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## Mikrobielle Nutzung von Ernteresten in Bodensäulen und Litterbags

Dissertation

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Die Arbeit beinhaltet 3 Artikel, welche bei internationalen, begutachteten Fachzeitschriften eingereicht wurden. Diese Artikel sind in die Kapitel 3, 4 und 5 eingearbeitet. Kapitel 1 ist die generelle Einleitung zum Themenbereich. Kapitel 2 stellt die Ziele dieser Arbeit heraus. In Kapitel 6 und 7 sind die Ergebnisse der Kapitel 3, 4 und 5 auf deutsch und englisch zusammengefasst, während Kapitel 8 einen Ausblick auf weitere Untersuchungen gibt.

Die folgenden Artikel sind Bestandteil der vorliegenden Arbeit:

Kapitel 3

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Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation selbständig und ohne unzulässige Hilfe angefertigt habe und keine anderen als in dieser Arbeit angegebenen Hilfsmittel benutzt habe. Die wörtlich oder sinngemäß angeführten und aus Veröffentlichungen oder unveröffentlichten Material entnommenen Zitate habe ich unter Angabe der Quellen kenntlich aufgeführt. Kein Teil dieser Arbeit liegt in einem anderen Promotions- oder Habilitationsverfahren vor.

Nils Rottmann



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## Abkürzungsverzeichnis

AFP	Luft gefülltes Porenvolumen (engl. air filled pore space)
Al	Aluminium
ANOVA	Varianzanalyse
BCA	Bicinchoninic acid
BMELF	Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz
C	Kohlenstoff
<sup>13</sup> C	Kohlenstoff Isotop mit der Masse 13
Ca	Kalzium
CaCl <sub>2</sub>	Calciumchlorid
CHCl <sub>3</sub>	Chloroform
CIRAS	Combined infrared gas analysis system
C <sub>mik</sub> (C <sub>mic</sub> )	Mikrobiell gebundener Kohlenstoff
CO <sub>2</sub>	Kohlendioxid
CV	Variationskoeffizient (engl. coefficient of variance)
d	Tag (engl. day)
δ <sup>13</sup> C	<sup>13</sup> C/ <sup>12</sup> C Verhältnis der Probe bezogen auf das <sup>13</sup> C/ <sup>12</sup> C Verhältnis des PDB Standards
DFG	Deutsche Forschungs Gesellschaft
dw	Trockengewicht (engl. dry weight)
e.g.	Zum Beispiel (lat. exempli gratia)
FAO-WRB	Food and Agriculture Organisation of the United Nations – World Reference Base for Soil Resources
Fe	Eisen
g	Gramm
GC	Gaschromatograph
GIS	Geographisches Informationssystem

## Abkürzungsverzeichnis (Fortsetzung)

h	Stunde (engl. hour)
H <sub>2</sub> O	Wasser
H <sub>2</sub> SO <sub>4</sub>	Schwefelsäure
ha	Hektar
HCl	Salzsäure
HNO <sub>3</sub>	Salpetersäure
hPa	Hektopascal
HPLC	Hochleistungsflüssigkeitschromatographie (engl. high performance liquid chromatography)
i.e.	das heißt (lat. id est)
ICP-AES	Induktiv gekoppeltes Hochfrequenzplasma – Atomemissions Spektrometer (engl. inductively coupled plasma atomic emission spectrometry)
IPCC	Zwischenstaatlicher Ausschuss für Klimaänderungen (engl. Intergovernmental Panel on Climate Change)
IR	Infrarot Gasanalysator
K	Kalium
K <sub>2</sub> SO <sub>4</sub>	Kaliumsulfat
K <sub>EC</sub>	extrahierbarer Anteil des Gesamtkohlenstoffs, gebunden in der mikrobiel- len Biomasse
kg	Kilogramm
M	mol/L
m <sup>2</sup>	Quadratmeter
m <sup>3</sup>	Kubikmeter
mg	Milligramm
Mg	Magnesium

## Abkürzungsverzeichnis (Fortsetzung)

min	Minute
ml	Milliliter
mm	Millimeter
Mn	Mangan
N	Stickstoff
Na	Natrium
NaOH	Natronlauge, Natriumhydroxid
nm	Nanometer
O <sub>2</sub>	Sauerstoff
P	Phosphor
PAS	Photoakustischen Spektroskopie
PDB	Pee Dee Belemnite (Standard für die Messung von $\delta^{13}\text{C}$ -Werten)
Pg	Petagramm ( $10^{15}$ Gramm)
pH	Säuregrad einer Lösung (lat. potentia Hydrogenii)
PLFA	Phospholipidfettsäuren-Analyse (engl. Phospholipid-derived fatty acids)
POM	Partikuläres Organisches Material (engl. particulate organic matter)
ppm	Teile von einer Million (engl. parts per million)
PVC	Polyvinylchlorid
rev	Umdrehungen
s	Sekunde
S	Schwefel
S	Siemens (elektrischer Leitwert, meist in Form von $\text{S m}^{-1}$ )
SOM	Organischer Material des Bodens (engl. soil organic matter)
SOC	Organischer Kohlenstoff des Bodens (engl. soil organic carbon)
Tg	Teragramm ( $10^{12}$ Gramm)

### **Abkürzungsverzeichnis (Fortsetzung)**

TS	Trockensubstanz
UK	United Kingdom
v/v	Volumen pro Volumen
%	Prozent
‰	Promille
µg	Mikrogramm
µl	Mikroliter
µm	Mikrometer
Σ	Summe



# 1 Einleitung

## 1.1 Streuabbau und mikrobielle Besiedlung

In tierbestandsfreien Landwirtschaftssystemen stellt die organische Düngung in Form von Ernteresten oder Stroh eine bedeutende, wenn nicht die bedeutendste und teilweise einzige Quelle organischer Bodensubstanz dar. Das „Potential des Bodens“, bzw. das Potential der Meso- und Mikrofauna des Bodens, Streu abzubauen, ist von großer Bedeutung um negative Effekte, wie die Immobilisierung von Stickstoff (Chesire et al., 1999; Henriksen and Breland, 1999b) und die Freisetzung von wachstumshemmenden niedermolekularen Phytotoxinen (Cochran et al., 1977; Koch et al., 1992) auf die anschließende Feldfrucht zu minimieren. Der Abbau von Streu und die daraus resultierende Mobilisierung von Nährstoffen sind zusätzlich von großer Bedeutung für die pflanzliche Nährstoffversorgung und stellen die Schlüsselfunktionen bodenbürtiger Mikroorganismen dar (Swift et al., 1979). Durch seine Rolle im Nährstoffkreislauf, sowie die Bildung von organischem Bodenmaterial (SOM) beeinflusst der Abbau von Streu bedeutend die Funktion und Struktur von Ökosystemen (Swift et al. 1979). Durch die Einarbeitung der Streu im Boden bilden sich „hot-spots“ mikrobieller Aktivität (Kandeler et al., 1999; Poll et al., 2007; Stemmer et al., 1999) mit erhöhtem Bedarf an Nährstoffen und Sauerstoff. Die Deckung eben dieses Bedarfs, sowie die mikrobielle Aktivität und Effizienz, werden neben der Streuqualität von der Bodenstruktur und der Bodenart beeinflusst. Die Bodenstruktur beeinflusst im Besonderen die Bildung von Mikrohabitaten aufgrund der kleinräumigen heterogenen Verteilung organischen Materials und mikrobieller Biomasse. Zusätzlich differenzieren sich Böden verschiedener Art und Struktur durch Unterschiede in ihren chemischen, physikalischen und chemo-physikalischen Eigenschaften. So weist sandiger Boden, in der Regel, eine höhere Sauerstoffverfügbarkeit, jedoch eine geringere Nährstoffverfügbarkeit auf als lehmiger oder toniger Boden (Smith et al., 2003; van Veen et al., 1984). Dieses resultiert zum

einen aus der höheren Porosität und geringeren Wasserhaltekapazität von sandigen Böden (Hutchinson and Rochette, 2003) und dem dadurch hohen Anteils an mit Luft gefüllten Porenvolumens (AFP; air filled pore space). Zum anderen weisen sandige Böden vor allem Zweischichttonminerale wie Kaolinit auf, welche aufgrund ihrer geringen Oberfläche nur eine geringe Kationen-Austausch-Kapazität besitzen. Lehmiger Boden hingegen, besitzt aufgrund eines generell höheren Anteils an Ton und im Besonderen an Dreischicht- und faserförmigen Tonmineralen eine höhere Kationen-Austausch-Kapazität. Des Weiteren wird davon ausgegangen, dass ein höherer Anteil an Ton zu einem höheren physikalischen Schutz von eingearbeiteter Streu führt (Bhuphinderpal et al., 2006) und somit den Abbau hemmen kann. Hinzu kommt, dass in der Landwirtschaft, in Abhängigkeit vom eingesetzten Bodenbearbeitungssystem, Streu in unterschiedliche Tiefen eingebracht wird, wodurch einzelne Effekte, wie z.B. eine eingeschränkte Sauerstoffverfügbarkeit, in Abhängigkeit des Bodens, noch verstärkt werden können (Kissele et al., 2001). Beim Einsatz von Bodenfräsen oder Kreiseleggen in reduzierten Bodenbearbeitungssystemen erfolgt die Einarbeitung der Streu in der Regel flachgründig (0-5 cm). Bei konventioneller Bodenbearbeitung mit dem Wendepflug, wird die Streu zum überwiegenden Teil in die Tiefe von ca. 20-30 cm eingearbeitet (Stockfisch et al., 1999). Die in der Landwirtschaft eingearbeitete Streu besteht in den meisten Fällen aus auf dem Feld verbleibenden Ernteresten sowie zerkleinertem Stroh der abgereiften Pflanzen. Meist handelt es sich dabei um Getreidepflanzen wie Mais oder Weizen von denen, im Jahr 2006, in Deutschland, ca. 22 Mio. und 3,2 Mio. Tonnen produziert wurden (BMELV, Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, 2009). Dieses Pflanzenmaterial wird hauptsächlich durch saprotrophe Pilze abgebaut (Bowen and Harper, 1990; Chesire et al., 1999). Gerade Pilze gelten als Organismen, die sich besonders gut an komplexe Strukturen, wie die Bodenmatrix, angepasst haben bzw. anpassen können (Boswell et al., 2003). Sie haben die Möglichkeit, durch das Ausbreiten von Hyphen durch Poren und Hohlräume der Bodenmatrix,

energiereiche und nährstoffreiche Mikrohabitate über die Detritussphäre hinaus zu erreichen und miteinander zu verbinden (Lindahl and Olsen, 2004). Dadurch zeigt sich die Wichtigkeit, neben dem Bereich der Detritussphäre auch den weiter entfernten, umgebenden Boden zu betrachten. Dieses ist unabdingbar, wenn das Wirkungsgefüge zwischen den chemo-physikalischen Bodeneigenschaften und der mikrobiellen Biomasse des Bodens verstanden werden soll. Des Weiteren muss überprüft werden, welchen Einfluss die Unterschiede der chemo-physikalischen Eigenschaften, auf die Entwicklung der mikrobiellen Gemeinschaft, den Streuabbau sowie die Sequestrierung von streubürtigem Kohlenstoff in die Fraktionen des Organischen Kohlenstoffes des Bodens (SOC), des extrahierbaren Kohlenstoffes, des Kohlenstoffes der mikrobiellen Biomasse ( $C_{\text{mik}}$ ) und des Kohlenstoffes des Kohlenstoffdioxides ( $\text{CO}_2\text{-C}$ ), haben.

## 1.2 Erfassung der Bodenrespiration

Im Zuge der zunehmenden Diskussion zum Klimawandel gegen Ende des vergangenen Jahrhunderts, rückten die Böden und im Speziellen landwirtschaftlich genutzten Böden, als Senke und Quelle klimarelevanter Gase ins Zentrum der Diskussion. Es wird erwartet, dass sich die weltweiten Bestände an organischem Kohlenstoff im Boden auf etwa 1500 Pg belaufen. Dieses entspricht in etwa dem Doppelten des atmosphärischen und dem Dreifachen des in pflanzlicher Biomasse gebundenen Kohlenstoffes (IPCC, 2001). Andere Studien schätzen die weltweiten Bestände sogar auf das Dreifache des atmosphärischen und das Fünffache des in pflanzlicher Biomasse gebundenen Kohlenstoffes (Schlesinger and Andrew, 2000). Da der organische Kohlenstoff im Boden nicht isoliert ist, sondern in enger Interaktion mit anderen Kompartimenten des globalen Kohlenstoffkreislaufes, wie der Atmosphäre und der Biosphäre, steht, stellt er einen bedeutenden Teil eben dieses Kreislaufes dar. Es wird davon ausgegangen, dass etwa 8 % der CO<sub>2</sub> Emissionen Großbritanniens und Nordirlands durch die Oxidation von organischem Kohlenstoff in den oberen 15 cm des Bodens entstehen (Bellamy et al., 2005). Dieses entspräche etwa 13 Tg. Global betrachtet ist die CO<sub>2</sub> Emission des Bodens (Bodenrespiration) einer der Hauptbestandteile des weltweiten Kohlenstoffkreislaufes (Raich and Schlesinger, 1992; Houghton, 2003) und wird einzig vom Anteil der Photosynthese übertroffen (Rustad et al., 2000). Die Bodenrespiration setzt sich aus heterotropher Atmung (Oxidation von organischem Material durch heterotrophe Organismen) und Wurzelatmung (Schlesinger, 1977) zusammen. Der genaue Anteil der Bodenrespiration am globalen Kohlenstoffkreislauf ist bisher jedoch nicht zu bestimmen. Grund dafür ist vor allem der Einsatz verschiedenster Messmethoden zur Bestimmung der Bodenrespiration (Raich and Schlesinger, 1992). Der Einsatz verschiedener Erfassungs- und Detektionsmethoden kann bereits innerhalb einer Fläche zu Unterschieden in der Abschätzung der Bodenrespiration führen. Darüber hinaus sind interareale Vergleiche erfasster Bodenrespiration durch Unterschiede in der Bodenart und Bodenstruktur,

sowie durch die daraus resultierenden methodenabhängigen Artefakte, erheblich erschwert. Somit ist selbst der Einsatz identischer Methoden noch kein Garant für vergleichbare Werte interarealer Messungen, sofern die Artefakte der eingesetzten Methode nicht vollständig bekannt sind. Aktuelle Studien (Hutchinson and Rochette, 2003; Pumpanen et al., 2004; Alavoine et al., 2008) zeigen deutliche Unterschiede beim Erfassen der Bodenrespiration mit verschiedenen Methoden wie der Kombination aus statischen und dynamischen Hauben mit Detektionsverfahren wie der Gaschromatographie, der Infrarot-Spektroskopie oder der chemischen Absorption von CO<sub>2</sub> durch Lauge. Alavoine et al. (2008) fanden beim Vergleich einer statischen Haube in Kombination mit chemischer Absorption von CO<sub>2</sub> und dem Einsatz einer dynamischen Haube in Kombination mit Infrarot-Spektroskopie identische Werte der Bodenrespiration bei einem lehmigen Boden. Der identische Einsatz dieser Methoden bei einem kalkigen Boden hingegen, lies mit dem Einsatz der dynamischen Haube in Kombination mit der Infrarot-Spektroskopie eine deutlich geringere Bodenrespiration erwarten als mit der Statischen Haube in Kombination mit chemischer Absorption von CO<sub>2</sub>. Normen et al. (1997) und Lund et al. (1999) beschrieben fünf Punkte die für die Auswahl der einzusetzenden Methode zur Bestimmung der Bodenrespiration wichtig sind. (1) der Bedarf an zeitlicher und räumlicher Auflösung der Probenahme; (2) die Sicherheit, dass die Methode im Feld eingesetzt werden kann; (3) die Methodenartefakte müssen bekannt sein; (4) die Genauigkeit der Methode muss bekannt sein; (5) die Mittel für den Einsatz der Methode müssen zur Verfügung stehen und das Equipment muss vorhanden sein. Dabei sind die Punkte drei und vier, welche das benötigte Wissen über methodenbedingte Artefakte, sowie die Methodengenauigkeit in den Vordergrund stellen, von größter Bedeutung. Um die Genauigkeit von Methoden zur Erfassung der Bodenrespiration abschätzen zu können wurde bereits eine Vielzahl von Labor- (Jensen et al., 1996; Janssens et al., 2000; Alavoine et al., 2008) und Feldversuchen (Reth et al., 2005; Keith and Wong, 2006, Müller et al., 2010) durchgeführt. Wie Hutchinson und Rochette (2003) jedoch zu Recht

kritisch anmerkten, ist die genaue Bodenrespiration in den meisten Studien, und im Besonderen in Feldstudien, nicht bekannt. Daher ist es nicht möglich, auf Grundlage dieser Untersuchungen beurteilen zu können, mit welcher Methode die Bodenrespiration am genauesten erfasst wird. Durch den direkten Vergleich verschiedener Methoden ist es zwar möglich deren Ergebnisse aufeinander zu beziehen, ob es sich dabei jedoch um die tatsächliche Bodenrespiration handelt ist ungewiss. In nur wenigen Studien (Martin et al., 2004; Butnor et al., 2005) ist die genaue Bodenrespiration bekannt und wird zur Genauigkeitsabschätzung, sowie zur Betrachtung des Einflusses unterschiedlicher Bodenarten auf die CO<sub>2</sub>-Detektion eingesetzt. In diesen Studien wurde der CO<sub>2</sub>-Fluss durch die Erhöhung der CO<sub>2</sub>-Konzentration unterhalb der Bodensäule erzeugt. Dadurch ist jedoch eine Betrachtung des Einflusses der Entfernung von CO<sub>2</sub>-hot-spots zur Bodenoberfläche, als ein potentielles Artefakt nicht mehr möglich. Da unterschiedliche Bodenbearbeitungssysteme, wie z.B. die Bodenfräse und der Pflug, durch die Inkorporation von Streu CO<sub>2</sub>-hot-spots in 0-5 cm bzw. 20-30 cm Tiefe erzeugen, ist das Wissen über dieses mögliche Artefakt jedoch von besonderer Bedeutung für die Beurteilung der Bodenbearbeitungssysteme. Um zusätzlich die tatsächliche Genauigkeit verschiedener Methoden zur Erfassung der Bodenrespiration bestimmen zu können, bedarf es Studien in denen die genaue Bodenrespiration bekannt ist und die Entfernung der CO<sub>2</sub>-hot-spots zur Bodenoberfläche variiert.

### **1.3 Streuabbau unter Subtropischen Klimaten**

Bereits 1974 formulierten Dickinson und Pugh, dass der Abbau von Streu durch eine Vielzahl von physikalischen, chemischen und biologischen Faktoren beeinflusst wird. Im Jahr 2003 unterschieden Knacker et al. drei Gruppen an Faktoren; Die erste Gruppe umfasst physikalisch-chemische Faktoren, zu denen u.a. die Position der Streu, die Eigenschaften des jeweiligen Bodens, sowie klimatische Bedingungen zählen. Die Zweite umfasst die Qualität der abzubauenen Streu, wie z.B. die Verfügbarkeit streubürtiger Nährstoffe für Mikroorganismen, sowie die Konzentration und die Zusammensetzung der Strukturkomponenten der pflanzlichen Zellwand, wozu Hemicellulose, Cellulose und Lignin zählen. Die dritte Faktorengruppe fokussiert die Zusammensetzung der Destruentengemeinschaft. Trotz unterschiedlicher Ergebnisse, bei denen zum einen das Klima den Streuabbau dominierend beeinflusst (Meentemeyer, 1978; Berg et al., 1993) oder zum anderen eher eine untergeordnete Rolle spielt (Johansson et al., 1995) besteht im allgemeinen der Konsens, dass auf lokaler Skala der Einfluss des Klimas irrelevant ist und stattdessen die Streuzusammensetzung, im Besonderen die Stickstoff-, Lignin- und Polyphenolgehalte den Streuabbau dominieren (Henriksen und Brealend, 1999c; Hättenschwiler und Vitousek, 2000). Auf überregionaler, nationaler und ins Besondere auf internationaler Skala, stellt der Klimaeinfluss jedoch den dominierende Abbaueinfluss dar (Berg, 2000). Um die Einflüsse der zweiten und dritten Faktorengruppe (Streuqualität und Zusammensetzung der mikrobiellen Gemeinschaft), in einem vom Arbeitsaufwand zu überblickenden Versuch, objektiv zu betrachten, ist es somit wichtig, die erste Faktorengruppe ausschließen zu können. Zur Betrachtung von Abbauprozessen ist es unabdingbar, die nach einer definierten Abbauezeit verbleibenden Bestandteile der zuvor eingearbeiteten Streu zurück zu gewinnen. Neben der Dichtefraktionierung, beschrieben bei Jacobs et al. (2009), und der Größenfraktionierung (Magid et al., 1997) gilt der Einsatz der, von Bocoock und Gilbert 1957 entwickelten, Litterbag – Methode für die Rückgewinnung von ausgebrachter Streu als sehr nützlicher An-

satz (Knacker et al., 2003; Joergensen et al., 2009). Durch die sehr einfache Wiederfindung ausgebrachter Streu, sowie die Möglichkeit durch das Variieren der Maschenweite des Litterbags, bestimmte Gruppen von Destruenten vom Abbauprozess ausschließen zu können (Joergensen et al., 2009), ist die Litterbag-Methode gerade für Feldversuche besonders gut geeignet. Aus diesem Grund wurde die Methode bereits in einer Vielzahl von Studien eingesetzt, um den Streuabbau unter Feldbedingungen in gemäßigten Klimaten zu untersuchen (Robinson et al., 1994; Joergensen et al., 2009). Sowohl unter den gemäßigten nördlichen Klimaten (Berg et al., 1996; Berg and Johanson, 2000) als auch unter den subtropischen und tropischen Klimaten (Alhamed et al. 2004; Xianiu und Hirata, 2005) waren die bisherigen Analysen zum Streuabbau im Litterbag zumeist auf Waldböden fokussiert. Auf landwirtschaftlich genutzten Flächen gemäßigter Klimate hingegen wurde der Abbau von Streu weniger intensiv untersucht (Christensen, 1985; Berg, 2000; Knacker et al., 2003). Über den Streuabbau auf landwirtschaftlichen Flächen unter den, sowohl für Destruenten und Feldfrüchte, extremen Bedingungen subtropischer Klimate ist bisher noch nichts bekannt. Auf landwirtschaftlich genutzten Flächen besteht durch die Zugabe unterschiedlicher Dünger sowie bei unterschiedlicher Feldfrucht die Wahrscheinlichkeit, dass der Streuabbau stark beeinflusst wird. Dieses ist durch Unterschiede in der Nährstoffzugabe, bzw. dem Nährstoffbedarf sowie eines möglichen Eintrages von Mikroorganismen durch z.B. organischen Dünger, begründet. Um die bestehenden Wissenslücken zum Streuabbau auf landwirtschaftlich genutzten Flächen in subtropischen Klimaten zu schließen, sowie den Einfluss von verschiedenen Düngern und Feldfrüchten auf den Streuabbau zu untersuchen, ist es wichtig, erste Versuche zu gestalten und durchzuführen, die eben diese Punkte fokussieren.



## 2 Ziele der Arbeit

### 2.1. Ziele des Versuches: Microbial use and decomposition of maize leaf straw incubated in packed soil columns at different depths. (Kapitel 3).

Da in Abhängigkeit der eingesetzten landwirtschaftlichen Bodenbearbeitungssysteme Streu sowie „grüner Dünger“ in unterschiedliche Tiefen eingebracht werden, was Bodenart abhängige Effekte, wie einen verminderten Sauerstofftransport, verstärken kann, wurde ein Gewächshausversuch unter kontrollierten Bedingungen ausgebracht. Dabei wurden 20 cm hohe Bodensäulen mit gestörtem sandigen, bzw. lehmigen Boden befüllt. In jeweils vier Parallelen wurde die Streu in die oberen 5 cm (0-5 cm) bzw. die unteren 5 cm (15-20 cm) eingearbeitet (Abb. 1). In die Kontrolle wurde keine Streu eingearbeitet. So das im Endeffekt 24 Bodensäulen (Sand: 4x Streu oben, 4x Streu unten, 4x Kontrolle; Lehm: 4x Streu oben, 4x Streu unten, 4x Kontrolle) ausgebracht wurden. Die Säulen wurden auf der Unterseite mit einem Nylongaze (1 mm Maschenweite) verschlossen und auf einem Sandbett ausgebracht (Abb. 2). An Hand der natürlichen  $^{13}\text{C}$  Anreicherung der eingearbeiteten Maisstreu konnte eine Verlagerung des streubürtigen Kohlenstoffes in die Fraktionen der mikrobiellen Biomasse, Streu Residuen,  $\text{CO}_2$  sowie extrahierbaren Kohlenstoff und SOC, selbst über den Bereich der Detritussphäre hinaus, verfolgt werden. Die Sequestrierung des Streubürtigen Kohlenstoffes diente nach Abschluss des Versuches zur Abschätzung der Genauigkeit von Messsystemen zur Erfassung der Bodenrespiration (Kapitel 4).

