Fig. 4-4.). The ratio of total C and N derived from rhizodeposition as well as the ratio of extractable C and N derived from rhizodeposition increased with increasing distance to the roots to values above 30. In contrast, the ratio of microbial biomass C and microbial biomass N derived from rhizodeposition decreased to values less than 5 at 2.6-4.2 mm distance to the roots, a value smaller than the total C/N ratio of the soil microbial biomass.

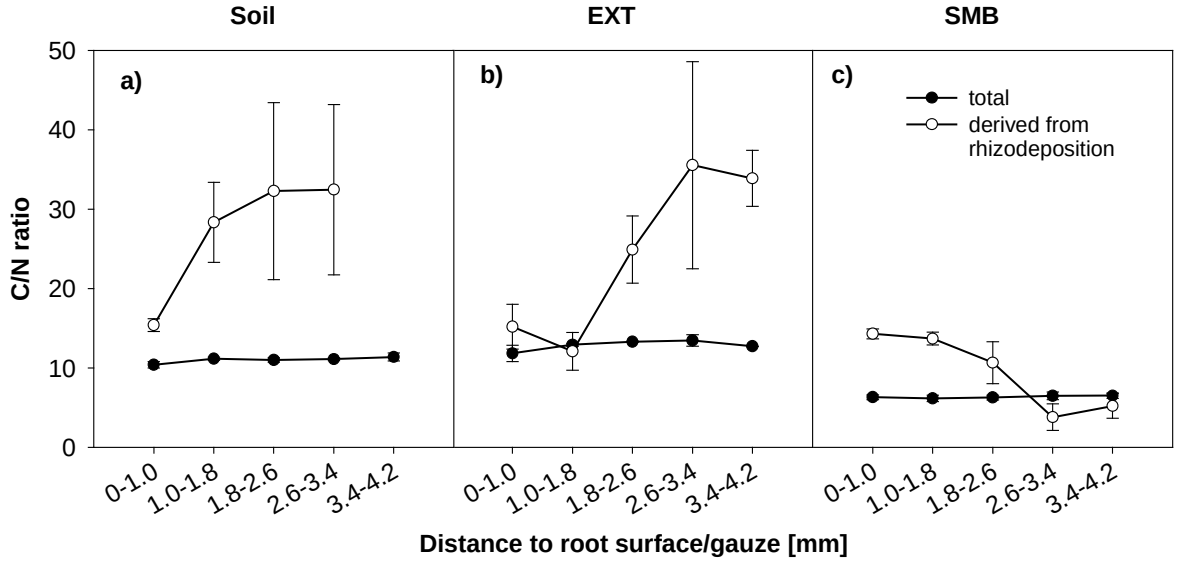

Fig. 4-4: Means of C/N ratios of total and of C and N derived from rhizodeposition in total soil (a), 0.05 M K_2SO_4-extractable fraction (b) and microbial biomass (c) in soil at different distances to an artificial root surface of ^{13}C- and ^{15}N-labelled *Lolium perenne*. I = standard error of mean (n = 4).

4.5 Discussion

4.5.1 Methodological considerations

The study reveals that the present rhizobox system can be used successfully for determining rhizodeposition C and N after ^{13}C and ^{15}N double labelling of plants. There was a reliable translocation of previously assimilated ^{13}C and ^{15}N into the newly growing root mat, indicated by high correlations of the isotopic abundance in the previously labelled plant parts and in the newly grown root mat. However, the depletion of previously ^{13}C labelled compounds into the newly grown root mat was high and leads to ^{13}C enrichment in roots in percent of ^{13}C enrichment in the previously labelled plant parts of only 12%. In contrast to

this, the ^{15}N enrichment in the newly grown root mat reached nearly 73% of the ^{15}N enrichment of the previously labelled plant parts, indicating a greater reallocation of previously assimilated N (structural-reserve-N) than of C, which is preferentially incorporated into structural carbon of the plant tissue. Certainly, adequate amounts of N derived from rhizodeposition were directly re-assimilated during penetration of the rooting compartment and growth of the root mat, while C was lost by root respiration. It can be assumed that any bias by the decomposition of available C followed by losses as CO_2 and N mineralisation is small, as they cannot exceed the constant turnover of these components in the rhizosphere compartment over the plant cultivation period. Consequently, the mass balance equation used for calculating the proportions of C and N derived from rhizodeposition according to BALESDENT and MARIOTTI (1996) underestimates the proportions only marginally.

The unplanted control soil was not affected by the labelling procedure. The observed high amounts of rhizodeposition derived C and N in the microbial biomass fraction in soil close to the artificial root surface of *L. perenne* may misleadingly suggest that root hair fragments in the sampled soil layer caused an overestimation of this fraction. However, this can definitely be excluded for the following three reasons: (1) The gauze including the penetrated root hairs was carefully removed from the rhizosphere soil before soil sampling, strictly avoiding the destruction of the root hairs. (2) Root hairs are single cells, if root hairs were destroyed by removing the gauze, the cell sap would percolate into the soil and be detectable in the non-fumigated fraction, followed by extremely enhanced isotopic signatures. This would result in much higher percentages of C and N derived from rhizodeposition in this fraction, even assuming a rapid turnover of rhizodeposits in the soil. The present data give no evidence to suggest that this is the case. In contrast to the outer distances from the root surface, the small reduction in extractable C derived from native soil organic matter close to the root in comparison with the unplanted soil indicates that the microbial biomass close to the root mainly assimilated C from the recently released compounds by rhizodeposition rather than from the native soil organic matter. (3) The estimation of microbial biomass cannot be affected by root hair fragments, because there is no additional opening of these cells by chloroform fumigation. Consequently, any isotope enrichments in the planted and thus root-affected soil could be attributed to rhizodeposition of ^{13}C- and ^{15}N-labelled *L. perenne* during the time of their release from the roots.

The cutting device for fresh soil was found to be a suitable tool for sampling of fresh soil at adequate trim thickness for investigations of processes at the soil-root interface. The sampling of fresh soil avoids the bias introduced by the microtome-freeze sectioning techniques (FITZ et al., 2003) and allows the direct handling of the samples for analysis without any freeze-thaw effects on microbial soil characteristics (DE NEERGAARD and MAGID, 2001; FRIEDEL et al., 2003).

The present approaches, including C and N isotope pre-labelling, are useful tools for estimation of C and N rhizodeposits to avoid several restrictions of other tracer techniques (MEHARG, 1994; WERTH and KUZYAKOV, 2008; WICHERN et al., 2008).

The difference in soil moisture between planted and unplanted treatments is a methodological problem in all studies (BADALUCCO et al., 1996). In the present study, this problem was reduced by supplying water from a mineral mat into the upper rhizosphere soil compartment using capillary rise. This led to a homogeneous soil water content throughout the entire investigated distance layers in planted and unplanted soil, but did not fully abolish the differences between planted and unplanted soil.

4.5.2 Distribution of ^{13}C and ^{15}N rhizodeposits

Effects of rhizodeposition on microbial growth and the related C and N pools were mainly detected up to 2.6 mm distance to the artificial root surface, although some effects, e.g. those on microbial biomass N and 0.05 M K_2SO_4-extractable C and N, remained visible in the layer with the greatest distance. Similarly, DE NEERGAARD and MAGID (2001) observed rhizosphere effects on microbial biomass C and microbial biomass N up to 1 to 3 mm from the root surface, depending on the amount of nutrients available for microorganisms. SAUER et al. (2006) found root-derived ^{14}C in soil even up to 12 mm distance from the root surface, depending on the plant species. Also TOUSSAINT et al. (1995) detected root-derived ^{15}N in soil at 5 to 10 mm distance from the roots. Differences in diffusion distance can be caused by differences in exudate composition and solubility (DARRAH, 1991a, b; MERBACH et al., 1999), by differences in the plant species (MERBACH et al., 1999), by differences in the plant physiological status (NEUMANN and ROEMHELD, 2007), by differences in density and length of root hairs (MERBACH et al., 1999) and most likely also by differences in the soil conditions.

The significant increase in microbial biomass C and N reveals distinct microbial growth induced by the rhizodeposits of *L. perenne*. On average, 26% (± 4% standard deviation) of the total C rhizodeposits and 35% (± 10%) of the total N rhizodeposits were incorporated into the soil microbial biomass within the 5 distance layers. Microbial growth was largest close to the artificial root surface. At 0-1.0 mm distance, the content of microbial biomass C from rhizodeposition was much larger (66%) than that based on the net increase (32%). A certain percentage of the autochthonous microbial biomass is replaced due to an increased turnover induced by the easily available rhizodeposits (MEHARG, 1994; PATERSON et al., 2007). In the 1.0-1.8 mm distance layer, the net increase in total microbial biomass was on a similar level to the increase in microbial biomass C derived from rhizodeposition, but only that based on changes in the $^{13}C/^{12}C$ ratio was significant. In contrast to C, the net increase in N incorporated into the microbial biomass was higher than the content of microbial incorporated N derived from rhizodeposition. Nearly 30% of the microbial biomass N close to the roots was derived from rhizodeposition, explaining the increase of microbial biomass N by nearly 59%. Moreover, the ratio of incorporated N rhizodeposits to the newly formed total constituted microbial biomass N decreased between the distances 1.0 and 4.2 mm from 17% to 8%. This indicates that (in addition to N from rhizodeposits) considerable amounts of soil organic matter-derived N were increasingly incorporated into the growing microbial biomass with increasing distance to the root surface.

The observed increase in the C/N ratio of C and N derived from rhizodeposition, but also in the 0.05 M K_2SO_4 extractable fraction, strongly indicates changes in the composition of the substrates derived from rhizodeposition in the soil with increasing distances to the roots of *L. perenne*. The low C/N ratio of 15 of C and N derived from rhizodeposition close to the roots in the present study was similar to that found by WICHERN et al. (2007) and may be caused by an immediate decomposition of easily available rhizodeposits of the growing microbial community at the soil root interface. The gross C/N ratio of rhizodeposits is hard to quantify, due to their fate in mineralisation and re-assimilation processes. However, the C/N ratio of rhizodeposits is presumably high, because passively released low molecular and water-soluble exudates were found to be dominated by N-free carbohydrates and organic acid with a low contribution of N-containing amino acids (MERBACH et al. 1999, HÜTSCH et al., 2002; BERTIN et al., 2003). However, any discussion about gross inorganic N released by roots may be disregarded, focusing on the distribution of rhizodeposits

within the rhizosphere, and may only be significant for calculations of net balances. Due to a strong diffusion gradient of inorganic N towards the roots and consequently high re-assimilation by plants, the majority of rhizodeposition induced processes will always be measured as a net outcome of several interrelationships at the plant-soil interface.

The majority of the organic exudates were immediately incorporated into the microbial biomass (FALCHINI et al., 2003; BUTLER et al., 2004), followed by a rapid transformation into microbial metabolites and residues close to the root surface or directly on the rhizoplane (NEUMANN and RÖMHELD, 2007). The more recalcitrant components with a larger C/N ratio, such as actively secreted phytohormones or allelochemicals (UREN, 2007), as well as parts of recently formed metabolites or residues, seem to diffuse into the more remote soil. Clearly, mineralised N from rhizodeposits was not detectable in our study, but it may be assumed that the majority of the recently mineralised N is subject to the same fate as the root exuded inorganic N: a strong flow towards the roots of the growing plants. Both processes are indicated by an increase in the C/N ratio derived from rhizodeposition and a lower response of the microbial biomass to substrate input with increasing distance to the artificial root surface. According to FONTAINE et al. (2003), it may be assumed that the substrate input of easily available organic components close to the root surface primarily stimulates the growth of r-strategists, whereas the main part of more recalcitrant compounds diffused into the more remote soil is utilised by successive slow growing K-strategists.

Net amounts of nearly 15% of K_2SO_4-extractable C and 60% of K_2SO_4-extractable N disappeared in the planted soil in comparison with the unplanted soil, doubling the C/N ratios of K_2SO_4-extractable components. This might be explained either by plant uptake of continuously mineralised N that remained in the unplanted soil or by uptake of organic N components from this pool by the microbial biomass, reducing the microbial biomass C/N ratio from 9 in the unplanted soil to 6 in the planted soil. This is in line with the observation of DE NEERGAARD and MAGID (2001), who found an increasing transfer of easily available ^{14}C-labelled organic components from a 2 M KCl-extractable pool into the microbial biomass pool in soil in the presence of growing plants and even with decreasing distance to the root surface.

A microbial biomass C/N ratio of 15 at 0-1.0 mm distance to the roots suggests that rhizosphere microorganisms reliant mainly on the substrate from rhizodeposition suffered

from N limitation in this layer. The observed decrease in the microbial C/N ratio derived from rhizodeposits in the outer layers may be caused either by a stronger C limitation by more recalcitrant rhizodeposits, by more microbial residues derived from rhizodeposition and/or by a more effective N recycling of previously formed microbial metabolites and residues (JENKINSON and PARRY, 1989). A microbial biomass C/N ratio below 5 suggests that the incorporated fraction derived from rhizodeposition consists mainly of protein, which has an average C/N ratio of 3.1. An actively growing microorganism in the outer parts of the rhizosphere usually contains a mosaic of C derived from autochthonous soil organic C and C derived from rhizodeposition.

4.6 Conclusions

The present approach using ^{13}C and ^{15}N double labelling of plants is useful for estimating rhizodeposition C and N in soil, depending on the distance to the root surface of plants and in different soil fractions, especially the microbial biomass. Rhizodeposition C and also N were ascertained as strong determinants for microbial growth along the rhizosphere gradient. Close to the root surface, high amounts of C and N derived from rhizodeposition were contributed to microbial biomass. As a consequence, an excessive microbial growth was triggered by the substrate input from rhizodeposition, leading to high proportions of microbial biomass C and N derived from rhizodeposition. With increasing distance to the roots, decreasing microbial response indicates decreasing availability of the substrates released by rhizodeposition. Moreover, increasing C/N ratios were observed in the fractions of C and N derived from rhizodeposition in total soil and the extractable fraction with increasing distance to the root surface, whereas the C/N ratio of the microbial biomass at this gradient decreased from 15 to 5. This suggests that more recalcitrant rhizodeposits and certain microbial metabolites diffuse into the more remote soil and contribute quite differently to the microbial population along the rhizosphere gradient, N limitation of the microbial community close to the root surface and N recycling from microbial residues during microbial growth in the outer soil layers. The simultaneous determination of rhizodeposition C and N in soil and their complex functions, especially with regard to microbial biomass and soil organic matter turnover, contributes to a better understanding of the processes occurring in the rhizosphere.

4.7 References

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5 C and N derived from rhizodeposition: Relation to microbial growth and C and N turnover as a function of their spatial distribution within the rhizosphere

5.1 Abstract

Two greenhouse rhizobox experiments were carried out to investigate the fate and turnover of ^{13}C- and ^{15}N-labelled rhizodeposits within a rhizosphere gradient from 0-8 mm distance to the roots of oat and wheat. Rhizosphere soil layers from 0-1, 1-2, 2-3, 3-4, 4-6 and 6-8 mm distance to separated roots of oat and wheat were analysed after a period of 27 and 53 days of roots affecting the rhizosphere soils, respectively. The soil samples from different distances to the separated roots of oat and wheat were investigated in an incubation experiment (42 days, 15°C) for changes in total C and N and C and N derived from rhizodeposition in total soil, in soil microbial biomass and in 0.05 M K_2SO_4-extractable soil fraction. Additionally, the CO_2-C respiration in total and that derived from rhizodeposition were measured from the rhizosphere soil samples up to 6 mm distance to the previously separated roots during the soil incubation. In total, 36% less C and 39% less N derived from rhizodeposition were found in the rhizosphere soil of oat in comparison to wheat within the rhizosphere gradients. Significant amounts of rhizodeposition C were revealed in rhizosphere soil up to 4-6 mm distance from the separated roots of oat and wheat. Rhizodeposition N was only revealed in the rhizosphere soils up to 1-2 mm and 3-4 mm distance from the roots of oat and wheat, respectively. Microbial biomass C and N increased significantly with increasing proximity to the separated roots in both experiments. In the rhizosphere soil of oat, the net increase of microbial biomass C close to the separated roots was smaller than the amount of incorporated rhizodeposition C. In contrast, the net increase of microbial biomass C in the soil close to roots of wheat accounted for only 82% of C derived from rhizodeposition. However, these values changed with increasing distance to the roots. During soil incubation, microbial biomass C derived from rhizodeposition decreased by about 50% and 22% of the previously incorporated C derived from rhizodeposition in oat and wheat soil samples, respectively. Beside rhizodeposit N, large amounts of unlabelled soil N (native SOM) were incorporated into the growing microbial biomass towards the roots in both experiments, indicating a distinct acceleration of soil organic matter decomposition and N immobilisation into the growing microbial biomass, even under the competition of plant growth. C decomposition of native soil organic matter was enhanced within the entire investigated rhizosphere gradients. This effect was less distinctive in the rhizosphere samples of oat. These relations indicate a complex interaction between microbial growth and turnover as well as substrate input derived from rhizode-

position and accelerated decomposition of native soil organic matter. The effects were different between the experiments with oat and wheat, presumably due to different amounts of rhizodeposits previously entering the rhizosphere soils during plant growth. The data indicate differential microbial response to the rhizodeposit input at a high spatial resolution from the roots. The turnover of rhizodeposits C and N and the acceleration of native soil organic matter decomposition (i.e. rhizosphere priming effects) as well as their relation to microbial growth within the rhizosphere are discussed.

Keywords: ^{13}C; ^{15}N; Rhizodeposition; Rhizodeposits; Soil microbial biomass; Rhizosphere priming effects; Rhizobox

5.2 Introduction

The biochemical processes of microbial substrate turnover in soil in the immediate vicinity of roots, i.e. in the rhizosphere, are fuelled by a multitude of root-derived substrates and differ significantly from those of the bulk soil (HÜTSCH et al., 2002; BERTIN et al., 2003, WICHERN et al., 2008). The substrate input by roots, so-called rhizodeposition, comprises highly diverse root exudates (diffusates, secretions and excretions), lysates and dead root cell material, and is a vital energy and substrate source for microbial activity and growth in the rhizosphere (PATERSON, 2003; UREN, 2007). The composition of the root released compounds varies widely depending on plant species, plant physiological status and varying conditions (NEUMANN and RÖMHELD, 2007). Taking into account that rhizodeposition mainly consists of organic C compounds (beside CO_2-C from root respiration), earlier investigations mainly focused on C derived from rhizodeposition (TOAL et al., 2000; KUZYAKOV and DOMANSKI, 2000; KUZYAKOV, 2002; NGUYEN, 2003; KUZYAKOV and SCHNECKENBERGER, 2004). Estimations of C released from roots via rhizodeposition accounted for up to 20% or even more of the total assimilated plant C during the vegetation period of different plant species (KUZYAKOV, 2002; HÜTSCH et al., 2002). However, beside C, significant amounts of rhizodeposit N, including inorganic N (NO_3, NH_4) and organic N compounds (amino-acids, different phytohormones, vitamins and allelochemicals), are released into the rhizosphere by legumes, cereals and grasses (JANZEN, 1990; TOUSSAINT et al., 1995; JENSEN, 1996; MAYER et al., 2003; WICHERN et al., 2007). Investigations on N derived from rhizodeposition are scarce and our knowledge about rhizodeposition of N is incomplete (WICHERN et al., 2008; JONES et al., 2009). Rhizodeposition N has yet to be accurately quantified, estimations varying widely from 4% up to 71% of the total assimilated plant N, depending on plant species, plant physiological status and varying conditions (WICHERN et al., 2008).

The main part of the released rhizodeposits from plant roots is subjected to continuous microbial metabolism in the adjacent rhizosphere soil. Significant amounts of C and N derived from rhizodeposition are transferred into the organic matter pool during plant growth (SCHMIDTKE, 2005; WICHERN et al., 2007), but the varying composition of the rhizodeposits suggests strongly that there are differences in (1) their decomposition rates and mobilisation/immobilization patterns and (2) their spatial distribution within the rhizosphere. Whereas the water-soluble fraction is subject to rapid microbial decomposition and immo-

bilisation close to the root surface or directly on the rhizoplane (NEUMANN and RÖMHELD, 2007), more recalcitrant components may allow diffusion into the more remote rhizosphere (UREN, 2007). Moreover, rhizodeposition is identified as a strong determinant for microbial community development in planted soils (PATERSON et al., 2007). Therefore, it can be assumed that continuous substrate input leads to changes in the microbial community and activity in the rhizosphere as a direct response to the structure and function of rhizodeposits' and their fate of microbial breakdown.

In accordance with this view, KANDELER et al. (2002) detected clear gradients of bacterial community composition between 0 to 2.2 mm and 2.2 to 5.0 mm distance from the root surface of maize, which were also significant in comparison to an unplanted soil. Changes in the functional diversity of the bacterial communities extend up to several millimetres from roots (KANDELER et al., 2002). However, these changes might be explained as a response of the microbial community to rhizodeposit input, whereas the composition of the substrate presumably also had a decisive impact on the microbial changes within the rhizosphere soil.

For estimating rhizodeposition C and N, ^{13}C, ^{14}C and ^{15}N isotope-tracing techniques were developed over the past decades, focussing on different soil pools such as total and extractable soil organic matter or microbial biomass (POTTHOFF et al., 2003). Due to methodological restrictions, investigations on the spatial distribution of processes at the root-soil interface mostly focus on artificial plant-root-soil systems called rhizobox or rhizosphere systems. HELAL and SAUERBECK (1981) and KUCHENBUCH and JUNGK (1982) introduced these methods, in which roots are separated from soil by a fine mesh for studying rhizosphere processes in soil layers at different proximities to roots. These separation techniques were improved for a wide range of investigations on plant-soil interactions (YOUSSEF and CHINO, 1988; GAHOONIA and NIELSEN, 1991; WENZEL et al., 2001). Only a few investigations have studied the distribution of microbial biomass in the rhizosphere as a function of the distance to the root surface (HELAL and SAUERBECK, 1986; YOUSSEF et al., 1989; DE NEERGAARD and MAGID, 2001). Sampling of soil layers at high spatial resolution is mainly based on slicing of frozen rhizosphere soil by microtome sectioning (KUCHENBUCH and JUNGK, 1982; GAHOONIA and NIELSEN, 1991; WENZEL et al., 2001; KANDELER et al., 2002; KUZYAKOV et al., 2003; SAUER et al., 2006). However, this type of sampling leads to erroneous reductions in microbial biomass C and microbial biomass N

(DE NEERGAARD and MAGID, 2001; FRIEDEL et al., 2003) and also causes artefacts in different soil chemical and physical properties (FITZ et al., 2003). Only very few studies have used cutting devices for slicing of non-frozen soil (BADALUCCO et al., 1996; NURUZZAMAN et al., 2006). Therefore, a rhizobox system enabling simultaneous ^{13}C and ^{15}N plant labelling and slicing of non-frozen soil has been developed by SCHENCK ZU SCHWEINSBERG – MICKAN et al. (2010). The system was used for investigating the processes stimulated by rhizodeposit input into the rhizosphere with increasing distance to the root surface of ^{13}C- and ^{15}N-labelled *Lolium perenne* (SCHENCK ZU SCHWEINSBERG – MICKAN et al., 2010). Based on the findings of this study, we concluded that the spatial distribution of continuously released rhizodeposits within the rhizosphere strongly depends on the microbial availability of the rhizodeposit composition. It might be assumed that the substrate input of easily available organic components close to the root surface primarily stimulates the growth of r-strategists, whereas more recalcitrant rhizodeposits and certain microbial metabolites diffuse into the more remote soil and contribute quite differently to the microbial population along the rhizosphere gradient. However, the knowledge about rhizodeposition C and N induced processes and their influence on microbial development and on C and N turnover within the rhizosphere is still limited and is basically insufficient for understanding the complexity of plant-soil interactions. Therefore, simultaneous investigations on the impact of rhizodeposition C and N on microbial growth and C and N turnover within the rhizosphere are necessary for a better understanding of the interdependency of the substrate C and N input by rhizodeposition and the related turnover processes occurring within the rhizosphere.

