Effects of tillage on processes of organic matter sequestration

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Eidesstattliche Erklärung

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Preface

This thesis is submitted to the Faculty of Organic Agricultural Sciences of the University of Kassel to fulfil the requirements for the degree Doktor der Naturwissenschaften (Dr. rer. nat.).

This dissertation is based on three papers as first author which are published, submitted, or in preparation for submission to international refereed journals. They are included in Chapters 4, 5, and 6.

Chapter 1 gives a general introduction to all parts of the present thesis. Chapter 2 contains the objectives of the work. A methodological annotation is made in Chapter 3.

Chapter 7 resumes the outcomes and conclusions of the three papers as a synthesis and gives the general conclusions. English and German summaries are given in Chapter 8.

The following papers contribute to this thesis:

Chapter 4:

Jacobs, A., Rauber, R., Ludwig, B. 2009. Impact of reduced tillage on carbon and nitrogen storage of two Haplic Luvisols after 40 years. Soil Tillage Res. 102,158-164.

Chapter 5:

Jacobs, A., Helfrich, M., Hanisch, S., Quendt, U., Rauber, R., Ludwig, B. 2010. Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter: Biol. Fert. Soils, accepted.

Chapter 6:

Jacobs, A., Helfrich, M., Dyckmans, J., Rauber, R., Ludwig, B. 2010. Soil aggregate dynamics in an incubation experiment with ^{15}N maize: effects of residue location, submission in preparation.

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Abbreviations

CEC	cation exchange capacity
C_{MB}	microbial biomass carbon (0.05 M K_2SO_4-extractable)
C_{mic}	microbial biomass carbon (0.5 M K_2SO_4-extractable)
C_{org}	organic carbon
CT	conventional tillage
F	fumigated
fPOM	free particulate organic matter
md	macroaggregates destroyed
MT	minimum tillage
N	total nitrogen
NF	non-fumigated
N_{MB}	microbial biomass nitrogen (0.05 M K_2SO_4-extractable)
N_{mic}	microbial biomass nitrogen (0.5 M K_2SO_4-extractable)
N_{min}	0.05 M K_2SO_4-extractable inorganic nitrogen
NT	no-tillage
OM	organic matter
oPOM	occluded particulate organic matter
POM	particulate organic matter
qCO_2	metabolic quotient
SPT	sodiumpolytungstate
WHC	water holding capacity
WRB	Word Reference Base for Soil Ressources

1. General introduction

To increase the organic matter (OM) content in the soil is one main goal in arable soil management since soil OM plays a key role for sustainable fertility and prevents degradation (e.g. soil erosion). Furthermore, in the context of global change, an enhanced stabilization of C and N in the soil is aimed in order to counteract the emissions of climate relevant gases, namely CO_2 and N_2O (Conant et al. 2007; White and Rice 2009).

In arable soils, the OM content can be influenced by various strategies, such as fertilization, crop management, and tillage (Paustian et al. 2000; Bronick and Lal 2005). Since changes in soil management go along with alterations in biological, physical, and chemical processes accountable for decomposition and storage of OM, an accurate understanding of the interactions between soil management and processes of OM transformation is required for the evaluation of strategies and tools for an enhanced and sustainable sequestration of OM (White and Rice 2009).

1.1. The role of tillage management for soil fertility

Generally, tillage affects soil OM transformation by (i) disrupting the soil structure periodically, (ii) incorporating organic residues and fertilizer into the soil, and by (iii) changing the climate and physical properties of soils (Balesdent et al. 2000; Conant et al. 2007). Conventional tillage (CT; ploughing to 20-30 cm) was evaluated to result in substantial losses of OM from soil (Reicosky 2003). The adoption of tillage systems which minimize soil disturbance by reducing tillage depth and/or frequency (reduced tillage) or by no-tillage (NT) (direct drilling, direct seeding, zero-tillage) alters the extent and/or intensity of the effects on soil OM transformations mentioned before. Studies investigating the effects of NT have been carried out in many soil types and climatic regions. In general, it was found that the implementation of NT may increase the

content of soil OM (e.g. Paustian et al. 2000; Six et al. 2000a; Six et al. 2000b; Kushwaha et al. 2001; White and Rice 2009). However, fewer investigations were done on reduced tillage systems. Especially results for tillage systems with a shallow tillage depth are scarce. Moreover, the underlying processes of these findings were discussed contradictorily.

Among reduced tillage systems, there is a large variability in machinery used, tillage frequency, tillage depth, and definitions (conservation tillage, minimum tillage, reduced tillage, shallow tillage). In the following, systems reaching a maximum depth of 10 cm will be referred to as minimum tillage (MT) systems. All other tillage systems of reduced tillage depth and/or frequency will be called “reduced tillage”.

1.2. Processes responsible for different storage of OM

The stabilization of OM in soils, i.e. the protection of OM against microbial decomposition, can mainly be attributed to three general mechanisms, namely (i) spatial inaccessibility, (ii) biochemical recalcitrance, and (iii) organo-mineral association (von Lützow et al. 2006). The importance of these mechanisms can differ for each soil horizon and depends on several factors such as soil type and texture, mineralogical composition and land use.

In the following, a short synthesis shall be given of the current knowledge about the interactions between tillage intensity and the general mechanisms of OM stabilization.

In arable soils, the formation of macroaggregates (>250 µm; Tisdall and Oades 1982), which results in the occlusion of fresh organic residues and OM-rich microaggregates, is regarded as the major process leading to spatial inaccessibility of OM for microorganisms (Oades 1984; Balesdent et al. 2000; Helfrich et al. 2008). Thus, macroaggregates play an important role in OM storage since mineralization kinetics depend on spacial accessibility of OM (Ashman et al. 2003; White and Rice 2009).

Abiven et al. (2009) stated in a review that aggregate stability is one of the main factors affecting soil fertility. Six et al. (1999) proposed a conceptual model of macroaggregate-turnover, where new macroaggregates are formed around fresh particulate organic matter (POM). Within the stable macroaggregates, POM decomposes slowly and becomes further encrusted into more recalcitrant microaggregates. When macroaggregates disrupt, highly recalcitrant residual POM and stable microaggregates (<250 µm) are released (simplified after Paustian et al. 2000). However, little is known about the temporal dynamics of the aggregate distribution in soils (Plante et al. 2002; De Gryze et al. 2006).

The physical impact of ploughing (CT) was evaluated to accelerate the macroaggregate-turnover leading to a decreased physical protection of OM in the soil (Jastrow 1996; Six et al. 1999; Kushwaha et al. 2001; Paustian et al. 2000; Six et al. 2000a; Six et al. 2000b; Bronick and Lal 2005). In this regard, two main effects of ploughing were considered to account for a decreased stability of macroaggregates: Firstly, perturbation exposes new soil periodically to dry-wet and thaw-freeze cycles on the soil surface, thereby increasing the susceptibility of macroaggregates to disrupt (Balesdent et al. 2000; Paustian et al. 2000). Secondly, the disruption of macroaggregates by the plough itself may expose young and labile OM to decomposition (Six et al. 1999; Balesdent et al. 2000).

A reduction in tillage intensity or the implementation of NT may therefore slow down the process of macroaggregate-turnover compared to CT. Indeed, several authors showed an increased occurrence of stable macroaggregates under reduced or NT systems (Six et al. 2000b; Wander and Bidart 2000; Hernández-Hernández and López-Hernández 2002; Denef et al. 2007; Oorts et al. 2007; Zotarelli et al. 2007). However, MT systems (maximum tillage depth of 10 cm) were rarely investigated. Kushwaha et al. (2001) studied aggregate stability in a MT system of an Inceptisol with a sandy loam texture. They reported that tillage reduction tended to increase the proportion of

macroaggregates. Daraghmeh et al. (2009) and Schjønning and Rasmussen (1989) found similar trends for a Danish sandy loam and a Danish fine loam, respectively.

Apart from the physical impact, the change in depth of residue incorporation by MT implementation affects several soil parameters (Holland and Coleman 1987; Balesdent et al. 2000, Peigné et al. 2007) which may result in an increased macroaggregation. In MT systems, the input of residues is more concentrated in the surface soil than under CT. Consequently, substrate availability to microorganisms is increased in the surface soil under MT (Kandeler et al. 1999b; Stockfisch et al. 1999; Kushwaha et al. 2001). An enhanced microbial activity increases the availability of microbial products which serve as binding agents for the formation and cementation of aggregates (Kandeler and Murer 1993; Martens 2000; Kushwaha et al. 2001; Hernández-Hernández and López-Hernández 2002; Oorts et al. 2007).

Little is known about the real contribution of macroaggregates to the storage of a surplus of OM in MT compared to CT. Balesdent et al. (2000) suggested that microaggregates may be the main agent of physical protection. Furthermore, the occurrence and stability of macroaggregates may vary seasonally depending on tillage operations (Álvaro-Fuentes et al. 2007; Daraghmeh et al. 2009).

As described above, the conceptual model of macroaggregate-turnover proposed by Six et al. (1999) assumes that biochemically recalcitrant POM-compounds, organo-mineral associations, and stable microaggregates “mature” mainly within the macroaggregates (Paustian et al. 2000). Consequently, a slower macroaggregate-turnover, as postulated for reduced tillage and NT systems, would give time for a higher production of recalcitrant OM fractions (Paustian et al. 2000). An elevated occurrence of mineral-associated OM in the surface soil of NT systems, compared to CT, was found by Fabrizzi et al. (2003), Oorts et al. (2007), and Zotarelli et al. (2007) using distinct physical fractionation procedures. However, to the best of my knowledge,

there are no data available on mineral-associated OM for soils under MT or other reduced tillage systems.

From another point of view, a lower mineralization of OM under reduced tillage or under NT may lead to an accumulation of young, biochemically labile OM, e.g. in the form of occluded POM (oPOM). A higher occurrence of oPOM and higher concentrations of C and N within the oPOM fraction were reported for NT compared to CT soils by Six et al. (1999), Six et al. (2000b), Wander and Bidart (2000), Oorts et al. (2007), and Zotarelli et al. (2007). Again, no data on the occurrence of oPOM in MT soils were found in the literature.

The biodegradability of OM under different tillage systems has rarely been investigated. However, there is evidence from incubation experiments that OM accumulates as a readily degradable fraction when reduced tillage is implemented (Alvarez et al. 1995; Salinas-Garcia et al. 1997; Wright et al. 2005; Wright et al. 2008; Van Den Bossche et al. 2009). Collins et al. (2000) found by chemical fractionation (acid hydrolysis) that the proportion of recalcitrant organic C (C_{org}) on total C_{org} remained similar or even slightly decreased in a NT compared to a CT soil.

2. Research needs and general objectives

Focussing on MT, an increase in OM concentration (Meyer et al. 1996; Salinas-Garcia et al. 1997; Kandeler et al. 1999b; Stockfisch et al. 1999; Wright et al. 2005), an enhanced physical protection of OM within macroaggregates (Kushwaha et al. 2001), and a higher microbial biomass (Kandeler et al. 1999b; Stockfisch et al. 1999) were recorded in contrast to CT soils. Furthermore, higher potential OM mineralization rates and higher contents of mineralizable C and N especially in MT surface soils were detected in incubation experiments (Salinas-Garcia et al. 1997; Ahl et al. 1998; Wright et al. 2005). Moreover, changes in the microbial community (Ahl et al. 1998) and in soil climate and soil physical properties (Balesdent et al. 2000; Holland 2004; Alvarez and Steinbach 2008) were reported for MT systems.

As specified above, several mechanisms which possibly contribute to an altered OM processing due to the implementation of MT were suggested. However, data on MT systems are scarce and the underlying processes are not yet clear. However, a better understanding of the interactions of MT implementation and changes in OM transformation is essential in order to evaluate the contribution of MT to a sustainable management of arable soils.

The present thesis, addresses the following research topics:

(i) Since information on OM dynamics in MT systems are scarce, it was aimed to survey selected parameters of OM sequestration in a MT field trial. Thus, OM concentrations, microbial biomass, the distribution of water-stable aggregate size classes, and the occurrence of oPOM were compared for MT and CT plots after fallow (Chapter 4).

(ii) The temporal variability of the distribution of aggregate size classes in the field was rarely investigated. Thus, the occurrence of water-stable aggregates of different

sampling dates, namely after fallow and directly after tillage, were compared for CT and MT plots (Chapter 5). Moreover, a microcosm experiment investigated the dynamics of macroaggregate formation and disruption in the short-term under controlled conditions (Chapter 6).

(iii) It remained unclear whether physical disruption by the plough or a lower formation rate is the dominant process leading to a lower occurrence of water-stable macroaggregates under CT. Therefore, the dynamics of macroaggregate formation following the addition of residues were studied in MT and CT systems simulated in a microcosm experiment under controlled conditions (Chapter 6). In the field, the occurrence of macroaggregates after fallow was compared with samples taken six months later directly after tillage for CT and MT soils (Chapter 5).

(iv) In literature, it is rarely discussed, which OM fraction (physical or chemical) is the major agent for storing the surplus of OM found under MT. Thus, the role of macroaggregates for OM storage was investigated in an incubation study (Chapter 5), in a microcosm experiment which focussed on the short-term aspect (Chapter 6), and in the field (Chapter 5). Furthermore, the proportions of biochemically stabilized C and N were analysed by acid hydrolysis for CT and MT soils (Chapter 5).

(v) There is a lack of investigations of the early processes of decomposition and of storage of recently added OM under different tillage regimes. Thus, the mineralization rates and the partitioning of C and N of recently added OM were analysed in CT and MT systems simulated in a microcosm experiment (Chapter 6).

3. Methodological annotations

I had the opportunity to investigate the soils of the long-term experimental sites “Garte-Süd” and “Hohes Feld” of the University of Göttingen. About 40 years ago, MT (rotary harrow; 5-8 cm) and CT (plough; 25 cm) have been implemented at these two sites. The sites are described in more detail in the Chapters 4 and 5.

The main objective of the present thesis was not to investigate the distribution of OM stocks among the entire soil profile but to focus on differences in the principles of OM processing within the layers influenced by the respective tillage regime. Therefore, sampling depths adequate for this approach were chosen (0-5 cm and 10-20 cm). For an estimation of the actual OM stocks under different long-term tillage systems, sampling to a deeper depth would have been required. This task was performed within the subproject 5 of the DFG Research Training Group 1397.

Investigations on samples from the field trial were combined with two laboratory incubations. Incubation experiments can be carried out under controlled conditions reducing environmental and abiotic effects and spacial variability found in the field. Therefore, selected mechanisms can be investigated more accurately. However, the transferability of microcosm experiments to field conditions must be handled with care.

4. Impact of reduced tillage on carbon and nitrogen storage of two Haplic Luvisols after 40 years

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Abstract

It is broadly accepted that reduced tillage increases soil organic carbon (C_{org}) and total nitrogen (N) concentrations in arable soils. However, the underlying processes of sequestration are not completely understood. Thus, our objectives were to investigate the impact of a minimum tillage (MT) system (to 5-8 cm depth) on aggregates, on particulate organic matter (POM), and on storage of C_{org} and N in two loamy Haplic Luvisols in contrast to conventional tillage (CT) (to 25 cm). Surface soils (0-5 cm) and subsoils (10-20 cm) of two experimental fields near Göttingen, Germany, were investigated. Each site (Garte-Süd and Hohes Feld) received both tillage treatments for 37 and 40 years, respectively. In the bulk soil of both sites C_{org}, N, microbial carbon (C_{mic}), and microbial N (N_{mic}) concentrations were elevated under MT in both depths. Likewise, water-stable macroaggregates (>0.25 mm) were on average 2.6 times more abundant under MT than under CT but differences in the subsoils were generally not significant. For surface soils under MT, all aggregate size classes <1 mm showed approx. 35% and 50% increased C_{org} concentrations at Garte-Süd and Hohes Feld, respectively. For greater macroaggregates (1-2, 2-10 mm), however, differences were inconsistent. Elevations of N concentrations were regular over all size classes reaching 61% and 52%, respectively. Density fractionation of the surface soils revealed that

tillage system affected neither the yields of free POM nor occluded POM nor their C_{org} and N concentrations. Moreover, more C_{org} and N (15-238%) was associated within the mineral fractions investigated under MT in contrast to CT. Overall, similar to no-tillage, a long-term MT treatment of soil enhanced the stability of macroaggregates and thus was able to physically protect and to store more organic matter (OM) in the surface soil. The increased storage of C_{org} and N did not occur as POM, as reported for no-tillage, but as mineral-associated OM.

Keywords

density fractionation; microbial biomass; minimum tillage; particulate organic matter (POM); water-stable aggregates

4.1. Introduction

The displacement of conventional tillage (CT) by practices which reduce the physical impact on the soil has been broadly reviewed in the context of energy saving, sustainable fertility and degradation of arable soils (Ahl et al. 1998; Kushwaha et al. 2001) as well as within the discourse of enhancing C sequestration in arable soils in order to reduce CO_2 emissions (Paustian et al. 2000).

The argument most frequently discussed for enhanced organic matter (OM) storage in no-tillage (NT) soils is the increase of aggregate stability (Kushwaha et al. 2001; Hernández-Hernández and López-Hernández 2002; Denef et al. 2007; Zotarelli et al. 2007). In CT soils, the physical impact caused by the plough leads to a disruption of macroaggregates (>0.25 mm; Tisdall and Oades 1982) (Kushwaha et al. 2001; Six et al. 2000a; Bronick and Lal 2005) and exposes microaggregates (<0.25 mm) and free OM to microbial decomposition (Six et al. 2000a; Zotarelli et al. 2007). This macroaggregate-turnover is supposed to be the primary mechanism leading to C-loss in cultivated soils (Jastrow 1996; Six et al. 2000b). Nutrient sequestration is decreased

in CT soils (Lupwayi et al., 1999) due to a loss of macroaggregates rich in OM (Six et al. 2000a; Six et al. 2000b). Thus, macroaggregates play an important role in protecting OM from degradation (Ashman et al. 2003). This leads to an accumulation of OM in NT systems (Oorts et al. 2007). For example, Six et al. (2000a) and Balota et al. (2004) found 38% and 45%, respectively, higher organic carbon (C_{org}) and total nitrogen (N) concentrations in the bulk soil (0-5 cm) of a NT treatment than in a CT soil whereas Oorts et al. (2007) measured an increase of 10-15% in C_{org} and N totals.

Due to the elevated macroaggregate-turnover, a CT soil forms less occluded particulate organic matter (oPOM) than a NT soil (Six et al. 2000b). Moreover, tillage systems also have an effect on C_{org}- and N-concentrations in POM fractions: Wander and Bidart (2000), Oorts et al. (2007), and Zotarelli et al. (2007) proved that there are higher OM concentrations under NT systems than under CT for light free POM (fPOM) and oPOM. Six et al. (1999) reported that the C-concentration of fine (0.053-0.25 mm) oPOM decreased about 51% in CT soils. Zotarelli et al. (2007) found less mineral-associated C in CT than in NT systems after 14 years but no differences were detected after 4 years of diverging tillage systems.

In contrast to CT-NT comparisons, there has been less research into those systems which are ploughless but do not completely abandon tillage. Moreover, such reduced tillage systems vary largely in terms of machinery used and of tillage depth. In the following, those systems reaching a maximum depth of 10 cm are defined as minimum tillage (MT) systems. In several studies, C_{org}, N, and the microbial status of MT soils were reviewed: Compared to NT systems, MT soils among various soil types and climatic regions accumulate OM to a similar extent (Meyer et al. 1996; Salinas-Garcia et al. 1997; Ahl et al. 1998; Kandeler et al. 1999a; Stockfisch et al. 1999; Wright et al. 2005). Also MT increased microbial C (C_{mic}) and microbial N (N_{mic}) (Kandeler et al. 1999a; Stockfisch et al. 1999) and potential C and N mineralization (Salinas-Garcia et al. 1997; Wright et al. 2005). Kushwaha et al. (2001) studied aggregate stability in a

MT system of an Inceptisol of a sandy loam texture revealing a trend that tillage reduction increased macroaggregate proportion.

Whether MT has similar impacts on aggregate stability and on POM fractions as described for NT remains still unclear. Thus, the aim of our study was to investigate the effect of a MT system (1) on C_{org} and N concentrations and (2) on aggregates and particulate organic matter (POM) in contrast to CT.

4.2. Materials and Methods

4.2.1. Sites, treatments, and sampling design

Our study was conducted on two long-term experimental sites located near Göttingen, Germany: Garte-Süd and Hohes Feld. Mean annual precipitation is 645 mm and mean annual temperature is 8.7°C (30-years' average 1961-1990 by Deutscher Wetterdienst, 2007). The soil type of both sites is a Haplic Luvisol (WRB) derived from loess (Ehlers et al. 2000; Reiter et al. 2002). A field-experiment was established in 1970 at Garte-Süd and in 1967 at Hohes Feld consisting of 4 and 3 field-replicates, respectively: Conventional tillage (CT) with a regular mouldboard plough to 25 cm depth and minimum tillage (MT) with a rotary harrow to 5-8 cm depth have been implemented. Before the start of the experiment soil had been mouldboard ploughed.

