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Effects of grassland conversion and tillage intensities on soil microbial biomass, residues and community structure

Dissertation

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by

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(Rajasekaran Murugan)

Preface

This thesis was prepared within the Research Training Group "Regulation of soil organic matter and nutrient turnover in organic agriculture" (Graduiertenkolleg 1397/2) and funded by the Deutsche Forschungsgemeinschaft (DFG). The thesis is submitted to the Faculty of Organic Agricultural Sciences to fulfil the requirements for the degree "Doktor der Agrarwissenschaften" (Dr. agr.). The cumulative dissertation is based on three papers as first author, which are published in or submitted to international refereed journals. The manuscripts are included in chapters 2, 3 and 4. The focus of the general introduction (chapter 1) is on theoretical and methodological issues, whereas specific introductions on the effect of tillage and grassland conversion intensities on soil are given in the following manuscripts (chapters 2, 3 and 4).

Chapter 2:

Murugan, R., Koch, H-J. Joergensen, R.G 2012. Long-term influence of different tillage intensities on soil microbial biomass, residues and community structure at different depths. Soil and Tillage Research (in revision)

Chapter 3:

Murugan, R., Loges, R., Taube, F., Joergensen, R.G 2013. Specific response of fungal and bacterial residues to one-season tillage and repeated slurry application in a permanent grassland soil. Applied Soil Ecology 72, 31-40.

Chapter 4:

Murugan, R., Loges, R., Taube, F., Joergensen, R.G 2013. Changes in soil microbial biomass, residues and functional diversity after conversion of permanent to modified grassland or maize crop. Microbial Ecology (under review)

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List of abbreviations

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
$BaCl_2$	Barium chloride
C	Carbon
Ca	Calcium
$CaCl_2$	Calcium chloride
$CHCl_3$	Chloroform
CLPP	Community level physiological profiles
CO_2	Carbon dioxide
CV	Coefficient of variation
DA	Discriminant analysis
DF	Discriminant function
DFG	Deutsche Forschungsgemeinschaft
GalN	Galactosamine
GlcN	Glucosamine
GRT	Grubber tillage
H_2O	Water
HCl	Hydrochloric acid
HNO_3	Nitric acid
HPLC	High performance liquid chromatography
ICP-AES	Inductively coupled plasma atomic emission
K	Potassium
K_2SO_4	Potassium sulphate
k_{EC},k_{EN},k_{ES}	Extractable portion of total C, N, S from microbial biomass
ManN	Mannosamine
MBT	Mouldboard tillage
MG	Modified grassland
MM	Maize monoculture
MurN	Muramic acid
N	Total nitrogen
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NH_4NO_3	Ammonium nitrate
NLFA	Neutral lipid fatty acid
NT	No tillage

O_2	Oxygen
OPA	Ortho-phthaldialdehyde
P	Total phosphorus
PG	Permanent grassland
PLFA	Phospholipid fatty acid
P2+	Permanent grassland + slurry in 2 year old trial
P2-	Permanent grassland - slurry in 2 year old trial
P5+	Permanent grassland + slurry in 5 year old trial
P5-	Permanent grassland - slurry in 5 year old trial
qCO_2	Metabolic quotient
r	Correlations coefficient
R2+	Re-established grassland + slurry in 2 year old trial
R2-	Re-established grassland - slurry in 2 year old trial
R5+	Re-established grassland + slurry in 5 year old trial
R5-	Re-established grassland - slurry in 5 year old trial
S	Total sulphur
SOC	Soil organic carbon
SOM	Soil organic matter
THF	Tetrahydrofuran
WHC	Water holding capacity

Summary

Tillage, grassland conversion and fertilisation are the major practices which have a strong impact on soil organic matter (SOM), microbial biomass stocks and microbial community structure in agro-ecosystems. The relationship between microbial biomass sulphur (S) and ergosterol might serve as an additional indicator for saprotrophic fungi. Due to the specificity, amino sugar analysis gives more important information on the relative contribution of fungal and bacterial residues to carbon (C) sequestration potential of soils. Recently, microbial residues (i.e. necromass) have been identified as a significant source of SOM. Effects of tillage, grassland conversion intensities and fertilisation on microbial biomass, residues and community structure with high vertical and horizontal spatial variation across land use system is still missing and remains unmapped, although tillage may have strong impact on microbial processes and C sequestration in the subsoil. To study the response of fungal, bacterial residues and their contribution to soil organic C sequestration, the present work was conducted with the following objectives.

1. To quantify the long-term influence of different tillage intensities on soil microbial biomass, residues and community structure at different depths in four large-scale experimental sites located across eastern and southern Germany.

2. To assess the impact of one-season tillage and repeated slurry application on specific response of fungal and bacterial residues in a permanent grassland soil following 2 years and 5 years after the tillage event.

3. To investigate the changes in soil microbial biomass, residues and functional diversity of microbial community after conversion of permanent to modified grassland or maize monoculture.

1. In the first experiment, the following three hypotheses were tested: (1) a reduction in tillage intensity from mouldboard plough (MBT) and grubber (GRT) to no tillage (NT) increases stocks of soil organic matter and especially microbial biomass. (2) In the top soil layers, these increases lead by the preferential accumulation of fungal residues. (3) In the bottom soil layers, a

reduction in tillage intensity generally promotes arbuscular mycorrhizal fungi (AMF) at the expense of saprotrophic fungi on loess-derived soils from four long-term tillage trials in Germany down to 40 cm. At 0-30 cm depth, the stocks of SOM (SOC, total N, P and S), soil microbial biomass C and N, fungal biomass as well as amino sugars were significantly higher in the GRT and NT treatments in comparison with the MBT treatment. In contrast, the stocks of SOM, soil microbial biomass, fungal biomass and amino sugars were significantly higher in the MBT treatment at 30-40 cm depth followed by the GRT treatment, while the lowest microbial biomass C stock was observed in the NT treatment but tillage had no effect on microbial biomass S in both top and bottom soil layers. The differences in microbial biomass stocks might have caused by the different development in the microbial community structure, which is reflected by the negative relationship between the ratios ergosterol to microbial biomass C and fungal C to bacterial C. Further, the positive correlations between ergosterol and microbial biomass S; ergosterol and fungal C could therefore be used as an indicator for the contribution of saprotrophic fungi to fungal C. At 0-5 cm depth, the presence of saprotrophic fungi was significantly increased in the GRT and NT treatments in comparison with the MBT treatment. In contrast, the GRT and NT treatments promoted AMF at the expense of saprotrophic fungi at 30-40 cm depth, suggesting the importance of saprotrophic fungi and AMF in SOM dynamics.

2. The second study was carried out with the objectives (1) to assess the effects of one-season cultivation of winter wheat in two field trials on the stocks of soil organic C (SOC), total N, microbial biomass, fungal biomass, and microbial residues in comparison with permanent grassland and (2) to determine the effects of repeated manure application to restore the negative tillage effects. One trial was started 2 years before sampling, and the other 5 years before sampling. Mouldboard ploughing decreased the stocks of SOC, total N, microbial biomass C, and microbial residues (muramic acid and glucosamine), but increased those of the fungal biomarker ergosterol in both trials. Slurry application increased stocks of SOC and total N only in the short-term (2 years) trial, whereas the stocks of microbial biomass C, ergosterol and microbial residues were generally increased in both trials, especially in combination with tillage. Generally, the ergosterol to microbial biomass C ratio was increased by tillage, and decreased by slurry application in both trials. The fungal C to bacterial C ratio was generally decreased by these two treatments indicating that AMF apparently respond more sensitive to tillage and

fertilisation than other soil microorganisms. The fungal C to bacterial C ratio revealed a significant positive linear relationship with the ergosterol to microbial biomass C ratio, indicating an increased formation of fungal residues along with increasing saprotrophic fungal biomass. Tillage promoted saprotrophic fungi at the expense of biotrophic AMF and bacteria as indicated by the increased ergosterol to microbial biomass C ratio and the constant fungal C to bacterial C ratio compared to undisturbed grassland soils suggesting that the saprotrophic fungal community did not return to pre-cultivation composition. In conclusion, if tillage of grassland soils cannot be avoided, the application of cattle slurry has positive effects on soil microorganisms and C sequestration.

3. The third experiment was aimed to investigate the effects of land use change caused by the boom in biogas production strongly based on maize monoculture on the dynamics of soil organic C (SOC), microbial biomass, fungal biomass, fungal and bacterial residues in a permanent grassland soil. Cattle slurry was applied to quantify the effects of fertilisation on change in microbial residues and functional diversity of microbial community across land use types. Maize monoculture (MM) decreased the stocks of SOC, microbial biomass C, N and S and microbial residues (muramic acid and fungal glucosamine) compared to the permanent grassland (PG) and modified grassland (MG) at 0-40 cm depth. The significantly higher ergosterol to microbial biomass C ratio and lower the microbial biomass C/S ratio, indicates greater accumulation of saprotrophic fungi in the MM treatment compared to the grassland treatments. The microbial biomass C/S ratio could therefore be used as an additional indicator for a shift in microbial community. In contrast, the PG treatment promoted ergosterol-free AMF as indicated by higher fungal C to bacterial C ratio and lower ergosterol to microbial biomass C ratio. The functional diversity of microbial community declined significantly under the tilled MM and MG treatment compared to the undisturbed PG treatment. The increase in the stocks of microbial biomass and amino sugars were the most sensitive indicators of slurry application. Similarly, the current experiment showed that the lost functional diversity of microbial community due to tillage and the rush into maize monoculture can be restored by slurry application.

In a nutshell, the combined analyses of microbial biomass and residue formation of both fungi and bacteria provided a unique opportunity to study the effect of tillage, grassland conversion and fertilisation on soil microbial dynamics. In top soil at 0-30 cm layer, a reduction in tillage intensity by the GRT and NT treatments increased the accumulation of saprotrophic fungi in comparison with the MBT treatment. In contrast, the GRT and NT treatments promoted AMF at the expense of saprotrophic fungi in the bottom soil layer at 30-40 cm depth. Slurry application generally increased microbial biomass C, bacterial residues and their contribution to SOC. In general, mouldboard ploughing in permanent grassland soil promoted saprotrophic fungi at the expense of biotrophic AMF and bacteria compared to undisturbed grassland soils. The ratios fungal C to bacterial C and ergosterol to microbial biomass C were positively correlated in the second experiment, in contrast to their negative relationship in the first and third experiment. I conclude that the differences in microbial community structure not only caused by the tillage and grassland conversion. The next challenge is to find the role of edaphic factors (soil texture, moisture, and temperature) in a shift in microbial community structure. The close relationships between living microbial fractions and dead microbial residual indices points to the importance of saprotrophic fungi and biotrophic AMF for agricultural management induced effects on microbial turnover and ecosystem C storage. The specific phospholipid fatty acid and neutral lipid fatty acid biomarkers are not as effective as previously thought to discriminate saprotrophic fungi and biotrophic AMF (Joergensen and Wichern, 2008; De Vries et al., 2012). Quantitative information on exact biomass estimates of these two important fungal groups in soil is inevitably necessary to understand their different roles in SOM dynamics.

Zusammenfassung

Bodenbearbeitung, die Umwandlung von Grünland in Ackerland und Düngung sind Verfahren, die in Agrarökosystemen den größten Einfluss auf den Umfang der organischen Bodensubstanz (SOM), der mikrobiellen Biomasse sowie auf die Zusammensetzung der mikrobiellen Gemeinschaft ausüben. Das Verhältnis zwischen mikrobiellem Biomasse S und Ergosterol, welches als spezifischer Biomarker für saprotrophe Pilze dient, könnte als zusätzlicher Indikator zur Unterscheidung zwischen saprotrophen Pilzen und arbuskulärer Mykorrhiza (AMF) herangezogen werden, da diese statt Ergosterol die Zellwandkomponente Glucosamin enthält. Die Analyse von Aminozuckern liefert aufgrund ihrer Spezifität wichtige Informationen über die Beteiligung pilzlicher und bakterieller Rückstände am Potential von Böden, Kohlenstoff zu binden. Mikrobielle Residuen (Nekromasse) sind unlängst als eine bedeutende Quelle organischer Bodensubstanz erkannt worden. Der Einfluss der oben genannten landwirtschaftlichen Bewirtschaftungspraktiken auf die mikrobielle Biomasse sowie mikrobielle Rückstände mit starken vertikalen und horizontalen Schwankungen zwischen verschiedenen Landnutzungssystemen ist bisher wenig erforscht. Um einen spezifischen Datensatz für pilzliche und bakterielle Rückstände sowie deren Beteiligung an der Speicherung von organischem Kohlenstoff im Boden bereitzustellen, wurde die vorliegende Arbeit mit folgenden Zielstellungen durchgeführt:

1. Quantifizierung des langfristigen Einflusses verschiedener Bodenbearbeitungsintensitäten auf die mikrobielle Biomasse, deren Rückstände sowie deren Gemeinschaftsstruktur in verschiedenen Tiefen auf 4 groß angelegten landwirtschaftlichen Versuchsflächen in Ost- und Süddeutschland.
2. Bewertung der Auswirkung einer einsaisonalen Bodenbearbeitung mit wieder-holter Gülleausbringung auf das spezifische Verhalten pilzlicher und bakterieller Rückstände in Dauergrünlandboden 2 und 5 Jahre nach dem Bearbeitungser-eigniss.
3. Untersuchung der Veränderung der mikrobiellen Biomasse im Boden, mikrobieller Residuen sowie der funktionellen Diversität der mikrobiellen Gemeinschaft nach der Umwandlung von Dauergrasland in modifiziertes Grünland und oder eine Maismonokultur.

1. In einem ersten Experiment wurden folgende Hypothesen getestet: (1) eine Verringerung der Bodenbearbeitungsintensität von Pflug (MBT) und Grubber (GRT) hin zu Direktsaat (NT) erhöht den Umfang der organischen Bodensubstanz und besonders der mikrobiellen Biomasse. (2) In den oberen Bodenschichten werden diese Zunahmen vorrangig durch eine Akkumulation pilzlicher Residuen hervorgerufen. (3) Eine Reduzierung der Bodenbearbeitungsintensität fördert im Allgemeinen die Entwicklung von AMF auf Kosten saprotropher Pilze in den unteren Bodenschichten bis 40 cm Tiefe in Lößböden von 4 Langzeit-Bodenbearbeitungsversuchen in Deutschland. In einer Tiefe von 0-30 cm war der Umfang an organischer Bodensubstanz (SOC, total N, P und S), dem mikrobiellen Biomasse C und N, der pilzlichen Biomasse sowie den Aminozuckern in den GRT- und NT-Behandlungen verglichen mit MBT signifikant erhöht. Im Gegensatz dazu waren diese in einer Tiefe von 30-40 cm in der MBT-Behandlung signifikant höher, gefolgt von GRT, während die niedrigsten Gehalte an mikrobiellem Biomasse C in der NT-Behandlung gemessen wurden. Die Bodenbearbeitung hatte keinen Effekt auf mikrobiellen S in den oberen und unteren Bodenschichten. Die Unterschiede im Umfang der mikrobiellen Biomasse sind Folge einer ungleichen Entwicklung innerhalb der mikrobiellen Populationsstruktur, welche durch die negative Korrelation zwischen den Verhältnissen Ergosterol zu mikrobiellem Biomasse C und pilzlichem C zu bakteriellem C reflektiert wird. Weiterhin könnte die positive Korrelation zwischen den Verhältnissen Ergosterol zu mikrobiellem Biomasse S und Ergosterol zu pilzlichem C als ein Indikator für die Beteiligung von saprotrophen Pilzen an pilzlichem C dienen. In 0-5 cm Tiefe war das Verhältnis Ergosterol zu mikrobiellem Biomasse C als ein Indikator für das Vorhandensein von saprotrophen Pilzen in den Behandlungen GRT und NT im Vergleich zu MBT signifikant erhöht. Im Gegensatz dazu förderten die Behandlungen GRT und NT in 30-40 cm Tiefe das Auftreten von AMF auf Kosten saprotropher Pilze, was die Bedeutung von saprotrophen Pilzen und AMF für die Dynamiken der organischen Bodensubstanz verdeutlicht.

2. Ein zweites Experiment wurde durchgeführt, um die Effekte einer einzelnen saisonalen Anbauperiode von Winterweizen in zwei Feldversuchen auf die Gehalte von organischem Bodenkohlenstoff (SOC), Gesamtstickstoff, mikrobieller Biomasse, pilzlicher Biomasse und mikrobieller Residuen im Vergleich mit Dauergrasland zu beurteilen und mögliche restaurative Auswirkungen einer wiederholten Gülleausbringung auf die negativen Effekte der

Bodenbearbeitung zu untersuchen. Die Feldversuche begannen jeweils 2 und 5 Jahre vor der Probenahme. Pflügende Bodenbearbeitung verringerte die Gehalte an SOC, Gesamtstickstoff, mikrobiellem Biomasse C und mikrobiellen Residuen (Muraminsäure und Glucosamin), steigerte jedoch die Menge des pilzlichen Biomarkers Ergosterol in beiden Feldversuchen. Das Ausbringen von Gülle erhöhte den Umfang von SOC und Gesamtstickstoff nur kurzzeitig (nach 2 Jahren), wohingegen die Gehalte an mikrobiellem Biomasse C, Ergosterol und mikrobiellen Residuen in beiden Feldversuchen im Allgemeinen, besonders aber in Kombination mit Bodenbearbeitung gesteigert wurden. Generell wurde das Verhältnis von Ergosterol zu mikrobiellem Biomasse C in beiden Versuchen durch die Bodenbearbeitung erhöht und durch die Gülleausbringung verringert. Das Verhältnis von pilzlichem C zu bakteriellem C wurde durch beide Behandlungen im Allgemeinen verringert, was darauf hindeutet, dass AMF empfindlicher auf Bodenbearbeitung und Düngung reagieren, als andere Bodenmikroorganismen. In den Bodenschichten zwischen 30 und 40 cm Tiefe führte jedoch das Ausbringen von Gülle, besonders aber die Bodenbearbeitung zu einem signifikant höherem Verhältnis von pilzlichem C zu bakteriellem C. Das Verhältnis von pilzlichem C zu bakteriellem C offenbarte eine signifikante positiv-lineare Beziehung zum Verhältnis von Ergosterol zu mikrobiellem Biomasse C, was auf eine erhöhte Bildung pilzlicher Residuen im Zusammenhang mit einem Anstieg der Biomasse saprotropher Pilze hinweist. Im Vergleich mit ungestörten Graslandböden förderte die Bodenbearbeitung saprotrophe Pilze auf Kosten biotropher AMF und Bakterien, was durch ein erhöhtes Verhältnis von Ergosterol zu mikrobiellem Biomasse C und einem konstanten Verhältnis von pilzlichem C zu bakteriellem C angezeigt wird. Dies verdeutlicht, dass die saprotrophe Pilzgesellschaft nicht in den Zustand vor der Bodenbearbeitung zurückkehren konnte. Die Ergebnisse des Versuches zeigen, dass bei einer nicht vermeidbaren Bearbeitung von Graslandböden das Ausbringen von Rindergülle eine stark positive Wirkung auf Bodenmikroorganismen, mikrobielle Residuen sowie die Kohlenstoffbindung im Boden haben kann.

3. Ziel des dritten Versuches war es, die Effekte einer Landnutzungsänderung hin zu Maismonokulturen, welche durch eine stark zunehmenden Biogasproduktion verursacht wird, auf die Dynamiken des organischen Bodenkohlenstoffes (SOC), der mikrobielle Biomasse, der pilzlichen Biomasse sowie auf pilzliche und bakterielle Residuen zu untersuchen. Rindergülle

wurde ausgebracht, um die Effekte der Düngung auf Veränderungen in den mikrobiellen Residuen und der funktionellen Diversität der mikrobiellen Gemeinschaft über verschiedene Landnutzungs-formen hinweg zu quantifizieren. Der Anbau einer Maismonokultur (MM) verringerte im Vergleich zu Dauergrasland (PG) und Modifiziertes grasland (MG) die Gehalte an SOC, mikrobiellem Biomasse C, N und S sowie der mikrobiellen Residuen (Muraminsäure und pilzliches Glucosamin) in 0-40 cm Tiefe. Ein signifikant höheres Verhältnis von Ergosterol zu mikrobiellem Biomasse C bei gleichzeitig signifikant niedrigerem Verhältnis von mikrobiellem Biomasse C zu Biomasse S deutet auf eine verstärkte Akkumulation saprotropher Pilze in der MM-Behandlung im Vergleich zu Grünland hin. Das Verhältnis von mikrobiellem Biomasse C zu Biomasse S könnte daher als ein zusätzlicher Indikator für eine Veränderung innerhalb der mikrobiellen Gemeinschaft dienen. Die PG-Behandlung förderte im Gegensatz dazu Ergosterol freie AMF, was durch ein höheres Verhältnis von pilzlichem C zu bakteriellem C und einem niedrigerem Ergosterol zu mikrobiellem Biomasse C Verhältnis angezeigt wird. Die funktionelle Diversität der mikrobiellen Gemeinschaft nahm in den MM- und MG-Behandlungen im Vergleich zu ungestörtem Dauergraslandboden signifikant ab. Ansteigende Gehalte von mikrobieller Biomasse und Aminozuckern waren die empfindlichsten Anzeiger der Gülleapplikation. Das vorliegende Experiment hat gezeigt, dass der Verlust der funktionellen Diversität der mikrobiellen Gemeinschaft aufgrund von Bodenbearbeitung und einem rasanten Umschwung hin zu Maismonokulturen durch das Ausbringen von Gülle in Regionen mit sandigen Böden in Norddeutschland wiederhergestellt werden kann.

Kurz gesagt: die kombinierte Analyse der mikrobiellen Biomasse und pilzlicher sowie bakterieller Residuenbildung bietet eine einzigartige Möglichkeit, die Effekte der Bodenbearbeitung, der Umwandlung von Grünland in Ackerland und der Düngung auf Dynamiken von Bodenmikroorganismen zu untersuchen. In den oberen Bodenschichten von 0-30 cm konnte durch die Reduzierung der Bodenbearbeitungsintensität in den Behandlungen GRT und NT im Vergleich zu MBT eine erhöhte Akkumulation saprotropher Pilze festgestellt werden. Im Gegensatz dazu förderten die Behandlungen GRT und NT das Auftreten von AMF auf Kosten saprotropher Pilze in den unteren Bodenschichten von 30-40 cm Tiefe. Das Ausbringen von Gülle führte hingegen zu einem generellen Anstieg des mikrobiellen Biomasse C, bakterieller Residuen und deren Beteiligung am organischen Bodenkohlenstoff. Das Pflügen von

Dauergraslandböden führte im Vergleich zu ungestörten Grünlandböden generell zu einem Anstieg saprotropher Pilze auf Kosten biotropher AMF und Bakterien. Die Verhältnisse von pilzlichem C zu bakteriellem C und Ergosterol zu mikrobiellem Biomasse C waren im Gegensatz zu ihrer negativen wechselseitigen Beziehung im ersten und dritten Experiment im zweiten Versuch positiv miteinander korreliert. Zusammenfassend kann gesagt werden, dass die Unterschiede in der Zusammensetzung der mikrobiellen Gemeinschaft nicht nur durch Bodenbearbeitung und Grünlandumwandlung verursacht werden. Die nächste Herausforderung wird sein, den Einfluss edaphischer Faktoren (Bodentextur, Bodenfeuchte, Bodentemperatur) auf Verschiebungen innerhalb der mikrobiellen Gemeinschaftsstruktur zu klären. Die Beziehungen zwischen mikrobieller Biomasse und Nekromasse-Indizes weisen auf die Bedeutung des Zusammenhangs von saprotrophen Pilzen und biotrophen AMF bei Effekten auf den mikrobiellen Umsatz und der Kohlenstoffspeicherung des Ökosystems hin, welche durch die Bewirtschaftungspraktiken verursacht werden. Die spezifischen PLFA-Biomarker sind nicht effektiv genug, um zwischen saprotrophen Pilzen und biotrophen AMF zu unterscheiden (Joergensen and Wichern, 2008; De Vries et al., 2012). Quantitative Informationen exakter Biomasseabschätzungen dieser beiden wichtigen Pilzgruppen sind unabdingbar, um die unterschiedlichen Funktionen beider Gruppen in den Dynamiken der organischen Bodenmaterie zu verstehen.

1 General introduction

Human impact became an important factor when he developed a systematic agriculture. Cultivation of soil led to an explosion in agricultural production, which eventually made large-scale agriculture possible by preparing a more suitable environment for plant growth. Since then, tillage became almost synonymous with agriculture. The severe environmental problems due to intensive agriculture in 20th century led to the regulation of soil organic matter (SOM) content as one of the main goal in agricultural management. Soil organic matter plays a key role for sustainable soil fertility and prevents degradation (e.g. soil erosion). Tillage, grassland conversion and fertilisation are the three major management practices that directly affect the levels of SOM stocks in agro-ecosystems (Six et al., 2006).

1.1 Tillage

Tillage is potentially the most unique and strongest agricultural practice that affects SOM. Agriculture began as no-tillage system but the intensification of agriculture with mouldboard plough tillage (MBT) system has caused a significant loss of SOM and serious soil degradation through increased soil erosion, degradation of soil structure and depletion of soil nutrients stocks (Ogle et al., 2003; Six et al., 2006; Prasuhn et al., 2012). As an early indicator, a change in microbial biomass C should provide early warning of changes in SOM, long before changes in total C and N become measurable (Powlson and Jenkinson, 1981). A reduction in tillage intensity reduces soil erosion, preserves soil microbial properties and can lead to accrual of much of the soil C lost during tillage (Ogle et al., 2003; Jacobs et al., 2009; Heinze et al., 2010a). Moreover, non-inversion tillage offers the potential to sequester organic C (Freibauer et al., 2004; Jacobs et al., 2009) and often increases the stocks of soil microbial biomass (Stockfisch et al., 1999; Heinze et al., 2010a), but not in all cases (Ahl et al., 1998). The two largest functional microbial subgroups in soil (i.e., bacteria and fungi) generally comprise > 90% of the total soil microbial biomass, and they are responsible for the majority of SOM decomposition (Strickland and Rousk, 2010). The impact of management practices on the flow of C and N through ecosystems is largely mediated through the soil microbial community, which is also called as ‘black box of soil’ (Cortois and de Deyn, 2012). Soil microbes also indirectly influence C

cycling by improving soil aggregation, which physically protects SOM (Guggenberger et al., 1999; Bailey et al., 2002).

Non-inversion tillage practices generally increase fungi, presumably because they decrease the disruption of hyphal networks and causes differences in the distribution of crop residues (Frey et al., 1999; Hendrix et al., 1986). This expected outcome is largely based on key differences in the growth forms of fungi and bacteria (Hendrix et al., 1986). An increased substrate use efficiency of fungi lead to the production of more biomass than do bacteria from the same amount of substrate (Holland and Coleman, 1987; Sakamoto and Oba, 1994). These results lead to the general hypothesis that non-inversion tillage practices may promote C storage by inducing a functionally dominant role for fungi in SOM dynamics (Six et al., 2006). In contrast to fungi, bacterial biomass is promoted by intensive tillage (Guggenberger et al., 1999; Frey et al., 1999), fertilisation (Mäder et al. 2000; Walsh et al., 2012) and land use change from grassland to arable land (Potthoff et al., 2006; DuPont et al., 2010; Wakelin et al., 2012). Strickland and Rousk (2010) reviewed the general promotion of fungi under no-till or reduced tillage practices compared to those under MBT practices but no consistent evidences were found (Heinze et al., 2010a; van Groenigen et al., 2010; Jacobs et al., 2011).