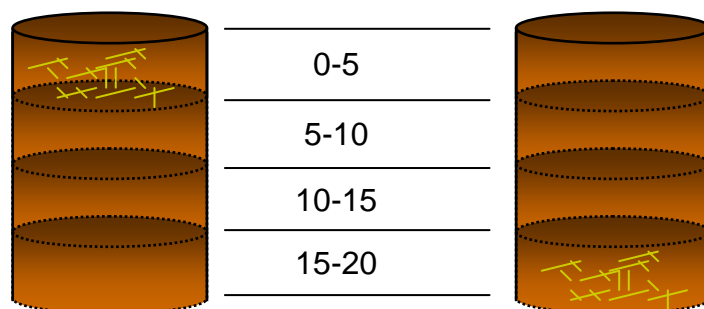


Abbildung 1: Skizze der Bodensäulen, links: Stroh oben, rechts: Stroh unten

Ziel des Versuches war es, den Einfluss der Einarbeitungstiefe von Streu in Abhängigkeit der Bodenart, auf den Abbau der Streu und die mikrobielle Nutzung hin zu untersuchen. Aufgrund der, in Kapitel 1.1 und 3.1 beschriebenen Bodeneigenschaften wurden folgende Erwartungen formuliert:

1. Trotz eines geringeren Umsatzes der eingearbeiteten Streu im lehmigen Boden, wird ein größerer Anteil der eingearbeiteten Streu in die mikrobielle Biomasse inkorporiert.
2. Streuabbau und Mineralisation der Streu sind im sandigen Boden höher als im lehmigen Boden.
3. Unterschiede in der mikrobiellen Nutzung und dem Abbau der Streu, nach der Einarbeitung in unterschiedliche Tiefen, werden im sandigen Boden geringer sein als im lehmigen Boden.



Abbildung 2: Versuchsaufbau, Versuch 1 (Foto Rottmann, 2007)

## **2.2. Ziele des Versuchs: Mesuring the CO<sub>2</sub> production from maize-straw amended soil columns - a comparison of four methods (Kapitel 4).**

Die Versuchsdurchführung war zeitlich und räumlich identisch zu der unter 2.1 und 3.2 beschrieben. Der Versuchsaufbau unterschied sich einzig darin, dass die Bodensäulen auf der Unterseite nicht, wie im Versuch unter 2.1 und 3.2 beschrieben, mit einem Nylon-gaze sondern einer PVC-Platte verschlossen waren. Dieses war nötig um zu verhindern, dass CO<sub>2</sub> am unteren Ende der Säulen austritt und somit bei der CO<sub>2</sub>-Detection nicht erfasst werden würde. Um die Genauigkeit der vier Messmethoden zur Bestimmung der Bodenrespiration: (1) Absorption von CO<sub>2</sub> durch Natronlauge in Kombination mit einer statischen Haube (NaOH); (2) Gaschromatographie in Kombination mit einer dynamischen Haube (GC); (3) Infrarot Spektroskopie in Kombination mit einer dynamischen Haube (IR) und (4) Photo-akustische Spektroskopie in Kombination mit einer dynamischen Haube (PAS), zu überprüfen, sowie eventuelle methodenbedingte Artefakte aufzuzeigen wurden diese zur Bestimmung der Bodenrespiration eingesetzt.

Ziel des Versuches war es zum Einen, den Einfluss der Distanz von CO<sub>2</sub>-hot-spots zur Bodenoberfläche in Abhängigkeit des Bodentyps und der eingesetzten Methoden zur Bestimmung der Bodenrespiration zu betrachten. Zum Anderen, sollte die Genauigkeit der eingesetzten Methoden, in Abhängigkeit der Bodenart, anhand der zuvor im Versuch „Microbial use and decomposition of maize leaf straw incubated in packed soil columns at different depths“ (Kapitel 3) ermittelten, zu erwartenden Bodenrespiration überprüft werden. Aufgrund der in Kapitel 1.2 beschriebenen Bodeneigenschaften und der bereits bekannten Methodenartefakte wurden folgende Erwartungen formuliert:

1. Eventuelle Turbulenzen, verursacht durch den Ventilator der dynamischen Haube, zeigen keinen Einfluss auf die Bestimmung der Bodenrespiration beim lehmigen Boden.
2. Eventuelle Turbulenzen, verursacht durch den Ventilator der dynamischen Haube, können im Vergleich zur statischen Haube kombiniert mit chemischer CO<sub>2</sub> Absorption durch Lauge, beim sandigen Boden eine höhere Bodenrespiration suggerieren.
3. Die Entfernung der CO<sub>2</sub>-hot-spots zur Bodenoberfläche beeinflusst lediglich die Bestimmung der Bodenrespiration mittels dynamischer Haube am sandigen Boden.
4. Die Bestimmung der Bodenrespiration am lehmigen Boden, durch den Einsatz von GC, IR und PAS in Kombination mit der dynamischen Haube ist genauer als die Bestimmung durch NaOH.

### 2.3 Ziele des Versuches: Litter decomposition in fertilizer treatments of vegetable crops under subtropical conditions (Kapitel 5).

Um die bestehenden Wissenslücken, bezüglich des Streuabbaus auf landwirtschaftlich genutzten Flächen, unter subtropischen Klimaten, schließen und den Einfluss verschiedener Dünger sowie verschiedener Feldfrüchte bewerten zu können, wurde ein Litterbag-Experiment auf den Flächen eines Feldexperimentes ausgebracht, welches die Effekte von organischer und mineralischer Düngung auf den Anbau von Mohrrüben (*Daucus carota* ssp. *Sativu*) und Blumenkohl (*Brassica oleracea* var. *botrytis* L) auf einer privaten Farm an der Nord-Ost Küste des Omans ( $24^{\circ}22'N$ ,  $56^{\circ}34'E$ ) (Abb.3) untersuchte (Siegfried et al., 2010).

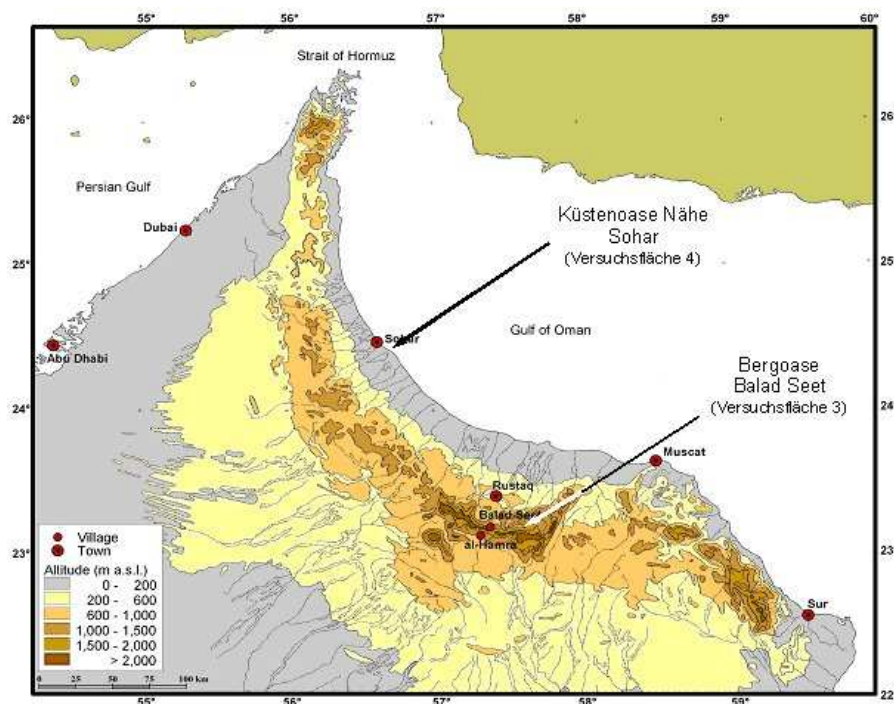


Abbildung 3: GIS-basierte Karte des Nordomans mit der Versuchsfäche Nähe Sohar „Küstenoase Nähe Sohar (Versuchsfäche 4)“ (Bürkert, 2007)

Insgesamt wurden 192 Litterbags mit qualitativ hochwertigem Streu wie Luzerne (*Medicago sativa* L. 'Verko'(50%) 'Plato'(50%)), bzw. Maiz (*Zea mays* L. 'Askann') oder mit Streu geringer Qualität wie Raps (*Brassica napus* L.), bzw. Weizen (*Tritiyum aesticum* L. 'Capo') befüllt und vertikal in 1-6 cm Tiefe eingebracht.



Abbildung 4: Streu im Litterbag vor Versuchsbeginn; v.l.n.r. Maiz, Weizen, Luzerne



Abbildung 5: Streu nach 81 Tagen; links: Maiz, rechts: Weizen

Das Versuchsfeld war in zwölf Plots á 17,5 m<sup>2</sup> aufgeteilt. Auf sechs Plots wurden Mohrrüben und auf sechs Plots Blumenkohl angebaut (Abb.6). Drei Plots beider Feldfrüchte wurden mineralisch, die anderen Drei jeweils organisch gedüngt.

Ziel war es, die unter 1.3 aufgezeigten Wissenslücken zum Streuabbau in landwirtschaftlichen Systemen unter subtropischen Klimaten in Abhängigkeit von Streuqualität, Düngung und Feldfrucht zu untersuchen. Aufgrund der in Kapitel 1.3 und 5.1 beschriebenen, Streuabbau beeinflussenden Faktoren wurden folgende Erwartungen formuliert:

1. Unterschiede im Gehalt von Stickstoff, Phosphor, Schwefel, sowie weiterer Nährstoffe und die Zusammensetzung von Zellstrukturkomponenten (Hemicellulose, Cellulose und Lignin) der Streu führen zu Unterschieden im Kohlenstoffabbau sowie in der Nährstoffabgabe und der mikrobiellen Besiedelung der Streu.
2. Organische Düngung führt zu einem erhöhten Kohlenstoffabbau aufgrund des Eintrages von saprotrophen Mikroorganismen (Henriksen and Breaand, 1999c).
3. Der Anbau von Mohrrüben führt aufgrund des hohen Nährstoffbedarfs zu einem erhöhten Kohlenstoff- und Nährstoffverlust der Litterbag Streu (Müller und von Fragstein, 2006)



Abbildung 6: Versuchsfeld nahe Sohar mit Blumenkohl und Mohrrüben als Feldfrucht

### **3 Microbial use and decomposition of maize leaf straw incubated in packed soil columns at different depths**

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## **Abstract**

An experiment was carried out to investigate the decomposition and microbial use of maize leaf straw incubated in packed soil columns at different depths. The straw was incorporated into the top layer at 0-5 cm depth and into the bottom layer at 15-20 cm depth of a sandy or a loamy soil. Microbial biomass C was significantly increased after adding straw to the bottom layer of both soils. After adding straw to the top layer, this increase was significantly lower in the sandy soil and significantly higher in the loamy soil. Maize straw application significantly increased the ergosterol-to-microbial biomass C ratio in both soils from 0.26% to a mean content of 0.72% after adding straw to the top layer and to a mean content of 1.11% after adding straw to the bottom layer. The calculation of the maize-derived CO<sub>2</sub> production revealed that the mineralization rates of maize C were always higher in the sandy soil, with a mean of 20%, than in the loamy soil, with a mean of 14%. The application of maize straw always significantly increased the soil organic matter derived CO<sub>2</sub> production. This increase was stronger in the loamy soil than in the sandy soil and stronger after application of the maize straw to the top layer than to the bottom layer. On average, 100% of the maize straw C was recovered in the different fractions analysed. In the layers with maize leaf straw application, 28% of the maize C was recovered as particulate organic matter (POM) > 2 mm and 32% as POM 0.4-2.0 mm, without a significant difference between the two soils and the depth of application. In the layers with maize leaf straw application, 19% of the maize C was recovered as microbial residue C and 3.1% as microbial biomass C. In the three layers without straw, the microbial biomass incorporated a further 2.4% of the maize C in the sandy soil, but only 0.9% in the loamy soil. Considerable amounts of substrate C were transferred within the microbial biomass over a decimetre distance. The finer pore space of the loamy soil seems to obstruct the transfer of maize-derived C. This was especially true if the maize leaf straw was added to the bottom layer.

### 3.1 Introduction

Decomposition of plant residues and the resulting release of nutrient elements are key functions of soil microorganisms [49]. Straw is an important organic residue added to soil in farming systems without livestock or straw-less livestock keeping. In these systems, the capability of straw decomposition is important to minimize negative effects on the following crop, caused by N immobilization [9, 20] and the release of growth-inhibiting low molecular substances [10, 27]. Straw is mainly decomposed by saprotrophic fungi [5, 9] and forms hot spots of high biological activity after incorporation into the soil [24, 37, 47], with an increased demand for nutrients and oxygen.

The oxygen supply for straw decomposition differs under field conditions depending on the depth of incorporation [26]. In reduced tillage systems using, for example, rotary cultivators, the straw is mixed into the first 5 to 10 centimetres, whereas the straw is buried mainly at 20 to 30 cm depth by mouldboard ploughing [48]. Nutrient and oxygen supply to decomposing soil microorganisms is also strongly affected by the soil texture, i.e. sandy soils provide larger pore space with more oxygen, but less nutrients to the microbial community [46, 51]. However, not only the detritosphere, i.e. the few millimetres of soil attached to the organic residues [24, 37, 47] is important for the decomposition of straw, also the surrounding bulk soil may contribute to the nutrient supply [7]. Fungi are able to transport nutrients over relatively large distances within their hyphal network, connecting soil microhabitats rich in energy and poor in nutrient supply with microhabitats poor in energy and rich in nutrients [30].

An experiment was carried out to investigate decomposition and microbial use of maize leaf straw incubated in packed soil columns incorporated at different depths. The straw was applied into the top layer at 0-5 cm depth or into the bottom layer at 15-20 cm depth of sandy or loamy soil. This resulted in the following four underlying hypotheses:

(1) a higher percentage of substrate is incorporated into the microbial biomass of the loamy soils due to the lower turnover, (2) decomposition and mineralization rates of an added substrate are faster in the sandy soil than in the loamy soils and (3) the differences in microbial use and decomposition of substrate due to the application of substrate to the top or bottom layer are less different in the sandy soil. Maize straw usually differs from soil organic matter in its  $\delta^{13}\text{C}$  value [38, 43, 44]. In combination with particulate organic matter analysis to recover unused substrate [31], this makes it possible to follow C sequestration into the fractions of microbial biomass and microbial residues, and  $\text{CO}_2$ . It also makes it possible to monitor the translocation of maize-derived C into layers distant from the detritusphere.

## 3.2 Material and methods

### 3.2.1 Soil and plant material

The soils used for the experiment were sampled in March 2007 from the upper 15 cm of an experimental site in Angerstein near Göttingen (Southern Lower Saxony, Germany) and an experimental site of the Institute of Biodynamic Research near Darmstadt (Southern Hesse, Germany). The loamy soil of Göttingen was classified as a Haplic Luvisol (FAO-WRB, Food and Agriculture Organisation of the United Nations – World Reference Base for Soil Resources) with the following characteristics: 16% sand, 66% silt, 18% clay, a pH in H<sub>2</sub>O of 7.8, 14.3 mg g<sup>-1</sup> total C, a soil organic  $\delta^{13}\text{C}$  value of -26.07‰, 1.2 mg g<sup>-1</sup> total N and a C/N ratio of 11.9. The sandy soil of Darmstadt was classified as a Haplic Cambisol (FAO-WRB) with 79% sand, 16% silt, 5% clay, a pH in H<sub>2</sub>O of 7.5, 11.9 mg g<sup>-1</sup> total C, a soil organic  $\delta^{13}\text{C}$  value of -26.47 ‰, 1.2 mg g<sup>-1</sup> total N and a C/N ratio of 9.9. The field moist soils were sieved (< 2 mm) before the experiment was started. Oven dried (60°C) maize leaf straw (*Zea mays* L.) was cut into small pieces of 2-4 mm. It contained 45.3% C with a  $\delta^{13}\text{C}$  value of -12.45‰, 0.57% N and 0.3  $\mu\text{g}$  ergosterol g<sup>-1</sup>.

### 3.2.2 Experimental design

The six treatments of the experiment were carried out in four replicates in PVC cylinders (15 cm diameter, 20 cm height) in a greenhouse for 57 days: (1) sandy soil with straw at 0-5 cm depth (sand straw top), (2) sandy soil with straw at 15-20 cm depth (sand straw bottom), (3) sandy soil without straw (sand control), (4) loamy soil with straw at 0-5 cm depth (loam straw top), (5) loamy soil with straw at 15-20 cm depth (loam straw bottom), and (6) loamy soil without straw (loam control). Depending on the treatment, the soils were mixed at a concentration of 20 mg g<sup>-1</sup> maize leaf straw into the respective depth layer. For the sandy and loamy treatments, 4444 g and 4161 g dry soil were used per column,

respectively, equivalent to 1111 g and 1040 g per layer. The water content was adjusted to 40% of water holding capacity (WHC), which was gravimetrically controlled and adjusted every seventh day, so that the water content did not decrease below 35% WHC. After destructive sampling at the end of the experiment, the water content was measured separately in each layer seven days after the last water addition, without detecting differences between the different layers. The temperature was kept constant at  $19 \pm 2$  °C and the bulk density was adjusted to  $1.4 \text{ g cm}^{-3}$ . The PVC cylinders stood on a sand bed and were closed at the bottom with a nylon gauze (1 mm mesh size) to afford a natural diffusion of CO<sub>2</sub> as much as possible and to exclude an artificially high CO<sub>2</sub> accumulation. For sampling, the soil columns were subdivided into layers of 5 cm thickness. In an identical experiment, the cylinders were closed at the bottom with a PVC lid for measuring CO<sub>2</sub>. This was necessary to catch all CO<sub>2</sub> for the exact estimation of C sequestration. Both experiments were carried out at the same time, in the same greenhouse room and therefore under the same temperature and light conditions.

### 3.2.3 *Particular organic matter (POM)*

Moist soil (400 g) was initially dispersed in 400 ml 5% sodium chloride, shaken by hand and allowed to stand for 45 min [31, 34]. Then the samples were poured gradually onto two sieves of 2 mm and 0.4 mm mesh size and washed with tap water. The aggregates were destroyed by pushing the soil through the sieve during the washing procedure until the water passing through the sieve became clear. The material retained on the sieve was transferred into a beaker. Tap water was added, the bucket was swirled and organic material was separated from the mineral material by flotation-decantation. Swirling and flotation-decantation was repeated several times, until organic particles were no longer visible in the mineral fraction. Then, the mineral fraction was discarded. The fractions POM 0.4-2

mm and POM > 2 mm were washed with distilled water and transferred into crucibles, dried at 60°C, weighed and milled for further analyses.

#### 3.2.4 *Carbon dioxide production*

Every third day, closed static PVC chambers (15 cm in diameter, 10 cm height) were placed on the cylinders and closed with a rubber-band for 24 h. Inside the chamber, a beaker containing up to 20 ml 2 M NaOH solution was placed on the soil surface. The amount of 2 M NaOH was adjusted according to the expected flux rate so that usually no more than 60% of the absorption capacity was utilised. Total CO<sub>2</sub> in the alkali traps was determined by back-titrating the excess NaOH to pH 8.3 with 2 M HCl after precipitation of carbonates with BaCl<sub>2</sub>. The  $\delta^{13}\text{CO}_2$  was determined in precipitated BaCO<sub>3</sub>. The titration solution was centrifuged at 4°C and 9800 m s<sup>-2</sup>. The supernatant was discharged and the pellet stirred in 5 ml of distilled water and centrifuged again. This was repeated three times before freeze-drying the pellet containing the precipitated carbon dioxide [28]. The mean  $\delta^{13}\text{CO}_2$  of  $\Sigma\text{CO}_2\text{-C}$  was calculated for each sampling day and averaged at the end of the experiment.

#### 3.2.5 *Microbial biomass indices*

Microbial biomass C was estimated by fumigation-extraction [50]. A sub-sample of 20 g soil was separated into two portions. One portion was fumigated at 25°C with ethanol-free CHCl<sub>3</sub>, which was removed after 24 h. Fumigated and non-fumigated 10-g samples were extracted with 40 ml of 0.05 M K<sub>2</sub>SO<sub>4</sub> by 30 min horizontal shaking at 200 rev min<sup>-1</sup> and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C in the extracts was measured as CO<sub>2</sub> by infrared absorption after combustion at 850°C using a Dimatoc 100 automatic analyser (Dimatec, Essen, Germany). Microbial biomass C was cal-

culated as follows  $E_C / k_{EC}$ , where  $E_C$  = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and  $k_{EC} = 0.45$  [54]. Microbial residue C was calculated as maize derived soil organic C without POM and maize-derived microbial biomass C.

The fungal cell-membrane component ergosterol was extracted with 100 ml ethanol from 2 g moist soil by 30 min oscillating shaking at 250 rev min<sup>-1</sup> [12]. Then, ergosterol was determined by reversed-phase HPLC analysis at 25 °C by using a column of 125 x 4 mm Spherisorb 5µ ODS II with a guard column (4 x 3 mm) and with 100% methanol as the mobile phase and a resolution of detection of 282 nm.

### 3.2.6 $\delta^{13}C$ values

Ball-milled samples of maize leaf straw, POM and soil were analysed for  $\delta^{13}C$  values by isotope-ratio mass spectrometry Delta C IRMS (Finnigan Mat, Bremen). Before analysis, diluted HCl (pH > 4.8) was added to the soil samples for 12 h, which were then oven-dried for 24 h (105°C) to remove CaCO<sub>3</sub>. For the determination of  $\delta^{13}C$  values in the 0.05 M K<sub>2</sub>SO<sub>4</sub> extracts, 10 ml aliquots were freeze-dried for about three days. The isotopic composition of a sample was calculated relative to the V-PDB standard [3, 38]. The C content of the fumigated extracts ( $C_f$ ) is the sum of the C content of the non-fumigated (control) extracts ( $C_c$ ) and the additionally extracted C from cell lyses by chloroform fumigation (chloroform-labile C,  $C_b$ ), which is converted to microbial biomass C. Accordingly, the  $\delta^{13}C$  of the fumigated extracts is [44]:

$$(\delta^{13}C_f \times C_f) = (\delta^{13}C_c \times C_c) + (\delta^{13}C_b \times C_b)$$

The chloroform-labile fraction can be converted to microbial biomass C assuming that the extractable and non-extractable fractions of the microbial biomass have the same  $\delta^{13}C$

value [14]. The part of maize straw-derived C (f%) was calculated for each single replicate of all treatments from the  $\delta^{13}\text{C}$  data by the following equation [3]:

$$f\% = 100 \times \frac{\delta^{13}\text{C}_{\text{sample}} - \delta^{13}\text{C}_{\text{control}}}{\delta^{13}\text{C}_{\text{maize}} - \delta^{13}\text{C}_{\text{control}}}$$

where  $\delta^{13}\text{C}_{\text{sample}}$  represents the carbon in soil organic C, particulate organic matter C (POM-C 0.4-2 mm and POM-C > 2 mm), 0.05 M  $\text{K}_2\text{SO}_4$  extractable C, and microbial biomass C at day 57, or the  $\text{CO}_2$  evolved at day 5, 12, 33, 38, 43, and 57;  $\delta^{13}\text{C}_{\text{control}}$  is the average  $\delta^{13}\text{C}$  value of the control treatments that did not receive maize leaf straw.

### 3.2.7 Statistical analysis

The data presented in tables and figures are arithmetic means and are given on an oven-dry basis (105°C, 24 h). Significance of treatment effects was tested by a one-way analysis of variance using the Scheffé post-hoc test. All statistical calculations were performed using JMP 7.0 (SAS Institute Inc.).



