Fachgebiet Bodenbiologie und Pflanzenernährung

Fachbereich Ökologische Agrarwissenschaften

Universität Kassel

Der Einfluss organischer Düngung auf die mikrobielle Biomasse und deren Residuen in unterschiedlichen Nutzungssystemen

Dissertation

zur Erlangung des akademischen Grades eines Doktors der Agrarwissenschaften
(Dr. agr.)

am Fachbereich Ökologische Agrarwissenschaften der Universität Kassel

vorgelegt von
André Erik Sradnick

Erstgutachter: Prof. Dr. R.G. Jörgensen

Zweitgutachter: Prof. Dr. B. Ludwig

Witzenhausen, Mai 2013

Die vorliegende Arbeit wurde vom Fachbereich Ökologische Agrarwissenschaften der Universität Kassel als Dissertation zur Erlangung des akademischen Grades eines Doktors der Agrarwissenschaften (Dr. agr.) angenommen.

Erstgutachter: Prof. Dr. R.G. Jörgensen
Zweitgutachter: Prof. Dr. B. Ludwig

Tag der mündlichen Prüfung: 13. 09. 2013

Eidesstattliche Erklärung

„Hiermit versichere ich, dass ich die vorliegende Dissertation selbstständig, ohne unerlaubte Hilfe Dritter angefertigt und andere als die in der Dissertation angegebenen Hilfsmittel nicht benutzt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten oder unveröffentlichten Schriften entnommen sind, habe ich als solche kenntlich gemacht. Dritte waren an der inhaltlich-materiellen Erstellung der Dissertation nicht beteiligt; insbesondere habe ich hierfür nicht die Hilfe eines Promotionsberaters in Anspruch genommen. Kein Teil dieser Arbeit ist in einem anderen Promotions- oder Habilitationsverfahren verwendet worden."

_____

(André Sradnick)

Vorwort

Die vorliegende Dissertation wurde im Rahmen des DFG-Graduiertenkollegs 1397 an der Universität Kassel im Fachbereich Ökologische Agrarwissenschaften im Fachgebiet Bodenbiologie und Pflanzenernährung angefertigt, um die Anforderungen des akademischen Grades des Doktors der Agrarwissenschaften (Dr. agr.) zu erfüllen. Die Arbeit besteht aus drei wissenschaftlichen Publikationen, von denen zwei bereits bei einer international, begutachteten Fachzeitschrift veröffentlicht wurden. Der andere Artikel wurde bereits eingereicht und befindet sich in der Begutachtung.

Die Artikel sind in Kapitel 3, 4 und 5 eingearbeitet. Kapitel 1 ist eine generelle Einleitung zum Thema, während Kapitel 2 die Ziele dieser Arbeit beschreibt. In Kapitel 6 und 7 werden die Ergebnisse aus Kapitel 3, 4 und 5 auf Deutsch sowie auf Englisch zusammenfassend dargestellt. Kapitel 8 beinhaltet eine Schlussfolgerung und einen Ausblick für weitere Untersuchungen, die sich innerhalb dieser Arbeit ergaben. Kapitel 9 beinhaltet die Quellen die für Kapitel 1, 2 und 8 benötigt wurden.

Folgende Publikationen sind in die Arbeit eingebettet:

Kapitel 3

    Sradnick, A., Murugan, R., Oltmanns, M., Raupp, J., Joergensen, R. G., 2013. Changes in functional diversity of the soil microbial community in a heterogeneous sandy soil after long-term fertilization with cattle manure and mineral fertilizer. Appl. Soil Ecol. 63, 23-28.

Kapitel 4

    Sradnick, A., Oltmanns, M., Raupp, J., Joergensen, R. G., 2013. Microbial residue indices down the soil profile after long-term addition of farmyard manure to a sandy soil. Geoderma. submitted

Kapitel 5

    Sradnick, A., Ingold M., Marold, J., Murugan, R., Buerkert A., Joergensen, R.G., 2013. Impact of activated charcoal and tannin amendments on microbial biomass and residues in an irrigated sandy soil under arid subtropical conditions. Biol. Fertil. Soils. DOI 10.1007/s00374-013-0837-z

Inhaltsverzeichnis

Abbildungsverzeichnis

Tabellenverzeichnis

Abkürzungsverzeichnis

^{13}C	Kohlenstoffisotop mit der Masse 13
^{15}N	Stickstoffisotop mit der Masse 15
ANCOVA	Kovarianzanalyse
ANOVA	Varianzanalyse
C	Kohlenstoff
$CaCO_3$	Calciumcarbonat
CH_4	Methan
CLPP	Community Level Physiological Profile (Substratnutzungsprofil)
C_{mik} (C_{mic})	Mikrobieller Biomasse Kohlenstoff
CO_2	Kohlendioxid
CV	Variationskoeffizient
DFA	Diskriminanzanalyse
DFG	Deutsche Forschungsgemeinschaft
EPS	Extrazelluläre polymere Substanzen
GalN	Galactosamin
GlcN	Glucosamin
H_2O	Wasser
HCl	Salzsäure
HPLC	Hochleistungsflüssigkeitschromatographie
K	Kalium
K_2SO_4	Kaliumsulfat
KCl	Kaliumchlorid
k_{EC}, k_{EN}	Extrahierbarer Teil des Gesamtkohlenstoffs und –stickstoffs gebunden in der mikrobiellen Biomasse

LSD	Kleinster signifikanter Unterschied
ManN	Mannosamin
MANOVA	Multivariate Varianzanalyse
Mg	Magnesium
mg	Milligramm
MIN	Mineralische Düngung
MurN	Muraminsäure
N (N_{tot})	Stickstoff (Stickstoff-Gesamtgehalt)
N_2O	Lachgas
ns	Nicht signifikant
$NaHCO_3$	Natriumhydrogencarbonat
NaOH	Natronlauge
N_{mik} (N_{mic})	Mikrobieller Biomasse Stickstoff
OC	Organischer Kohlenstoff
OPA	Ortho-Phthaldialdehyd
P	Phosphor
pH	Negativer dekadischer Logarithmus der Wasserstoffionenaktivität (*potentia Hydrogenii)*
PLFA	Phosphorlipidfettsäuren
r	Korrelationskoeffizient
CM	Rottemist-Düngung
CMBD	Rottemist-Düngung plus Zugabe biologisch-dynamischer Präparate
S	Schwefel
SIR	Substrat-Induzierte-Respiration
SOC/C_{org}	Organischer Kohlenstoff des Bodens

1. Einleitung

Der landwirtschaftlich genutzte Boden stellt ein Ökosystem dar, welches intensiver Bewirtschaftung ausgesetzt ist. Diese Nutzung ist meist mit einem Verlust von organischem Bodenmaterial durch Auswaschung, Entnahme von Feldfrüchten oder Ernterückständen verbunden (Jarecki und Lal, 2003, Tivy, 1987; Fließbach et al., 2007).

Vor allem die ökologische Landwirtschaft setzt sich zum Ziel, dieser Entwicklung entgegenzuwirken und durch ein angepasstes Bodenmanagement den Humusgehalt des Bodens zu erhalten (Fließbach et al., 2007). Als weiteres Ziel soll die Bodenqualität, definiert als die Kapazität des Bodens, pflanzenverfügbare Nährstoffe nachhaltig zur Verfügung zu stellen und landwirtschaftliche Erträge zu sichern, erhalten oder verbessert werden (IFOAM 1998). Die Bodenqualität ist hauptsächlich vom Ausgangsmaterial des Bodens abhängig, wird aber durch die Fruchtfolge, Bodenbearbeitung oder Düngung beeinflusst (Paustian et al., 2000, Carter et al., 1997). In Langzeitversuchen wurde bereits gezeigt, dass durch organische Düngung der Boden-pH Wert, die Wasserhaltkapazität oder die Kohlenstofffraktionen durch organische Düngung positiv beeinflusst werden (Fließbach et al., 2007; Johnston, 1986; Heinze et al., 2010; Heitkamp et al., 2009). Neben der positiven Beeinflussung der physikalischen und chemischen Bodeneigenschaften verändert sich auch die Zusammensetzung der organischen Bodensubstanz und ihre Verfügbarkeit für Mikroorganismen, aber auch die Mineralisation des organisch gebunden Kohlenstoffs im Vergleich zur mineralischen Düngung (Paustian et al., 2000).

Um Aussagen zur Bodenqualität und zum Nährstoffkreislauf zu treffen, wurden im Rahmen des Graduiertenkolleges 1397 der Einfluss von organischer und biodynamischer Düngung im Vergleich zur mineralischen Düngung auf die Speicherung von C, N, P und S in die mikrobielle Biomasse untersucht (Heinze et al., 2010). Das vorliegende Dissertationsprojekt ist innerhalb des interdisziplinären Projekts dieses Graduiertenkollegs angesiedelt, welches sich seit 2007 intensiv mit dem Humus- und Nährstoffkreislauf in der ökologischen Landwirtschaft befasst. Ergänzend dazu werden in der vorliegenden Arbeit Aspekte der funktionellen Diversität der mikrobiellen Gemeinschaft im Oberboden untersucht. Es werden ebenfalls die mikrobiellen Residuen im Unterboden, sowie die mikrobielle Biomasse und ihre Residuen unter subtropischen Gegebenheiten analysiert, um die Effekte organischer und mineralischer Düngung in den globalen Kohlenstoffkreislauf des Bodens einzuordnen und bewerten zu können.

1.1 Die mikrobielle Biomasse im landwirtschaftlich genutzten Oberboden

Mikroorganismen im Boden steuern den Umsatz der organischen Bodensubstanz und sind der Schlüsselfaktor für Bodenfruchtbarkeit und Nährstoffkreislauf. David Jenkinson beschrieb 1977 die mikrobielle Biomasse des Bodens als: „ the eye of the needle through which all the organic material must pass". So dient sie als Quelle von pflanzenverfügbaren Nährstoffen und schützt diese auch vor der Auswaschung (Brookes, 2001).

Es ist bekannt, dass Bodenorganismen auf die Quantität und Qualität des Düngemittels reagieren. So wird in organisch gedüngten Systemen die mikrobielle Biomasse im Vergleich zur konventionellen mineralischen Düngung meist signifikant beeinflusst (Six et al., 2006). In der Arbeit von Heinze et al. (2010) wurde beispielsweise gezeigt, dass der Kohlenstoff, welcher in der mikrobiellen Biomasse gespeichert ist, sich nach langzeitlicher organischer im Vergleich zur mineralischen Düngung erhöhte. Aber auch der mikrobiell gebundene Stickstoff-und Phosphor sowie die mikrobielle Aktivität wurden erhöht (Heinze et al., 2010). Zugleich konnte der organische Kohlenstoff im Boden im Vergleich zur mineralischen Düngung gesteigert werden. Weitere Untersuchungen zeigten, dass organische Düngung in Vergleich zur konventionellen, mineralischen Düngung Bodenbakterien fördert (Fließbach and Mäder, 2000). Auch eine Veränderung der pilzlichen Gemeinschaft durch eine Förderung arbuskulärer Mykorrhiza (Ngosong et al., 2010; Oehl et al., 2004) wurde beobachtet. Hingegen wirkte sich eine organische Düngung negativ auf die saprotrophen Pilze des Bodens gegenüber langfristiger mineralischer Düngung aus. Aber auch chemische Bodeneigenschaften, wie der pH-Wert, haben einen Einfluss auf den Gehalt der mikrobiellen Biomasse im Boden und deren Zusammensetzung (Heinze et al., 2010; Lauber et al., 2009; Rousk et al., 2010). Eine positive Korrelation des pH-Wertes mit mikrobiellen Parametern wurden von Heinze et al. (2010) beschrieben.

Die regulierenden Prozesse, welche auf die Aktivität, den Anteil der mikrobiellen Biomasse und ihre Zusammensetzung wirken, stehen heute im Interesse der Forschung (Zeller et al., 2001; Romaniuk et al., 2011). Ebenfalls rücken heute immer mehr die Effekte der organischen und mineralischen Düngung auf die mikrobiellen Residuen, wegen ihrer langen Verweildauer im Boden (Amelung, 2001) und ihrem hohen Anteil an der organischen Bodensubstanz, in den Fokus der Forschung (Ding et al., 2013; Joergensen et al., 2010).

1.2 Der Unterboden bei landwirtschaftlicher Nutzung

Der Unterboden ist eine der größten globalen Speicher an organischem Kohlenstoff im Boden. So zeigten Guo et al. (2006), dass im Unterboden (20-200cm Bodentiefe) mehr als doppelt so viel organischer Kohlenstoff gespeichert ist als im Oberboden. Nicht nur Wurzeln von Bäumen oder Sträuchern durchziehen den Unterboden, auch viele Gräser erreichen eine Bodentiefe von mehr als einem Meter (Craine et al., 2003). Die Annahme, dass der Anteil an stabilem Kohlenstoff in der Tiefe ansteigt, erklärten Fontaine et al. (2007) mit dem Fehlen mikrobiell verfügbaren Kohlenstoffs in den tieferen Bodenlagen. Zudem wurde eine Erhöhung der Umsatzzeit mit steigender Tiefe (Flessa et al., 2008) beobachtet. Auch sinkt die mikrobielle Biomasse durch die Abnahme an mikrobiell nutzbarem C und N mit zunehmender Tiefe (Taylor et al., 2002). In der vorliegenden Arbeit wurde der Unterboden als Bereich definiert, der nicht direkt durch landwirtschaftliche Bodenbearbeitung beeinflusst wird.

Bisher wurden die Auswirkungen von organischer Düngung auf den Unterboden kaum untersucht (Kautz et al., 2012). Will man aber den gesamten Nährstoffkreislauf des landwirtschaftlich genutzten Bodens verstehen, so ist eine genaue Erforschung des Unterbodens unabdingbar. Es wird ebenfalls vermutet, dass organische Düngung potentiell eine Erhöhung der organischen Substanz im Unterboden bewirkt (Kautz et al., 2012). Auch könnte sie sich auf den Bodenwasserhaushalt auswirken, was wiederum den Zugang zu Nährstoffen im Unterboden beeinflusst (Kautz et al., 2012). Eine Schlüsselrolle kommt hier der mikrobiellen Biomasse zu, welche die Abbauprozesse im Unterboden steuert. Es wurden Untersuchungen zur Zusammensetzung der mikrobiellen Biomasse im Unterboden durchgeführt, in denen gezeigt wurde, dass sich vor allem die Zusammensetzung der mikrobiellen Gemeinschaft mit zunehmender Bodentiefe zu verändern scheint (Ekelund et al., 2001; Fierer et al., 2003; Agnelli et al., 2004). Von größerem Interesse ist jedoch der Anteil an mikrobiellen Residuen in der Tiefe. Sie könnten sich im Unterboden anreichern und so einen signifikanten Beitrag zur langfristig gespeicherten organischen Substanz liefern (Rumpel und Kögel-Knabner, 2010; Liang und Balser, 2008). Vor allem die Bestimmung der Zusammensetzung der mikrobiellen Residualmasse in den tieferen Bodenschichten und deren Anteil am gesamtorganischen Kohlenstoff kann einen wichtigen Beitrag zum Verständnis der Rolle von Bakterien und Pilzen im globalen Kohlenstoffkreislauf leisten.

1.3 Organische Düngung in der subtropischen Landwirtschaft

Im Hinblick auf die globale Klimaveränderung gewinnt die Forschung zur organischen Düngung unter ariden subtropischen Bedingungen immer mehr an Bedeutung (Willer und Kilcher (Eds), 2010). Primär unter subtropischen Bedingungen ist die praktizierte organische Landwirtschaft durch einen starken Verlust von organischem Kohlenstoff und Stickstoff charakterisiert (Siegfried et al., 2011). Gründe hierfür können als erstes bei den Parametern Temperatur und Feuchtigkeit, welche eine erhöhte mikrobielle Aktivität bedingen, gesucht werden (Aerts, 1997). Um zum Beispiel die erhöhten Verluste an C und N auszugleichen, werden teilweise in Oasen des Omans bis zu 30 t ha^{-1} an organischem Dünger appliziert. Dies gewährleistet eine adäquate Versorgung mit pflanzenverfügbaren Nährstoffen (Buerkert et al., 2010). Zudem setzt man sich heute zum Ziel, die Nährstoffverluste mittels Zugaben von Aktivkohle oder Tanninen zu verringern (McHenry, 2010; Lehmann et al., 2011; Kraus et al., 2003). Vor diesem Hintergrund ist das Wissen über die mikrobielle Biomasse unter ariden subtropischen Bedingungen noch limitiert. Es konnte aber gezeigt werden, dass der Gehalt der mikrobiellen Biomasse bei gleichen Bodeneigenschaften in subtropischen Gefilden mit denen unter gemäßigten Klimaten vergleichbar ist (Wardle, 1998; Wichern et al., 2004). Aber welchen Einfluss klimatische Bedingungen, wie eine lange heiße Trockenperiode und starke Temperaturschwankungen auf die Zusammensetzung und Dynamik der mikrobiellen Gemeinschaft haben, ist nicht bekannt (Strickland und Rousk, 2010). Ebenfalls ist der Einfluss von organischer und mineralischer Düngung auf die Akkumulation und den Abbau der mikrobiellen Residuen unter diesen Bedingungen noch nicht untersucht.

1.4 Bestimmung von Düngeeffekten auf die mikrobielle Biomasse im Boden

Der Einfluss von organischer Düngung auf die mikrobielle Biomasse des Bodens kann mit vielfältigen Methoden bestimmt werden. Wie in den vorangegangenen Kapiteln beschrieben, wurden bereits weitreichende Untersuchungen der mikrobielle Biomasse und ihrer Zusammensetzung nach organischer Düngung durchgeführt. Im Folgenden werden ergänzende Methoden vorgestellt, die es ermöglichen, die Auswirkungen von organischer Düngung auf die Substratnutzung und auf die Zusammensetzung der mikrobiellen Residuen zu beschreiben.

1.4.1 Physiologisches Profil der mikrobiellen Gemeinschaft

Die Bestimmung der mikrobiellen Diversität mittels molekulargenetischer oder biochemischer Methoden ist in der Bodenbiologie weit verbreitet. Dabei werden die dominierenden Spezies der mikrobiellen Gemeinschaft erfasst (Loisel et al., 2006), was jedoch wenig über deren Funktionen im Boden aussagt (Leckie, 2005). Deshalb könnte eine Betrachtung der heterotrophen Funktionen der mikrobiellen Bodengemeinschaft Aussagen über ihre Relevanz im Bodenökosystem liefern (Zak et al., 1994). In den letzten zwei Jahrzehnten wurden mehrere Methoden entwickelt, die geeignet sind, das physiologische Profil der mikrobiellen Gemeinschaft im Boden auf Grundlage der Substratnutzung zu untersuchen (Garland and Mills, 1991; Campbell et al., 2003, Degens und Harris, 1997). Alle Methoden beruhen auf der Erfassung des Katabolismus individueller Substrate durch die mikrobielle Gemeinschaft. Dabei werden meist leicht verfügbare organische Kohlenstoffverbindungen zur mikrobiellen Biomasse hinzugefügt und ihr Umsatz gemessen.

Zusammen mit der Bestimmung eines physiologischen Substratnutzungsprofils, welches stark vom Bodentyp abhängig ist (Girvan et al., 2003), stellt die Erfassung der funktionellen Diversität einen wichtigen Punkt, bezüglich der Erfassung des Mineralisationsvermögen der mikrobiellen Gemeinschaft dar (Zak et al., 1994; Romaniuk et al., 2011). Wie Degens et al., (2001) zeigten, scheint die sinkende funktionelle Diversität des Bodens ein Hinweis auf eine Verschlechterung der „Bodengesundheit" zu sein.

Der Einfluss organischer Düngung auf die funktionelle Diversität, wurde auf dem Versuchsfeld in Darmstadt bereits untersucht (Raupp et al., 2004). Zwar konnte in der höchsten Düngestufe eine Erhöhung der funktionellen Diversität beobachtet werden. Doch mit der angewendeten Biolog-Methode (Garland and Mills, 1991) wurden keine Substrate identifiziert, welche dies erklären könnten. Die Biolog Methode misst die Substratnutzungsfähigkeit eines Bodenextraktes, was es schwierig macht, die Funktion der Mikroorganismen des ganzen Bodens zu erfassen (Konopka et al., 1998). Eine Alternative hierzu ist die von Degens und Harris im Jahre 1997 beschriebene Methode der substratinduzierten Respiration. Hier wird nach der direkten Zugabe verschiedener organischer Verbindungen und einer Inkubationszeit von bis zu sechs Stunden die CO_2 Respiration des Bodens gemessen (Anderson und Domsch, 1978; West and Sparling, 1986). In der vorliegenden Dissertation wird eine Modifikation dieser Technik, nach Campbell et al., (2003), angewendet. Sie beruht auf der Grundlage eines Mikrotiterplatten

basierenden Systems. Bei dieser Microresp[TM] Methode wird die substratinduzierte Respiration mittels colorimetrischem Indikatorgels gemessen. Neben dem Vorteil, dass hier die Substratnutzung im gesamten Boden gemessen wird, handelt es sich hierbei um eine schnelle und genaue Methode, die wenig Bodenmaterial benötigt (Lalor et al., 2007; Campbell et al., 2003). Ebenfalls wurde bisher gezeigt, dass die Microresp[TM] Methode sensitiv auf Veränderungen im Bodenökosystem in Abhängigkeit ihrer Nutzung reagiert (Waklin et al., 2008; Chapman et al., 2007; Lalor et al., 2007).

1.4.2   Mikrobielle Residuen im Boden

In der Bodenwissenschaft wird die abgestorbene mikrobielle organische Substanz als mikrobielle Residualmasse definiert. Die mikrobielle Residualmasse hat einen großen Anteil am organischen Bodenkohlenstoff (Joergensen und Wichern, 2008; Liang et al., 2011). Es ist davon auszugehen, dass ca. 50% der organischen Bodensubstanz den mikrobiellen Residuen zuzuordnen sind (Joergensen und Wichern, 2008).