1.2 One-season tillage effects

A strong decline in soil C and N, soil structure and change in microbial community structure occurs after repeated tillage (Six et al., 2006; Jacobs et al., 2009; Wakelin et al., 2012). Many studies have documented that the long-term tillage caused significant losses in SOM, microbial biomass and deterioration of soil aggregates (Guggenberger et al., 1999; Frey et al., 1999; Six et al., 2006). The short-term impacts of tillage on soil microbial biomass and activity and consequent loss of soil aggregate fractions and SOM are still ambiguous and the reasons have not been firmly explained (Conant et al., 2007). The loss of SOM due to permanent conversion has been repeatedly observed (DuPont et al., 2010; Wakelin et al., 2012) but less is known about ley-arable rotations (Vertés et al., 2007) and grasslands which are tilled occasionally. The soil microbial biomass and root biomass was greater in soil under native tussock than under cultivated pasture (Wakelin et al., 2012). Stockfisch et al. (1999) and Wortmann et al. (2008)

showed that not only permanent conversion but also one-time tillage of no-till soil had a strong negative impact on soil organic C (SOC) stocks, water stable aggregates and soil microbial biomass. A single cultivation event on permanent grassland soil caused a significant loss of water stable aggregates, root biomass and SOC stocks (Linsler et al., 2013a). Reduction in microbial biomass and AMF fungi caused by one-single tillage event were found in western Nebraska, persisting even after 5 years (Garcia et al., 2007; Wortmann et al., 2008). However, the inconsistency of one-time tillage effects 3 to 5 year after tillage across locations is unexplained, especially since all microbial groups were generally similarly affected within locations (Wortmann et al., 2010). In addition, the effects of one-season tillage on C stocks (Linsler et al., 2013a) and microbial residues in grassland soils are completely unknown.

1.3 Fertilisation

Organic agriculture strives to integrate human, environment and economically sustainable production systems. The term organic refer to the holistic and extensive interaction of the plants, soil, animals, and humans in the system rather than intensive interaction but does not necessarily refer to the types of inputs to the system (Mäder et al., 2000). Several long-term field experiments have demonstrated positive effects of organic fertiliser on the contents of SOM and microbial biomass. The amount and type of organic and mineral fertiliser added to soil is known to directly and indirectly influence the size, activity, and structure of the soil microbial community (Mäder et al., 2000; Heinze et al., 2010b; Joergensen et al., 2010; Murugan et al., 2013). This is especially true for grassland soils where the majority of top soil is rhizosphere soil, a large impact on the size and structure of the soil microbial community is expected to follow fertiliser applications. Fungi appear to be very sensitive to management practices (Zeller et al., 2000) such as mineral (Bardgett et al., 1996) or organic manure addition (Strickland and Rousk 2010; Walsh et al., 2012). In arable soils, manure application reduced the occurrence of saprotrophic fungi (Scheller and Joergensen, 2008; Heinze et al., 2010b), decreased AMF colonization (Mäder et al., 2000; Bradley et al., 2006; Murugan et al., 2013) and especially promoted the formation of bacterial residues, leading to an increased SOC stocks (Joergensen et al., 2010; Sradnick et al., 2013b). The balance between fungal and bacterial contribution to decomposition has been linked to plant productivity, nutrient cycling and C sequestration

potential of an ecosystem (Strickland and Rousk 2010). Consequently, to determine the effects of fertilisers, it is important to determine how they influence the active and growing soil microbial decomposer community. However, only few studies have attempted to explain differences in fungal:bacterial biomass ratios between fertilised and unfertilised grasslands and the mechanisms at play are likely to be very complex (De Vries et al., 2009).

1.4 Land use change

At present, grassland areas cover approximately 40% of the terrestrial land surface. In western and northern Europe and in the former USSR, grassland occupies 50–60% of the agricultural area. Due to intensive agriculture and land use change, temperate grasslands are now considered to be the most altered terrestrial ecosystems (Henwood, 2010). Since the amendment of Renewable Energy Sources Act (EEG) in the year 2004, agricultural biogas production is a fast growing market in many European countries. In Germany, the number of operating biogas plants increased from 274 in 1995 to about 3200 in the middle of 2006 and doubled biogas plants within next 5 years (2011) to become largest biogas producer in the European Union (Hermann, 2012). The maize acreage increased by 22% from the year 2006 (0.4 million ha) and expected to increase to 1.7 million ha by the year 2015 (Gömann et al., 2007). The other crops which are currently used in biogas plants are silage cereals, perennial grasses and grass-clover mixtures. The rush into biogas production strongly based on maize (*Zea mays ssp. mays*) monoculture is a cause of growing concern about potential damage to the terrestrial agro-ecosystems (Hermann, 2012).

Land use change from grassland to crop land led to an average loss of soil C stocks by 59% (Guo and Gifford, 2002) and may accelerate soil erosion (Prasuhn, 2012). A strong decline in SOC stock occurs after repeated tillage and cultivation of arable crops, while grasslands tend to support increased soil C and microbial biomass with greater spatial heterogeneity within the soil profile than in cultivated soils (Potthoff et al., 2006; Culman et al., 2010; Kösters et al., 2013). Grasslands are regarded as C and N sinks, high intensity arable cropping resulted in significant loss of microbial biomass C, water stable aggregates and root biomass down to 80 cm depth in compared to permanent grassland soil (Culman et al., 2010; DuPont et al., 2010). Prolonged cultivation of arable crops and tillage causes shift in the microbial community structure towards

Gram positive bacteria (Tian et al., 2012; Wakelin et al., 2012), while permanent grassland soils recorded two times higher fungi to bacteria biomass ratio compared to arable land (Bailey et al., 2002). The significance of soil microbial community structure and functional diversity in SOM dynamics under grassland ecosystems is still poorly understood (Bailey et al., 2002; Cortois and de Deyn, 2012).

1.5 Research needs

Extensive land use, for instance through the cessation of fertiliser use and adoption of no-tillage farming, may cause an improvement in C sequestration potential through a shift from bacterial to fungal dominated systems, albeit in the long term. In contrast, land use intensification, especially the application of fertiliser N and tillage, generally leads to a shift from fungal to bacterial dominated soil systems, although this shift is sometimes restricted to top soil (De Vries et al., 2009; Postma-Blaauw et al., 2010). The distribution of microbial community with depth has received substantially less attention (Möller et al., 2002; van Groenigen et al., 2010), despite the fact that 50–65% of the organic C contained in the top 1 m of soil is distributed below 30 cm (Rumpel and Kögel-Knabner, 2011). Tillage and grassland conversion may have strong impact on microbial processes and C sequestration in the subsoil (Frier et al., 2003; Wright et al., 2007). However, very few studies investigated the importance of the relationship between microbial biomass, residues and community structure for tillage, fertilisation and land use induced effects on microbial turnover and ecosystem C storage (De Vries et al., 2009; van Groenigen et al., 2010).

The non-living microbial residues represent a significant SOM pool much greater than the living biomass and they are highly related to SOM dynamics (Liang et al., 2011). The size of the microbial necromass C pool could be about 40 times that of the living microbial biomass carbon pool in soils (Simpson et al., 2007; Liang and Basler, 2011). It has been suggested that the microbial residues represented by amino sugars are refractory and could serve as time integrated bio-marker for their contribution to sequestration of C in soils (Liang et al., 2011). Meanwhile, the relationship between living and dead microbial tissues in soil and its ecological significance on SOM are not fully understood (Appuhn et al., 2006; van Groenigen et al., 2010). To approach a more complete understanding of how tillage, grassland conversion and fertilisation affect SOM

dynamics, we therefore need to consider their effect on the concentration, production and stabilization of microbial derived organic matter.

1.6 Research Objectives

Summarising the findings above, only very few studies investigated the effects on different tillage intensity on soil microbial residues and community structure below 30 cm soil depth with high vertical spacial resolution. The relationship between microbial biomass S and ergosterol as specific saprotrophic fungal biomarker might serve as an additional indicator to differentiate saprotrophic fungi from AMF which do not contain ergosterol but the cell wall component fungal glucosamine (Joergensen and Wichern, 2008). Recently, microbial residues have been identified as a significant source of SOM and are stabilised in soil. Therefore, they are very useful to identify the specific contribution of fungal and bacterial residues to the C sequestration potential of soils (Joergensen and Wichern, 2008; Miltner et al., 2011). To provide a unique data set of microbial biomass in combination with microbial residues at different soil depths, the first experiment leading to the objective 1.

Objective 1: Quantification of long-term influence of different tillage intensities on soil microbial biomass, residues and community structure at different depths in four large-scale experimental sites located across eastern and southern Germany.

Tillage of grassland soil and the replacement of perennial plant communities with annual crops are the two fundamental ways that affects the soil ecosystem. Very few studies have examined the rate or extent of re-establishment of fungal and bacterial residues following the restoration of permanent grassland (Lauer et al., 2011), but completely unknown are the effects of one-season tillage event. The application of cattle manure has demonstrated positive effects on SOC stocks in grassland (Vertès et al., 2007) and aggregates (Six et al., 2006) and microbial biomass (Walsh et al., 2012). However, nothing is known about the timescale and mechanisms regulating the possible response of microbial residues to manure application in combination with a one-season cultivation event in a grassland soil. This observed gap in knowledge open the floor for the second experiment leading to the objective 2.

Objective 2: Assessment of one-season tillage and repeated slurry application on specific response of fungal and bacterial residues in a permanent grassland soil following 2 years and 5 years after the tillage event.

The biogas boom induced change in land use from permanent grassland to maize monoculture led negative effects on SOC, microbial biomass stocks has been reported previously (Vertès et al., 2007; Liang et al., 2012). This is highly challenging for farms located on light sandy soils, as in northern Germany, where ploughing intensifies the degradation of SOM (Hermann, 2012). It has been suggested that AMF are more susceptible to disturbance and fertilisation than saprotrophic fungi (Kabir et al., 1997; Bradley et al., 2006). The land use change from perennial grass to arable cropping systems, including different biofuel crops such as maize, perennial grass-mixtures are known to alter soil microbial community structure (Strickland and Rousk, 2010; Liang et al., 2012) and functional diversity (Stevenson et al., 2004; Romaniuk et al., 2011) which are central to maintaining soil ecosystem services. Yet to the best of my knowledge, no studies have made a distinction between three different biofuel cropping effects on fungal and bacterial residual contribution to the C sequestration potential of soils. The third experiment was aimed to answer the unsolved research questions under the previously stated conditions, which leads to the objective 3.

Objective 3: Investigation of changes in microbial biomass, residues and functional diversity after conversion of permanent to modified grassland or maize monoculture

Overall, the aim of the dissertation was to deepen the understanding of the effects of tillage, grassland conversion and fertilisation on the distribution and relationship between microbial biomass and amino sugar derived microbial residues. An additional focus of my experiments was to consider the vertical spatial variability on shift of fungal and bacterial residues and their contribution to SOM accumulation within a specific ecosystem.

2 Long-term influence of different tillage intensities on soil microbial biomass, residues and community structure at different depths

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Abstract

The site-specific contribution of bacterial and especially fungal residues to a tillage-induced C sequestration is largely unknown, although microbial residues contribute a significant percentage to the soil organic C (SOC) pool. In the present study, we tested the following three hypotheses: (1) A reduction in tillage intensity from mouldboard plough (MBT) and grubber (GRT) to no tillage (NT) generally increases stocks of soil organic matter and especially microbial biomass. (2) In the top soil layers, these increases lead by the preferential accumulation of fungal residues. (3) In the bottom soil layers, a reduction in tillage intensity generally promotes AMF at the expense of saprotrophic fungi. In early April 2010, samples were taken on loess-derived soils (mainly Luvisols) from four long-term tillage trials in Germany down to 40 cm. The concentrations of SOC, total N, P, S and amino sugars declined by 50% from the 0-5 cm down to the 20-30 cm layer, whereas those of microbial biomass C, N, and S as well as fungal ergosterol declined by 70% in the GRT and NT treatments, while no depth gradient was observed in the MBT treatment. The GRT and NT treatments increased the stocks of SOC (+7%) and microbial biomass C (+20%) in comparison with the MBT treatment. The differences between the GRT and NT were small, but there were more positive effects for the GRT treatment in most cases. Our results indicate significant tillage effects in loess-derived silt loams suitable for rain-fed sugar beet production, although strong site-specific differences occurred for most of the soil chemical and biological properties analysed. In the GRT and NT treatments, the increased stocks of SOC were not caused by the preferential accumulation of fungal residues at 0-5 cm depth, whereas ergosterol-free biotrophic AMF was promoted at the expense of saprotrophic fungi at 30-40 cm depth. Our results suggest that the relationship between saprotrophic fungi and AMF is an important factor for tillage-induced changes in microbial turnover of SOC.

2.1 Introduction

The structural changes in Central European agriculture and the increase in price of fuel have forced the replacement of cost intensive mouldboard ploughing by simplified tillage systems (Nail et al., 2007; Koch et al., 2009). A reduction in tillage intensity by non-inversion systems promotes earthworms (Ehlers, 1975), which increases the water infiltration capacity of soils and reduces erosion risk (Tebrügge and Düring, 1999; Soane et al., 2012). This is especially important for sugar beet cultivation, which leaves large soil areas uncovered in late spring during a period when heavy rainfall events regularly occur in central Europe (Clemens and Stahr, 1994; Prasuhn, 2012). This problem is intensified by the fact that sugar beet is mainly cropped on silt loams, especially sensitive to water erosion (Koch et al., 2009). Moreover, non-inversion tillage offers the potential to sequester organic C (Freibauer et al., 2004; Jacobs et al., 2009) and often increases the stocks of soil microbial biomass (Stockfisch et al., 1999; Heinze et al., 2010a), but not in all cases (Ahl et al., 1998).

Non-inversion tillage systems generally seem to promote fungi (Hendrix et al., 1986; Frey et al., 1999), especially biotrophic arbuscular mycorrhizal fungi (AMF) (Kabir et al., 1998; Kabir, 2005), in most cases also saprotrophic fungi (Ahl et al., 1998), but not always (Heinze et al., 2010a; Strickland and Rousk, 2010; Jacobs et al., 2011). Fungi are generally able to take up large amounts of S into their biomass (Banerjee and Chapman, 1996) and, thus, a close relationship has been observed between microbial biomass S and the fungal biomarker ergosterol (Heinze et al., 2010b). The most important indicators for saprotrophic fungi are currently the cell-membrane components linoleic acid (18:2ω6,9) (Frostegård et al., 2011) and ergosterol (Joergensen and Wichern, 2008). However, ergosterol is more specific for saprotrophic fungi and simpler to measure than linoleic acid. Neither of these cell-membrane components are suitable to estimate the biomass of AMF (Olsson et al., 2003; Ruess and Chamberlain, 2010).

In contrast, the cell-wall component glucosamine occurs in the chitin of AMF and saprotrophic fungi, as well as in the murein of bacteria (Amelung, 2001; Appuhn and Joergensen, 2006). The bacterial contribution to the glucosamine content of soils can be estimated by the highly-specific muramic acid, which occurs only in bacteria (Engelking et al., 2007). As cell-wall components accumulate in soil organic matter (Amelung, 2001), they are very useful to identify the specific contribution of fungal and bacterial residues to the C

sequestration potential of soils (Joergensen and Wichern, 2008; Miltner et al., 2011). Fungi are said to have a higher C sequestration potential than do bacteria (Guggenberger et al., 1999, Bailey et al., 2002; Jastrow et al., 2007) due to the higher substrate use efficiency, i.e. the lower metabolic quotient of fungi (Sakamoto and Oba, 1994). Consequently, amino sugar analysis could serve as a time-integrated biomarker for the contribution of these two main microbial groups to soil organic matter in tillage experiments (van Groenigen et al., 2010; Martins et al., 2012). This is especially true for soil layers below 30 cm, which have received substantially less attention (Moritz et al., 2009), although tillage may have strong impact on microbial processes and C sequestration in the subsoil (Wright et al., 2007).

Our study is based on the following three hypotheses: (1) A reduction in tillage intensity (mouldboard plough > grubber > no tillage) generally increases stocks of soil organic matter and especially microbial biomass, independently of site-specific differences in environmental conditions. (2) In the top (0-5 cm) soil layers, these increases lead by the preferential accumulation of fungal residues. (3) In the bottom (30-40 cm) soil layers, a reduction in tillage intensity generally promotes AMF at the expense of saprotrophic fungi. Our objectives were to test these hypotheses by measuring fungal biomass (ergosterol) and C, N, and S stored in the soil microbial biomass and in soil organic matter, as well as by estimating the co-accumulation of fungal and bacterial residues in loess-derived soils down to 40 cm of a mature, 15-year-old on-farm tillage experiment (Koch et al., 2009). The experimental arable sites were located in eastern and southern Germany and characterized by large-scale plots. This made it possible to investigate the interactions between tillage treatments and site effects, which have been shown by Heinze et al. (2010a) to override tillage effects.

A specific focus of our experiment was to consider the vertical and horizontal spatial variability for all soil properties analysed. For this reason, samples were taken once with many replicates per site and tillage treatment and at a high resolution down the profiles in early spring. This is a period at field capacity, before strong root growth takes place and when the ambient temperature is close to the average annual temperature (Anderson and Domsch, 1989, 1990). For these reasons, early spring is recommended for taking representative soil samples in soil biological monitoring programmes (Höper and Kleefisch, 2001; Bloem et al., 2006).

2.2 Materials and methods

2.2.1 Experimental site and investigation design

Three tillage systems were investigated at four sites (Friemar, Grombach, Lüttewitz and Zschortau) established in the early 1990s by the agricultural division of Südzucker AG, Mannheim/Ochsenfurt, and the Institute of Sugar Beet Research, Göttingen, Germany, as on-farm long-term field experiments (Koch et al., 2009). The mean annual temperature and precipitation ranges from 7.8 to 9.3°C and 512 to 776 mm, respectively. Site characteristics, soil pH and texture of the sites are given in Table 1 and Koch et al. (2009).

Table 1. Climatic and soil characteristics (3-27 cm) of the four experiment sites

Site	Establishment	Altitude	Temperature	Precipitation	Soil pH	Clay	Silt	Sand	Soil type
		(m)	(°C)	(mm)	(H_2O)	($g\ kg^{-1}$)			(FAO, 2006)
Friemar	1992	310	7.8	517	8.1	290	680	30	Haplic Phaeozem
Grombach	1990	95	9.3	776	7.2	230	760	10	Haplic Luvisol
Lüttewitz	1992	290	8.6	572	7.4	160	810	30	Haplic Luvisol
Zschortau	1997	110	8.8	512	7.6	140	530	320	Gleyic Luvisol

At each site, three similar sized tillage treatment plots were formed on one large field with spatially homogeneous soil properties. The different tillage systems were: annual mouldboard ploughing to a depth of 30 cm (MBT), grubber tillage to a depth of 10-15 cm (GRT) and no-tillage (NT) with direct seed drilling. Before sugar beet sowing, 3-5 cm deep seedbed cultivation was introduced in the NT treatment to improve sugar beet crop establishment. Depending on the site, tillage plot size ranged from 2.5 to 8 ha per treatment. The crop rotation consisted of sugar beet (*Beta vulgaris* L.) / winter wheat (*Triticum aestivum* L.) / winter wheat at all sites for the past 20 years (Koch et al., 2009). White mustard (*Sinapis alba* L.) was sown after harvest of the second wheat as green manure. Crop residues were left on the field and sugar beet was sown in March–April using a single-seed drill adapted to crop residues lying on the soil surface. The crop management was carried out following the regional standards of agricultural practice, including the use of non-selective herbicides in MBT and NT treatments. Based on the infestation level

between treatments, sugar beet selective herbicides, molluscicides and rodenticides were used (Koch et al., 2009). Application of N fertilizer varied between the sites, but was identical for all treatments at one site. The mean annual crop yield and the mean annual N fertilization rate from 1999-2010 are presented in Table 2.

Table 2. Treatment-specific mean grain yields for winter wheat and taproot yields for sugar beet; mean N fertilization rates for all sites and treatments from the year 1999-2010 (in brackets N fertilization rates in 2010, the year of soil sampling).

Site	Treatment	Winter wheat	Sugar beet
Crop yield (t ha^{-1} a^{-1})			
Friemar	Mouldboard plough	7.8	71
	Grubber	8.1	67
	No tillage	7.9	61
Grombach	Mouldboard plough	7.5	62
	Grubber	7.6	69
	No tillage	7.3	59
Lüttewitz	Mouldboard plough	8.5	67
	Grubber	8.5	66
	No tillage	7.9	61
Zschortau	Mouldboard plough	8.1	62
	Grubber	8.0	62
	No tillage	7.9	54
N fertilizer (kg ha^{-1} a^{-1})			
Friemar		198 (212)	96
Grombach		180 (186)	139
Lüttewitz		180 (159)	98
Zschortau		172 (211)	68

2.2.2 Soil sampling and chemical analysis

All soil samples were taken in April 2010 when the sites were grown with winter wheat crop sown in October 2009. At each large-scale plot of the tillage treatment, 3 equal blocks were

randomly specified. Soil samples were taken from 3 points 10 m apart in a triangle within each of these 3 blocks, resulting in 9 samples per treatment per site. Samples were taken at 0-5, 5-10, 10-15, 15-20, 20-30, and 30-40 cm depth, using a steel corer with 4 cm diameter. All samples were passed through a 2 mm sieve and stored at 4°C for a few weeks until the assessment of biological properties (Anderson and Domsch, 1989; 1990). A field moist soil sample was used to analyse pH (1:2.5 soil water ratio). Dried (24 h at 105°C) and finely ground samples were used for chemical analyses (C, N, P and S). Total C and N were determined by gas chromatography using a Vario EL (Elementar, Hanau, Germany) analyser. Concentrations of total P and S were measured after HNO_3 / pressure digestion (Chander et al., 2008) by ICP-AES (Spectro Analytical Instruments, Kleve, Germany).

2.2.3 Microbial activity and biomass indices

The basal respiration of soil was measured by the incubation of 60 g soil sample for seven days at 22°C with 40% water holding capacity. The CO_2 evolved was trapped in 0.5 M NaOH and the excess NaOH was back-titrated using 0.5 M HCl after the addition of saturated BaCl2 solution. Fumigated (24 h with ethanol-free $CHCl_3$ at 25°C) and non-fumigated 5-g samples were extracted with 20 ml of 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany) to measure microbial biomass C and N (Brookes et al., 1985; Vance et al., 1987). 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 EC / k_{EC}, where EC = (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 EN / k_{EN}, where EN = (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). Fumigated and non-fumigated 5-g samples were extracted with 25 ml of 1 M NH_4NO_3 to measure microbial biomass S (Khan et al., 2009). Microbial biomass S was calculated as ES / k_{ES}, where ES = (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 from 2 g of moist soil with 100 ml ethanol (Djajakirana et al., 1996). Then, ergosterol was determined by reverse phase HPLC with 100% methanol as the mobile phase and detected at a wavelength of 282 nm. The amino sugars (muramic acid, mannosamine, glucosamine and galactosamine) were determined according to Appuhn et al. (2004), as described by Indorf et al. (2011). Moist samples of 0.5 g soil were weighed into 20 ml test tubes, mixed with 10 ml 6 M HCl and hydrolysed for 6 h at 105°C. The HCl was removal from the filtered hydrolysates in a vacuum rotary evaporator at 40°C and centrifuged. The samples were transferred to vials and stored at -18 °C until the HPLC measurement. Chromatographic separations were performed on a Phenomenex (Aschaffenburg, Germany) Hyperclone C_{18} column (125 mm length × 4 mm diameter), protected by a Phenomenex C_{18} security guard cartridge (4 mm length × 2 mm diameter) at 35°C. The HPLC system consisted of a Dionex (Germering, Germany) P 580 gradient pump, a Dionex Ultimate WPS 3000TSL analytical auto sampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector set at 445 nm emission and 330 nm excitation wavelengths. For the automated pre column derivatisation, 50 ml OPA and 30 ml sample were mixed in the preparation vial and after 120 s reaction time15 ml of the indole derivatives were injected. The mobile phase consisted of two eluents and was delivered at a flow rate of 1.5 ml min^{-1}. Eluent A was a 97.8/0.7/1.5 (v/v/v) mixture of an aqueous phase, methanol and tetrahydrofuran (THF). The aqueous phase contained 52 mmol sodium citrate and 4 mmol sodium acetate, adjusted to pH 5.3 with HCl. Then methanol and THF were added. Eluent B consisted of 50% water and 50% methanol (v/v).

2.2.4 Calculation and statistical analysis

The stocks of the soil nutrients and microbial indices at different depths were calculated on a volume basis by taking the bulk density of the respective soil layer into account. Bulk density was calculated from core dry weight divided by volume. Fungal glucosamine was calculated by subtracting bacterial glucosamine from total glucosamine as an index for fungal residues, assuming that muramic acid and glucosamine occur at a 1 to 2 ratio in bacteria (Engelking et al., 2007): fungal C (μg g^{-1} dry weight) = (mmol glucosamine – 2 × mmol muramic acid) × 179.2 $gmol^{-1}$ × 9, where 179.2 is the molecular weight of glucosamine and 9 is the conversion value of

fungal glucosamine to fungal C (Appuhn and Joergensen, 2006). Bacterial C (µg g^{-1} dry weight) was calculated as an index for bacterial residues by multiplying the concentration of muramic acid in µg g^{-1} dry weight by 45 (Appuhn and Joergensen, 2006). The values of three sampling points from three blocks per site were used to calculate mean concentrations and stocks for every site (n = 9).The results presented in the tables are arithmetic means of the stocks and are given on an oven dry basis (105°C, 24 h). The mean concentrations per site of each parameter served as field replicates. The concentrations and ratios presented in Figs. 2a, b and 3a-f are the arithmetic means of the tillage treatment in the respective depth from four sites as field replications (n=4). The mean value of the four field-replicates was used for a statistical evaluation of the tillage treatment effect (n=4). Data were checked for normal distribution by Chi-square test. When necessary, the data were ln-transformed. The significance of tillage treatment and site effects on stock parameters was analysed by a MANOVA, with depth as repeated measures and blocks as replicates. All statistical analyses were carried out using JMP 7.0 (SAS Inst. Inc.).

2.3 Results

2.3.1 Tillage effects at different depths

The concentrations of all elements for each block were not significant (Table 3). At 0-30 cm depth, mouldboard tillage (MBT) led to a homogeneous bulk density (Fig. 1) and a homogeneous distribution of soil organic C (SOC, Fig. 2a), total N, total P, and total S (Supplementary Table 1a,b), microbial biomass C (Fig. 2b), N, and S (Supplementary Table 2a,b) as well as microbial amino sugars (Supplementary Table 3a,b). In contrast, long-term grubber tillage (GRT) and no tillage (NT) led to a significant 20% increase in bulk density from the 0-5 cm layer down to the 20-30 cm layer (Fig. 1). The mean soil bulk density did not differ between the tillage treatments, but it was significantly higher in the MBT treatment at 0-5 cm depth and significantly lower at 20-30 cm depth in comparison with the GRT and NT treatments. The depth-specific differences between the GRT and NT treatments were in most cases insignificant for bulk density, for soil chemical properties (Fig. 2a) as well as for microbial biomass indices (Fig. 2b) and microbial residues (Supplementary Tables 1 to 3). At 30-40 cm, the concentrations of soil chemical and biological parameters were generally lower than at 20-30 cm depth (Fig. 2, Supplementary

Tables 1 to 3) and did not reveal clear tillage effects, although the bulk density of the GRT and NT treatments significantly exceeded that of the MBT treatment (Fig. 1).

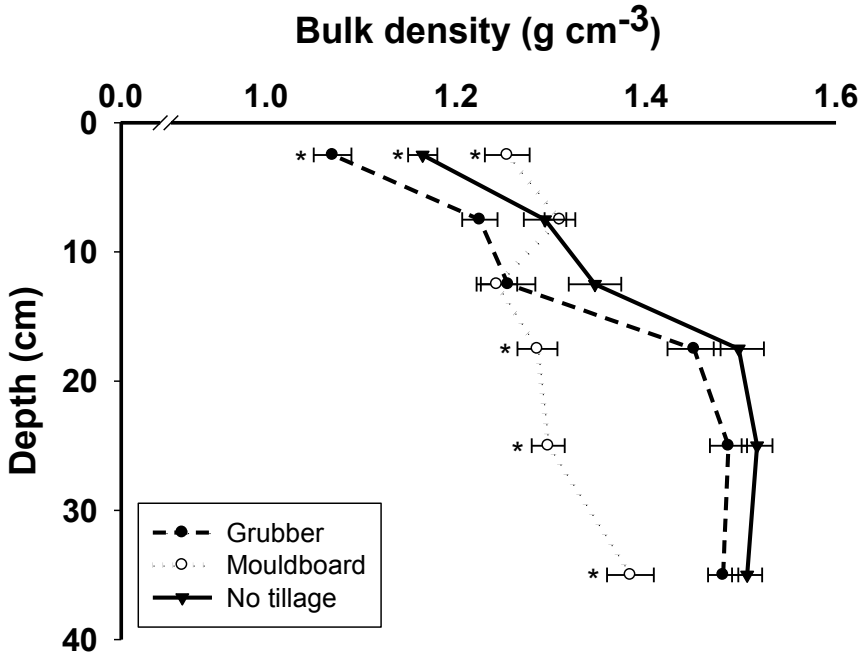

Fig. 1. Effect of soil tillage on mean bulk density at different soil depths (0-40 cm), means of four sites; * indicates a depth-specific significant difference between the three tillage treatments ($P < 0.05$, ANOVA repeated measurement); bars represent standard errors of the mean ($n = 4$).