### 3.3 Results

The content of soil organic C was slightly higher in the loamy soil than in the sandy soil, with a tendency to increase in the layers with maize straw application and a significant increase in sandy soil (Table 1). In contrast, the layers of sandy soil without maize straw contained significantly 80% more  $K_2SO_4$  extractable C than the respective layers of loamy soil. With maize straw application, the content of  $K_2SO_4$  extractable C increased by approximately  $30 \mu\text{g g}^{-1}$  in both soils.

Without maize straw application, the contents of microbial biomass C were similar at roughly  $200 \mu\text{g g}^{-1}$  soil in the two soils, regardless of soil depth, with the exception of the sand control treatment (Table 1). Microbial biomass C was significantly increased to roughly  $350 \mu\text{g g}^{-1}$  soil after adding straw to the bottom layer of both soils. After adding straw to the top layer, this increase was significantly lower in the sandy soil and significantly higher in the loamy soil. Without maize straw application, the contents of fungal ergosterol were always approximately  $0.5 \mu\text{g g}^{-1}$  soil. Maize straw application significantly increased not only the ergosterol content to an average of  $3.6 \mu\text{g g}^{-1}$  soil, with the exception of sand straw top, but also the ergosterol-to-microbial biomass C ratio. This ratio showed a nearly 3-fold increase to a mean of 0.72% after adding straw to the top layer of both soils and even a nearly 4-fold increase to a mean of 1.11% after adding straw to the bottom layer of both soils.

Maize straw application significantly increased the  $\delta^{13}\text{C}$  values of soil organic C on average by 2.1‰ and those of  $K_2SO_4$  extractable C on average by 4.4‰ (Table 2). In the layers with maize straw application, the  $\delta^{13}\text{C}$  values of the microbial biomass varied around a mean of -13.2‰ and were thus 11.4‰ higher than that of the sandy control soil and 8.1‰ higher than that of the loamy control soil. In the sandy soil, the  $\delta^{13}\text{C}$  values of microbial biomass C were on average 4.3‰ higher in the layers below or above the layers

Table 1

Soil organic C, K<sub>2</sub>SO<sub>4</sub> extractable C, microbial biomass C, ergosterol, and the ergosterol-to-microbial biomass C ratio at the end of a 57-d incubation period in four 5-cm layers of 20-cm soil columns

Treatment	Soil	K <sub>2</sub> SO <sub>4</sub>	Microbial		Ergosterol/
	organic C (mg g <sup>-1</sup> soil)	extractable C	biomass C	Ergosterol	microbial biomass C (%)
			(μg g <sup>-1</sup> soil)		
Sand straw top-S	16.0 a	66 a	270 c	1.50 c	0.66 b
Sand straw top-N	11.0 bc	35 bc	220 d	0.57 d	0.26 c
Sand straw bottom-S	15.2 a	69 a	350 b	3.79 a	1.09 a
Sand straw bottom-N	10.7 c	34 bcd	200 d	0.53 d	0.26 c
Sand control	11.3 bc	35 bc	120 e	0.40 d	0.35 c
Loam straw top-S	15.5 a	48 b	420 a	3.28 b	0.78 b
Loam straw top-N	14.4 abc	19 de	200 d	0.45 d	0.23 c
Loam straw bottom-S	15.3 a	47 b	340 b	3.72 a	1.13 a
Loam straw bottom-N	14.6 ab	17 e	210 d	0.50 d	0.24 c
Loam control	14.0 abc	23 cde	200 d	0.47 d	0.24 c
CV (± %)	11	12	10	14	16

-S = straw layer; -N = mean of three layers without straw, CV = mean coefficient of variation between replicate columns (n = 4); different letters within a column indicate a significant difference ( $P < 0.05$ , Scheffé-test)

Table 2

$\delta^{13}\text{C}$  in soil organic C,  $\text{K}_2\text{SO}_4$  extractable C, microbial biomass C and the cumulative  $\text{CO}_2$ -C production at the end of a 57-d incubation period in four 5-cm layers of 20-cm soil columns

	Soil organic C	$\text{K}_2\text{SO}_4$ extractable C	Microbial biomass C	$\text{CO}_2$ -C
$\delta^{13}\text{C}$ (‰)				
Sand straw top-S	-24.4 a	-21.4 d	-11.3 a	} -15.8 a
Sand straw top-N	-26.6 b	-24.2 e	-19.4 c	
Sand straw bottom-S	-24.6 a	-19.3 bc	-13.9 b	} -15.8 a
Sand straw bottom-N	-26.5 b	-24.4 e	-19.9 cd	
Sand control	-26.5 b	-24.6 e	-24.0 e	-23.2 c
Loam straw top-S	-23.8 a	-16.9 a	-14.1 b	} -15.6 a
Loam straw top-N	-26.3 b	-20.7 cd	-21.4 cd	
Loam straw bottom-S	-24.1 a	-18.4 ab	-13.7 b	} -15.4 a
Loam straw bottom-N	-26.3 b	-21.0 cd	-21.5 cd	
Loam control	-26.1 b	-22.3 d	-22.0 de	-19.2 b
CV ( $\pm$ %)	1.6	2.2	3.2	3.4

-S = straw layer; -N = mean of three layers without straw, CV = mean coefficient of variation between replicate extractions ( $n = 4$ ); different letters within a column indicate a significant difference ( $P < 0.05$ , Scheffé-test)

with maize straw application than in the control soil. In the loamy soil, there were no significant differences between the control soil and the layers without maize straw application. The  $\delta^{13}\text{C}$  values of the  $\text{CO}_2$  production from litter containing microcosms were on average 15.7‰ and thus 7.5‰ and 3.5‰ higher than that of microcosms with sandy and loamy control soils.

The total CO<sub>2</sub> production of both control soils was on a similar level of around 510 mg C pot<sup>-1</sup> (Fig. 7). After application of maize straw to the top layer, total CO<sub>2</sub> production was higher than after its application to the bottom layer. However, the difference between top and bottom layer was significant only in the sandy soil. The calculation of the maize-derived CO<sub>2</sub> production revealed that the mineralization rates of maize C were always higher in the sandy soil, with a mean of 20%, than in the loamy soil, with a mean of 14% (Table 3). The application of maize straw always significantly increased the soil organic matter derived CO<sub>2</sub> production (Fig. 7). This increase was stronger in the loamy soil than in the sandy soil and stronger after application of the maize straw to the top layer than to the bottom layer.

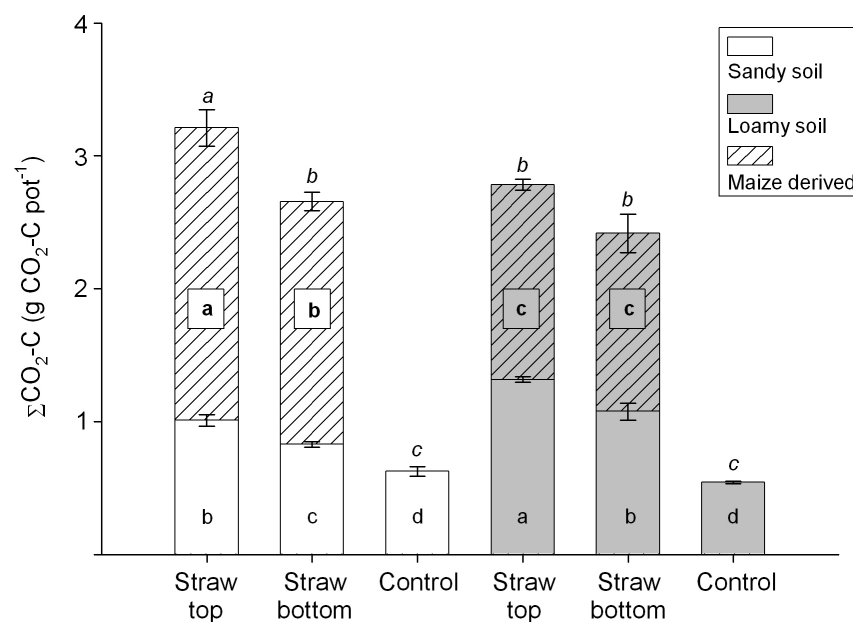


Figure 7: Cumulative CO<sub>2</sub>-C production at the end of a 57-day incubation period in 20-cm soil columns; error bars show  $\pm$  one standard error (n = 4); different letters in italics above the columns indicate a significant difference for the total CO<sub>2</sub>-C production ( $P < 0.05$ ); different bold letters in the patterned area indicate a significant difference for the maize derived CO<sub>2</sub>-C production ( $P < 0.05$ ), and different letters in the non-patterned area indicate a significant difference for the soil organic matter-derived CO<sub>2</sub>-C production ( $P < 0.05$ ).

On average, 100% of the maize straw C was recovered in the different fractions (Table 3). A balance gap occurred only in the loam straw bottom treatment. In the layers with maize leaf straw application, 28% of the maize C was recovered as particulate organic matter (POM) > 2 mm and 32% as POM 0.4-2.0 mm, without a significant difference between the two soils and the depth of application. In the layers with maize leaf straw application, 19% of the maize C was recovered as microbial residue C and 3.1% as microbial biomass C, equivalent to 270  $\mu\text{g C g}^{-1}$  soil (Fig. 8). In the three layers without straw, the microbial biomass incorporated a further 2.4% of the maize C in the sandy soil, but only 0.9% in the loamy soil (Table 3). The content of maize-derived microbial biomass C declined with increasing distance from the layer with maize straw application (Fig. 8). An exception was the high content of maize-derived microbial biomass C in the top layer of the sandy soil after application of maize straw to the bottom layer.

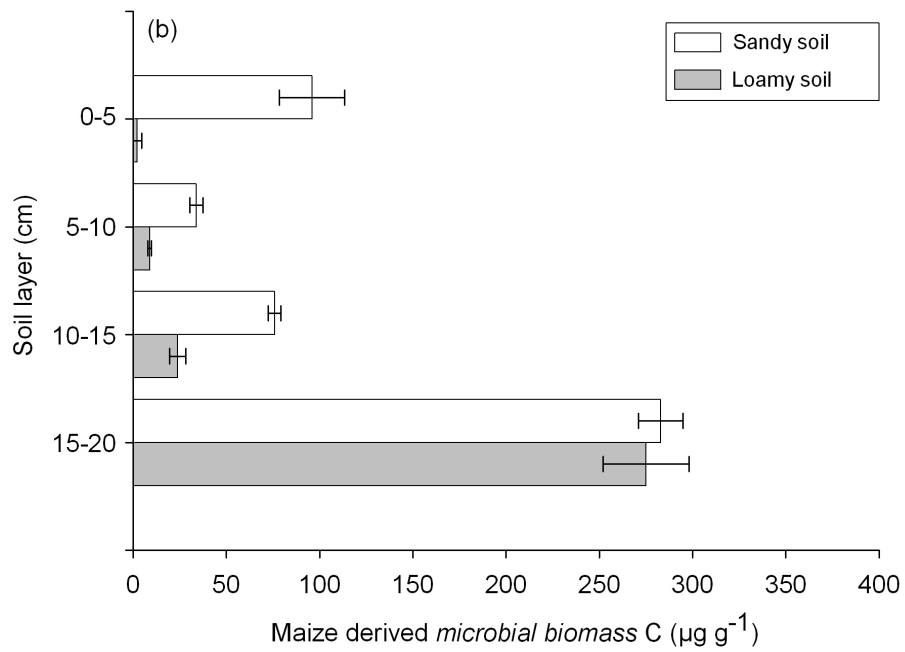
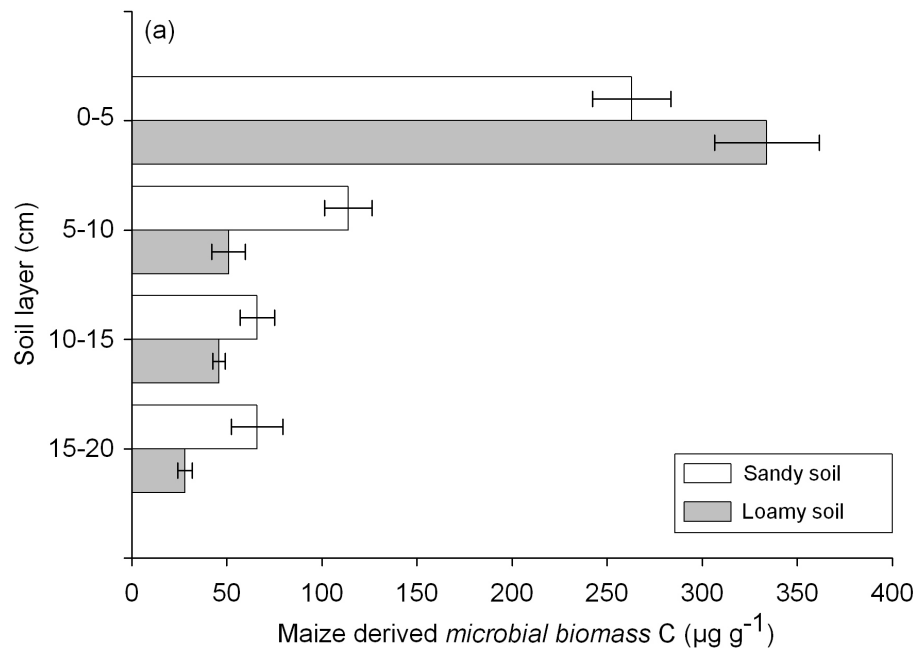


Figure 8: Maize-derived microbial biomass C after application of maize leaf straw (a) at 0-5 cm depth and (b) at 15-20 cm depth at the end of a 57-day incubation period in 20-cm soil columns; error bars show  $\pm$  one standard deviation

Table 3

Sequestration of maize-derived C on different fractions at the end of a 57-d incubation period in four 5-cm layers of 20-cm soil columns

	POM-C		$\Sigma\text{CO}_2\text{-C}$	Microbial residue C	Microbial biomass C	Recovery
	> 2 mm	0.4-2 mm				
	(%)		(%)	(%)	(%)	(%)
Sand straw top-S	24 a	29 a	} 21 a	23 a	2.9 a	103 $\pm$ 6
Sand straw top-N	ND			ND	2.6 b	
Sand straw bottom-S	29 a	33 a	} 18 b	19 ab	3.2 a	104 $\pm$ 4
Sand straw bottom-N	ND			ND	2.2 bc	
Loam straw top-S	28 a	35 a	} 14 c	17 b	3.4 a	99 $\pm$ 4
Loam straw top-N	ND			ND	1.2 bc	
Loam straw bottom-S	30 a	30 a	} 13 c	18 b	2.8 a	94 $\pm$ 2
Loam straw bottom-N	ND			ND	0.3 c	
CV ( $\pm$ %)	22	19	7.2	12	17	

S = straw layer; N = mean of three layers without straw, POM = particulate organic matter; CV = mean coefficient of variation between replicate extractions (straw layer n = 4; no-straw layer n = 12); ND = not determinable; different letters within a column indicate a significant difference ( $P < 0.05$ , Scheffé-test)

### 3.4 Discussion

In both soils, similar amounts of maize-derived C were incorporated into the microbial biomass in the layers with maize application, regardless of the soil depth at which the maize straw was added. In contrast, the transfer of maize-derived C into the layers without maize application depends (1) on the texture and (2) on the depth of application. In the sandy soil, a relatively high amount of maize-derived C was found in the three layers without straw. In contrast, a markedly lower percentage of maize-derived C was transferred to the layers without straw application in the loamy soil, especially after application to the bottom layer. Consequently, more substrate C was incorporated into the microbial biomass in the sandy soil, contrasting hypothesis 1. The transfer of substrate C by fungal hyphae has been shown over short distances from a litter layer to soil [7, 16], but not over the decimetre distance used in the present experiment. The finer pore space of the loamy soil seems to generally obstruct the transfer of maize-derived C by fungal hyphae. Boswell et al. [4] described fungi as well adapted organisms for living in complex structures like soil with hyphae growing through pores and across air gaps. It is likely that finer pores hinder hyphal growth. Consequently, maize-derived microbial biomass C decreased more strongly with increasing distance to the straw layer in the loamy soil than in the sandy soil. Furthermore, O<sub>2</sub> diffusion is certainly higher in the more porous sandy than in loamy soil, offering better conditions for microbial growth in deeper layers. Another reason for the lower transfer of maize-derived C into the microbial biomass of the loamy soil might be a better physical protection by the higher clay content [6]. This is supported by the increase of the absolute microbial biomass in the non-litter layer in the sandy soil. In contrast, microbial biomass C did not increase in the non-litter layer in the loamy soil. However, it remains unclear whether the differences in pore structure are also the main reason for the low transfer of maize-derived C from the bottom to the top layer. This transfer of saprotrophic fungi



via hyphae was apparently not accompanied by significant net increases in ergosterol and microbial residues. The shift in the  $\delta^{13}\text{C}$  values of soil organic C is too small and in the range of natural abundance. The incorporation of maize-derived C into ergosterol or amino sugars using component specific  $\delta^{13}\text{C}$  analysis would also be helpful to indicate changes in the microbial community structure and the compartment-specific transfer of C and N through bacteria and fungi [17, 32].

The increase in ergosterol, but especially in the ergosterol-to-microbial biomass C ratio was stronger after application of the maize straw to the bottom layer than to the top layer. Ergosterol is an important indicator for the presences of saprotrophic fungi, especially in arable soils [22]. The promotion of saprotrophic fungi by the decomposition of maize straw usually results in strong increases in soil ergosterol [19, 39, 40]. The depth-specific differences in ergosterol and ergosterol-to-microbial biomass C ratio have not been observed in field and incubation experiments until now. One reason might be the development of different fungal communities at different depths, i.e. a fungal community containing lower ergosterol concentrations in their biomass after straw addition to the top layer. A more likely explanation is the higher turnover of fungal biomass C as indicated by the higher  $\text{CO}_2$  production rate after adding the straw to the top layer, reducing the amount of ergosterol accumulated temporarily in dead fungal tissue [14, 33, 57]. The turnover of the fungal biomass might be especially high in the coarse textured sandy soil, leading to a significantly lower microbial biomass C content in comparison with the loamy soil. In agreement with the present study, Kisselle et al. [26] found a lower amount of microbial biomass at 0-5 cm than at 5-15 cm depth. The higher turnover of soil microorganisms, especially fungi, is most likely due to the better supply of oxygen in the top layer [26], which is necessary for lignin degradation [18, 25, 56].

The mineralization of maize leaf straw to  $\text{CO}_2$  was generally stronger in the sandy soil than in the loamy soil, as suggested by hypothesis 2. In contrast to hypothesis 3, this min-

eralization was significantly higher after adding the straw to the top layer in comparison with the bottom layer in the sandy soil only, lower contents of microbial biomass C and ergosterol indicating the strong turnover of added substrate. However, an average of 17% maize leaf straw C mineralised to CO<sub>2</sub> over a 57-day incubation period at 19°C is within the lower part of the range observed by others [1; 40]. The reasons for the relatively low C mineralization of the added maize straw are the low N content [20, 39, 41] and the low microbial colonization [40, 41, 45]. The larger size of the present maize straw particles and the resulting reduction in contact between straw particles and soil colloids might be an additional reason for low decomposition rates, although contrary results have been observed [2, 52].

According to the maize straw recovered as POM (0.4 – 2 mm; > 2 mm), 41% of the maize straw was decomposed. In agreement with the present data, Mueller et al. [35] observed that the total amount of decomposed plant residue C calculated from POM measurements was more than double that of the plant residue C evolved as CO<sub>2</sub> from the soil surface. The present POM data were also roughly in agreement with Kisselle et al. [26] and Wichern et al. [53], where a total of 111% (±43) maize straw derived C was recovered in a field experiment and 70-120% in a pot experiment, respectively. The variability was considerably lower in the present experiment, although the coefficient of variation for recovered POM >2 mm and 0.4-2 mm was 22% and 19%, respectively, as the sum of the two showed a coefficient of variation of only 7%.

According to van Veen et al. [51] and Joergensen et al. [23], the yield coefficient Y, as an indicator for substrate use efficiency can be calculated as follows:

$$Y = \text{substrate C in microbial products} / \text{utilised substrate C} = A / B$$

$$A = \text{substrate derived microbial biomass C} + \text{substrate derived microbial residue C}$$

$$B = A + \text{substrate derived CO}_2\text{-C}$$

This calculation results in an average yield coefficient  $Y = 0.58$  ( $A / B = 26.5\% / 46.0\%$ ) of the two layer treatments in the sandy soil and  $Y = 0.61$  ( $A / B = 21.4\% / 34.9\%$ ) in those of the loamy soil, suggesting a slightly but significantly higher substrate use efficiency. These yield coefficients are higher than those obtained by Muhammad et al. [34] for maize leaf straw, decomposing in saline soil, but similar to the yield coefficient of 0.55 for the metabolised remains of the original soil microbial biomass after a 10-day incubation [21] and somewhat below the yield coefficient of 0.70 for glucose after a 2-day incubation [8].

In the sandy soil, the addition of the maize straw caused significant (33% in straw bottom and 66% in straw top) increases in soil organic matter derived  $\text{CO}_2$  evolution (Fig. 7). In the loamy soil, the respective increases were considerably stronger, at 100% (straw bottom) and 140% (straw top). An increase in soil organic matter-derived respiration has been observed after the addition of cellulose [11, 15], but also after the addition of maize straw [36, 58]. This may be due to the mineralization of C already present in the microbial biomass [8, 55] or an accelerated mineralization of soil organic C, a so-called priming effect [29]. In the present experiment, the decrease in soil organic matter-derived microbial biomass accounted for roughly 60% of the cumulative increase in soil organic matter-derived  $\text{CO}_2$  evolution. The most likely explanation is that extracellular enzymes such as cellulases and lignin-modifying enzymes, produced by saprotrophic fungi to decompose added maize straw and freshly formed microbial residues, are partly efficient in degrading soil organic C [13, 42]. The lower increase in soil organic C in the straw layer of the loamy soil treatments in comparison with the sandy soil as well as the higher soil organic C-derived  $\text{CO}_2$  production in the loamy soil may be caused by differences in the microbial community structure, leading to a higher production of extracellular enzymes in the loamy soil. Several studies described N availability as a reason for a priming effect [8, 36], which was not detected specifically in the two soils of the present experiment. Consequently, N availability may be a reason for differences in soil organic matter C-derived  $\text{CO}_2$  production.

### **3.5 Conclusions**

We have shown for the first time that considerable amounts of substrate C were transferred within the microbial biomass, probably by fungi, over a decimetre distance within 57 days. The finer pore space of the loamy soil seems to generally obstruct the transfer of maize-derived C, especially if the maize leaf straw was added to the bottom layer. It is likely that the smaller pore space hindered hyphal growth and led to a better physical protection. However, the reasons for this observation remain unclear. The turnover of fungal biomass was stronger after application of maize leaf straw to the top layer at 0-5 cm in comparison with the application to the bottom layer at 15-20 cm, especially in the sandy soil. This was indicated by lower ergosterol-to-microbial biomass C ratios and higher CO<sub>2</sub> evolution rates from the added maize straw, but especially from the autochthonous soil organic matter.

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#### **4 Measuring the CO<sub>2</sub> production from maize-straw amended soil columns - a comparison of four methods.**

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## **Abstract**

A soil column experiment with maize straw application at different depths was carried out to investigate the accuracy of CO<sub>2</sub> measurement systems in a greenhouse experiment with sandy and loamy soils. The classical approach of CO<sub>2</sub> absorption in NaOH solution was compared in the present study with three other methods using dynamic chambers. These methods were gas chromatography, a portable infrared analyzer, and a portable photoacoustic system. The cumulative CO<sub>2</sub> production over the 57-day incubation period was significantly affected by the method and soil-specifically by the treatments. The NaOH and GC method always formed a pair of lowest cumulative CO<sub>2</sub> production in all treatments with maize straw addition. In the treatments with bottom application of the maize straw, IR and PAS methods gave values at identical levels in both soils. In the treatments with top application of the maize straw, the IR method gave significantly highest values in the sandy soil and the PAS method in the loamy soil. The correlation coefficients between the cumulative CO<sub>2</sub> production of the three dynamic chamber methods (GC, IR, and PAS) and the static NaOH method were all significant, with r-values between 0.90 and 0.93. The C balance can be used for testing the plausibility of CO<sub>2</sub> production data. Roughly 102% (NaOH and GC) and 114% (IR and PAS) were recovered, including the CO<sub>2</sub> production data in the C balance of the sandy soil. The respective data were 97% (NaOH and GC) and 104% (IR and PAS) for the loamy soil.

