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Organische Düngung und reduzierte Bodenbearbeitung als Steuerungsfaktoren für die C-, N-, P- und S-Speicherung von Mikroorganismen

Dissertation

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Eidesstattliche Erklärung

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

_____

(Stefanie Heinze)

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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 Naturwissenschaften (Dr. rer. nat.) zu erfüllen.
Die Arbeit besteht aus 3 Papern, von denen eins bereits bei einer international, begutachteten Fachzeitschrift veröffentlicht wurde und zwei weiteren, die zur Veröffentlichung eingereicht sind. Die Paper sind in Kapitel 4, 5 und 6 eingearbeitet. Kapitel 1 wird eine generelle Einleitung zum Thema liefern, während im Kapitel 2 die Langzeitversuche eingeführt und vorgestellt werden. Das Kapitel 3 stellt die Ziele dieser Arbeit heraus. Kapitel 7 fasst die Ergebnisse der Kapitel 4 ,5 und 6 zusammen, und wird in Kapitel 8 in Englisch aufgeführt. Kapitel 9 beinhaltet eine Schlussfolgerung, die sich aus den Untersuchungen innerhalb dieser Arbeit ergab. Während Kapitel 10 einen Ausblick für weitere Untersuchungen gibt. In Kapitel 11 sind die Quellen, die für das Kapitel 1, 2, 3 und 10 benötigt wurden aufgeführt.

Die folgenden Paper sind in die Arbeit eingebettet:

Kapitel 4

> 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 and Soil 328: 203-215.

Kapitel 5

> Heinze, S., Rauber, R., Joergensen, R.G.: Tillage systems and their relationships to microbial C, N, P, and S storage in two long-term experiments on loess-derived Luvisols. Applied Soil Ecology (submitted).

Kapitel 6

> Heinze, S., Raupp, J., Rauber, R., Joergensen, R.G., Ludwig, B.: Usefulness of near infrared spectroscopy for the prediction of soil properties: effects of sample pretreatment and calibration procedure. European Journal of Soil Science (submitted).

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Abkürzungsverzeichnis

Al	Aluminium
ANOVA	Varianzanalyse
ASL	Über dem Meeresspiegel
$BaCl_2$	Barriumdichlorid
C	Kohlenstoff
Ca	Kalzium
$CaCl_2$	Kalziumchlorid
$CHCl_3$	Chloroform
CO2	Kohlendioxid
C_{mik} (C_{mic})	Mikrobieller Biomasse Kohlenstoff
DFG	Deutsche Forschungsgemeinschaft
DOK	bio-dynamisch, bio-organisch, konventionell
H	Wasserstoff
H_2O	Wasser
HCl	Salzsäure
HNO_3	Salpetersäure
HPLC	Hochleistungsflüssigkeitschromatographie
IBDF	Institut für biologisch-dynamische Forschung
ICP-AES	Induktiv gekoppeltes Hochfrequenzplasma - Atomemissions Spektrometer
IFOAM	International Federation of organic agricultural movements
K	Kalium
K_2SO_4	Kaliumsulfat
k_{EC},k_{EN}, k_{EP}	Extrahierbarer Teil des Gesamtkohlenstoffs, -stickstoffs und -phosphors gebunden in der mikrobiellen Biomasse
MIN	Mineralische Düngung
Mg	Magnesium
Mn	Mangan
MPLS	Modified partial least squares regression
N (N_{tot})	Stickstoff (Stickstoff-Gesamtgehalt)
N_2O	Lachgas
$NaHCO_3$	Natriumhydrogencarbonat

NaOH	Natronlauge
NH_4NO_3	Ammoniumnitrat
NIR	Nahes Infrarot
NIRS	Nahinfrarot-Spektroskopie
N_{mik} (N_{mic})	Mikrobieller Biomasse Stickstoff
O_2	Sauerstoff
OM	Organisches Material
P (P_{tot})	Phosphor (Phosphor-Gesamtgehalt)
PLFA	Phosphorlipidfettsäuren
P_{mik} (P_{mic})	Mikrobieller Biomasse Phosphor
POM	Partikuläres organisches Material
qCO_2	Metabolischer Quotient
r	Korrelationskoeffizient
RM (CM)	Rottemist-Düngung
RMBD (CMBD)	Rottemist-Dünungn plus Zugabe biologisch-dynamischer Präparate
RPD	Standardfehler der Laborwerte geteilt durch den Standard-fehler der Vorhersage
RQ	Respiratorischer Quotient
RSC	Standardabweichung der Laborwerte geteilt durch den Standardfehler der Kreuzvalidierung
S (S_{tot})	Schwefel (Schwefel-Gesamtgehalt)
SD	Standardabweichung
SEC	Standardfehler der Kalibrierung
SECV	Standardfehler der Kreuzvalidierung
SEP	Standardfehler der Vorhersage
S_{mik} (S_{mic})	Mikrobieller Biomasse Schwefel
SNV	Standard normal variate
SOC	Organischer Kohlenstoff
VIS	Sichtbares Licht
WHK (WHC)	Wasserhaltekapazität

1. Einleitung

Im Laufe der 1980er Jahre rückte das Interesse an Bodenfruchtbarkeit und –qualität immer mehr in den Fokus der Wissenschaft und fand 1999 Ausdruck im Bundes Bodenschutzgesetz (Filip, 2002). Vor allem in intensiv genutzten Systemen, wie der Landwirtschaft, wird dem Kompartiment Boden und seiner nachhaltigen Nutzen immer größere Aufmerksamkeit geschenkt (Guerrero et al., 2007, Widmer et al., 2006). Die Bodenfruchtbarkeit, die laut IFOAM (1998) als Möglichkeit des Bodens, Nährstoffe für das Pflanzenwachstum nachhaltig bereit zu stellen und Erträge zu sichern beschrieben wird, gilt es durch schonende Nutzung zu sichern. Ebenso findet der Erhalt der Bodenqualität, welche die Fähigkeit des Bodens, Schlüsselfunktionen wie den Abbau und die Bildung organischen Materials erhalten soll, besonderere Berücksichtigung (IFOAM, 1998). Vor allem die ökologische Landwirtschaft, die nach dem Grundsatz „fertilising the soil rather than the plant is an organic farmers goal“ (Fließbach et al., 2007) handelt, achtet auf ein nachhaltiges Bodenmanagement. Dieses umfasst den Einsatz organischer Dünger, die Optimierung geschlossener Nährstoffkreisläufe, den Verzicht auf synthetische Dünger und Pestizide und die Erhöhung förderlicher biologischer Interaktionen und Prozesse (Schjønning et al. 2002, Fließbach et al. 2007). Einen wesentlichen Beitrag zum Erhalt der Bodenfruchtbarkeit und –qualität liefert die mikrobielle Biomasse im Boden. Sie stellt mit einem Anteil von 1-5% des Gesamtpools an Kohlenstoff (C), Stickstoff (N), Phosphor (P) und Schwefel (S) in der organischen Substanz zwar nur einen kleinen Teil dar, umfasst aber eine wesentliche Aufgabe in der Umwandlung organischen Materials in pflanzenverfügbare Nährstoffe (Balota et al., 2003, Wu et al., 1993). Die Schlüsselfunktion der Mikroorganismen wurde 1977 von Jenkinson sehr treffend mit „the eye of the needle through which all the organic material must pass” (Jenkinson, 1977) beschrieben. Durch die Umwandlung organischen Materials, welches durch Erntereste oder organische Düngung dem Boden zurückgegeben wird, fungiert die mikrobielle Biomasse als Quelle und Senke von essentiellen Pflanzennährstoffen, die innerhalb des labilen Pools der Mikroorganismen gespeichert und sukzessive an die Pflanzen abgegeben werden (Joergensen, 1995, Marumoto et al., 1982). So werden die Nährstoffe gegen Auswaschung geschützt und gehen dem System nicht verloren (Brookes, 2001). Bei der Bereitstellung der Nährstoffe und als Motor des Energieflusses sind vor allem Bakterien und Pilze von besonderer Bedeutung (Richards, 1987), die in den meisten terrestrischen Ökosystemen den Hauptanteil der mikrobiellen

Biomasse ausmachen (Schloter et al., 2003). Bakterien und Pilze sind durch eine unterschiedliche Substratnutzungseffizienz gekennzeichnet, wobei sich Pilze durch einen langsamen Energieumsatz auszeichnen (Blagodatskaya und Anderson, 1998) und eine hohe Effizienz besitzen, organisches Material zu nutzen und eigene Biomasse aufzubauen (Sakamoto und Oba, 1994). Wohingegen Bakterien eher schnelle Energieumsatzraten aufweisen und daher ein erhöhtes Nährstoffangebot benötigen, um eigene Biomasse aufzubauen, was zu einer geringeren Substratnutzungseffizienz im Vergleich zu Pilzen führt (Holland und Coleman, 1987, Sakomoto und Oba, 1994).

Über die Funktion des Nährstoffumsatzes hinaus, kann die mikrobielle Biomasse als „early warning“ (Brookes, 2001) für Veränderungen in den Bodeneigenschaften und somit der Bodenfruchtbarkeit herangezogen werden (Marinari et al., 2006, Lundquist et al., 1999). Eine Abnahme der mikrobiellen Biomasse in Böden kann zu einer Bodendegradation führen (Kushwaha et al., 2000), die bodenphysikalische und –chemische Verschlechterungen zur Folge haben. Somit können Veränderungen der mikrobiellen Aktivitätsrate, der quantitativen Biomasse und der Zusammensetzung der mikrobiellen Gemeinschaft herangezogen werden (Schloter et al., 2003), um eine langzeitliche Veränderung des Bodens durch Änderungen, wie zum Beispiel des Düngermanagements oder der Bodenbearbeitung, frühzeitig zu erkennen und ihr so entgegenzuwirken. Da einer dauerhaften Abnahme der Bodenqualität entgegengewirkt werden soll, müssen landwirtschaftliche Systeme hinsichtlich ihres Langzeiteinflusses auf die bodenphysikalischen, -chemischen und –biologischen Eigenschaften hin untersucht werden. Veränderungen der Bodenqualität erfolgen nur langsam und machen es daher notwendig, die Eingriffe auf den Boden in einem Langzeitversuch zu beobachten (Polwson und Johnston, 1994). Hier steht die langzeitliche Dauerbeobachtung der Pflanzenproduktion, des Nährstoffkreislaufs und des Umwelteinflusses durch die Landwirtschaft im Vordergrund (Fließbach et al., 2007). Die kontinuierliche Erhebung von Bodeneigenschaften innerhalb eines Langzeitversuchs stellt eine unverzichtbare Quelle wissenschaftlicher Erkenntnis dar, welche die Erhebung und das Verstehen von Veränderungen der Bodenqualität und -fruchtbarkeit im Zuge lanwirtschaftlicher Bearbeitung vorantreibt (Debreczeni und Körschens, 2003).

2. Langzeitversuche in der Landwirtschaft

Unter Langzeitversuchen werden systematisch angelegte landwirtschaftliche Feldversuche verstanden, die länger als 20 Jahre unter permanenter Nutzung stehen und Fragestellungen zur Pflanzenproduktion, dem Nährstoffkreislauf und dem Einfluss der Landwirtschaft auf die Umwelt bearbeiten (Fließbach et al., 2007, Rasmussen et al., 1998). Sie zeichnen sich durch periodische Probenahmen und ein umfangreiches Probenarchiv aus und ermöglichen somit Veränderungen des Bodens über einen zeitlichen Verlauf in einem dekadischen Maßstab zu erkennen und zu quantifizieren (Richter et al., 2007, Debreczeni und Körschens, 2003). Durch die ausführliche Dokumentation, die stetige Probenahme, die gut organisierte Archivierung der Daten und ihrer statistischen Auswertung, liefern Langzeitversuche eine umfassende Datenbank, die dem wissenschaftlichen Austausch zur Verfügung steht. Einer der am längsten bestehenden Langzeitversuche ist das Broadbalk Winter Wheat Experiment der Rothampsted Experiment Station, das 1843 von Sir John Lawes und Sir Henry Gilbert angelegt wurde. Im Fokus stand dabei die Frage, ob eine Mixtur von anorganischen Nährstoffen (P, K, Na, Mg) orientierend an dem Gehalt, der in Asche von Pflanzen gefunden wurde, ausreicht, um eine vollkommene Entwicklung der Weizenpflanze herbeizuführen, oder ob eine Zugabe von mineralischem N notwendig wäre (Jenkison, 1991). Neben dem schon seit 166 Jahren bestehenden Broadbalk-Feldversuch gibt es noch weitere Langzeit-Untersuchungen, wie den in Bad Lauchstädt (Deutschland, Powlson et al., 1998) 1902 oder in Askov Dänemark 1894 etablierten Langzeitversuch (Christensen, 2008, Edmeades, 2003), bei denen Düngungs- und Fruchtfolgeeffekte auf den Boden untersucht werden. Die Betrachtung der weltweiten Verbreitung von Langzeitversuchen zeigt, dass zwar 70% aller Flächen in Europa zu finden sind (Debreczeni und Körschens, 2003), aber ein stetig steigender Anteil an mehreren Jahrzehnten durchgeführten Langzeitversuchen befindet sich in China, Indien und Pakistan, die vor allem die Auswirkungen intensiven Reisanbaus auf die Ertragsstruktur und die Bodenfunktion im Auge haben (Nayak et al., 2007, Tirol-Padre und Ladha, 2006, Bhandari et al., 2002). Trotz der Vielzahl erfolgreich ablaufender Langzeitversuche und der aktuellen Fragestellungen, sind eine Etablierung und der Fortbestand mit Problemen behaftet. Vor allem die Initialisierung und das Aufrechterhalten dieser Langzeit-Untersuchungsflächen sind mit großem finanziellen und Kräfte zehrenden Aufwand verbunden. Oft sind die Versuche an Projekte geknüpft, die nur eine befristete

Finanzierung vorsehen (Richter et al., 2007). Eine weitere Schwierigkeit bei der dauerhaften Durchführung der Versuche ist die flexible Reaktion auf Veränderungen in der landwirtschaftlichen Praxis oder auf den Umschwung wissenschaftlicher Schwerpunkte. Darüber hinaus bedarf es bei der Durchführung und bei der Datenpflege einer sehr guten Organisation und der Zusammenarbeit der Versuchsansteller mit unterschiedlichsten, interdisziplinär arbeitenden Wissenschaftlern und zwar über Jahrzehnte hinweg. Nichts desto trotz stellen Langzeitversuche eine große Hilfe dar, den Einfluss der Landwirtschaft auf biologische und biogeochemische Prozesse zu verstehen und sie im globalen Zusammenhang einordnen zu können, um mit diesem Wissen eine umweltfreundliche und nachhaltige Nährstoffversorgung der Pflanzen zu entwickeln (Rasmussen et al., 1998).

2.1. Dauerdüngungsversuch Darmstadt

Der Langzeit-Versuch des **I**nstituts für **b**iologisch-**d**ynamische **F**orschung (IBDF) in Darmstadt wurde 1980 angelegt, um den Einfluss unterschiedlicher Dünger auf die Nahrungsqualität der Kulturpflanzen zu untersuchen (Abb. 1). Durch das Bundesministerium für Landwirtschaft erhielt der Dauerversuch für die ersten 4 Jahre finanzielle Unterstützung. Von 1988 bis 1991 wurde auf den Flächen der Einfluss unterschiedlicher Düngung auf bodenbiologische Messgrößen analysiert (Bachinger 1996), während zwischen 1992 und 1999 die Ertragsbildung und Langzeiteffekte in den Fokus der Betrachtung gerückt sind. Seit diesem Jahrhundert gelangten der Humus und seine Stabilität immer mehr in den Vordergrund wissenschaftlicher Forschung. Seit dem Jahr 2007 wird innerhalb des DFG-Graduiertenkollegs 1397 der Universität Kassel und Göttingen die Regulation des Humus- und Nährstoffkreislaufs in der ökologischen Landwirtschaft auf den Flächen dieses Langzeitversuchs untersucht. Bei diesem interdisziplinären Projekt werden sowohl Fragestellungen zur Stabilisierung des organischen Materials, CO_2- und N_2O-Flüssen bearbeitet als auch Untersuchungen zu bodenbiologischen Prozessen durchgeführt, deren Verständnis zur Sicherung der Bodenfruchtbarkeit und der Opti-mierung des geschlossenen Nährstoffkreislaufs besonders in Low-Input-Systemen, wie der ökologischen Landwirtschaft, beitragen sollen.

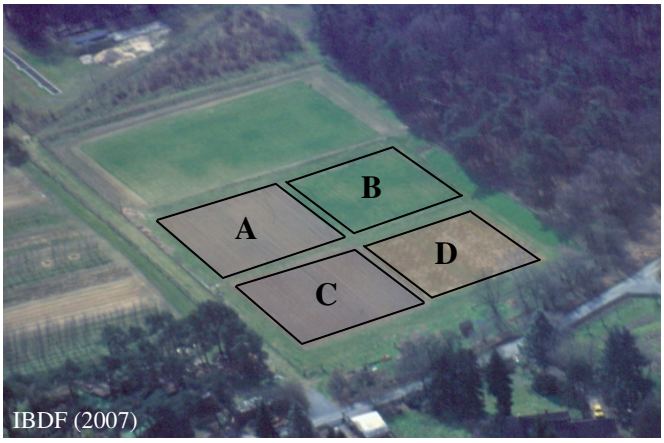

Abbildung 1: Luftbild der Untersuchungsfläche des Langzeit-Düngerversuchs des Instituts für biologisch-dynamische Forschung in Darmstadt. Feldwiederholungen A-D mit mineralischer, organischer und organische Düngung.plus biologisch-dynamische Präparatsbeimischung. Foto (IBDF, 2007).

2.1.1. Versuchsdesign

Der Langzeitversuch des IBDFs liegt circa 10 km südwestlich von Darmstadt (49° 50′ N, 8° 34′ O). Auf einer sandigen Braunerde über fluviatilen Sanden des Neckars (Haplic Cambisol, FAO-WRB, 2006), die aus 86% Sand, 9% Schluff und 5% Ton besteht, wurde ein geteiltes Blockdesign bestehend aus 4 Feldwiederholungen (A-D) angelegt, auf dem die Anwendung drei verschiedener Dünger in drei unterschiedlichen Düngerraten in vierfacher Wiederholung etabliert wurde (Abb. 2). Die mittlere Jahrestemperatur beträgt an diesem Standort 9,5°C bei mitteleren Jahresniederschlagssummen von 590 mm.

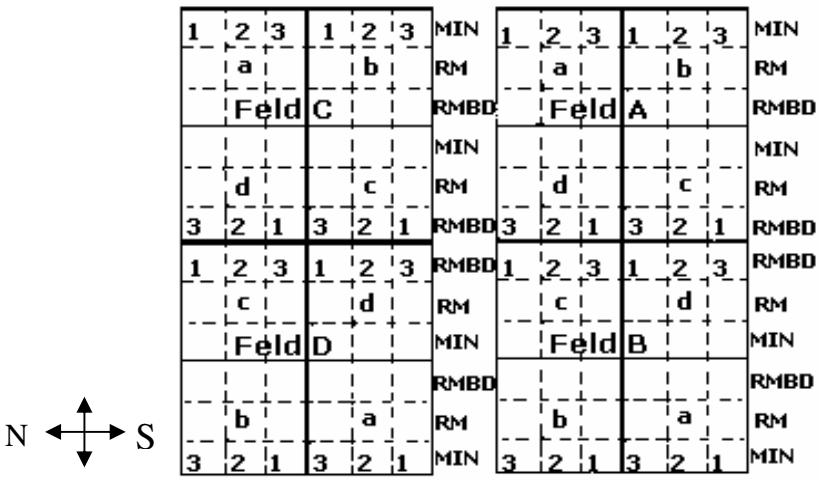

Abbildung 2: Geteiltes Blockdesign des Langzeitversuchs des IBDFs mit 4-facher Feldwiederholung (A-D). Vergleich der mineralischen (MIN) Düngung, der Düngung mit

Rottemist (RM) und Rottemist plus der Zugabe biologisch-dynamischer Präparate (RMBD). (Quelle: IBDF, 2007).

Die Zugabe der Dünger erfolgte zum einen als mineralischer Dünger (MIN) (Calcium-ammoniumnitrat, Superphosphat, Kaliumchlorid (seit 1996 Kaliummagnesium)) mit der Rückfuhr des geernteten Strohs, zum anderen als Rottemist (RM) und als dritte Düngervariante wurde der gleiche Rottemist mit der Zugabe biologisch-dynamischer Präparate (RMBD) hinzugegeben (Raupp, 2001). Der Rottemist wurde in Mistmieten über 3 Monate für die Zugabe bei Winterfrüchten und bis zu 6 Monate für Frühjahrssaaten gelagert (Raupp und Oltmanns, 2006). Die biologisch-dynamischen Präparate wurden zum einen als Kompostpräparat oder als Feldpräparat hinzugegebn. Die Kompostpräparate bestehend aus 0,5g *Archillea millefolium* , *Chamomilla recutita*, *Taraxacum officinale*, *Valeriana officinalis*, *Urtica dioica* und Rinde von *Quercus robur*, wurden separat in einer Tonne Mist zufällig verteilt (Koepf et al., 1990). Die Feldpräparate werden zum einen aus Hornmist hergestellt, der aus Kuhdung besteht und mit Wasser verdünnt nach der Bodenbearbeitung oder der Einsaat in einer Dosis von 200-300g ha^{-1} auf die Felder gespritzt wird. Und zum anderen wurde Hornkiesel verwendet, der aus gemahlenem Quarzstein besteht und mit Wasser verdünnt beim Auflaufen, der Blüte und bei der Kornfüllung in einer Dosis von 4g ha^{-1} auf die Pflanzen gespritzt wird (Koepf et al., 1990).

Die drei Düngervarianten wurden in drei unterschiedlichen Düngermengen hinzugegeben. Orientierend an der Stickstoffgabe erfolgte eine Zugabe von 60 kg N ha^{-1} in der niedrigsten Düngerstufe (1), in der mittleren Stufe (2) wurden 100 kg N ha^{-1} und in der höchsten (3) Applikation 140 kg N ha^{-1} auf die Felder gegeben. Bei der Kultivierung von Hackfrüchten wurde die Düngergabe leicht modifiziert, so dass bei der niedrigsten Düngerstufe 50 kg N ha^{-1}, bei der mittleren 100 und bei der höchsten Düngergabe 150 kg N ha^{-1} hinzugefügt wurden (Bachinger, 1996) Neben der kontrollierten Stickstoff-menge, wurden zudem Phosphor, Kalium und Schwefel jährlich in Form von Mist oder Gülle oder als mineralischer Dünger auf die Felder gegeben (Tabelle 1).

Tabelle 1: Gehalte an Phosphor (P), Kalium (K) und Schwefel (S) in kg ha^{-1} eingebracht durch jährliche Düngung in Form von Mist, Gülle oder mineralischen Düngern. Die Angaben von Rottemist (RM) und Rottemist mit biologisch-dynamischen Präparat (RMBD) beziehen sich auf die mittlere Düngerzugabe zwischen 1994 und 1998 (Raupp und Oltmanns, 2006).

	Niedrige Stufe (1)			Mittlere Stufe (2)			Hohe Stufe (3)		
	P	K	S	P	K	S	P	K	S
RM									
Mist	16	76	9	21	101	12	26	126	15
Gülle	0	0	0	1	33	0	2	66	0
RMBD									
Mist	17	81	9	23	108	11	29	135	14
Gülle	0	0	0	1	32	0	2	65	0
Mineralisch	50	75	73	75	100	102	100	125	132

Außer der variierenden Düngerbehandlung unterlagen alle Flächen der gleichen Bewirtschaftung mit einer Fruchtfolge aus Rotklee (*Trifolium pratense L.*) oder Luzerne (*Medicage sativa L.*), Sommerweizen (*Triticum aestivum L.*), Kartoffeln (*Solanum tuberosum L.*) oder Karotten (*Daucus carota* ssp. *Sativus (Hoffm.) Arcang.*) und Winterroggen (*Secale cereale L.*) und der herkömmlichen Pflug- und Saatbettbearbeitung.