The concentrations of SOC (Fig. 2a), but also those of total N, total P, and total S (Supplementary Table 1a,b) as well as those of microbial amino sugars (Supplementary Table 3a,b) declined by 50% from the 0-5 cm down to the 20-30 cm layer in the GRT and NT treatments, whereas no depth gradient was observed in the MBT treatment. The SOC concentration was 40% higher in the GRT and NT treatments in the 0-5 cm layer and 10% lower at 20-30 cm depth in comparison with the MBT treatment (Fig. 2a). Microbial biomass C was 150% higher in the GRT and NT treatments in the 0-5 cm layer and 30% lower at 20-30 cm depth in comparison with the MBT treatment (Fig. 2b). Microbial biomass C, N and S (Supplementary Table 2a, b) declined roughly by 70% from the 0-5 cm down to the 20-30 cm layer in the GRT and NT treatments, whereas again no depth gradient was observed in the MBT treatment.

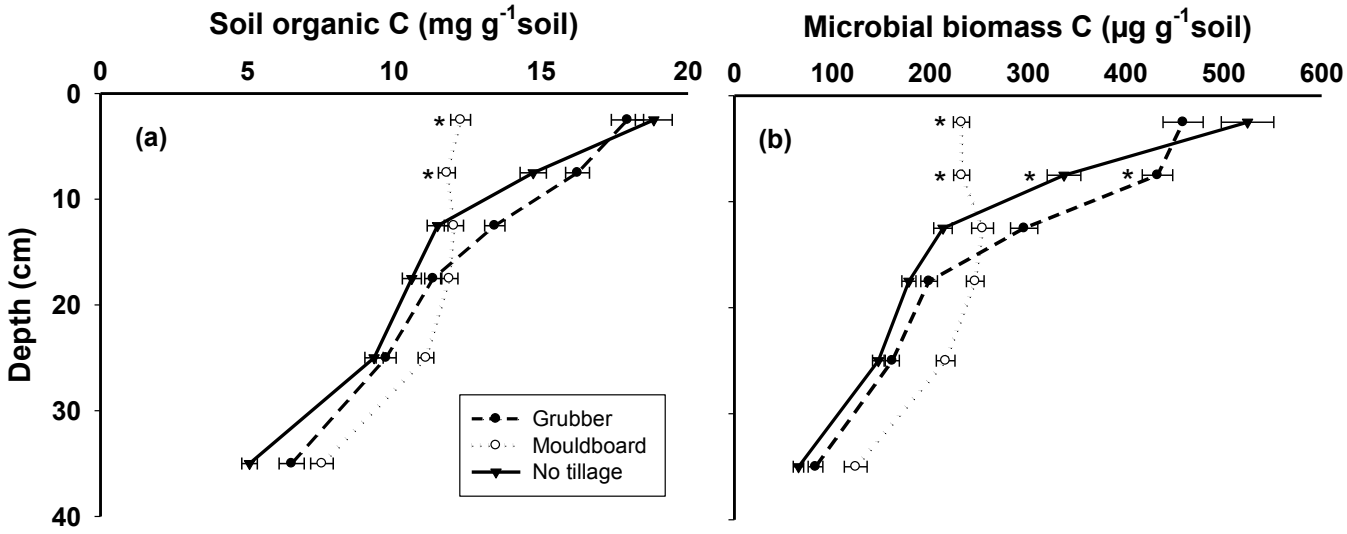

Fig. 2. Mean contents over four sites (n = 54 per site per depth) of (a) soil organic C and (b) soil microbial biomass C in soils from three tillage treatments at different depths; * indicates a depth-specific significant difference between the three tillage treatments ($P < 0.05$, ANOVA repeated measurement); bars represent standard errors of the mean (n = 4).

At 0-30 cm depth, the metabolic quotient (qCO_2) was the only ratio (Fig. 3d) that significantly increased by 50% (Fig. 3d). Long-term GRT and NT treatments did not affect the soil C/N ratio (overall mean 10.2, Fig. 3a), the amino-sugar based microbial C to SOC ratio (overall mean 0.75, Fig. 3b) and the fungal C to bacterial C ratio (overall mean 2.2). In contrast, the microbial biomass C to SOC ratio and the ergosterol to microbial biomass C ratio decreased by roughly 40% (Fig. 3c) and 60% (Fig. 3f), respectively, from the 0-5 cm down to the 20-30 cm layer in the GRT and NT treatments. The microbial biomass C to SOC ratio was 40% higher in the GRT and NT treatments in the 0-5 cm layer and 15% lower at 20-30 cm depth in comparison with the MBT treatment (Fig. 3c). Similarly, the ergosterol to microbial biomass C ratio was 40% higher in the GRT and NT treatments in the 0-5 cm layer and even 40% lower at 20-30 cm depth in comparison with the MBT treatment (Fig. 3f). At 30-40 cm, the soil C/N ratio (Fig. 3a), the qCO_2 (Fig. 3d) and the fungal C to bacterial C ratio (Fig. 3e) increased, whereas the ergosterol to microbial biomass C (Fig. 3f) and the microbial biomass C to SOC ratio (Fig. 3c) decreased in the GRT and NT treatment compared to the MBT treatment.

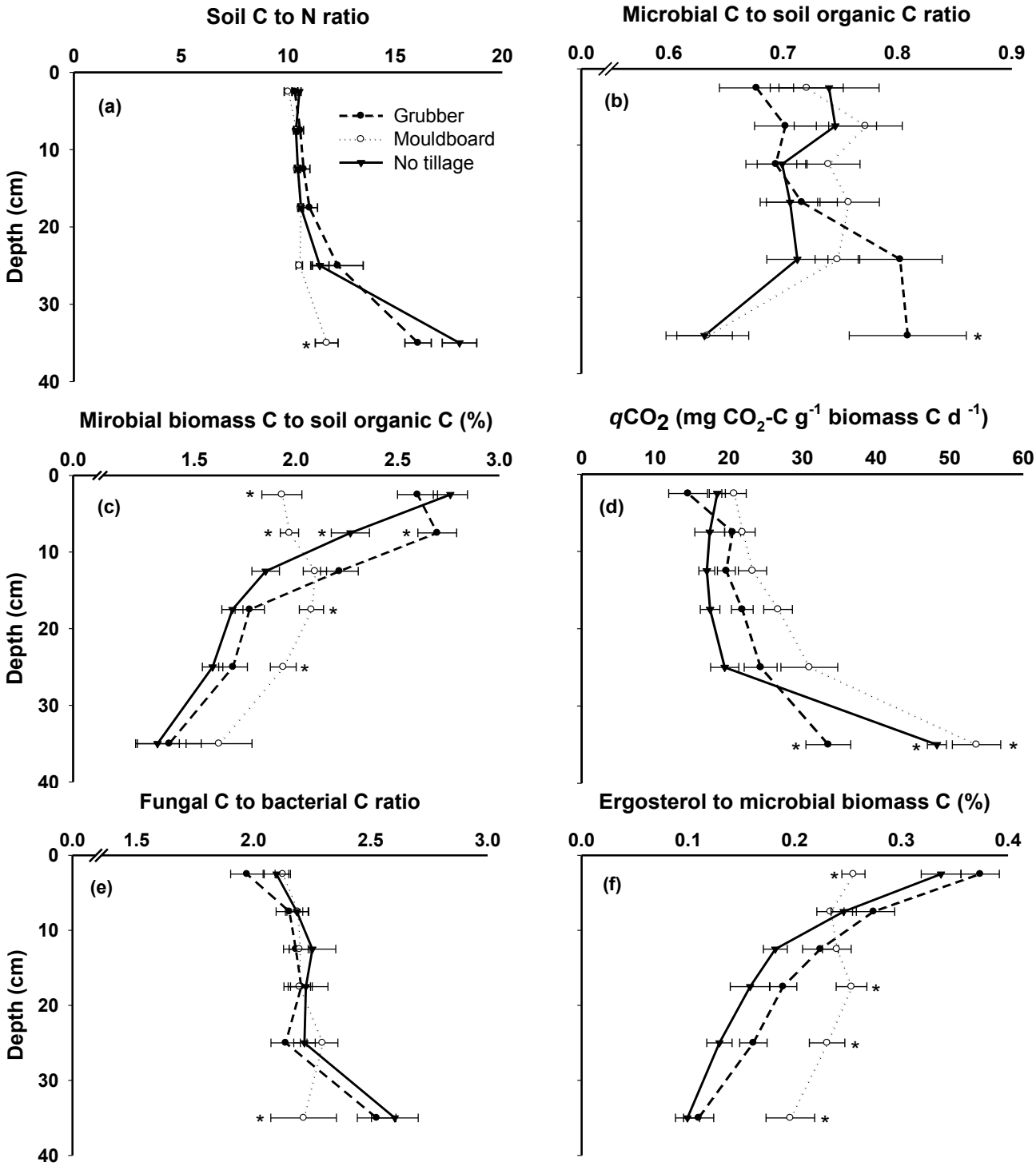

Fig. 3. Mean ratios over four sites (n = 54 per site per depth) of various microbial indices in soils with three tillage treatments at different depths; * indicates a depth-specific significant difference between the three tillage treatments ($P < 0.05$, ANOVA repeated measurement); bars represent standard errors of the mean (n = 4).

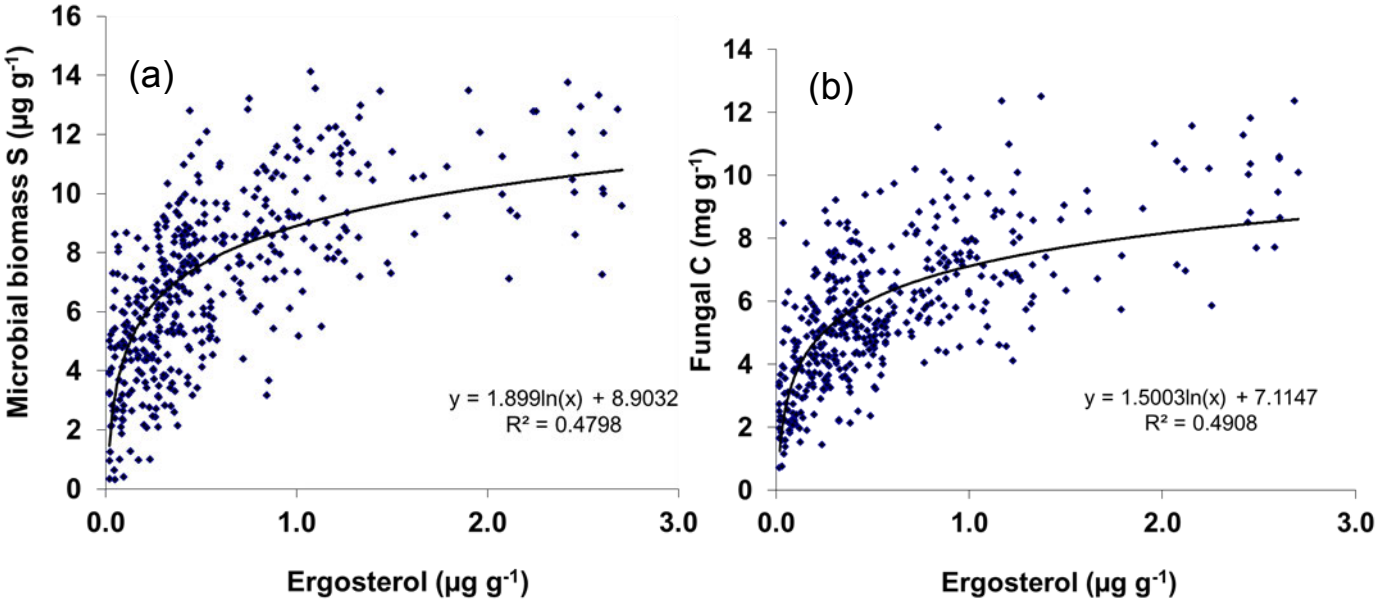

Fig. 4. The relationships between (a) soil microbial biomass S and ergosterol and (b) ergosterol and fungal C; data from four sites, three tillage treatments and all field replicates were combined (n = 648).

The fungal biomass indicator ergosterol showed significant non-linear relationships with the microbial biomass S (Fig. 4a) and amino sugar-based fungal C (Fig. 4b). A significant positive linear relationship was observed for the ergosterol to microbial biomass C ratio and the SOC concentration (Fig. 5a), whereas a significant negative relationship was found for the fungal C to bacterial C and the SOC concentration (Fig. 5b).

2.3.2 Tillage and site effects on stocks

At 0-30 cm depth, the stocks of SOC, total nutrient elements (N, P, and S; Table 3), soil microbial and fungal biomass indices (Table 4) as well as amino sugars and related indices (Table 5) were significantly higher in the GRT and NT treatments in comparison with the MBT treatment. Mean positive GRT and NT effects on soil chemical properties declined in the order total N = total S (14%) > SOC (7%) > total P (5%) (Table 3). Positive GRT and NT effects on soil microbial biomass indices were similar for biomass C, N, as well as ergosterol (+20%) and absent for biomass S (Table 4). Positive GRT and NT effects on amino sugars were similar for muramic acid, galactosamine, and glucosamine (+8%), and absent for mannosamine (Table 5).

The differences between NT and MBT were generally smaller than those between GRT and MBT and not always significant, i.e. for total P, total S (Table 3) and microbial biomass S (Table 4). The stock of mannosamine was significantly lowest in the NT treatment (Table 5).

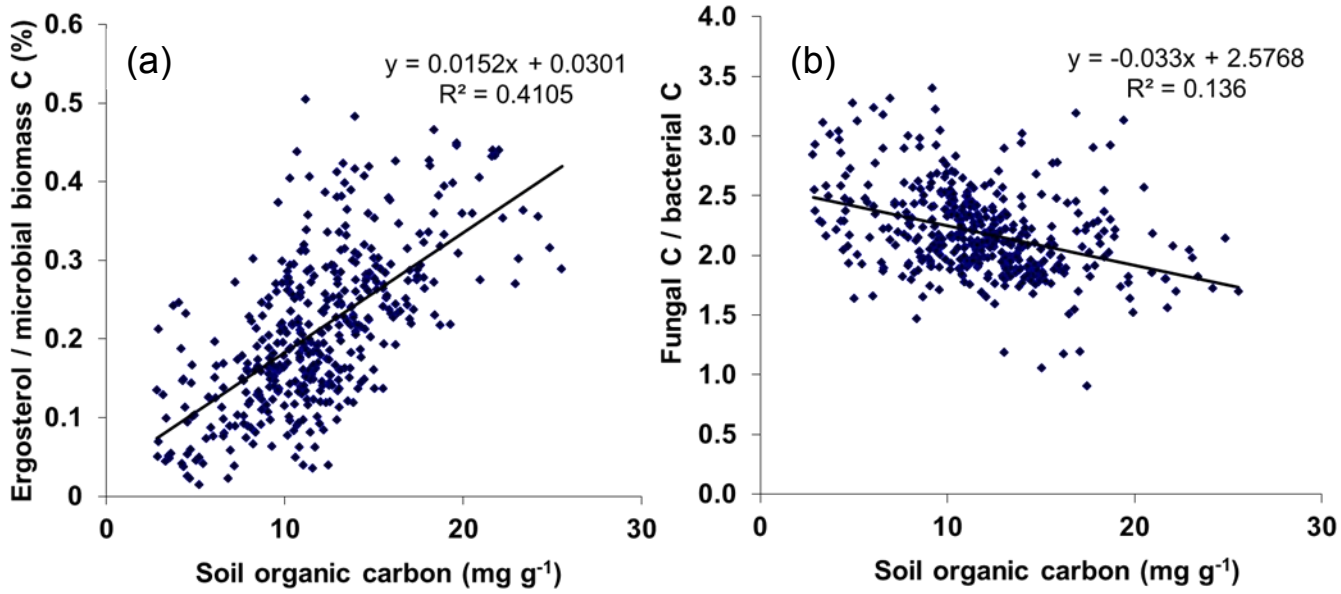

Fig. 5. The relationships between (a) soil organic C and ergosterol to microbial biomass C (%) and (b) soil organic C and fungal C to bacterial C ratio; data from four sites, three tillage treatments and all field replicates were combined (n = 648).

At 30-40 cm, the stocks of SOC, total N, and total P (Table 3) as well as the stocks of amino sugars and related indices (Table 5) were higher in the MBT and GRT treatments in comparison with the NT treatment. The stock of total S was highest in the GRT treatment (Table 3). The stock of soil microbial biomass C was significantly higher in the MBT treatment followed by the GRT treatment, while the lowest microbial biomass C stock was observed in the NT treatment (Table 4). The MBT treatment led to a significantly higher microbial biomass N and ergosterol stocks than the GRT and NT treatments. The stock of microbial biomass S was not significantly affected by the tillage treatments.

Table 3. Mean stocks of soil organic C, total N, total P and total S in soils from three tillage treatments and four investigation sites; probability values of a MANOVA with depth as repeated measurements.

Treatment	SOC	Total N	Total P	Total S
	(t ha^{-1})			
Tillage (0-30 cm)				
Mouldboard plough	55 b	5.9 b	2.8 b	1.1 b
Grubber	60 a	6.9 a	3.0 a	1.3 a
No tillage	58 a	6.5 a	2.9 ab	1.2 ab
Tillage (30-40 cm)				
Mouldboard plough	11 a	1.2 a	0.66 a	0.24 b
Grubber	10 a	1.4 a	0.68 a	0.29 a
No tillage	7 b	1.1 b	0.59 b	0.23 b
Site (0-40 cm)				
Friemar	68 a	7.6 a	3.0 a	1.3 a
Grombach	58 a	7.1 a	4.1 a	1.4 a
Lüttewitz	53 b	5.5 b	2.5 ab	0.9 b
Zschortau	52 b	5.6 b	2.0 b	1.0 b
Probability values (0-40 cm)				
Tillage	<0.01	<0.01	<0.01	<0.01
Site	<0.01	<0.01	<0.01	<0.01
Replicate	NS	NS	NS	NS
Site × tillage	0.03	<0.01	<0.01	<0.01
CV (±%)	12	12	10	13

CV = mean coefficient of variation between replicate samples within one block (n = 3); NS = not significant; different letters indicate a significant difference among the three tillage treatments in each depth interval and between four sites (Tukey's HSD test, $P < 0.05$)

Table 4. The effects of different tillage treatments on mean stocks of microbial biomass C, N and S, and ergosterol at varying soil depths and investigation sites. Mean stocks of microbial biomass C, N and S, and ergosterol in soils from three tillage treatments and four investigation sites.

Treatment	Microbial biomass C	N	S	Ergosterol
		(kg ha^{-1})		(kg ha^{-1})
Tillage (0-30 cm)				
Mouldboard plough	890 b	124 c	29 a	2.1 c
Grubber	1080 a	177 a	29 a	2.8 a
No tillage	1020 a	134 b	27 a	2.3 b
Tillage (30-40 cm)				
Mouldboard plough	172 a	23 a	5.8 a	0.29 a
Grubber	123 b	15 b	5.8 a	0.11 b
No tillage	99 c	13 b	5.4 a	0.10 b
Site (0-40 cm)				
Friemar	1263 a	196 a	39 a	3.5 a
Grombach	1106 b	131 c	37 a	3.0 ab
Lüttewitz	1009 c	162 b	34 ab	2.2 b
Zschortau	1132 b	158 b	27 b	2.6 ab
Probability values (0-40 cm)				
Tillage	<0.01	<0.01	NS	<0.01
Site	<0.01	NS	<0.01	<0.01
Replicate	NS	NS	NS	NS
Site × tillage	<0.01	NS	0.02	NS
CV (±%)	19	28	22	22

CV = mean coefficient of variation between replicate samples within one block (n = 3); NS = not significant; different letters indicate a significant difference among the three tillage treatments in each depth interval and between four sites (Tukey's HSD test, $P < 0.05$)

Table 5. The effect of different tillage treatments on the distribution of mean soil amino sugar stocks at varying soil depths and investigation sites.

Treatment	MurN	ManN	GalN	GlcN	Fungal C	Microbial C
	(kg ha^{-1})				(t ha^{-1})	
Tillage (0-30 cm)						
Mouldboard plough	240 b	94 a	1720 b	3020 b	23 b	34 b
Grubber	260 a	93 a	1920 a	3240 a	24 ab	36 a
No tillage	260 a	90 b	1950 a	3260 a	25 a	36 a
Tillage (30-40 cm)						
Mouldboard plough	49 a	25 a	400 a	590 a	4.4 a	6.6 a
Grubber	47 a	30 a	390 a	640 ab	4.9 a	7.0 a
No tillage	32 b	19 b	270 b	460 b	3.6 b	5.0 b
Site (0-40 cm)						
Friemar	400 a	190 a	2970 a	4710 a	35 a	53 a
Grombach	290 ab	120 ab	2260 b	3630 b	27 b	41 b
Lüttewitz	220 b	87 b	1840 c	3180 c	25 b	35 c
Zschortau	270 ab	77 b	1810 c	3410 b	26 b	38 c
Probability values (0-40 cm)						
Tillage	0.01	NS	<0.01	0.02	0.03	0.02
Site	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Replicate	NS	NS	NS	NS	NS	NS
Site × tillage	<0.01	NS	<0.01	<0.01	<0.01	<0.01
CV (±%)	20	24	20	18	18	18

CV = mean coefficient of variation between replicate samples within one block (n = 3); NS = not significant; different letters indicate a significant difference among the three tillage treatments in each depth interval and between four sites (Tukey's HSD test, $P < 0.05$)

At the site Friemar, the highest stocks were observed for SOC and total N (Table 3), microbial biomass C, N, S, and ergosterol (Table 4) as well as for amino sugars and related residue indices (Table 5). At the site Grombach, the highest stocks were found for total P and total S (Table 3). At the sites Lüttewitz and Zschortau, the lowest stocks were observed for SOC, total N, and total S (Table 3), ergosterol, microbial biomass N, and S (Table 4) as well as for muramic acid, mannosamine, and galactosamine (Table 5). The lowest stocks of microbial biomass C (Table 4) and glucosamine (Table 5) occurred at the site Lüttewitz, whereas the lowest total P stock was obtained at the site Zschortau (Table 3).

2.4 Discussion

After 15 years, a reduction in tillage intensity by the GRT and NT treatments increased stocks of soil organic matter and especially microbial biomass at 0-40 cm depth in comparison with the MBT treatment. Our results indicate significant tillage effects in loess-derived silt loams suitable for rain-fed sugar beet production and verify our first hypothesis. Similar effects of a reduction in tillage intensity on soil organic matter and microbial biomass stocks have been observed by Stockfisch et al. (1999) and by Heinze et al. (2010a), after 20 and 30 experimental years, respectively with the rotary cultivator and mouldboard plough treatments. Effects of a reduction in tillage depth on SOC stocks at 0-30 or 0-40 cm depth (Ahl et al., 1998; Stockfisch et al., 1999) might be due to differences in aboveground crop yield (Koch et al., 2009), but especially in belowground root development (Qin et al., 2006). Differences in C input are probably the main reason for the small but more positive effects of the GRT treatment on SOC and microbial biomass C stocks in comparison with the NT treatment, which has the lowest crop yields, especially for sugar beet (Table 2). The lowest sugar beet yields in the NT treatment were caused by low population density (Koch et al., 2009), presumably in combination with an unequal distribution of wheat straw by the combine harvester, which intensifies unfavourable conditions by direct seed drilling for embedding of seeds, field emergence and plant establishment (Koch et al., 2009).

Seriously compacted layers were not observed in any of the current tillage treatments, not even below 30 cm depth in the MBT treatment, which has been repeatedly observed in silt loams (Tessier et al., 1997; Ahl et al., 1998; Heinze et al., 2010a). Consequently, root penetration

resistance did not differ between the tillage treatments at the same experimental sites (Koch et al., 2009). Another reason for the increased SOC stocks in the current GRT and NT treatments might be an increased formation of macro-aggregates at 0-25 cm depth in comparison with the MBT treatment (Andruschkewitsch et al., 2013), which physically protect SOC against microbial decomposition and mechanical breakdown (Jacobs et al., 2009). However, this occlusion in macro-aggregates does not explain the increased stocks of soil microbial biomass in the GRT and NT treatments, due to the lower yields, especially in the NT treatment, and consequently the lower inputs by harvest and root residues compared to the MBT treatment (Table 2; Koch et al., 2009). Most likely reasons for the tillage-induced increase in stocks of microbial biomass are interactions between the turnover, substrate availability, water content, temperature, and bulk density (Taylor et al., 2004; Poll et al., 2008; Jacobs et al., 2011). The higher SOC concentration in the GRT and NT treatments certainly improves the conditions for microbial turnover in dry periods of the year. However, a higher water content in combination with higher bulk density and reduced pore space might reduce the microbial turnover, due to lower soil temperatures and a reduced O_2 transfer into deeper parts of the A horizon.

A tillage-induced reduction in microbial turnover in the GRT and NT treatments was indicated by the inverse relationship between the metabolic quotient qCO_2 and the microbial biomass C to SOC ratio, repeatedly observed in tillage experiments (Meyer et al., 1996; Hungria et al., 2009; Heinze et al., 2010a). The metabolic quotient qCO_2 describes the amount of substrate used to build up microbial biomass (Anderson and Domsch, 1990) and the microbial biomass C to SOC ratio is an important index for the SOC availability to soil microorganisms (Anderson and Domsch, 1989). For this reason, the decreased input of fresh plant material and an increased recalcitrance of SOC usually led to an inverse relationship between qCO_2 values and microbial biomass C to SOC ratios (Lavahun et al., 1996; Meyer et al., 1996; Goberna et al., 2006).

The differences in microbial biomass stocks may also be caused by the different development in the microbial community structure, which is reflected by the ergosterol to microbial biomass C ratio. The cell-membrane component ergosterol is an important indicator for saprotrophic fungi (Joergensen and Wichern, 2008), but does not occur in AMF (Olsson et al., 2003). A positive correlation between ergosterol and fungal C (Fig. 4b) has been observed in

silt loams that forms a gradient in soil organic matter (Appuhn et al., 2006). A positive correlation between ergosterol and microbial biomass S (Fig. 4a) has been found in a long-term fertilization experiment on a highly sandy soil (Heinze et al., 2010b), but not in two tillage experiments on silt loams (Heinze et al., 2010a). However, all three indices for the presence of fungi differed in their response to the tillage treatment, although they were generally interrelated.

The ergosterol to microbial biomass C ratio showed a significant positive linear relationship with SOC (Fig. 5a), indicating an increased supply of fresh root and harvest residues, which promotes saprotrophic fungi. Especially the regular incorporation of straw is known to have strong positive effects on the ergosterol concentration in soil (Scheller and Joergensen, 2008; Heinze et al., 2010b; Rousk et al., 2010). The higher contribution of fungi to the microbial community at higher SOC concentrations is in line with the view repeatedly stated by others (Bailey et al., 2002; Jastrow et al., 2007). The significantly higher ergosterol to microbial biomass C ratio and the SOC concentration means that more saprotrophic fungi occurred under the GRT and NT treatments at 0-10 cm depth in comparison with the MBT treatment. This also means that the presence of more saprotrophic fungi is combined with a lower turnover of the microbial biomass based on the inverse relationship between the microbial biomass C to SOC ratio and the qCO_2. This relationship has been observed by Sakamoto and Oba (1994) for soil fungi using direct microscopy. However, their measurements of fungal biomass included biotrophic AMF, which have a low saprotrophic capability, which leads to low basal respiration rates and consequently low qCO_2 values. This view is supported by the negative relationship between the ergosterol to microbial biomass C ratio and the fungal C to bacterial C ratio, which suggest that the residues of saprotrophic fungi have a stronger turnover than those of AMF.

