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Long-term effects of specific agricultural managements on soil water extractable organic matter and microbial communities in South Germany

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“If the soil is destroyed, then our liberty of action and choice are gone ...”

- W.C. Lowdermilk, 1953.

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ABBREVIATIONS AND ACRONYMS

ANOVA	analysis of variance
BIX	freshness index
C1, C2, C4	fulvic/humic-like substances;
C3	tryptophan-like substances.
EE	easily extractable
EEM	excitation emission matrix spectrum
EEM-PARAFAC	excitation emission matrix spectrum + parallel factor analysis
F_{max}	maximum fluorescence intensity
FI	fluorescence index
GalN	galactosamine
GlcN	glucosamine
HIX	humification index
IMT	integrated farming + minimum tillage treatment
IPL	integrated farming + plough tillage treatment
I-pop	integrated farming + poplar treatment
I-rob	integrated farming + robinia treatment
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
MurN	muramic acid
NH_4-N	ammonium
NO_3-N	nitrate
OMT	organic farming + minimum tillage treatment
OPL	organic farming + plough tillage treatment
O-pop	organic farming + poplar treatment
O-rob	organic farming + robinia treatment
PARAFAC	parallel factor analysis
PCA	principal component analysis
PLFA	phosphorus lipid fatty acid
RDA	redundancy analysis
SOC	soil organic carbon
$SUVA_{254}$	specific ultraviolet visual absorption at 254 nm
Total N	soil total nitrogen
WEOM	water extractable organic matter

WEOC	water extractable organic carbon
WEON	water extractable organic nitrogen
WEOC/N	ratio of water extractable organic carbon to water extractable organic nitrogen

ABSTRACT

Agricultural management strategies influence the inherent soil properties. Labile organic matter such like soil water extractable organic matter (WEOM), soil microbial communities and microbial residues are important for the soil processes and functions like nutrient turnover and carbon sequestration. In recent years, organic farming, minimum tillage and agroforestry cropping draw increasing attention of people. However, our understanding of their effects on soil WEOM, microbial communities and microbial residues is still insufficient to deduce knowledge, especially considering the decisive long-term effects and changes. Long-term field trial offer precious opportunities to investigate the long-term effects of specific agricultural managements on soil WEOM, microbial communities and microbial residues. Therefore, the WEOM, microbial communities and microbial residues were investigated in a case of long-term field trials considering two types of organic farming (high-input and low-input), minimum tillage and alley cropping agroforestry (poplar and robinia) at the experimental farm in Scheyern. In addition, a lab ^{13}C isotope tracing incubation with soils from the combined high-input organic farming and minimum tillage field trial was performed to investigate managements effects on the fate of labile organic matter.

In the first part of the PhD thesis, the lab incubation with ^{13}C labelled glucose indicated that a large portion of the labile organic carbon was immobilized by microorganisms or incorporated into stable organic fraction in the short term. Soil management influenced the labile organic carbon distribution by affecting soil microorganisms. This was indicated by the microbial immobilization of carbon and soil respiration. Thus, management enhancing soil microbial biomass and microorganism populations will influence the microbial contribution to soil organic matter. High-input organic farming combined with minimum tillage shows such advantage.

In the second part (publications I and II), soil WEOM was investigated to detect the long-term effects of organic farming (high-input and low-input), minimum tillage and robinia, and poplar-based alley cropping agroforestry on WEOM. The results revealed that both type of organic farming had negative effect on WEOM amount, but the effects on quality and components of WEOM revealed by EEM-PARAFAC were different. High-input organic farming led to soil WEOM with high stability and tended to enrich more humic-like components in WEOM, while WEOM of low-input organic farming soil contained less humic-like components and less humified. Minimum tillage had little effect on both the quantity and quality of soil WEOM, but resulted in a negative depth gradient, namely, higher WOEM content and more humic-like components accumulate in WEOM at the upper soils. Thus, the high-input organic farming dominated the change of WEOM in

system including both organic farming and minimum tillage. In comparison with poplar, the effect of robinia on the amount of soil WEOM was little, but robinia led to soil WEOM with more compounds with aromatic structure and enriched humic-like components in WEOM. Therefore, the results implied the effect of low-input organic farming and robinia alley cropping on WEOM interplayed, albeit on opposite direction. Regarding soil organic carbon (SOC), both organic farming and robinia alley cropping led to an increase, and minimum tillage increased SOC content in upper soil.

In the third part (publications III and IV), the soil microbial communities and microbial residues were investigated to detect their responses to organic farming (high-input and low-input), minimum tillage, and robinia and poplar-based alley cropping agroforestry. The results revealed positive effects of high-input organic farming on microbial biomass, bacteria and arbuscular mycorrhizal fungi (AMF) and microbial residues, while low-input organic farming system was observed with lower microbial biomass content and lower abundance of the functional guilds of microbes and its effect on microbial residues was little. Minimum tillage showed positive effects on microbial biomass and AMF abundance, while the effect on bacteria was insignificant. Minimum tillage resulted in a negative depth gradient of soil microbial properties. Robinia showed little effect on microbial biomass and microbial residues, but a positive effect on Gram (+) bacteria was observed.

Combining the findings of the second part and the third part, the results suggest that the effect of specific agricultural management on WEOM, microbial communities and microbial residues are independent. Organic farming dominated the development of soil properties development. Organic farming with additional organic input is more advantageous in optimizing soil microbial community and carbon sequestration than low-input organic farming. Minimum tillage favors the accumulation of SOC and optimizes microbial conditions. Robinia alley cropping has the potential to sequester more carbon. Among the investigated agricultural managements, high-input organic farming combined with minimum tillage and low-input combined with robinia-based alley cropping are more superior than other combinations of management strategies from the view of carbon sequestration and optimizing soil microbial community. Our findings can be used in setting up strategies and policies for agricultural carbon sequestration in soils and sustainable use of soil resources.

ZUSAMMENFASSUNG

Die landwirtschaftliche Bodennutzung beeinflusst inhärente Bodeneigenschaften. Die labilen organischen Stoffe, zumeist als Bodenwasser-extrahierbare organische Stoffe (WEOM) definiert, die mikrobiellen Gemeinschaften und die mikrobiellen Rückstände in Böden sind wichtig für Prozesse und Funktionen wie die Nährstoffumsätze und die Kohlenstoffbindung bzw. dem Haushalt der organischen Substanz. In den letzten Jahren wurden der ökologische Landbau, die minimale Bodenbearbeitung und die Agroforstwirtschaft als Alternativen stärker in Betracht gezogen. Dennoch ist unser Verständnis für deren Bedeutung für WEOM, die mikrobiellen Gemeinschaften und die mikrobiellen Rückstände nicht hinreichend zur Ableitung von Wissen, vor allem hinsichtlich der entscheidenden langfristigen Auswirkungen. Der langfristige Feldversuch in Scheyern, mit an einem Standort integriertem und ökologischem Landbau (High-Input und Low-Input), minimaler Bodenbearbeitung und Agroforstwirtschaft bietet eine hervorragende Gelegenheit, die langfristigen Auswirkungen der spezifischen landwirtschaftlichen Bewirtschaftungen auf die WEOM der Böden, die mikrobiellen Gemeinschaften und die mikrobiellen Rückständen (als Substrate der Bildung organischer Bodensubstanz und als Marker der mikrobiellen Diversität) zu untersuchen. Entsprechend vielfältige bodenchemische und bodenmikrobiologische Analysen der Oberböden wurden durchgeführt. Zusätzlich wurde per Inkubation von Bodenproben aus dem Feldversuch der ökologischen Landwirtschaft (hoher organischer input bzw. organische Düngung und minimale Bodenbearbeitung), versetzt mit 13C-markiertem Substrat erfasst, wie langfristige Bewirtschaftungen die Dynamik der labile organischen Substanz bedingen.

Im ersten Teil wird aus der Laborinkubation mit ^{13}C-markierter Glucose gezeigt, dass ein großer Teil des labilen organischen Kohlenstoffs durch Mikroorganismen immobilisiert oder kurzfristig in eine stabile organische Fraktion eingebaut wurde. Die Bodenbewirtschaftung veränderte der labile Teil des organischen Kohlenstoffs über eine Beeinflussung der Bodenmikroflora. Dies wurde durch die mikrobielle Immobilisierung von Kohlenstoff- und die Bodenatmung gezeigt. Eine erhöhte mikrobielle Biomasse und veränderte Mikroorganismenpopulationen beeinflussten die organischen Bodensubstanz. Der High-Input-ökologischen Landbau, mit minimaler Bodenbearbeitung kombiniert, zeigte diese positive Entwicklung.

Der zweiten Teil (Publikationen I und II) befasst sich mit den langfristigen Auswirkungen des ökologischen Landbaus (High-Input und Low-Input), der minimalen Bodenbearbeitung sowie der Robinien- und Pappel-Agroforstwirtschaft auf WEOM Eigenschaften. Die Ergebnisse zeigten, dass der ökologische Landbau einen negativen Effekt auf die WEOM-Menge hatte, aber die Auswirkungen auf die Qualität und die Komponenten von WEOM, die per EEM-PARAFAC ersichtlich wurden, unterschiedlich waren. Der ökologische Landbau bewirkte WEOM mit einer hohen Stabilität, mit einer Tendenz zu mehr humic-ähnlichen Komponenten in WEOM, während die WEOM vom ökologischen Landbau mit niedrigem Input weniger "humic" ähnliche Komponenten enthielt und geringer humifiziert war. Die minimale Bodenbearbeitung hatte wenige Einflüsse auf sowohl die Menge als auch die Qualität der WEOM, mit jedoch einen negativen Tiefengradient des WEOM, da im oberen Boden ein höherer WEOM-Gehalt und ein höherer Anteil humic-artiger Bestandteile

akkumuliert waren. So bewirkte der ökologische Landbau mit der minimalen Bodenbearbeitung die stärksten Veränderungen des WEOM. Im Vergleich zu Pappeln war die Wirkung der Robinie auf die Menge des WEOM geringer, jedoch entstand unter Robinie ein WEOM mit höherem Anteil an Verbindungen mit aromatischen Struktur, angereichert mit huminstoffartigen Komponenten. Somit zeigten die Ergebnisse, dass Low-Input-ökologische Landwirtschaft und Robinie-Agroforestry die Boden-WEOM entgegengesetzt beeinflussen. Hinsichtlich des organischen Kohlenstoffs (SOC) führten sowohl der ökologische Landbau als auch die Robinie-Agroforestry zu einer Zunahme, die minimale Bodenbearbeitung erhöhte die SOC-Gehalte nur im oberen Boden.

Im dritten Teil (Publikationen III und IV) wurden die Beeinflussungen der bodenmikrobiellen Gemeinschaften und die mikrobiellen Residuen durch den ökologischen Landbau (high-input and low-input), die Minimalbearbeitung, und die Robinie- und die Pappel-Agroforstwirtschaft ermittelt. Der high-input ökologische Landbau erhöhte die mikrobielle Biomasse, die Bakterien und arbuskulären Mykorrhizapilze (AMF), wie auch die mikrobiellen Rückstände. Der low-input ökologische Landbau führte zu niedrigeren mikrobielle Biomassegehalten und geringerer Häufigkeit der funktionellen mikrobiellen Einheiten während die Wirkung auf die mikrobiellen Residuen nur sehr gering ausfiel. Die minimale Bodenbearbeitung zeigte positive Einflüsse auf die mikrobielle Biomasse und AMF-Häufigkeit, während die Beeinflussung der Bakterien nicht signifikant war. Die minimale Bodenbearbeitung führte zu einem negativen Tiefengradient der mikrobiellen Eigenschaften des Bodens. Robinien hatten eine nur geringe Wirkung auf die mikrobielle Biomasse und die mikrobiellen Residuen, jedoch aber eine positive Wirkung auf die Gram (+) - Bakterien.

Kombiniert man die Ergebnisse des zweiten und des dritten Teils, zeigen die Ergebnisse unabhängige Wirkungen der Bewirtschaftungen auf WEOM, die mikrobiellen Gemeinschaften und die mikrobiellen Rückständen. Die ökologische Landwirtschaft zeigte die stärksten Auswirkungen auf die untersuchten Bodeneigenschaften. Der ökologische Landbau mit organischem Eintrag optimiert die mikrobiellen Gemeinschaften und erhöht die Kohlenstoff-Sequestrierung gegenüber dem ökologischen Landbau ohne organische Düngung. Die minimale Bodenbearbeitung begünstigte die Akkumulation von SOC und optimierte die mikrobiellen Vorausetzungen. Die Robinien-Agroforstbewirtschaftung hat offenbar das Potential für höhere Kohlenstoffanreicherung. Unter den erfassten Bewirtschaftungssystemen können dem High-Input-ökologischen Landbau kombiniert mit der minimalen Bodenbearbeitung, und dem Low-Input-ökologischen Landbau kombiniert mit Robinien-Agroforstbewirtschaftung höherere Potentiale hinsichtlich Kohlenstoff-Sequestrierung und Optimierung der Bedingungen zur mikrobiellen Diversität als den anderen Bewirtschaftungssystemen und -maßnahmen zugeschrieben werden. Die hier vorgestellten Ergebnisse bilden eine Grundlage hinsichtlich der Ableitung von Empfehlungen zur Maßnahmen der nachhaltigen, bodenkonservierenden und organischer Bodensubstanz erhaltenden Landwirtschaft.

List of publications and my contributions

Publications

I. Hanyin Sun, Philipp Koal, Georg Gerl, Reiner Schroll, Rainer Georg Joergensen, Jean Charles Munch, (2017) Water extractable organic matter and its fluorescence fractions in response to minimum tillage and organic farming in a Cambisol. (in press by Chemical and Biological Technologies in Agriculture)

II. Hanyin Sun, Philipp Koal, Georg Gerl, Reiner Schroll, Rainer Georg Joergensen, Jean Charles Munch, (2017) Response of water extractable organic matter and its fluorescence fractions to organic farming and tree species in poplar and robinia-based alley cropping agroforestry systems. Geoderma 290, 83-90.

III. Hanyin Sun, Philipp Koal, Dong Liu, Georg Gerl, Reiner Schroll, Andreas Gattinger, Rainer George Joergensen, Jean Charles Munch (2016) Soil microbial community and microbial residues respond positively to minimum tillage under organic farming in Southern Germany. Applied Soil Ecology, 108,16-24.

IV. Hanyin Sun, Philipp Koal, Georg Gerl, Reiner Schroll, Andreas Gattinger, Rainer Georg Joergensen, Jean Charles Munch (2016) Microbial communities and residues in Robinia and Poplar-based alley cropping systems under organic and integrated management. Agroforestry Systems, 1-12, DOI: 10.1007/s10457-016-0009-x.

My contributions:

I. I was involved in the planning and conducting the global sampling in the field and the analysis of all the samples in the lab. I analyzed the data and the manuscript is mainly based on my input.

II. I was involved in the planning and conducting the global sampling in the field and the analysis of all the samples in the lab. I analyzed the data and the manuscript is mainly based on my input.

III. I was involved in the planning and conducting the global sampling in the field and the analysis of all the samples in the lab. The phosphorus fatty acid analysis was done in the lab of Dr. Andreas Gattinger and the amino sugars were measured in the lab of Prof. Rainer Georg Joergensen after pre-treatment of all the samples in HMGU labs, after introduction in technics and data analysis by the lab heads. I analyzed the data and the manuscript is mainly based on my input.

IV. I was involved in the planning and conducting the global sampling in the field and the analysis of all the samples in the lab. The phosphorus lipid fatty acids analysis was measured in the

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1 INTRODUCTION

At a global scale, agriculture is a large source of greenhouse gases, often accompanied by soil degradation in the sense of losses of organic matter - a decisive compound of soils. It is estimated that agriculture releases 10-12% of the total anthropogenic greenhouse gases (Mangalassery et al., 2014). Under the background of global warming, measures to mitigate the greenhouse gases emission, to enhance carbon sequestration and to increase soil organic matter are drawing the interests of scientists and policy makers. Whereby, conservation tillage practices such like reduced tillage, minimum tillage or no-tillage, direct drilling and strip cropping are widely recommended (Mangalassery et al., 2014; Melero et al., 2009; Six et al., 1999). Besides tillage practices, agricultural managements like organic farming as well as agroforestry are also reported to have a large potential to sequester atmospheric carbon and to mitigate global warming (Albrecht and Kandji, 2003; Gadermaier et al., 2012; Gattinger et al., 2012; Lorenz and Lal, 2014; Skinner et al., 2014). The amount and quality of WEOM, the abundance and diversity of microorganisms as well as the structure of microbial communities all tangle with the release of greenhouse gases and carbon sequestration (Bailey et al., 2002; Bastida et al., 2013; Baumann et al., 2013; Berthrong et al., 2013; Garcia-Pausas and Paterson, 2011), and even new findings report that the necromass of microorganisms contribute to the sequestration of soil carbon (Glaser et al., 2006; Liang and Balser, 2008; Liang and Balser, 2011; Ludwig et al., 2015). Therefore, soil water extractable organic matter (WEOM) and microorganisms are of significance in the release of greenhouse gases and carbon sequestration. However, many previous studies only consider single factors, i.e. either tillage or organic farming, either agroforestry with one tree species or organic farming, and few studies test both tillage and organic farming, agroforestry and organic farming, especially in a long term field experiment. Therefore, our understanding on WEOM and microorganisms' long-term responses to specific agricultural managements or practices still needs to be strengthened.

The aims of this thesis are to study

(i) the long-term influence of minimum tillage, poplar- and robinia-based alley cropping and organic farming management on the content and properties/composition of WEOM;

(ii) the responses of microbial communities and microbial residues to long-term minimum tillage, poplar and robinia-based alley cropping and organic farming management; and

(iii) the fate of labile organic materials in soil under long-term minimum tillage and organic farming.

To realize these targets, fluorescence spectroscopic measurement of WEOM, PLFA and amino sugar analysis, isotope labelling technique were performed to realize the aims of this study.

1.1 Fate of carbon in soils

Soils are the largest C pool in terrestrial systems. Thus, any change in this C pool can affect significantly the atmospheric C pool and contributing to the direction of the global change. Therefore, the deep understanding of soil organic matter (SOM) can help to reveal the role of soil for CO_2 dynamics -as a sink or source, and can also give information about the functioning of ecosystems and global C-cycling, and help to predict and control SOM fluxes eventually (Marx et al., 2007a; Mganga and Kuzyakov, 2014).

Microbes play important roles in the C-cycling. They regulate the dynamics of SOM directly by organic matter degradation into plant nutrients and they are also concomitantly a source of basic compounds for the stable soil organic matter, the humus pool (Brant et al., 2006; Ludwig et al., 2015). Long-term effects of agricultural managements could alter the inherent soil microbial communities, especially the functional groups (Tiemann et al., 2015; Zhang et al., 2015b), thereby influencing the pattern of organic matter degradation and stabilization (Fontaine and Barot, 2005). Labile organic matter is the main energy source for soil microorganisms and many sources can contribute to this pool, such as root exudates, rhizodeposits and plant residues incorporated in soils by faunal activities (Hoyle et al., 2008; Kramer and Gleixner, 2006; Marx et al., 2007a; Marx et al., 2007b). Gunina et al. (2014) concluded that the stabilization of C in soil is mainly associated with its incorporation into microbial compounds of various stability and not with its initial microbial uptake through a study of the fate of low molecular weight organic substances (LMWOS) in an arable soil. Thus, the understanding of labile organic matter degradation in soils under various environmental conditions is very important for the understanding of global C-cycling. For example, Mganga and Kuzyakov (2014) reported that land uses determine the degradation of glucose and its incorporation into soil microbial biomass in Mountain Kilimanjaro ecosystems. Land use intensification in agroecosystems led to an average increase of glucose decomposition, and the decay rates of labile C pool in intensively used agricultural lands were up to three times higher compared with natural ecosystem. Study investigating effects of long-term compost and inorganic fertilizer amendments on the dynamics of glucose-derived ^{13}C incorporation into aggregates and uptake of glucose-derived ^{13}C into soil microbial communities showed that the exogenous easily decomposable organic C could be more effectively maintained in compost treated soil (organic C-rich) than in non-amended and synthesized fertilizer treated (organic C-poor soil) (Zhang et al., 2015a), and the mean amount of ^{13}C in bacterial, actinobacterial and fungal PLFAs in compost treated-soil during a 30 incubation were significantly higher than those in the synthesized fertilizer-treated and control soils (Zhang et al., 2013). On the other hand, a study of the microbial utilization of glucose, as a representative of labile organic carbon, in a semiarid soil showed bacteria

dominated glucose metabolism in comparison to fungi. Gram-negative populations were initially more involved in glucose assimilation than Gram-positive bacteria (Bastida et al., 2013). Gunina et al. (2014) also observed Gram-negative bacteria (identified by the PLFA 16:1ω7c and 18:1ω7c) were the most abundant and active in LMWOS utilization in an arable soil. However, still our understanding of the fate or flow of organic carbon in soils through microbial processes after imposing different long-term management practices is insufficient in the view of understanding the functioning of the soil ecosystem to deduce rules for sustainable soil use.

1.2 Soil water extractable organic matter

1.2.1 Significance of soil water extractable organic matter

Dissolved organic matter (DOM) in soils is a fraction of soil organic matter operationally defined as the organic matter present in soil solution passing 0.4~0.6 μm filter (Chantigny, 2003). However, strictly speaking, the term DOM can only be applied to organic matter in soil solutions extracted with lysimeters (Marschner and Kalbitz, 2003). Therefore, the more precise term soil water extractable organic matter (WEOM) was proposed by Zsolnay (1996) to clarify the potentially soluble organic matter obtained by extraction from soil with batch or percolation methods. Moreover, the magnitude of WEOM is generally larger than DOM (Zsolnay, 1996). Therefore, all throughout this dissertation, WEOM is used as the term to designate this fraction of soil organic matter as batch method was used in present work.

WEOM accounts for only a small portion of the total soil organic matter and probably the most bioavailable fraction of soil organic matter, since all microbial uptake mechanisms require a water environment. the soluble state is presumably a prerequisite for the diffusion of substrates through microbial cell membranes so that the degradation of solid phase organic matter or large molecules can only occur after dissolution or hydrolysis by exoenzyme (Chantigny, 2003; Marschner and Kalbitz, 2003). It has been commonly acknowledged that WEOM has strong influences on many ecological processes. In addition to being the substrate of microorganisms, a direct function of WEOM affecting nutrient cycling in terrestrial and aquatic ecosystem via soil respiration (Glatzel et al., 2003), methane production as well as other soil biochemical functions (Zsolnay, 2003). WEOM also interferes the solubility and transport of organic contaminants and heavy metal, mineral weathering and podsolization (Embacher et al., 2007). From the mentioned complex functions of WEOM above, the ecological significance of WEOM covers at least the following aspects as stated by Zsolnay (2003): global change, desertification, pollution impact as well as water quality. The interest in WEOM research strongly increased in the past two decades. However, to our knowledge, the study of WEOM responses to long-term soil management was scarce. In addition, previous

studies often offer fragmented information and mainly focused on study WEOM in forest soil or studied WEOM from different soils, lack of long-term study about the effects of certain agricultural management factor on WEOM, especially the study under organic farming agricultural management.

1.2.2 Characterization of soil water extractable organic matter

Batch extraction is widely used by researchers to extract water extractable organic matter at the absence of lysimeters or similar instruments. WEOM is commonly extracted with cold or hot ultrapure water and salt solutions by researchers at a different soil and solution ratios (Kalbitz et al., 2003; Nakanishi et al., 2012; Tian et al., 2012; Zsolnay, 2003). The extract of WEOM is usually quantified as water extractable organic carbon (WEOC) and water extractable organic nitrogen (WEON) directly or after special fractionation processes, such as separation according to polarity, acidity or molecular size (Aiken and Leenheer, 1993; Kalbitz, 2001; Leenheer, 1981; Malik and Gleixner, 2013; Marschner and Kalbitz, 2003; Tian et al., 2012). Moreover, with the advance in techniques, WEOM is characterized not only by its quantification, but also by its composition or quality through advanced spectroscopic means, such like UV spectra (Zsolnay, 2003), two dimensional or multidimensional fluorescence spectra (Chen et al., 2003; Kalbitz et al., 1999; Zsolnay, 2003), nuclear magnetic resonance spectra (Fernandez-Romero et al., 2016), diffuse reflectance infrared Fourier transform spectroscopy (Margenot et al., 2015), ultrahigh resolution Fourier transform ion cyclotron mass spectrometry (Stubbins et al., 2014) directly or after fractionation. Among the present means of WEOM characterization, fluorescence spectroscopy gets information on the chemical properties of fluorescing fraction of organic matter. According to the published peer studies, multidimensional fluorescence spectroscopy combines with advanced algebra technique, such like parallel factor analysis and regional integration, gains more popularity and is widely used in recent decades, especially in water science, for its advantage in less time-consumption, non-destructive sample preparation and high sensitivity (Borisover et al., 2012; Chen et al., 2003; Fellman et al., 2009; Fernandez-Romero et al., 2016; Ohno and Bro, 2006). The method has been approved to be valid for the characterization of aqueous samples extracted from soil and soil amendments (Ohno and Bro, 2006). The indices derived from fluorescence spectra, such as humification index (HIX), fluorescence index (FI) and freshness index (BIX), are used to indicate the quality or the origin of WEOM (Coble, 1996; Fellman et al., 2010; Wilson and Xenopoulos, 2009; Zsolnay et al., 1999). Besides the various spectroscopic characterization of WEOM, isotope labeling technique and batch incubation are also used to trace the source and fate of WEOM (De Troyer et al., 2011) and to explore the biodegradability of WEOM (Kalbitz et al., 2003; Wang et al., 2014a; Xu et al., 2013).

1.2.3 Effect of cropping system management on water extractable organic matter

Management of cropping system influences WEOM. Chantigny (2003) has reviewed the influence of land use and management practices on WEOM. However, the review only remained on the discussion of WEOM content and mainly reported data from temperate forest soils, whereas without considering the influence on WEOM quality, fractions or composition of WEOM. The data from arable soils were relatively scarce compared with that from forest soils (Chantigny, 2003; Embacher et al., 2007). Per the work of Chantigny (2003), research on soil DOM and WEOM as influenced by land use and management practices has offered fragmented and sometimes contradictory information, a normal issue considering the richness of soils and sites. In the short term, temporal and spatial variations in DOM and WEOM are complex and influenced by environmental conditions. Laboratory studies have shown that management practices, such as liming and N fertilization, can induce marked fluctuations in DOM and WEOM. Under field conditions, however, the net effect of management practices often remains unclear because many soil properties, which can interact and counterbalance, are influenced at the same time. Changes in DOM and WEOM upon management practices are generally of short duration, whereas long-term effects are more related to vegetation type and to the amount of plant litter returned to the soil.

Regarding to the influence of cropping system management on WEOM quality and composition, the WEOM-quality data from arable soils is more scarce than the quantity data, and the methods for quality/composition/characterization varies. The available data showed the cropping system managements' influences on WEOM quality differed. A study comparing different land-use effects on quantity and quality of dissolved organic matter in soil samples from both Canada and China showed land use (native forest, grassland, and arable land) did not affect the UV absorption at 280 nm and HIX, biodegradability of dissolved organic carbon (DOC) (Sun et al., 2013). Xu et al. (2013) reported that crop rotation and land use type significantly affected specific absorption ($SUVA_{254}$), an aromatic structure indicator of organic matter compounds, of WEOM. $SUVA_{254}$ of WEOM of crop rotation with perennial plant was lower than that of crop rotation with annual plants, and grassland and forest soils showed lower $SUVA_{254}$ than those of agricultural soils. The HIX index followed the same effects. Akagi and Zsolnay (2008) reported that long-term devegetation under field condition has small influence on the quality ($SUVA_{254}$ and HIX) of WEOM of the concerned agricultural soil. Some researcher observed that experimental N deposition altered the composition (identified by 3-D fluorescence spectra) of soil dissolved organic matter while the HIX remained unaffected (Fang et al., 2014). Whereas Sun et al. (2015) reported that nitrogen fertilization decreased the aromaticity ($SUVA_{254}$) of WEOM in two canadian soils, but the effects on WEOM condensation indicated by HIX index varied. Ohno et al. (2009) observed that N source

significantly affect fluorescent component identified by excication emission matrix fluorescence spectra with paralell factor analysis (EEM-PARAFAC). Zhang et al. (2011) reported that tillage and fertilization treatments did not affected $SUVA_{254}$ of WEOM, while the component revealed by EEM-PARAFAC differed from among treatments. From above, our understanding on the influence of cropping system management on WEOM still remains elusive.

1.3 Soil microorganisms

1.3.1 Microbial biomass and microbial communities

Soil is one of the habitats for microorganism in nature. Diverse populations of microorganisms live in the soil - a complex, heterogeneous and discontinuous system (Nannipieri et al., 2003). Soil microorganisms, mainly as bacteria, archaea and fungi, play pivotal roles in a multitude of biogeochemical cycles and are responsible for cycling of C and N, such as organic matter decomposition and N mineralization as well as immobilization (Berthrong et al., 2013; Kirk et al., 2004; Yevdokimov et al., 2008; Yevdokimov et al., 2012). In addition, soil microorganisms also influence plant growth, plant health, soil structure and aggregate formation, and soil fertility (Fontaine and Barot, 2005; Mäder et al., 2002; Rillig et al., 2002; Widmer et al., 2006). Soil microbial biomass is a sensitive indicator of changes in soil organic matter and soil quality changes caused by human activities (Araujo et al., 2012; Joergensen, 1996; Joergensen and Mueller, 1996; Lin and Brookes, 1996; Vance et al., 1987; Zelles, 1999). Soil microbial biomass, the living part of soil organic matter, can be determined by fumigation-extraction method, substrate-induced respiration, total amounts of phospholipid fatty acids and by soil adenosine 5'-triphosphate (ATP) content. Besides the general characterization of microorganisms as microbial biomass, microorganisms are investigated to study the structure of microbial community. Because merely no microbial-dependent soil processes are influenced or regulated by single microbes, and understanding structure, dynamics and functions of soil microbial communities represents one key to the understanding of soil fertility and soil quality as important for sustainable agriculture (Lupwayi et al., 1998; Widmer et al., 2006). It was also observed that the structure of microbial communities has important implications for the rates of soil processes, e.g. variation in microbial community structure in soils has been observed to influence rates of denitrification, nitrification and nitrogen fixation (Berthrong et al., 2013). Therefore, the investigation of microbial community structure and microbial diversity is increasingly popular (Frostegård et al., 1993; Li et al., 2015; Scheibe et al., 2015; Zhang et al., 2014c). With the development of new techniques, culture-independent approaches, such as phospholipid fatty acid (PLFA) analysis, nucleic acid techniques, phylogenetic analysis and fluorescent in situ hybridization (FISH), have been developed to characterize the composition, diversity and functional groups of microbial communities. Methods to

characterize microbial community have been reviewed by many authors from different perspectives (Hill et al., 2000; Kirk et al., 2004; Nannipieri et al., 2003; Torsvik and Ovreas, 2002; Zelles, 1999). However, most studies concentrate on description of microbial communities but not on functional aspects (Baveye et al., 2016).

1.3.2 Effect of cropping system management on soil microbial communities

Land use changes and management practices are key factors affecting soil microbial communities via changes in soil properties, such as the rhizosphere pH, soil moisture, soil temperature, nutrient availability and soil organic matter composition (Tian et al., 2012). Among the management practices applied by the farmers, the effects of tillage, fertilization, crop residue management, crop rotation and organic farming on soil microbial biomass and microbial community have been repeated studied (Esperschuetz et al., 2007; Helgason et al., 2010; Moeskops et al., 2010; Tian et al., 2014; Wang et al., 2014b). In general, the application of no-tillage, reduced tillage or minimum tillage promotes the increase of soil microbial biomass in upper soil zone (Kuntz et al., 2013; Sun et al., 2011; Wang et al., 2012) and changes the microbial community, especially tends to increase the abundance of fungi in bulk or soil aggregates (Frey et al., 1999; Helgason et al., 2010). However, some authors also observed that tillage cause immediate changes in microbial community structure, based on PLFA analysis, but little concomitant change in total microbial biomass in short term (Jackson et al., 2003). The crop rotation diversity enhances the belowground communities and functions and microbial biomass (McDaniel et al., 2014; Tiemann et al., 2015). Specifically, the effect of crop rotation on soil microbial community varies from studies for the exact difference of investigated crop rotation. Zhang et al. (2014c) reported crop rotation (monoculture of maize VS maize-soybean) had little effect on soil bacterial communities, but it significantly influenced soil fungal communities, particularly arbuscular mycorrhizal fungi. Soils under maize monoculture had higher fungal biomass than soils under maize-soybean rotation in a clay soil in northeast China. Bünemann et al. (2004) observed that maize-crotalaria rotation increased microbial biomass and the relative abundances of fungi and gram-negative bacteria PLFA indicators in comparison with continuous maize in western Kenya soils. Legume plants are increasingly incorporated in crop rotation, the legume-based crop rotations generally support the microbial biomass and microbial diversity (Lupwayi et al., 1998), while the new review reported the addition of legumes to rotation had no consistent effects on microbial diversity or richness derived from data determined by nucleic techniques (Venter et al., 2016), this technique analysis relies on the whole background of soil DNA, in opposite to techniques that extract only markers of metabolic active organisms. In order to develop sustainable agricultural systems and to promote recycling of elements within a system, crop residues are increasingly returned to soil. Both positive and insignificant effect of crop residue

retention of soil microbial communities were reported (Navarro-Noya et al., 2013; Wang et al., 2014b; Wang et al., 2012; Yang et al., 2013). Even it was reported that the effect of crop residue management on microbial biomass was more pronounced than that of tillage (Spedding et al., 2004). Organic farming, a holistic management practice of agriculture, is regarded as an alternative of conventional farming and has been studied in recent decades. Positive effects of organic farming on microbial biomass, microbial communities and functional microbial groups have been reported repeatedly (Berthrong et al., 2013; Esperschuetz et al., 2007; Kuffner et al., 2004; Mäder et al., 2002; Oehl et al., 2004; Santos et al., 2012). Under the background of organic farming, reduced tillage (minimum tillage) practices have become a challenging option for organic farmers (Kuntz et al., 2013). However, the absence of synthesized chemicals for weed control and limited nitrogen availability in the spring pose major challenges and may hamper successful implementation of reduced tillage (minimum tillage) systems in organic farming (Peigne et al., 2007; Sans et al., 2011). In addition, the incorporation of the grass clover in reduced tillage regimes is challenging in organic arable systems, due to the risk of second emergence of weeds after shallow tillage (Kuntz et al., 2013). Many reports have showed that the positive effect of minimum tillage on soils, such like soil carbon sequestration in top soil and soil microbial community. However, our understanding on the suitability of minimum tillage and the effect of minimum tillage on soil microorganisms under organic farming still needs more investigation.

1.4 Soil microbial residues

Microbes are responsible the formation and turnover of soil organic matter in related to microbial communities dynamics and the balance between production and degradation of microbial products (Ding et al., 2015). Because on one hand, microbes utilize available C for the build-up of microbial biomass and degrade organic matter comes from various sources (e.g. plant residues and manure). On the other hand, the rapid growth of microbial biomass is always concomitant with microbial death, and significant amounts of microbial residues are produced and then incorporated into soil organic matter. Therefore, the catabolic and anabolic activities of microorganisms heavily influence the dynamics of the terrestrial C pool (Liang et al., 2015). Recently, microbial residues are believed to represent a significant source of stable C pool. It was reported that the carbon in the dead microbial residues would account for 80% of the organic carbon/organic matter in soil (Liang and Balser, 2011). Thus, microbial residues may play a more critical role in long-term C sequestration in soils than thought before (Ding et al., 2015; Guggenberger et al., 1999; Liang et al., 2015; Simpson et al., 2004).

