Effects of different tillage treatments on labile soil organic matter pools and stabilization processes

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(Rouven Andruschkewitsch)

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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.) and was prepared within the Research Training Group “Regulation of soil organic matter and nutrient turnover in organic agriculture” (Graduiertenkolleg 1397), funded by the Deutsche Forschungsgemeinschaft (DFG).

The dissertation is on the basis of three scientific publications as first author, which are published in or submitted to international refereed journals. The papers are included in chapter 3, 4 and 5.

A general introduction about the history of aggregate fractionation and the factors influencing the aggregate size distribution in arable soils is given in chapter 1. Research objectives are included in chapter 2. In chapter 6, the entire thesis is summarized and a general conclusion is drawn.

The following papers are included in this thesis:

Chapter 3:

Andruschkewitsch, R., Geisseler, D., Koch, H.-J., Ludwig, B. (2013): Effects of tillage on contents of organic carbon, nitrogen, water-stable aggregates and light fraction for four different long-term trials. Geoderma 192, 368–377, http://www.sciencedirect.com/science/article/pii/S0016706112002753.

Chapter 4:

Andruschkewitsch, R.; Koch, H.-J.; Ludwig, B.: Effect of long-term tillage treatments on the temporal dynamics of water-stable aggregates and on macro-aggregate turnover at three German sites (in preparation for submission).

Chapter 5:

Andruschkewitsch, R.; Geisseler, D.; Dultz, S.; Joergensen, R.-G.; Ludwig, B. 2013. Rate of soil aggregate formation under different clay and organic matter amendments - a short-term incubation experiment. Journal of Plant Nutrition and Soil Science (submitted).

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List of abbreviations

$AlCl_3$	Aluminium chloride
ANOVA	Analysis of Variance
$BaCl_2$	Barium chloride
C	Carbon
$CaCl_2$	Calcium chloride
$CHCl_3$	Chloroform
Cmic	microbial biomass carbon
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate ion
C_{org}	Organic cabon
CT	Conventional tillage
DCB	Dithionite-citrate-bicarbonate
E_C	Extractable carbon of microbial biomass after fumigation
fLF	Free light fraction
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HF	Heavy fraction
HPLC	High-performance liquid chromatography
K	Potassium
K_2SO_4	Potassium sulphate
k_C	Proportion of extractable carbon bound in microbial biomass (0.41)
LF	Light fraction
Mg	Magnesium
MT	Mulch tillage
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NT	No tillage
N_{tot}	Total nitrogen
oLF	Occluded light fraction
OM	Organic matter
OM_1	Addition of pre-incubated wheat straw at a rate of 4.1 g C kg^{-1} soil
OM_2	Addition of pre-incubated wheat straw at a rate of 8.2 g C kg^{-1} soil

OM_{2_c}	Same as OM_2, whereat the clay content was increased to 25%
rpm	revolutions per minute
SOM	soil organic matter
Tukey HSD	Tukey’s honestly significant difference test
WEOC	Water extractable organic carbon

Summary

An improved understanding of soil organic carbon (C_{org}) dynamics in interaction with the mechanisms of soil structure formation is important in terms of sustainable agriculture and reduction of environmental costs of agricultural ecosystems. However, information on physical and chemical processes influencing formation and stabilization of water-stable aggregates in association with C_{org} sequestration is scarce. Long-term soil experiments are important in evaluating open questions about management induced effects on soil C_{org} dynamics in interaction with soil structure formation. The objectives of the present thesis were:

(i) to determine the long-term impacts of different tillage treatments on the interaction between macro-aggregation (>250 µm) and light fraction (LF) distribution and on C sequestration in plots differing in soil texture and climatic conditions.

(ii) to determine the impact of different tillage treatments on temporal changes in the size distribution of water-stable aggregates and on macro-aggregate turnover.

(iii) to evaluate the macro-aggregate rebuilding in soils with varying initial C_{org} contents, organic matter (OM) amendments and clay contents in a short-term incubation experiment.

Soil samples were taken in 0-5 cm, 5-25 cm and 25-40 cm depth from up to four commercially used fields located in arable loess-regions of eastern and southern Germany after 18-25 years of different tillage treatments with almost identical experimental setups per site. At each site, one large field with spatially homogenous soil properties was divided into three plots. One of the following three tillage treatments was carried in each plot: (i) Conventional tillage (CT) with annual mouldboard ploughing to 25-30 cm (ii) mulch tillage (MT) with a cultivator or disc harrow 10-15 cm deep, and (iii) no tillage (NT) with direct drilling. The crop rotation at each site consisted of sugar beet (*Beta vulgaris* L.) - winter wheat (*Triticum aestivum*

L.) - winter wheat. Crop residues were left on the field and crop management was carried out following the regional standards of agricultural practice.

To investigate the above mentioned research objectives, three experiments were conducted: Experiment (i) was performed with soils sampled from four sites in April 2010 (wheat stand). Experiment (ii) was conducted with soils sampled from three sites in April 2010, September 2011 (after harvest or sugar beet stand), November 2011 (after tillage) and April 2012 (bare soil or wheat stand). An incubation study (experiment (iii)) was performed with soil sampled from one site in April 2010.

Based on the aforementioned research objectives and experiments the main findings were:

(i) Consistent results were found between the four long-term tillage fields, varying in texture and climatic conditions.

Correlation analysis of the yields of macro-aggregate against the yields of free LF ($\varphi \leq 1.8$ g cm^{-3}) and occluded LF, respectively, suggested that the effective litter translocation in higher soil depths and higher litter input under CT and MT compensated in the long-term the higher physical impact by tillage equipment than under NT. The C_{org} stocks (kg C_{org} m^{-2}) in 522 kg soil, based on the equivalent soil mass approach (CT: 0–40 cm, MT: 0–38 cm, NT: 0–36 cm) increased in the order CT (5.2) = NT (5.2) < MT (5.7). Significantly ($p \leq 0.05$) highest C_{org} stocks under MT were probably a result of high crop yields in combination with reduced physical tillage impact and effective litter incorporation, resulting in a C_{org} sequestration rate of 31 g C^{-2} m^{-2} yr^{-1}.

(ii) Significantly higher yields of macro-aggregates (g kg^{-2} soil) under NT (732-777) and MT (680-726) than under CT (542-631) were generally restricted to the 0-5 cm sampling depth for all sampling dates. Temporal changes on aggregate size distribution were only small and no tillage induced net effect was detectable. Thus, we assume that the physical impact by tillage equipment was only small or the impact was compensated by a higher soil mixing and effective litter translocation into higher soil depths under CT, which probably resulted in a high re-aggregation.

(iii) The short-term incubation study showed that macro-aggregate yields (g kg^{-2} soil) were higher after 28 days in soils receiving OM (121.4-363.0) than in the control soils (22.0-52.0), accompanied by higher contents of microbial biomass carbon and ergosterol. Highest soil respiration rates after OM amendments within the first three days of incubation indicated that macro-aggregate formation is a fast process. Most of the rebuilt macro-aggregates were formed within the first seven days of incubation (42-75%). Nevertheless, it was ongoing throughout the entire 28 days of incubation, which was indicated by higher soil respiration rates at the end of the incubation period in OM amended soils than in the control soils. At the same time, decreasing carbon contents within macro-aggregates over time indicated that newly occluded OM within the rebuilt macro-aggregates served as C_{org} source for microbial biomass. The different clay contents played only minor role in macro-aggregate formation under the particular conditions of the incubation study.

Overall, no net changes on macro-aggregation were identified in the short-term. Furthermore, no indications for an effective C_{org} sequestration on the long-term under NT in comparison to CT were found. The interaction of soil disturbance, litter distribution and the fast re-aggregation suggested that a distinct steady state per tillage treatment in terms of soil aggregation was established. However, continuous application of MT with a combination of reduced physical tillage impact and effective litter incorporation may offer some potential in improving the soil structure and may therefore prevent incorporated LF from rapid decomposition and result in a higher C sequestration on the long-term.