2.1.2. Bisherige Ergebnisse

Im Mittelpunkt der bisherigen bodenkundlichen Erhebungen standen Fragestellungen hinsichtlich der Anreicherung und Umsetzung des organischen Materials durch mineralische und organische Düngung. Dabei wurde festgestellt, dass die langjährige Zugabe (18 Jahre) von Rottemist einen höheren Gehalt an organischem Material (OM) im Boden im Vergleich zur mineralischen Düngung aufweist, wobei die Zugabe von biologisch-dynamischen Präparaten zu den höchsten Gehalten führte und als einzige Düngergabe den Erhalt des OM, bezogen auf den Ausgangswert von 1980, bewirkte (Raupp und Oltmanns, 2006, Abele, 1987). Die unterschiedlichen Düngerraten hatten keinen Einfluss auf den Gehalt an organischem Kohlenstoff bei mineralischer Düngung, während die Zugabe von Rottemist zu erhöhten Gehalten mit steigender Düngerrate führte (Raupp und Oltmanns, 2006). Ein wesentlicher Einflussfaktor, der die Umsetzung organischen Materials steuert, ist die mikrobielle Biomasse und ihre Aktivität. Bachinger

(1996) fand heraus, dass der höhere Gehalt an organischem Kohlenstoff in den beiden organisch gedüngten Behandlungen, die mikrobielle Biomasse (C_{mik}) und ihre Aktivität, gemessen an Protease und Dehydrogenase, erhöhte. Die Zusammensetzung und die Qualität des organischen Materials wurde 2002 von Raupp und Oltmanns untersucht. Bei der Untersuchung des partikulären organischen Materials (POM) wurde deutlich, dass die organisch gedüngten Flächen einen geringeren Anteil der leichten Fraktion aufwiesen und somit Schlüsse auf eine schnellere Umsetzung des organischen Materials im Vergleich zu mineralisch gedüngten Flächen zuließen (Raupp und Oltmanns, 2002). Scheller und Raupp (2005) untersuchten die Auswirkungen unterschiedlicher Düngung auf den Aminosäuregehalt. Dabei wurde deutlich, dass organische Düngung den Gehalt an Aminosäuren zum einen durch den Input an Aminosäuren und Proteinen durch den Mist selbst und zum anderen durch die Förderung des anabolen Eiweißstoffwechsels gegenüber mineralischer Düngung erhöhte und somit zu einer effizienteren Nutzung des organischen Materials führte (Scheller and Raupp, 2005). Neben den Kohlenstoffpools untersuchten Heitkamp et al. (2009) mit Hilfe eines 2 Pool-Modells auch den Einfluss mineralischer und organischer Düngung auf den Stickstoffpool in den ersten 25 cm des Bodens. Dabei wurde deutlich, dass die sehr labilen Vorräte an C (Umsatzzeit: 17 Tage) und N (Umsatzzeit: 9 Tage) nur sehr gering waren und keinerlei Veränderung durch unterschiedliche Düngertypen und Düngerraten zeigten, während der labile Pool (Umsatzzeit für C: 462 und N: 153 Tage) mit zunehmender Düngerrate auch höhere C- und N- Vorräte aufwies. Der intermediäre C-Pool (Umsatzzeit: mehrere Dekaden) zeigte höhere Vorräte durch die Zugabe organischen Düngers, während der intermediäre N-Pool (Umsatzzeit: mehrere Dekaden) weder durch den Düngertyp noch die Düngerrate signifikant beeinflusst wurde (Heitkamp et al., 2009). Die passiven Pools (Umsatzeit > 600 Jahre) zeigten keine Reaktion auf die Düngergaben.

Im Wesentlichen zeigten die bisherigen Ergebnisse, dass langzeitliche organische Düngung im Vergleich zur Anwendung mineralischer Dünger zu einer Erhöhung des organischen Materials führte, die allerdings keine langzeitliche Steigerung des C-Vorrats (passive Pools) im Boden zur Folge hatte (Heitkamp et al., 2009). Die mikrobielle Biomasse (C_{mik} und N_{mik}) und ihre Aktivität (Protease, Dehydrogenase) wurden durch die organische Düngung gegenüber der Zugabe mineralischer Dünger erhöht.

2.2. Langzeit-Bodenbearbeitungsversuch Göttingen

Der Langzeitversuch zur Bodenbearbeitung, angelegt durch die Universität Göttingen, umfasst zwei landwirtschaftliche Flächen (Garte und Hohes Feld) ähnlichen Ausgangsmaterials, auf denen der Einfluss von Pflug- und Kreiseleggenbearbeitung auf bodenchemische, -physikalische und -biologische Messgrößen, auf die Ertragsbildung der Kulturpflanzen, die Unkrautregulierung und das Strohmanagement untersucht wurden. Die Untersuchungsfläche Garte (51° 29' 86 N, 9° 56' O, 163 m über NN) liegt auf dem Gelände des Versuchsguts Reinshof der Universität im Süden Göttingens und wurde im Jahr 1970 etabliert, während die Fläche Hohes Feld (51° 37' N, 9° 53' O, 151 m über NN) im Norden Göttingens bei Angerstein zu finden ist und bereits seit 1967 besteht. Der mittlere Jahresniederschlag auf beiden Flächen beträgt 645 mm bei einer jährlichen Durchschnittstemperatur von 8,7 °C. Die Böden beider Untersuchungsflächen sind Parabraunerden über Löss (Haplic Luvisol, FAO-WRB, 2006), die am Standort Garte in den ersten 30 cm durch 15,1 % Ton, 72,7 % Schluff und 12,2 % Sand gekennzeichnet sind (Reiter et al, 2002, Ehlers et al., 2000), während sich die Textur am Standort Hohes Feld aus 17,2 % Ton, 66,4 % Schluff und 16,4 % Sand zusammensetzt (De Mol, 1996). Beide Flächen weisen einen ähnlichen mittleren pH Wert auf, der in Garte 7,7 und im Hohen Feld 7,5 beträgt.

2.2.1. Versuchsdesign

Der langjährige Bodenbearbeitungsversuch am Standort Garte wurde in einem Blockdesign in zufälliger Verteilung der verschiedenen Bodenbearbeitungssysteme in 4-facher Wiederholung angelegt bei einer Plotgröße von 40 x 20 m. Am Standort Hohes Feld besteht seit mehr als 40 Jahren eine Spaltenanlage mit 3-facher Wiederholung der beiden Boendbearbeitungssysteme auf einer Plotgröße von 36 x 12,7 m (Abb. 3).

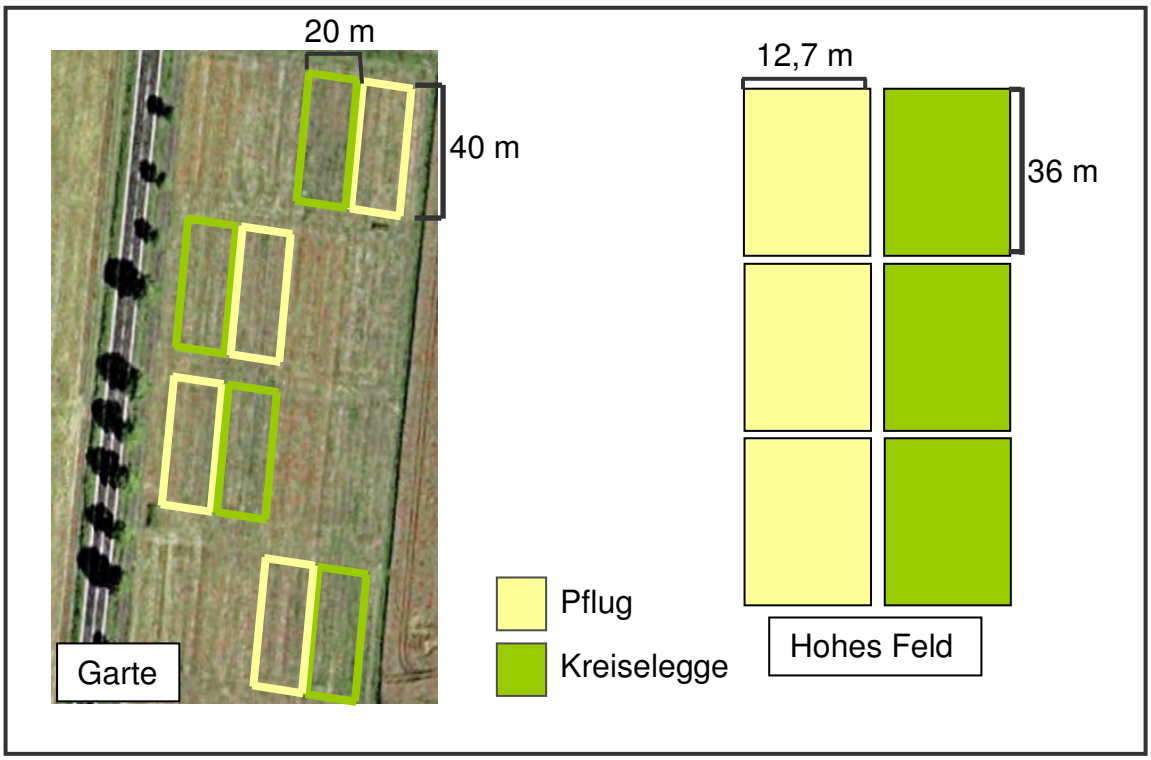

Abbildung 3: Versuchsdesign der Standorte Garte (Randomisiertes Blockdesign) und Hohes Feld (Streifenanlage) mit vierfacher und dreifacher Wiederholung der beiden Bodenbearbeitungsvarianten Wendepflug und Kreiselegge (verändert nach Rauber und Ehlers, o.J.).

Die beiden Bodenbearbeitungssysteme umfassen den in Europa am weitesten verbreiteten Wendepflug, der den Boden bis zu einer Tiefe von 25-30 cm wendet und die Kreiselegge, mit der der Boden nur flach bis zu einer maximalen Tiefe von 8 cm bearbeitet wird. Die Saatbettbereitung erfolgte auf allen Flächen mit der Kreiselegge. Die Fruchtfolge auf beiden Flächen ist Getreide basiert und bestand 2004 aus Erbse (*Pisum sativum* L.), gefolgt im Jahr 2004/2005 von Winterweizen (*Triticum aestivum* L.), 2006 Mais (Zea mays L.) und 2007 folgte die Ackerbohne (*Vicia faba* L.). Auf allen Flächen wurde das geerntete Stroh zurückgeführt und mit der entsprechenden Bodenbearbeitung eingearbeitet. Die Düngung basierte auf der Stickstoffgabe und erfolgte in einer Höhe von 164 kg N ha^{-1} im Mittel der letzten 10 Jahre. Des Weiteren wurden Phosphor, Kalium und Magnesium hinzugegeben, orientierend am Bedarf, der mittels Gesamtgehaltsanalyse des Bodens kontinuierlich bestimmt wurde (Tabelle 2).

Tabelle 2: Grunddüngung auf den Flächen Garte und Hohes Feld mit Phosphor (P_2O_5), Kalium (K_2O) und Magnesium (MgO) gemittelt über die vergangenen 10 Jahre in kg ha^{-1} (nach Rauber, 2009).

Standort	2004			2005			2006		
	P_2O	K_2O	MgO	P_2O	K_2O	MgO	P_2O	K_2O	MgO
Garte	0	60	9	0	60	9	0	169	25
Hohes Feld	46	60	9	46	60	9	0	169	25

Die Beikrautregulierung und der Pflanzenschutz erfolgte auf allen Flächen nach Bedarf mit entsprechenden und Herbi- und Fungiziden.

2.2.2. Bisherige Ergebnisse

Der langzeitliche Bodenbearbeitungsversuch der Universität Göttingen wurde in den vergangenen Jahren vor allem in Bezug auf Fragestellungen der Pflanzenproduktion und der Ertragsleistung untersucht. Ehlers et al. (2000) untersuchten unter Berücksichtigung der Gefügeveränderung die Auswirkungen mechanischer Bodenverdichtung auf die Ertragsleistung der Flächen unter Pflug- und Kreiseleggenbearbeitung. Hier wurde auf Teilparzellen der Einfluss künstlich erhöhter Verdichtung (gering = 2x 2,5 t, mittel = 2x 5 t, hoch = 6x 5 t) des Bodens durch den mehrfachen Einsatz schwerer Maschinen auf die unterschiedliche Verdichtungsempfindlichkeit der Böden unter Pflug- und Kreiseleggenbearbeitung und ihre Auswirkungen auf die Ertragsleistung der Flächen untersucht. Dabei wurde deutlich, dass auf den Parzellen mit Pflugbearbeitung schon eine geringe Belastung zu 50% geringeren Erträgen des Sommerweizens führte, während dieses bei Kreiseleggenbewirtschaftung erst mit hoher Belastung auftrat (Ehlers et al., 2000). Des Weiteren wurden die Gesamtwurzellänge und die Durchwurzelungstiefe auf Flächen mit Pflugbearbeitung schon bei geringer Belastung reduziert, während auf den Flächen mit der Bearbeitung durch die Kreiselegge nur die Wurzellänge bei starker Belastung beeinflusst wurde (Ehlers et al., 2000). Reiter et al. (2002) untersuchten den Einfluss der unterschiedlichen Bodenbearbeitung auf die N-Fixierung von Leguminosen (Erbse und Kleegras) bei wendender Pflugbearbeitung und unter dem Einsatz der Kreiselegge. Hier zeigte sich keinerelei Einfluss der Bodenbearbeitung auf die N-Fixierung (Reiter et al., 2002). Jacobs et al. (2009) untersuchten den Einfluss der beiden Bodenbearbeitungsverfahren ausschließlich auf bodenkundliche Kenngrößen. Dabei stand

der Einfluss der Pflug- und Kreiseleggenbearbeitung auf Bodenaggregate, POM und organische Kohlenstoff- und Stickstoffgehalte im Vordergrund. Der Einsatz der Kreiselegge führte im Oberboden (0-5 cm) zu einer Erhöhung des organischen Kohlenstoffs, des Stickstoffs und der mikrobiellen Biomasse (C_{mik}, N_{mik}) im Gegensatz zur Pflugbearbeitung. Diese Unterschiede waren im Unterboden (10-20 cm) allerdings nicht mehr signifikant (Jacobs et al., 2009). Die Bodenbearbeitung mittels Kreiselegge führte zu einer 2,6-mal höheren Bildung wasserstabiler Makroaggregate im Oberboden, die das organische Material besser vor mechanischer Zerstörung schützen (Jacobs et al., 2009). Alle Aggregatklassen zeigten höhere Gehalte an organischem Kohlenstoff und Stickstoff bei Kreiseleggenbearbeitung (Jacobs et al., 2009).

3. Ziele der Arbeit

Über die bereits erhobenen Ergebnisse hinaus leistet die vorliegende Arbeit einen tieferen Einblick in die Funktion und die Effektivität der mikrobiellen Biomasse im Nährstoffkreislauf landwirtschaftlicher Systeme. Anders als bei der Analyse durch Bachinger (1996) stand innerhalb dieser Arbeit auf dem Langzeit-Düngerversuch in Darmstadt, der Einfluss der durch das Ausgangsmaterial hervorgerufenen räumlichen Heterogenität vor allem des pH-Werts (H_2O) im Zusammenspiel mit mineralischer und organischer Düngung auf die Parameter der Bodenqualität im Vordergrund. Vor allem die Auswirkungen auf den Gehalt an organischem Kohlenstoff, Gesamt- Stickstoff, Phosphor, Schwefel, der mikrobiellen Biomasse (C_{mik}, N_{mik}, P_{mik}, S_{mik}) und ihrer Aktivität (CO_2-Abgabe und O_2-Verbrauch) wurden eingehend analysiert. Zudem bildete die Untersuchung der mikrobiellen Gemeinschaft einen Schwerpunkt, die durch die Analyse von Ergosterol, einem pilzlichen Biomarker, Aussagen über den Einfluss organischer und mineralischer Düngung auf die Substratnutzungseffizienz zulassen sollte.
Im Folgenden werden die dem Dauerdüngungsversuch unterliegenden Hypothesen im Überblick vorgestellt:

a) Organische Düngung führt zu erhöhten Gehalten an organischem Kohlenstoff und Gesamtgehalten an N, P und S und an mikrobieller Biomasse (C_{mik}, N_{mik}, P_{mik}, S_{mik}).
b) Die Aktivität der mikrobiellen Biomasse wird durch die Zugabe organischer Dünger erhöht.
c) Die räumliche Heterogenität - vor allem des pH-Werts - beeinflusst die Auswirkungen der unterschiedlichen Düngung auf die mikrobielle Biomasse.
d) Mineralische Düngung plus Strohrückführung und geringere pH-Werte führen zu einem erhöhten Anteil an saprotrophen Pilzen, welcher mit einer erhöhten Substratnutzungseffizienz einhergeht.

Innerhalb der Untersuchung der beiden Flächen des Bodenbearbeitungsversuchs in Göttingen werden die Auswirkungen des Einsatzes des Wendepfluges und der reduzierten Bearbeitung mittels Kreiselegge auf bodenchemische und bodenbiologische Messgrößen quantifiziert. Anders als bei den vorangegangenen Untersuchungen wurden die Messgrößen in dieser Arbeit auf die Vorräte im Boden über die gesamte

Bearbeitungstiefe bezogen, um Aussagen zum langzeitlichen Anreicherungspotential von organischem Kohlenstoff, Nährstoffen und mikrobieller Biomasse über den Oberboden hinaus treffen zu können. Erweiternd zu den mikrobiellen C und N Analysen von Jacobs et al. (2009) wurden in dieser Arbeit auch P_{mik} und S_{mik} bestimmt, um Aussagen über die Nährstoffverfügbarkeit und den Umsatz der mikrobiellen Biomasse treffen zu können. Des Weiteren waren Untersuchungen des Einflusses des Wendepfluges und der Kreiselegge, als reduzierte Bodenbearbeitung, auf die mögliche Veränderung der mikrobiellen Zusammensetzung von besonderer Bedeutung, um so Informationen zur Substratnutzungseffizienz zu erhalten. Im Folgenden sind die zu bearbeitenden Hypothesen zusammengefasst:

a) Pilze werden durch reduzierte Bodenbearbeitung mittels Kreiselegge gefördert.
b) Durch die Förderung der Pilze steigen die Phosphor- und Schwefelvorräte in der mikrobiellen Biomasse.
c) Durch eine erhöhte Substratnutzungseffizienz der Pilze kommt es zu einer Anreicherung des organischen Materials unter reduzierter Bodenbearbeitung.
d) Kreiseleggenbearbeitung führt zu einer Erhöhung der Vorräte an organischem Kohlenstoff, den Gesamtgehalten von Stickstoff, Phosphor und Schwefel und den mikrobiellen Messgrößen.

3.1. Methodische Ziele der Arbeit

Die Erhebung bodenkundlicher Messgrößen, vor allem in einem langzeitlichen Ansatz, erfordert ein hohes Maß an Kapital- und Arbeitsaufwand. Zudem werden oft große Probenmengen benötigt, die durch eine destruktive Beprobung nicht erneut Verwendung finden können. Um diese aufwendigen Messmethoden zu umgehen, soll innerhalb dieser Arbeit das Potential der Nahinfrarot-Spektroskopie (NIRS) zur Bestimmung bodenchemischer und -biologischer Messgrößen untersucht werden. Denn NIRS ermöglicht es in einer relativ kurzen Zeit, kostengünstig und materialschonend zeitgleich eine Vielzahl von Messgrößen zu erfassen (Odlare et al., 2005, Chang et al., 2001). NIRS beruht auf den spektralen Eigenschaften der zu messenden Materialien im Nahen Infrarot (750-2500 nm) und erweitert im Wellenlängenbereich des sichtbaren Lichts (400-750 nm). Durch nahinfrarote Strahlung entstehen in den C-H-, N-H- oder O-H- Gruppen, welche die Hauptkomponenten in organischen Bindungen darstellen, Schwingungen, Rotationen oder Deformierung (Shenk et al., 2001). Die monochromatischen Strahlen des

nahen Infrarots werden vom Probenmaterial reflektiert, gebrochen, absorbiert, zerlegt oder transmittiert, was zu einem Energieverlust der Strahlung führen kann (Shenk et al., 2001). Dieses Strahlungsverhalten führt zu Überlappungen oder Obertönen der entstandenen Reflektions- und Absorptionspeaks und verhindert so die Abbildung eines eindeutigen Spektrums (Shenk und Westerhaus, 1993). Um die Informationen des NIR-Spektrums weiter nutzen zu können, müssen die bestehenden Überlappungen und Obertöne im Spektrum durch vielfache mathematische Behandlungen, wie die Bildung von Ableitungen, entfernt werden (Chodak et al., 2007). Die Qualitätsabschätzung der Bestimmung von Messgrößen mittels NIRS erfolgt über multivariate statistische Modelle, die mittels Kalibrierungs- und Validierungsverfahren des spektral gemessenen Datensatzes die Vorhersage der im Labor ermittelten Werte überprüft (Shenk und Westerhaus, 1993).

Unter Berücksichtigung der Funktionsweise von NIRS trägt diese Arbeit dazu bei, das Potential der Bodenprobenvorbereitung in Form von Schockgefrieren und Gefriertrocknen in Anlehnung an Terhoeven-Urselmans et al. (2008) zur erfolgreichen Bestimmung von bodenchemischen und -biologischen Messgrößen zu prüfen. Des Weiteren soll mittels verschiedenen Kalibrierungs- und Validierungsverfahren für einen in der Probenanzahl variablen Datensatz zu erfolgreichen Vorhersageergebnissen gelangt werden.

So ergaben sich für die vorliegende Arbeit folgende Forschungsfragen bezüglich des Einsatzes von NIRS zur Bestimmung bodenchemischer und -biologischer Kenngrößen an den Böden beider Langzeitversuche (siehe Kapitel 2):

a) Führen das Schockgefrieren mit Flüssigstickstoff und das anschließende Gefriertrocknen in Anlehnung an Terhoeven-Urselmans et al. (2008) zu einer erfolgreichen Bestimmung chemischer und -biologischer Bodenparameter?
b) Führt die Einteilung eines großen Datensatzes in einen homogeneren, kleineren Datensatz innerhalb einer Kreuzvalidierung zu besseren Ergebnissen?
c) Durch welches Selektionsschema werden die besten Ergebnisse mittels separate Kalibrierung und Validierung erreicht?
d) Können bodenchemische und -biologische Messgrößen durch eine separate Kalibrieung und Validierung eines kleinen Datensatzes erfolgreich vorhergesagt werden?

4. Effects of fertilizer and spatial heterogeneity in soil pH on microbial biomass indices in a long-term field trial of organic agriculture

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Abstract

In the Darmstadt long-term fertilization trial, the application of composted cattle farmyard manure without (CM) and with (CMBD) biodynamic preparations was compared to mineral fertilization with straw return (MIN). The present study was conducted to investigate the effects of spatial variability, especially of soil pH in these three treatments, on soil organic matter and soil microbial biomass (C, N, P, S), activity (basal CO_2 production and O_2 consumption), and fungal colonization (ergosterol). Soil pH was significantly lower in the MIN treatments than in the organic fertilizer treatments. In the MIN treatments, the contents of soil organic C and total N were also significantly lower (13% and 16%, respectively) than those of the organic fertilizer treatments. In addition, the total S content increased significantly in the order MIN < CM < CMBD. The microbial biomass C content was significantly lower (9%) in the MIN treatments than in the organic fertilizer treatments. Microbial biomass N and biomass P followed microbial biomass C, with a mean C/N ratio of 7.9 and a mean C/P ratio of 23. Neither the microbial biomass C to soil organic C ratio, the metabolic quotient qCO_2, nor the respiratory quotient (mol CO_2/mol O_2) revealed any clear differences between the MIN and organic fertilizer treatments. The mean microbial biomass S content was 50% and the mean ergosterol content was 40% higher in the MIN treatments compared to the organic fertilizer treatments. The increased presence of saprotrophic fungi in the MIN treatments was indicated by significantly increased ratios of ergosterol-to-microbial biomass C and decreased ratio of microbial biomass C/S ratio. Our results showed that complex interactions between the effects of fertilizer treatments and natural heterogeneity of soil pH existed for the majority of microbial biomass and activity indices.

4.1. Introduction

A basic aim of organic agriculture is the improvement of soil fertility by maintaining or even improving soil organic matter levels by considering soil biological processes (Mäder et al., 2002). An important tool to achieve this aim is the application of farmyard manure (Raupp and Oltmanns, 2006a). Several other long-term field experiments have demonstrated the positive effects of organic fertilizer on the contents of soil organic matter and microbial biomass (Edmeades, 2003; Ludwig et al., 2007). The majority of these experiments were carried out on silt loams (Jenkinson, 1990; Mäder et al,. 2002; Hepperly et al., 2006; Marinari et al., 2006) and some on clay soils (Witter et al., 1993; Elfstrand et al., 2007). Information on the effects of farmyard manure on sandy soils is limited (Christensen, 1996; Ellmer et al., 2000), especially under organic agricultural management (Raupp and Oltmanns, 2006ab).