Amino sugars originating from cell wall of microbes and residing stably in soils, are taken as the biomarker of microbial residues (Amelung et al., 2001; Glaser et al., 2004; Guggenberger et al., 1999). They can reflect the historical and current community structure of microorganisms (Glaser et al., 2004; Liang and Balser, 2011) and serve as a time-integrated biomarker for microbial composition, effects of soil management on microbes, and microbial contribution to soil carbon sequestration (Ding et al., 2013a; Ding et al., 2013b; Glaser and Gross, 2005; Glaser et al., 2004; Murugan and Kumar, 2013). There are four detectable amino sugars, i.e. glucosamine (GluN), galactosamine (GalN), muramic acid (MurA) and mannosamine (ManN) (Zhang and Amelung, 1996). The origin of GalN in soil is still unclear, although it accounts for 30–50% of the amino sugar pool (Amelung et al., 2001; Engelking et al., 2007). The chitin of fungal cell walls is the major source of GluN, although bacterial cell walls and the exoskeletons of soil invertebrates also make some contribution (Amelung et al., 2001). MurA exclusively originates from bacteria, as a component of the peptidoglycan of the bacterial cell wall (Chantigny et al., 1997; Glaser et al., 2004). Currently, the amino sugars can be measured by gas-chromatography (GC) and high-pressure liquid chromatography (HPLC) (Appuhn et al., 2004; Indorf et al., 2011; Zhang and Amelung, 1996).

More attention has been paid on the study of amino sugars in recent years. Many studies have shown the influences of land use (Ding et al., 2013b; Murugan et al., 2014), including organic farming (Joergensen et al., 2010), tillage (Guggenberger et al., 1999; van Groenigen et al., 2010; Zhang et al., 2014b), crop rotation (Ding et al., 2011b; Martins et al., 2012; Zhang et al., 2014a), residue quality (Ding et al., 2011a; Liang et al., 2007b), fertilization (Ding et al., 2015; Ding et al., 2013c; Murugan and Kumar, 2013; Sradnick et al., 2014), tree species (Liang et al., 2008; Liang et al., 2007a) and deforestation (Turrion et al., 2002) on amino sugars. In general, reduced tillage, organic farming, diverse crop rotation, manure application, and crop residues returning can promote the accumulation of microbial residues in soils, although opposite results or insignificant effect were reported as well (van Groenigen et al., 2010; Zhang et al., 2014a). In addition, the response of individual amino sugar varied from the investigated factors and the effect of one factor on amino sugars may depend on another factor, for example, the fertilization effects on amino sugars may depend on the crop rotation and fertilizer type as reported by Murugan and Kumar (2013). Besides amino sugars, another important microbial residue is glomalin-related soil protein, produced by Arbuscular-Mycorrhizal Fungi (AMF) (Burrows, 2014), which is closely related to SOC sequestration and to aggregate stabilization (Rillig et al., 2002). The previous study mainly focused on the function of glomalin-related soil protein and its relation with plants and soil properties (Burrows, 2014; Purin and Rillig, 2007; Rillig et al., 2006; Wu et al., 2014), the effect of soil

management practices on glomalin-related soil protein is lacking, although there are some studies presenting the effect of land use (Bedini et al., 2007), crop straw returning (Nie et al., 2007), organic amendment (Zhang et al., 2014d) and fertilization (Turgay et al., 2015) on glomalin-related soil protein. Therefore, our understanding of agricultural management practices on microbial residues are still needed to be strengthened in view of soil conserving management, especially under different long-term agricultural management conditions.

1.5 Aim and hypotheses of the PhD thesis

Many previous studies have studied effects of agricultural management practices on soil WEOM, microbial communities as well as microbial residues via chamber incubation, including short-term or long-term field trials. However, studies considering both organic farming with minimum tillage and agroforestry are scarce. Moreover, long-term agricultural field experiments are particularly valuable to detect effects of management practices on soils. It has to be taken in consideration that changes in soils may develop slowly and may adjust to a new long-term steady state after a change of management or conversion to a different farming system. Regarding the study of soil WEOM, previous studies mainly concentrated on the content, while the quality study of soil WEOM is relatively less compared with quantitative study. Furthermore, the WEOM-qualities study mostly based on the measurement of SUVA and HIX of soil WEOM. In the current study, a technique combining excitation-emission spectra and parallel factor analysis can provide more information about the quality and composition of soil WEOM. PLFA analysis together with residues analysis can provide more information on microbial communities as well as microbial residues. Therefore, a long-term (21-year) field trial in current thesis provides unique opportunity to study the effects of long-term implementation of organic farming, minimum tillage as well as agroforestry (tree species) on soil WEOM (publications I and II), microbial community structure and microbial residues (publications III and IV). The specific hypotheses in this thesis are:

1) Minimum tillage and organic farming increase the content of SOM and WEOM and change quality and composition of WEOM, and organic farming affects more on WEOM quality than minimum tillage.

2) Organic farming increases SOC and WEOM contents, especially that of the fluorescent WEOM fractions. N_2-fixing robinia has stronger positive effects on SOC and WEOM than poplar, especially close to the hedgerow.

3) Organic farming increases microbial biomass, especially bacterial biomass, leading to an increased contribution of microbial residues to SOC. Minimum tillage increases microbial biomass,

especially fungal and AMF biomass. The combination of organic farming and minimum tillage would be the most effective management strategy, resulting in maximum contents of microbial biomass, with strongest effects on AMF and only intermediate effects on bacteria and saprotrophic fungi.

4) Organic farming increases SOC, soil microbial biomass, microbial functional guilds as well as microbial residues. N_2-fixing robinia biosystem has stronger positive effects on microbial community than the poplar system, especially when it is close to the hedgerow.

5) Long-term soil managements cause different responses of soil to added labile organic carbon and distribution of labile organic carbon in carbon pools.

2 MATERIALS AND METHODS

2.1 Filed trial

2.1.1 Field trial testing minimum tillage and organic farming

Field experiments have been carried out at the Scheyern Research Farm (TERENO site, www.tereno.net) located 40 km north of Munich, Germany (48.50°N, 11.45°E) since 1992. The altitude of the farm ranges between 445-500 m a.s.l. The mean annual precipitation and mean annual temperature are 803 mm and 7.4 °C, respectively (Schröder et al., 2002). The central part of the research station was divided into two parts: organic and integrated farming systems, each striving for ecological and economical sustainability as a farm. Moreover, specific detailed studies on tillage and fertilization induced changes were carried out in plots sub-divided into integrated and organic farming on two neighboring fields with same soil and site conditions (Schröder et al., 2002).

On the organic and integrated sites, plot experiments studying tillage-induced changes to the systems were set up. Two farming managements (Integrated (I) and Organic farming (O)) and two tillage systems (Plough tillage (PL) and Minimum tillage (MT)) were arranged in full factorial plot design (the third tillage system, no till, was not considered in this work, as more sample analysis was not possible and because the farm responsible will no longer propose this management form in such type of landscape, an outcome of the site research). The farming systems in the study represent two tillage intensities under the same soil and climate conditions. PL as a conventional tillage system means tilling the soil with a moldboard plow (25-30 cm). MT in the study means cultivating the soil with a chisel plow in the first 6-8 cm of soil. Therefore, there are four treatments in the present study in total and each has three replicates. Namely, 1) organic farming + plough tillage (OPL); 2) organic farming + minimum tillage (OMT); 3) integrated farming + plough tillage (IPL); 4) integrated farming + minimum tillage (IMT). The assignments of the treatments to the plots have

been kept constant since 1992. The IF plots are 12 × 12 m in size and the crop rotation is potato (*Solanum tuberosum* L.) + mustard (*Sinapis alba* L.) as catch crop, (2) winter wheat (*Triticum aestivum* L.), (3) maize (*Zea mays* L.) + mustard as catch crop, and (4) winter wheat. The OF plots are 12 × 12 m in size and the crop rotation is a seven-crop rotation: grass–clover–alfalfa (GCA) (*Lolium perenne* L. + *Trifolium pratense* L. + *Medicago sativa* L.), (2) potato + mustard as cover crop, (3) winter wheat, (4) sunflower (*Helianthus annuus* L.) + GCA as cover crop, (5) GCA, (6) winter wheat, and (7) winter rye (*Secale cereale* L.) + GCA as cover crop. The soil types are sandy to loamy Cambisols, derived from tertiary sediments. The soil in both field trials has a soil texture of silty loam (USDA) and the soil texture of top soil is 22% sand, 58% silt and 20% clay (Flessa et al., 2002; Kölbl and Kögel-Knabner, 2004).

In the IF system, N fertilization was done with UAN (50% urea N, 50% ammonium nitrate N), with a modified boom sprayer with tubes to conduct the solution directly to the soil surface. Fertilizer rates were fixed for the cultivated crops (135 kg N ha^{-1} for winter wheat, 105 kg N ha^{-1} for maize and 100 kg N ha^{-1} for potato), considering globally assessed credits due to effects of previous crop cultivation or mineralization in the soil. In the OF system trial, cattle farmyard manure was applied at a rate of 30 t ha^{-1} a^{-1} dry weight. The management summary of tillage systems under organic and integrated farming are listed in Table 2.1.

2.1.2 Field trial testing alley cropping system and organic farming

The agroforestry systems were located in the same research station. The climate and geographical background of agroforestry systems were the same to tillage trial systems under organic and integrated farming (2.1.1). The soils of organic and integrated field for agroforestry systems are similar in this hilly landscape with long time erosion driven soil modifications and have also a soil texture of silty loam (USDA). The soil texture of top soil was 27% sand, 54% silt and 19% clay for the organic field and 27% sand, 50% silt and 23% clay for the integrated field.

In 2009, agroforestry parcels were incorporated into both integrated and organic farming systems. Six swaths of trees, each comprising of several different species, were planted in an alley cropping for the purpose of bioenergy production (30 m length for each tree species). 30 m wide arable soil was left for crop production (Fig. 1).

The poplar (*Populus maximowiczii x P. nigra*) and Robinia (*Robinia pseudoacacia* L.) alley systems were chosen for this study in both organic and integrated farming systems. The experiment consisted of four treatments with three replications: organic farming + poplar (O-pop), organic farming + robinia (O-rob), integrated farming + poplar (I-pop), and integrated farming + robinia (I-

rob). The plot for each treatment is 30 × 30 m in size. The treed portions of both systems were not treated with fertilizer, neither in manure or mineral form. Neither did the treed portions receive weed control mechanically or via pesticide. The tree density of poplar and robinia were the same. The organic farming system was low-input, utilizing nitrogen fixing cover crops instead of mineral nitrogen or surplus inputs as green manures. Also no pesticide, esp. herbicide was applied. Soils were plowed with moldboard. A seven-field crop rotation was run in the organic farming system: (1) Grass–clover–alfalfa (GCA) (*Lolium perenne* L. + *Trifolium pratense* L. + *Medicago sativa* L.), (2) potatoes (*Solanum tuberosum* L.) + mustard (*Sinapis alba L.)* as cover crop, (3) winter wheat (*Triticum aestivum* L.), (4) sunflower (*Helianthus annuus* L.) + GCA as cover crop, (5) GCA, (6) winter wheat, and (7) winter rye (*Secale cereale* L.) + GCA as cover crop. In the integrated farming system, soils were tilled by harrowing and chiseling, and the tillage intensity was reduced to a level to control weed as well as to conserve soil. Pesticides were completely forbidden in organic farming, while in necessity it is applicable in the integrated farming system. A four-field crop rotation with cover crops was run in the integrated farming system: (1) winter wheat; (2) potatoes; (3) winter wheat; and (4) maize (*Zea mays* L.). The management was summarized in Table 1.

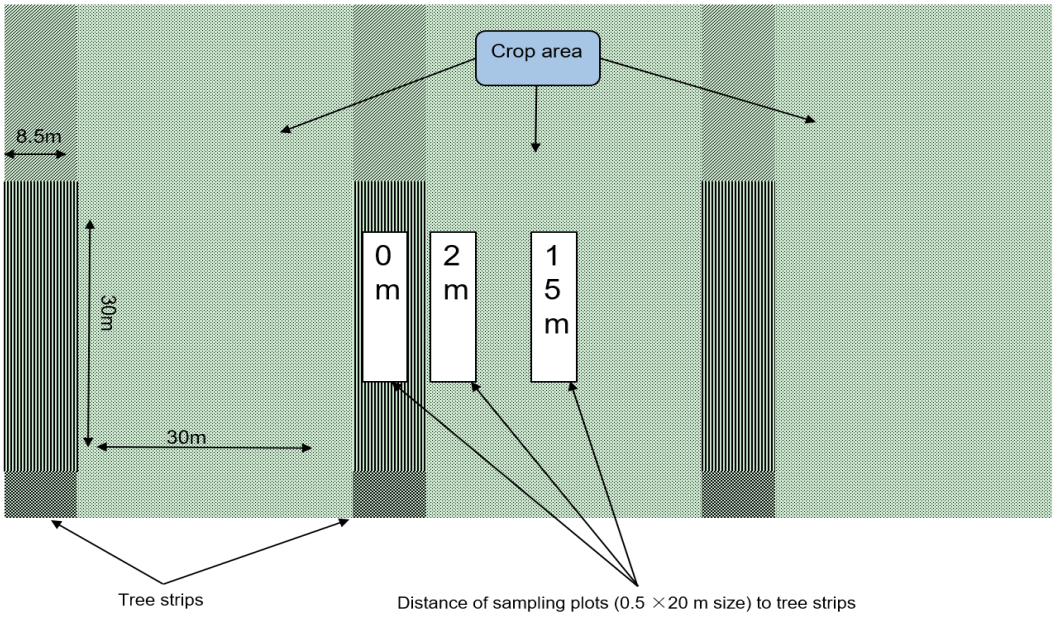


Figure 2.1 The experimental layout of alley cropping agroforestry systems and sampling sites (0 m, 2 m and 15 m). The entire field experiment comprises six wooden species.

Table 2.1 Summary of the agricultural management systems in the long-time field trial, chosen for this study

Farming	Tillage type	Tillage description	Soil type	Soil texture	Crop rotation and management	Fertilization
High-input Organic farming	plowing	till the soil with a moldboard plow (25-30 cm)	Cambisols	silty loam	(1) Grass–clover–alfalfa (GCA), (2) potatoes, (3) winter wheat, (4) sunflower (5) GCA, (6) winter wheat, and (7) winter rye. no pesticide and herbicide	cattle farmyard manure30 t ha^{-1} a^{-1} dry weight.
	minimum	cultivate the soil with a chisel plow in the first 6-8 cm of soil	Cambisols	silty loam	(1) Grass–clover–alfalfa (GCA), (2) potatoes, (3) winter wheat, (4) sunflower, (5) GCA, (6) winter wheat, and (7) winter rye, no pesticide and herbicide	cattle farmyard manure30 t ha^{-1} a^{-1} dry weight.
Integrated farming	plowing	till the soil with a moldboard plow (25-30 cm)	Cambisols	silty loam	(1) potato, (2) winter wheat, (3) maize, (4) winter wheat. pesticide and herbicide when necessary	N fertilization (135 kg N ha^{-1} for winter wheat, 105 kg N ha^{-1} for maize and 100 kg N ha^{-1} for potato)
	minimum	cultivate the soil with a chisel plow in the first 6-8 cm of soil	Cambisols	silty loam	(1) potato, (2) winter wheat, (3) maize (4) winter wheat. pesticide and herbicide when necessary	N fertilization (135 kg N ha^{-1} for winter wheat, 105 kg N ha^{-1} for maize and 100 kg N ha^{-1} for potato)
Low-input organic farming	Robinia (Black Locust)	plough tillage with moldboard (25-30cm)	Cambisols	silty loam	(1) Grass–clover–alfalfa (GCA), (2) potatoes, (3) winter wheat, (4) sunflower (5) GCA, (6) winter wheat, and (7) winter rye, no pesticide and herbicide	low-input, utilizing nitrogen fixing cover crops instead of mineral nitrogen or synthetic inputs as green manures

	Poplar	plough tillage with moldboard (25-30cm)	Cambisols	silty loam	(1) Grass–clover–alfalfa (GCA), (2) potatoes, (3) winter wheat, (4) sunflower, (5) GCA, (6) winter wheat, and (7) winter rye, no pesticide and herbicide	low-input, utilizing nitrogen fixing cover crops instead of mineral nitrogen or synthetic inputs as green manures
Integrated farming	Robinia (Black Locust)	tilled by harrowing and chiseling, and the tillage intensity was reduced to a level to control weed as well as to conserve soil.	Cambisols	silty loam	(1) potato, (2) winter wheat, (3) maize (4) winter wheat. pesticide and herbicide when necessary.	N fertilization (135 kg N ha^{-1} for winter wheat, 105 kg N ha^{-1} for maize and 100 kg N ha^{-1} for potato)
	Poplar	tilled by harrowing and chiseling, and the tillage intensity was reduced to a level to control weed as well as to conserve soil.	Cambisols	silty loam	(1) potato, (2) winter wheat, (3) maize (4) winter wheat. pesticide and herbicide when necessary.	N fertilization (135 kg N ha^{-1} for winter wheat, 105 kg N ha^{-1} for maize and 100 kg N ha^{-1} for potato)

2.2 Soil sampling and soil preparation

For the tillage system under organic and integrated farming, three replicates composite samples of the top soil composed of 20 augers (5-cm) were collected from plots of each treatment at two soil depths (0-8 cm-upper soil and 12-25 cm-deeper soil) separately in April 2013 just at the beginning of spring and soil reactivation after winter.

Regarding to the robinia and poplar-based alley cropping system under organic and integrated farming: Sampling plots were randomly set within the tree row (0 m), transition area (2 m from the tree row: 2 m) and middle of crop area (15 m from the tree row: 15 m) corresponding to poplar and robinia strips in organic and integrated farming system (Fig. 1). In May 2013 before vegetation developed after a unusually long winter, three replicate composite samples (from each 5-cm auger) were collected at a depth of 0-25 cm.

The field moist samples were transported to the lab on ice. The moist soils were sieved with 2 mm size sieve immediately. Subsamples for total organic carbon, total nitrogen, pH, and soil texture measurement were randomly taken from each composite sample and air-dried. Subsamples for phosphorous fatty acids analysis were preserved in the freezer under -20 °C until analysis. Subsamples for microbial residues and microbial biomass analysis were preserved under 4°C until analysis.

2.3 Lab incubation experiment

Soils used in the incubation were collected at the upper 8 cm from the tillage trials comprising of plough tillage (PL) and minimum tillage (MT) under organic (O) and integrated (I) farming management. Therefore, four soil materials were used for the incubation. Namely, OPL (Organic farming +Plough tillage), OMT (Organic farming +Minimum tillage), IPL (Integrated farming + Plough tillage) and IMT (Integrated farming +Minimum tillage) soils. The soil in both field trials had a soil texture of silty loam (USDA) and the texture of top soil was 22% sand, 58% silt and 20% clay. The basic soil properties are shown in Table 2.2.

Table 2.2 Some basic properties of soil materials taken for lab incubation

soil	Total organic carbon (mg g^{-1})	Total nitrogen (mg g^{-1})	pH ($CaCl_2$)	NO_3^- (µg g^{-1})	Microbial biomass carbon (µg g^{-1})
OPL	14.2	1.5	5.8	9.8	388.5
OMT	19.6	2.0	6.0	13.3	663.0
IPL	12.5	1.4	5.7	70.1	238.8
IMT	13.8	1.5	6.0	68.0	406.0

Two independent incubation experiments were set up. The first incubation evaluated the amount and form of the added carbon remaining in the labile and stable soil carbon pools. Soils were pre-incubated 3 weeks at 40% water holding capacity (WHC). Then, 50 g dry weight equivalents moist soil aliquot were weighed and putted into 250 ml incubators; 2ml deionized water (control) or with glucose (99% ^{13}C), to reach 200 µg C g^{-1} soil for the ^{13}C-glucose treated were applied into each soil sample. The experiment was designed with three replicates, and thereby 30 incubators for each soil in total. All incubators were closed with plastic wraps with needle-punctured holes to maintain aerobic conditions and then incubated at 14˚C in the darkness. Soil moisture kept constant at 60%

WHC by addition of deionized water every other day, by weighting them. At days 1, 3, 7, 11 and 15, destructive sampling with three replicates was conducted for each of the treatments (controls and ^{13}C-glucose treated soils) to analyze each on non-extractable SOC (the fraction of soil organic matter remaining in soils samples after removal of WEOC), $\delta^{13}C$- non-extractable SOC, WEOC, $\delta^{13}C$-WEOC, MBC, $\delta^{13}C$-MBC.

The second incubation was set up to monitor soil respiration and glucose mineralization. 12 ml vials containing 1g (dry weight) moist soil as used in the first incubation were used to measure the CO_2 development and their corresponding $^{13}C/^{12}C$ ratios evolved from soil samples. On day 0, 1, 2, 3, 5, 7, 10, 14 after addition of labelled glucose four vials of each treatment and each control were closed air-tight with a plastic lid with a rubber septum and placed in a water jacket adjusted to the incubation temperature 14 °C. The rack was specially built to be mounted on a CombiPAL autosampler (CTC, Zwingen, Switzerland).

2.4 Analysis

2.4.1 CO_2 and CO_2-^{13}C measurement

The values of CO_2 and respective $^{13}CO_2$ enrichment in the headspace of the vials were determined on-line with a GC/IRMS. Four measurements were determined on each sample. Samples were withdrawn from the vials headspaces by a syringe on the auto-sampler and injected into the GC/IRMS. All sample values were compared with reference CO_2 at different concentrations. A regression line was fitted to the measured points ($R_2 > 0.95$) to calculate the CO_2 production rate. From this rate the CO_2 amounts per day were estimated and subsequently cumulated to obtain the total CO_2 amounts evolved on the respective samplings. Regarding to the non-gas parameters, the soil parameters were briefly summarized in Table 2.3 and the detailed information was given as follows.

Table 2.3 Overview of main investigated soil chemical and biological properties

Parameters	Principle	References
WEOC (water extractable organic carbon)	extract soil with 10 mM $CaCl_2$ (2:1 v:m), filter with 0.45μm membrane filters, then measure with TOC analyzer	(Embacher et al., 2007)
WEON (water extractable organic nitrogen)	equals total nitrogen content minus mineral nitrogen content	(Embacher et al., 2007)
HIX (Humification index)	the ratio of integrated fluorescence emission peak at longer wavelength region (435-480 nm) over shorter wavelength region (300-345 nm) at 254 nm excitation wavelength.	(Zsolnay, 2003; Zsolnay et al., 1999)
SUVA (Specific UV absorption)	the ratio of absorption at 254 nm to WEOC concentration	(Corvasce et al., 2006)
EEM (Excitation and emission matrix) spectra	EEMs were obtained by scanning over excitation wavelength from 250 nm to 450 nm with an increment of 5 nm and emission wavelength from 300 nm to 600 nm with an increment of 5 nm.	(Borisover et al., 2012; Ohno and Bro, 2006)
Microbial community structure	extract soil lipids, separation of phosphorous lipids and measurement with GC-MS	(Gattinger et al., 2002; Zelles, 1999; Zelles and Bai, 1993)
Microbial residue	extract soil amino sugar, purify the extracts and measurement with HPLC	(Appuhn and Joergensen, 2006; Appuhn et al., 2004; Indorf et al., 2011)
Glomalin	extract soil glomalin and measurement with Bradford dye-binding assay	(Wright and Upadhyaya, 1996; Wright and Upadhyaya, 1998)
MBC (N) (Microbial biomass carbon/nitrogen)	fumigate moist soil sample with chloroform and extract the fumigated and non-fumigated samples, then measure C and N in the filtered extracts	(Joergensen, 1996; Joergensen and Mueller, 1996; Vance et al.,

		1987)
^{13}C-WEOC	after extraction of WEOC, the extracts were imposed to measurement of ^{13}C with LC-isoLINK	(Krummen et al., 2004a; Marx et al., 2007a; Marx et al., 2007b)
^{13}C-MBC	after extraction of MBC, the extracts were imposed to measurement of ^{13}C with LC-isoLINK	(Krummen et al., 2004a; Marx et al., 2007a; Marx et al., 2007b)

2.4.2 Soil physical-chemical analysis

Soil organic carbon (SOC) and total nitrogen was determined by a CN analyzer (EA3000 Eurovector) with an aliquot air dried soil samples. Soil texture was determined by wet sieving and pipet method (Gee and Bauder, 1986). Briefly, sand and silt fractions ≥ 20 µm were measured by sieving after treatment with H_2O_2 (hydrogen peroxide) to eliminate gently the organic matter, silt and clay < 20 µm by a pipette procedure. The soil pH was measured in a soil suspension with 0.01 M $CaCl_2$ solution (1:5, w/v).

2.4.3 WEOM extraction and spectroscopic characteristics

Soil WEOM was extracted according to method of Zsolnay (1996). Briefly, 2 mm mesh sieved soil samples were shaken overhead for 10 min in 0.01 M $CaCl_2$ at a ratio of 1:2 (soil: volume). After that, a 10-min centrifugation at 3000 *g* succeeded and the supernatants were filtered through 0.4 µm polycarbonate membrane filters. WEON was quantified by subtracting inorganic N from soluble total N. Soluble total N, NO_3-N and NH_4-N were quantified colorimetrically with an automated continuous flow analyzer (Skalar).

Specific UV absorption ($SUVA_{254}$), obtained by dividing the absorption at 254 nm by WEOC concentration, provides the information about the aromatic structures of WEOM. Absorption was determined using 1cm quartz cells with a Varian Cary 50 Bio UV-visible spectrophotometer (Corvasce et al., 2006).

As pH and molecular concentrations can influence fluorescence, dilution was made to have a WEOC absorbance < 0.1 cm^{-1} at 254 nm, and then WEOC extracts were acidified with 2 M HCl to pH 2 (Embacher et al., 2007) before measuring fluorescence with a Varian Cary Eclipse

spectrophotometer, using 1 cm quartz cells at 254 nm excitation. The fluorescence emission spectra were recorded (300-480 nm) at 21±1 °C and corrected by multiplication with factor e^A, where A is the absorbance in cm^{-1} at the excitation wavelength (Embacher et al., 2007). The humification index (HIX), indicating the complexity and condensation of WEOM, was calculated as ratio of integrated fluorescence emission peak at longer wavelength region (435-480 nm) and that of a shorter wavelength region (300-345 nm) according to Zsolnay et al. (1999).

EEMs were obtained by scanning over excitation wavelength from 250 to 450 nm with an increment of 5 nm and emission wavelength from 300 to 600 nm also with an increment of 5 nm. The slit wide was set as excitation slit 10 nm and emission slit 20 nm. Fluorescence data were corrected for inner-filter effect with absorption data as suggested by Lakowicz (2006):

$$I_{corr} = I_{obs} \times 10^{0.5(Aex + Aem)}$$

where I_{corr} and I_{obs} are corrected and uncorrected fluorescence intensities, and A_{ex} and A_{em} are the absorbance values at the excitation and emission wavelength of the fluorescence intensity value. The correction is based on the assumption that the average path length of excitation and emission light is 50% of the cuvette width, respectively.

Before the application of PARAFAC model, zero emission intensities were assigned for excitation wavelengths (λex) greater than emission wavelengths (λem). Raleigh scattering was minimized according to Andersen and Bro, and Borisover et al. (Andersen and Bro, 2003; Borisover et al., 2012). An EEM of the 0.01 M $CaCl_2$ solution was obtained and subtracted from the EEM of each sample in order to removed most of the Raman scatter peaks. Any negative value produced by the sub traction was converted to a missing value. The fluorescence was normalized by dividing the integrated area under Raman scatter peak of the corresponding Milli-Q water of each set of measurement (Lawaetz and Stedmon, 2009). All fluorescence intensities were reported in Raman units (R.U.).

2.4.4 PARAFAC modeling of excitation and emission spectra of WEOM

PARAFAC provides a way to reduce a dataset of EEM into tri-linear function of concentration of one component and its specific absorption/emission properties (Andersen and Bro, 2003; Borisover et al., 2012; Bro, 1997). Components extracted by PARAFAC can be ascribed to specific species of organic matter present in liquid samples, but they are more likely represent groups of organic compounds having similar fluorescence properties. While component scores indicate the relative concentrations of groups of organic fractions represented by the components, excitation and emission loadings indicate their characteristic excitation and emission spectra (Baghoth et al., 2011).

It is important to recognize that the concentrations are based on their fluorescence signal contributions that provide relative concentrations rather than their true chemical concentration contributions. The full description about PARAFAC can be referred to Bro (1997). Split-half analysis and examination of residual error plots were applied to get appropriate number of components and a series of PARAFAC models were generated with Matlab (2009a) by using the DOM-fluor toolbox specifically developed for PARAFAC analysis of DOM fluorescence (Stedmon and Bro, 2008).

2.4.5 Non-extractable soil organic carbon (SOC) and $\delta^{13}C$ of non-extractable SOC

As the non-extractable SOC is a stable fraction of SOC in soil, samples collected at the beginning and the end of the lab incubation were analyzed. The fraction after the removal of WEOM is taken as the non-extractable soil organic matter. After air-dry and grinding process of soil samples conserved at room temperature, the non-extractable SOC content and non-extractable $\delta^{13}C$-SOC were determined with an element analyzer (Eurovector CN) coupled with a gas chromatograph/isotope ratio mass spectrometer (GC/IRMS) (Finnigan MAT DeltaPlus, Bremen, Germany).

2.4.6 Microbial analysis

2.4.6.1 Microbial biomass

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined using the chloroform fumigation extraction method (Vance et al., 1987). In brief, 5 g (on an oven-dry basis) moist soil samples were fumigated for 24 h with $CHCl_3$, and then a 5 g fumigated and a separate 5 g non-fumigated sample were extracted with 20 ml 0.01 M $CaCl_2$ solution (Joergensen, 1995). Organic C and total N in the extracts were measured using a TOC/TN_b analyzer (Dimatec, Essen, Germany). MBC and MBN were calculated using a k_{EC} value of 0.45 (Joergensen, 1996) and a k_{EN} value of 0.54 (Joergensen and Mueller, 1996), respectively.

2.4.6.2 Microbial community structure

PLFA were determined according to Zelles and Bai (1993) and Gattinger et al. (2002). In brief, a fresh soil sample equivalent to 15 g dry soil was extracted in accordance to the modified Bligh Dyer method (1v methanol, 2v chloroform and 0.8v phosphate buffer mixture). The extracts were fractionated into neutral lipids, glycolipids and phospholipids on a SI column (SPE-SI; Bond Elute, Varian, Palo Alto, USA) by chloroform, acetone and methanol as elution liquids, respectively. The phospholipid was subjected to the mild alkaline methanolysis to get fatty acid methyl esters (FAMEs) and a SCX column impregnated with silver nitrate was applied to fractionate the unsubstituted FAMEs into saturated, monounsaturated and poly-unsaturated FAMEs and

abbreviated as SATFA, MUFA and PUFA, respectively (Zelles, 1999). The SATFA and PUFA fraction were measured directly using GC/MS with nonadecylacidmethylester as internal standard solution. MUFA fraction was measured after dimethyl disulphide (DMDS) derivatization, using the GC/MS operating condition given in Zelles and Bai (1993). The first temperature program of the oven began at 70 °C (for 2 min) and increased to 160°C at 40 °C min^{-1}, followed by 280 °C at 3 °C min^{-1} (injector temperature 290 °C). The second program started at 100 °C and enhanced to 210 °C at 50 °C min^{-1}, followed by 300 °C at 3 °C (injector temperature 300 °C). The latter operational variables were only used to measure DMDS derivatives of FAMES. The identification of individual compounds was based on comparison of retention time and mass spectral data obtained from standard compounds, monocultures and environmental samples (Zelles, 1999). The quantification was achieved using chromatography software (HP ChemStation, SOVLVIT, CH) by considering the ratio of target compound and the internal standard with respect to peak areas, as well as the response for each, which was estimated experimentally with standard mixtures (Gattinger et al., 2002). The PLFA n15:0, i15:0, a15:0, i16:0, i17:0, a17:0 are used as biomarker for Gram (+) bacteria (Frostegård and Bååth, 1996; Zelles, 1997); the PLFA 16:1ω7t, 16:1ω7c, 16:1ω9, 16:1ω6, 18:1ω7, cy17:0 and cy19:0 for Gram(-) bacteria (Colaco et al., 2007; Frostegård and Bååth, 1996; Zelles, 1997); 18:2ω6,9 for fungi (Joergensen and Wichern, 2008); 16:1ω5 for arbuscular mycorrhizal fungi (AMF) (Olsson et al., 1995). Ratios of (i15:0+i17:0) / (a15:0+a17:0) were used as index for nutritional or environmental stress on Gram (+) bacteria (Zhang et al., 2014).

2.4.6.3 Microbial residues

The amino sugars, muramic acid (MurN), galactosamine (GalN), 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 hydrolyzed for 6 h at 105 °C. 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 C18 column (125 mm length × 4 mm diameter), protected by a Phenomenex C18 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 derivatization, 50 µl ortho-phthaldialdehyde (OPA) and 30 µl sample were mixed in the preparation vial and after 120 s reaction time, 15 µl 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).

Fungal C was estimated by multiplying the content of fungal GlcN in by 9 (Appuhn and Joergensen, 2006). Fungal GlcN was calculated by subtracting bacterial GlcN from total GlcN, assuming that MurN and GlcN occur at a 1:2 molar ratio in bacteria (Engelking et al., 2007). Bacterial C was calculated by multiplying the content of MurN by 45 (Appuhn and Joergensen, 2006).

2.4.6.4 Glomalin

Replicate 0.25 g samples of dry-sieved 1-2 mm aggregates were extracted with 2 ml of extractant. Easily extractable glomalin (EEG) was extracted with 20 mM citrate (pH 7.0) at 121°C for 30 min. Total glomalin (TG) was extracted with 50 mM citrate (pH 8.0) at 121°C. For sequential extractions, the supernatant was removed by centrifugation at 10,000 g for 5 min, 2 mL of 50 mM citrate, pH 8.0 was added to the residue and samples were autoclaved for 60 min. Extraction of a sample continued until the supernatant showed none of the red-brown color typical of glomalin. Extracts from each replicate were pooled and then analyzed. After extraction cycles were completed, samples were centrifuged to remove the soil particles (10,000 g for 5 min), and protein in the supernatant was determined by the Bradford dye-binding assay (Bradford assay (Sigma-Aldrich Inc.)) with bovine serum albumin as the standard (Wright and Upadhyaya, 1996, 1998).

2.4.7 ^{13}C in WEOC and MBC extracts

The concentration and $^{13}C/^{12}C$ ratios in liquid samples were determined by liquid chromatograph/isotope ratio mass spectrometer (LC/IRMS) (Thermo Finnigan LC IsoLink and MAT 253, Bremen, Germany) by an on-line method developed by Krummen et al. (2004b) as described in previous work of our working group (Marx et al., 2007b). The LC IsoLink worked in a mode that allows the bulk isotopic analysis of all water-soluble material. On the LC-IsoLink, the samples were processed as follows: organic substances in the extracts were oxidized quantitatively to CO_2 by 0.45M $Na_2S_2O_8$ and 8.5% H_3PO_4 solutions in a reaction chamber at 99.9°C. The CO_2 was separated from the liquid phase with a gas exchange membrane and admitted to the IRMS in a stream of helium via an open split. The ^{13}C values of the soil extracts determined were compared with values of an internal laboratory standard (benzoic acid solution) and a blank (solvent used for soil extraction), both of which were included in all sample measurements at regular intervals. The C concentrations of the standards were chosen according to the given concentrations of the soil extracts. The accuracy of this method has been presented for soil extracts by Marx et al. (2007). The

measurements were reproducible over time and sensitive in measuring ^{13}C in extracts with very small C concentrations around 1mg C l^{-1}. The detection limit is 0.5mg C l^{-1}. The external precision of the $\delta^{13}C$ measurements is 0.3‰ VPDB.