The soil texture (0-30 cm) at Garte-Süd consists of 15.1% clay, 72.7% silt, and 12.2% sand (Ehlers et al. 2000) and at Hohes Feld of 17.2% clay, 66.5% silt, 16.4% sand (De Mol 1996). Table 1 shows some further site properties. C_{org} stocks at Garte-Süd were slightly higher under MT (0-5 cm: 10.2 t ha^{-1}; 10-20 cm: 17.6 t ha^{-1}) than under CT (0-5 cm: 9.0 t ha^{-1}; 10-20 cm: 15.1 t ha^{-1}). Further, N stocks were 0.78 and 1.66 t ha^{-1} at MT and 0.67 and 1.42 t ha^{-1} at CT plots, respectively. The same trends were detected for C_{org} stocks at Hohes Feld (0-5 cm: 12.2 t ha^{-1}; 10-20 cm: 19.3 t ha^{-1} under MT and 11.6 t ha^{-1}; 14.0 t ha^{-1} under CT, respectively). N stocks at Hohes Feld were 0.95 and 1.87 t ha^{-1} under MT and 0.67 and 1.53 t ha^{-1} under CT, respectively.

The crop growing was the same on both sites and crops were in the last five years as follows: forage maize, winter wheat/mustard, pea, winter wheat, winter wheat. All residues were incorporated by the respective tillage operations.

Each tillage system and each field-replicate was sampled on the 20th of March (Garte-Süd) and the 26th of March (Hohes Feld) 2007. For each field-replicate a composite sample out of three sub-plots was taken from 0-5 cm (surface soil) and from 10-20 cm (subsoil). This sampling approach was chosen in order to distinguish accurately between those layers which were affected by MT and those which were not. After sampling, the samples were soon stored in plastic bags at 4°C. To avoid any influence of preparation on microbial biomass parameters and on aggregates, all samples were gently mixed by hand, large chunks were broken and large plant material was picked out.

Table 1: Site properties (bulk soil <2 mm) of Garte-Süd and Hohes Feld for different tillage systems and depths; mean values and standard errors (n = 4 for Garte-Süd, n = 3 for Hohes Feld).

Site	Depth (cm)	Tillage system	C_{org} (g kg^{-1})	N (g kg^{-1})	C_{mic} (mg kg^{-1})	N_{mic} (mg kg^{-1})	C_{mic}:C_{org} (%)	bulk density (g cm^{-3})	pH (H_2O)	CEC ($mmol_c kg^{-1}$)
Garte Süd	0-5	conventional	13.3 (2.4)	0.98 (0.03)[a]	147 (15)[a]	24.0 (2.3)[a]	1.19 (0.21)	1.36 (0.09)	7.0 (0.1)	151 (26)
	10-20		10.2 (0.9)	0.95 (0.04)	183 (7)	31.5 (1.8)	1.70 (0.10)	1.49 (0.01)[a]	7.0 (0.1)	156 (28)
	0-5	minimum	16.3 (1.1)	1.24 (0.07)[b]	357 (47)[b]	62.0 (5.1)[b]	2.23 (0.37)	1.25 (0.04)	6.9 (0.1)	149 (21)
	10-20		11.5 (0.4)	1.08 (0.10)	234 (32)	41.5 (5.4)	2.03 (0.21)	1.54 (0.01)[b]	7.1 (0.1)	159 (20)
Hohes Feld	0-5	conventional	16.9 (4.9)	1.00 (0.09)[a]	201 (28)	37.5 (2.5)[a]	1.31 (0.26)	1.32 (0.08)	7.0 (0.1)	163 (13)
	10-20		9.9 (0.8)	1.07 (0.03)	206 (7)	35.0 (2.7)	2.12 (0.26)	1.42 (0.05)	7.0 (0.1)	171 (12)
	0-5	minimum	17.4 (0.5)	1.36 (0.08)[b]	359 (73)	77.3 (3.2)[b]	2.08 (0.44)	1.40 (0.04)	6.7 (0.1)	173 (8)
	10-20		12.4 (0.6)	1.20 (0.82)	249 (17)	42.8 (3.5)	2.01 (0.14)	1.56 (0.02)	7.1 (0.0)	176 (6)

Letters indicate comparison among tillage systems; exclusively those values which are significantly different at $p \leq 0.05$ are followed by different letters.

4.2.2. Separation of water-stable aggregates

Water-stable aggregates were separated according to John et al. (2005) prior to analysis of C_{org} and N. Briefly, field-moist composite samples were gently passed through a 10 mm mesh and dried at 40°C for 48 h. One hundred grams of dry soil were placed on a 2 mm sieve and submerged into distilled water for 10 min to allow slaking. Thereafter, the sieve was moved up and down into the water with 50 repetitions. Water-stable aggregates remaining on the mesh (large macroaggregates: 2-10 mm) were collected, vacuum filtered (<0.45 µm) to remove water, dried at 40°C for 48 h on the filter, and weighed. Aggregates which passed the 2 mm sieve were poured onto the next smaller mesh size and the fractionation-procedure was continued as described above. Mesh-sizes used were: 1 mm for medium macroaggregates, 0.25 mm for small macroaggregates, and 0.053 mm for microaggregates. Finally, the supernatant (silt and clay together with finest microaggregates <0.053 mm) was precipitated with 0.5 M $AlCl_3$ (5 ml on 2 l of supernatant). To recover the <0.053 mm-fraction after precipitation, the water was siphoned off and the deposit was dried at 40°C for 48 h. All fractions were ball-milled (Retsch, Haan, Germany) and stored.

4.2.3. Separation of aggregate size classes by moist sieving

The procedures described by Mendes et al. (1999) were used for separation of aggregate size classes through moist sieving and determination of C_{mic} and N_{mic}. As a pretreatment, a pre-incubation of 10 days at room temperature was carried out to ensure that living roots were decomposed (Jenkinson 1988) and would not interact with the determination of C_{mic} and N_{mic} through the chloroform fumigation extraction method (Vance et al. 1987). Pre-incubated samples were allowed to air-dry for 24 h to reach a water content of 13 to 21% which was convenient for the sieving procedure and wet enough to minimize an impact on the determination of microbial biomass (Brookes et al. 1985). Four sieves of different mesh size were piled to separate >10 mm clods

from large macroaggregates (1-10 mm), from small macroaggregates (0.25-1 mm), and from microaggregates (<0.25 mm). Portions of 500 g of soil material were placed on the top sieve and piled sieves were fixed on a shaker (SM-30, Edmund Bühler, Hechingen, Germany). To separate aggregate size classes, the shaker operated for 10 min at 210 oscillations per minute. Visible roots and plant material were picked out, the yield of each size class obtained was determined by weighing, and water content was analysed. Out of each aggregate size class a sub-sample of 50 g was stored in a plastic bag for 24 h at room temperature to equilibrate prior to chloroform fumigation extraction procedure. The remaining soil material of each size class was oven-dried at 40°C for 48 h and stored.

4.2.4. Separation of density fractions

Field-moist aliquots of surface soil samples were gently passed through a 2 mm mesh prior to density fractionation (John et al. 2005) into the following fractions: free particulate organic matter (fPOM) and occluded particulate organic matter (oPOM) with a density <1.6 g cm^{-3}, and the mineral fraction with a density >1.6 g cm^{-3} (mineral). A soil sample (10 g) was placed into a 70 ml centrifugation tube together with 40 ml of a sodiumpolytungstate (SPT) solution (Sometu, Berlin, Germany) with a density of 1.6 g cm^{-3}. The tube was gently shaken 5 times by hand and the sample was allowed to equilibrate for 30 min. Afterwards it was centrifuged for 30 min at 4000 *g* (Multifuge 3 S-R, Heraeus, Hanau, Germany). After centrifugation, the supernatant (fPOM) was vacuum filtered (<0.45 µm) and washed with 2 l of distilled water in order to avoid contamination by SPT. To gain the oPOM fraction, the pellet remaining in the tube had to be disaggregated: 10 glass beads (5 mm) and 40 ml of the SPT solution were added, the pellet was brought into solution and then shaken for 18 h at 175 rotations per minute (SM-30, Edmund Bühler, Hechingen, Germany). Again, centrifugation and filtration took place as described above. The mineral fraction

remaining in the centrifugation tube was washed three times by bringing the pellet into solution with distilled water and than centrifuging. Thereafter, the mineral fraction was separated into sand (mineral >53 µm) and silt together with clay (mineral <53 µm) by a sieve. The mineral <53 µm fraction was precipitated with 0.5 M $AlCl_3$ (5 ml on 2 l of supernatant) and the water was siphoned off. Finally, all fractions gained were oven dried at 40°C for 48 h, weighed, ball-milled (Retsch, Haan, Germany) and stored.

4.2.5. Chemical and biological analysis

For determination of C_{mic} and N_{mic} fumigation-extraction after Vance et al. (1987) was performed of each aggregate size class (moist sieving) and of the bulk soil. Therefore, 10 g of moist soil were exposed to ethanol-free $CHCl_3$ for 24 h and then extracted with 40 ml of 0.5 M K_2SO_4. For each sample a control was performed by extracting it under the same conditions but not fumigated. Carbon and nitrogen were measured in the K_2SO_4-extracts (Dima-TOC 100, Dima-N, Dimatec, Essen, Germany). C_{mic} and N_{mic} were calculated as follows: $C_{mic} = E_C / k_{EC}$ where E_C was (organic C extracted from fumigated samples) – (organic C extracted from non-fumigated samples) and k_{EC} was 0.45 (Joergensen et al. 1995). The same equation was applied for N_{mic} but using $k_{EN} = 0.54$ (Joergensen et al. 1995).

C_{org} and N concentrations were determined in all water-stable aggregate size classes as well as in all density fractions obtained and in the bulk soil. Carbonates in the bulk soil and in the fractions were destroyed by 10% HCl prior to analysis of C_{org} and N (Elementar Vario El, Heraeus, Hanau, Germany).

For the determination of the cation exchange capacity (CEC) and the pH value, standard procedures were applied (König and Fortmann 1996)

4.2.6. Calculations and statistical analysis

C_{org} and N concentrations within the aggregate size classes were expressed on a sand-free basis as suggested by Elliott et al. (1991) and Six et al. (2000b). The

following equation [1] adopted from John et al. (2005) was used for C_{org} and N, respectively:

$$C_{org}\,\text{sand-free}\left(g\ kg^{-1}\right) = \frac{C_{org}\left(g\ kg^{-1}\right)}{1-\text{sandproportion}\ (\%)/100} \qquad [1]$$

Means and standard errors were calculated for each parameter measured within each site, sampling depth, tillage system, and fraction. A one-way ANOVA (GLM of Statistica 7, Statsoft) was used to detect differences between CT and MT among all parameters measured. The level of significance was fixed at $\alpha = 0.05$.

4.3. Results

4.3.1. Analysis of bulk soil

For both sites, C_{org} concentrations (Table 1) of surface soils and subsoils were higher under MT, but differences were not significant, whereas the increase of N in 0-5 cm of MT was significant for both sites. However, C_{org}:N ratios were not affected by tillage. Likewise, C_{mic} and N_{mic} concentrations were increased under MT compared to CT treatments by similar factors. Thus, C_{mic}:N_{mic} ratios did not differ among tillage systems. The increase in microbial biomass on MT plots was more pronounced in the surface soil than in the subsoil: Surface soils showed 2.6 and 2.0 times higher concentrations of C_{mic} and N_{mic} at Garte-Süd and Hohes Feld, respectively, whereas the increase in subsoil had factors of 1.4 and 1.2, respectively. Again, elevation of N_{mic} was significant for surface soils. C_{mic}:C_{org} ratio increased with depth in CT and decreased in MT. None of the C_{mic}:C_{org} ratios differed significantly among tillage systems but there was a trend of higher values in MT soils at both depths. Bulk density indicated a more compacted subsoil under MT than under CT. The pH value, and the CEC were not affected by tillage system.

4.3.2. Occurrence of water-stable aggregates and OM concentrations within size classes

On both sites and in both depths sampled, the percentage yields of water-stable macroaggregates (>0.25 mm) were on average 2.6 times higher under MT and differences were significant in most cases for the surface soils (Figure 1).

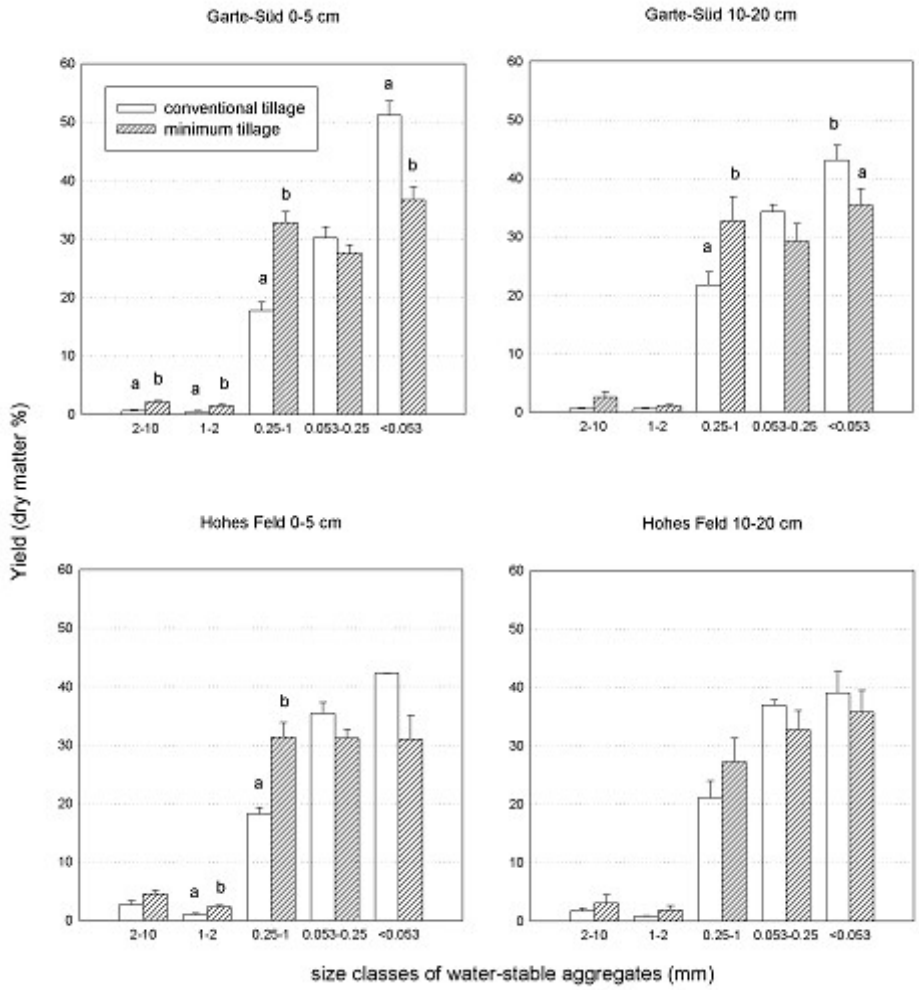

Figure 1: Total yield of water-stable aggregate size classes among tillage systems, sites, and depths; mean values and standard errors (n = 4 for Garte-Süd, n = 3 for Hohes Feld).
Letters indicate comparison among tillage systems; exclusively those bars which are significantly different at p ≤ 0.05 are marked by different letters.

In the macroaggregates, C_{org} concentrations were higher than in microaggregates (Table 2). Among all size classes, there was a general accumulation of C_{org} and N in the surface soils under MT in contrast to CT. Some of the differences between tillage

systems were significant, especially N was more abundant under MT systems in the surface soils of both sites. However, two exceptions were found: C_{org} concentrations of the 1-2 mm size class of Garte-Süd and of the 2-10 mm of Hohes Feld. Thus, for larger macroaggregates, differences in C_{org} between the tillage systems were inconsistent and variability was considerable (Table 2). The C_{org} concentrations of the sand-free <0.053 mm fractions, of the microaggregates, and of the small macroaggregates increased about 35% at Garte-Süd and 50% at Hohes Feld. Sand-free N was regular: overall sand-free size classes the N concentrations of surface soils were elevated about 61% and 52%, respectively.

The subsoils did not show any clear trend of diverging C_{org} and N concentrations within water-stable aggregate size-classes among tillage systems.

4.3.3. Aggregates and microbial biomass after moist sieving

Moist sieving of surface soils did not yield any microaggregates (<0.25 mm) (Table 3). For both sites, most of the soil material was collected in the 1-10 mm fraction. Small macroaggregates (0.25-1 mm) were more abundant under MT in both sites, whereas this difference was significant for Hohes Feld. The recovered 0.25-1 mm material was insufficient in which to measure C_{mic} and N_{mic}. C_{mic} and N_{mic} concentrations were similar among size classes and showed significantly higher values under MT versus CT, similar to the bulk soil behaviour (Table 1). Thus, no trend was detected in distribution among aggregate size classes.

Table 2: C_{org}, C_{org} in sand-free aggregates, and N concentrations among water-stable aggregate size classes, different tillage systems, sites, and depths; mean values and standard errors (n = 4 for Garte-Süd, n = 3 for Hohes Feld).

		Conventional tillage			Minimum tillage		
Site and depth	Water-stable aggregates (mm)	C_{org} (g kg^{-1})	C_{org} (g kg^{-1}) sand-free	N (g kg^{-1}) sand-free	C_{org} (g kg^{-1})	C_{org} (g kg-1) sand-free	N (g kg^{-1}) sand-free
Garte-Süd 0-5 cm	>2	24.6 (1.3)	27.7 (1.5)	0.68 (0.21)[a]	31.5 (5.2)	38.6 (6.4)	2.01 (0.27)[b]
	1-2	35.8 (3.5)	59.4 (6.0)	1.80 (0.29)	35.9 (4.0)	44.4 (5.0)	2.41 (0.31)
	0.25-1	10.0 (1.3)[a]	15.0 (2.0)[a]	1.56 (0.07)[a]	17.3 (0.7)[b]	22.2 (0.9)[b]	1.99 (0.08)[b]
	0.053-0.25	11.5 (0.6)	16.8 (1.3)	1.49 (0.12)	15.9 (2.0)	20.9 (2.8)	1.96 (0.19)
	<0.053	8.6 (1.5)	8.6 (1.5)	0.93 (0.07)	11.3 (0.5)	11.3 (0.5)	1.06 (0.03)
Garte-Süd 10-20 cm	>2	22.9 (1.9)	26.2 (2.2)	0.64 (0.20)	18.1 (2.0)	22.6 (2.5)	1.23 (0.29)
	1-2	19.1 (2.8)	28.9 (4.3)	1.05 (0.18)	27.0 (4.6)	35.8 (6.1)	1.46 (0.42)
	0.25-1	18.3 (6.2)	24.2 (8.2)	1.44 (0.13)	12.5 (1.5)	15.3 (1.8)	1.04 (0.17)
	0.053-0.25	13.0 (1.3)	18.2 (1.8)	1.27 (0.08)	11.0 (1.3)	14.9 (1.8)	1.23 (0.21)
	<0.053	12.5 (1.5)	12.5 (1.5)	0.75 (0.08)	9.0 (0.8)	9.0 (0.8)	0.82 (0.06)
Hohes Feld 0-5 cm	>2	28.6 (7.4)	35.7 (9.2)	1.42 (0.16)	22.0 (1.2)	26.3 (1.5)	1.84 (0.28)
	1-2	15.8 (2.4)[a]	24.2 (3.7)	1.30 (0.13)	27.2 (1.5)[b]	34.8 (2.0)	2.20 (0.44)
	0.25-1	11.8 (0.3)[a]	18.2 (0.5)[a]	1.63 (0.04)[a]	17.5 (1.1)[b]	23.3 (1.6)[b]	2.15 (0.08)[b]
	0.053-0.25	10.9 (0.4)	15.7 (0.5)	1.44 (0.05)	23.0 (7.2)	33.4 (10.5)	2.80 (0.71)
	<0.053	10.1 (0.3)	10.1 (0.3)	1.03 (0.04)	11.0 (0.1)	11.0 (0.1)	1.39 (0.22)
Hohes Feld 10-20 cm	>2	18.6 (2.4)	23.5 (3.0)	0.87 (0.15)	15.4 (0.7)	22.4 (1.0)	0.85 (0.36)
	1-2	15.2 (0.8)	30.1 (1.8)	1.02 (0.15)	15.8 (1.2)	23.5 (1.9)	1.15 (0.20)
	0.25-1	12.2 (0.9)	17.6 (1.3)	1.38 (0.02)	13.5 (1.3)	17.3 (0.6)	1.06 (0.15)
	0.053-0.25	12.2 (1.2)	18.0 (1.7)	1.31 (0.03)	12.7 (1.3)	18.1 (1.9)	1.07 (0.23)
	<0.053	10.6 (0.8)	10.6 (0.8)	0.89 (0.10)	14.6 (4.3)	14.6 (4.3)	0.46 (0.21)

Letters indicate comparison among tillage systems; exclusively those values which are significantly different at $p \leq 0.05$ are followed by different letters.