Zur Bestimmung der mikrobiellen Residualmasse wird in der Bodenbiologie die Aminozuckeranalyse verwendet (Amelung, 2001, Joergensen und Wichern, 2008). Aminozucker sind Bestandteile von bakteriellen und pilzlichen Zellwänden. Sie kommen aber auch in Vertebraten, als Bestandteil des Chitins vor. Eine besondere Bedeutung haben Aminozucker im Boden, da sie zwischen fünf und zwölf Prozent des Bodenstickstoffs (Stevensen, 1982) und ca. drei Prozent des Bodenkohlenstoffs (Joergensen und Meyer, 1990) binden. Zellwandbestandteile wie Ergosterol, Phospholipidfettsäuren (PLFA) oder Bestandteile des Zellinneren wie DNA oder RNA sterben nach dem Zelltod relativ schnell ab. Dagegen werden Aminozucker langfristig im Boden akkumuliert (Amelung, 2001; Guggenberger et al., 1999; Glaser et al., 2004). Im Boden können die Aminozucker: Muraminsäure, Glucosamin, Galaktosamin und Mannosamin nachgewiesen werden. Der Ursprung und die Funktion von Galaktosamin und Mannosamin sind noch unbekannt. Zwar kommen sie bis zu 4% bzw. 15% in Bakterien und Pilzen vor (Engelking et al., 2007) und nehmen einen Anteil von 30 bis 50 Prozent der gesamten Aminozuckermasse im Boden ein. Doch bis auf die Vermutung, dass diese ein Bestandteil von Schleimstoffen im Boden sind (Turck et al., 1993; Xu et al., 2004), ist über sie noch wenig bekannt. Das Mannosamin ist nur in geringen Mengen im Boden vorhanden und wurde bereits in Pilzen und Bakterien nachgewiesen (Wasylnka et al., 2011; Ferrero und Aparicio, 2010). Es konnte aber dort kein direkter Ursprung nachgewiesen werden. Das Glucosamin ist

dagegen ein Bestandteil von Zellwänden der Bodenpilze. Darüber hinaus aber auch ein Baustein bakterieller Zellwände. Setzt man voraus, dass Bakterien ein Muramin- zu Glucosamin Verhältnis von 1:2 haben, so ist es möglich, den Anteil des Glucosamins aus den Zellwänden der Pilze zu berechnen (Engelking et al., 2007). Dies geschieht durch Multiplikation des Muramins mit 45 und des pilzlichen Glucoamins mit neun (Appuhn und Joergensen, 2006; Engelking et al., 2007). Dadurch ist es möglich, den Anteil der mikrobiellen Residualmasse des Bodens zu ermitteln. Auch wurde bereits gezeigt, dass Aminozucker Indikatoren genutzt werden können, um Effekte der landwirtschaftlichen Bewirtschaftung wie Bodenbearbeitung oder Düngung aufzuzeigen (Guggenberger et al., 1999, Joergensen et al., 2010). Die Aminozucker können aus einem Bodenextrakt mittels Hochleistungsflüssigkeitschromatographie gemessen werden (Appuhn et al., 2004; Indorf et al., 2011).

2. Ziele der Arbeit

Die Wirkung organischer Düngung auf die mikrobielle Biomasse im Oberboden wurde bereits in den letzten Jahren umfangreich erforscht (Heinze et al., 2010/2011; Heitkamp et al., 2009; Ngosong et al., 2010). Im Rahmen des DFG-Graduiertenkollegs 1397 "Steuerung des Humus- und Nährstoffhaushalts in der Ökologischen Landwirtschaft" soll in der vorliegenden Arbeit die Wirkung von organischer Düngung auf das physiologische Profil, den Unterboden und unter subtropischen Bedingungen untersucht werden.

Um die Auswirkungen langfristiger organischer und mineralischer Düngung auf die Substratnutzungseffizienz im Oberboden zu beurteilen, soll in der vorliegenden Arbeit mittels Erstellung eines physiologischen Substratnutzungsprofils die mikrobielle Gemeinschaft in Bezug auf funktionelle Unterschiede zwischen organischer und mineralischer Düngung untersucht werden. Weiterhin wird geprüft, inwieweit diese methodische Herangehensweise sensitiv genug ist, um Effekte unterschiedlicher Düngeintensitäten und biodynamischer Präparate aufzuzeigen. Im Fokus stehen hier auch die pH-Heterogenität der Versuchsfläche und die Rolle des organischen Kohlenstoffs auf die Substratnutzung.

Ebenfalls ist über den Anteil und die Zusammensetzung der mikrobiellen Residuen im Oberboden zwischen 0 und 25 cm, nach langfristiger organischer und mineralischer Düngung, nur wenig bekannt. Ein zweiter Aspekt, welcher in Darmstadt bisher nur sehr wenig untersucht wurde, ist der Einfluss einer langfristigen Gabe organischen Düngung im Vergleich zur mineralischen Düngung auf den Unterboden bis zu einem Meter Tiefe. In der vorliegenden Arbeit rücken neben dem organischen Kohlenstoff und Stickstoff im Bodenprofil, vor allem die mikrobiellen Residuen in den Vordergrund, da sie als Indikator für langfristige Veränderungen der mikrobiellen Biomasse stehen (Joergensen und Wichern, 2008), und sich potentiell im Unterboden anreichern können (Liang und Balser, 2008). Es soll gezeigt werden, wie sich die Zusammensetzung der mikrobiellen Residuen mit steigender Tiefe verhält, und ob es einen Einfluss organischer Düngung auf den Anteil der mikrobieller Residuen vor dem Hintergrund der pH-Heterogenität auf der Versuchsfläche gibt.

Im letzten Teil der vorliegenden Dissertation soll die Gabe von Ziegenmist auf mikrobielle Parameter nach zweijähriger Düngung untersucht werden. Das Versuchsfeld befindet sich in Sohar (Oman) unter trockenen subtropischen Bedingungen mit einem hohen Verlust von C und N (Siegfried et al., 2011). Unter diesen Bedingungen ist die Wirkung von

unterschiedlicher Düngung auf die mikrobielle Biomasse und ihrer Residuen bisher wenig erforscht. Tannine und Aktivkohle sind potentiell in der Lage, den Verlust organischer Substanz zu vermindern. Speziell wird hier untersucht, wie sich die Zugabe von Tanninen und Aktivkohle als Futterzusätze und Feldapplikationen, zusammen mit Ziegenmist im Vergleich zur mineralischen Düngung verhält. Im Fokus dieses Teilprojektes steht die Untersuchung von Kurzzeiteffekten der organischen Düngung auf die Akkumulation und Zusammensetzung mikrobieller Biomasse und ihrer Residuen.

Das Ziel der vorliegenden Dissertation ist es, den Einfluss organischer Düngung auf die Funktion und die Zusammensetzung der mikrobiellen Biomasse und ihrer Residuen in ihrer Rolle im Nährstoffkreislauf zu erforschen und zu bewerten.

3. Changes in functional diversity of the soil microbial community in a heterogeneous sandy soil after long-term fertilization with cattle manure and mineral fertilizer

André Sradnick [1], Rajasekaran Murugan [1], Meike Oltmanns [2], Joachim Raupp [3], Rainer Georg Joergensen [1]

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

[2]   *Research Association for Biodynamic Agriculture, Brandschneise 5, 64295 Darmstadt, Germany*

[3]   *Agric-science.org, Geinsheimer Str. 3, 64521 Gross-Gerau, Germany*

Abstract

The effects of cattle without and with biodynamic preparations on functional diversity of the soil microbial community were investigated in comparison with mineral fertilization (+ straw incorporation) at low and high application rates to a sandy soil of the long-term fertilization trial in Darmstadt, Germany. The multi-SIR method was used for investigating the respiratory response to 17 individual substrates in surface arable soils (0-5 cm). Multivariate analysis made it possible to significantly separate mineral fertilizer and manure treatments, mainly on the basis of carbohydrates and amino acids. Correlation analysis of the extracted discriminant function showed that the changes in the catabolic profiles of soil microorganisms between the mineral fertilizer and manure treatments were partly caused by differences in soil pH and soil organic C content. The functional diversity of soil microorganisms caused by differences in the community level physiological profile decreased as a result of long-term mineral fertilization with straw incorporation in comparison with farmyard manure application.

3.1 Introduction

Fertilization with cattle manure is an important means for improving soil fertility under arable conditions (Maeder et al., 2002), by increasing the contents of soil organic matter and microbial biomass in comparison with mineral fertilization (Edmeades, 2003; Esperschuetz et al., 2007; Heinze et al., 2010). It has been suggested that soil quality is directly linked to biodiversity of soil microorganisms, as their metabolic capabilities are linked to most of the soil functions, such as nutrient cycling (Nannipieri et al., 2003; Bastida et al., 2008). The ability of soil microorganisms to catabolise a range of different organic substrates is known as the community level physiological profile (Garland and Mills, 1991; Degens and Harris, 1997; Campbell et al., 2003), giving information on those involved in the carbon cycle (Degens et al., 2001). The "insurance hypothesis" predicts that only certain species are essential for ecosystem functioning under steady state conditions, whereas the main part is involved in the stabilisation processes in the case of changing environments (Loreau et al., 2001).

The addition of relevant, low molecular and easily available C sources to soil, similar to root exudates or microbial decomposition products, followed by their mineralization to CO_2, has been used to describe differences between management (Nsabimana et al., 2004) or fertilization treatments (Buyer and Drinkwater, 1997; Romaniuk et al., 2011). Two main measuring methods exist for determining the community level physiological profile (CLPP) of a soil. Garland and Mills (1991) developed a micro-plate based method (Biolog), containing a tetrazolium dye plus substrate, which resulted in a CO_2-induced colour change after adding a serially diluted soil extract. The multi-SIR (substrate-induced-respiration) method of Degens and Harris (1997) is based on the SIR method of Anderson and Domsch (1978), but uses a large variety of different substrates and not only glucose. The "whole soil" method of Degens and Harris (1997) was combined by Campbell et al. (2003) with the advantage of a multi plate system of the Biolog method (Chapman et al., 2007; Creamer et al., 2009). In the multi-SIR method, the soil is weighed into a deep well plate and the CO_2 respiration of the soil is measured by a colour change in the $NaHCO_3$ and a pH indicator dye contained in the detector gel.

The long-term fertilization trial in Darmstadt was established to compare the effects of mineral fertilization with those of composted farmyard cattle manure application with and without biodynamic preparations on soil and crop quality (Abele, 1987), based on the anthroposophic farming aim to stimulate nutrient transformation processes (Zaller and

Koepke, 2004). The biodynamic preparations have been the subject of controversial debate; they sometimes seem to have positive effects on C sequestration, microbial biomass content, amino acid metabolism, or soil organic matter turnover (Scheller and Raupp, 2005; Turinek et al., 2009). In other cases, only minor or even no effects were observed in comparison with simple organic management (Carpenter-Boggs et al., 2000; Heinze et al., 2010). One reason might be that soil organic matter and total microbial biomass indices were not sensitive enough to detect the sometimes contrasting effects of the biodynamic preparations (Heinze et al., 2011). The aim of the current study was to investigate whether community level physiological profiling provides sensitive indices for detecting and evaluating the effects of mineral fertilization on microbial function in comparison with those of farmyard manure application without, but also with biodynamic preparations.

3.2   Materials and methods

3.2.1   Site characteristics and experimental layout

Soil samples were taken from the long-term field trial of the Institute of Biodynamic Research, Darmstadt, Germany (49° 50'N, 8° 34'E, 100 m a.s.l). The site is characterised by a mean annual temperature of 9.5 °C and a mean annual precipitation of 590 mm. In 1980, the experimental field was established on a Haplic Cambisol (WRB 2006) with 86% sand, 9% silt and 5% clay (in topsoil) on an alluvial fine sand of the former river bed of the river Neckar. A continuous application of composted farmyard cattle manures with (CMBD) and without (CM) biodynamic preparations in comparison to mineral fertilizer (MIN) was conducted for 29 years (1980-2009). Field A, one of the four experimental fields, was chosen for sampling because the fertilization treatments had remained unchanged since 1980.

Field A was divided into 36 plots with the three fertilizer and three different application rates: (1) low = 50 kg N ha^{-1} for root crops and 60 N kg ha^{-1} for cereals, (2) medium = 100 kg N ha^{-1} and (3) high = 140 kg N ha^{-1} (cereals) and 150 kg N ha^{-1} (root crops). The mean annual C-input was around 10% higher (sum over all fertilizer rates) in the two organic treatments in comparison with the CM and MIN treatments, where the straw was returned to the soil (Heitkamp et al., 2009). No differences in the contents of N, S, and Mg were determined in the organic fertilizer treatments, while P (0.02 – 0.5%) and K (1.4 – 1.6%)

were higher in the CMBD treatment. No fertilizer was applied in the years with legume cropping. The farmyard cattle manure was composted for three and six months before application to winter rye and spring wheat, respectively. To half of the manure, biodynamic compost preparations were added, containing *Achillea millefolium, Chamomilla recutita, Taraxacum officinale, Valeriana officinalis, Urtica dioica* and the bark of *Quercus robur* with a concentration of 0.5 g t^{-1} fresh manure. The field preparations horn manure and horn silica were applied at rates of 200 to 300 g ha^{-1} and 4 g ha^{-1}, respectively (Koepf et al., 1990). The fertilizer was incorporated before sowing by mouldboard ploughing down to 25 cm depth. Crop rotation was red clover (*Trifolium pratense L.*) or alfalfa (*Medicago sativa L.*), spring wheat (*Triticum aestivum L.*), potatoes (*Solanum tuberosum L.*) or carrots (*Daucus carota* L.), and winter rye (*Secale cereale L.*). Except for fertilization, all management techniques were the same in all treatments.

3.2.2   Sampling and soil chemical analysis

Four soil samples were taken in September 2009 at 0-5 cm depth per plot of the four field replicates (n = 96), sieved < 2 mm, and stored at 4°C. Samples from the lowest and highest application rate were selected for the current experiment. Soil pH was measured in a soil water ratio of 1 to 2.5 (Table 1). Total C and total N were determined using a Vario MAX elemental analyser (Elementar, Hanau Germany). Organic C was total C minus carbonate C, obtained by gas-volumetric determination (Table 1).

Table 1: Soil pH and soil organic C content in mineral (MIN), cattle manure (CM) and cattle manure plus biodynamic preparation (CMBD) in different applications (high, low) treatments.

	pH-H$_2$O	Soil organic C (mg g^{-1} soil)
MIN-low	6.6 b	6.3 c
MIN-high	6.6 b	6.8 bc
CM-low	7.0 a	6.9 bc
CM-high	7.0 a	7.5 b
CMBD-low	7.1 a	7.6 ab
CMBD-high	7.2 a	8.5 a

Different letters within a column show significant differences ($P < 0.05$, Tukey-test)

3.2.3 Functional diversity by CLPP

The physiological profiles were determined by the multi-SIR approach using the MicroResp^{TM} method (Campbell et al., 2003). The soil was adjusted to a water holding capacity of 50% and stored for 7 days in the dark at 25°C, prior to CLPP analysis. Into each well (1.1 ml deep-well microtitre plate (Nunc, Thermo Electron LED, Langenselbold, Germany)), 350 mg of moist soil was added before applying aqueous solutions of the different C sources and sealing the wells with a CO$_2$ trap.

The physiological profiles were determined by applying aqua dest. (basal respiration), 5 amino acids (γ-aminobutyric acid, L-leucine, L-alanine, DL-aspartic acid, and L-glutamine), 2 amino sugars (D-glucosamine and N-acetyl-glucosamine), 5 neutral sugars (D-galactose, L-arabinose, D-fructose, D-glucose, and D-trehalose), and 5 carboxylic acids (protocatechuic acid, L-malic acid, ascorbic acid, citric acid, and oxalic acid). The substrates were selected to present a cross section of root exudates (Campbell et al., 1997) and microbial components and products (Amelung et al., 2001; Nehls et al., 2001; Miwa et al., 2009; Taylor et al., 2012). The analysis was started with 8 mg g^{-1} dry soil of each substrate. Because of the low solubility at higher concentrations, only 2 mg g^{-1} soil of L-leucine and L-glutamine and 0.8 mg g^{-1} soil of protocatechuic acid and DL-aspartic acid

were applied. After application of the aqueous solutions, soil moisture increased to 60% and 70% water holding capacity.

The colorimetric CO_2 trap contained 1% Noble agar, 150 mM KCl, 2.5 mM $NaHCO_3$ and 12.5 µg g^{-1} cresol red (Campbell et al., 2003). The warm gel (150 µl) was applied to a microtitre plate (Nunc), which was stored for 72 h before the measurement in a closed PVC bag, containing soda lime and water. The colour development of the CO_2 trap was measured immediately before sealing and after 6 h incubation (25°C) at 572 nm (FLUOstar, BMG, Offenburg, Germany). For calibrating the CO_2 trap, five different soils were incubated with and without 8 mg g^{-1} glucose in five replicates each for 6 h at 22ºC in the dark, before measuring the CO_2 evolution with a gas chromatograph (Shimadzu) and with the MicroResp[TM] system. The resulting regression line was fitted to the following power function:

$$\mu l\ CO_2 = 51 \text{ x } (0.2 + ABS)^3, r = 0.98$$
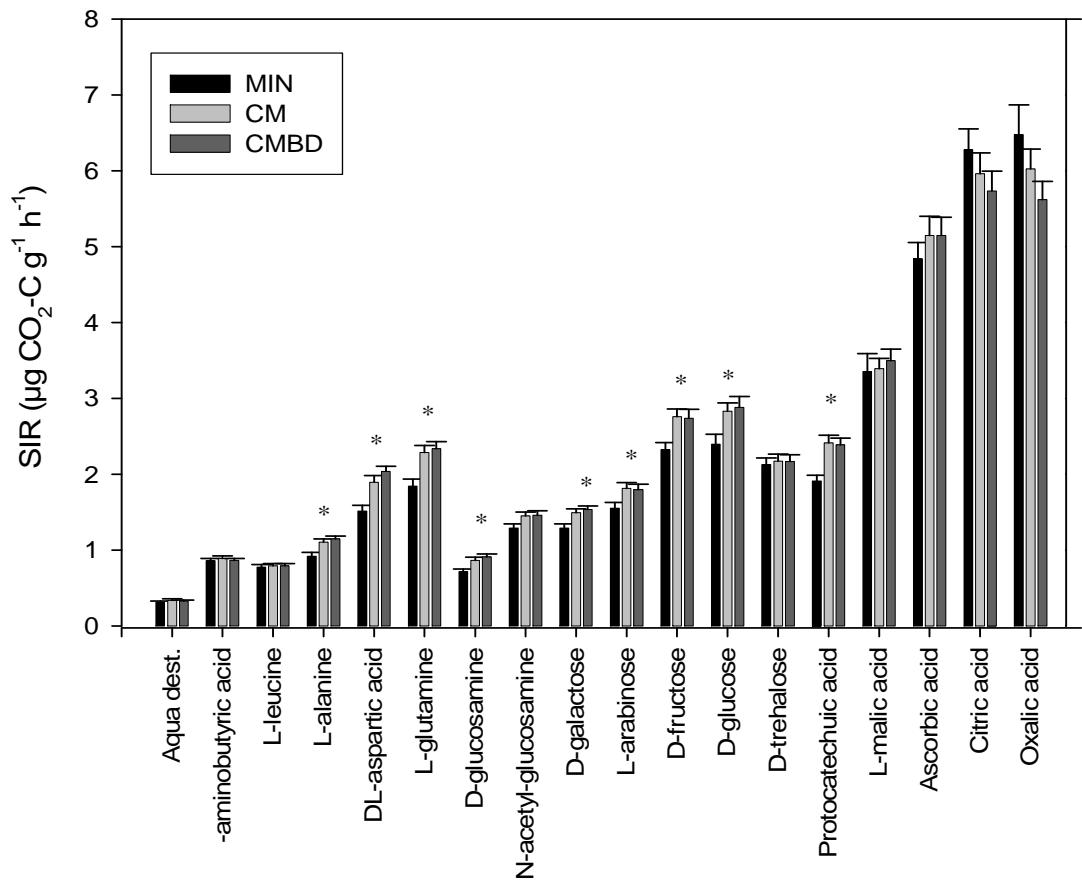
where ABS is the difference in absorption (572 nm) between T1 minus T0. Due to the interaction between soil $CaCO_3$ after adding non-acid and acid substrates (Oren and Steinberger, 2008), samples that were affected by the lime band (Heinze et al., 2010) were excluded (24 of 96).

3.2.4 Statistics

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (about 24 h at 105°C). Fertilizer treatment effects on soil pH soil organic C were determined by analysis of variance (ANOVA), using the Tukey post-hoc test. Fertilizer treatments effects on multi SIR were tested by multivariate statistics using multivariate analysis (MANOVA). Discriminant function analysis (DFA) was carried out after canonical variate analysis of all 17 organic substrates and aqua dest. as variables and the specific fertilization treatments as groups using STATISTICA 9.0 (StatSoft). To describe the discrimination, the substrate-specific respiration was correlated with the canonical scores of the first discriminant function (DF) 1 using SPSS 17.0. Pearson correlation coefficients were used to express this significance. The Shannon diversity index was calculated according to Zak et al. (1994), where p_i is the particular activity of the sum of all activities.

3.3 Results

The basal respiration ranged from 0.26 to 0.49 and from 0.18 to 0.40 μg CO_2-C g^{-1} soil h^{-1} in the low and the high application rate treatments, respectively. The application of C sources always led to a higher respiration rate in comparison with the basal respiration rate (Figure 1). The highest respiration rate was measured after application of oxalic acid and citric acid. Significant fertilizer treatment effects were determined mainly for amino acids and neutral sugars ($P < 0.01$), without any significant fertilizer treatment + application rate interactions ($P > 0.2$).

Figure 1: Mean catabolic response for 17 C sources and aqua dest. in fertilization treatments with minerals (MIN), cattle manure (CM) and cattle manure plus biodynamic preparations (CMBD) (averages of low and high application). Stars are significant differences ($P < 0.05$; Tukey test) between mineral and organic fertilization treatments; error bars = standard errors.

Discriminant function analysis significantly (Wilks' Lambda: 0.064 approx. F = 1.97, $P < 0.001$) separated the composted manure from the MIN treatments (Table 2, Figure 2), but not the CM from the CMBD treatments. However, the strongest quadratic metabolic distances were observed between the CMBD-high and the MIN-low treatment (Table 2). The CMBD-high treatment was also significantly separated from the CMBD-low treatment (Table 2).

Table 2: F values and significant indices of quadratic metabolic distances between mineral (MIN), cattle manure (CM) and cattle manure plus biodynamic preparation (CMBD) in different applications (high, low) treatments of canonical variates.