Zusammenfassung

Nach dem marinen und dem geologischen Kohlenstoff-Vorrat sind in den Böden global die größten Kohlenstoff-Vorräte gespeichert, welche im weltweiten Mittel auf 100 Mg ha^{-1} geschätzt werden. Zum Vergleich, 15 Mg ha^{-1} Kohlenstoff sind schätzungsweise in der oberirdischen Biomasse gespeichert. Innerhalb dieser terrestrischen Pools ist der größte Anteil des Kohlenstoffs in Wald- und Grassland-Ökosystemen gespeichert. Diese weltweit vernetzten Systeme befinden sich in einem Zustand des kontinuierlichen Austausches über Kohlenstoff Ein- bzw. Austräge. Veränderungen der Landnutzung und die Erweiterung landwirtschaftlicher Flächen innerhalb der letzten Jahrzehnte erhöhten einerseits den Kohlenstoff-Austrag aus diesen Systemen durch verstärkte Störung des Bodens und deshalb erhöhten Abbauraten der organischen Bodensubstanz und verminderten andererseits den Kohlenstoff-Eintrag in diese Systemen durch die Abfuhr der Biomasse als Erntegut.

Um landwirtschaftlich genutzte Böden in eine Netto-Kohlenstoffsenke umzuwandeln, ist es nötig die Einträge an organischem Material zu erhöhen und ebenso dessen Abbauraten zu erniedrigen. Das kann durch angepasste landwirtschaftliche Bewirtschaftungsmethoden, z.B. Anbauintensität, Pflanzentyp, (Grün-)Düngung und Bodenbearbeitung, erfolgen. Des Weiteren führt eine Erhöhung des Kohlenstoff-Gehaltes in landwirtschaftlichen Böden zu weiteren begleitend auftretenden begünstigenden Effekten, die sich positiv auf fast alle Bodeneigenschaften auswirken. Um erfolgreich und langfristig Kohlenstoff in Böden zu speichern und um die Änderungen der Vorräte anhand von Modellen zu berechnen ist es unerlässlich die Mechanismen der Kohlenstoff-Stabilisierung und -Freisetzung in Böden zu verstehen.

Ein Großteil der Modelle zur Berechnung der Umsatzraten der organischen Bodensubstanz beruhen auf konzeptuellen Vorräten die anhand spezifischer Umsatzraten und Vorrat-Größen definiert werden. Die Größe des aktiven Vorrats, direkt verfügbar für mikrobiellen Abbau, wird über kurzzeitige Inkubation in Abbauexperimenten bestimmt, wohingegen die biologisch stabile Vorratsgröße über chemische Oxidation erfasst wird. Allerdings gibt die chemische Oxidation nicht unbedingt einen homogenen Vorrat in Bezug auf die Abbaubarkeit an.

Neben der theoretischen Einteilung der organischen Bodensubstanz in konzeptionelle Vorräte mit jeweils spezifischen Umsatzraten unterliegt die Kohlenstoffspeicherung in Böden mehreren Mechanismen und Prozessen: (i) primäre und sekundäre Rekalzitranz, (ii) räumliche Trennung von Substrat und Zersetzter, z.B. innerhalb von Aggregaten, und (iii) die Interaktion von organischem Material mit Bodenmineralen und Metall-Ionen. Jedoch ist das Verhältnis zwischen diesen verschiedenen Prozessen und der Speicherung des Bodenkohlenstoffes unklar.

Die Wirkung von selektivem Schutz vor mikrobiellem Abbau durch Rekalzitranz wurde lange als wichtigster Prozess bei der Kohlenstoff-Stabilisierung im Boden angesehen, wird aber mittlerweile als überschätzt angesehen. Durch ^{13}C-Markierung oder ^{14}C-Datierung wurde gezeigt, dass organische Bodensubstanz die nicht in Aggregaten eingeschlossen oder an Minerale gebunden ist, schnelle Abbauraten hat bzw. jünger als 50 Jahre ist. Wohingegen organische Bodensubstanz, die innerhalb von Aggregaten eingeschlossen oder an Minerale gebunden ist, signifikant längere Abbauraten bzw. ein höheres Alter zeigte. Diese Ergebnisse deuten an, dass die Stabilisierungsmechanismen einzelner Fraktionen der organischen Bodensubstanz mit unterschiedlichen Abbauraten nicht nur durch Rekalzitranz erklärt werden können. Stattdessen kann die Zersetzbarkeit der organischen Bodensubstanz u.a. durch die Verfügbarkeit von Wasser, Nährstoffen, Sauerstoff und räumliche (Un-)Erreichbarkeit erklärt werden, welche durch die Sorption der organischen Bodensubstanz an Bodenpartikeln und den Einschluss in Aggregaten beeinflusst wird. Diese Annahme, dass der Umsatz der organischen Bodensubstanz von einem Zusammenspiel physikalischer Eigenschaften abhängt, erhöhte das Interesse an der räumlichen Verteilung der organischen Bodensubstanz als einen Mechanismus, der erheblich zur Kohlenstoffstabilisierung beiträgt.

Durch ein verbessertes Verständnis bestimmter Stabilisierungsmechanismen, die durch die Interaktion von Boden-Mineralen und –Struktur bestimmt werden, können funktionelle Kohlenstoff-Vorräte mit homogenen Umsatzraten definiert werden. Diese könnten zu einer weiteren Verbesserung zur Berechnung der Kohlenstoffumsätze und dem Potential der Kohlenstoffsequestrierung im Boden mittels Modellierung beitragen.

Die organische Bodensubstanz kann in drei funktionelle Vorrats-Gruppen eingeteilt werden: (i) nicht komplexierte organische Bodensubstanz, (ii) primäre organo-mineralische Komplexe und (iii) sekundäre organo-mineralische Komplexe.

Nicht komplexierte organische Bodensubstanz befindet sich in der Übergangsphase zwischen frisch eingetragenem organischem Material (z. B. Streu) und organischer Bodensubstanz. Organische Bodensubstanz kommt im Boden durch die (physikalische) Interaktion mit Bodenmineralen (z. B. Ligandenaustausch oder van der Waals-Kräfte) u.a. als primäre organo-mineralische Komplexe vor. Nicht komplexierte organische Bodensubstanz kann durch eine Dichtefraktionierung mit Flüssigkeiten von einer Dichte zwischen 1,6 bis 1,9 g cm^{-3} vom Mineralboden abgetrennt werden, weshalb sie auch als leichte Fraktion bezeichnet wird. Sie tritt im Boden frei und okkludiert innerhalb von sekundären organo-mineralischen Komplexen auf und kann durch landwirtschaftliche Bewirtschaftungsmethoden beeinflusst werden. Sekundäre organo-mineralische Komplexe, auch als Aggregate bezeichnet, bestehen aus Partikeln der Schluff- und Ton-Fraktion, die durch organische Bindemittel aus lebender und toter Biomasse (mechanisch) zusammengehalten werden.

Aufgrund des hierarchischen Aufbaus der Aggregat-Struktur wird ein System angenommen, in welchem die Makro-Aggregate (>250 µm) aus Mikro-Aggregaten (250-53 µm) und kurzlebigen organischen Bindemitteln wie Pilzhyphen, Wurzeln und anderen Pflanzenresten aufgebaut sind. Mikro-Aggregate, aufgebaut aus Partikeln der Schluff- und Ton-Fraktion in Verbindung mit mikrobiellen bzw. pflanzlichen Polysacchariden und primären organo-mineralischen Komplexen, gelten als bedeutend weniger beeinflussbar durch äußere Einflüsse, wie landwirtschaftliche Bewirtschaftungsmethoden, als Makro-Aggregate. Die Aggregatgrößen-Verteilung in landwirtschaftlichen Böden kann in vielerlei Hinsicht beeinflusst werden: Bodenbearbeitung (Streuverteilung, Bodenmischung), Pflanzenart, Düngung, mikrobielle Aktivität, externe Faktoren (Temperatur, Niederschlag), Bodenwassergehalt und Frost/Tau-Zyklen.

Ein verbessertes Prozessverständnis der Abbaudynamiken von organischer Bodensubstanz in Zusammenhang mit der Bodenstruktur ist Grundlage einer nachhaltigen Landwirtschaft bei gleichzeitiger Reduktion der ökologischen Kosten landwirtschaftlicher Systeme.