2.5 Calculations

The $\delta^{13}C$ values of the samples were expressed relative to the international VPDB standard:

$$\delta^{13}C\ (‰\ VPDB) = \left(\frac{R_{sample} - R_{VPDB}}{R_{VPDB}}\right) * 1000$$

where $R=^{13}C/^{12}C$ and $R_{VPDB}=0,\ 0111802$. We calculated the $\delta^{13}C$ (‰ VPDB) of MBC from the following mixing equation:

$$\delta^{13}MBC=\frac{(C_{fum}\times\delta^{13}C_{fum})-(C_{nfum}\times\delta^{13}C_{nfum})}{(C_{fum}-C_{nfum})}$$

where C_{fum} and C_{nfum} is the concentration (μg g^{-1}) of C in the fumigated and non-fumigated soils, respectively.

The fraction of C originating from glucose ($f_{\text{glucose-C13}}$) in MBC, WEOC, CO_2-C, or non-extractable C_{org} was calculated from

$$f_{\text{glucose-C13}}=\frac{(\delta^{13}C_{sample}-\delta^{13}C_{soil})}{(\delta^{13}C_{glucose}-\delta^{13}C_{soil})}$$

where $\delta^{13}C_{sample}$ is the $\delta^{13}C$ (‰ VPDB) of the sample, $\delta^{13}C$soil is the $\delta^{13}C$ (‰ VPDB) of soil and $\delta^{13}C_{glucose}$is the $\delta^{13}C$ (‰ VPDB) of glucose.

The contribution of glucose-^{13}C to WEOC, MBC, CO_2-C, or non-extractable C_{org} total concentration (μg g^{-1}) was calculated from

$$C_{glucose}= f_{glucose} \times C_{total}$$

where C_{total} is the total concentration (μg g^{-1}) of WEOC, MB-C, CO_2-C, or non-extractable C_{org}, respectively.

2.5.1 Statistics

ANOVA with repeated measurement was applied to analyze organic matter fractions, glucose-derived organic fractions and recovery of ^{13}C in each organic matter fractions. One-way ANOVA and Pairwise Sample T-test was used to test the difference of soil respiration, and glucose mineralization between soils.

3 Results

3.1 Impacts of minimum and plough tillage under long-term organic and integrated management on the fate of labile carbon in soils

3.1.1 Changes in organic matter fractions

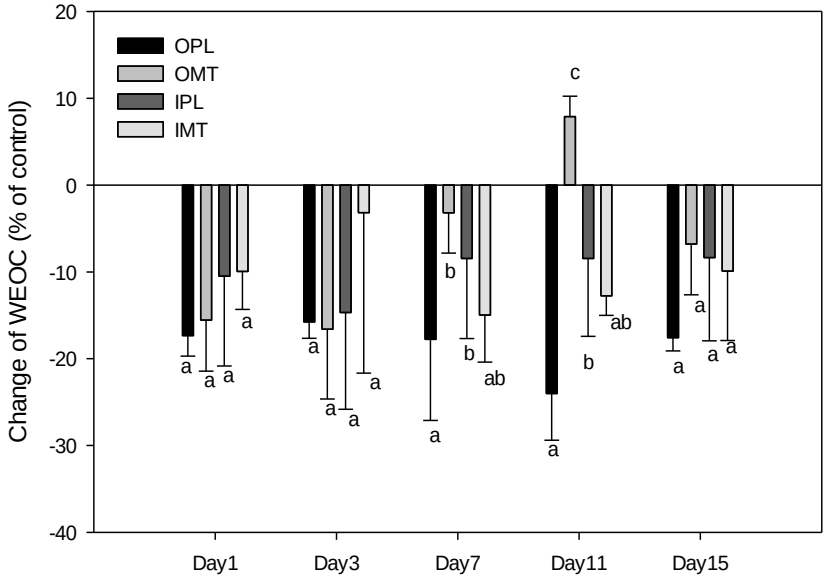
Changes in total water extractable organic carbon (WEOC)

In the course of first incubation, the content of total WEOC decreased in short term except on day 7 for OMT soil (Fig. 3.1). Soil management significantly influenced the change of total WEOC content. The sampling time had no main influence on change of WEOC content (Table 3.1, Fig. 2). The change of WEOC content fluctuated during the incubation time for all four soils (Fig.3.1). The greatest mean change occurred in OPL soil. For soils in the same farming system, change of WEOC in PL soils was greater than MT soil over the incubation. For soils of the same tillage practice, change of WEOC in organic PL soil was greater than integrated PL soil over the incubation. Whereas the change of WEOC for MT soil varied over the incubation and organic MT was lower than integrated MT at the end of incubation. At the beginning of incubation (1day after glucose addition), the change of total WEOC followed an order: OPL > OMT > IPL > IMT, while at the end of the incubation, the change of WEOC content of the four soils followed an order: OPL > IMT > IPL > OMT, although the difference was not significant at both sampling ($p<0.05$).

Table 3.1 ANOVA with repeated measurement analysis to the investigated soil organic matter fractions

	Change of WEOC		Glucose derived WEOC		^{13}C in WEOC	
	F	P	F	P	F	P
Soil (S)	10.8	0.005	8.74	0.009	8.79	0.009
Sampling(S)	0.579	NS	31.4	<0.001	31.30	<0.001
S × S	1,60	NS	31.5	<0.001	31.5	<0.001
	Change of MBC		Glucose derived MBC		^{13}C in MBC	
	F	P	F	P	F	P
Soil (S)	18.5	0,001	299	<0.001	299	<0.001
Sampling(S)	17.0	<0,001	6.09	0,007	6.09	0,007
S × S	2.28	NS	21.9	<0.001	21.9	<0.001
	Change of non-extract SOC		Glucose derived non-extract SOC		^{13}C in non-extract SOC	

	F	P	F	P	F	P
Soil (S)	0.55	NS	9.63	0.005	18.8	0.001
Sampling(S)	0.64	NS	38	<0.001	19.4	0.002
S× S	0.41	NS	4.10	0.05	2.30	NS



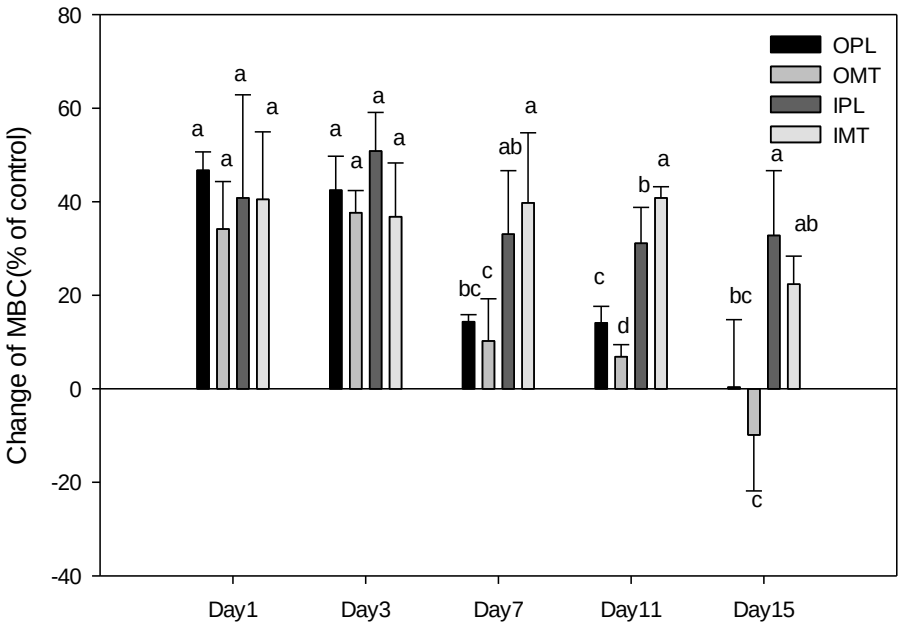
OPL: organic farming + plough tillage; OMT: organic farming + minimum tillage; IPL: integrated farming + plough tillage; IMT: integrated farming + minimum tillage

Figure 3.1 Change of water extractable organic carbon in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose relative to controls without the glucose addition

Change of total microbial biomass carbon

In the course of first incubation, the content of MBC generally increased for each soil except a decrease for OMT soil at the end of incubation (Fig. 3.2). In general, the change of MBC content decreased with time all through the incubation for OPL and OMT, while the change fluctuated for IPL and IMT soil. The change of MBC content was negligible for OPL soil and even negative for OMT soil at the end of incubation. IPL soil was observed with the highest mean change of MBC. For soils in the same farming system, the average change of MBC was higher in PL than MT soil. For soils of same tillage practices, the average change of MBC in integrated farming was higher than organic farming. At the beginning of incubation, the change of MBC followed an order: OPL > IPL > IMT > OMT, but the difference was insignificant ($P<0.05$). At the end of incubation, the

change of MBC followed an order: IPL > IMT > OPL > OMT, the change of MBC in IPL soil was significantly different from the other soils ($P<0.05$).

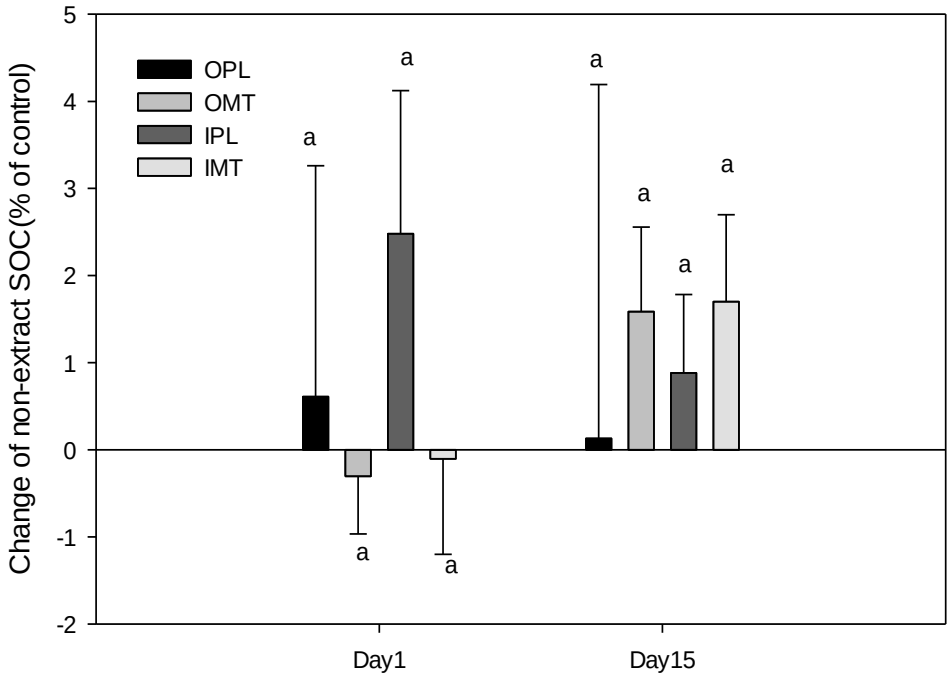


OPL: organic farming + plough tillage; OMT: organic farming + minimum tillage; IPL: integrated farming + plough tillage; IMT: integrated farming + minimum tillage.

Figure.3.2 Change of microbial biomass in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose relative to controls without the glucose addition

Change in total non-extractable soil organic carbon

The change in non-extractable SOC for each soil was insignificant although positive change was observed for OPL and IPL soil at the first sampling and for all soils at the end of incubation (Fig. 3.3). Here, it has to be highlighted that although positive effect of glucose addition on non-extractable SOC for each soil was observed at the end of incubation, the standard error especially for data as presented in the change of non-extractable SOC for all soils at first sampling and OPL soil at the end of incubation (relative difference between the treatment and control) was very large. The positive effect for OPL soil cannot be assured with current data.



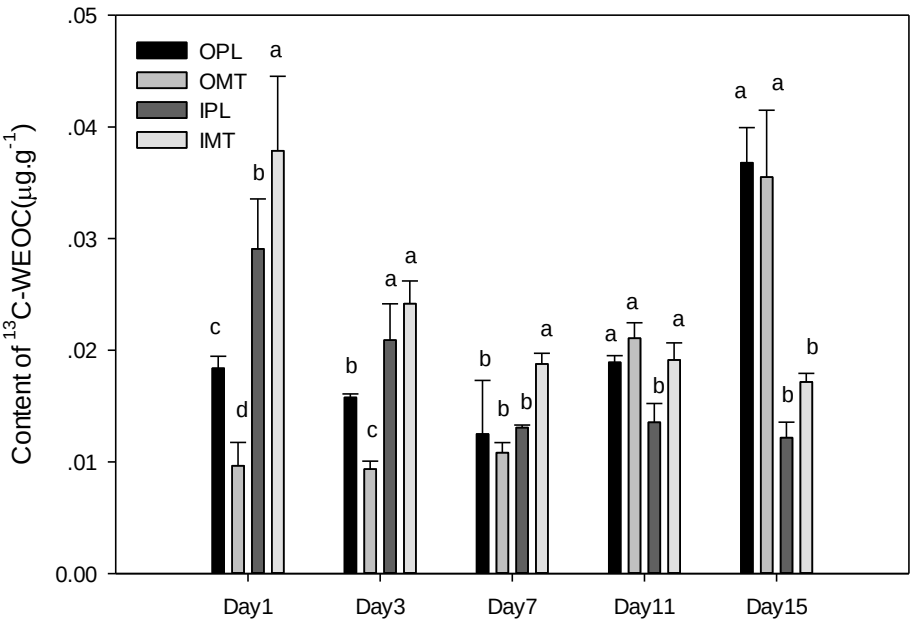
OPL: organic farming + plough tillage; OMT: organic farming + minimum tillage; IPL: integrated farming + plough tillage; IMT: integrated farming + minimum tillage.

Figure 3.3 Change of non-extractable organic carbon in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose and relative to controls without the glucose addition

3.1.2 Content of ^{13}C-in the organic matter fractions

^{13}C in water extractable organic carbon

The content of ^{13}C in WEOC is negligible in comparison to the content of total WEOC (Fig. 3.4). However, different response patterns of ^{13}C in WEOC can be observed. The content of ^{13}C in WEOC generally increased with time for OPL and OMT soil while decreased with time for IPL and IMT soil. The average content of ^{13}C in WEOC content was highest in IMT over the whole incubation. For soils in the same farming system, the content of ^{13}C in WEOC in organic PL was higher than that in organic MT soil, whereas integrated MT was higher than integrated PL. For soils of same tillage practice, the average content of ^{13}C in WEOC of organic PL soil was higher than integrated PL, whereas integrated soil was higher than organic soil of MT. At the beginning of incubation, more ^{13}C in WEOC was observed for IPL and IMT soil than OPL and OMT soil, while the results changed to opposite direction at the end of incubation.

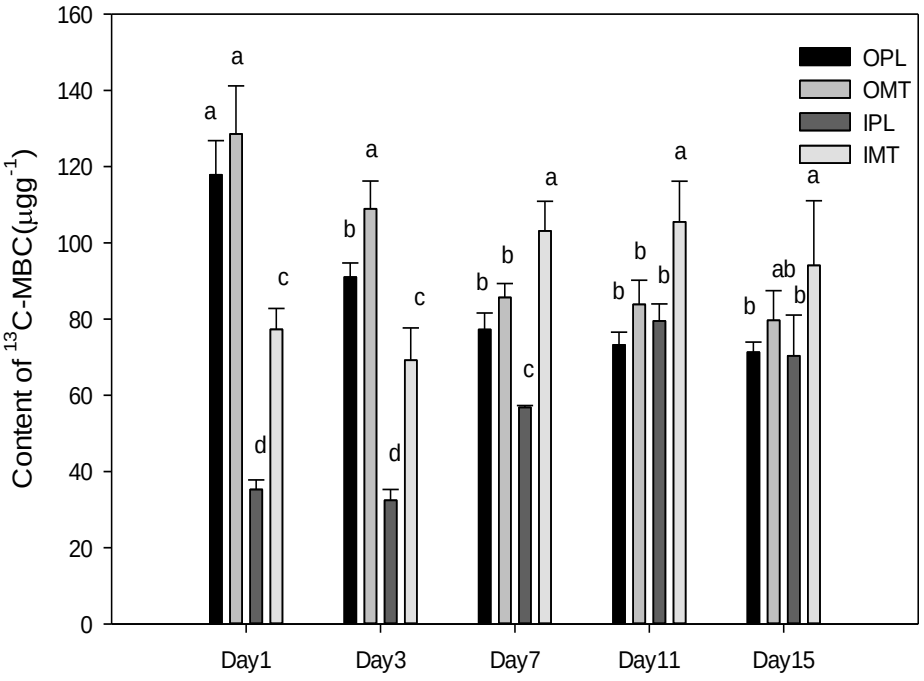


OPL: organic farming + plough tillage; OMT: organic farming + minimum tillage; IPL: integrated farming + plough tillage; IMT: integrated farming + minimum tillage.

Figure 3.4 Content of added ^{13}C remains in water extractable organic matter in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose

^{13}C in microbial biomass

The ^{13}C in microbial biomass with up to 60% of the added glucose at day 1 decreased with time for OPL and OMT soil over the incubation, while it tended to increase for IPL and IMT soil (Fig. 3.5). The average content of ^{13}C in microbial biomass over the whole incubation was highest in OMT soil. For soils in the same farming system, the content of ^{13}C in microbial biomass was higher in MT than in PL soils. For soils of same tillage practice, the content of ^{13}C in microbial biomass was higher in organic soils than integrated soils. At the beginning of incubation (1 day after glucose addition), the content of ^{13}C in microbial biomass ranked by OMT > OPL > IMT > IPL and the difference between IPL and IMT was significant ($p<0.05$). The content of ^{13}C in microbial biomass ranked by IMT > OMT > OPL >IPL and the difference between IMT and OPL and IPL were significant ($P<0.05$).

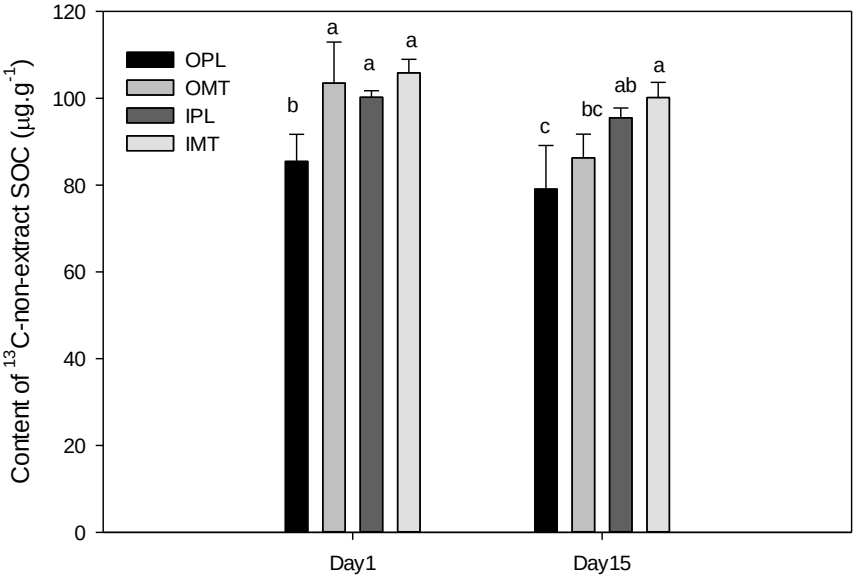


OPL: organic farming + plough tillage; OMT: organic farming + minimum tillage; IPL: integrated farming + plough tillage; IMT: integrated farming + minimum tillage.

Figure 3.5 Content of added ^{13}C remains in microbial biomass in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose

^{13}C in non-extractable soil organic carbon

The content of ^{13}C in non-extractable SOC tended to decrease over the incubation (Fig.3.6). The average content of ^{13}C in non-extractable SOC over the whole incubation was highest in IMT soil. For soils in the same farming, the content of ^{13}C in non-extractable SOC in MT was higher than that in PL soil. For soils of same tillage practice, the content of ^{13}C in non-extractable SOC of integrated soil was higher than that of organic soils. At the first sampling, the ^{13}C in non-extractable SOC ranked by IMT > OMT > IPL > OPL and ^{13}C-glucose-derived non-extractable SOC in OPL was significantly lower than that in other soils ($P<0.05$). At the end of incubation, the pattern changed and ^{13}C in non-extractable SOC ranked by IMT > IPL > OMT > OPL. The difference between IMT and IPL, between OMT and OPL was insignificant ($P<0.05$) and ^{13}C in non-extractable SOC in OPL was significantly lower than that in IMT and IPL soil ($P<0.05$).



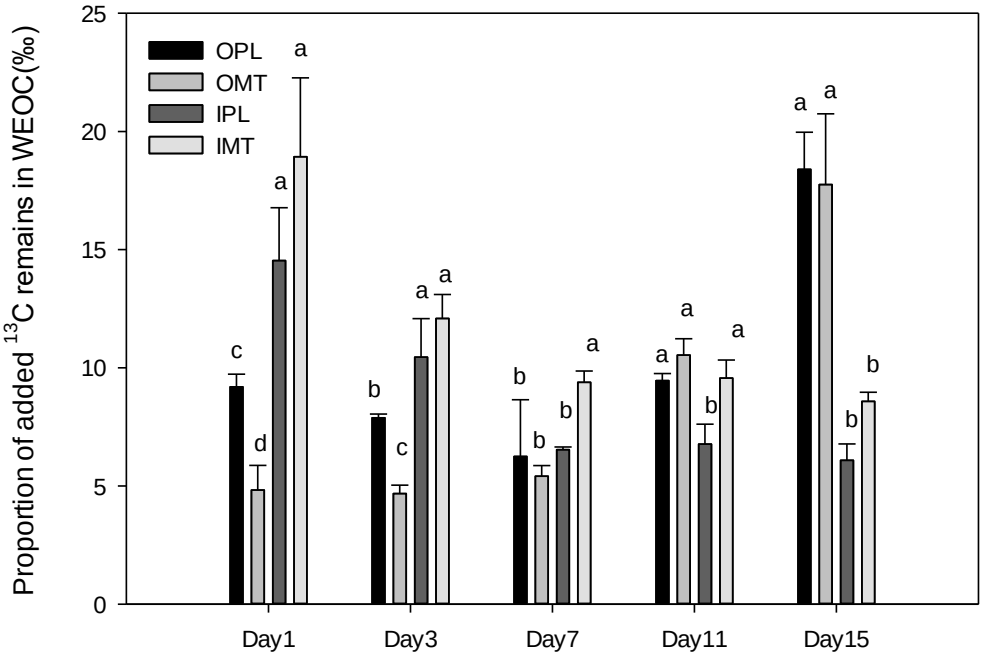
OPL: organic farming + plough tillage; OMT: organic farming + minimum tillage; IPL: integrated farming + plough tillage; IMT: integrated farming + minimum tillage.

Figure 3.6 Content of added ^{13}C remains bound in non-extractable organic carbon in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose

3.1.3 Proportion of added ^{13}C remaining in the organic matter fractions

Proportion of added ^{13}C remaining in water extractable organic carbon

The average proportion of added ^{13}C remains in WEOC over all soils was 9.6‰. The proportion of added ^{13}C remains in WEOC generally increased for OPL and OMT soil and decreased for IPL and IMT soil over the whole incubation (Fig.3.7). The proportion was highest in IMT soil over the whole incubation. For soil in the same farming system, the proportion was higher in PL than MT in organic farming system, whereas MT was higher than PL in integrated farming system. For soils of same tillage practice, the proportion was higher in organic soil than integrated soil of PL, whereas integrated soil was higher than organic soil of MT. At the beginning of incubation (1 day after glucose addition), the proportion of added ^{13}C remains in WEOC was higher in IPL and IMT than that in OPL and OMT and ranked IMT > IPL > OPL > OMT, whereas the pattern changed to OPL > OMT > IMT > IPL at the end of incubation. The difference between OPL and OMT, between IPL and IMT were insignificant at the end of incubation ($P<0.05$).

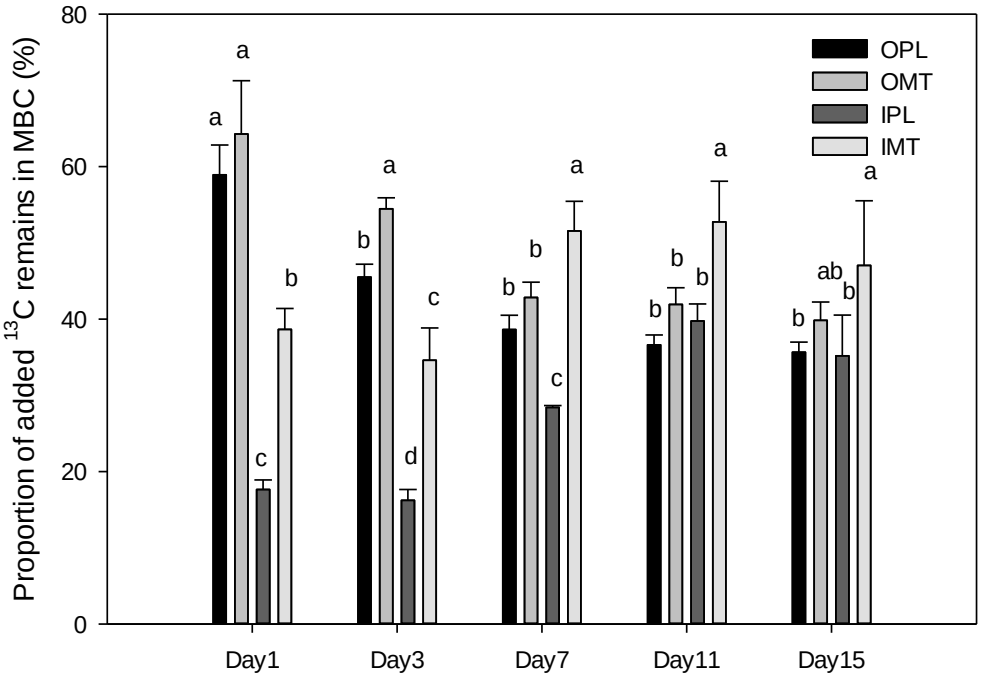


OPL: organic farming + plough tillage; OMT: organic farming + minimum tillage; IPL: integrated farming + plough tillage; IMT: integrated farming + minimum tillage.

Figure 3.7 Parts of added ^{13}C bound in water extractable organic carbon in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose

Proportion of added ^{13}C remaining in microbial biomass

The average proportion of added ^{13}C remains in MBC over all soil was 41.03% (Fig. 3.8). The proportion of added ^{13}C remains in MBC decreased for OPL and OMT soil while increased for IPL and IMT soil over the incubation. The highest average proportion of added ^{13}C remains in microbial biomass over the incubation was in OMT soil. For soils in the same farming, the proportion of added ^{13}C remains in MBC was higher in MT soil than PL soil. For soils of the same tillage practice, the proportion of added ^{13}C remains in MBC in organic soils was higher than integrated soils. At the beginning of incubation (1 day after glucose addition), the proportion of added ^{13}C remains in MBC was greater in OPL and OMT soil than that in IPL and IMT soil and ranked OMT > OPL > IMT > IPL. The pattern changed to IMT > OMT > OPL > IPL at the end of incubation.

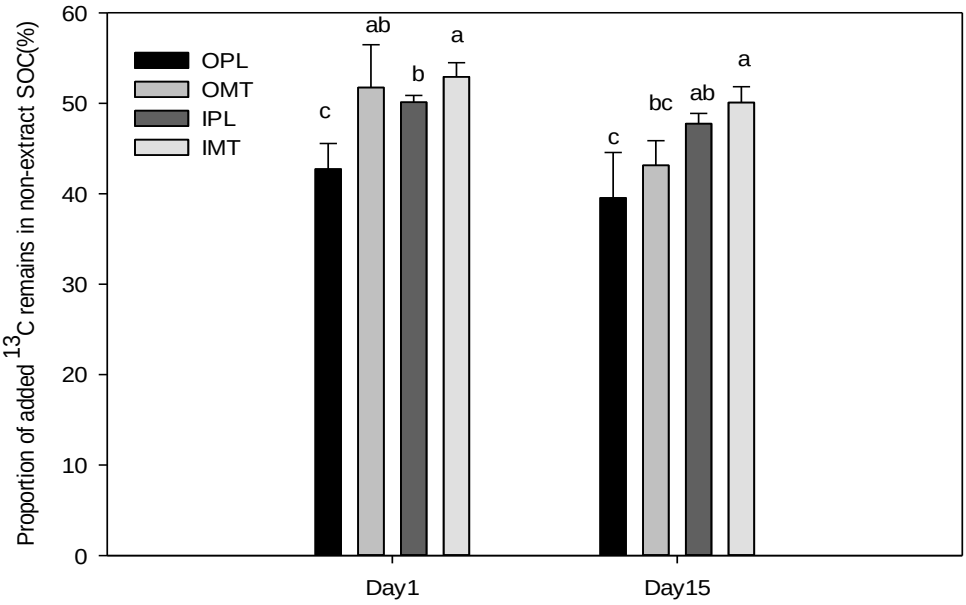


OPL: organic farming + plough tillage; OMT: organic farming + minimum tillage; IPL: integrated farming + plough tillage; IMT: integrated farming + minimum tillage.

Figure 3.8 Parts of added ^{13}C bound in microbial biomass in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose

Proportion of added ^{13}C remaining in non-extractable organic carbon fraction

The average proportion of added ^{13}C remains in non-extractable SOC over all soils was 47.3% (Fig. 3.9). The proportion of added ^{13}C remains in non-extractable SOC decreased over the incubation for all soils. The average proportion of added ^{13}C remains in non-extractable SOC over the incubation was highest in IMT soil. For soils in the same farming, the proportion of added ^{13}C remains in non-extractable SOC of MT was higher than that of PL soil. For soils of same tillage practice, the proportion of added ^{13}C remains in non-extractable SOC of organic soil was lower than integrated soil. At the beginning of incubation (1day after glucose addition), the proportion of added ^{13}C remains in non-extractable SOC ranked IMT > OMT > IPL > OPL and the difference between OPL and OMT, between IPL and IMT was significant ($P<0.05$). At the end of incubation, pattern changed to IMT > IPL > OMT > OPL. The difference between OPL and OMT, between IPL and IMT was insignificant ($P<0.05$).



OPL: organic farming + plough tillage; OMT: organic farming + minimum tillage; IPL: integrated farming + plough tillage; IMT: integrated farming + minimum tillage

Figure 3.9 Parts of added ^{13}C bound in non-extractable organic matter in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose

3.1.4 Soil respiration and glucose mineralization

The soil respiration and glucose mineralization were significantly influenced by soil (Table 3.2). After 2-week incubation, the total respiration reached 342.9, 426.8, 453.8 and 602.9 μg g^{-1} soil in IPL, OPL, IMT and OMT soil, respectively (Fig.3.10). The mineralization of ^{13}C-glucose was already detectable at the start of incubation after addition of glucose. Over the whole incubation, the total mineralization of ^{13}C-glucose was highest in OMT soil and followed by IMT, OPL and IPL soil. At the end of incubation, the mineralization of ^{13}C-glucose reached 33.6%, 38.2%, 39.9% and 42.1% in IPL, OPL, IMT and OMT soil, respectively (Fig. 3.11).

Table 3.2 Pairwise comparison of soil respiration and glucose mineralization after 15 days of incubation of soils from four long-term specific managements applied with ^{13}C-glucose using t-test

Comparison	Soil respiration		Glucose mineralization	
	t	p	t	p
OPL-OMT	-9.00	<0.001	-40.93	<0.001
OPL-IPL	10.12	<0.001	52.67	<0.001
OPL-IMT	-30.68	<0.001	-17.09	<0.001
OMT-IPL	9.34	<0.001	125.51	<0.001
OMT-IMT	7.51	<0.001	61.53	<0.001
IPL-IMT	-13.04	<0.001	-97.18	<0.001

Note: OPL: organic farming + plough tillage; OMT: organic farming + minimum tillage; IPL: integrated farming + plough tillage; IMT: integrated farming + minimum tillage.

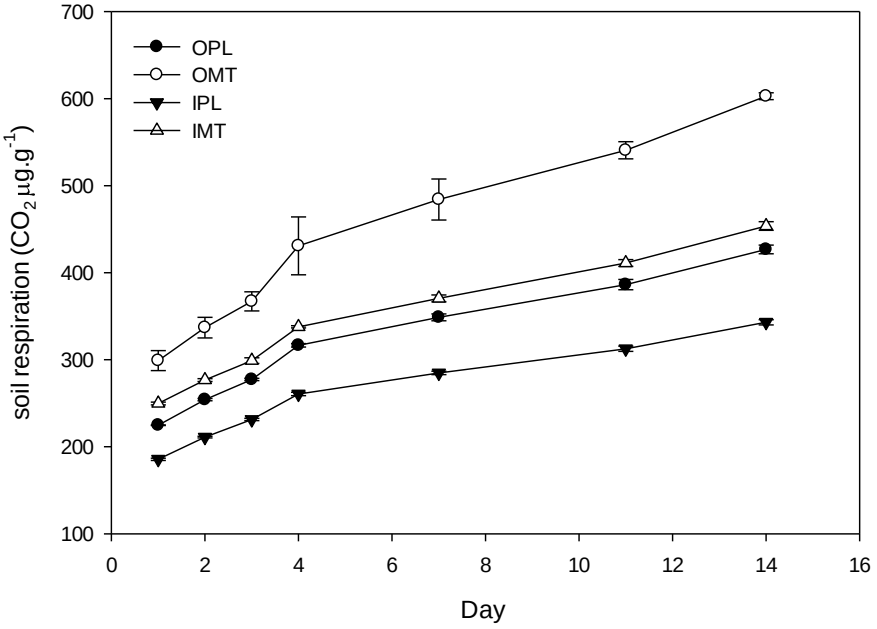


Figure 3.10 Soil respiration in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose

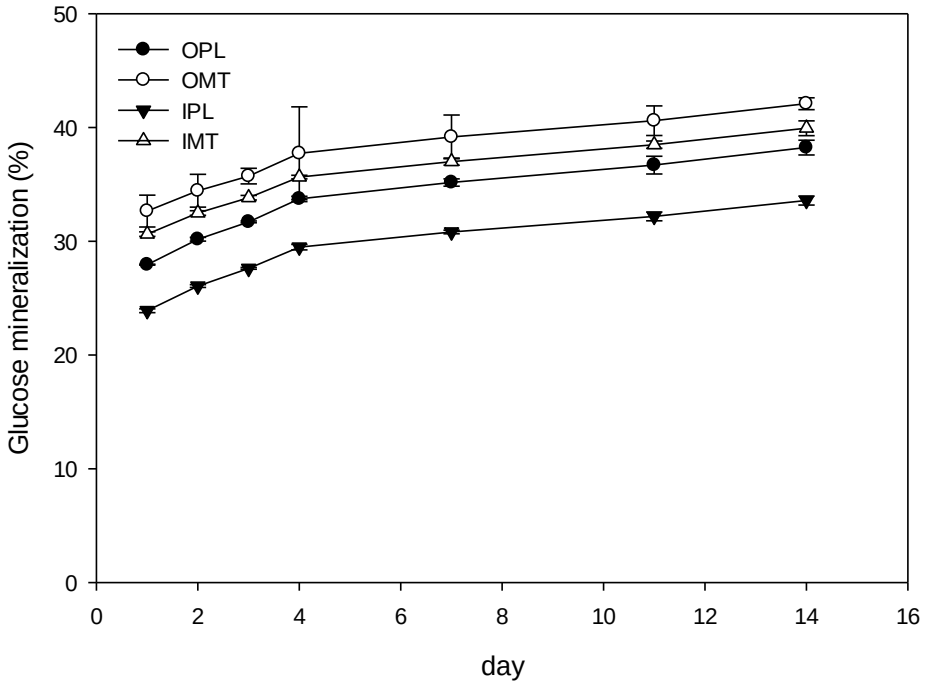


Figure 3.11 Development of cumulative mineralization of ^{13}C-glucose in the course of incubation of soils from four long-term specific managements supplied with ^{13}C-glucose

3.1.5 Discussion

In agriculture systems, the labile organic carbon mainly comes from the exudates of plants and degradation of plant residues and microbes/fauna. This fraction of soil organic matter can be used directly by fauna and microbes as a carbon and energy source. After a change or establishment of a new soil management practice, a stable and new microbial community will develop. Therefore, the transformations of organic matter could be altered as a response to soil management practice leading to also other inputs of plant residues. From this point of view, it is important to get insight in the respective response of labile organic carbon after a long-term soil management practices under the background of global C cycling.