## 4.1 Introduction

Microbial CO<sub>2</sub> production from the decomposition of soil organic matter and organic residues is an important driver of global warming (*Raich and Schlesinger, 1992; Rustad et al. 2000*). In agricultural research, numerous decomposition studies have mostly been carried out in closed laboratory systems (*Hoffmann et al., 2010; Zareitalabad et al., 2010*) and less often in open, dynamic systems based on soil columns (*Heitkamp et al., 2009*) or pot experiments in the presence of growing plants (*Muhammad et al. 2007ab*). In their pot experiments, *Muhammad et al. (2007a)* observed that a portable dynamic chamber method with infrared CO<sub>2</sub> detection (*Blanke, 1996*) led to strong underestimation of the CO<sub>2</sub> evolution rates at very high rates after application of easily available alfalfa residues (*Muhammad et al. 2007ab*). In contrast, the mineralization of biogenic compost was successfully monitored with this portable system (*Muhammad et al., 2007b*).

However, other methods are available for measuring the CO<sub>2</sub> evolution rate from the soil surface, which may also lead to more consistent results in soil columns or pots. In these larger, but still controlled soil systems, the incubation conditions are more similar to the field situation than incubation studies in microcosms. In soil columns, lateral CO<sub>2</sub> fluxes and CO<sub>2</sub> fluxes down the bottom can be prevented, enabling more precise information on the effects of differences in application depth and on decomposition and C mineralization processes. In field experiments, there is an ongoing discussion about the depth from the soil surface to which soil microbial activity contributes to CO<sub>2</sub> fluxes (*Lund et al., 1999; Janssens et al., 2001; Reth et al., 2005a*). In a column experiment with maize straw, it has been shown that it is possible to monitor decomposition by the recovery of non-decomposed plant residues (*Rottmann et al., 2010*). For this purpose, the contribution of maize-derived C was analyzed in soil organic C, particulate organic matter and CO<sub>2</sub> by monitoring the shifts in the <sup>13</sup>C/<sup>12</sup>C ratio (*Zareitalabad et al., 2010*). This shift is caused by

the differences in  $\delta^{13}\text{C}$  values between  $\text{C}_4$ -plant maize and soil organic matter, mainly derived from  $\text{C}_3$ -plants (Boutton, 1996). In the experiment by Rottmann et al. (2010), the evolved  $\text{CO}_2$  was absorbed in NaOH solution using a static chamber. However, the results of  $\text{CO}_2$  production and the recovery of non-decomposed maize straw were not fully congruent in a loamy soil, contrasting the results of sandy soil. For this reason, the classical approach of  $\text{CO}_2$  absorption in NaOH solution was compared in the present study with three other methods using dynamic chambers repeatedly used in field experiments during the last decade. These methods were gas chromatography (Müller et al., 2010), a portable infrared analyzer (Blanke, 1996; Norman Müller et al., 2010) and a portable photo-acoustic system (Ambus and Robertson, 1998, Pumpanen et al., 2004; Reth et al., 2005b). An underlying hypothesis was that the dynamic chamber methods would lead to more accurate results, especially in loamy soils with higher gas diffusion barriers in comparison with a sandy soil (Butnor and Johnsen 2004; Martin et al., 2004; Butnor et al., 2005). It was assumed that the circulation of chamber air would lead to greater accuracy, due to a more homogeneous distribution of the  $\text{CO}_2$  evolved. However, dynamic chambers may lead to overestimates in the sandy soil, with lower gas diffusion barriers in comparison with a loamy soil (Kimball and Lemon, 1971; Hanson et al. 1993; Le Dantec et al. 1999, Pumpanen et al. 2004; Butnor et al. 2005). As yet, there has been a lack of research on the effect and possible artefacts of  $\text{CO}_2$  measuring methods affected by the distance of hot spots of  $\text{CO}_2$  production to the soil surface. However, this knowledge is important for evaluating effects of different management systems like reduced and non-reduced tillage on soil respiration (Frank et al., 2006)

## 4.2 Material and methods

### 4.2.1 Soil and plant material

The soils used for the experiment were sampled in March 2007 from the upper 15 cm of an experimental site in Angerstein near Göttingen (Southern Lower Saxony, Germany) and an experimental site of the Institute of Biodynamic Research near Darmstadt (Southern Hesse, Germany). The loamy soil of Göttingen was classified as a Haplic Luvisol (FAO-WRB, Food and Agriculture Organization of the United Nations – World Reference Base for Soil Resources) with the following characteristics: 16% sand, 66% silt, 18% clay, a pH in H<sub>2</sub>O of 7.8, 14.3 mg g<sup>-1</sup> total C, 1.2 mg g<sup>-1</sup> total N and a C/N ratio of 11.9. The sandy soil of Darmstadt was classified as a Haplic Cambisol (FAO-WRB) with 79% sand, 16% silt, 5% clay, a pH in H<sub>2</sub>O of 7.5, 11.9 mg g<sup>-1</sup> total C, 1.2 mg g<sup>-1</sup> total N and a C/N ratio of 9.9. The field moist soils were sieved (< 2 mm) before the experiment was started. Oven dried (60°C) maize leaf straw (*Zea mays* L.) was cut into small pieces of 2-4 mm. It contained 45.3% C and 0.57% N.

### 4.2.2 Experimental design

The six treatments of the experiment were carried out in four replicates in PVC cylinders (15 cm diameter, 20 cm height, closed at the bottom with a PVC lid) in a greenhouse for 57 days: (1) sandy soil with straw at 0-5 cm depth (sand straw top), (2) sandy soil with straw at 15-20 cm depth (sand straw bottom), (3) sandy soil without straw (sand control), (4) loamy soil with straw at 0-5 cm depth (loam straw top), (5) loamy soil with straw at 15-20 cm depth (loam straw bottom), and (6) loamy soil without straw (loam control). Depending on the treatment, the soils were mixed with 20 mg maize leaf straw g<sup>-1</sup> soil at the respective depth. The bulk density was adjusted to 1.4 g cm<sup>-3</sup>, which was close to field situation, and the water content to 40% of water holding capacity (WHC), which was equivalent to a wa-

ter content of 164 mg g<sup>-1</sup> dry soil and 180 mg g<sup>-1</sup> dry soil for the sandy and the loamy soil, respectively. This was gravimetrically controlled and adjusted every seven days, so that the water content did not decrease below 35% WHC. Because of the stimulation of microbial biomass activity by watering, the CO<sub>2</sub> evolution rate was not measured in the following 36 h. The temperature was kept constant at 19 ±2°C.

The CO<sub>2</sub> evolution rate was measured twice a week for each treatment, with the following four measurement methods using the same columns: (1) NaOH method with a static chamber, (2) GC (gas chromatography) method with a dynamic chamber, (3) IR (infra-red gas analyzer) method with a dynamic chamber and (4) PAS (photo acoustic gas analyzer) with a dynamic chamber. The days of measurement for the different methods were randomized in each week and the missing values were calculated by linear extrapolation for the other days. The three different dynamic chamber methods were analyzed intensively 20 times at a day during the experiment in both sandy treatments to exclude an effect of the course of a day. The CO<sub>2</sub> evolution rate data were corrected for the difference in temperature between time of measuring on the sampling day and the mean daily temperature during the experimental period. The rate-modifying factor ( $y = 47.9 / (1 + e^{(106 / (x + 18.3))})$ ) of the RothC model was used for this temperature correction (Jenkinson et al., 1987).



#### *4.2.3 NaOH static-chamber method*

Twice a week, closed static PVC chambers (15 cm in diameter, 10 cm height, volume = 1670 ml, area = 178 cm<sup>2</sup>) were placed on the cylinders and closed with a rubber-band for 24 h. Inside the chamber, a beaker (4 cm in diameter) containing up to 20 ml 2 M NaOH solution was placed on the soil surface. The amount of 2 M NaOH was adjusted according to the expected flux rate, so that usually 30 to 60% of the absorption capacity was utilized. The CO<sub>2</sub> was determined by back-titrating the excess NaOH to pH 8.3 with 2 M HCl after precipitation of carbonates with BaCl<sub>2</sub>. One measurement was carried out per cylinder per sampling date.

#### *4.2.4 GC – dynamic-chamber method*

For the GC (gas chromatography) dynamic-chamber method, the same chamber construction was used as for the NaOH static-chamber method. Additionally a brushless fan (Akasa, DFS802512L; 8 x 8 x 2.5 cm) with a circulation rate of 39 m<sup>3</sup> air h<sup>-1</sup> was installed under the top of the chamber and connected to a 9 V block battery. A further two 3-layer septa (laminated silicone rubber, Hamilton Company, Nevada, USA) with a thickness of 3.81 mm were installed in the top of the chamber for sampling by cannula. Soil air samples were taken with a steel cannula (7 cm length), attached to a 10 ml plastic syringe with luer-lock tip (Terumo Cooperation, Tokyo, Japan). The CO<sub>2</sub> enrichment was consecutively measured after time-shifted chamber closing. The intervals of the sampling were: at the beginning (0 min), after 10, 20, 30 and 40 min. Five air samples were taken per chamber. Additionally, three samples of room air were taken at the beginning, in the middle and at the end of the sampling. The samples were analyzed immediately using a gas chromatograph GC-14B (Shimadzu Corporation, Kyoto, Japan). The five consecutive measurements per cylinder were used to calculate the gradient of the enrichment curve. The CO<sub>2</sub>-

evolution rates ( $\text{mg m}^{-2} \text{h}^{-1}$ ) were calculated from the gradient, the volume and the enclosed soil surface area of the chamber as well as the air temperature.

#### *4.2.5 Infrared Analyzer – dynamic-chamber method*

For the infrared dynamic chamber method, the  $\text{CO}_2$  evolution rate was measured with the portable gas analyzer CIRAS-1 (Combined Infrared Gas Analysis System, PP Systems, Hitchin, UK). The analyzer consisted of a cylindrical chamber (height = 15 cm, diameter = 10 cm, volume = 1100 ml, area =  $78.5 \text{ cm}^2$ ), with a small fan, connected to the gas analyzer with a data logger and a probe for measuring soil temperature (Blanke 1996). For the  $\text{CO}_2$  measurements, the steel ring at the bottom of the cylindrical chamber was pushed about 2 cm into soil. In the chamber,  $\text{CO}_2$  enrichment began and was measured for 120 sec or until an increase about 50 ppm  $\text{CO}_2$  was achieved. Before each measurement, the CIRAS calibrated itself with ambient air. One measurement was carried out per cylinder per sampling date.

#### *4.2.6 Photo Acoustic System – dynamic-chamber method*

For the photo acoustic analyzing system (INNOVA 1312 AirTech Instruments, LumaSense Technologies AS, Ballerup, Denmark), the same dynamic chamber construction was used as for the GC method. Soil air samples were taken with a steel cannula (7 cm length), attached with a silicone tube to the photo acoustic analyzing device. The analyzed air sample was restored by a second silicone tube attached with a cannula and a septum. The  $\text{CO}_2$  enrichment measurement took place consecutively by time-shifted chamber closing. The intervals of the sampling were: at the beginning (0 min), after 10, 20, 30 and 40 min. Five air samples were taken per pot. After each fourth sample the room air was analyzed. The  $\text{CO}_2$  evolution rates ( $\text{mg m}^{-2} \text{h}^{-1}$ ) were calculated similarly to GC-DC.

#### 4.2.7 *Statistical analysis*

The significance between the measuring methods was tested by a two-way analysis of variance (ANOVA). Post hoc comparisons were made using Tukey/Kramer HSD test ( $p < 0.05$ ). Pearson correlation with additional pair wise correlation was used to determine relationships between the methods used and air temperature. All statistical analyses were performed using JMP 7.0 (SAS Institute Inc., Cary, USA).

### 4.3 Results

In the non-amended control treatments, the CO<sub>2</sub> evolution rates from the columns varied over the 57-day incubation period between 5 and 75 mg C m<sup>-2</sup> h<sup>-1</sup> around a mean of 25 mg C m<sup>-2</sup> h<sup>-1</sup>, averaging the data of all four methods for measuring CO<sub>2</sub> in the sandy soil and between 5 and 125 mg C m<sup>-2</sup> h<sup>-1</sup> around a mean of 22 mg C m<sup>-2</sup> h<sup>-1</sup> in the loamy soil. The variations in CO<sub>2</sub> evolution rates were not correlated with the temperature. The addition of maize straw generally led to an increase in CO<sub>2</sub> evolution rates and stronger temporal variations, which ranged now from 50 to 320 mg C m<sup>-2</sup> h<sup>-1</sup>. Throughout the intensive measuring period at day 18, none of three methods tested (GC, IR, and PAS) showed significant temporal variations of CO<sub>2</sub> evolution rate in the treatments with maize straw addition to the top (Fig. 9a) and to the bottom (Fig. 9b). However, the IR and PAS methods resulted in 1.8-fold higher values than the GC method. It was not possible to use the NaOH method for the required temporal resolution.

The cumulative CO<sub>2</sub> production over the 57-day incubation period was significantly affected by the method and soil-specifically by the treatments (Fig. 10a/b). The NaOH method gave values at the lowest level in all treatments. In the non-amended control treatments, the IR values did not differ significantly from the NaOH values in either of the soils. The same was true for the PAS values only in the sandy soil, whereas the GC method was at the highest level in both soils. In contrast, NaOH and GC method always formed a pair of lowest cumulative CO<sub>2</sub> production in all treatments with maize straw addition. In the treatments with bottom application of the maize straw, IR and PAS method gave values at identical levels in both soils. In the treatments with top application of the maize straw, the IR method gave significantly highest values in the sandy soil (Fig. 10a) and the PAS method in the loamy soil (Fig. 10b). However, the PAS method showed higher values than the GC method in both soils. The differences between the methods occurred from day 17 in

the sandy soil (Fig. 11a) and from day 8 in the loamy soil (Fig. 11b), as revealed by the cumulative respiration curves. These were more or less linear for most methods, only the cumulative respiration curve of the GC method showed a decline from day 21 in both soils (Fig.11a/b).

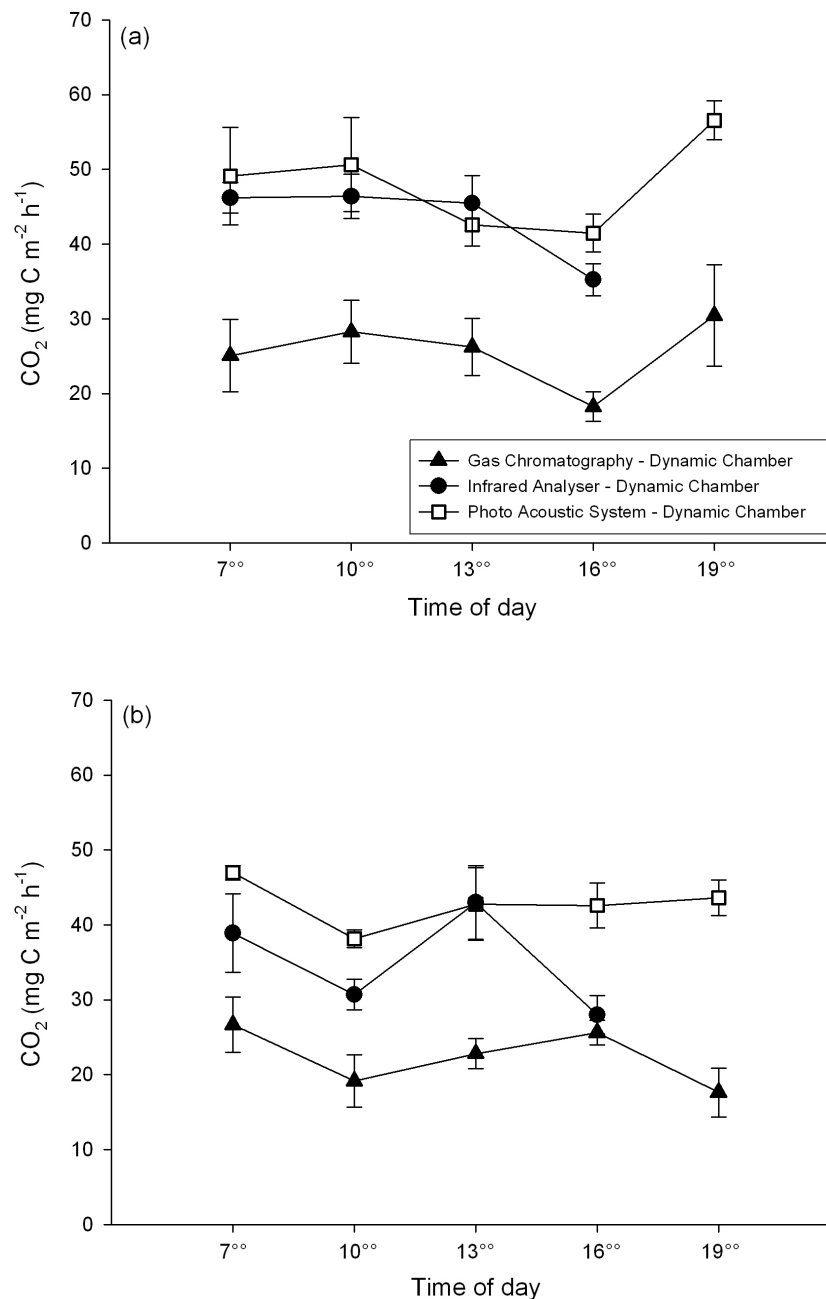


Figure 9: CO<sub>2</sub>-C evolution rate at day 18 in the sandy soil with maize application (a) at the top (0-5 cm) or (b) at the bottom (15-20 cm); error bars show ± standard error (n = 4).

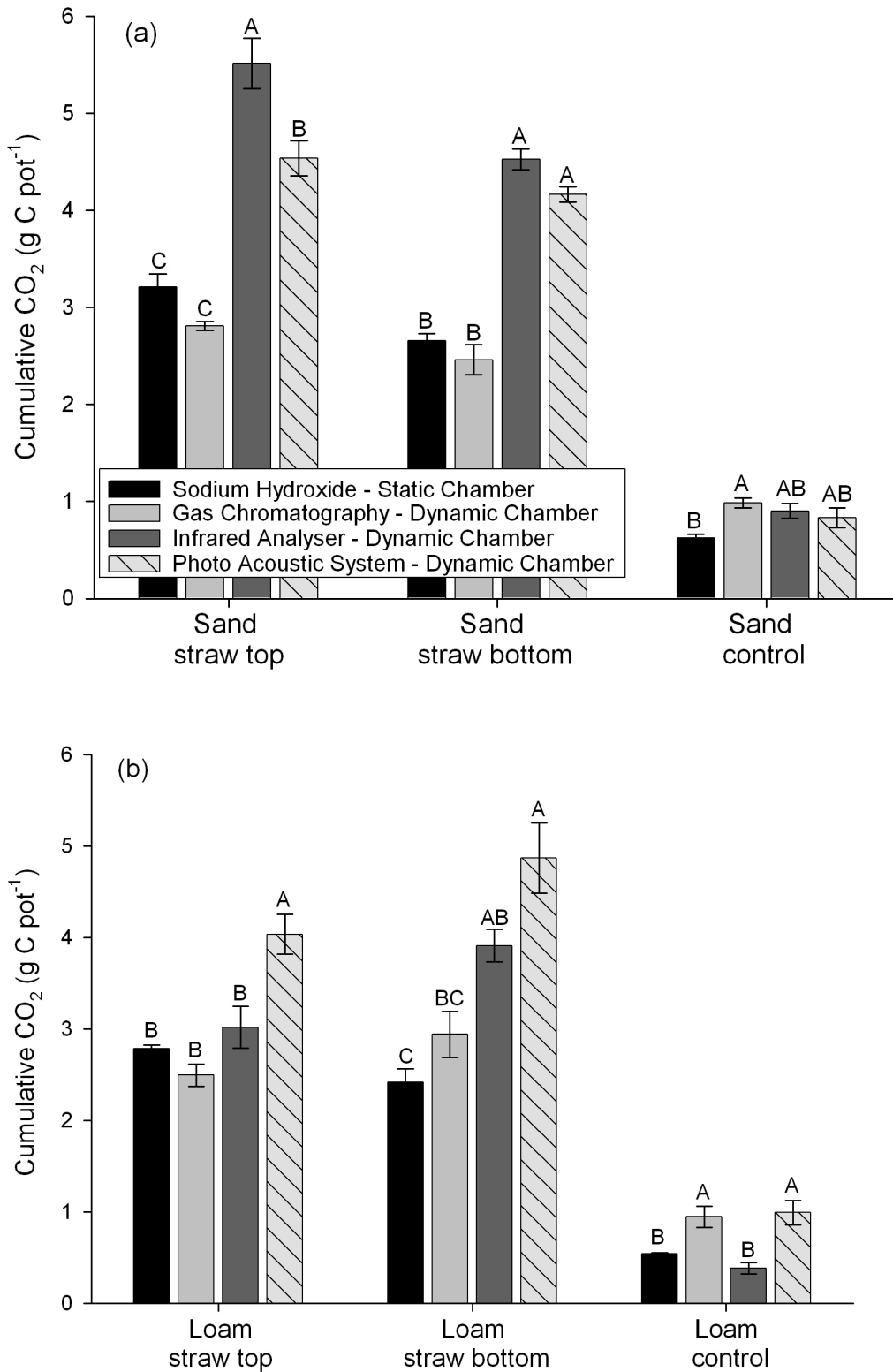


Figure 10: Cumulative CO<sub>2</sub>-C production at the end of a 57-day incubation period in (a) sandy treatments (n = 4) and (b) loamy treatments (n = 4); error bars show ± standard error (n = 4); different letters indicate significant soil- and treatment-specific differences (*P* < 0.05, Tukey/Kramer HSD test).

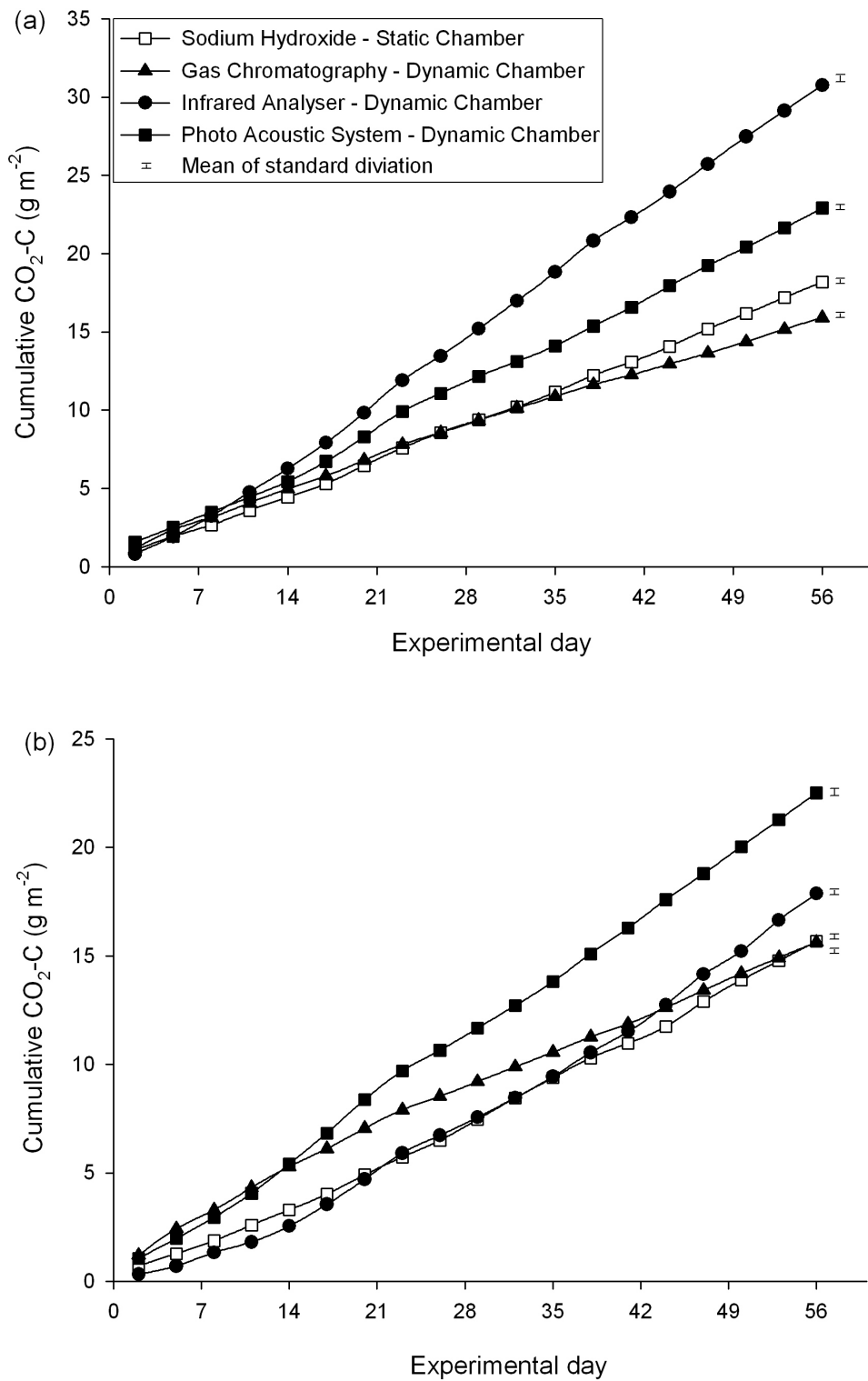


Figure 11: Cumulative CO<sub>2</sub>-C production during a 57-day incubation period (a) into the sandy soil with maize-straw application at 0-5 cm and (b) into the loamy soil with maize-straw application at 0-5 cm; values were calculated by mean respiration rates of three days; error bars show  $\pm$  mean standard deviation (n = 4).