In contrast to the ergosterol to microbial biomass C ratio, the ratio of fungal C to bacterial C showed a significant negative linear relationship with SOC (Fig. 5b), despite the general positive interrelationships between the three fungal indices. This is mainly due to the strong increase in the fungal C to bacterial C ratio at 30-40 cm depth in the GRT and NT treatments and the relatively low values of this ratio at 0-5 cm depth in all treatments, which contrasts our second hypothesis. At 30-40 cm depth, the increase in the fungal C to bacterial C ratio is combined with a strong increase in the soil C/N ratio, which indicates accumulation of less degraded recalcitrant SOC in comparison with the soil at 0-30 cm depth (John et al., 2005; Yamashita et al., 2006;

Lauber et al., 2008). The high metabolic quotient qCO_2 and the lower microbial C to SOC ratio are additional indications for the presence of recalcitrant organic matter at 30-40 cm depth in the GRT and NT treatments (Meyer et al., 1996). At 30-40 cm depth, the higher fungal C to bacterial C ratio and lower ergosterol to microbial biomass ratio in the GRT and NT treatments is likely caused by a shift within the fungal community structure towards AMF, which confirms our third hypothesis. In contrast, the MBT treatment with straw incorporation led to increased saprotrophic fungi at 30-40 cm depth. All soil microbial indices clearly demonstrate the absence of a plough pan in the GRT and NT treatment, whereas no significant differences in the bulk density were observed between 20-30 and 30-40 cm depth, both being considerably higher than that in the MBT treatment.

It is more difficult to explain the low fungal C to bacterial C ratio at 0-5 cm soil depth in the presence of the highest ergosterol to microbial biomass C ratios. The reverse could be easily explained by the difference in the fungal community structure, as AMF do not contain ergosterol but glucosamine (Olsson et al., 2003; Joergensen and Wichern, 2008). One explanation might be that, in the strong presence of saprotrophic fungi, a disproportionate increase in the turnover of fungal biomass may have led to a lower accumulation of fungal residues in comparison with bacterial residues. Differences in the turnover between fungal and bacterial cell-wall residues have been repeatedly claimed in the literature (Guggenberger et al., 1999; Amelung, 2001; Six et al., 2006; Strickland and Rousk, 2010), although not really proven on the basis of bacterial and fungal cells added to soil (Jenkinson, 1976). However, cultured bacteria and fungi might behave differently in soil than autochthonous soil microorganisms. One way to obtain more accurate information is to use amino-sugar specific $\delta^{13}C$ analysis (Amelung et al., 2008; Bodé et al., 2009) in the range of natural abundance (Indorf et al., 2012). The fractionation of $\delta^{13}C$ sometimes observed between different fungal tissue, organic substrates and soil organic matter (Hobbie et al., 2004; Zeller et al., 2007) might give information on differences in the turnover of fungal and bacterial cell wall residues.

Nearly all elemental and physiological ratios obtained in the present experiment are in the range obtained in the respective literature, such as the microbial biomass C/N ratio (Joergensen and Emmerling, 2006), the microbial biomass C/S ratio (Heinze et al., 2010a/b), the ergosterol to microbial biomass C ratio (Djajakirana et al., 1996; Joergensen and Wichern, 2008); the

microbial biomass C to SOC ratio (Meyer et al., 1996; Müller and Höper, 2004; Heinze et al., 2010a/b), the metabolic quotient qCO_2 (Müller and Höper, 2004) as well as the fungal C to bacterial C ratio (Joergensen and Wichern, 2008). An exception is to some extent the microbial C to SOC ratio, which reached on average 75% and was considerably higher than the mean value of 55% obtained by Appuhn et al. (2006). Solomon et al. (2001) obtained a mean of 32% in Chromic Luvisols under different land-use systems and van Groenigen et al. (2010) only 22% in Irish arable Haplic Luvisols, comparing MBT and GRT treatments. As the soil C/N ratio of most soils was very similar, methodological constraints of amino sugar hydrolysis or determination method cannot be fully excluded. Also the conversion values proposed by Appuhn and Joergensen (2006) and Engelking et al. (2007) might be questionable for different soils. However, the question of the conditions under which microbial residues or, in reverse, plant residues are sequestered as soil organic matter apparently remains unsolved.

The site effects on soil chemical and biological properties often exceeded those of the tillage treatments, with numerous significant interactions, which suggest that the tillage effects varied often in a site-specific way. The strong site effects of the current research are in agreement with Heinze et al. (2010a). However, in contrast to this experiment, the maximum site effects at the site Friemar can be explained by a combination of the following four site factors: (1) highest clay content, (2) lowest mean temperature due to the highest altitude, (3) lowest annual mean precipitation, (4) and the highest soil pH in comparison to the other three sites. The highest sand content in combination with a high groundwater table at the site Zschortau apparently had only minor effects on the soil chemical and biological properties of the silt dominated sites. This is especially true in comparison with the site Lüttewitz, which had soil properties very similar to those of the site Zschortau. Consequently, it is not possible to determine the dominant factor on the basis of the current data pool. However, the absence of small-scale block effects for most of the properties analysed suggest that climatic effects might be more important, especially in combination with differences in management by the different farmers. A considerably larger number of sites would be necessary to assess the relative importance of one specific factor.

2.5 Conclusions

A reduction in tillage intensity by the GRT and NT treatments increased stocks of soil organic matter and especially microbial biomass in comparison with the MBT treatment. Our results indicate significant tillage effects in loess-derived silt loams suitable for rain-fed sugar beet production, although strong site-specific differences occurred for most of the soil chemical and biological properties analysed. A higher C input is the main reason for the small but more positive effects of the GRT treatment on soil organic matter and microbial biomass stocks in comparison with the NT treatment. In the top soil layer at 0-5 cm depth, the increased stocks of SOC are not caused by the preferential accumulation of fungal residues, although the ergosterol to microbial biomass C ratio, an index for the presence of saprotrophic fungi, was significantly increased in the GRT and NT treatments in comparison with the MBT treatment. In contrast, the GRT and NT treatments promoted AMF at the expense of saprotrophic fungi in the bottom soil layer at 30-40 cm depth. The negative relationship between the ergosterol to microbial biomass C ratio and the fungal C to bacterial C ratio points to the importance of the relationship between saprotrophic fungi and biotrophic AMF for tillage-induced changes in microbial turnover of SOC.

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3 Specific response of fungal and bacterial residues to one-season tillage and repeated slurry application in a permanent grassland soil

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Abstract

The study was carried out with the objective of assessing the effects of one-season cultivation of winter wheat in two field trials on the stocks of soil organic C (SOC), total N, microbial biomass, fungal biomass, and microbial residues in comparison with permanent grassland, to determine the effects of repeated manure application performed to restore negative tillage effects. One trial was started 2 years before sampling and the other 5 years before sampling. Mouldboard ploughing decreased the stocks of SOC, total N, microbial biomass C, and microbial residues (muramic acid and glucosamine), but increased those of the fungal biomarker ergosterol in both trials. Slurry application increased stocks of SOC and total N only in the short-term, whereas the stocks of microbial biomass C, ergosterol and microbial residues were generally increased in both trials, especially in combination with tillage. The ergosterol to microbial biomass C ratio was increased by tillage, and decreased by slurry application in both trials. The fungal C to bacterial C ratio was generally decreased by these two treatments. However, the negative effects were significant only in the short term. The metabolic quotient qCO_2 showed a significant negative linear relationship with the microbial biomass C to SOC ratio and an even stronger significant positive relationship with the soil C/N ratio. The fungal C to bacterial C ratio revealed a significant positive linear relationship with the ergosterol to microbial biomass C ratio, but a negative one with the SOC content. In conclusion, if tillage of grassland soils cannot be avoided, the application of cattle slurry has positive effects on soil microorganisms and C sequestration.

3.1 Introduction

Conversion of perennial grassland into agricultural cropland typically results in the degradation of soil and water quality as well as in dramatic shifts in soil biota and the ecosystem services they provide (DuPont et al., 2010; Strickland and Rousk, 2010; Wakelin et al., 2012). Effects of permanent conversion have been the focus of most research, while information on grass-arable rotation and occasional ploughing is rare, amino sugar derived microbial residues in particular. Tillage of grassland soil and the replacement of perennial plant communities with annual crops are the two fundamental practices that affect the soil ecosystem. A strong decline in soil organic C (SOC), total N, and soil structure as well as a change in microbial community structure towards Gram positive bacteria occurs after repeated tillage (Six et al., 2006; Quincke et al., 2007; Wortmann et al., 2008). A loss of 2.6 t CO_2-C ha^{-1} was observed three months after cultivation of grassland compared to 1.4 t CO_2-C ha^{-1} in the undisturbed grassland (Eriksen and Jensen, 2001). A single mouldboard ploughing after 20 years of minimum tillage reduced the SOC stocks by 5.3 t ha^{-1} within five months after the tillage event (Stockfisch et al., 1999).

Repeated tillage has especially negative impacts on microbial biomass C (Quincke et al., 2007; Wortmann et al., 2008) and causes a shift in the microbial community structure towards Gram positive bacteria (Wakelin et al., 2012). Other investigations revealed a high resilience of the original microbial biomass and community structure to tillage-induced manipulations of the grassland vegetation (Potthoff et al., 2006). In accordance with this, Linsler et al. (2013a) did not detect significant effects of a single cultivation event on the SOC stocks at 0-40 cm depth, although a significant decrease occurred at 0-10 cm depth. However, this discrepancy may be due to different site characteristics, e.g. clay content, soil pH and climate. Also, differences in sampling strategy might contribute to the presence or absence of tillage effects.

The fraction of non-living microbial residues contributes to long-term sequestration of C in soil and represents a significant SOC pool, larger than the living biomass (Strickland and Rousk, 2010). Microbial residues can be easily assessed by measuring amino sugars, which are exclusively microbial products in soil (Amelung et al., 2008). The determination of fungal glucosamine and bacterial muramic acid gives an additional opportunity to estimate the contribution of these two main microbial groups to C sequestration (Joergensen and Wichern, 2008). Very few studies have examined the rate or extent of re-establishment of fungal and

bacterial residues following the restoration of permanent grassland (Lauer et al., 2011), while the effects of one-season cultivation events are completely unknown. This leads to the hypotheses that (1) microbial residues respond faster than SOC but slower than the microbial biomass to tillage and (2) fungal residues react more sensitively than bacterial residues.

The application of cattle manure repeatedly resulted in positive effects on SOC in grassland soil (Vertès et al., 2007) and in aggregate formation in no-till soils (Six et al., 2006). However, nothing is known about the timescale or mechanisms regulating the possible response of microbial residues to manure application in combination with a one-season cultivation event in a grassland soil. In arable soils, manure application reduced the occurrence of saprotrophic fungi (Scheller and Joergensen, 2008; Heinze et al., 2010a) and especially promoted the formation of bacterial residues, leading to increased SOC stocks (Joergensen et al., 2010; Sradnick et al., 2012). This results in the hypotheses that (3) manure application intensifies the negative effects of tillage on soil fungi, but (4) has positive effects on the stocks of SOC and especially microbial biomass C.

These four hypotheses were investigated in two field trials with the objectives (i) to assess the effects of one-season winter wheat cultivation on the stocks of SOC, total N, microbial biomass, fungal biomass, and microbial residues in comparison with permanent grassland and (ii) to determine the effects of manure application performed to restore the negative effects of tillage. One trial was started 2 years before sampling and the other 5 years before sampling.

3.2 Material and methods

3.2.1 Study site and experimental layout

The experimental site Lindhof is located north of Kiel, (54°27 `N, 9°57 `E), Germany, near the Baltic Sea. The mean annual temperature in the area is 8.9 °C and precipitation is 768 mm (Linsler et al., 2013a). In 1994, the arable land at the site was converted to permanent grassland. The grass was generally cut four times each year for forage production (Schmeer et al., 2009). Fertilization was done during the year 2007 and 2009 and amounted to 100 kg ha^{-1} K, 24 kg ha^{-1} Mg and 68 kg ha^{-1} S and 45 kg ha^{-1} P in the form of rock phosphate in each treatment. The site has been managed organically since 1993.

Tillage trial 5 years before sampling: In 2005, a field experiment was initiated to determine the long-term effects of fertilisation of grassland soils with cattle slurry on N fluxes and C storage. In addition, a treatment with one-season cultivation of winter wheat was included to investigate the effects on nitrate leaching and on the use efficiency of mineralised N. After winter wheat cultivation, grassland was re-established to investigate the dynamics of soil organic carbon (SOC). The 4 treatments were (i) permanent grassland with cattle slurry application (240 kg N ha^{-1} a^{-1}) (P5+), (ii) permanent grassland without slurry application (P5-), (iii) re-established grassland with cattle slurry application (240 kg N ha^{-1} a^{-1}) (R5+) and (iv) re-established grassland without slurry application (R5-). All four treatments were arranged in a randomised block design with three replicates (block size 3 × 18 m). The R5+ and R5- plots were ploughed in October 2005 and winter wheat (*Triticum aestivum* L. variety Bussard) was sown. After wheat harvest, the plots were ploughed again to incorporate the straw (approximately 7 t ha^{-1}) in September 2006 and a grass clover mixture was sown. The mixture contained 67% perennial ryegrass (*Lolium perenne* L.), 17% timothy-grass (*Phleum pratense* L.), 10% smooth meadow-grass (*Poa pratensis* L.) and 6% white clover (*Trifolium repens* L.). At the time of sampling, the surface of the treatments P5+ and R5+ was covered with 29 and 13% clover plants, respectively, whereas that of the treatments P5- and R5- was covered with 23 and 31% clover plants, respectively (Shimeng Chen, personal communication). The soil of this first trial contained 53% sand, 29% silt and 18% clay at 0 - 40 cm depth and was classified as Eutric Cambisol (FAO, 2006).

Tillage trial 2 years before sampling: The previous trial was repeated in an adjacent area about 50 m away with tillage events in October 2008 and September 2009. The four treatments were (i) permanent grassland with cattle slurry application (240 kg N ha^{-1} a^{-1}) (P2+), (ii) permanent grassland without slurry application (P2-), (iii) re-established grassland with cattle slurry application (240 kg N ha^{-1} a^{-1}) (R2+) and (iv) re-established grassland without slurry application (R2-). All four treatments were arranged in a randomised block design with three replicates (block size 3 × 18 m). The soil of this second trial contained 66% sand, 21% silt and 13% clay at 0 - 40 cm depth and was also classified as Eutric Cambisol (FAO, 2006).

3.2.2 Soil sampling and chemical analysis

All soil samples were taken in October 2010 in a grid design with four replicates from each block at 0-5, 5-10, 10-20, 20-30, 30-40 cm depth using a steel corer with 4 cm diameter. This resulted in 12 samples per treatment and depth in both tillage trials. All samples were passed through a 2 mm sieve and stored at 4 °C until the assessment of biological properties. Bulk density was calculated from core dry weight divided by volume. Flint stones (sedimentary cryptocrystalline form of the mineral quartz) were observed in both tillage trials, especially in the second trial. Therefore, volume of soil (cm^{-3}) was calculated by subtracting the volume of stones or the volume of the fraction of fragments > 2 mm from the total soil volume taken. A field moist soil sample was used to analyse pH (1:2.5 soil water ratio). Dried (24 h at 105°C) and finely ground samples were used for chemical analyses (C and N). Total C and N were determined by gas chromatography using a Vario EL (Elementar, Hanau, Germany) analyser.

3.2.3 Microbial activity and biomass indices

The basal respiration of soil was measured by the incubation of 60 g soil sample for seven days at 22 °C with 40% WHC. The emitted CO_2 was trapped in 0.5 M NaOH and the excess NaOH was back titrated using 0.5 M HCl after the addition of saturated $BaCl_2$ solution. Fumigated (24 h with ethanol-free $CHCl_3$ at 25 °C) and non-fumigated 5-g samples were extracted with 20 ml of 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany) to measure microbial biomass C and N (Brookes et al., 1985; Vance et al., 1987). 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 EC / k_{EC}, where EC = (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 EN / k_{EN}, where EN = (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).

The fungal cell membrane component ergosterol was extracted from 2 g of moist soil with 100 ml ethanol (Djajakirana et al., 1996), determined by reverse phase HPLC using 100% methanol as the mobile phase and detected at a wavelength of 282 nm. The amino sugars muramic acid (MurN), mannosamine (ManN), galactosamine (GlcN), and glucosamine (GlcN) were determined according to Appuhn et al. (2004) as described by Indorf et al. (2011). Moist samples of 0.5 g soil were weighed into 20 ml test tubes, mixed with 10 ml 6 M HCl and hydrolysed for 6 h at 105 °C. The HCl was removed by rotary evaporator; the residue was dissolved in water and centrifuged. The samples were transferred to vials and stored at -18 °C until the HPLC measurement. Chromatographic separations were performed on a Phenomenex (Aschaffenburg, Germany) Hyperclone C_{18} column (125 mm length × 4 mm diameter), protected by a Phenomenex C_{18} security guard cartridge (4 mm length × 2 mm diameter) at 35°C. The HPLC system consisted of a Dionex (Germering, Germany) P 580 gradient pump, a Dionex Ultimate WPS 3000TSL analytical auto sampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector set at 445 nm emission and 330 nm excitation wavelengths. For the automated pre column derivatisation, 50 ml OPA and 30 ml sample were mixed in the preparation vial and after 120 s reaction time15 ml of the indole derivatives were injected. The mobile phase consisted of two eluents and was delivered at a flow rate of 1.5 ml min^{-1}. Eluent A was a 97.8/0.7/1.5 (v/v/v) mixture of an aqueous phase, methanol and tetrahydrofuran (THF). The aqueous phase contained 52 mmol sodium citrate and 4 mmol sodium acetate, adjusted to pH 5.3 with HCl. Then methanol and THF were added. Eluent B consisted of 50% water and 50% methanol (v/v).

3.2.4 Calculation and statistical analysis

The stocks of the soil nutrients and microbial indices at different depths were calculated on a volume basis by taking the bulk density of the respective soil layer into account. Fungal glucosamine was calculated by subtracting bacterial glucosamine from total glucosamine as an index for fungal residues, assuming that muramic acid and glucosamine occur at a 1 to 2 ratio in bacteria (Engelking et al., 2007): fungal C (μg g^{-1} dry weight) = (mmol glucosamine – 2 × mmol muramic acid) × 179.2 $gmol^{-1}$ × 9, where 179.2 is the molecular weight of glucosamine and 9 is the conversion value of fungal glucosamine to fungal C (Appuhn and Joergensen, 2006).

Bacterial C (µg g^{-1} dry weight) was calculated as an index for bacterial residues by multiplying the concentration of muramic acid in µg g^{-1} dry weight by 45 (Appuhn and Joergensen, 2006). The results presented in the tables are arithmetic means of treatments at the respective depths (n = 12), given on an oven dry basis (105 °C, 24 h). Data were checked for normal distribution by Chi-square test. The significance of treatment effect was analysed by a two way-ANOVA using tillage and slurry application as independent factors and depth as repeated measurements. All statistical analyses were carried out using SPSS statistical software (SPSS 16.0).

3.3 Results

3.3.1 Slurry and tillage effects on mean values and stocks

The mean bulk density at 0-40 cm depth was not significantly affected by any treatment in either trial (Table 6). Slurry application had a positive effect on the mean soil pH, whereas tillage had inconsistent effects on soil pH, i.e. positive effects in the short term (trial 2 years before sampling) and negative effects in the long term (trial 5 years before sampling). Tillage decreased the stocks of SOC and total N in both trials, whereas slurry application had a positive effect on these two soil properties only in the short term. However, the soil C/N ratio significantly increased only in the long term (Table 6).

Tillage generally had no effects on the stocks of microbial biomass N, but significantly decreased those of microbial biomass C and the mean basal respiration (Table 7). In contrast, tillage increased the stocks of ergosterol in the long term. Slurry application generally increased stocks of microbial biomass C and those of ergosterol, especially in the long term, whereas the mean basal respiration was increased in the short term, but decreased in the long term. Tillage decreased the stocks of all amino sugars (MurN, ManN, GalN and GlcN) and consequently also that of microbial C in the long term and also in the short term, with the exception of ManN (Table 8). Slurry application had in most cases significant positive effects on the indicator for microbial residues, especially in combination with tillage, leading to numerous significant interactions in both trials. The ergosterol to microbial biomass C ratio was increased by tillage and decreased by slurry application in both trials. The fungal C to bacterial C ratio was generally decreased by these two treatments. However, the negative effects were significant only in the short term.

Table 6. Mean stocks of soil organic C (SOC), total N and mean soil pH, bulk density and soil C / N ratio among different treatments for 0–40 cm soil profiles in a permanent grassland from a tillage trial 2 and 5 years before sampling; two-way factorial ANOVA (tillage, slurry application)

Treatment	Soil pH	Bulk density ($kg\ m^{-3}$)	SOC ($t\ ha^{-1}$)	Total N ($t\ ha^{-1}$)	Soil C/N
Tillage trial 2 years before sampling					
P2+	6.2	1.39	73	6.5	11.2
P2-	6.0	1.40	70	6.2	11.2
R2+	6.2	1.40	69	6.2	11.1
R2-	6.2	1.42	66	5.9	11.1
Probability values					
Tillage	0.03	NS	0.04	0.02	NS
Slurry	<0.01	NS	0.04	0.04	NS
Tillage × slurry	0.01	NS	0.06	0.06	NS
CV (±%)	2	8	12	12	15
Tillage trial 5 years before sampling					
P5+	6.5	1.40	74	6.9	10.5
P5-	6.4	1.38	74	7.0	10.6
R5+	6.4	1.44	67	6.2	11.2
R5-	5.9	1.46	66	6.1	11.1
Probability values					
Tillage	<0.01	NS	0.05	0.03	0.02
Slurry	<0.01	NS	NS	NS	NS
Tillage × slurry	<0.01	NS	NS	NS	NS
CV (±%)	2	8	12	13	17

CV = mean coefficient of variation between replicate samples within a plot (n = 4); NS = not significant.

Table 7. Mean stocks of microbial biomass C and N, ergosterol and mean respiration rate among different treatments for 0–40 cm soil profiles in a permanent grassland from a tillage trial 2 and 5 years before sampling; two-way factorial ANOVA (tillage, slurry application)

Treatment	Microbial biomass			
	C	N	Ergosterol	Basal respiration
	($kg\ ha^{-1}$)			($kg\ CO_2\text{-}C\ d^{-1}\ ha^{-1}$)
Tillage trial 2 years before sampling				
P2+	1470	200	2.2	42
P2-	1180	180	1.9	33
R2+	1270	210	2.1	36
R2-	1110	180	1.9	32
Probability values				
Tillage	0.04	NS	0.06	0.05
Slurry	0.01	0.02	<0.01	0.03
Tillage × slurry	<0.01	0.03	0.04	<0.01
CV (±%)	20	27	25	20
Tillage trial 5 years before sampling				
P5+	1370	130	1.4	49
P5-	1300	140	1.4	51
R5+	1280	140	1.8	41
R5-	1240	110	1.6	47
Probability values				
Tillage	0.04	NS	0.01	0.01
Slurry	NS	NS	0.07	<0.01
Tillage × slurry	NS	NS	NS	0.05
CV (±%)	25	32	27	17

CV = mean coefficient of variation between replicate samples within a plot (n = 4); NS = not significant.

Table 8. Mean stocks of amino sugars and mean ratios of ergosterol / microbial biomass C (%) and fungal C / bacterial C among different treatments for 0–40 cm soil profiles in a permanent grassland from a tillage trial 2 and 5 years before sampling; two-way factorial ANOVA (tillage, slurry application)

Treatment	MurN	ManN	GalN	GlcN	Microbial C	Ergosterol / Microbial	Fungal C /
	(kg ha^{-1})				(t ha^{-1})	biomass C (%)	Bacterial C
Tillage trial 2 years before sampling							
P2+	360	36	2630	4820	53	0.15	2.3
P2-	350	31	2850	4980	54	0.18	2.4
R2+	390	32	2770	4970	55	0.17	2.1
R2-	310	37	2410	4290	47	0.18	2.4
Probability values							
Tillage	0.04	NS	0.01	<0.01	0.01	0.06	0.01
Slurry	<0.01	0.06	0.01	NS	NS	0.05	<0.01
Tillage × slurry	0.01	NS	0.03	0.04	0.03	NS	0.03
CV (±%)	15	29	14	14	14	26	8
Tillage trial 5 years before sampling							
P5+	440	48	3070	5360	60	0.11	2.0
P5-	430	43	3080	5300	59	0.12	2.2
R5+	380	31	2560	4650	52	0.14	2.0
R5-	340	20	2200	4140	47	0.15	2.1
Probability values							
Tillage	<0.01	<0.01	<0.01	0.02	<0.01	0.03	NS
Slurry	<0.01	<0.01	0.01	<0.01	<0.01	0.04	NS
Tillage × slurry	NS	<0.01	0.05	0.06	0.06	NS	NS
CV (±%)	18	37	17	16	16	28	12

CV = mean coefficient of variation between replicate samples within a plot (n = 4); NS = not significant.

3.3.2 Decline of soil chemical and microbial properties

Bulk density and soil pH both significantly increased with depth (Supplementary Table 4). All other soil chemical and microbial properties analysed decreased with depth (Fig. 6a, b; Fig. 7a-d; Supplementary Tables 4 to 6). The strongest decline occurred from the 0-5 cm to the 5-10 cm layer in most treatments, with the exception of the R2+ and R2- treatments. There, the SOC content declined only moderately down to 15 cm (Fig. 6a), whereas the microbial biomass C content already showed a considerably stronger decline with depth (Fig. 7a). The differences between the slurry and tillage treatments were non-significant for the majority of soil properties below 10 cm. An exception was the SOC content in the bottom layer at 30-40 cm depth (Fig. 6b), which was significantly lower in the R5+ and R5- treatments in comparison with the respective permanent grassland treatments. Another marked exception was the relatively high ergosterol content in the 10-20 cm layer of the re-established grassland treatment in the short term (Fig. 7c). Slurry application did not lead to consistent effects on the depth decline of any soil property analysed in contrast to the tillage trials.

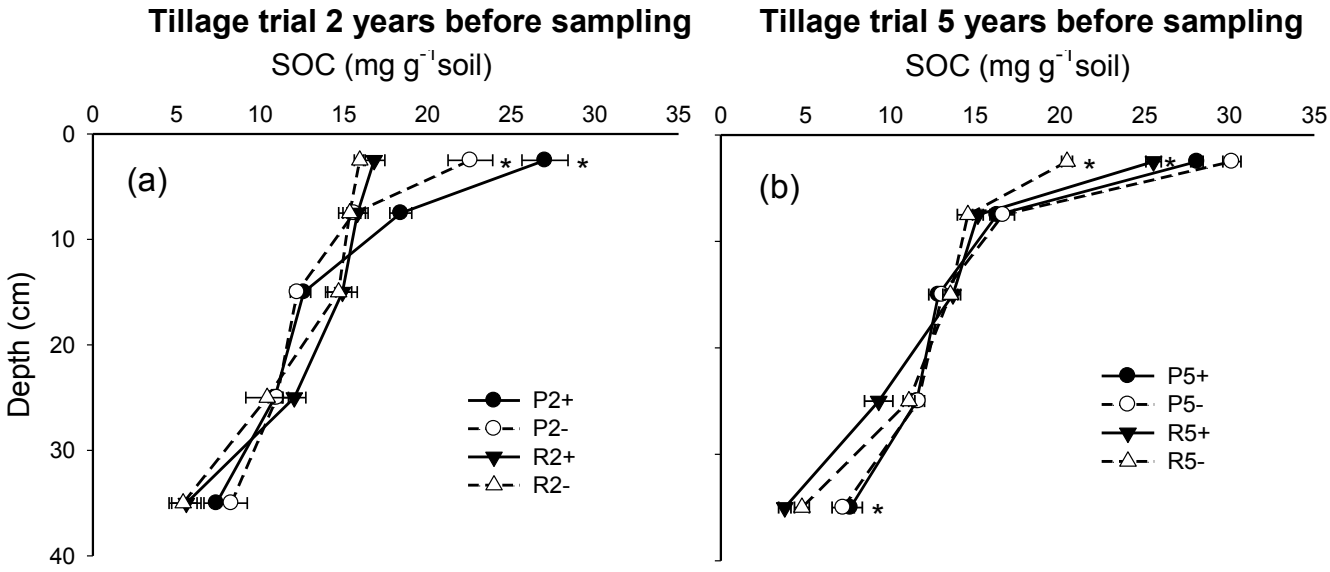

Fig. 6. Mean concentrations of SOC among different treatments (permanent grassland with slurry application (P+); permanent grassland without slurry application (P-); re-established grassland with slurry application (R+); re-established grassland without slurry application (R-)) and soil depths in a permanent grassland from a tillage trial 2 (a) and 5 (b) years before sampling. * indicates a depth specific difference between treatments ($P < 0.05$, ANOVA repeated measurement); error bars show standard error of mean (n = 12).