Langzeitfeldversuche stellen nach wie vor ein wichtiges Element dar bei der Bearbeitung offener Fragen in Bezug auf den Einfluss von landwirtschaftlichen Bewirtschaftungsmethoden auf den Kohlenstoff- und Nährstoffkreislauf dar. Durch den realitätsnahen Maßstab können praxisorientiert nachhaltige Wege zu ge-

steigerten Ernteerträgen untersucht werden. Insbesondere Untersuchungen an solch trägen Systemen wie der Bodenstruktur und Kohlenstoffdynamik betonen den Nutzen von Langzeitfeldversuchen, da kleine Fehler aus Kurzzeitfeldversuchen, extrapoliert auf viele Jahre, die Ergebnisse von Hochrechnungen sehr stark beeinflussen können. Die Möglichkeit, standortspezifische Effekte herauszufiltern bietet die Auswertung von Daten verschiedener Flächen, um so die wichtigsten Faktoren in großem Maßstab zu erfassen. Allerdings sind Daten aus Langzeitfeldversuchen die den Effekt unterschiedlicher Bodenbearbeitung auf Dichtefraktionen und Größenverteilung, Bildung und Umsatz wasserstabiler Aggregate in Böden unterschiedlicher Textur und klimatischen Bedingungen untersuchen selten. Die obigen Ergebnisse zusammenfassend kann postuliert werden, dass Informationen über den Einfluss physikalischer und chemischer Prozesse der Aggregat-Bildung und -Stabilisierung in Zusammenhang mit Kohlenstoffsequestrierung in realitätsnahem Maßstab nur sehr begrenzt verfügbar sind.

Aufgrund der genannten Wissenslücken wurden die folgenden Forschungsziele dieser Arbeit formuliert:

(i) Bestimmung des Langzeiteinflusses unterschiedlicher Bodenbearbeitungsmethoden auf die Interaktion zwischen der Bildung von Makro-Aggregaten und der Verteilung der leichten Fraktion und auf die Kohlenstoffsequestrierung auf Standorten unterschiedlicher Textur und klimatischen Bedingungen.

(ii) Bestimmung von kurz- und langfristigen Effekten unterschiedlicher Bodenbearbeitungsmethoden auf die Größenverteilung wasserstabiler Aggregate.

(iii) Erfassung der Neubildungsrate der Makro-Aggregate in Böden mit unterschiedlichen Kohlenstoff-Gehalten, Zugaben an organischem Material und Ton-Gehalten in einem Kurzzeit-Inkubationsexperiment.

Bodenproben wurden in 0-5 cm, 5-25 cm und 25-40 cm Tiefe von bis zu vier Flächen entnommen. Diese waren kommerziell genutzte Flächen in den fruchtbaren Lössgebieten von Ost- und Süddeutschland, welche seit 18-25 Jahren mit unterschiedlichen Bodenbearbeitungsmethoden bearbeitet werden, die sich allerdings nur

unwesentlich zwischen den jeweiligen Flächen unterscheiden. Auf jeder Fläche wurde ein großes Feld in drei Schläge unterteilt. Jeder Schlag wurde mit einem der drei folgenden Bodenbearbeitungsmethoden behandelt: (i) Pflug-Verfahren mit jährlichem Pflügen in 25-30 cm Tiefe, (ii) Mulch-Verfahren mit jährlichem Grubbern in 10-15 cm Tiefe und (iii) Direktsaat-Verfahren. Die Fruchtfolge bestand auf jeder Fläche aus Zuckerrübe (*Beta vulgaris* L.) - Winterweizen (*Triticum aestivum* L.) - Winterweizen. Erntereste verblieben auf den Feldern und die Pflanzenproduktion erfolgte nach den regionalen Leitlinien der „Guten landwirtschaftlichen Praxis“.

Um die oben genannten Forschungsziele zu untersuchen, wurde zu jedem Forschungsziel jeweils ein Experiment durchgeführt: Experiment (i) mit Bodenproben von vier Flächen, beprobt im April 2010 (Weizenbestand). Experiment (ii) mit Bodenproben von drei Flächen, beprobt im April 2010, September 2011 (nach der Ernte oder Zuckerrübenbestand), November 2011 (nach der Bodenbearbeitung) und April 2012 (Brache oder Weizenbestand). Ein Inkubationsversuch (Experiment (iii)) mit Bodenproben einer Fläche, beprobt im April 2010.

Basierend auf den Forschungszielen dieser Arbeit können folgende Schlussfolgerungen gezogen werden:

(i) Die Langzeitfeldversuche zeigten zwischen den vier Standorten mit unterschiedlicher Textur und klimatischen Bedingungen konsistente Ergebnisse.

Eine Korrelationsanalyse der Erträge der Makro-Aggregate gegen die Erträge der Dichtefraktionen ($\varphi \leq 1{,}8$ g cm^{-3}) zeigte, dass eine effektive Streu-Verteilung und ein erhöhter Streu-Eintrag innerhalb der Pflug- und Mulch-Varianten langfristig den höheren physikalischen Einfluss der Geräte zur Bodenbearbeitung im Vergleich zu Direktsaatverfahren ausgleichen können. Die Kohlenstoff-Vorräte (kg C m^{-2}) in 522 kg Boden, basierend auf dem Ansatz der äquivalenten Bodenmassen, nahmen in der Reihenfolge CT (5,2) = NT (5,2) < MT (5,7) zu. Die signifikant ($p \leq 0{,}05$) höheren Kohlenstoff-Vorräte der Mulch-Variante waren wahrscheinlich durch eine Kombination von hohen Ernteerträgen und reduzierter Bodenbearbeitung bei gleichzeitig effektiver Streu-Einarbeitung erzeugt, was zu einer Kohlenstoffsequestrierungsrate von 31 g C^{-2} m^{-2} yr^{-1} führt.

(ii) Signifikant höhere Erträge der Makro-Aggregate (g kg^{-2} Boden) der Direktsaat- (732-777) und Mulch-Variante (680-726) im Vergleich zu der Pflug-Variante (542-631) waren bei allen Probenahmeterminen auf 0-5 cm Bodentiefe beschränkt. Veränderungen innerhalb des Probenahme-Zeitraumes waren nur gering und es konnte kein Netto-Effekt auf die Größenverteilung der Aggregate durch die Bodenbearbeitung festgestellt werden. Wahrscheinlich führte ein geringer physikalischen Einfluss der Geräte zur Bodenbearbeitung oder eine hohe Aggregatneubildungsrate, angeregt durch Bodenmischung und effektive Streu-Einarbeitung in höhere Bodentiefen innerhalb der Pflugvariante.

(iii) Der Inkubationsversuch ergab höhere Makro-Aggregaterträge (g kg^{-2} Boden) in Böden die eine Zugabe an organischem Material erhielten (121,4-363,0) als in den Kontroll-Böden (22,0-52,0), begleitet von höheren Gehalten an mikrobiellem Kohlenstoff und Ergosterol. Die Neubildung von Makro-Aggregaten war ein schnell ablaufender Prozess, was durch die höchsten Raten der Bodenatmung innerhalb der ersten drei Tage nach Zugabe des organischen Materials und der Wiederbefeuchtung des Bodens angezeigt wurde. Der Großteil der Makro-Aggregate in den Böden, die organisches Material erhalten hatten, wurde innerhalb der ersten sieben Tage des Inkubationsexperimentes gebildet (42-75%). Dennoch war der Prozess der Aggregatbildung fortlaufend während der gesamten Inkubationsdauer, was durch eine erhöhte Bodenatmung im Vergleich zu den Kontroll-Böden angezeigt wurde. Gleichzeitig nahm der Kohlenstoffgehalt innerhalb der Makro-Aggregate ab was darauf schließen lässt, dass das organische Material in den neu gebildeten Makro-Aggregaten eine Kohlenstoff-Quelle für die mikrobielle Biomasse darstellt. Die unterschiedlichen Ton-Gehalte spielten bei den gegebenen Konditionen des Inkubationsexperiments nur eine untergeordnete Rolle bei der Neubildung der Makro-Aggregate.