The aim of the present study was to investigate the impact of rhizodeposition C and N on microbial growth and turnover within the rhizosphere of oat and wheat. In detail, we focused on (1) the extent of ^{13}C- and ^{15}N-labelled rhizodeposits within the rhizosphere and the relation of rhizodeposits C and N (C/N ratio) with increasing distance to the root, (2) the spatial pattern of rhizosphere microbial biomass response to ^{13}C- and ^{15}N-labelled rhizodeposits and (3) its relation to the C and N turnover within the rhizosphere.

For these investigations, two experiments with oat and wheat were carried out in a rhizobox system according to GAHOONIA and NIELSEN (1991), which was modified for ^{13}C and ^{15}N double labelling of plants by $^{13}CO_2$ labelling and simultaneous application of ^{15}N-nutrient solution in a closed chamber (SCHENCK ZU SCHWEINSBERG – MICKAN et al., 2010).

In addition, the system was combined with a device that enabled slicing of fresh soil with increasing distance to an artificial root surface (gauze) as proposed by FITZ et al. (2003).

5.3 Material and Methods

5.3.1 Soil and growing conditions

Sandy loam soil (74% sand, 14% silt, 12% clay; C_t 0.8%, N_t 0.08%, pH_{H20} 6.2) was collected from the top 10 cm of arable land 10 km south of Göttingen, Germany. After removing visible organic matter, the soil was incubated for 2 weeks at 15°C, then air-dried and sieved to 1 mm and stored in the dark at 4°C until the experiments started. The experiments were conducted in a conditioned greenhouse cabin (temperature: 16 ± 3°C night, 20 ± 4°C day, light exposure: 12 h day^{-1}, 43.3 ± 2.2 lm m^2s).

5.3.2 Rhizobox system

Three-compartment rhizoboxes based on the system developed by GAHOONIA and NIELSEN (1991) were modified for simultaneous ^{13}C and ^{15}N pre-labelling of plants without contact to the later rhizosphere soil (SCHENCK ZU SCHWEINSBERG – MICKAN, 2010). The boxes were also adapted for use of a cutting device for fresh sampling of rhizosphere soil at specific distances to the roots, separated by a 30 µm nylon gauze. During the first stage of plant growth, plants were pre-cultivated in sand/perlite substrate in the upper labelling compartment (compartment 1: L x W x H = 22 x 6 x 10 cm) of the rhizobox system inside a gastight labelling chamber made of Plexiglas (L x W x H = 130 x 80 x 120 cm) with a capacity for 30 systems. Inside this chamber, the plants were $^{13}CO_2$-C labelled and simultaneously labelled via a wick-system with ^{15}N-nutrient solution. After plant labelling, the labelling compartments were taken out of the chamber and tightly attached to the rooting and rhizosphere compartments, which were filled with unlabelled test soil. The rooting compartment (compartment 2: L x W x H = 22 x 6 x 1 cm) and the rhizosphere soil compartment (compartment 3: L x W x H = 22 x 6 x 3 cm) were horizontally separated by a 30 µm nylon gauze. The two compartments were held in place by a removable frame, which also secured the labelling compartment later to be transferred onto the rooting/rhizosphere compartments. In the following stage of plant development, roots grew into the rooting

compartment, but could not penetrate the gauze, thus creating an artificial rhizosphere (root affected soil) below the gauze in the rhizosphere compartment.

5.3.3 Plant cultivation, labelling and sampling

For each experiment, 33 labelling compartments closed at the bottom by a removable 500 µm mesh were filled with 1600 g sand/perlite mixture (ratio 2/1) and wetted to 100% water holding capacity. In addition, each 6 compartments were evaluated for plant growth status. These compartments were destructively sampled during the experimental period. Oat and wheat seeds were pre-cultivated until germination in conventional pre-cultivation substrate. After complete germination of the seeds, 5 selected seedlings were planted in each of the prepared 24 compartments and 9 compartments were left unplanted. The compartments were supplied with Hoaglands` nutrient solution via wicks. At growth stage BBCH-code 12-14 (LANCASHIRE et al., 1991), 16 planted labelling compartments and the unplanted references were transferred into the labelling chamber and supplied via wicks with Hoaglands` nutrient solution labelled with NH_4NO_3 10.26 atom% ^{15}N. The modified Hoaglands` nutrient solution included 2.66 atom% $^{15}N$ in the experiment with wheat and 1.33 atom% $^{15}N$ in the experiment with oat. Planted unlabelled reference compartments were kept outside of the chamber and were supplied with unlabelled Hoaglands` nutrient solution. ^{13}C-labelling of oat was conducted in two periods starting 10 and 25 days after planting. During these periods, the chamber was closed for 4 days and 12 ml 7.5 M lactic acid were added through a pipe to 2.8 g $NaHCO_3$ with 99 atom% ^{13}C placed in a 25 ml beaker inside the chamber. After the first labelling period, the compartments were relocated inside the chamber and the second labelling period was started as described above. Wheat was ^{13}C-labelled during one period 17 days after planting using 2.2 mg $NaHCO_3$ with 99 atom% ^{13}C as described above. Oat and wheat were removed from the chamber 35 days and 27 days after planting, respectively, and the wick systems were removed. The sand/perlite substrate was then carefully rinsed with 4 l H_2O to wash out potential adherent labelled nutrient solution from roots and the substrate. After complete drainage, the 500 µm nylon meshes, closing the compartments underneath, were removed and the labelling compartments were tightly attached onto the prepared rooting and rhizosphere soil compartments, filled with 210 g and 900 g of the dry test soil (1.3 g cm^{-3}), respectively. The soil in the compartments was wetted via capillary action from watered porous mineral mats (thickness: 5 cm), which were placed under the boxes in water filled dishes, where the wa-

ter was regularly replenished. Plants and soil were collected at similar plant growth stage (BBCH-code: 59-61; LANCASHIRE et al., 1991). At the time of harvest, roots of oat and wheat had grown into the later transferred rooting compartments for 27 and 53 days, respectively.

During harvest, plants were separated into above ground plant material (in the following: shoots) and roots grown inside the rooting compartment (in the following: roots). Roots growing inside the labelling compartment were not analyzed. The rooting compartment including the gauze was carefully removed from the rhizosphere soil compartment. The rhizosphere soil was sliced using a modified cutting device, slicing fresh soil at specified trim thickness (FITZ et al., 2003) at 0-1, 1-2, 2-3, 3-4, 4-6 and 6-8 mm distances from the separated rooting compartment. According to FITZ et al. (2003), the cutting device allows for slicing fresh soil at a spatial resolution of 250 µm and less. In the present experiment, the cutting device was used for slicing soil at a margin of 1.0 mm trim thickness.

5.3.4 Incubation of rhizosphere soil samples and measurements

Immediately after sampling of the rhizosphere soil, sub samples (65 g) of soil from 0-1, 1-2, 2-3, 3-4, 4-6 and 6-8 mm soil layers and the unplanted soil were collected in incubation glasses (250 ml) and then incubated in a climate cabinet at 15°C for 42 days. During the incubation period, the soil was aerated and controlled for continuous water content (50-60% Whc_{max}) regularly. At the beginning and the end of the incubation period, contents and delta signatures of total soil C and N, 0.05 M K_2SO_4 extractable C and N and microbial biomass C and N were measured. Additionally, at day 1, 7, 21 and 42 of the incubation period, the amounts and delta signatures of CO_2-C released from the incubated soil layers were measured.

5.3.5 Analysis and calculations

For each plant and soil analysis, samples of 4 compartments were merged. For determination of plant dry matter, C and N concentration and ^{13}C and ^{15}N delta signatures, the plant material was dried at 60°C until weight constancy. For determination of soil water content, C and N concentrations and ^{13}C and ^{15}N delta signatures in soils of different distances to the rooting compartment, aliquots of 5 g of each soil layer were dried at 105°C until weight constancy. Soil microbial biomass C and N and soil microbial biomass ^{13}C and ^{15}N delta signatures were estimated using the chloroform-fumigation-extraction method (BROOKES

et al., 1985; VANCE et al., 1987) modified for simultaneously isotope determination of $^{12}C/^{13}C$ and $^{14}N/^{15}N$ in the extracts (POTTHOFF et al., 2003). Immediately after harvest, two portions of 10 g fresh soil of each soil layer were taken from separated samples. One portion was fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Following fumigant removal, the soil was extracted with 40 ml 0.05 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered. The other non-fumigated portion was extracted similarly at the time fumigation commenced. Sub-samples (17 ml) of the extracts were freeze-dried until weight constancy.

The delta signatures (isotopic abundance) and the total ^{13}C and ^{15}N concentrations in all samples (plant material, soil, freeze-dried K_2SO_4 extracts) were measured by isotope ratio mass spectroscopy using a Delta plus IRMS (Finnigan Mat, Bremen, Germany) after combustion in combination with an NA 2500 elemental analyzer (CE-Instruments, Milano, Italy). The ^{13}C and ^{15}N abundances of the samples were expressed in delta signatures relative to the Pee Dee Belemnite standard in case of ^{13}C and relative to atmospheric nitrogen in case of ^{15}N (SLATER et al., 2001). Soil microbial biomass C (SMB-C) was calculated as follows: SMB-C = EC / k_{EC}, where EC = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (WU et al., 1990; JOERGENSEN, 1996). Soil microbial biomass N (SMB-N) was calculated as follows: SMB-N = EN / k_{EN}, where EN = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (BROOKES et al., 1985; JOERGENSEN and MÜLLER, 1996). The delta signatures for soil microbial biomass C ($\delta^{13}C_{SMB}$) and N ($\delta^{15}N_{SMB}$) were calculated by using the following equations (RYAN and ARAVENA, 1994):

$$\delta^{13}C_{SMB} = \frac{(\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{unfum} \times C_{unfum})}{C_{fum} - C_{unfum}} \text{ and}$$

$$\delta^{15}N_{SMB} = \frac{(\delta^{15}N_{fum} \times N_{fum} - \delta^{15}N_{unfum} \times N_{unfum})}{N_{fum} - N_{unfum}} \quad (1)$$

where $\delta^{13}C$ and $\delta^{15}N$ are the isotope signatures of the fumigated (fum) and unfumigated (unfum) soil extracts, respectively. C and N are the corresponding amounts of extractable C and N in the extracts. Extractable C and N fractions from soil (EXT-C and EXT-N) were measured as 0.05 M K_2SO_4 extractable organic C and total N from the non-fumigated soil,

respectively. The proportions of C (C_{dfr}) and N (N_{dfr}) derived from rhizodeposition in total soil, extractable fractions and soil microbial biomass were calculated by using the following equations according to BALESDENT and MARIOTTI (1996):

$$C_{dfr}[\%] = \frac{\delta^{13}C_{sample} - \delta^{13}C_{control}}{\delta^{13}C_{rhizodeposition} - \delta^{13}C_{control}} \times 100 \text{ and}$$

$$N_{dfr}[\%] = \frac{\delta^{15}N_{sample} - \delta^{15}N_{control}}{\delta^{15}N_{rhizodeposition} - \delta^{15}N_{control}} \times 100 \quad (2)$$

where $\delta^{13}C_{sample}$ and $\delta^{15}N_{sample}$ are the isotope signatures of total C and N, extractable C and N or microbial biomass C and N in the planted soil and $\delta^{13}C_{control}$ and $\delta^{15}N_{control}$ are the isotope signatures of the related pools in the unplanted control soil. $\delta^{13}C_{rhizodeposition}$ and $\delta^{15}N_{rhizodeposition}$ are the isotope signatures of the roots of the labelled plants, assuming that these isotope signatures also are representative for the rhizodeposition (BALESDENT and MARIOTTI, 1996).

For measuring the CO_2-C evolution (soil microbial activity) and the delta ^{13}C signatures of the released CO_2-C from soil, sub samples (each 2-3 g) were weighed into 12 ml *headspace* vials from the 0-1, 1-2, 2-3 and 4-6 mm incubated soil layers and from the unplanted soil at day 1, 7, 21 and 42 of the incubation period. The vials were closed by an airtight septum and flushed for 2 min by carbon-free synthetic air to remove CO_2 completely from the vials. To measure increasing CO_2-C concentration inside the headspace vials at detectable concentrations, the samples were incubated on a temperature rack (15°C) for max. 24 h, which enabled direct measurement of aliquots taken from the headspace of the vials using an automated gas-sampler. The concentrations and the delta ^{13}C signatures of CO_2-C were measured by GC/IRMS using a TraceGC (Thermo Finnigen, Milano, Italy) in combination with IRMS Delta Plus (Thermo Finnigen MAT, Bremen, Germany).

The microbial CO_2-C production was calculated by using the following equation:

$$CO_2\text{-}C[\mu g\,C\,g^{-1}\,soil\,dm\,day^{-1}] = C_{CO_2} \times \frac{M_C}{8.3145 \times \frac{T}{p} \times 1000} \times \frac{V_{headspace}}{dm} \times \frac{24}{\Delta t} \quad (3)$$

where C_{CO2} is the measured volumetric concentration of CO_2 [ppmv], M_C is the molar mass of C [g mol^{-1}], T is the temperature during the incubation [°K], p is the atmospheric pressure [kPa], $V_{headspace}$ is the volume of the *headspace* [ml], dm is the dry weight of the incubated soil sample [g], and Δt is the time of soil incubation inside the closed vials [h].

The proportion of CO_2-C derived from rhizodeposition (CO_2-C_{dfr}) was calculated by using the following equation:

$$CO_2\text{-}C_{dfr}[\%] = \frac{\delta^{13}CO_2\text{-}C_{sample} - \delta^{13}CO_2\text{-}C_{control}}{\delta^{13}C_{rhizodeposition} - \delta^{13}CO_2\text{-}C_{control}} \times 100 \quad (4)$$

where $\delta^{13}CO_2$-C_{sample} is the isotopic signature of CO_2-C from microbial respiration in the root affected soil and $\delta^{13}CO_2$-$C_{control}$ is the isotopic signature of CO_2-C from microbial respiration in the unplanted soil. $\delta^{13}C_{rhizodeposition}$ is the isotope signature of the roots of the labelled plants, assuming that these isotope signatures also are representative for the rhizodeposition (BALESDENT and MARIOTTI, 1996).

The priming effect (PE) on soil organic matter C was calculated by using following equation:

$$PE[\%] = \frac{CO_2\text{-}C_{dfSOM\ sample} - CO_2\text{-}C_{control}}{CO_2\text{-}C_{control}} \times 100 \quad (5)$$

where CO_2-$C_{dfSOM\ sample}$ is the amount of soil organic matter derived CO_2-C in the rhizosphere samples, calculated from the difference of total CO_2-C and CO_2-C derived from rhizodeposition released from the rhizosphere samples, and CO_2-$C_{control}$ is the total amount of CO_2-C released from the unplanted soils.

5.3.6 Experimental design and statistical analysis

All treatments were carried out in a completely randomized design with four experimental parallels. To adjust potential light and assimilation differences during the period inside the chamber, the boxes of the labelled treatments were relocated regularly. Differences of means between rhizosphere soil and unplanted control soil were tested for significance using an unpaired t-test. All delta signatures were tested one-sided, assuming a potential increase in case of isotope labelling (rhizosphere vs. unplanted control soil) or an increase with increasing proximity to the labelled root surface. All other tests were evaluated two-sided. All statistics were computed using the JMP 7.0 statistical software package (SAS Institute, 2007).

5.4 Results

5.4.1 Plant development and labelling

At the time of plant and soil sampling (BBCH-code: 59-61; LANCASHIRE et al., 1991), the total growing periods were 62 days and 80 days after planting for oat and wheat, respectively. At this time, roots of oat and wheat had grown for 27 and 53 days, respectively, into the later transferred rooting compartments, which were tightly rooted, although there were only individual roots attached to the gauze, without creating dense root mats. There were no significant differences in dry matter of shoots and roots inside the rooting compartments between the two experiments (shoots oat: 5.7 g ± 0.3 standard deviation, shoots wheat: 5.5 ± 0.7 g; roots oat: 0.54 ± 0.04 g, roots wheat: 0.49 ± 0.04 g). The C to N ratio of roots was 28.8 ± 3.0 and 29.8 ± 1.5 for oat and wheat, respectively.

The ^{13}C- and ^{15}N-double-labelling led to significant enrichment of ^{13}C and ^{15}N in shoots and roots of the labelled plants in comparison to the unlabelled control plants (Tab. 5-1). ^{13}C enrichment in the roots inside the rooting compartment in percent of ^{13}C enrichment in shoots was 18.4 ± 1.9% and 54.8 ± 10.4% in oat and wheat, respectively. ^{15}N enrichment in roots in percent of ^{15}N enrichment in shoots was 77 ± 2.5% and 65.2 ± 2.2% in oat and wheat, respectively. The enrichment of ^{13}C and ^{15}N in the newly grown roots in the rooting compartment was highly correlated with enrichment of ^{13}C and ^{15}N in the previously labelled shoots for oat and wheat (^{13}C oat: $R^2 = 0.77$; ^{13}C wheat: $R^2 = 0.75$; ^{15}N oat: $R^2 = 0.73$; ^{15}N wheat: $R^2 = 0.93$).

Tab. 5-1: Enrichment of ^{13}C and ^{15}N [‰] in shoots and roots in the rooting compartments of ^{13}C- and ^{15}N-labelled oat and wheat.

	enrichment ^{13}C [‰]	**enrichment ^{15}N [‰]**
oat		
shoots	429 ± 91	2744 ± 116
roots	78.4 ± 14.1	2114 ± 128
wheat		
shoots	68.7 ± 25.9	1429 ± 166
roots	37.3 ± 13.1	933 ± 117

mean ± standard deviation (n = 4)

5.4.2 Soil moisture, total and extractable C and N contents

At the time of plant and soil sampling, soil moisture of the unplanted soil was slightly higher than in the rhizosphere soils (unplanted soil: 24.1 ± 3.4%; rhizosphere soil: 20.8 ± 3.4%). There was no significant change in soil moisture depending on the distance to the gauze in the rhizosphere soils. The contents of total C and N in the planted soils did not differ between different distances to the gauze in oat and wheat and the related unplanted soil (rhizosphere soil: 8750 ± 608 µg C g^{-1} soil, 834 ± 44 µg N g^{-1} soil; unplanted soil: 8494 ± 925 µg C g^{-1} soil, 812 ± 24 µg N g^{-1} soil). The contents of 0.05 M K_2SO_4 extractable C and N did not show any clear gradient from the soil close to the roots up to the more remote soil (data not shown). Compared to the unplanted control soil, extractable C was about 27.9 ± 8.6% lower in the rhizosphere soil of oat (20.6 ± 2.6 µg C g^{-1} soil) and 62.5 ± 3.8% lower in the rhizosphere soil of wheat (15.8 ± 1.6 µg C g^{-1} soil). Both differences were significant. The contents of extractable N were small in both rhizosphere soils. Extractable N in oat and wheat rhizosphere soil was similar (1.7 ± 0.3 µg N g^{-1} soil) and about 84.2 ± 3.5% lower in comparison to the unplanted soils.

5.4.3 Delta values, C and N derived from rhizodeposition

The $\delta^{13}C$ and $\delta^{15}N$ values in total soil, extractable and microbial biomass increased in the rhizosphere soil of oat and wheat with increasing proximity to the 30 µm gauze. The ^{13}C and ^{15}N enrichment in all fractions followed the pattern total soil < extractable fraction < microbial biomass. With the exception of extractable C, the isotopic enrichment in the different soil fractions was higher for oat than for wheat (Fig. 5-1). For oat and wheat, similar amounts of C and N derived from rhizodeposition were detectable in the soil close to the rooting compartments (Fig. 5-2). In the rhizosphere soil of oat the detectable amounts of C and N derived from rhizodeposition decreased more strongly than in the rhizosphere soil of wheat along the investigated rhizosphere gradient, although the growth period of the latter was almost twice as long (Fig. 5-2).

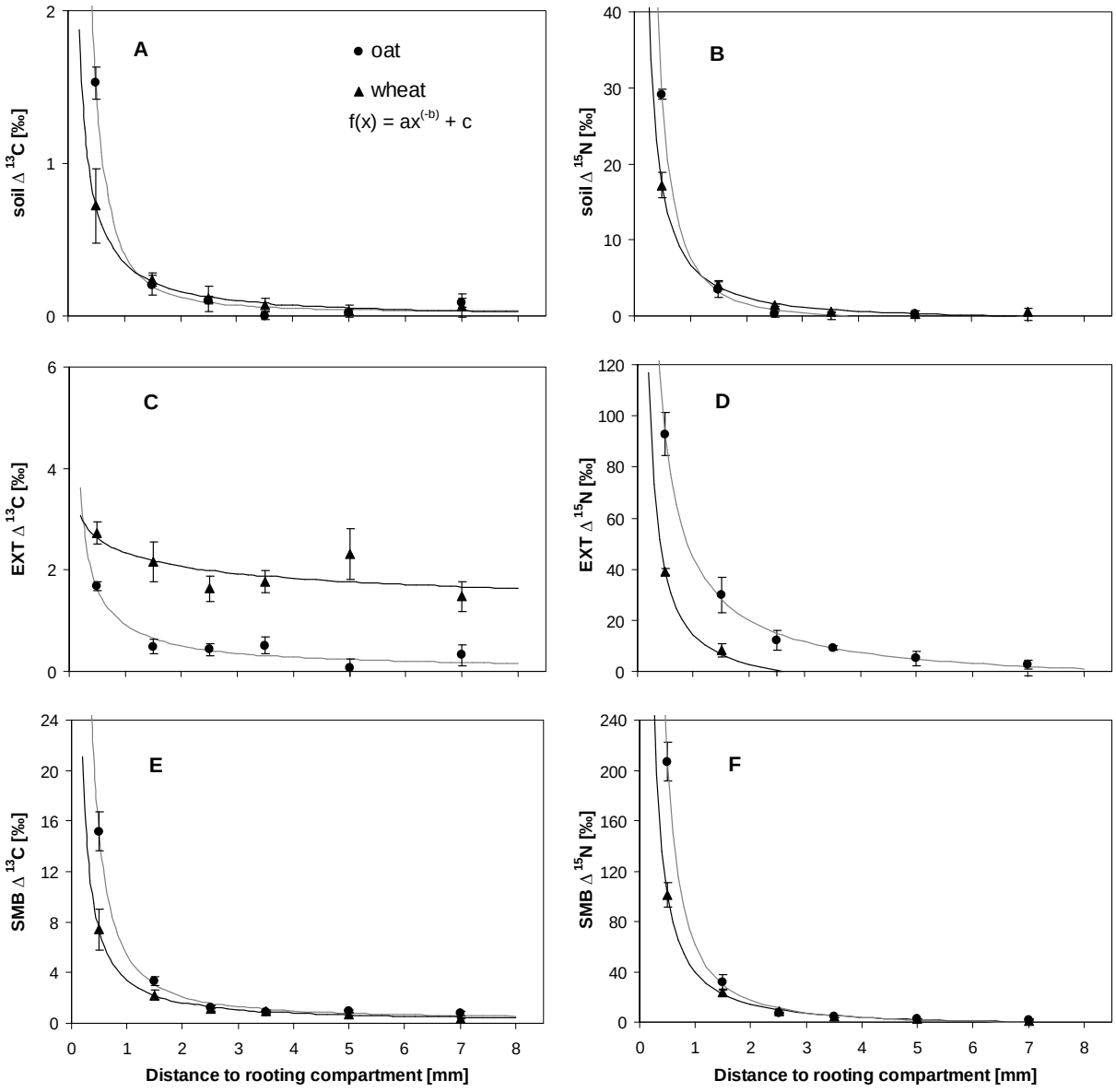

Fig. 5-1: Enrichment (Δ) of ^{13}C and ^{15}N [‰] in total soil (A, B), 0.05 M K_2SO_4 extractable compounds (C, D) and soil microbial biomass (E, F) at different distances to a separated rooting compartment of ^{13}C- and ^{15}N-labelled oat and wheat. Symbols = means of measured values; I = standard error; n = 4; Lines = estimated values by nonlinear regression: $f(x) = ax^{(-b)} + c$.