The results of incubation experiment with labelled glucose showed that the difference between glucose treatment and the control treatment (without glucose addition) in WEOC, MBC and non-extractable SOC generally decreased in the course of incubation after the addition of labelled glucose. The supply of labelled glucose decreased the WEOC content as compared with the control. This can be explained by the growth of microbial biomass after application of glucose: WEOC was used by microbes. At the end of 15-day incubation, the difference in changes between soils was only significant for MBC. The insignificant difference of WEOC change could be explained by that WEOC is an easily available substance can be used rapidly by microorganisms (Marx et al., 2007b)

and non-extractable organic carbon is very recalcitrant to the degradation of microbes. These results also indicate microbial biomass is more sensitive to the introduction of external input of labile organic carbon in comparison with WEOC and the non-extractable organic carbon and can be sensitive indicator of soil changes. However, when consider soils under same farming management or same tillage, farming management had greater effect on the change of MBC than the effect of tillage at the end of incubation and led to minor increase or even decrease of MBC in comparison with control. The difference could be attributed to the differences in microbial community structure of the soils and initial microbial biomass of each soil. The differences in microbial biomass and microbial community structure of soils from the tested four soils have been approved in publication III. The microbial biomass was significant higher in organic farming than that in integrated farming. With the increasing microbial biomass, carbon source might change to a limiting factor with time. This could lead to the starvation of microbes to return initial level again.

Although WEOC is only a small amount of SOC, we still observed a difference of glucose-derived WEOC content between soils at the beginning and end of incubation experiment. At the beginning of incubation, the glucose-derived WEOC content differed between soils subjected to minimum tillage and plough tillage under the same farming management, and the glucose-derived WEOC content of organic soil was significantly lower than that of integrated soil. However, at the end of incubation, there was no significant difference between soils of minimum tillage and plough tillage in the glucose-derived WEOC content. The same pattern also occurred in the recovery of ^{13}C in WEOC. The difference in glucose-derived WEOC content at the beginning of incubation could be attributed to the difference of microbial biomass in soils. Higher content of microbial biomass led to use more glucose, therefore, less glucose contributed to WEOC. At the end of incubation, microbial biomass might become the source of ^{13}C-WEOC. This can be verified by the data of Glucose–derived MBC (Figs 3.5 and 3.8).

Regarding to the glucose - derived MBC, at the beginning of incubation, it was up more 60% glucose-^{13}C was immobilized in microbial biomass for organic soils, while it was significantly lower for integrated soils (Figs. 3.5 and 3.8). At the end of incubation, the glucose – derived MBC decreased for organic soils while a slight increase for integrated soils. The difference of glucose–derived MBC between minimum tillage soil and plough soil was still significant for integrated soils at the end of incubation (Figs. 3.5 and 3.6). This can be explained by the microbial biomass differences in the investigated soils. In organic soils, the initial microbial biomass was higher than that in integrated soils, and microbial biomass in soil of minimum tillage was higher than that of plough tillage (publication III). With the development of incubation, the growth of microbes in

organic soils was depressed for the C source limitation at the end of incubation. In contrast, C source did limit the growth of microbes in the integrated soils at the end of incubation (Fig. 3.5 and 3.8). The different responses of minimum tillage soil and plough tillage soil to glucose addition at the end of incubation might be attributed to the differences in microbial composition.

The cumulative soil respiration and mineralization of supplied glucose was highest for minimum tillage soils under organic farming and lowest for plough tillage soil under integrated soils (Fig. 3.10 and 3.11). The minimum tillage soil under both organic farming and integrated farming had higher cumulative soil respiration and glucose mineralization than plough tillage soil. It was up to almost 40% added glucose was mineralized for minimum tillage soil under organic farming. This can be explained by the initial microbial biomass in respective soils (see also publication III). After a 15-day incubation, more ^{13}C was remaining in MBC and non-extractable SOC, and followed by CO_2-C pool. However, it should be noted that the MBC pool and non-extractable SOC pool might overlap, because of the non-extractable SOC is the fraction of SOC after the remove of WEOC with mild $CaCl_2$ solution. Thus, MBC pool cannot be excluded from non-extractable SOC pool in present study for the methodological restriction.

From above, most of added glucose as a model compound of labile organic carbon was immobilized in microbial biomass and respired in microbial energy transfer metabolism of microorganisms. The extent of immobilization and respiration of glucose was determined by the contents of microbial biomass which is a result of soil management practices.

3.2 Effect of organic farming and minimum tillage on soil water extractable organic matter

This paper reports and analyses the effects of minimum tillage and organic farming management on soil water extractable organic matter as revealed by excitation emission matrix (EEM) spectroscopy. Soil samples from the plots imposed under the same climate conditions and same soil (Cambisol), with long-term minimum tillage and plough tillage under both organic farming and integrated farming were analyzed. Therefore, two factors, namely, tillage and farming management were tested. The results indicated organic farming enhanced the positive effect of minimum tillage on soil organic matter in a Cambisol but not the effect on the fluorescence fractions of water extractable organic matter.

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Water-extractable organic matter and its fluorescence fractions in response to minimum tillage and organic farming in a Cambisol

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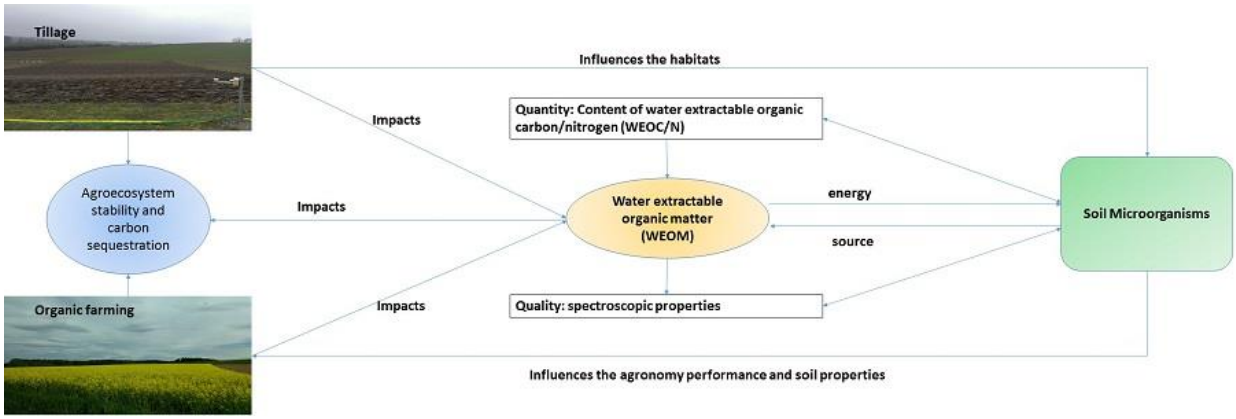
ABSTRACT

Minimum tillage (MT) and organic farming (OF) are increasingly conducted in agricultural managements from the interest of optimizing soil conditions and developing sustainable agriculture. Our understanding on their effects on water-extractable organic matter (WEOM) is still insufficient. To study the effects of MT and OF on WEOM, we analyzed soil materials sampled at two depths (0-8cm-upper soil and 12-25cm-deeper soil) from long-term field experiments with different farming and tillage methods. The content, composition and quality of WEOM were examined. The results showed organic farming significantly decreased water-extractable organic carbon (WEOC) and nitrogen (WEON), but had positive effect on WEOM humic-like components (C1 and C3) revealed by parallel factor analysis (PARAFAC) with excitation emission matrix (EEM), soil organic carbon (SOC), total nitrogen (TN) as well as SOC/TN. In addition, organic farming increased the aromaticity and condensation of WEOM as indicated by specific UV absorption (SUVA) and humification index (HIX). MT had no effect on WEOM both quantitatively and qualitatively but significantly decreased SOC and TN of the whole investigated soil profile. The depth effect was significant with strong stratification of WEOM, WEOM components as well as SOC and total N in upper soil. Moreover, the WEOM spectroscopic quality showed sharp differences between upper and deeper soils. The results indicated that in the combined presence of both tillage and farming management, farming management imposed more influence on WEOM than tillage, and organic farming may facilitate the transformation of WEOM and lead to formation of WEOM with high stability. MT significantly changed the distribution of SOC and WEOM in soil profile but did not increase the contents of SOC and WEOM. However, the presence of larger pool of WEOM in MT+OF treatment at upper soil is likely to fuel possibly greater microbial activity and more rapid nutrient cycling in soil which can be favorable practice with potential in improving soil conditions in the view of developing a sustainable ecosystem in the studied site.

Key words: Agricultural soil management, Water-extractable organic matter, Minimum tillage, Organic farming, Excitation–emission matrix spectroscopy, Parallel factor analysis

Figure abstract and abbreviations



BIX	freshness index
C1 and C3	humic-like substance in WEOM
C2	trytophan-like substance
EEM	excitation-emission matrix
FI	fluorescence index
F*max*	maximum fluorescence intensity
HIX	humification index
IF	integrated farming
MT	minimum tillage
OF	organic farming
PARAFAC	parallel factor analysis
PL	plow tillage
SUVA254	specific ultraviolet visual absorption 254 nm
WEOM	water-extractable organic matter
WEOC	water-extractable organic carbon
WEON	water-extractable organic nitrogen

1 Background

Agriculture is a potential source of negative impacts on ecosystems. Tillage is basically used to prepare soil for plant growth, while organic farming, as a holistic management practices in agriculture, is conducted for achieving the objectives of protecting human health, conserving natural resources and preserving the quality of environment while being economically sustainable [1]. Minimum tillage and organic farming are reported as having a potential of retaining more soil organic matter and enhancing microbial biomass and activities [2-5]. Water-extractable organic matter (WEOM) is the soluble fraction of organic matter extracted from the soil under various laboratory conditions, the most active and mobile fraction of soil organic matter (SOM). Although it is only a small part of soil organic matter, it has a strong influence on several ecologically relevant processes in soil [6-8]. It is a potential carbon source for soil microorganisms, modulating soil microbial community via the changes of quality and quantity of carbon compounds and their bioavailability [9-10].

Land-use changes and management activities influence the dynamics of WEOM [6]. Reduced tillage and organic farming as alternative practice of conventional tillage and farming were reported with an effect of increasing WEOC content by reducing soil disturbance and the addition of organic matter [11-14], while negative or neutral effect also existed [15-18]. However, most of the previous study focused less on the quality of WEOM. Actually, the concentration, composition, and structural complexity of WEOM affect soil chemical reactions; soil physicochemical and physical degradation in soil ecosystem; and WEOM bioavailability and biodegradability [19, 20]. The characterization of both concentration and composition will contribute to a better understanding of processes in soil and function of soil WEOM in agricultural system. Therefore, the study of WEOM can be of paramount importance to understand the dynamics of soil organic carbon pool and its implication in microbial activities, carbon fluxes, and climate change.

Characterization of WEOM has been performed using specific absorbance [8], the humification index [21], and fluorescence spectroscopy [22]. Furthermore, the advancement in fluorescence spectroscopy enables more detailed characterization on the chemical properties of fluorescing fraction of organic matter. Multidimensional fluorescence spectroscopy is a rapid, nondestructive and highly sensitive method [7, 19]. More spectral information could be obtained from multidimensional fluorescence spectroscopy than traditional fluorescence approaches. The fluorescence index (FI) strongly correlated with the structural conjugation and aromaticity and was used to differentiate source of dissolved organic matter [23]. Biological/freshness index (BIX) or β/α index is an indicator of relative contribution of recently microbially produced dissolved organic

matter [24, 25]. With the development of parallel factor analysis, it is also possible to decompose excitation-emission matrix (EEM) data into chemically meaningful components rather than only extract the information by peak picking method [7].

The availability of soils from long-term (21-year) field experiment in adjacent fields with the same soil formation offers an opportunity to evaluate management and tillage effects on SOM and WEOM. Further, the EEM combined with PARAFAC analysis provides a new insight into evaluating WEOM changes in response to tillage. Therefore, we hypothesized that (1) minimum tillage and organic farming increased content and changed quality of WEOM, and (2) organic farming affects more on WEOM quality compared with minimum tillage.

2 Materials and methods

2.1 Field experiment

Field experiments were carried out since 1992 at the Scheyern Research Farm (TERENO site, http://www.tereno.net) located 40 km north of Munich, Germany (48.50°N, 11.45°E). The altitude of the farm ranges between 445 and 500 masl. The mean annual precipitation and mean annual temperature are 803 mm and 7.4 °C, respectively. The central part of the research station was divided into two parts: organic and integrated, each striving for ecological and economical sustainability. Moreover, detailed studies on management-induced changes were carried out in plots subdivided in integrated and organic farming [26]. The soil types are sandy-to-loamy Cambisols, derived from tertiary sediments and partly covered by loess, and most of the soils have loamy texture [27, 28].

Plot experiments to study tillage-induced [Plow tillage (PL) and minimum tillage (MT)] and management-induced [Integrated farming (IF) and Organic farming (OF)] changes to the systems were set up in two adjacent fields, with the same soil formation and basic soil properties. The soils in both fields have a soil texture of silty loam (USDA), and the soil texture of top soil is 22% sand, 58% silt, and 20% clay. The field trial was arranged in a randomized factor design with three replicate treatments for each factor. The tillage practices in the study represent two possible tillage intensities under the local soil and climatic conditions. PL as a conventional tillage system means to till the soil with moldboard plow (25-30 cm). MT in the study was cultivated soil with chisel mixing in the first 6-8 cm of soil.

For OF system, organic manure (as cattle manure 30t ha^{-1}, dry weight) was applied instead of synthetic N-fertilizer, and no pesticides were applied. The OF plots are 12 × 12 m in size, and the crop rotation is a seven-crop rotation: (1) Grass–clover–alfalfa (GCA) (*Lolium perenne* L. +

Trifolium pratense L. + *Medicago sativa* L.), (2) potatoes (*Solanum tuberosum* L.) + mustard (*Sinapis alba* L.) as cover crop, (3) winter wheat (*Triticum aestivum* L.), (4) sunflower (*Helianthus annuus* L.) + GCA as cover crop, (5) GCA, (6) winter wheat, and (7) winter rye (*Secale cereale* L.) + GCA as cover crop. In IF system, nitrogen fertilization was conducted with UAN (50% urea N + 50% ammonium nitrate N) with a modified boom sprayer with tubes to conduct the solution directly to the soil surface. Pesticide and herbicide was used for pest and weed control. Fertilizer rates were fixed for the cultivated crops (135 kg N ha^{-1} for winter wheat, 105 kg N ha^{-1} for maize and 100 kg N ha^{-1} for potato), irrespective of credits due to effects of previous crops cultivation or mineralization in the soil. The IF plots are 12 × 12m in size and the crop rotation was (1) potatoes + mustard as catch crop, (2) winter wheat, (3) maize (*Zea mays* L.) + mustard as catch crop, and (4) winter wheat. Therefore, there are four treatments in the present study in total: (1) organic farming + plough tillage (OPL); (2) organic farming + minimum tillage (OMT); (3) integrated farming + plough tillage (IPL); (4) integrated farming + minimum tillage (IMT). The assignments of the treatments to the plots were kept constant since 1992.

2.2 Sampling and soil properties

Three replicates of composite samples (each a mixture from 5 cores taken randomly) of top soil were collected from plowing tillage and minimum tillage plots at two soil depths (0-8-cm-upper soil and 12-25-cm-deeper soil) in integrated and organic-farming system in April 2013. Soil organic carbon (SOC) and total nitrogen were determined by a CN analyzer (EA3000 Eurovector) with an aliquot air-dried soil samples. Soil texture was determined by wet sieving and pipette method [29]. Briefly, sand and silt fractions ≥ 20 μm were measured by sieving after treatment with H_2O_2, silt and clay < 20 μm by the pipette procedure. The soil pH was measured in a soil suspension with 0.01 M $CaCl_2$ solution (1:5, w/v).

2.3 WEOM extraction and spectroscopic characteristics

Soil WEOM was extracted according the method of Zsolnay [30]. In brief, 2 mm mesh-sieved soil samples were shaken with 0.01 M $CaCl_2$ at a ratio of 1:2 (soil: volume) using an overhead shaker for 10 min. After 10-min centrifugation at 3000 *g*, the supernatant was filtered through 0.4 μm polycarbonate membrane filter. WEON was quantified by subtracting inorganic N from soluble total N. Soluble total N, NO_3-N and NH_4-N were quantified using an automated continuous flow analyzer (Skalar).

Specific UV absorption (SUVA), obtained by dividing the absorption at 254 nm by WEOC concentration, provides the information about the aromatic structures of WEOM. Absorption was determined using 1-cm quartz cells with a Varian Cary 50 Bio UV-Visible spectrophotometer [22].

Both pH and molecular concentrations can influence fluorescence [21, 31]. Therefore, to avoid concentration artifacts, dilution was made to have WEOC absorbance <0.1 cm^{-1} at 254 nm [21], and then WEOC extracts were acidified until reaching a constant standard pH of 2 with 2 M HCl [8, 32]. At low pH, most metal complexes disassociate, which should minimize the quenching of fluorescence due to metal complexation [23]. The pH has been used for fluorescence spectra [8, 23, 32]. PARAFAC analysis was also used to decompose the EEMs measurements at a pH range of 2-12.5 [20, 33-35]. In present study, EEMs spectra and fluorescence spectra for humification index calculation were measured separately, and a round of whole measurement procedure including pH adjustment was finished within half an hour, and no precipitation was observed in the samples. All the fluorescence measurements for humification index were performed on a Varian Cary Eclispe fluorescence spectrophotometer using 1-cm quartz cells at 254-nm excitation. The fluorescence spectra were recorded (300 to 480 nm with 2-nm increment) at 21 ± 1 °C. Even though the optical density was adjusted by dilution in advance, the fluorescence emission spectra were corrected for "inner filter effect" [36] according to Zsolnay [21] with a simplified formula that measured fluorescence emission multiplies e^A, where A is the absorbance in cm^{-1} at the 254-nm excitation wavelength [8, 37]. Humification index (HIX) indicating the complexity and condensation (H/C ratios) of WEOM was calculated as the ratio of integrated fluorescence emission peak at longer wavelength region (435-480 nm) over shorter wavelength region (300-345 nm) at 254 nm excitation wavelength [21].

EEMs were obtained by scanning over excitation wavelengths from 250 nm to 450 nm with an increment of 5 nm and emission wavelength from 300 to 600 nm with an increment of 5 nm. The slit widths were set as follows: excitation slit 10 nm, and emission slit 20 nm. Fluorescence data were corrected for inner-filter effect with absorption data as suggested by Lakowicz [38]:

$$I_{corr} = I_{obs} \times 10^{0.5(Aex+Aem)}$$

where I_{corr} and I_{obs} are corrected and uncorrected fluorescence intensities; and A_{ex} and A_{em} are the absorbance values at the excitation and emission wavelengths of the fluorescence intensity values. The correction is based on the assumption that the average path length of excitation and emission lights is 50% of the cuvette width, respectively. Although the correction of EEMs and fluorescence spectra for humification index calculation were different, this will not affect the results in a comparison study. First, EEMs spectra and fluorescence spectra for humification index calculation were measured separately, the correction for each was done in the same way, respectively. Second, the two correction formulas are both simplified from the same original formula and based on the same theory as reported by Gauthier et al. [36].

The fluorescence index (FI), strongly correlated with the structural conjugation and aromaticity and used to differentiate the sources of DOM, was calculated as the ratio of emission intensity at 470 and 520 nm at fixed excitation wavelength of 370 nm [23]. Biological/freshness index (BIX) or β/α index, an indicator of the relative contribution of recently microbially produced DOM, was calculated as the ratio of emission intensity at 380 nm (β) to the maximum emission intensity observed between 420 and 435 nm (α) for an excitation wavelength of 310 nm [24, 25].

2.4 PARAFAC modeling

PARAFAC provides a way to decompose a dataset of EEM into individual fluorescence components [39, 40]. An EEM can be reduced to trilinear terms and a residual array by

$$X_{ijk}=\sum_{n=1}^{F} a_{in} b_{jn} c_{kn} + \varepsilon_{ijk}$$

where, for the EEM fluorescence data, X_{ijk} is the fluorescence intensity of the ith sample at the kth excitation and jth emission wavelength. a_{in} is directly proportional to the concentration (here defined as scores) of the nth fluorophore in the ith sample. b_{jn} and c_{kn} are estimates of emission and excitation spectra (loadings) of nth fluorophore at the wavelengths j and k, respectively. F is the number of components, and ε_{ijk} is the residual matrix of the model which represents unexplained variability by the model. For the full description about PARAFAC, reader can refer to Bro [39]. Components extracted by PARAFAC can be ascribed to specific species of organic matter present in liquid samples, but they are more likely to represent groups of organic compounds having similar fluorescence properties. The PARAFAC model identifies the number of components as well quantifies the scores for each component that is directly proportional to the component's (fluorophore) concentration in the sample. This concentration can be converted into actual concentration if specific absorption coefficient or quantum yields associated with excitation and emission of each fluorophore are known [41]. The concentration scores of the PARAFAC components were expressed as maximum fluorescence intensity (F_{max}) (R.U.) [42] for each modeled component in current study. F_{max} gives estimates of the relative concentrations of each component; however, direct comparison of relative concentrations between different components depends on the magnitude of their quantum efficiencies as well as on their individual responses to quenching effects [43].

Before the application of PARAFAC model, zero emission intensities were assigned for excitation wavelengths (λex) greater than emission wavelengths (λem). Raleigh scattering was minimized according to [19, 40]. An EEM of the 0.01 M $CaCl_2$ solution was obtained and subtracted from the EEM of each sample in order to remove most of the Raman scatter peaks; the any negative values produced by the subtraction were converted to missing values and the fluorescence was normalized

by dividing the integrated area (Ex: 350 nm, Em: 371-428 nm) under Raman scatter peak of the corresponding Milli-Q water of each set of measurement. This calibration procedure is universal, and the Raman signal of water can be used irrespective of sample solvent and matrix, and no spectral changes will occur from applying this method [44] and the fluorescence intensities were reported in Raman units (R.U.). In total, the dataset contained 36 corrected and standardized EEMs. After an initial exploratory analysis (calculating its leverage using DOMFluor), one an outlier was identified and removed from the dataset. Therefore, 35 EEMs were performed with PARAFAC analysis in the end. Split-half analysis and examination of residual error plots were applied to get the appropriate number of components. A series of PARAFAC models were generated with Matlab (2009a) by means of DOMfluor toolbox specifically developed for PARAFAC analysis of DOM fluorescence [45].

2.5 Statistics

The results presented in the tables are geometric means and expressed on dry-weight basis (for about 24 h at 105°C). The significance of treatment effects was tested by a two-way ANOVA using tillage and farming management as independent factors and soil depth as repeated measure. ANOVA and Pearson correlation coefficients were calculated using the vegan package of R [46].

3 Results

3.1 Soil total organic matter and dissolved organic matter

The contents of SOC and total N as well as the SOC/total N and WEOC/WEON were significantly higher in organic farming in comparison with integrated farming system, while the contents WEOC, WEON, NO_3-N as well as NH_4-N were lower (Table 1). The contents of WEOC and WEON as well as the SOC/total N and WEOC/WEON ratios did not differ between the tillage treatments, while MT significantly decreased the contents of SOC and total N but increased that of NO_3-N and NH_4-N. The accumulation of SOC, total N, WEOC, WEON, NO_3-N and NH_4-N in the 0-8 cm layer was significant in comparison with 12-25 cm layer, usually leading to significant tillage × soil-depth interactions, except WEON and NH_4-N. The ratio of SOC/total N was significantly higher while WEOC/WEON was significantly lower at 0-8 cm in comparison with 12-25 cm. The significant interactions of farming × tillage on SOC (Fig. 1), but also those on total N, SOC/total N, WEON, NO_3-N, and NH_4-N were caused by the much stronger tillage effects in the organic farming than in the integrated system.

3.2 Spectroscopic properties of WEOM

SUVA and HIX were significantly higher, while BIX was significantly lower in organic farming in comparison with integrated farming (Table 2). The FI did not differ between farming systems.

Tillage had no effect on any WEOM spectroscopic property. The SUVA values were significantly higher at 0-8 cm than at 12-25 cm, which was especially true for the organic farming system (Fig. 2). The HIX values were significantly lower at 0-8 cm than at 12-25 cm, which was again most pronounced in the organic-farming system (Fig. 3). In contrast, the BIX values did not change with depth and farming system (Table 2).

3.3 PARAFAC components

Three EEM-PARAFAC components (C1, C2, C3), which account for 99.28% of the fluorescence variability, were extracted from the dataset (Fig. 4). The C1 component had two excitation maxima at 240 nm and 325 nm and one emission maxima at 435 nm, similar to the humic-like substance identified by [47-49]. The C2 component had two excitation maxima at < 240 nm and 260 nm and one emission maxima at 360 nm, similar to the component 5 in [50-52] or peak N in [53] and C4 component in [49]. The C2 component is characterized by tryptophan-like substance associated with biological production and one shoulder could be found on the emission spectrum due to different fluorescence characteristics [54]. The C3 component had one excitation maxima at 265 nm and one emission maxima at 505 nm. The C3 component is similar to humic-like substance reported before [41, 49, 53, 54].

Concentrations of three components as indicated by the concentration scores followed an order of C1 > C2 > C3 (Table 2). The two humic-like substances (C1 and C3) were significantly higher in organic farming in comparison with integrated farming, while the tryptophan-like substance (C2) did not differ between farming systems. The effect of tillage on the three EEM-PARAFAC components was insignificant. The three EEM-PARAFAC components were significantly higher at 0-8 cm than those at 12-25 cm. All three EEM-PARAFAC components were significantly correlated to SOC and total N at both depths (Table 3), although the relationships were always closer at 12-25 cm. This was also true for the relationships to WEOC, which were already insignificant for C1 and C2 at 0-8 cm. The relationships to WEON were only significant for C1 and C2 at 12-25 cm.

4 Discussion

4.1 Soil organic carbon and nitrogen

In the present study, our results showed MT and organic farming significantly increased SOC and TN in upper soil (Table1). The results agreed with the results of [11, 12, 55]. Higher content of SOC and TN in MT at upper soil could be attributed to that MT reduces the mineralization of SOM [14] and improves the physical protection of SOM via reducing the disruption of soil aggregates and avoiding physically the access of SOM by microbes [56] compared with conventional tillage.

Further, crop residues accumulate in the upper layer of soil, while plowing tillage incorporates crop residues into the whole tillage horizon of soil more uniformly resulting in dilution effects of C and N [57, 58]. When the two soil depths were considered, the effect of MT was negative, this perhaps because of dilution effect in deeper soil part. The results agreed with the findings of Luo et al. [59] that no-tillage changed distribution of C in the soil profile significantly, but did not increase the total SOC, the increase of SOC in surface soil compensates for the loss of SOC in deeper soil. In terms of organic farming, similar findings [60, 61] were reported, although some inconsistent findings occurred where no obvious improvement of SOC was observed [2, 62]. The difference between studies can be attributed to the difference in the climatic conditions of the studied sites. In the study of Parras-Alcantara et al. [62], their trial site (Los Pedroches Valley) is characterized by cold winters and warm, dry summers with temperatures ranging from -2 to 40 °C (extreme temperatures) and an average annual rainfall of 600 mm, while the climate of our study is more mild with mean precipitation of 803 mm and mean temperature of 7.4°C. Therefore, different degradations and turnovers of organic matter might occur in the two study sites. Further, their soils were sandier. This supports the hypothesis that the tillage–induced changes of soil are soil and site specific [63]. The beneficial effect of organic farming could be associated with the continuous input of organic matter by manure, plants, and legume catch-crop-based crop rotations [60]. It is also recognized that the addition of organic manure increases soil water repellency, thus increasing the aggregate stability [64] and carbon sequestration [65]. In addition, the diverse microbial community under organic farming facilitates the degradation of plant materials, thus increasing the SOM from the decayed plant debris [64].

4.2. WEOM components

WEOM is believed to originate from plant roots, litter, and soil humus and is a labile substrate for microbial support and transformations [11]. In the present study, organic farming significantly decreased the contents of WEOC and MT resulting in significant accumulation of WEOC in upper soil. The decreased WEOM in organic-farming treatments could be attributed to the sampling time. Bausenwein et al. [9] reported significant effect of sampling time on WEOC and the higher WEOC was observed in October in the same research station as ours but with samples from different fields. Our samples were collected in 2013 after a long winter when the snow ended in May. In addition, we also observed increased microbial biomass compared with integrated farming [3]. Therefore, due to more WEOC consumption than its production, the production and consumption of WEOC became unbalanced. Organic farming significantly decreased the content of WEON. One explanation could be that microbial communities in organic farming are more N-depleted and the N fertilization in integrated farming changed microbial community's structure thus resulting in

alteration of organic N mineralization [66]; this agreed with Embacher et al.'s report that the fertilizer itself is not the direct source of WEOM but affects WEOM via the biota [32]. The increased WEOC in upper soil under MT treatments was consistent with other findings [11, 12], although some authors also reported tillage had no significant effect at any soil depth [15]. The reason behind this might be that, on one hand, the MT reduced the mineralization of WEOM [13], and that, on the other hand, the diverse microbial community in MT [67] increased the degradations of SOM and the plant residues accumulated in upper soil contributed to WEOM.

Organic farming significantly increased the components 1 and 3 (humic-like substance components). This is in line with results of Zhang et al. [16] who observed that the application of manure increased humic-like substances identified by EEM-PARAFAC, but contrary to results reported by Ohno et al. [68] that tillage and cropping management did not affect the humic-like substance components 1 and 5 and amino acid-like component 4. But they found N sources (NH_4NO_3 and poultry litter) significantly affected the identified components of WEOM. In the present study, organic farming caused significantly lower NO_3-N in comparison with integrated farming. Thus, the increased humic-like substance in organic-farming treatments indicated that WEOM in organic farming was highly microbial processed which can also be reflected by SUVA and HIX of WEOM (Table 2). Besides, EEM-PARAFAC components of upper soil were significantly higher than those of deeper soil. This can be explained by the accumulation and degradation of accumulated organic matter by MT in top soil which can be proved by the close association of EEM-PARAFAC components with SOC and total N (Table 3). Compared with humic-like substance, C2 as tryptophan-like substance with simpler chemical structure can be degraded more easily by microbes and will return to background level; this can be a reason for the tillage and organic-farming practices having had insignificant effects on C2.

4.3. Spectroscopic properties of WEOM and relations to soil characteristics

In comparison with tillage effect, farming practices are the main factor that influenced the quality of WEOM. Increased values of SUVA and HIX in organic farming in the present study implied that WEOM in organic farming is more aromatic and condensed compared with that in integrated farming. This might be because of the altered diverse microbial community's structure in organic farming [69], which might promote the degradation of WEOM in comparison with integrated farming. What's more, the different crop rotations which affected WEOM by crop root exudates, and the return of crop residues might be another reason to explain the different SUVA and HIX values of WEOM. Our result suggested that complex crop rotations release WEOM with higher aromaticity and humification. This is opposite to that of Xu et al. [18] who reported the inclusion of

a perennial crop alfalfa in a crop rotation, which releases WEOM with lower aromaticity and lower extent of humification. This may be attributed to simpler crop rotations in their study and different crop species they planted compared with our study. Although FI values of WEOM at upper and deeper soils were statistically different, according to McKnight et al. [23], FI value of WEOM around 1.4 indicates WEOM derives from terrestrial source, while FI around 1.9 indicates WEOM derives from microbial source. Therefore, FI values in the present study were around 1.6 indicating the influence of both terrestrial and microbial source on WEOM. The lower BIX (freshness index) in organic farming indicated less recently microbial-derived organic matter contributed to WEOM in organic farming, while the higher BIX in MT implied more freshly WEOM contributed WEOM in MT.

5. Conclusions

In the present agricultural long-term field study, soil WEOM responded diversely to the long-term applications of MT and OF. MT resulted in negative depth gradients of soil properties and SOC, total N as well as WEOM and its components accumulated in upper soil. In contrast, MT had no effect on the quality and WEOM components. OF had significant effects on SOM and WEOM. Specifically, OF increased SOC, total N content as well as the humic-like components and the complexity of WEOM, while the content of WEOM was lowered by OF. The results indicated that the minimum tillage changed the distribution of SOM in soil profile rather than increasing SOM in field-trial region of the present study, and organic farming dominated the quality change of WEOM. The combined organic farming plus minimum tillage would be a better option of agricultural practice to improve soil conditions as indicated by the improved soil WEOM at upper soil and is likely to fuel possibly greater microbial activity, favoring the development of nutrient cycling in view of sustainable agricultural practice at the site of present study. Minimum tillage may be considered in the concerned landscape as more acceptable way in realizing the balance between the yield loss and improved soil conservation [70].

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Delarations

Abbreviations

MT: minimum tillage; PL: plough tillage; OF: organic farming; IF: integrated farming; WEOM: water extractable organic matter; WEOC: water extractable organic carbon; WEON: water extractable organic nitrogen; EEM: excitation emission matrix; PARAFAC: parallel factor analysis; C1 and C3: humic-like substance in WEOM; C2: tryptophan-like substance. $SUVA_{254}$: specific ultraviolet visual absorption at 254 nm; HIX: humification index; FI: fluorescence index; BIX: freshness index. F_{max}: maximum fluorescence intensity.