Zusammenfassend lassen diese Ergebnisse darauf schließen, dass durch das Zusammenspiel der Störung und Streu-Verteilung innerhalb der einzelnen Bodenbearbeitungsverfahren und durch die Fähigkeit der raschen Neubildung von Makro-Aggregaten, sich ein jeweiliger Gleichgewichtszustand bezüglich des Gehalts und Umsatzes der Makro-Aggregate einstellte. Deshalb konnten keine kurz- bzw.

langzeitlichen Netto-Effekte, beeinflusst durch die Bodenbearbeitung, auf die Erträge der Makro-Aggregate festgestellt werden. Allerdings könnte die kontinuierliche Streu-Einarbeitung bei gleichzeitig reduziertem physikalischem Einfluss der Geräte zur Bodenbearbeitung der Mulch-Variante eine Möglichkeit liefern, erfolgreich die Bodenstruktur zu verbessern. Dabei könnte die eingearbeitete leichte Fraktion durch den Einbau in Makro-Aggregate vor unmittelbarem Abbau geschützt werden, was möglicherweise eine langfristige Kohlenstoffsequestrierung zur Folge hätte.

1 General introduction

The global carbon (C) pools (atmosphere, vegetation, soil and ocean) are in a state of continuous exchange via carbon inputs and losses. Besides oceanic and geologic C pools, global carbon stocks in soil are a large store of C and are estimated to be in the order of 100 Mg ha^{-1}. In contrast, only about 15 Mg ha^{-1} of carbon is estimated to be stored in aboveground biomass (Janzen, 2004). For these terrestrial pools, most of the carbon is stored in forest and grassland ecosystems. Land use change and the increase of agricultural area in the last centuries increased the decomposition rate of soil organic matter (SOM) due to soil disturbance and reduced the input of fresh organic material by harvesting the biomass (Janzen, 2004).

For a transformation of agricultural soils into a net carbon sink, it is necessary to increase organic matter (OM) inputs and decrease its decomposition rates by adapted agricultural management techniques i.e. cropping intensity, crop type, fertilization, green manuring and tillage (Ostle et al., 2009; Ragot and Schubert, 2008). Furthermore, an increase of C contents in arable soils may provide several accompanying benefits that are beneficial for almost all soil properties (Powlson et al., 2011a). To successfully sequester C in soils and to calculate C stock changes with the help of modelling it is crucial to understand the mechanisms regarding the stabilization and release of C (Powlson et al., 2011a; Wiseman and Puttmann, 2005).

Most of the SOM turnover models are based on conceptual pools defined by different specific turnover times and pool sizes (Von Lützow et al., 2007). Pool size of the active fraction, available for microbial decomposition, is quantified by short-term incubation in decomposition experiments, while the biologically resistant passive pool size is determined by chemical oxidation (Von Lützow et al., 2007). However, chemical oxidation may not provide a homogenous pool in terms of biodegradability (Helfrich et al., 2007; Mikutta and Kaiser, 2011).

Apart from the theoretical division of SOM in conceptual pools, C storage in soils underlies several mechanisms and processes: (i) primary and secondary recalcitrance, (ii) spatial inaccessibility of OM to decomposing microbial biomass, i.e. within soil aggregates, and (iii) interaction of OM with soil minerals and metal ions (Von Lützow et al., 2006). However, the proportion in which way these different processes are responsible for SOM stabilization is not clear (Mikutta et al., 2006).

Selective preservation by recalcitrance was assumed to be a major process in stabilizing SOM against microbial decomposition, however, it might have been previously overestimated in the literature (Marschner et al., 2008). It was shown with natural ^{13}C labelling or by determination of ^{14}C ages, that SOM, which is not associated with minerals or occluded within aggregates, has short turnover times or is younger than 50 years, whereas SOM associated with minerals and occluded within aggregates may have significantly longer turnover times and higher ages (John et al., 2005; Kaiser et al., 2002). These findings indicate that the stabilization mechanisms of SOM fractions with different turnover times were not only due to recalcitrance. In contrast, decomposability of SOM may depend on the supply of water, nutrients, oxygen and spatial (in-)accessibility which is influenced by sorption of SOM to soil particles and occlusion within aggregates and clay layers (Dungait et al., 2012). The assumption that SOM turnover is regulated by physical inaccessibility of substrates to decomposing microorganisms increased the interest on spatial distribution of SOM as a more important stabilization mechanism for SOM turnover than the chemical structure (Christensen, 2001).

Specific stabilization mechanisms with homogenous turnover times by interaction with minerals and soil structure define functional SOM pools. These functional SOM pools could help to improve modelling of C turnover, so far based on conceptual pools defined by different specific turnover times and pool sizes (Von Lützow et al., 2007) and for estimations of C sequestration potentials (Prechtel et al., 2009). Therefore, functional SOM pools with homogeneous turnover times and a specific stabilization mechanism must be identified.

Christensen (2001) divided SOM in three different physical and functional pools: (i) uncomplexed SOM, (ii) primary organo-mineral complexes and (iii) secondary organo-mineral complexes. Uncomplexed SOM is the transitory pool between fresh litter and SOM associated with the mineral phase in primary organo-mineral complexes. It is separated from bulk soil by density fractionation with heavy liquids with densities ranging from 1.6 to 1.9 g cm^{-3}, therefore termed as light fraction (LF) (Gregorich et al., 2006; Von Lützow et al., 2007). It occurs in soils as free light fraction (fLF) in between primary and secondary organo-mineral complexes and as occluded light fraction (oLF) within secondary organo-mineral complexes (Christensen, 2001) and it is sensitive to different cropping and tillage intensities (Gregorich et al., 1994; Sequeira et al., 2011; Soon et al., 2007). Secondary

organo-mineral complexes also termed aggregates consist of silt and clay sized particles and organic binding agents (Christensen, 2001; Tisdall and Oades, 1982).

Tisdall and Oades (1982) were the first who introduced an aggregate formation theory at different hierarchical stages, whereupon living and dead organic biomass acts as binding agent to form aggregates. According to the hierarchical aggregate formation theory by Tisdall and Oades (1982), macro-aggregates (>250 µm) are formed of micro-aggregates (250-53 µm) and temporary and transient organic binding agents like fungal hyphae and roots and microbial- and plant-derived poly-saccharides, respectively. The micro-aggregate fraction, consisting of silt and clay sized particles and humified OM and polyvalent metal cation complexes, is much less susceptible to external influences, like agricultural management, than macro-aggregates (Christensen, 2001; Oades, 1988; Six et al., 2000a). The aggregate size distribution in arable soils can be influenced in several ways: tillage (litter distribution, soil mixing), crop types, manuring, microbial activity, external influences (temperature, precipitation), soil water content and freeze/thaw cycles (Alvaro-Fuentes et al., 2008b; Bossuyt et al., 2002; Layton et al., 1993; Luo et al., 2010).

2 Research objectives

A better understanding of SOM dynamics in interaction with the mechanisms of soil structure formation is crucial for sustainable agriculture and reduction of environmental costs of agricultural ecosystems. Summarising the findings above, information on physical and chemical processes influencing aggregate formation and stabilization in association with C sequestration is limited (De Jonge et al., 2009; Powlson et al., 2011a).

Long-term soil experiments are crucial in evaluating questions on controlling C and nutrient cycling by management on a larger scale than under laboratory conditions. Further they may promote sustainable increases in crop yields. Especially research on changes in such slow-moving systems like soil structure and C dynamics emphasizes the use of long-term trials, as small errors extrapolated across many years from short-term soil experiments can easily bias long-term predictions (Richter Jr. et al., 2007). To level out site specific effects, collecting data from several sites may offer a potential to identify the main variables on a larger scale (Alvarez, 2005). However, field data from long-term studies investigating the effect of different tillage practices on density fractions and water-stable aggregate size distribution, formation and turnover are rare in soils varying in texture and climatic conditions.