The effects of organic farming and biodynamic farming on soil organic matter and soil biological properties have been most intensively investigated on a silt loam in the DOK (bio-Dynamic, bio-Organic and conventional) trial in Therwil, Switzerland (Mäder et al., 2002; Fließbach et al., 2007; Birkhofer et al., 2008). In this DOK trial, highest levels of soil organic matter, microbial biomass and soil biological activities were usually observed in the biodynamic treatment (Fließbach et al., 2007; Birkhofer et al., 2008). Biodynamic agriculture is based on the anthroposophical concept of Rudolf Steiner, it is the oldest form of organic farming with a history of more than 80 years and still has considerable importance (Koepf et al., 1990, Podolinsky, 2000, Turinek et al., 2009). It is characterized by the unique use of compost and field preparations to stimulate the nutrient transformation processes (Zaller and Koepke, 2004). However, the results of the DOK trial are biased by the fact that the farmyard manure is obtained from two different farms so that not only could the biodynamic preparations cause differences but also could the different manure qualities. It is also unknown whether the results of the DOK trial are valid for sandy soils under warmer and dryer climatic conditions. This aspect could be addressed using the long-term field experiment carried out since 1980 by the Institute for Biodynamic Research on a fluviatile sediment in the south-west of Darmstadt, Hessia, Germany (Abele, 1987; Bachinger, 1996; Raupp and Oltmanns, 2006ab). In this experiment, the application of composted cattle farmyard manure without and with biodynamic preparations was compared to mineral fertilization with straw return. Both farmyard manure treatments were of the same origin and differed only in the addition of biodynamic preparations to the compost and to the field (Raupp, 2001).

Treatment effects on soil organic C, total N and microbial biomass C and N have been analyzed repeatedly over the last 27 years in the Darmstadt long-term fertilization trial (Bachinger, 1996; Raupp and Oltmanns, 2006ab; Heitkamp et al., 2009). However, virtually nothing is known about microbial biomass P and microbial biomass S in the present experiment, which is also true of the majority of other long-term experiments (Oehl et al., 2004). Knowledge of the behavior of P and S in long-term experiments might help us to use the nutrients held in organic matter more efficiently, due to the interactions with N turnover (Marschner, 1995; Schnug et al., 1993; Karamanos et al., 2005). A balanced supply of N, P, and S to crops may additionally help to improve crop health (Marschner, 1995). In other studies, significant differences have been observed for the respiratory quotient (mol CO_2/mol O_2) between organic and conventional farming systems (Dilly, 2001). Also the contents of the fungal cell-membrane component ergosterol and the ergosterol-to-microbial biomass C ratio were significantly lower in soils under organic management than conventional farming systems (Scheller and Joergensen, 2008).

Usually fluviatile soils exhibit a much higher spatial variability in soil properties than soils derived from aeolic sediments such as loess (Iqbal et al., 2005; Magid et al., 2006; Wälder et al., 2008). This spatial variability was not in the focus of the previous sampling strategies investigating the Darmstadt long-term fertilization trial. For this reason, the aim of the present research was to investigate the effects of spatial variability in high resolution, especially those of soil pH on soil organic matter (C, N, P, S) and soil microbial biomass (C, N, P, S), activity (basal CO_2 production and O_2 consumption), and community composition (ergosterol) in the Darmstadt trial using mineral fertilizer with straw addition and farmyard manure with and without biodynamic preparations as treatments.

4.2. Materials and Methods

4.2.1. Experimental site

The soil samples were taken from a long-term field trial of the Institute of Biodynamic Research, Darmstadt, Hessia, Germany (49° 50’ N, 8° 34’ E) at 100 m above see level. The long-term experiment was established in 1980 on a Haplic Cambisol (FAO-WRB, 2006) with 86% sand, 9% silt, and 5% clay, which has been developed from alluvial

sediments of the river Neckar (Abele, 1987; Bachinger, 1996). The mean annual temperature is 9.5°C and the mean annual precipitation is 590 mm.

The long-term experiment was initially built up of four fields (A, B, C, D). Out of these four, field A was chosen in this project because here the treatments remained unchanged since 1980 and edge effects due to a nearby forest did not occur. The field consisted of four field replicates (a, b, c, d) of the following three fertilizer types (horizontally) (Fig. 4: (i) mineral fertilizer (MIN, i.e. calcium ammonium nitrate, superphosphate, potassium chloride (since 1996 potassium magnesia) with the return of straw, (ii) composted cattle farmyard manure (CM) and (iii) composted cattle farmyard manure with the addition of biodynamic compost and field preparations (CMBD) both without straw return (Raupp, 2001).

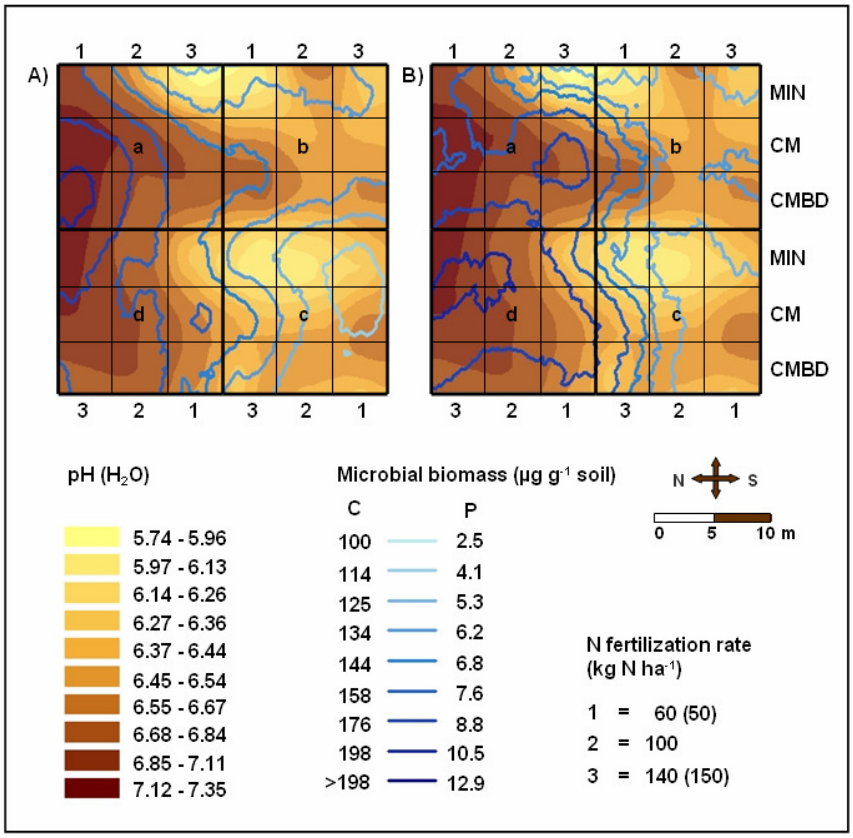

Figure 4: Spatial distribution of (A) microbial biomass C and (B) microbial biomass P in comparison with the spatial distribution of pH with different fertilizer types (MIN = mineral, CM = composted cattle manure, CMBD = composted cattle manure with biodynamic preparations) and fertilizer rates; the spatial distribution resulted from Ordinary Kriging with n = 144 obtained by grid sampling.

These three fertilizer types were added at three fertilizer rates (vertically) corresponding to an equivalent amount of total N (Table 3), i.e. (1) low = 60, (2) medium = 100, and (3) high = 140 kg N ha^{-1} for cereals and (1) low = 50, (2) medium = 100, and (3) high = 150 kg N ha^{-1} for root crops (Bachinger 1996). The mean annual C-input differed only slightly between the mineral and organic treatments (Table 3) so that both organic treatments showed around 10% (summed up over all fertilizer rates) higher mean annual C input rates in comparison with the MIN treatment where straw was returned to the field.

Table 3: Mean annual C and N inputs (kg ha^{-1}) into the different treatments by composted farmyard manure (CM and CMBD) or straw and mineral N (MIN) at the three N fertilization levels from 1981 to 2006 (Heitkamp et al., 2009).

N fertilization level	Mean annual C input		Mean annual N input	
	MIN	CM +CMBD	MIN	CM + CMBD
Low	800	630	43	45
Medium	880	950	77	82
High	930	1300	111	121

MIN = mineral fertilizer, CM = composted farmyard manure; CMBD = farmyard manure with the addition of biodynamic preparations.

The organic fertilizers were composted before application for three months if added to winter rye, or for six months if added to spring wheat and root crops (Raupp and Oltmanns, 2006b). The biodynamic compost preparation, 0.5 g of *Achillea millefolium*, *Chamomilla recutita*, *Taraxacum officinale*, *Valeriana officinalis, Urtica dioica* and the bark of *Quercus robur* were added separately to 1 t of composted farmyard manure (Koepf et al., 1990). The field preparations, horn-manure (200 to 300 g ha^{-1}) which consisted of cow dung was spread after tillage, sowing and stem elongation, while horn-silica (4 g ha^{-1}), which was produced by ground quartz (e.g. rock crystal) was spread at tillering, flowering and corn filling according to the usual biodynamic farming practice (Koepf et al., 1990). Except fertilization, all fields were managed identically, with a crop rotation of red clover (*Trifolium pratense* L.) or lucerne (*Medicago sativa* L.), spring wheat (*Triticum aestivum* L.), potatoes (*Solanum tuberosum* L.) or carrots (*Daucus carota* ssp. *Sativus* (Hoffm.) Arcang.) and winter rye (*Secale cereale* L.).

4.2.2. Sampling and soil chemical analysis

All soil samples were taken on 27 February 2007 before fertilizer application and sowing. The soil was collected out of the first 5 cm from the nine treatments, which were repeated four times in the field using a steel core (diameter 4 cm) in a grid design with four replicates per plot (25 m^2, Fig. 4). This resulted in 144 soil samples. The soils were sieved (< 2 mm), adjusted to 40% water holding capacity (WHC) and stored in polyethylene bags at 4°C until soil biological analysis was carried out. A sub-sample was dried and finely ground for chemical analyses. 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) analyzer. Total S and total P were analyzed after HNO_3-pressure digestion as described by Chander et al. (2008) by ICP-AES (Spectro Analytical Instruments, Kleve, Germany).

4.2.3. Basal respiration

For measuring basal respiration, 60 g moist soil adjusted to 40% water holding capacity were weighed into 80 ml incubation cylinders made of stainless steel nets (Hoffmann et al., 2009). The cylinders were placed into 500 ml Pyrex glass jars containing 5 ml 0.5 M NaOH at the bottom and incubated for 7 days at 22°C in the dark. The CO_2 evolved was determined by back-titration to pH 8.3 of the excess NaOH with 0.5 M HCl after addition of 0.5 M $BaCl_2$ solution. The O_2 consumed was measured at the same time using an Aqualytic (Darmstadt, Germany) tension-recording device (Robertz et al., 1999). The incubated soil was used for measuring all microbial biomass indices.

4.2.4. Microbial biomass and ergosterol

Microbial biomass C, N, P and S were estimated by fumigation extraction. For determining microbial biomass C and N (Brookes et al., 1985; Vance et al., 1987), 10 g moist soil was fumigated at 25°C with ethanol-free $CHCl_3$, which was removed after 24 h. Fumigated and non-fumigated 10-g samples were extracted with 40 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 as CO_2 by infrared absorption after combustion at 850°C using a Dimatoc 100 automatic analyzer (Dimatec, Essen, Germany). Microbial biomass C was calculated as E_C / k_{EC}, where E_C =

(organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990). Total N in the extracts was measured using a Dima-N chemoluminescence detector (Dimatec). Microbial biomass N was calculated as E_N / k_{EN}, where E_N = (total N extracted from fumigated soil) – (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996).

For determining microbial biomass P (Brookes et al., 1982), three portions equivalent to 2.5 g oven-dry soil were each extracted with 50 ml of 0.5 M $NaHCO_3$ (pH 8.5) after different pre-treatment. The first portion was used for the fumigated treatment (see above), the second portion for the non-fumigated treatment, and the third portion for estimating P fixation by the addition of 25 µg P g^{-1} soil to the extractant. P was analyzed by an ammonium molybdate-ascorbic acid method as described by Joergensen et al. (1995). Microbial biomass P was calculated as E_P / k_{EP} / recovery, where E_P = (PO_4-P extracted from fumigated compost) – (PO_4-P extracted from non-fumigated compost) and k_{EP} = 0.40 (Brookes et al., 1982).

For determining microbial biomass S (Khan et al., 2009), fumigated and non-fumigated 10-g samples were extracted with 50 ml 1 M NH_4NO_3 by 60 min horizontal shaking at 200 rev min^{-1} and filtered (hw3). All laboratory ware was washed with 1 M HCl. After filtration, the extracts were acidified with 0.5 ml 65% HNO_3 and stored at 4°C until total S was measured by ICP-AES. Microbial biomass S was calculated as E_S / k_{ES}, where E_S = (total S extracted from fumigated soil) – (total S extracted from non-fumigated soil) and k_{ES} = 0.35 (Saggar et al., 1981; Wu et al., 1994).

The fungal cell-membrane component ergosterol was extracted with 100 ml ethanol from 2 g moist soil by 30 min oscillating shaking at 250 rev min^{-1} (Djajakirana et al., 1996). Then, ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and detection at 282 nm.

4.2.5. Statistical analysis and interpolation

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (about 24 h at 105°C). Normality of distribution was tested using the χ^2-test. Data were log-transformed if they did not fit to a normal distribution for statistical analysis. The significance of differences between the treatments and the effects of treatment and pH and their interactions were tested by a one-way analysis of variance (ANOVA). The effects of soil pH on the relationship between treatments and other soil chemical and soil

biological properties were analyzed by analyses of covariance. All statistical analyses were performed using JMP 7.0 (SAS Inst. Inc.).

The interpolation of the spatial distribution of the pH, microbial biomass C and microbial biomass P were calculated on the basis of the 144 sampling points by Ordinary Kriging in ESRI ArcGIS 9, ArcMap 9.1. The function of the Geostatistical Analyst of ArcMap 9.1 was chosen for interpolation of the spatial distribution, while the input data such as sampling position and pH values, microbial biomass C and P contents were recorded in the attribute table. The Kriging method took the distance of the overall spatial arrangement of the measured values (autocorrelation) into account (Bonmati et al., 1991; Stutter et al., 2004). The multistep process of Kriging included exploratory statistical analysis of data, variogram modeling, creating the surface and exploring a variance surface (Kitanidis, 1997). The Kriging method is a best linear unbiased estimator (BLUE), which suggests that there is a linear combination between the interpolated values of an unsampled location $\hat{Z}_{(0)}$ and the mean measured values (Kitanidis, 1997; Liu et al., 2006), like

$$\hat{Z}_{(0)} = \sum_{i=1}^{n} \tilde{\lambda}_{0,i} * Z_{(i)}$$

where $\tilde{\lambda}_{0,i}$ is the unknown weight of the measured values at the i^{th} location, $Z_{(i)}$ is the measured value at the i^{th} location and n is the number of measurements (Blöschel, 2006).

4.3. Results

4.3.1. Soil chemical properties

Soil pH ranged from 5.74 to 7.35 and showed a specific spatial distribution in the field, which is characterized by highest pH values in the northern block ‘a’ and lowest in southern block ‘c’ (Fig. 4). In the MIN treatments, the mean pH over the three N levels was significantly lower (5%) than in the manure treatments (Table 4). No significant differences could be observed for pH (results not shown), soil organic C or any other soil chemical property between the three N levels (results not shown). In the MIN treatments, the mean contents of soil organic C and total N were also significantly lower than those of both manure treatments, which resulted in increases of 13% and 16%, respectively, whereas the mean contents of total P did not differ significantly between the treatments (Table 4).

Table 4: Mean soil pH, contents of soil organic C, total N, total P, and total S contents of the soils with different fertilizer treatment over all fertilizer rates (n = 144), effects of soil pH as covariate.

Treatment	pH (H_2O)	Soil organic C	Total N	Total P	Total S
		(mg g^{-1} soil)			
MIN	6.4 b	8.0 b	0.73 b	0.42 a	0.13 c
CM	6.7 a	9.0 a	0.85 a	0.41 a	0.14 b
CMBD	6.7 a	9.2 a	0.87 a	0.42 a	0.16 a
Probability values					
Treatment		0.71	0.96	0.01	0.01
Soil pH		0.01	0.01	<0.01	<0.01
Treatment × pH		0.78	0.98	0.01	0.01
CV (± %)	1.5	12	14	8.2	17

CV = mean coefficient of variation between replicates within one plot (n = 4); different letters within a column show significant differences ($P < 0.05$); MIN = mineral fertilizer, CM = composted farmyard manure; CMBD = farmyard manure with the addition of biodynamic preparations.

In contrast, the mean contents of total S increased significantly in the order MIN < CM < CMBD. This led to a 7% and 19% lower total S content in MIN in comparison to CM and CMBD, respectively. The differences in soil organic C and total N content were mainly caused by differences in soil pH according to the analysis of covariance. In contrast, the contents of total S and total P revealed complex interactions of soil pH and fertilizer treatments. These interactions completely masked any fertilizer effect on total P. Soil pH effects on the total storage pool of organic C, N, P, and S were underlined by significant correlation coefficients between these properties (Table 5).

Table 5: Correlation coefficients of pH and microbial biomass indices (n=144).

Indices	pH-H_2O	Microbial biomass C
Soil organic C	0.37**	0.25**
Total N	0.35**	0.19*
Total P	0.39**	0.32**
Total S	0.55**	0.50**
Microbial biomass C	0.64**	
Microbial biomass N	0.61**	0.76**
Microbial biomass P	0.39**	0.33**
Microbial biomass S	-0.07	0.03
Basal CO_2 production	0.37**	0.42**
Basal O_2 consumption	0.17*	0.16*

* $P < 0.05$; ** $P < 0.01$

The mean contents of K_2SO_4 extractable C were significantly higher (18%) in both manure treatments in comparison to MIN (Table 6). It was the only extractable fraction that was strongly influenced by soil pH. The mean contents of K_2SO_4 extractable N were also higher in the manure treatments, but only the MIN and CM treatments differed significantly. In contrast, the mean contents of $NaHCO_3$ extractable P were significantly highest in the MIN treatments. Also the mean contents of NH_4NO_3 extractable S were highest in the MIN treatments, but only the MIN and CMBD treatments differed significantly. Like K_2SO_4 extractable C, NH_4NO_3 extractable S showed significant interactions between fertilizer treatments and soil pH as covariate.

Table 6: Mean contents of 0.5 M K_2SO_4 extractable C, 0.5 M K_2SO_4 extractable N, 0.5 M $NaHCO_3$ extractable P and 1 M NH_4NO_3 extractable S in the soils with different fertilizer treatment over all fertilizer rates (n = 144); effects of soil pH as covariate.

Treatment	K_2SO_4 extractable C	K_2SO_4 extractable N	$NaHCO_3$ extractable P	NH_4NO_3 extractable S
	($\mu g\ g^{-1}$ soil)			
MIN	47 b	20 b	27 a	14 a
CM	54 a	24 a	24 b	13 ab
CMBD	57 a	23 ab	25 b	12 b
Probability values				
Treatment	<0.01	0.64	0.96	0.01
Soil pH	<0.01	0.97	0.77	0.20
Treatment × pH	<0.01	0.57	0.98	0.02
CV (± %)	13	24	12	17

CV = mean coefficient of variation between replicates within one plot (n = 4); different letters within a column show significant differences ($P < 0.05$); MIN = mineral fertilizer, CM = composted farmyard manure; CMBD = farmyard manure with the addition of biodynamic preparations

4.3.2. Microbial biomass and activity

Microbial biomass C varied around a mean of 144 $\mu g\ g^{-1}$ soil (Table 7) and ranged from 83 to 263 $\mu g\ g^{-1}$ soils. No significant differences could be observed for microbial biomass C or any other soil biological property between the three N levels (results not shown). In the MIN treatments, the mean microbial biomass C content over the three N levels was significantly lower (9%) than in the manure treatments (Table 7).

Table 7: Mean contents of microbial biomass C, N, P and S, ergosterol and basal respiration of the soils with different fertilizer treatment over all fertilizer rates (n = 144), effects of soil pH as covariate.

Treatment	Microbial biomass					Basal respiration	
	C	N	P	S	Ergosterol	CO_2-C	O_2
	($\mu g\ g^{-1}$ soil)				($\mu g\ g^{-1}$ soil)	($\mu g\ d^{-1}\ g^{-1}$)	
MIN	136 b	18 b	7.0 a	4.2 a	0.70 a	5.3 ab	17.9 a
CM	149 a	20 ab	8.0 a	2.9 b	0.48 b	5.0 b	17.6 a
CMBD	149 a	21 a	7.2 a	2.7 b	0.52 b	5.4 a	18.0 a
Probability values							
Treatment	0.01	0.06	0.35	<0.01	<0.01	<0.01	<0.01
Soil pH	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01
Treatment × pH	0.01	0.06	0.31	0.02	<0.01	<0.01	<0.01
CV (± %)	16	25	32	27	24	13	14

CV = mean coefficient of variation between replicates within one plot (n = 4); different letters within a column show significant differences ($P < 0.05$); MIN = mineral fertilizer, CM = composted farmyard manure; CMBD = farmyard manure with the addition of biodynamic preparations

Not only the fertilizer treatments, but also the soil pH had significant effects on the microbial biomass C and N contents (Fig. 5), as revealed by the analysis of covariance. The strong connection between soil pH and microbial biomass is obvious in Fig. 4 and 5 and additionally expressed by a strong correlation coefficient (Table 5). The highest mean microbial biomass C contents were detected in the plots with highest pH values. This led to complex significant interactions between fertilizer treatments and soil pH for the majority of microbial biomass and activity indices. The microbial biomass C-to-soil organic C ratios varied around a mean of 1.68, ranging from 0.76 to 3.33, and it was slightly higher (4%) in the MIN treatments (Table 8).

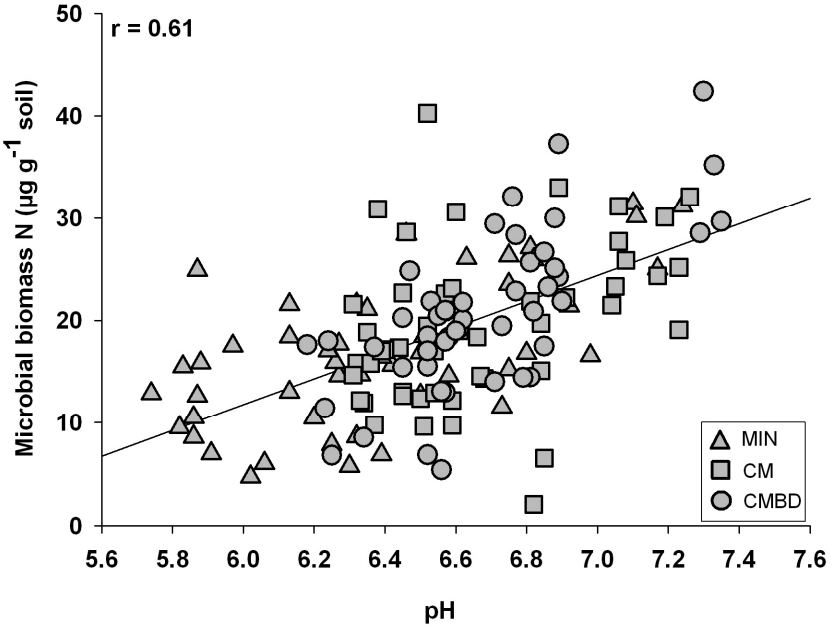

Figure 5: Correlation and correlation coefficient (r) of microbial biomass N and pH (n = 144) separated into fertilizer treatments (MIN = mineral, CM = composted cattle manure, CMBD = composted cattle manure with biodynamic preparations).

Microbial biomass N followed microbial biomass C, with a mean C/N of 7.9 and it was lowest in the CMBD treatments (Tables 7 and 8). Microbial biomass P followed microbial biomass C, with a mean C/P ratio of 23, without any fertilizer effect, but strong small-scale variability as shown by highest coefficients of variation within the replicate plots. The mean content of microbial biomass S was 50% higher in the MIN treatments (Table 7), leading to a significantly 44% lower microbial biomass C/S ratio in the manure treatments (Table 8). The mean ergosterol content was 40% higher in the MIN treatments compared to both manure treatments (Table 7), leading to a 39% higher ergosterol/microbial biomass C ratio which differed significantly (Table 8).