	MIN-low	MIN-high	CM-low	CM-high	CMBD-low
MIN-high	2.2*				
CM-low	2.9***	4.8***			
CM-high	2.4**	2.7***	1.1		
CMBD-low	2.1*	3.7***	1.0	0.5	
CMBD-high	6.0***	5.7***	1.5	1.1	2.0*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$
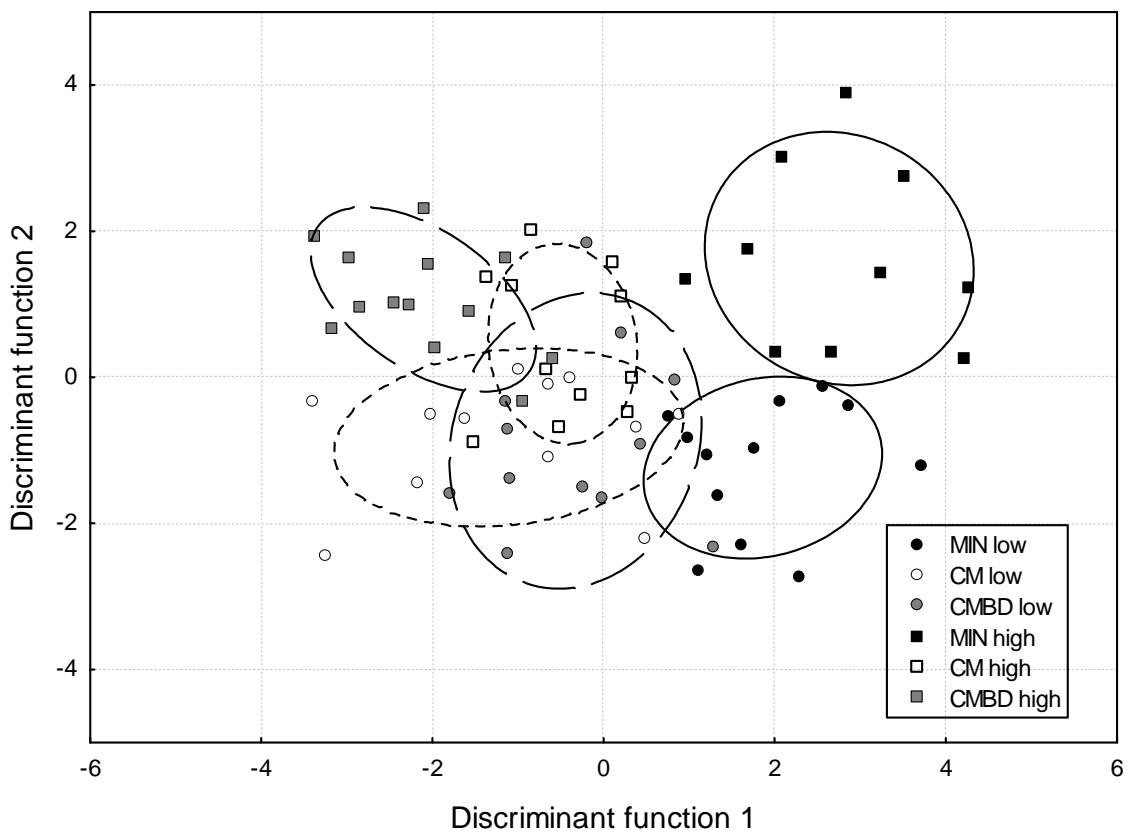
The same was true for the metabolic distances between the MIN-high and MIN-low treatments. The discrimination was mainly caused by DF 1 ($P < 0.001$), whereas DF 2 was not significant ($P > 0.2$), i.e. this function did not separate any treatment. The correlation coefficients between the substrate-induced respiration rates revealed that DF 1 was mainly caused by L-alanine, DL-aspartic acid, L-glutamine, D-glucosamine, D-galactose, L-arabinose, D-fructose, D-glucose and protocatechuic acid (Table 3). Soil pH and soil organic C content correlated with the individual substrates similarly to DF 1.

Table 3: Pearson correlation between substrate utilisation of individual substrates and the canonical discriminant function (DF) 1, soil pH and soil organic C content.

	DF 1	Soil pH	Soil organic C
Aqua dest.	-0.05	-0.03	-0.04
γ- aminobutyric acid	0.02	-0.07	-0.08
L-leucine	0.04	-0.06	0.03
L-alanine	-0.43**	0.43**	0.37**
DL-aspartic acid	-0.51**	0.59**	0.45**
L-glutamine	-0.45**	0.50**	0.38**
D-glucosamine	-0.43**	0.50**	0.41**
N-acetyl-glucosamine	-0.23	0.19	0.14
D-galactose	-0.38**	0.46**	0.32*
L-arabinose	-0.32*	0.37**	0.22
D-fructose	-0.37**	0.35**	0.28*
D-glucose	-0.34**	0.43**	0.37**
D-trehalose	0.05	0.17	0.12
Protocatechuic acid	-0.48**	0.43**	0.26*
L-malic acid	-0.03	0.40**	0.31*
Ascorbic acid	-0.11	0.24	0.21
Citric acid	0.25	-0.02	-0.02
Oxalic acid	0.27	-0.17	-0.09
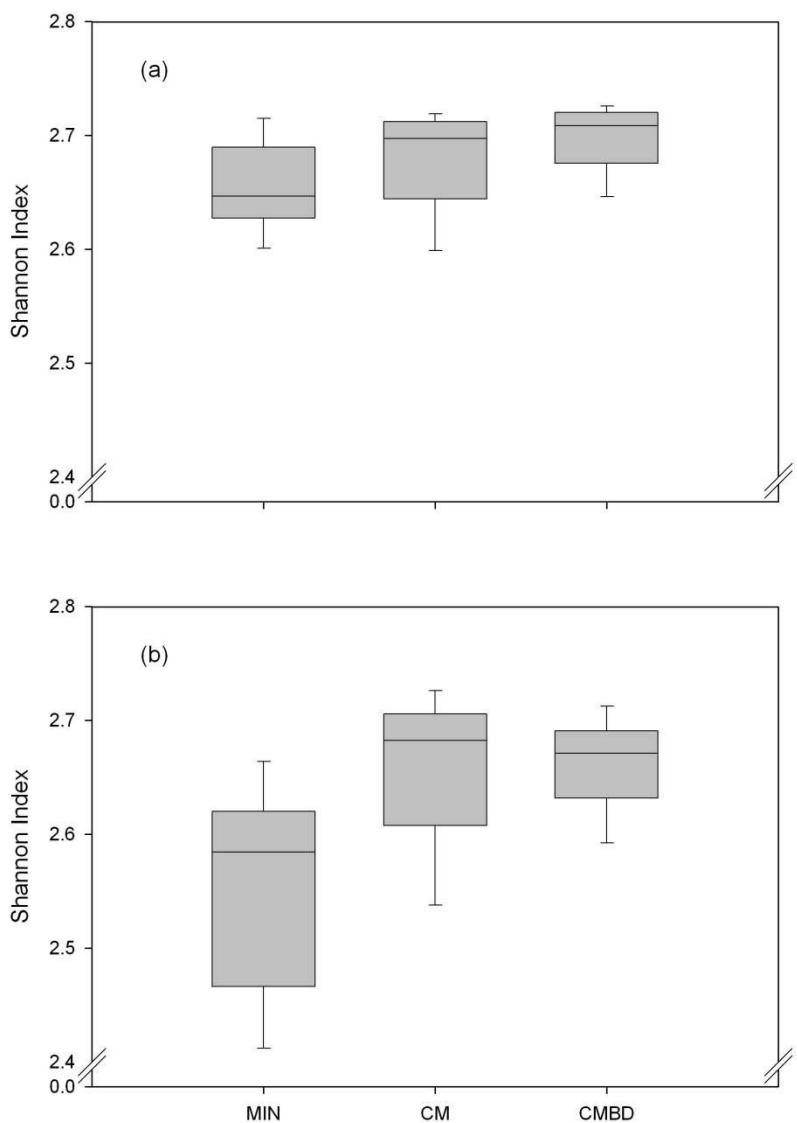
*$P < 0.05$; ** $P < 0.01$

In the MIN treatments, the high application rates led to a significantly lower catabolic diversity index (Shannon index) with higher standard deviations, but no significant differences were estimated between MIN-low and the four cattle manure treatments (Figure 3).

Figure 2: Discrimination function analysis (DFA) of catabolic response of 17 substrates plus aqua dest. of soil after fertilization with minerals (MIN), cattle manure (CM) and cattle manure plus biodynamic preparations (CMBD) in the low application (low) and high application (high) treatments. The scatter plot shows ellipses with confidence ranges of $\alpha = 0.05$.

The mean catabolic diversity in the CMBD treatments was characterized by higher values and standard deviations in comparison with the MIN treatments. The correlation coefficients between the individual substrates and the catabolic diversity index demonstrated that the carboxylic acids, mainly citric and oxalic acid, negatively correlated with the diversity index. In contrast, positive interactions were obtained between the amino acids and the diversity index (Table 4). In the MIN treatments, a wide range of substrates was correlated with the diversity index, although the soil pH did not affect the catabolic diversity.

Figure 3: Boxplot of the Shannon diversity index after fertilization with minerals (MIN), cattle manure (CM) and cattle manure plus biodynamic preparations (CMBD), divided into low (a) and high (b) application treatments.

Table 4: Pearson correlation of catabolic response of soil pH, aqua dest., and the 17 different organic substrates with the Shannon Index for each fertilization treatment; mineral (MIN), cattle manure (CM) and cattle manure plus biodynamic preparation (CMBD)

	MIN	CM	CMBD
Soil pH	0.23	-0.03	-0.19
Aqua dest.	0.62*	0.60*	0.58*
γ- aminobutyric acid	0.61*	0.36	0.15
L-leucine	0.58*	0.69*	0.43*
L-alanine	0.75*	0.54*	0.13
DL-aspartic acid	0.50*	0.34	0.09
L-glutamine	0.44*	0.42*	-0.16
D-glucosamine	0.56*	0.68*	0.42*
N-acetyl-glucosamine	0.73*	0.58*	0.17
D-galactose	0.62*	0.54*	0.18
L-arabinose	0.58*	0.39	0.03
D-fructose	0.17	-0.14	-0.41*
D-glucose	0.18	0.20	-0.40
D-trehalose	0.44*	0.36	0.12
Protocatechuic acid	0.47*	0.22	0.08
L-malic acid	-0.21	-0.46*	-0.44*
Ascorbic acid	-0.39	-0.62*	-0.70*
Citric acid	-0.82*	-0.67*	-0.76*
Oxalic acid	-0.66*	-0.82*	-0.69*

* $P < 0.05$

3.4 Discussion

The catabolic substrate utilization profile under the cattle manure treatments clearly differed from those on the mineral fertilizer treatments. This discrimination was mainly based on amino acids or neutral sugars, all described as components of root exudates (Campbell et al., 1997), but also ascribed to microbial products, such as glucosamine (Amelung et al., 2001). The strong correlation coefficients of these sensitive C substrates indicate that soil microorganisms with similar function were related to C sources, promoted by fertilization with composted farmyard manure. According to the insurance hypothesis (Loreau et al., 2001), the utilization profile of C sources reflects changes in the ecological function under organically fertilized conditions. Several studies confirm that neutral sugars are sensitive in discriminating not only different ecosystems (Stevenson et al., 2004; Lalor et al., 2007), but also different arable fertilization strategies (Romaniuk et al., 2011). An underlying reason might be that composted farmyard manure increased the soil microbial biomass in comparison with mineral fertilizers (Heitkamp et al., 2009; Heinze et al., 2010). This would explain the generally discriminating effect of higher neutral sugar-induced respiration, as the mineralization, e.g. of glucose is usually strongly correlated with the microbial biomass content in soil (Lin and Brookes, 1999; Carpenter-Boggs et al., 2000). It has been suggested that predominantly highly active microorganisms, such as r-strategy bacteria, influence the catabolic response in soil (Degens et al., 2000; Wakelin et al., 2008). Also an increase in biotrophic arbuscular mycorrhizal fungi, described in the same field for the cattle manure plots (Ngosong et al., 2009), can affect the catabolic response of the soil microbial community. Most of the carboxylic acids, except the phenolic protocatechuic acid, were not affected by the application of composted farmyard manure, for unknown reasons. This is even more surprising, since the carboxylic acids, especially citric acid and oxalic acid, were strongly related to the Shannon Index.

Dependent on the N application rate, the calculated C-input was larger in the cattle manure treatments (Heitkamp et al., 2009). Especially the intermediate available C fraction increased in the composted farmyard manure treatments, whereas the labile and passive C pools remained unaffected by fertilization (Heitkamp et al., 2009). Nevertheless, the correlation of soil organic C content with the catabolic response of the individual substrates suggests an influence of this soil property. However, the catabolic response profile did not always indicate clear differences dependent on the application rate, but the differentiation was much stronger than that of the microbial biomass indices (C, N, P, and S) or ergosterol (Heinze et al., 2010), an indicator for the biomass of saprotrophic fungi (Scheller and Joergensen, 2008). In addition, the link between the size of the total microbial biomass and catabolic response to organic substrates has been questioned (Kemmitt et al., 2008) and has led to controversial discussion (Kuzyakov et al., 2009; Paterson, 2009).

Not only the soil organic C content, but also the soil pH is a major reason for differences in the catabolic response profile of soil microorganisms, even in the rather small range between pH 6.3 and 7.3 measured in the present study. Winding and Hendriksen (2007) and Wakelin et al. (2008) described a significant relationship between soil pH and the potential of microorganisms to use low molecular substrates, such as aspartic acid, with decreasing acidity. However, the soil pH showed also significant positive relationships with the contents of microbial biomass C and soil organic C and a negative relationship with the ergosterol content (Heinze et al., 2010, 2011). The correlation pattern of the DF 1 with soil pH points to its importance for the discrimination of fertilization treatments. In particular, the composition of the soil bacterial community is strongly controlled by soil pH (Lauber et al., 2009) and may differ between mineral fertilizer and manure treatments. However, no clear differences in the bacterial PFLA profiles have been observed at the long-term Darmstadt fertilization trial to date (Ngosong et al., 2009, 2010), despite a shift towards saprotrophic fungi being detected (Ngosong et al., 2009; Heinze et al., 2010). In the current investigation, the application of biodynamic preparations did not indicate clear effects and was generally similar to the simple cattle manure treatments, but clearly different from the mineral fertilizer treatments. This is in accordance with earlier results of Heinze et al. (2010) and with a Swiss experiment comparing tillage systems and additionally organic fertilizers with and without the preparations (Berner et al., 2008).

Organic fertilization increased the catabolic diversity in soil and was partly affected by the application rate. This is in accordance with Romaniuk et al. (2011), who demonstrated a

strong increase of catabolic evenness in manure treatments with increasing experimental time. The relatively small differences in the current experiment may have been caused by the absence of insecticides and other pesticide applications in the mineral fertilizer treatments, which might have inhibited the catabolic response of the microbial community. An increase in microbial diversity has been repeatedly observed in manure treatments in comparison with mineral fertilizer treatments (Maeder et al., 2002; Esperschuetz et al., 2007). An earlier study in the same field experiment also showed that microbial diversity (Shannon index) significantly decreased with increasing application rate of mineral fertilizers, whereas diversity in the manure treatments corresponded to the higher results obtained with mineral fertilizers at low and medium rate (Raupp et al., 2004). This study was carried out with the Biolog method (EcoPlate^{TM}, Biolog Inc., Hayward, USA). Interestingly, none of the fertilization treatments showed a preference for any of the 31 C sources offered by the Biolog method.

This suggests that organic farming supports the development of a microbial community with higher complexity than mineral fertilization. It was demonstrated in the same field experiment that the amount of crop yield in years of poor conditions was much higher in the manure treatment in comparison with the mineral fertilizer treatments (Raupp, 2001). Consequently, the higher diversity index, especially at the high application, could be ascribed to an increased soil fertility, whereas the depletion of soil organic C declined concomitantly again with a reduction of catabolic diversity (Degens et al., 2001). The catabolic diversity was negatively correlated with the respiration of citric and oxalic acid and with lower respiration for some sugars and amino acids, but was independent of pH. It seems that the microbial community in soils with a strong respiratory response to organic acids has a low catabolic diversity.

3.5 Conclusion

The differences in microbial community composition caused by mineral N fertilization with straw incorporation and farmyard manure application was reflected by differences in the community level physiological profile using the multi-SIR method. The current multi-SIR method was unable to separate the manure treatments without and with biodynamic preparations. However, the multi-SIR method was able to differentiate between high and low application rates of mineral fertilizer and between high and low application rates of composted farmyard manure with biodynamic preparations. It was thus more sensitive than the total microbial biomass and soil organic matter indices. The discriminating effects of the multi-SIR method were most likely caused by shifts in microbial community structure interacting with differences in soil pH and soil organic matter content. The functional diversity of soil microorganisms caused by differences in the community level physiological profile decreased as a result of long-term mineral fertilization with straw incorporation in comparison with farmyard manure application.

Acknowledgements

3.6 References

Abele, U., 1987. Produktqualität und Düngung - mineralisch, organisch, biologisch-dynamisch. Schriftenreihe des Bundesministers für Ernährung, Landwirtschaft und Forsten. Heft 345. Münster Hiltrup.

Amelung, W., Miltner, A., Zhang, X., Zech, W., 2001. Fate of microbial residues during litter decomposition as affected by minerals. Soil Sci. 166, 598–606.

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215–221.

Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008. Past, present and future of soil quality indices: a biological perspective. Geoderma 147, 159–171.

Berner, A., Hildermann, I., Fliessbach, A., Pfiffner, L., Niggli, U., Maeder, P., 2008. Crop yield and soil fertility response to reduced tillage under organic management. Soil Till. Res. 101, 89-96.

Buyer, J.S., Drinkwater, L., 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. J. Microbiol. Methods 30, 3–11.

Campbell, C.D., Grayston, S.J., Hirst, D.J., 1997. Use of rhizosphere C sources in sole carbon source tests to discriminate soil microbial communities. J. Microbiol. Methods 30, 33–41.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from C substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl. Environ. Microbiol. 69, 3593–3599.

Carpenter-Boggs, L., Kennedy, A.C., Reganold, J.P., 2000. Organic and biodynamic management: effects on soil biology. Soil Sci. Soc. Am. J. 64, 1651–1659.

Chapman, S.J., Campbell, C.D., Artz, R.R.E., 2007. Assessing CLPPs using MicroResp™ - A comparison with Biolog and multi-SIR. J. Soils Sediments 7, 406-410.

Creamer, R.E., Bellamy, P., Black, H.I.J., Cameron, C.M., Campbell, C.D., Chamberlain, P., Harris, J., Nisha Parekh, N., Pawlett, M., Poskitt, J., Stone, D., Ritz, K., 2009. An inter-laboratory comparison of multi-enzyme and multiple substrate-induced respiration assays to assess method consistency in soil monitoring. Biol. Fertil. Soils 45, 623-633.

Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biol. Biochem. 29, 1309–1320.

Degens, B.P., Schipper, L.A., Sparling, G.P., Vojvodic-Vukovic, M., 2000. Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. Soil Biol. Biochem. 32, 189–196.

Degens, B.P., Schipper, L.A., Sparling, G.P., Duncan, L.C., 2001. Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biol. Biochem. 33, 1143–1153.

Edmeades, D.C., 2003. The long-term effects of manures and fertilisers on soil productivity and quality: a review. Nutr. Cycl. Agroecosyst. 66, 165–180.

Esperschuetz, J., Gattinger, A., Maeder, P., Schloter, M., Fließbach, A., 2007. Response of soil microbial biomass and community structures to conventional and organic farming systems under identical crop rotations. FEMS Microbiol. Ecol. 61, 26–37.

FAO-WRB, 2006. World reference base for soil resources 2006. World Soil Resources Reports No 103. FAO, Rome

Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57, 2351–2359.

Heinze, S., Raupp, J., Joergensen, R.G., 2010. Effects of fertilizer and spatial heterogeneity in soil pH on microbial biomass indices in a long-term field trial of organic agriculture. Plant Soil 328, 203–215.

Heinze, S., Oltmanns, M., Joergensen, R.G., Raupp, J., 2011. Changes in microbial biomass indices after 10 years of farmyard manure and vegetal fertilizer application to a sandy soil under organic management. Plant Soil 343, 221-234.

Heitkamp, F., Raupp, J., Ludwig, B., 2009. Impact of fertilizer type and rate on carbon and nitrogen pools in a sandy Cambisol. Plant Soil 319, 259–275.

Kemmitt, S.J., Lanyon, C.V., Waite, I.S., Wen, Q., Addiscott, T.M., Bird, N.R., O'Donnell, A.G., Brookes, P.C., 2008. Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass - a new perspective. Soil Biol. Biochem. 40, 61–73.

Koepf, H.H., Pettersson, B.D., Schaumann, W., 1990. Bio-dynamic Agriculture: an Introduction. Anthroposophic Press. Spring Valley, New York.

Kuzyakov, Y., Blagodatskaya, E., Blagodatsky, S., 2009. Comments on the paper by Kemmitt et al., 2008 'Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass - A new perspective' [Soil Biology & Biochemistry 40, 61-73]: The biology of the Regulatory Gate. Soil Biol. Biochem. 41, 435-439.

Lalor, B.M., Cookson, W.R., Murphy, D.V., 2007. Comparison of two methods that assess soil community level physiological profiles in a forest ecosystem. Soil Biol. Biochem. 39, 454–462.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75, 5111–5120.

Lin, Q., Brookes, P.C., 1999. Comparison of substrate induced respiration, selective inhibition and biovolume measurements of microbial biomass and its community structure in unamended, ryegrass-amended, fumigated and pesticide-treated soils. Soil Biol. Biochem. 31, 1999–2014.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D., Wardle, D.A., 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294, 804–808.

Maeder, P., Fließbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil fertility and biodiversity in organic farming. Science 296, 1694-1697.

Miwa, H., Ahmed, I., Yokota, A., Fujiwara, T., 2009. *Lysinibacillus parviboronicapiens* sp. nov., a low-boron-containing bacterium isolated from soil. Int. J. Syst. Evol. Microbiol. 59, 1427-1432.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670.

Nehls, U., Mikolajewski, S., Magel, E., Hampp, R., 2001. Carbohydrate metabolism in ectomycorrhizas: gene expression, monosaccharide transport and metabolic control. New Phytol. 150, 533-541.

Ngosong, C., Raupp, J., Scheu, S., Ruess, L., 2009. Low importance for a fungal based food web in arable soils under mineral and organic fertilization indicated by Collembola grazers. Soil Biol. Biochem. 41, 2308–2317.

Ngosong, C., Jarosch, M., Raupp, J., Neumann, E., Ruess, L., 2010. The impact of farming practice on soil microorganisms and arbuscular mycorrhizal fungi: Crop type versus long-term mineral and organic fertilization. Appl. Soil Ecol. 46, 134-142.