Based on the above mentioned gaps of knowledge, the research objectives of this thesis were to:

(i) determine the long-term impact of different tillage treatments on the interaction between macro-aggregation and light fraction distribution, on water extractable organic carbon (WEOC) and on carbon sequestration on plots differing in soil texture and climatic conditions;

(ii) determine the impact of a different tillage treatments on the short and the longer-term in water-stable aggregate size distribution and on macro-aggregate turnover;

(iii) evaluate the macro-aggregate rebuilding in soils with varying initial C contents, organic matter amendments and clay contents in a short-term incubation experiment.

3 Effects of tillage on contents of organic carbon, nitrogen, water-stable aggregates and light fraction for four different long-term trials

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Abstract. Soil management may affect C and N dynamics in soils, but the underlying processes are not well understood. Our objective was to quantify the impact of different tillage treatments on the amount and distribution of free and occluded light fractions (fLF and oLF, respectively), on the water-stable macro-aggregate (>250 µm) contents, and on organic carbon (C_{org}) storage. Four long-term tillage trials were carried out on loess soils in Germany with sugar beet followed by two years of winter wheat as crop rotations. The different tillage treatments trialled were regular conventional tillage (CT, to 30 cm), mulch tillage (MT, to 10 cm) and no-tillage (NT). Soils were sampled in 0-5 cm, 5-25 cm and 25-40 cm depth after 18-25 years of the different tillage treatments. These four long-term tillage trials on plots differing in soil texture and climatic conditions revealed consistent results between them. Average crop yields of sugar beet and winter wheat from 2004 to 2010 were higher under CT and MT than under NT. The NT and MT treatments produced significantly higher C_{org} contents than the CT treatment in 0–5 cm soil depth. The C_{org} stocks in the sampled profile, based on the equivalent soil mass approach (CT: 0-40, MT: 0-38, NT: 0-36 cm), were significantly higher for the MT treatment than for the CT and NT treatments. The fLF, oLF, and macro-aggregate contents were significantly higher for the NT and MT treatments than for the CT treatment in the top 5 cm, whereas in 5-25 cm depth, the oLF contents were significantly higher for the CT and MT treatments. The correlation of the macro-aggregate content against the fLF and oLF contents suggested that the macro-aggregate content is not directly influenced by the different tillage treatments but by the contents of available biomass, presumably due to the

higher biomass input via higher crop yields under CT and MT and the vertical distribution of the residue input by mulching and plowing. Stepwise multiple linear regression analysis suggested that the C_{org} content was the most important factor influencing the macro-aggregate content in the soils of the four long-term trials, whereas the contents of fLF and silt were negatively related to the macro-aggregate content.

3.1 Introduction

Concerns about climate change have led to an increased interest in the potentials of carbon sequestration in agricultural soils. Land use conversion and soil cultivation have resulted in a depletion of organic carbon (C_{org}) contents in soils contributing to the increase in atmospheric CO_2 during the last 150 years (Lal, 2009). The concept of C sequestration aims to partially reverse this trend via higher net primary production and/or decreased decomposition rates in soils (Janzen, 2004; Paustian et al., 1997). Increased contents of C_{org} may also provide additional benefits (ecosystem services) such as retention of water, increased soil biodiversity, reduced risks of soil erosion, and improved soil structure and productivity (Christensen, 2001; Janzen, 2004; Lal and Kimble, 1997).

Soil management, particularly tillage, has a strong influence on the stabilization mechanisms and dynamics of C_{org} in arable soils (Balesdent et al., 2000; Von Lützow et al., 2008). The lower physical impact of conservation tillage increases aggregate stability, leading to lower aggregate turnover rates and therefore improved physical protection of C_{org} from decomposition and thus higher C_{org} stocks in arable soils. In contrast, conventional tillage (CT) disrupts macro-aggregates and formerly incorporated C_{org} is exposed to microbial decomposition (Balesdent et al., 2000; Cambardella and Elliott, 1993; Mikha and Rice, 2004; Six et al., 2000a; Tan et al., 2007; Tisdall and Oades, 1982; Zotarelli et al., 2007). In agreement with this, Six et al. (2000b) and Jacobs et al. (2009) found for long-term agricultural field experiments a decrease of macro-aggregate contents under CT in comparison with no-tillage (NT) and reduced tillage (rotary harrow to 5-8 cm depth), respectively.

Besides the physical tillage impact, tillage also affects litter placement and thus decomposition dynamics (Coppens et al., 2006a; Hermle et al., 2008; Jacobs et al., 2010; Oorts et al., 2007b). For instance, Coppens et al. (2006a) reported that 55% of the litter incorporated was mineralized after 9 weeks of incubation compared to 18%

of mineralized litter in the treatment with litter placed on the soil surface. Overall, organic material may accumulate on the soil surface of NT systems due to a reduced contact between crop residues and soil, whereas litter distribution in plowed soil layers is relatively uniform in CT soils and decomposition rates of the organic material are higher (Lenz and Eisenbeis, 1998; Oorts et al., 2007b).

Turnover of C_{org} in soils is regulated by various factors, such as selective preservation of recalcitrant compounds, spatial inaccessibility by biogenic aggregation or interaction with mineral surfaces (Von Lützow et al., 2008). However, the chemical composition is less important for the C_{org} dynamics in soils than the location and physical protection of C_{org} (Balesdent, 1996; Von Lützow et al., 2008). Physical fractionation methods which consider the accessibility of organic material to decomposers (Christensen, 2001) and the character of organic material *in situ* (Golchin et al., 1994) may facilitate an improved understanding of C dynamics. The fractionation of water-stable aggregates and density fractionation may thus be helpful for an improved understanding of C dynamics affected by soil management, since aggregate and density fractions are more sensitive to changes in soil management than total C_{org} (Oades, 1988; Pikul et al., 2007; Puget et al., 2000; Von Lützow et al., 2006). For instance, Puget et al. (2000) reported that water-stable macro-aggregates were enriched in younger organic material and have faster turnover times than micro-aggregates.

The light fraction (LF) is generally seen as a temporary pool between fresh incorporated litter and mineral associated C_{org} (Christensen, 2001; Six et al., 2001). It mainly consists of recently incorporated organic material, such as microbial- and plant-derived polysaccharides, roots, fungal hyphae, and charcoal (Six et al., 2001; Tisdall and Oades, 1982). Most of the light fraction (except for charcoal particles in this fraction) is generally readily accessible for microbial decomposition (Balesdent et al., 2000). It occurs as loose organic particles and adhering to the exterior of secondary organo-mineral complexes as free light fraction (fLF) and entrapped within secondary organo-mineral complexes (i.e. aggregates) as occluded light fraction (oLF) (Christensen, 2001). The oLF has undergone more decomposition during its protection within aggregates than the fLF (Golchin et al., 1994).

Several researchers related increased macro-aggregate contents to higher inputs of fresh organic material due to increased microbial activity and the production of microbial and fungal derived binding agents (Denef and Six, 2005; Mikha and Rice,

2004). For example, Kiem and Kandeler (1997) reported for soils with different texture that there was an increased aggregate stability with increasing microbial biomass after fertilization. This effect was highest in sandy soils and lowest in clays. However, this was explained by an already high aggregate stability of the clay soils before fertilization (Kiem and Kandeler, 1997). Soil texture may rather affect aggregate stabilization than aggregate formation (De Gryze et al., 2006a).

The impact of different tillage treatments on the factors affecting C turnover and therefore on C sequestration in arable soils was investigated by several authors in different climatic regions. It was shown that the transformation rate of organic material as fLF into oLF and HF is connected with the aggregate turnover in soils (Yoo et al., 2011). The reduced turnover of macro-aggregates under NT treatment (Alvaro-Fuentes et al., 2009; Six et al., 2000a) induces stabilization of LF within aggregates, which was shown for arable soils in temperate climates (Tan et al., 2007; Yoo and Wander, 2008), as well as in tropical climates (Buurman and Roscoe, 2011; Zotarelli et al., 2007). However, long-term studies with different tillage practices on soils differing in soil texture and climatic conditions are rare.

To identify the main variables that are involved in C sequestration it is not sufficient to focus on site specific tillage effects; rather, information from different sites is needed (Alvarez, 2005). Also, it is often argued that the shallow sampling depth of many studies is not sufficient to state a conclusion about management-induced changes in C_{org} (Lal, 2009; Prechtel et al., 2009) and that the conclusion of conservation tillage techniques to sequester C is just an artefact of a shallow sampling methodology (Baker et al., 2007).