Table 8: Mean microbial biomass quotients, ergosterol to microbial biomass C ratio and metabolic quotient of the soils with different fertilizer treatment over all fertilizer rates (n = 144), effects of soil pH as covariate.

Treatment	Microbial biomass C/soil organic C (%)	Microbial biomass			Ergosterol/ microbial biomass C (%)	qCO_2 (µg CO_2-C g^{-1} biomass C d^{-1})	RQ CO_2/O_2 (mol mol^{-1})
		C/N	C/P	C/S			
MIN	1.71 a	8.1 ab	22 a	35 b	0.54 a	41 a	0.92 a
CM	1.66 ab	8.3 a	23 a	53 a	0.33 b	34 b	0.81 b
CMBD	1.65 b	7.2 b	25 a	63 a	0.36 b	37 ab	0.90 ab
Probability values							
Treatment	0.04	0.75	0.11	0.06	0.03	<0.01	<0.01
Soil pH	<0.01	<0.01	0.38	0.03	<0.01	0.41	<0.01
Treatment × pH	0.05	0.79	0.10	0.10	0.07	<0.01	<0.01
CV (%)	20	21	38	27	26	21	17

CV = mean coefficient of variation between replicates within one plot (n = 4); different letters within a column show significant differences ($P < 0.05$); MIN = mineral fertilizer, CM = composted farmyard manure; CMBD = farmyard manure with the addition of biodynamic preparations

The basal respiration expressed as CO_2 production and O_2 consumption varied around means of 5.2 µg CO_2-C d^{-1} g^{-1} and 17.9 µg O_2 d^{-1} g^{-1}, respectively, without clear differences between MIN and manure treatments, although highly significant differences were observed (Table 7). Lowest basal respiration rates were measured in the CM treatments and highest in the CMBD treatments. Soil pH seemed to mask treatment effects, as indicated by highly significant fertilizer treatment and soil pH interactions. The metabolic quotient qCO_2 varied around a mean of 37 µg CO_2-C g^{-1} microbial biomass C d^{-1} and the respiratory quotient (RQ) varied around a mean of 0.88 mol CO_2 mol^{-1} O_2 (Table 8). These two quotients did not reveal any clear differences between MIN and both manure treatments, like the basal respiration rates. Lowest qCO_2 and RQ values were measured in the CM treatments and highest in the MIN treatments.

4.4. Discussion

The soil of the experimental area exhibits very good conditions for rapid decomposition of plant residues and microbial turnover: the clay content is low, aeration is good, mean soil pH is moderately acidic, annual mean temperature is relatively high, and the site is irrigated during drought periods in the summer. For these reasons, the contents of soil organic C and microbial biomass C in the sandy soil of the field trial are in the lower part of the range reported for Central European arable soils (Anderson and Domsch, 1989).

The application of mineral fertilizers resulted in a significantly decreased soil pH. This has been repeatedly observed in long-term fertilization trials with application of ammonium fertilizers (Witter et al., 1993; Christensen, 1996; Birkhofer et al., 2008), although calcium ammonium nitrate has often been judged to have only little effect on acidification (Watson et al., 1995). In contrast, the application of farmyard manure usually leads to higher soil pH, caused by enrichment of cations during storage (Bachinger, 1996). However, the fertilizer effects on soil pH in the present experiment formed complex interactions with the natural heterogeneity in pH caused by lime-bands of shells in the fluviatile sediments (Abele, 1987; Bachinger, 1996). This led to higher pH values in the northern field replicates 'a' and 'd' (Fig. 4). The differences in soil pH seemed to be the major driving force for differences in soil organic matter and microbial biomass and activity between the different treatments. This was reflected by the spatial distribution of microbial biomass C and microbial biomass P, where the highest contents occurred at plots with highest pH values (Fig. 4). As it is known, long-term application of

farmyard manure led to higher soil organic C and total N levels, respectively compared to mineral fertilization (Christensen, 1988). This was reflected here by 13% and 16% higher contents of soil organic C and total N, in that order with organic fertilization. On the one hand this could be caused by the 10% higher C-input due to manure fertilization (Table 1) and on the other hand by the differing quality of composted manure which had a more complex chemical structure than straw which was more easily decomposable (Haynes and Naidu, 1998, Guerrero et al., 2007). This lead to higher recalcitrance of composted manure against microbial decomposition as suggested by Fließbach et al. (2007). Another reason might be the different effects of farmyard manure and straw, which was returned in the MIN treatments, on soil microorganisms (Schnürer et al., 1985; Lejon et al., 2007; Scheller and Joergensen, 2008), especially in combination with lowering of soil pH by mineral fertilization (Böhme et al., 2005).

Similarly to the DOK trial (Mäder et al., 2002; Fließbach et al., 2007; Birkhofer et al., 2008), neither mineral nor manure fertilization lead to a long-term increase of soil organic C over the 27 years of manure application compared with the initial level (Table 9). The soil organic C level was maintained despite the increase in pH, contrasting the commonly held opinion that an increase in pH intensifies the mineralization of soil organic C (Chan and Heenan, 1999). For this reason, the increase in soil organic matter with increasing pH suggests better conditions for plant growth, resulting in an increased C input by roots (Bruun et al., 2003), which was favored especially during the clover-grass cropping (Heitkamp et al., 2009). Bachinger (1996) measured 7.9, 9.2 and 10.6 mg organic C g^{-1} soil at 25 cm depth for the treatments MIN, CM and CMBD in 1989 and 1990, very similar compared to the results of 2007 with 8.0, 9.0 and 9.2 mg organic C g^{-1} soil at 5 cm depth (Table 9).

Table 9: Mean soil organic carbon contents (mg g^{-1}) of the three fertilizer types over all rates; [a)] initial level, no separation into fields (Raupp, 2001), [b)] first field separated soil organic carbon detection, used as initial level (Heitkamp et al., 2009), [c)] according to Bachinger (1996).

Treatment	1980 [a)]	1982 [b)]	1989/90 [c)]	2007
MIN	10.9	9.2	7.9	8.0
CM	10.9	9.3	9.2	9.0
CMBD	10.9	10.5	10.6	9.2

This suggests that the sampling depth has only minor effects on the organic C contents in a soil homogenized by mouldboard plowing. The stabilization of soil organic C due to manure fertilization in sandy soils was also detected by Ellmer et al. (2000), but contrary to our results here mineral fertilization with straw return resulted in a 20-30% decrease of soil organic C. This emphasizes the need for farmyard manure to maintain soil fertility, especially in arable sandy soils (Ellmer et al., 2000). In contrast to the DOK trial (Mäder et al., 2002; Fließbach et al., 2007) and to earlier results of the present experiment (Bachinger, 1996; Raupp, 2001), no significant differences were observed in the contents of soil organic C and N between the farmyard manure without and with addition of the biodynamic preparations. Reasons might be the higher resolution of sampling and the fact that exclusively field A was sampled in the present study.

Similar to soil organic C and total N, the content of total S was significantly increased in the two manure treatments, compared to the MIN treatment with straw return. This increase is remarkable because the S input that was 8 times higher with mineral fertilization (73 kg S ha^{-1} a^{-1}) than the input in the manure treatments (9 kg S $ha^{-1}a^{-1}$) (Raupp, 2001). The 7% and 19% higher total S content in CM and CMBD, respectively is most likely due to the sequestration of farmyard manure-derived S into soil organic matter, as explained above (Eriksen and Mortensen, 1999; Reddy et al., 2002). Total S was the only soil property analyzed in which the biodynamic preparations had a significant positive effect. In contrast to soil organic C, total N and total S, no effects of fertilization on the total P content were observed. This indicates similar P input and output rates in the MIN, CM and CMBD treatments, although this was not explicitly analyzed. The differences between the contents of microbial biomass C, microbial biomass N, and microbial biomass P in the three treatments reflected the contents of the total storage pool in soil. This means that no treatment effects on microbial biomass P were observed, but also no effects on the microbial biomass C/N and the microbial biomass C/P ratio. These two ratios are within the range reported by Joergensen and Emmerling (2006). The generally low microbial biomass C/S ratios indicate the absence of any S deficiency in the present experiment. The mean microbial biomass C/S ratio of 56 is similar to that reported by Khan et al. (2009), but only half of that reported by Wu et al. (1993, 1994) for English grassland soils. This is in part caused by the use of 1 M NH_4NO_3 as extractant, which renders more amino acids extractable than 0.01 M $CaCl_2$ (Khan et al., 2009). However, Chapman (1987) also measured a mean microbial biomass C/S ratio of 53 in acidic Scottish arable soil using 0.01 M $CaCl_2$ as extractant.

Microbial biomass C was significantly lower in the mineral fertilizer treatment, which is in accordance to Heitkamp et al. (2009). In contrast, microbial biomass S was significantly higher in this treatment. As a consequence, the microbial biomass C/S ratio was 44 % lower than in the manure treatments. A likely explanation is a shift in the microbial community structure towards saprotrophic fungi at the expense of ergosterol-free arbuscular mycorrhizal fungi (Scharfy et al., 2005; Joergensen and Wichern, 2008). This is indicated by the increased ergosterol content and especially by the increased ergosterol-to-microbial biomass C ratio (Scheller and Joergensen, 2008). Fungi which were grown under laboratory conditions in culture media were able to increase their S concentration with increasing S supply by about 50-130%, while bacteria could only enhance their S concentration by about 20% (Saggar et al., 1981; Banerjee and Chapman, 1996). In the present field experiment, a low microbial biomass C/S ratio obtained by the fumigation extraction method seems to be an additional good indicator for a shift in the microbial community, contrasting the indicative properties of high microbial biomass C/N ratios (Joergensen and Emmerling, 2006). Straw is strongly colonized by saprotrophic fungi (Bowen and Harper, 1990; Cheshire et al., 1999; Scheller and Joergensen, 2008) and the regular incorporation of straw into soils promotes saprotrophic fungi (Allison and Killham, 1988), causing not only a rapid straw mineralization but may also increase the mineralization rate of soil organic matter as observed by Scheller and Joergensen (2008). The high saprotrophic potential of the microbial community in the mineral fertilizer treatments is revealed by its higher content of extractable S, indicating a high S mineralization capacity, which led in the long-term to lower contents of total S and soil organic C.

The present fertilizer treatments had no clear effects on the microbial biomass C-to-soil organic C ratio, the respiratory quotient or the metabolic quotient qCO_2. All three ecophysiological ratios were within the range reported in the literature (Anderson and Domsch, 1989, 1990; Dilly, 2001, 2005). The microbial biomass-to-soil organic C ratio is an indicator for organic matter availability to soil microorganisms (Anderson and Domsch, 1989) and was often increased by farmyard manure addition (Mäder et al., 2002; Marinari et al., 2006; Melero et al., 2006). The metabolic quotient qCO_2 was often decreased by farmyard manure addition (Mäder et al., 2002; Marinari et al., 2006; Melero et al., 2006; Scheller and Joergensen, 2008), indicating a lower catabolic demand and thus a higher average age of the soil microbial community (Anderson and Domsch, 1990; Dilly, 2005). Also the respiratory quotient remained unaffected by organic farming in

contrast to Dilly (2001), indicating the absence of nutrient and other substrate quality effects on microbial ecophysiology (Dilly, 2001, 2003). The effects on these ecophysiological ratios seem to be masked by the very good conditions for rapid decomposition of plant residues and microbial turnover in the present sandy soil generally low in soil organic matter and the identical crop rotation in all treatments.

4.5. Conclusion

Mineral fertilizer application decreased soil pH. In combination with straw return, this promoted saprotrophic fungi and led to significant decreases in soil organic C, total N and especially total S compared to the farmyard manure treatments. The biodynamic treatment only showed significant differences for the total S content. The spatial variability of pH caused by the heterogeneity of fluviatile sandy sediments masked possible further treatment effects on microbial biomass and activity indices as well as their ratios. The increased presence of saprotrophic fungi in the mineral fertilizer treatments was indicated by significantly increased ratios of ergosterol-to-microbial biomass C and significant decrease of the microbial biomass C/S ratio. It would be interesting to investigate whether this ratio can be used as an index for a shift in the microbial community structure of agricultural soils towards saprotrophic fungi.

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5. Tillage systems and their relationships to microbial C, N, P, and S storage in two long-term experiments on loess-derived Luvisols

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Abstract

The nutrient-specific effects of tillage on microbial activity (basal respiration), microbial biomass (C, N, P, S) indices and the fungal cell-membrane component ergosterol were examined in two long-term experiments on loess derived Luvisols, comparing the effects of mouldboard ploughing with a maximal tillage depth of 30 cm versus rotary harrow going down to maximal 8 cm over a period of around 40 years. The rotary harrow treatment led to a significant 8% increase in the mean stocks of soil organic C, 6% of total N and 4% of total P at 0-30 cm depth, but had no main effect on the stocks of total S. The tillage effects were identical at both sites, but site effects between the two experiments were usually stronger than the effects of the two tillage treatments. The rotary harrow treatment led to a significant increase in the mean stocks of microbial biomass C (+18%), N (+25%), and P (+32%) and to a significant decrease in the stocks of ergosterol (-26%) at 0-30 cm depth, but had no main effect on the stocks of microbial biomass S or on the mean basal respiration rate. The mean microbial biomass C/N (6.4) and C/P (25) ratios were not affected by the tillage treatments. In contrast, the microbial biomass C/S ratio was significantly increased from 34 to 43 and the ergosterol-to-microbial biomass C ratio significantly decreased from 0.20% to 0.13% by the rotary harrow treatment. The microbial biomass C-to-soil organic C ratio varied around 2.1% in the plough treatment and declined from 2.6% at 0-10 cm depth to 2.0 at 20-30 cm depth in rotary harrow treatment. The metabolic quotient qCO_2 revealed exactly the inverse relationships with depth and treatment to the microbial biomass C-to-soil organic C ratio. Rotary harrow management caused a reduction in the microbial turnover in combination with an improved microbial substrate use efficiency and a lower contribution of

saprotrophic fungi to the soil microbial community. This contrast the view reported elsewhere and points to the need of more information on tillage-induced shifts within the fungal community in arable soils.

5.1. Introduction

Tillage is one of the major tools for farmers to control the growth of crops and weeds and to regulate the microbial process of plant residue decomposition and the resulting nutrient release (Lal et al., 1990; Dick, 1992). A reduction in tillage intensity has often been discussed in terms of energy saving, increasing rainfall infiltration, thus reducing erosion, and enhancing C sequestration to reduce CO_2 emissions (Frede et al., 1994; Doran et al., 1998; Paustian et al., 2000). Mouldboard ploughing is still the most widespread method used in humid Central Europe (Derpsch, 1998; Etana et al., 1999; Holland, 2004), especially in organic farming (Kouwenhoven et al., 2002). This type of ploughing can be replaced in the order of decreasing intensity by rotary cultivators (Meyer et al., 1996; Ahl et al., 1998), rotary harrows (Stockfisch et al., 1999; Jacobs et al., 2009) or grubbers (Berner et al., 2008). No-tillage systems are rare in Central Europe (Frede et al., 1994; Børrensen, 1999), but widespread all over the world (Balota et al., 2003; Alvear et al., 2005; Reeleder et al., 2006). These studies focussed mainly on soil organic C and microbial biomass C (Stockfisch et al., 1999; Balesdent et al., 2000; Kushwaha et al., 2001; Wright et al., 2008) as indicators for tillage effects, often in combination with total N and microbial biomass N (Jacobs et al., 2009). Information on tillage-specific effects on total P and microbial biomass P are rare (Saffigna et al., 1989; Meyer et al., 1996), whereas that on total S and microbial biomass S do not exist.

Microbial biomass C and microbial biomass N are closely related in C-limited agricultural systems (Dilly et al., 2003; Joergensen and Emmerling, 2006) where N rarely limits microbial growth (Jenkinson, 1988; Joergensen and Mueller, 1996). Microbial biomass P and microbial biomass S are less intimately connected with microbial biomass C (Heinze et al., 2010). One reason is that different storage components, such as polyphosphates and teichoic acids for P (Grant, 1979; Gächter and Meyer, 1993; Nielsen et al., 2002) are known, which are affected by the fertilizer management and the ratio of fungi to bacteria (Heinze et al., 2010). Saprotrophic fungi are apparently able to store large amounts of S in their biomass (Saggar et al., 1981; Banerjee and Chapman, 1996). As the tillage intensity is reported to have a strong impact on soil fungi (Ahl et al., 1998),

the analysis of microbial biomass S would give additional information on tillage induced nutrient turnover.

In Göttingen, we had the unique opportunity to investigate two long-term tillage experiments on loess-derived Luvisols, which have been comparing mouldboard ploughing with a combination of rotary harrow and rototiller over a period of around 40 years. This enabled us to examine the nutrient-specific effects of these two tillage systems on microbial activity (basal respiration), microbial biomass (C, N, P, S) indices and the fungal cell-membrane component ergosterol. The underlying hypothesis was that the promotion of fungi by a reduction in tillage intensity will specifically increase P and S storage within the microbial biomass and increase C sequestration by improving the substrate use efficiency.

5.2. Materials and Methods

5.2.1. Experimental site and investigation design

Two tillage systems were investigated at two research sites (Garte and Hohes Feld) close to Göttingen (Lower Saxony, Germany). The experiment at the site Garte (51° 29' N, 9° 56' E, 163 m ASL) was established in 1970 and that at the site Hohes Feld (51° 37' N, 9° 53'' E, 151 m ASL) in 1967. The average mean annual precipitation is 645 mm with a mean annual temperature of 8.7 °C. The soil type at the two sites was characterized as Haplic Luvisol (FAO-WRB, 2006) derived from loess. The mean texture at 0-30 cm depth was 15.1% clay, 72.7% silt, and 12.2% sand at the site Garte (Ehlers et al., 2000; Reiter et al., 2002) and 17.2% clay, 66.4% silt, and 16.4% sand at the site Hohes Feld (De Mol, 1996). The mean soil pH-H_2O was 7.7 at the site Garte and 7.5 at the site Hohes Feld. The soils had been mouldboard ploughed for many years before the experiments started.

Tillage was always carried out after harvest. One tillage treatment was regular mouldboard ploughing down to 25-30 cm depth in autumn followed by seedbed preparation with a rotary harrow. The other tillage treatment consisted of shallow cultivation down to 6-8 cm depth with a rotary harrow and a rototiller for stubble cultivation and seedbed preparation (Ehlers et al., 2000; Reiter et al., 2002). At the site Garte, the experimental design was a randomised complete block design with four replicate plots (40 × 20 m). At the site Hohes Feld, a split plot design with three replicate

plots (36 × 12.7 m) was established due to a smaller dimension of the field. The crop rotation was based on cereals in the long-term and identical at both sites (Reiter et al., 2002). However, in the four years before soil sampling the crop rotation was balanced between cereals and legumes with peas (*Pisum sativum* L.), winter wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and broad beans (*Vicia faba* L.). The experimental sites were fertilized with 164 kg N ha^{-1} a^{-1} at an average over the past 10 years with the exception of the cultivated legumes. Additionally, 60 kg K ha^{-1} and 9 kg Mg ha^{-1} fertilizers were applied to both sites in 2004 and 2005, with 169 kg K ha^{-1} and 25 kg Mg ha^{-1} added in 2006. The site Hohes Feld was fertilized with 46 kg P ha^{-1} in 2004 and 2005, whereas the site Garte has not received any P fertilizer since 1997. The chopped straw remained on the plots and was incorporated into the soil by the respective tillage systems.

5.2.2. Soil sampling and chemical analysis

All soil samples were taken on 8 March 2007 when the sites laid fallow before sowing of broad beans. The soils were sampled in a grid design with 4 replicates from each plot at 0-5, 5-10, 10-20, 20-30, and 30-40 cm depth using a steel core with 4 cm diameter at 0-5 and 5-10 cm depth and 8 cm diameter in the deeper layers. This resulted in 16 samples per treatment and depth at the site Garte and 12 replicate samples per treatment and depth at the site Hohes Feld. All samples were sieved (< 0.2 mm), adjusted to 40% water holding capacity (WHC) and stored in polyethylene bags at 4°C until soil biological analysis was carried out. A sub-sample was dried (105°C) and finely ground for chemical analysis. The pH was determined in water with 1:2.5 (w:v). Total contents of C and N were detected by gas chromatography using a Vario MAX (Elementar, Hanau, Germany) elemental analyser. Total S and P were analysed by an HNO_3-pressure digestion as described by Chander et al. (2008) by ICP-AES (Spectro Analytical Instruments GmbH, Kleve, Germany).

5.2.3. Microbial activity and biomass indices

For measuring basal respiration, 60 g moist soil adjusted to 40% water holding capacity was weighed into 80 ml incubation cylinders made of stainless steel nets (Hoffmann et al., 2010). The cylinders were placed into 500 ml Pyrex glass jars containing 5 ml 0.5 M NaOH at the bottom and incubated for 7 days at 22°C in the dark.

The CO_2 evolved was determined by back-titration of the excess NaOH to pH 8.3 using 0.5 M HCl after addition of 5 ml of a saturated $BaCl_2$ solution.

Microbial biomass C, N, P and S were estimated by fumigation extraction. For determining microbial biomass C and N (Brookes et al., 1985; Vance et al., 1987), 10 g moist soil was fumigated at 25°C with ethanol-free $CHCl_3$, which was removed after 24 hours. 10 g of fumigated and non-fumigated soil were extracted with 40 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 as CO_2 by infrared absorption after combustion at 850°C using a Dimatoc 100 automatic analyser (Dimatec, Essen, Germany). Microbial biomass C was calculated as E_C / k_{EC}, where E_C = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990). Total N in the extracts was measured using a Dima-N chemoluminescence detector (Dimatec). Microbial biomass N was calculated as E_N / k_{EN}, where E_N = (total N extracted from fumigated soil) – (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996).

For determining microbial biomass P (Brookes et al., 1982), three portions equivalent to 2.5 g oven-dry soil were each extracted separately with 50 ml of 0.5 M $NaHCO_3$ (pH 8.5) after different pre-treatment. The first portion was used for the fumigated treatment (see above), the second portion for the non-fumigated treatment, and the third portion for estimating P fixation by the addition of 25 µg P g^{-1} in form of KH_2PO_4 soil to the extractant. P was analysed by an ammonium molybdate-ascorbic acid method as described by Joergensen et al. (1995). Microbial biomass P was calculated as E_P / k_{EP} / recovery, where E_P = (PO_4-P extracted from fumigated soil) – (PO_4-P extracted from non-fumigated soil) and k_{EP} = 0.40 (Brookes et al., 1982).

For determining microbial biomass S (Khan et al., 2009), fumigated and non-fumigated 10-g samples were extracted with 50 ml 1 M NH_4NO_3 by 60 min horizontal shaking at 200 rev min^{-1} and filtered (hw3). After filtration, the extracts were acidified with 0.5 ml 65% HNO_3 and stored at 4°C until total S was measured by ICP-AES. Microbial biomass S was calculated as E_S / k_{ES}, where E_S = (total S extracted from fumigated soil) – (total S extracted from non-fumigated soil) and k_{ES} = 0.35 (Saggar et al., 1981; Wu et al., 1994).

The fungal cell-membrane component ergosterol was extracted with 100 ml ethanol from 2 g moist soil by 30 min oscillating shaking at 250 rev min^{-1} (Djajakirana et al.,

1996). Then, the ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and a resolution of detection of 282 nm.

5.2.4. Statistical analysis

The results presented in the tables are arithmetic means of the stocks at 0-30 cm depth and expressed on an oven-dry basis (about 24 h at 105°C). Normality of distribution was tested using the χ^2-test. The significant influence of the factors treatment, sites, depth and their interactions on soil physical, chemical, and biological properties were analysed by a two-way analysis of variance (ANOVA) with depth as repeated measures. All statistical analyses were performed using JMP 7.0 (SAS Inst. Inc.).

5.3. Results

Lowest bulk density was generally found at 0-5 cm depth and highest at 10-20 cm depth (Fig. 6). At 0-5 cm and at 30-40 cm depth, bulk density was significantly lower in the rotary harrow treatment in comparison with the plough treatment at both sites. The mean bulk density over all 5 depths was significantly lower ($P < 0.01$) at the site Garte (1.29 g cm^{-3}), than at the site Hohes Feld (1.32 g cm^{-3}).