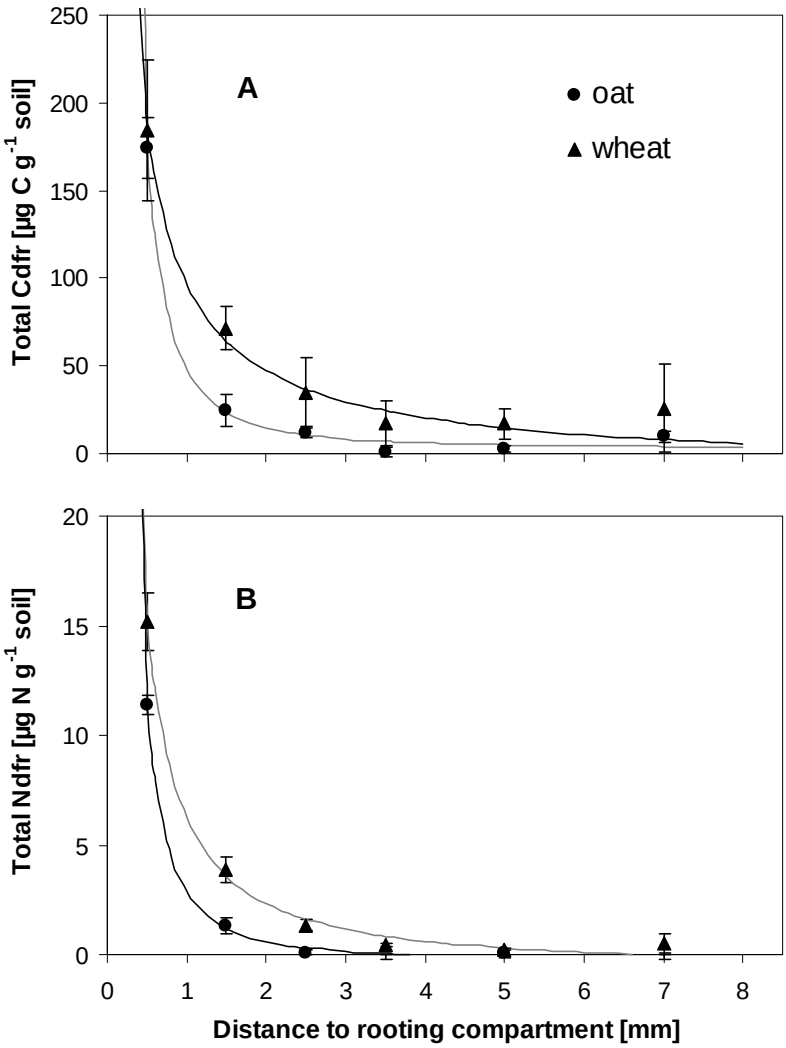

Fig. 5-2: Distribution of C (A) and N (B) derived from rhizodeposition (Cdfr, Ndfr) in soil at different distances to a separated rooting compartment of ^{13}C- and ^{15}N-labelled oat and wheat. Symbols = means of measured values; I = standard error; n = 4. Lines = estimated values by nonlinear regression: $f(x) = ax^{(-b)} + c$.

In total, 36% less Cdfr and 39% less Ndfr were found in the rhizosphere soil of oat in comparison to wheat within the rhizosphere gradient up to 8 mm from the rooting compartments (Fig. 5-2). The spatial diffusion gradients of ^{13}C- and ^{15}N-enrichment within the rhizosphere soils were estimated by nonlinear regression ($f(x) = ax^{-b} + c$; for x = distance) (Tab. 5-2). Significant amounts of rhizodeposition C were revealed up to rhizosphere soil layers of oat and wheat 4-6 mm from the rooting compartments, and significant amounts of rhizodeposition N were revealed at 1-2 mm and 3-4 mm from the rooting compartments in the rhizosphere soils of oat and wheat, respectively (Tab. 5-2). The C to N ratios of total C and N derived from rhizodeposition in soils close to the rooting compartments were 15.3 ± 3.2 and 12.2 ± 5.9 in oat and wheat, respectively, and increased with increasing distance to the rooting compartments (Tab. 5-2).

Tab. 5-2: Estimated means (± standard deviation) of total C [µg C g^{-1} soil dm] and total N [µg N g^{-1} soil dm] derived from rhizodeposition (Cdfr and Ndfr) and the related Cdfr/Ndfr ratios in rhizosphere soil at different distances to a separated rooting compartment of ^{13}C- and ^{15}N-labelled oat and wheat. Values estimated by nonlinear regression: $f(x) = ax^{-b} + c$, for x = distance. p-values indicate significance for $p<0.05$ (t-test, one-sided, for $t\geq0$.).

	Oat				
	Total Cdfr		**Total Ndfr**		**Cdfr/Ndfr**
Distance [mm]	[µg g^{-1} soil]	p-value	[µg g^{-1} soil]	p-value	
0 - 1	174 ± 34.1	0.001	11.4 ± 0.91	0.000	15.3 ± 3.2
1 - 2	23.2 ± 17.1	0.036	1.17 ± 0.64	0.018	19.9 ± 18.3
2 - 3	11.4 ± 6.26	0.018	0.31 ± 0.36	0.096	37.2 ± 48.8
3 - 4	7.38 ± 3.30	0.010	0.05 ± 0.05	0.366	142 ± 155
4 - 6	4.82 ± 3.56	0.037	n.d.		n.d.
6 - 8	3.35 ± 4.62	0.122	n.d.		n.d.
	Wheat				
	Total Cdfr		**Total Ndfr**		**Cdfr/Ndfr**
Distance [mm]	[µg g^{-1} soil]	p-value	[µg g^{-1} soil]	p-value	
0 - 1	186 ± 79.1	0.009	15.2 ± 2.63	0.001	12.2 ± 5.6
1 - 2	63.4 ± 24.8	0.007	3.56 ± 1.01	0.003	17.8 ± 8.6
2 - 3	39.3 ± 18.8	0.012	1.60 ± 0.53	0.005	24.6 ± 14.3
3 - 4	28.5 ± 16.7	0.021	0.82 ± 0.35	0.009	34.6 ± 25.1
4 - 6	19.7 ± 16.0	0.045	0.27 ± 0.32	0.097	73.0 ± 106
6 - 8	13.3 ± 17.1	0.108	n.d.		n.d.

mean ± standard deviation (n = 4); n.d. = not detectable

The majority of C and N derived from rhizodeposition was detectable in the non-extractable soil organic matter fraction and accounted for 79.5 ± 1.1% and 80.3 ± 4.2% of total C derived from rhizodeposition and 84.4 ± 1.2% and 84.7 ± 2.2% of total N derived from rhizodeposition in oat and wheat, respectively. Only marginal proportions (< 1%) of total C and N derived from rhizodeposition were detectable in the 0.05 M K_2SO_4 extractable fraction in both experiments. The proportions of total C and N derived from rhizodeposition in the soil microbial biomass were also similar in both experiments and accounted for 19.9 ± 1.1% and 18.0 ± 3.5% of total C and 14.7 ± 1.0% and 15.0 ± 2.2% of total N derived from rhizodeposition in oat and wheat, respectively.

5.4.4 Microbial biomass

Microbial biomass C and N increased significantly with increasing proximity to the rooting compartments of oat and wheat (Fig. 5-3). Related to the unplanted soils, microbial biomass C increased by 24.6 ± 3.8 and 52.4 ± 8.2 µg C g^{-1} soil in the rhizosphere soil close to the rooting compartment in oat and wheat, respectively. Microbial biomass N increased by 2.9 ± 1.1 and 7.9 ± 2.0 µg N g^{-1} soil in the rhizosphere soil close to the rooting compartment in oat and wheat, respectively. In the outer distances from the root zones of oat and wheat, reductions of microbial biomass C and N were significant for microbial biomass N in the rhizosphere soil of oat in comparison to the related unplanted control soil. In the rhizosphere soil of wheat, there was a strong increase of the total microbial biomass C and N based upon high amounts of unlabelled soil C and N (native SOM) incorporated into the growing microbial biomass towards the roots. This effect was also clear but less distinct in the treatment with oat. In soil close to the rooting compartment of oat, 20.4 ± 3.1% of the microbial biomass C and 9.7 ± 1.1% of microbial biomass N were derived from rhizodeposition. In this soil, the net increase of microbial biomass C was smaller than the amount of incorporated rhizodeposition C. In contrast, the net increase of microbial biomass N in this soil was higher than the amount of incorporated rhizodeposition N-compounds, explaining the increase only by 63.3 ± 23.8%. In the soil close to the rooting compartment of wheat, 23.6 ± 2.5% of microbial biomass C and 10.8 ± 1.6% of microbial biomass N, were derived from rhizodeposition, explaining the total increase of microbial biomass in this zone by 82.1 ± 10.7% and 32.7 ± 1.7% for C and N, respectively.

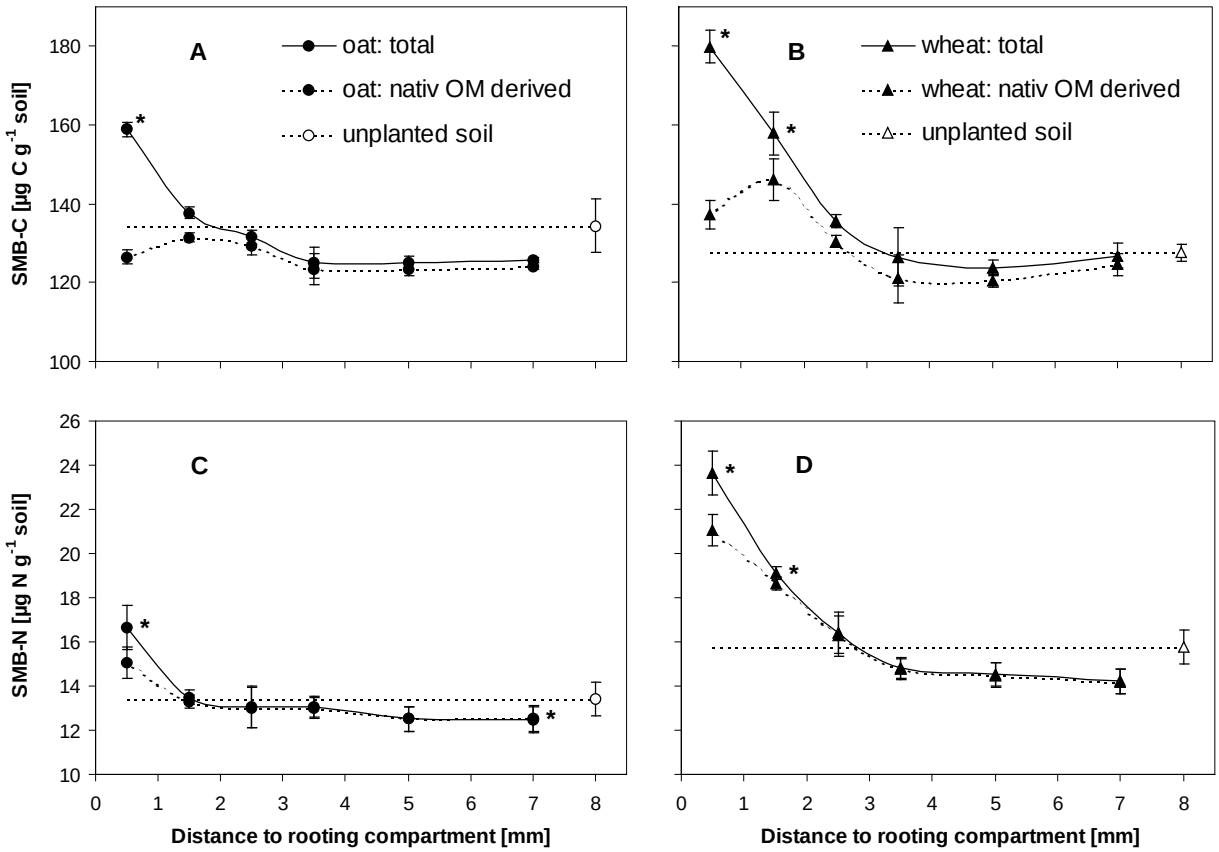

Fig. 5-3: Total microbial biomass C [MBC; µg C g^{-1} soil] and N [MBN; µg N g^{-1} soil] and the proportions of C and N derived from native soil organic matter (OM) and C and N derived from rhizodeposition (= total microbial biomass – microbial biomass derived from native soil organic matter) in soil at different distances to a separated rooting compartment of ^{13}C- and ^{15}N-labelled oat and wheat and unplanted control soil (unplanted soil). **A**: MBC in rhizosphere soil of oat; **B**: MBC in rhizosphere soil of wheat; **C**: MBN in rhizosphere soil of oat; **D**: MBN in rhizosphere soil of wheat. Symbols = means of measured values; I = standard error; n = 4.

5.4.5 C and N turnover in the incubated rhizosphere layers

Microbial CO_2-C released from the incubated rhizosphere soil samples of oat and wheat increased with increasing proximity to the former rooting compartments and decreased continuously during the 42 days of soil incubation (Fig. 5-4). In the rhizosphere soil of oat, the increased CO_2-C evolution was significant in the soil samples from 0-1.0 mm distance to the former rooting compartment during the 42 days of soil incubation, related to the unplanted control soil (Fig. 5-4).

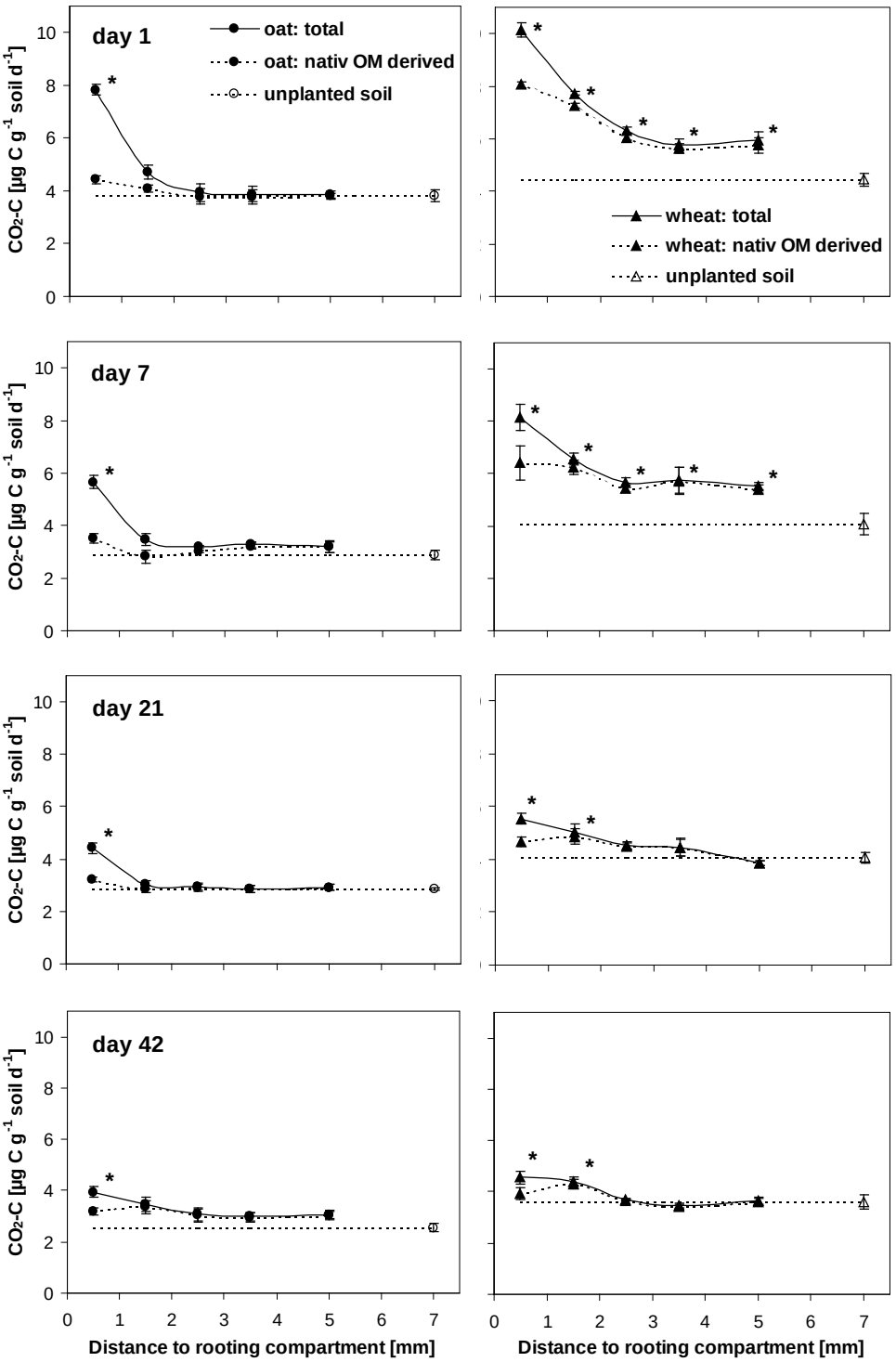

Fig. 5-4: Total CO_2-C [µg C g^{-1} soil] and the proportion of C derived from soil organic matter (OM) and C derived from rhizodeposition (= total CO_2-C - CO_2-C derived from soil organic matter) released from soil samples from different distances to a separated rooting compartment of ^{13}C- and ^{15}N-labelled oat and wheat and unplanted soil at the beginning (day 1) and at 7, 21 and 42 days of soil incubation (42 days; 15°C). * indicates significant differences in total released CO_2-C in the rhizosphere soil samples related to the unplanted control soil; I = standard error; n = 4.

In the rhizosphere soil of wheat, the increased CO_2-C evolution related to the unplanted control soil was significant within the entire investigated distance until day 7 and significant during the total incubation period within 2 mm distance from the former rooting compartment (Fig. 5-4). In wheat and oat, the proportions of C derived from rhizodeposition on total CO_2-C decreased with increasing distance from the former rooting compartments and were smaller in wheat than in oat. Close to the former rooting compartment of oat, the proportions of CO_2-C derived from rhizodeposition declined during the incubation from 43.3 ± 6.1 to 19.7 ± 1.5% of total released CO_2-C. Close to the former rooting compartment of wheat, the proportions of CO_2-C derived from rhizodeposition declined during the incubation from 20.6 ± 1.8 to 13.7 ± 0.7% of total released CO_2-C. The amounts of CO_2-C derived from native soil organic matter soil increased with increasing proximity to the rooting compartments of oat and wheat, indicating primed C decomposition of native soil organic matter in the rhizosphere samples, related to the unplanted control soils. This effect was more distinct in the incubated rhizosphere soil samples of wheat than of oat.

The cumulative CO_2-C release from the wheat rhizosphere soil samples within 0 to 6 mm distance from the rooting compartment was 48% higher than from the oat rhizosphere soil samples (Tab. 5-3). Related to the unplanted control soils, the cumulative CO_2-C release was 48.8 ± 6.1 and 28.0 ± 10.8 µg C g^{-1} soil higher in the rhizosphere soil of wheat and oat, respectively. Total CO_2-C derived from rhizodeposition was 13.2 ± 1.9 µg C g^{-1} soil and 16.6 ± 2.7 µg C g^{-1} soil in wheat and oat, respectively, explaining the increased total CO_2-C respiration in the rhizosphere soils by nearly 30% in the wheat rhizosphere soil and up to 65% in the oat rhizosphere soil. C decomposition of native soil organic matter C was enhanced within the entire investigated rhizosphere gradient in the rhizosphere samples from the wheat rooting compartments. This effect was less distinctive in the rhizosphere samples of oat (Tab. 5-3).

Tab. 5-3: Cumulative soil CO_2-C release [µg C g^{-1} soil dm] of total C (CO_2-C_{total}), derived from native soil organic matter C (CO_2-C_{SOM}) and derived from rhizodeposition C (CO_2-C_{dfr}) and the calculated priming effects on soil organic matter C decomposition during incubation of rhizosphere soil samples (42 days, 15°C) from different distances to a separated rooting compartment of ^{13}C- and ^{15}N-labelled oat and wheat and unplanted soil.

	oat					
Distance [mm]	**CO_2-C_{total}** [µg C g^{-1} soil]	**CO_2-C_{SOM}** [µg C g^{-1} soil]	**CO_2-C_{SOM}** [% of total]	**CO_2-C_{dfr}** [µg C g^{-1} soil]	**CO_2-C_{dfr}** [% of total]	**CO_2-C_{primed}** [%]
0 - 1	206 ± 16.9	142 ± 7.5	69.1 ± 2.3	63.8 ± 9.6	30.9 ± 2.3	18.4 ± 6.3
1 - 2	143 ± 6.6	129 ± 7.6	90.7 ± 2.3	13.3 ± 3.1	9.3 ± 2.3	7.9 ± 6.3
2 - 3	131 ± 10.7	128 ± 8.9	97.5 ± 1.3	3.4 ± 1.9	2.5 ± 1.3	6.5 ± 7.4
3 - 4	129 ± 9.2	128 ± 9.0	98.9 ± 0.2	1.4 ± 0.3	1.1 ± 0.2	6.7 ± 7.5
4 - 6	131 ± 12.9	130 ± 12.4	99.4 ± 0.3	0.8 ± 0.5	0.6 ± 0.2	8.2 ± 10.4
Ø (0 – 6)	148 ± 10.8	131 ± 8.4	88.9 ± 1.3	16.5 ± 2.7	11.1 ± 1.2	9.5 ± 7.0
unplanted	120 ± 4.0	120 ± 4.0	100			
	wheat					
Distance [mm]	**CO_2-C_{total}** [µg C g^{-1} soil]	**CO_2-C_{SOM}** [µg C g^{-1} soil]	**CO_2-C_{SOM}** [% of total]	**CO_2-C_{dfr}** [µg C g^{-1} soil]	**CO_2-C_{dfr}** [% of total]	**CO_2-C_{primed}** [%]
0 - 1	268 ± 16.5	220 ± 20.3	82.0 ± 2.7	47.9 ± 4.6	18.0 ± 2.7	31.1 ± 12.1
1 - 2	232 ± 16.7	222 ± 17.9	95.8 ± 1.0	9.5 ± 1.7	4.2 ± 1.0	32.5 ± 10.7
2 - 3	200 ± 7.0	196 ± 6.2	97.7 ± 1.0	4.6 ± 2.1	2.3 ± 1.0	17.0 ± 3.7
3 - 4	196 ± 1.7	195 ± 0.2	99.2 ± 0.9	1.7 ± 1.8	0.8 ± 0.9	16.3 ± 0.1
4 - 6	186 ± 1.4	183 ± 1.3	98.7 ± 0.3	2.5 ± 0.6	1.3 ± 0.3	9.3 ± 0.8
Ø (0 – 6)	216 ± 6.0	203 ± 7.6	93.9 ± 1.0	13.2 ± 1.9	6.0 ± 1.0	21.2 ± 4.6
unplanted	168 ± 8.1	168 ± 8.1	100			

mean ± standard deviation (n = 4)

At the end of the incubation period, the concentrations of extractable C in the incubated rhizosphere soil samples were decreased only slightly, on average by about 2.5 ± 0.7 µg C g^{-1} soil, and were lower in the incubated rhizosphere soil samples than in the related incubated unplanted soils (oat: 17.8 ± 1.0 µg C g^{-1} soil; wheat: 13.6 ± 0.6 µg C g^{-1} soil; unplanted soils: 24.8 ± 3.5 µg C g^{-1} soil). Total extractable C and C derived from rhizodeposition showed no clear differences within the rhizosphere gradient (data not shown). On average, C derived from rhizodeposition accounted for only 1.1 ± 0.4% and 5.1 ± 2.2% of total extractable C in the rhizosphere samples of oat and wheat, respectively.