The objectives of this study were (i) to determine the impact of different tillage systems on the amount and distribution of labile C_{org} pools (fLF, oLF), water extractable organic carbon (WEOC) and macro-aggregates in different soil layers, including the subsoil, and (ii) to investigate how these fractions interact and affect C sequestration in soils of different tillage treatments.

3.2 Material and Methods

3.2.1 Experimental sites and treatments

Four commercial fields cultivated by the agricultural division of the Südzucker AG Mannheim/Ochsenfurt were selected in the early 1990s to establish a series of

long-term soil tillage experiments. The sites are located in arable loess-regions of eastern and southern Germany. Further details about the climatic conditions, experimental setup, tillage treatments and crop management of the locations are given in Table 3.1 and in Koch et al. (2009).

Table 3.1: Site characteristics, pH and texture are mean values of pseudoreplicates with standard deviation in brackets (n = 3), data refer to the 0-25 cm depth.

Site (Soil type)	Year trial started	Annual precipitation (mm)	Mean annual temperature (℃)	pH	Clay (%)	Silt (%)	Sand (%)
Friemar (Haplic Phaeozem)	1992/93	517	7.8	7.1 (0.25)	31 (5)	65 (6)	5 (2)
Grombach (Haplic Luvisol)	1990/91	776	9.3	6.3 (0.58)	26 (6)	72 (6)	2 (1)
Luettewitz (Haplic Luvisol)	1992/93	572	8.6	6.7 (0.31)	14 (3)	78 (3)	12 (1)
Zschortau (Gleyic Luvisol)	1997/98	512	8.8	7.1 (0.18)	16 (2)	56 (2)	28 (3)

At each site one large field with spatially homogenous soil properties was divided into three plots, with each plot being assigned to one of the three tillage treatments: (i) CT with annual mouldboard ploughing to 25-30 cm (ii) mulch tillage (MT) with a cultivator or disc harrow 10-15 cm deep, and (iii) NT with direct drilling. To assure successful germination, 3-5 cm deep seedbed cultivation before sugar beet sowing was carried out in the NT treatment. At each site the crop rotation consisted of sugar beet (*Beta vulgaris* L.) – winter wheat (*Triticum aestivum* L.) – winter wheat. White mustard (*Sinapis alba* L.) was sown after harvest of the second wheat crop as a catch crop. Crop residues were left on the field and the crop management was carried out following the regional standards of agricultural practice, including the use of non-selective herbicides in MT and NT treatments. Sugar beet selective herbicides, molluscicides and rodenticides were used, the applications depending on the infestation level (Koch et al., 2009). The nitrogen fertilization varied between the sites but the different treatments at each site were fertilized equally. The grain yields for winter wheat, taproot yields of sugar beet and N fertilization rates for the years 2009 and 2010 are given in Table 3.2.

Table 3.2: Grain yields for winter wheat, taproot yields for sugar beet and N fertilization rates for all sites and treatments in the year before soil sampling (2009) and in the year of soil sampling (2010).

Site	Treatment[a]	Crop and yield[b] (t ha^{-1})		N fertilization (kg ha^{-1})	
		2009 Sugar beet	2010 Winter wheat	2009	2010
Friemar	CT	75.8	9.1		
	MT	71.7	8.7	93	212
	NT	66.7	8.3		
Grombach	CT	69.6	6.9		
	MT	67.7	7.6	129	186
	NT	58.8	7.2		
		Winter wheat	Winter wheat		
Luettewitz	CT	7.4	7.9		
	MT	7.7	7.6	181	159
	NT	7.4	7.5		
Zschortau	CT	8.7	8.1		
	MT	8.8	7.8	157	211
	NT	8.2	6.6		

[a] CT: conventional tillage, MT: mulch tillage, NT: no-tillage

[b] Yields are given in dry matter for winter wheat (including 14% water content) and in taproot fresh matter for sugar beet

3.2.2 Soil sampling and soil properties

Soil samples were taken in April 2010. Every plot of each tillage treatment was divided into three subplots. From each subplot a composite sample of five cores was taken with a core sampler of 8 cm diameter. The samples were taken from 0 to 5 cm, 5-25 cm, and 25-40 cm. The fresh soil samples were sieved to pass a 10 mm sieve and homogenized. A representative subsample was sieved to pass a 2 mm sieve for texture analysis, pH determination, water content (dried for 24 h at 105°C), C_{org} and total N (N_{tot}) analysis. Texture was determined by wet-sieving and sedimentation following DIN ISO 11277 (2002). The pH was measured in $CaCl_2$ with a soil:solution ratio of 1 g:2.5 mL. Dry combustion (Elementar Vario El, Heraeus, Hanau, Germany) was used to determine total C and N_{tot} contents. Organic C content was calculated as the difference between the contents of total C and inorganic C. Inorganic C content

was determined using the Scheibler method. After adding about 5-10 mL 10% HCl to a bulk soil sample the evolving CO_2 was measured volumetrically.

The C_{org} stocks of the different soil layers were calculated for an equivalent soil mass described by Jacobs et al. (2010). This approach takes into account differences in bulk density of the respective soil layers.

3.2.3 Water-stable aggregate fractionation

Because differences in C_{org} and N_{tot} stocks were mainly explained by larger amounts of organic matter occluded within aggregates >250 µm (Oorts et al., 2007a), we focused on the separation in macro- (>250 µm) and micro-aggregates (<250 µm).

To determine the amount of water-stable aggregates, the fractionation method of John et al. (2005) was adapted. Briefly, 50 g of sieved (≤10 mm) and oven dried soil (48 h at 40℃) were placed on a 250 µm sieve and submerged into deionized water to allow slaking for 10 min. After removing and dipping the sieve back into the water for 50 times the water-stable aggregates remaining on the sieve (>250 µm) were collected, vacuum filtered and dried for 48 h at 40°C. While collecting the macro-aggregates remaining on the sieve, at first the bottom of the mesh was carefully rinsed with deionized water until the running off water became clear to ensure that all particles <250 µm were rinsed through the sieve. The particles <250 µm were precipitated with 2.5 mL of 0.5 M $AlCl_3$ solution on 1 L of supernatant (Merck, Germany) and dried for 48 h at 40℃ after d ecantation of the supernatant.

The C_{org} and N_{tot} contents of the aggregate fractions were determined by dry combustion as described for the bulk soil above. The results were not corrected for sand content, as this does not change the relative contribution of the aggregate size classes on C_{org} storage (John et al., 2005).

3.2.4 Density fractionation

To separate the fLF and oLF from the heavy fraction the soil samples were treated following the fractionation method of Balesdent et al. (1991) and Golchin et al. (1994). The exact timing, solution density and soil to solution ratio were determined after preliminary tests to identify the conditions that would end up with a sufficient separation result and the highest LF yields. Densities ranging from 1.6 to 1.8 g cm^{-3} and soil to solution ratios from 1:3 to 1:4 (weight-to-volume) were tested. The used density in our experimental setup of 1.8 g cm^{-3} is close to the recom-

mended density for numerous soil types and textures of 1.6 g cm^{-3} by Cerli et al. (2012) and also within the range of 1.6 to 2.0 g cm^{-3}, mentioned as commonly used in the review of Gregorich et al. (2006).

Shaking the soil sample with glass beads to release the oLF faces the problem that it is difficult to apply precise dispersive energies (Cerli et al., 2012). However, Elliott and Cambardella (1991) mentioned in their review about physical separation of soil organic matter fractions that sonification might redistribute the soil organic matter fractions. Balesdent et al. (1991) showed that ultrasonic treatment failed in destroying aggregates >0.2 µm, whereas glass beads are able to destroy the LF rich aggregates >0.2 µm. In comparison with the method of Balesdent et al. (1991), the revolutions per minute of the shaking procedure were increased, as recommended by Paul et al. (2008) to increase the effectiveness in aggregate disruption.