4.3.4. Density fractions and OM concentrations

For both sites and both tillage systems merely low yields of both POM fractions were obtained for the surface soils, revealing that small amounts of young organic matter were present in the soil at sampling date (Table 4). Moreover, the yields of POM and mineral fractions were equal among tillage systems. Likewise, C_{org} and N concentrations of the POM fractions were not affected by tillage. However, there was a significant effect of MT on C_{org} and N concentrations detected for the mineral fractions. Here, most of the increase occurred in the mineral >53 µm fraction: C_{org} and N values were elevated by 125-238%, whereas elevation in the mineral <53 µm fraction reached 15-59% in the MT soils.

Table 3: Total yield, C_{mic}, and N_{mic} concentrations among aggregate size classes after moist sieving of the surface soil for different tillage systems and sites; mean values and standard errors (n = 4 for Garte-Süd, n = 3 for Hohes Feld).

		Conventional tillage			Minimum tillage		
Site	Size class (mm)	Yield (dry matter %)	C_{mic} (µg g^{-1})	N_{mic} (µg g^{-1})	Yield (dry matter %)	C_{mic} (µg g^{-1})	N_{mic} (µg g^{-1})
Garte Süd	>10	34.2 (7.6)	151 (24)[a]	25.2 (2.8)[a]	28.8 (2.2)	345 (46)[b]	57.8 (5.2)[b]
	1-10	64.8 (7.5)	161 (14)[a]	24.0 (2.9)[a]	68.7 (2.5)	357 (41)[b]	67.1 (1.3)[b]
	0.25-1	0.6 (0.3)	nd	nd	1.2 (0.4)	nd	nd
Hohes Feld	>10	45.0 (7.5)	169 (22)[a]	27.9 (2.2)[a]	21.6 (6.4)	355 (57)[b]	70.8 (7.9)[b]
	1-10	54.3 (7.1)	222 (30)	35.2 (0.5)[a]	71.2 (6.0)	341 (75)	76.5 (4.1)[b]
	0.25-1	0.3 (0.1)a	nd	nd	6.1 (1.1)[b]	335 (76)	71.8 (5.5)

Letters indicate comparison among tillage systems; exclusively those values which are significantly different at $p \leq 0.05$ are followed by different letters.
nd = not determined

Table 4: Total yield, C_{org}, and N concentrations among density fractions of the surface soil for different tillage systems and sites; mean values and standard errors (n = 4 for Garte-Süd, n = 3 for Hohes Feld).

		Conventional tillage			Minimum tillage		
Site	Density fraction	Yield (dry matter %)	C_{org} (g kg^{-1})	N (g kg^{-1})	Yield (dry matter %)	C_{org} (g kg^{-1})	N (g kg^{-1})
Garte-Süd	fPOM	0.8 (0.3)	323 (29)	15.9 (0.7)	0.7 (0.2)	266 (12)	15.1 (0.9)
	oPOM	1.4 (0.5)	334 (18)	19.4 (1.1)	1.3 (0.3)	372 (20)	21.2 (1.0)
	mineral >53 µm	15 (1)	6.6 (1.0)[a]	0.28 (0.02)[a]	14 (1)	14.8 (1.7)[b]	0.94 (0.09)[b]
	mineral <53 µm	87 (2)	10.1 (1.2)	0.69 (0.11)[a]	87 (0)	11.6 (0.8)	1.09 (0.05)[b]
Hohes Feld	fPOM	0.6 (0.3)	249 (26)	9.60 (1.59)	0.8 (0.2)	250 (6)	11.8 (0.9)
	oPOM	1.0 (0.2)	355 (6)	21.3 (0.6)	1.0 (0.2)	350 (7)	23.4 (0.7)
	mineral >53 µm	19 (1)	5.1 (0.6)[a]	0.25 (0.02)[a]	18 (1)	12.3 (0.3)[b]	0.67 (0.10)[b]
	mineral <53 µm	84 (0)	10.0 (0.1)[a]	0.99 (0.05)[a]	81 (2)	14.6 (0.9)[b]	1.36 (0.11)[b]

Letters indicate comparison among tillage systems; exclusively those values which are significantly different at $p \leq 0.05$ are followed by different letters.

4.4. Discussion

4.4.1. Analysis of bulk soil

C_{org} and N concentrations showed increased values for both depths and on both sites investigated under MT compared to CT after about 40 years of different tillage systems (Table 1). This finding was supported by researchers investigating MT (maximum tillage depth of 10 cm) for a number of soil types and textures (Meyer et al. 1996; Ahl et al. 1998; Stockfisch et al. 1999; Kandeler et al. 1999a; Kushwaha et al. 2001). In our study, significance was reached only for the N concentration in the surface soils (0-5 cm). Thus, the impact of MT was more pronounced within the soil layer affected by residue input and physical impact of tillage. Kandeler et al. (1999a) and Wright et al. (2005) also detected very low effects in terms of OM concentrations in subsoils. However, some studies reported elevation of C_{org} and N concentrations down to deeper soil layers (Salinas-Garcia et al. 1997; Ahl et al. 1998).

Likewise, the increase in microbial biomass in MT soils (Table 1), measured as 2.6 and 2.0 times higher C_{mic} and N_{mic} concentrations, respectively, was also detectable in the subsoils (10-20 cm) but much less pronounced than in the surface soils of both sites investigated. Several studies analysed microbial parameters with regard to MT. In agreement with our findings, most of them suggested a general gradient of tillage reduction and increasing microbial biomass in the surface soil (Meyer et al. 1996; Salinas-Garcia et al. 1997; Ahl et al. 1998; Kandeler et al. 1999a; Stockfisch et al. 1999; Kushwaha et al. 2001; Spedding et al. 2004; Wright et al. 2005). This can be explained by an increased substrate availability to microorganisms in MT provided through the accumulation of crop residues in the surface soil (Kandeler et al. 1999a; Stockfisch et al. 1999; Kushwaha et al. 2001). An increased substrate availability can be demonstrated by a high C_{mic}:C_{org} ratio (Stockfisch et al. 1999). In our study, the

C_{mic}:C_{org} ratio increased with depth in CT and decreased in MT (Table 1) indicating that C-availability to microorganisms was the best in the soil layer which received the highest residue input.

4.4.2. Occurrence of water-stable aggregates and OM concentrations within size classes

In our study, macroaggregates (>0.25 mm) were 2.6 times more abundant under MT compared to CT among both depths sampled and on both sites (Figure 1). Thus, macroaggregates were more stable in MT systems possibly improving the physical protection of OM against breakdown. Currently, only one study investigating a MT system comparable to our experiment (maximum tillage depth of 10 cm) has reported the effects of MT on aggregate stability: Kushwaha et al. (2001) found that MT increased the proportion of macroaggregates after one year of diverging treatment of a tropical sandy loamy Inceptisol. The higher macroaggregate stability under MT systems detected in our study and in the one of Kushwaha et al. (2001) can probably be attributed (1) to a lower physical impact of tillage machinery on surface soils and subsoils or (2) to a higher availability of binding agents caused by higher microbial biomass which enhanced formation and cementation of aggregates mainly in the surface soil (Kandeler and Murer 1993; Kushwaha et al. 2001; Hernández-Hernández and López-Hernández 2002; Oorts et al. 2007).

In our study, concentrations of C_{org} within water-stable aggregates decreased with decreasing size class whereas N was evenly distributed (Table 2). This was in agreement with other studies which focused on aggregates in arable soils (John et al. 2005; Manna et al. 2006; Oorts et al. 2007). In surface soils of our study, N in particular was more abundant under MT systems among all size classes compared to CT: Garte-Süd had values raised about 61% and Hohes Feld about 52%. C_{org} concentrations of macroaggregates >1 mm showed inconsistent trends but all size

classes <1 mm exhibited about 35% (Garte-Süd) and 50% (Hohes Feld) higher values for MT. After one year of MT treatment, Kushwaha et al. (2001) measured slightly raised C_{org} and N concentrations only in microaggregates. Thus, the marked differences in our study probably result from the long-term experimental treatment.

Overall, MT does not only improve aggregate stability but also increases the concentrations of C_{org} and N within aggregates.

4.4.3. Aggregates and microbial biomass after moist sieving

Separation of different aggregate size classes by moist sieving after Mendes et al. (1999) did not reveal any trend concerning any effects of MT on aggregates in arable soil (Table 3). Furthermore, in comparison with the results of water-stable aggregate fractionation which showed clear trends among tillage systems and which detected microaggregates, it seemed that the method of moist sieving was not suitable for separation of aggregates for the silt loam soils of our study. However, a sieving procedure of the moist soil was the only approach for separation of aggregate size classes in which microbial parameters were to be measured to avoid any influences of drying and re-wetting on the microbial community (Schutter and Dick 2002). Since a water content of ≥40% of water-holding capacity was required for determination of C_{mic} and N_{mic} (Friedel et al. 2002), soil was possibly too wet to be fractionated properly. We suggest that the sieving procedure after Mendes et al. (1999) prior to determination of microbial parameters is useful only for sandy soils which have lower water-holding capacities.

4.4.4. Occurrence of density fractions and OM concentrations within fractions

In our study, investigated fPOM and oPOM of the surface soils did not reveal any effect of tillage system on either site (Table 4). Since no comparable studies were available, we expected findings similar to NT systems or to reduced tillage treatments not comparable to the present MT (maximum tillage depth of 10 cm): Six et al. (2000b),

Pinheiro et al. (2004), and Oorts et al. (2007) suggested slower aggregate turnover in NT or reduced tilled soils protecting more OM as oPOM. However, these results were not proved by our MT study. This finding can possibly be explained by the sampling date: During the winter 2006/2007 the experimental fields lay fallow and the last residue-input was half a year ago. Thus, breakdown of POM residues was already well advanced.

Moreover, the present C_{org} and N concentrations of the POM fractions were not affected by tillage (Table 4). Again, NT studies reported a different trend: Wander and Bidart (2000) and Zotarelli et al. (2007) measured higher C_{org} and N concentrations in the fPOM and in the oPOM of NT soils compared to CT using distinct fractionation procedures. Thus, the present MT system did not store more OM as POM in a long-term aspect, despite a higher aggregate stability in the MT soils.

However, significant results were detected for the mineral fractions: MT soils had higher C_{org} and N concentrations in these fractions than CT ones. Most of the increase occurred in the mineral >53 µm fraction: C_{org} and N were 125-238% higher under MT. An elevated storage of mineral-associated OM in the surface soil was confirmed by some studies of NT systems using distinct fractionation procedures (Fabrizzi et al. 2003; Oorts et al. 2007; Zotarelli et al. 2007).

Overall, our data indicate that OM transformation processes in MT soils do not preserve POM from degradation but favour the storage of OM in mineral-associated forms.

4.5. Conclusions

Forty years of MT treatment led to an increased storage of OM in MT soils measured as higher concentrations of C_{org} and N. Water-stable macroaggregates were more abundant in MT soils possibly protecting OM from degradation. However, no increased storage of POM, as it had been suggested for NT soils, was detected in the surface

soils of MT. Raised C_{org} and N concentrations found under MT occurred as OM associated with minerals being further degraded than POM. Thus, breakdown of OM in MT soils follows different processes than in CT soils. Overall, our study showed that a MT treatment can have beneficial effects on arable soils in terms of stability of macroaggregates and C_{org} and N storage, as it had been reported for NT by other researchers.

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5. Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter

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Abstract

Objectives were to investigate (i) to which extent water-stable macroaggregates sequester organic matter (OM) in a minimum tillage (MT) system compared to a conventional tillage (CT) system and (ii) if the content of biochemically stabilized OM differs between both tillage systems; and (iii) to study the temporal dynamics of the distribution of aggregate size classes and of storage of OM within aggregates in the field. Surface soils (0-5 cm) and subsoils (10-20 cm) were sampled after fallow (March 2007) and directly after tillage (November 2007) from a 37 years' old long-term experimental field near Göttingen, Germany. Macroaggregates (>0.25 mm) were in general less abundant after fallow than directly after tillage. In March 2007, only 21% (CT) and 45% (MT) of C_{org} was stored within macroaggregates in the surface soil, whereas in November 2007, the percentages increased to 58% and 73%, respectively. In order to investigate the importance of physical protection within macroaggregates for OM storage, CT and MT soils of both depths were incubated (i) as bulk soil (CT_{bulk}, MT_{bulk}) and (ii) with macroaggregates disrupted (<0.25 mm) (CT_{md}, MT_{md}) for 28 days at 22°C and a water content of 50% of the maximum water holding capacity. For the MT_{bulk} and MT_{md} surface soils, C mineralization was significantly higher compared to

the CT soils. Incubation of md soils did not generally result in a significantly higher C mineralization compared to the respective bulk soils, except for the MT_{md} subsoil. Acid hydrolysis showed that the proportion of biochemically stabilized, non-hydrolysable, C_{org} to total C_{org} was lower in the MT than in the CT soils. Overall, the data indicate that (i) the effect of physical stabilization of OM stored in the macroaggregates should not generally be seen as a mechanism protecting very labile C with a turnover time of weeks, but that longer preservation is likely after macroaggregate transformation into microaggregates; (ii) biochemically stabilized OM is not the main reason for the surplus of OM found in the surface soil of MT; and (iii) the temporal variability of distribution of aggregate size classes in the field is large.

Keywords

acid hydrolysis, incubation, macroaggregates, potential mineralization, water-stable aggregates

5.1. Introduction

A prerequisite for sustaining the fertility of arable soils and for reducing CO_2 emissions from soils in general is the evaluation of mechanisms leading to a sustainable sequestration of organic matter (OM). It was shown for various soil types and climatic regions that tillage systems which minimize soil disturbance (conservation tillage, minimum tillage (MT), reduced tillage, and no-tillage (NT)) generally increase the storage of soil OM compared to conventionally tilled (CT) soils (Paustian et al. 2000; Six et al. 2000a; Six et al. 2000b; Kushwaha et al. 2001; Jacobs et al. 2009b). However, the processes underlying this increased OM sequestration are not completely understood.

In general, the stabilization of OM in soils, i.e., the protection of OM against microbial decomposition, may be mainly attributed to the three mechanisms, namely (i) spatial inaccessibility, (ii) biochemical recalcitrance, and (iii) organo-mineral association (von Lützow et al. 2006). However, the importance of each of these three mechanisms for OM stabilization can differ for each soil horizon and depends on several factors such as soil type and texture, mineralogical composition and land use.

In arable soils, the formation of macroaggregates, whereby fresh organic residues are enclosed within aggregates, is regarded as an important process leading to the spatial inaccessibility of OM (Oades 1984; Balesdent et al. 2000). Recently, results by Helfrich et al. (2008) emphasized the importance of microaggregates to spatial inaccessibility of OM. In their study, macroaggregate formation implied rapid incorporation and thereby short-term protection of maize-derived C and N. Moreover, macroaggregates allowed a transfer of maize-derived OM into microaggregates within macroaggregates, which prevented the release of significant amounts of free particulate OM upon macroaggregate breakdown.

Tillage systems with minimized soil disturbance (MT, here defined as tillage reaching a maximum depth of 10 cm) were reported to increase the occurrence and the stability of macroaggregates compared to CT soils (Paustian et al. 2000; Six et al. 2000a, 2000b; Kushwaha et al. 2001; Jacobs et al. 2009b) which was ascribed to various physical and biological factors, such as a reduced physical impact of machinery leading to less disruption of aggregates and a higher formation of macroaggregates due to a higher concentration of OM in the surface soil (Balesdent et al. 2000; Kushwaha et al. 2001; Bronick and Lal 2005; Jacobs et al. 2009b). However, little is known about the temporal dynamics of the aggregate distribution in soils (Plante et al. 2002; De Gryze et al. 2006; Álvaro-Fuentes et al. 2007; Olchin et al. 2008).

In order to investigate the role of macroaggregates for protection of OM against microbial decomposition, several incubation experiments have been carried out using

soils from CT and NT experiments. For instance, Beare et al. (1994) incubated undisrupted and disrupted macroaggregates of CT and NT soils and showed that the disruption of macroaggregates increased the OM mineralization from NT surface soils but had little effect on CT soils. In contrast, Oorts et al. (2006), who incubated undisrupted soil and soil after disruption of macroaggregates, found an increased mineralization due to the disruption of macroaggregates only for an NT subsoil. They suggested that the surplus of organic C (C_{org}) and N in the surface soil under NT was either mainly located in a non-highly protected form or situated in recalcitrant OM; i.e. OM which is biochemically stabilized by its structural composition. Indeed, an increasing size of a recalcitrant OM pool (isolated by acid hydrolysis) under NT compared to CT plots was reported by Collins et al. (2000). This increase can be attributed mainly to increasing SOC stocks. However, the contribution of recalcitrant to total C_{org} (% of C_{org}) remained similar or even slightly decreased in the NT compared to CT plots (Collins et al. 2000).

Jacobs et al. (2009b) suggested that long-term MT resulted in an enhanced physical protection of OM accounting for the higher concentrations of C_{org} and N found in MT soils. However, density fractionation of the surface soil did not reveal more particulate OM occluded within aggregates but higher C_{org} and N concentrations in the mineral fractions of MT than of CT soils (Jacobs et al. 2009b). These findings were supported by the results of Jastrow (1996) and Zotarelli et al. (2007) who suggested that most of OM is sequestered as a mineral-associated fraction which is further occluded into aggregates.

Overall, several mechanisms which possibly contribute to an increased OM sequestration were suggested by various studies and are still discussed controversially. Further, information on MT systems is scarce, since mainly NT systems have been investigated.

The objectives of our study were to investigate (i) to which extent water-stable macroaggregates sequester OM in a MT system compared to a CT system and (ii) if the content of biochemically stabilized OM differs between the two tillage systems; and (iii) to study the temporal dynamics of the distribution of aggregate size classes among tillage systems in the field.

5.2. Material and Methods

5.2.1. Sampling site and sample processing

Our study was conducted on a long-term experimental site near Göttingen, Germany (Garte-Süd). The mean annual precipitation and temperature are 645 mm and 8.7°C, respectively. The soil type is a Haplic Luvisol (WRB) derived from loess (15.1% clay, 72.7% silt, 12.2% sand in 0-30 cm, Ehlers et al. 2000). In 1970, two different tillage treatments were established with 4 field-replicates for each treatment: conventional tillage (CT) by mouldboard ploughing to 25 cm depth, and minimum tillage (MT) using a rotary harrow to 5–8 cm depth. Before 1970, the field had been mouldboard ploughed. The crops grown in the previous years were winter wheat (2003), pea (2004) and wheat/mustard (2005). All crop residues were incorporated by the respective tillage operations. In 2006, forage maize was grown until September, where aboveground parts were harvested. The maize stubble was not incorporated and the fields were bare fallows until the end of March 2007. Thereafter, the tillage operations were carried out and field bean was sown at the 29th of March 2007. Harvest was at the 28th of August. Tillage operations were carried out on the st (CT) and 2nd (MT) of November 2007. Further site characteristics are given by Ehlers et al. (2000) and Jacobs et al. (2009b).

Samples were taken in March 2007 after fallow (and before the tillage in the end of March) and at the 7th of November 2007, five (MT) and six (CT) days after tillage. In these six days, daily mean soil temperature was constant at 12°C (detected at 5, 10, and 20 cm depth). From each field replicate, a composite sample out of three sub-plots

was taken from 0–5 cm (surface soil) and 10–20 cm (subsoil) depth. Directly after sampling, samples were stored at 4°C. Samples were carefully broken apart and sieving <10 mm was done at room temperature as soon as possible. Directly after sieving, samples were dried at 40°C for 48 hours. In the following days, fractionation was carried out as described below.

The sampling depths 0-5 cm and 10-20 cm were chosen in order to distinguish accurately between those layers which were affected by MT and those which were not. However, one has to keep in mind that the 10-20 cm depth was not entirely representative of the plough layer of the CT treatment. At the sampling date of November, an accumulation of the harvest residues was visible in the lower third of the Ap horizon of the CT treatment.

5.2.2. Separation of water-stable aggregates and OM within aggregate size classes

The temporal dynamics of water-stable aggregates was studied by using a sampling date in March 2007 (after fallow, Jacobs et al. 2009b) and directly after tillage in November 2007. Briefly, one hundred grams (LP6200S, Sartorius, Göttingen, Germany) of dry soil (<10 mm) were placed on a 2 mm sieve and submerged into distilled water for 10 min to allow slaking. Thereafter, the sieve was moved up and down into the water with 50 repetitions. Water-stable aggregates remaining on the mesh (large macroaggregates: 2-10 mm) were collected, vacuum filtered (<0.45 µm) to remove water, dried at 40°C for 48 h on the filter, and weighed (LP3200D, Sartorius, Göttingen, Germany). Aggregates which passed the 2 mm mesh were poured onto the next smaller mesh-size and the fractionation-procedure was continued as described above. Mesh-sizes used were: 1 mm for medium macroaggregates, 0.25 mm for small macroaggregates, and 0.053 mm for microaggregates. Finally, the supernatant (silt and clay together with finest microaggregates <0.053 mm) was precipitated with

0.5 M $AlCl_3$ (5 ml on 2 l of supernatant). To recover the <0.053 mm-fraction after precipitation, the water was siphoned off, the deposit was dried at 40°C for 48 h, and weighed (LP3200D, Sartorius, Göttingen, Germany). All fractions were ball-milled (Retsch, Haan, Germany) and C_{org} and N concentrations were determined in all water-stable aggregate size classes obtained and in the bulk soil. Carbonates were destroyed by 10% HCl prior to analysis (Elementar Vario El, Heraeus, Hanau, Germany).