At the end of the incubation period, the concentrations of extractable N in the incubated rhizosphere soil samples were lower than in the related unplanted soils (Tab. 5-4). During the soil incubation, the concentrations of extractable N increased in all samples. In the rhizosphere soil samples of oat, total extractable N and the net increase of extractable N showed no clear differences within the rhizosphere gradient. The net increase of extractable N did not differ between the rhizosphere soil of oat and the related unplanted soil (oat: 5.7 ± 0.9 µg N g^{-1} soil; unplanted soil: 6.4 ± 3.3 µg N g^{-1} soil).

Tab. 5-4: Total 0.05 M K_2SO_4 extractable soil-N (EXT-N_{total}) [µg N g^{-1} soil dm] and the net increased N concentrations of total extractable N, extractable N derived from native soil organic matter (EXT-N_{SOM}) and extractable N derived from rhizodeposition (EXT-N_{dfr}) after incubation of rhizosphere soil samples (42 days, 15°C) from different distances to a separated rooting compartment of ^{13}C- and ^{15}N-labelled oat and wheat and unplanted soil.

	oat					
	EXT-N_{total}	**Net-EXT-N increase**				
		EXT-N_{total}	**EXT-N_{SOM}**	**EXT-N_{SOM}**	**EXT-N_{dfr}**	**EXT-N_{dfr}**
Distance [mm]	[µg N g^{-1} soil]	[µg N g^{-1} soil]	[µg N g^{-1} soil]	[% of total]	[µg N g^{-1} soil]	[% of total]
0 - 1	8.0 ± 1.6	6.2 ± 1.4	5.4 ± 1.2	86.2 ± 0.8	0.87 ± 0.24	13.8 ± 0.8
1 - 2	7.0 ± 1.3	5.4 ± 1.0	5.2 ± 1.0	97.9 ± 0.6	0.12 ± 0.05	2.1 ± 0.6
2 - 3	6.7 ± 1.7	4.9 ± 1.2	4.9 ± 1.2	99.6 ± 0.2	0.02 ± 0.01	0.4 ± 0.2
3 - 4	6.8 ± 1.5	5.0 ± 1.1	5.0 ± 1.1	99.8 ± 0.1	0.01 ± 0.01	0.2 ± 0.1
4 - 6	8.1 ± 1.5	6.3 ± 1.4	6.3 ± 1.4	100	n.d.	n.d.
6 - 8	8.3 ± 1.3	6.8 ± 1.2	6.8 ± 1.2	100	n.d.	n.d.
unplanted	19.5 ± 4.5	6.8 ± 4.3	6.8 ± 4.3	100	--	--
	wheat					
	EXT-N_{total}	**Net-EXT-N increase**				
		EXT-N_{total}	**EXT-N_{SOM}**	**EXT-N_{SOM}**	**EXT-N_{dfr}**	**EXT-N_{dfr}**
Distance [mm]	[µg N g^{-1} soil]	[µg N g^{-1} soil]	[µg N g^{-1} soil]	[% of total]	[µg N g^{-1} soil]	[% of total]
0 - 1	15.1 ± 1.2	13.7 ± 1.2	12.2 ± 1.1	88.7 ± 0.8	1.56 ± 0.18	11.3 ± 0.8
1 - 2	12.1 ± 0.6	10.5 ± 0.4	10.2 ± 0.4	97.3 ± 0.5	0.29 ± 0.06	2.7 ± 0.5
2 - 3	10.3 ± 0.3	8.8 ± 0.3	8.8 ± 0.3	99.2 ± 0.2	0.07 ± 0.02	0.8 ± 0.2
3 - 4	9.3 ± 0.6	7.8 ± 0.6	7.8 ± 0.6	99.8 ± 0.1	0.01 ± 0.01	0.2 ± 0.1
4 - 6	8.1 ± 0.3	6.3 ± 0.3	6.3 ± 0.3	100	n.d.	n.d.
6 - 8	8.0 ± 0.6	6.5 ± 0.6	6.5 ± 0.6	100	n.d.	n.d.
unplanted	14.8 ± 1.9	5.9 ± 3.0	5.9 ± 3.0	100	--	--

mean ± standard deviation (n = 4); n.d. = not detectable

In the rhizosphere soil samples of wheat, total extractable N and the net increase of extractable N increased significantly within the rhizosphere gradient with increasing proximity to the former rooting compartment. The net increase of extractable N concentrations in the rhizosphere soil samples of wheat and oat was mainly attributable to N derived from native soil organic matter (Tab. 5-4). The proportions of the net increase in extractable N derived from rhizodeposition during the soil incubation accounted for only 0.2% in the soil samples from 3-4 mm distance and a maximum of 11.3 and 13.8% in soil samples from close to the former rooting compartments of oat and wheat, respectively.

During the soil incubation, the microbial biomass C declined in all soil samples (Tab. 5-5). In the unplanted soils, soil microbial biomass C declined approximately by 26% (34.3 ± 13.3 µg C g^{-1} soil), related to the microbial biomass C contents at the beginning of the soil incubation. In the oat and wheat affected rhizosphere soil (0-8 mm) microbial biomass C declined on an average by 17% (22.8 ± 1.1 µg C g^{-1} soil) and 21% (30.2 ± 2.7 µg C g^{-1} soil), respectively. At the end of the soil incubation, microbial biomass C contents were higher in the rhizosphere soils than in the unplanted soils within the entire investigated rhizosphere gradients (Tab. 5-5). Microbial biomass C declined most strongly in the rhizosphere soil samples from close to the rooting compartment of wheat. With increasing distance to the former rooting compartments of oat and wheat, microbial biomass C accounted for increasing proportions of C derived from rhizodeposition at the end of the soil incubation. During soil incubation, microbial biomass C derived from rhizodeposition decreased by about 50% and 22% of the previously incorporated C derived from rhizodeposition in oat and wheat soil samples, respectively. Close to the former rooting compartments the decrease of microbial biomass C accounted for nearly 46% and 33% of previously incorporated C derived from rhizodeposition in oat and wheat, respectively. These percentages were higher than the proportions of previously incorporated rhizodeposition C into the microbial biomass and indicate a faster decrease of parts of the microbial biomass that grew on rhizodeposition input than that based on native soil organic matter. In the outer distances the decrease accounted for nearly 90% to 100% of microbial biomass C derived from native soil organic matter (Tab. 5-5).

Tab. 5-5: Soil microbial biomass C [µg C g^{-1} soil dm] in total (MBC_{total}), the proportions of C derived from rhizodeposition (MBC_{dfr}) and the net decrease/increase of total soil microbial biomass C, microbial biomass C derived from native soil organic matter (MBC_{SOM}) and microbial biomass C derived from rhizodeposition after incubation of rhizosphere soil samples (42 days, 15°C) from different distances to a separated rooting compartment of ^{13}C- and ^{15}N-labelled oat and wheat and unplanted soil.

	oat						
	MBC_{total}	**MBC_{dfr}**	**Net-MBC: decrease (-), increase (+)**				
			MBC_{total}	**MBC_{SOM}**		**MBC_{dfr}**	
Distance [mm]	[µg C g^{-1} soil]	[% of total]	[µg C g^{-1} soil]	[µg C g^{-1} soil]	[% of total]	[µg C g^{-1} soil]	[% of total]
0 - 1	134 ± 6.2	15.9 ± 2.1	-24.9 ± 3.8	-13.8 ± 5.0	54.1 ± 13.5	-11.1 ± 2.4	45.9 ± 13.5
1 - 2	110 ± 4.1	3.1 ± 0.8	-28.1 ± 3.5	-25.2 ± 3.4	89.7 ± 3.2	-2.9 ± 1.0	10.3 ± 3.2
2 - 3	103 ± 7.9	1.0 ± 0.4	-28.2 ± 4.4	-26.9 ± 3.7	95.6 ± 2.7	-1.3 ± 1.0	4.4 ± 2.7
3 - 4	109 ± 6.2	0.8 ± 0.6	-15.6 ± 5.9	-14.9 ± 5.9	94.9 ± 2.8	-0.7 ± 0.3	5.1 ± 2.8
4 - 6	106 ± 1.7	0.6 ± 0.3	-19.3 ± 4.6	-18.3 ± 4.9	94.2 ± 6.0	-1.0 ± 0.8	5.8 ± 6.0
6 - 8	105 ± 5.5	0.6 ± 0.3	-20.7 ± 4.5	-19.9 ± 4.2	96.2 ± 4.0	-0.8 ± 0.8	3.8 ± 4.0
unplanted	102 ± 5.4		-31.9 ± 6.4	-31.9 ± 6.4	100	--	--
	wheat						
	MBC_{total}	**MBC_{dfr}**	**Net-MBC: decrease (-), increase (+)**				
			MBC_{total}	**MBC_{SOM}**		**MBC_{dfr}**	
Distance [mm]	[µg C g^{-1} soil]	[% of total]	[µg C g^{-1} soil]	[µg C g^{-1} soil]	[% of total]	[µg C g^{-1} soil]	[% of total]
0 - 1	133 ± 9.5	20.8 ± 3.0	-46.7 ± 5.4	–31.9 ± 8.2	67.5 ± 11.9	–14.8 ± 3.8	32.5 ± 11.9
1 - 2	128 ± 12.5	7.8 ± 2.7	-29.4 ± 10.5	–27.6 ± 10.5	93.3 ± 7.0	–1.8 ± 1.6	6.7 ± 7.0
2 - 3	109 ± 5.1	4.7 ± 1.2	-26.8 ± 3.7	–26.8 ± 3.9	100 ± 3.8	–0.0 ± 1.1	0.0 ± 3.8
3 - 4	99.1 ± 2.9	5.1 ± 4.7	-27.3 ± 14.2	–27.1 ± 13.6	102 ± 6.4	–0.2 ± 0.6	-1.7 ± 6.4
4 - 6	103 ± 3.8	3.7 ± 1.7	-20.5 ± 2.7	–21.2 ± 1.7	104 ± 7.5	0.7 ± 1.4	-3.9 ± 7.5
6 - 8	96.0 ± 3.2	4.5 ± 2.9	-30.9 ± 3.9	–32.9 ± 2.3	107 ± 11.3	2.0 ± 3.0	-7.3 ± 11.3
unplanted	87.1 ± 8.4		-38.0 ± 7.5	-38.0 ± 7.5	100	--	--

mean ± standard deviation, (n = 4)

In contrast to microbial biomass C, microbial biomass N declined only slightly during the soil incubation in all samples. In the unplanted soils, microbial biomass N declined by approximately 41% (6.0 ± 1.7 µg N g^{-1} soil), and only by 6% (0.8 ± 0.5 µg N g^{-1} soil) and 15% (2.7 ± 1.7 µg N g^{-1} soil) in the rhizosphere samples of oat and wheat, respectively, related to the microbial biomass N contents at the beginning of the soil incubation. At the end of the soil incubation, microbial biomass N contents were also higher in the rhizosphere soils than in the unplanted soils within the entire investigated rhizosphere gradients (Tab. 5-6). No or only a marginal decrease of previously microbially incorporated rhizodeposition N (max. value in wheat soil sample from close to the former rooting compartment: < 0.7 ± 0.5 µg N g $soil^{-1}$) was detectable at the end of the soil incubation.

Tab. 5-6: Soil microbial biomass N [µg N g^{-1} soil dm] in total (MBN_{total}), the proportions of N derived from rhizodeposition (MBN_{dfr}) and the net decrease of total soil microbial biomass N after incubation of rhizosphere soil samples (42 days, 15°C) from different distances to a separated rooting compartment of ^{13}C- and ^{15}N-labelled oat and wheat and unplanted soil.

	oat		
Distance [mm]	**MBN_{total}** [µg N g^{-1} soil]	**MBN_{dfr}** [% of total]	**Net decrease MBN_{total}** [µg N g^{-1} soil]
0 - 1	16.2 ± 0.7	8.7 ± 0.9	0.4 ± 1.1
1 - 2	12.7 ± 1.1	1.4 ± 0.4	0.8 ± 1.1
2 - 3	11.7 ± 1.5	0.4 ± 0.1	1.4 ± 1.5
3 - 4	11.9 ± 0.7	0.2 ± 0.2	1.2 ± 0.6
4 - 6	11.9 ± 1.1	0.2 ± 0.1	0.6 ± 1.3
6 - 8	12.3 ± 1.4	0.1 ± 0.1	0.3 ± 1.6
unplanted	8.3 ± 2.0	--	5.5 ± 0.9
	wheat		
Distance [mm]	**MBN_{total}** [µg N g^{-1} soil]	**MBN_{dfr}** [% of total]	**Net decrease MBN_{total}** [µg N g^{-1} soil]
0 - 1	17.2 ± 1.8	11.2 ± 1.3	6.5 ± 3.2
1 - 2	15.8 ± 1.2	3.2 ± 0.5	3.3 ± 1.7
2 - 3	13.9 ± 0.9	1.5 ± 0.2	2.5 ± 2.4
3 - 4	12.8 ± 0.8	1.1 ± 0.3	2.0 ± 1.3
4 - 6	14.2 ± 0.6	0.7 ± 0.2	0.3 ± 1.3
6 - 8	13.5 ± 0.5	0.6 ± 0.1	0.7 ± 0.5
unplanted	9.2 ± 1.4	--	6.6 ± 2.3

mean ± standard deviation (n = 4)

5.4.6 Fate of rhizodeposition C and N

After 42 days of soil incubation, changes in C and N amounts, $\delta^{13}C$- and $\delta^{15}N$-signatures and proportions of C and N derived from rhizodeposition in total soil were not clearly detectable in the separated rhizosphere samples from different distances to the rooting compartment (data not shown). 38.7 ± 6.4% and 20.8 ± 2.1% of total rhizodeposition C detectable at the beginning of the soil incubation in the rhizosphere samples were released as CO_2-C from the rhizosphere soils samples from 0-6 mm distance to the former rooting compartment during soil incubation of oat and wheat, respectively (Fig. 5-5).

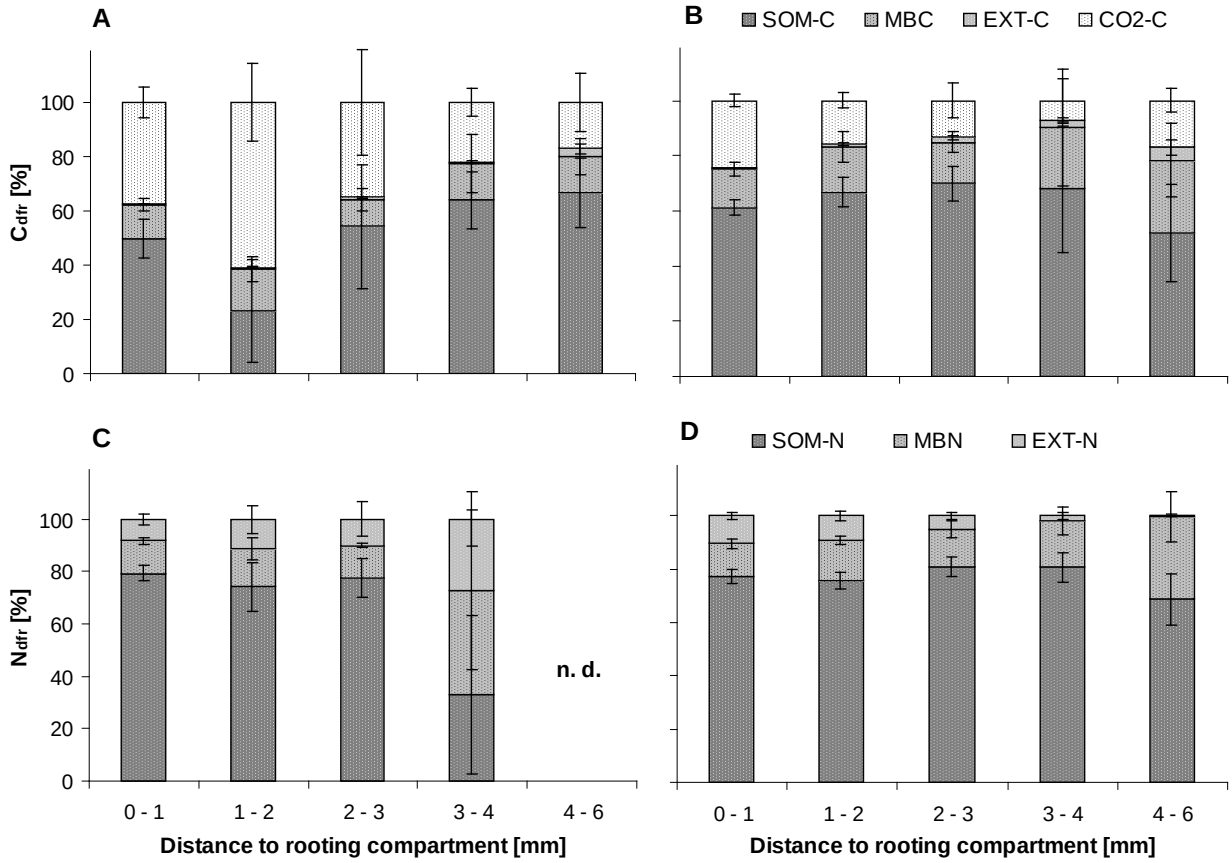

Fig. 5-5: Proportions of total C and N derived from rhizodeposition (C_{dfr}, N_{dfr}) in soil organic matter C and N (SOM-C, SOM-N), soil microbial biomass C and N (MBC, MBN)), 0.05 M K_2SO_4 extractable soil-C and soil-N (EXT-C, EXT-N) and the CO_2-C release (CO_2-C) after incubation of rhizosphere soil samples (42 days, 15°C) from different distances to a separated rooting compartment of ^{13}C- and ^{15}N-labelled oat and wheat. **A**: Proportion of C_{dfr} in soil samples from oat rhizosphere; **B**: Proportion of C_{dfr} in soil samples from wheat rhizosphere; **C**: Proportion of N_{dfr} in soil from oat rhizosphere **D**: Proportion of N_{dfr} in soil from wheat rhizosphere. I = standard error; n = 4.

On average, rhizodeposition C present in the microbial biomass accounted for 21.1 ± 5.5% and 19.1 ± 4.0% of the remaining total soil C derived from rhizodeposition and 13.3 ± 1.7% and 14.2 ± 1.4% of the remaining total N derived from rhizodeposition in the oat and wheat rhizosphere soils, respectively, at the end of the soil incubation. Only marginal proportions of C derived from rhizodeposition (< 1%) and approximately 9 to 10% of N derived from rhizodeposition remained in the extractable fraction.

5.5 Discussion

5.5.1 Methodological considerations - plant development and labelling

The present approaches can be used successfully for determining rhizodeposition C and N in soil after ^{13}C and ^{15}N double labelling of plants. A direct comparison of the effects on rhizodeposition C and N between oat and wheat was difficult, due to general differences in plant development. The different growth periods of wheat and oat plants until time of comparable plant development status in the presented experiments led to a different duration of root impact and, consequently, to different amounts of rhizodeposition input affecting the adjacent rhizosphere soil. The period in which the roots influenced the rhizosphere soil within the rhizosphere compartments was nearly twice as long for wheat (53 days) as for oat (27 days). This consequently had an impact on (1) total amounts of rhizodeposition C and N released into the adjacent soil, (2) extent of translocation of rhizodeposition C and N and its metabolites from the root surface into the adjacent soil (i.e.: rhizosphere extent), (3) growth and stimulation of the affected microbial community and (4) decomposition of rhizodeposits and turnover processes in the affected soils.

^{13}C enrichment in roots growing inside the rooting compartments in percent of ^{13}C enrichment in shoots was smaller in wheat (18.4% ± 1.9 standard deviation) than in oat (54.8% ± 10.4). These different relations between ^{13}C enrichment in the previously enriched plant material and the newly grown roots inside the rooting compartments indicate either a different translocation of previously assimilated ^{13}C in the different periods between the CO_2-C labelling and the time of determination of root labelling in oat (27 days after labelling) and wheat (53 days after labelling) or a stronger dilution of the ^{13}C enrichment in the above ground plant material in wheat than in oat during the growing period subsequent to the pulse labelling procedure. In contrast to this, the ^{15}N enrichment in the newly grown roots

was nearly equal between the two experiments and reached 77% (± 2.5) and 65.2% (± 2.2) of the ^{15}N enrichment of the previously labelled plant parts in oat and wheat, respectively, indicating a greater reallocation of previously assimilated N (structural-reserve-N) than of C, which might be preferentially incorporated into structural carbon of the plant tissue. Nevertheless, in both experiments, there was a reliable translocation of previously assimilated ^{13}C and ^{15}N into the newly growing roots inside the rooting compartment, indicated by high correlations of the isotopic abundance in the previously labelled plant parts and the newly grown roots. Independently of time and intensity of the labelling, the different procedures led to significant enrichment of the roots and traceable isotopic signals of rhizodeposits into the root affected soil. Certainly, adequate amounts of N derived from rhizodeposition were directly re-assimilated during the penetration of the growing roots within the rooting compartments, while C was lost by root respiration, which may cause a marginal underestimation of the proportions of C and N derived from rhizodeposition by using the mass balance equation for calculating the proportions according to BALESDENT and MARIOTTI (1996).