Authors' contributions

HYS:writing-original draft preparation, project management and realization. PK: co-field work, advice and help on lab work and draft; GR: field trial management; RS: advice on lab work and original draft preparation, supervision; RGJ: writing---review and editing, supervision; JCM, writing---review and editing, supervision. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1. Effect of tillage and farming practices on soil organic carbon, nitrogen and water extractable organic matter

Table 2. Effect of tillage and farming practices on spectrum characteristics and spectrally identified composition of water extractable organic matter of all treatments

Table 3. Correlations between PARAFAC components of WEOM and soil characteristics for all treatments at varying soil depths

Table 1. Effect of tillage and farming practices on soil organic carbon, nitrogen and water extractable organic matter

Main effects	SOC	Total N	SOC/	WEOC	WEON	WEOC/	NO_3-N	NH_4-N
	(mg g^{-1} soil)		Total N	(µg g^{-1} soil)		WEON	(µg g^{-1} soil)	
Organic farming	14.0	1.5	9.1	55	1.6	36.8	10.6	0.5
Integrated	11.9	1.3	8.9	62	5.0	27.4	37.9	0.6
Minimum tillage	12.7	1.4	9.0	59	3.6	32.9	25.5	0.7
Plough	13.2	1.5	9.1	58	3.0	31.3	23.0	0.4
0-8 cm depth	15.0	1.6	9.2	65	5.3	23.3	40.3	0.7
12-25 cm	10.9	1.2	8.9	52	1.3	40.9	8.1	0.4
Probability values								
Farming	<0.01	<0.01	0.05	0.09	<0.01	0.07	<0.01	NS
Tillage	0.02	0.03	NS	NS	NS	NS	0.02	0.07
Depth	<0.01	<0.01	0.01	<0.01	<0.01	0.01	<0.01	0.09
Farming × tillage	<0.01	0.01	NS	0.09	NS	NS	NS	0.06
Farming × depth	<0.01	<0.01	0.03	NS	<0.01	<0.01	<0.01	0.04
Tillage × depth	<0.01	<0.01	0.02	<0.01	NS	<0.01	<0.01	NS
CV (± %)	2	3	2	14	17	24	9	20

CV = mean coefficient of variation between replicate plots (n=4).

The results presented in the table are geometric means of all plots within each main factor treatments.

Table 2. Effect of tillage and farming practices on spectrum characteristics and spectrally identified composition of water extractable organic matter of all treatments

Main effects	SUVA	HIX	C1	C2	C3	FI	BIX
	(m^{-1} mg^{-1} C l)			(R.U.)			
Organic farming	1.18	4.61	1.90	0.94	0.75	1.60	0.74
Integrated	0.79	3.25	1.53	0.93	0.60	1.62	0.77
Minimum tillage	0.95	3.94	1.76	0.93	0.69	1.60	0.75
Plough	1.02	3.92	1.67	0.94	0.66	1.61	0.76
0-8 cm depth	1.14	3.53	1.98	1.18	0.80	1.58	0.76
12-25 cm	0.82	4.33	1.45	0.69	0.55	1.64	0.75
Probability values							
Farming	<0.01	<0.01	0.01	NS	0.01	NS	0.06
Tillage	NS	NS	NS	NS	NS	NS	NS
Depth	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	NS
Farming × tillage	0.08	NS	NS	NS	NS	NS	NS
Farming × depth	NS	NS	NS	NS	NS	NS	NS
Tillage × depth	NS	0.07	<0.01	<0.01	<0.01	0.06	0.09
CV (± %)	9	6	8	7	9	1	2

CV = mean coefficient of variation between replicate plots (n = 3); SUVA = Specific Absorption; HIX = humification index; FI = Fluorescence Index; BIX = Freshness Index. The results presented in the table are geometric means of all plots within each main factor treatments

Table 3. Correlations between PARAFAC components of WEOM and soil characteristics for all treatments at varying soil depths (n=12)

0-8cm	SOC	Total N	WEOC	WEON	Clay
C1	0.82**	0.74**	NS	NS	-0.72**
C2	0.69*	0.64*	0.60*	NS	-0.86**
C3	0.81**	0.73**	NS	NS	-0.74**
12-25cm					
C1	0.94**	0.94**	0.85**	0.61*	0.68*
C2	0.94**	0.91**	0.95**	NS	0.93**
C3	0.95**	0.95**	0.84**	0.60*	0.68*

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

SOC: soil organic carbon; WEOC: water extractable organic carbon; WEON: water extractable organic nitrogen; C1, C2, C3: EEM-PARAFAC components with C1 and C3 are humic-like substance and C2 is tryptophan-like substance; NO_3-N: nitrate; NH_4-N: ammonium.

Legends to the figures

Figure 1 Soil organic carbon content of at 0-8 cm and 12-25 cm soil of all treatments

Figure 2 Specific UV absorption of water extractable organic matter at 0-8 cm and 12-25cm soil of all treatments

Figure 3 Humification index of water extractable organic matter at 0-8 cm and 12-25 cm soil of all treatments

Figure 4 Split-half analysis of the three components of WEOM extracted by PARAFAC model for all treatments

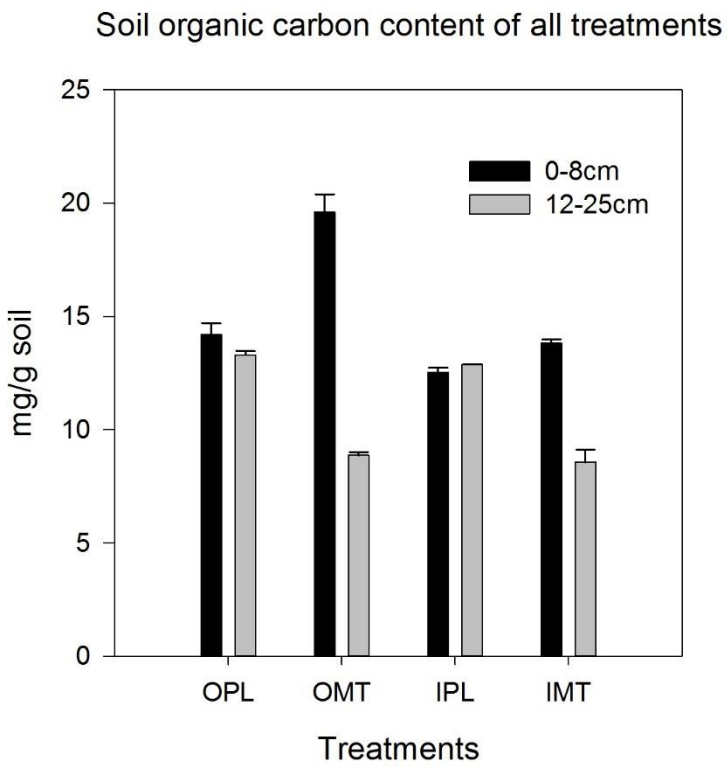

Fig. 1

OPL: organic farming + plowing tillage

OMT: organic farming + minimum tillage

IPL: integrated farming + plowing tillage

IMT: integrated farming + minimum tillage

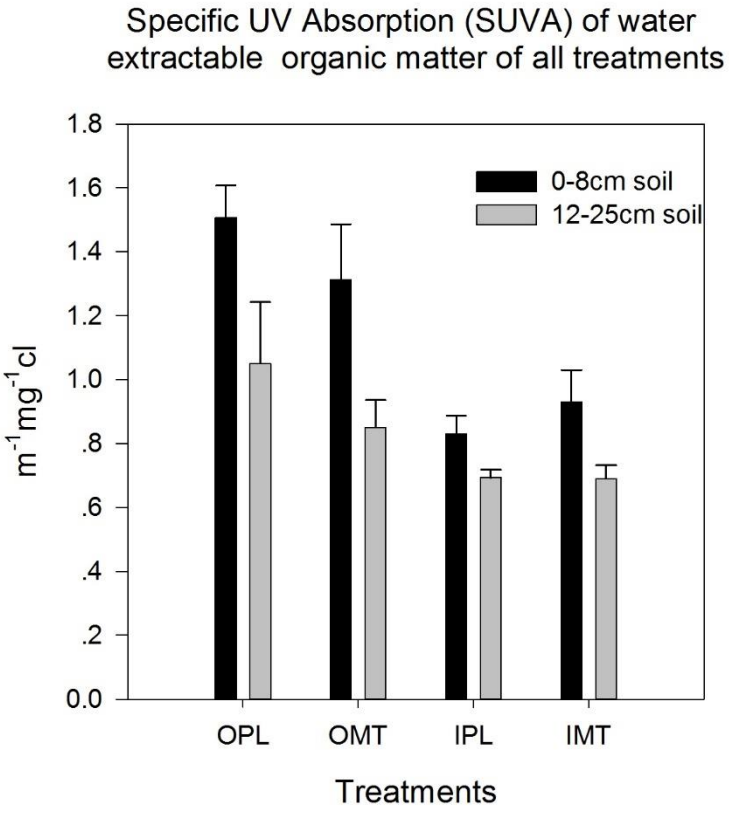

Fig. 2

OPL: organic farming + plowing tillage

OMT: organic farming + minimum tillage

IPL: integrated farming + plowing tillage

IMT: integrated farming + minimum tillage

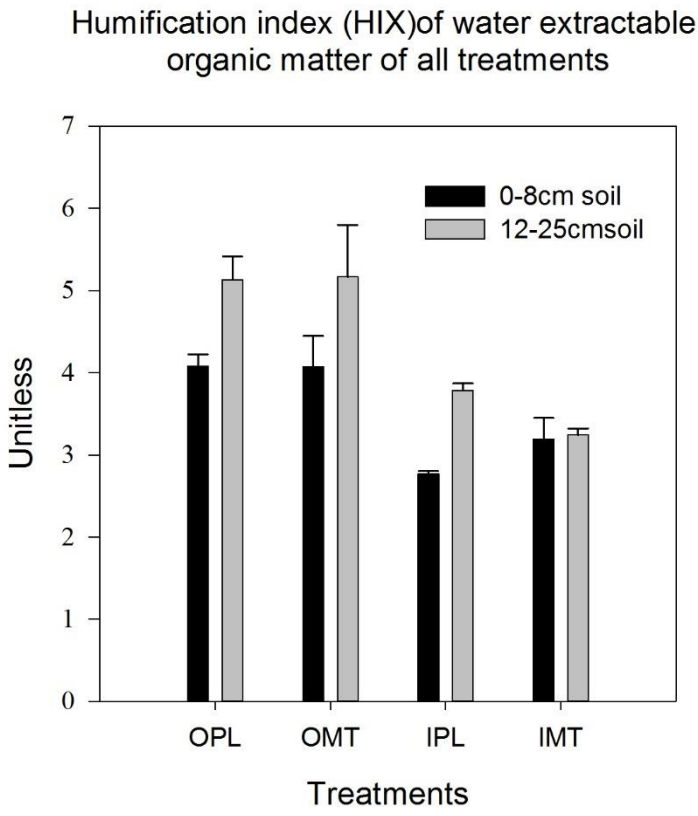

Fig. 3

OPL: organic farming + plowing tillage

OMT: organic farming + minimum tillage

IPL: integrated farming + plowing tillage

IMT: integrated farming + minimum tillage

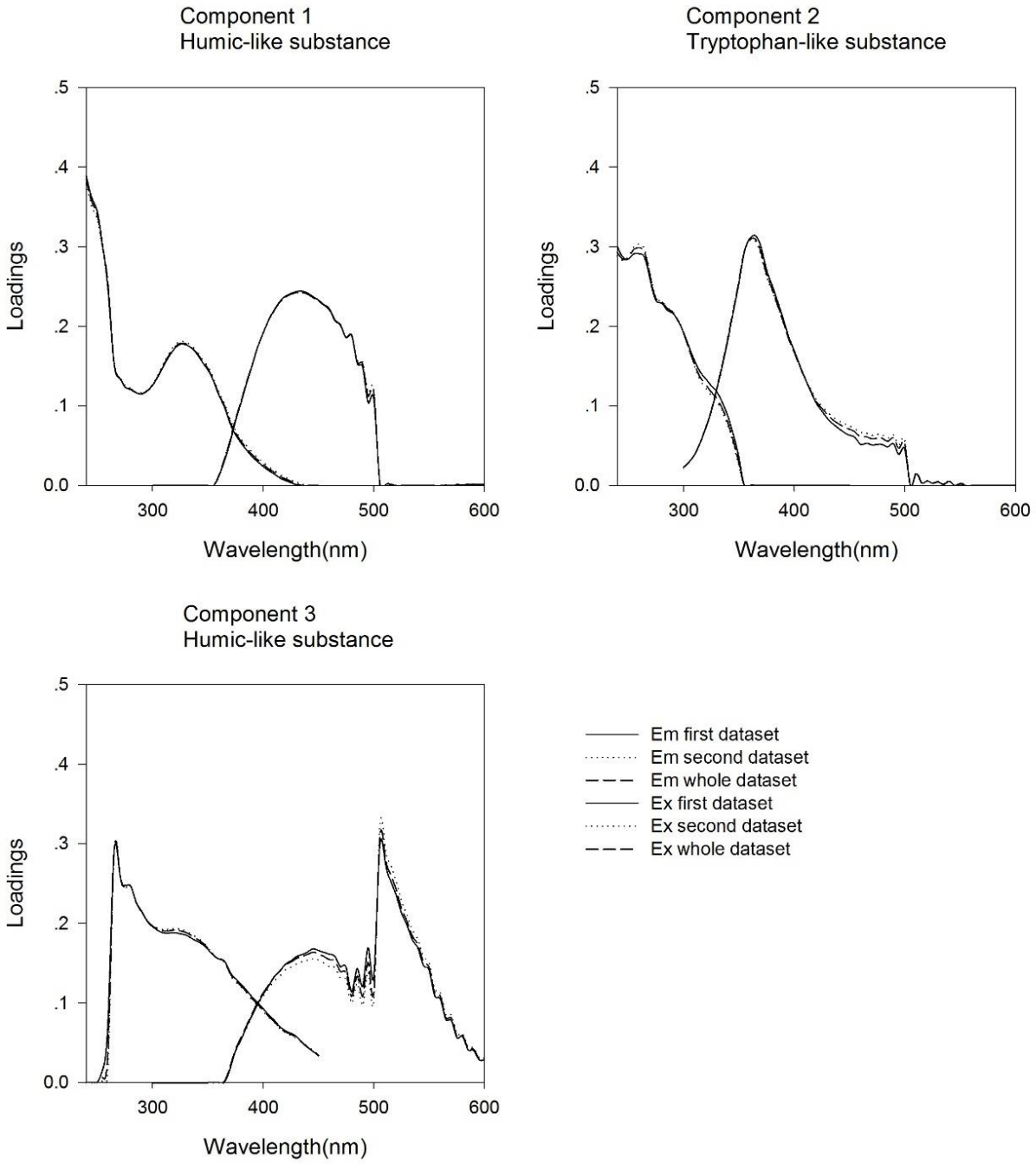

Fig.4 Split-half analysis of the three components of WEOM extracted by PARAFAC model for all treatments
Em: Emission wavelength; Ex: Excitation wavelength

3.3 Effect of robinia-based alley cropping and low-input organic farming on water extractable organic matter

This paper reports and analyses the changes of WEOM after a long-term implementation of low-input organic and integrated farming as well as shortly introduction of poplar and robinia-alley cropping to the existed farming systems. Samples from three distances (0m, 2m, and 15m) to the poplar and robinia tree rows under low-input organic farming and integrated farming were analyzed. Farming methods, tree species as well as the sampling distance were considered in study. The results showed low-input organic farming and robinia are beneficial to the accumulation of soil organic carbon, but low-input organic farming is adverse to the content of WEOM, while robinia had no effect on the content of WEOM. Tree species influenced significantly the quality of WEOM and robinia resulted in WEOM with more aromatic structure. The combination of organic farming and robinia trees are both important means for developing sustainable agricultural systems and soil carbon sequestration.

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Response of water extractable organic matter and its fluorescence fractions to organic farming and tree species in poplar and robinia-based alley cropping agroforestry systems

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ABSTRACT

Organic farming and agroforestry both have the potential to develop sustainable and environmental-friendly agroecosystems and to sequester more soil organic C (SOC). In a long-term field trial, we evaluated the effect of 21-year organic farming and 4-year agroforestry (Robinia and Poplar-based alley cropping system) on water extractable organic matter (WEOM). The technique combining excitation emission matrix (EEM) spectra with parallel factor analysis (PARAFAC) was used to reveal the components of WEOM. In addition, WEOM was characterized by UV absorbance and fluorescence spectra. Organic farming generally increased SOC and total N contents but decreased the WEOM content as well as the WEOM components indicated by the maximum fluorescence intensity (F_{max}). Specific UV absorbance (SUVA) and humification index (HIX) of WEOM in organic farming implied WEOM in the organic farming had more components with aromatic structure but less humified. Higher fluorescence (FI) and freshness indices (BIX) of WEOM in organic farming system indicated that a higher percentage of WEOM was microbial-derived in the organic than in the integrated farming system. Robinia showed positive effect on SOC and total N contents in comparison with poplar and had stronger effects on the WEOM components, although the WEOM content did not differ between the two tree species. The significant farming × trees interactions on SOC and water extractable organic carbon (WEOC) indicated that the robinia effects were more pronounced in the organic farming system. Thus, the change of SOC was the result of interactive effect of farming and hedgerow trees in an agroforestry system. The low-input organic farming and robinia tended to result in change of quality of WEOM and led to enrichment of substances of high stability in WEOM. From above, the combination of organic farming and robinia trees is an important means for developing sustainable agricultural systems and soil carbon sequestration.

1. Introduction

Soil WEOM is an active, mobile and complex fraction of soil organic matter (SOM) and is sensitive to land use and management practices (Dong et al., 2009; Mao et al., 2012; Xu et al., 2013). WEOM participates in multiple soil processes, such as SOM translocation and mineralization, denitrification and trace gas production, solubility, transportation and toxicity of organic contaminants (Chantigny, 2003; Stark et al., 2007; Xu et al., 2013). It was also reported that WEOM modulates soil microbial community as a feedback to the production of WEOM from microbial activities (Bausenwein et al., 2008). Moreover, land-use and management practices were repeatedly reported to influence the dynamics of WEOM and biodegradability by affecting soil biochemical properties and structure and WEOM composition (Chantigny, 2003; Marschner and Kalbitz, 2003; Xu et al., 2013).

Agroforestry systems combine agriculture and forestry into a production system and are recognized as integrated approaches for sustainable land use aside from their contribution to climate change adaptation and mitigation (Ramachandran Nair et al., 2009; Lorenz and Lal, 2014). Agroforestry promotes C sequestration in tropical and temperate regions (Montagnini and Nair, 2004; Nair et al., 2010), depending on tree species and management of the agroforestry system (Lorenz and Lal, 2014). Trees, especially broadleaf trees having deep and extensive root systems and high belowground to aboveground biomass ratios, enhancing the potential for soil C sequestration (Laganiere et al., 2010; Lorenz and Lal, 2014). However, positive, neutral, and negative effects of trees on SOC pool have been observed in the meta-

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analysis of Laganiere et al. (2010). They reported that positive effects of planting conifer trees other than *Pinus* spp. on SOC pools may be negligible. In contrast, the planting of trees with N-fixers symbiosis for afforestation can increase the SOC pool as indicated by the >30% increase in SOC pools (Johnson and Curtis, 2001; Lorenz and Lal, 2014). Moreover, compared with studies on SOC stocks, research on the effects of agroforestry systems on SOM quality and composition, for example on labile WEOM is scarce.

Many studies showed the positive effects of organic farming on SOC stocks (Gattinger et al., 2012) and the biomass and diversity of soil microorganisms (Mäder et al., 2002; Fließbach et al., 2007; Birkhofer et al., 2008). The application of cattle farmyard manure (Heinze et al., 2010) and a more diverse crop rotation strengthens nutrient cycling in organic farming. However, no information exists whether the combination of organic farming and agroforestry results in an additive positive impact. The current agroforestry trial offers the unique possibility to investigate the effects of tree species (poplar and robinia) and organic farming on WEOM, investigating the following hypotheses: (1) Organic farming increases SOC and WEOM contents, especially that of the components of WEOM revealed by EEM-PARAFAC technique. (2) N_2-fixing robinia has stronger positive effects on SOC and WEOM than poplar, especially close to the hedgerow.

2. Materials and methods

2.1. Field experiment

Filed experiments have been carried out conducted in Scheyern Research Farm (TERENO site) located 40 km north of Munich (Germany) (48.50°N, 11.45°E) since 1992. The altitude of the farm ranges between 445 and 500 m (a.s.l.). The mean annual precipitation is 803 mm and mean annual temperature is 7.4 °C (Schröder et al., 2002). The central part of the research station was divided into two parts (in 1992): organic and integrated farming system, each striving for ecological and economical sustainability. Moreover, detailed studies on management-induced changes were carried out in plots sub-divided in integrated and organic farming (Schröder et al., 2002). The soil types are sandy to loamy Cambisols, derived from tertiary sediments and partly covered by loess and most of the soils have loamy texture (Flessa et al., 2002; Kölbl and Kögel-Knabner, 2004). The soils of current organic and integrated alley-cropping farming (agroforestry) field are comparable and have a soil texture of silty loam (USDA) and the soil particle distribution of top soil is 27% sand, 54% silt and 19% clay for organic field and 27% sand, 50% silt and 23% clay for integrated field.

In 2009, agroforestry parcels were incorporated into fields under both integrated and organic farming systems. Three swaths of trees, each comprised of several different species were planted in an alley cropping for the purpose of bioenergy production (30 m length for each tree species) and leaving 30 m wide arable soil for crop production (Fig. 1). The poplar (*Populus maximowiczii* × *P. nigra*) and Robinia (*Robinia pseudoacacia* L.) strip systems were chosen for this study in both organic and integrated systems as they are commonly used tree species in German agroforestry systems. The experiment consisted of four treatments with three replications: organic farming + poplar (O-pop), organic farming + robinia (O-rob), integrated farming + poplar (I-pop), and integrated farming + robinia (I-rob). The plot for each treatment is 30 × 30 m in size. The treed portions of both systems do not receive fertilizer either in manure or mineral form and do not receive weed control via mechanical means or pesticide. The tree density of poplar and robinia are the same.

The organic farming system is low-input, utilizing nitrogen fixing cover crops instead of mineral nitrogen or synthetic inputs as green manures. Also no pesticide or herbicide was applied. Soils were tilled with moldboard. A seven-field crop rotation was run in the organic farming system: (1) Grass–clover–alfalfa (GCA) (*Lolium perenne* L. + *Trifolium pratense* L. + *Medicago sativa* L.), (2) potatoes (*Solanum tuberosum* L.) + mustard (*Sinapis alba* L.) as cover crop, (3) winter wheat (*Triticum aestivum* L.), (4) sunflower (*Helianthus annuus* L.) + GCA as cover crop, (5) GCA, (6) winter wheat, and (7) winter rye (*Secale cereale* L.) + GCA as cover crop. In the integrated farming system, soils were tilled by harrowing and chiseling, and the tillage intensity was reduced to a level to control weed as well as to conserve soil. Pesticides were completely forbidden in organic farming system while in necessity it is applicable in the integrated farming system. A four-field crop rotation with cover crops was run in the integrated farming system: (1) winter wheat; (2) potatoes; (3) winter wheat; and (4) maize (*Zea mays* L.).

2.2. Sampling and soil properties

Sampling plots were randomly set in the tree row (0 m), transition area (2 m from the tree row: 2 m) and middle of crop area (15 m from the tree row: 15 m) corresponding to poplar and robinia strips in organic and integrated farming system (Fig. 1). In May 2013, before vegetation developed after a special long winter time, three replicate composite samples were collected at a depth of 0–25 cm. SOC and total N

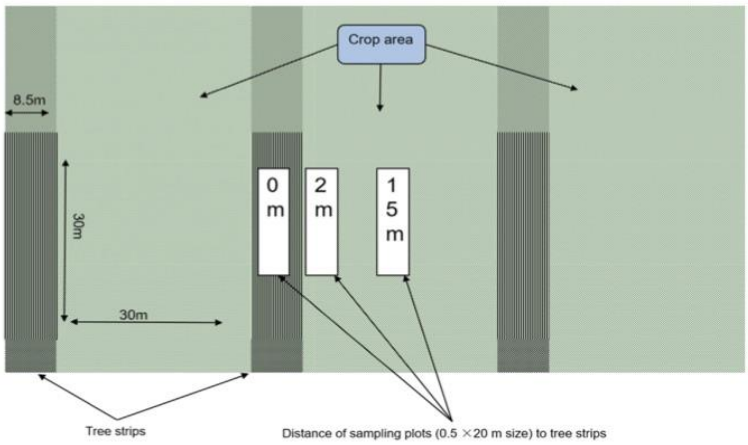

Fig. 1. Experimental layout of alley cropping agroforestry systems and sampling sites (0 m, 2 m and 15 m).

was determined by a CN analyzer (EA3000 Eurovector) with an aliquot air-dried soil samples. Soil texture was determined by wet sieving and pipet method (Gee and Bauder, 1986). Briefly, sand and silt fractions ≥20 μm were measured by sieving after treatment with H_2O_2, silt and clay <20 μm by a pipette procedure.

2.3. WEOM extraction and spectroscopic characteristics

Soil WEOM was extracted according to the method of Embacher et al. (2007). Briefly, 2 mm mesh sieved air-dried soil samples were shaken with 0.01 M $CaCl_2$ at a ratio of 1 to 2 (soil to volume) by using an overhead shaker for 10 min. After centrifugation (3000*g* 10 min), the supernatant was filtered through 0.4 μm polycarbonate membrane filters (Whatman). WEOC concentrations were quantified using catalytic high temperature combustion (680 °C) with a Shimadzu® TOC 5050A analyzer and expressed as mg kg^{-1} dry matter. Dissolved total N, NO_3-N, and NH_4-N of the extracts were quantified photometrically with an automated continuous flow analyzer (Skalar®), and WEON was quantified by subtracting inorganic N from dissolved total N.

Specific UV absorption (SUVA), obtained by dividing the absorption at 254 nm by WEOC concentration, provides the information about the aromatic structures (aromaticity) of WEOM components. Absorption was determined using 1 cm quartz cells with a Varian Cary 50 Bio UV–visible spectrophotometer (Corvasce et al., 2006).

Both pH and molecular concentrations can influence fluorescence (Zsolnay, 2003; Zsolnay et al., 1999). Therefore, to avoid concentration artifacts, dilution was made to have WEOC absorbance <0.1 cm^{-1} at 254 nm (Zsolnay et al., 1999), and then WEOC extracts were acidified to reach a constant standard pH of 2 with 2 M HCl (Embacher et al., 2007, 2008). The pH has been used for fluorescence spectra (Embacher et al., 2007; Embacher et al., 2008; McKnight et al., 2001). Moreover, at low pH, most metal complexes dissociate, which should minimize the quenching of fluorescence due to metal complexation (McKnight et al., 2001). PARAFAC analysis were also used to decompose the EEMs measurement at a pH range of 2 to 12.5 (Chen and Kenny, 2007; Cuss et al., 2014; Fellman et al., 2008; Yang and Hur, 2014). In present study, EEMs spectra and fluorescence spectra for humification index calculation were measured separately and a round of whole measurement procedure including pH adjustment was finished within half an hour and no precipitation was observed in the samples. All the fluorescence measurements for humification index were done with a Varian Cary Eclispe fluorescence spectrophotometer using 1 cm quartz cells at 254 nm excitation. The fluorescence spectra were recorded (300 to 480 nm with 2 nm increment) at 21 ± 1 °C. Even though the optical density was adjusted by dilution in advance, the fluorescence emission spectra were corrected for "inner filter effect" (Gauthier et al., 1986) according to Zsolnay et al. (1999) with a simplified formula that measured fluorescence emission multiplies e^A, where A is the absorbance in cm^{-1} at the 254 nm excitation wavelength (Akagi and Zsoinay, 2008; Embacher et al., 2007). Humification index (HIX) indicating the complexity and condensation (H/C ratios) of WEOM was calculated as the ratio of integrated fluorescence emission peak at longer wavelength region (435–480 nm) over shorter wavelength region (300–345 nm) at 254 nm excitation wavelength (Zsolnay et al., 1999).

EEMs were obtained by scanning over excitation wavelength from 250 nm to 450 nm with an increment of 5 nm and emission wavelength from 300 nm to 600 nm with an increment of 5 nm. The slit wide was set as excitation slit 10 nm and emission slit 20 nm. Fluorescence data were corrected for inner-filter effect with absorption data as suggested by Lakowicz (2006):

$$I_{corr} = I_{obs} \times 10^{0.5(Aex+Aem)}$$

where I_{corr} and I_{obs} are corrected and uncorrected fluorescence intensities, and A_{ex} and A_{em} are the absorbance values at the excitation and emission wavelength of the fluorescence intensity value. The correction is based on the assumption that the average path length of excitation and emission light is 50% of the cuvette width, respectively. Although the correction formula for EEMs and for fluorescence spectra for humification index calculation were different, this will not affect the results in a comparison study. Firstly, EEMs spectra and fluorescence spectra for humification index calculation were measured separately. Meanwhile, the correction for HIX calculation was done according to the published method, and EEM correction was also done followed the commonly reported method. Thus, the correction of fluorescence spectra for each parameter were done in the same way, respectively. Secondly, the two correction formulas are both simplified from the same original formula and base on the same theory as reported by Gauthier et al. (1986).

The fluorescence index (FI), strongly correlated with the structural conjugation and aromaticity and used to differentiate source of DOM, was calculated as the ratio of emission intensity at 470 and 520 nm at fixed excitation wavelength of 370 nm (McKnight et al., 2001). Biological/freshness index (BIX) or β/α index, an indicator of the relative contribution of recently microbially produced DOM, was calculated as the ratio of emission intensity at 380 nm (β) to the maximum emission intensity observed between 420 and 435 nm (α) for an excitation wavelength of 310 nm (Huguet et al., 2009; Wilson and Xenopoulos, 2009).

2.4. PARAFAC modeling

PARAFAC provides a way to decompose a dataset of EEM into individual fluorescence components (Bro, 1997; Andersen and Bro, 2003). An EEM can be reduced to trilinear terms and a residual array by

$$X_{ijk} = \sum_{n=1}^{F} a_{in} b_{jn} c_{kn} + \varepsilon_{ijk}$$

where, for EEM fluorescence data X_{ijk} is the fluorescence intensity of the *i*th sample at the *k*th excitation and *j*th emission wavelength. a_{in} is directly proportional to the concentration (here defined as scores) of the *n*th fluorophore in the *i*th sample. b_{jn} and c_{kn} are estimates of emission and excitation spectra (loadings) of *n*th fluorophore at wavelength *j* and *k*, respectively. F is the number of components and ε_{ijk} the residual matrix of the model which represents unexplained variability by the model. The full description about PARAFAC can be referred to Bro (1997). Components extracted by PARAFAC can be ascribed to specific species of organic matter present in liquid samples, but they are more likely represent groups of organic compounds having similar fluorescence properties. The PARAFAC model identifies the number of components as well quantifies the scores for each component that is directly proportional to the component's (fluorophore) concentration in the sample. This concentration can be converted into actual concentration if specific absorption coefficient or quantum yield associated with excitation and emission of each fluorophore is known (Singh et al., 2010). The concentration scores of the PARAFAC components were expressed as maximum fluorescence intensity (F_{max}) (R.U.) for each modeled component in current study (Murphy et al., 2013). F_{max} gives estimates of the relative concentrations of each component; however, direct comparison of relative concentrations between different components depends on the magnitude of their quantum efficiencies as well as on their individual responses to quenching effects (Baghoth et al., 2011).

Before the application of PARAFAC model, zero emission intensities were assigned for excitation wavelengths (λex) greater than emission wavelengths (λem). Raleigh scattering was minimized according to (Andersen and Bro, 2003; Borisover et al., 2012). An EEM of the 0.01 M $CaCl_2$ solution was obtained and subtracted from the EEM of each sample in order to removed most of the Raman scatter peaks, the any negative values produced by the sub traction were converted to missing value and the fluorescence was normalized by dividing the integrated area (Ex: 350 nm, Em: 371– 428 nm) under Raman scatter peak of the corresponding Milli-Q water of each set of measurement. This calibration procedure is universal and the Raman signal of water

Table 1
Effect of farming practices (1992–2013) and tree species (2009–2013) on SOC, total N and water extractable organic matter in poplar and robinia-based alley cropping systems.

Main effects	SOC (mg g^{-1} soil)	Total N	SOC/total N	WEOC (mg g^{-1} soil)	WEON	WEO-C/N	NO_3-N (μg g^{-1} soil)	NH_4-N
Organic farming	13.0	1.42	9.1	53	1.2	45	5.5	0.50
Integrated	12.3	1.37	9.0	70	1.4	53	11.2	0.55
Poplar	11.3	1.27	8.9	62	1.3	50	7.5	0.48
Robinia	14.0	1.52	9.2	61	1.3	49	9.1	0.56
0 m distance	12.5	1.38	9.0	68.	1.3	53	9.1	0.52
2 m	12.1	1.34	9.0	56	1.2	49	8.2	0.54
15 m	13.3	1.46	9.1	60	1.3	46	7.6	0.50
Probability values								
Farming	<0.01	0.01	0.01	0.01	0.05	NS	<0.01	<0.01
Tree species	<0.01	<0.01	0.04	NS	NS	NS	<0.01	<0.01
Sampling distance	<0.01	<0.01	NS	NS	0.07	NS	0.02	NS
Farming × tree	<0.01	<0.01	0.02	0.01	NS	0.03	NS	NS
Farming × distance	<0.01	<0.01	0.03	NS	0.08	NS	<0.01	0.03
Tree × distance	0.02	<0.01	NS	NS	0.06	0.04	NS	NS
CV (±%)	2	2	2	17	10	20	13	9

CV = mean coefficient of variation between replicate plots (n = 3); SOC: soil organic C; WEOC: water extractable organic carbon; WEON: water extractable organic N; NS: not significant.

can be used irrespective of sample solvent and matrix and no spectral changes will occur from applying this method (Lawaetz and Stedmon, 2009) and the fluorescence intensities was reported in Raman units (R.U.). In total, the dataset contained 36 corrected and standardized EEMs. After an initial exploratory analysis (calculate its leverage using DOMFluor), one an outlier was identified and removed from the dataset. Therefore, 35 EEMs were performed with PARAFAC analysis in the end. Split-half analysis and examination of residual error plots were applied to get the appropriate number of components. A series of PARAFAC models were generated with Matlab (2009a) (The MathWorks, Natick, MA) by using DOMfluor toolbox specifically developed for PARAFAC analysis of DOM fluorescence (Stedmon and Bro, 2008).

2.5. Statistics

The results presented are geometric means and expressed on dry weight basis. The significance of treatment effects was tested by a two-way ANOVA using tree species and farming managements as independent factors and sampling distance as a repeated measure. ANOVA and Pearson correlation coefficients were calculated using the vegan package (Oksanen et al., 2015) in R (R Core Team, 2015).

3. Results

3.1. Soil organic matter and water extractable organic matter

Mean SOC and total N contents as well as the SOC/total N ratio were significantly higher in the organic than in the integrated farming system, whereas the WEOC, WEON, NO_3-N and NH_4-N contents as well as the WEO-C/N ratio were significantly lower (Table 1). Mean WEOC and WEON contents as well as their ratio were not affected by tree species, whereas the SOC, total N, NO_3-N, and NH_4-N contents as well as the SOC/total N ratio were significantly higher in the robinia than in the polar agroforestry system (Table 1). The effects on SOC were stronger in the organic farming system (Fig. 2), as shown by significant farming × tree interactions (Table 1). Mean SOC, total N and WEON contents at 15 m distance from the tree strips were significantly higher than at 0 m and 2 m distance, whereas the mean NO_3-N content was significantly lowest at 15 m distance (Table 1), especially in the organic farming system, leading to significant farming × distance interactions effect on SOC (Fig. 2) and WEON (Fig. 3). Mean WEOC, and NH_4-N contents as well as mean SOC/total N and WEO-C/N ratios were not affected by any sampling distance from the tree strip (Table 1).

3.2. Spectroscopic properties of WEOM

Mean SUVA, FI, and BIX values were significantly higher in the organic than in the integrated farming system whereas the mean HIX value was lower (Table 2). Robinia significantly increased mean SUVA values in comparison with poplar but did not affect FI and HIX. However, this increase was solely observed in the organic farming system (Fig. 4), leading to significant farming × tree interactions (Table 2). In contrast, the significant farming × tree interactions on the HIX values were caused by significantly higher values in the integrated poplar agroforestry system (Fig. 5). The difference of SUVA and HIX between sampling distance was significant (Table 2). Mean SUVA and HIX values increased in the order 0 m < 2 m < 15 m distance. For the HIX values, this order was mainly caused by the robinia agroforestry system as indicated by the significant tree × distance interactions (Table 2, Fig. 5).