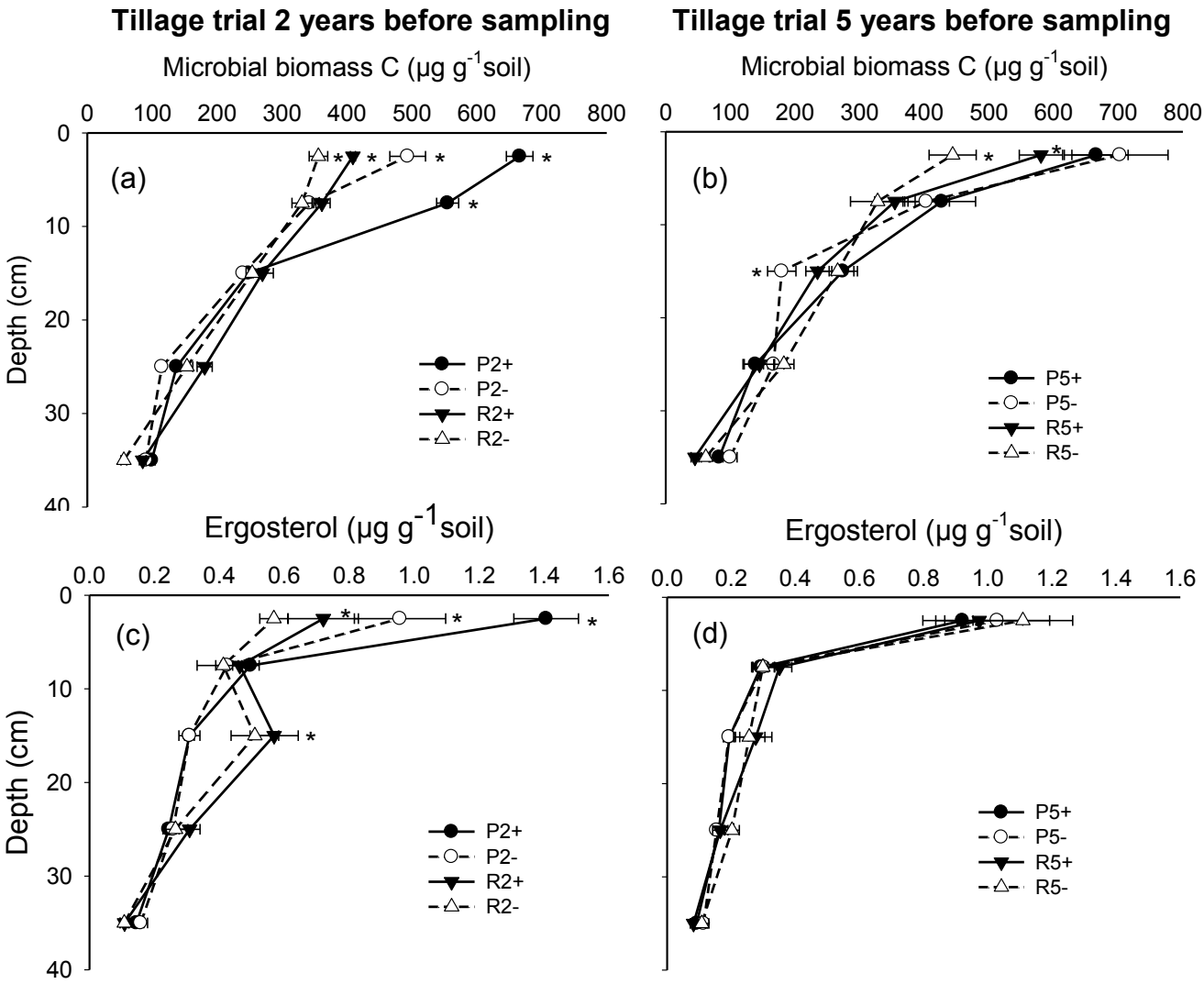

Fig. 7. Mean concentrations of (a,b) microbial biomass C and (c,d) ergosterol among different treatments (permanent grassland with cattle slurry application (P+); permanent grassland without cattle slurry application (P-); re-established grassland with cattle slurry application (R+); re-established grassland without cattle slurry application (R-)) and soil depths in a permanent grassland from a tillage trial 2 and 5 years before sampling. * indicates a depth specific difference between treatments ($P < 0.05$, ANOVA repeated measurement); error bars show standard error of mean ($n = 12$).

The mean soil C/N ratio varied between 10.4 and 12.2 (Fig. 8a,b), it declined at first to minimum values at 5 to 20 cm depth, followed by an increase to maximum values at 30-40 cm. The soil C/N ratio was significantly lower at 0-5 cm depth in the R2+ and R2- treatments in comparison with the respective permanent grassland treatments (Fig. 8a), whereas slurry application did not reveal depth-specific effects, despite the general increase in the P5+ and P5- treatments (Fig. 8b, Table 6). The microbial biomass C to SOC ratio varied around 2.5 to 1.1% and declined with depth. Slurry application had a significant positive effect on this ratio at 30-40 cm depth in the short term, whereas tillage had no obvious effects. The metabolic quotient qCO_2 values showed a significant negative linear relationship with microbial biomass C to SOC ratio ($r = -0.45$, $P < 0.01$, $n = 360$) and an even stronger significant positive relationship with the soil C/N ratio ($r = 0.52$, $P < 0.01$, $n = 360$).

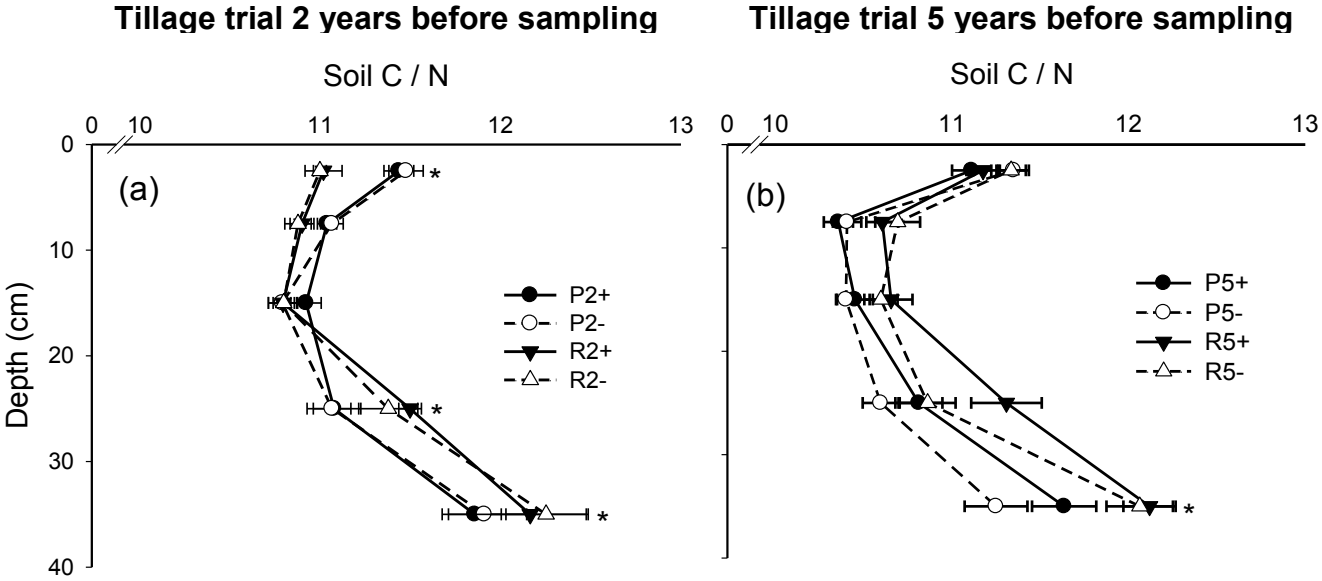

Fig. 8. Mean soil C/N among different treatments (permanent grassland with cattle slurry application (P+); permanent grassland without cattle slurry application (P-); re-established grassland with cattle slurry application (R+); re-established grassland without cattle slurry application (R-)) and soil depths in a permanent grassland from a tillage trial 2 (a) and 5 (b) years before sampling. * indicates a depth specific difference between treatments ($P < 0.05$, ANOVA repeated measurement); error bars show standard error of mean ($n = 12$).

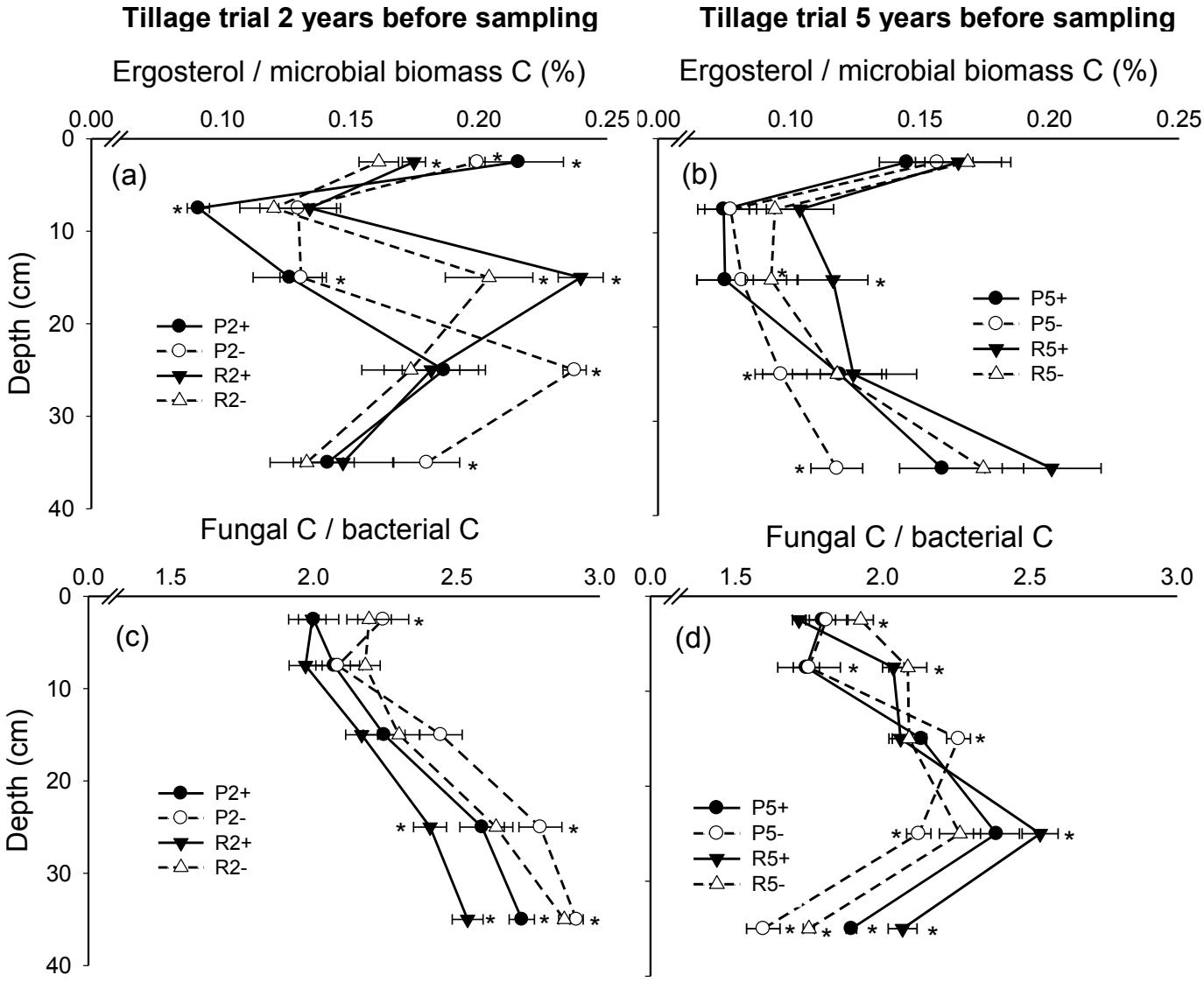

Fig. 9. Mean ratios of (a,b) ergosterol / microbial biomass C (%), (c,d) fungal C / bacterial C among different treatments (permanent grassland with cattle slurry application (P+); permanent grassland without cattle slurry application (P-); re-established grassland with cattle slurry application (R+); re-established grassland without cattle slurry application (R-)) and soil depths in a permanent grassland from a tillage trial 2 and 5 years before sampling. * indicates a depth specific difference between treatments ($P < 0.05$, ANOVA repeated measurement); error bars show standard error of mean (n = 12).

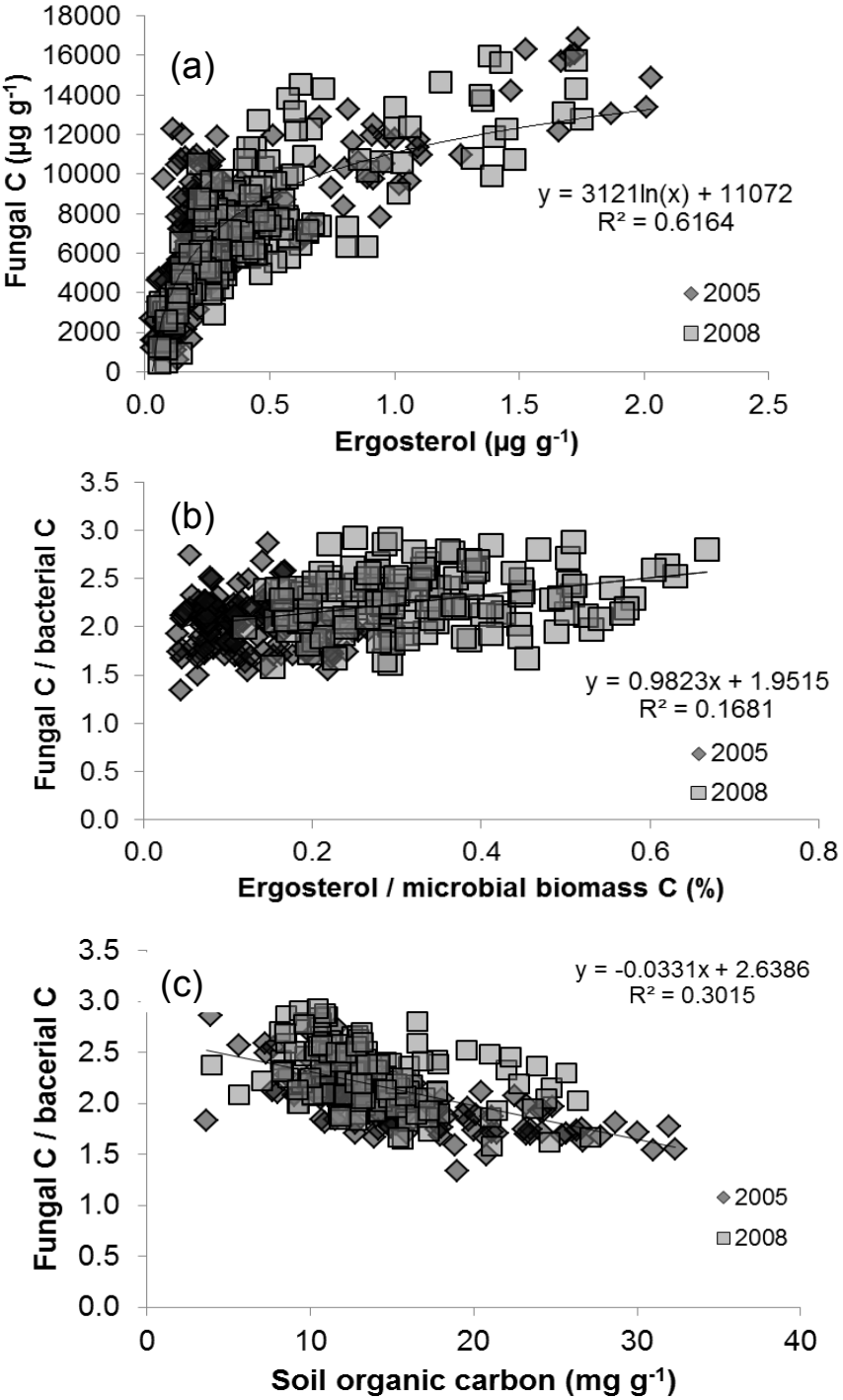

Fig. 10. The relationships between (a) ergosterol and fungal C, (b) fungal C / bacterial C and ergosterol / microbial biomass C (%) and fungal C / bacterial C and soil organic carbon; data from the long and short-term trial, all treatments and all field replicates were combined (n = 360).

The ergosterol to microbial biomass C ratio declined from values around 0.22% at 0-5 cm depth (Fig. 9a,b) to minimum values of 0.07% at 5-10 cm depth and increased again to the values of the surface layer at 30-40 cm in the long-term (Fig. 9b) and at 10-40 in the short term (Fig. 9a). Therefore, the ergosterol to microbial biomass C ratio was characterized by an extraordinarily high variability, contrasting all other ratios. The fungal C to bacterial C ratio increased continuously from values around 2.2 at 0-5 cm depth to values around 2.9 at 30-40 cm depth in the short term (Fig. 9c). In the long term, the fungal C to bacterial C ratio increased from values around 1.7 at 0-5 cm depth to values around 2.5 at 20-30 cm depth, followed by a decline to values of the surface layer at 30-40 cm depth (Fig. 9d). However, at 30-40 cm, slurry application and especially tillage led to a significantly higher fungal C to bacterial C ratio. Amino sugar-based fungal C showed a significant non-linear relationship with the fungal biomarker ergosterol (Fig. 10a). The fungal C to bacterial C ratio revealed a significant positive linear relationship to the ergosterol to microbial biomass C ratio (Fig. 10b), but a negative one to the SOC content (Fig. 10c).

3.4 Discussion

3.4.1 Effects of tillage

One-season cultivation of winter wheat with two tillage events led to a significant loss in the stocks of SOC and total N at 0-40 cm depth in comparison with the permanent grassland, not only in the short term, but also in the long term. This decrease was mainly caused by significant decreases at 0-5 cm depth. At the same experimental site, Linsler et al. (2013a) found similar significant differences in stocks of SOC and total N at 0-10 cm depth in the short term, but not in the long term, using a sampling scheme with a lower spatial resolution. A significant decline in SOC stocks was detectable at 0-5 cm depth (Quincke et al., 2007; Wortmann et al., 2010) and 0-7.5 cm depth (Kettler et al., 2000) even 4 to 5 years after a single mouldboard ploughing event. The organic matter input is higher in the permanent grassland, due to the perennity of grassland species, leading to a high belowground allocation of assimilates (Qin et al., 2006; Vertès et al., 2007). In addition, the SOC in permanent grassland is protected by macro-aggregate formation

against microbial decomposition (Six et al., 2000; Vertès et al., 2007). At the same site, the accrual of water stable aggregates (250-1000 µm) was significantly higher, especially at 0-10 cm, but also at 25-40 cm depth in the permanent grassland in comparison with the re-established grassland in the short term, but also in the long term (Linsler et al., 2013a). At 0-10 cm depth, they also found a positive correlation between water stable aggregates and root biomass.

The loss in SOC was accompanied by reduced stocks of microbial biomass C in the long-term, which is in line with the results of Stockfisch et al. (1999) and Wortmann et al. (2010) after one single mouldboard ploughing event. However, the tillage induced loss in microbial biomass C was roughly 40% less in the long term than in the short term of the current experiment, indicating a recovery process as observed by Potthoff et al. (2005) during grassland restoration. The immediate formation of a depth gradient in the microbial biomass C content in the short term indicates that the living fraction responds considerably faster to changes in the C input than SOC. This has already been proposed by Powlson et al. (1987), but has not always been demonstrated as clearly as in the present experiment (Joergensen and Emmerling, 2006). The content of ergosterol, an indicator for saprotrophic fungal biomass (Joergensen and Wichern, 2008), was increased at 10-20 cm depth in the short term: This indicates that straw and stubble incorporation by mouldboard ploughing specifically promoted this group of soil fungi (Wortmann et al. 2008; Heinze et al. 2010a; Rousk and Bååth, 2011). Tillage promoted saprotrophic fungi at the expense of biotrophic arbuscular mycorrhizal fungi (AMF) and bacteria, as indicated by the increased ergosterol to microbial biomass C ratio and the constant fungal C to bacterial C ratio. AMF do not contain ergosterol in their cell membranes (Olsson et al., 2003), but GlcN in the chitin of their cell walls (Joergensen and Wichern, 2008).

The tillage induced changes in microbial residues were stronger in the short term than in the long term and verify our first hypothesis. However, this might due to the differences in soil properties, e.g. the higher clay content and the higher soil pH of the first tillage trial. These differences led to a stronger tillage-induced increase in saprotrophic fungi and apparently to a stronger decline in microbial residues, which confirms our second hypothesis. No data are available for comparing the effects of a single tillage or cultivation event on the loss of microbial residues. However, some information can be found on the conversion of native grassland into arable use (Solomon et al., 2007; Amelung et al., 2002) and also on the restoration of such sites

into grassland (Lauer et al., 2011), suggesting that the saprotrophic fungal community did not return to a pre-cultivation composition and that above and below ground linkages may be affected by the land use history for long periods (Smith et al., 2009; DuPont et al., 2010).

3.4.2 Effects of slurry application

At 0-40 cm depth, the application of cattle slurry led to significant 5 to 15% increases in the stocks of SOC, total N, microbial biomass C, and microbial biomass N in comparison with the non-fertilized treatments in the short-term, especially after tillage, but not in the long-term. The slurry effects are apparently transient and only strong after disturbance until equilibrium with SOC has been reached again. The amounts of organic C and microbial biomass C added by the manure itself may result in some increases in the respective soil fractions (Scheller and Joergensen, 2008), especially under depleted soil conditions. The manure might be the source of the general increase in MurN, i.e. bacterial residue C in both trials and the increase in microbial residue C in the long term. Manure application has been repeatedly shown to promote bacteria (Scheller and Joergensen, 2008; Sradnick et al., 2012), as indicated by the decreases in both the ergosterol to microbial biomass C ratio and the fungal C to bacterial C ratio in the present experiment, which supports our third hypothesis. Bardgett et al. (1998) and Grayston et al. (2004) observed a lower proportion of fungi relative to bacteria in fertilised grassland compared to an improved grassland. Similarly, suppressed saprotrophic fungal community and increased Gram positive bacteria were observed after 5 years of fertiliser application in comparison with non-fertilised grassland soils (Bradley et al., 2006; Rousk and Bååth, 2011). Another possible reason is that the 15% lower white clover population promoted fungal biomass in the re-established in comparison with the permanent grassland (Denef et al., 2009; Walsh et al., 2012).

Another, even more important reason for the short-term increases in SOC and microbial biomass C may be the increased plant C input into arable soils (Heinze et al., 2010a), but also into grassland soils (Conant et al., 2001). This explains the increase in soil microbial biomass and enzyme activities by slurry application to grassland as observed by Kandeler and Eder (1993). Application of slurry did not increase the amount of water stable aggregates in comparison with the non-fertilised grassland soil, but showed a trend towards higher SOC contents in macro- and micro-aggregates at 0-10 cm depth (Linsler et al., 2013b).

3.4.3 Relationships between microbial community indices

Ergosterol, the indicator of saprotrophic fungal biomass (Joergensen and Wichern, 2008), and fungal C, the GlcN-based indicator of fungal residues (Amelung et al., 2008), showed a significant non-linear relationship in the present grassland management experiment. This has been similarly observed by Heinze et al. (2010a) in an arable long-term fertilization experiment and by Murugan et al. (2012) in an arable long-term tillage experiment. The relationship between the ergosterol to microbial biomass C ratio and the fungal C to bacterial C ratio was positive in the current experiment, indicating an increased formation of fungal residues along with increasing saprotrophic fungal biomass. However, this contrasts with the results of our tillage experiment (Murugan et al., 2012), probably due to the high contribution of AMF to the fungal biomass in the current grassland experiment. This is indicated by the generally very low ergosterol to microbial biomass C ratios (Djajakirana et al., 1996), especially in the trial 5 years before sampling. These were considerably lower than those of the long-term tillage trial in central and southern Germany (Murugan et al., 2012). AMF apparently respond more sensitively to tillage than other soil microorganisms in long-term field experiments (Kabir et al., 1998; Heinze et al., 2010b). Decreases in AMF fungi caused by one single tillage event were found in western Nebraska, persisting even after 5 years (Garcia et al., 2007; Wortmann et al., 2008). The negative relationship between the fungal C to bacterial C ratio and the SOC content suggests, again in agreement with Murugan et al. (2012), that the SOC turnover of saprotrophic fungi is faster than that of biotrophic AMF, reducing the amount of C sequestered as SOC.

The metabolic quotient qCO_2 and microbial biomass C to SOC ratio revealed a significant negative relationship, as repeatedly observed by others in different long-term field experiments (Meyer et al., 1996; Hungria et al., 2009; Heinze et al., 2010a,b; Murugan et al. 2012). The microbial biomass C to SOC ratio is an index for the availability of substrate to soil microorganisms (Anderson and Domsch, 1989). The qCO_2 value indicates the substrate use efficiency of soil microorganisms, i.e. the amount of substrate necessary to match the energetic demand (Anderson and Domsch, 1990). In the present grassland experiment, the qCO_2 is positively related to the soil C/N ratio and to the ergosterol to microbial biomass C ratio. These relationships support the view that an increasing soil C/N ratio is combined with an increasing fungal dominance (Scheller and Joergensen, 2008; Strickland and Rousk, 2010), but contradict

the view that saprotrophic fungi are more efficient in substrate use (Sakamoto and Oba, 1994; Jastrow et al., 2007).

Not only the treatments affect the soil properties analysed in the current experiment, in some cases differences in soil properties might also affect the relationships between the microbial community indicators. The higher clay content and the higher soil pH in the first trial generally led to lower ergosterol to microbial biomass C ratios, lower fungal C to bacterial C ratios, but also to higher microbial biomass C/N ratios and metabolic quotients. For these reasons, it cannot be excluded that the marked difference in the ergosterol to microbial biomass C ratios and in the fungal C to bacterial C ratios at 30-40 cm depth between the two trials might be due to legacy effects and may not be caused by tillage and slurry application.

3.5 Conclusions

At the present grassland site, tillage of a one-season winter wheat cultivation decreased stocks of SOC and total N in the short term (trial 2 years before sampling) but also in the long term (trial 5 years before sampling). Microbial biomass C responded faster than SOC in the short term and showed some recovery in the long term. However, the stocks of microbial residues were the most sensitive indicators of negative tillage effects. Tillage promoted saprotrophic fungi but had no specific effects on the stocks of fungal or bacterial residues, indicating negative effects on AMF. Slurry application generally reduced the presence of saprotrophic fungi and decreased the fungal C to bacterial C ratio. Some effects might be masked by the strong spatial variability of all soil properties at the sandy experimental site. Slurry application has strong positive effects on the formation of microbial residues after tillage, which was not reflected by significant increases in the stocks of SOC and total N during the present experimental periods. However, if tillage of grassland soils cannot be avoided, the application of cattle slurry has positive effects on soil microorganisms and C sequestration.