5.5.2 Diffusion and distribution of rhizodeposits C and N within the rhizosphere

In the rhizosphere soils of oat and wheat, microbial growth was affected significantly up to 2 mm from the separated roots. Microbial biomass N, 0.05 M K_2SO_4 extractable C and N and microbial C turnover (CO_2-C respiration) were even affected over the entire investigated gradients from the separated rooting compartments. Similar effects on microbial growth and the related C and N pools were found in a previous study with *Lolium perenne* (SCHENCK ZU SCHWEINSBERG – MICKAN et al., 2010) and by DE NEERGAARD and MAGID (2001), who observed rhizosphere effects on microbial biomass C and microbial biomass N up to 1 to 3 mm from an artificial root surface of *Lolium perenne* after 15 and 25 days of root influence. However, these experiments included a closed root mat, covering the separating gauze, and the penetration of root hairs into the adjacent rhizosphere soils. For estimating the amounts of rhizodeposits C and N translocated within the rhizosphere gradient, without any affect from the root hair zone, the creation of a closed root mat and the penetration of root hairs into the rhizosphere soil was strictly avoided in the present study. Nevertheless, significant amounts of C derived from rhizodeposition were revealed in total soil up to 6 mm from the separated roots of wheat and oat, whereas N derived from rhizode-

position only was revealed up to 4 mm and up to 2 mm from the separated roots of wheat and oat, respectively. SAUER et al. (2006) found root-derived ^{14}C in soil even up to 12 mm from the root surface of different plants, depending on the plant species. Also TOUSSAINT et al. (1995) detected root-derived ^{15}N in soil at 5 to 10 mm from the roots of wheat. Rhizodeposition, thus, moved from the rooting compartments into the adjacent rhizosphere soil and stimulated strong dynamic processes in the microbial community (rhizosphere effects), the extent and intensity of which depend on the amount of substrate input as well as the variety of substrate quality. Differences in the distance moved by rhizodeposits can be explained by differences in their diffusion ability, and can be caused by differences in exudate composition and solubility (DARRAH, 1991ab; MERBACH et al., 1999), by differences in plant species (MERBACH et al., 1999), by differences in plant physiological status (NEUMANN and RÖMHELD, 2007), by differences in density and length of root hairs (MERBACH et al., 1999) and most likely also by differences in soil conditions, total amounts of rhizodeposition and duration of root influence. At the time of soil sampling, total amounts of rhizodeposition C and N detectable within the entire rhizosphere soil gradient accounted for 36% C and 39% N less in oat than in wheat. This might be explained by higher amounts of total rhizodeposition moved/diffused from the rooting compartment into the adjacent rhizosphere soil compartment during the longer experimental phase of the wheat experiment, in comparison to the oat experiment. Apart from this, the distribution of rhizodeposition between different soil pools was equal in both experiments. In both experiments, nearly 80% and around 84 to 85% of C and N, respectively, derived from rhizodeposition were present in the non-extractable soil organic matter fraction. A proportion of 18 to 20% and nearly 15% of C and N, respectively, derived from rhizodeposition was present in the microbial biomass pool. Only marginal proportions were present in the 0.05 K_2SO_4 extractable pool. These patterns varied only minimally between several previous studies investigating the fate of continuously transferred rhizodeposition C and/or N into the rhizosphere soil pools during the growing phase of different plants under different conditions (MAYER et al., 2003; YEVDOKIMOV et al., 2006; WICHERN et al., 2008; SCHENCK ZU SCHWEINSBERG – MICKAN et al., 2010).

The fact that only marginal amounts of C and N derived from rhizodeposition were present in the extractable soil pool reflects the immediate transfer of recently released easily available rhizodeposits from this pool into the stimulated microbial biomass, non-extractable

soil pools and/or microbial residues (DE NEERGAARD and MAGID, 2001; BUTLER et al., 2004; YEVDOKIMOV et al., 2006). However, the half-life of rhizodeposits present in this pool is hard to quantify under conditions of plant growth due to methodological restrictions in separating root respiration and substrate induced microbial respiration and its direct transformation even close to the root surface or directly on the rhizoplane (NEUMANN and RÖMHELD, 2007). C lost by respiration as well as a strong diffusion gradient of inorganic N towards the roots, and consequently high re-assimilation of mineralised N derived from rhizodeposits during growth of the plant roots, restricted the calculation of any balances of substrate in- and output in the rhizosphere. Therefore, the majority of rhizodeposition induced processes will always be measured as a net outcome of several interrelationships at the plant-soil interface.

However, the C/N ratio of rhizodeposits is presumably high, because passively released low molecular and water-soluble exudates were found to be dominated by N-free carbohydrates and organic acid, with a low contribution of N-containing amino acids (MERBACH et al. 1999, HÜTSCH et al., 2002; BERTIN et al., 2003). The observed low C/N ratio of C and N derived from rhizodeposition about 15 and less close to the rooting compartments found in the present study was similar to that found by WICHERN et al. (2007) and a previous study with *Lolium perenne* (SCHENCK ZU SCHWEINSBERG – MICKAN et al., 2010) and may be caused by an immediate decomposition of easily available rhizodeposits of the growing microbial community at the soil root interface. On the one hand, the majority of the organic exudates were immediately incorporated into the microbial biomass (FALCHINI et al., 2003; BUTLER et al., 2004), followed by a rapid transformation into microbial metabolites and residues close to the root surface or directly on the rhizoplane (NEUMANN and RÖMHELD, 2007). On the other hand, it might be assumed that more recalcitrant components with a larger C/N ratio, such as actively secreted phytohormones or allelochemicals (UREN, 2007), as well as parts of recently formed metabolites or residues, diffused into the more remote soil. Mineralised N from rhizodeposits was not detectable in our study, but it may be assumed that the majority of the recently mineralised N is subject to the same fate as the root exuded inorganic N: a strong flow towards the roots of the growing plants. Both processes are indicated by an increase in the C/N ratio derived from rhizodeposition and a lower response of the microbial biomass to substrate input with increasing distance to the artificial rooting compartments.

5.5.3 Fate of rhizodeposition in relation to microbial processes

Within the rhizosphere, the affected microbial biomass is subject to a strong dynamic process, depending on the distance from the roots. In the zones nearer to the rooting compartments (< 3 mm distance), the significant increase in microbial biomass C and N reveals distinct microbial growth induced by continuously released rhizodeposits of oat and wheat during plant growth. In these soil layers, on average, microbial biomass C and N increased by 6.3% (± 0.8) C and 4.6% (± 3.0) N and even 23.7% (± 5.0) C and 25.4% (± 9.3) N in the rhizosphere soils of oat and wheat, respectively. Microbial biomass in these soil layers accounted for approximately 10% to 13% of C and 4% to 5% of N derived from rhizodeposition. In the outer distances (> 3 mm distance) total microbial biomass C and N clearly declined (but only significantly for MBN in the rhizosphere soil of oat), related to the unplanted soil, presumably caused by an increasing competition between plant and microbes for available nutrients and a clear deficiency of easily available substrates such as rhizodeposits. At these distances, the microbially incorporated rhizodeposition was marginal and accounted for less than 3% and less than 0.03% of microbial biomass C and N, respectively. The observed micro scale gradients of the processes occurring within the rhizosphere may explain why previous experiments had such great difficulty in establishing adequate sampling methods for rhizosphere soil. Due to severe methodological restrictions, in many investigations plant affected soil was divided into undefined rhizosphere soil and bulk soil after hand sampling of the roots (BUTLER, et al., 2004; WICHERN et al., 2007; MAYER et al., 2003). However, these methods either overestimate the proportions of the rhizodeposition, due to root hair fragments, or strongly underestimate the effects at the soil-plant interface, due to dilution of soil from outside of the main rhizosphere zone.

Microbial growth was largest closest to the separated rooting compartments. Close to the rooting compartment of oat, the content of microbial biomass C from rhizodeposition was larger (20.4 ± 3.1%) than that based on the net increase (15.4 ± 2.0%). A certain percentage of the autochthonous microbial biomass may be replaced due to an increased turnover induced by the easily available rhizodeposits in this soil (MEHARG, 1994; PATERSON et al., 2007). However, in the previous study with *Lolium perenne*, as much as 66% of the autochthonous microbial biomass C was replaced close to the simulated artificial rhizoplane, including the root hair zone (SCHENCK ZU SCHWEINSBERG – MICKAN et al., 2010). In contrast to this, the net increase in C incorporated into the microbial biomass

close to the rooting compartment of wheat was higher than the content of microbially incorporated C derived from rhizodeposition. In this soil, nearly 24% of the microbial biomass C close to the roots was derived from rhizodeposition, explaining the increase of microbial biomass C by only 85%. The net increase in N incorporated into the microbial biomass was higher than the content of microbially incorporated N derived from rhizodeposition in both experiments. Nearly 10% of the microbial biomass N close to the rooting compartment of oat and wheat were derived from rhizodeposition, explaining the increase of microbial biomass N by only 63% and 33% in oat and wheat, respectively. With increasing distance to the roots, the percentages of C and N derived from rhizodeposition explaining the growth of microbial biomass decreased rapidly. This indicates that, in addition to C and N from rhizodeposits, considerable amounts of soil organic matter-derived C and N were increasingly incorporated into the growing microbial biomass even under conditions of the growing plants.

Close to the roots, the distinct microbial growth may be attributed to large amounts of recently assimilated C rhizodeposits, most of which were accounted for by easily available low molecular weight substances with a high proportion of carbohydrates (HÜTSCH et al., 2002). The majority of rhizodeposits entering the rhizosphere are subject to fast microbial decomposition and pass the plant/soil system at a very rapid pace (BUTLER et al., 2004). Under these conditions of high amounts of readily decomposable C source, it is not surprising that N becomes limited in this highly active rhizosphere zone closer to the roots. Additionally, N is utilized from available native N pools even under the conditions of plant-microbe competition, whereas more recalcitrant rhizodeposits entering the rhizosphere and certain microbial metabolites are allowed to diffuse into the more remote soil and may contribute quite differently to the microbial population along the rhizosphere gradient.

According to FONTAINE et al. (2003), it may be assumed that the substrate input of easily available organic components close to the root surface primarily stimulates the growth of r-strategists, whereas the main part of more recalcitrant compounds diffused into the more remote soil is utilised by successive slow growing K-strategists. However, this view would correspond to the observed changes in microbial biomass within the rhizosphere gradient as well as in comparison with the two different experiments: a fast growth of r-strategists in the 27-days root-affected soil of oat primarily induced by readily decomposable C sub-

strate and a delayed growth of K-strategists in the 53-days root-affected soil of wheat induced by increasing amounts of more recalcitrant compounds such as metabolites present in soil after previous breakdown, and/or more recalcitrant rhizodeposits entering the rhizosphere continuously during plant growth. KANDELER et al. (2002) found a clear gradient in the bacterial community composition within the first 2.2 mm from the root surface of maize as well as differences in enzyme activities within the entire investigated rhizosphere gradient (5 mm) and the unplanted control soil even after only 12 days of root influence. Therefore, in accordance with these rapid changes observed in the microbial community along the rhizosphere gradient, the differences in microbial growth in relation to the rhizodeposit C and N input into the rhizosphere of wheat and oat might be explained as a different response of the autochthonous soil microbial population to the substrates input along the rhizosphere gradient.

5.5.4 Rhizodeposition input and its relation to C and N turnover within the rhizosphere

The distinct increase in microbial CO_2-C respiration observed with increasing proximity to the separated rooting compartments of oat and wheat is in line with previously observed factors (1) diffusion of rhizodeposition as well as (2) stimulated microbial growth within the rhizosphere gradient. During the soil incubation (42 days, 15°C), total released CO_2-C from the incubated rhizosphere soil samples was increased by 23.4% (±9.0) and 29.2% (±3.6) in oat and wheat, respectively, related to the unplanted control soils. The cumulative CO_2-C respiration accounted on average of the investigated rhizosphere zone (0-6 mm) for 8.9% (±1.1) and 5.3% (±1.1) of C derived from rhizodeposition in the oat and wheat experiments. The amounts of CO_2-C derived from rhizodeposition explain the total enhanced CO_2-C respiration by 65% and 30% in the incubated rhizosphere samples of oat and wheat, respectively. The large amounts of CO_2-C derived from rhizodeposition C in the oat rhizosphere samples may indicate that a high percentage of the rhizodeposits previously entering the soil are subject to a faster decomposition rate in relation to the rhizodeposit input in the wheat affected soil. However, the immediate turnover of recently released easily available exudates presumably reduces the net outcome of the induced effect of the rhizodeposit input measured in this study. The observed differences in the proportion between microbial CO_2-C respiration derived from native soil organic matter pool and organic C compounds derived from the substrate input by rhizodeposition in the two experi-

ments strongly indicate differences in microbial substrate utilization in these experiments, which may be attributed to the type, amount and duration of rhizodeposit input. MARX et al. (2007) investigated the fate of ^{13}C labelled maize and wheat exudates in an agricultural soil during a short-term incubation (25 days). They found increasing amounts of microbial biomass C and CO_2-C soil respiration during soil incubation experiments with constantly applied previously collected ^{13}C-labelled water soluble maize and wheat exudates. However, the C to N ratios of the applied exudates were very low (maize exudate solution: 1.5, wheat exudate solution: 2.8; MARX et al., 2007). The newly formed microbial biomass and the major part of the CO_2-C soil respiration consisted mainly of C derived from the constantly applied exudates in these investigations (MARX et al., 2007). These high percentages of C derived from the applied exudates in microbial biomass and CO_2-C soil respiration surely indicate the input of soluble exudates to soil as a favoured substrate for microbial growth and energy source and may only partially simulate the processes in the immediate vicinity of the root surface.

The fact that the increase in total CO_2-C respiration in the rhizosphere soil samples of oat and wheat was only partially accounted for by the substrate input of the labelled rhizodeposition C, related to the unplanted soil, indicates a stimulated microbial utilization of native soil C (i.e. positive priming effects; KUZYAKOV et al., 2000) during the soil incubation in both experiments. These positive priming effects on native soil organic matter decomposition observed from the incubated rhizosphere soil samples increased with increasing proximity to the former rooting compartments in oat and wheat. Related to the cumulative CO_2-C release from the soil samples along the rhizosphere gradient, the priming effects on native soil organic matter accounted for 8 to 18% and 9 to 31% of the soil CO_2-C release from the unplanted soil samples during soil incubation with increasing proximity to the former rooting compartments of oat and wheat, respectively.

The mechanisms of such priming effects occurring in the rhizosphere are still unclear (KUZYAKOV et al., 2000; KUZYAKOV, 2002; FONTAINE et al., 2003). However, within the rhizosphere, priming effects on native soil organic matter decomposition are obviously complex interactions. As revealed in this study, the promotion of CO_2-C derived from soil organic matter in the rhizosphere soils (1) decreased with increasing distance to the roots, (2) decreased with increasing duration of soil incubation and (3) was higher in the wheat affected soil, than in the oat affected soil. Due to the multitude of interactions within the

rhizosphere (e.g.: C and N input from rhizodeposition, nutrient and water uptake by growing plants, local changes of pH, differences in the relationship between nutrients due to diffusion or mass flow within the soil matrix to the roots, changes of microbial community structures) more investigations are still necessary to clarify these complex mechanisms of rhizosphere priming effects. However, different mechanisms of priming effects have also been discussed after addition of different complex substrates similar to single components of rhizodeposition (DE NOBILI et al., 2001; FALCHINI et al., 2003; MARX et al., 2007). The metabolic activity was found to be increased after the addition of glucose, amino acids, exudates and root extracts (DE NOBILI et al., 2001; FALCHINI et al., 2003; MARX et al., 2007). In contrast to our results, negative priming effects were dominant in soil after addition of wheat and maize exudates, indicating the preferential use of easily available substrate C input with a very low C to N ratio versus the native soil organic matter C in an unplanted soil (MARX et al., 2007). FALCHINI et al. (2003) assumed that a varying substrate input is utilized by different microbial communities and possibly leads to respiration of different soil C sources. In line with this view, FONTAINE et al. (2003) also attributed the appearance of priming effects to changes in the response of microbial communities to the substrate affected soil, which was based on competition between r- and K-strategists. They assumed that an increase in K-strategists in the rhizosphere is of great importance for plant fertility because K-strategists make soil organic matter N available to plants, whereas the growth and substrate utilization of r-strategists is limited under scarce or reduced nutrient (N) conditions (FONTAINE et al., 2003). This might explain why the occurrence of priming effects in the rhizosphere on microbial biomass activity and the acceleration of native soil organic matter mineralisation is strongly related to the availability of substrate input to a diversely responding microbial community in soil, including community changes. However, varying substrate input and a strong limitation of nutrient (N) availability for microbial growth are the case under the conditions of plant growth in the rhizosphere.

N mineralisation was not measured during the soil incubation. However, the net increase of 0.05 M K_2SO_4-extractable soil N in the incubated rhizosphere soil samples was not different between the unplanted soils, the rhizosphere soil of oat and the outer soil samples from the former rhizosphere compartments of wheat (4-8 mm). However, with increasing proximity to the former rooting compartments of wheat, the net increase of extractable soil N increased from 6.3 to 13.7 µg N g^{-1} soil. In all samples, the net increase in extractable soil

N mainly (< 90%) based on N derived from soil organic matter, indicating a distinct acceleration of native soil organic matter N mineralisation in comparison to previously released N derived from rhizodeposition. Even if the total amounts of extractable N were relatively small in comparison to soil C mineralisation, the pattern of the net soil N transfer into the extractable soil N pool may indicate the same priming effects on native soil organic N mineralisation in the affected rhizosphere soil of oat and wheat as discussed above for soil C. In addition to this, the observed pattern of net soil N transfer into the extractable soil N pool was strongly correlated to the observed pattern of microbial growth and C turnover in both experiments.

During soil incubation, the total microbial biomass C declined on average by about 17% and 21% in the incubated rhizosphere samples of oat and wheat, respectively. In the unplanted soils, the decline of microbial biomass C was higher and accounted for approximately 26%, compared to the microbial biomass C contents in these samples at the beginning of the incubation period. The total microbial biomass N contents in the incubated rhizosphere soil samples declined less than in the unplanted soil samples, leading to higher microbial biomass N contents at the end of the soil incubation in the rhizosphere soil samples than in the unplanted soil samples. Most of the decline in soil microbial biomass C and N during soil incubation was attributable to autochthonous biomass (native soil microbial biomass fraction). In wheat, differences in decline of total microbial biomass C and N were detectable depending on the distance to the former rooting compartments within the rhizosphere gradient during soil incubation. With increasing proximity to the former rooting compartments, the net decline in microbial biomass C contents during the soil incubation was attributable to increasing proportions of C from previously incorporated rhizodeposits in both experiments. In oat these percentages were higher than in wheat, presumably due to a higher proportion of fast growing r-strategists in this soil with increasing proximity to the rooting compartments of oat in comparison to wheat. At the end of the incubation, the contents of N derived from rhizodeposition in the microbial biomass differ only marginally, compared to the contents at the beginning of the incubation in both experiments. Nearly all of the N derived from rhizodeposition previously transferred into the microbial biomass during plant growth remained in the microbial biomass during the incubation. It might be assumed that the initial growth of microbial populations in the rhizosphere at the earlier stage of rhizodeposition input during plant growth, which is pre-

sumably the case in the experiment with oat, was mainly attributable to fast growing and presumably easily available substrate utilizing microbial populations. Under conditions of continuous rhizodeposit input as simulated in these experiments, in a second phase, which might be the case in the experiment with wheat, populations utilizing more recalcitrant compounds may become active and may induce a distinct decomposition of available compounds from the native soil organic matter within the rhizosphere, leading to drastic changes of C and N turnover in plant affected soil even in spatial distant from the roots and in comparison to unplanted soil. However, as observed in the soil incubation experiments, the processes of microbial biomass growth and C and N decomposition within the rhizosphere were significantly stimulated compared to the unplanted soils and these stimulated processes within the rhizosphere were visible even 42 days after the rhizodeposit input.

In total, approximately 40% and 20% of rhizodeposition C previously released during the plant growth period, detected in the adhering rhizosphere soil up to 6 mm from the rooting compartments at the time of soil sampling, were released from the rhizosphere soil samples during soil incubation in oat and wheat, respectively. Beside these differences in the utilization of C compounds from rhizodeposition for energy charge, indicated by strong differences in the release of CO_2-C derived from rhizodeposition between the rhizosphere soil samples from the rooting compartments of oat and wheat, the proportions of rhizodeposition C and N remaining in the soil samples after the incubation experiment were similar to the proportions at the beginning of the incubation. Approximately 20% of C and 13 to 14% of N derived from rhizodeposition remained in the microbial biomass pool in the oat and wheat experiments. Only marginal proportions of C derived from rhizodeposition (< 1%) and slightly increasing proportions of N derived from rhizodeposition (approx. 9 to 10%) remained in the extractable fraction. The main proportions of C and N derived from rhizodeposition seemed to be transferred into a relatively stable pool of the non-extractable soil organic matter fraction within the rhizosphere. However, these relations in the rhizosphere soil samples were similar to that found by MARX et al. (2007) after the continuous addition of artificially collected root exudates of wheat and maize with a very low C to N ratio to soil without any plant influence after 25 days of soil incubation. Related to the total C derived from rhizodeposition, 64% remained in the non-extractable C fraction,

18 to 20% were released as CO_2-C, 7 to 8% were present in MBC and 7 to 10% were present in the water extractable fraction (MARX et al., 2007).

However, the present study revealed that the continuous substrate input from rhizodeposition during plant growth affected the microbial growth and the transformation processes on soil organic matter C and N within the rhizosphere up to several millimetres distance from the roots. The occurrence of the processes promoted within the rhizosphere is presumably attributable to highly complex interactions between microbial substrate availability for C and N, microbial response to the substrate input and competition for nutrients between the growing plant and microbial biomass within the rhizosphere.

5.6 Conclusions

Rhizodeposition C and N were found to be strong determinants of microbial growth along the rhizosphere gradient. With increasing proximity to the roots, large amounts of C and N derived from rhizodeposition were contributed to microbial biomass. As a consequence, an excessive microbial growth was triggered by the substrate input from rhizodeposition, leading to high proportions of microbial biomass C and N derived from rhizodeposition. With increasing distance to the roots, decreasing microbial response indicated decreasing availability of the substrates released by rhizodeposition. Beside rhizodeposit N, large amounts of unlabelled soil N (native SOM) were incorporated into the growing microbial biomass towards the roots in both experiments, indicating a distinct acceleration of soil organic matter decomposition and N immobilisation into the growing microbial biomass even under the competition of plant growth. C decomposition of native soil organic matter was enhanced within the entire investigated rhizosphere gradients. This effect was less distinct in the rhizosphere samples of oat. The differential microbial response to the rhizodeposit input at a high spatial resolution from the roots indicates a complex interaction between microbial growth, turnover, substrate input derived from rhizodeposition and accelerated decomposition of native soil organic matter. The effects also differed between the experiments with oat and wheat, presumably due to different amounts of rhizodeposits previously entering the rhizosphere soils during plant growth. Moreover, the previous rhizodeposit input strongly affected the microbial growth and substrate turnover within the rhizosphere in comparison to the unplanted soil during the rhizosphere soil incubation ex-

periment, indicating that the microbial community feeds upon the stimulated processes induced by rhizodeposit C and N input within the rhizosphere during plant growth, even after the substrate input. The rhizodeposits thus have a meaningful function in the regulation of soil microbial biomass and the processes of soil organic matter composition and decomposition in soils, even during and after the plants' growing periods. The simultaneous determination of rhizodeposition C and N in soil and their complex functions within the rhizosphere, especially with regard to microbial biomass and soil organic matter turnover, contribute to a better understanding of the complex interactive processes occurring between plant and soil at the soil-root interface.