Nsabimana, D., Haynes, R.J., Wallis, F.M., 2004. Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. Appl. Soil Ecol. 26, 81–92.

Oren, A., Steinberger, Y., 2008. Coping with artefacts induced by $CaCO_3$-CO_2-H_2O equilibria in substrate utilization profiling of calcareous soils. Soil Biol. Biochem. 40, 2569–2577.

Paterson, E., 2009. Comments on the regulatory gate hypothesis and implications for C-cycling in soil. Soil Biol. Biochem. 41, 1352-1354.

Raupp, J., 2001. Manure fertilization for soil organic matter maintenance and its effects upon crops and the environment, evaluated in a long-term trial. In: Rees, R.M., Ball, B.C., Campbell, C.D., Watson, C.A. (Eds.), Sustainable Management of Soil Organic Matter. CABI, Wallingford, pp. 301–308.

Raupp, J., Niehus, A., Oltmanns, M., 2004. Die Diversität der Boden-Mikroflora ist bei Rottemistdüngung höher als bei Mineraldüngung. Mitt. Ges. Pflanzenbauwiss. 16, 149-150.

Romaniuk, R., Giuffre, L., Costantini, A., Nannipieri, P., 2011. Assessment of soil microbial diversity measurements as indicators of soil functioning in organic and conventional horticulture systems. Ecol. Indic. 11, 1345–1353.

Scheller, E., Raupp, J., 2005. Amino acid and soil organic matter content of topsoil in a long term trial with farmyard manure and mineral fertilizers. Biol. Agric. Hortic. 22, 379–397.

Scheller, E., Joergensen, R.G., 2008. Decomposition of wheat straw differing in N content in soils under conventional and organic farming management. J. Plant Nutr. Soil Sci. 171, 886-892.

Stevenson, B.A., Sparling, G.P., Schipper, L.A., Degens, B.P., Duncan, L.C., 2004. Pasture and forest soil microbial communities show distinct patterns in their catabolic respiration responses at a landscape scale. Soil Biol. Biochem. 36, 49–55.

Taylor, C.R., Hardiman, E.M., Ahmad, M., Sainsbury, P.D., Norris, P.R, Bugg, T.D.H. 2012. Isolation of bacterial strains able to metabolize lignin from screening of environmental samples. J. Appl. Microbiol. 113, 521–530.

Turinek, M., Grobelnik-Mlakar, S., Bavec, M., Bavec, F., 2009. Biodynamic agriculture research progress and priorities. Renew. Agr. Food Syst. 24, 146–154.

Wakelin, S.A., Macdonald, L.M., Rogers, S.L., Gregg, A.L., Bolger, T.P., Baldock, J.A., 2008. Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. Soil Biol. Biochem. 40, 803–813.

Winding, A., Hendriksen, N.B., 2007. Comparison of CLPP and enzyme activity assay for functional characterization of bacterial soil communities. J. Soils Sediments 7, 411–417.

Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G., 1994. Functional diversity of microbial communities: A quantitative approach. Soil Biol. Biochem. 26, 1101–1108.

Zaller, J.G., Koepke, U., 2004. Effects of traditional and biodynamic farmyard manure amendment on yields, soil chemical, biochemical and biological properties in a long-term field experiment. Biol. Fertil. Soils 40, 222–229.

4. Microbial residue indices down the soil profile after long-term addition of farmyard manure to a sandy soil

André Sradnick [1], Meike Oltmanns [2], Joachim Raupp [3], Rainer Georg Joergensen [1]

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

[2]   *Institute for Biodynamic Research, Brandschneise 5, 64295 Darmstadt, Germany*

[3]   *Agric-science.org, Geinsheimer Str. 3, 64521 Gross-Gerau, Germany*

Abstract

Long-term organic fertilization may control the accumulation of organic matter in subsoil. The objective of this study was to evaluate the effects of long-term farmyard manure application in comparison with mineral fertilization on the accumulation of amino sugars as indices for microbial residues down to 1 m depth at a sandy site that exhibits highly heterogeneous pH conditions. The soil C/N ratio increased from 11 to values around 16 in all treatments and the relative contribution of microbial residue C to SOC decreased with depth from 68% at 0-25 cm to 24% at 50-100 cm. The stocks of microbial residue C, specifically bacterial residues, were increased by organic fertilization in the subsoil in comparison with mineral fertilization. However, manure application did not increase SOC sequestration in the subsoil, suggesting that manure-induced priming effects increase the microbial turnover at 50-100 cm depth. The fungal C to bacterial C ratio decreased from 2.6 at 0-25 cm depth to 2.1 at 50-100 cm depth. The importance of microbial residues in subsoil C sequestration cannot be explained by the current data and warrants further investigations.

4.1 Introduction

Fertilization with cattle manure is an important means for improving soil fertility under arable conditions, by increasing the stocks of soil organic carbon (SOC) and microbial biomass in comparison with mineral fertilization (Heinze et al., 2010). Most studies on the effects of organic fertilizers have focused exclusively on the topsoil down to 10 or 30 cm depth, where the contents of SOC and densities of microorganisms and roots are highest (Heinze et al., 2010). The proportion of SOC stored at 30 to 100 cm depth ranges between roughly 40 and 60% of the SOC stock at 0 to 30 cm depth (Batjes, 1996). The composition of the microbial community probably changes with depth, especially the ratio of fungi to bacteria, i.e. the two microbial groups that dominate the biomass of soil microorganisms by more than 95% (Joergensen and Emmerling, 2006).

Organic fertilization might also have significant effects on microbial processes and SOC storage, like those observed in tillage experiments (Wright et al., 2007), caused by promoting bacteria (Scheller and Joergensen, 2008), a higher formation of dissolved organic C (Kaiser and Kalbitz, 2012), and increased root growth (Chirinda et al., 2012). However, the input of easily available C by these two processes may lower the SOC contents, as priming effects make stable subsoil organic matter available to soil microbial decomposition (Fontaine et al., 2007). Several studies suggest that the relative contribution of microbially derived components to SOC increases with soil depth (Boström et al., 2007; Rumpel and Kögel-Knabner, 2011). Amino sugars are useful indicators for the accumulation of microbial residues also in the subsoil (Liang and Balser, 2008). Fungal cell walls are the major source of glucosamine (Joergensen and Wichern, 2008), whereas bacterial cell walls, especially in the murein skeleton of Gram-positive species, are the exclusive source of muramic acid (Appuhn and Joergensen, 2006). Amino sugar analysis gives important information on the relative contribution of fungi and bacteria to the fraction of soil microbial residues (Joergensen and Wichern, 2008), and is a sensitive tool for investigating the effects of organic fertilizers (Scheller and Joergensen, 2008) and soil pH (Rousk et al., 2010).

The underlying hypotheses are that (1) the relative contribution of microbial residues to SOC increases with depth, especially in the manure treatment, (2) the increased formation of microbial residues does not lead to an increased subsoil organic carbon (OC) sequestration in the manure treatment, and (3) the ratio of fungal to bacterial residues is lower in the manure treatment and generally declines with depth.

4.2 Materials and methods

4.2.1 Site characteristics, sampling and soil chemical analysis

Depth profiles were taken from field A of a long-term field trial of the Institute of Biodynamic Research, Darmstadt, Hessia, Germany (49° 50' N, 8° 34' E) at 100 m above sea level (Heinze et al., 2010), during September 2009. The long-term experiment was established in 1980 on a Haplic Cambisol (WRB, 2006) with 86% sand, 9% silt, and 5% clay, which has been developed from alluvial sediments of the river Neckar. The mean annual temperature is 9.5°C and the mean annual precipitation is 590 mm. The experiment was set up as a strip design with four replicates, the treatments being fertilizer type and rate, applied at three different rates. For the current study, two fertilizer types, given at the highest rate, were compared: mineral fertilizer (MIN, i.e. calcium ammonium nitrate, superphosphate, potassium chloride, since 1996 potassium magnesia) with the return of straw, composted cattle farmyard manure (CM), without straw return. The application rate was 140 kg N ha^{-1} for cereals and 150 kg N ha^{-1} for root crops. The samples were collected at 5 cm steps down to 100 cm depth, using a steel corer (Eijkelkamp SC/SE diameter 4 cm). More information on the soil chemical properties, microbial properties and soil management can be obtained from (Heinze et al., 2010).

The soils were sieved (< 2 mm), adjusted to 50% water holding capacity and stored in polyethylene bags at 4°C for several weeks until soil biological analysis. The pH was determined in water using a soil to water ratio of 1 to 2.5. Total C and N were measured by gas chromatography using a Vario EL (Elementar, Hanau, Germany) analyser. For determining mobile C, 5-g samples were extracted with 20 ml of 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C in the extracts was measured using a Dimatoc 100 automatic analyser (Dimatec, Essen, Germany).

4.2.2 Amino sugar analysis

The amino sugars muramic acid (MurN), mannosamine (ManN), glucosamine (GlcN), and galactosamine (GalN) were determined according to Appuhn et al. (2004) as described by Indorf et al. (2011) using OPA (o-phthalaldehyd) derivatisation. Moist samples of 0.5 g soil were hydrolysed with 10 ml 6 M HCl, for 6 h at 105°C. Chromatographic separations
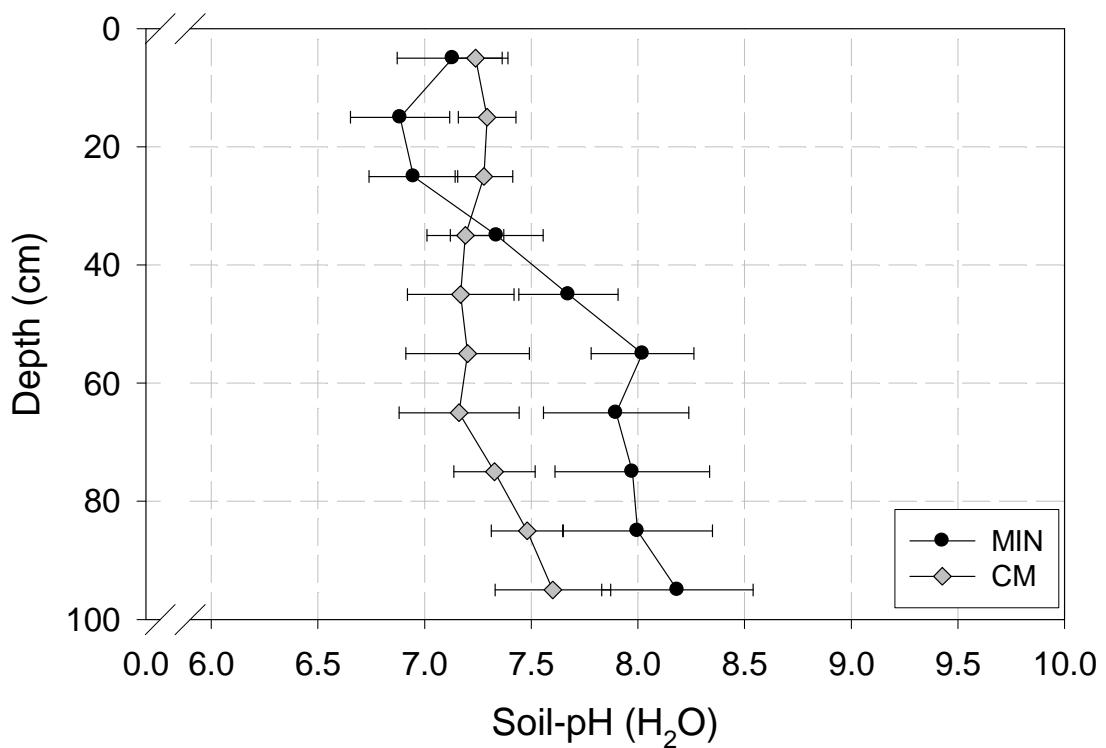
were performed on a Hyperclone C_{18} column (125 mm length × 4 mm diameter) at 35°C, using a Dionex (Germering, Germany) P 580 gradient pump, a Dionex Ultimate WPS – 3000TSL analytical autosampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector set at 445 nm emission and 330 nm excitation wavelengths. Fungal C and bacterial C were calculated according to Engelking et al. (2007) and Appuhn and Joergensen (2006). Microbial residue C was estimated as the sum of fungal C and bacterial C.

4.2.3 Statistics

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (about 24 h at 105°C). The significance of differences between the fertilizer treatments was determined by analyses of covariance (ANCOVA), by normalizing the fertilizer treatment effects for differences in soil pH and depth. For this analysis, the results of the different depth steps were recalculated into stocks using bulk density data and summarized to three depth zones: topsoil (0-25 cm), intermediate zone (25-50 cm) and subsoil (50-100 cm). Differences between means were tested using least significant differences (LSD) at $P < 0.05$, using SPSS, version 16.
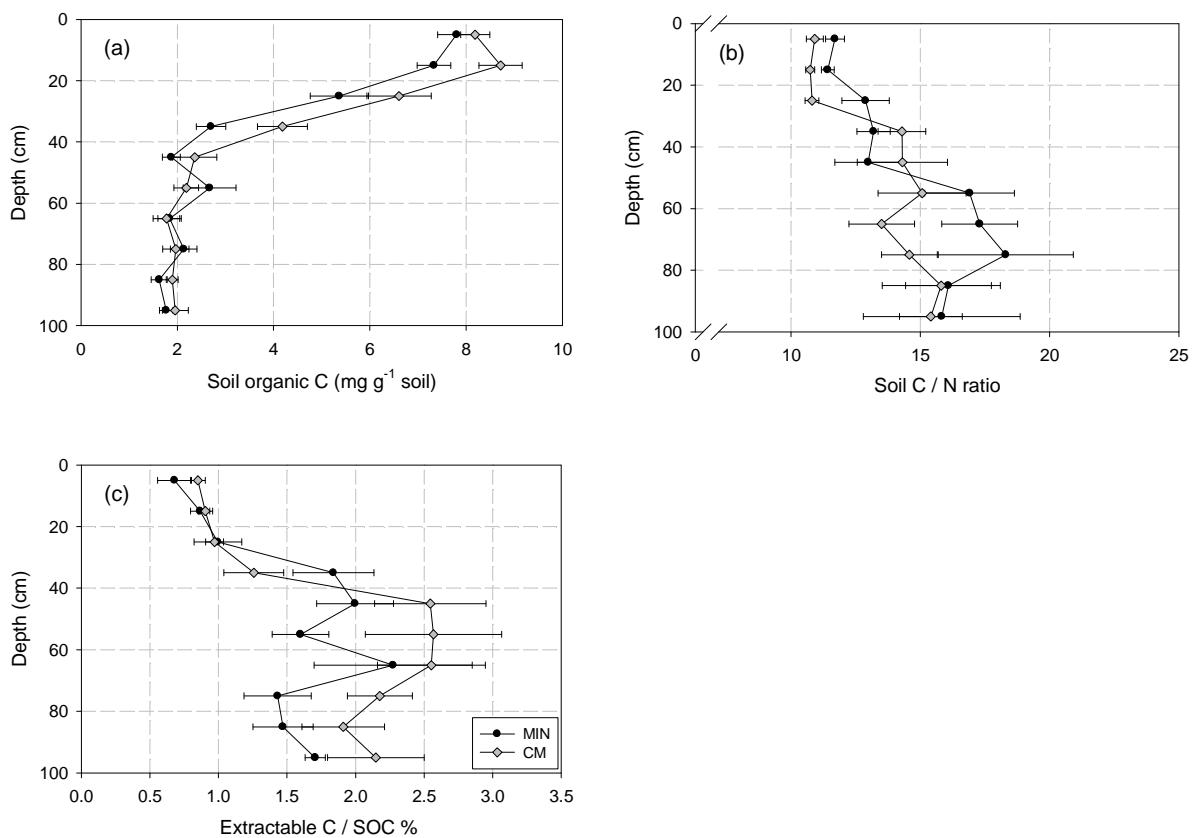
4.3 Results

Soil pH values increased from about 7 in the topsoil to 7.4-8.0 in subsoil (Figure 4). Maximum amounts of SOC and total N and microbial residue C were always found in the top 25 cm. The strongest decline occurred at 25-50 cm depth, followed by a small decline at 50-100 cm depth. At 90-100 cm depth, the SOC content decreased to roughly 24% (Figure 5a) and the total N content to 16% of the maximum values, leading to a significantly increased soil C/N ratio with depth ($P < 0.01$) from 11 to values around 15 in all treatments (Figure 5b).

Figure 4: Soil pH-H$_2$O at 10 depths. MIN = mineral fertilizer, CM = composted farmyard manure; bars show one standard error of mean (n = 8).

The ratio of K$_2$SO$_4$ extractable C to SOC increased from values of 0.6% to values between 1.4 and 2.5% in the subsoil at 50 to 100 cm depth (Figure 5c). The mean value of this ratio was 2.3% in the manure treatment and thus significantly ($P <0.01$) larger than the mean value of 1.4% in the mineral fertilizer treatment. Also for MurN and fungal GlcN, the strongest decline occurred in the intermediate zone (Figure 6). At 90-100 cm depth, the MurN content decreased to roughly 8% (Figure 6a) and the fungal GlcN content to 6% (Figure 6b) of the maximum values, leading to a significant decrease ($P < 0.01$) in the fungal C to bacterial C ratio with depth (Figure 6c). The microbial residue C to SOC ratio decreased from 68% at 0-25 cm depth down to 24% at 50-100 cm depth, averaged over all fertilizer treatments (Figure 6d; Table 5).

Figure 5: (a) Soil organic C, (b) soil C/N ratio and (c) K_2SO_4 extractable C to soil organic C ratio at 10 depths. MIN = mineral fertilizer, CM = composted farmyard manure; bars show one standard error of mean (n = 8).
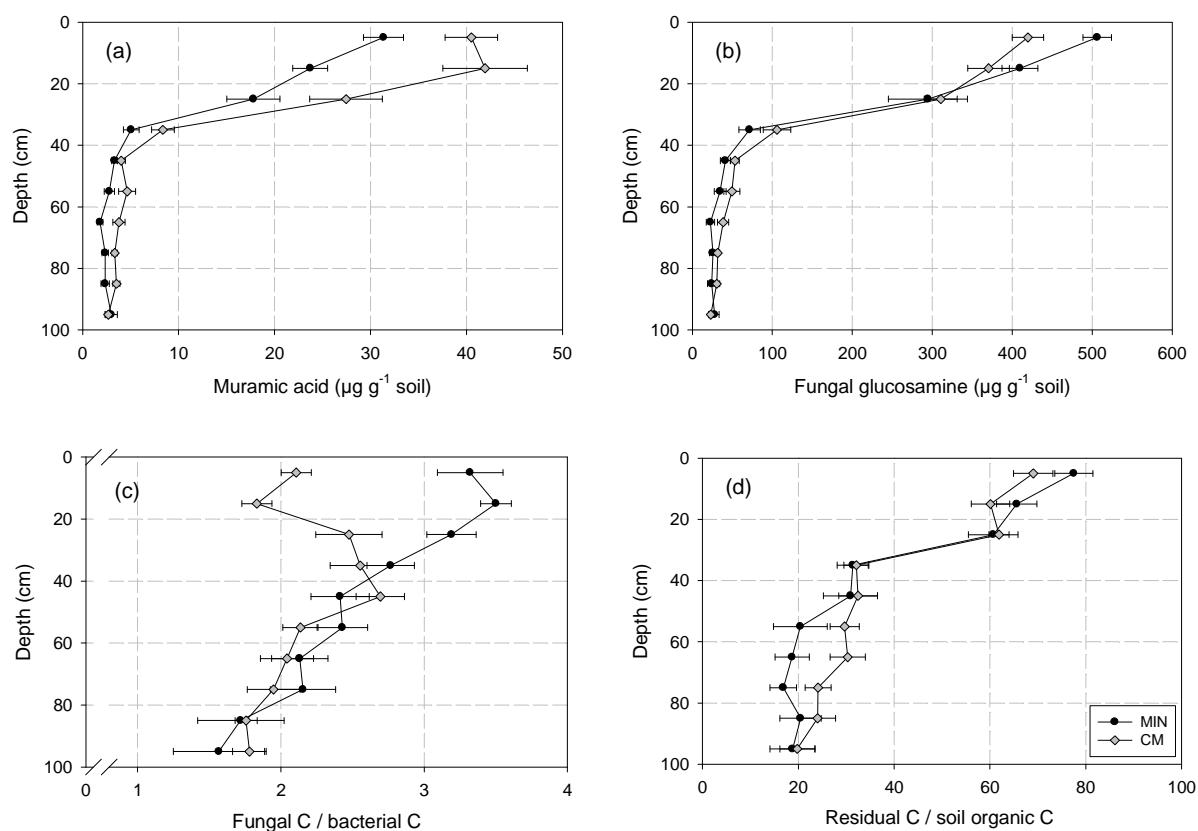
At 0-25 cm depth, the MIN treatment led to significantly lower soil pH ($P < 0.05$), and MurN (Table 5) as well as an increased fungal C to bacterial C ratio in comparison with the CM treatment. At 25-50 cm depth, significant differences between the fertilizer treatments were rare due to the strong depth-specific variability in soil (Table 5). However, a strong tendency was observed for MurN and fungal GlcN to be lower in MIN than in the CM treatments (Table 5).

Table 5: Mean stocks of muramic acid (MurN), fungal glucosamine (GlcN), galactosamine (GalN), and mannosamine (ManN) as well as the ratios fungal C to bacterial C and microbial residue C to SOC in three different depth zones, effects of pH and depth as covariate.

Treatment	MurN	Fungal GlcN	GalN	ManN	Fungal C / bacterial C	Microbial residue C/ SOC (%)
		(kg ha^{-1})				
Topsoil (0-25cm)						
MIN	90 b	1450 a	860	35	3.4 a	72 a
CM	130 a	1280 b	870	38	2.0 b	63 b
Probability values						
pH	<0.01	0.53	<0.01	0.02	<0.01	Ns
Depth	ns	ns	<0.01	ns	ns	0.04
CV(± %)	23	14	17	34	14	15
Intermediate zone (25-50cm)						
MIN	21 b	290	210	15	2.7	35
CM	30 a	410	280	18	2.7	39
Probability values						
pH	ns	ns	ns	0.02	ns	ns
Depth	<0.01	<0.01	<0.01	0.25	<0.01	<0.01
CV(± %)	24	30	28	37	20	32
Subsoil (50-100cm)						
MIN	18 b	200	150 b	13 b	2.0	20 b
CM	25 a	240	190 a	18 a	1.9	26 a
Probability values						
pH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Depth	<0.01	<0.01	<0.01	0.04	<0.01	0.05
CV(± %)	38	41	37	48	22	36

CV = mean coefficient of variation between field replicates (n = 4); different letters within a column show a depth-specific significant difference ($P < 0.05$, LSD-test); MIN = mineral fertilizer, CM = composted farmyard manure.