The correlation coefficients between the cumulative CO<sub>2</sub> production of the three dynamic chamber methods (GC, IR, and PAS) and the static NaOH method were all significant with r-values between 0.90 and 0.93 (Fig. 12). The regression coefficients reveal that the IR method resulted in 63% higher values and the PAS method in 46% higher values than the NaOH method.

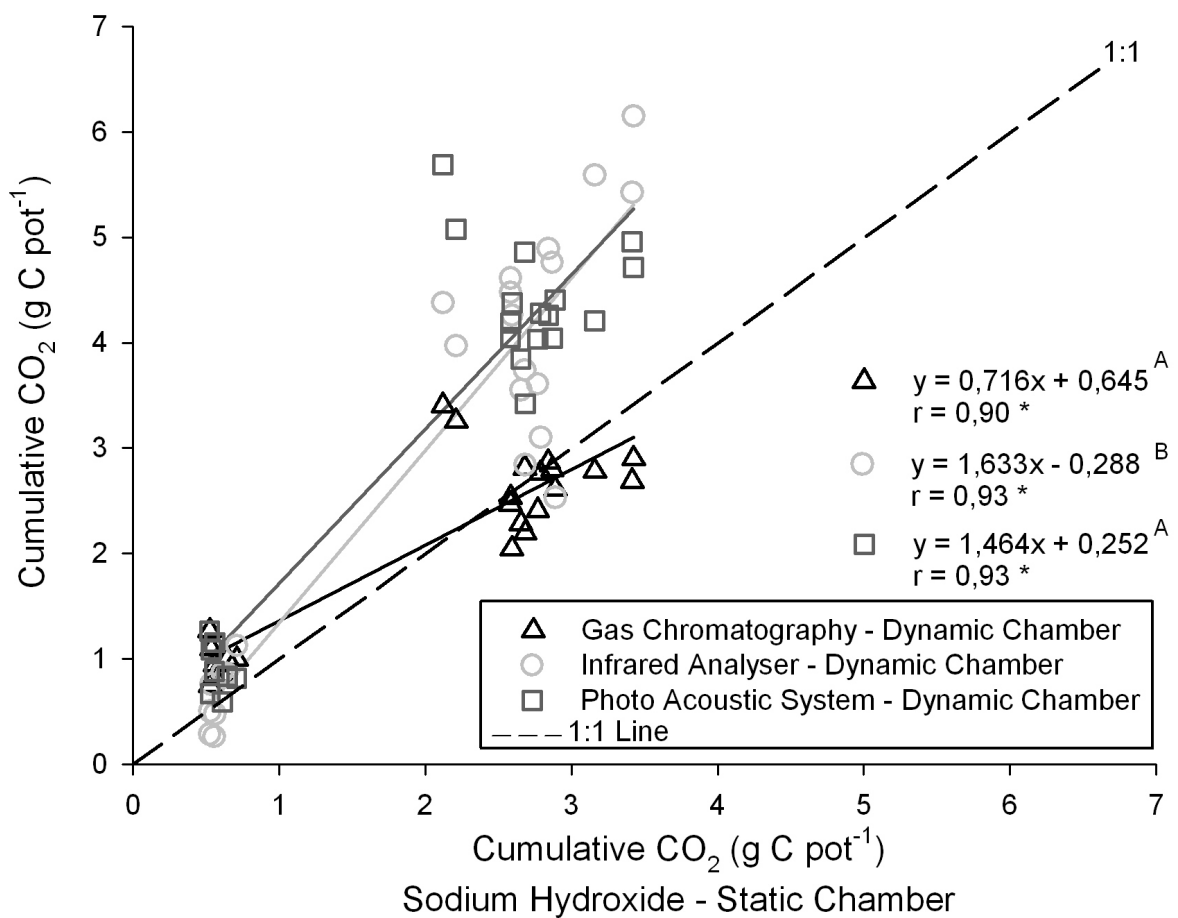


Figure 12: Correlation of cumulative CO<sub>2</sub>-C production measured by different methods at the end of a 57-day incubation period in the different treatments of the sandy and loamy soil (n = 24) (\*  $P < 0.0001$ ); different letters indicate differences in intersection with y axis (n = 4) ( $P < 0.05$ , Tukey/Kramer HSD test).



The C balance can be used for testing the plausibility of CO<sub>2</sub> production data (Fig. 13). Initially, 10.4 g straw-C were incorporated into the column of each maize straw treatment, between 8.5 g and 8.9 mg of straw-derived C were recovered as soil organic C and particulate organic C (Rottmann et al., 2010). Roughly 102% (NaOH and GC) and 114% (IR and PAS) were recovered, including the CO<sub>2</sub> production data in the C balance of the sandy soil. The respective data were 97% (NaOH and GC) and 104% (IR and PAS) for the loamy soil.

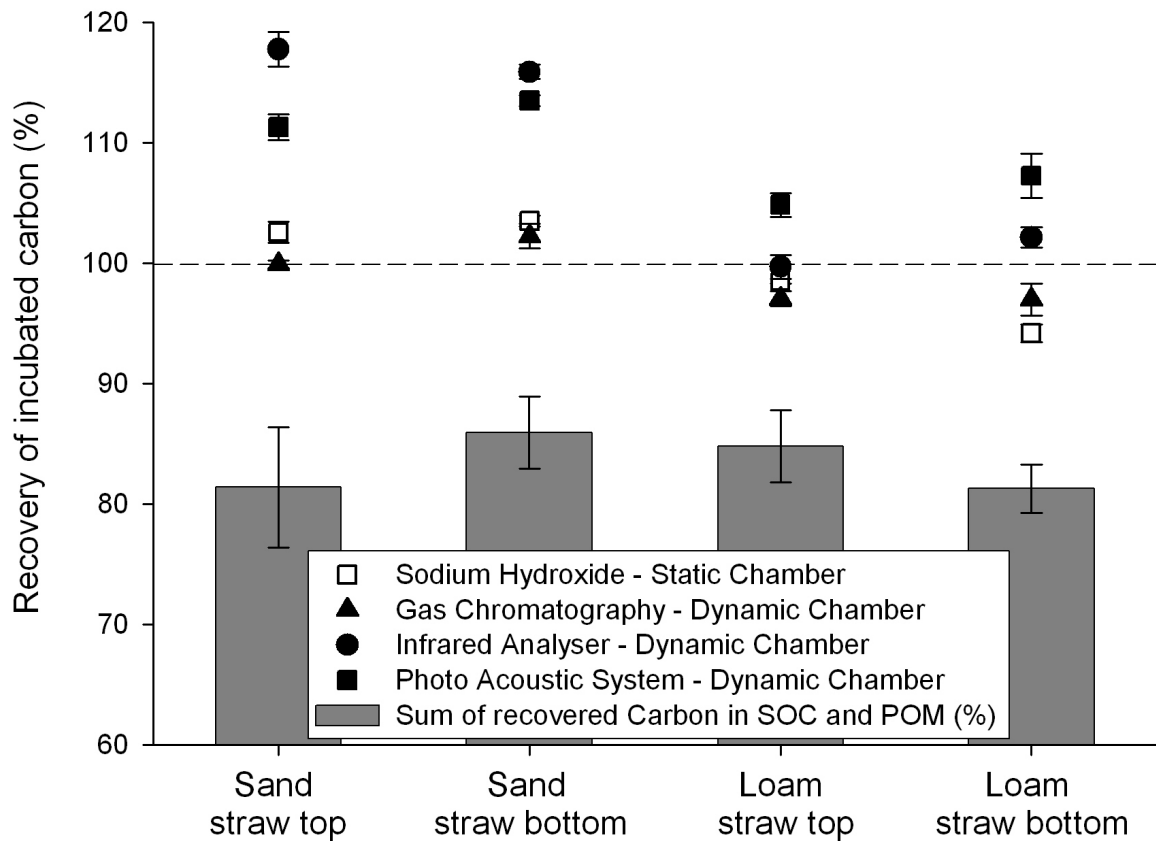


Figure 13: Plausibility-test of recovered C in % C added. The sum of recovered C was calculated as recovered maize derived POM-C and maize-derived soil organic C (n = 4); error bars show  $\pm$  carbon dioxide measurement system derived standard error (n = 4).

#### 4.4 Discussion

All four methods were highly correlated and each method has advantages and drawbacks. The data were affected by the depth of straw application and by the soil, i.e. especially gas diffusion properties, because the deeper layers of the soil also contributed actively to the CO<sub>2</sub> efflux from the soil surface. The general pattern of cumulative CO<sub>2</sub> production suggests a higher C mineralization for maize straw application to the top layer than for that to the bottom layer in the sandy soil and the reverse in the loamy soil. This is in accordance with recovery of maize-derived C as soil organic C and particulate organic matter (*Rottmann et al., 2010*). This general pattern is clearly reflected by all three dynamic chamber methods (GC, IR and PAS), but not by the NaOH-static chamber method. This might be explained by soil properties. During the 24 h measuring period with the NaOH method, CO<sub>2</sub> is transferred to the chamber by diffusion. However, CO<sub>2</sub> diffusion is lower in a loamy soil than in sandy soil, due to a lower porosity, a higher water content at the same matrix potential and consequently a lower air-filled pore space (*Hutchinson and Rochette, 2003; Martin et al., 2004; Butnor et al., 2005*). This is simply because the standard diffusion coefficient of CO<sub>2</sub> is nearly 10<sup>5</sup> times higher in air than in water (*Jensen et al. 1996*) and smaller soil particles increase the diffusion barrier (*Martin et al., 2004*). The absorption of CO<sub>2</sub> to soil water and the transport of dissolved CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> can be ignored, due to their small contribution (*Hutchinson and Rochette, 2003; Martin et al., 2004*). The lower CO<sub>2</sub> diffusion may be the main reason why the NaOH static chamber method led to a moderate underestimation of the CO<sub>2</sub> evolved when the maize straw was incubated in the bottom layer. However all methods revealed a strong participation of all layers, i.e. also the deeper layers to the CO<sub>2</sub> efflux from the soil surface.

The higher CO<sub>2</sub>-C production obtained by the IR-dynamic chamber method in comparison with the NaOH method is most likely due to fan-induced turbulence. This explanation

would be in accordance with *Pumpanen et al. (2004)*, who observed an overestimation of an equivalent IR system (PP Systems, Hitchin, UK) in combination with an identical chamber by nearly 27% for a dry sandy soil. Also *Hutchinson and Rochette (2003)*, *Martin et al. (2004)*, *Butnor et al. (2005)* as well as *Keith and Wong (2006)* described the same effect for sandy and highly porous soils. The higher difference between the IR and PAS-dynamic chamber method for top application of maize straw than for bottom application in the sandy soil might be caused by higher amounts of fan-extracted CO<sub>2</sub>-C. For the GC and PAS method, higher and larger chambers were used than for the IR method. Consequently, the turbulence induced by the fan was probably lower, reducing the effect on the CO<sub>2</sub>-C efflux from soil. Nevertheless, the significant higher values detected with PAS compared to GC could not be induced by chamber differences because for both methods the same chambers were used. However, the overestimation of CO<sub>2</sub> by PAS contradicts to other studies (e.g. *Ambus and Robertson, 1998*, *Pumpanen et al., 2004*; *Reth et al. 2005*), which suggest no or only small differences. Just before the experiment, the multi-gas monitor for the PAS method was calibrated by the manufacturer for compensating cross-interferences of gases and water vapor with CO<sub>2</sub>. Consequently, an equipment defect is unlikely.

However, a reduced diffusion did not affect the short-time measurements by the dynamic chamber systems. With decreasing porosity and air-filled pore space, the fan-induced turbulence loses its effects on CO<sub>2</sub> efflux (*Hutchinson and Rochette, 2003*). Therefore, the IR method did not show any differences to the NaOH and GC method in the loamy soil with maize straw addition to the top layer and also no difference to the GC method in the loamy soil with maize straw addition to the bottom layer. This is again in accordance with *Pumpanen et al. (2004)*, who observed only an overestimation of 5% by the IR method in a wet sandy soil, 22% less in comparison with a dry sandy soil.

The general overestimation of the CO<sub>2</sub>-C production by the PAS method, especially in the treatment for both sandy soils and both loamy soils, contradicts other studies (*Ambus and Robertson, 1998, Pumpanen et al., 2004; Reth et al., 2005b*), which detected no or only small differences between PAS, GC and/or IR as well as NaOH. Chamber effects could not have led to differences between GC and PAS values, because the same type of chamber was used. However, data obtained by the PAS method resulted in excessive amounts of annual CO<sub>2</sub> production in a field experiment (*Předotová et al., 2010*). In contrast to the maize straw treatments, the non-amended control treatments did not result in higher CO<sub>2</sub>-C production measured with the IR method. This is in agreement with *Jensen et al. (1996)* and *Keith and Wong (2006)*, who suggested an increasing CO<sub>2</sub>-C efflux by fans for treatments with a moderate or high efflux, but not for treatments with a low efflux.

In few studies (*Martin et al., 2004; Butnor et al., 2005*) is the real respiration known and used to estimate CO<sub>2</sub> measurement systems accurately. In most studies (*Jensen et al., 1996; Janssens et al., 2000; Alavoine et al., 2008*) and especially in field experiments (*Reth et al., 2005; Keith and Wong, 2006; Müller et al., 2010*), the real soil respiration is unknown and CO<sub>2</sub> measurements are compared with each other without a real reference. In this study, independent reference values were calculated by the difference of maize straw added minus recovered maize-derived C, as described by *Rottmann et al. (2010)*. In the loamy soil, all four methods gave values for the CO<sub>2</sub> efflux that were in the range proposed by the balance gap between added and recovered maize straw C. In this sandy soil, only the NaOH and GC method fitted into this range, whereas the IR and PAS method led to a clear overestimation of the CO<sub>2</sub> efflux.

## 4.5 Conclusions

The NaOH and GC method both have the advantage that they can be combined with  $\delta^{13}\text{C}$  measurements (*Engelking et al., 2007; Rottmann et al., 2010*). The NaOH method does not require any expensive equipment and integrates longer measurement periods, reducing the temporal variability. However, the length of the measurement period may be a drawback in the field, due to the effects on the microclimate, especially humidity and temperature (*Le Dantec et al., 1999*). The GC method needs repeated gas sampling and is the most laborious method, which reduces its applicability in the field (*Müller et al., 2010*). The measurement period is also considerably longer than that of the IR dynamic chamber methods. However, the data were the most consistent in relation to the C balance obtained from the recovery of added maize straw (*Rottmann et al., 2010*). The short 2 min measurement intervals of the IR method allow accurate rapid measurements of the  $\text{CO}_2$  efflux in the field, especially in loamy soils (*Müller et al., 2010*). Also the PAS method needs repeated gas sampling, but has the advantage of enabling the simultaneous measurement of  $\text{NH}_3$  and  $\text{N}_2\text{O}$ . However, the accuracy of the IR and PAS methods was more strongly affected by the properties of the two soils used than the NaOH and GC methods. In extremely calcareous soils, also the NaOH method might be strongly affected by the soil properties.

## Acknowledgements

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## **5 Litter decomposition in fertilizer treatments of vegetable crops under subtropical conditions**

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## **Abstract**

In the coastal Batinah plain of Oman, a litterbag experiment was carried out in an irrigated field, investigating the effects of organic fertilization and mineral fertilization on the cultivation of carrots and cauliflower. Two straw varieties and two green-harvested crops were used, simulating the properties of green manures. The loss of C in the litterbags declined in the order maize (-94%) > alfalfa (-89%) > wheat (-80%) > canola (-69%). For all these materials, the concentration of muramic acid, as an indicator of bacterial C, as well as galactosamine was generally increased in comparison with the initial values. In contrast, fungal glucosamine and consequently also the ratio of fungal C/bacterial C declined for canola and wheat straw. The loss of N, P, and S was generally smaller than that of C and showed strong substrate-specific patterns. Fertilization and crop cultivation had no effect on C losses. Organic fertilization resulted in significant increases in S, Mg and Al in the litterbags in comparison with mineral fertilization. Cultivation of carrots led to significantly lower ash, N, P, Ca, K, Na, and Al concentrations than cultivation of cauliflower. Organic fertilization and carrot cultivation both led to stronger fungal colonization of the litter retained in the litterbags in comparison with mineral fertilization and cauliflower cultivation, respectively. More information is required on the interactions between initial plant surface colonizing microorganisms and soil-derived colonizers.

## 5.1 Introduction

The decomposition of plant residues integrates numerous physical, chemical and biological factors (Dickinson and Pugh 1974). Knacker et al. (2003) distinguished three factor groups for decomposition: (1) the physico-chemical environment, e.g. the location of litter, soil properties and the climate; (2) the initial litter quality, e.g. nutrient availability as well as the concentration and composition of structural components like hemicellulose, cellulose and lignin, and (3) the decomposer community of microorganisms and soil animals. For investigating decomposition processes of plant residues under field conditions, the litterbag method developed by Bock and Gilbert (1957) is still considered to be a useful approach (Knacker et al. 2003; Joergensen et al. 2009). An important advantage of this method is the relatively simple recovery of litter transferred to the field and the possibility of excluding specific groups from the decomposition process (Joergensen et al. 2009).

Numerous studies have described litter decomposition under field conditions of humid temperate climate (Robinson et al. 1994; Joergensen et al. 2009), boreal and wet tropical conditions (Tam et al. 1990; Saini 1989). The majority of these studies have been carried out in forest soils under semi-natural conditions (Alhamed et al. 2004; Xianiu and Hirata 2005). Litterbags have been less intensively used for studying the decomposition of green manure and harvest residues in arable fields (Christensen 1985; Berg 2000; Knacker et al. 2003). Fertilizer management (mineral versus organic fertilization) and cultivation of different crops (cauliflower versus carrots) may have strong effects on the decomposition of plant residues, due to differences in nutrient inputs by the fertilizers and differences in nutrient demand by the crops. This is especially true for irrigated vegetable fields in hot arid regions. Virtually nothing is known about the decomposition of plant residues in this harsh environment for soil microorganisms and cultivated crops.

In the coastal Batinah plain of Oman, a litterbag experiment was carried out in a field experiment (Siegfried et al. 2010), mainly designed for investigating the effects of organic fertilization and mineral fertilization on the cultivation of carrots and cauliflower. Four different plant materials, all presently cultivated in Oman, were used for the present litterbags, two straw varieties (canola and wheat) and two green-harvested crops (alfalfa and maize), simulating the properties of green manures. The first underlying hypothesis was that the differences in nutrient (especially N, but also P, S, and cations) and organic matter composition (hemicellulose, cellulose, lignin) of the plant material lead to differences in C loss, nutrient release, and microbial colonization. The second hypothesis was that organic fertilization increases C loss, due to the application of additional decomposing microorganisms (Henriksen and Breland 1999c). The third hypothesis was that carrot cultivation increases C and nutrient losses from the litterbags, due to the high demand of carrots for nutrients (Müller and von Fragstein und Niemsdorff 2006).

## 5.2 Material and methods

### 5.2.1. Experimental site and conditions

The experiment was carried out on a private experimental farm (24°22'N, 56°34'E) on the northwestern Batinah coast of the Sultanate of Oman. The climate is characterized by two distinct seasons: a very hot summer with temperatures up to 45°C from May to September and a moderate period from October to April with temperatures declining below 20°C. The experiment started on 21 November 2007 and ended 81 days later on 8 February 2008. During the experiment, the temperature decreased from initially 26°C to 18°C (Fig. 14). The mean temperature was around 21°C ( $\pm 4^\circ\text{C}$ ), with total precipitation of 18 mm. Therefore, the experimental field was irrigated every fourth day so that the soil moisture did not drop below -250 hPa (Fig. 14). The experimental field was located 10 m above sea level. The soil was characterized as Irragic Cambisol (FAO – World Reference Base for Soil Resources), with 82% sand, 16% silt, 5% clay, 0.6% soil organic C and a pH-H<sub>2</sub>O of 8.7; bulk density was 2 g cm<sup>-3</sup> and the electrical conductivity was 60 mS m<sup>-1</sup> in the mineral fertilizer treatment and 53 mS m<sup>-1</sup> in the organic fertilizer treatment at the end of the experiment. The soil of both fertilizer treatments contained the following total nutrient contents (mg g<sup>-1</sup> soil  $\pm$  standard deviation): phosphor 0.49  $\pm$ 0.05; sulfur 0.63  $\pm$ 0.04; sodium 0.72  $\pm$ 0.02; potassium 1.71 mg  $\pm$ 0.14; calcium 37.1 mg g<sup>-1</sup>  $\pm$ 1.6; magnesium 127.9  $\pm$ 2.9; manganese 0.68 mg g<sup>-1</sup>  $\pm$  0.01; iron 45.1  $\pm$ 0.8; 18.8 mg g<sup>-1</sup>  $\pm$ 0.48.

The experimental field was divided into 12 plots (2.5 m  $\times$  7.0 m). Six plots were cultivated with approximately 40 individuals of cauliflower (*Brassica oleracea* var. *botrytis*) per plot, which resulted in a yield of 5300 kg dry weight ha<sup>-1</sup>. The other six plots were cultivated with approximately 150 individuals of carrots (*Daucus carota* ssp. *sativus*) per plot, which gave a yield of 2100 kg dry weight ha<sup>-1</sup>. One half of the plots cultivated with cauliflower and carrots was fertilized with mineral fertilizer (mixture of ammonium sulfate,

triple super phosphate, potassium sulfate and gypsum), the other with water buffalo manure, leading to three field replicates of the following four treatments: (1) cauliflower with mineral fertilization, (2) cauliflower with organic fertilization, (3) carrots with mineral fertilization, and (4) carrots with organic fertilization. The organic fertilization treatments consisted of 6700 kg ha<sup>-1</sup> air-dried water-buffalo manure with a C/N ratio of 17 and an additional supply of 42 kg ha<sup>-1</sup> K and 30 kg ha<sup>-1</sup> S as K<sub>2</sub>SO<sub>4</sub>. The mineral fertilization treatments received equivalent amounts of N, P, and K to the organic fertilization treatments, i.e. 160 kg N ha<sup>-1</sup>, 61 kg P ha<sup>-1</sup>, 57 K kg ha<sup>-1</sup>, with additional supply of 30 kg ha<sup>-1</sup> S. Two days before starting the litterbag experiment and one day before planting, the fertilizers were incorporated by plowing at 0-15 cm depth. All plant material, green-harvested alfalfa (*Medicago sativa* L.) and maize (*Zea mays* L.) leaves as well as mature canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) straw, grown in Germany, were chopped to 2-5 mm. Five grams of oven dried (60°C) plant material were filled in polyethylene litterbags (5 × 10 cm, 1 mm mesh). Four replicates per litter and field replicate were placed vertically at 1–6 cm depth on the plots of the field experiment, i.e. a total number of 192 litterbags were buried. The litterbag position in each plot and the plot position in the experimental area were both randomized. After 81 days, the litterbags were removed, dried and transported to Germany.