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6 Generelle Diskussion

6.1 Hintergrund und Methodik

Rhizodeposite stellen eine bedeutende Quelle für das mikrobielle Wachstum und den Stoffumsatz in der Rhizosphäre dar (LYNCH und WHIPPS, 1990; HÜTSCH et al., 2002; WICHERN et al., 2008). Das Wissen über diese durch Rhizodeposite induzierten Prozesse im wurzelangrenzenden Boden ist jedoch noch immer weitgehend begrenzt. Im Besonderen ist über den Verbleib und den Umsatz des Kohlenstoffs und Stickstoffs mit Herkunft aus der Rhizodeposition in der Rhizosphäre wenig bekannt (UREN, 2007). Zur Erweiterung des Verständnisses der mikrobiellen Prozesse und der Prozesse des C und N Umsatzes an der Schnittstelle Pflanze-Boden wurden daher Untersuchungen zum Verbleib und zum Umsatz des Rhizodepositions C und N in der Rhizosphäre in Abhängigkeit vom Abstand zur Rhizoplane bzw. zur Wurzel von *Lolium perenne, Triticum aestivum* und *Avena sativa* durchgeführt. Zur Untersuchung der Prozesse in der Rhizosphäre wurden artifizielle Pflanzen-Boden Systeme, sogenannte Rhizosphärensysteme eingesetzt.

Als methodische Voraussetzung für die Experimente wurde dazu ein Rhizosphärensystem nach dem Vorbild des von GAHOONIA und NIELSEN (1991) entwickelten Rhizosphärensystems konzipiert und angefertigt, welches sich für den Einsatz in ^{13}C- und ^{15}N-Doppelmarkierungsexperimenten eignet. Das System wurde zudem an eine Bodenbeprobungstechnik nach einem Vorbild von FITZ et al. (2003) angepasst, die es ermöglicht, frische Bodenproben in definiertem Abstand zur Rhizoplane zu gewinnen, ohne, wie bei bisherigen Untersuchungen gebräuchlich, aufwendige und die Bodeneigenschaften beeinflussende Gefriermikrotomtechniken einzusetzen (vgl.: GAHOONIA und NIELSEN, 1991; DE NEERGAARD und MAGID, 2001; KANDELER et al., 2002).

In den mit diesem System durchgeführten Experimenten konnte gezeigt werden, dass sich das modifizierte Rhizosphärensystem in Verbindung mit den durchgeführten ^{13}C- und ^{15}N-Markierungsmethoden und der eingesetzten Beprobungstechnik des Rhizosphärenbodens zur Untersuchung von C und N mit Herkunft aus der Rhizodeposition im Boden in Abhängigkeit vom Abstand zur simulierten Rhizoplane eignet. Die in dieser Arbeit entwickelten Methoden erweitern daher die Möglichkeiten zur Untersuchung spezifischer Wechselwirkungen zwischen Pflanzen und Boden im Bereich der Schnittstelle Boden-Wurzel unter Einsatz von Isotopen-Tracer-Techniken (YOUSSEF und CHINO, 1988; GAHOONIA und

NIELSEN, 1991; WENZEL et al., 2001). Wie im Versuch mit *Lolium perenne* gezeigt, kam es zu keiner Beeinflussung des unbepflanzten Bodens durch die eingesetzten Methoden der ^{15}N- und ^{13}C-Anreicherung zur Isotopenmarkierung von Pflanzen in den Anzuchtgefäßen des entwickelten Rhizosphärensystems (Kapitel. 4). Die in den verschiedenen untersuchten Bodenfraktionen (Gesamtboden, nicht extrahierbare organische Bodensubstanz, extrahierbarer und mikrobieller C und N) gemessenen Isotopenanreicherungen konnten daher vollständig auf die Rhizodeposition der zuvor ^{13}C und ^{15}N angereicherten Pflanzen zurückgeführt werden. Die eingesetzte Separationstechnik sowie die differenzierende Beprobung des Rhizosphärenbodens ermöglichte zudem die Simulation einer Rhizoplane, durch die Möglichkeit, dass Wurzelhaare die 30 µm Gaze durchdringen und in den angrenzenden Boden hineinreichen konnten, als auch die Simulation eines durch die Diffusion von Rhizodepositen und deren Metabolite beeinflussten Bodens in weiterer Entfernung zur eigentlichen Wurzel. Durch den Verzicht auf eine hochauflösende differenzierte Beprobung des Rhizosphärenbodens mittels Gefriermikrotomtechniken (KUCHENBUCH und JUNGK, 1982), ließen sich umfangreich diskutierte Artefakte durch die Beprobungstechnik auf die physikalischen und biologischen Eigenschaften des Bodens vermeiden (DE NEERGAARD und MAGID, 2001; FRIEDEL et al., 2003; FITZ et al., 2003). Die differenzierte Beprobung des Rhizosphärenbodens im Bereich von 0,8 bis 1,0 mm Beprobungsweiten, erwies sich als hinreichend genau, um die Prozesse an der Schnittstelle Boden/Wurzel zu erfassen. Bei der Simulation einer geschlossenen Rhizoplane erwies sich die Probenahme frischer (ungefrorener) Proben ebenfalls als vorteilhaft, da die in den Rhizosphärenboden hineinwachsenden Wurzelhaare mit dem Entfernen der Gaze vom Rhizosphärenboden ebenfalls leicht und ohne sichtbare Verletzungen der Wurzelhaare vom angrenzenden Rhizosphärenboden entfernt werden konnten. Hinweise auf eine mögliche Überschätzung der Effekte durch Wurzelhaarfragmente auf die Gehalte des C und N mit Herkunft aus der Rhizodeposition im direkt an die separierende Gaze angrenzenden Boden konnte in den Versuchen daher nicht gefunden werden. Als generelle methodische Problematik der Versuche ist allerdings die Bewässerung der Pflanzen in den künstlichen Systemen hervorzuheben (BADALLUCO et al., 1996). Um die ungewollte Verlagerung von Wurzelausscheidungen durch Gießwasser in tiefere Schichten der Rhizosphärenkompartimente während der Bewässerung zu vermeiden, wurde die Bewässerung der Pflanzen nur über die Kapillarität des Bodens gewährleistet. Dabei zeigte sich kein Einfluss der Bewässerung auf den Bodenwassergehalt in Abhängigkeit zum Abstand zur Gaze. In den unbepflanzten Gefäßen waren die Bodenwasser-

gehalte in den Rhizosphärenkompartimenten jedoch in allen Versuchen signifikant höher als in den bepflanzten Gefäßen, was sich auf den nicht zu kontrollierenden Wasserentzug durch die Pflanzen im Vergleich zum Boden ohne Pflanzen in diesem System zurückführen ließ.

In den Versuchen wurden in Abhängigkeit zum Isotopenangebot während der Markierungsphase unterschiedliche ^{13}C und ^{15}N Isotopenanreicherungen in den Pflanzen erreicht. In allen Experimenten wurde während der Markierungsphase assimilierter ^{13}C und ^{15}N aus der nunmehr ^{13}C und ^{15}N angereicherten Pflanze in die sich im Anschluss an die Markierungsphase neugebildete Wurzel verlagert. Die ^{13}C Anreicherung in den Wurzeln bezogen auf die ^{13}C Anreicherung im Aufwuchs betrug zum Zeitpunkt der Probennahmen 12% in *Lolium perenne*, 18% in *Avena sativa* und bis zu 55% in *Triticum aestivum*. Die ^{15}N Anreicherung in den Wurzeln bezogen auf die ^{15}N Anreicherung im Aufwuchs lag mit 73% in *Lolium perenne*, 77% in *Avena sativa* und 65% in *Triticum aestivum* vergleichsweise höher. Die unterschiedlich ausgeprägte Verlagerung der ^{13}C-Assimilate sowie die deutlich höhere Verlagerung der ^{15}N-Assimilate aus den während der Isotopenmarkierung gewachsenen Pflanzenteilen in die neugebildete Wurzel ging vermutlich auf die höhere Mobilität des Stickstoffs im Vergleich zum Kohlenstoff hervor, der vermutlich vornehmlich immobil in Strukturkomponenten der Pflanze eingebaut wurde. Mögliche Einflussfaktoren auf die Verlagerung zuvor assimilierten Kohlenstoffs und Stickstoffs hinsichtlich der Dauer der Isotopenmarkierungsperiode und des Zeitpunktes der Isotopenmarkierung der Pflanzen wurden in dieser Arbeit jedoch nicht weiter untersucht, was keine Aussage über Verdünnungseffekte aufgrund unterschiedlichen Sprosswachstums der Pflanzen zuließ. In Untersuchungen auf die Verteilung und die Verlagerung von ^{13}C-Assimilaten nach Puls-Markierungen zu verschiedenen Zeitpunkten der Entwicklung von *Lolium multiflorum* zeigten BUTLER et al. (2004) jedoch, dass zumindest auf die Verteilung von ^{13}C-Assimilaten innerhalb der Pflanze der ^{13}C-Markierungszeitpunkt in der Periode im Übergang vom aktiven Wurzelwachstum zum aktiven Sprosswachstums und während des aktiven Sprosswachstums nur marginalen Einfluss hat.

Der Einsatz von einmaligen und auch mehrmaligen Puls-Markierungsverfahren erfasst als Rhizodeposition nur kürzlich fixierte Assimilate (LYNCH und WHIPPS, 1990). Die kürzlich fixierten ^{13}C-Assimilate gelangen dabei innerhalb weniger Minuten bis Stunden in die verschiedene Pools in der Rhizosphäre und unterliegen dort einem raschen mikrobiellen Umsatz (CHENG et al., 1993; BUTLER et al., 2004; DILKES et al., 2004). Zur Schätzung der ge-

samten Rhizodeposition einschließlich komplexerer Rhizodeposite eignen sich dagegen nur Verfahren mit kontinuierlicher Markierung während des Wachstums der Pflanze oder *natural abundance* Methoden (LYNCH und WHIPPS, 1990). Der Einsatz und die Vergleichbarkeit der verschiedenen Markierungstechniken ist dabei nur unter bestimmten Einschränkungen möglich (WERTH und KUZYAKOV, 2008). Die in den vorliegenden Untersuchungen eingesetzte vorausgehende Isotopenmarkierung der Testpflanzen (Pre-labelling-Methode) setzt daher voraus, dass (1) die neugebildeten Wurzelmatten der zuvor markierten Pflanzen über die Dauer der Wurzelentwicklung anteilig aus einer gleichmäßigen Remobilisation und schließlich Translokation des zuvor während der Markierungsperiode in den Pflanzen fixierten ^{13}C und ^{15}N gebildet wurden, und (2) das Isotopenverhältnis in den neugebildeten Wurzelmatten dem Isotopenverhältnis der Rhizodeposition zum Zeitpunkt der Probenahme gemäß den Prinzipien kontinuierlicher Markierungsmethoden bzw. *natural abundance* Methoden entsprach (BALESDENT und MARIOTTI, 1996; KUZYAKOV und SCHNECKENBERGER, 2004). Durch den nichterfassbaren Anteil der schon während des Wurzelwachstums in den separierten Wurzelraum des Rhizosphärensystems stattfindenden Wurzelatmung einschließlich des schnellen Umsatzes mikrobiell leicht verfügbarer Rhizodeposite direkt auf der Wurzeloberfläche und die vermutlich direkte Wiederaufnahme von kürzlich mineralisiertem N in der Wurzelnähe, lässt sich der Rhizodepositionseintrag in den angrenzenden Rhizosphärenboden nur als Netto-Bilanz erfassen.

6.2 Verteilung der C- und N-Rhizodeposition im Rhizosphärenboden

In den Experimenten mit Weizen und Hafer waren außerhalb des Wurzelraums der Pflanzen signifikante Mengen an Rhizodepositions C bis maximal 6 mm Entfernung von der Gaze und Rhizodepositions N bis maximal 4 mm Entfernung von der Gaze im Boden nachweisbar. Im Vergleich dazu fanden SAUER et al. (2006) wurzelbürtigen ^{14}C in vergleichbaren Versuchen schon nach 4 Tagen in Entfernungen bis zu 12 mm vom Wurzelraum verschiedener Pflanzen. Ebenso fanden TOUSSAINT et al. (1995) wurzelbürtigen ^{15}N in Bodenkompartimenten in 5-10 mm Entfernung von der Wurzel. DARRAH (1991 a, b) und FALCHINI et al. (2003) wiesen dazu Unterschiede in der Diffusion einzelner anorganischer und organischer Verbindungen aus der Rhizodeposition im Boden nach. In den Versuchen von FALCHINI et al. (2003) zeigten sich nach 3 Tagen Diffusionsgradienten von Glucose und Glutaminsäure bis zu 14 mm im Boden. Unterschiede im Diffusiongradienten von Rhizodepositen können dabei auf Unterschiede in der Zusammensetzung der Rhizodeposition (DARRAH, 1991 a, b; MERBACH et al., 1999), Unterschiede in der physiologischen

Entwicklung der Pflanzen (NEUMANN und RÖMHELD, 2007), Unterschiede in der Ausprägung der Wurzelhaardichte und -länge (MERBACH et al., 1999) aber auch auf Unterschiede verschiedener Bodeneigenschaften zurückgeführt werden.

In direkter Wurzelnähe lagen die C/N-Verhältnisse der Rhizodeposition bei 11-15 und stiegen auf Werte über 30 im Versuch mit *Lolium perenne* und bis auf Werte über 100 in weiterer Entfernung zum Wurzelraum in den Getreideversuchen an. Die niedrigen C/N-Verhältnisse kleiner 15 in direkter Wurzelnähe sind vergleichbar mit denen von WICHERN et al. (2007) gemessenen Werten und sicherlich die Folge eines stetigen Umsatzes leicht verfügbarer Rhizodeposite in direkter Wurzelnähe oder gleich auf der Wurzeloberfläche. Die hohen Anteile mit bis zu 83% an N-freien Kohlenhydraten und Karbonsäuren in dieser Fraktion (MERBACH et al., 1999) lassen ein hohes C/N Verhältnis der Gesamt-Rhizodeposition vermuten. Das brutto C/N-Verhältnis der Rhizodeposition ist jedoch aufgrund des hohen und schnellen mikrobiellen Umsatzes im Boden kaum zu quantifizieren. MERBACH et al. (1999) konnten zeigen, dass schon in direkter Wurzelnähe 43 bis 86% des C aus der Rhizodeposition einer schnellen mikrobiellen Veratmung unterliegen. Niedermolekulare organische Verbindungen machen den bedeutensten Anteil der Rhizodeposition aus. In Untersuchungen von MERBACH et al. (1999) lagen in direkter Wurzelnähe bis zu 75% der ^{14}C markierten und bis zu 95% der ^{15}N markierten wurzelbürtigen Verbindungen als wasserlösliche Fraktion, größtenteils dominiert von Kohlenhydraten und Karbonsäuren jedoch mit geringerem Anteil an Aminosäuren, vor. Mit zunehmender Entfernung zur Wurzel nahm diese wasserlösliche Fraktion jedoch stark ab, während der wasserunlösliche Anteil relativ zunahm. Es lässt sich daher vermuten, dass der zu beobachtende Anstieg der C/N-Verhältnisse der Rhizodeposition im Boden mit zunehmender Entfernung zur Wurzel auf Unterschiede in der Diffusion und der mikrobiellen Substratverfügbarkeit der Rhizodeposite zurückzuführen sein kann: Während leicht verfügbare Verbindungen der in den Boden abgegebenen Rhizodeposition schon in direkter Wurzelnähe dem mikrobiellen Umsatz unterliegen, diffundieren mehr komplexere und weniger mikrobiell ansprechende Verbindungen wie aktiv ausgeschiedene Phytohormone oder allellopathische Verbindungen (UREN, 2007) auch mit höherem C/N Verhältnis sowie gebildete Metabolite in weitere Bodenschichten. Hinsichtlich des mineralisierten N kann dagegen angenommen werden, dass dieser unter den Bedingungen des Pflanzenwachstums einen ausgeprägten Diffusionsgradienten zur Wurzel hin aufweist und dort einer raschen Aufnahme durch die wachsenden Pflanzen unterliegt.

Zum Zeitpunkt der Probennahme wurden in allen Experimenten nur marginale Mengen an C und N mit Herkunft aus der Rhizodeposition in der 0,05 M K_2SO_4-extrahierbaren Fraktion des Bodens gefunden. Die Gehalte des extrahierbaren C und N mit Herkunft aus der Rhizodeposition zeigten im Versuch mit *Lolium perenne* eine hohe Streuung im Rhizosphärenboden und betrugen im Durchschnitt ca. 4% des gesamten im Boden gemessenen Rhizodepositions-C und -N. In den Versuchen mit Getreide lagen die Gehalte des extrahierbaren C mit Herkunft aus der Rhizodeposition bei unter 1% des gesamten im Boden gemessenen Rhizodepositions-C. Die Gehalte des extrahierbaren Rhizodepositions-N lagen dabei teilweise an der Nachweisgrenze. In diesen Untersuchungen wurden 75% der gesamt Kohlenstoffdeposition und 95% der gesamt Stickstoffdeposition im Boden bis 3 mm von der Rhizoplane gefunden. Im Durchschnitt wurden dabei ca. 80% des im Boden wiedergefundene Rhizodepositions-C in der nicht-extrahierbaren organischen Bodensubstanz wiedergefunden. In den Getreideversuchen betrug der Anteil des nicht-extrahierbaren Rhizodepositions-N im Boden bis zu 85% des gesamten im Boden wiedergefundenen N mit Herkunft aus der Rhizodeposition. Die Anteile des in der mikrobielle Biomasse immobilisierten C und N mit Herkunft aus der Rhizodeposition betrugen in den Getreideversuchen ca. 20% für C und ca. 15% für N und lagen damit in vergleichbaren Größenordnungen bisheriger Studien zur C und/oder N Rhizodeposition in verschiedenen Bodenpools (MAYER et al., 2003; YEVDOKIMOV et al., 2006; WICHERN et al., 2007). In Freilandversuchen mit Hafer und Erbsen betrugen die am Ende der Vegetationsperiode im Pool der mikrobiellen Biomasse des durchwurzelten Bodens wiedergefundenen Anteile des C und N mit Herkunft aus der Rhizodeposition 14-21% für C und 11-23% für N (WICHERN et al., 2008). Die bei WICHERN et al. (2008) wiedergefundenen Anteile der Rhizodeposition in der mikrobiellen Biomasse des Bodens im Freilandversuch zeigten trotz erheblicher methodischer Unterschiede eine Vergleichbarkeit mit den Ergebnissen der eigenen Versuche. Bei den Versuchen fanden sich zudem am Ende der Vegetationsperiode 15-25% des von den Pflanzen assimilierten C (netto) und bis zu 30% des assimilierten N (netto) in der Rhizodeposition im durchwurzelten Boden wieder (WICHERN et al., 2008).

Im Versuch mit *Lolium perenne* nahm der Anteil des mikrobiell immobilisierten Rhizodepositions-C und -N entlang des Rhizosphärengradienten von der Wurzeloberfläche bis in den weiter entfernteren Boden kontinuierlich von 33 auf 4% des Gesamt-Rhizodepositions-C und von 37 auf 16% des Gesamt-Rhizodepositions-N ab, was ebenfalls auf einen möglichen höheren Anteil rekalzitranter Verbindungen und/oder Metabolite im weiteren Rhi-

zosphärenbereich der Wurzel hindeuten kann, während die Prozesse in nächster Wurzelumgebung inklusive der Wurzelhaarzone vom stetigen Eintrag leichtverfügbaren Substrats gekennzeichnet waren. Hinsichtlich des erst kürzlich assimilierten C fanden DOMANSKI et al. (2001), dass schon 6 h nach einer ^{14}C-Puls-Markierung von *Lolium perenne* 4,9% des gesamten von der Pflanze assimilierten ^{14}C in die mikrobiellen Biomasse des Rhizosphärenbodens inkorporiert wurde. 1,1% des gesamt assimilierten ^{14}C befanden sich zu dieser Zeit in der extrahierbaren Fraktion des Bodens (0.5 M K_2SO_4). Ab dem 4 Tag betrug der Anteil des in die mikrobielle Biomasse inkorporierten ^{14}C 1,3%-1,8% der Gesamt-^{14}C-Assimilation und blieb bis zum Versuchsende in dieser Höhe. DOMANSKI et al. (2001) vermuten daher, dass sich in dieser Zeit ein Gleichgewicht zwischen Rhizodeposition, mikrobieller Aufnahme und mikrobiellen Umsatz des ^{14}C eingestellt haben könnte. In vergleichbaren Versuchen nach ^{14}C-Puls-Markierung von *Lolium perenne* fanden RATTRAY et al. (1994) die höchste Inkorporation des zuvor assimilierten ^{14}C in die mikrobielle Biomasse in der Rhizosphäre schon nach 3 h mit 74% der Gesamt-^{14}C-Rhizodeposition im Boden. Die Autoren geben den Anteil des in der Rhizosphäre wiedergefundenen zuvor assimilierten ^{14}C zu diesem Zeitpunkt jedoch mit lediglich 0,77% des gesamt assimilierten ^{14}C an (RATTRAY et al., 1994). BUTLER et al. 2004 fanden in Versuchen mit *Lolium multiflorum* zum Umsatz von Rhizodepositions-^{13}C aus kürzlich assimiliertem ^{13}C, dass im Boden innerhalb von 8 Tagen nach der Pflanzenmarkierung der Anteil des in der mikrobiellen Biomasse gefundenen ^{13}C von 85-97% (Tag 1 nach der ^{13}C-Markierung) auf 27-42% (Tag 8 nach der ^{13}C-Pflanzenmarkierung) abnahm. Im extrahierbaren Pool (0.05 M K_2SO_4) blieben die ^{13}C-Werte dagegen unverändert (BUTLER et al. 2004). In vergleichbaren Untersuchungen mit ^{14}C-Markierung und verschiedenen Pflanzen fanden DE NEERGAARD und GORISSEN (2002) 30 Tage nach der Pflanzenmarkierung ebenfalls eine Abnahme des in die mikrobielle Biomasse inkorporierten ^{14}C auf 30-40% des Gesamt ^{14}C im Boden. In den Versuchen beider Autorengruppen nahmen die Gehalte des mikrobiellen Biomasse C in den wurzelbeeinflussten Böden mit zunehmender Versuchsdauer zu (BUTLER et al., 2004; DE NEERGAARD und GORISSEN, 2002).

6.3 Mikrobielles Wachstum in der Rhizosphäre

Der Rhizosphärenraum der Testpflanzen war geprägt von einem signifikanten Anstieg der mikrobiellen Biomasse mit zunehmender Nähe zur Wurzel. Die mikrobiellen Wachstumsprozesse in der Rhizosphäre ließen sich auf den kontinuierlichen Eintrag von Rhizodepositen in den wurzelangrenzenden Boden während des Pflanzenwachstums zurückführen.

Diese substratinduzierten Wachstumsprozesse der mikrobiellen Biomasse im Rhizosphärenboden der Pflanzen waren bis zu 2 mm Entfernung vom Wurzelraum der verschiedenen Pflanzen im Vergleich zum unbepflanzten Testboden signifikant. Im Versuch mit *Lolium perenne* stiegen die Gehalte des mikrobiellen C und N um jeweils 70% innerhalb des untersuchten Gradienten mit zunehmender Nähe zur Rhizoplane. Im Vergleich zum unbepflanzten Boden stieg der mikrobielle C im Boden direkt an der Rhizoplane um 49% an, während der mikrobielle C im Boden in 4,2 mm Entfernung um 16% unter dem mikrobiellen C des unbepflanzten Bodens lag. Im Boden direkt an der Rhizoplane stammten im Durchschnitt 66% des mikrobiellen Biomasse C aus der Rhizodeposition. In 1,8 mm Entfernung betrug der Anteil nur noch 13%. In direkter Wurzelnähe (Wurzelhaarzone) ließ sich daher vermuten, dass ein bedeutender Anteil der autochthonen mikrobiellen Biomasse durch den permanenten Eintrag mikrobiell leicht umsetzbarer Rhizodeposite durch neugebildete mikrobielle Biomasse ersetzt wurde (MEHARG, 1994; PATERSON et al., 2007). Im Boden direkt an der Rhizoplane war der mikrobielle N um 100% höher als im unbepflanzten Boden. Der Anstieg ließ sich jedoch nur zu 59% aus in die mikrobielle Biomasse inkorporiertem N mit Herkunft aus der Rhizodeposition erklären. Im Boden direkt an der Rhizoplane stammten nur 30% des mikrobiellen N aus der Rhizodeposition und in 1-1,8 mm Entfernung zur Rhizoplane nur noch 6% aus der Rhizodeposition. Was zeigte, dass im Vergleich zu C in dieser Zone zusätzlich zum Substrateintrag N aus der Rhizodeposition eine bedeutende Menge an N aus der nativen organischen Substanz des Bodens in die wachsende mikrobielle Biomasse überführt wurde.