For this site, Jacobs et al. (2009b) showed that the calculation of the C_{org} and N concentrations within aggregate size classes on a sand-free basis (Elliott et al. 1991; Six et al. 2000b) did not have a significant effect on the distribution of OM among the size classes investigated. Thus, in this study, C_{org} and N concentrations of the respective aggregate size classes are expressed on a non-sand-free basis.

Operator variability was small to moderate for the five aggregate fractions. For the surface soil sampled in November, yields (dry matter %, means and standard errors, n = 4) in the fractions 2-10 mm, 1-2 mm, 0.25-1 mm, 0.053-0.25 mm and <0.053 mm of the MT treatments were 6.2 (0.9), 6.1 (1.0), 53.5 (1.1), 20.6 (1.9) and 16.4 (3.0) (Figure 1) obtained by U. Quendt. A. Sawallisch obtained the following yields (dry matter %, means and standard errors, n = 4) of the named fractions: 7.0 (0.7), 5.0 (0.9), 52.3 (1.2), 18.2 (0.7) and 16.9 (2.0), indicating a small operator variability. For the surface soil of the CT treatment, yields were affected to a moderate extent, but generally within the range of the standard errors. Yields (dry matter %, means and standard errors, n = 4) of the named fractions were 0.4 (0.1), 0.6 (0.1), 55.3 (2.0), 24.5 (2.0) and 18.3 (0.3) obtained by A. Sawallisch compared to 0.6 (0.2), 0.8 (0.1), 53.7 (5.0), 28.2 (4.5) and 17.1 (1.3) obtained by U. Quendt (Figure 1).

5.2.3. Incubation experiment

The incubation was carried out with the samples taken in November 2007 (sieved <10 mm and dried at 40°C for 48 hours) in the following treatments for surface soils

and subsoils: (1) CT, bulk soil <10 mm (CT_{bulk}), (2) CT, macroaggregates destroyed (CT_{md}), (3) MT, bulk soil <10 mm (MT_{bulk}), (4) MT, macroaggregates destroyed (MT_{md}). For the CT_{md} and MT_{md} treatments, all naturally occurring macroaggregates were gently destroyed by a mortar and the soil was passed through a 0.25 mm mesh prior to incubation in order to guarantee complete macroaggregate disruption.

For each treatment, 40 g of soil (either md or bulk soil) were incubated in glass jars at 50% of the maximum water holding capacity and 22°C for 28 days in the dark. Soil moisture was controlled regularly by weighing and corrected if necessary by adding distilled water.

Carbon and N concentrations were determined on all soil samples before (day 0) and at the end of incubation (day 28) by dry combustion (Vario Max, Elementar, Hanau, Germany). Carbonates were determined by the Scheibler method.

Accordingly, soil microbial biomass was determined as microbial C (C_{mic}) and N (N_{mic}) after 28 days of incubation. The chloroform-fumigation-extraction method after Vance et al. (1987) was used. Two portions equivalent to 10 g dry soil were taken from each soil sample. One portion was fumigated for 24 h with ethanol-free $CHCl_3$ and was extracted with 40 ml 0.5 M K_2SO_4. A non-fumigated portion was extracted at the same time fumigation commenced. After determination of the C and N concentrations in the extract (Dima-TOC 100, Dima-N, Dimatec, Essen, Germany), the C_{mic} and N_{mic} concentration were calculated as follows: $C_{mic} = E_C / k_{EC}$ where E_C was (C_{org} extracted from fumigated samples) – (C_{org} extracted from non-fumigated samples) and k_{EC} was 0.45 (Joergensen et al. 1995). The same equation was applied for N_{mic} but using $k_{EN} = 0.54$ (Joergensen et al. 1995).

For the determination of basal respiration (Heinze et al. 2009b), all incubation jars contained a CO_2 trap with 5 ml 0.5 M NaOH. The CO_2 traps were replaced after 1, 3, 7, 10, 14, 21, and 28 days and the amount of CO_2 trapped was determined titrimetrically: The CO_2 trapped was precipitated as $BaCO_3$ using 5 ml of saturated $BaCl_2$ solution.

The rate of CO_2 evolved was measured by titration of residual NaOH to pH 8.3 using 0.5 M HCl. The amount of CO_2 evolved (µg (g soil)$^{-1}$ day^{-1}) was calculated as follows:

CO_2-C emission = [((B - S) x M x E / DW) × 1000] / days of incubation [2],

where B is the amount (ml) of acid needed for the titration of the NaOH of the blank; S is the amount of acid (ml) needed for the titration of NaOH in the samples, M is the molarity of the HCl, E is the equivalent weight (6) of C to OH^- ions in the titration reaction, DW is the dry weight of the soil (g).

The metabolic quotient qCO_2 (mg CO_2-C (g C_{mic})$^{-1}$ day^{-1}) after 28 days of incubation was calculated using the following equation (Anderson and Domsch 1993):

$qCO_2 = CO_2\text{-}C / C_{mic}$ / days of incubation × 1000 [3],

where CO_2-C is the cumulative amount of CO_2 evolved in 28 days per gram soil (µg CO_2-C (g soil)$^{-1}$) and C_{mic} is the amount of microbial C per gram soil after 28 days of incubation (µg C_{mic} (g soil)$^{-1}$).

5.2.4. Acid Hydrolysis

In order to determine the biochemically stabilized OM pool, the samples taken in November 2007 were subjected to acid hydrolysis as described by Plante et al. (2006), but slightly modified. Briefly, 0.5 g of sample was treated with 25 ml 6 M HCl for 16 hours under reflux at 100°C. Thereafter, samples were washed three times with 40 ml of distilled H_2O, the supernatant was discarded after centrifugation at 5200 g for 15 minutes and the soil was freeze-dried. The remaining, non-hydrolysable, C_{org} and N fractions were determined by dry combustion (Vario Max, Elementar, Hanau, Germany).

5.2.5. Statistical analysis

Means and standard errors were calculated for each parameter detected within each sampling depth and for each treatment. For the investigation of water-stable aggregate

size classes and of the proportion of biochemically stabilized OM, a t-test was performed to determine differences between both tillage systems. For the investigation of the temporal variation of water-stable aggregates and C_{org} stored within the aggregate size classes between the two sampling dates, a paired t-test was performed for each tillage systems and sampling depth. For the incubation, an ANOVA followed by a Student-Newman-Keuls test including tillage system and aggregate treatment as fixed factors was performed. The different sampling depths were investigated separately. The level of significance was fixed at $\alpha = 0.05$. All statistical analyses were carried out using GLM of Statistica 7, Statsoft.

5.3. Results and Discussion

5.3.1. Total contents of C_{org} and N in soils

Thirty seven years of two different tillage systems resulted in significant differences in C_{org} and N contents in the surface soil: 15.8 g kg^{-1} C_{org} was present directly after tillage in November 2007 in the 0-5 cm depth of the MT system compared to 9.3 g kg^{-1} of the CT system (Table 5). This difference was more pronounced than for the sampling date in March 2007, where 16.3 (MT) and 13.3 (CT) g kg^{-1} were present (Jacobs et al. 2009b). The same pattern as for the C_{org} contents was observed for the N contents in the surface soils. However, in the subsoils, no significant differences between tillage systems were observed for C_{org} and N for either sampling dates. The main reason for the higher C_{org} content in the surface soil in March 2007 was probably that maize stubble had not been incorporated after harvest of maize in September 2006.

In November, five (MT) to six days (CT) after tillage, an intensive turnover of C_{org} in the soil was likely due to the incorporation of harvest residues and due to a possible break-up of aggregates by tillage. Thus, one sampling date with such intensive turnover may result in difficulties in the interpretation of long-term effects of tillage systems on C_{org}

storage. However, in our study, spatial variability of C_{org} contents in November was small (Table 5).

Table 5: Site properties (bulk soil <2 mm) at November 2007 for different tillage systems and sampling depths (means and standard errors, n = 4).

Depth (cm)	Tillage system	C_{org} (g kg^{-1})	N (g kg^{-1})
0-5	conventional	9.3 (0.3)^{A}	1.02 (0.03)^{A}
10-20		9.4 (0.4)^{A}	1.04 (0.03)^{A}
0-5	minimum	15.8 (0.4)^{B}	1.58 (0.02)^{B}
10-20		9.2 (0.7)^{A}	0.95 (0.03)^{A}

Letters indicate significant differences ($p \leq 0.05$) between tillage systems.

A calculation of the C_{org} stocks in the surface soils revealed that between March and November 2007 2.7 (CT) and 0.3 (MT) t C ha^{-1} were lost in the surface soil (Table 6). Such large loss within eight months as observed for the CT treatment is unlikely, since information on systems in a steady state suggest much smaller inputs and outputs. For instance, Ludwig et al. (2007) reported C inputs in the range of 0.9 to 2.4 t C ha^{-1} for winter wheat and 0.6 to 1.2 t C ha^{-1} for spring barley in the Bad Lauchstädt trial and since stocks were approximately constant (with a large spatial variability) in the fertilization treatments, outputs were in the same order. In our study, it has to be noted that spatial variability was large for the CT surfaces soil in March 2007 (Jacobs et al. 2009b). We suggest that spatial heterogeneity was the main reason for the large deviation in C_{org} stocks between sampling dates.

In order to assess the effect of different tillage systems on the entire soil profiles, greater depth ranges than used in our study have to be considered. However, for a site (Garte-Nord) close to our study site (Garte-Süd), Stockfisch et al. (1999) reported slightly higher C_{org} stocks in the 0-50 cm profile in MT than in CT after 20 years of different tillage systems.

Table 6: Bulk densities (n = 4 in March and n = 2 in November) and stocks of C_{org} (n = 4) on an equivalent soil mass approach for the surface soils and subsoils of different tillage systems (CT = conventional tillage; MT = minimum tillage) and two sampling dates.

Tillage system	Sampling date in 2007	Depth (cm)	Bulk density (g cm^{-3})	C_{org} stock (t ha^{-1})
CT	March	0 - 5[1]	1.36 (0.09)	9.04 (1.74)
	November	0 - 5[1]	1.36 (0.19)	6.32 (0.91)
MT	March	0 - 5.4[1]	1.25 (0.04)	11.08 (0.83)
	November	0 - 5.1[1]	1.33 (0.09)	10.74 (0.78)
CT	March	10 - 20[2]	1.49 (0.01)	15.20 (1.34)
	November	10 - 20.1[2]	1.48 (0.01)	14.01 (0.60)
MT	March	10 - 19.7[2]	1.54 (0.01)	17.14 (0.61)
	November	10 - 19.6[2]	1.55 (0.01)	13.71 (1.05)

[1]The volumes of the soils in the considered depths range from 500 to 544 m^3 and the mass is 680 t.
[2]The volumes of the soils in the considered depths range from 961 to 1007 m^3 and the mass is 1490 t.

5.3.2. Temporal variability of occurrence of different water-stable aggregate size classes and OM stored within size-classes

The separation of the soil samples taken in November 2007 into different size classes of water-stable aggregates showed a higher occurrence of macroaggregates (>0.25 mm) in the surface soil (0-5 cm) of MT than of CT (Figure 2c). This was pronounced especially for the size classes 1-2 and 2-10 mm. The subsoil followed the same trend but with no significant differences between the tillage treatments (Figure 2d), thus confirming those detected in March 2007 for the same site (Figure 2a, b; Jacobs et al. 2009b) and also for a tropical agroecosystem (Kushwaha et al. 2001). Jacobs et al. (2009b) outlined that a higher occurrence of water-stable macroaggregates under MT can be attributed (i) to a reduced physical impact by the machinery leading to less disruption of aggregates than under CT or (ii) to a higher formation of macroaggregates due to a higher concentration of OM in the surface soil.

In November 2007 directly after tillage (Figure 2c, d), macroaggregates, especially the size class 0.25-1 mm, were significantly more abundant under MT than in March 2007 after fallow (Jacobs et al. 2009b; Figure 2a, b). The increase of the contents of smaller macroaggregates (0.25-1 mm) with respect to March was larger in CT than MT. This was pronounced in the surface soil but the subsoil followed the same trend. The marked contents of smaller macroaggregates in the CT treatment after tillage suggest that an intense destruction through tillage is unlikely to be the major factor leading to less abundant macroaggregates of the size class 0.25-1 mm. An effect of physical disruption of larger macroaggregates by the plough cannot be ruled out. Moreover, the data suggest that during the cropping of field bean, differences in the aggregate distribution between the two tillage systems have levelled off. The slightly larger contents of macroaggregates under MT suggest that more macroaggregates were formed directly after tillage due to the input of fresh plant residues.

Daraghmeh et al. (2009) reported a significant temporal variation in wet aggregate stability (WAS) over the year for a Danish sandy loam under different tillage systems. They found that WAS was lowest in winter (December) and higher shortly after tillage (October) and in summer. They assigned the low WAS in winter to the interaction of freeze/thaw cycles with soil moisture and the recovery of WAS from spring onwards to increasing biological activity.

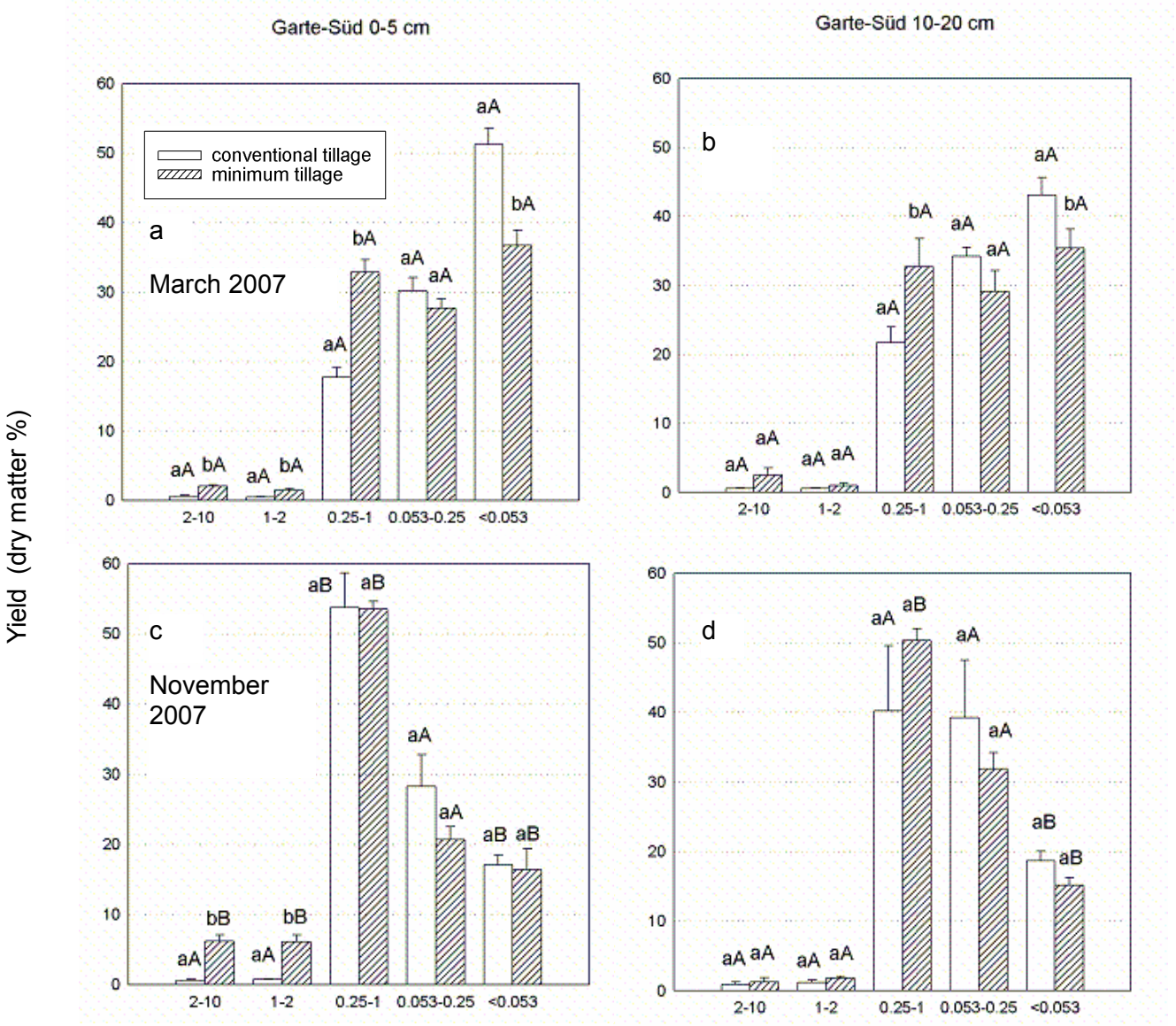

Figure 2: Total yield of water-stable aggregate size classes (means and standard errors, n = 4) among tillage systems and sampling depths for March 2007 (Jacobs et al. 2009; subfigure a, b) and November 2007 (subfigure c, d). Lowercase letters indicate significant differences (p ≤ 0.05) among tillage systems. Uppercase letters indicate significant differences (p ≤ 0.05) between sampling dates.

The calculation of the C_{org} concentration in the size classes of water-stable aggregates in g kg^{-1} soil indicated that the major part of C_{org} (58% and 73% for CT and MT surface soils, respectively, and 59% and 57% for CT and MT subsoils, respectively) was sequestered within macroaggregates (>0.25 mm) in November 2007 (Figure 3b). The respective proportions of N were analogous to C_{org} (data not shown). Thus, macroaggregates, especially the size class 0.25-1 mm, were the most important fraction for sequestration of OM directly after tillage (Figure 3b). However, it has to be noted that OM sequestration within macroaggregates is rather a short-term effect as discussed below. The comparison between the tillage systems revealed a significantly higher amount of OM stored within the macroaggregate fractions of MT than of CT surface soils (Figure 3b). In a comparable tillage system, Kushwaha et al. (2001) also found a high importance of macroaggregates as a location for storing the surplus of OM in MT surface soils. The same trend was reported by Beare et al. (1997) for a NT soil. In March 2007 after fallow, microaggregates (<0.25 mm) were more abundant and more important as a location of C_{org} storage (79% and 55% for CT and MT surface soils, respectively, and 72% and 53% for CT and MT subsoils, respectively) (Figure 2a, b; Figure 3a). However, comparing CT and MT surface soils, the surplus of C_{org} stored under MT was located within the macroaggregate fractions.

Overall, our data suggest that directly after tillage (and thus after residue incorporation), a considerable number of new macroaggregates were formed (as shown for our sampling in November). With ongoing time after tillage (as observed for the sampling in March 2007, which was 10 months after the last tillage and residue incorporation), macroaggregates disrupt into microaggregates, which are assumed to provide longer-term protection for OM (Paustian et al. 2000). This is in accordance with the previous finding that the turnover of macroaggregates is rapid if fresh residue inputs are lacking (e.g. Helfrich et al. 2008). However, two sampling dates are not adequate to provide the basis for accurate quantitative estimates of aggregate

turnover. For instance, Plante et al. (2002) reported that soil macroaggregate mean residence times in different soils ranged from 4 to 95 days, where aggregate dynamics were generally two to three times more rapid in a Gray Luvisol compared to a Black Chernozem. De Gryze et al. (2006) estimated turnover times for macroaggregates and microaggregates to be 30 and 88 days, respectively.