In the subsoil at 50-100 cm depth, lowest stocks in MurN, fungal GlcN, but also GalN and ManN were always found in the MIN treatment. In the topsoil and subsoil, stocks of all amino sugars were significantly affected by soil pH, except fungal GlcN in the topsoil. The differences to the CM were significant in most cases. Consequently, also the ratio of microbial residue C to SOC was significantly lowest in the MIN treatment (Table 5).

Figure 6: (a) Muramic acid, (b) fungal glucosamine, (c) the fungal C to bacterial C ratio and (d) the microbial residue C to SOC ratio at 10 depths. CM = composted farmyard manure; bars show one standard error of mean (n = 8).

4.4 Discussion

A decline in the microbial residue C to SOC ratio suggests an increased proportion of plant residues at lower depths. This view is supported by the strong negative relationship (r = 0.59, n = 180, *P* < 0.001) between the soil C/N ratio and the microbial residue C to SOC ratio. An increased soil C/N ratio is often considered to be an index for the accumulation of less decomposed organic material (Jenkinson et al., 2008; Rumpel and Kögel-Knabner, 2011). The decrease in the contribution of microbial residue C to SOC contrasts our first hypothesis, derived from the view that subsoil OC is depleted in energy-rich plant material in comparison with topsoil OC (Boström et al., 2007; Liang and Balser, 2008; Rumpel and Kögel-Knabner, 2011).

The increased contribution of plant residues might be due to a contribution of chemically recalcitrant OC (Rasse et al., 2005; Rumpel and Kögel-Knabner, 2011), or the low legacy of old geogenic organic matter, which is often an important component of subsoil OC (Rumpel and Kögel-Knabner, 2011). The current sandy soils have been developed from young river sediments and exhibit a low clay content, reducing the formation of SOC associations with soil minerals and also reducing the protection of SOC in aggregates. These processes are considered to be highly important for SOC stabilisation in subsoils (Rumpel and Kögel-Knabner, 2011). In particular, clay-bound amino sugars have been found to be resistant against further microbial decomposition (Lauer et al., 2011). The origin of the parent material seems to have strong effects on the pool size of the microbial residues. A decreasing contribution of amino sugar to organic matter with depth has also been observed in neutral and alkaline Cambisols, developed from recent floodplain sediments (Roth et al., 2011). This was explained by a markedly higher turnover of microbial residues in comparison with soil organic matter in the subsoil. This cannot be conclusively excluded by the current data and indicates a need to measure differences in the δ^{13}C signature of amino sugars and soil organic matter (Indorf et al., 2012).

With increasing depth, the contribution of microbial residue C to SOC in the manure treatment increased in comparison with the mineral fertilizer treatment, confirming the second part of our first hypothesis. This observation might be partly explained by the differences in soil pH, caused for example by natural differences in the carbonate content of the river sediments (Heinze et al., 2010). An increase in soil pH may reduce the microbial C turnover (Baldock and Skjemstad, 2000). Another reason might be the transfer of manure-derived soluble components to the subsoil, as suggested by the strong and

significant increase in 0.5 M K_2SO_4 extractable C to SOC ratio in the manure treatment at 50-100 cm depth in comparison with the mineral fertilizer treatment. A part of these soluble components seems to be available to the soil microbial community, as indicated not only by the increased microbial residue C to SOC ratio but also by the declined soil C/N ratio. In the subsoil at 50-100 cm depth the stocks of bacterial MurN, GalN and ManN were significantly higher in manure than in the mineral fertilizer treatment. However, the function and indicative value of GalN and ManN, probably mainly parts of microbial mucous substances (Indorf et al., 2011), remain unknown. The long-term application of composted cattle farmyard manure did not result in an increased C sequestration in the subsoil, in contrast to the topsoil, confirming our second hypothesis. This may also support the view that the increased transfer of microbially available C does not necessarily increase C sequestration in the subsoil (Fontaine et al., 2007).

In the topsoil, long-term manure application led to a significantly lower fungal C to bacterial C ratio in comparison with mineral fertilization in accordance with the first part of our third hypothesis. This was mainly due to a considerably higher contribution of bacterial C to the microbial residues. Cattle faeces are already dominated by bacteria (Jost et al., 2011), which are further promoted during the heating period of farmyard manure composting (Danon et al., 2008). The fungal C to bacterial C ratio increased with depth in the manure treatment and decreased in the mineral fertilizer treatment, contradicting and supporting the second part of our third hypothesis, respectively. Soil microorganisms were no longer directly affected by straw or manure application, but by decaying plant roots (Rasse et al., 2005), rhizodeposition (Rumpel and Kögel-Knabner, 2011), or dissolved organic C (Kaiser and Kalbitz, 2012). Instead of treatment effects, the fungal C to bacterial C ratio may simply reflect a complex mixture of root colonizing (Wu et al., 2008) and root decomposing microorganisms (Baumann et al., 2011).

In the subsoil at 50-100 cm depth, the fungal C to bacterial C ratio generally decreased, caused by the increasing scarcity of fresh root residues. This decrease might be overemphasized by the constant conversion value, proposed by Appuhn and Joergensen (2006) for calculating bacterial C from MurN if the ratio Gram-positive to Gram-negative bacteria increases with depth. However, such a shift has not been observed on the basis of PLFA data in subsoil down to 1 m depth (Fierer et al., 2003; Schütz et al., 2009). Similar or even stronger decreases in the GlcN to MurN ratio have been observed by others (Moritz et al., 2009; Roth et al., 2011). The reduction in fungal biomass and residues might contribute to the accumulation of less decomposed plant residues with increasing depth.

In conclusion, the long-term application of cattle farmyard manure affected the accumulation of soil microbial and plant residues in the subsoil. The contributions of microbial residue C and 0.5 M K_2SO_4 extractable C to SOC increased with depth, whereas the soil C/N ratio decreased in comparison with mineral fertilization. In contrast, the decrease in the fungal C to bacterial C ratio seemed to be mainly controlled by shifts in pH or unknown changes in other subsoil environmental conditions.

Acknowledgements

4.5 References

Appuhn, A., Joergensen, R.G., 2006. Microbial colonisation of roots as a function of plant species. Soil Biol. Biochem. 38, 1040-1051.

Appuhn, A., Joergensen, R.G., Raubuch, M., Scheller, E., Wilke, B., 2004. The automated determination of glucosamine, galactosamine, muramic acid, and mannosamine in soil and root hydrolysates by HPLC. J. Plant Nutr. and Soil Sci. 167, 17-21.

Baldock, J.A., Skjemstad, J.O., 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic Geochemistry 31, 697-710.

Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. Euro. J. Soil Sci. 47, 151-163.

Baumann, K., Marschner, P., Kuhn, T.K., Smernik, R.J., Baldock, J.A., 2011. Microbial community structure and residue chemistry during decomposition of shoots and roots of young and mature wheat (*Triticum aestivum* L.) in sand. Euro. J. Soil Sci. 62, 666-675.

Boström, B., Comstedt, D., Ekblad, A., 2007. Isotope fractionation and ^{13}C enrichment in soil profiles during the decomposition of soil organic matter. Oecologia 153, 89-98.

Chirinda, N., Olesen, J.E., Porter, J.R., 2012. Root carbon input in organic and inorganic fertilizer-based systems. Plant Soil 359, 321-333.

Danon, M., Franke-Whittle, I.H., Insam, H., Chen, Y., Hadar, Y., 2008. Molecular analysis of bacterial community succession during prolonged compost curing. FEMS Microbiol. Ecol. 65, 133-144.

Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biol. Biochem. 39, 2111-2118.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35, 167-176.

Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277-280.

Heinze, S., Raupp, J., Joergensen, R.G., 2010. Effects of fertilizer and spatial heterogeneity in soil pH on microbial biomass indices in a long-term field trial of organic agriculture. Plant Soil 328, 203-215.

Indorf, C., Dyckmans, J., Khan, K.S., Joergensen, R.G., 2011. Optimisation of amino sugar quantification by HPLC in soil and plant hydrolysates. Biol. Fertil. Soils 47, 387-396.

Indorf, C., Stamm, F., Dyckmans, J., Joergensen, R.G., 2012. Determination of saprotrophic fungi turnover in different substrates by glucosamine-specific $\delta^{13}C$ liquid chromatography/isotope ratio mass spectrometry. Fungal Ecol. 5, 694-701.

Jenkinson, D.S., Poulton, P.R., Bryant, C., 2008. The turnover of organic carbon in subsoils. Part 1. Natural and bomb radiocarbon in soil profiles from the Rothamsted long-term field experiments. Euro. J. Soil Sci. 59, 391-399.

Joergensen, R.G., Emmerling, C., 2006. Methods for evaluating human impact on soil microorganisms based on their activity, biomass, and diversity in agricultural soils. J. Plant Nutr. and Soil Sci. 169, 295-309.

Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol. Biochem. 40, 2977-2991.

Jost, D.I., Indorf, C., Joergensen, R.G., Sundrum, A., 2011. Determination of microbial biomass and fungal and bacterial distribution in cattle faeces. Soil Biol. Biochem. 43, 1237-1244.

Kaiser, K., Kalbitz, K., 2012. Cycling downwards – dissolved organic matter in soils. Soil Biol. Biochem. 52, 29-32.

Lauer, F., Kösters, R., Du Preez, C.C., Amelung, W., 2011. Microbial residues as indicators of soil restoration in South African secondary pastures. Soil Biol. Biochem. 43, 787-794.

Liang, C., Balser, T.C., 2008. Preferential sequestration of microbial carbon in subsoils of a glacial-landscape toposequence, Dane County, WI, USA. Geoderma 148, 113-119.

Moritz, L.K., Liang, C., Wagai, R., Kitayama, K., Balser, T.C., 2009. Vertical distribution and pools of microbial residues in tropical forest soils formed from distinct parent materials. Biogeochemistry 92, 83-94.

Rasse, D.P., Rumpel, C., Dignac, M.-F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269, 341-356.

Roth, P.J., Lehndorff, E., Cao, Z.H., Zhuang, S., Bannert, A., Wissing, L., Schloter, M., Kögel-Knabner, I., Amelung, W., 2011. Accumulation of nitrogen and microbial residues during 2000 years of rice paddy and non-paddy soil development in the Yangtze River Delta, China. Glob. Change Biol. 17, 3405-3417.

Rousk, J., Brookes, P.C., Bååth, E., 2010. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biol. Biochem. 42, 926-934.

Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter – a key but poorly understood component of terrestrial C cycle. Plant Soil 338, 143-158.

Scheller, E., Joergensen, R.G., 2008. Decomposition of wheat straw differing in nitrogen content in soils under conventional and organic farming management. J. Plant Nutr. and Soil Sci. 171, 886-892.

Schütz, K., Nagel, P., Vetter, W., Kandeler, E., Ruess, L. 2009. Flooding forested groundwater recharge areas modifies microbial communities from top soil to groundwater table. FEMS Microbiol. Ecol. 67, 171-182.

WRB, 2006. World reference base for soil resources. World Soil Resources Reports No 103. FAO, Rome.

Wright, A.L., Dou, F.G., Hons, F.M., 2007. Crop species and tillage effects on carbon sequestration in subsurface soil. Soil Sci. 172, 124-131.

Wu, T., Chellemi, D.O., Graham, J.H., Rosskopf, E.N., 2008. Assessment of fungal communities in soil and tomato roots subjected to diverse land and crop management systems. Soil Biol. Biochem. 40, 1967-1970

5. Impact of activated charcoal and tannin amendments on microbial biomass and residues in an irrigated sandy soil under arid subtropical conditions

André Sradnick [1], Mariko Ingold [2], Johanna Marold[1] Rajasekaran Murugan [1], Andreas Buerkert [2], Rainer Georg Joergensen [1]

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

[2] *Department of Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics, University of Kassel, Steinstr. 19, 37213 Witzenhausen, Germany*

Abstract

Effects of goat manure application combined with charcoal and tannins, added as feed additives or mixed directly, on microbial biomass, microbial residues and soil organic matter were tested in a 2-year field trial on a sandy soil under Omani irrigated subtropical conditions. Soil microbial biomass C revealed the fastest response to manure application, followed by microbial residue C, estimated on the basis of fungal glucosamine and bacterial muramic acid and finally soil organic C (SOC), showing the slowest, but still significant response. At the end of the trial, microbial biomass C reached 220 µg g^{-1} soil, i.e. contents similar to sandy soils in temperate humid climate, and showed a relatively high contribution of saprotrophic fungi, as indicated by an average ergosterol to microbial biomass C ratio of 0.35% in the manure treatments. The mean fungal C to bacterial C ratio was 0.55, indicating bacterial dominance of microbial residues. This fraction contributed relatively low concentrations of between 20 and 35% to SOC. Charcoal added to manure increased the SOC content and the soil C/N ratio, but did not affect any of the soil microbial properties analyzed. Tannins added to manure reduce the 0.5 M K_2SO_4 extractable N to N total ratio compared to manure control. These effects occurred regardless of whether charcoal or tannins were supplied as feed additive or directly mixed to the manure.

5.1 Introduction

In arid subtropical areas, such as the Batinah coastal region of Oman where irrigated vegetable farming prevails, the application of farmyard manure is especially important to maintain soil organic C (SOC) levels (Siegfried et al. 2011). Farmyard manure application also has positive effects on the contents of soil microbial biomass and microbial residues in different regions of the world (Marinari et al. 2006; Badalucco et al. 2010; Heinze et al. 2010; Joergensen et al. 2010; Murugan and Kumar 2013). These effects may be even enhanced by the addition of charcoal or tannins.

Charcoal has an aromatic structure and can be stabilised in soil (Preston and Schmidt 2006). The use of charcoal as a feed additive seems to improve stock growth and C sequestration in soil (McHenry 2010), but an increase in SOC storage has only sometimes been observed (Glaser et al. 2002; Dempster et al. 2012; Lentz and Ippolito 2012). The addition of charcoal to soils can provide a habitat for soil microorganisms (Pietikäinen et al. 2000; Lehmann et al. 2011) and affects the soil microbial community composition, e.g. by increasing the relative abundance of Gram-positive actinobacteria (Khodadad et al. 2011) and regulating the presence of mycorrhizal fungi (Warnock et al. 2007), especially under dry conditions (Blackwell et al. 2010).

Tannins, hydrolysable or condensed polyphenolic compounds (Haslam 1981), are redox-active and form complexes with protein (Hagerman et al. 1997; Halvorson et al. 2011). They influence SOC turnover and availability of soil N by inhibiting activity of mineralising enzymes (Kraus et al. 2003; Joanisse et al. 2007; Selvakumar et al. 2007). Tannins also have anti-nutritional effects, reducing feed intake and nutrient utilisation of ruminants (Kumar and Vaithiyanathan 1990). The effects of combined application of tannin or charcoal and manure on soil microbial biomass or activity have rarely been studied (Steiner et al. 2007), while the effects on soil microbial residues are completely unknown.

Microbial biomass and the fungal cell-membrane component ergosterol are sensitive indicators for monitoring the effects of farmyard manure application to soil (Heinze et al. 2010). As microbial cell-wall components, amino sugars are recalcitrant and, consequently, serve as slow responding indices for the contribution of microbial residues to the sequestration of SOC (Amelung 2001; Guggenberger et al. 1999; Glaser et al. 2004). Fungi are the main source of glucosamine (Joergensen and Wichern 2008), whereas bacteria are the exclusive source of muramic acid (Millar and Casida 1970; Appuhn and Joergensen

2006), making it possible to assess the specific contribution of these two main microbial groups to SOC (Joergensen et al. 2010). It has been suggested that bacterial muramic acid has a faster turnover than fungal glucosamine (Guggenberger et al. 1999; Six et al. 2006; Liang et al. 2007; He et al. 2011), contrasting observations by Appuhn et al. (2006).

The objective of the current investigation was to improve C and N balances under irrigated agriculture and high ambient temperatures. It is based on the following hypotheses: (1) The positive effects of farmyard manure application increase in the order microbial biomass C > microbial residue C > SOC in comparison with mineral fertilizer application. (2) In the manure treatments, the addition of charcoal as feed additive has positive effects on the accumulation of microbial biomass C, microbial residue C, and SOC. (3) The addition of tannin as feed additive has similar positive effects on the accumulation of microbial residue C and SOC to those of charcoal, but negative effects on microbial biomass C. (4) The effects of direct mixing of charcoal and tannin to the farmyard manure are similar to the addition as feed.

5.2 Materials and methods

5.2.1 Sampling site, soil and manure properties

Samples were taken in the northwestern Batinah coast from an experimental farm of the Sultanate of Oman (24°22′ N, 56°34′ E). The local climate is characterized by a hot summer with temperatures up to 45°C and a period from October to April with temperatures declining below 20°C. The mean (1983-2010) annual temperature is 27.0 °C and the mean annual precipitation is 109 mm, ranging from 10 to 341 mm per year. The growing season therefore lasts from September to May. In 2010 annual rainfall was about 20 mm. The experimental field was located 10 m above sea level on a soil characterized as a hyperthermic typic Torrifluvent (US Soil Taxonomy), with 82% sand, 13% silt and 5% clay. The experimental field was divided into 24 plots (2.5×7.0 m) laid out in a completely randomized design with four field replicates and three replicated per plot (n = 72 per sampling date).

The six treatments included a (1) mineral fertilization, (2) goat manure, (3) goat manure and 2.5% charcoal (AquaSorb® CP1) as feed additive, which corresponds to 1.65 t ha^{-1}, (4) goat manure and 3.6% tannin (quebracho extract) as feed additive, which correspondence to 2.2 t ha^{-1}, (5) goat manure mixed with charcoal (1.7 t ha^{-1}) before field application and

(6) goat manure mixed with tannin (2.2 t ha^{-1}) before field application. Roughly 15 t dry weight ha^{-1} of goat manure was incorporated by plowing at 0–15 cm depth. Mineral NPK fertilizer were applied as $(NH_4)_2SO_4$, $(H_2PO_4)_2$ and K_2SO_4. Sweet corn (*Zea mays* L.) was grown between October and January followed by radish (*Raphanus sativus* L.) in each cultivation period. For sweet corn 200 kg N ha^{-1}, 56 kg P ha^{-1} and 130 kg K ha^{-1} were applied in three split doses. For radish 135 kg N ha^{-1}, 38 kg P ha^{-1} in 2010/2011 and 60 kg P ha^{-1} in 2011/2012 and 90 kg K ha^{-1} were applied as basal dose, for all fertilizer treatments.

Three soil samples were collected at 0-10 cm depth from each plot in August 2010, six weeks before the experiment started, as well as in April 2011 and April 2012 after cropping. Soil samples were sieved (< 2 mm), stored in polypropylene bags at 4 °C, and transported to Germany for analyses. For measuring microbial biomass and activity indices, the samples were adjusted to 50% of water holding capacity and pre-incubated for one week before the analysis started. A subsample was stored at -20 °C for measuring amino sugars and ergosterol. Another subsample was dried at 105 °C for 24 h and milled for measuring soil chemical properties. Total C and total N in soils and organic amendments were determined after combustion, using a Vario Max CN analyzer (Elementar, Hanau, Germany). Carbonate was measured gas-volumetrically after the addition of 10% HCl (Chapman and Pratt 1961). Soil organic C (SOC) was determined as total C minus carbonate C. Before the experiment started in 2010, the contents of SOC and total N did not differ between the plots. The mean soil pH, measured at a soil to water ratio of 1 to 2.5, was 8.8 and not affected by any treatment. The mean electrical conductivity (EC) was estimated using a soil to water suspension of 1:5 and converted to a respective value in saturation extract (EC$_e$), which was slightly saline at 2.6 ds m^{-1}.

5.2.2 Microbial biomass indices

Microbial biomass C (Vance et al. 1987) and microbial biomass N (Brookes et al. 1985) were estimated by fumigation-extraction. Organic C and total N in the 0.5 M K_2SO_4 extracts was measured using an automatic analyzer (Multi N/C 2100, Analytik Jena, Germany). Microbial biomass C was calculated as E_C / k_{EC}, where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al. 1990). Microbial biomass N was calculated as E_N / k_{EN}, where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} =

0.54 (Brookes et al. 1985). The fungal cell-membrane component ergosterol was extracted from 2 g soil with 100 ml ethanol by oscillated shaking at 250 rev min^{-1} for 30 min according to Djajakirana et al. (1996). Ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and detected at a wavelength of 282 nm.

5.2.3 Microbial residues

The amino sugars muramic acid (MurN), glucosamine (GlcN), and galactosamine (GalN) were determined according to Appuhn et al. (2004) as described by Indorf et al. (2011) using OPA (o-phthalaldehyd) derivatisation. Moist samples of 0.5 g soil were hydrolysed with 10 ml 6 M HCl, for 6 h at 105°C. Chromatographic separations were performed on a Hyperclone C_{18} column (125 mm length x 4 mm diameter) at 35°C, using a Dionex (Germering, Germany) P 580 gradient pump, a Dionex Ultimate WPS – 3000TSL analytical autosampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector set at 445 nm emission and 330 nm excitation wavelengths. Fungal C was calculated by subtracting bacterial GlcN from total GlcN as an index for fungal residues, assuming that MurN acid and GlcN occur at a 1 to 2 molar ratio in bacterial cells (Engelking et al. 2007): mmol fungal C g^{-1} dry weight = (mmol GlcN – 2 × mmol MurN) × 9. Bacterial C was calculated as an index for bacterial residues by multiplying the concentration of MurN by 45 (Appuhn and Joergensen 2006). Microbial residue C was estimated as the sum of fungal C and bacterial C.