Die Intensität des mikrobiellen Wachstums war in den Getreideversuchen unterschiedlich ausgeprägt und ließ einen deutlichen Zusammenhang zu den Mengen der im Boden verbliebenden C und N Rhizodeposition zum Zeitpunkt der Probenahme erkennen. Im Rhizosphärenboden des Hafers (0 bis 8 mm) waren im Durchschnitt die Gehalte an Rhizodepositions C 36% und an Rhizodepositions N 39% geringer als im Rhizosphärenboden des Weizens. In den Experimenten differenzierte sich während des Pflanzenwachstums ein deutlicher Gradient zunehmender Gehalte der mikrobiellen Biomasse in der Rhizosphäre mit zunehmender Nähe zum Wurzelraum der Pflanzen. In direkter Wurzelnähe (0-1 mm) betrugen die C-Gehalte der mikrobiellen Biomasse am Tag der Probenahme im Rhizosphärenboden des Weizens 179.9 ± 8.2 µg C g^{-1} Boden TM und im Hafer 158.8 ± 3.8 µg C g^{-1} Boden TM. In beiden Böden waren die Gehalte des mikrobiellen Biomasse C signifikant höher als im unbepflanzten Boden. Im Vergleich zum unbepflanzten Boden wurden in di-

rekter Wurzelnähe am Tag der Probenahme im Rhizosphärenboden des Weizens 41% höhere Gehalte an mikrobiellen Biomasse C gemessen. Im Vergleich zum unbepflanzten Boden wurden in direkter Wurzelnähe am Tag der Probenahme im Rhizosphärenboden des Hafers 18% höhere Gehalte an mikrobiellen Biomasse C gemessen. Im Rhizosphärenboden des Weizens betrug der Anteil des mikrobiell inkorporierten C mit Herkunft aus der Rhizodeposition im Boden bis 1 mm Entfernung von der Rhizoplane 24%. Mit zunehmender Entfernung nahmen die Anteile von 7.8% auf weniger als 2% ab. Ab 2-3 mm von der Rhizoplane kam es zudem zu einem Anstieg des mikrobiellen Biomasse C mit Herkunft aus bodenbürtiger organischer Substanz oder reinkorporierter residualer mikrobieller Biomasse. In direkter Wurzelnähe (0-1 mm) war damit der Anteil des C mit Herkunft aus der Rhizodeposition an der neugebildeten Biomasse (bezogen auf die Gehalte im unbepflanzten Boden) mit 84% am höchsten. Im Rhizosphärenboden des Hafers war dieser Effekt deutlich schwächer ausgeprägt. Der Anteil des mikrobiell inkorporierten C mit Herkunft aus der Rhizodeposition betrug im Boden bis 1 mm Entfernung von der Rhizoplane bis zu 20%. Mit zunehmender Entfernung nahmen die Anteile von 4.5% auf weniger als 1.2% ab. Im Rhizosphärenboden des Hafers wurden in allen Entfernungen zur Rhizoplane zudem geringere C-Gehalte in der mikrobiellen Biomasse gemessen, die auf die ursprüngliche angestammte mikrobielle Biomasse oder mit Herkunft aus bodenbürtiger organischer Substanz zurückzuführen waren. In direkter Wurzelnähe (0-1 mm) war der Anteil des C mit Herkunft aus der Rhizodeposition größer als der Anstieg der mikrobiellen Biomasse bezogen auf die Gehalte im unbepflanzten Boden (128.4 ± 9.7%). Inwieweit es vor Beginn des substratinduzierten mikrobiellen Wachstums der mikrobiellen Biomasse demnach in der Rhizosphäre zu einer generellen Abnahme der autochtonen mikrobiellen Biomasse aufgrund möglicher Konkurrenzbedingungen im wurzelbeeinflußten Boden kam, ließ sich hierbei nicht statistisch absichern. Der Entzug von Nährstoffen aus der unmittelbaren Umgebung der Wurzeln durch die wachsenden Pflanzen läßt jedoch stark vermuten, dass das mikrobielle Wachstum in der Rhizosphäre im Vergleich zu einem unbepflanzten Boden einer direkten Beeinflussung unterliegt. Die Nutzungseffizienz des rhizodeposalen Substrateintrags durch die beeinflusste mikrobielle Biomasse standen dabei in enger Beziehung zur Verfügbarkeit eines ausreichenden Nährstoffangebotes zum Umsatz des mikrobiellen Wachstums (MERCKX et al., 1987).

In Bezug auf N zeigte sich erwartungsgemäß eine hohe Ausnutzung des in der extrahierbaren Fraktion vorliegenden Stickstoffs in den Rhizosphärenbodenproben des Weizens und

des Hafers am Tag der Probenahme. Trotz der im Vergleich zum unbewachsenen Boden herrschenden N-Limitation im Rhizosphärenboden der Versuchspflanzen, stieg der mikrobielle Biomasse N mit zunehmender Nähe zum Wurzelraum der Versuchspflanzen an. Ebenfalls ansteigend war der mikrobielle Biomasse N mit Herkunft aus der Rhizodeposition. Was zeigte, dass trotz erwarteter N-Limitation in der Rhizosphäre der Versuchspflanzen relevante Mengen an N-Rhizodeposition in die mikrobielle Biomasse überführt wurden. Dieser Effekt war im Rhizosphärenboden des Weizens stärker ausgeprägt als im Rhizosphärenboden des Hafers. Im Rhizosphärenboden des Weizens betrug der Anteil des mikrobiell inkorporierten N mit Herkunft aus der Rhizodeposition im Boden bis 1 mm Entfernung von der Rhizoplane 10%. Der Anstieg des mikrobiellen Biomasse N ging in dieser Zone jedoch nur mit bis zu 33% (bezogen auf die Gehalte im unbepflanzten Boden) aus der Aufnahme von N mit Herkunft aus der Rhizodeposition hervor. Im Rhizosphärenboden des Hafers betrug der Anteil des mikrobiell inkorporierten N mit Herkunft aus der Rhizodeposition im Boden bis 1 mm Entfernung von der Rhizoplane ebenfalls bis zu 10%. Der Anstieg des mikrobiellen Biomasse N ging in dieser Zone ebenfalls nur teilweise, maximal mit bis zu 63%, aus der Aufnahme von N mit Herkunft aus der Rhizodeposition hervor. Aufgrund des hohen Umsatzes des Rhizodepositions-C Eintrags im Boden, schlossen YEVDOKIMOV et al. (2006), dass C aus der Rhizodeposition trotz des geringeren Mengeneintrags im Vergleich zu C aus der bodenbürtigen organischen Substanz, ein mikrobiell vordergründig genutztes Substrat in der Rhizosphäre sei. In den Untersuchungen betrug der Anteil des mikrobiellen C, der auf die Herkunft aus der bodenbürtigen organischen Substanz zurückzuführen zwar 81-91% und war somit die bedeutendste Quelle für das mikrobielle Wachstums in der Rhizosphäre und 9-19% des mikrobiell genutzten Substrates stammten dabei aus der Rhizodeposition (YEVDOKIMOV et al., 2006).

In Abhängigkeit von der eingetragenen Menge und Dauer des Wurzeleinflusses auf den angrenzenden Boden kam es zu substratinduzierten mikrobiellen Wachstumsprozessen in der Rhizosphäre, die einerseits entweder auf die eingetragenen Mengen der C- und N-Rhizodeposition zurückzuführen waren, oder auf zusätzliche C- und N-Nutzungen aus der nativen organischen Bodensubstanz zurückzuführen waren. Der im Experiment mit *Lolium perenne* beobachtete hohe Umsatz der nativen mikrobiellen Biomasse in direkter Wurzelnähe (0-1 mm) war im Rhizosphärenbereich der Getreidepflanzen weniger stark ausgeprägt. Der Zusammenhang ging jedoch vermutlich auf den geringeren Rhizodepositionseintrag und der nicht vollständig geschlossenen Wurzelmatten in den Getreideversuchen

im Vergleich zum Versuch mit *Lolium perenne* zurück. Die ausgeprägte Dynamik der Substratnutzung und des mikrobiellen Wachstums im gesamten Entfernungsgradienten des beprobten Rhizosphärenbereichs der Testpflanzen ließen zudem eine Änderung der Populationsstrukturen in Abhängigkeit zum rhizodeposalen Substrateintrag vermuten. PATERSON et al. (2007) fanden einen deutlichen Einfluss des Rhizodepositionseintrags auf die Entwicklung der mikrobiellen Populationsstruktur in der Rhizosphäre von *Lolium perenne* im Vergleich zu unbepflanztem Boden. Im Entfernungsgradienten von 0 bis 2,2 mm und 2,2 bis 5,0 mm Entfernung zur Wurzeloberfläche von Mais fanden KANDELER et al. (2002) ebenfalls deutliche Änderungen der bakteriellen Population. Mit zunehmender Nähe zur Rhizoplane nahm zudem die funktionale Diversität der mikrobiellen Population zu (KANDELER et al., 2002). FALCHINI et al. (2003) fanden vergleichbare Gradienten nach dem Eintrag leichtmolekularer Exudatkomponenten (Glucose, Oxalsäure, Glutaminsäure) in ein simuliertes Rhizosphärenkompartiment und führten diese auf das unterschiedliche Ansprechen der bakteriellen Gemeinschaft in der Rhizosphäre und auf die verschiedenen Diffusionsgradienten der zugegebenen leichtmolekularen Exudate im Boden zurück.

Die Ergebnisse der Versuche ließen daher vermuten, dass die Änderungen der mikrobiellen Biomasse in der Rhizosphäre auf die unterschiedliche Verfügbarkeit des C- und N-Substrats mit Herkunft aus der Rhizodeposition in den verschiedenen Entfernungen zur Wurzel zurückgeführt werden könnten. Die Dynamik dieser Prozesse schließt dabei den gesamten untersuchten Rhizosphärenbereich bis 8 mm Entfernung zur Rhizoplane ein. Es ließ sich vermuten, dass die Prozesse von der Diffusion und des Umsatzes der eingetragenen Rhizodeposite sowie gebildeter Residuen und Metabolite geprägt wurden. In weitester Entfernung zur Rhizoplane kam es zu einer Abnahme der mikrobiellen Biomasse im Boden im Vergleich zu einem unbepflanzten Boden, vermutlich aufgrund zunehmender Nährstoffkonkurrenz zwischen der wachsenden Pflanze und der durch Rhizodeposite stimulierten mikrobiellen Biomasse in der Rhizosphäre und vermutlich einem zunehmenden Mangel an verfügbarem Substrat (Rhizodeposite). Die Ergebnisse zeigten zudem, dass undifferenzierte Probenahmen von Rhizosphärenboden in Form von Abschütteln und/oder Handselektion des wurzelanhaftenden Bodens nur ein unzureichendes Bild über die mikrobielle Biomasse und die Prozesse in der Rhizosphäre liefern können.

6.4 C und N Umsatz in der Rhizosphäre

Das ausgeprägte mikrobielle Wachstum in direkter Wurzelnähe der Testpflanzen, besonders im Versuch mit *Lolium perenne*, deutete auf einen stetigen mikrobiellen Umsatz der eingetragenen Rhizodeposite in die Rhizosphäre hin. Der mikrobiell leicht verfügbare Substrateintrag ist nach HÜTSCH et al. (2002) gekennzeichnet durch größtenteils niedermolekularen organischen Verbindungen mit einer hohen Anteil an Kohlehydraten, die im Boden einem schnellen mikrobiellen Umsatz unterliegen (BUTLER et al., 2004). Mögliche Änderungen der mikrobiellen Populationsstrukturen im Entfernungsgradienten innerhalb der Rhizosphäre könnten sich daher aus dem stetigen Eintrag einer bedeutenden Menge leichtverfügbarer Rhizodeposite in den Boden in direkter Wurzelnähe und vordergründig stimuliertes Wachstum von substratspezifischen r-Strategen ableiten (FONTAINE et al., 2003). Im Gegensatz dazu kann vermutet werden, dass ein ebenso nicht unbedeutender Anteil rekalzitranter Verbindungen und Metabolite im Boden in weitere Entfernung von der Wurzel diffundieren und dort fortlaufend einer mikrobiellen Nutzung einer vorwiegend langsam wachsenden mikrobiellen Gemeinschaft unterlagen (K-Strategen). Gemessen an den Gehalten und den Isotopensignaturen des Gesamt-C und Gesamt-N im Boden ließen sich die C- und N-Umsatzprozesse während der relativ kurzen Inkubationsdauer (42 Tage) aufgrund der hohen Gesamt-C und Gesamt-N Gehalte relativ zu den geringen Rhizodepositionsmengen im Boden nicht quantifizieren. In den beiden Fraktionen des extrahierbaren C und N (0,05 M K_2SO_4 extrahierbarer C und N sowie mikrobieller Biomasse C und N) zeigten sich jedoch in unterschiedlichen Entfernungen vom Wurzelraum der Getreidepflanzen und in den Versuchen selbst Unterschiede im Umsatz des C und N aus der nativen organischen Bodensubstanz und aus dem Pool des zuvor durch Rhizodeposition eingetragenen C und N im Boden. Der extrahierbare C (0,05 M K_2SO_4) nahm während der Bodeninkubation (42 Tage, 15°C) in beiden Versuchen nur marginal ab (Hafer: -2,9 ± 0,8 µg C g^{-1} Boden; Weizen: -2,2 ± 0,4 µg C g^{-1} Boden). In den Rhizosphärenbodenproben des Weizens nahm der Anteil des aus der Rhizodeposition stammenden C im Vergleich zum Gesamtanteil in dieser Fraktion (5-7% C mit Herkunft aus der Rhizodeposition) jedoch verhältnismäßig stark ab (18%), was auf einen bevorzugten Umsatz des C mit Herkunft aus der Rhizodeposition im Vergleich zum Umsatz des C mit Herkunft aus der nativen organischen Substanz in dieser Fraktion hindeutete (YEVDOKIMOV et al., 2006). Bezüglich des C-Umsatzes in dieser Fraktion zeigte sich kein Einfluss innerhalb des gemessenen Entfernungsgradienten. Die Tatsache, dass ohnehin schon zum Zeitpunkt der Probenahme nur

geringe Mengen an Rhizodepositions C in dieser Fraktion zu finden waren, kennzeichnet deutlich den umgehenden Übergang von kürzlich in diesen Pool eingetragenen Rhizodepositen in die wachsende mikrobielle Biomasse, in den nicht extrahierbaren Pool der organischen Bodensubstanz und/oder in mikrobielle Residualmasse (DE NEERGAARD und MAGID, 2001; BUTLER et al., 2004; YEVDOKIMOV et al., 2006). In den inkubierten Rhizosphärenbodenproben nahmen die Gehalte des mikrobiellen Biomasse C und N weniger stark ab als in den zuvor unbepflanzten Böden. Die in der mikrobiellen Biomasse des Bodens zum Zeitpunkt der Probenahme wiedergefundenen Mengen an Rhizodepositions C unterlagen in den Rhizosphärenbodenproben von Weizen und Hafer im Entfernungsgradienten zur Wurzel einem differenzierten Abbau in den Inkubationsexperimenten. Der zuvor in der mikrobiellen Biomasse immobilisierte C mit Herkunft aus der Rhizodeposition unterlag im Versuch mit Weizen mit zunehmender Wurzelnähe einem höheren Umsatz, als der im Boden mit Hafer. Im Durchschnitt wurden im Rhizosphärenboden bis 6 mm Entfernung zum ehemaligen Wurzelraum des Weizens mit 217 ± 32 µg C g^{-1} Boden TM 31% mehr CO_2-C freigesetzt als im unbepflanzten Boden mit 166 ± 8 µg C g^{-1} Boden TM. In den Bodenproben aus direkter Wurzelnähe (0-1 mm) wurden mit 264 ± 14 µg C g^{-1} Boden TM sogar 60% mehr CO_2-C freigesetzt als im unbepflanzten Boden. In den Rhizosphärenbodenproben des Hafers wurden während der gesamten Inkubationsperiode nur bis 2 mm Entfernung von der ehemaligen Rhizoplane signifikant höhere Mengen an CO_2-C aus dem Boden freigesetzt als im unbepflanzten Boden. Im Rhizosphärenboden bis 6 mm Entfernung zur ehemaligen Rhizoplane des Hafers wurden im Durchschnitt 23% mehr CO_2-C freigesetzt als im unbepflanzten Boden. In den Bodenproben aus direkter Wurzelnähe (0-1 mm) wurden 73% mehr CO_2-C freigesetzt als im unbepflanzten Boden. Im Haferversuch verließen bis zu 52% des zuvor inkorporierten Rhizodepositions C den Pool der mikrobielle Biomasse während der Inkubation. Der mikrobielle Biomasse C in den wurzelbeeinflussten Bodenproben aus unterschiedlicher Entfernung vom Wurzelraum des Hafers nahm im Durchschnitt bis zu 20% während der Inkubation ab. Mit zunehmender Nähe zur ehemaligen Wurzel ging die Abnahme von 95 bis zu 50% auf die native mikrobielle Biomasse des Bodens zurück, während keine signifikanten Unterschiede im Umsatz des gesamten mikrobiellen Biomasse C im Entfernungsgradienten zu beobachten waren. Die signifikant höhere Abnahme der mikrobiellen Biomasse C Gehalte in direkter Nähe zum Wurzelraum von Weizen im Vergleich zur Abnahme in weiterer Entfernung zur Wurzel schien nahezu vollständig auf einen höheren Umsatz von zuvor inkorporierten Rhizodepositions C aus dem

Pool der mikrobiellen Biomasse zurückzugehen. Die Abnahme des gesamt mikrobiellen Biomasse C ging in dieser Entfernung zur Wurzel zudem zu mehr als 30% auf während des Pflanzenwachstums in die mikrobielle Biomasse des Bodens inkorporierten Rhizodepositions C zurück. Der Anteil des mikrobiellen Biomasse C mit Herkunft aus der nativen organischen Bodensubstanz nahm gleichzeitig mit zunehmender Nähe zur ehemaligen Wurzel von 107% bis auf 70% ab. Dies lässt vermuten, dass in direkter Nähe zur Wurzel ein bedeutender Teil der neugebildeten substratspezifischen mikrobiellen Biomasse einer höheren Umsatzrate unterlag, als in weiterer Entfernung zur Wurzel. Zusätzlich zum C wurden bedeutende Mengen an Rhizodepositions N in die wachsende mikrobielle Biomasse, in den nicht extrahierbaren Pool der organischen Bodensubstanz und/oder in mikrobielle Residualmasse überführt. Die im Haferversuch geringer ausgeprägten Rhizosphäreneffekte hinsichtlich des C Umsatzes erklären auch die nur marginalen Umsätze des mikrobiell immobilisierten N in den inkubierten Rhizosphärenbodenproben. Der mit zunehmender Nähe zum ehemaligen Wurzelraum des Weizens zuvor in der mikrobiellen Biomasse immobilisierte N war während der Bodeninkubation stabil in diesem Pool eingelagert. Im Vergleich zum unbepflanzten Boden verließen nur marginale Anteile des zuvor unter dem Einfluss des Wurzelwachstums in die mikrobielle Biomasse inkorporierten N diesen Pool. Bemerkenswerterweise wurden nur geringe Anteile des zuvor immobilisierten N mit Herkunft aus der Rhizodeposition umgesetzt (< 5% in direkter Wurzelnähe). Mit zunehmender Entfernung zum ehemaligen Wurzelraum der Pflanze wurden dagegen bis zu 137% des zuvor in der mikrobiellen Biomasse immobilisierten N mit Herkunft aus der nativen organischen Bodensubstanz umgesetzt. Die mikrobielle C und N Dynamik im Entfernungsgradienten zur Wurzel und im besonderen zum unbepflanzten Boden ließen daher vermuten, dass die Anteile der nativen mikrobielle Biomasse im Rhizosphärenboden einem stärkeren Abbau unterlagen als die unter dem Einfluss des Wurzelwachstums neugebildete mikrobielle Biomasse. Es kann in der Folge auch weiter vermutet werden, dass sich unter wachsenden Pflanzen mit zunehmender Nähe zur Wurzel und mit zunehmender Dauer des Substrateintrags durch Rhizodeposition eine substratspezifizierte (Rhizodeposite) mikrobielle Gesamtpopulation im Boden entwickelt, die sich in bedeutender Weise von der nativen mikrobiellen Gemeinschaft im Boden differenziert. Der Eintrag der Rhizodeposite in den wurzelbeeinflussten Boden (hier gemessen anhand von C und N) scheint die Umsatzprozesse im Boden zugunsten eines geringeren Abbaus der stimulierten mikrobiellen Biomasse aber auch zugunsten eines erhöhten C und N Umsatzes aus der nativen organischen Bo-

densubstanz zu fördern. Die rhizodeposalen Effekte reichen dabei bis zu mehreren Millimetern in den Boden hinein und bilden einen signifikanten Entfernungsgradienten von der Wurzel in den angrenzenden Boden hinein.

In dem durch stark stimuliertes mikrobielles Wachstum und mikrobiellen Umsatz geprägten untersuchten Rhizosphärengradienten nahmen in den Inkubationsversuchen die Gehalte an extrahierbaren N (0,05 M K_2SO_4) im Boden signifikant zu, was auf einen stetigen N-Mineralisationsprozess in den Rhizosphärenbodenproben hindeutete. Die Anteile des N in dieser Fraktion stammten dabei im Durchschnitt bis zu mehr als 97% aus der nativen organischen Bodensubstanz. Lediglich 11 bis 14% stammten in direkter Nähe zum Wurzelraum des Weizens und des Hafers aus der Rhizodeposition, was vermuten lässt, dass N mit Herkunft aus der Rhizodeposition bereits relativ stabil in der nicht extrahierbaren organischen Bodensubstanz und/oder in der mikrobiellen Biomasse und Residualmasse des Bodens während des Pflanzenwachstums festgelegt wurde.