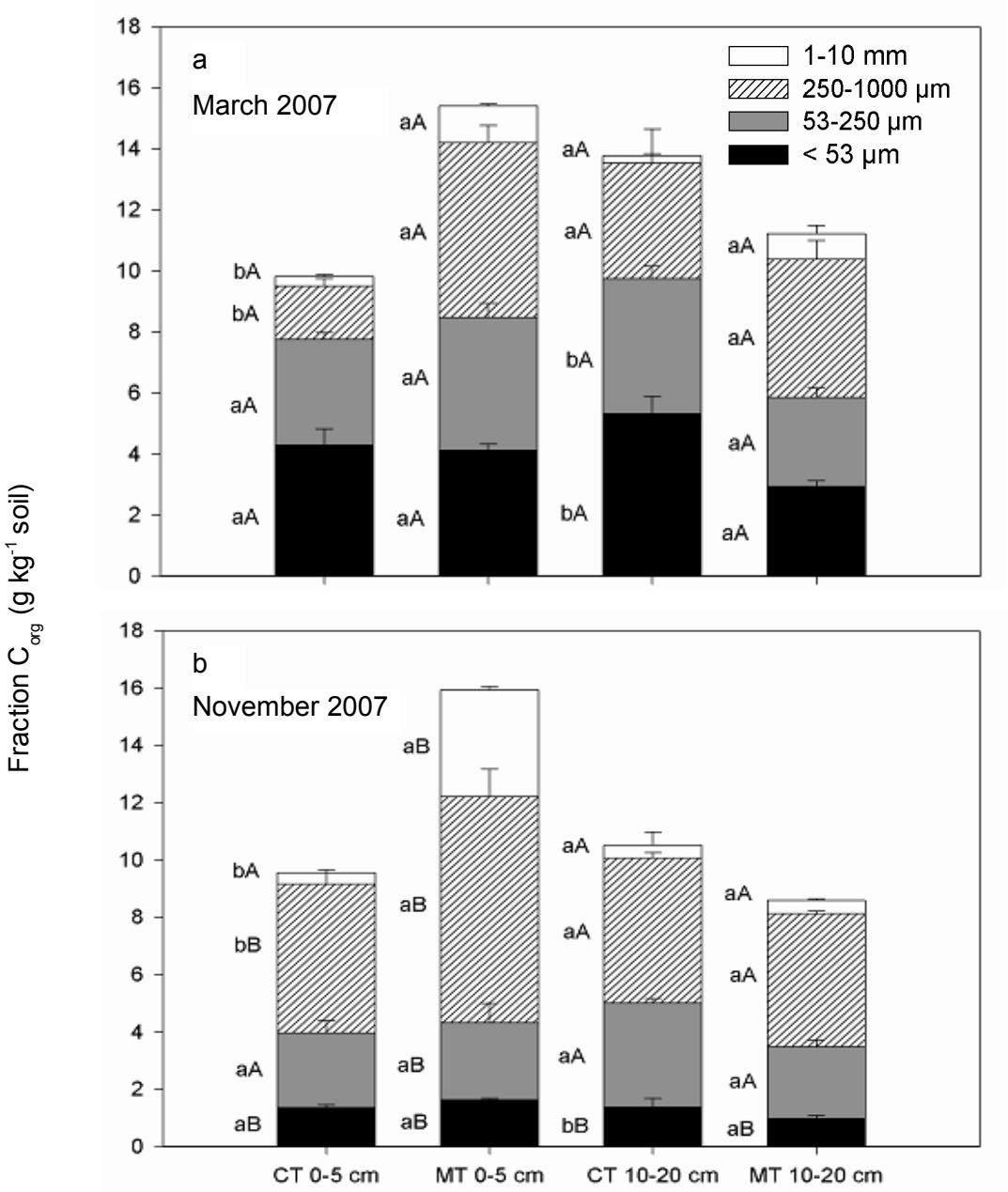

Figure 3: C_{org} concentration of water-stable aggregate size classes (<10 mm) per kg soil (means and standard errors, n = 4) among tillage systems and sampling depths for March 2007 (modified after Jacobs et al. 2009; subfigure a) and November 2007 (subfigure b). Lowercase letters indicate significant differences (p ≤ 0.05) among tillage systems. Uppercase letters indicate significant differences (p = 0.05) between sampling dates.

5.3.3. Effects of tillage system and macroaggregate disruption on OM decomposition and microbial activity within the incubation experiment

After 28 days of incubation at 22°C and a water content of 50% of the maximum water holding capacity, cumulative CO_2-C emission was significantly higher from the MT (MT_{bulk}, MT_{md}) than from the CT (CT_{bulk}, CT_{md}) surface soils (Figure 4). Further, the specific respiration (CO_2-C:C_{org}) expressed a higher C mineralization in MT_{bulk} surface soils (Table 7). Higher potential OM mineralization in MT or NT surface soils (compared to CT) were also found by Alvarez et al. (1995), Salinas-Garcia et al. (1997), Wright et al. (2005), and Oorts et al. (2006). Thus, it is well established that a higher concentrated input of organic residues into the surface soil under MT results in an increased potential mineralization. In the subsoil, the cumulative and the specific respiration of the MT system were lower than in the surface soil while it generally remained at the same level for CT soils (Figure 4, Table 7). Overall, C mineralization in the subsoils was higher from CT than from MT with significant differences between CT_{bulk} and MT_{bulk}. The main reason for this is that harvest residues are concentrated in the surface soil in the MT treatments. In the CT treatment, an accumulation of the harvest residues occurred mainly in the lower third of the Ap horizon.

The incubation of md soils (MT_{md}, CT_{md}) did not generally result in a significantly higher C mineralization compared to the respective bulk soils, except for the MT_{md} subsoil (Figure 4). These results indicate that the effect of physical stabilization of OM stored in the macroaggregates should not principally be seen as a mechanism protecting very labile C with a turnover time of weeks. A different explanation could be that the formation of new macroaggregates is sufficiently fast to diminish the effect of destroying macroaggregates within a few days. For instance, Bossuyt et al. (2001) found 25% of the bulk soil in new-built macroaggregates after 6 days of incubation. However, for the latter explanation, marked differences in the respiration would be

expected in the initial phase of the incubation which were not observed for the MT surface soil (Figure 4).

In their review, Balesdent et al. (2000) showed for most incubated soils an increase of mineralization rates after destruction of macroaggregates, except for soils with very low clay contents. Ratios of C mineralization of crushed soils to uncrushed soils generally ranged from 0.75 to 2.3 with an extreme value of 10.8. Recently, Oorts et al. (2006) reported a reduction of potential C mineralization after destruction of macroaggregates in a NT surface soil. Overall, different incubation conditions and crushing methods make interpretations difficult and indicate the need for standardization of aggregate destruction and of incubation procedure to achieve any truly reliable quantitative understanding of OM protection in soils.

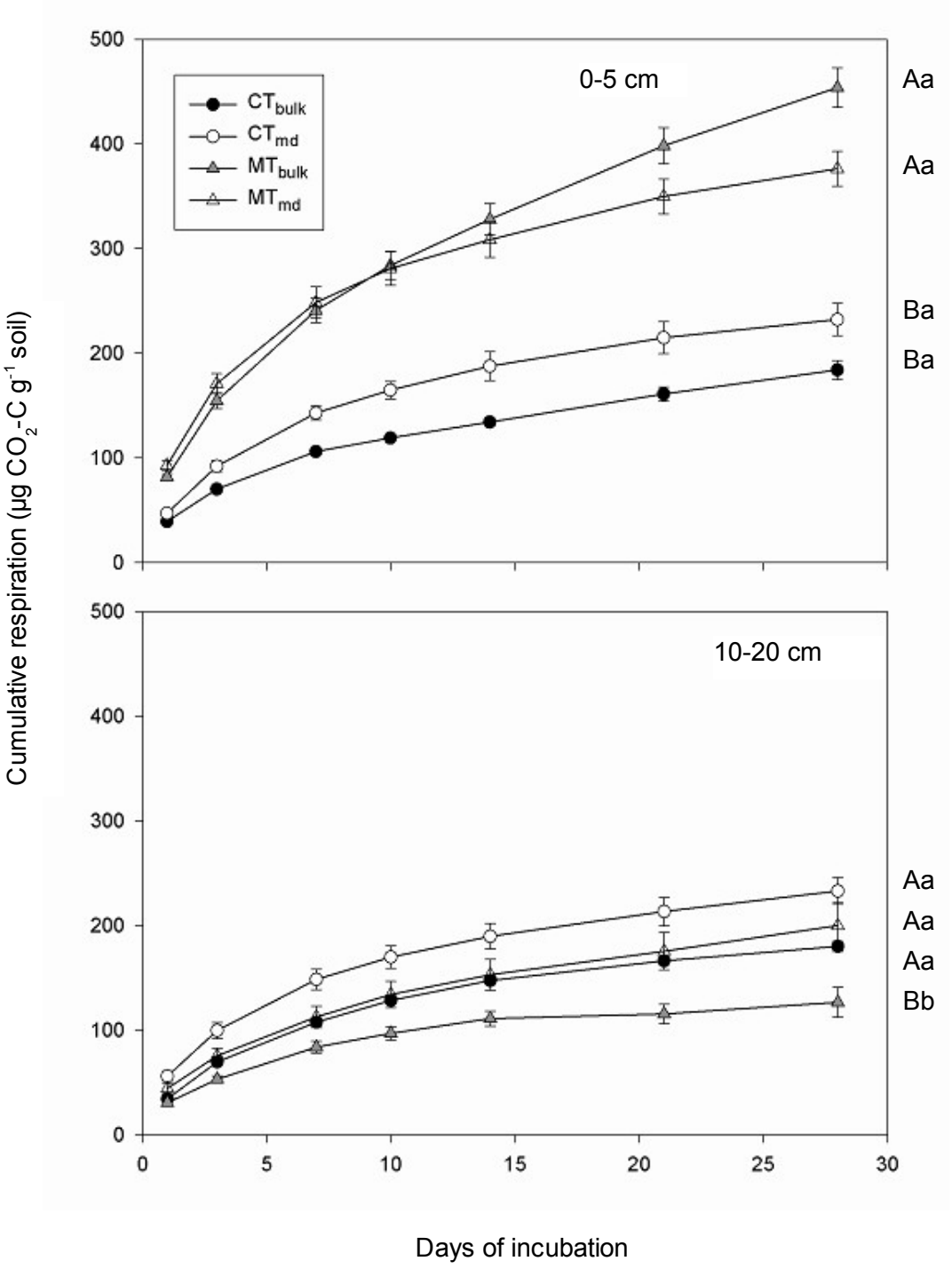

Figure 4: Cumulative basal respiration during incubation among tillage systems, aggregate treatments, and sampling depths (means and standard errors, n = 4). Capital letters indicate significant differences (p ≤ 0.05) between tillage systems with the same aggregate treatment (CT_{bulk} vs MT_{bulk}, CT_{md} vs MT_{md}); lowercase letters indicate significant differences (p ≤ 0.05) between aggregate treatments of the respective tillage treatment (CT_{bulk} vs CT_{md}, MT_{bulk} vs MT_{md}). Probability values were: aggregate treatment 0.41, tillage system <0.01, aggregate treatment x tillage system <0.01 at 0-5 cm; and aggregate treatment <0.01, tillage system 0.01, aggregate treatment x tillage system 0.39 at 10-20 cm.

The determination of the microbial biomass showed large differences between the tillage systems after 28 days of incubation. In the surface soil, C_{mic} was 3.6 and 4.0 times greater in the MT_{bulk} and MT_{md} treatments, respectively, compared to the respective CT treatments. In the subsoil, CT soil showed higher values but differences between tillage treatments were much less pronounced than in the surface soil (Table 7). Further, substrate availability to microorganisms, indicated by the C_{mic}:C_{org} ratio, was significantly higher in MT_{bulk} and MT_{md} than in the respective CT surface soils, while no differences between tillage systems were found in the subsoil (Table 7). The differences found between MT and CT in the distribution of OM (Table 5) and of microbial biomass (Table 7) agree well with findings of various other studies (e.g. Ahl et al. 1998; Kushwaha et al. 2001; Spedding et al. 2004) and are assigned to the difference in the depth of incorporation of plant residues between both tillage systems (Kandeler et al. 1999a; Stockfisch et al. 1999; Kushwaha et al. 2001; Jacobs et al. 2009b).

Table 7: Microbial biomass and different indices of organic matter decomposition after 28 days of incubation (means and standard errors, n = 4) for the incubation experiment with CT (conventional tillage) and MT (minimum tillage) bulk soils (CT_{bulk}, MT_{bulk}) and soils with destroyed macroaggregates (CT_{md}, MT_{md}).

Sampling depth (cm)	Soil	C_{mic} (µg g^{-1})	N_{mic} (µg g^{-1})	C_{mic}:C_{org} (%)	CO_2-C/C_{org} (%)	qCO_2
0-5	CT_{bulk}	99 (12)^{Aa}	17 (3)^{Aa}	1.01 (0.09)^{Aa}	2.03 (0.17)^{Aa}	70 (8)^{Aa}
	CT_{md}	56 (11)^{Aa}	11 (2)^{Aa}	0.57 (0.10)^{Ab}	2.49 (0.21)^{Aa}	168 (39)^{Ab}
	MT_{bulk}	358 (27)^{Bc}	44 (7)^{Bb}	2.32 (0.15)^{Bc}	2.85 (0.10)^{Ba}	45 (1)^{Aa}
	MT_{md}	222 (17)^{Bb}	28 (6)^{Aa}	1.49 (0.09)^{Bd}	2.50 (0.13)^{Aa}	62 (5)^{Ba}
	probability values					
	aggregate treatment	<0.01	0.05	<0.01	0.72	0.01
	tillage system	<0.01	<0.01	<0.01	0.02	<0.01
	aggregate treatment x tillage system	0.02	0.32	0.09	0.03	0.06
10-20	CT_{bulk}	131 (4)^{Aa}	16 (2)^{Aa}	1.38 (0.07)^{Aa}	1.92 (0.07)^{Aa}	49 (3)^{Aa}
	CT_{md}	117 (15)^{Aa}	11 (2)^{Ab}	1.17 (0.14)^{Aa}	2.32 (0.07)^{Aa}	74 (11)^{Ab}
	MT_{bulk}	118 (6)^{Aa}	13 (1)^{Ab}	1.38 (0.10)^{Aa}	1.41 (0.22)^{Ab}	38 (3)^{Aa}
	MT_{md}	84 (6)^{Bb}	8 (0)^{Ab}	0.95 (0.05)^{Ab}	2.32 (0.25)^{Aa}	86 (9)^{Ab}
	probability values					
	aggregate treatment	0.02	<0.01	<0.01	<0.01	<0.01
	tillage system	0.03	0.08	0.26	0.16	0.98
	aggregate treatment x tillage system	0.27	0.94	0.27	0.16	0.13

Capital letters indicate significant differences ($p \leq 0.05$) between tillage systems with the same aggregate treatment (CT_{bulk} vs MT_{bulk}, CT_{md} vs MT_{md}); lowercase letters indicate significant differences ($p \leq 0.05$) between aggregate treatments of the respective tillage treatment (CT_{bulk} vs CT_{md}, MT_{bulk} vs MT_{md}).

The qCO_2 was generally lower in MT than in CT soils, suggesting a higher efficiency of C_{org} incorporation into the microbial biomass for these treatments. Moreover, macroaggregate disruption increased the qCO_2 in both depths and for both tillage treatments (Table 7). This may have been mainly due to stress of the microbial community by physical disruption (Anderson and Domsch 1989) and an increased accessibility to OM which led to higher respiration of the microorganisms. However, changes in qCO_2 may also be caused by shifts in the composition of the microbial community since fungi respire less C per unit of biomass C than bacteria (Nannipieri et al. 2003). Furthermore, other factors (e.g. changes in pH; Wardle and Ghani 1995) may influence the qCO_2. Thus, the qCO_2 is generally used when systems are at or near equilibrium.

5.3.4. Effect of long-term tillage on recalcitrant, biochemically stabilized OM

In the surface soil, the content of non-hydrolysable C was slightly greater in the MT soil than in the CT soil (Figure 5). Much more pronounced, however, was the large content of hydrolysable C in the MT soil compared to the CT soil. The proportion of non-hydrolysable, C_{org} to total C_{org} was even lower in the MT than in the CT soils (Figure 5). Hydrolysable and non-hydrolysable N followed the same trend (Figure 5). The non-hydrolysable fraction is regarded as a biochemically stabilized, recalcitrant SOM pool (Paul et al. 2006). Thus, our data for the surface soils indicate that biochemical stabilization of OM is not the main reason for the surplus of OM found in the surface soil of MT.

In the subsoil, the proportion of non-hydrolysable C_{org} to total C_{org} was significantly lower in MT than in CT while no differences were detected for N. The lower contribution of non-hydrolysable, biochemical recalcitrant, C_{org} to total C_{org} found in MT soils of our study were in line with the values described by Collins et al. (2000) who found similar or slightly decreased values for NT than for CT soils. Further, our findings are

consistent with the general view that the proportion of resistant SOM will increase with SOM depletion (e.g. Kiem et al. 2000; Plante et al. 2004).

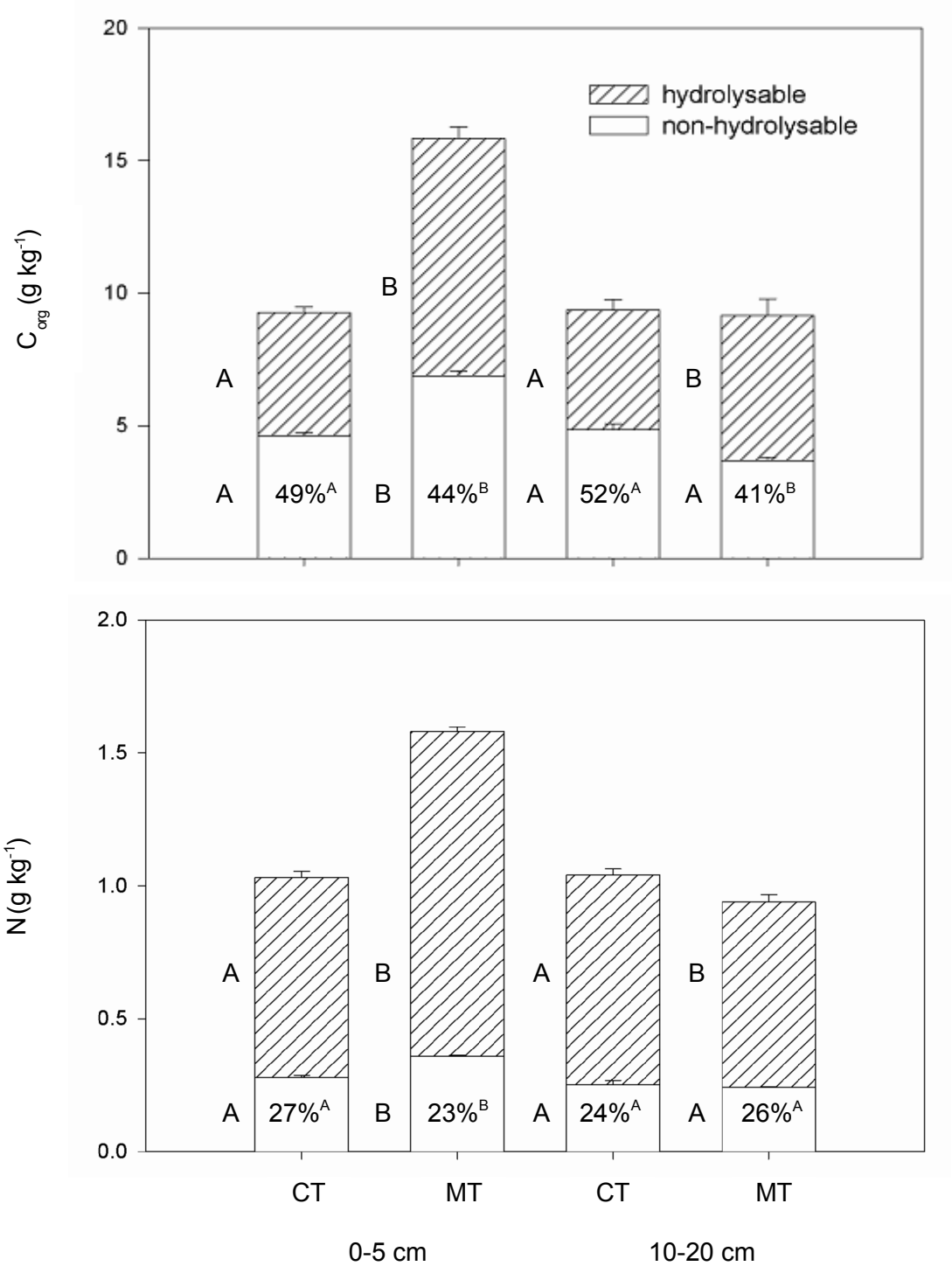

Figure 5: Concentrations of hydrolysable and non-hydrolysable C_{org} and N among tillage systems and depths (means and standard errors, n = 4). Percentages give the contribution of non-hydrolysable C_{org} and N to total C_{org} and total N. Letters indicate significant differences ($p \leq 0.05$) among tillage systems.

5.4. Conclusions

This study indicates that a lower occurrence of water-stable macroaggregates in a CT soil compared to a MT soil cannot be mainly attributed to a physical disruption of aggregates by ploughing. Overall, our data suggest that directly after tillage and residue incorporation, considerable amounts of new macroaggregates were formed, especially in the MT surface soil due to the high concentration of crop residues. In the short-term, these water-stable macroaggregates are an important fraction storing the surplus of OM found under MT compared to CT systems. Further, it was shown that the surplus of OM in MT is stored in a biochemically degradable fraction, especially in the surface soil. With ongoing time after tillage, macroaggregates disrupt into stabilized microaggregates which provide longer preservation of OM.