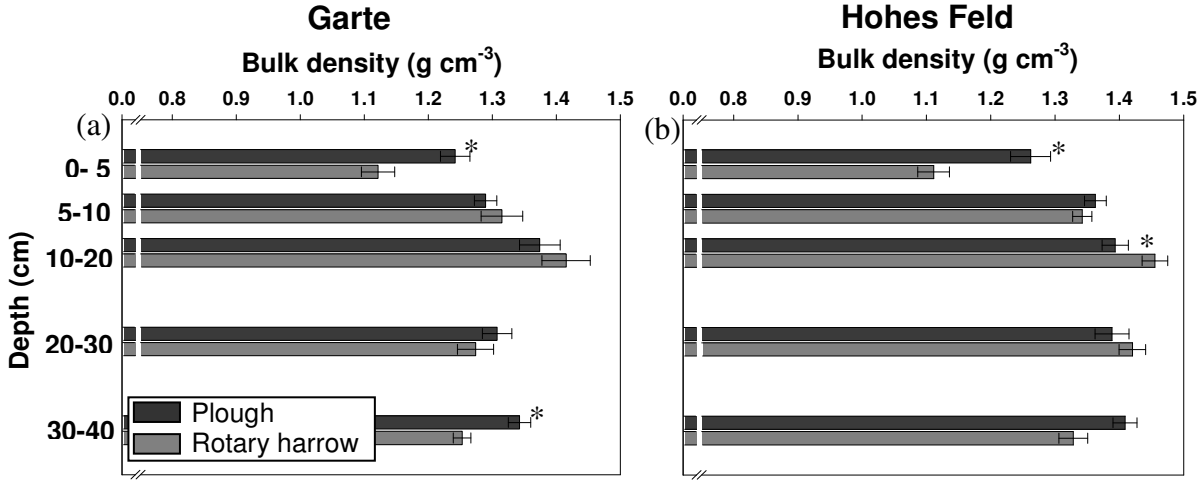

Figure 6: Mean bulk density at the sites (a) Garte (n = 32) and (b) Hohes Feld (n = 24) under two tillage treatments at different depths; * indicates a depth-specific significant difference between the two tillage treatments ($P < 0.01$, t-test); error bars show ± standard error.

The contents of soil organic C and total N showed a strong decrease with depth in the rotary harrow treatment (Fig. 7a/b). This decrease was less pronounced for total P and total S (Fig. 7c/d). In the plough treatment, no significant depth gradient was observed for soil organic C, total N, or total P at 0-30 cm depth. Only total S showed a moderate decline with depth. At 0-5 cm depth, the contents of soil organic C and total N were 35% and 22% higher in the rotary harrow treatment in comparison with the plough treatment. The respective figures for total P and total S were only 8% and 10%. At 5-10 cm depth, the contents of soil organic C, total N, and total P were 12%, 11% and 15%, respectively higher in the rotary harrow treatment than with plough management. In the next two layers at 10-30 cm depth, the differences between the two tillage treatments changed to insignificance for total P and total S and to significantly higher contents for soil organic C and total N in the plough treatment. At 30-40 cm, the content of all elements did not differ between the tillage treatments.

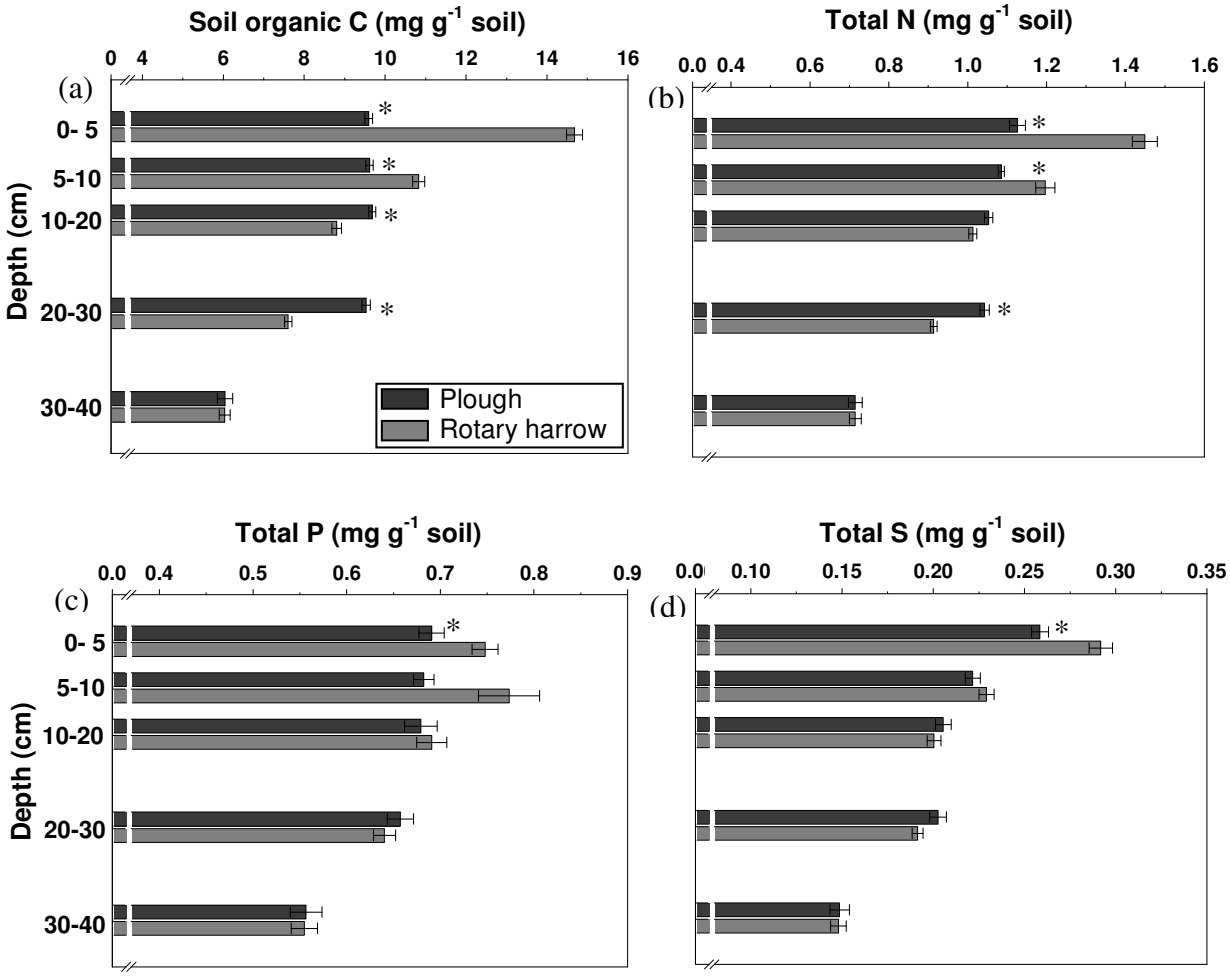

Figure 7: Mean contents over both sites (Garte and Hohes Feld) of (a) organic carbon, (b) total N, (c) total P, and (d) total S in soils from two tillage treatments at different depths; * indicates a

depth-specific significant difference between the two tillage treatments ($P < 0.01$, t-test); error bars show ± standard error (n = 56).

The rotary harrow treatment led to a significant 8% increase in the mean stock of soil organic C, total N (6%) and P (4%) at 0-30 cm depth, but had no main effect on the stocks of S or on the extractable fractions of P and S (Table 10). The tillage effects were usually identical at both sites as revealed by the absence of significant site × treatment effects. Only the stock of extractable P was significantly higher in the plough treatment of the site Garte, which was not confirmed at the site Hohes Feld. At this site, the stocks of all total and extractable elements exceeded those of the site Garte by 8% (total N) to 38% (total P).

Table 10: Mean stocks of soil organic C, total N, total P, total S, $NaHCO_3$ extractable P, and NH_4NO_3 extractable S in soils from two tillage treatments at 0-30 cm depth for the sites Garte (n = 64) and Hohes Feld (n = 48); probability values of a 2-factorial ANOVA with depth as repeated measurements.

Site, treatment	Soil organic C	Total N	Total P	Total S	$NaHCO_3$ extractable P	NH_4NO_3 extractable S
	(t ha^{-1})				(kg ha^{-1})	
Garte, plough	37	4.2	2.3	0.83	134	78
Garte, rotary harrow	39	4.3	2.4	0.82	119	78
Hohes Feld, plough	40	4.4	3.2	0.95	168	99
Hohes Feld, rotary harrow	44	4.8	3.3	1.0	177	96
Probability values						
Treatment	<0.01	<0.01	<0.01	0.27	0.11	0.77
Site	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Depth	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Site × treatment	0.55	0.06	0.25	0.29	0.02	0.85
Depth × treatment	<0.01	<0.01	<0.01	<0.01	<0.01	0.63
Site × depth	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
CV (±%)	8.7	8.9	8.6	9.2	13	11

The contents of microbial biomass C, N, P, and S as well as ergosterol showed a strong decrease with depth in the rotary harrow treatment (Fig. 8a/b/c/d/e). In the plough treatment, no depth gradient was observed for microbial biomass C and N as well as ergosterol at 0-30 cm depth, whereas microbial biomass P showed a moderate decline with depth and microbial biomass S a sharp drop after 10 cm depth

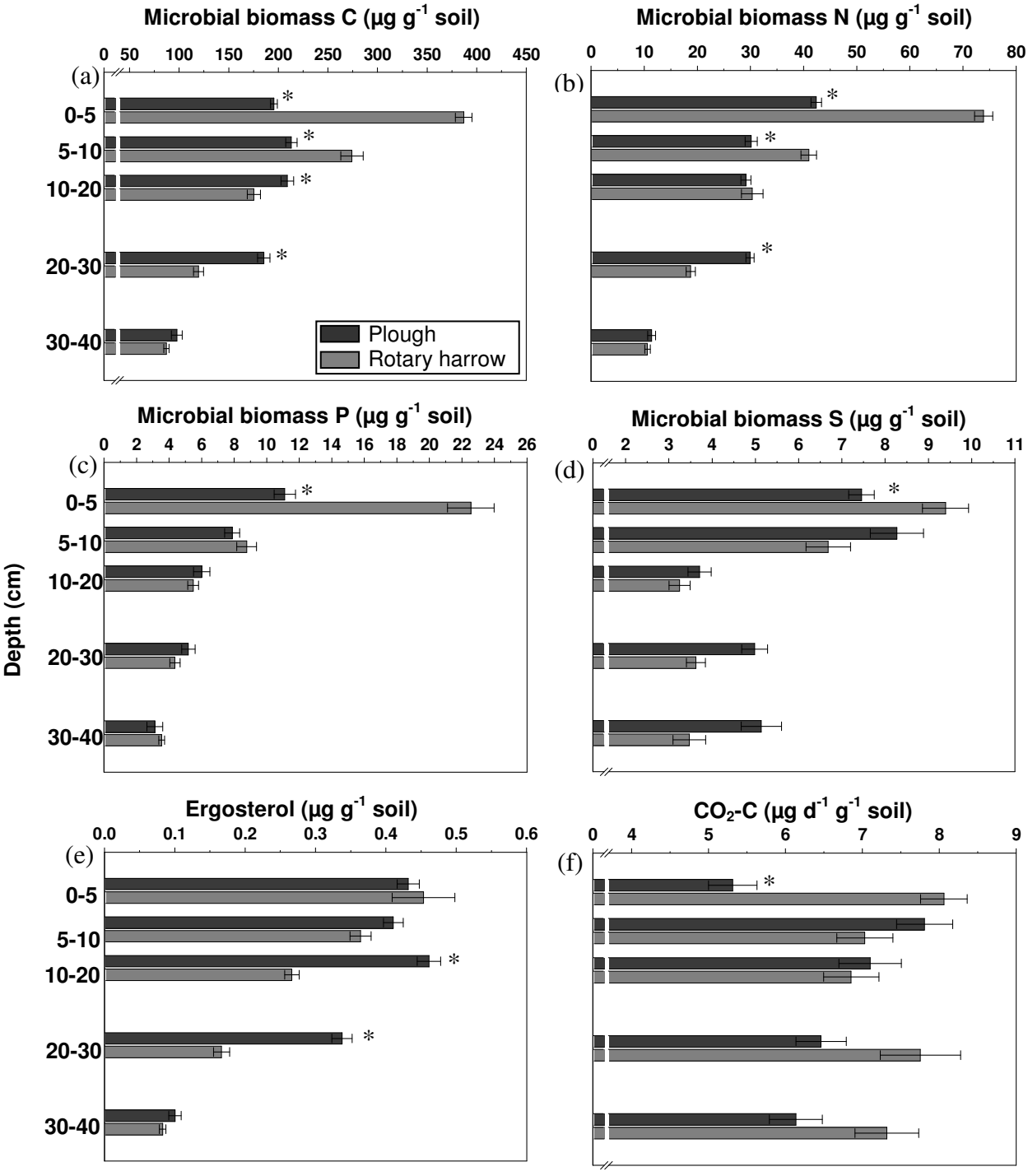

Figure 8: Mean contents over both sites (Garte and Hohes Feld) of (a) microbial biomass C, (b) microbial biomass N, (c) microbial biomass P, (d) microbial biomass S, (e) ergosterol, and (f) basal respiration rate in soils from two tillage treatments at different depths; * indicates a depth-

specific significant difference between the two tillage treatments ($P < 0.01$, t-test); error bars show ± standard error (n = 56).

Basal respiration remained more or less constant throughout the soil profile, with significantly higher rates in the rotary harrow treatment, especially at 0-5 cm depth and also at 20-40 cm depth (Fig. 8f). At 0-5 cm depth, the contents of microbial biomass C, N, and P were roughly 100% higher in the rotary harrow treatment in comparison with the plough treatment. In contrast, the respective figures for microbial biomass S and ergosterol were only 25% and 5%. At 5-10 cm depth, the contents of microbial biomass C and N were still higher in the rotary harrow treatment in comparison with the plough treatment. In the next layer at 10-20 cm depth, the differences between the two tillage treatments changed to insignificance for microbial biomass N, P, and S and to significantly higher contents for microbial biomass C and ergosterol in the plough treatment. At 20-30 cm depth, not only microbial biomass C and ergosterol, but also microbial biomass N and S were significantly higher. At 30-40 cm depth, the content of all microbial biomass indices did not differ between the tillage treatments.

The rotary harrow treatment led to a significant increase in the mean stocks of microbial biomass C (+18%), N (+25%), and P (+32%) and to a significant decrease in the stock of ergosterol (-26%) at 0-30 cm depth, but had no main effect on the stocks of microbial biomass S or on the mean basal respiration rate (Table 11). Tillage effects on microbial biomass C and N were stronger at the site Hohes Feld as indicated by the significant second-order interactions of sites and tillage treatments. In contrast, no site-specific treatment effects were detected on the other microbial biomass indices. At the site Hohes Feld, the stocks of microbial biomass C and P exceeded those of the site Garte by roughly 40%. In contrast, stocks of ergosterol and also the mean basal respiration rate were both 27% lower at the site Hohes Feld.

Table 11: Mean stocks of microbial biomass C, N, P, and S, ergosterol, and mean basal respiration rate in soils from two tillage treatments at 0-30 cm depth for the sites Garte (n = 64) and Hohes Feld (n = 48); probability values of a 2-factorial ANOVA with depth as repeated measurements

Site, treatment	Microbial biomass				Ergosterol	CO_2-C
	C	N	P	S		
	($kg\ ha^{-1}$)					($kg\ d^{-1}\ ha^{-1}$)
Garte, plough	698	132	25	24	1.76	30
Garte, rotary harrow	782	150	35	21	1.41	33
Hohes Feld, plough	934	128	37	24	1.44	22
Hohes Feld, rotary harrow	1146	174	47	24	0.96	24
Probability values						
Treatment	<0.01	<0.01	<0.01	0.79	<0.01	0.22
Site	<0.01	<0.01	<0.01	0.73	<0.01	<0.01
Depth	<0.01	<0.01	<0.01	<0.01	<0.01	0.17
Site × treatment	<0.01	<0.01	0.93	0.36	0.45	0.82
Depth × treatment	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Site × depth	0.08	<0.01	<0.01	<0.01	<0.01	0.14
CV (±%)	14	16	17	27	19	17

All soil organic matter-related elements were significantly correlated. The relationships between soil organic C and total N as well as total S and total N were especially strong (Table 12). All microbial biomass indices were significantly interrelated. Significant relationships were also found between the microbial biomass indices and most of the soil organic matter-related elements. Especially significant correlations were observed between microbial biomass C and soil organic C.

Table 12: Significant correlation coefficients ($P < 0.01$) between microbial biomass indices, soil organic C, and total contents of N, P, and S (n = 280)

	Microbial biomass C	Microbial biomass N	Microbial biomass P	Microbial biomass S	Ergosterol	Soil organic C	Total N	Total P
Microbial biomass N	**0.83**							
Microbial biomass P	0.71	0.72						
Microbial biomass S	0.41	0.37	0.35					
Ergosterol	0.43	0.49	0.32	0.27				
Soil organic C	**0.90**	**0.85**	0.70	0.40	0.53			
Total N	**0.80**	0.79	0.63	0.34	0.46	**0.87**		
Total P	0.62	0.42	0.42			0.49	0.52	
Total S	0.72	0.72	0.61	0.31	0.42	0.76	**0.87**	0.61

The mean microbial biomass C/N and C/P ratios were not affected by the tillage treatments (Table 13). In contrast, the microbial biomass C/S ratio was significantly increased and the ergosterol-to-microbial biomass C ratio significantly decreased by the rotary harrow treatment. At the site Hohes Feld, the microbial biomass C/N and C/S ratios were significantly increased and the microbial biomass C/P and ergosterol-to-microbial biomass C ratios significantly decreased in comparison with the site Garte.

Table 13: Mean microbial biomass quotients and ergosterol-to-microbial biomass C ratio and metabolic quotient (qCO_2) in soils from two tillage treatments at 0-30 cm depth for the sites Garte (n = 64) and Hohes Feld (n = 48); probability values of a 2-factorial ANOVA with depth as repeated measurements.

Site, treatment	Microbial biomass			Ergosterol/microbial biomass C	qCO_2 ($\mu g\ CO_2$-C g^{-1} biomass C d^{-1})
	C/N	C/P	C/S		
Garte, plough	5.3	28	29	0.25	43
Garte, rotary harrow	6.1	22	37	0.18	42
Hohes Feld, plough	7.3	25	39	0.15	24
Hohes Feld, rotary harrow	6.6	24	48	0.08	21
Probability values					
Treatment	0.22	0.11	0.04	<0.01	0.06
Site	<0.01	<0.01	<0.01	<0.01	<0.01
Depth	<0.01	<0.01	0.03	0.05	<0.01
Site × treatment	0.05	0.02	0.04	0.72	0.11
Depth × treatment	<0.01	0.10	0.10	0.13	<0.01
Site × depth	<0.01	<0.01	0.04	0.79	<0.01
CV (±%)	13	19	27	20	18

The microbial biomass C-to-soil organic C ratio decreased with depth in the rotary harrow treatment and varied around a mean of 2.1% in the plough treatment (Fig. 9a). Consequently, in the rotary harrow treatment, this ratio significantly exceeded that of the plough treatment at 0-10 cm depth and significantly went below the plough treatment at 10-40 cm depth. On a stock basis, the mean microbial biomass C-to-soil organic C ratio was significant 7% higher at the site Hohes Feld in comparison with the site Garte (data not shown). The metabolic quotient qCO_2 revealed exactly the reverse relationships with depth to the microbial biomass C-to-soil organic C ratio (Fig. 9b), which led to a significant inverse correlation between these two indices (r = -0.62, P <0.01). The mean qCO_2 was significantly 37% lower at the site Hohes Feld in comparison with the site Garte, whereas no effects of the tillage treatments could be detected (Table 13).

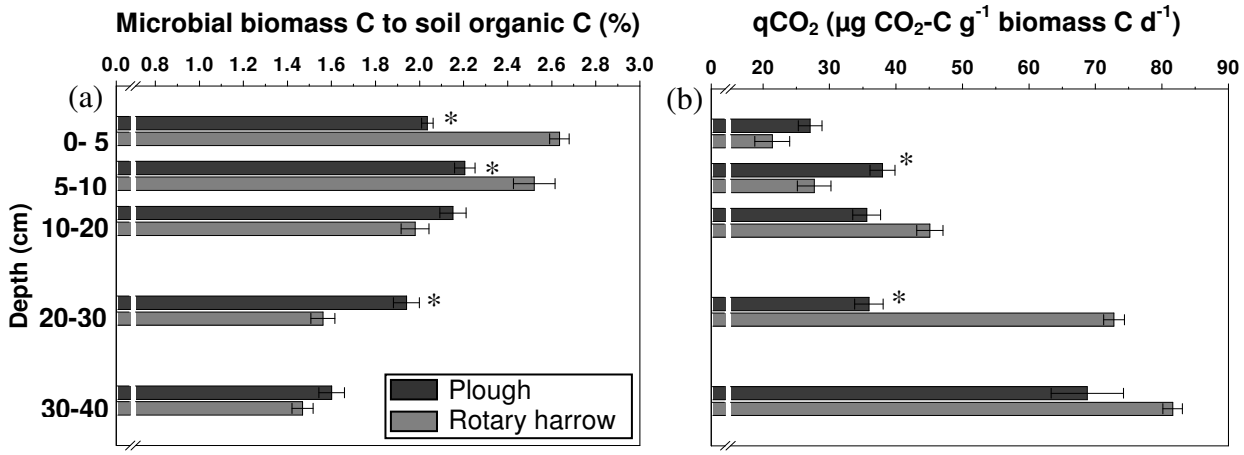

Figure 9: Mean ratios over both sites (Garte and Hohes Feld) of (a) soil organic carbon-to-microbial biomass C ratio and (b) the mean metabolic quotient qCO_2 in soils from two tillage treatments at different depths; * indicates a depth-specific significant difference between the two tillage treatments ($P < 0.01$, t-test); error bars show ± standard error.

5.4. Discussion

The reduction in tillage intensity by replacing the plough by a rotary harrow had small but significant positive effects on C sequestration, as indicated by the increased C stock at the present two sites. This significant increase has sometimes been found (Alvarez, 2005), so on a neighbouring site (Stockfisch et al., 1999), but not always (Ahl et al., 1998). For assessing the effects of different tillage systems on C sequestration and on nutrient storage, it is indispensable to calculate the total stocks in the tillage-affected layer, which is at least 0-30 depth. However, numerous studies still fail to take into account bulk density and tillage depth appropriately (Jacobs et al., 2009; Kandeler et al., 1999). A sole investigation of the top two layers would result in strong effects of doing without soil tillage. This would lead to an increased accumulation of soil organic C in the top 10 cm, followed by a strong decline with depth similar to grassland soils (Lavahun et al., 1996). Reasons for this depth gradient are an increased C input by plant roots into the top layers, but also by straw and stubble remaining on the field surface and being incorporated by the rotary harrow. The significant interrelationships between soil organic C, total N, total P and total S showed that a large percentage of these nutrient elements were strongly bound to soil organic components (Haynes, 2000). This led to a significant increase in the stocks of soil organic C, total N and P with rotary harrow management.

Reasons for the presence or absence of a net-increase in the soil organic C stock might be differences in the effects on plant yield, but especially on root development. Ahl et al. (1998) observed a strong compaction of the soil at 7-14 cm depth, which was tilled with a rotary cultivator at 0-7 cm depth. This harrow pan severely obstructed root growth. Conversely, a compacted layer was not observed at the present experimental sites, although the rotary harrow led to moderately higher bulk density at 10-20 cm depth. The differences in bulk density between the tillage treatments at 30-40 cm depth were more distinct than the differences in this mid-positioned layer and clearly indicate the presence of a plough pan as described elsewhere (Meyer et al., 1996; Stockfisch et al., 1999; Tebrügge and Düring, 1999). Another reason for the increased soil organic C stock in the rotary harrow treatment of the present experiments might be the increased formation of macro-aggregates, which protect organic matter physically against microbial decomposition and mechanical breakdown (Hernandéz-Hernández and López-Hernández, 2002; Jacobs et al., 2009).

The reduction in tillage intensity had much stronger increasing effects on microbial biomass C, N, and P than on the total storage pools of the respective elements in soil. This observation clearly supports the view stated a long time ago that microbial biomass indicates changes due to soil management, long before other measures such as soil organic C or total nitrogen (Powlson et al., 1987; Jenkinson, 1988). The increased stocks of microbial biomass C, N, and P in the rotary harrow treatments are apparently caused by a reduction in the microbial turnover, for example by a lower water content in the surface layer, assuming similar C inputs in the two tillage treatments. These results show a more efficient use of the annual C input in the rotary harrow treatment as indicated by the inverse relationship between metabolic quotient qCO_2 and microbial biomass C-to-soil organic C ratio. In the surface layer of the present experiments, the qCO_2 is lower in the rotary harrow treatment than in the plough treatment and the microbial biomass C-to-soil organic C ratio is higher. The qCO_2 is an indicator for the maintenance energy requirements (Anderson and Domsch, 1990; Meyer et al., 1996) and the microbial biomass C-to-soil organic C is an indicator for the availability of organic matter to soil microorganisms (Anderson and Domsch, 1989).