Die Ergebnisse der Inkubationsversuche ließen daher den Schluss zu, dass die Ausgeprägtheit der zu erwartenden Rhizosphäreneffekte hinsichtlich des C und N Umsatzes im Wurzelraum der Pflanzen erheblich auf die Gesamtmenge und Dauer des Eintrags von C und N mit Herkunft aus der Rhizodeposition während der Wachstumsperiode der Pflanzen zurückgeführt werden konnte. Wie die Herkünfte des mikrobiellen C zeigten, gingen im Haferversuch die beobachteten Wachstumsprozesse der mikrobiellen Biomasse auf die Nutzung der eingetragenen ^{13}C-Rhizodeposition zurück, während im Weizenversuch die mikrobielle Biomasse zusätzlich C und N aus der nativen organischen Bodensubstanz inkorporierte. Zudem zeigten sich im Versuch mit Weizen ausgeprägte Priming-Effekte in den Rhizosphärenbodenproben. Der an der mikrobiellen CO_2-C-Produktion im Rhizosphärenboden des Weizens gemesse C-Umsatz in den Proben zeigte einen induzierten C-Umsatz aus der nativen organischen Bodensubstanz im Vergleich zum unbepflanzten Boden. Im Rhizosphärenboden des Hafers ließ sich der C-Umsatz dagegen fast vollständig auf den Rhizodepositionseintrag zurückführen. Priming-Effekte in der Rhizosphäre könnten dabei als Folge eines substrat-induzierten oder -stimulierten Umsatzes der organischen Bodensubstanz durch den Eintrag von Rhizodepositen in den wurzelnahen Boden auftreten (KUZYAKOV, 2002). Die auslösenden Prozesse für die Ausprägung von Priming-Effekten sind jedoch nicht vollständig geklärt. Mögliche systemische Zusammenhänge werden umfangreich von FONTAINE et al. (2003) und KUZYAKOV et al. (2000) dargestellt. Die besonderen Mechanismen von Priming-Effekten in der Rhizosphäre (*Rhi-*

zospere-Priming-Effects: RPE) werden von KUZYAKOV (2002) umfangreich diskutiert. Dabei handelt es sich um einen positiven Priming-Effekt, wenn der Abbau der nativen organischen Bodensubstanz beschleunigt ist und um einen negativen Priming-Effekt, wenn der Abbau der organischen Bodensubstanz verlangsamt ist (KUZYAKOV, 2002). In Bezug auf den Priming-Effekt in der Rhizosphäre bedeutet dies einen schnelleren oder verlangsamten Abbau der organischen Bodensubstanz im wurzelbeeinflussten Boden im Vergleich zu unbewachsenen Boden (KUZYAKOV, 2002). Die Richtung und Ausprägung des Effekts kann dabei von der Qualität der Rhizodeposite und dem Nährstoffangebot in der Rhizosphäre abhängig sein. LILJEROTH et al. (1994) fanden in Untersuchungen mit Weizen bei hohem mineralischem N-Angebot einen geringeren Priming-Effekt in der Rhizosphäre als bei niedrigem N-Angebot. In Versuchen mit Mais, wurden dagegen keine Unterschiede im Priming-Effekt in Abhängigkeit zum N-Angebot im Boden gefunden (LILJEROTH et al., 1994). Nach DE NOBILI et al. (2001) führen schon relativ geringe Mengen an mikrobiell leicht verfügbaren Substratzugaben (Glucose, Aminosäuren, Exudate) zum Boden zu einer kurzfristigen 2- bis 5-fachen Erhöhung der mikrobiellen CO_2-C Produktion im Boden. Bei den Versuchen von DE NOBILI et al. (2001) war der Effekt auf die mikrobielle Aktivität bei Zugabe von Wurzelextrakten größer als nach Zugabe von Glucose oder Aminosäuren. DE NOBILI et al. (2001) schlossen daraus, dass durch das komplexere Substrat auch eine breitere Population der gesamten mikrobiellen Biomasse im Boden angesprochen wurde. Dabei führten DE NOBILI et al. (2001) ebenso wie DALENBERG und JAGER (1989) und auch WU et al. (1993) diese zu beobachtenden Effekte nach Zugabe leicht verfügbarer organischer Substrate lediglich auf den erhöhten Umsatz der nativen mikrobiellen Biomasse zurück und differenzierten damit den beobachteten Effekt von Effekten, die auf einen Anstieg des mikrobiellen Abbaus der organischen Bodensubstanz zurückzuführen sein können. FONTAINE et al. (2003) vermuten, dass Priming-Effekte daher hauptsächlich auf der Dynamik der die organische Bodensubstanz mineralisierenden Mikroorganismen (K-Strategen) zurückzuführen zu sein muss und nicht allein durch die mikrobielle Population, die vereinzelt auf den Eintrag leichtverfügbarer Substrate reagiert (r-Strategen). Es kann daher vermutet werden, dass daher der mögliche Priming-Effekt ausgeprägter ist, je diverser die biochemische Struktur des eingetragenen organischen Substrates in den Boden ist (WU et al., 1993).

7 Schlussfolgerung und Ausblick

In dieser Studie konnte gezeigt werden, dass sich das entwickelte Rhizosphärensystem zur Untersuchung von C- und N-Rhizodepositen im Boden in Abhängigkeit vom Abstand zur Wurzeloberfläche eignet. Die in den verschiedenen untersuchten Bodenfraktionen (Gesamtboden, extrahierbarer und mikrobieller C und N) gemessenen Isotopenanreicherungen gingen dabei vollständig auf die Rhizodeposition der zuvor ^{13}C und ^{15}N angereicherten Pflanzen zurück. Die hohe Korrelation der ^{13}C- und ^{15}N-Anreicherung im markierten Pflanzenmaterial und der Anreicherung in der nach dem Markierungsverfahren neu gebildeten Wurzelmatte zeigte zudem, dass es zu einer gleichmäßigen Verlagerung des zuvor assimilierten ^{13}C und ^{15}N in die neugebildete Wurzel kommt. Die so erreichte kontinuierliche Markierung der neugebildeten Wurzel erlaubte so eine Schätzung der gesamten von der Pflanze abgegebenen Rhizodeposite, während sich bei Puls-Markierungsverfahren die Translokation auf kürzlich angereicherte ^{13}C- und ^{15}N-Assimilate in die Wurzel und als Rhizodeposition abgegebene Verbindungen beschränkt.

Kohlenstoff und Stickstoff mit Herkunft aus der Rhizodeposition waren im Boden bis zu mehreren Millimetern von der Wurzeloberfläche nachweisbar. Die Verteilung der eingetragenen Rhizodeposition schien dabei von den Gesamtmenge und von der Dauer des Eintrags abhängig zu sein. In den Untersuchungen wurde Rhizodeposition bis zu 8 mm Entfernung von der Rhizoplane im angrenzenden Boden gefunden. Beträchtliche Anteile der gesamt Rhizodeposition verblieben jedoch im wurzelangrenzenden Boden im Bereich von 0-3 mm von der Wurzeloberfläche. 75% der gesamt Kohlenstoffdeposition und 95% der gesamt Stickstoffdeposition wurden im Boden bis 3 mm von der Wurzeloberfläche gefunden. Im Durchschnitt wurden ca. 80% des gesamt Rhizodepositions-C und bis zu 85% des Rhizodepositions-N in der organische Bodensubstanz wiedergefunden. Nur marginale Anteile (<1%) waren in der extrahierbaren Fraktion nachzuweisen. Die verbliebenen Anteile fanden sich als relativ stabiler Anteil im Pool der mikrobiellen Biomasse wieder. Im wurzelbeeinflussten Boden kam es zu einem ausgeprägten Anstieg der mikrobiellen Biomasse mit zunehmender Nähe zur Wurzeloberfläche. In Abhängigkeit von der eingetragenen Menge der Rhizodeposition während des Wurzeleinflusses auf den angrenzenden Boden kam es zu substratinduzierten mikrobiellen Wachstumsprozessen in der Rhizosphäre. Der Eintrag schien einerseits in direkter Nähe zur Wurzel eine substratspezifische Population der mikrobiellen Biomasse im Boden zu induzieren, wobei dieser auch nach längerem kon-

tinuierlichen Substrateintrag in den Wurzelraum und mit zunehmender Entfernung zur Wurzel zunehmend auch den mikrobiellen Umsatz der nativen organischen Bodensubstanz stimulierte. Es zeigten sich daher auch differenziert ausgeprägte Priming Effekte auf den C und N Umsatz mit Herkunft aus der nativen organischen Substanz in den Rhizosphärenböden der Testpflanzen. Unter diesen Bedingungen stellte die organische Bodensubstanz zumindest eine bedeutende Stickstoff-Quelle für das mikrobielle Wachstum in der Rhizosphäre bereit. Inwieweit dieser Umsatz die zusätzliche Nutzung von bereits zuvor gebildeter mikrobieller Residualmasse mit einschloss, konnte nicht erfasst werden. Im Vergleich zu einem unbewachsenen Boden zeigte sich zudem, dass die bodenbürtige mikrobielle Gemeinschaft im wurzelbeeinflussten Boden mit zunehmender Entfernung abnahm. Es wurde daher vermutet, dass die mikrobielle und die pflanzliche Konkurrenz um verfügbare Nährstoffe in diesem Bereich zu einer Abnahme der bodenbürtigen mikrobiellen Biomasse führen kann und damit die Bildung mikrobieller Residualmasse im wurzelbeeinflussten Boden fördert. In bedeutender Weise war die C-Umsatzaktivität dagegen in diesem Bereich im Vergleich zu einem unbepflanzten Boden dennoch höher. Zudem zeigte sich im Inkubationsexperiment, dass die mikrobielle Biomasse im zuvor wurzelbeeinflussten Boden einem geringeren Abbau unterlag als im unbepflanzten Boden. Dadurch verblieb mehr Kohlenstoff und Stickstoff im mikrobiellen Pool des zuvor wurzelbeeinflussten Bodens im Vergleich zum unbepflanzten Boden. Dieser Effekt war für den mikrobiellen Kohlenstoff umso ausgeprägter, je höher der Rhizodepositions-C Eintrag in den Boden war. Der Effekt war für den mikrobiellen Stickstoff über den gesamten Rhizosphärenbereich ausgeprägt und selbst in den Rhizosphärenbodenproben, in denen keine Rhizodeposition mehr nachzuweisen war, signifikant.

Rhizodeposition übt daher eine bedeutende Funktion bei der Regulation der mikrobiellen Biomasse und der Prozesse des Auf-, Ab- und Umbaus der organischen Substanz im Boden aus. Die während des Pflanzenwachstums durch den Eintrag der Rhizodeposite stimulierten Prozesse im Boden scheinen auch nach dem Ereignis des Eintrages weiterhin prägend auf den Umsatz der mikrobiellen Biomasse und die organische Bodensubstanz zu sein. Die Kenntnisse über die Funktionen von C und N mit Herkunft aus der Rhizodeposition im Boden, gerade im Hinblick auf das Wachstum und die Aktivität der mikrobiellen Biomasse und den Umsatz der organischen Substanz innerhalb der Rhizosphäre, führen damit zu einem besseren Verständnis der komplexen Wechselwirkungen zwischen Pflanze und Boden.

8 Zusammenfassung

Ziel dieser Arbeit war es, die Bedeutung des C und N mit Herkunft aus der Rhizodeposition für das mikrobielle Wachstum und den C- und N-Umsatz in der Rhizosphäre in Abhängigkeit von der Entfernung zur Wurzel zu untersuchen. Dazu wurde als wesentliche methodische Voraussetzung ein künstliches Rhizosphärensystem entwickelt, um die durch die Rhizodeposite induzierten Prozesse an der Grenzfläche zwischen Wurzeloberfläche und Boden zu untersuchen. Dieses eingesetzte Rhizosphärensystem wurde nach einem Vorbild eines in schon vielfältigen Untersuchungen der Rhizosphäre eingesetzten Systems von GAHOONIA und NIELSEN (1991) konzipiert. Zur Verfolgung der pflanzlichen C- und N-Rhizodeposition im Boden wurden ^{13}C- und ^{15}N-Tracer-Isotopen-Techniken zur Isotopenmarkierung der Testpflanzen eingesetzt. Zur Probenahme des Rhizosphärenbodens in räumlichen Abstand zur künstlichen Rhizoplane wurde eine Schneidvorrichtung nach FITZ et al. (2003) eingesetzt, die es ermöglicht frische Bodenproben in definiertem Abstand zur künstlichen Rhizoplane zu schneiden.

Das Rhizosphärensystem wurde in ^{13}C- und ^{15}N-Doppelmarkierungsexperimenten mit *Lolium perenne*, *Triticum aestivum* und *Avena sativa* eingesetzt. Im Experiment mit *Lolium perenne* wurde die Eignung des Rhizosphärensystems und die Verteilung des C und N mit Herkunft aus der Rhizodeposition im Gesamtboden, der mikrobiellen Biomasse und der extrahierbaren Fraktion (0,05 M K_2SO_4) in Abhängigkeit von der Entfernung zur Rhizoplane untersucht. Der Rhizosphärenboden wurde in Abständen von 0-1, 1-1,8, 1,8-2,6, 2,6-3,4 und 3,4-4,2 mm von der Rhizoplane von *Lolium perenne* untersucht. In den Experimenten mit *Triticum aestivum* und *Avena sativa* wurde der Verbleib und der Umsatz des ^{13}C- und ^{15}N-markierten Rhizodepositionseintrags im Rhizosphärengradienten von 0-8 mm Abstand von den Wurzeln untersucht. In diesen Versuchen wurde Rhizosphärenboden in Abständen von 0-1, 1-2, 2-3, 3-4, 4-6 und 6-8 mm von der künstlichen Rhizoplane des Hafers nach 27 Tagen und des Weizens nach 53 Tagen beprobt. Im Anschluss daran wurden die Rhizosphärenbodenproben in Inkubationsexperimenten (15°C, 42 Tage) hinsichtlich der Verlagerung und des Umsatzes von C und N aus der Rhizodeposition in der mikrobiellen Biomasse, in der nicht extrahierbare organische Bodensubstanz und in der extrahierbaren C und N Fraktion im Boden in Abhängigkeit vom ehemaligen Abstand zum Wurzelraum untersucht. Zur Messung des C-Umsatzes mit Herkunft aus der Rhizodeposition und mit Herkunft aus der nativen organischen Bodensubstanz wurde zudem die

mikrobielle CO_2-C und die $^{13}CO_2$-C Respiration aus den Rhizosphärenbodenproben bis 6 mm Entfernung zur ehemaligen Rhizoplane zu mehreren Terminen gemessen.

In den Versuchen mit *Lolium perenne* gingen 4,2% der gesamt C und 2,8% des gesamt N im Boden bis 1 mm von der Rhizoplane auf die Rhizodeposition zurück. Mit zunehmender Entfernung zur Rhizoplane nahmen diese Gehalte stark ab. Die Gehalte des mikrobiellen Biomasse C und N im Boden stiegen signifikant mit zunehmender Nähe zur Rhizoplane an. Im Boden bis 1 mm Entfernung zur Rhizoplane stammten im Durchschnitt 66% des mikrobiellen Biomasse C und 29% des mikrobiellen N aus der Rhizodeposition. Diese Anteile nahmen mit zunehmender Entfernung zur Wurzel stark ab, waren aber im Boden bis zu 4,2 mm Abstand von der Wurzel nachweisbar. In der extrahierbaren Fraktion waren nur geringe Mengen an C und N mit Herkunft aus der Rhizodeposition zu finden. Der extrahierbare C und N mit Herkunft aus der Rhizodeposition variierte dabei stark um 4% des gesamt nachweisbaren Rhizodepositions-C und -N im Boden und zeigte nur einen marginalen Anstieg mit zunehmender Wurzelnähe. Das C/N-Verhältnis des C und N aus der Rhizodeposition im Gesamtboden und in der extrahierbaren Fraktion stieg mit zunehmender Entfernung von der Rhizoplane auf Werte über 30 an. Im Gegensatz dazu nahm das C/N-Verhältnis des mikrobiell inkorporierten Rhizodepositions-C und -N auf Werte unter 5 im Boden von 2,6 bis 4,2 mm Entfernung zur Rhizoplane ab.

Im Versuch mit *Avena sativa* waren im Rhizosphärenboden 36% weniger C und 39% weniger N zu finden als im Versuch mit *Triticum aestivum*. Signifikante Mengen des Rhizodepositions-C waren in beiden Versuchen in 4 bis 6 mm Entfernung von der Wurzel nachweisbar. Rhizodepositions-N war im Versuch mit Hafer nur bis 2 mm und im Versuch mit Weizen nur bis 4 mm nachweisbar. Die Gehalte an mikrobiellen C und N stiegen mit zunehmender Nähe zur Wurzel in beiden Versuchen an. Im Versuch mit Hafer war die Netto-Zunahme an mikrobiellen Biomasse C in direkter Wurzelnähe kleiner als die Menge des mikrobiell inkorporierten Rhizodepositions-C. Im Gegensatz dazu betrug der Anteil des C aus der Rhizodeposition am Netto-Anstieg der mikrobiellen Biomasse C in direkter Nähe zur Wurzel des Weizens nur 82%. Die Werte nahmen mit zunehmender Entfernung zur Wurzel ab. Während der Bodeninkubation nahm der während des Pflanzenwachstums in die mikrobielle Biomasse inkorporierten Rhizodepositions-C im Rhizoshärenboden des Hafers um 50% und im Rhizosphärenboden des Weizens um 22% ab. Neben Rhizodepositions-N wurden in beiden Versuchen bedeutende Mengen unmarkierten Boden N (native organische Bodensubstanz) in die mit zunehmender Nähe zur Wurzel anwachsende mikro-

bielle Biomasse inkorporiert. Was zeigte, dass es mit zunehmender Wurzelnähe zu einem angeregten Umsatz der bodenbürtigen organischen Substanz und einer N Immobilisation durch die wachsende mikrobielle Biomasse unter den Bedingungen des Pflanzenwachstums kam. Der Abbau von C aus der bodenbürtigen organischen Substanz war über den gesamten Entfernungsgradienten erhöht. Diese Effekte waren im Versuch mit Hafer weniger stark ausgeprägt als im Versuch mit Weizen. Die unterschiedliche Ausprägung der Effekte ging dabei möglicherweise auch auf den Eintrag unterschiedlicher Gesamtmengen an Rhizodepositen in den beiden Versuchen zurück.

Das unterschiedliche Ansprechen der mikrobiellen Gemeinschaft auf den Substrateintrag in unterschiedlicher Entfernung zur Wurzel zeigte komplexe Wechselwirkungen in Bezug auf das mikrobielle Wachstum, den mikrobiellen Umsatz, den Substrateintrag aus der Rhizodeposition und den stimulierten Abbau der nativen organischen Bodensubstanz. Der Eintrag von Rhizodepositen stellt eine bedeutende Funktion in der Regulation der mikrobiellen Biomasse und der Prozesse des Umsatzes der organischen Bodensubstanz während und wahrscheinlich bis nach der Vegetationsperiode dar. Die simultane Verfolgung von Rhizodepositions-C und -N im Boden und deren Funktionen innerhalb der Rhizosphäre, gerade im Hinblick auf die mikrobielle Biomasse und den Umsatz der organischen Substanz im Boden, führt zu einem besseren Verständnis der komplexen wechselseitigen Prozesse zwischen Pflanze und Boden.

9 Summary

The aim of the present study was to investigate the impact of rhizodeposition C and N on microbial growth and C and N turnover within the rhizosphere. Therefore, an artificial rhizosphere system, which was developed by GAHOONIA and NIELSEN (1991), was modified to investigate the processes induced by rhizodeposit C and N input at spatial distance from an artificial root surface. For estimating C and N derived from rhizodeposition in soil, ^{13}C and ^{15}N isotope-tracing techniques were used. For sampling of fresh rhizosphere soils at spatial distance to the artificial root surface a cutting device according to FITZ et al. (2003) was employed.

The system was used in ^{13}C and ^{15}N double-labelling experiments with *Lolium perenne*, *Triticum aestivum* and *Avena sativa*. The experiment with *Lolium perenne* was conducted to investigate the general suitability of the employed system as well as the distribution of rhizodeposits C and N and their translocation in total soil, soil microbial biomass and the 0.05 M K_2SO_4 extractable soil pool within the rhizosphere up to 4.2 mm distance from the root surface. The rhizosphere soil gradient was investigated at 0-1, 1-1.8, 1.8-2.6, 2.6-3.4 and 3.4-4.2 mm distance from an artificial root surface of *Lolium perenne*. The experiments with *Triticum aestivum* and *Avena sativa* were conducted to investigate the fate and turnover of ^{13}C- and ^{15}N-labelled rhizodeposit input within a rhizosphere gradient from 0-8 mm distance. Rhizosphere soil layers from 0-1, 1-2, 2-3, 3-4, 4-6 and 6-8 mm distance to the separated roots of oat and wheat were analysed after a period of 27 and 53 days of roots affecting the rhizosphere soils, respectively. The soil samples then were investigated in incubation experiments (42 days, 15°C) for changes in C and N in total and that derived from rhizodeposition in total soil, in soil microbial biomass and in 0.05 M K_2SO_4-extractable soil fraction. Additionally, the CO_2-C respiration in total and that derived from rhizodeposition were measured from the rhizosphere soil samples up to 6 mm distance to the previously separated roots during the soil incubation.

In the rhizosphere soil of *Lolium perenne* C and N derived from rhizodeposition accounted for 4.2% of total C and 2.8% of total N at 0-1.0 mm distance and decreased rapidly with increasing distance. Microbial biomass C and N increased significantly towards the roots. At 0-1.0 mm distance microbial biomass C and N accounted for 66% and 29% of C and N derived from rhizodeposition, respectively. These percentages declined with increasing distance to the roots, but were still traceable up to 4.2 mm distance. Only small amounts of

root released C and N were found in the 0.05 M K_2SO_4-extractable fraction. Extractable C and N derived from rhizodeposition varied around means of 4% of total C and N derived from rhizodeposition and increased only marginally with increasing distance to the roots. The C/N ratio of total C and N derived from rhizodeposition as well as that of extractable C and N derived from rhizodeposition increased with increasing distance to the roots above 30. In contrast, the C/N ratio of rhizodeposition C and N incorporated in the microbial biomass decreased to values less than 5 at 2.6-4.2 mm distance.

In total, 36% less C and 39% less N derived from rhizodeposition were found in the rhizosphere soil of oat in comparison to wheat within the rhizosphere gradients. Significant amounts of rhizodeposition C were present in rhizosphere soil up to 4-6 mm distance from the separated roots of oat and wheat. Rhizodeposition N was only present in the rhizosphere soils up to 1-2 mm and 3-4 mm distance from the roots of oat and wheat, respectively. Microbial biomass C and N increased significantly with increasing proximity to the separated roots in both experiments. In the rhizosphere soil of oat, the net increase of microbial biomass C close to the separated roots was smaller than the amount of incorporated rhizodeposition C. In contrast, the net increase of microbial biomass C in the soil close to roots of wheat accounted for only 82% of C derived from rhizodeposition. However, these values changed with increasing distance to the roots. During soil incubation, microbial biomass C derived from rhizodeposition decreased by about 50% and 22% of the previously incorporated C derived from rhizodeposition in oat and wheat soil samples, respectively. Beside rhizodeposit N, large amounts of unlabelled soil N (native SOM) were incorporated in the growing microbial biomass towards the roots in both experiments, indicating a distinct acceleration of soil organic matter decomposition and N immobilisation in the growing microbial biomass, even under the competition of plant growth. C decomposition of native soil organic matter was enhanced within the entire investigated rhizosphere gradients. The effects were different between the experiments with oat and wheat, presumably due to different amounts of rhizodeposits previously entering the rhizosphere soils during plant growth.

The differential microbial response to rhizodeposit input in different distance from the roots indicated complex interactions between microbial growth, turnover, substrate input derived from rhizodeposition and accelerated decomposition of native soil organic matter within the rhizosphere. The rhizodeposits thus have a meaningful function in the regulation of soil microbial biomass and the processes of soil organic matter composition and decom-

position in soils, even during and after the plants' growing periods. The simultaneous determination of rhizodeposition C and N in soil and their functions within the rhizosphere, especially with regard to microbial biomass and soil organic matter turnover, contribute to a better understanding of the complex interactive processes occurring between plant and soil at the rhizosphere-root interface.

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