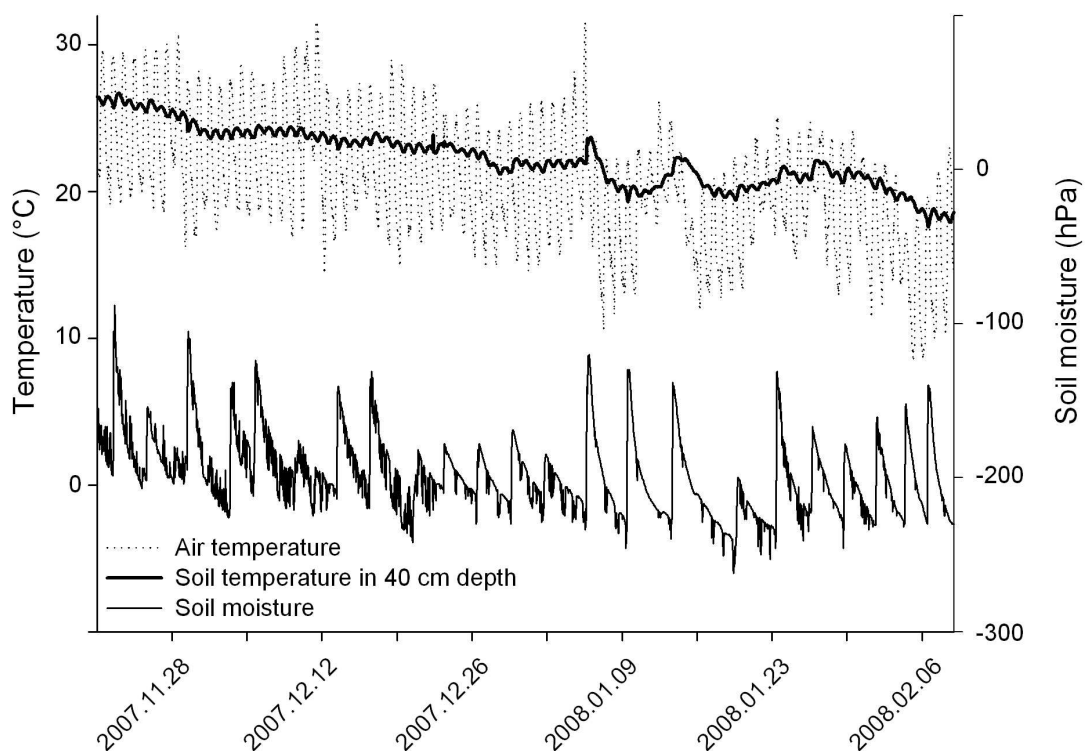


Figure 14: Air temperature, soil temperature and soil moisture during the experiment, interval of measurements every 30 minutes in Sohar.

### 5.2.2. Analytical procedures

The whole litterbag was dried at 60°C and homogenized by grinding in an agate stone mill. Total concentrations of C and N were determined by gas chromatography using a Vario MAX (Elementar, Hanau, Germany) elemental analyzer. The concentrations of total P, S, Na, K, Mg, Ca, Mn, Fe, and Al were analyzed after HNO<sub>3</sub>-pressure digestion as described by Chander et al. (2008) by ICP-AES (Spectro Analytical Instruments, Kleve, Germany). Ash was determined by heating 1 g of a separate sub-sample at 700°C for 6 h.

Before determining the concentrations of hemicellulose, cellulose and lignin, lipids were extracted from 500 mg oven dried (60°C) samples in a Soxhlet apparatus for 3 h with 80% ethanol and for 1 h with 100% chloroform. Then, the samples were air-dried in a fume cupboard and dried again at 60°C. For the determination of hemicellulose, 1 ml 0.75



M H<sub>2</sub>SO<sub>4</sub> was added to a lipid-free 200 mg sub-sample at room temperature. After 1 h, the suspension was refluxed for a further 1 h, cooled, and vacuum filtered through a Whatman GF/A glass-fiber filter. For the determination of cellulose, 1 ml 12 M H<sub>2</sub>SO<sub>4</sub> was added to another 200 mg lipid-free sub sample at room temperature for 2 h. Then, the suspension was diluted to 0.75 M by adding H<sub>2</sub>O, refluxed for 12 h, cooled and vacuum filtered through a Whatman GF/A glass-fiber filter (Joergensen and Meyer 1990). The carbohydrates in both extracts were measured by the reduction of Cu<sup>2+</sup> to Cu<sup>+</sup>, which was monitored with a BCA reagent at pH 11.25 and a wavelength of 562 nm (Joergensen et al. 1996). The concentration of cellulose was calculated by subtracting the hemicellulose-derived carbohydrates from this extract.

Lignin was determined according to Iiyama and Wallis (1988); a lipid-free 1.2 mg sub-sample (5 replicates) was mixed with 300 µl 25% acetyl-bromide (v/v in glacial acetic acid) and 10 µl 70% perchloric acid. Subsequently, the samples were shaken and incubated for 30 min at 70°C. After 10, 20 and 30 min, the samples were shaken again. Then, the samples were rapidly cooled on ice, mixed with 300 µl 2 M NaOH, and centrifuged for 5 min at 3000 g. A 250 µl aliquot of the supernatant was mixed with 1.25 ml glacial acetic acid. The absorbance of the solution was determined at 280 nm. Calibration curves were generated with increasing concentrations of coniferyl alcohol (0, 5, 10, 15 and 20 mmol l<sup>-1</sup>) obtained from Sigma (No. 27740), dissolved in UV-adequate ethanol processed by the same procedure. The molar extinction coefficient of coniferyl alcohol was 8.5.

Glucosamine, galactosamine and muramic acid were determined according to Appuhn et al. (2004). A moist sample of 500 mg plant material was weighed into a 20 ml test tube, mixed with 10 ml 6 M HCl, and heated for 6 h at 105 °C. After HCl removal and centrifugation (10000 g), the samples were transferred to vials and stored at -18 °C before the HPLC measurements. After derivatization with ortho-phthaldialdehyde, fluorometric emission of amino sugars was measured at a wavelength of 445 nm after excitation at a wave-

length of 340 nm. Fungal glucosamine ( $\text{mg g}^{-1}$  dry weight) was calculated by subtracting bacterial glucosamine from total glucosamine, assuming that muramic acid and glucosamine occur at a molar 1/1 ratio in bacterial cell walls (Zelles and Alef 1995):  $\text{mg glucosamine g}^{-1}$  dry weight  $(\text{mmol glucosamine} - \text{mmol muramic acid}) \times 179.2 \times 9$ , where 179.2 is the molecular weight of glucosamine and 9 the conversion value of fungal glucosamine to fungal C (Appuhn and Joergensen 2006). Bacterial C ( $\text{mg g}^{-1}$  dry weight) was calculated by multiplying the concentration of muramic acid in  $\text{mg g}^{-1}$  dry weight by 45 (Appuhn and Joergensen 2006).

### 5.2.3 *Statistical analysis*

Effects of plant material, fertilization and crop on the concentrations of individual elements and ash as well as on hemicellulose, cellulose, lignin, amino sugar, fungal C, microbial C and the fungal C/bacterial C ratio were tested by a three factorial ANOVA. Significance within plant material effects was tested by a one-way analysis of variance using the Tukey-Kramer HSD (Honestly Significant Difference) test. All statistical calculations were performed using JMP 7.0 (SAS Institute Inc.).

## 5.3 Results

### 5.3.1 Quality of the organic substrates

Alfalfa and maize residues were characterized by high concentrations of N, leading to a C/N ratio of roughly 20, and canola straw by high concentrations of S, but low concentrations of N (Table 4). The ash concentration declined in the order maize > wheat > alfalfa > canola. Wheat straw always exhibited the lowest nutrient concentrations, although the ash concentration was relatively high. Wheat straw was also characterized by maximum concentrations of hemicellulose (136 mg g<sup>-1</sup> ash-free dw) and lignin (290 mg g<sup>-1</sup> ash-free dw), maize residue by maximum concentrations of cellulose (330 mg g<sup>-1</sup> ash-free dw) in combination with high lignin concentrations (240 mg g<sup>-1</sup> ash-free dw). Canola and wheat straw contained highest concentrations of muramic acid (21 µg g<sup>-1</sup> ash-free dw), glucosamine (1500 µg g<sup>-1</sup> ash-free dw) and galactosamine (85 mg g<sup>-1</sup> ash-free dw), exceeding those in the green-harvested alfalfa and maize residues two- to nine-fold. Bacterial muramic acid and consequently bacterial C was not detected in the maize residues (Fig. 16). Based on glucosamine data, fungal C dominated the microbial colonization of the straw with a ratio of fungal C/bacterial C of nearly 14 (Fig. 17).

Table 4

Composition of green-harvested alfalfa and maize residues as well as canola and wheat straw at the beginning of the experiment

	Alfalfa	Maize	Canola	Wheat	CV ( $\pm\%$ )
C (mg g <sup>-1</sup> dw)	430 c	440 b	420 d	440 a	1.3
N (mg g <sup>-1</sup> dw)	23 a	22 a	5.6 b	4.1 c	2.0
P (mg g <sup>-1</sup> dw)	2.6 a	1.9 b	0.67 d	1.4 c	2.1
S (mg g <sup>-1</sup> dw)	1.2 c	1.6 b	3.0 a	0.54 d	1.2
Ca (mg g <sup>-1</sup> dw)	10.3 a	9.5 b	9.4 b	2.1 c	0.9
Mg (mg g <sup>-1</sup> dw)	5.3 b	2.0 c	5.8 a	0.66 d	1.3
K (mg g <sup>-1</sup> dw)	29 a	16 c	23 b	11 d	1.3
Na (mg g <sup>-1</sup> dw)	0.15 b	0.17 b	0.41 a	0.05 c	6.0
Mn ( $\mu\text{g g}^{-1}$ dw)	40 c	50 b	58 a	14 d	1.9
Fe (mg g <sup>-1</sup> dw)	1.5 b	0.23 c	1.9 a	0.15 d	1.3
Al (mg g <sup>-1</sup> dw)	1.4 b	0.2 c	2.1 a	0.05 d	2.0
Ash (mg g <sup>-1</sup> dw)	116 c	195 a	106 d	180 b	1.9
Hemicellulose (mg g <sup>-1</sup> ash-free dw)	55 bc	30 c	76 b	136 a	16
Cellulose (mg g <sup>-1</sup> ash-free dw)	230 c	330 a	230 c	290 b	5.1
Lignin (mg g <sup>-1</sup> ash-free dw)	180 c	240 b	190 c	290 a	10
Muramic acid ( $\mu\text{g g}^{-1}$ ash-free dw)	11 b	nd	21 a	18 a	11
Glucosamine ( $\mu\text{g}^{-1}$ ash-free dw)	291 b	163 b	1500 a	1500 a	14
Galactosamine (mg g <sup>-1</sup> ash-free dw)	19 d	28 c	85 a	73 b	8.1
Fungal C (mg g <sup>-1</sup> ash-free dw)	2.6 b	1.5 b	13.1 a	10.7 a	10
Microbial C (mg g <sup>-1</sup> ash-free dw)	3.0 b	1.5 b	14.0 a	11.5 a	10
Fungal C/bacterial C	5.2	nd	13.9	13.6	11

CV = pooled coefficient of variation between replicate analyses (n = 5); dw = dry weight; nd = not detectable; different letters within a row indicate a significant difference between the plant materials (Tukey-Kramer,  $P < 0.05$ , n = 5).

### 5.3.2 Loss of organic matter and changes in nutrients

The loss of C in the litterbags during the 81-day burial period declined in the order maize residues (-94%) > alfalfa residues (-89%) > wheat straw (-80%) > canola straw (-69%) (Table 5). The losses of N, P, and S were generally smaller than that of C and showed strong substrate-specific patterns (Table 5). The wheat straw strongly retained N (61% initial dw) and S (66% initial dw), whereas canola straw retained N (53% initial dw) and P (47% initial dw). The S-rich canola straw was characterized by strong S losses (86%). Fertilization and crop cultivation had no effect on C losses. In contrast, mineral fertilization led to a significantly higher retention of P in the litterbags (Fig. 15), and organic fertilization to a higher retention of S. Cultivation of carrots led to a significantly lower retention of N and P, but not of S in comparison with cultivation of cauliflower. Strong interactions occurred for P between plant material, fertilization and crop cultivation (Fig. 15).

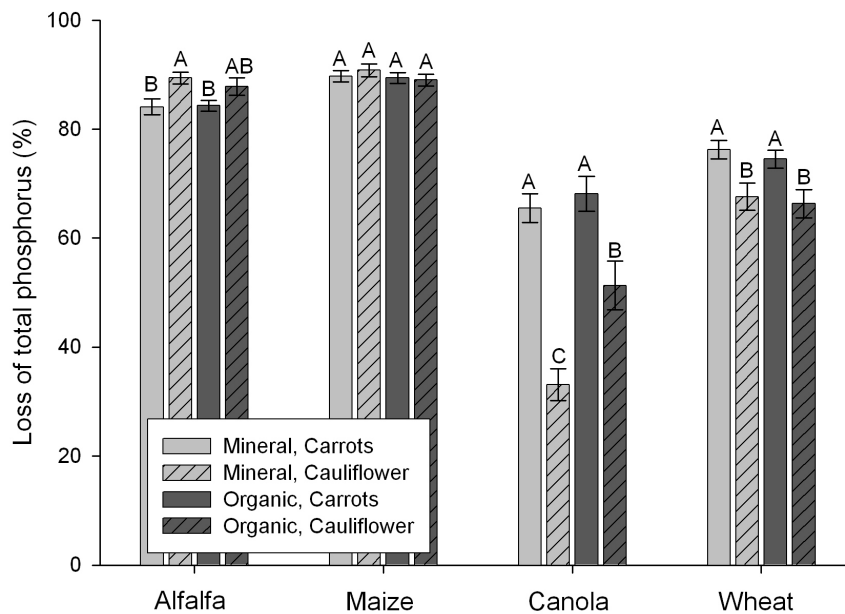


Figure 15: Loss of total P from different plant materials recovered at the end of a 81-day bury period in Sohar; Mineral = mineral fertilizer; Organic = organic fertilizer; error bars show  $\pm$  one standard error of mean ( $n = 12$ ); different letters above the columns indicate a significant differences (Tukey-Kramer HSD test,  $P < 0.05$ ).

Table 5

Amounts of C, N, P, and S ash in percent of the initial weight in the litterbags with different plant materials recovered at the end of a 81-day bury period in Sohar; probability levels for a three-factorial ANOVA using plant materials, fertilization and crop as independent factors.

	C	N	P	S
	(% initial weight)			
Plant materials				
Alfalfa	11 c	12 c	15 c	23 b
Maize	6 d	9 d	9 d	14 c
Canola	31 a	53 b	47 a	14 c
Wheat	20 b	61 a	24 b	66 a
Fertilization				
Mineral	17	36	28	27
Organic	17	32	22	32
Crop				
Cauliflower	17	37	27	30
Carrots	17	31	22	29
Probability levels				
Plant materials	<0.01	<0.01	<0.01	<0.01
Fertilization	0.54	0.08	0.05	0.02
Crop	0.84	0.02	0.01	0.40
PR × F	0.31	0.01	0.01	0.01
PR × C	0.06	0.03	0.01	0.05
F × C	0.32	0.61	1.00	0.92
CV (±%)	26	28	23	20

CV = pooled coefficient of variation between replicate litterbags (n = 4); different letters within a column indicate a significant difference of the one-way ANOVA between the plant materials (Tukey-Kramer,  $P < 0.05$ , n = 48).

The loss of ash in the litterbags during the 81-day burial period declined in the order: maize residues (-92%) > alfalfa residues (-85%) > wheat straw (-80%) > canola straw (-66%) (Table 6). The order was identical to the C losses, the percentages also being on similar levels. However, the elemental composition of the ash revealed strong changes during the burial period. Potassium was the only nutrient that was generally lost in the litterbags by between 99% (alfalfa) and 93% (wheat). Calcium was lost by 24% and 51% from alfalfa and maize residues, respectively, but increased in the canola and especially in the Ca-poor wheat straw. The concentrations of Mg, Na, Mn and especially Fe and Al were always increased by between 140 and 7600% (Al in wheat straw). Organic fertilization resulted in significant increases in Mg from 350% (maize) up to 550% (wheat) and Al from 230% (alfalfa) up to 7600 % (wheat) in the litterbags and thus to a significantly decreased ash to Al ratio in comparison with the mineral fertilization of 5.3 and 6.1, respectively. Cultivation of carrots led to significant decreases in ash, Ca, K, Na, and Al and to a significantly increased ash to Al ratio in the litterbags in comparison with cultivation of cauliflower. Strong interactions occurred for Al between plant material, fertilization and crop cultivation

### 5.3.3 *Changes in organic matter quality*

After the 81-day burial period, the hemicellulose concentration was similar in all four plant materials (Table 7). In contrast, cellulose and lignin concentrations formed two pairs, alfalfa and maize residues with relatively low cellulose ( $140 \text{ mg g}^{-1}$  ash-free dw) and high lignin concentrations ( $320 \text{ mg g}^{-1}$  ash-free dw) and canola and wheat straw with relatively high cellulose ( $220 \text{ mg g}^{-1}$  ash-free dw) and low lignin ( $180 \text{ mg g}^{-1}$  ash-free dw) concentrations. In comparison with the initial values (Table 7), the concentrations of hemicellulose were generally decreased in the range of  $3 \text{ mg g}^{-1}$  ash-free dw for maize and  $113 \text{ mg g}^{-1}$  ash-free dw for wheat. In contrast, the concentrations of cellulose were decreased and those of lignin were increased in the alfalfa and maize residues. The reverse was observed in the canola and wheat straw. Mineral fertilization significantly increased hemicellulose and decreased lignin in comparison with organic fertilization, whereas carrot cultivation led to significantly higher cellulose concentrations in comparison with cauliflower cultivation.



#### 5.3.4 *Changes in microbial colonization*

After the 81-day burial period, maize residues revealed highest concentrations of muramic acid ( $79 \mu\text{g g}^{-1}$  ash-free dw), glucosamine ( $1380 \mu\text{g g}^{-1}$  ash-free dw) and galactosamine ( $450 \mu\text{g g}^{-1}$  ash-free dw) and consequently also highest concentrations of fungal C ( $12 \text{ mg g}^{-1}$  ash-free dw) and microbial C ( $15.5 \text{ mg g}^{-1}$  ash-free dw). The concentrations of muramic acid and galactosamine were significantly lowest and on similar levels in canola and wheat straw in comparison with green-harvested alfalfa and maize residues (Table 7). In these materials, the concentration of all three amino sugars was generally increased in comparison with the initial values. The same was also true for muramic acid and galactosamine in canola and wheat straw, whereas the concentration of glucosamine and consequently also the ratio of fungal/bacterial C declined. Organic fertilization significantly decreased the concentration of muramic acid (Table 7) and thus bacterial C (Fig. 16), but increased the concentrations of glucosamine (Table 7), galactosamine, fungal C and microbial C and increased the ratio of fungal C/bacterial C in comparison with mineral fertilization (Fig. 17). Cultivation of carrots had no significant effect on the concentration of muramic acid, but significantly increased the concentrations of glucosamine, galactosamine, fungal C and microbial C and the ratio of fungal C/bacterial C in comparison with cultivation of cauliflower. Significant interactions between plant material, crop and fertilization were mainly detected for muramic acid and thus for bacterial C.

Table 6: Amounts of cations and ash in percent of the initial weight in the litterbags as well as the ratio ash/Al in the litterbags with different plant materials recovered at the end of a 81-day bury period in Sohar; probability levels for a three-factorial ANOVA using plant materials, fertilization and crop as independent factors.

	Ca	Mg	K	Na	Mn	Fe	Al	Ash	Ash/Al
	(% initial weight)								
Plant materials									
Alfalfa	76 bc	350 b	1.3 c	260 b	220 b	1000 c	230 c	15 c	6.2 a
Maize	49 c	340 b	1.6 c	160 c	140 c	1100 c	970 b	8 d	5.4 a
Canola	130 b	500 a	2.8 b	140 c	250 b	3400 a	320 c	34 a	6.0 a
Wheat	510 a	550 a	6.1 a	700 a	1070 a	2700 b	7600 a	20 b	5.1 a
Fertilization									
Mineral	170	370	2.7	320	370	1800	2000	20	6.1
Organic	190	470	3.1	310	440	2200	2600	19	5.3
Crop									
Cauliflower	200	450	3.4	330	430	2000	2700	21	4.9
Carrots	170	400	2.5	300	380	2000	1900	18	6.4
Probability levels									
Plant materials	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.14
Fertilization	0.60	0.04	0.21	0.21	0.32	0.38	0.02	0.80	0.02
Crop	0.02	0.14	0.01	0.02	0.11	0.53	0.01	0.02	<0.01
PR × F	0.24	0.29	0.28	0.13	0.60	0.93	0.01	0.03	0.97
PR × C	0.41	<0.01	0.08	<0.01	0.13	0.37	0.01	<0.01	0.03
F × C	0.62	0.17	0.67	0.67	0.06	0.20	0.35	0.56	0.65
CV (±%)	23	28	28	18	20	24	24	17	18

CV = pooled coefficient of variation between replicate litterbags (n = 4); different letters within a column indicate a significant difference of the one-way ANOVA between the plant materials (Tukey-Kramer,  $P < 0.05$ , n = 48).

Table 7: Concentrations of hemicellulose, cellulose, lignin, muramic acid, glucosamine, galactosamine, fungal C and microbial C (fungal C + bacterial C) and the fungal C to bacterial C ratio in the litterbags with different plant materials recovered at the end of a 81-day bury period in Sohar; probability levels for a three-factorial ANOVA using plant materials, fertilization and crop as independent factors.

	Hemicellulose (mg g <sup>-1</sup> ash-free dw)	Cellulose	Lignin	MurN (μg g <sup>-1</sup> ash-free dw)	GlcN	GalN	Fungal C (mg g <sup>-1</sup> ash-free dw)	Microbial C	Fungal C/ bacterial C
Plant materials									
Alfalfa	23 b	150 b	320 a	63 b	970 b	240 b	8.4 b	11.3 b	3.0 b
Maize	27 a	130 b	320 a	79 a	1380 a	450 a	12.0 a	15.5 a	3.4 b
Canola	22 b	240 a	150 c	43 c	1050 b	150 c	9.2 b	11.1 b	5.0 a
Wheat	23 b	200 a	200 b	43 c	1030 b	130 c	9.0 b	10.1 b	5.1 a
Fertilization									
Mineral	27	190	240	60	1030	220	8.9	11.7	3.2
Organic	21	170	260	54	1180	260	10.3	12.7	4.3
Crop									
Cauliflower	24	160	260	53	930	190	8.1	10.4	3.5
Carrots	24	200	250	61	1290	280	11.3	14.0	4.2
Probability levels									
Plant materials	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fertilization	<0.01	0.08	<0.01	0.03	0.06	0.04	0.05	0.23	<0.01
Crop	0.85	<0.01	0.51	0.06	<0.01	<0.01	<0.01	<0.01	0.01
PR × F	0.46	0.66	0.13	0.04	0.40	0.08	0.42	0.30	0.02
PR × C	0.67	0.12	0.01	0.02	0.14	0.01	0.14	0.12	0.14
F × C	0.68	0.05	0.65	0.01	0.12	0.29	0.12	0.10	0.17
CV (±%)	12	18	21	24	19	21	19	18	20

CV = pooled coefficient of variation between replicate litterbags (n = 4); dw = dry weight; MurN = muramic acid. GlcN = glucosamine; GalN = galactosamine; different letters within a column indicate a significant difference of the one-way ANOVA between the plant materials (Tukey-Kramer,  $P < 0.05$ , n = 48).

## 5.4 Discussion

### 5.4.1 *Effects of substrate quality on loss processes*

The loss of organic material in the litterbags was generally high in the irrigated vegetable field over the 81-day burial period in comparison with data obtained from arable fields under humid temperate climate (Christensen 1985; Robinson et al. 1994). Litterbag data from vegetable home gardens are rare (Isaac and Nair 2006). The same is true for litterbags buried in soils from arid regions (Cepeda-Pizarro 1993). Decomposition data from litterbags buried in irrigated arid regions are not available.