The effects of differences in substrate use efficiency are also revealed by the increase of qCO_2 values and the decrease of microbial biomass C-to-soil organic C ratios with depth, with decreasing input of fresh plant material and increasing recalcitrance of soil organic matter, as observed by Lavahun et al. (1996) and Meyer et al. (1996). Differences

in substrate use efficiency are also the reason for the different levels of soil organic matter and microbial biomass between the two experimental sites. The site Hohes Feld has a 2.1% higher clay content, and substrate use efficiency is usually related to the clay content (Carter, 1991; Müller and Höper, 2004). However, it is unknown whether this small difference in texture could explain the strong differences observed between the two sites, which are in most cases much stronger than the tillage effects. The higher stocks of total P and $NaHCO_3$ extractable P at the site Hohes Feld might be a consequence of the higher application rate of P fertilizers in the past 10 years. Due to the higher contents of N, P, and in the soil at the site Hohes Feld, the yields were increased by 8%, which might have led to a higher input of plant residues. Also, the clay content was slightly higher at the site Hohes Feld than at the site Garte.

The most apparent difference between the two sites is the much higher metabolic quotient at the site Garte and thus a much lower substrate-use efficiency as explained above in comparison to the site Hohes Feld. This difference seem to be caused by different development in the microbial community structure, which is reflected by differences in the ergosterol-to-microbial biomass C ratio and the microbial biomass C/S ratio. The microbial biomass C/S ratio was significantly higher at the site Hohes Feld and also in the rotary harrow treatment, while the opposite effect was observed for the ergosterol-to-microbial biomass C ratio and the qCO_2. Therefore, the ergosterol-to-microbial biomass C and the qCO_2 were inversely related to the microbial biomass C/S ratio. The cell-membrane component ergosterol is an important indicator for saprotrophic fungi (Joergensen and Wichern, 2008), but does not occur in arbuscular mycorrhizal fungi (Olsson et al., 2002). Saprotrophic fungi grown under laboratory conditions in culture media were able to increase their S concentration with increasing S supply by about 50-130%, while bacteria could only enhance their S concentration by about 20% (Saggar et al., 1981; Banerjee and Chapman, 1996). Thus, in the present field experiment, a low microbial biomass C/S ratio obtained by the fumigation extraction method is an additional good indicator for the presence of saprotrophic fungi. The microbial biomass C/S ratio varied around a mean of 38 and was similar to that detected by Heinze et al. (2010) with mineral fertilization on a sandy soil, but reached only a third of the ratios reported by Wu et al. (1993, 1994) for English grassland soils. This might be in part explained by the use of 1 M NH_4NO_3 as extractant, which probably more S-containing amino acids renders extractable than 0.01 M $CaCl_2$ (Khan et al., 2009).

The present results on the relationship between the ergosterol-to-microbial biomass C ratio and the microbial biomass C/S ratio are in agreement with the results reported by Heinze et al. (2010) analysing the effects of organic and inorganic fertilizer application in a long-term field experiment. This would also mean that soils with a lower contribution of saprotrophic fungi exhibit a lower microbial biomass C/N ratio. However, this was not obvious in the present results where the site Hohes Feld showed the lowest contribution of saprotrophic fungi in combination with the highest microbial biomass C/N ratio. This again contradicts the indicative properties of the microbial biomass C/N ratio for shifts in the ratio of fungi to bacteria (Joergensen and Emmerling, 2006). A lower substrate use efficiency in the greater presence of saprotrophic fungi is in line with the observations of Scheller and Joergensen (2008), but contradicts the view that fungi are generally more efficient in substrate use than bacteria, as repeatedly stated (Sakamoto and Oba, 1994; Bailey et al., 2001; Jastrow et al., 2007). Even more surprising is the observation that mouldboard ploughing promotes saprotrophic fungi in the present experiments, especially in the surface layer. A reduction in tillage intensity is usually reported to promote fungal hyphae expansion, due to lower mechanical destruction (Beare et al., 1997; Frey et al., 1999). Nevertheless, the soils of the rotary harrow treatment may contain a higher contribution of arbuscular mycorrhizal fungi, which sensitively react highly to tillage intensity (Kabir, 2005). This might be especially true for the soil at 10 to 30 cm depth, which is not affected by tillage anymore in the rotary harrow treatment.

5.5 Conclusions

The rotary harrow treatment led to small increases in the stocks of soil organic C, total N and total P, but to strong increases in the stocks of microbial biomass C, N, and P. This strong increase is caused by a reduction in the microbial turnover in combination with an improved microbial substrate use efficiency. Tillage had no specific effects on the storage of N and P within the microbial biomass as indicated by their constant C/N and C/P ratios. Tillage affected the microbial community structure as indicated by the inverse changes in the microbial biomass C/S ratio and the ergosterol-to-microbial biomass C ratio. This means that a decrease in the microbial biomass C/S ratio is also a strong indicator for a shift in the microbial community structure towards saprotrophic fungi. Rotary harrow management apparently led to a lower contribution of saprotrophic fungi to the soil microbial community, probably at the expense of arbuscular mycorrhizal fungi.

This contrast the view reported elsewhere and points to the need of more information on tillage-induced shifts within the fungal community in arable soils, for example by measuring the contribution of biotrophic arbuscular mycorrhizal fungi to the soil microbial biomass.

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6. Usefulness of near infrared spectroscopy for the prediction of soil properties: effects of sample pretreatment and calibration procedure

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Summary

The prediction accuracy of near infrared spectroscopy (NIRS) for soil chemical and biological parameters has been variable and the effects of sample pretreatment and calibration procedures have not yet been tested sufficiently. Objectives were to test, (i) whether a sample pretreatment which involves shock freezing followed by freeze-drying results in an accurate prediction of soil parameters, (ii) how different calibration procedures affect the accuracy of the prediction equations, and (iii) how the population size affects the results of a cross-validation procedure compared to a calibration/validation procedure. Six hundred and ten samples from three arable soils in Germany were sampled, their chemical and biological properties determined and their reflectance spectra in the VIS-NIR region recorded after shock-freezing followed by freeze-drying. Cross-validation and calibration/validation were carried out for the entire population as well as for each population from the respective sites. Excellent or good prediction accuracies were found for pH, the contents of soil organic carbon (SOC), total N, P, S, K, Mg, Mn, Fe and Al. However, prediction accuracy for biological properties was less: contents of microbial C and N were predicted approximately quantitative, whereas contents of microbial P and S could not be predicted. The comparison of the sample selection strategies for the calibration set (either based on the SOC content, on the H-value or random) indicated that the SOC-based selection strategy had slightly more excellent, good and approximate quantitative predictions in the validation procedure for most of the populations. Cross-validation results were markedly more favourable than the validation results independently of sample size, indicating that cross-validation results may only be regarded as rough estimates (valid only approximately and only for a closed

population) and should not be viewed as an adequate surrogate for an independent validation.

6.1. Introduction

Methods of soil analyses may be complex, time-consuming and expensive (Chang *et al.*, 2001). Especially for long-term monitoring of soil properties it would be useful to establish a rapid, reliable, non-destructive method able to measure many constituents simultaneously (Foley *et al.*, 1998; Font *et al.*, 2004). Near infrared spectroscopy (NIRS) meets these requirements and is an established technique in many scientific areas (Norris *et al.*, 1976; Fahey Jr. and Hussein, 1999). Its importance is increasing for ecological studies (Foley *et al.*, 1998) and soil science research (Ben-Dor & Banin, 1995; Coûteaux *et al.*, 2003; Zornoza *et al.*, 2008).

Several studies showed the possibility of NIRS to predict a variation of soil properties like soil organic carbon (SOC), total nitrogen and exchangeable nutrients (Dalal & Henry, 1986; Sheperd & Walsh, 2002; Russell, 2003; Terhoeven-Urselmans *et al.*, 2008). The estimation of soil biological properties is more complex than the prediction of soil chemical properties, since biological properties depend not only on the vibrations of the main constituents in the near infrared (NIR) region but also on other factors such as moisture, pH, temperature, and micronutrients. Rinnan & Rinnan (2007) reached with 72 oven dried (70°C) soil samples from 0-5 cm and 36 samples from 5-10 cm a promising prediction accuracy for ergosterol content ($r > 0.9$), microbial biomass C (C_{mic}) and P (P_{mic}) content and the microbial community structure (PLFA) with r between 0.78-0.79. Furthermore, Ludwig *et al.* (2002) showed for 120 dried soil samples from Australia that NIRS had the potential to predict C_{mic} and microbial activity parameters like cumulative respiration and N mineralization, potentially mineralisable C and Olson P quite usefully ($r \geq 0.8$, $0.8 < a \leq 1.2$). These results are surprising, since drying affects the contents of several biological constituents. For instance, ergosterol is destroyed during drying, which indicates that the prediction was indirectly due to a correlation of ergosterol contents with other absorbing constituents. Because of the effect of drying on the biological constituents, it is not surprising that the performance of NIRS was variable for the prediction of biological parameters if dried samples were used (Ludwig *et al.*, 2002; Terhoeven-Urselmans *et al.* 2006).

Few studies have tested different sample pretreatments. Ergosterol content was predicted well ($r = 0.9$) with NIRS for freeze-dried samples of forest organic layers

(Pietikainen & Fritze, 1995). Terhoeven-Urselmans *et al.* (2008) achieved good NIRS predictions with 116 shock frozen and freeze-dried samples for microbial biomass C and N (N_{mic}), basal respiration, ergosterol and even the metabolic quotient (qCO_2) and suggested that future research should address this potential to build calibrations for open populations.

Cross-validation has been used in many studies, especially in those with a sample size < 100. It has been suggested that it may be a promising approach for small datasets (Cozzolino & Morón, 2006). However, an extensive comparison of the results of cross-validation compared to a calibration/validation has not yet been made for the prediction of soil properties. Thus, it remains unclear, whether cross-validation is a surrogate for a calibration/validation procedure.

For a calibration/validation procedure, there exist several sample selection strategies for the calibration set. In place of a random selection strategy, Williams (2001) suggested a strategy covering the range of contents of a constituent approximately uniformly. In contrast, Shenk *et al.* (2001) suggested a selection strategy according to spectral features using the Mahalanobis distance (H-value). These suggestions have not yet been examined in detail.

Objectives were to test, (i) whether a sample pretreatment which involves shock freezing followed by freeze-drying results in an accurate prediction of soil parameters, (ii) how different calibration procedures affect the accuracy of the prediction equations, and (iii) how the population size affects the results of a cross-validation procedure compared to a calibration/validation procedure.

6.2. Materials and Methods

6.2.1. Sites and soil samples

Soil samples were taken from two long-term field experiments in Göttingen, Lower Saxony and Darmstadt, Hesse. The long-term field experiment in Göttingen consists of two research areas (Garte and Hohes Feld) where the influence of different soil tillage management systems on soil properties and crop yields were analyzed for more than 40 years. The soils in Garte and Hohes Feld are Haplic Luvisols (FAO-WRB, 2006) and their textures consist of 15% clay, 73% silt and 12% sand (Garte) and 17% clay, 66% silt and 16% sand (Hohes Feld). Additional information is provided by Jacobs *et al.* (2009).

The other long-term experiment in Darmstadt consists of two neighbouring investigation sites which were established in 1980 and since that time the influence of mineral, organic fertilizer and plant-based organic fertilizer on soil properties and crop yields has been analyzed (Raupp & Oltmanns, 2006). The soil is a Haplic Cambisol (FAO-WRB, 2006) and its texture consists of 5% clay, 9% silt and 86% sand.

6.2.2. Soil sampling and chemical analysis

Three hundred and thirty soil samples were taken in February 2007 in Darmstadt at 0-5 cm. Each plot (5 x 5 m) was sampled in a grid design with 4 replicates and resulted for the field with the comparison of mineral fertilizer and farmyard manure application in a sum of 144 samples (Heinze *et al.*, 2009). An additional 186 samples were collected from the area where mineral fertilizer, plant-based organic fertilizer and farmyard manure were added.

In March 2007, the depths 0-5, 5-10, 10-20, 20-30, 30-40 cm were sampled at Garte (n = 160) and Hohes Feld (n = 120) using a steel corer. Samples were also taken in a grid design with 4 replicates per plot with a dimension of 40 × 20 m (Garte) and 36 × 12.7 m (Hohes Feld).

All samples were sieved (< 0.2 mm), adjusted to 40% water holding capacity (WHC) and stored in polyethylene bags at 4°C until soil biological analysis were carried out. A sub-sample was dried (105°C) and finely ground for chemical analysis. The pH was determined in water with a soil to water ratio of 1 to 2.5. Total contents of N were detected by gas chromatography using a Vario EL and Vario MAX (Elementar, Hanau, Germany) elemental analyzer. Total contents of P, S, K, Ca, Mg, Mn, Fe, and Al were analyzed by an HNO_3-pressure digestion as described by Chander *et al.* (2008) by ICP-AES (Spectro Analytical Instruments, Kleve, Germany).

6.2.3. Soil biological analysis

For the measurement of basal respiration, 60 g moist soil adjusted to 40% water holding capacity were weighed into 80 ml incubation cylinders made of stainless steel nets (Hoffmann *et al.*, 2009). The cylinders were placed into 500 ml Pyrex glass jars containing 5 ml 0.5 M NaOH at the bottom and incubated for 7 days at 22°C in the dark. The evolved CO_2 was determined by back-titration to pH 8.3 of the excess NaOH with

0.5 M HCl after addition of saturated $BaCl_2$ solution. The incubated soil was used for measuring all microbial biomass indices. Contents of C_{mic}, N_{mic}, P_{mic} and microbial biomass S (S_{mic}) were estimated by fumigation extraction (Joergensen *et al.*, 1995; Khan *et al.*, 2009). The fungal cell-membrane component ergosterol was extracted with ethanol from 2 g moist soil (Djajakirana *et al.*, 1996). Subsequently, ergosterol was determined by reversed-phase HPLC and detection at 282 nm.

6.2.4. Near infrared spectral reflectance measurement and cross-validation

Field-moist soil samples were filled into scintilization vessels, shock-frozen with liquid N_2, freeze-dried over 3 to 5 days and then ground and stored in desiccators until NIRS measurements were carried out. The absorbance spectra (log [1 / reflectance]) of each sample were recorded in the VIS-NIR range (400-2500 nm) in 2 nm steps using a Foss NIRSystems spectrometer (Silver Spring, USA). Each sample was measured twice and averaged afterwards. If the spectra differences exceeded a root mean square of 3000, the measurement was repeated maximal 5 times.

The spectra were transformed by using the standard normal variate (SNV) and detrend to remove scatter effects, which can be caused by particle size or linear and quadratic trends in the spectra. The development of the cross-validation equation was performed by using the derivate of the first to third order; the gaps and the smoothing width ranged between 1 and 20. The cross-validation equation was calculated by the modified partial least squares regression method (MPLS) (Shenk & Westerhaus, 1991). Samples, which had a spectrum out of the global population of spectra (H-outliers) and which had a standardized H-value > 3.0 (Mahalanobis distance) were defined as outliers (entire population: 6 outliers, Garte: 3, Darmstadt, Hohes Feld: 1) and eliminated (Shenk & Westerhaus, 1991). Samples with a difference between reference and predicted values exceeding the standard error of cross-validation (SECV; t-outliers) by 2.5 were also eliminated for the MLPS procedure, as suggested by the WINSIS II Software and in accordance to Tillmann (2000). For the linear regression of predicted against measured values and for the calculation of the coefficients of determination (r^2), the outliers were included again to avoid an overestimation of the NIRS potential.

The best mathematical treatment was selected by trial and error procedure, involving the first to third order of the derivate. The gaps and the smoothing over which the derivates were calculated ranged from 1 to 20 in five steps, so that 45 different

mathematical treatments were calculated. The choice of the best mathematical treatment was orientated to the smallest SECV and to the highest RSC value (ratio of standard deviation from laboratory results and SECV).

6.2.5. Separate calibration/validation

For the separation of the whole dataset into a calibration and validation sample set three different selection strategies were conducted.

The first selection operation consisted of a randomized selection with a selection ratio of 2.5 for calibration to 1 for validation according to Williams (2004).

The second selection strategy ensured that sample properties (ranges, means and standard deviation) were more similarly distributed in-between the calibration and validation sample sets as suggested by Williams (2001). The samples were sorted by their SOC content from the lowest to the highest values and additionally selected with a ratio 2.5 to 1 like for the random sample selection.

The third selection procedure took the Mahalanobis distance (H-value) into account (Shenk *et al.*, 2001). Here the spectra information of all samples were ordered by their H-value from the lowest to the highest so that a similar distribution of the spectra ranges in between the calibration and validation data set was reached.

After each selection procedure calibration was carried out by elimination of the t- and H-outliers with the limits of 2.5 and 3, respectively. For the calculation of r^2 the outliers were included again to avoid an overestimation. The equations obtained in the respective calibration procedures were then used for the respective validation sample sets (SOC based, H-value based and random based) and the RPD (ratio of standard deviation from laboratory results and standard error of prediction) values were calculated.

6.2.6. Evaluation of cross-validation and calibration/validation results

The accuracy of NIRS prediction was ranked according to Saeys *et al.* (2005) who identified excellent prediction with an RSC (or RPD) > 3.0 and a r^2 > 0.91, good prediction with a RSC (or RPD) between 3.0-2.5 and r^2 between 0.90-0.82, approximate quantitative prediction with RSC (or RPD) between 2.5-2.0, r^2 between 0.81-0.66, the possibility of prediction to distinguish between high and low values with RSC (or RPD) between 2.0-1.5 and a r^2 between 0.65-0.50 and all smaller values stood for no usable prediction.

6.3. Results and discussion

6.3.1. Chemical and biological soil properties

The soil properties covered a considerable range at the three experimental sites with highest mean element contents, pH and microbial biomass at both loamy sites Hohes Feld and Garte (Table 14 shows the data for the entire population and the subpopulation Hohes Feld).

Table 14: Chemical and biological soil properties (ranges, means and standard deviation (SD)) of the entire population (n=610) and of the subpopulation Hohes Feld (n=120). All contents are expressed on an oven dried basis

Constituent	Entire population			Subpopulation Hohes Feld		
	Ranges	Means	SD	Ranges	Means	SD
pH (H_2O)	5.3-8.7	7.0	0.78	6.4-8.3	7.5	0.44
SOC (mg g^{-1})	3.4-18	8.8	2.4	5.1-18	9.7	2.6
N_{tot} (mg g^{-1})	0.42-1.9	0.86	0.26	0.55-1.9	1.07	0.25
P (mg g^{-1})	0.29-1.0	0.53	0.16	0.54-1.8	0.78	0.13
S (mg g^{-1})	0.05-0.56	0.18	0.07	0.12-0.37	0.23	0.05
C_{mic} (µg g^{-1})	54-531	167	75	60-531	230	104
N_{mic} (µg g^{-1})	2.1-94	25	16	4.3-94	33	20
P_{mic} (µg g^{-1})	0.50-51	8.0	5.6	3.0-51	9.5	7.0
S_{mic} (µg g^{-1})	0.10-17	4.7	3.3	0.23-17	5.4	3.9
Ergosterol (µg g^{-1})	0.03-1.2	0.41	0.20	0.04-0.49	0.26	0.13
Basal respiration (µg CO_2-C $g^{-1}d^{-1}$)	0.43-16	6.1	2.5	2.2-10	5.6	1.6
qCO_2 (mg CO_2-C d^{-1} g^{-1} C_{mic})	2.9-195	42	26	11-124	29	18
Ergosterol/C_{mic} (%)	0.02-0.91	0.27	0.15	0.03-0.28	0.12	0.05
K (mg g^{-1})	2.4-8.1	4.3	1.67	5.9-8.1	6.8	0.44
Ca (mg g^{-1})	1.8-40	4.5	4.3	3.7-7.7	5.2	0.71
Mg (mg g^{-1})	1.5-6.0	2.8	1.2	3.4-6.0	4.9	0.55
Mn_t (mg g^{-1})	0.32-1.2	0.52	0.14	0.59-0.94	0.70	0.05
Fe (mg g^{-1})	6.9-22	12	3.7	15-22	17	1.2
Al (mg g^{-1})	11-30	17	5.2	21-30	24	1.5

Several constituents were markedly correlated with the content of soil organic carbon (SOC) (Table 15). However, correlation coefficients were different for each subpopulation (Garte, Hohes Feld or Darmstadt). For instance, contents of C_{mic}, N_{mic} and N_{tot} revealed stronger correlation coefficients at the loamy sites than at the sandy

Darmstadt site or in the entire population (Table 15). Thus, different accuracies of predictions were expected for those constituents predicted indirectly due to the correlation with SOC or other constituents.

Table 15: Correlation coefficients for the contents of measured indices with the SOC contents (mg g^{-1}) for the entire population (n=610) and the subpopulations Hohes Feld (n=120), Garte (n=160), and Darmstadt (n=330)

Constituents	Entire population	Hohes Feld	Garte	Darmstadt
pH (H_2O)	0.21	-0.69	-0.43	0.70
N_{tot} (mg g^{-1})	0.67	0.95	0.80	0.47
P (mg g^{-1})	0.46	0.45	0.71	0.54
S (mg g^{-1})	0.76	0.87	0.66	0.81
C_{mic} (µg g^{-1})	0.73	0.90	0.92	0.40
N_{mic} (µg g^{-1})	0.71	0.85	0.87	0.44
P_{mic} (µg g^{-1})	0.55	0.65	0.71	0.33
S_{mic} (µg g^{-1})	0.37	0.60	0.24	0.12
Ergosterol (µg g^{-1})	0.24	0.57	0.61	0.19
Basalrespiration (µg CO_2-C $g^{-1}d^{-1}$)	0.24	0.44	0.11	0.33
qCO_2 (mg CO_2-C d^{-1} g^{-1} C_{mic})	-0.34	-0.56	-0.61	n.s.
Ergosterol/C_{mic} (%)	-0.16	n.s.	n.s.	n.s.
K (mg g^{-1})	0.26	n.s.	n.s.	0.52
Ca (mg g^{-1})	0.50	n.s.	n.s.	0.73
Mg (mg g^{-1})	0.36	n.s	n.s.	0.76
Mn_t (mg g^{-1})	0.26	n.s	0.42	n.s.
Fe (mg g^{-1})	0.21	-0.24	n.s.	0.39
Al (mg g^{-1})	0.17	-0.40	n.s.	0.11

n.s.: not significant

6.3.2. Spectral fingerprint

The spectra of the three different soils with differing SOC contents showed typical absorbance characteristics for soils (Fig. 10). The peaks in the 1400 nm, 1900 nm and 2200 nm region occurred from the association with clay minerals, especially free water at 1400 nm and 1900 nm and absorption of OH-bonds in crystal lattice at 2200 nm (Chang *et al.*, 2001; Shenk *et al.*, 2001). The higher absorbance of the soil samples of the site Darmstadt resulted from the coarser structure of the sandy soil which was also the case after grinding. The peaks of all soils in the 450 nm region might be the result of the absorbance of iron oxides like goethite (Russell, 2003).