Acknowledgements

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6. Soil aggregate dynamics in an incubation experiment with ^{15}N-maize: effects of residue location

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Abstract

Differences in the mechanisms of storage and decomposition of organic matter (OM) between minimum tillage (MT) and conventional tillage (CT) are generally attributed to differences in the physical impact through tillage but less is known about the effects of residue location. We conducted an incubation experiment at a water content of 60% of the maximum water holding capacity and at 15°C with soils from CT (0-25 cm tillage depth) and MT (0-5 cm tillage depth) fields with ^{15}N-labelled maize straw incorporated to different depths (CT simulations: 0-15 cm; MT simulations: 0-5 cm) for 28 days. Aims were to determine the effects of the tillage simulation on (i) mineralization of recently added residues, (ii) the dynamics of macroaggregate formation and physical protection of OM, and (iii) the partitioning of maize-derived C and N within soil OM fractions. The MT simulations showed lower relative C-losses and the amount of maize-C mineralized after 28 days of incubation was slightly, but significantly lower in the MT simulations with maize added (MT_{maize}) than in the respective CT (CT_{maize}) simulations. The formation of new water-stable macroaggregates occurred during the phase of the highest microbial activity with a maximum peak 8 days after the start of incubation. The new-formed macroaggregates were an important location for the short-term

stabilization of C and N with a higher importance for MT_{maize} than for CT_{maize} simulations. In conclusion, our results indicate that the higher amounts of OM found in MT soils compared with CT soils result from an increased macroaggregate formation and from a higher efficiency of C retention due to a more concentrated residue input in the surface horizon.

Keywords

conventional tillage, minimum tillage, partitioning of C and N, tillage simulation, water-stable aggregates, ^{15}N, ^{13}C

6.1. Introduction

The replacement of conventional tillage (CT) by reduced tillage systems is broadly discussed due to the associated increase in soil quality. Tillage systems with reduced physical impact and shallower depth of residue incorporation were found to increase OM concentration (Meyer et al. 1996; Salinas-Garcia et al. 1997; Kandeler et al. 1999a; Stockfisch et al. 1999; Wright et al. 2005), to enhance physical protection of OM within macroaggregates (Kushwaha et al. 2001; Jacobs et al. 2009b), and to result in a higher microbial biomass (Kandeler et al. 1999a; Stockfisch et al. 1999) compared to CT systems. Higher potential C and N mineralization rates were found for various soils under reduced tillage management (Alvarez et al. 1995; Salinas-Garcia et al. 1997; Ahl et al. 1998; Wright et al. 2005; Wright et al. 2008). Thus, decreasing tillage intensity was found to result in an accumulation of readily degradable OM, especially in the surface soil (Van Den Bossche et al. 2009). Furthermore, changes in the microbial community (Ahl et al. 1998) and in soil physical properties (Balesdent et al. 2000; Alvarez and Steinbach 2008) were reported.

The increased occurrence of stable macroaggregates under reduced or no-tillage systems has been reported as an important factor for stabilization of a surplus of OM

by physical protection (Wander and Bidart, 2000; Six et al. 2000b; Oorts et al. 2007; Zotarelli et al. 2007). Despite seasonal variations (Yoo and Wander 2008; Jacobs et al. 2009a), macroaggregates were shown to be more important as a location of storage of OM under minimum tillage (MT; maximum depth of 10 cm) than under CT (Jacobs et al. 2009a) and it is broadly assumed that macroaggregate-turnover is accelerated under CT due to the physical impact of the plough which disrupts macroaggregates (Six et al. 2000b; Bronick and Lal 2005).

Besides the physical impact, CT and MT differ also in the depth of residue incorporation. In tillage systems with a shallow tillage depth (e.g. MT; maximum depth of 10 cm), the input of residues into the surface soil is more concentrated compared to CT soils where the residues are more or less distributed among the tilled horizon. Consequently, substrate availability to microorganisms is increased in the surface soils under MT (Kandeler et al. 1999a; Stockfisch et al. 1999; Kushwaha et al. 2001). An enhanced microbial activity in turn increases the availability of microbial products which serve as binding agents for the formation and the cementation of aggregates (Kandeler and Murer 1993; Kushwaha et al. 2001; Hernández-Hernández and López-Hernández 2002; Martens 2000; Oorts et al. 2007). However, detailed studies on the effect of the location of residue input on OM mineralization and stabilization are scarce.

We conducted a short-term laboratory incubation with differently tilled soils and with labelled maize residues (^{15}N enriched, ^{13}C natural abundance) incorporated to different depths. The objectives of our study were to follow the initial mechanisms of residue transformation and translocation in these soils by investigation of (i) mineralization dynamics, (ii) dynamics of macroaggregate formation, and (iii) the partitioning of residue derived C and N added into aggregate size classes and CO_2-C in CT and MT systems simulated.

6.2. Materials and Methods

6.2.1. Site and soils

Soil samples from the long-term experimental site “Hohes Feld” (near Göttingen, Germany) were used in this study. Mean annual precipitation of the site is 645 mm and mean annual temperature is 8.7°C (30-years' average 1961-1990 by Deutscher Wetterdienst 2007). The soil type is a Haplic Luvisol (WRB) derived from loess (Ehlers et al. 2000; Reiter et al. 2002). The soil texture (0-30 cm) consists of 17.2% clay, 66.5% silt, and 16.4% sand (De Mol 1996). Two treatments are implemented in the field since 1967: CT with a regular mouldboard plough to a depth of 25 cm and MT with a rotary harrow to a depth of 5-8 cm. Soil samples were taken from the tilled layer (CT: 0-25 cm, MT: 0-5 cm) in August 2008 directly after the harvest of winter wheat and air dried. Macroaggregates were physically broken by a mortar and the soil was passed through a 250 µm mesh in order to destroy all naturally occurring macroaggregates and to allow a homogeneous application of residues.

6.2.2. Plant material

Maize plants were grown in pots under greenhouse conditions. The plants were watered with a nutrient solution containing 200 mg l^{-1} $^{15}NH_4NO_3$ (10% labelled ammonium) in order to obtain two stable isotope tracers for the incubation experiment (^{15}N from the nutrient solution and ^{13}C of natural abundance). Maize leaves were harvested when all plants had reached the flowering-stage and oven-dried at 60°C. Leaves were chopped <1000 µm (SM1 Retsch, Haan, Germany) and sieved >250 µm.

6.2.3. Incubation experiment

Microcosms consisted of a soil column of 15 cm height in glass jars (1500 cm^3) containing 1022.7 g dried and sieved (<250 µm) CT and MT soil, respectively. Bulk density was similar to field conditions after tillage (1.0 g cm^{-3}). In order to match the varying conditions in the field, incorporation of maize residues was varied for CT and

MT microcosms. Thus, the following treatments with 16 replications each were applied (i) "CT_{maize}" (CT soil with maize residues homogeneously distributed among the entire microcosm), (ii) "$CT_{control}$" (CT soil without addition of residues), (iii) "MT_{maize}" (MT soil with maize residues homogeneously distributed within in the 0-5 cm layer (equivalent to 340.9 g dry soil)), and (iv) "$MT_{control}$" (MT soil without addition of residues). For the maize treatments, 6.18 g of maize residues (2.72 g maize-C) were added to each microcosm. This addition is equivalent to 2.66 g maize-C kg^{-1} soil and 0.24 g maize-N kg^{-1} soil and corresponds to twice the estimated amount as incorporated under field conditions (Denef et al. 2001). Since the maize residues were homogeneously distributed in the CT_{maize} treatment, each of the soil layers 0-5 cm, 5-10 cm, and 10-15 cm received 2.66 g maize-C kg^{-1} soil. In the MT_{maize} treatment, the addition was 7.99 g maize-C kg^{-1} in the 0-5 cm layer and nil in the 5-10 and 10-15 cm layers. Dry soil and maize residues were mixed before filled into the glass jars. Microcosms were slowly and homogeneously brought to 60% of the maximum water holding capacity with distilled water and were incubated in a climate chamber at 15°C for 28 days. The jars were covered with an air-permeable foil to allow aeration and to prevent desiccation of the microcosm. Water content was controlled during the incubation period by weighing the jars and was corrected with distilled water when necessary. At days 3, 8, 14, 28 after the start of incubation, four microcosms of each treatment were sampled destructively from the trial and the soil was analysed for several characteristics of decomposition dynamics (see below). At each sampling date, the soil of the respective microcosms was divided into two portions: (i) the 0-5 cm layer and (ii) the 10-15 cm layer. Initial soil properties were determined at day 0 as starting point of incubation (Table 8).

Table 8: Concentrations of C, N, isotopic data, and 0.05 M K_2SO_4-extractable microbial biomass (C_{MB}, N_{MB}) of differently tilled soils and of maize leaves used for the incubation; means and standard errors (soil: n = 2; maize: n = 3).

	Tillage system	C (g kg^{-1})	$\delta^{13}C$ (‰ V-PDB)	N (g kg^{-1})	^{15}N (atom%)	C_{MB} (µg g^{-1})	N_{MB} (µg g^{-1})
soil	conventional	12.3 (0.3)	-27.6 (1.9)	1.22 (0.00)	0.37 (0.0)	53 (4)	13 (1)
	minimum	18.1 (0.0)	-26.5 (0.8)	1.77 (0.01)	0.37 (0.0)	126 (6)	21 (1)
maize leaves		440.6 (5.9)	-13.5 (0.5)	39.82 (1.44)	1.64 (0.02)		

6.2.4. Gas measurements

Four replicates of each treatment were connected to an automated gas chromatographic system as described by Loftfield et al. (1997). The headspace of the microcosm was continuously flushed by fresh air (40 ml min^{-1}) through an air inlet port. CO_2 concentration emitted from the microcosm and of the fresh air were analysed every 2.25 hours for the entire period of incubation through an air outlet port (KNK-3000-HRGO, Konik, Barcelona, Spain). The CO_2 and the $^{13}CO_2$ concentrations of the fresh air were analysed the same way by using blank microcosms without soil. After 1, 2, 3, 6, 9, 14, and 27 days of incubation, gas for $^{13}CO_2$ determinations was sampled through a branch connection tube with a 50 ml syringe and was injected into gas vials (10 ml). The $\delta^{13}C$ values (‰ V-PDB) of all gas samples were determined by GC-IRMS (GC-GP Interface, Finnigan MAT, Bremen, Germany; Delta plus, Finnigan MAT, Bremen, Germany). The $\delta^{13}C$ values of the CO_2 produced (δ_{resp}) by the soil incubated was calculated as follows (Eq.4):

$$\delta_{resp} = \frac{\left(\delta_{out} - \delta_{in} \times \frac{CO_2 in}{CO_2 out}\right)}{CO_2 out - CO_2 in} \times CO_2 out, \quad [4],$$

where δ_{out} was the $\delta^{13}C$ value (‰ V-PDB) of the gas sample, δ_{in} was the $\delta^{13}C$ value (‰ V-PDB) of the fresh air flushed into the microcosm, CO_2in was the CO_2 concentration (ppm) of the fresh air flushed into the microcosm, and CO_2out was the CO_2 concentration (ppm) of the gas sample. After four weeks of incubation, the CO_2 concentration of the gas emitted from the microcosms was too low for accurate determination of $^{13}CO_2$. Thus, microcosms were closed for 20 minutes for CO_2-enrichment prior to sampling.

6.2.5. Analysis of microbial C and microbial N

An aliquot of the the 0-5 cm and of the 10-15 cm layer, respectively, was analysed for 0.05 M K_2SO_4-extractable microbial C (C_{MB}) and N (N_{MB}) by chloroform fumigation-

extraction after Vance et al. (1987). Briefly, 10 g of soil were exposed to ethanol-free $CHCl_3$ for 24 h and then extracted with 40 ml of 0.05 M K_2SO_4. Differently from the standard method, 0.05 M K_2SO_4 was used for extraction in order to reduce the quantity of salt in the extracts which would have hampered the determination of the isotopic composition within the extracts (Potthoff et al. 2005). For each sample, a control was performed by extracting a non-fumigated sample. C and N concentrations and the isotopic composition of C and N were detected in fumigated and non-fumigated freeze-dried extracts by EA-IRMS (EuroVektor, HEKAtech GmbH, Wegberg, Germany; Delta plus, Finnigan MAT, Bremen, Germany). C_{MB} concentrations were calculated as follows: C_{MB} = (organic C extracted from fumigated samples) – (organic C extracted from non-fumigated samples). The same equation was applied correspondingly for N_{MB}. A conversion factor, as usually required for the calculation of microbial biomass C and N after Joergensen et al. (1995), was not applied since these factors are validated for extractions using 0.5 M K_2SO_4 only.

The $\delta^{13}C$ of the C_{MB} was calculated using mass balance:

$$\delta C_{MB} = \frac{(\delta C_F \times C_F - \delta C_{NF} \times C_{NF})}{C_{MB}} \quad [5],$$

where δC_F is the $\delta^{13}C$ value (‰ V-PDB) of the extract after fumigation, C_F is the C concentration (µg g^{-1}) in the extract after fumigation, δC_{NF} is the $\delta^{13}C$ value (‰ V-PDB) of the non-fumigated extract, and C_{NF} is the C concentration (µg g^{-1}) in the non-fumigated extract. The ^{15}N values (atom%) of the 0.05 M K_2SO_4-extractable microbial biomass N were calculated correspondingly.

To calculate the specific respiration, the C_{MB} concentrations of the entire microcosms of day 28 were used. For the CT_{maize} and the control simulations, the mean value of the 0-5 cm and the 10-15 cm layer was used to calculate C_{MB} concentrations of the entire microcosm, while for the MT_{maize} simulations, it was assumed that the 5-10 cm layer had the same C_{MB} concentrations as the 10-15 cm layer.

6.2.6. Separation of different water-stable aggregate size classes

All soil portions were oven-dried at 40°C for 48 hours before separation into different aggregate size classes. The fractionation procedure described by Jacobs et al. (2009b) was performed in order to investigate the following size classes: >250 µm (macroaggregates; Tisdall and Oades 1982), 53-250 µm (micoraggregates), and <53 µm fraction (finest microaggregates + silt and clay). For this site, Jacobs et al. (2009b) showed that the calculation of the C and N concentrations within aggregate size classes on a sand-free basis (Elliott et al. 1991) did not have a significant effect on the results of the distribution of OM among the size classes investigated. Thus, in this study, C and N concentrations of the respective aggregate size classes are expressed on a non-sand-free basis. In order to determine the maize-derived C and N occluded within the aggregates, the free particulate organic matter (fPOM) outside of the aggregates was separated from the macroaggregate fraction (Angers et al. 1997) by performing a density fractionation as described by Jacobs et al. (2009b). Briefly, all macroaggregates were soaked in 40 ml of a sodiumpolytungstate solution (SPT) (Sometu, Berlin, Germany) with a density of 1.6 g cm^{-3}. The sample was allowed to equilibrate for 30 minutes and then centrifuged at 4000 x g for 30 minutes (Multifuge 3 S-R, Heraeus, Hanau, Germany). The supernatant (fPOM) was vacuum filtered (<0.45 µm; 72 Whatmann OE67, cellulose acetate) and washed with 2 l of distilled water in order to avoid a contamination by SPT. The fPOM gained was dried at 40°C for 48 hours and weighed. The amount of fPOM recovered was corrected for contamination by soil particles of a density <1.6 g cm^{-3} by determination of the ash content (5 h at 550°C) within the fraction. The pellet remaining in the centrifugation tube was washed three times with distilled water to remove the SPT. In order to precipitate the silt and clay particles, 0.5 M $AlCl_3$ (2 drops on 40 ml) was added. After 24 hours, the supernatant was siphoned off, the pellet was freeze-dried, and weighed. The freeze-dried pellets were ball milled (Retsch, Haan, Germany), dried at 105°C and

analysed for the concentrations and the isotopic compositions of C and N (EuroVektor, HEKAtech GmbH, Wegberg, Germany; Delta plus, Finnigan MAT, Bremen, Germany).

6.2.7. Calculations of maize-derived C and N

The percentage contribution of the maize-derived C in the fractions investigated was calculated by Eq. 6 according to Balesdent and Mariotti (1996):

$$f = \frac{(\delta_{maize} - \delta_{control})}{(\delta_{maizestraw} - \delta_{soil})} \times 100 \quad [6],$$

where f (%) corresponds to the proportion of maize-derived C in the fraction, δ_{maize} is the $\delta^{13}C$ value (‰ V-PDB) in the fraction of the maize treatment, $\delta_{control}$ is the $\delta^{13}C$ value (‰ V-PDB) in the respective fraction of the control treatment, $\delta_{maize\ straw}$ is the $\delta^{13}C$ value (‰ V-PDB) of the maize straw added to the maize treatments, and δ_{soil} is the $\delta^{13}C$ value (‰ V-PDB) of the soil of the respective control treatment and sampling day. The proportion of maize-derived N was calculated correspondingly.

6.2.8. Statistical analysis

Means and standard errors were calculated for each parameter detected for the different treatments and layers. Significant effects of the factor “tillage simulation” were calculated by a t-test for those parameters regarding the entire microcosm only. In this case, statistics for maize and control treatments were calculated separately. The level of significance was set to $\alpha = 0.05$. All statistical analysis were carried out using GLM of Statistica 7, Statsoft.

6.3. Results

6.3.1. CO_2 emission and OM mineralization in different tillage simulations

During the initial three days of the incubation, the MT_{maize} and the $MT_{control}$ simulations showed a more pronounced peak in CO_2-C evolution than the CT_{maize} and the $CT_{control}$ simulations, respectively (Figure 6). During the further course of incubation,

respiration rates of CT and MT simulations were at a similar level. Due to the higher starting peak, the cumulative CO_2-C emissions after 27 days of incubation from the $MT_{control}$ and MT_{maize} simulations were higher ($p \leq 0.05$) by 3% than from the respective CT simulations (Table 9). However, the cumulative emission of maize-derived CO_2-C was lower ($p \leq 0.05$) by 10% from MT_{maize} than from CT_{maize} simulations (Table 9, Table 10). The difference between the cumulative emission of total CO_2-C and of the maize-CO_2-C for the maize treatments showed that the addition of maize residue also increased the mineralization of non-maize-derived C compared to the control treatments (priming effect) (Table 9).

The total and the maize-derived specific respirations CO_2-C:C, CO_2-C:C_{MB}, and maize-derived-CO_2-C:C_{MB} were significantly lower in the MT than in the CT simulations for the control and for the maize treatments (Table 9).

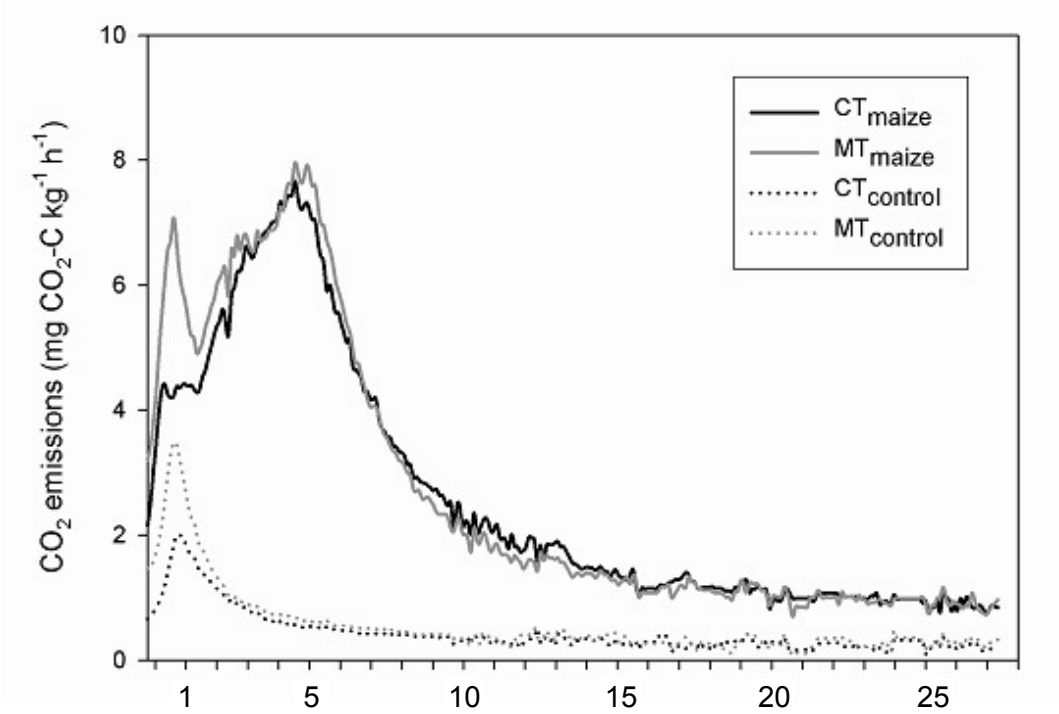

Figure 6: CO_2 emission rate from the microcosms (0-15 cm) during 28 days of incubation for the control treatments ($MT_{control}$, $CT_{control}$) and the maize treatments (MT_{maize}, CT_{maize}) in minimum and conventional tillage simulations; means of n = 4.