The observation that green-harvested alfalfa and maize residues with a low C/N ratio had a stronger C loss than canola and wheat straw with a high C/N ratio is in agreement with findings already reported (Powlson et al. 2001). The stronger C loss of the maize than of the alfalfa residues might be due to the stronger microbial colonization during the 81-day burial period, as suggested by Allison and Killham (1988) and Henriksen and Breland (1999b). However, the reasons for this stronger colonization from initially the lowest contents of microbial biomass and especially fungal C cannot be explained by the present data. It has been reported that legume biomass contains substances such as polyphenols that inhibit microbial (Bending et al. 1998) and especially fungal colonization (Hättenschwiler and Vitousek 2000). The composition of the plant material certainly affects its palatability to mesofaunal decomposers (Loranger-Merciris et al. 2007), leading to a selective transport and/or grazing of microorganisms (Taylor et al. 2004; Aneja et al. 2006). It is also difficult to explain the higher C loss of the wheat straw in comparison with the canola straw. The wheat straw contained higher lignin and lower nutrient concentrations. Inhibitory organic components such as glucosinolates might have slowed down microbial decomposition, especially during early stages (Hättenschwiler and Vitousek 2000).

In green-harvested alfalfa and maize residues, the strong decrease in cellulose concentration might be explained by the high initial N concentration, which has been shown to increase the microbial production and activity of exo-cellulases, endo-cellulases and xylanases (Henriksen and Breland 1999b) and consequently cellulose degradation (Recous et al. 1995; Berg 2000; Henriksen and Breland 1999a). In canola and wheat straw, another reason for the lower decrease in the cellulose concentration than alfalfa and maize might be the higher protection of cellulose by lignin (Robinson et al. 1994). The hemicellulose concentrations of the different plant materials decreased to a similar level. This is in accordance with Henriksen and Breland (1999c) who did not find an effect of litter quality on the hemicellulose concentration at the end of a 100-day incubation. Murayama (1984) suggested that the remaining fraction of hemicellulose was biased by microbially synthesized components.

Differences in nutrient composition between the different plant materials apparently have only minor effects on decomposition. However, such effects might be masked by the input of soil material into the litterbags, as indicated by increases above 100% of initial weight for most nutrients. Soil and soil-derived nutrients can be incorporated in different ways, e.g. transport by mesofaunal organisms (Potthoff and Loftfield 1998, Joergensen et al. 2009), or by root growth and mycelial colonization (van Hees et al. 2004). Rottmann et al. (2009) observed a transport of labeled C by fungal hyphae even over a distance of decimeters. The input of soil material increases the retention capacity of nutrients, especially P (Joergensen 1991). The absence of significant differences in the Al to ash ratio between the plant materials indicates that the absolute transfer of soil material was not affected by the litter quality, although the relative increases differed markedly.

#### 5.4.2 *Effects of fertilization and crops on loss processes*

Organic fertilization led to a significantly higher microbial, especially fungal colonization of the plant material retained in the litterbags than mineral fertilization. In contrast, the higher N availability in the mineral fertilizer treatments specifically increased bacterial colonization, as indicated by the higher muramic acid concentrations. This higher fungal colonization had no general effects on C loss, but led to some shifts in composition of organic and inorganic material retained in the litterbags. The concentration of hemicellulose was lower, that of lignin, Mg and Al higher in organic than in mineral fertilization. The effects on the nutrient concentrations might be explained by enhanced addition of nutrients with organic fertilization and by effects on fungal transport processes as explained above. Decreased lignin degradation efficiency by manure-derived microorganisms has been observed by Henriksen and Breland (1999c). This can be explained by the lower support of saprotrophic fungi by farmyard manure (Scheller and Joergensen 2008; Joergensen et al. 2010), contradicting the present higher fungal colonization of the plant material retained in the litterbags. This might be due to differences in the fungal community composition, to interacting effects with the mesofaunal decomposers or to artifacts of the litterbags. However, litterbag experiments under similar fertilizer treatments are not available for comparison and are urgently needed to verify the observed effects.

Cultivation of carrots led to a significantly higher microbial, especially fungal colonization of the plant material retained in the litterbags than cultivation of cauliflower. Similar to the fertilizer effects, this higher fungal colonization had no general effects on C loss. However, in contrast to the fertilizer effects, the shifts in composition of organic components solely affected the cellulose fraction and not the fractions of hemicellulose and lignin, for unknown reasons. These results point to the importance of differences in root growth, rhizodeposition and nutrient uptake for the decomposition of plant material and the

retention of nutrients in the litterbags. The significantly lower retention of N, P, Ca, Na, and especially K suggests that carrots have a higher nutrient demand than cauliflowers. The higher ash to Al ratio and the lower Al concentration in the litterbags of the carrot treatment additionally indicate a lower transfer of soil material into the litterbags of the carrot treatment. However, litterbag experiments under different crop treatments are not available for comparison.

#### *5.4.3 Changes of amino sugars as microbial indicators*

After a burial period of 81 days, the microbial colonization was similar in all plant materials, despite strong initial differences. Green, i.e. actively growing plant leaves exhibit a significant but small microbial composition. In contrast, the two straw varieties were strongly colonized by microorganisms, especially by fungi. It is an interesting feature that this fungal colonization decreased during the burial period in the soil by 30%. This decrease was not observed by Cheshire et al. (1999) in litterbags with straw or by Potthoff et al. (2008) in maize straw recovered as particulate organic matter by sieving. The final concentrations in glucosamine were similar in the present experiment to those obtained by Potthoff et al. (2008). However, the initial values were considerably higher than those observed by Scheller and Joergensen (2008) in wheat straw and by Potthoff et al. (2008) in maize straw, suggesting that the further development of a straw colonizing microbial community depends on the colonizing condition of the standing crop in the field. The amino sugar data give clear evidence that fungi dominate the decomposition of straw, as suggested by others (Bowen and Harper 1990; Cheshire et al. 1999). A fungal C/bacterial C ratio of 14 initially observed in the canola and wheat straw means that the microbial tissue consisted of 93% fungal and 7% bacterial mass. A fungal C/bacterial C ratio of 5 observed in this straw after a burial period of 81 days in litterbags means that the microbial

tissue consisted of 83% fungal and 17% bacterial mass. A fungal C/bacterial C ratio of 3.2 observed in the alfalfa and maize residues in litterbags means that the microbial tissue consisted of 76% fungal and 24% bacterial mass.

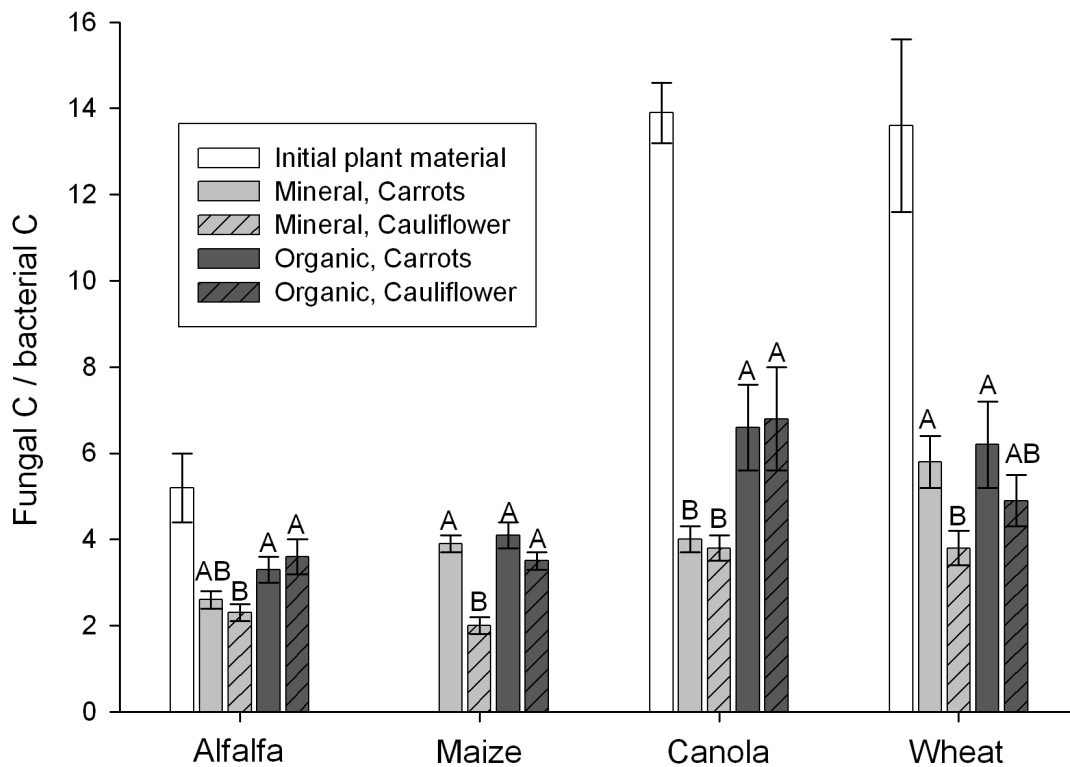


Figure 16: Ratio of fungal C-to-bacterial C different plant materials recovered at the end of a 81-day bury period in Sohar; Mineral = mineral fertilizer; Organic = organic fertilizer; error bars show  $\pm$  one standard error of mean ( $n = 12$ ); different letters above the columns indicate a significant differences (Tukey-Kramer HSD test,  $P < 0.05$ )

At the end of the experiment, the fraction of amino sugars consisted of 80% glucosamine, 16% galactosamine, and 4% muramic acid. These percentages are only slightly different to those obtained by others in soil samples of different origin measured by gas chromatography (Glaser et al. 2004; Amelung et al. 2008) or HPLC (Engelking et al. 2007). Between 0 and 50% galactosamine have been found in laboratory cultures of bacteria and fungi (Engelking et al. 2007). Galactosamine contributed on average 4% to the total amino sugar concentration in cultured bacteria and 15% in cultured fungi. The differences



between the plant materials suggest a close relationship with muramic acid, i.e. bacteria, the fertilization and crop treatments a close relationship with glucosamine, i.e. fungi. However, the function of galactosamine within microbial cells or as metabolites and consequently the processes behind galactosamine formation during decomposition of straw and plant residues are still unknown.

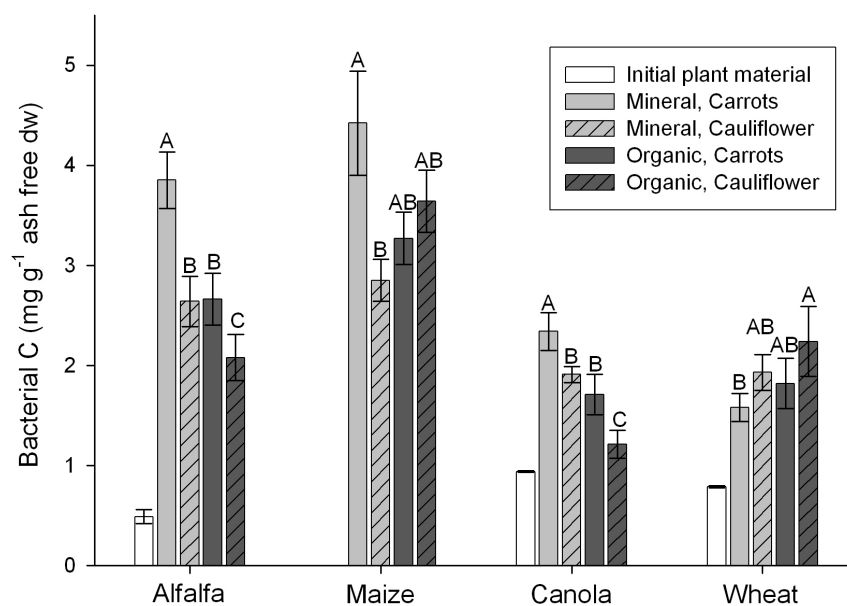


Figure 17: Bacterial C based on muramic acid data in different plant materials recovered at the end of a 81-day bury period in Sohar; Mineral = mineral fertilizer; Organic = organic fertilizer; error bars show  $\pm$  one standard error of mean ( $n = 12$ ); different letters above the columns indicate a significant differences (Tukey-Kramer HSD test,  $P < 0.05$ ).

## **5.5 Conclusions**

The C/N ratio of the plant material has the strongest effects on the C loss in litterbags. Concentrations of lignin and other nutrient elements are less important. Fertilization and crop cultivation also led to complex interacting effects on the nutrients retained in the litterbags. This might be due to differences in microbial, especially fungal colonization of green manure and straw in the litterbags over the experimental period. More information is necessary on the interactions between initial plant surface colonizing microorganisms and soil-derived colonizers. Organic fertilization and carrot cultivation both led to stronger fungal colonization of the litter retained in the litterbags in comparison with mineral fertilization and cauliflower cultivation, respectively. The interacting effects of litter quality, microbial colonization, fertilization, and crop cultivation, observed in the present field experiments need to be verified by other litterbag experiments under similar, but also under different soil and climatic conditions.

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## 6 Zusammenfassung

Die vorliegende Arbeit untersuchte die Einflüsse der Bodenart und Einarbeitungstiefe von Streu auf die mikrobielle Nutzung und ihren Abbau. Anhand einer Kohlenstoffsequestrierung wurde die Verlagerung streubürtigen Kohlenstoffes in die Fraktionen  $\text{CO}_2\text{-C}$ , SOC, extrahierbaren Kohlenstoff,  $C_{\text{mik}}$  und POM-C betrachtet. Aufgrund der Analyse der  $\delta^{13}\text{C}$ - $\text{CO}_2$  Werte der Bodenrespiration, im Rahmen der Sequestrierung des streubürtigen Kohlenstoffes, war der Anteil der streubürtigen Bodenrespiration und somit die gesamte, zu erwartende Bodenrespiration bekannt. Durch die, bei der Kohlenstoffsequestrierung, ermittelten Werte konnte eine Plausibilitätsprüfung an vier Methoden zur Erfassung der Bodenrespiration, auf ihre Genauigkeit und mögliche Artefakte hin, durchgeführt werden. Des Weiteren wurden in einem anschließenden Freilandversuch unter subtropischen Bedingungen die Einflüsse verschiedener Dünger und Feldfrüchte, in Abhängigkeit der Streuqualität, auf den Streuabbau und die mikrobielle Besiedelung hin untersucht.

Im ersten Versuch (Kapitel 3), wurde anhand eines Säulenversuches der Einfluss der Einarbeitungstiefe, in Anhängigkeit der Bodenart, auf den Streuabbau untersucht. Dieses ist von großer Bedeutung, da auf landwirtschaftlich genutzten Flächen Streu und so genannte „Grüne Dünger“ durch den Einsatz unterschiedlicher Bodenbearbeitungssysteme, wie z.B. der Kreiselegge oder dem Wendepflug, in unterschiedliche Tiefen eingearbeitet werden. Die Verlagerung streubürtigen mikrobiellen Kohlenstoffes per Pilzhyphen, über eine Distanz von bis zu 20 cm wurde innerhalb dieser Arbeit das erste Mal gezeigt. Bisherige Studien zeigten einzig einen Transport von streubürtigem Kohlenstoff per Pilzhyphen, über eine kurze Distanz von der Detritussphäre in den angrenzenden Boden. Der höhere Anteil streubürtigen mikrobiellen Kohlenstoffes innerhalb der von der Streuschicht weiter entfernten Schichten im sandigen Boden, im Vergleich zum lehmigen Boden zeigte, dass das feine Porenvolumen des lehmigen Bodens den Transport Streubürtigen Kohlenstoffes per Pilzhyphen grundsätzlich behindert. Diese Annahme wurde durch die stärkere Abnah-



me des Anteils streubürtigen mikrobiellen Kohlenstoffes, mit zunehmender Entfernung zur Streuschicht, im lehmigen Boden im Vergleich zum sandigen Boden unterstützt. Es ist davon auszugehen, dass der sandige Boden zusätzlich durch die höhere Porosität eine erhöhte Sauerstoffdurchlässigkeit und somit, in den tieferen Schichten bessere Wachstumsbedingungen für Mikroorganismen bietet als der lehmige Boden. Durch die Ausbreitung substratbürtigen mikrobiellen Kohlenstoffes wurde im sandigen Boden mehr streubürtiger Kohlenstoff durch Mikroorganismen inkorporiert als im lehmigen Boden. Ein weiterer Grund für die geringere Verlagerung von streubürtigem Kohlenstoff in die mikrobielle Biomasse des lehmigen Bodens ist wahrscheinlich der bessere physikalische Schutz durch den höheren Tonanteil. Durch die Einarbeitung der Streu stieg in allen Ansätzen der Gehalt an Ergosterol, welcher ein wesentlicher Indikator für die Präsenz saprotropher Pilze ist. Besonders stark ausgeprägt war der Anstieg des Ergosterolgehaltes, sowie des Ergosterol / mikrobielle Biomasse C – Quotienten, wenn Streu in die untere Schicht (15 - 20 cm) eingearbeitet wurde. Diese tiefenspezifischen Unterschiede wurden bisher in noch keinem weiteren Versuch beobachtet und können auf die Entwicklung unterschiedlicher pilzlicher Gemeinschaften zurück zu führen sein. Es ist jedoch wahrscheinlicher, dass pilzliche Nekromasse in den oberen Bodenschichten schneller umgesetzt wird und somit bei der Ergosterolbestimmung nicht mit erfasst wird. Da der Umsatz der pilzlichen Nekromasse im porösen sandigen Boden, aufgrund der höheren Sauerstoffverfügbarkeit und des geringeren physikalischen Schutzes, vermutlich höher ist als im lehmigen Boden, wird diese Annahme durch den im sandigen Boden geringeren Gehalt an mikrobiellen Kohlenstoff unterstützt. Wie erwartet, überstieg die Mineralisation der Streu im sandigen Boden die der im lehmigen Boden. Jedoch anders als erwartet, unterschied sich die Mineralisation in Abhängigkeit der Einarbeitungstiefe, mit einer erhöhten Mineralisation bei Einarbeitung der Streu in 0 - 5 cm Tiefe, einzig im sandigen Boden. Die Berechnung des Ertragskoeffizienten zeigte, dass die Substratsnutzungseffizienz der Mikroorganismen im sandigen Boden signifikant

geringer war als die im lehmigen Boden. Die Zugabe von Streu führte in beiden Böden, verstärkt jedoch im lehmigen Boden, zu einem positiven Priming Effekt, der in beiden Böden stärker ausgeprägt war, als Streu in 0–5 cm Tiefe eingearbeitet wurde. Trotz Abnahme der SOC-bürtigen mikrobiellen Biomasse stieg die Mineralisation des SOC stark an. Es ist anzunehmen, dass extrazelluläre Enzyme wie Cellulase und Lignin modifizierende Enzyme, produziert von saprotrophen Pilzen, zum Abbau von Cellulose und Lignin der Streu, zum Teil sehr effizient SOC abbauen.

Im zweiten Versuch (Kapitel 4) wurde anhand des gleichen Säulenversuches (Versuch 1; Kapitel 3) der Einfluss der Entfernung von CO<sub>2</sub>-hot-spots im Boden zur Bodenoberfläche, in Abhängigkeit der Bodenart, auf vier verschiedene Methoden zur Erfassung der Bodenrespiration betrachtet. Zusätzlich wurde durch eine Plausibilitätsprüfung anhand der Kohlenstoffbilanz, basierend auf der in Versuch 1 durchgeführten Kohlenstoffsequestrierung, die Genauigkeit der vier Methoden in Abhängigkeit der Bodenart überprüft. Für beide Ansätze mit sandigem Boden zeigen IR und PAS eine deutliche Überschätzung der mit NaOH und GC bestimmten Bodenrespiration. Die Überschätzung durch IR ist dabei auf die durch die dynamische Haube verursachten Turbulenzen und deren Auswirkungen auf den porösen sandigen Boden zurück zu führen. Bei geringen Respirationsraten, wie bei der Kontrolle, zeigt die Messung mittels IR trotz Turbulenzen, verursacht durch den Ventilator der Haube, keine Überschätzung. Die Überschätzung durch PAS hingegen kann nicht auf Turbulenzen, verursacht durch die dynamische Haube, zurück geführt werden, da bei den Analysen mit PAS und GC identische Hauben, höher und größer als bei IR, eingesetzt wurden und die Bodenrespiration durch GC nicht überschätzt wurde. Im Gegensatz zu beiden sandigen Ansätzen überschätzt IR die Bodenrespiration im lehmigen Boden nicht. NaOH hingegen unterschätzt die Bodenrespiration, wenn Streu in 15-20 cm Tiefe des lehmigen Bodens eingearbeitet ist. Dieses ist dadurch zu erklären, dass, bedingt durch die geringere Porosität sowie das höhere Wasserhaltevermögen und dem daraus resultierenden

geringeren Luft gefüllten Porenvolumen, die Diffusion von CO<sub>2</sub> im lehmigen Boden langsamer ist als im sandigen Boden. Nach Absorption des CO<sub>2</sub> der Haubenluft diffundiert das CO<sub>2</sub> des CO<sub>2</sub>-hot-spots in 15-20 cm Tiefe, entlang des Diffusionsgradienten, aufgrund des Diffusionswiderstandes in lehmigen Boden langsamer zur Oberfläche als im sandigen Boden oder wenn der CO<sub>2</sub>-hot-spot direkt unter der Bodenoberfläche liegt. Da bei der Messung mit der dynamischen Haube diese nur kurz auf der Fläche verbleibt, beeinflusst der Diffusionsgradient diese Messungen nicht. Hinzukommt, dass bei den Messsystemen, die in Kombination mit der dynamischen Haube eingesetzt werden, im Gegensatz zur Absorption durch Lauge keine CO<sub>2</sub> Abreicherung stattfindet und die Diffusion von CO<sub>2</sub> aus dem Boden über lange Zeit bis zu hohen CO<sub>2</sub> Konzentration in der Haube linear bleibt. Alle drei mit einer dynamischen Haube kombinierten Methoden zeigen mit Korrelationskoeffizienten zwischen 0,90 und 0,93 starke Korrelationen mit NaOH. Während PAS die Bodenrespiration im Verhältnis zu NaOH immer überschätzt, tritt eine Überschätzung durch GC nur bei Mineralisationsraten unter 500 mg m<sup>-2</sup> h<sup>-1</sup> und für IR bei Mineralisationsraten über 40 mg m<sup>-2</sup> h<sup>-1</sup> ein. Die Plausibilitätsprüfung zeigt, dass für sandigen Boden, mit NaOH und GC eine sehr exakte Wiederfindung von Kohlenstoff erreicht wird, wohingegen IR und PAS in der Wiederfindung von Kohlenstoff bei deutlich über 100 % liegen. Für den lehmigen Boden hingegen ist nach Entfernung der CO<sub>2</sub>-hot-spots zur Bodenoberfläche zu differenzieren. Befindet sich der CO<sub>2</sub>-hot-spot direkt unter der Bodenoberfläche ist die Wiederfindung von Kohlenstoff für NaOH, GC und IR sehr exakt. Befindet sich der CO<sub>2</sub>-hot-spot jedoch in 15-20 cm Tiefe, ist die Wiederfindung des Kohlenstoffes durch NaOH deutlich unter 100 %. Die Wiederfindung durch PAS liegt sowohl für den sandigen als auch für den lehmigen Boden immer deutlich über 100 %.