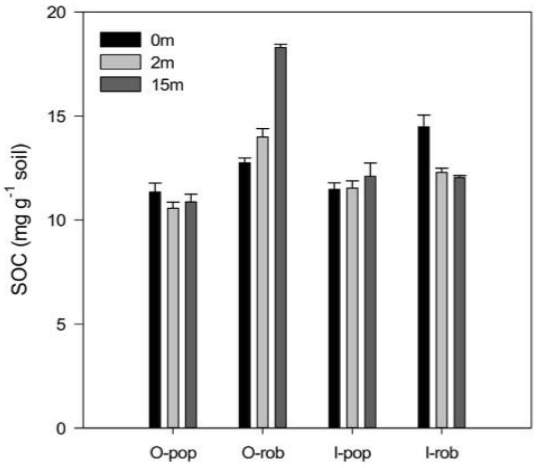

Fig. 2. The soil organic carbon (SOC) content of all treatments in the alley cropping agroforestry field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites. (O-pop: organic farming + poplar; O-rob: organic farming + robinia; I-pop: integrated farming + poplar; I-rob: integrated farming + robinia).

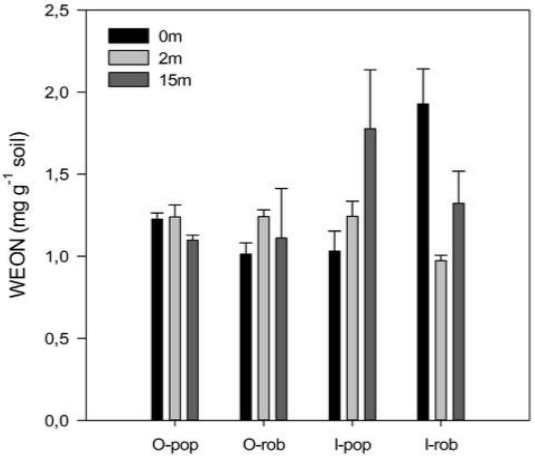

Fig. 3. The water extractable organic N (WEON) content of all treatments in the alley cropping agroforestry field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites. (O-pop: organic farming + poplar; O-rob: organic farming + robinia; I-pop: integrated farming + poplar; I-rob: integrated farming + robinia).

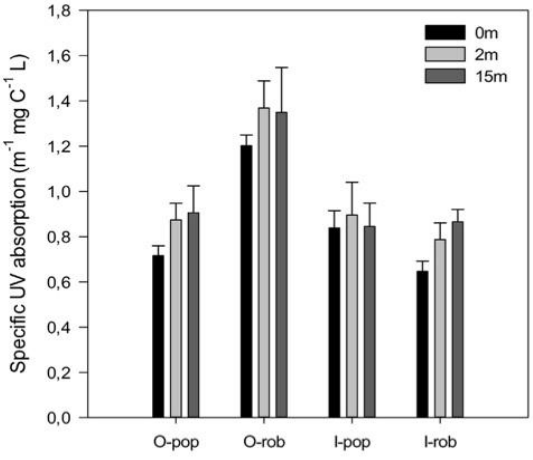

Fig. 4. The specific UV absorbance (SUVA) of water extractable organic matter (WEOM) of all treatments in the alley cropping agroforestry field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites. (O-pop: organic farming + poplar; O-rob: organic farming + robinia; I-pop: integrated farming + poplar; I-rob: integrated farming + robinia).

3.3. PARAFAC components of WEOM

After PARAFAC and split-half analysis of the excitation and emission matrix, four EEM-PARAFAC components were extracted (Fig. 6). The four components C1 to C4 explained 99.7% variability of samples. C1 has one excitation maxima at 325 nm and one emission maxima at 420 nm, resembling the humic-like PARAFAC-derived C1 in soil WEOM (Ohno and Bro, 2006; Borisover et al., 2012). C2 had one excitation maxima at ≤250 nm and one emission maxima at 435 nm. This component is similar to the fulvic-like DOM of boreal soils (Olefeldt et al., 2013) and fulvic-like C9 of marine DOM (Murphy et al., 2008). C3 with an excitation peaks at 260 nm and an emission peak at 360 nm resembled the tryptophan-like PARAFAC-derived component in soil WEOM (Borisover et al., 2012; Ohno and Bro, 2006). C4 in had two excitation maxima at 270 nm and 380 nm, and one emission maximum at 475 nm. This PARAFAC-derived component is similar to the humic-like substance, identified in soil WEOM (Borisover et al., 2012).

The F_{max} of the PARAFAC-derived components followed an order of C2 (fulvic-like) > C1 (humic-like) > C3 (tryptophan-like) > C4 (humic-like) (Table 2). C1, C2 and C4 values were significantly lower in the organic than in the integrated farming system, whereas C3 did not differ between the two farming system. All four components were significantly larger in the robinia than in the poplar agroforestry system. Only the C2 values were significantly affected by the sampling distance, following the 0 m < 2 m < 15 m. The C1, C2 and C4 values were significantly correlated with the HIX values and negatively correlated with the FI

Table 2
Effects of farming practices (1992–2013) and tree species (2009–2013) on spectral characteristics and spectrally identified components of water extractable organic matter in poplar and robinia-based alley cropping systems.

Main effects	SUVA	HIX	Fmax (R.U.)				FI	BIX
			C1	C2	C3	C4		
Organic farming	1.07	2.8	1.00	1.57	0.89	0.32	1.61	0.77
Integrated	0.81	3.3	1.15	1.78	0.84	0.39	1.52	0.74
Poplar	0.85	3.1	1.00	1.57	0.82	0.32	1.55	0.76
Robinia	1.04	3.0	1.15	1.78	0.91	0.39	1.57	0.75
0 m distance	0.85	2.7	1.06	1.57	0.91	0.34	1.55	0.76
2 m	0.98	3.1	1.02	1.62	0.81	0.33	1.59	0.75
15 m	0.99	3.3	1.12	1.83	0.87	0.40	1.54	0.75
Probability values								
Farming	<0.01	<0.01	<0.01	0.01	NS	<0.01	<0.01	0.02
Tree species	<0.01	NS	<0.01	<0.01	0.02	<0.01	NS	NS
Sampling distance	0.05	0.02	NS	0.03	NS	NS	NS	NS
Farming × tree	<0.01	<0.01	0.03	0.01	0.01	NS	<0.01	NS
Farming × distance	NS	NS	NS	NS	NS	NS	0.02	NS
Tree × distance	NS	0.03	NS	0.04	NS	0.03	0.09	NS
CV (±%)	10	6	9	8	11	13	2	2

CV = mean coefficient of variation between replicate plots (n = 3); SUVA = specific UV absorption; HIX = humification index; FI = fluorescence index; BIX = freshness index; C1, C2, C4: fulvic/humic-like substances; C3: tryptophan-like substances; NS: not significant.

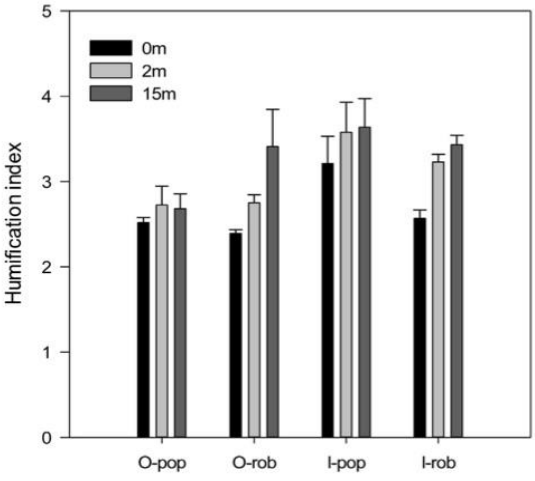

Fig. 5. The humification index (HIX) of water extractable organic matter (WEOM) of all treatments in the alley cropping agroforestry field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites. (O-pop: organic farming + poplar; O-rob: organic farming + robinia; I-pop: integrated farming + poplar; I-rob: integrated farming + robinia).

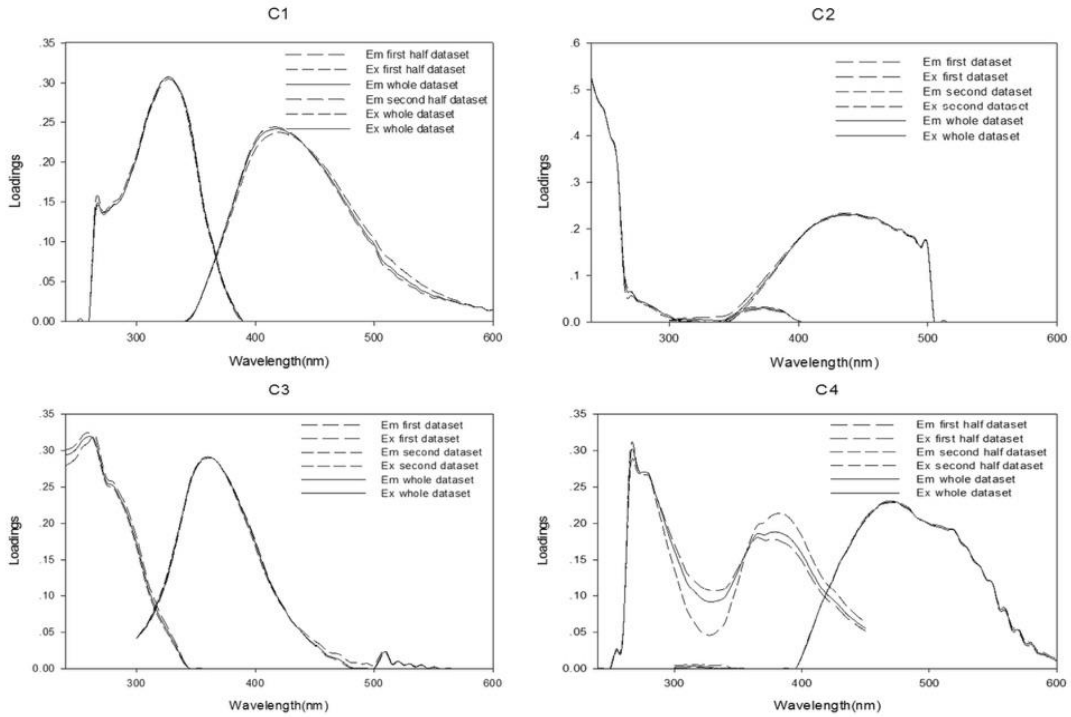

Fig. 6. Split-half analysis of the three components of WEOM extracted by PARAFAC model for all treatments. C1: humic-like substance; C2: fulvic-like substance; C3: tryptophan-like substance; C4 : humic-like substance.

and BIX values (Table 3). The C3 values were only significantly correlated with SUVA.

4. Discussion

4.1. Farming practice

Organic farming had positive effects on SOC and total N contents. This is in agreement with our first hypothesis and with previous studies, which reported organic farming tends to retained more SOM, especially in the upper soil (Pulleman et al., 2003; Marriott and Wander, 2006; Gomiero et al., 2011; Gattinger et al., 2012). This can be explained by the quality of the organic input as the inclusion of legume-based and more diverse crop rotation in the organic farming in comparison with the integrated system and agrees with Marriott and Wander (2006) that the legume-based organic management is as capable of building SOM as systems receiving manure or compost.

Table 3
Correlations between PARAFAC components of water extractable organic matter and soil spectral indices.

	SUVA	HIX	BIX	FI
C1	0.02	0.42*	−0.54**	−0.58**
C2	0.14	0.54**	−0.55**	−0.58**
C3	0.34*	−0.25	−0.12	−0.27
C4	−0.13	0.51**	−0.57**	−0.51**

C1, C2, C4: fulvic/humic-like substances; C3: tryptophan-like substances; SUVA: specific UV absorbance; HIX: humification index; BIX: freshness index; FI: fluorescence index.
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Long-term organic farming decreased the WEOC and WEON contents in the current study, contrasting our first hypothesis and previous studies (Chantigny, 2003; Xu et al., 2013). However, they observed only immediate effects, directly after application of crop residues and manure (Chantigny, 2003) or alfalfa (Xu et al., 2013). The lower WEOM contents in the organic farming system might be caused by a larger consumption than production rate of WEOM, since the degradation of organic matter is the main source of WEOM in organic farming system and the organic farming system is low-input and no external organic input was added. Moreover, strong seasonal effects on WEOM were repeatedly reported by others (Bausenwein et al., 2008; Embacher et al., 2007; Liang et al., 2011).

The increased SUVA, FI and BIX indicated WEOM in organic farming contained more aromatic components and more new WEOM was produced in the organic farming system. The increased spectral indices also indirectly reflected the high activities of microorganisms in the organic farming system. The decreased F_{max} of fulvic- and humic-like components contrast our first hypothesis and the results of Zhang et al. (2011) who observed that manure addition increased the humic-like component identified by EEM-PARAFAC at surface soil. The difference might attribute to the sources of WEOM in the current organic farming system were more diverse and of more easily degradable due to the presence of legumes in the crop rotation. Whereas in the study of Zhang et al. (2011), the crop rotation is a simple corn/cotton rye rotation. Moreover, inorganic N seems to promote the formation of water-soluble, brown and recalcitrant compounds (Fog, 1988), which is in line with the increase in humic/fulvic-like substances of the current integrated farming system. The SUVA indicates the content of aromatic compounds, while according to Zsolnay et al. (1999), HIX shows the condensation (H/C ratio) of organic matter. Organic farming showed opposite effects on SUVA and HIX of WEOM, although the two indices

both reflect the aromaticity of WEOM. Since HIX is a ratio of the upper quarter (435–480 nm) to lower quarter (300–345 nm) of the emission spectra, the existence of protein-like components might interfere the HIX as the corresponding peak for protein-like compounds lies in the lower quarter of emission spectra (Cuss and Guéguen, 2015) could potentially explain the opposite effects. The SUVA and HIX values might also indicate WEOM in organic farming contains more aromatic components but less condensed.

4.2. Tree species

Robinia had general positive effects on SOC and total N contents at 0–25 cm depth as well as on SUVA values and PARAFAC components in comparison with poplar, confirming our second hypothesis. Similarly, Nii-Annang et al. (2009) observed higher SOC contents at 0–3 cm depth in a robinia than in poplar stands of an alley cropping system, re-cultivating quaternary deposits for 9 years. Also Medinski et al. (2015) observed significantly higher SOC stocks at 0–10 cm depth in robinia than in poplar agroforestry systems. The higher residue input indicated by the biomass of robinia in previous years (Supp.-Table 1), may be one reason for the higher SOM content in the robinia agroforestry system. Another reason might be the higher N and lower lignin contents of robinia leaf litter and root residues in comparison with poplar (Kaleeem Abbasi et al., 2015; Mafongoya et al., 1998; Thippayarugs et al., 2008). This leads to an increased formation of microbial residues and in the long-term to higher SOM contents and points to the demand of sufficient N supply for soil C sequestration (Khan et al., 2016). This view is in line with the current observation that robinia had stronger effects on the PARAFAC components of WEOM than on the WEOC and WEON contents.

4.3. Sampling distance

SOC was not significantly accumulated in hedgerow of the current alley cropping system in comparison with the sampling points 2 and 15 m apart. This contrasts our second hypothesis and the results of Nii-Annang et al. (2009) and Medinski et al. (2015), but is in agreement with Udawatta et al. (2014) who did not observe significant differences in SOC and total N between hedgerows and crop areas, 21 years after establishing an alley cropping agroforestry system. They attributed the results to the maturity of their system. In the current case, the short-term establishment of hedgerows for 4 years may explain the absence of effects on SOC and total N contents between the sampling points. The disturbance of soil during tree planting may cause SOC loss (Six et al., 1998) and the rooting behavior of tree is different to crops, i.e. a larger volume is less densely rooted by robinia and poplar in comparison whit arable crops. This might also explain the lower SUVA and HIX values of WEOM in the hedgerow in comparison with the sampling points 2 and 15 m apart. In addition, the agronomic performance, for example of winter wheat was worse than that at 15 m distance during the sampling periods.

4.4. The relationship between WEOM components revealed by EEM-PARAFAC and spectroscopic indices of WEOM

SUVA, HIX, BIX and FI characterize WEOM from various aspects. SUVA indicates the compounds with aromatic structure in WEOM (Akagi et al., 2007); HIX describes the condensation of WEOM reflected by the H/C ratio (Zsolnay et al., 1999); FI strongly correlates with the structural conjugation and aromaticity of WEOM and can be used to differentiate the source of substance in WEOM, terrestrial-derived (higher FI value) or microbially-derived (low FI value) (Johnson et al., 2011), and BIX is an indicator of the relative contribution of recently produced WEOM through microbial activities (Huguet et al., 2009). Since the fulvic/humic-like substances are more chemically condensed and complex than tryptophan-like substances and have complex structure (Hudson et al., 2007). The positive correlation between SUVA and C3 (tryptophan-like substances) implied the C3 component mainly contributed to the SUVA of WEOM in current study. The positive correlation between C1, C2, C4 and the fluorescence-derived indices indicated the fulvic/humic-like substances contribute positively to the humification index of WEOM. This partially supports humification is a process decompose organic materials to structurally condensed substances. The negative correlation between BIX, FI and C1, C2, C4 indicated the higher fulvic/humic like substance in WEOM under natural conditions, the more WEOM might be microbially-derived and microbial process of organic materials leads to condensation of organic matter. Microbial activities and microorganism per se are important source of WEOM. From above, the WEOM components revealed by EEM-PARAFAC can also be used to judge the quality of WEOM.

5. Conclusions

Our data showed that long-term low-input organic farming had positive effects on SOC and total N as well as general negative effects on WEOM and its PARAFAC components. The spectral indices indicated the higher presence of more fresh and microbially-derived organic components of WEOM in the organic farming than in the integrated farming system. Robinia as hedgerow tree for 4 years showed positive effect on SOC and total N contents in comparison with poplar and had stronger effects on the PARAFAC components of WEOM, although the WEOM content did not differ between the two tree species. The robinia effects were more pronounced in the organic than in the integrated farming system. The correlation analysis suggested the WEOM component revealed by EEM-PARAFAC can be used to judge the quality of WEOM directly. Consequently, low-input organic farming and robinia tended to result in change of quality of WEOM and led to enrichment of WEOM of high stability. The alley-cropping agroforestry system combining robinia with organic farming has a great potential for sequestering SOC and developing a sustainable agroecosystem.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.geoderma.2016.12.014.

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The following are the supplementary data related to this article.

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3.4 Effect of organic farming and minimum tillage on microbial communities and microbial residues

The paper reports and analysis the responses of microbial communities and microbial residues to long-term minimum tillage and high-input organic farming. The two factors tillage and farming methods were tested in the study. Phosphorous lipid fatty acids analysis was used to indicate soil microbial communities. Amino sugar analysis and soil glomalin measurement were used to indicate soil microbial residues. The results showed that soil microbial communities and microbial residues respond positively to minimum tillage under organic farming. The combination of minimum tillage and organic farming appears to be an effective agricultural strategy that enhance soil microbial biomass, microbial residues as well as bacterial and fungal abundance. The positive effects of minimum tillage on microbial communities can be enhanced by organic farming.

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Soil microbial community and microbial residues respond positively to minimum tillage under organic farming in Southern Germany

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ABSTRACT

In a field trial comprising organic farming and minimum tillage management strategies in Scheyern, Germany, we evaluated the long-term (21-year) effects of organic farming (use of a diverse crop rotation with legume cover crop and without application of synthetic fertilizer or pesticides) and minimum tillage (6–8 cm depth) on the microbial community structure and microbial residues in Cambisols. Organic farming had a positive effect on microbial biomass, total phospho-lipid fatty acids (PLFA), Gram (+) bacteria, Gram (−) bacteria and the arbuscular mycorrhizal fungi (AMF) indicator PLFA 16:1ω5 and amino sugars. The increase in presence of Gram (+) bacteria when compared to integrated farming was also reflected by increased content of bacterial muramic acid (MurN), i.e. an increased formation of bacterial residues. Minimum tillage significantly increased microbial biomass N and the fungal PLFA 18:2ω6,9, averaging the values of upper (0–8 cm) and deeper (12–25 cm) soil, but had no effects on PLFA 16:1ω5. Minimum tillage generally resulted in a negative depth gradient of almost all microbial properties analyzed. The only important exception was fungal galactosamine (GlcN), which led to increases in the fungal C/bacterial C ratio and in the contribution of microbial residue C to SOC in the deeper soil. Significant second order tillage × management interactions indicated that minimum tillage effects on microbial biomass and PLFA indices (Gram (+) and (i15:0 + i17:0)/(a15:0 + a17:0)) were much stronger in the organic farming system than in the integrated farming system. Redundancy analysis (RDA) showed SOC and H_2O content predominantly affected the microbial community structure in the present study. Minimum tillage in combination with organic farming appears to be an effective agricultural strategy that enhances soil microbial biomass, microbial residues and bacterial and fungal abundances. The results indicate that the positive effects of minimum tillage on microbial community can be enhanced by organic farming. Microbial residues as a fraction of SOC respond faster to farming management than to tillage.

1. Introduction

The maintenance of viable, diverse populations and functioning microbial communities is essential to agricultural ecosystems (Kennedy and Smith, 1995), because soil microbial communities are critical to achieving many important ecological functions and services, to maintaining soil quality, to reflecting inherent soil fertility and to developing sustainable agriculture. However, it is subjected to the influence of agricultural management strategies and sensitive to soil perturbation and land use changes (Bending et al., 2004; Fließbach et al., 2007). Cropping system management strategy is one of the most significant anthropogenic activities that greatly alter soil characteristics, including physical, chemical, and biological properties (Jangid et al., 2008). Therefore, the development and implementation of sustainable agricultural strategies is necessary and it is important to understand the impacts of

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different cropping system management strategies on microbial communities in agricultural soils. Organic farming and minimum tillage are two alternative agricultural management strategies of intensive agriculture (Mäder et al., 2002; Kuntz et al., 2013).

The effects of organic farming, characterized by no use of synthetic fertilizer or pesticides and use of diverse crop rotations and manure, on soil microorganisms have been repeatedly analyzed (Mäder et al., 2002; Fließbach et al., 2007; Birkhofer et al., 2008). The application of cattle farmyard manure has usually positive effects on the soil microbial biomass (Heinze et al., 2010b), bacterial biomarkers (Bausenwein et al., 2008) and amino sugars (Scheller and Joergensen, 2008; Joergensen et al., 2010; Sradnick et al., 2014). Organic farming tends to shift the microbial community structure to bacteria, because of the application of farmyard manure (Joergensen et al., 2010), and bacteria respond most strongly to farming management compared with other microbial groups (Esperschütz et al., 2007). Also the effects of minimum tillage or reduced tillage on soil microorganisms have been repeatedly investigated (Heinze et al., 2010a; Jacobs et al., 2011). The use of a grubber instead of a soil-turning plough usually benefits fungi (Frey et al., 1999; Spedding et al., 2004), saprotrophic fungi due to an increased presence of organic residues in the deeper soil (Berner et al., 2008; Heinze et al., 2010b) and AMF due to decreased disturbance (Klein and Paschke, 2004; Kabir, 2005; Sale et al., 2015). Both organic farming and minimum tillage have potential to improve the soil microbial community, and thereby nutrient cycling. As minimum tillage is a management strategy that is rare in organic farming systems (Mäder and Berner, 2012; Armengot et al., 2015), limited information exists on the interactions of these two factors. This is especially true considering the confounding effects of climate and soil properties on tillage and management-induced changes in microbial properties (Six et al., 2006; Navarro-Noya et al., 2013). As most previous studies on the effects of agricultural practices have focused on active microorganisms (Liang et al., 2008), limited knowledge exists on the changes in microbial residues caused by the combination of minimum tillage and organic farming.

The biomass, i.e. the active fraction of microorganisms, can be estimated by fumigation extraction (Brookes et al., 1985; Vance et al., 1987) and the phospholipid fatty acids (PLFA) as cell membrane components give additional information on the biomass of functional microbial guilds (Zelles and Bai, 1993; Gattinger et al., 2002), such as Gram (+) and Gram (−) bacteria and saprotrophic and arbuscular mycorrhizal fungi (AMF) (Joergensen and Wichern, 2008).

Microbial cell wall-derived amino sugars are routinely used as biomarkers for microbial residues (Liang et al., 2013; Sradnick et al., 2014), with muramic acid and glucosamine being highly specific for bacteria and fungi, respectively (Joergensen and Wichern, 2008). Another important component of microbial residues is the glomalin-related soil protein, produced by AMF (Burrows, 2014), closely related to soil organic C (SOC) sequestration and to aggregate stabilization (Rillig et al., 2002; Baez-Perez et al., 2010; Singh et al., 2013).

Long-term agricultural field experiments are particularly valuable for detecting effects of management or tillage systems on soil (Widmer et al., 2006). In the current field trial, we evaluated the effects of long-term (21-year) implementation of organic farming and minimum tillage on the microbial community structure and microbial residues. Our investigations are based on the following hypotheses (1) Organic farming increases microbial biomass, especially bacterial biomass, leading to an increased contribution of microbial residues to SOC. (2) Minimum tillage increases microbial biomass, especially fungal and AMF biomass. (3) The combination of organic farming and minimum tillage would be the most effective management strategy, resulting in maximum contents of microbial biomass, with strongest effects on AMF and only intermediate effects on bacteria and saprotrophic fungi.

2. Materials and methods

2.1. Field experiment

Field experiments have been carried out at the Scheyern Research Farm (TERENO site) located 40 km north of Munich, Germany (48.50°N, 11.45°E) since 1992. The altitude of the farm ranges between 445 and 500 m a.s.l. The mean annual precipitation and mean annual temperature are 803 mm and 7.4 °C, respectively (Schröder et al., 2002). The central part of the research station was divided into two parts: organic and integrated systems, each striving for ecological and economical sustainability. Moreover, detailed studies on management-induced changes were carried out in plots sub-divided into integrated and organic farming (Schröder et al., 2002).

On the organic and integrated sites, plot experiments studying tillage-induced changes to the systems were set up. Two farming managements (Integrated (I) and Organic farming (O)) and two tillage systems (Plough tillage (PL) and Minimum tillage (MT)) were arranged in full factorial plot design. The tillage systems in the study represent two possible tillage intensities under the existing soil and climate conditions. PL as a conventional tillage system means tilling the soil with a moldboard plow (25–30 cm). MT in the study means cultivating soil with a chisel plow in the first 6–8 cm of soil. Therefore, there are four treatments in the present study in total and each has three replicates. Namely, 1) organic farming + plough tillage (OPL); 2) organic farming + minimum tillage (OMT); 3) integrated farming + plough tillage (IPL); 4) integrated farming + minimum tillage (IMT). The assignments of the treatments to the plots have been kept constant since 1992. The IF plots are 12 × 12 m in size and the crop rotation is potato (*Solanum tuberosum* L.) + mustard (*Sinapis alba* L.) as catch crop, (2) winter wheat (*Triticum aestivum* L.), (3) maize (*Zea mays* L.) + mustard as catch crop, and (4) winter wheat. The OF plots are 12 × 12 m in size and the crop rotation is a seven-crop rotation: grass–clover–alfalfa (GCA) (*Lolium perenne* L. + *Trifolium pratense* L. + *Medicago sativa* L.), (2) potato + mustard as cover crop, (3) winter wheat, (4) sunflower (*Helianthus annuus* L.) + GCA as cover crop, (5) GCA, (6) winter wheat, and (7) winter rye (*Secale cereale* L.) + GCA as cover crop. The soil types are sandy to loamy Cambisols, derived from tertiary sediments. The soil in both field trials has a soil texture of silty loam (USDA) and the soil texture of top soil is 22% sand, 58% silt and 20% clay (Flessa et al., 2002; Kolbl and Kögel-Knabner, 2004).

In the IF system trial, N fertilization was done with UAN (50% urea N, 50% ammonium nitrate N), with a modified boom sprayer with tubes to conduct the solution directly to the soil surface. Fertilizer rates were fixed for the cultivated crops (135 kg N ha^{-1} for winter wheat, 105 kg N ha^{-1} for maize and 100 kg N ha^{-1} for potato), irrespective of credits due to effects of previous crop cultivation or mineralization in the soil. In the OF system trial, cattle farmyard manure was applied at a rate of 30 t ha^{-1} a^{-1} dry weight.

2.2. Sampling and soil properties

Three composite samples (20 augers, 5 cm in diameter) were separately collected at two soil depths (0–8 cm-upper soil and 12–25 cm-deeper soil) in April 2013 from each experimental plot. Soil moisture (WC) was determined by oven-drying fresh soil at 105 °C to a constant weight. Soil pH was measured in a soil-0.01 M $CaCl_2$ suspension (1:2.5 v:v). SOC and total N were measured using

an elemental analyzer (EuroVector EA3000 Series). Ammonium and nitrate was determined with an automated continuous flow analyzer (Skalar) after extracting soil with 0.01 M $CaCl_2$ (1:4 v:v). The environmental factors used to constrain the soil PLFA profile were determined according to the methods shown in the Supplementary materials (Appendix 1).

2.3. Microbial biomass and phospholipid fatty acid profile

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined using the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). In brief, 5 g (on an oven-dry basis) moist soil samples were fumigated with $CHCl_3$ for 24 h, and then a 5 g fumigated and a separate 5 g non-fumigated sample were extracted with 20 ml 0.01 M $CaCl_2$ solution (Joergensen, 1995). Organic C and total N in the extracts were measured using a TOC/TNb analyzer (Dimatec, Essen, Germany). MBC and MBN were calculated using a k_{EC} value of 0.45 (Joergensen, 1996) and a k_{EN} value of 0.54 (Joergensen and Mueller, 1996), respectively.

PLFA were determined according to Zelles and Bai (1993) and Gattinger et al. (2002). In brief, a fresh soil sample equivalent to 15 g dry soil was extracted in accordance with the modified Bligh Dyer method (1 v methanol, 2 v chloroform and 0.8 v phosphate buffer mixture). The extracts were fractionated into neutral lipids, glycolipids and phospholipids on an SI column (SPE-SI; Bond Elute, Varian, Palo Alto, USA) by chloroform, acetone and methanol as elution liquids, respectively. The phospholipid was subjected to mild alkaline methanolysis to obtain fatty acid methyl esters (FAMEs) and an SCX column impregnated with silver nitrate was applied to fractionate the unsubstituted FAMEs into saturated, monounsaturated and poly-unsaturated FAMEs, abbreviated as SATFA, MUFA and PUFA, respectively (Zelles, 1999). The SATFA and PUFA fraction were measured directly using GC/MS with nonadecylacidmethylester as internal standard solution. MUFA fraction was measured after dimethyl disulphide (DMDS) derivatization, using the GC/MS operating condition given in Zelles and Bai (1993). The first temperature program of the oven began at 70 °C (for 2 min) and increased to 160 °C at 40 °C min^{-1}, followed by 280 °C at 3 °C min^{-1} (injector temperature 290 °C). The second program started at 100 °C and increased to 210 °C at 50 °C min^{-1}, followed by 300 °C at 3 °C (injector temperature 300 °C). The latter operational variables were only used to measure DMDS derivatives of FAMES. The identification of individual compounds was based on comparison of retention time and mass spectral data obtained from standard compounds, monocultures and environmental samples (Zelles, 1999). The quantification was achieved using chromatography software (HP ChemStation, SOVLVIT, CH) by considering the ratio of target compound and the internal standard with respect to peak areas, as well as the response for each, which was estimated experimentally with standard mixtures (Gattinger et al., 2002). The PLFA n15:0, i15:0, a15:0, i16:0, i17:0, and a17:0 are used as biomarkers for Gram (+) bacteria (Frostegård and Bååth, 1996; Zelles, 1997); the PLFA 16:1ω7t, 16:1ω7c, 16:1ω9, 16:1ω6, 18:1ω7, cy17:0 and cy19:0 for Gram(−) bacteria (Colaco et al., 2007; Frostegård and Bååth, 1996; Zelles, 1997); 18:2ω6,9 for fungi (Joergensen and Wichern, 2008); 16:1ω5 for arbuscular mycorrhizal fungi (AMF) (Olsson et al., 1995). Ratios of (i15:0 + i17:0)/(a15:0 + a17:0) were used as an index for nutritional or environmental stress on Gram (+) bacteria (Zhang et al., 2014).

2.4. Amino sugars

The amino sugars muramic acid (MurN), galactosamine (GalN), 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 hydrolyzed for 6 h at 105 °C. 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 C18 column (125 mm length × 4 mm diameter), protected by a Phenomenex C18 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 derivatization, 50 μl orthophthaldialdehyde (OPA) and 30 μl sample were mixed in the preparation vial and after 120 s reaction time, 15 μl 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).

Fungal C was estimated by multiplying the content of fungal GlcN by 9 (Appuhn and Joergensen, 2006). Fungal GlcN was calculated by subtracting bacterial GlcN from total GlcN, assuming that MurN and GlcN occur at a 1:2 molar ratio in bacteria (Engelking et al., 2007). Bacterial C was calculated by multiplying the content of MurN by 45 (Appuhn and Joergensen, 2006).

2.5. Glomalin

Replicate 0.25 g samples of dry-sieved 1–2 mm aggregates were extracted with 2 ml of extractant. Easily extractable glomalin (EEG) was extracted with 20 mM citrate (pH 7.0) at 121 °C for 30 min. Total glomalin (TG) was extracted with 50 mM citrate (pH 8.0) at 121 °C. For sequential extractions, the supernatant was removed by centrifugation at 10,000*g* for 5 min, 2 ml of 50 mM citrate, pH 8.0 was added to the residue and samples were autoclaved for 60 min. Extraction of a sample continued until the supernatant showed none of the red-brown color typical of glomalin. Extracts from each replicate were pooled and then analyzed. After extraction cycles were completed, samples were centrifuged to remove the soil particles (10,000*g* for 5 min), and protein in the supernatant was determined by the Bradford dye-binding assay (Bradford assay (Sigma-Aldrich Inc.)) with bovine serum albumin as the standard (Wright and Upadhyaya, 1996, 1998).

2.6. Statistical analysis

A two-way repeated measures ANOVA analysis was conducted to test the effects of minimum tillage and organic farming on soil microbial indices (Gueorguieva and Krystal, 2004). Principal component analysis (PCA) was done to analyze the soil microbial community based on a covariance matrix (Thoms et al., 2010). Redundancy analysis (RDA) was used to examine the relationship among soil physicochemical variables (soil moisture, pH, clay, NH_4-N, NO_3-N, SOC, DOC, DON, SUVA, HIX, PARAFAC components of DOM-C1, C2 and C3) (Supplementary materials Table 1) and soil microbial community composition. The data used in PCA and RDA were expressed as mol percent. The PLFA data were Helliger transformed prior to being subject to PCA and RDA (Andersen et al., 2010). Before RDA, all soil physicochemical variables were standardized and forward selection of soil physicochemical variables was conducted for response variable (PLFAs) to get a

Table 1
Mean contents of SOC, microbial biomass and total PLFA in main factor treatment in a long-term field trial. Two-way ANOVA with repeated measures (Farming, Tillage).