So et al. (1997) showed that soil dispersion with end over shaking (without glass beads) is mainly affected by the soil to liquid ratio, shaking period, container size, and the size of the air-gap above the suspension, whereas the effects of temperature and soil texture on dispersion were not significant. Those important parameters were kept constant in this work. However, it cannot be excluded that some minerals might adhere to the LF after the density fractionation procedure. The density fractionation gives only indications about the intensity of the association between organic particles and minerals, and therefore about the access for microbial decomposition.

Briefly, 10 g of field fresh soil (≤2 mm) were placed in a 70 mL centrifugation tube and adjusted to the same water content by adding deionized water. Then 40 mL of sodiumpolytungstate solution (Sometu, Berlin, Germany) with a density of 1.8 g cm^{-3} was added. The tube with the suspension of the soil and sodiumpolytungstate solution was then gently shaken five times by hand. After allowing the suspension to settle for 30 min it was centrifuged at 2000 g for 30 min (Multifuge 3S-R, Heraeus, Hanau, Germany). The supernatant of the sample was vacuum filtered (<0.45 µm) and washed with 2 L of deionized water to obtain the fLF. The oLF was released from the remaining sample material in the centrifugation tube by orbital shaking at 175 rpm (SM-30, Edmund Bühler, Hechingen, Germany) with 10 glass beads (5 mm) in 40 mL of sodiumpolytungstate solution for 18 h. After a first centrifugation and decantation step the pellet was suspended again in 40 mL sodiumpolytungstate solution and centrifuged. This second suspension step was performed to assure a complete separation of remaining oLF from the heavy fraction. The supernatants of both sus-

pension steps were then combined and vacuum filtered. The remaining pellet, consisting of the heavy fraction with a density of >1.8 g cm^{-3}, was transferred into a beaker together with 1.5 L of deionized water to wash out the sodiumpolytungstate. This suspension was then precipitated with 2.5 mL of 0.5 M $AlCl_3$ solution on 1 L of supernatant (Merck, Germany) and the supernatant water was decanted. The heavy fraction was filtered and washed again with 0.5 L of deionized water to remove the remaining sodiumpolytungstate. Following the methodological adaption by John et al. (2005) of the original method by Golchin et al. (1994), the density fractions were oven dried at a reduced temperature of 40℃ for at minimum 48 h before weighing. The C_{org} and N_{tot} content of every fraction was determined by dry combustion (Elementar Vario El, Heraeus, Hanau, Germany).

3.2.5 Water extractable organic carbon

Water extractable organic carbon was extracted following the method of Zhao et al. (2008) with the exception that a soil to solution ratio of 1:2 (weight-to-volume), recommended by Kalbitz et al. (2003) for arable soil, was used. Bottles containing 75 g of field moist soil (≤2 mm) and 150 mL $CaCl_2$-solution (10 mM) were placed on an orbital shaker for 0.5 h at 175 rpm. After sedimentation of larger soil particles for 1 h, the supernatants were vacuum filtered through pre-rinsed <0.45 µm cellulose acetate filters (OE 67, Schleicher and Schuell) and the filtrate was stored in the freezer at −20℃. Dissolved organic C was determined by infrared detection of CO_2 and for total N by chemoluminescence detection after combustion at 850℃ (Dima-TOC 100, Dima-N, Dimatec, Essen, Germany), respectively.

3.2.6 Statistical evaluation

All statistical evaluations were conducted with the statistic software R (R Development Core Team, 2010).

The three pseudoreplicates per site were used to carry out correlation analyses, the multiple linear regression analyses and to calculate mean C_{org}, N_{tot}, fLF and oLF contents for every site (n=3), without further statistical evaluation of the mean values per site.

The mean contents per site of each parameter served as field-replicates. The mean value of the four field-replicates was used for a statistical evaluation of the tillage treatment effect (n=4). The analysis of variance was conducted with the nlme

package (Pinheiro et al., 2011) on the basis of a linear mixed-effects model with the different sites as nested random effect. Pairwise comparisons were performed with a Tukey-HSD test of the multcomp package (Hothorn et al., 2008). Tillage treatment effects were considered significant at <0.05. No statistical analysis of the different soil depths was conducted.

For comparison of the sugar beet and winter wheat yields of the different tillage treatments the average yields of sugar beet and winter wheat of Germany (German Federal Statistical Office) were set as 100% (mean yields 2004-2007; grain yield of winter wheat: 7.5 t dry matter (including 14% water content) ha^{-1}, taproot yield of sugar beet: 60.5 t fresh matter ha^{-1}). The relative differences for the tillage treatments were calculated for every year from 2004 to 2010 and were expressed as mean values from the six years and four sites per treatment. Statistical analyses were conducted in the same way as for the mean values of the four field-replicates.

Stepwise multiple linear regression was applied on the dataset to identify the soil properties explaining macro-aggregate formation with the AIC (Akaike Information Criterion) as selection criterion. The independent parameters used were C_{org}, fLF, oLF, silt, clay and WEOC.

3.3 Results

3.3.1 Crop yields, organic carbon contents and stocks under different tillage treatments

Grain yields for winter wheat and taproot yields for sugar beet were generally higher under CT and MT than NT with only two exceptions (winter wheat in 2010 in Grombach and in 2009 in Luettewitz, Table 3.2). Overall, the relative yields of the main field crops in comparison with the German average yields from 2004 to 2007 calculated as mean values (with standard deviations) of the four sites from 2004 to 2010 were higher for the MT and CT treatments with 109.6% (±17.0%) and 109.2% (±18.4%), respectively, than for the NT treatment with 102.4% (±18.2%).

At all four sites, despite their differences in texture, climate and the duration of the long-term trial (Table 3.1), the C_{org} and N_{tot} contents in the soils, calculated as the mean values of the three pseudoreplicates, followed the same pattern (data not shown).

The crop residues accumulated on the soil surface under MT and NT due to the reduced litter incorporation. Therefore, the C_{org} contents in the top 5 cm of the soil layer, calculated from the four field replicates, were significantly higher for the MT and NT treatments with 17.1 and 17.8 g (kg soil)$^{-1}$, respectively, than for the CT treatment with 11.1 g (kg soil)$^{-1}$. In 5-25 cm soil depth, the C_{org} content of the NT treatment decreased and only the MT treatment had a significantly higher C_{org} content than the CT and NT treatments. The C_{org} contents in 25-40 cm soil depth were significantly higher for the CT treatment than for the NT treatment (Table 3.3).

Table 3.3: Average contents of organic carbon (C_{org}) and total nitrogen (N_{tot}), C_{org}/N_{tot} ratio, water extractable organic carbon (WEOC) contents and C_{org} stocks, calculated from mean values of the three pseudoreplicates per site, standard deviation in brackets (n = 4).

Depth (cm)	Treatment[a]	C_{org} content (g kg^{-1} soil)	N_{tot} content (g kg^{-1} soil)	C_{org}/N_{tot} ratio	WEOC (mg kg^{-1} soil)
0-5	CT	11.1 (2.1) b	1.2 (0.2) b	9.2 (0.5) b	9 (1) b
	MT	17.1 (2.7) a	1.7 (0.2) a	9.8 (0.2) a	13 (6) ab
	NT	17.8 (2.5) a	1.8 (0.3) a	9.8 (0.4) a	16 (3) a
5-25	CT	11.0 (2.4) b	1.2 (0.2)	9.4 (0.6) ab	11 (3)
	MT	12.1 (1.9) a	1.3 (0.1)	9.6 (0.7) a	11 (4)
	NT	10.9 (1.5) b	1.2 (0.1)	9.3 (0.7) b	12 (2)
25-40	CT	8.1 (2.2) a	0.9 (0.2)	9.3 (0.5)	12 (4)
	MT	7.6 (2.9) ab	0.8 (0.3)	9.6 (0.7)	11 (4)
	NT	6.2 (1.4) b	0.7 (0.1)	8.9 (0.9)	11 (2)
		Depth in the equivalent soil mass (cm)		C_{org} stocks (kg C_{org} m^{-2} in 522 kg soil)	
	CT	0-40		5.2 (1.2) b	
	MT	0-38		5.7 (1.2) a	
	NT	0-36		5.2 (0.8) b	