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4 Changes in soil microbial biomass, residues and functional diversity after conversion of permanent to modified grassland or maize crop

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Abstract

The effects of permanent grassland conversion to modified grassland or maize monoculture on the dynamics of soil organic C (SOC), microbial biomass, fungal biomass, fungal and bacterial residues in grassland soils were investigated. Cattle slurry was applied to quantify the effects of fertilisation on change in microbial residues and functional diversity of microbial community across land use types. Maize monoculture (MM) decreased the stocks of SOC, microbial biomass C, N and S and microbial residues (muramic acid and fungal glucosamine) compared to permanent grassland (PG) and modified grassland (MG) at 0-40 cm depth. The MM treatment led to a greater accumulation of saprotrophic fungi, as indicated by the lower microbial biomass C/S ratio and higher ergosterol to microbial biomass C ratio in comparison with the grassland treatments. In contrast, the reduced presence of saprotrophic fungi and the higher fungal C to bacterial C ratio in grassland treatments compared to the MM treatment suggest a shift in the fungal community structure towards AMF, which do not contain ergosterol but glucosamine. Slurry application generally increased the stocks of SOC, microbial biomass C, N and S and especially microbial residues, but reduced the presence of saprotrophic fungi and the fungal C to bacterial C ratio. The functional diversity of the microbial community was diminished in the MG and MM treatments compared to the undisturbed PG treatment. Our results indicate that the microbial diversity lost due to tillage and maize monoculture can be restored by slurry application.

4.1 Introduction

Soil quality assessment is necessary to detect changes in soil properties under different agricultural management practices. Germany, the largest biogas producer in the European Union strongly promotes maize (*Zea mays ssp. mays*) cultivation due to high methane yield per kg organic matter and high biomass yield, which led to a strong increase in conversion of grassland to acreage cultivated with maize in monoculture even on less productive sites. This rush into biogas production strongly based on maize monoculture is a cause of growing concern about potential damage to the terrestrial agro-ecosystems (Hermann, 2012).

Land use change from grassland to crop land has led to an average loss of soil C stocks by 59% (Guo and Gifford, 2002) and may accelerate soil erosion (Prasuhn, 2012). Soil organic C (SOC), macroaggregates, catabolic diversity and associated living and dead microbial biomass decreased remarkably under cotton (*Gossypium hirsutum* L.) monoculture (Acosta-Martinez et al., 2010) and prolonged cultivation of maize in grassland soils (Lauer et al., 2011; Kösters et al., 2013). A strong decline in SOC stock occurs after repeated tillage and cultivation of arable crops, while grasslands tend to support increased soil C and microbial biomass with greater spatial heterogeneity within the soil profile than in cultivated soils (Culman et al., 2010; Kösters et al., 2013). High intensity arable cropping resulted in a significant loss of microbial biomass C, water stable aggregates and root biomass down to 80 cm depth compared to permanent grassland soil (Culman et al., 2010). Prolonged cultivation of arable crops and tillage causes a shift in the microbial community structure towards Gram positive bacteria (Tian et al., 2012; Wakelin et al., 2012), while permanent grassland soils recorded a two times higher fungi to bacteria biomass ratio compared to arable land (Bailey et al., 2002).

The biodiversity of the soil microbial community plays an important role in maintenance of soil ecosystem function, such as C sequestration (Nannipieri et al., 2003; Degens et al., 2000). Crop mixtures in grassland produced greater plant biomass, microbial biomass C and C utilisation by microbial communities than single species, due to variations in the quantity and quality of root exudates (Grayston et al., 2004, De Vries et al., 2009). The native grassland soil showed greater functional diversity than in modified grassland soil (Wakelin et al., 2012).

The non-living microbial residues represent a significant SOM pool much greater than the living biomass and they are highly related to SOM dynamics (Liang et al., 2011). The size of the

microbial necromass C pool in soil may be about 40 times that of the living microbial biomass (Simpson et al., 2007; Liang and Basler, 2011). The microbial residues can be assessed by measuring amino sugars, which are exclusively microbial products in soil (Amelung et al., 2001). The determination of highly-specific muramic acid and glucosamine are useful indicators for the specific contribution of bacterial and fungal residues, respectively, to the C sequestration potential of soils (Appuhn and Joergensen, 2006; Joergensen and Wichern, 2008). The microbial community structure and its diversity have been suggested as a sensitive means of assessing soil quality (Nannipieri et al., 2003). The modified MicroResp^{TM} method (Campbell et al., 2003) based on Degens and Harris (1997) multiple carbon source substrate induced respiration (multi-SIR) has been reported to be a reliable and sensitive method for measuring the change in microbial community level physiological profiles (CLPP) due to land use change and fertilisation (Stevenson et al., 2004; Romaniuk et al., 2011).

A close relationship has been observed between microbial biomass S and the fungal biomarker ergosterol (Heinze et al., 2010a; Murugan et al., 2012a), due to the ability of saprotrophic fungi to store large amounts of S in their biomass (Banerjee and Chapman, 1996). The application of cattle slurry can have considerable positive effects on the nutrient dynamics of ecosystems (Vertès et al., 2007), resulting in a higher catabolic diversity and more dynamic microbial system than in inorganically fertilised soil (Romaniuk et al., 2011; Sradnick et al., 2013). In addition, slurry application affected the microbial community structure and the fungal and bacterial contribution to C sequestration in grassland ecosystems (Walsh et al., 2012; Murugan et al., 2012b). Slurry treated grassland soils showed a positive effect on bacterial biomass, but a negative one on fungal biomass (Bitmann et al., 2005; Walsh et al., 2012) and on the fungal C to bacterial C ratio (Murugan et al., 2012b). Further manure application reduced the presence of saprotrophic fungi in both arable (Scheller and Joergensen, 2008; Heinze et al., 2010a) and grassland soils (Murugan et al., 2012b) and especially promoted the formation of bacterial residues, leading to increased SOC stocks (Joergensen et al., 2010).

Only limited information is available about the cultivation effects of different biogas plants on soil microbial residues and functional diversity. Based on these results, we tested the following four hypotheses: (1) Slurry application has positive effects on bacterial residues, the stocks of SOC and especially microbial biomass C. (2) Grassland conversion to maize

monoculture leads to a decrease in SOC stocks and soil fungi, which react more sensitively to management practises than do bacteria. (3) Differences in plant species composition and species-dominance between grasslands are likely to exert strong selective pressures on the soil microbial community and functional diversity. (4) Slurry application promotes the functional diversity of the microbial community.

In grasslands, productivity and soil quality are related to root characteristics. Wardle et al. (1997) suggest that the aboveground effect of plant litter on soil biota is of less importance than that of plant parts belowground. However, less is known about concomitant changes in soil microbial community composition (fungal and bacterial residues) and microbial diversity due to fertilisation. The specific objectives of this study were (i) to measure the effects of grassland conversion on the stocks of SOC, total N, microbial biomass, fungal biomass, and microbial residues in comparison with permanent grassland, (ii) to assess the effect of land use on functional diversity of the microbial community and (iii) to quantify the effects of manure application on change in microbial CLPP across land use types.

4.2 Material and methods

4.2.1 Study site and experimental layout

The experimental site Lindhof is located north of Kiel, (54°27 `N, 9°57 `E), Germany, near the Baltic Sea. The mean annual temperature in the area is 8.9 °C and precipitation is 768 mm (Linsler et al., 2013). In 1994, the arable land at the site was converted to permanent grassland. The grass was generally cut four times each year for forage production (Schmeer et al., 2009). Fertilisation was done during 2007 and 2009 and amounted to 100 kg ha^{-1} K, 24 kg ha^{-1} Mg and 68 kg ha^{-1} S and 45 kg ha^{-1} P in the form of rock phosphate in each treatment. The site has been managed organically since 1993.

During 1994, the arable land at that site was converted to permanent grassland (PG). Between 1994 and 2005, the grassland was cut 1–2 times per year for silage production. In 2005, a field experiment was initiated to determine the short and long-term effects of tillage and fertilisation of grassland soils with cattle slurry on N fluxes and C storage. In Germany, there has been a boom in biogas production in many regions, maize being the main crop substrate and

often being grown in monoculture. This applies, for instance, to the sandy soil regions of northern Germany, where dairy farming has traditionally been the prevailing farming activity and the share of maize on arable land was already high before the biogas boom took hold. During 2010, in one treatment the permanent grassland was 'modified' by sowing a mixture of permanent grass species immediately after tillage (0-20 cm). The mixture contained 67% perennial ryegrass (*Lolium perenne* L.), 17% timothy-grass (*Phleum pratense* L.), 10% smooth meadow-grass (*Poa pratensis* L.) and 6% white clover (*Trifolium repens* L.). In another treatment, permanent grassland was converted to maize monoculture cropping to investigate the monoculture effects on nitrate leaching, the use efficiency of N mineralised and to investigate the dynamics of SOC. The soil in the first replicate contained 53% sand, 29% silt and 18% clay, the second replicate contained 66% sand, 21% silt and 13% clay and the third replicate contained 73% sand, 16% silt and 11% clay at 0 - 40 cm depth and was classified as Eutric Cambisol (FAO, 2006).

The blocks were split and received cattle slurry 240 kg N ha^{-1} a^{-1} or no fertilisation. Four cuts followed in these grassland treatments for forage production, and slurry was applied in four split doses following each cut. The six treatments were (i) permanent grassland with slurry application (PG+), (ii) permanent grassland without slurry application (PG-), (iii) modified grassland with slurry application (MG+), (iv) modified grassland without slurry application (MG-), (v) maize monoculture with slurry application (MM+) and (vi) maize monoculture without slurry application (MM-). To investigate the role of microbial residues and community structure in SOC dynamics, all six treatments were arranged in a randomized block design with three replicates (block size 3×18 m).

4.2.2 Soil sampling and chemical analysis

All soil samples were taken in April 2012 at three sampling points from each treatment from 0-10, 10-20, 20-30, 30-40 cm depth using a steel corer with 4 cm diameter. This resulted in 9 samples per treatment and depth. All samples were passed through a 2 mm sieve and stored at 4°C for a few weeks until the assessment of biological properties. A field moist soil sample was used to analyse pH (1:2.5 soil water ratio). Dried (24 h at 105°C) and finely ground samples were used for chemical analyses (C and N). Bulk density was calculated from core dry weight

divided by volume. Flint stones (sedimentary cryptocrystalline form of the mineral quartz) were observed in the second and third replicates. Therefore, volume of soil (cm^{-3}) was calculated by subtracting the volume of stones or the volume of the fraction of fragments > 2 mm from the total soil volume taken. Field moist soil sample was used to analyse pH (1:2.5 soil water ratio). Total C and N were determined by dry combustion in a CNS Analyser (Elementar Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany).

4.2.3 Microbial activity and biomass indices

The basal respiration of soil was measured by the incubation of 60 g soil sample for seven days at 22 °C with 40% WHC. The emitted CO_2 was trapped in 0.5 M NaOH and the excess NaOH was back titrated using 0.5 M HCl after the addition of saturated $BaCl_2$ solution. Fumigated (24 h with ethanol-free $CHCl_3$ at 25 °C) and non-fumigated 5-g samples were extracted with 20 ml of 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany) to measure microbial biomass C and N (Brookes et al., 1985; Vance et al., 1987). Simultaneous determination of total organic C and T was measured by infrared absorption after catalytic high temperature combustion up to 800 °C, multi N/C® 2100 automatic analyser (AnalytikJena, Jena, Germany). Microbial biomass C was calculated as EC / 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). 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). Fumigated and non-fumigated 5-g samples were extracted with 25 ml of 1 M NH_4NO_3 to measure microbial biomass S (Khan et al., 2009). 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 from 2 g of moist soil with 100 ml ethanol (Djajakirana et al., 1996), determined by reverse phase HPLC using 100% methanol as the mobile phase and detected at a wavelength of 282 nm. The amino sugars muramic acid (MurN), mannosamine (ManN), galactosamine (GlcN), and glucosamine (GlcN) were determined as described by Indorf et al. (2011). Moist samples of 0.5 g soil were weighed

into 20 ml test tubes, mixed with 10 ml 6 M HCl and hydrolysed for 6 h at 105 °C. The HCl was removed by rotary evaporator; the residue was dissolved in water and centrifuged. The samples were transferred to vials and stored at -18 °C until the HPLC measurement. After derivatisation with ortho-phthaldialdehyde, fluorometric emission of amino sugar was measure at a wavelength of 445 nm after excitation at a wavelength of 330 nm. The HPLC system consisted of a Dionex (Germering, Germany) P 580 gradient pump, a Dionex Ultimate WPS – 3000TSL analytical autosampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector.

4.2.4 Catabolic response profiling

The catabolic response profile was obtained by the MicroResp^{TM} method of Campbell et al. (2003). The soil (0.3 g) was adjusted to a water holding capacity of 50%, before weighed into each deep well (1.1 ml deep-well microtitre plate (Nunc, Thermo Electron LED, Langenselbold, Germany)) and stored for 7 days in the dark at 25°C, prior to CLPP analysis. Besides water, the following 17 substrates were used: 5 amino acids (γ-aminobutyric acid, L-alanine, DL-aspartic acid, L-glutamine and L-leucine), 2 amino sugars (N-acetyl-glucosamine and D-glucosamine), 5 carbohydrates (L-arabinose, D-galactose, D-glucose, D-fructose, and D-trehalose), and 5 carboxylic acids (ascorbic acid, citric acid, L-malic acid, protocatechuic acid and oxalic acid). These substrates were chosen to present a cross section of root exudates (Campbell et al., 2003) and microbial components and products (Amelung et al., 2001). A substrate concentration of 8 mg g^{-1} dry soil was used by placing 25 µl of solution in the deep well plate before incubating the soil for 4 hrs at 25°C. Only 2 mg g^{-1}soil of L-leucine and L-glutamine and 0.8 mg g^{-1} soil of protocatechuic acid and DL-aspartic acid were used to due to their low solubility at higher concentrations (Sradnick et al., 2013a). The indicator gel and calibration were carried out as described by Sradnick et al. (2013a). The indicator gel plates were regenerated for reuse by storing them for 36 hours in a plastic box containing soda lime and wet tissue paper. For calibrating the CO_2 trap, five different soils were incubated with and without 8 mg g^{-1} glucose in five replicates each for 4 h at 25°C in the dark, before measuring the CO_2 evolution with a gas chromatograph (Shimadzu) and with the MicroResp^{TM} system. The resulting regression line was fitted to the following power function: µl CO_2 g^{-1} soil = 51 × $(0.2 + ABS)^3$, r = 0.98 where ABS is the difference in absorption (572 nm) between T1 and T0.

4.2.5 Calculation and statistical analysis

The stocks of the soil nutrients and microbial indices at different depths were calculated on a volume basis by taking the bulk density of the respective soil layer into account. Fungal glucosamine was calculated by subtracting bacterial glucosamine from total glucosamine as an index for fungal residues, assuming that muramic acid and glucosamine occur at a 1 to 2 ratio in bacteria (Engelking et al., 2007): fungal C (μg g^{-1} dry weight) = (mmol glucosamine – 2 × mmol muramic acid) × 179.2 $gmol^{-1}$ × 9, where 179.2 is the molecular weight of glucosamine and 9 is the conversion value of fungal glucosamine to fungal C (Appuhn and Joergensen, 2006). Bacterial C (μg g^{-1} dry weight) was calculated as an index for bacterial residues by multiplying the concentration of muramic acid in μg g^{-1} dry weight by 45 (Appuhn and Joergensen, 2006). The values of three sampling points from three blocks replicates of each treatment were used to calculate mean concentrations and stocks (n = 9). The results presented in the figures 1-2 are arithmetic means of treatments in the respective depths (n = 9), given on oven dry basis (105°C, 24 h). Data were checked for normal distribution by Chi-square test. When necessary, the data were ln-transformed. The significance of treatment effect was analysed by a two way-ANOVA using land use and slurry application as independent factors and depth as repeated measurements. Pairwise LSD test ($P < 0.05$) was used to discriminate factor specific treatment pairs. A discriminant analysis (DA) was used to determine whether the substrate-specific respiration could be used to discriminate between the treatments. The Shannon diversity index was calculated according to Zak et al. (1994), where pi is the particular activity of the sum of all activities. All statistical analyses were carried out using SPSS statistical software (SPSS 16.0).

4.3 Results

4.3.1 Land use and slurry effects on mean values and stocks

The mean soil pH at 0-40 cm depth was generally similar in all land use and fertilisation treatments (Table 9). Slurry application had no significant effect on the mean soil bulk density, whereas maize monoculture led to the lowest bulk density in comparison with the grassland soils. The stocks of SOC and total N (Table 9), basal respiration, soil microbial and fungal biomass indices (Table 10) as well as amino sugars and related indices (Table 11) were

significantly lower in the MM treatment in comparison with the grassland treatments. Slurry application generally increased the mean basal respiration and stocks of microbial biomass C and S, and ergosterol, and showed strong positive effects on amino sugars stocks as well (Table 10, 11), but had no main effects on stocks of SOC, total N, or microbial biomass N. The differences between the PG and MG treatments were not significant for bulk density, for soil chemical properties (Table 9) as well as for microbial biomass indices (Table 10) and microbial amino sugars (Table 11), but not always, i.e. the stocks of total N, microbial biomass N and fungal C to bacterial C ratio (Table 12) were significantly higher in the PG in comparison with the MG treatment.

Table 9. Main effects of the factor land use and slurry on mean stocks of soil organic C (SOC), total N and mean soil pH, bulk density and soil C / N ratio at 0–40 cm soil depth.

Treatment	soil pH	Bulk density	SOC	Total N	Soil C/N
		($g\ cm^{-3}$)	($t\ ha^{-1}$)		
Land use					
Permanent grassland	6.5 a	1.7 a	91 a	8.2 a	11.3 a
Modified grassland	6.4 a	1.6 a	80 a	7.1 b	11.4 a
Maize monoculture	6.5 a	1.5 b	65 b	5.6 c	11.7 a
Slurry application					
+ Slurry	6.5 a	1.5 a	78 a	7.0 a	11.4 a
– Slurry	6.4 a	1.6 a	79 a	6.9 a	11.6 a
CV (±%)	3	7	12	12	3

CV = mean coefficient of variation between replicate samples within a plot (n = 3); different letters within a column indicate a factor-specific significant difference between pairs (PLSD test, $p < 0.05$).

Table 10. Main effects of the factor land use and slurry on mean stocks of microbial biomass C, N and S, ergosterol and mean respiration rate at 0–40 cm soil depth.

Treatment	Microbial biomass			Ergosterol	Basal respiration
	C	N	S		
	(kg ha^{-1})				(kg CO_2-C d^{-1} ha^{-1})
Land use					
Permanent grassland	1691 a	221 a	17 a	1.8 a	79 a
Modified grassland	1452 a	168 b	16 a	1.6 b	74 a
Maize monoculture	997 b	134 c	14 b	1.4 c	59 b
Slurry application					
+ Slurry	1414 a	177 a	18 a	1.7 a	74 a
– Slurry	1346 b	172 a	15 b	1.5 b	62 ab
CV (±%)	20	22	21	24	15

CV = mean coefficient of variation between replicate samples within a plot (n = 3); different letters within a column indicate a factor-specific significant difference between pairs (PLSD test, $p < 0.05$).

4.3.2 Soil chemical and microbial properties at different depths

The soil pH and bulk density significantly increased with depth. The contents of ergosterol (Fig. 11a,b), SOC, total N, microbial biomass C and S and as well as those of microbial amino sugars showed a strong decrease with depth in the PG treatment, while this decrease was less pronounced in the MG and MM treatments (Supplementary Tables 7-9). In PG treatments, the highest contents of SOC, microbial biomass C, N and S and ergosterol were found at 0-10 cm depth in comparison with the MG and MM treatments. In contrast, the MG and MM treatments led to significantly higher ergosterol (Fig. 11a,b), SOC, microbial biomass C, N and S contents in the next two layers at 10-20 and 20-30 cm depths (Supplementary Tables 7-9). At 0-10 cm depth, slurry application led to a significant increase in the contents of microbial C, N and S and ergosterol contents roughly by 15% but had no consistent effects on the depth decline of any soil property analysed.

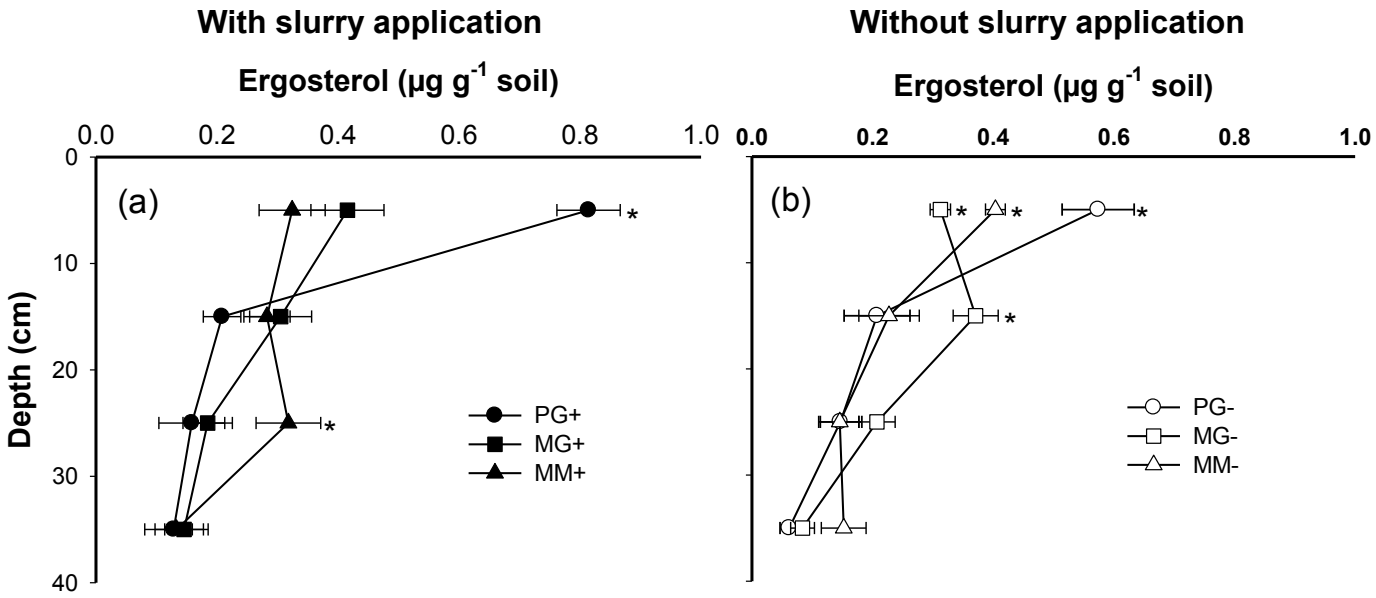

Fig. 11. Mean (a,b) ergosterol content among different land use and slurry treatments (permanent grassland with slurry application (PG+), permanent grassland without slurry application (PG-), (iii) modified grassland with slurry application (MG+), modified grassland without slurry application (MG-), (v) maize monoculture with slurry application (MM+) and (vi) maize monoculture without slurry application (MM-)) and soil depths. * indicates a depth specific difference between treatments ($P < 0.05$, ANOVA repeated measurement); error bars show standard error of mean (n = 9).

The microbial biomass C to SOC ratio strongly decreased with depth in the PG treatment and showed higher values at 10-20 and 20-30 cm depths in MG and MM treatments. The mean microbial biomass C to SOC ratio was significantly 17% higher in the grassland treatments in comparison with MM treatments. The qCO_2 values showed a significant negative linear relationship with the microbial biomass C to SOC ratio (r = -0.40, $P < 0.01$, n = 218) and strong significant positive relationship with the soil C/N ratio (r = 0.60, $P < 0.01$, n = 218). The ergosterol to microbial biomass C ratio declined from values around 0.27% at 0-10 cm depth to minimum values of 0.07% at 20-30 cm depth and increased again to the values of 0.20% at 30-40 cm (Fig. 12a,b). The fungal C to bacterial C ratio increased continuously from values around 2.4 at 0-10 cm depth to values around 2.8 at 20-30 cm depth, followed by a decline to values of the surface layer at 30-40 cm depth (Fig. 12c,d). The mean microbial biomass C/S ratio (+56%) was significantly increased in the grassland treatments compared to the MM treatment

(Table 12). In contrast, the ergosterol to microbial biomass C ratio (-27%) was decreased, whereas the fungal C to bacterial C ratio (+17%) was increased in the grassland treatments compared to the MM treatment (Fig. 12a-d; Table 12). A significant negative linear relationship was observed between the ergosterol to microbial biomass C ratio and the ratios microbial biomass C/S (Fig. 13a) and fungal C to bacterial C (Fig. 13b).

Table 11. Main effects of the factor land use and slurry on mean stocks of amino sugars at 0–40 cm soil depth.

Treatment	MurN	ManN	GalN	Fungal GlcN	Microbial residual C
	(kg ha^{-1})				(t ha^{-1})
Land use					
Permanent grassland	511 a	199 a	3936 a	6235 a	79 a
Modified grassland	464 a	147 a	3158 a	5171 a	67 a
Maize monoculture	335 b	88 b	1959 b	3334 b	45 b
Slurry application					
+ Slurry	464 a	170 a	3398 a	5461 a	70 a
– Slurry	409 b	120 b	2638 b	4366 b	58 b
CV (±%)	20	18	20	19	19

CV = mean coefficient of variation between replicate samples within a plot (n = 3); different letters within a column indicate a factor-specific significant difference between pairs (PLSD test, $p < 0.05$).

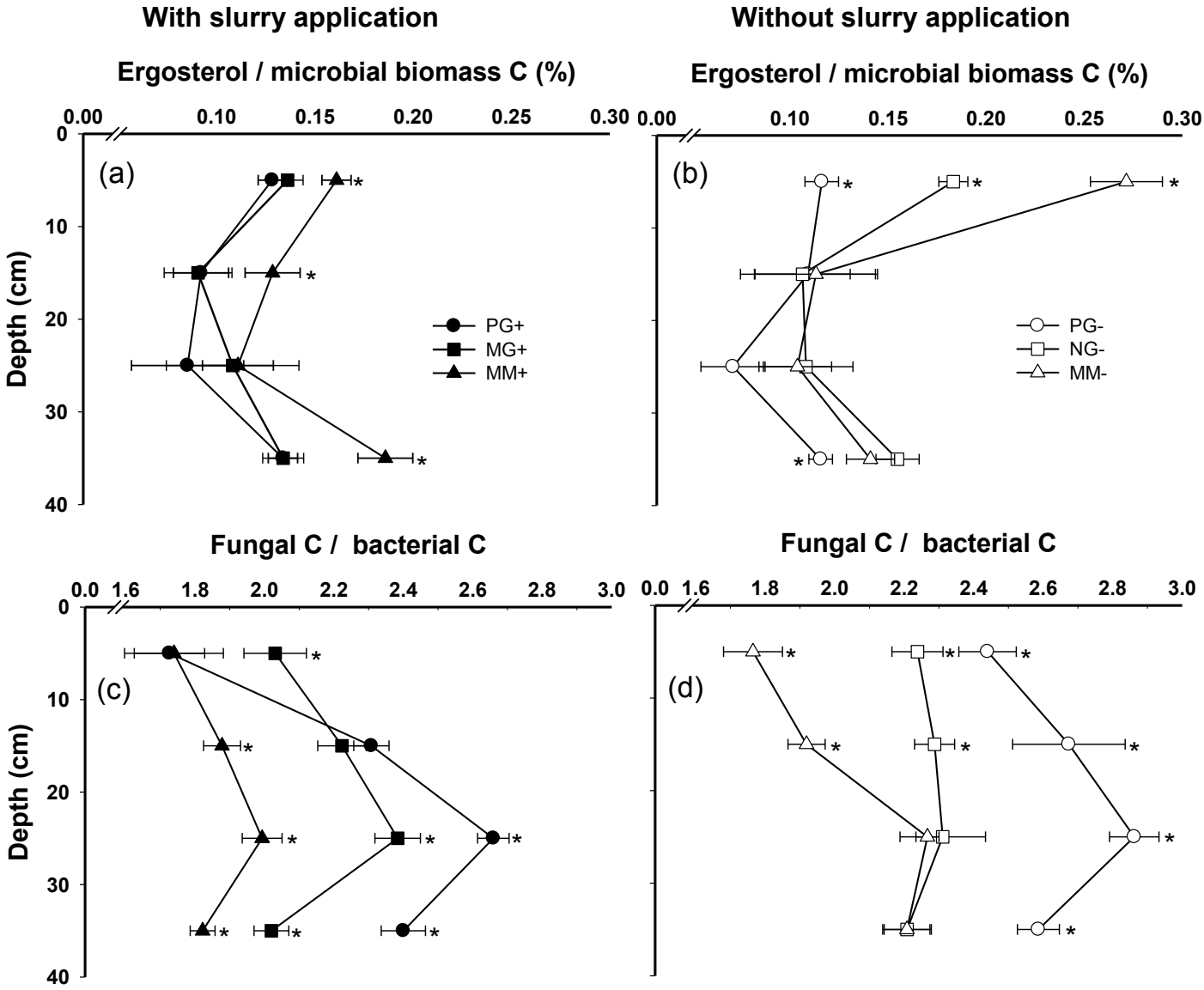

Fig. 12. Mean ratios of (a,b) ergosterol / microbial biomass C (%) and (c,d) fungal C / bacterial C among different land use and slurry treatments (permanent grassland with slurry application (PG+), permanent grassland without slurry application (PG-), (iii) modified grassland with slurry application (MG+), modified grassland without slurry application (MG-), (v) maize monoculture with slurry application (MM+) and (vi) maize monoculture without slurry application (MM-)) and soil depths. * indicates a depth specific difference between treatments ($P < 0.05$, ANOVA repeated measurement); error bars show standard error of mean ($n = 9$).