5.2.4 Statistics

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (24 h at 105°C). Residuals of all datasets fit to normal distribution. To test the effects of fertilization systems, simple contrast tests of were used to compare mineral versus manure control, tannin (manure fed tannin, manure mix tannin) versus charcoal (manure fed charcoal, manure mix charcoal), and feeding (manure fed charcoal, manure fed tannin) versus mixing (manure mix charcoal, manure mix tannin) treatments. The tested contrasts were performed across the first plus second growing season. All the analyses were carried out using the software package SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

5.3 Results

During the first growing season, goat manure application increased the contents of SOC and total N by roughly 15% in comparison with mineral fertilized plots (Table 6). This difference was more than doubled at the end of the second growing season, but on a considerably lower level of SOC and total N. The highest SOC contents and soil C/N ratios were always observed in the two charcoal treatments. The soil C/N ratio was significantly higher in the mix than in the feed treatment, and was thus the only property significantly affected by this contrast. The contribution of K_2SO_4 extractable N to total N decreased during the growing season from 9.6% to a mean of 5.2%, and was significantly lowest in the tannin treatments, with a mean of 4.4%, leading to the highest C/N ratio of 3.5 in this mobile fraction.

Table 6: Mean contents for soil organic C (SOC), total N, the soil C/N ratio, K_2SO_4-extractable N as % total N and the C/N ratio of K_2SO_4 extractable material in different fertilizer treatments of a sandy subtropical soil from the Batinah region of Oman.

	SOC	Total N	Soil C/N	K_2SO_4 extractable N	C/N
	mg g^{-1} soil			(% total N)	
Initial content	7.6 (0.2)	0.40 (0.01)	19.4 (0.3)	9.6 (0.4)	3.5 (0.1)
First growing season					
Mineral	8.5 (0.3)	0.54 (0.02)	15.9 (0.8)	4.8 (0.7)	3.3 (0.5)
Manure	9.1 (0.5)	0.63 (0.03)	14.4 (0.5)	5.4 (0.9)	2.8 (0.4)
Manure fed charcoal	10.6 (0.8)	0.67 (0.04)	15.9 (0.8)	5.4 (0.7)	2.7 (0.4)
Manure mix charcoal	10.5 (0.5)	0.64 (0.03)	16.5 (0.5)	5.5 (0.5)	2.3 (0.3)
Manure fed tannin	9.6 (0.6)	0.70 (0.04)	13.7 (0.6)	4.5 (0.6)	3.3 (0.4)
Manure mix tannin	10.3 (0.5)	0.68 (0.04)	15.3 (0.4)	4.7 (0.8)	3.3 (0.3)
Second growing season					
Mineral	5.7 (0.2)	0.32 (0.02)	18.4 (0.9)	4.6 (0.2)	4.9 (0.3)
Manure	7.6 (0.3)	0.54 (0.03)	14.3 (0.5)	6.2 (0.3)	2.5 (0.2)
Manure fed charcoal	9.3 (0.4)	0.54 (0.02)	17.1 (0.3)	5.8 (0.3)	2.5 (0.1)
Manure mix charcoal	9.1 (0.5)	0.46 (0.04)	20.4 (0.6)	6.2 (0.4)	3.0 (0.4)
Manure fed tannin	7.8 (0.4)	0.57 (0.03)	13.8 (0.4)	4.4 (0.4)	3.2 (0.1)
Manure mix tannin	8.0 (0.5)	0.54 (0.03)	15.0 (0.8)	4.7 (0.2)	3.8 (0.3)
Contrasts					
Mineral vs. Manure	<0.01	<0.01	<0.01	0.06	<0.01
Charcoal vs. Tannin	0.02	ns	<0.01	<0.01	<0.01
Feed vs. mix	ns	ns	<0.01	ns	ns
Initial vs. First GS	<0.01	<0.01	<0.01	<0.01	<0.01
First GS vs. Second GS	<0.01	<0.01	<0.01	ns	0.05
CV (±%)	16	15	10	27	23

CV = mean coefficient of variation between replicates within one plot (n=4), GS = growing season. The values in parentheses represent the standard error of the means (initial contents: n= 72, treatments n=12)

During the first growing season, goat manure application increased the contents of microbial biomass C (mean 180 µg g^{-1} soil) and microbial biomass N (mean 26 µg g^{-1} soil) by roughly 40 and 60%, respectively, in comparison with mineral fertilized plots (Table 7). These discrepancy increased by roughly 80% for both microbial biomass (means 220 µg C g^{-1} soil and 40 µg N g^{-1} soil) indices at the end of the second growing season, i.e. on a considerably higher level. For this reason, the microbial biomass C to SOC ratio increased from 1.8% after the first to 2.7% after the second growing season. During the first growing season, goat manure application increased the ergosterol content by roughly 50% (mean 0.6 µg g^{-1} soil) in comparison with mineral fertilized plots. This alteration increased to 110% (mean 0.7 µg g^{-1} soil) at the end of the second growing season, mainly caused by the strong decline in the mineral fertilizer treatment. The mean ergosterol to microbial biomass C ratio was 0.35% in the manure treatments and was thus somewhat higher than the 0.32% in the mineral fertilizer treatments. Neither charcoal nor tannin additions had significant effects on any microbial biomass index in soil.

Table 7: Contents for microbial biomass C, microbial biomass N and ergosterol as well as the contribution of microbial biomass C to SOC in different fertilizer treatments of a sandy subtropical soil from the Batinah region of Oman.

| | Microbial biomass | | | Ergosterol |
| | C | N | C | |
	(μg g^{-1} soil)		(% SOC)	(μg g^{-1} soil)
Initial contents	106 (4)	12 (1)	1.4 (0.05)	0.61 (0.02)
First growing season				
Mineral	125 (8)	16 (2)	1.5 (0.13)	0.40 (0.02)
Manure	152 (13)	24 (2)	1.7 (0.16)	0.53 (0.04)
Manure fed charcoal	176 (14)	26 (4)	1.7 (0.19)	0.58 (0.05)
Manure mix charcoal	183 (15)	23 (2)	1.8 (0.14)	0.59 (0.06)
Manure fed tannin	181 (12)	29 (3)	1.9 (0.11)	0.72 (0.05)
Manure mix tannin	205 (27)	26 (2)	2.0 (0.21)	0.74 (0.12)
Second growing season				
Mineral	120 (15)	22 (2)	2.1 (0.26)	0.32 (0.02)
Manure	224 (13)	41 (1)	3.0 (0.13)	0.66 (0.04)
Manure fed charcoal	244 (18)	44 (3)	2.6 (0.13)	0.73 (0.05)
Manure mix charcoal	206 (16)	36 (3)	2.2 (0.12)	0.64 (0.05)
Manure fed tannin	238 (24)	40 (2)	3.1 (0.29)	0.72 (0.05)
Manure mix tannin	201 (19)	39 (3)	2.5 (0.19)	0.71 (0.07)
Contrasts				
Mineral vs. Manure	<0.01	<0.01	0.02	<0.01
Charcoal vs. Tannin	ns	ns	0.06	0.07
Feed vs. mix	ns	ns	ns	ns
Initial vs. First GS	<0.01	<0.01	<0.01	ns
First GS vs. Second GS	<0.01	<0.01	<0.01	ns
CV ($\pm$%)	23	26	22	24

CV = mean coefficient of variation between replicates within one plot (n=4), GS = growing season. The values in parentheses represent the standard error of the means (initial contents: n= 72, treatments n=12)

During the first growing season, goat manure application increased the contents of MurN, fungal GlcN, and GalN by roughly 20, 30, and 20%, respectively, in comparison with mineral fertilized plots (Table 8). These differences increased to roughly 60% for MurN and 75% for GlcN and GalN, respectively, at the end of the second growing season. The contribution of microbial residue C increased from values around 20% SOC to a mean value of nearly 35% SOC, especially due to the increased percentages in the manure treatments. The fungal C to bacterial C ratio varied around 0.55 and was on average increased by 10% in the manure treatments after the first and by 13% after the second growing season. Again, neither charcoal nor tannin additions had significant effects on any microbial residue index in soil.

Table 8: Mean contents for muramic acid (MurN), fungal glucosamine (Fungal GlcN), and galactosamine (GalN) as well as the ratios of microbial residue C to SOC and fungal C to bacterial C in different fertilizer treatments of a sandy subtropical soil from the Batinah region of Oman.

	MurN	Fungal GlcN	GalN	Microbial residue C	Fungal C / bacterial C
		($\mu g\ g^{-1}$ soil)		(% SOC)	
Initial contents	26 (0.7)	80 (3)	31 (0.9)	25 (0.7)	0.62 (0.01)
First growing season					
Mineral	23 (1.7)	53 (5)	26 (1.6)	18 (1.4)	0.46 (0.03)
Manure	28 (2.4)	70 (9)	32 (3.2)	20 (1.0)	0.50 (0.05)
Manure fed charcoal	29 (2.2)	64 (7)	30 (2.5)	18 (1.3)	0.44 (0.03)
Manure mix charcoal	27 (2.6)	70 (7)	31 (2.6)	18 (1.2)	0.54 (0.05)
Manure fed tannin	30 (2.4)	80 (8)	34 (2.5)	22 (1.3)	0.54 (0.04)
Manure mix tannin	28 (2.5)	66 (6)	31 (3.0)	18 (0.9)	0.49 (0.05)
Second growing season					
Mineral	25 (2.3)	66 (5)	24 (1.7)	30 (2.3)	0.53 (0.02)
Manure	41 (2.6)	127 (8)	44 (2.5)	40 (1.3)	0.62 (0.02)
Manure fed charcoal	44 (2.9)	126 (6)	45 (2.2)	34 (1.4)	0.59 (0.02)
Manure mix charcoal	36 (2.5)	109 (9)	38 (2.6)	29 (1.9)	0.60 (0.02)
Manure fed tannin	38 (2.5)	117 (6)	41 (2.0)	36 (1.8)	0.63 (0.02)
Manure mix tannin	42 (2.7)	116 (6)	41 (2.7)	38 (1.6)	0.59 (0.02)
Contrasts					
Mineral vs. Manure	<0.01	<0.01	<0.01	0.05	0.05
Charcoal vs. Tannin	ns	ns	ns	0.06	ns
Feed vs. mix	ns	ns	ns	ns	ns
Initial vs. First GS	ns	<0.01	ns	<0.01	<0.01
First GS vs. second GS	<0.01	<0.01	<0.01	<0.01	<0.01
CV (±%)	17	20	18	16	16

CV = mean coefficient of variation between replicates within one plot (n=4), GS = growing season. The values in parentheses represent the standard error of the means (initial contents: n= 72, treatments n=12)

5.4 Discussion

The SOC content of 7-11 mg g^{-1} soil at 0-10 cm depth was considerably higher than the mean SOC content of 4 mg g^{-1} for the Batinah region given by Cookson and Lepiece (1996). The higher contents of the current investigation were most likely caused by continuous application of organic matter to soil (Siegfried et al. 2011). For this reason, the soil microbial biomass C contents were in the range of a sandy arable site in temperate climatic conditions (Heinze et al. 2010). This shows again that the contents of SOC and soil microbial biomass are in the same range in irrigated soils from arid areas in comparison with those from humid temperate regions if soils are similar in texture and adequately supplied with organic matter (Wardle 1998; Wichern et al. 2004).

The numerous significant differences in the majority of soil properties between the initial values and between the two growing seasons indicate that microbial biomass C, microbial residue C, and SOC are all highly dynamic, responding quickly to changes in environmental conditions and organic matter supply. The general positive effects of manure application on SOC and especially on microbial biomass C are in agreement with numerous investigations on sandy soils all over the world (Christensen 1988; Marinari et al. 2006; Murugan and Kumar 2013), but this has rarely been shown in arid regions with slightly saline soils (Siegfried et al. 2011). As stated in the first hypothesis, microbial biomass C revealed the fastest response, followed by microbial residue C and finally SOC, showing the slowest response to the cropping practices carried out in the present experiment.

After only 2 years of manure fertilization with charcoal, soils have significantly higher contents of SOC and soil C/N ratios, due to the recalcitrance of charcoal C (Preston and Schmidt 2006). The percentage of SOC present as microbial biomass C or as microbial residue C can be simply explained by the accumulation of charred material. No additional effects of charcoal on microbial biomass or microbial residues were detected after application of charcoal-enriched goat manure, contradicting the second hypothesis stated in the introduction (Pietikäinen et al. 2000; Blackwell et al. 2010). One reason may be that manure effects on the microbial community masked any charcoal effect. The same might be true for the use of tannins as goat manure additives, which did not show any direct effect on any soil chemical or soil biological property analyzed, contradicting also the third hypothesis stated in the introduction.

However, effects of charcoal and tannins as feed or manure additives on SOC sequestration and soil microbial biomass and residues are only one aspect for vegetable production under irrigated subtropical conditions, on calcareous and slightly saline sandy soils, which are prone to N_2O gas emission and nitrate leaching. The significantly lower values of the K_2SO_4 extractable N to N total in samples from April 2012 (Table 1) are probably due to the ability of tannins to form complexes in the rumen (Patra and Saxena 2011) and immobilize mineral N in soil (Kraus et al. 2003). The reduction in CH_4 and N_2O emissions from the ruminants (Patra and Saxena 2011) and later the reduction in N_2O emissions from soil to the atmosphere are an even more important rationale behind these treatments. Charcoal and tannins might also reduce nitrate leaching to the subsoil in irrigated agriculture (Kraus et al. 2003). However, the absence of clear charcoal and tannin effects on most of the microbial indices does not allow any clear statement concerning our fourth hypothesis, whether feeding or direct mixing are more appropriate means for SOC sequestration.

It is a striking feature of the present results that microbial residue C is dominated by bacterial residues, contrasting the situation in soils from temperate humid climate (Appuhn et al. 2006; Joergensen and Wichern 2008; van Groenigen et al. 2010). The reasons may be that bacteria dominate the soil microbial community or that the turnover of fungal residues is much faster than that of bacterial residues. Rottmann et al. (2011) concluded that litter decomposition in Oman is controlled by fungi, as indicated by the very high fungal C to bacterial C ratios initially developing on crop residues buried in nylon mesh-bags at the same experimental site. The ergosterol to microbial biomass C ratio is in the higher range of sandy soils, developed under temperate climatic conditions (Ellmer et al. 2000; Heinze et al. 2010; Murugan and Kumar 2013). This indicates a similar presence of saprotrophic fungi and suggests the absence of any relationship between fungal biomass and fungal residues, contrasting the view stated of Appuhn et al. (2006). As the application of ruminant manure has been shown to promote bacteria (Scheller and Joergensen 2008; Walsh et al. 2012), the dominance might be caused by the high application rates of ruminant manure to a soil initially very low in organic matter.

Interestingly the fungal C to bacterial C ratio in the current soil is similar to that of cattle feces (Jost et al. 2013), but did not seriously differ between the mineral and manure treatments. This supporting the view of Řzáčová et al. (2007) that fungal biomass is not affected by different types of fertilization. A shift in microbial community structure towards bacteria with an increasing salinity has been repeatedly shown, using linoleic acid

(Pankhurst et al. 2001) or ergosterol (Sardinha et al. 2003) as indicators for fungi. This has been explained by the higher sensitivity of fungi to osmotic stress. The low fungal C to bacterial C ratio in the alkaline soils of the Omani Batinah region might also be due to a stronger turnover of fungal residues than of bacterial residues, e.g. by the chitinolytic Gram positive actinobacteria, highly abundant at high pH (Lauber et al. 2009) and containing roughly 3 times higher concentrations of MurN in their cell walls in comparison with Gram negative bacteria (Appuhn and Joergensen 2006). However, a stronger turnover of fungal residues than of bacterial residues is in clear contradiction to the common view stated by others (Guggenberger et al. 1999; Amelung 2001; Six et al. 2006; Liang et al. 2007; He et al. 2011). It seems that under the recent conditions the role of fungal cell walls in SOC accumulation is lesser than those of bacterial origin. The responses of microbial residues to recent management changes might be masked by the legacy effects of SOC accumulation in the past (Stromberger et al. 2007). Therefore, the use of amino sugar specific analysis of ^{15}N and ^{13}C will make it possible to elucidate the turnover of fungal and bacterial residues in the near future (He et al. 2011; Indorf et al. 2012; Bai et al. 2013).

The contribution of microbial residue C to SOC is relatively low, which may be caused again by the rapid turnover of microbial residues or by their specific elution with the excess irrigation water. In soils of temperate Europe values between 50 and 70%, using amino sugar analysis to determine microbial residues, are common (Appuhn et al. 2006), but have not always been observed (van Groenigen et al. 2010). Not only was the contribution of microbial residue C to SOC relatively low, but the contribution of GalN (17% w/w) to the total sum of amino sugars was only roughly half of the usual percentage observed in soil hydrolysates (Guggenberger et al. 1999; Glaser et al. 2004; Indorf et al. 2011). GalN in soil is still considered to be mainly of bacterial origin (Amelung et al. 2008), which has sometimes been supported by correlation analysis (Rottmann et al. 2011). However, the function of GalN for soil microorganisms and consequently the origin and processes behind the GalN formation during decomposition processes are still unknown (Indorf et al. 2011). Mucins and mucous substances are most likely the dominating source of GalN (Turck et al. 1993; Xu et al. 2004), forming extracellular polymeric substances (EPS) surrounding microbial cells, most likely also in soil (Costerton et al. 1981; Wright et al. 1996; Aguilera et al. 2008). This suggests that soil microbial EPS make a minor contribution to SOC in the soil of the Omani Batinah region, for unknown reasons. As the K_2SO_4 extractable fraction is relatively high, especially before the growing season after a long period of drought, elution, but also the numerous drying and rewetting cycles may be

a reason for the low GalN content of the current soils. After rewetting, dead microbial tissue such as EPS could be rapidly mineralized by reactivated soil microbial community (van Gestel et al. 1993). However, this view needs considerably more experimental evidence.

5.5 Conclusions

Goat manure revealed significant positive effects an all soil organic matter and soil microbial indices analyzed, even after a short 2-year application period to a calcareous and slightly saline sandy soil under irrigated subtropical conditions. This makes an important contribution to the stabilization of organic material in C and N balances. Soil microbial biomass C revealed the fastest response, followed by microbial residue C and finally SOC, showing the slowest response to the cropping practices carried out in the present experiment. At the end of the experiment, the microbial biomass reached contents similar to those of sandy soils under temperate humid climate and showed a relatively high contribution of saprotrophic fungi. In contrast, microbial residues, based on amino sugar measurements, were dominated by bacterial residues and contributed relatively low concentrations to SOC. Charcoal added to manure has increasing effects only on the SOC content and the soil C/N ratio, but not on any of the soil microbial properties analyzed. Tannins added to manure did not show further effects on any property analyzed. It made no difference whether charcoal or tannins were supplied as feed additive or directly mixed into the manure. In the future, the analysis of long-term effects of tannin and charcoal applications to fields will help to detect their role in N and C cycling. The determination of amino sugar specific isotopic signature (^{15}N and ^{13}C) will identify the dynamic of microbial residue turnover under arid subtropical conditions.

Acknowledgements

5.6 References

Aguilera, M., Quesada, M.T., del Águila, V.G., Morillo, J.A., Rivadeneyra, M.A., Ramos-Cormenzana, A., Monteoliva-Sánchez, M., 2008. Characterisation of *Paenibacillus jamilae* strains that produce exopolysaccharide during growth on and detoxification of olive mill wastewaters. Biores. Technol. 99, 5640-5644

Amelung, W., 2001. Methods using amino sugars as markers for microbial residues in soil. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (eds) Assessment methods for soil carbon. Adv. Soil. Sci. 100, 233-270

Amelung, W., Brodowski, S., Sandhage-Hofmann, A., Bol, R., 2008. Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. Adv. Agron. 100, 155-250

Appuhn, A., Joergensen, R.G., 2006. Microbial colonisation of roots as a function of plant species. Soil Biol. Biochem.38, 40-51

Appuhn, A, Joergensen, R.G., Scheller, E., Wilke, B., 2004. The automated determination of glucosamine, galactosamine, muramic acid and mannosamine in soil and root hydrolysates by HPLC. J. Plant Nutr. Soil Sci. 167, 17-21

Appuhn, A., Scheller, E., Joergensen, R.G., 2006. Relationships between microbial indices in roots and silt loam soils forming a gradient in soil organic matter. Soil Biol. Biochem. 38, 2557-2564

Badalucco L., Rao M., Colombo C., Palumbo G., Laudicina V.A., Gianfreda L. 2010. Reversing agriculture from intensive to sustainable improves soil quality in a semiarid south Italian soil. Biol. Fertil. Soils 46:481-489

Bai, Z., Bodé, S., Huygens, D., Zhang, X., Boeckx, P., 2013. Kinetics of amino sugar formation from organic residues of different quality. Soil Biol. Biochem. 57, 814-821

Blackwell, P., Krull, E., Butler, G., Herbert, A., Solaiman, Z., 2010. Effect of banded biochar on dryland wheat production and fertiliser use in south-western Australia: an agronomic and economic perspective. Aust. J. Soil Res. 48, 531-545

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837-842

Chapman, H.D., Pratt, P.F., 1961. Methods of Analysis for Soils, Plants and Waters. University of California, Division of Agricultural Sciences, Riverside, USA

Christensen, B.T., 1988. Effects of animal manure and mineral fertilizer on the total carbon and nitrogen contents of soil size fractions. Biol. Fertil. Soils 5, 304-30

Cookson, P., Lepiece., A.G., 1996. Urease enzyme activities in soils of the Batinah region of the Sultanate of Oman. J. Arid Environ. 32, 225-238

Costerton, J.W., Irvin, R.T., Cheng, K.J., 1981. The bacterial glycocalyx in nature and disease. Ann. Rev. Microbiol. 35, 299-324

Dempster, D.N., Jones, D.L., Murphy, D.V., 2012. Organic nitrogen mineralisation in two contrasting agro-ecosystems is unchanged by biochar addition. Soil Biol. Biochem. 48, 47-50

Djajakirana, G., Joergensen, R.G., Meyer, B. 1996. Ergosterol and microbial biomass relationship in soil. Biol. Fertil. Soils 22, 299-304

Ellmer, F., Peschke, H., Köhn, W., Chmielewski, F.M., Baumecker, M., 2000. Tillage and fertilizing effects on sandy soils. Review and selected results of long-term experiments at Humboldt-University Berlin. J. Plant Nutr. Soil Sci. 163, 267-272

Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biol. Biochem. 39, 2111-2118

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. Biol. Fertil. Soils 35, 219-230

Glaser, B., Turrión, M.B., Alef, K., 2004. Amino sugars and muramic acid—biomarkers for soil microbial community structure analysis. Soil Biol. Biochem. 36, 399-407

van Groenigen, K.J., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bodé, S., Forristal, D., Jones M.B., 2010 Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. Soil Biol. Biochem. 42, 48-55

van Gestel M., Merckx R., Vlassak K. 1993. Soil drying and rewetting and the turnover of 14C-labeled plant residues: first order decay rates of biomass and non-biomass 14C. Soil Biol Biochem 25:125-134

Guggenberger, G., Frey, S.D., Six, J., Paustian, K., Elliott, E.T., 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Sci. Soc. Am. J 63, 1188-1198

Hagerman, A.E., Zhao, Y., Johnson, S., 1997. Methods for determination of condensed and hydrolyzable tannins. In: Shahidi F (ed) Antinutrients and phytochemicals in food. American Chemical Society, Washington D.C, pp 209-222

Halvorson, J.J., Gonzalez, J.M., Hagerman, A.E., 2011. Repeated applications of tannins and related phenolic compounds are retained by soil and affect cation exchange capacity. Soil Biol. Biochem. 43, 1139-1147

Haslam, E., 1981. Vegetable tannins. In: Conn EE (ed) The biochemistry of plants. Secondary plant products, vol. 7. Academic Press, New York, pp 527-556

He, H.B., Li, X.B., Zhang, W., Zhang, X.D., 2011. Differentiating the dynamics of native and newly immobilized amino sugars in soil frequently amended with inorganic nitrogen and glucose. Eur. J. Soil Sci. 62, 144-151

Heinze, S., Raupp, J., Joergensen, R.G., 2010. Effects of fertilizer and spatial heterogeneity in soil pH on microbial biomass indices in a long-term field trial of organic agriculture. Plant Soil 328, 203-215

Indorf, C., Dyckmans, J., Khan, K.S., Joergensen, R.G., 2011. Optimisation of amino sugar quantification by HPLC in soil and plant hydrolysates. Biol. Fertil. Soils 47, 387-396

Indorf, C., Stamm, F., Dyckmans, J., Joergensen, R.G., 2012. Determination of saprotrophic fungi turnover in different substrates by glucosamine-specific δ^{13}C liquid chromatography/isotope ratio mass spectrometry. Fungal Ecol. 5, 694-701

Joanisse, G.D., Bradley R.L., Preston, C.M., Munson, A.D., 2007. Soil enzyme inhibition by condensed litter tannins may drive ecosystem structure and processes: the case of *Kalmia angustifolia*. New Phytol. 175, 535-546

Joergensen, R.G., Mäder, P., Fließbach, A., 2010. Long-term effects of organic farming on fungal and bacterial residues in relation to microbial energy metabolism. Biol. Fertil. Soils 46:303-307

Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil Soil Biol. Biochem. 40, 2977-2991.