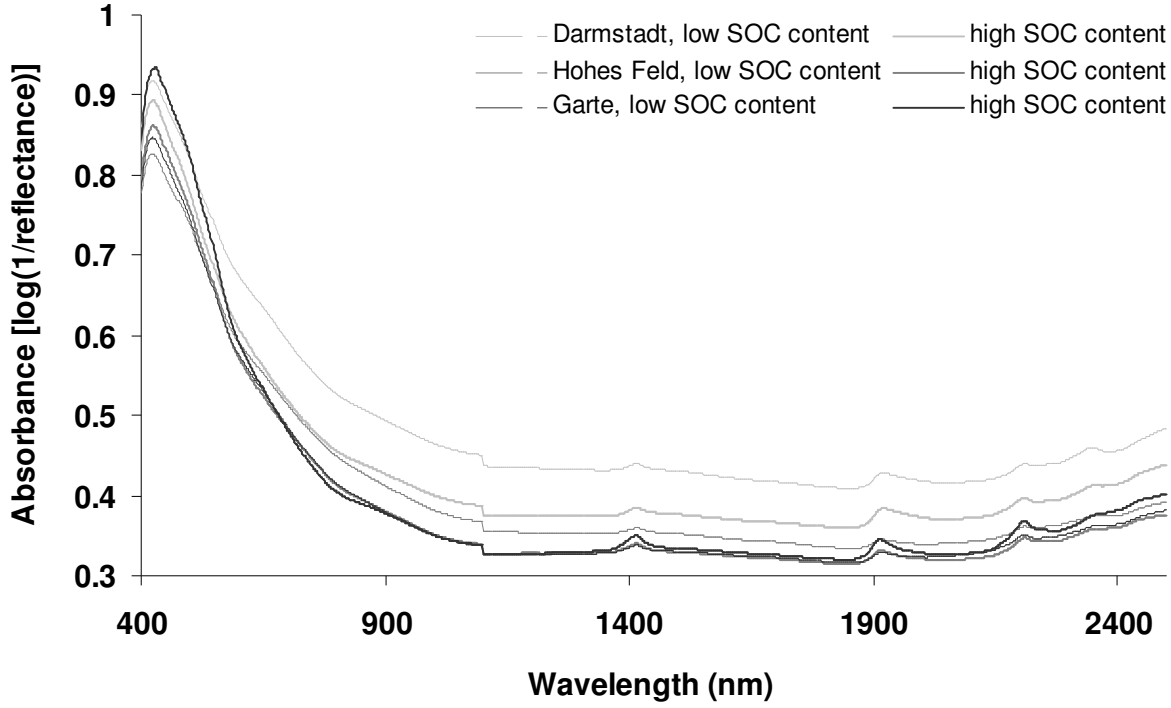

Figure 10: Near-infrared absorbance spectra of soils from the sites Darmstadt, Hohes Feld and Garte. For each site, spectra of soils with low and high SOC contents are shown as examples.

6.3.3. Prediction of soil chemical properties using cross-validation

The main constituents, the contents of SOC, N, P and S and pH were predicted in the cross-validation procedure with excellent accuracy (RPD > 3.0, $r^2 > 0.91$) when the entire population (n = 610 samples) was considered (Table 16, Fig. 11). Excellent predictions of SOC and N_{tot} contents were also reported by Terhoeven-Urselmans *et al.* (2008) with shock frozen, freeze-dried and ground soil samples using cross-validation (n = 116) and

reached a RSC of 3.8 and 3.5 for these constituents, respectively. Zornoza *et al.* (2008) used air-dried samples (n=393) and also predicted the SOC and N contents with excellent accuracy with RSC of 5.8 and 4.7 which underlines the good prediction possibility for these constituents using cross-validation. Overall, the comparison of our data with those of Zornoza *et al.* (2008) indicates that shock freezing/freeze-drying is not required for the prediction of the main constituents SOC and N.

The excellent prediction of pH in our study for the entire population and for the subpopulation Darmstadt is consistent with the results reported by Terhoeven-Urselmans *et al.* (2008) who found only a slightly worse accuracy (good prediction accuracy (RSC = 2.7, r = 0.94) according to Saeys *et al.* (2005)). In contrast, the use of air dried soils in the study of Zornoza *et al.* (2008) resulted only in a differentiation between high and low $pH_{(H_2O)}$.

The prediction of the contents of the metals, which are predicted indirectly via correlations with absorbing constituents was excellent for K, Mg, Mn, Fe and Al and approximate quantitative for Ca when the entire population was used (Table 16). For the smaller subpopulation Hohes Feld, results were more variable, ranging from excellent (K, Mg) and good (Fe, Al) to only a differentiation between high and low (Ca, Mn, Table 17). Indirect predictions have been reported before (Ben-Dor & Banin, 1995). However, they always bear the risk that NIRS predictions may fail, since there is not necessarily a causal relationship between NIRS predictions and such constituents. For instance, it is not surprising that NIRS failed to predict contents of Pb and Zn in a heavy-metal polluted area (Chodak *et al.*, 2007).

Table 16: Statistics of validation with SOC based selection and cross-validation of the entire population (n=610); shown are the mathematical treatments, the standard error of calibration (SEC), the standard error of prediction (SEP), the ratio of standard deviation (SD) of laboratory results to SEP (RPD), the coefficients of determination (r^2), the standard error of cross-validation (SECV) and the ratio of SD of laboratory results to SECV (RSC); accuracy levels are indicated by subscripted numbers in accordance to Saeys et al. 2005

	Cross-validation procedure				Calibration/validation procedure				
Constituent (unit)	Math treatment	SECV	RSC	r^2	Math treatment	SEC	SEP	RPD	r^2
pH (H_2O)	1-1-1	0.23	3.3 [1]	0.82	2-20-15	0.21	0.27	2.9 [2]	0.88
SOC (mg g^{-1})	1-5-5	0.63	3.2 [1]	0.82	1-10-1	0.54	0.99	2.3 [3]	0.82
N_{tot} (mg g^{-1})	1-20-1	0.07	3.5 [1]	0.84	1-20-20	0.07	0.11	2.3 [3]	0.81
P (mg g^{-1})	1-20-15	0.04	3.9 [1]	0.86	1-20-20	0.04	0.05	2.9 [2]	0.88
S (mg g^{-1})	1-10-5	0.02	3.2 [1]	0.88	1-20-20	0.02	0.03	2.6 [2]	0.85
C_{mic} (µg g^{-1})	2-20-10	29	2.3 [3]	0.80	2-15-10	26	37	2.0 [3]	0.78
N_{mic} (µg g^{-1})	1-15-15	6.4	2.0 [3]	0.70	3-10-10	5.9	8.2	1.8 [4]	0.71
P_{mic} (µg g^{-1})	1-1-1	3.4	1.2	0.41	1-5-1	3.3	4.1	1.3	0.43
S_{mic} (µg g^{-1})	1-1-1	2.0	1.3	0.37	1-5-5	1.8	2.3	1.4	0.48
Ergosterol (µg g^{-1})	1-5-1	0.12	1.6 [4]	0.51	1-20-5	0.10	0.16	1.4	0.50
Basal respiration (µg CO_2-C $g^{-1}d^{-1}$)	1-5-5	1.6	1.4	0.33	1-15-10	1.5	2.0	1.2	0.36
qCO_2 (mg CO_2-C d^{-1} g^{-1} C_{mic})	1-5-5	13	1.4	0.44	1-15-5	13	18	1.4	0.49
Ergosterol/C_{mic} (%)	1-10-5	0.09	1.6 [4]	0.58	1-20-5	0.08	0.09	1.6 [4]	0.63
K (mg g^{-1})	2-10-10	0.16	10 [1]	0.97	2-20-20	0.16	0.24	6.8 [1]	0.98
Ca (mg g^{-1})	1-15-15	0.58	2.4 [3]	0.85	1-20-10	0.53	1.2	3.2 [1]	0.92
Mg (mg g^{-1})	1-5-1	0.15	8.1 [1]	0.97	2-20-15	0.15	0.22	5.5 [1]	0.97
Mn_t (mg g^{-1})	1-1-1	0.02	5.4 [1]	0.90	2-10-1	0.02	0.03	4.1 [1]	0.94
Fe (mg g^{-1})	3-20-1	0.46	7.8 [1]	0.95	2-15-15	0.48	0.80	4.6 [1]	0.95
Al (mg g^{-1})	1-5-5	0.58	8.6 [1]	0.95	2-15-1	0.56	1.1	4.8 [1]	0.96

[1] Excellent: RPD > 3.0, r^2 > 0.91
[2] Good: RPD 3.0-2.5, r^2 0.90-0.82
[3] Approximate quantitative: RPD 2.5-2.0, r^2 0.81-0.66
[4] High and low distinction: RPD 2.0-1.5, r^2 0.65-0.50

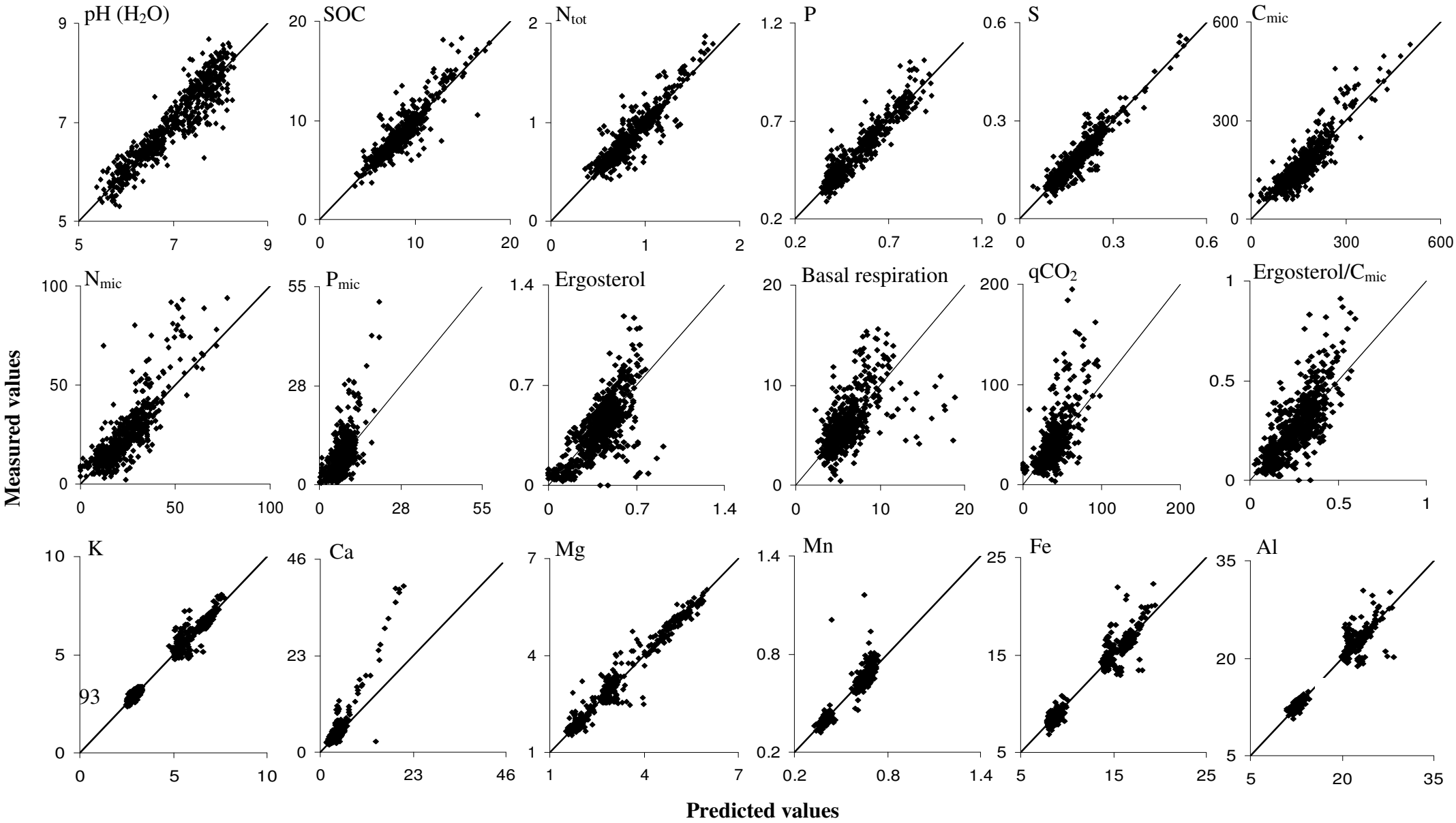

Figure 11: Cross-validation scatter plot of measured and predicted values of the entire population (n=610) for pH(H_2O), SOC, N_{tot}, P, S (mg g^{-1}), C_{mic}, N_{mic}, P_{mic}, ergosterol (µg g^{-1}), Basal respiration (µg CO_2-C g^{-1} d^{-1}), qCO_2 (mg CO_2-C d^{-1} $g^{-1}C_{mic}$), ergosterol/C_{mic} ratio (%) and the contents of metal cations K, Ca, Mg, Mn, Fe, and Al (mg g^{-1}). All contents are expressed on an oven dried basis (105°C). The 1:1 line is indicated in each figure.

Table 17: Validation statistics (SOC based selection) and cross-validation statistics of the dataset Hohes Feld (n=120); shown are the mathematical treatments, the standard error of calibration (SEC), the standard error of prediction (SEP), the ratio of standard deviation (SD) of laboratory results to SEP (RPD), the coefficients of determination (r^2), the standard error of cross-validation (SECV) and the ratio of SD of laboratory results to SECV (RSC); accuracy levels are indicated by subscripted numbers in accordance to Saeys et al. 2005

	Cross-validation procedure				Calibration/validation procedure				
Constituent (unit)	Math treatment	SECV	RSC	r^2	Math treatment	SEC	SEP	RPD	r^2
pH (H_2O)	1-20-20	0.24	1.9 [4]	0.71	2-20-15	0.20	0.28	1.5 [4]	0.60
SOC (mg g^{-1})	1-5-5	0.27	8.8 [1]	0.94	1-5-1	0.18	0.53	4.7 [1]	0.96
N_{tot} (mg g^{-1})	2-20-20	0.02	9.1 [1]	0.99	3-20-5	0.01	0.04	6.8 [1]	0.98
P (mg g^{-1})	2-20-10	0.04	1.9 [4]	0.42	3-20-1	0.04	0.07	1.4	0.53
S (mg g^{-1})	3-10-1	0.02	3.0 [2]	0.90	3-10-1	0.01	0.02	2.6 [2]	0.87
C_{mic} (µg g^{-1})	1-20-5	38	2.4 [3]	0.79	3-5-5	29	48	2.0 [3]	0.76
N_{mic} (µg g^{-1})	1-5-5	7.7	2.2 [3]	0.74	1-1-1	6.5	9.7	2.0 [3]	0.77
P_{mic} (µg g^{-1})	3-1-1	2.0	1.4	0.26	2-5-1	1.3	5.5	1.2	0.27
S_{mic} (µg g^{-1})	2-5-5	2.2	1.4	0.67	1-10-1	1.7	3.3	1.2	0.35
Ergosterol (µg g^{-1})	2-5-1	0.06	1.9 [4]	0.79	3-10-1	0.02	0.11	1.2	0.47
Basal respiration (µg CO_2-C $g^{-1}d^{-1}$)	3-10-1	1.3	1.1	0.41	3-15-1	0.49	1.6	1.0	0.25
qCO_2 (mg CO_2-C d^{-1} g^{-1} C_{mic})	1-5-1	7.2	1.4	0.39	1-5-1	6.1	17	1.1	0.17
Ergosterol/C_{mic} (%)	2-20-15	0.03	1.5 [4]	0.49	1-1-1	0.02	0.04	1.1	0.22
K (mg g^{-1})	3-15-5	0.11	3.7 [1]	0.93	3-15-15	0.08	0.14	3.0 [2]	0.91
Ca (mg g^{-1})	2-10-10	0.43	1.5 [4]	0.72	2-15-5	0.31	0.59	1.0	0.38
Mg (mg g^{-1})	2-10-10	0.14	3.8 [1]	0.94	2-10-5	0.10	0.17	3.8 [1]	0.93
Mn_t (mg g^{-1})	2-5-5	0.03	1.5 [4]	0.42	2-5-5	0.02	0.04	1.1	0.41
Fe (mg g^{-1})	2-10-1	0.36	2.8 [2]	0.89	2-10-1	0.21	0.43	1.8 [4]	0.77
Al (mg g^{-1})	1-1-1	0.46	2.7 [2]	0.89	2-20-10	0.37	0.80	1.3	0.57

[1] Excellent: RPD > 3.0, r^2 > 0.91
[2] Good: RPD 3.0-2.5, r^2 0.90-0.82
[3] Approximate quantitative RPD 2.5-2.0, r^2 0.81-0.66
[4] High and low distinction: RPD 2.0-1.5, r^2 0.65-0.50

6.3.4. Prediction of soil biological properties using cross-validation

For the entire population, the microbial biomass constituents C_{mic} and N_{mic} were predicted approximate quantitatively with an RSC of 2.3 and 2.0, respectively (Table 16). The prediction for ergosterol (RSC = 1.6) and ergosterol to C_{mic} ratio (RSC = 1.6) could only distinguish between high and low values. No prediction was possible for P_{mic}, S_{mic}, the basal respiration and the qCO_2 (Table 16). Overall, the prediction of soil biological properties was less successful than the results by Terhoeven-Urselmans *et al.* (2008) who also used shock freezing/freeze-drying. In their study, a good prediction accuracy was obtained for C_{mic} (RSC = 2.6), whereas for N_{mic} (RSC = 2.3) the results were similar to ours (Table 16). Moreover, Terhoeven-Urselmans *et al.* (2008) reported more successful results for P_{mic} (distinguishment between high and low) and basal respiration (approximate quantitative prediction). The use of air-dried soils for the estimation of C_{mic} and N_{mic} gave variable results in several studies. Terhoeven-Urselmans *et al.* (2006) reported an unsatisfactory prediction for C_{mic}. Chang *et al.* (2001) obtained an RPD value of 1.5 (which allows a distinction between high and low according to Saeys *et al.* 2005). Chodak *et al.* (2003) reported an RPD value of 2.2 (approximate quantitative prediction according to Saeys *et al.* 2005) for C_{mic} in organic layers of forest soils. Finally, Coûteaux *et al.* (2003) and Zornoza *et al.* (2009) reported excellent prediction accuracy for C_{mic}. Thus, the prediction accuracy for biological properties depends considerably on the population.

The importance of the population for the prediction of biological parameters is stressed in our study (Table 18): Contents of C_{mic} and N_{mic} were either predicted with good accuracy (subpopulation Garte), approximate quantitatively (subpopulation Hohes Feld, entire population) or the accuracy allowed only a distinction between high and low (subpopulation Darmstadt). Notably, this decrease in prediction accuracy follows the same order of correlation of C_{mic} with SOC contents: Garte ($r = 0.92$) > Hohes Feld ($r = 0.90$) > entire population ($r = 0.73$) >> Darmstadt ($r = 0.40$, Table 2).

Similar to the contents of C_{mic}, accuracy of the ergosterol predictions varied (Table 18). Moreover, useful predictions for qCO_2 and the basal respiration were possible only for the subpopulation Garte (Table 18).

Overall, our data indicates that the variability of accuracy of predictions of biological properties depends largely on the population investigated. The sample pretreatment shock freezing/freeze drying proved to be useful for a NIRS prediction of C_{mic} and N_{mic} contents

in a range of distinction between high and low to good in our study (Table 18) and up to excellent in the study by Terhoeven-Urselmans *et al.* (2008). In contrast, use of air-dried soils resulted in a larger range of accuracy in the NIRS prediction from not useful to excellent (Terhoeven-Urselmans *et al.* 2006; Chang *et al.* 2001; Chodak *et al.* 2003; Coûteaux *et al.* 2003; Zornoza *et al.* 2009).

6.3.5. Comparison of the results of cross-validation and calibration/validation procedures

A comparison of the cross-validation results for the main constituents with the validation results of a calibration/validation procedure with a SOC-based selection strategy indicated a decrease of accuracy from excellent down to approximate quantitative (SOC, total N) or to good (P, S) (Table 16, Fig. 12) for the entire population. In contrast to our findings, Morón & Cozzolino (2004) reported very similar RPD and RSC values of 3.0 and 3.6 for SOC (thus good or excellent predictions according to Saeys *et al.*, 2005) and 4.0 and 4.5 for total N (thus excellent predictions), and 2.3 and 2.2 for potentially mineralizable N (which is approximate quantitative).

For the biological constituents, the comparison of the cross-validation results with the validation results of the calibration/validation procedure for the entire population indicates the same accuracy for C_{mic} (approximate quantitative, Table 16) and the ergosterol/C_{mic} ratio (distinction between high and low, Table 16), but a decrease of accuracy for N_{mic} and ergosterol (Table 16).

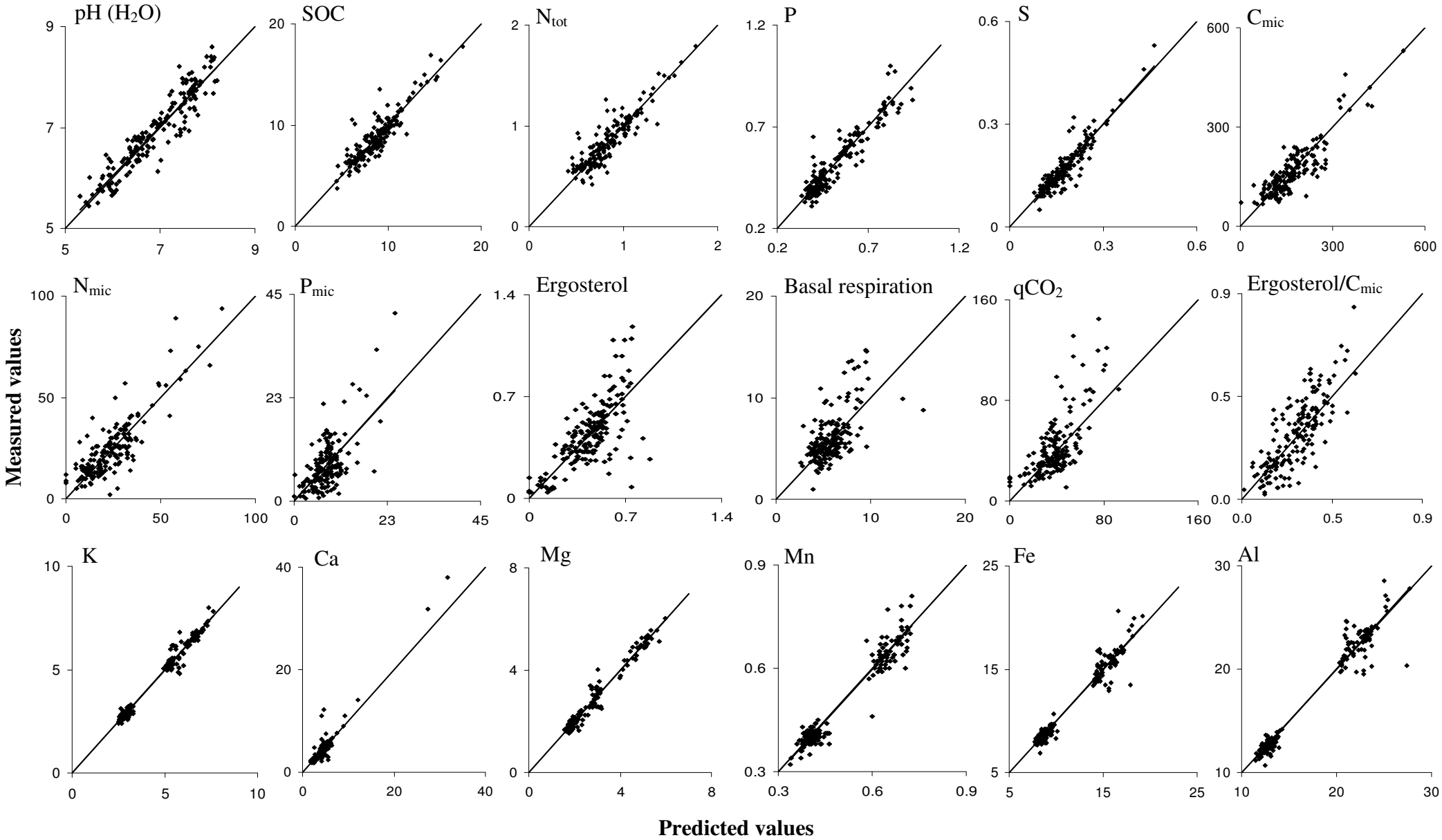

Figure 12: Validation scatter plot of measured and predicted values with the SOC based selection of the entire population (n=174) for pH(H_2O), SOC, N_{tot}, P, S (mg g^{-1}), C_{mic}, N_{mic}, P_{mic}, ergosterol (µg g^{-1}), Basal respiration (µg CO_2-C g^{-1} d^{-1}), qCO_2 (mg CO_2-C d^{-1} $g^{-1}C_{mic}$), ergosterol/C_{mic} ratio (%) and the contents of metal cations K, Ca, Mg, Mn, Fe, and Al (mg g^{-1}). All contents are expressed on an oven dried basis (105°C). The 1:1 line is indicated in each figure.