Table 12. Main effects of the factor land use and slurry on mean values of different microbial community indices at 0–40 cm soil depth.

Treatment	Microbial biomass C / SOC	*q*CO2	Microbial biomass C/N	Microbial biomass C/S	Ergosterol/ Microbial biomass C (%)	Fungal C/ Bacterial C	Microbial residual C / SOC
Land use							
Permanent grassland	1.8 a	54 b	8.1 b	115 a	0.10 b	2.5 a	0.88 a
Modified grassland	1.8 a	65 a	9.4 a	106 a	0.13 ab	2.2 b	0.82 a
Maize monoculture	1.5 b	77 a	8.1 b	71 b	0.16 a	2.0 c	0.69 b
Slurry application							
+ Slurry	1.7 a	69 a	9.3 a	105 a	0.12 b	2.1 b	0.76 b
– Slurry	1.7 a	62 a	7.8 a	90 b	0.14 a	2.3 a	0.84 a
CV (±%)	23	21	21	26	29	6	19

CV = mean coefficient of variation between replicate samples within a plot (n = 3); different letters within a column indicate a factor-specific significant difference between pairs (PLSD test, $p < 0.05$).

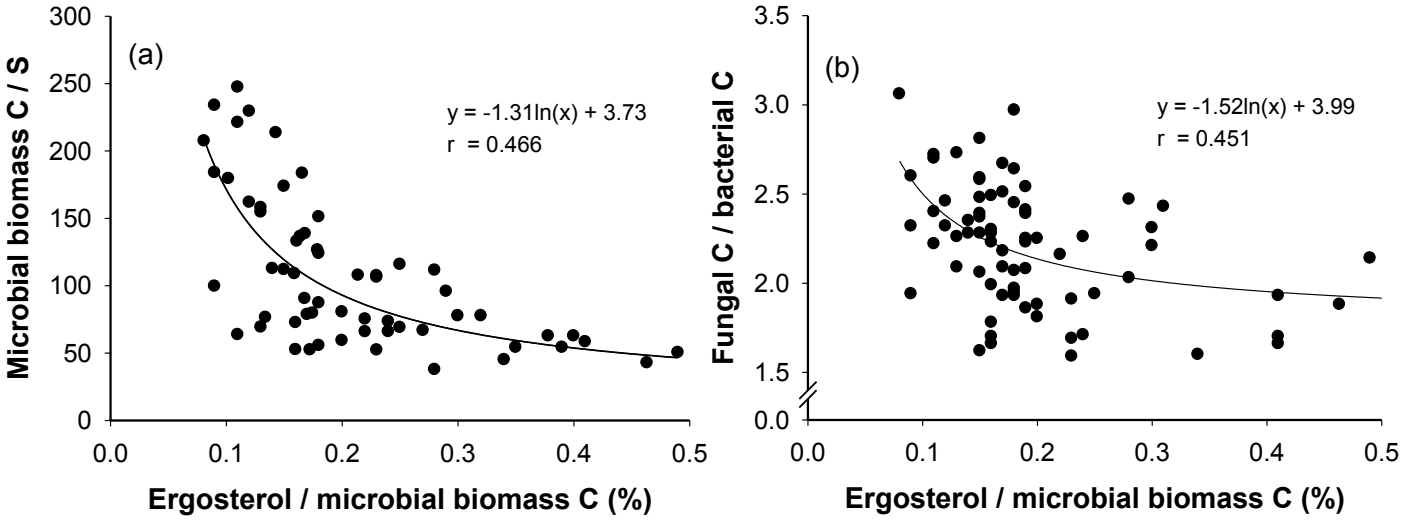

Fig. 13. The relationships between (a) ergosterol / microbial biomass C (%) and microbial biomass C / S and (b) ergosterol / microbial biomass C (%) and fungal C / bacterial C; data from all treatments and field replicates were combined (n = 218).

4.3.3 Microbial functional diversity by CLPP

Discriminant function (DF) analysis significantly (Wilks' Lambda: 0.0014 approx. F = 5.84, $P < 0.001$) separated the PG and MG treatments from the MM treatment and PG+ and MM+ treatments from the PG- and MM- treatments, respectively (Fig. 14). By far, DF1 contributed with the largest proportion of variation (65%), with significant main effects (ANOVA, $P < 0.0001$) for both land use and slurry application. The DF2 explains 22% of the variation and separates the MG+ treatment from MG- treatment and the PG- treatment from the MG- and MM- treatments. The correlation coefficients between the substrate-induced respiration rates revealed that DF1 was mainly caused by L-glutamine, N-acetyl glucosamine, L-arabinose, D-galactose, D-glucose, citric and oxalic acid. The DF2 had higher utilisation of L-alanine, γ-aminobutyric acid, DL-aspartic acid, L-leucine, D-glucosamine, D-trehalose and ascorbic acid (Table 13).

Table 13. Pearson correlation between substrate utilisation of individual substrates and the canonical discriminant function (DF) 1, 2, Shannon index, soil pH and ergosterol to microbial biomass C ratio.

	DF1	DF2	Shannon Index	Soil pH	Ergosterol /microbial biomass C
Aqua	0.12	0.16	-0.07	0.10	0.05
L-alanine	-0.26	0.72**	0.88**	0.53**	-0.52**
γ-aminobutric	-0.22	0.74**	0.79**	0.36*	-0.34*
DL-aspartic acid	-0.17	0.69**	0.74**	0.56**	-0.60**
L-glutamine	-0.34*	0.70**	0.79**	0.50**	-0.37*
L-leusine	-0.07	0.74**	0.70**	0.37*	-0.46**
N-acetyl glucosamine	-0.39*	0.64**	0.88**	0.51**	-0.50**
D-glucosamine	-0.17	0.54**	0.59**	0.64**	-0.23
L-arabinose	-0.48**	0.66**	0.80**	0.56**	-0.37*
D-fructose	-0.22	0.02	0.23	0.02	-0.24
D-galactose	-0.63**	0.63**	0.62**	0.42*	-0.34*
D-glucose	-0.59**	-0.42*	0.05	0.06	0.20
D-Trehalose	-0.17	0.65**	0.69**	0.34*	-0.24
Ascorbic acid	0.32	-0.73**	-0.95**	-0.54**	0.59**
Citric acid	0.59**	-0.45**	-0.62**	-0.39*	-0.01
L-malic acid	-0.08	0.17	-0.03	-0.14	0.24
Protocatechuic acid	-0.04	0.23	0.42*	0.51**	-0.46**
Oxalic acid	0.72**	0.62**	-0.23	-0.07	-0.19

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

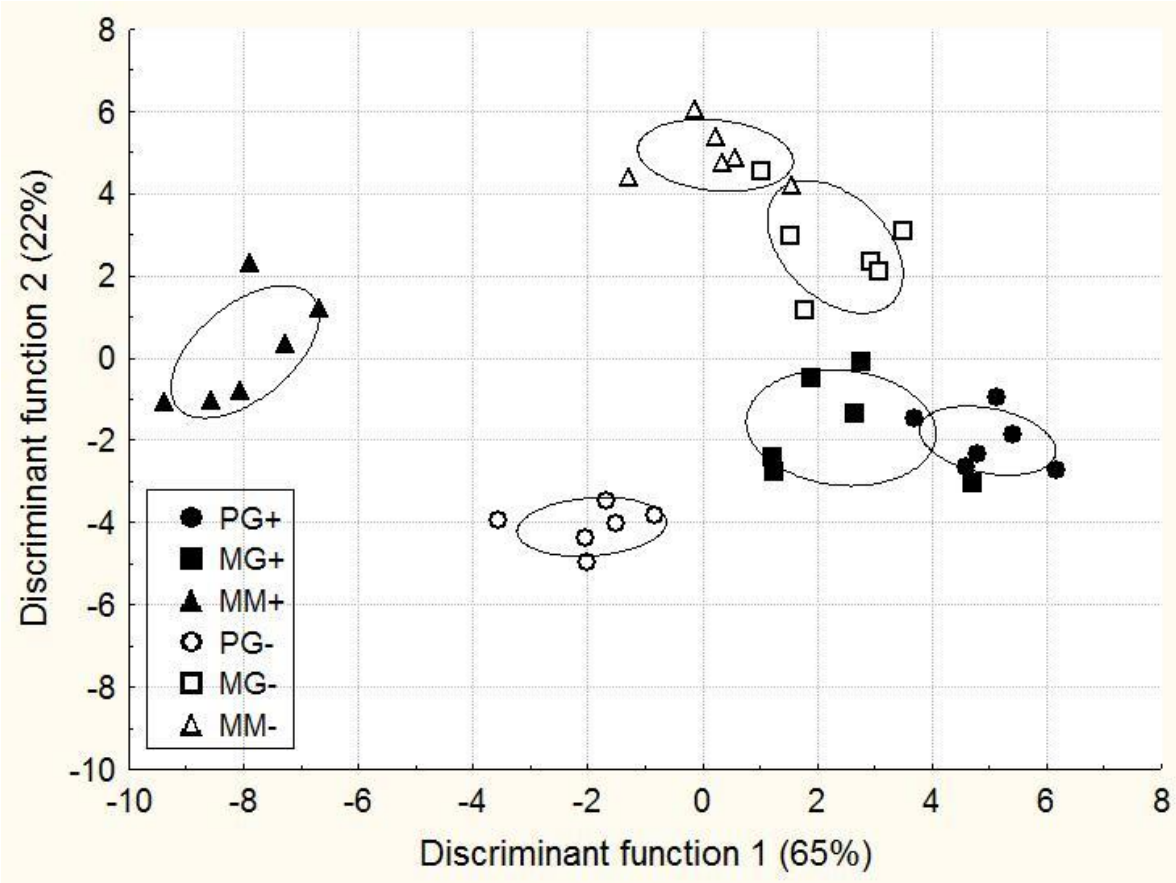

Fig. 14. Discrimination function analysis of catabolic response of 17 substrates plus aqua dest at 0-10 cm soil depth for different land use and slurry treatments (permanent grassland with slurry application (PG+), permanent grassland without slurry application (PG-), (iii) modified grassland with slurry application (MG+), modified grassland without slurry application (MG-), (v) maize monoculture with slurry application (MM+) and (vi) maize monoculture without slurry application (MM-)). The scatter plot shows ellipses with confidence ranges of $\alpha = 0.05$.

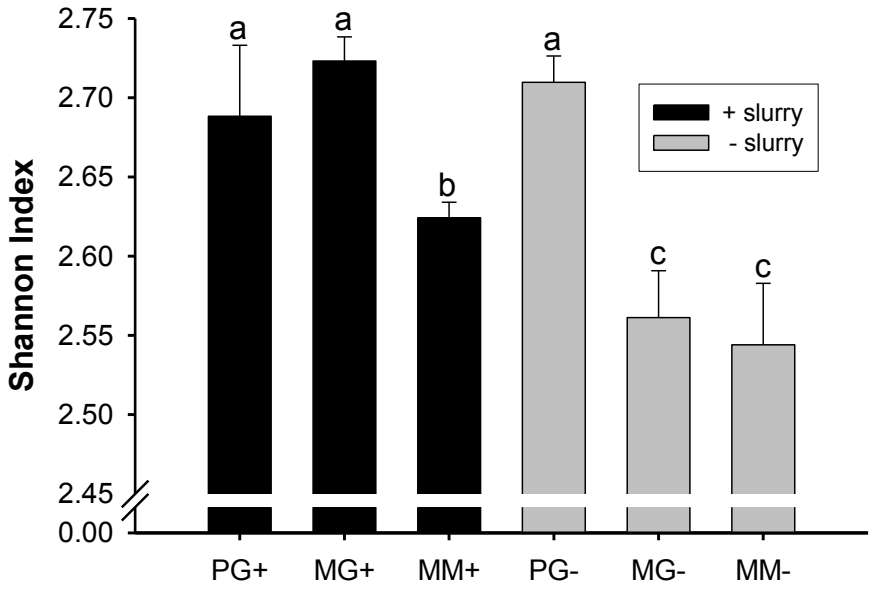

Fig. 15. Response of microbial catabolic diversity profiles (Shannon index) among different land use treatments (permanent grassland = PG, modified grassland = MG, maize monoculture = MM) with and without slurry application. Error bars show standard error of mean (n = 3). Different letters represent significance differences between treatments ($P < 0.01$).

The PG treatment led to a significantly higher catabolic diversity index (Shannon index), while the MM treatments presented the lowest values. Slurry application generally led to a higher catabolic diversity in comparison with the non-slurry treatments, but had no significant differences between the PG+ and PG- treatments (Fig. 15). The carboxylic acids, mainly ascorbic and citric acid negatively correlated with the diversity index. In contrast, positive interactions were obtained between the amino acids, amino sugars and the diversity index (Table 13).

4.4 Discussion

4.4.1 Effects of land use

The MM treatment significantly decreased the stocks of SOC and total soil N by 33% at 0-40 cm depth in comparison with the perennial and new grassland soils. Maize monoculture decreased the stocks of SOC, due to macro-aggregate destruction and lower below ground input than perennial grasses (Ludwig et al., 2003; Bleken et al., 2009). The single tillage event in

the MG treatment significantly decreased the SOC stocks at 0-10 cm, mainly due to a significant loss of SOC content compared to the PG treatment (Supplementary Table 7; Linsler et al., 2013), although they did not differ in the whole soil profile (0-40 cm). This was most likely caused by the destruction of macro-aggregate formation (Vertès et al., 2007; Linsler et al., 2013), death of living biomass and subsequent decomposition after the tillage operation and also by lower root biomass in the modified grasslands than in the permanent pastures (Bolinder et al., 2002; Culman et al., 2010).

The microbial biomass in the surface layer (0-10 cm) of the PG treatment was three times greater than the MG and MM treatments (Supplementary Table 8), which supports the findings of others (Jenkinson and Powlson, 1976; Culman et al., 2010). In contrast, an increased C input by tillage led to a strong increase in microbial biomass C at 10-20 and 20-30 cm depths in the MG and MM treatments compared to the PG treatment (Supplementary Table 8). This indicates greater sensitiveness of microbial biomass than SOC to changes in C input caused by different land use systems (Joergensen and Emmerling, 2006; Murugan et al., 2012b). The ergosterol content (Fig. 11a,b) increased similarly to that of SOC and microbial biomass C. The saprotrophic fungal indicator ergosterol showed a significant positive linear relationship with SOC, which was similarly observed in an arable long-term tillage experiment (Murugan et al., 2012a), indicating strong positive effects of fresh root and harvest residues on the ergosterol concentration in soil (Scheller and Joergensen, 2008; Heinze et al., 2010a).

The grassland treatments led to a strong positive effect on microbial residues as indicated by roughly 75% higher amino sugar stocks in comparison with the MM treatment. The carbon derived through amino sugar based microbial residues (necromass) is considered to be a major contributor (80%) to the SOC sequestration (Liang and Basler, 2011; Miltner et al., 2011). This was evidenced by a significant positive linear relationship between SOC and amino sugar derived microbial residual C found in our study ($r^2 = 0.68$; $P < 0.01$) and other reports (Amelung et al., 2001; Miltner et al., 2011).

4.4.2 Effects of slurry application

The stocks of amino sugars were the most sensitive indicators of slurry application (Sauheitl et al., 2005), as indicated by a significant 27% increase in microbial residual C compared to unfertilised treatments. Slurry application increased stocks of SOC, total N and especially had significant positive effects on microbial biomass C and S (+10%) at 0-40 cm depth. Similarly, application of slurry caused a strong increase in the stocks of bacterial residues, i.e. MurN, which confirms our first hypothesis. The slurry derived organic C, microbial biomass C and MurN might have resulted in some increases in the respective soil fractions (Sauheitl et al., 2005; Rousk and Bååth, 2011). The positive effects of manure application on bacterial C and SOC has been reported in both long (Joergensen et al., 2010; Johansen et al., 2013) and short-term (Murugan et al., 2012b) experiments. Slurry application had a strong negative effect on soil fungi, as indicated by a significant decrease in the ergosterol to microbial biomass C ratio and the fungal C to bacterial C ratio, which is in agreement with our grassland restoration experiment (Murugan et al., 2012b). A likely reason is differences in the availability of labile organic substrates due to slurry addition (Rousk and Bååth, 2011). The response of soils treated with dairy manure promoted Gram-negative bacteria, which grow faster on labile organic substrates in the manure than fungi (Grayston et al., 2004; Johansen et al., 2013).

4.4.3 Microbial community indices as affected by land use

The cell-membrane component ergosterol is an important indicator for saprotrophic fungi (Joergensen and Wichern, 2008), but does not occur in AMF (Olsson et al., 2003). The fungal biomass indicator ergosterol showed significant non-linear relationships with amino sugar-based fungal C ($r = 0.45$, $P < 0.01$, $n = 218$) and microbial biomass S ($r = 0.47$, $P < 0.01$, $n = 218$), which has been found in a long-term tillage (Murugan et al., 2012a) and fertilisation experiment on a highly sandy soil (Heinze et al., 2010a), but not in two tillage experiments on silt loams (Heinze et al., 2010b). A striking feature of this experiment was the greater accumulation of saprotrophic fungi in the MM treatment compared to the grassland treatments, as indicated by the 27% higher ergosterol to microbial biomass C ratio and 56% lower microbial biomass C/S ratio, which contradict our second hypothesis. The saprotrophic fungi can able to store six times

higher S concentration in their biomass with increasing S supply compared to bacteria (Banerjee and Chapman, 1996). In addition, a strong increase in the ergosterol to microbial biomass C ratio in the subsoil layer (30-40 cm) was observed in the MM treatment. A likely explanation is a shift in the microbial community structure towards saprotrophic fungi at the expense of ergosterol-free arbuscular mycorrhizal fungi (AMF) (Joergensen and Wichern, 2008), due to an increased supply of fresh root and harvest residues by mouldboard plough (Scheller and Joergensen, 2008; Heinze et al., 2010a; Murugan et al., 2012a). Our results suggest that the relationship between the ratios ergosterol to microbial biomass C and microbial biomass C/S can be used as an additional indicator of a shift in microbial community.

The higher fungal C to bacterial C ratio and lower ergosterol to microbial biomass C ratio in grassland treatments compared to the MM treatment suggests a shift in fungal community structure towards AMF, which do not contain ergosterol but glucosamine (Olsson et al., 2003; Joergensen and Wichern, 2008). Similarly, our previous long-term tillage experiment showed that the proliferation of AMF is at the expense of saprotrophic fungi in the subsoil layer at 30-40 cm depth (Murugan et al., 2012a). The fungal C to bacterial C ratio was the only microbial index to show a significant difference between the PG and MG treatments (t-test, $P > 0.01$). The absence of a white clover population in the PG treatments might have caused a greater accumulation of fungal residues (presumably AMF), as indicated by the significant 14% higher fungal C to bacterial C ratio in the PG treatments compared to the MG treatment (Denef et al., 2009; De Vries et al., 2009; Walsh et al., 2012). The lower metabolic quotients in the PG treatment compared to the MG and MM treatments may partly explain the larger contribution of biotrophic AMF to the microbial residues with increasing fungal C to bacterial C ratio (Sakamoto and Oba, 1994). Due to the denser root system, the perennial ryegrass soils contained larger hyphal lengths of mycorrhizal fungi per gram of soil, although the mycorrhizal infection per root length was higher in white clover (Tisdall and Oades, 1979; De Vries et al., 2009). The intensive management, including tillage, fertilisation and addition of white clover, are known to adversely affect AMF in grassland and arable soils (Kabir et al., 1998; Mäder et al., 2000; De Vries et al., 2009). Although not measured here, AMF can be measured by quantifying the PLFA 16:1ω5, but this PLFA also occurs in Gram-negative bacteria (Zelles, 1999). The neutral lipid fatty acid (NLFA) 16:1ω5 is mainly present in AM fungal spores (Olsson, 1999).

Thus, a combination of several methods is needed for a complete picture. The PLFA and NLFA biomarkers are a more specific indicator for the presence, but not always the biomass of saprotrophic and AM fungi (Olsson, 1999). And they are not as effective as previously thought for discriminating saprotrophic fungi and biotrophic AMF (Joergensen and Wichern, 2008; De Vries et al., 2012). Quantitative information on exact biomass estimates of these two important fungal groups in soil is inevitably necessary to understand their different roles in SOM dynamics.

4.4.4 Response of microbial functional diversity to land use and slurry application

The CLPP of the microbial communities between the grassland treatments and the MM treatment showed clear discrimination (DF1), mainly based on carbohydrates and carboxylic acids. Discrimination analysis (DF2) showed that the mineralization of amino acids and amino sugars was mainly caused by the differences between slurry and non-slurry treatments. This suggests that the higher labile C present in the grassland and slurry treatments promoted bacterial residues compared to the MM and non-slurry treatments, respectively. Carbohydrates and carboxylic acids were discriminated different land use and grassland types (Grayston et al., 2004; Stevenson et al., 2004). Similarly, an increased utilisation of various amino acids and amino sugars showed them to be sensitive indicators to discriminate different fertilisation strategies (Romaniouk et al., 2011; Sradnick et al., 2013). The differences between CLPP of the PG- and MG- treatments indicate that crop species diversity plays an important role in C utilisation by microbial communities, which supports our third hypothesis. The only other difference between the PG and MG treatments was a lower fungal C to bacterial C ratio in the MG treatments that contain a grass–clover mixture. This is probably caused by the lower AMF growth and biomass, which is characteristic of soils with grass-clover mixture (De Vries et al., 2009; Walsh et al., 2012). Variation in microbial diversity between fertilisation treatments may also be caused by differences in pH, as indicated by the positive correlation between DF2, pH and a negative one with the ergosterol to microbial biomass C ratio. The relatively higher pH and soil fertility of the slurry treatments favoured bacterial growth compared to the non-slurry treatments (Grayston et al., 2004; Sradnick et al., 2013), as indicated by higher bacterial residues and lower saprotrophic fungi accumulation.

The higher catabolic diversity of grassland treatments compared to the MM treatment is most likely caused by the presence of heterogenic organic matter, which leads to the development of a more complex microbial community for decomposition (Mäder et al., 2000; Grayston et al., 2004). In general, application of slurry increased the catabolic diversity compared to unfertilised soils, which has been reported previously (Mäder et al., 2000; Romaniuk et al., 2011). The catabolic diversity of soils in the PG- and MG- treatments showed significant differences, but not between the PG+ and MG+ treatments, which confirms our fourth hypothesis that the lost microbial diversity due to tillage and maize monoculture can be restored by slurry application in arable and grassland soils. The higher microbial diversity under grassland and slurry applied soils may be ascribed to an increased soil fertility compared to arable land and unfertilised soils, respectively (Degens et al., 2000).

4.5 Conclusions

The rush into bioenergy production by maize monoculture decreased the stocks of SOC, especially microbial biomass and residues in comparison to the grassland treatments. The MM treatment caused a shift in the microbial community structure towards saprotrophic fungi, as indicated by the inverse changes in the microbial biomass C/S ratio and the ergosterol to microbial biomass C ratio. Thus, the lower microbial biomass C/S ratio in the MM treatment led to greater accumulation of saprotrophic fungi compared to the grassland treatments. In contrast, the PG treatment promoted ergosterol-free arbuscular mycorrhizal fungi as indicated by higher fungal C to bacterial C ratio and lower ergosterol to microbial biomass C ratio. The increase in the stocks of microbial biomass and amino sugars were the most sensitive indicators of slurry application. The functional diversity diminished in all the cases under the MM treatment compared to the undisturbed PG treatment. We conclude that the lost microbial diversity due to tillage and the booming maize monoculture can be restored by slurry application.

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5 General conclusions

The combined analyses of microbial biomass and residue formation of both fungi and bacteria applied to soil samples of the present three field experiments enhanced the understanding the contribution of microbial community to SOM regulation under different long-term tillage, grassland conversion and fertilisation experiments. The striking feature of the present results is the close relationship between the composition of microbial residues and the living fraction in soil. The significant correlations between the ratios, ergosterol to microbial biomass C and fungal C to bacterial C indicates a link between saprotrophic fungi and biotrophic AMF and their role in SOM dynamics.

1. A reduction in tillage intensity by the GRT and NT treatments increased stocks of soil organic matter and especially microbial biomass in comparison with the MBT treatment. In contrast to the ergosterol to microbial biomass C ratio, the ratio of fungal C to bacterial C showed a significant negative linear relationship with SOC, despite the general positive interrelationships between the three fungal indices i.e. microbial biomass S, ergosterol and fungal C. This is mainly due to the strong increase in the fungal C to bacterial C ratio combined with a strong increase in the soil C/N ratio at 30-40 cm depth in the GRT and NT treatments and the relatively low values of this ratio at 0-5 cm depth in all treatments. The negative relationship between the ergosterol to microbial biomass C ratio and the fungal C to bacterial C ratio points to the importance of the relationship between saprotrophic fungi and biotrophic AMF for tillage-induced changes in microbial turnover of SOC.

2. One-season cultivation of winter wheat with two tillage events led to a significant loss in the stocks of SOC and microbial biomass C at 0-40 cm depth in comparison with the permanent grassland, not only in the short term, but also in the long term. However, the tillage induced loss in microbial biomass C was roughly 40% less in the long term than in the short of the current experiment, indicating a recovery process during grassland restoration. Tillage promoted saprotrophic fungi at the expense of biotrophic AMF and bacteria as indicated by the increased ergosterol to microbial biomass C ratio and the constant fungal C to bacterial C ratio. Slurry application generally reduced the presence of saprotrophic fungi and decreased the fungal C to bacterial C ratio. The strong positive effects of slurry application on the formation of microbial

residues after tillage were not reflected by significant increases in the stocks of SOC and total N during the present experimental periods. These results indicate that the negative effects of one-season tillage on soil microorganisms and C sequestration can be restored by slurry application.

3. The rush into bioenergy production by maize monoculture decreased the stocks of SOC, especially microbial biomass and residues, consequently led to a shift in the microbial community structure towards saprotrophic fungi, as indicated by the lower microbial biomass C to microbial biomass S ratio and higher ergosterol to microbial biomass C ratio in comparison to the grassland treatments. In contrast, significant increase in the fungal C to bacterial C ratio and decrease in the ergosterol to microbial biomass C ratio indicates greater accumulation of ergosterol-free biotrophic AMF in grassland treatments compared to the maize monoculture treatment. The increase in the stocks of microbial biomass and amino sugars were the most sensitive indicators of slurry application. Slurry application promoted bacterial residues as indicated by the decreases in both, the ergosterol to microbial biomass C ratio and the fungal C to bacterial C ratio. Our results showed that the lost microbial functional diversity due to tillage and maize monoculture was restored by slurry application both in arable and grassland soils.