Table 9: Cumulative emission of total and maize-derived CO_2 from the microcosms (0-15 cm) and the specific respiration (CO_2-C:C; CO_2-C:C_{MB}) for total and for maize-derived (maize) CO_2 for control ($CT_{control}$, $MT_{control}$) and maize treatments (CT_{maize}, MT_{maize}) of tillage simulations after 27 days of incubation; means and standard errors (n = 4).

Tillage simulation	Total CO_2 (mg CO_2-C kg^{-1} soil)	Maize-CO_2 (mg CO_2-C kg^{-1} soil)	Total CO_2-C:C (%)	Total CO_2-C:C_{MB}	Maize-CO_2-C:C_{MB}
CT_{maize}	1699 (13)^{A}	1276 (16)^{A}	11.36 (0.09)^{A}	360 (3)^{A}	270 (3)^{A}
$CT_{control}$	284 (4)^{A}		2.31 (0.03)^{A}	142 (2)^{A}	
MT_{maize}	1751 (5)^{B}	1153 (8)^{B}	8.50 (0.02)^{B}	299 (1)^{B}	197 (1)^{B}
$MT_{control}$	355 (4)^{B}		1.96 (0.02)^{B}	95 (1)^{B}	

Letters indicate a comparison among tillage simulations where maize (CT_{maize}, MT_{maize}) and control treatments ($CT_{control}$, $MT_{control}$) were investigated separately; values which were significantly different at $p \leq 0.05$ are followed by different letters.

6.3.2. Dynamics of the microbial biomass and assimilation of recently added OM in different tillage simulations

Concentrations of C_{MB} and N_{MB} were higher in the MT than in the CT soil used for incubation (Table 8, Figure 7a, b). During the course of incubation, the control treatments did not show marked dynamics in the total C_{MB} and N_{MB} concentrations for both soils (data not shown). Furthermore, in the MT_{maize} and CT_{maize} simulations, the amount of non-maize-derived C_{MB} resembled the respective control treatments after 28 days of incubation (Figure 7a). Thus, in the MT_{maize} and CT_{maize} simulations, the dynamics of the C_{MB} concentration in the 0-5 cm layers occurred mainly via the incorporation of maize-C into the microbial biomass (Figure 7a). Eight days after the start of the incubation, the C_{MB} concentrations in the MT_{maize} and CT_{maize} simulations reached a maximum (Figure 7a) and were 4.2 and 2.7 times higher, respectively, than in the respective control treatments.

The progress of the N_{MB} concentration showed the same trend. However, in contrast to C_{MB}, the addition of maize residues resulted in a marked increase in the non-maize-derived N_{MB} fraction (Figure 7b) possibly through the assimilation of soil-derived mineral N. Moreover, in the 0-5 cm layer, the microbial biomass incorporated a higher proportion of the added maize-N (7-11% for CT_{maize} and 5-9% for MT_{maize}) than of maize-C (4-6% for CT_{maize} and 3-5% for MT_{maize}) (data not shown).

Generally, the concentrations of C_{MB} and N_{MB} detected for the 10-15 cm layer of the CT_{maize} simulations were equal to the respective 0-5 cm layer while the values of the 10-15 cm layer of the MT_{maize} simulations were equal to the $MT_{control}$ simulations (data not shown).

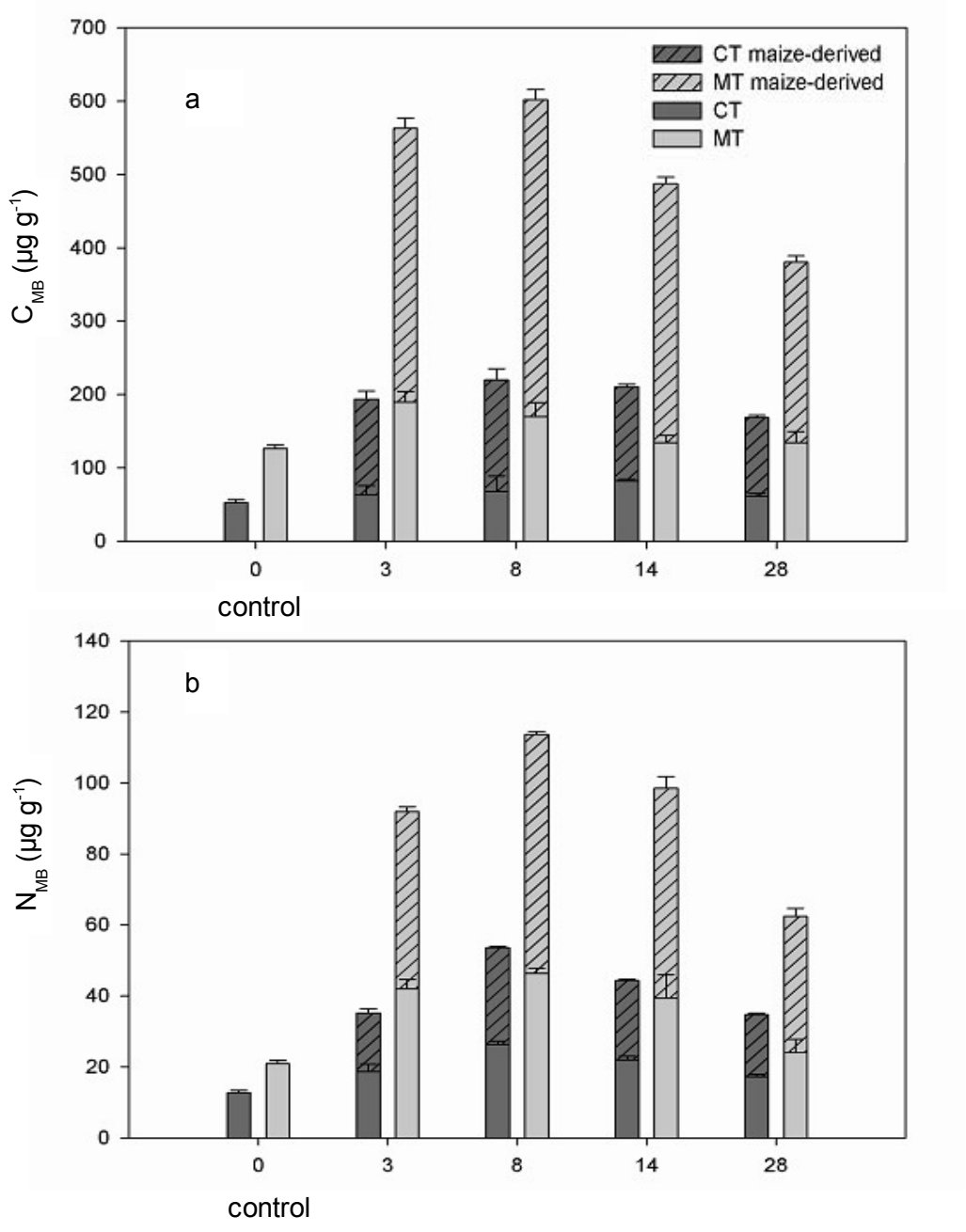

Figure 7: Total and maize-derived 0.05 M K_2SO_4-extractable microbial carbon (C_{MB}) (a) and nitrogen (N_{MB}) (b) in the surface layers (0-5 cm) of maize treatments of minimum and conventional tillage simulations (MT_{maize}, CT_{maize}) after 3, 8, 14, and 28 days of incubation; for day 0, the control treatments ($MT_{control}$, $CT_{control}$) are shown; means and standard errors (n = 4).

6.3.3. Formation of macroaggregates and stabilization of OM within in water-stable aggregates in different tillage simulations

The formation of macroaggregates in the 0-5 cm layers of the microcosms occurred immediately after the start of incubation and differed markedly between the maize treatments of the tillage simulations (Figure 8). After eight days of incubation, 42% and 10% of the soil were recovered as macroaggregates in MT_{maize} and in CT_{maize} simulations, respectively. Afterwards, disintegration of the macroaggregates prevailed with macroaggregate yields of 22% (MT_{maize}) and 7% (CT_{maize}) after 28 days (Figure 8). In the control treatments, a slight macroaggregate dynamic was noted with a maximum yield after 14 days of incubation (Figure 8).

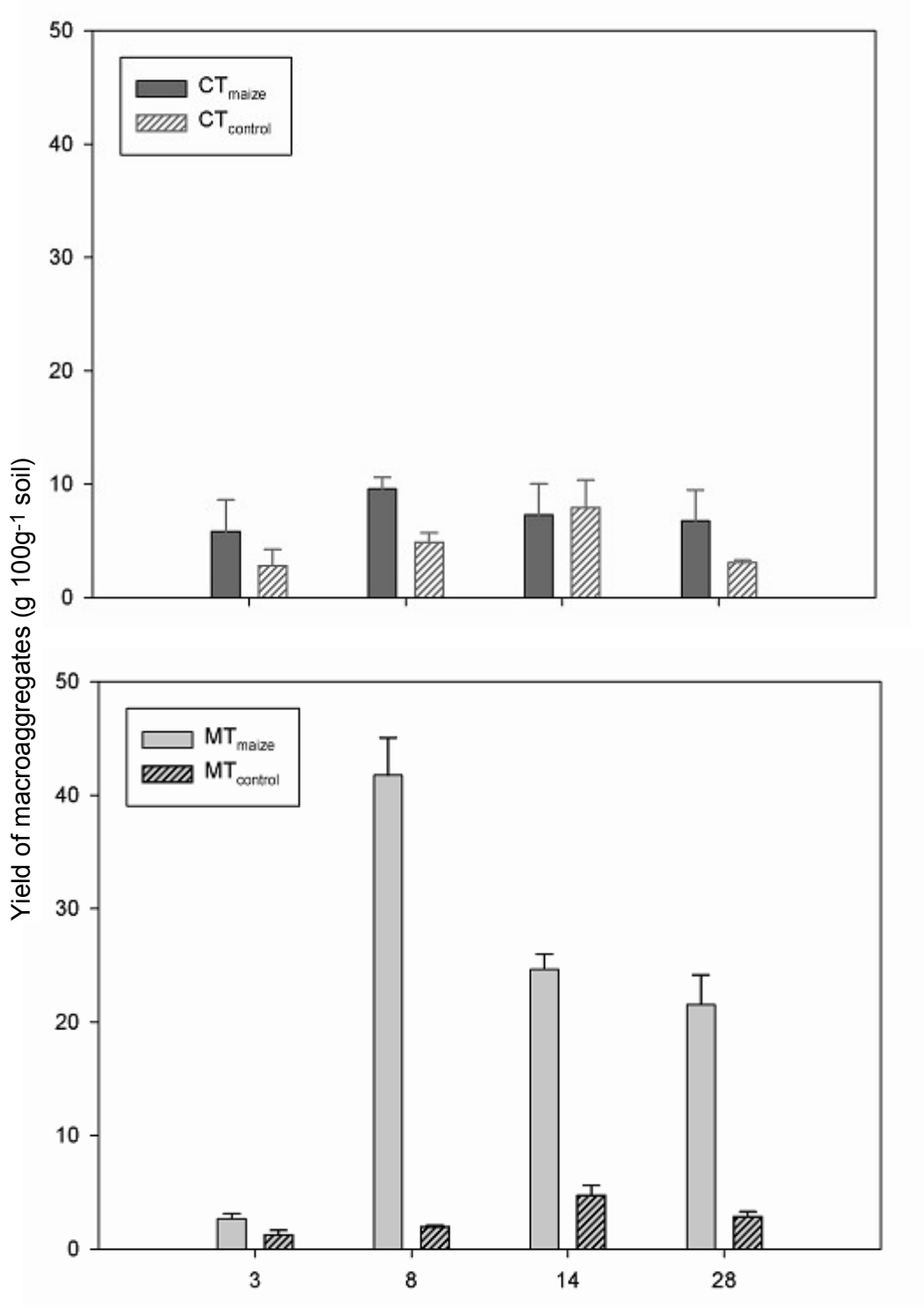

Figure 8: New-built macroaggregates (>250 µm) in the surface layers (0-5 cm) of maize (MT_{maize}, CT_{maize}) and control treatments ($MT_{control}$, $CT_{control}$) of minimum and conventional tillage simulations after 3, 8, 14, and 28 days of incubation; means and standard errors (n = 4).

In the 0-5 cm layers, the concentrations of C in the different aggregate size classes expressed in g C per kg soil indicated a large temporal variability in the MT_{maize} simulations (Figure 9). The contribution of macroaggregate-C to total C increased markedly between day 3 and day 8 of incubation associated with decreases in the contribution of C in the <53 µm and the microaggregate fraction to total C. For CT_{maize}, however, temporal changes were not pronounced. After 28 days of incubation, differences between the treatments were still marked with a relative contribution of macroaggregate-C to the total C of 27% at MT_{maize} and 10% at CT_{maize} simulations (Figure 9). The relative contribution of macroaggregate-N to total N followed the same trend (Figure 9).

In the end of the incubation, the percentage contribution of maize-C to the total macroaggregate-C was higher at CT_{maize} (41%) than in MT_{maize} (28%) simulations, since total macroaggregate-C was much smaller at CT_{maize} (Figure 9).

After 28 days of incubation, 21% and 36% of the added maize-C and maize-N, respectively, were associated with micro- and macroaggregates in the 0-5 cm layer of the MT_{maize} simulations (Table 10). Similarly, in the CT_{maize} simulations, 18% and 34% of the added maize-C and maize-N, respectively, were associated with these aggregate size classes (Table 10).

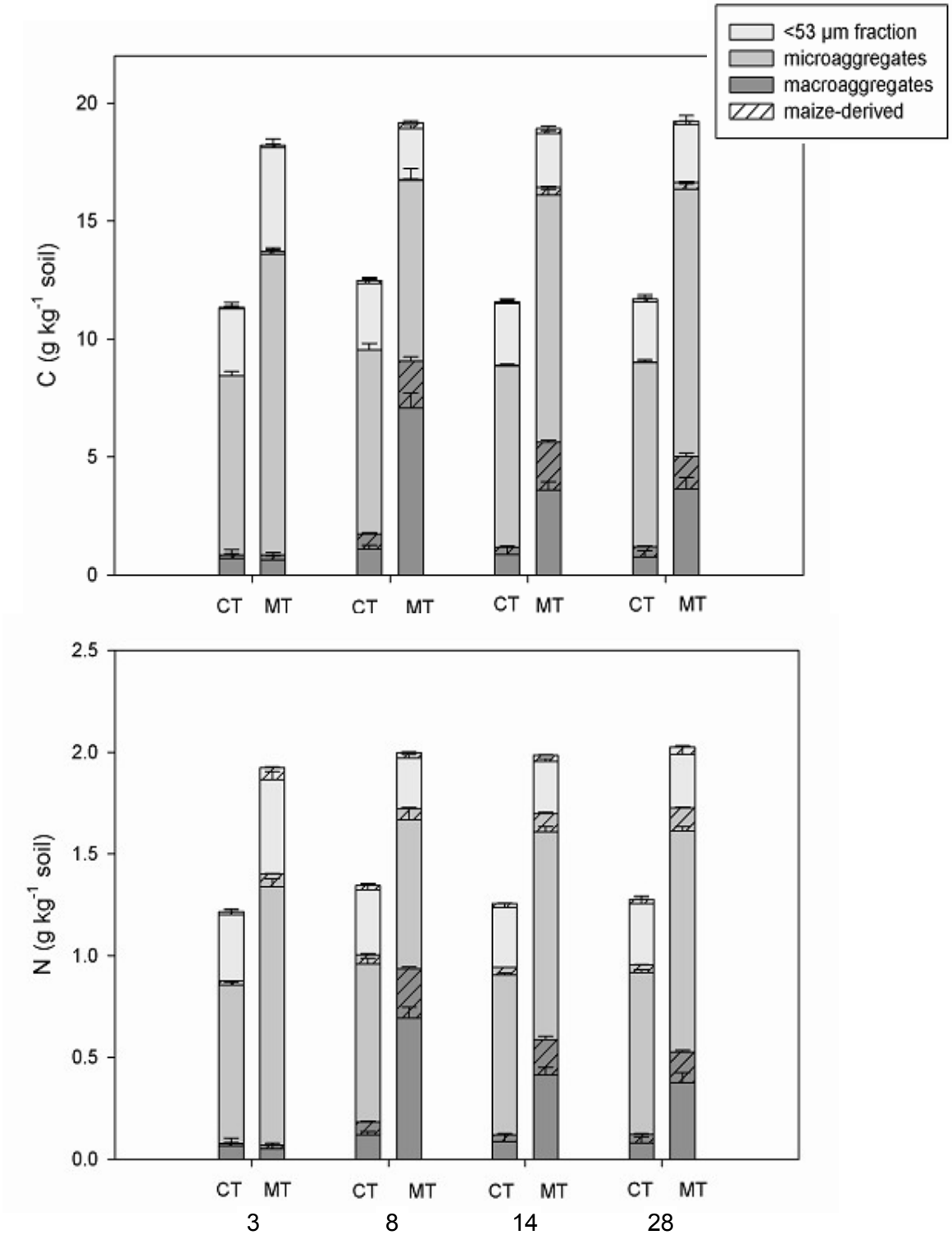

Figure 9: Concentrations of total and of maize-derived C and N of water-stable aggregates (macroaggregates >250 µm, microaggregates 53-250 µm, <53 µm fraction) per kg soil (means and standard errors, n = 4). Results are shown for the surface layers (0-5 cm) of maize treatments of minimum (MT_{maize}) and conventional (CT_{maize}) tillage simulations after 3, 8, 14, and 28 days of incubation.

Table 10: Partitioning of maize-C and maize-N added at the beginning of the incubation into different fractions after different times of incubation and for different tillage simulations (CT_{maize} and MT_{maize}). The fractions include C mineralized in the entire microcosm, free particulate organic matter (fPOM), and water-stable aggregate size classes (macroaggregates >250 µm, microaggregates 53-250 µm, <53 µm fraction) in the surface layer (0-5 cm); means and standard errors (n = 4).

		CT_{maize}				MT_{maize}			
	Time of incubation (days)	3	8	14	28	3	8	14	28
Maize-C	mineralized (%)[a]	8 (0)	29 (1)	39 (0)	48 (1)	8 (0)	28 (0)	36 (0)	43 (0)
	bulk soil (%)[b]	93 (4)	85 (3)	64 (4)	49 (3)	82 (4)	84 (3)	74 (2)	80 (4)
	fPOM (%)[b,c]	86 (4)	57 (2)	48 (5)	33 (10)	78 (7)	56 (1)	35 (2)	58 (4)
	macroaggregates (%)[b]	5 (3)	24 (2)	12 (2)	17 (1)	3 (1)	25 (2)	25 (1)	17 (2)
	microaggregates (%)[b]	0 (0)	0 (0)	2 (1)	1 (1)	2 (1)	1 (0)	4 (1)	4 (0)
	<53 µm fraction (%)[b]	2 (2)	5 (2)	3 (2)	5 (3)	1 (1)	3 (1)	2 (1)	2 (0)
Maize-N	bulk soil (%)[b]	79 (4)	78 (2)	81 (4)	81 (1)	75 (2)	74 (1)	74 (2)	57 (1)
	$fPOM_{calculated*}$ (%)[b,c]	59 (5)	25 (3)	43 (5)	39 (1)	56 (3)	29 (1)	34 (2)	16 (2)
	macroaggregates (%)[b]	5 (2)	27 (2)	14 (2)	18 (1)	3 (1)	34 (1)	24 (2)	21 (1)
	microaggregates (%)[b]	9 (0)	18 (3)	15 (0)	16 (0)	9 (1)	8 (1)	12 (1)	15 (1)
	<53 µm fraction (%)[b]	7 (1)	8 (1)	9 (1)	9 (1)	8 (1)	4 (0)	4 (0)	5 (1)

[a]The percentages refer to the mineralization of maize-C emitted as maize-CO_2-C from the microcosms (0-15 cm).

[b]The percentages refer to the 0-5 cm layer, thus 100% equals 2.66 g C kg^{-1} for CT_{maize} and 7.99 g C kg^{-1} for MT_{maize}.

[c]Maize-C and maize-N in the fPOM fraction was calculated as the difference between the maize contents in the bulk soil fraction and in all water-stable aggregate size classes, since fPOM had to be burnt for a correction of the contamination by soil particles of a density <1.6 g cm^{-3} and could not be used for isotope measurements.