Table 18: Number (N) of predicted constituents in the different accuracy levels with cross-validation of the entire population (n=610), Hohes Feld (n=120), Garte (n=160) and Darmstadt sample set (n=330) and the number of predicted constituents with calibration/validation for the entire population, Hohes Feld, Garte and Darmstadt samples set with SOC based, H-value and random based selection

Prediction accuracy	Cross-validation procedure		Calibration/Validation procedure					
			SOC based		H-value based		Random based	
	N	Constituent	N	Constituent	N	Constituent	N	Constituent
Entire population (n=610)								
Excellent	10	pH, SOC, N_{tot}, P, S, K, Mg, Mn, Fe, Al	6	K, Ca, Mg, Mn, Fe, Al	7	P, K, Ca, Mg, Mn, Fe, Al	7	P, K, Ca, Mg, Mn, Fe, Al
Good	0		3	pH, P, S	1	pH	2	pH, S
Approximate quantitative	3	C_{mic}, N_{mic}, Ca	3	SOC, C_{mic}, N_{tot}	4	C_{mic}, SOC, N_{tot}, S	2	SOC, N_{tot}
Distinction between high and low	2	Ergosterol, Ergosterol/C_{mic}	2	N_{mic}, Ergosterol/C_{mic}	2	N_{mic}, Ergosterol/C_{mic}	2	C_{mic}, N_{mic}
Sum	**15**		**14**		**14**		**13**	
Hohes Feld (n=120)								
Excellent	4	SOC, N_{tot}, K, Mg	3	SOC, N_{tot}, Mg	2	SOC, N_{tot}	2	N_{tot}, K
Good	3	S, Fe, Al	2	S, K	1	Mg	2	SOC, Mg
Approximate quantitative	2	C_{mic}, N_{mic}	2	C_{mic}, N_{mic}	4	S, K, Fe, Al	1	Fe
Distinction between high and low	6	pH, Ergosterol, P, Ca, Mn, Ergosterol/C_{mic}	2	pH, Fe	2	pH, C_{mic}	4	N_{mic}, P, S, Al
Sum	**15**		**9**		**9**		**9**	
Garte (n=160)								
Excellent	1	SOC	1	SOC	1	SOC	1	SOC
Good	3	C_{mic}, N_{mic}, N_{tot}	0		0		1	C_{mic}
Approximate quantitative	1	P	2	C_{mic}, N_{tot}	1	C_{mic}	0	
Distinction between high and low	8	pH, P_{mic}, Ergosterol, Basal respiration, S, K, Fe, qCO_2	3	N_{mic}, S, qCO_2	5	N_{mic}, Basal respiration, N_{tot}, P, qCO_2	6	pH, N_{mic}, Basal respiration, N, P, qCO_2
Sum	**13**		**6**		**7**		**8**	
Darmstadt (n=330)								
Excellent	2	pH, Ca	3	S, Ca, Mg	1	Ca	3	pH, S, Ca
Good	1	S	1	pH	2	S, Mg	1	Mg
Approximate quantitative	2	K, Mg	0		1	pH	0	
Distinction between high and low	6	SOC, C_{mic}, N_{mic}, Ergosterol, N_{tot}, Fe	2	SOC, K	2	SOC, K	3	SOC, N_{mic}, K
Sum	**11**		**6**		**6**		**7**	

We tested whether the sample size affected the performance of a calibration/validation procedure compared to a cross-validation procedure. We assumed that results of cross-validation would match results of validation more closely with increasing sample size of the population. In agreement with this assumption, the sum of useful predictions (sum of excellent, good, approximate quantitative predictions and those with a distinction between high and low) was almost identical for cross-validation (15, Table 18) compared to the calibration/validation procedures (13 to 14, Table 18) when the entire population was used. However, the comparison of the number of excellent predictions (10 in the cross-validation compared to 6 to 7 in the calibration/validation procedure) indicates that results of cross-validation are not an adequate surrogate for a calibration/validation procedure.

For the subpopulations with the sample sizes of 120, 160 and 330 samples, the calibration/validation procedure performed markedly less accurately compared to the cross-validation procedure (Tables 17 and 18). The sum of useful predictions was 11 to 15 in the cross-validation procedures compared to 6 to 9 in the calibration/validation procedures (Table 18).

The sample selection strategy for the calibration set affected the validation results only slightly. The sums of useful predictions were either identical for all three strategies (SOC-based, based on the H-value or random) as for Hohes Feld or differed by up to two constituents (Table 18). If the emphasis is put on the excellent, good and approximate quantitative predictions, then the SOC-based selection strategy performed slightly more favorably (26 excellent, good and approximate quantitative predictions) than the H distance procedure (25) and the random selection (22, Table 18).

6.4. Implications

The use of the sample pretreatment shock freezing/freeze-drying was useful for C_{mic}, N_{mic} and the ergosterol to C_{mic} ratio as indicated by the calibration/validation procedure. However, except for the subpopulation Garte, prediction of the other biological parameters failed. Thus, the shock freezing/freeze-drying sample pretreatment may be recommended, if approximate quantitative data for C_{mic} and a distinction between high and low for N_{mic} and ergosterol to C_{mic} ratio are needed.

Cross-validation gave markedly better estimates than the calibration/validation procedure for a large population as well as for the smaller subpopulations. Overall, for all

populations shown in Table 5, the accuracy of prediction in the comparison of cross-validation versus SOC-based calibration/validation decreased by one level of accuracy (24 constituents) or remained on the same level (19 constituents). For few constituents, the decrease of accuracy was even more than one level (6 constituents), whereas for 3 constituents, accuracy increased. Thus, cross-validation results should not be regarded as an adequate surrogate for an independent validation.

The choice of the sample selection strategy for the calibration set (either based on the SOC content or on the H-value or randomly selected) had only little impact on the accuracy of predictions in the independent validation data set. Overall, the SOC-based selection strategy is recommended because of slightly more excellent, good and approximate quantitative predictions in the validation procedure for most of our populations.

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7. Zusammenfassung

In dieser Arbeit wurden zum einen die Auswirkungen der mineralischen und organischen Düngung und zum anderen der Einfluss reduzierter Bodenbearbeitung im Vegleich zum Einsatz des Wendepfluges auf die mikrobielle Biomasse als Motor des Nährstoffkreislaufs in umfassendem Maße untersucht. Im Fokus der Untersuchungen stand auf der einen Seite die Beeinflussung bodenchemischer und -biologischer Messgrößen im Zusammenspiel mit der räumlichen Heterogenität des pH-Werts und mineralischer und organischer Düngung. Auf der anderen Seite wurde das Augenmerk auf unterschiedliche Bodenbearbeitungssysteme gelegt, welche ebenfalls hinsichtlich ihrer Auswirkungen auf bodenchemische und -biologische Messgrößen untersucht wurden.

Die vergleichende Betrachtung zweier Langzeitversuche machte deutlich, dass sowohl die organische Düngung, als auch die Bodenbearbeitung mittels Kreiselegge, durch die eine langzeitliche Verbesserung der Bodenqualität erwartet wurde, diese Vermutung weitestgehend bestätigten. Trotz unterschiedlicher Bewirtschaftungsansätze konnten beide Systeme zeigen, dass auf der einen Seite organische Düngung, und auf der anderen Seite die reduzierte Bodenbearbeitung mittels Kreiselegge die Messgrößen der Bodenqualität, wie den organischen Kohlenstoff oder Indizes der mikrobiellen Biomasse, im Vergleich zur mineralischen Düngung und der Anwendung des Wendepfluges erhöhten.

Die Anwendung mineralischer und organischer Dünger im Zusammenspiel mit der herrschenden Heterogenität auf den Flächen des Lanzeitversuchs in Darmstadt, zeigte, dass nicht allein die Heterogenität der fluviatilen Sande, sondern auch die Anwendung mineralischer Dünger im Vergleich zu organischem Input zu einer Abnahme des $pH_{(H_2O)}$-Werts führten. Des Weiteren lagen die Gehalte an organischem Kohlenstoff, Gesamtstickstoff und -schwefel bei mineralischer Düngung signifikant unter denen mit organischer Düngung. Die Gesamtgehalte an Phosphor zeigten keine Veränderungen durch die unterschiedliche Düngung. Die Gehalte der mikrobiellen Biomasse (C_{mik}, N_{mik}) wurden durch organische Düngung erhöht, während S_{mik} und Ergosterol durch mineralische Düngung gefördert wurden. Die Quotienten zur Abschätzung der Substratnutzungseffizienz (qCO_2 und C_{mic}/C_{org}) zeigten keinerlei Reaktion auf die unterschiedliche Düngung. Der Ergosterol/C_{mik}-Quotient war in den mineralisch gedüngten Flächen erhöht, während der C_{mik}/S_{mik}-Quotient niedrigere Werte im Vergleich zu den organisch gedüngten Flächen aufwies. Durch die Tatsache, dass mineralische Düngung plus Strohrückführung und die dadurch verstärkte Abnahme des $pH_{(H_2O)}$-Wertes, den Anteil saprotropher Pilze an der mikrobiellen Gemeinschaft begünstigte und somit

eine erhöhte Menge zugeführten Schwefels inkorporiert wurde, kann der geringere C_{mik}/S_{mik}-Quotient als Anzeichen für eine Veränderung in der mikrobiellen Gemeinschaft zu Gunsten saprothropher Pilze gesehen werden.

Die Untersuchung der Auswirkungen der Pflug- und Kreiseleggenbearbeitung auf die Verteilung und die Vorräte bodenchemischer und -biologischer Messgrößen zeigte eine Erhöhung der Gehalte des organischen Kohlenstoffs, des Gesamtstickstoffs und -phosphors und des mikrobiellen Biomasse C, N und P in den ersten 30 cm des Bodenprofils bei reduzierter Bodenbearbeitung. Wohingegen die Bodenbearbeitung keinerlei Auswirkungen auf die Gesamtgehalte an S, den mikrobielle Biomasse S und die Basalatmung in den 30 cm des Bodens zeigte. Die Ergebnisse des Ergosterols als pilzlicher Biomarker zeigten, dass Pflugbearbeitung saprotrophe Pilze auf diesen Flächen förderte. Einhergehend mit dem hohen Ergosterolgehalt unter dieser Bodenbearbeitung, zeigte sich ein hoher Ergosterol/C_{mik}-Quotient, der im Zusammenhang mit dem geringeren C_{mik}/S_{mik}-Quotient auch hier eine Veränderung der mikrobiellen Gemeinschaft zu Gunsten saprotropher Pilze anzeigte. Dieses Ergebnis steht im Wiederspruch zu zahlreichen Untersuchungen anderer Bodenbearbeitungsversuche, in denen eine Förderung von Pilzen durch eine flachere Bodenbearbeitung herbeigeführt wurde. Weiterhin zeigten die Untersuchungen dieser Arbeit, dass entgegen der allgemeinen Annahme, der erhöhte Pilzanteil zu einer Abnahme der Substratnutzungseffizienz führte, was mit einem erhöhten metabolischen Quotienten einherging.

Die Analyse der an den Böden beider Langzeitversuche bestimmten bodenchemischen und -biologischen Konstituenten mittels Nahinfrarot Spektroskopie (NIRS) zeigte sowohl für die Kreuzvalidierung als auch für die separate Kalibrierung und Validierung des Gesamtdatensatzes und der Untergruppe Hohes Feld exzellente Vorhersagen (RPD-Werte > 3 und r > 0.91) für den organische Kohlenstoff, Gesamt-N und teilweise für die mittels HNO_3-Druckaufschluss bestimmten Nährstoffe (K, Ca, Mg, Mn, Fe, Al). Bei der Vorhersage der mikrobiellen Messgrößen C_{mik} und N_{mik} führte NIRS ausschließlich zu einer ungefähren Abschätzung der Quantität (RPD-Werte zwischen 2.5 und 2.0, r zwischen 0.81 und 0.66) oder war nur in der Lage zwischen hohen und niedrigen Werten zu unterscheiden (RPD-Werte zwischen 2 und 1.5, r zwischen 0.65 und 0.50). NIRS führte bei P_{mik}, S_{mik}, der Basalatmung und dem qCO_2 zu keiner brauchbaren Abschätzung, während es für Ergosterol eine Abschätzung von hohen und niedrigen Werten innerhalb der Kreuzvalidierung erlaubte. Obwohl die Kreuzvalidierung für alle Konstituenten zu den besten Ergebnissen führte, war eine Separierung des Datensatzes in

einen unabhängigen Kalibrierungs- und Validierungsdatensatz unabdingbar, um die Güte der Kalbration an einer offenen Population zu überprüfen und somit die Möglichkeit zu besitzen, den Kalibrierungsdatensatz mit anderen Proben zu erweitern. Die unterschiedlichen Selektionsverfahren zeigten keinen großen Einfluss auf die Vorhersagegenauigkeit. Jedoch wies die SOC-basierte Selektion leicht mehr exzellente, gute oder nahezu quntitative Vorhersagen auf. Die Annahme, dass eine Reduzierung des Gesamtdatensatzes auf einen homogeneren Datensatz, wie dem des Hohen Feldes, zu einer erhöhten Abschätzungsgenauigkeit mittels NIRS führe, hat sich bei den Untersuchungen innerhalb dieser Arbeit mit der Kreuzvalidierung nicht bestätigt, während die Qualität der Vorhersage mittels separierter Kalibrierung und Validierung für den Gehalt des organischen Kohlenstoffs, C_{mik}, N_{mik} und die Gesamtgehalte des Stickstoffs gesteigert wurden oder mindestens gleich blieben.

Zusammenfassend bleibt festzuhalten, dass die organische Düngung und die reduzierte Bodenbearbeitung mittels Kreiselegge zur Erhöhung der Bodenqualität erhaltenden Messgrößen wie organischem Kohlenstoff, Gesamtstickstoff und mikrobiellem Biomasse C und N führten. Beide Systeme wiesen einen im Vergleich zur mineralischen Düngung und Pflugbearbeitung geringeren Anteil der Pilze an der mikrobiellen Biomasse auf. Dies führte zu einem erhöhten C_{mic}/S_{mik}-Quotienten und zeigte, einhergehend mit dem geringeren qCO_2, dass die organische Düngung und die reduzierte Bodenbearbeitung mittels Kreiselegge zu einer höheren Substratnutzungseffizienz führte. Daher kann eine Kombination organischer Düngung und reduzierter Bodenbearbeitung die C-Speicherung im Boden erhöhen.

8. Summary

The thesis was conducted to investigate the effects of fertilization (mineral vs. organic fertilizer) and tillage (mouldboard plough vs. reduced tillage with a rotary harrow) on the microbial biomass.

The comparison of the influence of mineral and organic fertilizer in relationship to the spatial heterogeneity resulting from fluviatile sandy sediments, showed that not only the variability was influencing the pH but also the fertilization had an impact on soil pH with a lowering effect under mineral fertilization. Furthermore mineral fertilization led to a significant lower content of soil organic carbon, total N and S, while total P was not influenced by fertilization treatment. Microbial biomass indices (C_{mic}, N_{mic}) were increased significantly with organic fertilization in comparison to the mineral treatments, while S_{mic} and ergosterol were enhanced by the addition of mineral fertilizer. The microbial biomass quotients (qCO_2 and C_{mic}/C_{org}), which are used as indices for subtsrate use efficiency by microorganisms, showed no clear influence by fertilizer treatment. However, the ergosterol to C_{mic} quotient was significant higher, while the C_{mic}/S_{mic} quotient was significantly lower with mineral fertilization in comparison to the organic fertilizer treatment. As the mineral fertilization treatment, in which straw was returned, lowered soil pH, saprotrophic fungi, which are able to increase their S-incorporation by about 130%, have been promoted. The C_{mic}/S_{mic} ratio could therefore be used as an indicator for a shift in microbial community.

Soil organic carbon, total N and P and microbial biomass C, N and P in the top 30 cm of the soil profile were significantly increased with reduced tillage compared to plough tillage. In contrast, total S, and S_{mic} were not affected by tillage treatment. Ergosterol, which acts as a fungal biomarker, was enhanced by plough tillage in comparison to reduced tillage. The high ergosterol content was associated with a high ergosterol to C_{mic} quotient in the soil under plough tillage which indicates, in correspondence with the lower C_{mic}/S_{mic} quotient, that plough tillage favored saprotrophic fungi. This is in contradiction to other studies which found that fungi were promoted by reduced soil management. Furthermore, the assumption that a higher amount of fungal biomass enhances the substrate use efficiency was not confirmed by our results. In contrast, a higher content of fungal biomass with mineral fertilization and plough tillage led to a lower substrate use efficiency, as indicated by the higher metabolic quotient under these treatments.

The analysis of soil chemical and biological constituents with near infrared spectroscopy (NIRS) showed that not only the cross-validation procedure but also the

separated calibration and validation of the whole dataset as well as for a smaller subgroup (Hohes Feld) led to an excellent prediction (RPD value > 3, r >0.91) of soil organic carbon, total N and especially for some nutrients (K, Ca, Mg, Mn, Fe, and Al) analysed by HNO_3-pressure digestion. The prediction accuracy of C_{mic} and N_{mic} allowed only an approximate quantitative estimation (RPD between 2.5 and 2.0, r value between 0.81 and 0.66) or differentiation between high and low values (RPD value between 2. and 1.5, r between 0.65 and 0.50). The contents of P_{mic}, S_{mic}, CO_2 and qCO_2 could not be predicted with NIRS, while the prediction of ergosterol allowed an estimation of high and low values with the cross-validation procedure. Although the results of the cross-validation procedure showed the highest prediction accuracy for all constituents, the separation of an independent calibration and validation dataset was required for proving and expanding open populations. The choice of the sample selection strategy for the calibration set (either based on the SOC content or on the H-value or randomly selected) had only little impact on the accuracy of predictions in the independent validation data set. Overall, the SOC-based selection strategy is recommended because of slightly more excellent, good and approximate quantitative predictions in the validation procedure for most of our populations. The hypothesis that a reduction of the whole dataset to a smaller, more homogeneous subgroup would enhance the prediciton accuracy of the constituents was not confirmed in our investigation for the cross-validation procedure but led, together with a separation of the calibration and validation dataset, to a higher or equal prediction accuracy for soil organic carbon, C_{mic}, N_{mic}, total N.

In summary, organic fertilization and reduced tillage enhanced key factors of soil quality, such as soil organic carbon, C_{mic} and N_{mic}, in comparison to the compromising treatment. Organic fertilization and reduced tillage by rotary harrow resulted in a lower content of saprotrophic fungi in comparison to mineral fertilization and plough tillage. This led to an increased C_{mic}/S_{mic} ratio which, together with a lower metabolic quotient, indicated a higher substrate use efficiency by the use of organic fertilization and reduced tillage. Therefore, preferentially a combined use of reduced tillage and organic fertilization supports C sequestration.

9. Schlussfolgerung

Der Einsatz organischer Düngung und der reduzierten Bodenbearbeitung mittels Kreiselegge stellt im Gegensatz zu der mineralischen Düngung und dem Einsatz des Wendepfluges ein vielversprechendes landwirtschaftliches Managementsystem dar, das sogar auf sandigen Böden zu einem Erhalt der Bodenqualität in Form von organischem Kohlenstoff und mikrobieller Biomasse führen kann. Trotz des erhöhten Anteils an Pilzen an der mikrobiellen Gemeinschaft unter mineralischer Düngung und unter Pflugbearbeitung wurde, anders als in der Literatur beschrieben, die Substratnutzungseffizienz unter diesen Managementsystemen reduziert. Während die Förderung der Pilze innerhalb des Langzeitversuchs in Darmstadt durch den pH-Wert senkenden Effekt der mineralischen Dünger und die Einarbeitung des Strohs begründet liegt, kann der erhöhte Pilzanteil unter Pflugbearbeitung nicht vollkommen aus den in dieser Arbeit erhobenen Daten erklärt werden und muss durch weitere Untersuchungen bezüglich der mikrobiellen Zusammensetzung analysiert werden. Nichts desto trotz zeigte sich in den Untersuchungen beider Langzeitversuche, dass der C_{mik}/S_{mik}-Quotient als Anzeichen für eine Veränderung der mikrobiellen Gemeinschaft herangezogen werden kann, der bei geringen Werten auf eine Dominanz saprotropher Pilze hinweist.

Die Erfassung der chemischen und biologischen Messgrößen mittels NIRS an den schockgefrorenen und gefriergetrockneten Bodenproben beider Langzeitversuche zeigte, dass sowohl die Kreuzvalidierung als auch die separierte Kalibrierung und Validierung des Gesamtdatensatzes und des kleineren Datensatzes zur erfolgreichen Vorhersage des organischen Kohlenstoffs und der Nährstoffkationen führte. Der in der mikrobiellen Biomasse inkorporierte Kohlenstoff und Stickstoff konnte zwar für alle Kalibierungs- und Validierungsverfahren vorhergesagt werden, führte aber nur zu einer annähernd quantitativen Bestimmung. Die Vorhersage der Quotienten der mikrobiellen Biomasse als Aktivitäts- und Effizienzmessgröße konnten durch kein Verfahren zufrieden stellend vorhergesagt werden. Daher muss festgehalten werden, dass sowohl die Probenaufbereitung als auch die unterschiedlichen Verfahren der Kalibrierung und Validierung für eine Bestimmung von indirekten Aktivitäts- und Effizienzparametern, die komplexen Wirkungszusammenhängen unterliegen, zu keinen zufriedenstellenden Vorhersagen an den verwendeten Böden beider Langzeitversuche mittels NIRS führten und weitere Untersuchungen in diese Richtung notwendig sind.

Abschließend hat diese Arbeit gezeigt, dass die mikrobielle Biomasse und ihre Funktion im Nährstoffkreislauf auch langzeitlich von landwirtschaftlichen Systemen

beeinflusst werden. Organische Düngung (seit 27 Jahren) und reduzierte Bodenbearbeitung (seit 40 Jahren) erhöhten die Speicherung von C, N und P in die mikrobielle Biomasse und trugen so zu einem stärkeren Erhalt der Bodenqualität, im Vergleich zur Anwendung mineralischer Dünger oder der Pflugbearbeitung bei. Die Sicherung der Bodenqualität durch eine intakte mikrobielle Gemeinschaft gilt es durch angepasstes landwirtschaftliches Management, wie einer Kombination organischer Düngung und reduzierter Bodenbearbeitung, zu erhalten und gezielt nachhaltig zu fördern.

10. Ausblick

Die Ergebnisse dieser Arbeit haben unter anderem die Veränderungen des Pilzanteils an der mikrobiellen Biomasse durch unterschiedliche landwirtschaftliche Bearbeitung aufgewiesen. Dabei zeigte sich, dass entgegen anderer Studien, die Pilze nicht zu einer erhöhten Substratnutzungseffizienz führten. Vor allem die Förderung der Pilze unter Pflugbearbeitung wurde aufgrund gegenläufiger Ergebnisse aus der Literatur nicht erwartet und konnte innerhalb dieser Arbeit nicht vollkommen erklärt werden. Um diese Zusammenhänge besser verstehen zu können, wäre eine vollständige Untersuchung der mikrobiellen Gemeinschaftsstruktur wünschenswert. Dies kann unter anderem durch die Bestimmung von Muraminsäure als bakteriellen und Glucosamin als pilzlichen Biomarker (Apphun, 2004) erreicht werden. Hierdurch kann der Anteil bakterieller Biomasse zu der pilzlichen ins Verhältnis gesetzt werden und stellt somit einen weiteren Indikator für Veränderungen der Substratnutzung ausgehend von Änderungen in der landwirtschaftlichen Praxis dar (Joergensen und Wichern, 2008). Darüber hinaus wäre die Analyse des Anteils biotropher arbuskulärer Mykorrhiza von Vorteil, da sie einen wesentlichen Beitrag zur Nährstoffversorung in landwirtschaftlichen Systemen liefern, aber innerhalb dieser Arbeit durch das Ergosterol nicht erfasst werden konnten. Um die Anwendung des C_{mik}/S_{mik}-Quotienten zu verifizieren, könnten Untersuchungen in Bezug auf die Schwefelinkorporation unterschiedlicher Schwefelverbindungen in die mikrobielle Biomasse von Vorteil sein, um Aussagen zur allgemeinen Anwendbarkeit dieses Quotientens im Hinblick auf die Zusammensetzung der mikrobiellen Gemeinschaft treffen zu können.

Für die erfolgreiche Anwendung von NIRS auch für bodenbiologische Aktivitäts- und Effektivitätsparameter sollten Untersuchungen bezüglich der Probenaufbereitung ausgebaut und auf das Verhalten der mikrobiellen Biomasse angepasst werden. Dabei könnte ein kleinerer Probensatz aus möglichst feinkörnigem, homogenem Bodenmaterial (mit geringerer spektralen Varianz) mit unterschiedlicher Probenvorbereitung, wie feldfrische, gesiebte Bodenproben im Vergleich zu luftgetrockneten und gemahlenen Proben, gemessen werden.

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