Main effects	SOC (mg g^{-1} soil)	Microbial biomass C (μg g^{-1} soil)	N	C/N	C (% SOC)	N (% total N)	Total PLFA (nmol g^{-1} soil)
Organic farming	14.0 ± 0.28a	370 ± 44a	55 ± 9a	7.7 ± 0.8a	2.5 ± 0.3a	3.3 ± 0.6a	88 ± 12a
Integrated	11.9 ± 0.24b	257 ± 31b	26 ± 4b	9.9 ± 1.1b	2.1 ± 0.3a	1.9 ± 0.4b	60 ± 8b
Minimum tillage	12.7 ± 0.25a	346 ± 42a	47 ± 8a	8.5 ± 0.9a	2.5 ± 0.3a	3.0 ± 0.6a	74 ± 10a
Plough	13.2 ± 0.26b	281 ± 34b	34 ± 5b	9.2 ± 1.0a	2.1 ± 0.3a	2.3 ± 0.4a	74 ± 10a
0–8 cm depth	15.0 ± 0.30a	424 ± 51a	56 ± 9a	8.6 ± 0.9a	2.7 ± 0.4a	3.0 ± 0.6a	94 ± 13a
12–25 cm	10.9 ± 0.22b	203 ± 24b	25 ± 4b	9.0 ± 1.0a	1.8 ± 0.2b	2.0 ± 0.4b	54 ± 8b
Probability values							
Farming	<0.01	<0.01	<0.01	<0.01	NS	<0.01	<0.01
Tillage	0.02	0.06	<0.01	NS	NS	NS	NS
Depth	<0.01	<0.01	<0.01	NS	<0.01	<0.01	<0.01
Farming × tillage	<0.01	0.04	0.03	NS	NS	NS	NS
Farming × depth	<0.01	<0.01	<0.01	0.02	NS	0.02	0.03
Tillage × depth	<0.01	<0.01	<0.01	NS	NS	NS	<0.01
CV (±%)	2	12	16	11	13	19	14

CV = mean coefficient of variation between replicate plots (n = 6). NS: not significant. The different letters following the mean indicate a significant difference within same factor.

parsimonious set of explanatory variables based on a double stopping rule of both alpha level ($p < 0.05$) and adjusted R^2 (Blanchet et al., 2008; Bowles et al., 2014). All the statistical analysis was conducted using the vegan package (Oksanen et al., 2015) based on R program (R Core Team, 2015).

3. Results

3.1. Microbial biomass and total PLFA

The contents of SOC, MBC, MBN and total PLFAs as well as the contribution of MBN to total N were significantly higher in the organic than in the integrated farming system, while organic farming significantly decreased the MB-C/N ratio (Table 1). MBC as a percentage of SOC did not differ between organic and integrated farming. Minimum tillage significantly increased the MBC and MBN contents in comparison with plough tillage, while all other microbial indices remained unaffected. In contrast, the MB-C/N ratio was the only microbial property that did not differ between the upper and lower soil, where all others significantly declined with depth. The significant tillage × farming interactions of MBC (Table 1, Fig. 1), but also those of MBN, MB-C/N, MBC as a percentage of SOC, MBN as a percentage of total N and total PLFA (Table 1, Fig. 2) showed much stronger tillage effects in the organic than in the integrated farming system.

3.2. Microbial lipid groups and microbial community structure

The contents of total bacterial, Gram (+), Gram (−), and the AMF signature PLFA 16:1ω5 as well as the ratio of Gram (+)/Gram (−) PLFA were significantly higher in the organic than in the integrated farming system, while organic farming decreased the (i15:0 + i17:0)/(a15:0 + a17:0) ratio (Table 2). The PLFA biomarker for saprotrophic fungi 18:2ω6,9 did not differ between organic and integrated farming. Minimum tillage resulted in significantly higher 18:2ω6,9 contents and (i15:0 + i17:0)/(a15:0 + a17:0) ratios than plough tillage, whereas all other PLFA indices remained unaffected. All PLFA based indices significantly declined with depth, except the Gram (+)/Gram (−) PLFA ratio. The significant farming × tillage interactions of Gram (+) PLFA (Fig. 3), but also those of Gram (−) and total bacteria showed much stronger tillage effects in the organic than in the integrated farming system. All functional PLFA groups were significantly correlated with MBC and total PLFA, ranging from 0.62 to 0.99 (Table 3). The strongest correlations were found for Gram (+) PLFA and the lowest for the fungal PLFA 18:2ω6,9.

The first principal component (PC) explained 41.7% of the total variance and separated tillage effects (Fig. 4). The bacterial PLFA i15:0, a15:0, i17:0, cy19:0, 16:1ω9 and 16:1ω7c as well as the AMF indicator PLFA 16:1ω5 contributed most to PC1. The second PC explained 33.1% of the total variance and separated management effects. The Gram (−) bacterial PLFA 16:1ω9, 16:1ω7c, and 18:1ω7 as well as the fungal PLFA 18:2ω6c were strongly represented in PC2. The first RDA explained 32.0% and the second 22.3% of the total variance of the PLFA data (Fig. 5). All the explanatory variables retained in the model were significant ($p < 0.05$) in constraining the PLFA data based on permutation tests. Clay content and soil C/N ratio were more strongly associated with the first RDA axis, while SOC, WC, DOC and DON were more strongly associated with the second RDA axis. SOC and WC predominantly affected the microbial community structure. Higher SOC, WC, DOC and C/N was associated with Gram (−) PLFA, 18:2ω6c and 16:1ω5. A high

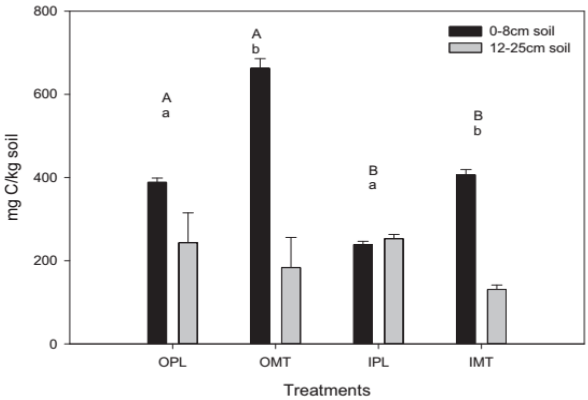

Fig. 1. Mean soil microbial biomass of all treatments at upper and deeper soil of the field trial with two farming (Integrated and Organic farming) and two tillage systems (ploughing and minimum tillage) (O: organic farming; I: integrated farming; PL: plough tillage; MT: minimum tillage). The different uppercase and lowercase letters indicate a significant difference between farming or tillage treatments within the same farming system at each soil depth, respectively. Data are shown as mean +SD (n = 3).

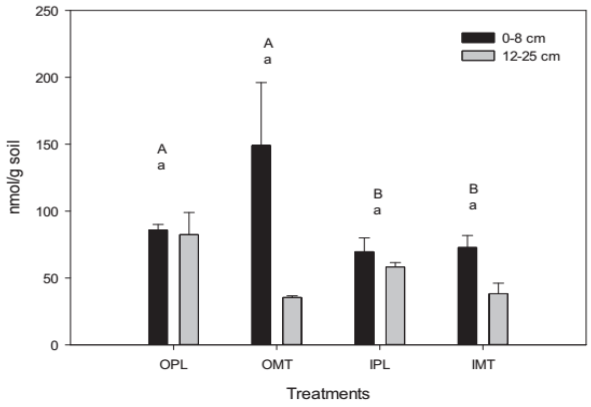

Fig. 2. Mean soil total PLFA of all treatments at upper and deeper soil in the field trial with two farming (Integrated and Organic farming) and two tillage systems (ploughing and minimum tillage) (O: organic farming; I: integrated farming; PL: plough tillage; MT: minimum tillage). The different uppercase and lowercase letters indicate a significant difference between farming or tillage treatments within the same farming system at each soil depth, respectively. Data are shown as mean +SD (n = 3).

DON content was associated with the Gram (−) PLFA 18:1ω7, the clay content was associated with the Gram (−) PLFA 16:1ω9.

3.3. Amino sugars and glomalin

The contents of MurN, GalN, fungal GlcN, and total glomalin were higher in the organic than in the integrated farming system (Table 4), whereas the farming system did not affect the contents of easily extractable glomalin and microbial residue C or the fungal C/bacterial C ratio. Minimum tillage significantly increased the microbial residue C content, but decreased that of easily extractable glomalin in comparison with plough tillage. The contents of amino sugars and total glomalin as well as the fungal C/bacterial C did not differ between minimum and plough tillage. The contents of MurN, microbial residue C, total and easily extractable glomalin significantly declined with depth, those of GalN and fungal GlcN remained unaffected, whereas the fungal C/bacterial C ratio significantly increased with depth. The significant tillage × farming interactions of total glomalin were caused by the less strong tillage effects in the organic in comparison with the integrated farming system.

4. Discussion

4.1. Management effects

Organic farming had positive effects on all microbial biomass and PLFA indices, with specific positive effects on the presence of Gram (+) bacteria and the AMF indicator PLFA 16:1ω5. This is in line with previous studies, which reported that organic farming systems increase the soil microbial biomass (Mäder et al., 2002; Esperschütz et al., 2007; Bausenwein et al., 2008; Heinze et al., 2010a), especially N storage within the microbial biomass (Joergensen et al., 2010). This can be explained by the input of cattle manure, which supplies additional available organic matter to soil as well as cattle manure-derived microorganisms (Widmer et al., 2006; Jost et al., 2011; Sradnick et al., 2014). Moreover, the diverse crop rotation in organic farming, especially the inclusion of legume pre-crops and the lay phase, supplies more high quality organic inputs with low C/N and lignin to soil and also supports microbial development (Lupwayi et al., 1999; Grayston et al., 2001; Oehl et al., 2003, 2004; Bending et al., 2004; Six et al., 2006).

The AMF specific PLFA 16:1ω5 was increased significantly in the current organic farming system in comparison with integrated farming. As nutrients in organic fertilizer are less available to plants than those in mineral fertilizers, crops rely to a much larger extent on AMF (Bossio et al., 1998; Moeskops et al., 2010), and the importance of AMF in unfertilized control treatments is even stronger than treatments with manure application (Zhang et al., 2012a, 2012b). Also the colonization potential of AMF is higher in organic than in conventional farming systems (Mäder et al., 2002). Another reason is that OF increases the AMF inoculum potential in the soil (Oehl et al., 2003, 2004; Gosling et al., 2006).

Gram (+) bacteria tends to use labile organic carbon in comparison to Gram (−) bacteria (Zhang et al., 2015). It has been repeatedly reported that cattle manure specifically increases the abundance of Gram(+) bacteria (Birkhofer et al., 2008; Ngosong et al., 2010; Ai et al., 2012; Zhang et al., 2015), due to their high

Table 2
Mean contents of microbial lipid groups and ratios of specific PLFAs in main factor treatment in a long-term field trial. Two-way ANOVA with repeated measures (Farming, Tillage).

Main effects	Bacterial PLFA	Gram(+) PLFA	Gram(−) PLFA	Gram(+)/ Gram(−)	(i15:0 + i17:0)/ (a15:0 + a17:0)	18:2ω6,9	16:1ω5
	(nmol g^{-1} soil)					(nmol g^{-1} soil)	
Organic Farming	42 ± 5a	17 ± 2a	25 ± 5a	0.72 ± 0.12a	2.2 ± 0.1a	0.57 ± 0.16a	3.9 ± 1.1a
Integrated	30 ± 4b	11 ± 1b	19 ± 3b	0.59 ± 0.09b	2.3 ± 0.1b	0.57 ± 0.16a	2.2 ± 0.6b
Minimum tillage	35 ± 5a	14 ± 2a	22 ± 4a	0.67 ± 0.11a	2.4 ± 0.1a	0.85 ± 0.24a	3.1 ± 0.9a
Plough	37 ± 5a	14 ± 2a	23 ± 4a	0.64 ± 0.10a	2.1 ± 0.1b	0.30 ± 0.08b	3.0 ± 0.9a
0–8 cm depth	45 ± 6a	17 ± 2a	28 ± 5a	0.65 ± 0.10a	2.1 ± 0.1a	0.95 ± 0.27a	3.9 ± 1.1a
12–25 cm	27 ± 4b	10 ± 1b	17 ± 3b	0.66 ± 0.11a	2.4 ± 0.1b	0.19 ± 0.05b	2.3 ± 0.7b
Probability values							
Farming	<0.01	<0.01	0.03	0.03	0.06	NS	0.01
Tillage	NS	NS	NS	NS	<0.01	<0.01	NS
Depth	<0.01	<0.01	<0.01	NS	<0.01	<0.01	0.02
Farming × tillage	NS	0.02	NS	NS	0.01	NS	NS
Farming × depth	0.03	<0.01	NS	NS	0.06	NS	0.10
Tillage × depth	<0.01	<0.01	<0.01	0.08	<0.01	<0.01	0.01
CV (±%)	13	11	18	6	3	29	28

CV = mean coefficient of variation between replicate plots (n = 6). NS: not significant. The different letters following the mean indicate a significant difference within same factor.

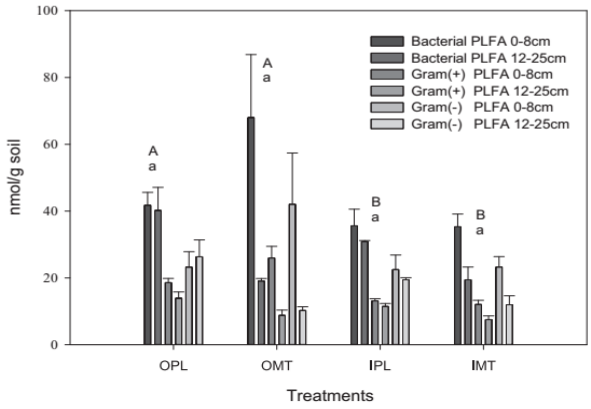

Fig. 3. Mean soil bacterial PLFAs of all treatments at upper and deeper soil of the field trial with two farming (Integrated and Organic farming) and two tillage systems (ploughing and minimum tillage) (O: organic farming; I: integrated farming; PL: plough tillage; MT: minimum tillage). The different uppercase and lowercase letters indicate a significant difference between farming or tillage treatments within same farming system at each soil depth, respectively. Data are shown as mean +SD (n = 3).

Table 3
Correlation between soil microbial properties.

	Microbial biomass C	Total PLFA
Total PLFA	0.86	
Bacterial PLFA	0.86	0.99
Gram(+)PLFA	0.88	0.94
Gram(−)PLFA	0.81	0.98
18:2ω6,9	0.75	0.62
16:1ω5	0.75	0.96

All correlations are significant at the 0.01 level (2-tailed).

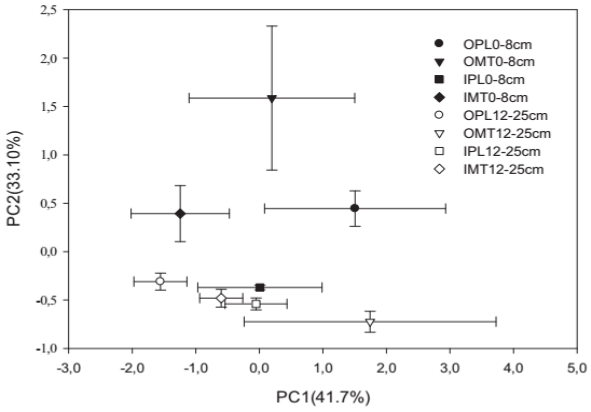

Fig. 4. Principal Component analysis of soil PLFA profile of all treatments in the field trial with two farming (Integrated and Organic farming) and two tillage systems (ploughing and minimum tillage) (O: organic farming; I: integrated farming; PL: plough tillage; MT: minimum tillage). Data are shown as mean +SD (n = 3).

presence in cattle manure, which provides a large amount of labile organic carbon (Frostegård and Bååth, 1996; Gattinger et al., 2007). The increase in SOC content in the organic farming system enhances also the soil moisture content, which may additionally promote Gram(+) bacteria (Steinberger et al., 1999; Ma et al., 2015).

The increased presence of Gram (+) bacteria is reflected by the increased content of MurN, i.e. an increased formation of bacterial residues in the long-term. Gram (+) bacteria contain on average 3.7 times more MurN than Gram (−) (Appuhn and Joergensen, 2006). Strong positive effects of cattle manure application on the MurN content have been observed in the DOK trial in Switzerland (Joergensen et al., 2010), in the Darmstadt long-term fertilization trial (Sradnick et al., 2014), but also in a long-term experiment on a tropical rice field in India (Murugan and Kumar, 2013). However, in all three experiments, these results were not directly supported by Gram (+) PLFA data. The manure effects on MurN observed by Ding et al. (2013a, 2013b, 2015) were less clear, presumably due to the combined application of manure with mineral fertilizer and the considerably shorter experimental period of 10 and 12 years, respectively.

4.2. Tillage effects

Minimum tillage generally resulted in a negative depth gradient of almost all microbial properties analyzed. This stratification of microbial properties has been often observed by others (Bausenwein et al., 2008; Helgason et al., 2009; Zhang et al., 2012a, 2012b; Kuntz et al., 2013). Specifically, minimum tillage leaves large amounts of crop residues and organic fertilizers in the upper soil, whereas ploughing tillage distributes these organic amendments uniformly throughout the plow layer (Chivenge et al., 2007). For this reason, minimum tillage strongly promotes saprotrophic fungi in the upper soil of the current long-term field experiment, as indicated by the disproportionately positive main effects on the fungal PLFA 18:2ω6,9, averaging the values of upper and deeper soil. Another factor that promotes fungi in minimum tillage may be the increased soil moisture, since fungi positively respond to increases in soil water holding capacity (Frey et al., 1999). Soil moisture was the major factor influencing microbial community structure across seven biogeoclimatic zones in western Canada (Brockett et al., 2012). The minimum tillage treatment increased the PLFA ratio of (i15:0 + i17:0)/(a15:0 + a17:0), which has been used as an indicator of physiological stress to bacteria (Zhang et al., 2014). This stress might be caused by competition between saprotrophic fungi and bacteria for organic substrate and nutrients in the upper soil.

The minimum tillage treatment had no positive effects on AMF indicator PLFA 16:1ω5, contrasting the view stated by others that minimum tillage promotes AMF (Heinze et al., 2010a, 2010b; Murugan et al., 2014). Fewer disturbances by minimum tillage is usually more favorable than soil turning ploughing for the fine hyphal AMF networks (Klein and Paschke, 2004). One reason for the current results might be that the positive effects of cattle manure application blur the positive effects of minimum tillage. Another reason might be the interference of Gram (+) bacteria derived from cattle manure, which contain PLFA 16:1ω5 (Zelles, 1997). However, the relevance of this interference is a still a subject of considerable debate (Joergensen and Wichern, 2008; Frostegard et al., 2011), as little data is available on the occurrence of the PLFA 16:1ω5 in Gram (+) bacterial species.

Minimum tillage increased not only the fungal PLFA 18:2ω6,9 but also microbial biomass N. This suggests a close relationship between saprotrophic fungi and microbial N storage, clearly contrasting the relationship between bacteria and MBN in organic farming systems (Tables 1 and 2). This means that the MB-C/N ratio responds in complex patterns to the microbial community

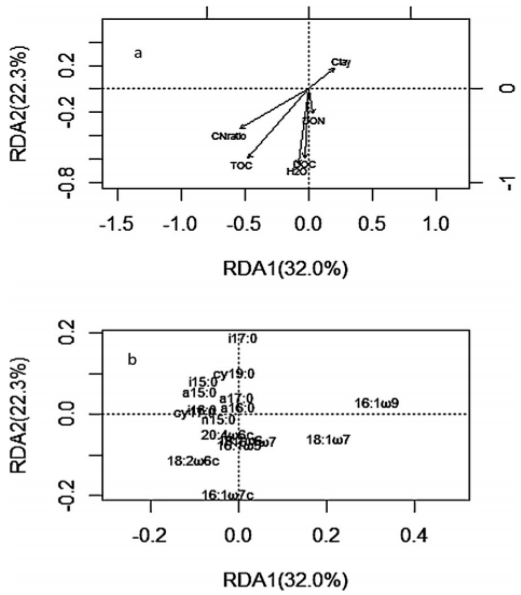

Fig. 5. Redundancy analysis of soil PLFA profile of all treatments in the field trial with two farming (Integrated and Organic farming) and two tillage systems (ploughing and minimum tillage). (a: the figure of constrained factors, b: the pattern of PLFA profile).

structure and nutrient availability and should not be used as an indicator of change of relative abundance of fungi and bacteria.

Positive minimum tillage effects on microbial biomass and PLFA indices were much stronger in the organic than in the integrated farming system. This indicates the additive benefits of both management factors to the living fraction. In contrast, tillage and management effects on microbial residues were small, indicating a slow response of this important SOC fraction, in line with van Groenigen et al. (2010). However, the absence of tillage effects on fungal GlcN led to a strong and significant increase in the fungal C/bacterial C ratio and in the contribution of microbial residue C to SOC in the deeper soil. The increase in fungal C/bacterial C might be caused by an increased turnover of bacterial residues, as suggested by others (Guggenberger et al., 1999; Roth et al., 2011). However, the strong increase in the contribution of microbial residue C to SOC with depth is certainly due to an increased turnover of plant residues in the deeper soil (Six et al., 2006; Jacobs et al., 2011), which receives less fresh plant residues in the minimum tillage treatment.

4.3. Interaction of management and tillage

The interaction effect between management and tillage on SOC, MBC, MBN and abundance of Gram (+) bacteria was significant (Tables 1 and 2). These results suggest that minimum tillage with organic farming would result in significantly higher SOC content, microbial biomass and abundance of Gram (+) bacteria than any other management and tillage combinations in the current study. These partially support our third hypothesis and agree with Kuntz et al. (2013) that reduced tillage enhances inherent soil biota under organic arable farming. The single tillage effect on Gram (+) bacteria was insignificant, but its significance became apparent after interaction with management factor. This indicates that the tillage effect was modified by management system. Thus, organic farming in combination with minimum tillage appear to be an effective cropping system management strategy that enhances soil organic matter and soil microbial biomass and promotes a more abundant microbial community.

5. Conclusions

In the present long-term experiment, our results showed that both organic farming and minimum tillage had positive effects on microbial biomass and the microbial community. Moreover, interaction effects of organic farming and minimum tillage occurred on microbial biomass and Gram (+) bacteria. Microbial residues and microbial guilds respond to organic farming and minimum tillage in various ways. Microbial residues were more sensitive to organic farming rather than minimum tillage. Bacterial guilds and AMF were more influenced by organic farming, whereas saprotrophic fungi were more sensitive to tillage. Moreover, minimum tillage effects on microbial biomass and PLFA indices

Table 4
Mean content of amino sugars, microbial residue C and glomalin in main factor treatment in a long-term field trial. Two-way ANOVA with repeated measures (Farming, Tillage).

			Fungal		Microbial	Glomalin	
	MurN	GalN	GlcN	Fungal C/	residue C	Total	EE
Main effects	($\mu g\,g^{-1}$ soil)			bacterial C	(% SOC)	($mg\,g^{-1}$ soil)	
Organic Farming	63 ± 11a	438 ± 74a	841 ± 151a	2.5 ± 0.2a	69 ± 11a	2.4 ± 0.1a	1.3 ± 0.2a
Integrated	48 ± 8b	366 ± 62b	671 ± 121b	2.5 ± 0.2a	65 ± 10a	2.2 ± 0.1b	1.4 ± 0.2a
Minimum tillage	60 ± 10a	425 ± 72a	816 ± 147a	2.5 ± 0.2a	75 ± 12a	2.3 ± 0.1a	1.2 ± 0.2a
Plough	50 ± 9a	379 ± 64a	697 ± 125a	2.4 ± 0.2a	59 ± 9b	2.4 ± 0.1a	1.5 ± 0.2b
0–8 cm depth	62 ± 11a	417 ± 71a	782 ± 141a	2.2 ± 0.2a	58 ± 9a	2.7 ± 0.1a	1.5 ± 0.2a
12–25 cm	49 ± 8b	387 ± 66a	731 ± 132a	2.7 ± 0.2b	76 ± 12b	1.9 ± 0.1b	1.1 ± 0.2b
Probability values							
Farming	0.03	0.08	0.06	NS	NS	<0.01	NS
Tillage	NS	NS	NS	NS	0.05	NS	0.02
Depth	0.04	NS	NS	<0.01	0.03	<0.01	<0.01
Farming × tillage	NS	NS	NS	NS	NS	<0.01	NS
Farming × depth	NS	NS	NS	0.01	NS	0.05	NS
Tillage × depth	<0.01	<0.01	<0.01	NS	NS	<0.01	NS
CV (±%)	17	17	18	7	16	5	15

CV = mean coefficient of variation between replicate plots (n = 6); EE = easily extractable; NS: not significant. The different letters following the mean indicate a significant difference within same factor.

were much stronger in the organic than in the integrated farming system. The combination of organic farming and minimum tillage appears to be an effective cropping system management strategy that enhances soil organic matter and soil microbial biomass and promotes a more abundant microbial community. The results indicate that the positive effects of minimum tillage on the microbial community can be enhanced by organic farming. Microbial residues as a fraction of SOC respond faster to farming management than to tillage.

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Appendix A. Supplementary data

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3.5 Effect of robinia-based alley cropping and low-input organic farming on soil microbial communities and microbial residues

This paper reports how soil microbial communities and microbial residues respond to tree species and organic farming after a short introduction of poplar and robinia-based alley cropping to long-term organically and integratedly managed systems. Same soil microbial properties were analyzed as those of tillage trial systems. The results showed the hedgerow tree did not show significantly positive effect on soil organic carbon and microbial properties except the abundance of fungi and Gram (+) bacteria. Tree species-specific effect on microbial community was stronger in the organic farming than that in the integrated farming. The short-term introduction of trees into existing agricultural system will not substantially change the microbial biomass but it has certain influence on the abundance of specific microbial groups in the hedgerow. Although low-input organic farming did not show positive effect on overall microbial indices, we still see positive effect on soil organic carbon (SOC) after 21 years organic farming and its additive effect with robinia on SOC. It is expected that alley-cropping agroforestry system that combine organic farming and robinia hedgerow has a great potential for sequestering SOC and developing sustainable agroecosystems.

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Microbial communities and residues in robinia- and poplar-based alley-cropping systems under organic and integrated management

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Abstract Organic farming and agroforestry are considered as sustainable alternative agricultural practices for intensive agriculture. In a long-term field trial in Scheyern Germany, we evaluated the effects of 21-year organic farming and 4-year agroforestry (robinia and poplar) on microbial community and microbial residues. Microbial biomass and microbial community were determined by fumigation–extraction method and the analysis of phospholipid fatty acid (PLFA), respectively. Microbial residues were evaluated by the measurement of amino sugars. The results showed that organic farming had significantly positive effect on soil organic carbon (SOC) but that it tended to decrease microbial biomass C (MBC), PLFA functional guilds, muramic acid (MurN), and glucosamine (GlcN). Robinia system, however, significantly increased SOC and had the potential to enhance MBC, PLFA functional guilds especially Gram (+), but it tended to decrease MurN and GlcN, in comparison with poplar system. The hedgerow tree did not show significantly positive effect on SOC and microbial properties except the abundance of fungi and Gram (+) bacterial, after 4-year establishment period. The principal component analysis of the PLFA profile showed that in comparison with other investigated treatments, robinia system under organic farming had significantly a different microbial community structure. It also indicated tree species-specific effect on microbial community in the organic farming was stronger than that in the integrated farming. In summary, the short-term introduction of trees into an existing agricultural system will not substantially change the microbial biomass, but it has certain

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influence on the abundance of specific microbial groups in the hedgerow. Although organic farming did not show positive effect on overall microbial indices, we still see positive effect on SOC after 21-year organic farming and its additive effect with robinia on SOC in current study. We expect that alley-cropping agroforestry system that combines organic farming and robinia hedgerow has a great potential for sequestering SOC and developing sustainable agroecosystems with time.

Keywords Organic farming · Agroforestry · Poplar · Robinia · Microbial community · Microbial residue

Introduction

Soil microorganisms are important drivers for a variety of soil processes and for the sustainable soil–plant systems (Thoms and Gleixner 2013). Both microbial biomass and residues are involved in the degradation and stabilization of soil organic carbon (SOC) (Ludwig et al. 2015).

Agroforestry systems, integrating trees and crops, are recognized as the integrated approaches for sustainable land use in the recent decades (Lorenz and Lal 2014). It has been repeatedly reported that agroforestry promotes SOC sequestration (Nair et al. 2010) and that it improves soil's physical (Udawatta et al. 2009), chemical (Zaia et al. 2012), and biological qualities (Kaur et al. 2000). These effects are more significant in tree strips than in the alley-cropping area, although the differences regarding microbial biomass, microbial diversity as well as enzyme activities were not always observed (Bardhan et al. 2013; Udawatta et al. 2014). It is also recognized that carbon sequestration potential varies from trees, especially broadleaf trees with deep and extensive root systems and high belowground to aboveground biomass ratios may enhance the potential for C sequestration (Lorenz and Lal 2014), and furthermore, tree species also affect soil microbial community (Thoms et al. 2010). Liang et al. (2007) observed significant tree species-specific effects on microbial residues in an upper Michigan old-growth forest system, whereas Medinski et al. (2015) reported no remarkably different effects on microbial biomass and activity was observed between poplar and robinia areas in a German alley-cropping system. These contradictory results indicate that there is a lack of our understanding of tree species' effects on microbial properties in agroforestry systems at the moment.

The benefits of organic farming on soil carbon sequestration, the biomass, and the diversity of soil microorganisms have been frequently reported (Esperschütz et al. 2007; Gattinger et al. 2012), and a more diverse crop rotation strengthens nutrient cycling in organic farming (Mäder et al. 2002). However, whether the combination of organic farming and agroforestry could produce an additive positive impact still remains unclear. The analysis of phospholipid fatty acids (PLFA) reveals additional information on the biomass of functional microbial guilds (Gattinger et al. 2002), such as Gram (+) and Gram (−) bacteria, and arbuscular mycorrhiza fungi (AMF). The amino sugars, muramic acid and glucosamine, are highly specific for bacterial and fungal residues, respectively (Joergensen and Wichern 2008). Produced by AMF (Burrows 2014), the glomalin-related soil protein is another important microbial residue, which is closely related to SOC sequestration and to aggregate stabilization (Rillig et al. 2002). The current agroforestry trial offers the unique opportunity to investigate the effects of tree species (poplar and robinia) and organic farming on soil microbial community and microbial residues. The following hypotheses can be tested: (1) Organic farming increases SOC, soil microbial biomass, microbial functional guilds as well as microbial residues. (2) N_2-fixing robinia has stronger positive effects on microbial community than the poplar system, especially when it is close to the hedgerow.

Materials and methods

Field experiment

The field experiments have been carried out at the Scheyern Research Farm (TERENO site) located 40 km north of Munich, Germany (48.50°N, 11.45°E) since 1992. The altitude of the farm ranges between 445 and 500 m a.s.l. The mean annual precipitation is 803 mm, and the mean annual temperature is 7.4 °C (Schröder et al. 2002). The central part of the research station was divided into two parts: organic and integrated farming system, each striving

for ecological and economic sustainability. The soil types are sandy-to-loamy Cambisols, derived from tertiary sediments and partly covered by loess (Koelbl and Kögel-Knabner 2004). The soils of organic and integrated field for present study are similar and have a soil texture of silty loam (USDA). The soil texture of top soil is 27 % sand, 54 % silt, and 19 % clay for the organic field; and 27 % sand, 50 % silt, and 23 % clay for the integrated field.

In 2009, agroforestry parcels were incorporated into both integrated and organic farming systems. Three swaths of trees, each comprising varieties of species, were planted in an alley cropping for the purpose of bioenergy production (30-m length for each tree species), and 30-m-wide arable soil was left for crop production (Fig. 1).

The poplar (*Populus maximowiczii x P. nigra*) and Robinia (*Robinia pseudoacacia* L.) alley systems were chosen for this study in both organic and integrated farming systems. The experiment consisted of four treatments with three replications: organic farming + poplar (O-pop), organic farming + robinia (O-rob), integrated farming + poplar (I-pop), and integrated farming + robinia (I-rob). The plot for each treatment is 30 × 30 m in size. The treed portions of both systems were not treated with fertilizer, either in manure or mineral form. Neither did the portions and the received weed control mechanically or via pesticide. The tree densities of poplar and robinia are the same. The organic farming system is of low-input type, utilizing nitrogen fixing cover crops instead of mineral nitrogen or synthetic inputs as green manures. Also no pesticide or herbicide was applied. Soils were plowed with moldboard. A seven-field crop rotation was run in the organic farming system: (1) Grass–clover–alfalfa (GCA) (*Lolium perenne* L. + *Trifolium pratense* L. + *Medicago sativa* L.), (2) potatoes (*Solanum tuberosum* L.) + mustard (*Sinapis alba L.)* as cover crop, (3) winter wheat (*Triticum aestivum* L.), (4) sunflower (*Helianthus annuus* L.) + GCA as cover crop, (5) GCA, (6) winter wheat, and (7) winter rye (*Secale cereale* L.) + GCA as cover crop. In the integrated farming system, soils were tilled by harrowing and chiseling, and the tillage intensity was reduced to a level to control weed as well as to conserve soil. Pesticides were completely forbidden in organic farming system, while in case of necessity, they were applied in the integrated farming system. A four-field crop rotation with cover crops was run in the integrated farming system: (1) winter wheat; (2) potatoes; (3) winter wheat; and (4) maize (*Zea mays* L.).

Sampling and soil properties

Sampling plots were randomly set in the tree row (0 m), transition area (2 m from the tree row: 2 m), and middle of crop area (15 m from the tree row: 15 m) in each treatment (Fig. 1). In May 2013 before

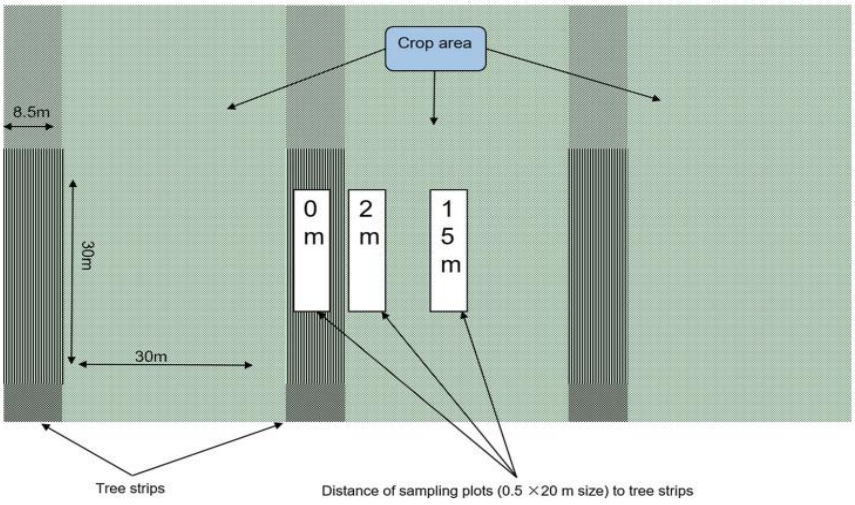

Fig. 1 Experimental layout of alley-cropping agroforestry systems and sampling sites (0, 2, and 15 m)

vegetation development but after a long cold winter, three replicate composite samples consisting of 20 augers (5-cm diameter) subsamples were collected at depths in the range of 0–25 cm in each sampling plot (0.5 m × 20 m). SOC and total N were determined by a CN analyzer (EA3000 Eurovector) with an aliquot of air-dried soil samples.