Jost, D.I., Joergensen, R.G., Sundrum, A., 2013. Effect of cattle faeces with different microbial biomass content on soil properties, gaseous emissions and plant growth. Biol. Fertil. Soils 49, 61-70

Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. Soil Biol. Biochem. 43, 385-392

Kraus, T.E.C., Dahlgren, R.A., Zasoski, R.J., 2003. Tannins in nutrient dynamics of forest ecosystems - a review. Plant Soil 256, 41-66

Kumar, R., Vaithiyanathan, S., 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. *Anim. Feed Sci. Technol.* 30, 21-38

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microb. 75, 5111-5120

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D. 2011. Biochar effects on soil biota – A review. Soil Biol. Biochem. 43, 1812-1836

Lentz, D., Ippolito, A., 2012. Biochar and manure affect calcareous soil and corn silage nutrient concentrations and uptake. J. Environ. Qual. 41, 1033-1043

Liang, C., Zhang, X., Rubert, K.F., Balser, T.C., 2007. Effect of plant materials on microbial transformation of amino sugars in three soil microcosms. Biol. Fertil. Soils 43, 631-639

Marinari, S., Mancinelli, R., Campiglia, E., Grego, S., 2006. Chemical and biological indicators of soil quality in organic and conventional farming systems in central Italy. Ecol. Indic. 6, 701-711

McHenry, M.P., 2010. Carbon-based stock feed additives: a research methodology that explores ecologically delivered C biosequestration, alongside live weights, feed use efficiency, soil nutrient retention, and perennial fodder plantations. J. Sci. Food Agric. 90, 183-187

Millar, W.N., Casida, L.E., 1970. Evidence for muramic acid in soil. Can. J. Microbiol. 16, 299-304

Murugan, R., Kumar, S., 2013. Influence of long-term fertilisation and crop rotation on changes in fungal and bacterial residues in a tropical rice-field soil. Biol. Fertil. Soils: DOI 10.1007/s00374-013-0779-5

Pankhurst C.E., Yu S., Hawke B.G., Harch B.D. 2001. Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biol. Fertil. Soils 33:204-217

Patra, A.K., Saxena, J. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric. 91, 24-37

Pietikäinen, J., Kiikkilä, O., Fritze, H., 2000. Charcoal as a habitat for microbes and its effects on the microbial community of the underlying humus. Oikos 89:231-242

Preston, C.M., Schmidt, M.W.I., 2006. Black (pyrogenic) carbon in boreal forests: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3, 397-420

Rottmann, N., Siegfried, K., Buerkert, A., Joergensen, R.G., 2011. Litter decomposition in fertilizer treatments of vegetable crops under irrigated subtropical conditions. Biol. Fertil. Soils 47, 71-80

Řzáčová, V., Baldrian, P., Hrselova, H., Larsen, J., Gryndler, M., 2007. Influence of mineral and organic fertilization on soil fungi, enzyme activities and humic substances in a long-term field experiment. Folia Microbiol. 52, 415-421

Scheller, E., Joergensen, R.G., 2008. Decomposition of wheat straw differing in N content in soils under conventional and organic farming management. J. Plant Nutr. Soil Sci. 171, 886-892

Sardinha M., Müller T., Schmeisky H., Joergensen R.G. 2003. Microbial performance in a temperate floodplain soil along a salinity gradient. Appl. Soil Ecol. 23:237-244

Selvakumar, G., Saha, S., Kundu, S., 2007. Inhibitory activity of pine needle tannin extracts on some agriculturally resourceful microbes. Indian J. Microbiol. 47, 267-270

Siegfried, K., Dietz, H., Schlecht, E., Buerkert, A., 2011. Nutrient and carbon balances in organic vegetable production on an irrigated, sandy soil in northern Oman. J. Plant Nutr. Soil Sci. 174, 678-689

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555-569

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., de Macedo, J.L.V., Blum, W.E.H., Zech, W., 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant Soil 291:275-290

Stromberger, M., Shah, Z., Westfall, D., 2007. Soil microbial communities of no-till dryland agroecosystems across an evapotranspiration gradient. Appl. Soil Ecol. 35, 94-106

Turck, D., Feste, A., Lufschitz, C.H., 1993. Age and diet affect the composition of porcine colonic mucins. Pediatric Res. 33, 565-576

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

Walsh, J.J., Rousk, J., Edwards-Jones, G., Jones, D.L., Williams, A.P., 2012. Fungal and bacterial growth following the application of slurry and anaerobic digestate of livestock manure to temperate pasture soils. Biol. Fertil. Soils 48, 889-897

Wardle, D.A., 1998. Controls of temporal variability of the soil microbial biomass: a global scale synthesis. Soil Biol. Biochem. 30, 1627-1637

Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to biochar in soil – concepts and mechanisms. Plant Soil 300, 9-20

Wichern, F., Mueller, T., Joergensen, R.G., Buerkert, A., 2004. Effects of manure quality and application forms on soil C and N turnover of a subtropical oasis soil under laboratory conditions. Biol. Fertil. Soils 39, 165-171

Wright, S.F., Franke-Snyder, M., Morton, J.B., Upadhyaya, A., 1996. Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant Soil 181, 193-203

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction - an automated procedure. Soil Biol. Biochem. 22, 1167-1169

Xu, R., Hanson, S.R., Zhang, Z., Yang, Y.Y., Schultz, P.G., Wong, C.H., 2004. Site-Specific incorporation of the mucin-type N-acetylgalactosamine-α-o-threonine into protein in *Escherichia coli*. J. Am. Chem. Soc. 126, 15654-15655

6. Zusammenfassung

Die Gabe von organischem Dünger beeinflusst die Menge und die Zusammensetzung der organischen Substanz im landwirtschaftlich genutzten Boden. Dies wirkt sich auch auf den Umfang der mikrobiellen Biomasse im Boden und ihrer Gemeinschaftsstruktur aus. Neben einem Anstieg der mikrobiellen Biomasse C und N, nach langfristiger organischer Düngung, wird auch der mikrobiell gebundene Schwefel, der Ergosterolgehalt sowie die mikrobielle Aktivität im Vergleich zur mineralischen Düngung beeinflusst.

Multivariate Verfahren, welche das Substratnutzungsmuster der mikrobiellen Gemeinschaft des Bodens bestimmen, gewinnen immer mehr an Bedeutung. So ist es möglich die Auswirkungen von landwirtschaftlicher Bewirtschaftung auf die physiologische Substratnutzungsdiversität zu untersuchen, um so die Funktion der mikrobiellen Gemeinschaft zu bestimmen.

Ein weiterer Aspekt der bisher nur sehr wenig untersucht wurde, ist der Einfluss landwirtschaftlicher Bewirtschaftung auf den Unterboden. Die mikrobiellen Residuen tragen hier einen bedeutenden Anteil zum organischen Kohlenstoffgehalt (>50%) des Bodens bei. Gemessen als Aminozucker können sie durch ihre Spezifität, die Rolle von bakteriellem und pilzlich Residuen in der Kohlenstoffspeicherung beschreiben.

Des Weiteren soll die Dynamik der mikrobiellen Biomasse und ihrer Residuen unter ariden subtropischen Bedingungen untersucht werden. Dieses Nutzungssystem unterscheidet sich durch ihre Temperatur und Feuchtigkeit von mittleren Klimaten, und kann so neue Erkenntnisse bezüglich der Zusammenhänge zwischen der mikrobiellen Biomasse und ihrer Residuen liefern.

Mit dem Ziel, die Auswirkungen der landwirtschaftlichen Bewirtschaftung auf die Funktion der mikrobiellen Gemeinschaft, sowie den Anteil und die Zusammensetzung der mikrobiellen Residuen auf verschiedene Bereiche des Bodens und Nutzungssystemen zu untersuchen, wurden folgende Untersuchungen durchgeführt.

1. Die Erstellung eines Substratnutzungsprofils nach 29 jähriger Rottemistdüngung mit und ohne biodynamischen Präparaten im Vergleich zur mineralischen Düngung, in zwei unterschiedlichen Düngeintensitäten.

2. Untersuchung der mikrobiellen Residualmasse im Bodenprofil bis zu einem Meter Tiefe bezüglich ihrer Akkumulation und spezifischen Zusammensetzung nach 29 jähriger mineralischer Düngung und Rottemistdüngung.

3. Analyse der Dynamik der mikrobiellen Biomasse und der mikrobiellen Residuen nach Düngung mit unterschiedlichen Ziegenmistvarianten, innerhalb 2 -jähriger Bewirtschaftung unter subtropischen Bedingungen, im Vergleich zur mineralischen Düngung.

1. Das erste Experiment beschäftigte sich mit der Fragestellung, wie das physiologische Profil der mikrobiellen Gemeinschaft durch die langfristige Rottemistdüngung im Vergleich zur mineralischen Düngung beeinflusst wird. Es sollte geprüft werden, inwieweit es möglich ist, zwischen geringen (50 kg N ha^{-1}) und hohen (150 kg N ha^{-1}) Düngerstufen zu unterscheiden. Daneben sollte untersucht werden, ob Hinweise zu finden sind, die Funktionsunterschiede zwischen den Behandlungen mit und ohne Zugabe biodynamischer Präparate zeigen. Hierzu wurden Bodenproben vom Langzeitversuch in Darmstadt (29 Jahre) aus dem Oberboden (0-5cm) genommen und die Respiration des Bodens, mittels MicroResp^{TM} nach Zugabe von 17 unterschiedlichen Substraten untersucht.

Es konnte in der vorliegenden Arbeit gezeigt werden, dass es mit der multi-SIR Methode möglich ist, signifikant zwischen Rottemistdüngung und mineralischer Düngung zu unterscheiden. Diese Trennung wurde hauptsächlich durch Kohlenhydrate und Aminosäuren gesteuert. Ebenso korrelierte die extrahierte Diskriminantsfunktion mit dem Boden pH-Wert und dem organischen Kohlenstoff des Bodens. Ergänzend dazu wurde der Substratnutzätsdiversitätsindex nach langjähriger mineralischer Düngung gegenüber der Rottemistdüngung verringert.

Auch wenn die multi-SIR Methode keine Effekte von biodynamischen Präparaten auf die Funktion der mikrobiellen Gemeinschaft zeigen konnte, so war eine Trennung zwischen den Düngeintensitäten bei Rottemistdüngung mit biodynamischen Präparaten und mineralischer Düngung möglich. In Bezug dazu, war die multi-SIR Methode empfindlicher, als die Bestimmung der mikrobiellen Biomasse oder die Bestimmung des organischen Kohlenstoffs im Boden.

2. Im zweiten Experiment wurde getestet, inwieweit die Düngung mit Rottemist die Speicherung des organischen Materials (C_{org} und N) im Unterboden beeinflusst. Die Proben wurden auf der Versuchsfläche in Darmstadt auf den Parzellen mit der höchsten Düngestufe genommen.

Die Akkumulation von Aminozuckern als Anzeiger für die mikrobiellen Residuen wurde unter organischer und mineralischer Düngung bis zu einer Tiefe von einem Meter untersucht. Folgende Hypothesen wurden dazu getestet: (1) der Anteil der mikrobiellen Residuen am gesamten organischen Kohlenstoff steigt mit der Tiefe, (2) der höhere Anteil an mikrobiellen Residuen muss zwangsläufig mit einer Steigerung des organischen Kohlenstoffs im Boden zusammenhängen und (3) das Verhältnis der pilzlichen zu bakteriellen Residuen wird durch organische Düngung im Oberboden verringert und nimmt mit steigender Tiefe ab.

Mit zunehmendem C/N Verhältnis von Oberboden zu Unterboden im sandigen Bodenprofil verringerte sich der Anteil der mikrobiellen Residuen am gesamten organischen Kohlenstoff mit steigender Tiefe. Langfristige organische Düngung führte zu einem deutlich niedrigen Verhältnis von pilzlichen zu bakteriellen Residuen im Vergleich zur mineralischen Düngung im Oberboden. Es konnte auch gezeigt werden, dass die langzeitliche organische Düngung den Gehalt an bakteriellen Residuen im Unterboden gegenüber mineralischer Düngung erhöhte. Da keine Steigerung des gesamten organischen Kohlenstoffs im Unterboden gezeigt werden konnte, kann ein Anstieg des mikrobiellen Umsatzes im Unterboden bei organischer Düngung vermutet werden. Auch eine Erhöhung des 0,5 M K_2SO_4 extrahierbaren Kohlenstoffs unter organischer Düngung unterstützte diese Vermutung. Daneben sank das Verhältnis der pilzlichen zu bakteriellen Residuen von 2,6 im Oberboden auf 2,1 im Unterboden. Diese Veränderung könnte, durch einen pH-Anstieg in der Tiefe oder andere nicht identifizierte Veränderungen des Unterbodens zu erklären sein. Weitere Untersuchungen sind hier noch nötig um die Rolle von mikrobiellen Residuen im Unterboden zu zeigen.

3. Im dritten Versuch wurden die Auswirkungen der Gabe von Ziegenkot, der mit Aktivkohle oder Tanninen behandelt wurde, auf die mikrobielle Biomasse, ihrer Residuen und des organischem Bodenmaterials hin untersucht. Die Applikation der Aktivkohle sowie der Tannine erfolgte als Futterzusätze und durch direkte Verteilung auf dem Feld. In einem zweijährigen Feldversuch wurden unter subtropischen Bedingungen im Oman Bodenproben genommen. Es wurde vermutet, dass die Gabe von Ziegenmist sich positiv

auf die mikrobielle Biomasse, die mikrobielle Residuen und den organischen Bodenkohlenstoff, im Vergleich zur mineralischen Düngung auswirkt. Daneben könnte die Gabe von Aktivkohle einen positiven Effekt auf die Akkumulation von mikrobiellem Kohlenstoff, von mikrobieller Biomasse und von organischem Kohlenstoff im Boden haben. Für die Gabe von Tanninen werden ähnliche Auswirkungen vermutet. Doch könnte es negative Effekte auf die mikrobielle Biomasse, wegen ihrer antimikrobiellen Eigenschaft geben.

Nach zweijähriger Versuchsphase stieg die mikrobielle Biomasse im Boden nach organischer Düngung am stärksten gegenüber der mineralischen Düngung an. Danach folgten die mikrobiellen Residuen und der organische Kohlenstoffgehalt. Nach Beendigung des Versuches erreicht die mikrobielle Biomasse einen Gehalt, der vergleichbar ist mit Böden ähnlicher Textur und Nutzung in gemäßigten Klimaten. Der Anteil des Ergosterols an der gesamten mikrobiellen Biomasse deutete darauf hin, dass der Boden durch einen hohen Gehalt an saprotrophischen Pilzen gekennzeichnet war. Zudem zeigten die Untersuchungen der mikrobiellen Residuen, dass diese von der bakteriellen Residualmasse dominiert waren und einen relativen geringen Anteil am gesamten organischen Kohlenstoff ausmachten. Die Aktivkohle hatte einen positiven Einfluss auf die organische Bodensubstanz und die Zugabe von Tanninen erhöhte den extrahierbaren Stickstoff im Boden. Doch gab es keine Effekte durch die unterschiedlichen Applikationsmethoden auf dem Versuchsfeld.

Zusammenfassend ist zu sagen, dass die organische Düngung sich positiv auf die Bodenqualität auswirkt. Nicht nur das physiologische Profil der mikrobiellen Gemeinschaft wird positiv beeinflusst, sondern auch die mikrobiellen Residuen im gesamten Bodenprofil werden durch organische Düngung im Vergleich zur mineralischen Düngung erhöht. Die Rolle der mikrobiellen Biomasse und ihrer Residuen als Anzeiger für die Veränderungen in der mikrobiellen Gemeinschaftsstruktur wurde in der vorliegenden Arbeit dargestellt. So wirkten sich eine unterschiedliche langzeitliche Düngung, die Tiefe des Bodenprofils (0 – 100 cm) und das Klima (gemäßigt und subtropisch) auf das Verhältnis von pilzlichen zu bakteriellen Residuen aus. Dies zeigt einmal mehr, dass diese als Indikator für Veränderungen in der mikrobiellen Gemeinschaftsstruktur herangezogen werden können. Speziell in Nutzungssystemen mit einem schnellen Umsatz der organischen Substanz, wie unter ariden subtropischen Bedingungen, bildet die Bestimmung der mikrobiellen Biomasse und ihrer Residuen eine Möglichkeit, die

Dynamik der mikrobiellen Biomasse und der organischen Substanz durch Düngung zu dokumentieren.

7. Summary

The application of organic fertilizer affects the quantity and composition of organic matter in agricultural soils. This also has an impact on the amount of soil microbial biomass and its community structure. Long-term organic fertilization increased the microbial biomass C and N compared to mineral fertilization.

Multivariate methods, which determine the substrate utilization profile of the soil microbial community, becoming more and more important. So it is possible to determine the effects of agricultural management on the substrate utilization diversity and to measure the functional diversity of the microbial community.

Another aspect which has been studied very little is the impact of agricultural management in subsoil. The microbial residues contribute a specified percentage yield (>50%) of soil organic carbon. Highly specific amino sugars are very useful indicators to differentiate the contribution of bacterial and fungal residues to C sequestration in soil.

Furthermore, the dynamics of soil microbial biomass and residues are determined under arid subtropical conditions, due to their differences in temperature and humidity compared to temperate conditions. This may provide new insights on the relationship between soil microbial biomass and microbial residues.

The effects of agricultural management in different soil depths and agriculture systems were studied under temperate and tropical conditions. With the objective to investigate the microbial community function as well as the proportion and composition of the microbial residuals, the following investigations were carried out.

1 Measurement of substrate utilization profile after 29 years of farmyard manure with and without biodynamic preparations compared to mineral fertilization, in two different fertilization intensities.

2 Investigation of microbial residuals in a soil profile down to one meter depth on the specific composition of the bacterial and fungal residuals after 29 years of fertilization with manure and mineral fertilizer.

3 Analysis of the dynamics of soil microbial biomass and microbial residues after fertilization with different goat manure qualities under subtropical conditions within two years of cultivation.

1. The effects of cattle manure without and with biodynamic preparations on functional diversity of the soil microbial community were investigated in comparison with mineral fertilization (+ straw incorporation) at low (50 kg N ha^{-1}) and high (150 kg N ha^{-1}) application rates. The soil samples were taken from long-term trial (29 years) in Darmstadt, Germany, from the topsoil (0-5cm). The substrate utilization profiles of microbial community were measured using the Microresp^{TM} system after the addition of 17 different substrates. In this study it was demonstrated that it is possible to distinguish significantly between manure and mineral fertilizer. This separation was controlled mainly by carbohydrates and amino acids. The extracted discriminate function was correlated well with the soil pH and organic carbon content. In addition, the Shannon diversity index reduced after long-term mineral fertilization compared to manure application. The current multi-SIR method was unable to separate the manure treatments without and with biodynamic preparations. In contrast, the multi-SIR method was able to differentiate between high and low application rates of mineral fertilizer and between high and low application rates of composted farmyard manure with biodynamic preparations. Our results suggest that the multi-SIR method is more sensitive indicator than the total microbial biomass and soil organic matter indices.

2. In the second experiment it was tested whether the fertilization with manure affects the accumulation of organic matter (SOC and N) in the subsoil. The samples were taken from the experimental area in Darmstadt on the plots with the highest fertilizer level.

The accumulation of amino sugars as the indicator for microbial residuals was examined by long-term organic and mineral fertilization down to a depth of one meter. The following hypotheses were tested to: (1) the relative contribution of microbial residues to SOC increases with depth, especially in the manure treatment, (2) the increased formation of microbial residues does not lead to an increased subsoil organic carbon sequestration in the manure treatment, and (3) the ratio of fungal to bacterial residues is lower in the manure treatment and generally declines with depth.

In the topsoil, long-term manure application led to a significantly lower fungal C to bacterial C ratio in comparison with the mineral fertilization. With increasing C/N ratio from topsoil to subsoil in this sandy soil profile, the proportion of microbial residues reduced of total organic carbon decreases with increasing depth. It was shown that a long time organic fertilization increases the bacterial residues in the subsoil compared with mineral fertilization. Since no increase in the total organic carbon was found in the subsoil,

an increase of microbial turnover is suspected in the subsoil under organic fertilization. An increase of 0.5 M K_2SO_4-extractable carbon under organic fertilization shows their influence on the subsoil. In addition, the ratio of fungal to bacterial residuals decreased from 2.6 in the topsoil to 2.1 in the subsoil. This was mainly explained by a pH increase in the depth, or other changes in subsoil environmental conditions. Further studies will be necessary to show here the role of microbial residues in the subsoil.