6.4. Discussion

6.4.1. Mineralization of organic matter and its assimilation by the microbial biomass in different tillage simulations

The total and maize-derived specific respiration (CO_2-C:C, CO_2-C:C_{MB}, and maize-derived-CO_2-C:C_{MB}) was lower in the MT_{maize} and $MT_{control}$ simulations than the respective CT simulations (Table 9), despite the fact that the potential mineralization, determined as the cumulative CO2-C emission, was slightly increased by 3% (Table 9). The amount of maize-C mineralized after 28 days of incubation was slightly lower (by 10%) in MT_{maize} than in CT_{maize} simulations (Table 10). Furthermore, the proportion of maize-C assimilated by the microbial biomass after 28 days of incubation in the 0-5 cm layer of the microcosms was slightly lower in the MT_{maize} than in CT_{maize} simulations simulations (data not shown). These differences are probably due to the lower residue to soil ratio in the 0-5 cm layer of the CT_{maize} than of the MT_{maize} simulations and thus a more pronounced contact of residues with soil and soil-derived decomposers compared to the MT_{maize} simulations. For instance, Potthoff et al. (2005) found an increased C-mineralization in the presence of soil and Henriksen and Breland (2002) observed a faster decomposition of residues due to an increased contact between straw and soil particles.

A second explanation for the differences between the CT and MT simulations reported above may be an increased occurrence of fungi in the MT soil used for incubation. Generally, fungi are considered being more efficient substrate users than bacteria (Holland and Coleman 1987; Sakamoto and Oba 1994; Hodge et al. 2000) which is reflectable in a lower metabolic quotient qCO2. However, for the site studied, Heinze et al. 2009a reported a higher ergosterol concentration in the CT soil, but stressed that only saprotrophic and no arbuscular mycorrhizal fungi are detectable by egosterol. Under field conditions, Ahl et al. (1998), who investigated a MT system using a

horizontal axis rotary cultivator on a silt loam and on a sand loam (Northern Hesse, South-Eastern Lower Saxony, Germany), reported a higher fungal biomass, detected by the biomarker ergosterol, for MT soils. Heinze et al. (2009a), who worked on the same site as we did, found a higher ergosterol concentration in the CT soil but stressed that only saprotrophic and no arbuscular mycorrhizal fungi are detectable through ergosterol.

6.4.2. Macroaggregate formation and stabilization of OM within water-stable aggregate size classes as affected by tillage simulation

After 8 days of incubation, we found the maximum occurrence of macroaggregates in the 0-5 cm layers of the microcosms. In the MT_{maize} simulations, macroaggregate formation was markedly higher (42%) than in the CT_{maize} simulations (10%) (Figure 8). Similarly, Bossuyt et al. (2001) and Helfrich et al. (2008) reported for incubation experiments with either wheat straw, maize leaves, or maize roots and soils that maximum macroaggregation occurred after 12 and 14 days of incubation, respectively. The maximum peak of macroaggregation in our study was accompanied by a peak in microbial biomass and microbial activity, as observed by the CO_2-C emission rate (Figure 6, 7). Since microbial products serve as binding agents for the cementation of macroaggregates it was expected that macroaggregation occurred during the decomposition of the easily available compounds of the fresh maize residues with a peak during or just after the highest microbial activity (Bossuyt et al. 2001; Helfrich et al. 2008; Abiven et al. 2009).

In our study, the high amount of maize straw added to the 0-5 cm layer of the MT_{maize} microcosms induced a strong and accelerated formation of macroaggregates. This is in accordance with Oades (1984) and De Gryze et al. (2005) who stated that the amount of macroaggregates formed in a soil increased with the addition of decomposable OM. However, after 28 days of incubation, there was still maize-fPOM available in the

CT_{maize} simulation (21% by weight; data not shown). Thus, the availability of maize residues was not the limiting factor for the lower formation of macroaggregates within 28 days of incubation in the CT_{maize} simulations.

The percentage contribution of aggregate-C to the total C was higher in the macroaggregate size class of the MT_{maize} than of the CT_{maize} simulations (Figure 9). This is in accordance to studies investigating different tillage systems under field conditions (Kushwaha et al. 2001; Jacobs et al. 2009a).

In our incubation study, there was less maize-derived C and N found in the microaggregate and in the <53 µm fraction than in the macroaggregates (Figure 9). This is in line with a range of field experiments (Yamashita et al. 2006; John et al. 2005) and of microcosm studies (Bossuyt et al. 2001; Helfrich et al. 2008) which revealed that a higher proportion of young or recently added OM is associated with macroaggregates than with smaller size classes. The findings of our study and of other researchers confirm the conceptual model of macroaggregate-turnover proposed by Six et al. (1999) who postulated that first macroaggregates are formed around fresh organic particles and that microaggregates form then within the macroaggregates.

In our study, for both tillage simulations, new macroaggregates did mainly consist of non-maize-derived and thus older C and N (Figure 9). The few data on the contribution of recently added OM to macroaggregate-OM available in literature for short-term incubations in the lab and in the field were in a range of 5-60% (Angers et al. 1997; Helfrich et al. 2008). Further studies are required to determine which factors (soil types and textures, quality, quantity, and particle size of the given substrate, temperature, moisture, and composition of decomposer community) are mainly responsible for this large range.

The transformation of OM in the aggregate size classes investigated were slightly different between maize-C and maize-N. The accumulation of maize-N within microaggregates and the <53 µm fraction occurred earlier and to a higher proportion

than for maize-C (Figure 9). A preferential accumulation of maize-N was also found for the <53 µm fraction by Angers et al. (1997), Coppens et al. (2006a), and Helfrich et al. (2008). This may be explained by the relatively high concentration of soluble N in organic residues which accumulates more rapidly in the small aggregate size classes (Coppens et al. 2006a; Helfrich et al. 2008).

Overall, we showed that new macroaggregates formed within a few days after the incorporation of residues are a substantial location for the stabilization of C and N with a higher importance for MT_{maize} than for CT_{maize} simulations indicating the importance of the residue location (and thus substrate concentration) for the formation of macroaggregates. Macroaggregation played a key role in the stabilization of recently added OM in both tillage simulations.

6.5. Conclusions

In our study, two main differences between CT and MT simulations were evident, namely the higher efficiency of C retention and the higher formation of macroaggregates in the MT_{maize} simulations compared to the CT_{maize} simulations. Both characteristics indicate a higher presence of fungal biomass in the MT soil used or incubation. Thus, the better contact of soil particles with the organic residues led to a better access of decomposers to maize residues resulting in a faster processing of the recently added maize-C and maize-N in the CT_{maize} simulations.

Acknowledgements

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7. Synthesis and general conclusions

Within the present thesis, substantial differences in parameters of OM transformation between soils under MT and CT management were described. Differences found between CT and MT were most pronounced in the surface soil (0-5 cm) which was affected by the rotary harrow used under MT. Furthermore, for some of the parameters investigated, the MT system showed similar attributes as described in literature for NT systems.

In the following, the main results and the general conclusions of the present thesis are described:

(i) The analysis of the soil samples taken after fallow (before tillage operations) revealed that about 40 years of MT regime led to higher concentrations of microbial biomass, C_{org}, and N compared to the CT which was most pronounced in the surface soil (0-5 cm). Furthermore, beneficial effects of MT in terms of a higher abundance of stable macroaggregates rich in OM were confirmed. However, no increased amount of oPOM was detected. Rather, higher C_{org} and N concentrations were found in the mineral-associated fraction in MT compared to CT (Chapter 4).

(ii) In the field, a large temporal variability in the distribution of aggregate size classes was found depending on management events (Chapter 5). The microcosm experiment demonstrated that the process of macroaggregate formation and disruption is very rapid and dynamic (Chapter 6). It was therefore assumed that macroaggregates can provide physical protection of OM against degradation rather in a short-term aspect. Generally, seasonal variations among sampling dates should be considered for the investigation of aggregate size classes in the field.

(iii) Both, microcosm and field data showed the formation of a considerable amount of water-stable macroaggregates (especially the size class 0.25-1 mm) shortly after the

incorporation of organic residues in CT as well as in MT soils (Chapters 5 and 6). Thus, the higher amount of macroaggregates found under MT compared to CT cannot be attributed to a higher physical disruption by the plough (CT) but to a higher formation rate in MT soils.

(iv) By field data and the microcosm experiment, it could clearly be demonstrated that water-stable macroaggregates are an important location for the storage of the surplus of OM found under MT compared to CT (Chapters 5 and 6). However, macroaggregates were considered to provide physical protection of OM only in a short-term. Consequently, macroaggregates were not found to protect the very labile OM against mineralization which was revealed in the incubation experiment (Chapter 5). Nevertheless, the surplus of OM detected after tillage in the MT compared to the CT soil comprised of biochemically degradable OM (Chapter 5).

(v) By the microcosm experiment, it was shown that the early formation of new macroaggregates was higher while the specific respiration was lower in the simulated MT than in the simulated CT systems. Two factors were considered to account for differences in the early OM processing between both tillage simulations: Firstly, the contact of one unit of residue to the soil was lower in the MT simulations due to a higher concentrated input into the top layer. Hence, the use of recently added residues was less efficient than in the CT simulations. Secondly, it may be that a lower specific respiration and a higher macroaggregate formation were caused by a shift in the microbial community towards fungi in the MT soil used for incubation (Chapter 6).

Summarizing the findings of the present work, the long-term implementation of MT was found to have several beneficial effects on the soil structure and on the storage of OM especially in the surface soil. Although macroaggregates were not considered to store OM in a long-term, they play a key role for the enhanced OM sequestration under MT compared to CT. A higher formation of macroaggregates directly after residue

incorporation in the MT soil may provide physical protection to a higher amount of OM than in CT soils.

The reported differences in the early processes after the incorporation of residues may be crucial for differences between CT and MT soils. Further investigations should especially focus on short-term processes and on the temporal variability of soil parameters as driven by management events. It was suggested that shifts in the microbial community towards fungi due to the implementation of MT may contribute considerably to differences in OM transformation processes compared to CT. Further studies should therefore include an intense consideration of microbial aspects. The present thesis showed that the higher concentration of residues in the surface soil under MT than under CT has a pronounced impact on the processes of storage and decomposition of OM. Therefore, further investigations should emphasise analysis of the residue-soil-interface as affected by tillage management.

8. Summary

8.1. Summary

To increase the organic matter (OM) content in the soil is one main goal in arable soil management. The adoption of tillage systems with reduced tillage depth and/or frequency (reduced tillage) or of no-tillage was found to increase the concentration of soil OM compared to conventional tillage (CT; ploughing to 20-30 cm). However, the underlying processes are not yet clear and are discussed contradictorily. So far, few investigations were conducted on tillage systems with a shallow tillage depth (minimum tillage = MT; maximum tillage depth of 10 cm). A better understanding of the interactions between MT implementation and changes in OM transformation in soils is essential in order to evaluate the possible contribution of MT to a sustainable management of arable soils.

The objectives of the present thesis were (i) to compare OM concentrations, microbial biomass, water-stable aggregates, and particulate OM (POM) between CT and MT soils, (ii) to estimate the temporal variability of water-stable aggregate size classes occurring in the field and the dynamics of macroaggregate (>250 µm) formation and disruption under controlled conditions, (iii) to investigate whether a lower disruption or a higher formation rate accounts for a higher occurrence of macroaggregates under MT compared to CT, (iv) to determine which fraction is the major agent for storing the surplus of OM found under MT compared to CT, and (v) to observe the early OM transformation after residue incorporation in different tillage systems simulated.

Two experimental sites (Garte-Süd and Hohes Feld) near Göttingen, Germany, were investigated. Soil type of both sites was a Haplic Luvisol. Since about 40 years, both sites receive MT by a rotary harrow (to 5-8 cm depth) and CT by a plough (to 25 cm depth). Surface soils (0-5 cm) and subsoils (10-20 cm) of two sampling dates

(after fallow and directly after tillage) were investigated for concentrations of organic C (C_{org}) and total N (N), different water-stable aggregate size classes, different density fractions (for the sampling date after fallow only), microbial biomass, and for biochemically stabilized C_{org} and N (by acid hydrolysis; for the sampling date after tillage only). In addition, two laboratory incubations were performed under controlled conditions: Firstly, MT and CT soils were incubated (28 days at 22°C) as bulk soil and with destroyed macroaggregates in order to estimate the importance of macroaggregates for the physical protection of the very labile OM against mineralization. Secondly, in a microcosm experiment simulating MT and CT systems with soil <250 µm and with ^{15}N and ^{13}C labelled maize straw incorporated to different depths, the mineralization, the formation of new macroaggregates, and the partitioning of the recently added C and N were followed (28 days at 15°C).

Forty years of MT regime led to higher concentrations of microbial biomass and of C_{org} and N compared to CT, especially in the surface soil. After fallow and directly after tillage, a higher proportion of water-stable macroaggregates rich in OM was found in the MT (36% and 66%, respectively) than in the CT (19% and 47%, respectively) surface soils of both sites (data shown are of the site Garte-Süd only). The subsoils followed the same trend. For the sampling date after fallow, no differences in the POM fractions were found but there was more OM associated to the mineral fraction detected in the MT soils. A large temporal variability was observed for the abundance of macroaggregates. In the field and in the microcosm simulations, macroaggregates were found to have a higher formation rate after the incorporation of residues under MT than under CT. Thus, the lower occurrence of macroaggregates in CT soils cannot be attributed to a higher disruption but to a lower formation rate. A higher rate of macroaggregate formation in MT soils may be due to (i) the higher concentrated input of residues in the surface soil and/or (ii) a higher abundance of fungal biomass in contrast to CT soils. Overall, as a location of storage of the surplus of OM detected

under MT compared to CT, water-stable macroaggregates were found to play a key role. In the incubation experiment, macroaggregates were not found to protect the very labile OM against mineralization. Anyway, the surplus of OM detected after tillage in the MT soil was biochemically degradable. MT simulations in the microcosm experiment showed a lower specific respiration and a less efficient translocation of recently added residues than the CT simulations. Differences in the early processes of OM translocation between CT and MT simulations were attributed to a higher residue to soil ratio and to a higher proportion of fungal biomass in the MT simulations.

Overall, MT was found to have several beneficial effects on the soil structure and on the storage of OM, especially in the surface soil. Furthermore, it was concluded that the high concentration of residues in the surface soil of MT may alter the processes of storage and decomposition of OM. In further investigations, especially analysis of the residue-soil-interface and of effects of the depth of residue incorporation should be emphasised. Moreover, further evidence is needed on differences in the microbial community between CT and MT soils.

8.2. Zusammenfassung

Die Erhöhung der organischen Substanz (OS) in Böden ist ein Hauptziel des ackerbaulichen Bodenmanagements. Es wurde festgestellt, dass die Anwendung von Bodenbearbeitungs-Systemen mit einer reduzierten Bearbeitungstiefe und/oder -Frequenz (reduzierte Bearbeitung) oder von Direktsaat-Systemen die Konzentration von OS in Böden im Vergleich zu konventioneller Bearbeitung (KB; Pflug mit 20-30 cm Tiefe) erhöht. Die zu Grunde liegenden Prozesse sind jedoch nicht abschließend verstanden und werden kontrovers diskutiert. Bisher wurden nur wenige Untersuchungen von Bearbeitungssystemen mit einer flachen Bearbeitungstiefe

(Minimal-Bearbeitung = MB; maximale Bearbeitungstiefe von 10 cm) durchgeführt. Ein besseres Verständnis der Interaktion von MB-Anwendung und Veränderungen von Prozessen der OS in Böden ist essentiell für die Bewertung eines möglichen Beitrages von MB zum nachhaltigen Management von Ackerböden.

Die Ziele der vorliegenden Arbeit waren (i) die Konzentrationen und das Vorkommen von OS, mikrobieller Biomasse, wasserstabiler Aggregate und partikulärer OS (POS) zwischen KB- und MB-Böden zu vergleichen, (ii) die zeitliche Variabilität des Vorkommens von Größenklassen wasserstabiler Aggregate im Feld und die Dynamik von Bildung und Zerfall von Makroaggregaten (>250 µm) unter kontrollierten Bedingungen abzuschätzen, (iii) zu untersuchen, ob eine geringere Zerfalls- oder eine höhere Bildungsrate für das höhere Vorkommen von Makroaggregaten unter MB im Vergleich zu KB verantwortlich ist, (iv) zu bestimmen, in welcher Fraktion der Überschuss an OS, der unter MB im Vergleich zu KB gefunden wurde, hauptsächlich gespeichert wird und (v) die frühe Transformation der OS nach Einarbeitung von Strohresten in verschiedenen, simulierten Bearbeitungssystemen zu beobachten.

Zwei Versuchsflächen (Garte-Süd und Hohes Feld) in der Nähe von Göttingen, Deutschland, wurden untersucht. Der Bodentyp beider Flächen war eine schluffige Parabraunerde. Seit etwa 40 Jahren erhalten beide Flächen eine MB durch eine Kreiselegge (5-8 cm Tiefe) und KB durch einen Pflug (bis 25 cm Tiefe). Oberboden (0-5 cm) und Unterboden (10-20 cm) von zwei Beprobungsterminen (nach Brache und direkt nach der Bearbeitung) wurden auf Konzentrationen von organischem C (C_{org}) und Gesamt-N (N), verschiedene wasserstabile Aggregat-Größenklassen, verschiedene Dichtefraktionen (nur für den Beprobungstermin nach der Brache), mikrobielle Biomasse und auf biochemisch stabilisierten C_{org} und N (durch saure Hydrolyse; nur für den Beprobungstermin nach der Bearbeitung) untersucht. Zusätzlich wurden zwei Laborinkubationen unter kontrollierten Bedingungen durchgeführt: Zum

Einen wurden MB- und KB-Böden als ungestörter Boden und mit zerstörten Makroaggregaten inkubiert (28 Tage bei 22°C), um die Wichtigkeit von Makroaggregaten für den physikalischen Schutz der sehr labilen OS vor Mineralisierung abzuschätzen. Zum Anderen wurden in einem Mikrokosmen-Experiment, das MB- und KB-Systeme mit Boden <250 µm und mit ^{15}N und ^{13}C markiertem Maisstroh in verschiedenen Einarbeitungstiefen simulierte, die Mineralisierung, die Bildung neuer Makroaggregate und die Partitionierung von dem kürzlich zugefügten C und N verfolgt (28 Tage bei 15°C).

Besonders im Oberboden führten 40 Jahre MB zu höheren Konzentrationen von mikrobieller Biomasse und von C_{org} und N im Vergleich zu KB. Nach Brache und direkt nach der Bearbeitung wurden höhere Anteile an OS-reichen wasserstabilen Makroaggregaten in den MB- (36 bzw. 66%) als in den KB- (19 bzw. 47%) Oberböden auf beiden Flächen gefunden (nur Daten der Fläche Garte-Süd genannt). Die Unterböden folgten dem gleichen Trend. Für den Beprobungstermin nach der Brache wurden keine Unterschiede in den POS-Fraktionen festgestellt, jedoch wurde mehr mineralisch-assoziierte OS in den MB-Böden gemessen. Eine hohe zeitliche Variabilität des Vorkommens von Makroaggregaten wurde beobachtet. Im Feld und in den Mikrokosmen-Simulationen wurde eine höhere Bildungsrate von Makroaggregaten nach Einarbeitung von Strohresten unter MB als unter KB festgestellt. Daher kann ein geringeres Vorkommen von Makroaggregaten in KB-Böden nicht einem höheren Zerfall, sondern einer geringeren Bildungsrate zugeschrieben werden. Eine höhere Bildungsrate von Makroaggregaten in MB-Böden dürfte durch (i) den höher konzentrierten Eintrag von Strohresten in den Oberboden und/oder (ii) ein höheres Vorkommen pilzlicher Biomasse im Vergleich zu KB-Böden begründet sein. Insgesamt wurde festgestellt, dass wasserstabile Makroaggregate eine Hauptrolle bei der Speicherung des gemessenen Überschusses an OS in MB- im Vergleich zu KB-Böden

spielen. Im Inkubationsversuch wurde nicht festgestellt, dass Makroaggregate die sehr labile OS vor Mineralisierung schützen. Dennoch war der Überschuss an OS, der in MB-Böden nach der Bearbeitung gemessen wurde, biochemisch abbaubar. Die MB-Simulationen des Mikrokosmenexperiments zeigten eine niedrigere spezifische Respiration und eine weniger effiziente Translokation des kürzlich zugefügten Strohs als die KB-Simulationen. Unterschiede in den frühen Prozessen der OS-Translokation zwischen KB- und MB-Simulationen wurden zurückgeführt auf das höhere Verhältnis von Stroh zu Boden und auf ein höheres Vorkommen von pilzlicher Biomasse in den MB-Simulationen.

Insgesamt wurde festgestellt, dass MB mehrere vorteilhafte Effekte auf die Bodenstruktur und auf die Speicherung von OS insbesondere im Oberboden hat. Des Weiteren wurde gefolgert, dass die hohe Konzentration von Strohresten im MB-Oberboden die Prozesse von Speicherung und Abbau von OS ändern kann. In weiteren Untersuchungen sollten besonders Analysen der Stroh-Boden-Grenzfläche und Effekte der Einarbeitungstiefe von Strohresten berücksichtigt werden. Darüber hinaus sind weitere Nachweise von Unterschieden in der mikrobiellen Gemeinschaft zwischen KB- und MB-Böden nötig.

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