Microbial biomass and phospholipid fatty acid profile

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined using the chloroform fumigation–extraction method (Vance et al. 1987). In brief, 5 g each of oven-dried moist soil samples was fumigated for 24 h with $CHCl_3$, and then a 5 g fumigated sample and a separate 5 g nonfumigated sample were extracted with 20 ml 0.01 M $CaCl_2$ solution (Joergensen 1995). Organic C and total N in the extracts were quantified using a TOC/TNb analyzer (Dimatec, Essen, Germany). MBC and MBN contents were calculated using a k_{EC} value of 0.45 (Joergensen 1996) and a k_{EN} value of 0.54 (Joergensen and Mueller 1996), respectively.

PLFAs were determined according to Zelles and Bai (1993) and Gattinger et al. (2002). In brief, a fresh soil sample equivalent to 15 g dry soil was extracted in accordance with the modified Bligh Dyer method (methanol, chloroform, and phosphate buffer mixture at a 1:2:0.8 volume ratio). The extracts were fractionated into neutral lipids, glycolipids, and phospholipids on a SI column (SPE-SI; Bond Elute, Varian, Palo Alto, USA) by chloroform, acetone, and methanol as elution liquids, respectively. The phospholipid was subjected to the mild alkaline methanolysis to get fatty acid methyl esters (FAMEs), and a SCX column impregnated with silver nitrate was applied to fractionate the unsubstituted FAMEs into saturated, monounsaturated, and poly-unsaturated FAMEs and abbreviated as SATFA, MUFA, and PUFA, respectively (Zelles 1999). The SATFA and PUFA fractions were measured directly using GC/MS with nonadecylacidmethylester as internal standard solution, whereas MUFA fraction was measured after dimethyl disulfide (DMDS) derivatization, as per the GC/MS operating condition given in Zelles and Bai (1993). The identification of individual compounds was performed by the comparison of retention time and mass spectral data obtained from standard compounds, monocultures, and environmental samples (Zelles 1999). The quantification was achieved using chromatography software (HP ChemStation, SOVLVIT, CH) by considering the ratio of target compound and the internal standard with respect to peak areas, as well as the response for each, which was estimated experimentally with standard mixtures (Gattinger et al. 2002). The PLFAs, n15:0, i15:0, a15:0, i16:0, i17:0, a17:0, were used as biomarkers for Gram(+) bacteria (Frostegård and Bååth 1996; Zelles 1997); the PLFAs 16:1ω7t, 16:1ω7c, 16:1ω9, 16:1ω6, 18:1ω7, cy17:0, and cy19:0 for Gram (−) bacteria (Colaco et al. 2007; Frostegård and Bååth 1996; Zelles 1997); 18:2ω6,9 for fungi (Joergensen and Wichern 2008); 16:1ω5 for arbuscular mycorrhizal fungi (AMF) (Olsson et al. 1995). Ratios of (i15:0 + i17:0)/(a15:0 + a17:0) were used as index for nutritional or environmental stress on Gram (+) bacteria.

Amino sugars

The amino sugars muramic acid (MurN), galactosamine (GalN), and glucosamine (GlcN) were determined according to Appuhn et al. (2004) as described by Indorf et al. (2011). Moist soil samples each weighing 0.5 g were collected, mixed with 10 ml 6 M HCl, and hydrolyzed for 6 h at 105 °C. HCl was removed by rotary evaporator, and 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 C18 column (125-mm length × 4-mm diameter), protected by a Phenomenex C18 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 derivatization, 50 µl ortho-phthaldialdehyde (OPA) and 30 µl sample were mixed in the preparation vial, and after 120-s reaction time, 15 µl of the indole derivatives was 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).

Fungal C was estimated by multiplying the content of fungal GlcN by 9 (Appuhn and Joergensen 2006). Fungal GlcN was calculated by subtracting bacterial GlcN from total GlcN, assuming that MurN and GlcN occur at a 1:2 molar ratio in bacteria (Engelking et al. 2007). Bacterial C was calculated by multiplying the content of MurN by 45 (Appuhn and Joergensen 2006).

Glomalin

Replicate 0.25 g samples of dry-sieved 1–2-mm aggregates were extracted with 2 ml of extractant. Easily extractable glomalin (EEG) was extracted with 20 mM citrate (pH 7.0) at 121 °C for 30 min. Total glomalin (TG) was extracted with 50 mM citrate (pH 8.0) at 121 °C. For sequential extractions, the supernatant was removed by centrifugation at 10,000 g for 5 min, and 2 mL of 50 mM citrate, pH 8.0 was added to the residue, and then the samples were autoclaved for 60 min. Extraction of the sample continued until the red-brown color typical of glomalin entirely vanished from the supernatant. Extracts from each replicate were pooled and then analyzed. After the extraction cycles were completed, samples were centrifuged to remove the soil particles (10,000 g for 5 min), and protein in the supernatant was determined by the Bradford dye-binding assay [Bradford assay (Sigma-Aldrich Inc.)] with bovine serum albumin as the standard (Wright and Upadhyaya 1996, 1998).

Statistical analysis

A two-way repeated measure ANOVA analysis was conducted to test the effects of organic farming and tree species on soil microbial indices. Principal component analysis (PCA) was carried out to analyze soil microbial community based on a covariance matrix (Thoms et al. 2010). PLFA data were Helliger transformed prior to being subjected to PCA (Andersen et al. 2010). All statistical analysis procedures were conducted using the vegan package (Oksanen et al. 2015) based on the R program (R Core Team 2015).

Results

Microbial biomass and total PLFA

Means of MBC, MBN, and total PLFA contents as well as the contributions of MBC to SOC and MBN to total N were significantly lower in the organic than in the integrated farming system, whereas the SOC content was significantly higher (Table 1). The MB-C/N ratio remained unaffected. Tree species had little effect on MBC, MBN, total PLFA contents as well as the MB-C/N ratio and the contribution of MBN to total N (Table 1). The mean SOC content was significantly higher in the robinia than in the poplar system, whereas the contribution of MBC to SOC was lower (Table 1). In contrast, MBC and MBN were the only microbial biomass indices that differed between sampling distances. The means of MBC and MBN contents were the highest at 15-m distance in comparison with 0- and 2-m distances, especially in the robinia system, leading to significant tree species × sampling distance interaction (Table 1; Fig. 2).

Microbial lipid groups and microbial community structure

The mean signature PLFA contents for Gram (−) bacteria and for saprotrophic fungi content were significantly lower in the organic farming than in the integrated farming system, whereas the (i15:0 + i17:0)/(a15:0 + a17:0) ratio was higher (Table 2). The other microbial groups remained unaffected by the organic farming, in comparison with the integrated farming system. The mean signature PLFA contents for Gram (+) as well as (i15:0 + i17:0)/(a15:0 + a17:0) ratio were significantly higher in the robinia system than those in the poplar system (Table 2). Total bacterial PLFA, Gram (−) bacterial PLFA, 18:2ω6,9, as well as 16:1ω5 remained unaffected by tree species. Gram (+) bacterial PLFA and 18:2ω6,9 were the only functional PLFA guilds, which were significantly affected by the sampling distance, i.e., Gram (+) bacterial PLFA contents were the lowest at 2 m, and 18:2ω6,9 declined in the order 0 m > 2 m > 15 m distance.

All the functional PLFA groups were significantly correlated with MBC and total PLFA, except total PLFA and PLFA 18:2ω6,9 (Table 3). The first two

Table 1 Contents of soil organic carbon (SOC), microbial biomass, and total PLFA of all treatments associated with organic and integrated farming (21-year) and tree species (4-year)

Main effects	SOC (mg g^{-1} soil)	Microbial biomass					Total PLFA
		C (μg g^{-1} soil)	N	C/N	C (% SOC)	N (% total N)	(nmol g^{-1} soil)
Organic farming	13.0	168	19	9.8	1.3	1.3	70
Integrated	12.3	222	24	9.4	1.8	1.8	77
Poplar	11.3	193	21	9.6	1.7	1.6	71
Robinia	14.0	197	22	9.6	1.4	1.5	76
0 m distance	12.5	189	20	9.6	1.5	1.5	75
2 m	12.1	184	19	10.7	1.6	1.4	70
15 m	13.3	212	26	8.4	1.6	1.8	75
Probability values							
Farming	<0.01	0.01	0.08	NS	<0.01	0.01	0.08
Tree species	<0.01	NS	NS	NS	0.03	NS	NS
Sampling distance	<0.01	0.04	0.01	NS	NS	NS	NS
Farming × tree	<0.01	NS	NS	NS	0.08	NS	NS
Farming × distance	<0.01	NS	NS	NS	NS	0.02	NS
Tree × distance	0.02	0.02	0.04	NS	NS	NS	0.1
CV (±%)	2	12	20	13	13	24	11

CV mean coefficient of variation between replicate plots

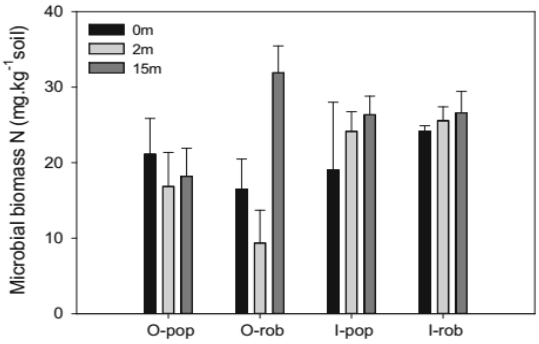

Fig. 2 The mean microbial biomass N contents of all treatments in the field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites ($n = 3$) (O-pop, organic farming + poplar; O-rob, organic farming + robinia; I-pop, integrated farming + poplar; I-rob, integrated farming + robinia)

components of PCA explained 81.4 % of the total variance of soil PLFA profile, i.e., the first component accounted for 56.3 % and the second component explained 25.1 %. The organic farming with robinia system was clearly separated from the other investigated systems by PC2. The poplar system was clearly separated from the robinia system only the in organic farming system (Fig. 3).

Amino sugars and glomalin

The mean GalN content was significantly higher in the organic farming than that in the integrated farming system, whereas easily extractable glomalin of organic farming was lower (Table 4). The other microbial residue indices remained unaffected by farming practices, although organic farming tended to decrease MurN and GlcN. In contrast, tree species had little effect on the mean contents of individual amino sugar although the robinia system tended to decrease MurN and GlcN (Table 4). This led to a significantly lower average fungal C-to-bacterial C ratio in robinia system regardless of farming method, and the fungal C-to-bacterial C ratio in robinia system was lower in the integrated farming system (Table 4; Fig. 4). The mean total glomalin content was significantly higher in the robinia than that in the poplar system, especially in the organic farming system, leading to a significant farming × tree interaction (Table 4; Fig. 5). The

Table 2 Contents of signature PLFAs of bacteria, fungi, and ratios of signature PLFAs of all treatments associated with organic and integrated farming (21-year) and tree species (4-year)

Main effects	Bacterial PLFA (nmol g^{-1} soil)	Gram (+) PLFA	Gram (−) PLFA	Gram (+)/Gram(−)	(i15 + i17)/(a15 + a17)	18:2ω6,9 (nmol g^{-1} soil)	16:1ω5
Organic farming	34	12	22	0.53	2.32	0.81	3.2
Integrated	37	12	25	0.49	2.26	1.29	3.5
Poplar	34	11	23	0.49	2.25	1.15	3.3
Robinia	37	12	24	0.53	2.33	0.95	3.4
0 m distance	36	12	24	0.52	2.33	1.30	3.3
2 m	34	11	23	0.47	2.26	1.00	3.3
15 m	36	12	24	0.53	2.28	0.87	3.5
Probability values							
Farming	NS	NS	0.08	NS	0.08	<0.01	NS
Tree species	NS	0.03	NS	NS	0.02	NS	NS
Sampling distance	NS	<0.01	NS	NS	NS	0.01	NS
Farming × tree	NS	0.02	NS	NS	<0.01	NS	NS
Farming × distance	NS	NS	NS	NS	NS	NS	NS
Tree × distance	0.05	NS	0.03	0.02	NS	NS	0.06
CV (±%)	10	8	14	12	3	23	18

CV mean coefficient of variation between replicate plots

Table 3 Correlation between soil microbial properties

	Microbial biomass C	Total PLFA
Total PLFA	0.51**	1
Bacterial PLFA	0.45**	0.99**
Gram (+) PLFA	0.337*	0.66**
Gram (−) PLFA	0.41*	0.96**
18:2w6,9	0.37*	0.06
16:1w5	0.34*	0.88**

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

mean content of easily extractable glomalin remained unaffected by treatment.

Discussion

Organic farming positively influenced the SOC content, which is in agreement with our first hypothesis as well as the previous studies (Marriott and Wander 2006; Gattinger et al. 2012). It can be explained by the inclusion of more diverse legume-based crop rotation in the organic farming when no external C was introduced to the system other than the plant residues after harvest in current study. Marriott and Wander (2006) also found that the legume-based organic management is as capable of building SOM as systems receiving manure or compost even though intensive and frequent cultivation is usually applied in organic farming system. Moreover, the difference of the quality of plant residues might also influence the C level in soil (Fließbach et al. 2007).

In contrast to our first hypothesis and many previous studies (Esperschütz et al. 2007; Joergensen et al. 2011), organic farming had no positive effects on MBC, total PLFA Gram (+) bacterial PLFA, 16:1ω5, and microbial residues in the present study. Since the substrates' supply could influence microbial growth (Kuntz et al. 2013), substrates' limitation in organic farming could be an explanation for current results. Because present organic farming system offers low input, organic materials' degradation is the main source of substrates and N only comes from the legume plants; however, in comparison, integrated farming system received additional nutrients in the

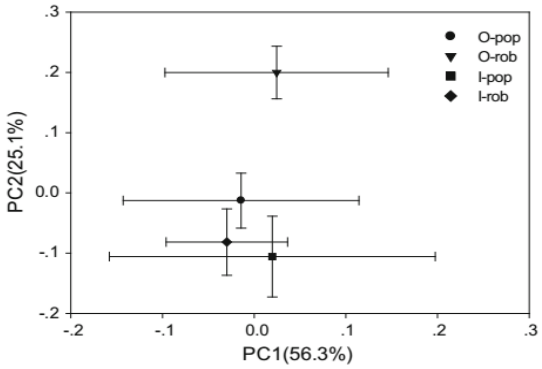

Fig. 3 The principal component analysis for phospholipid fatty acid's profile of samples from all treatments associated with farming (organic and integrated farming) and trees (robinia and poplar) ($n = 3$) (O-pop, organic farming + poplar; O-rob, organic farming + robinia; I-pop, integrated farming + poplar; I-rob, integrated farming + robinia)

Table 4 Contents of amino sugars, microbial residue C, and glomalin of all treatments associated with organic and integrated farming (21-year) and tree species (4-year)

Main effects	Fungal			Fungal C/ bacterial C	Microbial residue C	Glomalin	
	MurN (μg g^{-1} soil)	GalN	GlcN		(% SOC)	Total (mg g^{-1} soil)	EE
Organic farming	40.1	302	617	2.8	54	2.4	1.0
Integrated	42.7	275	658	2.8	59	2.4	1.2
Poplar	41.9	288	672	2.9	65	2.2	1.0
Robinia	41.0	289	604	2.7	48	2.6	1.2
0 m distance	42.6	280	625	2.7	56	2.3	1.0
2 m	39.6	298	634	2.9	58	2.4	1.0
15 m	42.2	287	654	2.8	56	2.5	1.3
Probability values							
Farming	NS	0.02	NS	NS	NS	NS	0.09
Tree species	NS	NS	NS	<0.01	0.02	<0.01	0.08
Sampling distance	NS	NS	NS	NS	NS	0.09	0.09
Farming × tree	NS	<0.01	NS	<0.01	NS	<0.01	NS
Farming × distance	NS	<0.01	0.04	NS	0.02	<0.01	NS
Tree × distance	NS	0.02	NS	NS	NS	0.02	NS
CV (±%)	11	7	11	6	9	6	19

CV mean coefficient of variation between replicate plots, *EE* easily extractable

MurN muramic acid, *GalN* galactosamine, *GlcN* glucosamine

form of synthetic fertilizer and the higher input of harvest residue. This is also supported by the lower mineral nitrogen content and water-soluble organic matter in organic farming in our study (Supplementary Table 1). Nutrient limitation, especially N, decreases microbial growth efficiency (Six et al. 2006), thereby decreasing the microbial biomass. Another reason can be the reduced tillage intensity of the integrated plots,

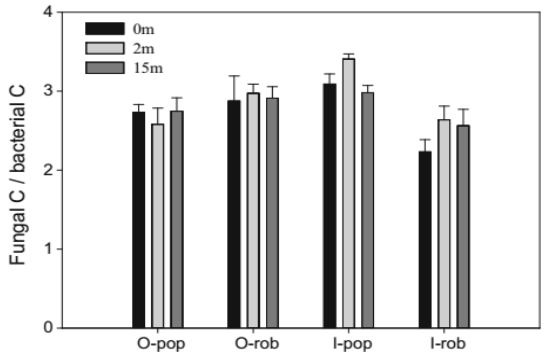

Fig. 4 The ratios of fungal C and bacterial C of all treatments in the field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites ($n = 3$) (O-pop, organic farming + poplar; O-rob, organic farming + robinia; I-pop, integrated farming + poplar; I-rob, integrated farming + robinia)

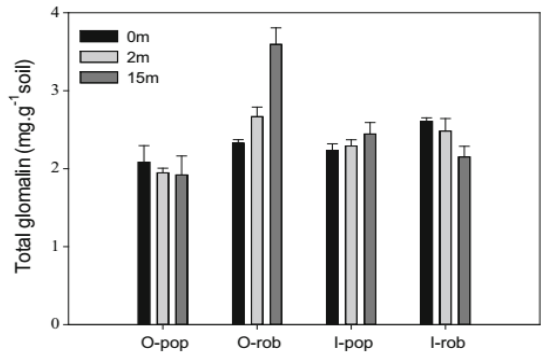

Fig. 5 The mean total glomalin contents of all treatments in the field trial associated with farming (organic and integrated farming) and trees (robinia and poplar) at 3 sampling sites ($n = 3$) (O-pop, organic farming + poplar; O-rob, organic farming + robinia; I-pop, integrated farming + poplar; I-rob, integrated farming + robinia)

leading to the increased SOC contents close to the surface (Murugan et al. 2014), which might be over-represented if the samples were not taken exactly at the 0–25-cm depth. Any positive organic farming effects on soil microorganism might be diluted by the tree row area, since a lower C input in the topsoil and a higher C input into the subsoil occurs in growing forest (Davidson et al. 2002; Raich et al. 2014). This is in line with the lowest MBC contents in and close to the treed area of the current experiment. Moreover, the sampling was performed only shortly after winter snow; therefore, one reason could be assumed that plow tillage in organic farming could increase soil infiltration (Lipiec et al. 2006), resulting in more water from melted snow that stayed in the plow layer and that soil water filled more soil pores, thereby limiting the air supply in soil and leading to reduction in microbial activity and microbial growth in the organic farming system. However, this reason needs to be verified with further comprehensive work. In addition, seasonal variation also cannot be fully excluded according to Monokrousos et al. (2008), who observed in May that the MBC contents were lower under organic farming than those under conventional farming. Consequently, the effects of present farming on soil microbial properties should not be stressed too much.

Robinia system had significantly positive effects on SOC and Gram (+) PLFA in the current study, partly supporting our second hypothesis. Robinia also significantly decreased the fungal C-to-bacterial C ratio, and this might be in line with the reduction in fungal biomass and the increase of bacteria in the presence of legumes (Habekost et al. 2008). Substrate quality affects soil microbial community significantly. In comparison with poplar, the litter quality of robinia is higher, characterized by a lower C/N ratio (Supplementary Table 1), and lower lignin contents in robinia leaf litter and root residues favor the growth of microorganisms (Kaleeem Abbasi et al. 2015). Similar to the application of inorganic nitrogen to soil, addition of N to soil by robinia via its N-fixation capability might result in increased bacteria, especially Gram (+) in current study. Yevdokimov et al. (2012) also reported that moderate addition of nitrogen increased the abundances of Gram (+) and Gram (−) bacteria. The markedly higher contents of glomalin in robinia system and the hedgerow indicate that the robinia hedgerow tree increases the activity of AMF although its effect on the abundance of AMF was comparatively minor, thereby favoring the retention of SOC. From these results, however, we expect that the tree species' effect on microbial community will be of more significance with the growth of trees in the agroforestry system in a long run, since trees affect soil in proportion with their standing age (Gupta et al. 2009).

The hedgerow trees did not generally increase SOC and soil microbial properties, except the growth saprotrophic fungi as indicated by PLFA 18:2ω6,9 and Gram (+) bacteria, in comparison with 2-m and

15-m distances. These results are in contrast with our second hypothesis, and the results of Muñoz et al. (2007) and Unger et al. (2013). However, insignificant effects on soil microbial properties were measured 9-years and 21-years after establishing alley-cropping systems in Eastern Germany (Nii-Annang et al. 2009) and in Northeast Missouri (Bardhan et al. 2013), respectively. Even negative effects of robinia hedgerow on MBC contents in comparison with poplar hedgerow have been observed in a German alley-cropping system in a sandy soil (Medinski et al. 2015). Hedgerow tree effects on SOC and soil microbial properties may vary with the duration and the site of a cropping system. In the current case, the short-term establishment of hedgerow for 4-year period may explain the absence of effect on SOC and microbial community as well as microbial residues. However, the interactions between climate, soil properties, and plant residue quality are a more likely explanation for the current observation.

Conclusions

Organic farming and robinia significantly increased SOC, and an additive effect of organic farming and robinia hedgerow on SOC was observed. This indicates that alley-cropping agroforestry system comprising organic farming management and robinia hedgerows would have more potential to sequester C in soil. In contrast, the organic farming and robinia hedgerow effects on soil microbial biomass, microbial residues, and microbial guilds were diverse. Organic farming showed negative effect on the microbial indices in contradiction with the previous studies, while robinia hedgerows showed positive effect in comparison with integrated farming and poplar hedgerow, respectively. The hedgerow tree did not show positive effect on SOC and microbial properties except the abundance of fungi and Gram (+) in such 4-year short-term introduction compared with alley area. These results indicate the short-term (4-year) introduction of hedgerow trees into an organic farming crop land with the long history might lessen the positive influence of organic farming on microbial communities and residues. Short-term introduction of trees into the existing agricultural system will not change the microbial biomass but only the abundance of specific microbial groups in the hedgerow. However, future work under other site conditions needs to be undertaken in order to further verify our conclusion.

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4 General discussion

The study based on long-term field trial located in TERENO experimental farm in Scheyern. The effects of organic farming, tillage and agroforestry on soil properties and system sustainability are the main goals of the project. Two types of organic farming were considered in current study (Table 1), one is high-put and the other is low-put. The high-input organic farming system receives additional manure input while the low-input organic farming system receives no additional organic input except organic matter residues produced within the system. Long-term field trial can show the influences of tested factors on soil properties that cannot be detected in a short term. In fact, the change of soil from one initial state to another stable state takes time. This is especially important for study on soil chemical and biological properties. Water extractable organic matter and soil microbial communities are important for understanding soil carbon cycling as they directly take part in the cycling by providing substrates for soil microorganisms and by assimilation and dissimilation activities of microorganisms, respectively. In the recent decades, organic farming, minimum tillage and agroforestry draw the interests of scientists and farmers. The hope is that organic farming and minimum tillage as well as agroforestry are thought have a potential to store "atmospheric" carbon into soil, thereby influencing the global carbon cycling. In addition, those agricultural managements have a potential to improve soil sustainably. Therefore, a long-term filed trial is very precious that can provide us insights of soil changes after a long-term implementation of soil/ agricultural management practices, especially the long-term organic farming trial is unique in the world, not only because of duration of this trial, but also the specific management operation. Thus, this can provide precious guide for agricultural management practices.

The Ph.D thesis focused on the study of long-term specific agricultural management practices (organic farming, minimum tillage and agroforestry) influences on soil properties, i.e. WEOM, microbial communities as well as microbial residues as substrates for new SOM. Therefore, samples from field trial were analyzed for WEOM, microbial communities and microbial residues, besides the selected basic soil properties. In addition, in order to know whether a long-term minimum tillage and organic farming change the metabolism of inherent microorganisms, that can be reflected by the fate of a certain substance. A lab incubation with labelled compound

(glucose), which can be taken as the model compound of WEOM, was performed to trace the fate of labile organic carbon soils imposed with long term high-input organic farming and minimum tillage.

4.1 Soil organic matter and soil water extractable organic matter

Soil water extractable organic matter (WEOM) provides the most direct substrate for the activities of soil microorganisms, also influencing the emission of greenhouse gases and soil organic carbon sequestration. Therefore, both the content and the quality or composition of soil water extractable organic matter count. The excitation emission matrix spectra with parallel factor analysis offer a new and easy way to detect the composition of WEOM, thereby giving insights on WEOM responses to environment changes such like soil management practices and agricultural practices. The results in publication I and publication II indicated organic farming either high-put or low-input had the same influence on the content of WEOM. However, high-input organic farming increased the complexity of WEOM, i.e. WEOM was more humified and with more aromatic structure compounds, and more humic-like component in WEOM in comparison with integrated farming. This indicates high-input organic farming lead WEOM to be more stable than WEOM in integrated farming. In contrast, the effect of low-input organic farming on WEOM differed. The difference of organic farming effect on WEOM content might largely resulted from the organic input, as the high-input organic farming received additional manure. The low-input receive is a self-supported system and the tree rows might also affect the performance of low-input organic farming via residue inputs and nutrients competition. In addition, the tillage in publication II was only plough tillage, this can also contribute to the decrease of WEOM. However, the influence on the content of WEOM might vary from concrete organic farming operation to another concrete organic farming operation. Compared with low-input organic farming, organic farming with additional organic input likely to lead more stable WEOM.

Minimum tillage reduces the intensity of soil disturbance in comparison with plough tillage. The results in publication I showed minimum tillage showed little effect on WEOM either in quantity or quality. But minimum tillage caused higher WEOM, quality indicators and humic-like component at upper soil, namely, a negative depth gradient. Thus, high-input organic farming dominates the development of WEOM in a system where minimum tillage and high-input organic farming coexists.

Robinia and poplar are common tree species grown in German agroforestry systems. Compared with poplar, robinia has the ability to support nitrogen fixation, therefore, providing nitrogen to soil by associated biological fixation and thus, this is the only external nitrogen source in current low-input organic farming system. The results in publication II showed the newly established tree rows didn't affect the content of WEOM but enriched more substance with aromatic structure in WEOM, leading WEOM to be more stable. Therefore, the results indicated robinia are able to enrich WEOM with higher stability, which may contribute to soil carbon sequestration. There is no difference between the effect of robinia and poplar on soil WEOM content. However, the tree rows only stood in the systems 4 years until the sampling. Perhaps, the time duration is too short to observe the soil properties at stable state after the introduction of trees into the systems. More observation should be taken to verify the tree species-specific effect on WEOM. Thus, when investigating the influence of agroforestry and organic farming on soil WEOM, tree species and the standing age of trees in an agroforestry system and the concrete practice of organic farming, such like high-input or low-input, should be considered.

Minimum tillage and robinia both enriched more soil organic carbon in comparison with plough tillage and poplar, respectively. From above, high-input organic farming combines with minimum tillage and low-input organic farming combines with robinia-based alley cropping are capable of sequestering carbon into soil. From the point of sequestering organic carbon, organic farming with additional organic input would be better than low-input organic farming for its performance in enriching more stable WEOM components.

4.2 Soil microbial communities and microbial residues

Soil microorganisms directly participate in the global carbon cycling. The influences of agricultural managements on soil microorganisms are critical for people to understand global carbon cycling and to find solutions to mitigate global climate change. On the other hand, healthy soil is also characterized by rich microbial biomass and diversity because of the microbial role in fostering soil fertility and promoting sustainable use of soil resources. The results in publication III and IV showed high-input and low-input organic farming imposed contrasting influence on soil microorganisms. Positive effects of high-input organic farming on microbial biomass, bacteria and arbuscular mycorrhizal fungi (AMF) were observed while low-

input organic farming system was observed with low microbial biomass content and low abundance of the functional guilds of microbes indicated phosphorous lipid fatty acids. This can be partially explained by the low substrate supply in low-input organic farming (publication II). Minimum tillage showed positive effects on microbial biomass and AMF abundance while the effect on bacteria was insignificant (publication III). Robinia showed little effect on microbial biomass, but a positive effect on Gram (+) bacteria was observed (publication IV). This might because the short time duration of trees growth until sampling.

The highest microbial biomass and abundance of functional guilds were observed when organic farming was coupled with minimum tillage. In addition, the microbial community structure was totally different from the other treatments, i.e. organic farming + plough tillage, integrated farming + minimum tillage and integrated farming + plough tillage (publication III). The positive effects of minimum tillage and high-input organic farming on soil microorganisms are additive, and minimum tillage is applicable for organic farming. For the agroforestry system, although either low-input organic farming or robinia showed little effect on soil microorganisms, robinia combines low-input organic farming caused a shift of microbial community structure (publication IV) resulting in a different microbial community structure from other managements. It also should be noted that the current result of microbial biomass in system is just a snapshot of microbial biomass, also sampling was timed after winter and before vegetation growths to get a representative insight in biomass parameters for the soils without direct vegetation impacts, and temporal effect cannot be excluded totally. Especially, the sampling in current thesis faced extreme long winter in 2012. The climate effect can also not be fully excluded. However, from the point of carbon sequestration, robinia-based alley cropping system under low-input organic farming has the higher potential to store soil organic carbon. Because the duration of the alley cropping is kind of short, therefore further observation needs to be done to verify the effect of tree species on microorganisms.

There is increasing evidence that microbial residues, the necromass of dead microbes, are significant parts of soil organic matter and have been found contribute more to soil organic matter than expected before. To understand well on microbial residues responses to soil management practices is critical to understand the microbial contribution to soil organic matter and soil carbon sequestration. In addition,

microbial residues characterized by amino sugars can also serve as a historic biomarker of microbial communities. In present study, high-input organic farming showed positive influence on microbial residues indicated by amino sugars and total glomalin, and the bacteria residues biomarker was more sensitive to organic farming in comparison with residue biomarkers (publication III). In contrast, the effect of low-input organic farming on microbial residues was little, only galactosamine was significantly affected (publication IV). Minimum tillage had little influence on microbial residues. Therefore, microbial residues are more sensitive to the change of farming management than to change of tillage. This indicate the farming management alters the composition of soil organic matter. The application of high-input organic farming can increase the microbial contribution to soil organic matter. Tillage change may not influence the direct microbial contribution to soil organic matter in the form of microbial necromass. Tree species-specific effect was not significant in current study. However, the temporal effect also cannot be fully excluded, because the trees only grow 4 years before sampling. Long term observation and further work needs to be done to verify the tree species-specific effect on microbial residues.

4.3 The fate of labile organic carbon

The results of lab incubation indicated long-term soil managements caused a difference in the distribution of labile organic matter in the investigated pools of soil organic carbon (WEOC, MBC, not-extractable SOC and CO_2). After a 15-day incubation, more ^{13}C was observed remaining in MBC and non-extractable SOC, and followed by CO_2-C pool. Although non-extractable SOC overlap with MBC pool in present study because of the methodological restriction that MBC cannot remove from the bulk pool, the results indicated most of the labile organic matter from plants exudates, soil fauna and degradation of plant residues are immobilized by active microorganisms or incorporated in the stable, non-water extractable organic fraction. The microbial immobilization of labile organic matter and incorporation into stable fraction were relevant to the microbial biomasses which were influenced by soil managements. Thus, the agricultural management influencing soil microbial biomass will alter the fate of labile organic matter. However, the method in present study cannot give a high-resolution picture of which active microbial species or functional groups dominant the immobilization of labile organic matter or there is no significant difference between microbial species or functional groups. In addition, glucose is a

simple compound and cannot really represent the real labile organic matter in soils, despite it has a general occurrence after exo-enzymatic cleavage of carbohydrates with higher molecular weight, such like cellulose. Thus, high resolution study on the functional groups in the labile organic matter transformation under different environments could answer the questions. Anyway, the results imply proper agricultural management can promote the immobilization of labile organic matter thereby enhancing microbial contribution to soil organic pool.

5 CONCLUSIONS AND OUTLOOK

Proper agricultural management practices can not only promote the crops performance but also must have the potential to use land sustainably by retaining more soil organic matter and optimizing soil microbial biomass and diversity. Based on the long-term field trial considering organic farming (high-input and low-input), minimum tillage and alley cropping agroforestry system, water extractable organic matter, microbial community as well as microbial residues were evaluated.

Our results revealed that as a holistic agricultural management, the influence of organic farming on water extractable organic matter quality varied according to the concrete practice of organic farming, while the WEOM of less usual low-input organic farming was less humified than that of integrated farming system. This is consistent with effect on the components of WEOM revealed by excitation emission matrix spectra and parallel factor analysis. High-input organic farming leads to higher stability of WEOM and is beneficial to carbon sequestration. However, both organic farming decreased the content of water extractable organic matter regardless of the concrete organic farming practice. This is out of expectation. However, the temporal effect and climate factor cannot be excluded in present study. Thus, further verifications at more sites should be included to verify the effect. Minimum tillage showed little effect on the content and quality of water extractable organic matter. However, minimum tillage enriched water extractable organic matter content and humic-like components in the upper soil. This result indicates that minimum tillage did not affect WEOM but caused a negative depth gradient of soil properties. In comparison with poplar, robinia is beneficial to enrich humic-like components of WEOM while showed little effect on the content of water extractable organic matter. This indicated robinia tended to lead WEOM with higher stability, which is favorable of carbon sequestration.

Regarding to the effects on microorganisms, our results revealed the effects varied with the concrete practice of organic farming. High-input organic farming with minimum tillage increased the content of microbial biomass, microbial residues, and it also shifted the microbial community structure. The positive effect of minimum tillage on microbial communities could be enhanced by organic farming management. However, when low-input organic farming was conducted with agroforestry, no positive effects of organic farming on microbial communities were observed but tended to decrease the microbial biomass. However, only one sampling cannot fully sure low-input organic farming exerts negative effect on microorganisms and microbial residues when trees are newly (4 years) introduced into a long-term organic farming field. Therefore, the different effects of high-input and low-input organic farming on microorganisms are to be clarified additively. Nevertheless, organic farming in both conditions was observed with the potential to increased soil organic carbon. From above, we expect high-input organic farming with minimum tillage practice have the potential to realize the object of sustainable use of soils by enriching soil organic matter and optimizing soil microbial community. Low-input organic farming with robinia also has potential to increase soil organic matter even though the effect on microbial biomass is little. Specific organic farming practices like additional manure application, crop rotation, soil properties should be considered as important factors affecting water extractable organic matter and microbial communities.

The results revealed also the difference of distribution of labile organic carbon in different soil organic matter fractions. A large portion of labile organic matter was immobilized in microorganisms or incorporated into the stable non-extractable organic fraction. However, the information about the functional species of microorganisms cannot be reflected by only measuring microbial biomass. Therefore, additional high-resolution work is necessary to explore the responses of functional species in soils after long-term specific management to labile organic carbon. In addition, microbial carbon use efficiency could be done to explore the mechanism of organic farming in recruiting organic carbon in the future.

The results show also the general importance of long term field experiments. The coupling with actual analytical possibilities allows new insights and possibilities to address new challenges.

Our findings can be used as basis for setting up strategies and policies for agricultural carbon sequestration and sustainable use of soil resources (e.g. establish high-input organic farming system, establish robinia-based alley cropping and use minimum tillage in organic farming system). Therefore, the agricultural strategies can mitigate climate change and improve soil stability. Farmers can reduce the cost of farming. Questions of yield stabilities were however not in consideration here, but are known to be answered positively for agroforestry systems in Germany.

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