3. As a third test the effects, of goat manure treated with activated charcoal and tannins on microbial biomass properties there residuals and the organic soil material in comparison to mineral fertilization, were investigated. The application of the activated carbon and the tannins were performed as feed additives and direct distribution in the field. In a two-year field experiment soil samples were taken in Oman under subtropical conditions. It has been suggested that the administration of goat manure have an influence on the microbial biomass, microbial residues and soil organic carbon. It can be that activated carbon has a positive effect on the microbial accumulation of carbon, the microbial biomass and the organic carbon in the soil. For the addition of tannins similar effects are suspected, but there could be negative effects on the microbial biomass, due there antimicrobial properties.

After a two-year trial period, the microbial biomass increased after organic fertilization compared to mineral fertilization, followed by the increases of microbial residues and soil organic carbon content. After completion of the experiment, the microbial biomass reached a level that is comparable with similar soil texture and use in temperate climates. The proportion of ergosterol on total microbial biomass indicates a high proportion of saprotropic fungi under the present subtropical conditions. The composition of microbial residues showed that the microbial community was dominated by bacteria but account for a relative small proportion of the total organic matter. However the activated carbon had a positive impact on soil organic matter content and the addition of tannins increases the extractable nitrogen in soil, but had no significant effect by feeding or the direct addition on the trial field.

In summary it can be said that the organic fertilizer has a positive effect on soil quality. Not only the physiological profile of the microbial community is positively affected, but also the microbial residues in the entire soil profile (0 - 100 cm) were increased by organic fertilization compared to mineral fertilization. The role of microbial biomass and its residuals as an indicator of the changes in the microbial community structure was shown in

the present work. Thus, a different long-time fertilization, depth (of the soil profile) and climate (temperate and subtropical) had an effect on the ratio of fungal to bacterial residuals. This shows that they can be used as an indicator of changes in the microbial community structure. Especially in subtropical arid conditions, a system with a rapid turnover of soil organic matter, the microbial biomass and residues can serve as a useful indicator to document the role of microbial community on the dynamics of soil organic matter under different fertilization regimes.

8. Schlussfolgerung und Ausblick

Wie in der vorliegenden Dissertation gezeigt wurde, führt die organische Düngung im Vergleich zur konventionellen mineralischen Düngung zu einer Veränderung der Bodeneigenschaften. Nicht nur der Gehalt der mikrobiellen Biomasse und deren Residuen, sondern auch das Substratnutzungsprofil im Oberboden und die mikrobiellen Residuen im Unterboden wurden durch die Qualität des Düngemittels verändert, was zum Erhalt der Bodenqualität, auch unter ariden subtropischen Bedingungen beiträgt. Das Verhältnis von pilzlichen Residuen zu bakteriellen Residuen wurde in dieser Arbeit als Indikator für Veränderungen der mikrobiellen Gemeinschaft hervorgehoben.

Die Bestimmung des Substratnutzungsprofils und der funktionellen Diversität mittels MicroResp^{TM} sind hilfreiche Indikatoren, um die Auswirkungen langfristiger Düngegaben zu beschreiben. Vor allem die positive Wirkung der Rottemistdüngung konnte mit Hilfe dieser Methode dokumentiert werden. Trotzt der gemessenen Unterschiede war eine direkte Interpretation der Substratnutzung ohne Kenntnis der funktionellen Gruppen der mikrobiellen Gemeinschaft, welche für Funktionsunterschiede verantwortlich sind, allein mit dieser Methode nicht möglich. Gründe hierfür liegen in erster Line an der fehlenden Verbindung zwischen mikrobieller und funktioneller Diversität des Bodens (Nannipieri, et al. 2003). Bis heute wurde keine Methode entwickelt, welche dieses fehlende Bindeglied schließen könnte. Eine Kombination von selektiven Inhibitionsmethoden mit einem Substratnutzungsprofil und einer molekulargenetischen Bestimmung der mikrobiellen Gemeinschaft könnte eine Möglichkeit sein, diese Lücke zu schließen. Ebenfalls könnte eine genaue Evaluierung der potentiellen Kurzzeitsubstratnutzung von arbuskulären Mykorrhizapilzen und saprotrophischen Pilzen, sowie von grampositiven und gramnegativen Bakterien, eine Interpretation der Ergebnisse erleichtern.

Wie in der vorliegenden Arbeit gezeigt wurde, scheint die Gabe von organischen und mineralischen Düngemitteln sich auf das Bodenprofil bis zu einem Meter Tiefe auszuwirken. Vor allem die mikrobiellen Residuen bakteriellen Ursprungs erhöhten sich im gesamten Bodenprofil durch die organische Düngung im Vergleich zur mineralischen Düngung, was sich aber nicht auf den Gehalt des organischen Kohlenstoffs des Bodens auswirkte. Bei der beobachteten Verringerung des Verhältnisses pilzlicher zu bakterieller Residuen, konnte nicht ausgeschlossen werden, dass der pH-Wert des Bodens oder andere nicht identifizierte Mechanismen hierfür verantwortlich sind. Eine Analyse der mikrobiellen Residualmasse im Unterboden anhand anderer Langzeitversuche, die den

Einfluss organischer und mineralischer Düngung untersuchen, könnten die gewonnenen Vermutungen erhärten. Damit könnte die Rolle von mikrobiellen Residuen, als Indikator für die Qualitätsveränderung der organischen Substanz im Unterboden und den Einfluss von Düngung auf die Nährstoffmobilisierung im Unterboden, bewertet werden. Auch eine Evaluierung der Funktion der mikrobiellen Gemeinschaft mittels Streubeuteleintrag könnte hier Antworten liefern (Rottmann et al. 2011). Zudem könnte eine aminozucker-spezifische δ^{13}C-Analyse, wie von Indorf et al. (2011) und Bodé et al. (2009) beschrieben, die Dynamik von mikrobiellen Residuen im Unterboden aufzeigen.

Auch wurde gezeigt, dass sich die mikrobielle Biomasse und ihre Residuen schon nach kurzer Versuchsdauer, unter subtropischen Bedingungen signifikant erhöhten. Ebenso wurde hier eine Dominanz der bakteriellen Residuen dokumentiert. Interessanterweise suggerierte das Verhältnis von Ergosterol und mikrobiellen Biomassekohlenstoff einen bedeutenden Anteil saprotrophischer Pilze im Boden. Eine Analyse des Bodens mittels PLFA oder molekulargenetische Untersuchungen könnte die Diskrepanz zwischen mikrobielle Residuen und der mikrobiellen Biomasse erklären. Zwar konnte in dieser Arbeit kein bedeutender Einfluss auf die mikrobiellen Parameter durch Aktivkohle und Tanninen nachgewiesen werden, doch könnte eine längere Versuchsdauer deutlichere Effekte zeigen.

9. Literaturverzeichnis

Aerts, R., 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79, 439-449.

Agnelli, A., Ascher, J., Cortia, G., Ceccherini, M. T., Nannipieri, P., Pietramellara, G. 2004. Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil Biol. Biochem. 36, 859-868

Amelung W., 2001. Methods using amino sugars as markers for microbial residues in soil. In: Lal R., Kimble, J.M., Follett, R.F., Stewart, B.A. (eds) Assessment methods for soil carbon. Adv. Soil Sci. 100, 233-270

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215-221.

Appuhn, A, Joergensen, R.G., Scheller, E., Wilke, B., 2004. The automated determination of glucosamine, galactosamine, muramic acid and mannosamine in soil and root hydrolysates by HPLC. J. Plant Nutr. Soil Sci. 167, 17-21

Appuhn, A., Joergensen, R.G., 2006. Microbial colonisation of roots as a function of plant species. Soil Biol. Biochem. 38, 40-51

Bodé, S., Denef, K., Boeckx, P., 2009. Development and evaluation of a high-performance liquid chromatography isotope ratio mass spectrometry methodology for δ13C analysis of amino sugars in soil. Rapid Comm. Mass Spec. 23, 2519-2526.

Brookes, P.C., 2001. The soil microbial biomass: Concept, Measurement and Applications in soil ecosystem research. Microb. Environ. 16, 131-140.

Buerkert, A., Jahn, H., Golombek, S. D., Al Rawahi, M. N., Gebauer, J., 2010. Carbon and nitrogen emissions from stored manure and cropped fields in irrigated mountain oases of Oman. J. Agri. Res. Trop. Subtrop. 111, 55-63.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from C substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl. Environ. Microbiol. 69, 3593-3599.

Carter, M.R., Gregorich, E.G., Anderson, D.W., Doran, J.W., Janzen, H.H., Pierce, F.J., 1997. Concepts of soil quality and their significance. In: Gregorich, E.G., Carter, M.R. (Eds.), Soil Quality for Crop Product. Elsevier Science Publisher, Amsterdam, Netherlands, pp. 1-19.

Chapman, S.J., Campbell, C.D., Artz, R.R.E., 2007. Assessing CLPPs using MicroResp™ - A comparison with Biolog and multi-SIR. J. Soils Sediments 7, 406-410.

Craine, J. M., Wedin, D. A., Chapin, F. S., Reich, P. B. 2003. Relationship between the structure of root systems and resource use for 11 North American grassland plants. Plant Ecol. 165, 85-100.

Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biol. Biochem. 29, 1309-1320.

Degens, B.P., Schipper, L.A., Sparling, G.P., Duncan, L.C., 2001. Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biol. Biochem. 33, 1143-1153.

Ding, X., Han, X., & Zhang, X., 2013. Long-term impacts of manure, straw, and fertilizer on amino sugars in a silty clay loam soil under temperate conditions. Biol. Fertil. Soils 10.1007/s00374-012-0768-0.

Ekelund, F., Rønn, R., Christensen, S., 2001. Distribution with depth of protozoa, bacteria and fungi in soil profiles from three Danish forest sites. Soil Biol. Biochem. 33, 475-481.

Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biol. Biochem 39, 2111-2118.

Ferrero, M.Á., Aparicio, L.R., 2010. Biosynthesis and production of polysialic acids in bacteria. Appl. Microbiol. Biotechnol. 86,1621-1635

Fierer, N., Schimel, J. P., Holden, P. A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35, 167-176

Flessa, H., Amelung, W., Helfrich, M., Wiesenberg, G. L. B., Gleixner, G., Brodowski, S., Rethemeyer, J., Kramer, C., Grootes, P. M., 2008. Storage and stability of organic matter and fossil carbon in a Luvisol and Phaeozem with continuous maize cropping: A synthesis. J. Plant Nutr. Soil Sci. 171, 36-51.

Fließbach, A., Oberholzer, H.R., Gunst, L., Mäder, P., 2007. Soilorganic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agric. Ecosyst. Environ. 118, 273-284

Fließbach, A., Mäder, P., 2000. Microbial biomass and size-density fractions differ between soils of organic and conventional agricultural systems. Soil Biol. Biochem. 32, 757-768.

Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277-280.

Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57, 2351-2359.

Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl. Environ. Microbiol. 69, 1800–1809.

Glaser, B., Turrión, M.B., Alef, K., 2004. Amino sugars and muramic acid - biomarkers for soil microbial community structure analysis. Soil Biol. Biochem. 36, 399-407

Guggenberger, G., Frey, S.D., Six, J., Paustian, K., Elliott, E.T., 1999. Bacterial and fungal cell wall residues in conventional and no-tillage agroecosystems. Soil Sci. Soc. Am. J. 63, 1188-1198

Guo, Y., Amundson, R., Gong, P., Yu, Q., 2006. Quantity and spatial variability of soil carbon in the Conterminous United States. Soil Sci. Soc. Am. J. 70, 590-600.

Heinze, S., Oltmanns, M., Joergensen, R.G., Raupp, J., 2011. Changes in microbial biomass indices after 10 years of farmyard manure and vegetal fertilizer application to a sandy soil under organic management. Plant Soil 343, 221-234.

Heinze, S., Raupp, J., Joergensen, R.G., 2010. Effects of fertilizer and spatial heterogeneity in soil pH on microbial biomass indices in a long-term field trial of organic agriculture. Plant Soil 328, 203-215.

Heitkamp, F., Raupp, J., Ludwig, B., 2009. Impact of fertilizer type and rate on carbon and nitrogen pools in a sandy Cambisol. Plant Soil 319, 259-275

IFOAM (International Federation of organic agricultural movements) 1998. Basic standards of organic production and processing. Belgium. European communities.

Jarecki, M., Lal, R., 2003. Crop management for soil carbon sequestration. Crit. Rev. Plant Sci. 22, 471-502.

Jenkinson, D.S., 1977. The soil biomass. In: Brookes, P.C., 2001. The soil microbial biomass: Concept, Measurement and Applications in soil ecosystem research. Microb. Environ. 16, 131-140.

Joergensen, R.G., Mäder, P., Fließbach, A., 2010. Long-term effects of organic farming on fungal and bacterial residues in relation to microbial energy metabolism. Biol. Fertil. Soils 46, 303-307

Joergensen, R.G., Meyer, B., 1990. Chemical change in organic matter decomposing in and on a forest Rendzina under beech (*Fagus sylvatica L.*). J. Soil. Sci. 41, 17-27.

Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol. Biochem. 40, 2977-2991.

Johnston, A. E., 1986. Soil organic matter, effects on soils and crops. Soil Use Manage. 2, 97-105.

Kautz, T., Amelung, W., Ewert, F., Gaiser, T., Horn, R., Jahn, R., Javaux, M., Kemna A., Kuzyakov, Y., Munch, J-C., Pätzold, S., Peth, S., Scherer, H.W., Schloter, M., Schneider, H., Vanderborght, J., Vetterlein, D., Walter, A., Wiesenberg, G.L.B., Köpke, U (2012). Nutrient acquisition from arable subsoils in temperate climates: A review. Soil Biol. Biochem. 57, 1003-1022

Konopka, A., Oliver, L., Turco, R. F., 1998. The use of carbon substrate utilization patterns in environmental and ecological microbiology. Microb. Ecol. 35, 103–115

Kraus, T.E.C., Dahlgren, R.A., Zasoski, R.J., 2003. Tannins in nutrient dynamics of forest ecosystems - a review. Plant Soil 256, 41-66

Lalor, B.M., Cookson, W.R., Murphy, D.V., 2007. Comparison of two methods that assess soil community level physiological profiles in a forest ecosystem. Soil Biol. Biochem. 39, 454-462.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microb. 75, 5111-5120

Leckie, S.E., 2005. Methods of microbial community profiling and their application to forest soils. Forest Ecol. Manag. 220, 88-106.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – A review. Soil Biol. Biochem. 43, 1812-1836

Liang, C., Balser, T.C., 2008. Preferential sequestration of microbial carbon in subsoils of a glacial-landscape toposequence, Dane County, WI, USA. Geoderma 148, 113–119.

Loisel, P., Harmand, J., Zemb, O., Latrille, E., Lobry, C., Delgenes, J.P., Godon, J.J., 2006. Denaturing gradient electrophoresis (DGE) and single-strand conformation polymorphism (SSCP) molecular fingerprintings revisited by simulation and used as a tool to measure microbial diversity. Environ. Microbiol, 8, 720-731

McHenry, M.P., 2010. Carbon-based stock feed additives: a research methodology that explores ecologically delivered C biosequestration, alongside live weights, feed use efficiency, soil nutrient retention, and perennial fodder plantations. J. Sci. Food Agric. 90, 183-187

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655-670.

Ngosong, C., Jarosch, M., Raupp, J., Neumann, E., Ruess, L., 2010. The impact of farming practice on soil microorganisms and arbuscular mycorrhizal fungi: Crop type versus long-term mineral and organic fertilization. Appl. Soil Ecol. 46, 134-142.

Oehl, F., Sieverding, E., Mader, P., Dubois, D., Ineichen, K., Boller, T., Wiemken, A., 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138, 574-583.

Paustian, K., Six, J., Elliott, E.T., Hunt, H.W., 2000. Management options for reducing CO_2 emissions from agricultural soils. Biogeochemistry 48, 147-16

Raupp, J., Niehus, A., Oltmanns, M., 2004. Die Diversität der Boden-Mikroflora ist bei Rottemistdüngung höher als bei Mineraldüngung. Mitt. Ges. Pflanzenbauwiss. 16, 149-150.

Romaniuk, R., Giuffre, L., Costantini, A., Nannipieri, P., 2011. Assessment of soil microbial diversity measurements as indicators of soil functioning in organic and conventional horticulture systems. Ecol. Indic. 11, 1345-1353.

Rottmann, N., Siegfried, K., Buerkert, A., Joergensen, R.G., 2011. Litter decomposition in fertilizer treatments of vegetable crops under irrigated subtropical conditions. Biol. Fertil. Soils 47, 71-80

Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant and Soil 338, 143-158.

Siegfried, K., Dietz, H., Schlecht, E., Buerkert, A., 2011. Nutrient and carbon balances in organic vegetable production on an irrigated, sandy soil in northern Oman. J. Plant. Nutr. Soil. Sci. 174, 678-689

Six, J., Frey, S.D., Thiet, R.K., & Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555-569.

Stevenson, F.J., 1982. Organic forms of soil nitrogen. In: Stevenson, F.J. (Ed.), Nitrogen in Agricultural Soils. American Society of Agronomy, Madison, pp. 101–104.

Strickland, M.S., Rousk, J., 2010. Considering fungal: bacterial dominance in soils–Methods, controls, and ecosystem implications. Soil Biol. Biochem. 42, 1385-1395.

Taylor, J.P., Wilson, B., Mills, M.S., Burns, R.G., 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biol. Biochem. 34, 387-401.

Tivy, J., 1987. Nutrient cycling in agro-ecosystems. Appl. Geogr. 7, 93-113.

Turck, D., Feste, A., Lufschitz, C.H., 1993. Age and diet affect the composition of porcine colonic mucins. Pediatric Res. 33, 565-576

Wakelin, S.A., Macdonald, L.M., Rogers, S.L., Gregg, A.L., Bolger, T.P., Baldock, J.A., 2008. Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. Soil Biol. Biochem.40, 803-813

Wardle, D.A., 1998. Controls of temporal variability of the soil microbial biomass: a global scale synthesis. Soil Biol. Biochem. 30, 1627-1637

Wasylnka, J.A., Simmer, M.I., Moore, M.M., 2001. Differences in sialic acid density in pathogenic and non-pathogenic Aspergillus species. Microbiol. 147, 869-877

West, A.W., Sparling G.P., 1986. Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. J. Microbiol. Methods 5, 177-189.

Wichern, F., Mueller, T., Joergensen, R.G., Buerkert, A., 2004. Effects of manure quality and application forms on soil C and N turnover of a subtropical oasis soil under laboratory conditions. Biol. Fertil. Soils 39, 165-171

Willer, H., Kilcher, L. (Eds.), 2010. The World of Organic Agriculture - Statistics and Emerging Trends 2010. IFOAM, Bonn (Germany), and FiBL, Frick (Switzerland).

Xu, R., Hanson, S.R., Zhang, Z., Yang, Y.Y., Schultz, P.G., Wong, C.H. 2004. Site-Specific incorporation of the mucin-type N-acetylgalactosamine-α-o-threonine into protein in Escherichia coli. J. Am. Chem. Soc. 126, 15654-15655

Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G., 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biol. Biochem. 26, 1101-1108

Zeller, V., Bardgett, R.D., Tappeiner, U., 2001. Site and management effects on soil microbial properties of subalpine meadows: a study of land abandonment along a north–south gradient in the European Alps. Soil Biol. Biochem. 33, 639–649.

10. Danksagung

Zunächst möchte ich mich bei meinem Betreuer Herrn Prof. Dr. Rainer Georg Jörgensen für die Möglichkeit der Bearbeitung der vorliegenden Arbeit bedanken. Seine fachliche Kompetenz, seine Geduld und Unterstützung beim Verfassen der wissenschaftlichen Publikationen, gaben mir den nötigen Rückhalt diese Arbeit zu schreiben.

Herrn Prof. Dr. Bernard Ludwig möchte ich für die Möglichkeit der Arbeit mit der Nahinfrarotspektroskopie und sein Engagement für das Weiterbestehen des Graduiertenkollegs danken.

Bei Gabi Dormann bedanke ich mich für die fachliche Betreuung im Labor. Sie zerstreute stets meine Selbstzweifel und unterstützte mich bei der Einarbeitung neuer Methoden.

Auch für die Unterstützung in der Laborarbeit durch Margit Rode, Anja Sawallisch, Andrea Gerke, Elsa Zwicker und Sabine Ahlers möchte ich mich bedanken.

Ein besonderer Dank geht an meine Kollegen des Graduiertenkollegs, mit denen ich zusammen bei vielen Veranstaltungen lustige Momente erlebte. Hier geht ein besonderer Dank an Rajasekaran Murugan und Mariko Ingold für die fachliche Unterstützung.

Des Weiteren danke ich meinen Kollegen des Fachbereichs Bodenbiologie und Pflanzenernährung für die unterhaltsamen und interessanten Frühstücks- und Mittagspausen.

Ein großes Dankeschön geht hier an die ehemaligen und aktuellen Auszubildenden: Sabine Werk, Sophie Trümper, Matthias Wollrath, Ann-Katrin Becker, Sabine Schröter und Luisa Bierwirth. Aber auch den Kollegen aus den anderen Fachgebieten möchte ich für die Unterstützung danken.

Außerdem geht ein großer Dank an „meine" Projektstudenten: Sibylle Faust, Peter Pilz und Johanna Marold. Sie bearbeiteten ihre Projekte sehr gewissenhaft und mit viel Engagement.

Schließlich möchte ich meiner Familie danken, die mich immer unterstützt und motiviert hat. Meinen Eltern und Großeltern danke ich für die schönen Telefonate in die Heimat und die Unterstützung in der Endphase der Dissertation. Meinen Schwiegereltern für die das Vertrauen und die aufmunternden Worte. Meinen Bruder Jan und seiner Frau Anja, danke ich für die Zeit die sie sich für Kontrolllesungen genommen haben. Meinen Bruder Falk und seiner Freundin Katharina danke ich für den Zuspruch.

Meiner lieben Frau Mar gilt ganz besonderer Dank. Du wagtest mit mir das Abenteuer nach Witzenhausen zuzziehen und gabst mir immer Mut für die Dissertation. Dafür, dass du immer für mich da bist, liebe ich dich.