Im dritten Versuch (Kapitel 5), wurde anhand eines Litterbag-Versuches im Norden des Omans, der Einfluss verschiedener Dünger und Feldfrüchte auf den Abbau von Streu auf landwirtschaftlich genutzten Flächen in Abhängigkeit der Streuqualität betrachtet. Bei

dem Großteil bisheriger Streuabbauversuche, unter gemäßigten und subtropischen Klimaten, stand der Abbau von Streu im Wald im Fokus der Betrachtung. Die wenigen Versuche zum Streuabbau auf landwirtschaftlich genutzten Flächen beschränken sich auf die gemäßigten Klimate. Wohingegen der Abbau von Streu, sowie der Einfluss von Dünger und Feldfrucht unter subtropischen Bedingungen, zum ersten mal mit der vorliegenden Arbeit fokussiert wurde. Der Verlust an organischem Material war verglichen mit Versuchen unter gemäßigten Klimaten, bei allen vier Streuarten, generell hoch. Der höhere Abbau von Luzernen- und Maisstreu im Vergleich zu Raps- und Weizenstreu ist auf Unterschiede der Streuqualität zurückzuführen. Neben der Verwertbarkeit durch Mikroorganismen beeinflusst die Streuqualität zusätzlich die „Schmackhaftigkeit“ der Streu für Organismen der Mesofauna. Wodurch ein selektiver Transport und/oder Grazing von Mikroorganismen stattfindet. Der geringere Abbau der Luzernenstreu verglichen mit Maisstreu jedoch ist nicht auf die Streuqualität sondern auf die geringere mikrobielle Besiedelung der Luzernenstreu während der Versuchszeit zurückzuführen. Der Unterschied im Grad der mikrobiellen Besiedelung kann durch die erhobenen Daten nicht erklärt werden. Es ist jedoch davon auszugehen, dass Leguminosen Substanzen wie z.B. Polyphenole enthalten, welche die mikrobielle Biomasse und im Besonderen die pilzliche Biomasse in beachtlichem Umfang inhibieren. Ebenso wenig ist der höhere Abbau von Weizenstreu verglichen mit Rapsstreu durch die Streuqualität zu begründen. Eine mögliche Erklärung für den geringeren Abbau der Rapsstreu kann ihr hoher Aluminium Gehalt sein. Es ist jedoch wahrscheinlicher, dass die Rapsstreu organische Substanzen wie Glucosinolate enthält, welche den mikrobiellen Streuabbau inhibieren. Während der Hemicellulosegehalt am Ende des Versuches nicht durch die Streuqualität beeinflusst war, zeigten Cellulose und Lignin qualitätsabhängige Effekte. Der stärkere Abbau von Cellulose bei Luzernen- und Maisstreu ist auf den anfänglich höheren Stickstoffgehalt zurückzuführen, wodurch die Produktion und Aktivität von Cellulose degradierenden Enzymen, wie Exo-Cellulase, Endo-Cellulase und

Xylanase, anstieg. Es ist davon auszugehen, dass die Differenzen im Celluloseabbau von Luzernen- und Maisstreu im Vergleich zu Raps- und Weizenstreu, neben Unterschieden im anfänglichen Stickstoffgehalt, auf den höheren Schutz von Cellulose durch Lignin in Raps- und Weizenstreu zurückzuführen sind. Während der initial geringe Stickstoffgehalt den Ligninabbau in Raps- und Weizenstreu unterstützt, ist die relative Anreicherung von Lignin in Luzernen- und Maisstreu hingegen auf den initial hohen Stickstoffgehalt zurückzuführen. Dem entgegen hat die Zusammensetzung weiterer Nährstoffe einen sehr geringen Effekt. Es ist jedoch möglich, dass stärkere Effekte durch den Eintrag von Boden in die Litterbags durch Organismen der Mesofauna, Wurzelwachstum oder physikalische Verlagerung überdeckt werden. Während unter organische Düngung, die pilzliche Biomasse ansteigt, fördert der leicht verfügbare Stickstoff der mineralischen Düngung die Bildung bakterieller Biomasse. Der höher Gehalt an pilzlicher Biomasse unter organischer Düngung zeigte keinen generellen Effekt auf den Abbau von Kohlenstoff. Er führte jedoch zu einer Veränderung in der Streuzusammensetzung. Die verringerte Abnahme bzw. verstärkte Zunahme der Nährstoffgehalte bei organischer Düngung ist durch den Eintrag düngerbürtiger Nährstoffe, im Besonderen durch die verstärkte Bildung pilzlicher Hyphen in die Litterbags hinein, zu erklären. Trotz höherer Gehalte an pilzlicher Biomasse war der Ligningehalt am Ende des Versuches unter organischer Düngung höher als unter mineralischer Düngung. Diese ist auf den Eintrag düngerbürtiger Pilze zurückzuführen, welche eine geringere Lignindegradierungseffizienz aufweisen. Der Einfluss der Feldfrucht auf den Streuabbau äußert sich durch höhere Gehalte mikrobieller und im Besonderen pilzlicher Biomasse, und durch geringere Gehalte an N, P, Ca, Na und K in, im Litterbag verbleibender Streu, unter dem Anbau von Mohrrüben. Der Anstieg der pilzlichen Biomasse führt, ebenso wie bei der organischen Düngung zu keinem generellen Anstieg der Kohlenstoffdegradation, zeigt jedoch einen selektiven Effekt auf den Abbau von Cellulose. Der Einfluss, sowohl auf die mikrobielle Biomasse, als auch auf den Nährstoffgehalt, zeigt die

Bedeutung der Unterschiede im Wurzelwachstum, der Rhizodeposition sowie des Nährstoffbedarfs in Abhängigkeit der Feldfrucht. Trotz großer Unterschiede der Streuarten im anfänglichen Gehalt mikrobieller Biomasse war dieser am Ende des Versuches für alle Streuarten identisch. Dieses war Folge eines starken Anstiegs der pilzlichen Biomasse bei Luzernen- und Maisstreu sowie einer Abnahme der pilzlichen Biomasse bei Raps- und Weizenstreu, welche zuvor noch nicht beobachtet wurde. Dieses macht den Einfluss der anfänglichen mikrobiellen Biomasse auf deren Entwicklung während des Streuabbauprozesses im Boden deutlich. Es ist anzunehmen, dass ein Teil der anfänglichen pilzlichen Biomasse der Raps- und Weizenstreu, welche sich unter gemäßigten Klimaten entwickelte, unter subtropischen Bedingungen nicht überlebensfähig war. Generell war der Streuabbau durch Pilze dominiert. Es zeigte sich jedoch, dass Unterschiede im Pflanzenmaterial einen Einfluss auf die bakterielle Biomasse hatten, Unterschiede in Düngung und Feldfrucht hingegen die pilzliche Biomasse und die bakterielle Biomasse beeinflussten.

## **6.1 Kurzzusammenfassung Versuch 1**

Abschließend zusammengefasst zeigte Versuch 1 (Kapitel 3):

1. zum ersten Mal den Transport von streubürtigem Kohlenstoff innerhalb der mikrobiellen Biomasse über eine Distanz von ca. 20 cm. Dabei wird von einem Transport per Pilzhyphen ausgegangen.
2. dass das geringere Porenvolumen des lehmigen Bodens die Ausbreitung von Pilzhyphen behindert. Im Besonderen, wenn Streu in eine Tiefe von 15 – 20 cm eingearbeitet ist.
3. dass der Umsatz der Streu durch saprotrophe Pilze, besonders im sandigen Boden, wenn Streu in 0 - 5 cm eingearbeitet wurde stärker war als wenn sie in 15 – 20 cm eingearbeitet wurde.

## 6.2 Kurzzusammenfassung Versuch 2

Abschließend zusammengefasst zeigte Versuch 2 (Kapitel 4):

1. die Überschätzung der Bodenrespiration von sandigem Boden durch IR. Vermutlich verursacht durch Luftturbulenzen in der dynamischen Haube.
2. die Unterschätzung der Bodenrespiration von lehmigem Boden durch NaOH, wenn sich die CO<sub>2</sub>-hot-spots in 15–20 cm Tiefe befinden.
3. die exakte Bestimmung der Bodenrespiration durch GC für beide Böden
4. die generelle Überschätzung der Bodenrespiration durch PAS für beide Böden
5. dass GC und PAS von der Bodenart, NaOH und IR von Bodenart und Entfernung der CO<sub>2</sub>-hot-spots beeinflusst werden.



### 6.3 Kurzzusammenfassung Versuch 3

Abschließend zusammengefasst zeigte Versuch 3 (Kapitel 5):

1. dass Unterschiede im C/N –Verhältnis den Streuabbau am stärksten deutlich beeinflussen. Wohingegen andere Qualitätsmerkmale, wie der Ligningehalt und Nährstoffgehalte, von geringerer Bedeutung sind. Zusätzlich zu den Qualitätsmerkmalen spielen Faktoren wie die mikrobielle Besiedelung sowie noch unbekannte Komponenten eine bedeutende Rolle für den Streuabbau.
2. dass die organische Düngung zu einem Anstieg der pilzlichen Biomasse, die mineralische Düngung hingegen zu einem Anstieg der bakteriellen Biomasse führt und dass trotz höherer Gehalte pilzlicher Biomasse der generelle Kohlenstoffabbau nicht durch die organische Düngung beeinflusst wird, jedoch ein selektiver Abbau bestimmter Strukturelemente stattfindet.
3. dass der Anbau von Mohrrüben im Vergleich zum Anbau von Blumenkohl zu einem stärkeren Anstieg der mikrobiellen und im Besonderen der pilzlichen Biomasse führt und verschiedene Feldfrüchte den Streuabbau zusätzlich durch Wurzelwachstum, Rizodeposition und Nährstoffbedarf unterschiedlich stark beeinflussen .

## 7 Summary

The present thesis focused on the effects of soil type and incorporation depth on the decomposition of straw and its microbial use. Furthermore, the fate of the carbon (C) derived from the straw was studied during its decomposition by measuring CO<sub>2</sub>, SOC, extractable C, C<sub>mik</sub> and POM-C. As <sup>13</sup>C-enriched straw was used, it was possible to determine the amount of straw-derived CO<sub>2</sub>. These results were used to determine the accuracy of four methods to detect the soil respiration. Additionally, possible artefacts of the methods due to soil type and incorporation depth of straw were considered. In a subsequent field experiment the effects of litter quality, fertilization and crop species on the decomposition of straw and its microbial colonisation were studied under subtropical conditions. The first experiment was carried out in soil columns and focused on the effect of straw incorporated at different depths. In contrast to former studies, where straw-derived carbon was found to be transported within the microbial biomass for only a few millimetres, in the present study straw-derived carbon was shown to be transported within microbial biomass (possibly by fungi) over a decimetre distance. In the sandy soil, higher contents of straw-derived microbial biomass C were observed in layers further away from the straw layer compared to the loamy soil. This indicates that the finer pore space of the loamy soil limited the transfer of straw-derived C, especially if the straw was added to the bottom layer. It is likely that the smaller pore space hindered hyphal growth and led to a better physical protection of the added straw. This assumption was supported by a stronger decrease of straw-derived microbial biomass C with increasing distance from the straw layer in loamy soil than in sandy soil. Furthermore, the higher O<sub>2</sub> diffusion rate in the more porous sandy soil led to better conditions for microbial growth in deeper layers than in the loamy soil. The sum of straw-derived carbon incorporated into the microbial biomass was higher in sandy soil than in loamy soil due to the higher diffusion rate of straw-derived carbon in

sandy soil. Another reason for the lower transfer of straw derived C into the microbial biomass of the loamy soil might be a better physical protection by the higher clay content. However, the content of ergosterol, which was determined as an indicator of saprotrophic fungi, increased in all treatments after the addition of straw. This increase as well as the increase in the ergosterol to microbial biomass C ratio was especially high when straw was added to the deeper layer of 15-20 cm compared to the top layer (0-5 cm). The depth-specific differences in ergosterol and ergosterol to microbial biomass C ratio have not been observed so far neither in field studies, nor in incubation experiments. These observations may be explained by the development of different fungal communities at different depths. However, a more likely explanation is the higher turnover in the top layer of fungal biomass, which reduced the amount of ergosterol accumulated temporarily in dead fungal tissue. Therefore, the turnover of the fungal biomass might be especially high in the coarse textured sandy soil due to a better oxygen supply as well as a lower physical protection by clay particles. This hypothesis is supported by generally lower contents of microbial biomass C in sandy soil compared to loamy soil. The mineralization of added straw was higher in sandy soil than in loamy soil. However, a difference in the mineralization rate between the two soil types could only be observed when straw was added to the top 0-5 cm. In addition, the yield coefficient showed a lower efficiency of the microbial biomass in sandy soil than in loamy soil. The addition of straw caused a positive priming effect in both soils, especially in the loamy soil. This effect was higher when straw was added to the top layer than to the bottom layer. Despite the decrease in SOC-derived microbial biomass, the mineralization of SOC was strongly increased. Extracellular enzymes, such as cellulases and lignin modifying enzymes, produced by saprotrophic fungi to decompose the added straw and freshly formed microbial residues, may also have contributed to the degradation of SOC.

The second experiment used the same experimental setup as the first experiment, but focused on the effect of the distance between CO<sub>2</sub>-hot-spots and the soil surface as affected by soil type. Four soil respiration measurement systems were compared. To determine the accuracy of the soil respiration measurement systems for the different soil types, the results were compared with the carbon balance, which was based on the carbon sequestration detected in experiment 1. In both sandy treatments, soil respiration was overestimated with the IR and PAS methods compared to NaOH and GC methods. The overestimation by IR may be explained by the effect of turbulences for porous sandy soil induced by the fan of the dynamic chamber. Despite the turbulences, IR did not overestimate low respiration rates as observed in the unamended control. However, the overestimation by PAS could not be explained by chamber effects because the same type of chamber was used with the GC method, which did not result in an overestimation. IR did not overestimate the soil respiration of the loamy treatments compared to NaOH. However, NaOH underestimated the soil respiration for loamy soil when straw was added to the bottom layer of 15-20 cm. This could be explained by soil properties. During the 24 h measuring period with the NaOH method, CO<sub>2</sub> is transferred by diffusion to the chamber. In the loamy soil, CO<sub>2</sub> diffusion was lower than in the sandy soil due to a lower porosity, a higher water content at the same matrix potential, which resulted in a lower air-filled pore space. However, a reduced diffusion did not affect the short-time measurements by the dynamic chamber systems. In addition to the short measurement time, GC, IR and PAS did not decrease the CO<sub>2</sub> concentration in the chamber air. In contrast to a decrease in chamber-air-CO<sub>2</sub>, an increase did not seem to affect the CO<sub>2</sub> efflux from the soil, because the efflux remained constant over a long time. The correlation coefficients between the cumulative CO<sub>2</sub> production of the three dynamic chamber methods (GC, IR, and PAS) and the static NaOH method were significant with r-values between 0.90 and 0.93. PAS showed a general overestimation of soil respiration compared to NaOH, whereas GC and IR only overestimated respiration

rates lower than  $500 \text{ mg m}^{-2} \text{ h}^{-1}$  and higher than  $40 \text{ mg m}^{-2} \text{ h}^{-1}$ , respectively. The proof of plausibility for sandy soil showed an exact carbon recovery with NaOH and GC, whereas the carbon recovery with IR and PAS was significantly higher than 100 %. In contrast to the sandy soil the distance of  $\text{CO}_2$ -hot-spots to the soil surface affected the accuracy in the loamy soil. If straw was added to the top layer, NaOH, GC and IR measured the  $\text{CO}_2$  emissions correctly, while the addition of straw to the bottom layer reduced the accuracy of the NaOH method. The PAS method significantly overestimated the  $\text{CO}_2$  emissions in both soils.

The third experiment, a litterbag field experiment, focused on the effect of litter quality, fertilization and crop species on straw decomposition in agricultural systems. The majority of studies which have described litter decomposition under field conditions of humid temperate, boreal and wet tropical conditions focused on the decomposition of straw in forest soils under semi-natural conditions. The few studies which focused on the decomposition of straw in agricultural systems were carried out under temperate climates. Therefore, the decomposition of straw and the effects of fertilization and crop species under subtropical conditions, were considered for the first time in this thesis. Litter mass loss was higher with alfalfa and maize straw than with canola and wheat straw. In general however, litter mass loss was higher for all four kinds of straw compared to decomposition studies carried out under temperate climate. Litter quality may have affected the “palatability” of straw for mesofaunal organisms. This might be caused by selective transport and/or grazing of microorganisms by mesofaunal organisms. However, the lower decomposition rate for alfalfa than for maize could not be explained by litter quality. Legume derived substances, e.g. polyphenols, may have inhibited to some extent fungal, and to a lesser degree bacterial, colonization during decomposition. Furthermore, differences in the decomposition rate between canola and wheat may be explained by high Al concentrations in the canola straw or by inhibitory organic components such as glucosinolates. In contrast to hemi-

cellulose, the content of cellulose and lignin at the end of the experiment was affected by straw quality. The cellulose concentration decreased faster in the high qualitative alfalfa and maize straw than in the low qualitative canola and wheat straw. This could be explained by the higher initial N concentration, which increased the production and activity of exo-cellulases, endo-cellulases and xylanases and consequently cellulose degradation. A further reason for the lower decrease of the cellulose content of rape and wheat straw could be the higher protection of cellulose by lignin. In contrast to canola and wheat straw, where lignin degradation was not inhibited by initially low contents of nitrate, high contents of nitrate may have inhibited lignin degradation from alfalfa and maize, causing a relative enrichment. Differences in nutrient composition between the different plant materials have apparently only minor effects on the decomposition. However, such effects might be masked by the input of soil material into the litterbags by different ways, e.g. transport by mesofaunal organisms, root growth and physical translocation. With organic and mineral fertilization the contents of fungal biomass and bacterial biomass increased. The increase of bacterial biomass was supported by the high amount of easy available nitrate under mineral fertilization. The higher fungal colonization under organic fertilization had no general effects on the C loss but lead to some shifts in composition of straw material retained in the litterbags. The lower decrease or rather the stronger increase of nutrient concentration under organic fertilization could be explained by enhanced addition and by effects on fungal transport processes. Despite higher contents of fungal biomass the lignin degradation was lower under organic than under mineral fertilization. This was caused by the addition of manure-derived microorganisms with a lower lignin degradation efficiency. However, the cultivation with carrots indicated the effects of crop with lower amounts of N, P, Ca, Na and K in the straw, remaining in the litterbag. Further, the cultivation with carrots followed in higher amounts of microbial and especially fungal biomass. The increase in fungal biomass did not affect the general carbon degradation but resulted in a selective increase in

cellulose degradation. The effects of crop species on the microbial colonization and the nutrient contents pointed to the importance of differences in root growth, rhizodeposition and nutrient uptake for the decomposition of plant material and the retention of nutrients in the litterbags. However, despite initially high differences in microbial colonization at the end of the experiment all four kinds of straw had identical contents of microbial biomass. This was due to a strong increase of microbial and especially fungal biomass for alfalfa and maize and a decrease for canola and wheat, which was not observed before. This highlights the importance of initial microbial colonization for the further development of a straw colonizing microbial community. A part of the fungi, which colonized the straw under humid temperate conditions in Germany, had difficulties to survive under the hot conditions of the irrigated vegetable field in Oman. However, the decomposition was generally dominated by fungi. Furthermore, the kind of straw material only affected the bacterial biomass whereas crop and fertilization affected both, the fungal and bacterial biomass.

## **7.1 Outlines experiment 1**

Summarized, experiment 1 showed:

1. for the first time the transport of straw derived carbon within the microbial biomass, probably by fungi was possible, over a decimetre distance.
2. that the fine pore space of loamy soil hindered hyphal growth, especially if straw was added to the bottom layer of 15-20 cm.
3. that the turnover of incubated straw by saprotrophic fungi was higher if straw was added in 0-5 cm than in 15-20 cm, especially in sandy soil.



## 7.2 Outlines experiment 2

Summarized, experiment 2 showed:

1. an overestimation of soil respiration by IR for sandy soil, probably induced by turbulences of the chamber fan.
2. an underestimation of soil respiration by NaOH for loamy soil, if CO<sub>2</sub>-hot-spots were in 15-20 cm depth.
3. exact detection of soil respiration by GC for both soils.
4. a general overestimation of soil respiration by PAS for both soils.
5. that GC and PAS were affected only by soil type, whereas NaOH and IR were affected by soil type as well as by the distance of CO<sub>2</sub>-hot-spots to the soil surface.

### 7.3 Outlines experiment 3

Summarized, experiment 3 showed:

1. that the C/N ratio, i.e. the protein concentration of the plant material has the strongest effects on the C loss in litterbags. Concentrations of lignin and other nutrient elements have less importance. Other factors like microbial colonization, but also unknown components may play an important role for the straw decomposition.
2. that the organic fertilization led to stronger microbial, especially fungal colonization of the litter retained in the litterbags in comparison with mineral fertilization. Further, the higher amounts of fungal biomass under organic fertilization did not cause a generally higher carbon degradation but a shift of straw composition.
3. that the carrot cultivation led to stronger microbial colonization, especially fungal colonization of the litter retained in the litterbags in comparison with cauliflower cultivation and further, the importance of the effect of root growth, rhizodeposition and nutrient demand by different crops species.

## 8 Ausblick

Die Ergebnisse der vorliegenden Arbeit zeigen unter anderem, dass die Einarbeitungstiefe von Streu in Abhängigkeit der Bodenart sowohl auf den Streuabbau und die mikrobielle Nutzung als auch die Bestimmung der Bodenrespiration mit verschiedenen Methoden einen signifikanten Einfluss hat.

Die in Versuch 1 beobachteten tiefenspezifischen Unterschiede im Verhältnis der pilzlichen Biomasse zur bakteriellen Biomasse wurden in der vorliegenden Arbeit zum ersten Mal beobachtet. Mögliche Gründe, wie die unterschiedliche Entwicklung der mikrobiellen Gemeinschaften in Abhängigkeit der Tiefe, sowie der verstärkte Umsatz pilzlicher Nekromasse bei erhöhter Sauerstoffverfügbarkeit und geringerem physikalischen Schutz durch Ton, sollten durch weiterführende Studien und anhand geeigneter Methoden zielgerichtet untersucht werden. Um die Zusammensetzung der mikrobiellen Gemeinschaft, über die Einteilung in bakterielle Biomasse und pilzliche Biomasse hinaus, differenzierter betrachten zu können, bestünde die Möglichkeit, die mikrobielle Zusammensetzung mittels PLFA oder DNA Analysen zu bestimmen. Des Weiteren sollte der Einfluss der Bodenart auf den Sauerstofftransport in tiefer liegende Schichten mittels Bodensonden, und der Einfluss sowohl der Sauerstoffverfügbarkeit als auch des physischen Schutzes durch Ton auf die Umsetzung pilzlicher Nekromasse betrachtet werden.

Es ist wichtig, dass die im zweiten Versuch ermittelten Ergebnisse zur Überschätzung und Unterschätzung durch die einzelnen Methoden, sowie deren Abhängigkeit zur Bodenart in weiteren Säulenversuchen verifiziert werden. Für die objektive Betrachtung der Genauigkeit der Methoden zur Bestimmung der Bodenrespiration, ist dabei das Wissen über die erwartete Bodenrespiration grundlegend. Zusätzlich wären Freilandversuche zu begrüßen, welche auf identischen Flächen die Einarbeitung von Streu in unterschiedliche Tiefen und die daraus resultierende Bodenrespiration betrachten. Um dabei die Genauigkeit der Methoden sowie eventuelle Methodenartefakte bewerten zu können, ist der Einsatz stabiler

Isotope, wie z.B.  $^{13}\text{C}$ , unabdingbar. Außerhalb der Analytik stabiler Isotope muss die starke Überschätzung der Bodenrespiration durch die photoakustische Spektroskopie, welche allgemein als sehr genaues Detektionsverfahren gilt, untersucht werden. Dabei sollten verschiedene Hauben und verschiedene PAS-Systeme verwendet werden, um Hauben und System bedingte Fehler beschreiben zu können.

Wie im dritten Versuch deutlich wird, spielen neben der Streuqualität pflanzenbürtige Substanzen wie Polyphenole und Glucosinolate aufgrund ihrer inhibierenden Wirkung auf die mikrobielle Biomasse eine bedeutende Rolle im Streuabbau. Um die Unterschiede im Streuabbau verstehen zu können, ist es wichtig die Funktion sowie das Vorkommen dieser Substanzen zu untersuchen sowie weitere noch unbekannte, die mikrobielle Besiedelung beeinflussende, pflanzenbürtige Substanzen zu ermitteln. Die starke Abnahme anfänglich hoher Gehalte an pilzlicher Biomasse, bei Raps- und Weizenstreu, sind höchst wahrscheinlich darauf zurück zu führen, dass ein Großteil, der sich unter gemäßigten Klimaten entwickelten mikrobiellen Biomasse, unter subtropischen Klimaten nicht überlebensfähig ist. Um diese Annahme zu überprüfen, sollten weitere Versuche zum Streuabbau unter subtropischen Klimaten ausgebracht werden. Dabei ist es wichtig, Pflanzenmaterial aus gemäßigten Klimaten und subtropischen Klimaten zu verwenden. Des Weiteren wäre es von Vorteil, das Wurzelwachstum, die Rhizodiposition und den Nährstoffbedarf gängiger Feldfrüchte zu bestimmen. Ziel wäre es dabei, den Einfluss dieser Faktoren auf die mikrobielle Biomasse und somit auf den Streuabbau und die Kohlenstoff- und Nährstoffverlagerung abschätzen zu können.

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