A reduction in tillage intensity, permanent grassland and slurry application increased stocks of SOM and especially microbial biomass in comparison to the compromising treatments. The microbial biomass C to microbial biomass S ratio can be used as an additional indicator for a shift in microbial community. Slurry application generally increased bacterial residues, as indicated by the decreases in both, the ergosterol to microbial biomass C ratio and the fungal C to bacterial C ratio. The close relationships between microbial biomass and necromass indices points to the differential role of saprotrophic fungi and biotrophic AMF in SOC sequestration in top soil and subsoil layers.

6 Outlook

The striking feature of this study is the close relationships between microbial biomass and necromass indices points to the differential role of saprotrophic fungi and biotrophic AMF in SOC sequestration at top soil and subsoil layers. It is more difficult to explain the low fungal C to bacterial C ratio at 0-5 cm depth in the presence of the highest ergosterol to microbial biomass C ratios. The reverse could be easily explained by the difference in the fungal community structure, as AMF do not contain ergosterol but glucosamine. Generally, the ergosterol to microbial biomass C ratio showed negative linear relationship with fungal C to bacterial C ratio but exhibited a positive correlation in the second experiment. This could be attributed to the fact that both these ratios were characterized by an extraordinarily high variability, contrasting all other ratios. Differences within the fungal community structure may partly explain such variation under heterogeneous soil conditions.

One way to obtain more accurate information is to use amino-sugar specific $\delta^{13}C$ analysis in the range of natural abundance. The fractionation of $\delta^{13}C$ sometimes observed between different fungal tissues, organic substrates and soil organic matter might give information on differences in the turnover of fungal and bacterial cell wall residues. Although not measured here, AMF can be measured by quantifying the PLFA 16:1ω5, although this PLFA also occurs in Gram-negative bacteria (Zelles, 1999). The neutral lipid fatty acid (NLFA) 16:1ω5, which is mainly present in AM fungal spores (Olsson, 1999). Thus, a combination of several methods is needed is needed to estimate the real contribution of AMF to the microbial biomass, which should be a focus of future research. The PLFA and NLFA biomarkers are a more specific indicator for the presence, but not always the biomass of saprotrophic and AM fungi (Olsson, 1999). And they are not as effective as previously thought to discriminate saprotrophic fungi and biotrophic AMF (Joergensen and Wichern, 2008; De Vries et al., 2012). Shifts within the soil fungal community, especially from biotrophic mycorrhizal fungi to saprotrophic fungi apparently have a stronger influence on soil biological processes than a general shift from fungi-to-bacteria (Joergensen and Wichern, 2008; Strickland and Rousk, 2010). Quantitative information on exact biomass estimates of these two important fungal groups in soil is inevitably necessary to understand their different roles in SOM dynamics.

The site effects on soil chemical and biological properties often exceeded those of the tillage treatments, with numerous significant interactions, which suggest that the tillage effects varied often in a site-specific way. The strong site effects of the current research are in agreement with Heinze et al. (2010a). However, in contrast to this experiment, the maximum site effects at the site Friemar can be explained by a combination of the following four site factors: (1) highest clay content, (2) lowest mean temperature due to the highest altitude, (3) lowest annual mean precipitation, (4) and the highest soil pH in comparison to the other three sites. However, the absence of small-scale block effects for most of the properties analysed suggest that climatic effects might be more important, especially in combination with differences in management by different farmers. A considerably larger number of sites would be necessary to assess the relative importance of one specific factor.

Once again in the second experiment, in some cases differences in soil properties might have influenced the relationships between the microbial community indicators. The higher clay content and the higher soil pH in the first trial led generally to lower ergosterol to microbial biomass C ratios, lower fungal C to bacterial C ratios, but also to higher microbial biomass C/N ratios and metabolic quotients. Differences in clay and silt contents between soils are likely to create a range of soil environments (e.g. soil moisture and temperature) and strongly mediate microbial community composition (Frey et al., 1999; Amelung et al., 2002; Jastrow et al., 2007; Neumann et al., 2013).

As bacteria, saprotrophic fungi and biotrophic AMF do have distinct functional roles in SOM dynamics, a more robust understanding of the specific effects of agricultural management and edaphic factors on these microbial groups will shed more light to predict the specific effects of tillage, land use change and fertilisation on the contribution of those microbial groups in SOC sequestration.

7 Supplementary materials

Supplementary Table 1a. Physical and chemical characteristics of three tillage treatments from different sites and soil depths

Site	Treatment	Depth (cm)	Bulk density (g cm^{-3})	SOC (mg g^{-1})	Total N (mg g^{-1})	Total P (mg g^{-1})	Total S (mg g^{-1})
Friemar	Mouldboard	0-5	1.1	13.9	1.5	0.62	0.26
		5-10	1.4	14.2	1.6	0.63	0.27
		10-15	1.3	14.9	1.6	0.65	0.27
		15-20	1.3	14.2	1.6	0.62	0.26
		20-30	1.3	11.9	1.4	0.60	0.24
		30-40	1.4	6.8	1.0	0.48	0.20
	Grubber	0-5	1.0	21.5	2.3	0.69	0.36
		5-10	1.1	19.0	2.1	0.61	0.31
		10-15	1.2	15.2	1.6	0.59	0.29
		15-20	1.5	13.2	1.5	0.58	0.26
		20-30	1.5	12.4	1.4	0.57	0.26
		30-40	1.4	9.0	1.1	0.47	0.18
	No tillage	0-5	1.1	24.1	2.5	0.71	0.38
		5-10	1.3	16.8	1.7	0.57	0.29
		10-15	1.4	14.3	1.5	0.55	0.26
		15-20	1.5	13.3	1.4	0.56	0.25
		20-30	1.5	11.9	1.4	0.54	0.25
		30-40	1.4	6.3	0.8	0.41	0.17
Grombach	Mouldboard	0-5	1.2	11.9	1.0	0.68	0.19
		5-10	1.2	10.8	1.0	0.68	0.19
		10-15	1.3	10.8	1.0	0.68	0.24
		15-20	1.3	10.4	1.0	0.67	0.19
		20-30	1.4	10.5	1.0	0.69	0.20
		30-40	1.5	8.6	1.0	0.65	0.22
	Grubber	0-5	1.0	18.9	2.0	0.91	0.32
		5-10	1.2	16.3	1.7	0.86	0.30
		10-15	1.3	13.9	1.6	0.84	0.31
		15-20	1.6	11.7	1.4	0.85	0.30
		20-30	1.6	9.5	1.4	0.79	0.31
		30-40	1.5	5.6	1.4	0.69	0.33
	No tillage	0-5	1.1	18.5	1.9	0.83	0.28
		5-10	1.3	14.9	1.5	0.78	0.24
		10-15	1.4	11.6	1.2	0.70	0.22
		15-20	1.6	11.0	1.2	0.74	0.22
		20-30	1.6	8.8	1.1	0.70	0.24
		30-40	1.5	5.1	1.2	0.60	0.26

Supplementary Table 1b. Physical and chemical characteristics of three tillage treatments from different sites and soil depths.

Site	Treatment	Depth (cm)	Bulk density (g cm^{-3})	SOC (mg g^{-1})	Total N (mg g^{-1})	Total P (mg g^{-1})	Total S (mg g^{-1})
Lüttewitz	Mouldboard	0-5	1.3	12.7	1.3	0.58	0.21
		5-10	1.3	11.8	1.2	0.60	0.21
		10-15	1.2	12.0	1.2	0.58	0.21
		15-20	1.2	12.0	1.3	0.58	0.21
		20-30	1.2	11.6	1.2	0.58	0.21
		30-40	1.3	7.1	0.8	0.45	0.13
	Grubber	0-5	1.1	17.8	1.7	0.50	0.24
		5-10	1.3	16.4	1.6	0.49	0.23
		10-15	1.2	11.5	1.1	0.46	0.19
		15-20	1.5	9.7	1.0	0.46	0.17
		20-30	1.4	9.0	0.9	0.46	0.16
		30-40	1.5	5.8	0.6	0.38	0.11
	No tillage	0-5	1.2	16.8	1.7	0.51	0.23
		5-10	1.4	13.0	1.3	0.46	0.21
		10-15	1.3	9.1	0.9	0.44	0.16
		15-20	1.5	8.8	0.9	0.50	0.16
		20-30	1.5	8.2	0.8	0.44	0.18
		30-40	1.5	4.1	0.5	0.29	0.08
Zschortau	Mouldboard	0-5	1.3	10.5	1.1	0.39	0.20
		5-10	1.3	10.4	1.1	0.39	0.20
		10-15	1.2	10.4	1.1	0.38	0.20
		15-20	1.3	10.9	1.2	0.37	0.20
		20-30	1.3	10.4	1.1	0.38	0.19
		30-40	1.4	7.6	0.8	0.31	0.15
	Grubber	0-5	1.2	13.6	1.4	0.43	0.23
		5-10	1.3	13.3	1.4	0.44	0.23
		10-15	1.2	13.0	1.4	0.43	0.23
		15-20	1.3	10.6	1.1	0.42	0.20
		20-30	1.4	8.1	0.9	0.39	0.17
		30-40	1.5	5.6	0.6	0.30	0.14
	No tillage	0-5	1.1	16.0	1.7	0.42	0.25
		5-10	1.3	14.3	1.5	0.43	0.24
		10-15	1.2	10.9	1.2	0.42	0.21
		15-20	1.5	9.3	1.0	0.42	0.19
		20-30	1.5	8.4	0.9	0.38	0.17
		30-40	1.6	4.8	0.5	0.27	0.10

Supplementary Table 2a. The effects of different tillage treatments on mean contents of basal respiration, microbial biomass C, N and S, and ergosterol in soils from different sites and soil depths.

Site	Treatment	Depth	Basal respiration	Microbial biomass			Ergosterol
		(cm)	CO_2-C (µg g^{-1} d^{-1})	C (µg g^{-1})	N (µg g^{-1})	S (µg g^{-1})	(µg g^{-1})
Friemar	Mouldboard	0-5	9.5	289	36	9.4	0.89
		5-10	9.8	276	38	8.8	0.86
		10-15	12.4	332	47	10.3	1.05
		15-20	10.7	272	37	8.8	0.88
		20-30	10.4	208	27	6.7	0.53
		30-40	7.1	128	16	4.3	0.11
	Grubber	0-5	11.6	517	42	10.5	2.90
		5-10	11.4	455	124	9.9	2.00
		10-15	4.9	272	68	8.9	0.97
		15-20	3.0	187	49	7.6	0.40
		20-30	2.1	166	49	6.1	0.31
		30-40	2.0	89	8	5.4	0.07
	No tillage	0-5	15.8	744	87	13.8	2.78
		5-10	6.6	387	52	9.9	0.89
		10-15	4.7	266	36	7.6	0.52
		15-20	3.9	226	29	7.3	0.38
		20-30	3.8	184	23	5.9	0.28
		30-40	4.3	84	11	5.0	0.09
Grombach	Mouldboard	0-5	3.6	208	8	9.3	0.48
		5-10	3.4	216	18	8.2	0.37
		10-15	3.3	220	22	7.9	0.39
		15-20	3.9	220	22	8.3	0.35
		20-30	3.7	222	23	7.6	0.36
		30-40	3.4	156	17	5.9	0.30
	Grubber	0-5	8.7	539	71	11.0	1.55
		5-10	7.1	429	57	10.4	0.86
		10-15	6.3	352	47	9.2	0.43
		15-20	6.5	235	34	5.8	0.26
		20-30	6.3	184	26	4.6	0.14
		30-40	5.9	68	10	3.6	0.03
	No tillage	0-5	6.1	419	40	10.8	1.84
		5-10	3.1	250	29	8.2	0.52
		10-15	2.8	178	20	7.1	0.24
		15-20	3.1	153	19	6.0	0.18
		20-30	3.0	119	17	5.5	0.10
		30-40	2.1	47	8	3.6	0.03

Supplementary Table 2b. The effects of different tillage treatments on mean contents of basal respiration, microbial biomass C, N and S, and ergosterol in soils from different sites and soil depths.

Site	Treatment	Depth	Basal respiration	Microbial biomass			Ergosterol
		(cm)	CO_2-C (µg g^{-1} d^{-1})	C (µg g^{-1})	N (µg g^{-1})	S (µg g^{-1})	(µg g^{-1})
Lüttewitz	Mouldboard	0-5	4.9	247	28	7.5	0.45
		5-10	4.7	235	42	7.2	0.44
		10-15	6.0	255	47	7.3	0.42
		15-20	5.6	246	46	7.6	0.50
		20-30	6.1	235	41	7.4	0.48
		30-40	3.6	96	17	3.6	0.12
	Grubber	0-5	8.1	447	52	11.0	1.76
		5-10	8.3	415	75	9.0	1.07
		10-15	4.4	221	39	8.0	0.44
		15-20	3.2	151	24	6.7	0.26
		20-30	3.1	130	20	6.2	0.24
		30-40	2.4	69	11	5.6	0.10
	No tillage	0-5	8.1	459	61	12.6	1.31
		5-10	4.6	331	60	8.0	0.96
		10-15	2.2	157	26	6.9	0.28
		15-20	1.7	140	22	5.0	0.24
		20-30	2.0	126	19	3.8	0.16
		30-40	2.0	38	7	3.2	0.07
Zschortau	Mouldboard	0-5	2.4	183	24	5.3	0.50
		5-10	3.3	202	34	5.1	0.45
		10-15	4.1	206	34	6.0	0.54
		15-20	7.4	246	43	8.8	0.68
		20-30	4.8	198	34	7.4	0.51
		30-40	3.1	116	18	3.0	0.29
	Grubber	0-5	6.2	389	44	11.5	1.02
		5-10	8.5	430	66	10.1	1.16
		10-15	7.2	339	51	9.2	0.90
		15-20	4.8	222	33	3.7	0.59
		20-30	3.5	165	22	2.6	0.30
		30-40	2.8	106	10	1.1	0.11
	No tillage	0-5	9.3	476	65	7.9	1.26
		5-10	7.2	379	52	6.4	0.83
		10-15	5.2	251	34	4.9	0.51
		15-20	3.9	194	24	4.1	0.36
		20-30	2.6	159	19	3.3	0.28
		30-40	2.1	93	9	2.7	0.09

Supplementary Table 3a. The effect of different tillage treatments on the distribution of soil amino sugar contents at varying soil depths and investigation sites.

Site	Treatment	Depth (cm)	MurN (µg g^{-1})	ManN (µg g^{-1})	GalN (µg g^{-1})	GluN (µg g^{-1})
Friemar	Mouldboard	0-5	89	37	604	1054
		5-10	95	43	645	1098
		10-15	90	35	577	1007
		15-20	98	43	663	1124
		20-30	74	36	501	837
		30-40	35	20	283	421
	Grubber	0-5	130	45	861	1431
		5-10	98	38	749	1249
		10-15	76	34	624	1016
		15-20	76	36	622	963
		20-30	79	45	685	1021
		30-40	62	45	533	720
	No tillage	0-5	117	33	797	1325
		5-10	101	37	728	1184
		10-15	82	41	598	941
		15-20	79	42	570	906
		20-30	64	27	497	797
		30-40	29	18	263	378
Grombach	Mouldboard	0-5	69	28	492	862
		5-10	65	25	491	839
		10-15	63	25	488	830
		15-20	59	26	446	764
		20-30	54	20	445	740
		30-40	39	18	326	313
	Grubber	0-5	81	21	548	894
		5-10	71	24	526	844
		10-15	57	23	446	702
		15-20	48	19	393	600
		20-30	60	20	430	652
		30-40	24	15	210	358
	No tillage	0-5	110	27	795	1344
		5-10	79	26	592	1013
		10-15	52	21	406	677
		15-20	57	23	428	724
		20-30	44	20	346	577
		30-40	17	14	153	272

Supplementary Table 3b. The effect of different tillage treatments on the distribution of soil amino sugar contents at varying soil depths and investigation sites.

Site	Treatment	Depth (cm)	MurN (µg g^{-1})	ManN (µg g^{-1})	GalN (µg g^{-1})	GluN (µg g^{-1})
Lüttewitz	Mouldboard	0-5	46	20	349	589
		5-10	47	20	371	621
		10-15	51	21	387	693
		15-20	51	21	388	653
		20-30	48	22	429	698
		30-40	26	16	233	397
	Grubber	0-5	72	17	528	996
		5-10	76	23	592	1101
		10-15	55	19	433	785
		15-20	40	14	339	586
		20-30	37	12	302	542
		30-40	20	10	180	340
	No tillage	0-5	77	19	644	1062
		5-10	65	21	588	939
		10-15	38	16	371	586
		15-20	33	15	338	522
		20-30	33	16	270	498
		30-40	15	10	137	256
Zschortau	Mouldboard	0-5	54	14	358	716
		5-10	54	14	366	728
		10-15	47	12	322	644
		15-20	49	16	345	696
		20-30	50	17	352	697
		30-40	41	20	305	569
	Grubber	0-5	79	19	460	906
		5-10	73	18	426	849
		10-15	75	19	430	849
		15-20	64	17	401	770
		20-30	46	15	301	558
		30-40	22	10	150	315
	No tillage	0-5	85	17	577	1018
		5-10	75	14	541	942
		10-15	58	12	409	722
		15-20	50	14	339	598
		20-30	46	11	316	553
		30-40	24	9	177	323

Supplementary Table 4. Mean contents of soil organic C (SOC), total N and mean soil pH, bulk density and soil C / N ratio among different treatments and soil depth profiles in a permanent grassland from a tillage trial 2 and 5 years before sampling

Treatment	Depth (cm)	Soil pH	Bulk density (kg m^{-3})	SOC (mg g^{-1})	Total N (mg g^{-1})	soil C/N
Tillage trial 2 years before sampling						
P2+	0-5	6.6	1.17	27	2.4	11.4
	5-10	5.7	1.33	18	1.7	11.0
	15-20	6.1	1.43	13	1.2	10.9
	20-30	6.2	1.47	11	1.0	11.1
	30-40	6.4	1.56	7	0.6	11.9
P2-	0-5	5.8	1.27	23	2.0	11.5
	5-10	5.5	1.39	16	1.4	11.1
	15-20	6.0	1.41	12	1.1	10.8
	20-30	6.2	1.41	11	1.0	11.1
	30-40	6.4	1.49	8	0.7	11.9
R2+	0-5	6.8	1.33	17	1.5	11.0
	5-10	5.7	1.38	16	1.5	10.9
	15-20	6.0	1.35	15	1.4	10.8
	20-30	6.2	1.43	12	1.1	11.0
	30-40	6.3	1.50	7	0.6	12.2
R2-	0-5	5.9	1.38	16	1.4	11.0
	5-10	6.0	1.33	15	1.4	10.9
	15-20	6.2	1.35	15	1.4	10.8
	20-30	6.4	1.49	10	0.9	11.4
	30-40	6.4	1.54	6	0.5	12.3
Tillage trial 5 years before sampling						
P5+	0-5	6.0	1.09	28	2.5	11.1
	5-10	6.0	1.36	16	1.6	10.4
	15-20	6.4	1.50	13	1.2	10.5
	20-30	7.1	1.52	12	1.1	10.8
	30-40	6.7	1.52	8	0.7	11.6
P5-	0-5	6.1	1.08	30	2.7	11.3
	5-10	6.0	1.35	17	1.6	10.4
	15-20	6.4	1.48	13	1.3	10.4
	20-30	7.0	1.48	12	1.1	10.6
	30-40	6.6	1.51	7	0.7	11.2
R5+	0-5	6.6	1.16	26	2.3	11.2
	5-10	6.0	1.46	15	1.4	10.6
	15-20	6.2	1.47	14	1.3	10.7
	20-30	6.8	1.54	9	0.8	11.3
	30-40	6.5	1.64	4	0.3	12.1
R5-	0-5	5.5	1.27	20	1.8	11.3
	5-10	5.6	1.43	15	1.4	10.7
	15-20	5.7	1.46	14	1.3	10.6
	20-30	6.7	1.52	11	1.0	10.9
	30-40	6.2	1.61	5	0.4	12.1

Supplementary Table 5. The mean contents of basal respiration, microbial biomass C and N, and ergosterol in soils among different treatments and soil depth profiles in a permanent grassland from a tillage trial 2 and 5 years before sampling

Treatment	Depth	Basal respiration	Microbial biomass		Ergosterol
	(cm)	CO_2-C (μg g^{-1} d^{-1})	C (μg g^{-1})	N (μg g^{-1})	(μg g^{-1})
Tillage trial 2 years before sampling					
P2+	0-5	23	666	71	1.41
	5-10	8	555	63	0.50
	15-20	7	248	41	0.31
	20-30	5	138	21	0.24
	30-40	4	99	16	0.14
P2-	0-5	17	493	52	0.96
	5-10	6	342	39	0.42
	15-20	5	240	38	0.31
	20-30	4	115	25	0.26
	30-40	3	90	20	0.16
R2+	0-5	18	409	36	0.72
	5-10	7	361	61	0.46
	15-20	7	270	50	0.57
	20-30	4	181	38	0.31
	30-40	3	85	15	0.11
R2-	0-5	13	356	33	0.57
	5-10	6	331	54	0.41
	15-20	7	254	45	0.51
	20-30	4	153	32	0.26
	30-40	3	56	12	0.11
Tillage trial 5 years before sampling					
P5+	0-5	20	666	47	0.92
	5-10	10	427	37	0.29
	15-20	8	274	28	0.19
	20-30	7	128	16	0.16
	30-40	6	83	12	0.09
P5-	0-5	25	703	38	1.03
	5-10	10	403	39	0.30
	15-20	8	180	25	0.19
	20-30	7	167	22	0.15
	30-40	6	100	15	0.11
R5+	0-5	22	580	36	0.97
	5-10	9	354	24	0.35
	15-20	7	235	25	0.28
	20-30	6	145	20	0.17
	30-40	5	45	6	0.08
R5-	0-5	15	444	35	1.11
	5-10	9	328	30	0.30
	15-20	7	266	29	0.26
	20-30	6	183	25	0.20
	30-40	4	62	9	0.11

Supplementary Table 6. The distribution of soil amino sugar contents among different treatments and soil depth profiles in a permanent grassland from a tillage trial 2 and 5 years before sampling

Treatment	Depth (cm)	MurN (μg g^{-1})	ManN (μg g^{-1})	GalN (μg g^{-1})	GlcN (μg g^{-1})
Tillage trial 2 years before sampling					
P2+	0-5	117	9	758	1384
	5-10	131	5	881	1615
	15-20	61	7	440	810
	20-30	43	6	352	641
	30-40	35	6	298	545
P2-	0-5	138	6	959	1777
	5-10	85	6	581	1045
	15-20	47	5	397	677
	20-30	55	7	509	873
	30-40	44	4	381	645
R2+	0-5	102	6	664	1215
	5-10	80	6	532	942
	15-20	88	7	609	1137
	20-30	73	7	592	1031
	30-40	28	4	214	362
R2-	0-5	71	4	510	916
	5-10	98	11	679	1253
	15-20	75	7	532	988
	20-30	46	6	424	717
	30-40	23	5	189	316
Tillage trial 5 years before sampling					
P5+	0-5	174	18	963	1909
	5-10	96	13	598	1020
	15-20	66	10	487	837
	20-30	87	6	706	1205
	30-40	33	4	247	378
P5-	0-5	160	15	895	1747
	5-10	82	11	526	858
	15-20	99	7	765	1318
	20-30	67	6	497	841
	30-40	31	5	259	413
R5+	0-5	113	9	576	1198
	5-10	93	6	568	1134
	15-20	68	2	457	836
	20-30	46	3	360	603
	30-40	24	1	144	294
R5-	0-5	128	13	775	1479
	5-10	79	7	520	976
	15-20	60	3	422	736
	20-30	76	6	557	1008
	30-40	27	2	147	260

Supplementary Table 7. Mean contents of soil organic C (SOC), total N and mean soil pH, bulk density and soil C / N in different land use and fertilisation treatments at 0–40 cm soil depth.

Treatment	Depth (cm)	Soil pH	Bulk density (g cm^{-3})	SOC (mg g^{-1})	Total N (mg g^{-1})	soil C/N
With slurry application						
Permanent grassland	0-10	6.7	1.4	24	2.1	11.8
	10-20	6.3	1.6	13	1.1	11.1
	20-30	6.5	1.7	12	1.1	11.2
	30-40	6.7	1.7	8	0.7	11.4
Modified grassland	0-10	6.7	1.4	15	1.3	11.1
	10-20	6.2	1.6	15	1.4	11.0
	20-30	6.4	1.6	13	1.2	11.0
	30-40	6.4	1.7	10	0.9	11.2
Maize monoculture	0-10	6.4	1.3	14	1.2	11.4
	10-20	6.6	1.4	13	1.1	11.4
	20-30	6.7	1.4	13	1.1	11.4
	30-40	6.8	1.6	8	0.7	12.6
Without slurry application						
Permanent grassland	0-10	6.4	1.5	18	1.6	11.4
	10-20	6.5	1.7	15	1.4	10.9
	20-30	6.5	1.9	13	1.2	11.0
	30-40	6.7	1.8	10	0.8	11.7
Modified grassland	0-10	6.5	1.6	13	1.1	11.5
	10-20	6.1	1.6	15	1.3	11.5
	20-30	6.3	1.7	12	1.0	11.8
	30-40	6.7	1.6	8	0.6	12.5
Maize monoculture	0-10	6.3	1.4	13	1.1	11.5
	10-20	6.3	1.3	13	1.1	11.7
	20-30	6.4	1.7	10	0.9	11.2
	30-40	6.8	1.6	9	0.8	12.0

Supplementary Table 8. The mean contents of basal respiration, microbial biomass C, N and S, and ergosterol in different land use and fertilisation treatments at 0–40 cm soil depth.

Treatment	Depth	Basal respiration	Microbial biomass			Ergosterol
	(cm)	CO_2-C (µg g^{-1} d^{-1})	C (µg g^{-1})	N (µg g^{-1})	S (µg g^{-1})	(µg g^{-1})
With slurry application						
Permanent grassland	0-10	28	646	83	5.6	0.81
	10-20	11	239	35	2.9	0.21
	20-30	10	180	19	1.8	0.16
	30-40	8	102	13	1.5	0.13
Modified grassland	0-10	17	306	36	5.0	0.42
	10-20	13	352	42	2.4	0.31
	20-30	12	203	14	2.1	0.18
	30-40	8	104	17	1.5	0.15
Maize monoculture	0-10	12	205	33	5.5	0.32
	10-20	11	237	32	2.4	0.28
	20-30	12	209	23	2.8	0.32
	30-40	8	100	13	2.7	0.13
Without slurry application						
Permanent grassland	0-10	18	442	47	4.6	0.57
	10-20	11	246	38	2.7	0.21
	20-30	9	203	32	1.4	0.15
	30-40	7	121	18	1.5	0.06
Modified grassland	0-10	13	234	32	2.5	0.31
	10-20	15	361	34	2.3	0.37
	20-30	11	229	26	2.1	0.21
	30-40	7	68	12	0.9	0.08
Maize monoculture	0-10	11	201	36	3.2	0.40
	10-20	13	252	24	3.3	0.23
	20-30	8	104	17	1.5	0.15
	30-40	7	103	13	2.1	0.15

Supplementary Table 9. The distribution of soil amino sugar contents among different treatments and soil depth profiles in different land use and fertilisation treatments.

Treatment	Depth (cm)	MurN (μg g^{-1})	ManN (μg g^{-1})	GalN (μg g^{-1})	Fungal GluN (μg g^{-1})
With slurry application					
Permanent grassland	0-10	100	17	568	860
	10-20	112	26	784	1292
	20-30	55	24	452	723
	30-40	43	25	328	509
Modified grassland	0-10	63	10	419	636
	10-20	97	22	664	1077
	20-30	88	32	658	1043
	30-40	55	28	424	661
Maize monoculture	0-10	58	14	344	571
	10-20	94	18	483	872
	20-30	61	17	390	609
	30-40	14	0	69	130
Without slurry application					
Permanent grassland	0-10	59	11	458	718
	10-20	75	23	606	983
	20-30	109	63	891	1417
	30-40	57	36	539	825
Modified grassland	0-10	54	9	349	615
	10-20	84	22	547	914
	20-30	87	34	534	914
	30-40	42	21	283	480
Maize monoculture	0-10	67	13	347	595
	10-20	105	20	556	992
	20-30	55	28	424	661
	30-40	31	13	187	350

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