[a]CT: conventional tillage, MT: mulch tillage, NT: no-tillage

For each parameter, different letters indicate significant differences between treatment means within one depth ($p < 0.05$)

The mean C_{org} stocks in the top 5-6 cm, based on the equivalent soil mass approach and calculated from the field replicates for 63 kg soil m^{-2} (CT: 0-5 cm, MT: 0-6 cm, NT: 0-5 cm), showed significant differences in the order NT (1.12 kg C_{org} m^{-2}) > MT (1.07 kg C_{org} m^{-2}) > CT (0.69 kg C_{org} m^{-2}). In the subsurface soil (CT: 5-25 cm, MT: 6-25 cm, NT: 5-23 cm), the order changed and the soils under MT had

significantly higher C_{org} stocks in 256 kg soil m^{-2} than those of the CT and NT treatments. The C_{org} stocks in the subsoil (CT: 25-40 cm, MT: 25-38 cm, NT: 23-36 cm) and in 203 kg soil m^{-2} followed the order CT > MT > NT with significant differences between the CT and NT treatment (data not shown). Over the whole soil profile the C_{org} stocks in 522 kg soil, based on the equivalent soil mass approach (CT: 0-40 cm, MT: 0-38 cm, NT: 0-36 cm), were significantly increased for the MT treatment (5.7 kg C_{org} m^{-2}) in comparison to the CT (5.2 kg C_{org} m^{-2}) and NT (5.2 kg C_{org} m^{-2}) treatments, respectively (Table 3.3).

The C_{org}/N_{tot} ratio for the bulk soil (Table 3.3) was significantly higher in 0-5 cm soil depth for the NT and MT treatment and in 5-25 cm soil depth for the MT treatment, no significant differences were found in 25-40 cm soil depth.

In accordance with the C_{org} contents, the WEOC contents increased in the top 5 cm in the order CT (9 mg (kg soil)$^{-1}$) > MT (13 mg (kg soil)$^{-1}$) > NT (16 mg (kg soil)$^{-1}$), with significant differences between the CT and NT treatment (Table 3.3). In deeper soil layers no significant differences between the treatments were found. Water extractable organic carbon accounted for 0.1% of C_{org} content.

3.3.2 Density fractions

The fLF and oLF contents in the soils differing in texture, climate and the duration of the long-term trial (Table 3.1), calculated as the mean values of the three pseudo-replicates, followed the same pattern at all four sites (data not shown).

Reflecting the different extent of litter incorporation, the average fLF contents were significantly higher for the MT treatment than for the CT treatment and decreased in the order MT (3.9 g (kg soil)$^{-1}$) > NT (2.7 g (kg soil)$^{-1}$) > CT (1.5 g (kg soil)$^{-1}$) in 0-5 cm soil depth (Table 3.4). The fLF contents decreased with increasing soil depth. In 5-25 cm and 25-40 cm soil depths, the fLF contents followed a different order than in the top 5 cm with higher contents under MT and CT due to litter incorporation and the lowest contents under NT. However, only the difference between MT and NT in 25-40 cm soil depth was statistically significant.

The oLF content was significantly higher for the NT and MT treatments, with 7.4 g (kg soil)$^{-1}$ and 6.7 g (kg soil)$^{-1}$, respectively, than for the CT treatment with 4.1 g (kg soil)$^{-1}$ in 0-5 cm soil depth. In 5-25 cm soil depth, the CT and MT treatments showed no significant difference with oLF contents of 5.1 g (kg soil)$^{-1}$ and 5.3 g (kg soil)$^{-1}$, respectively, but both were significantly increased compared to the NT treatment with

3.9 g (kg soil)$^{-1}$. The same order (except that oLF tended to be highest under CT) was found in 25-40 cm soil depth, but without significant differences (Table 3.4).

Table 3.4: Average contents of the free light fraction (fLF), the occluded light fraction (oLF), the fLF/oLF ratio and the organic carbon (C_{org}) content of the fLF and oLF, calculated from mean values of the three pseudoreplicates per site, standard deviation in brackets (n = 4).

Depth (cm)	Treatment[a]	fLF content (g kg^{-1} soil)	oLF content (g kg^{-1} soil)	fLF/oLF ratio	C_{org} content (g kg^{-1} fLF)	C_{org} content (g kg^{-1} oLF)
0-5	CT	1.5 (0.7) b	4.1 (1.1) b	0.4 (0.3)	237 (67)	294 (7) a
	MT	3.9 (1.4) a	6.7 (1.3) a	0.6 (0.3)	180 (16)	284 (9) b
	NT	2.7 (1.2) ab	7.4 (1.5) a	0.4 (0.3)	207 (9)	285 (3) b
5-25	CT	0.8 (0.4)	5.1 (1.4) a	0.2 (0.1)	243 (71)	299 (7)
	MT	1.0 (0.6)	5.3 (1.2) a	0.2 (0.1)	194 (66)	293 (3)
	NT	0.6 (0.1)	3.9 (1.3) b	0.2 (0)	254 (59)	295 (14)
25-40	CT	0.5 (0.3) ab	3.8 (1.4)	0.1 (0.1)	226 (72)	298 (8)
	MT	0.6 (0.3) a	3.6 (2.0)	0.2 (0.1)	191 (51)	277 (28)
	NT	0.3 (0.1) b	3.0 (2.3)	0.2 (0.1)	216 (90)	284 (19)

[a]CT: conventional tillage, MT: mulch tillage, NT: no-tillage

For each parameter, different letters indicate significant differences between treatment means within one depth ($p \leq 0.05$)

The C_{org} content of the fLF fraction decreased in 0-5 cm and 25-40 cm soil depths in the order: CT > NT > MT and in 5-25 cm soil depth in the order NT > CT > MT. However, differences between the tillage treatments were not significant (Table 3.4).

For each soil depth, soils of the CT treatment showed an increased C_{org} content of the oLF fraction, and the MT and NT treatments ranged around the same contents. However, the differences were statistically significant only in 0-5 cm soil depth (Table 3.4).

A slightly increased C_{org} content was measured for the oLF fraction in comparison with the fLF fraction, but no treatment dependent differences were found in the distinct soil depths. On average, 2.5 and 12.2% of C_{org} of the bulk soil were recovered in the fLF and oLF fractions, respectively, the rest consisting of mineral associated C in the heavy fraction (data not shown) (Table 3.4).

3.3.3 Distribution of water-stable macro-aggregates over soil depth

Macro-aggregate (>250 µm) contents in the top 5 cm were significantly higher for soils of the NT and MT treatments than for soils of the CT treatment and decreased in the order NT (711 g (kg soil)$^{-1}$) > MT (666 g (kg soil)$^{-1}$) > CT (518 g (kg soil)$^{-1}$) (Table 3.5). Differences between the treatments were less pronounced in deeper soil layers. For MT and NT treatments the macro-aggregate contents decreased with depth. However, for the CT treatment it was higher in the 5-25 cm soil layer than in the top 5 cm, which is in line with the oLF contents.

The C_{org} contents in the macro-aggregates in 0-5 cm depth were significantly higher in soils of the MT and NT treatment with 19 and 20 g (kg aggregates)$^{-1}$, respectively, than the contents in the soils of the CT treatment with 13 g (kg aggregates)$^{-1}$ (Table 3.5). With increasing depth the C_{org} contents in the macro-aggregates of the MT and NT treatments decreased to the level of the CT treatment. In 25-40 cm soil depth the CT treatment showed even a significantly higher C_{org} content in the macro-aggregates (10 g (kg aggregates)$^{-1}$) than the NT treatment (7 g (kg aggregates)$^{-1}$).

In accordance with the macro-aggregate contents and the C_{org} contents of the macro-aggregates the relative contribution of C_{org} stored in macro-aggregates was significantly higher in 0-5 cm soil depth for the NT treatment than for the CT treatment. It decreased in the order NT (81% of C_{org} bulk soil) > MT (75% of C_{org} bulk soil) > CT (60% of C_{org} bulk soil). Differences were less pronounced in 5-25 cm and 25-40 cm soil layers and without significant differences between the tillage treatments.

The C_{org}/N_{tot} ratio of the macro-aggregates in 5-25 cm soil depth was significantly increased for the CT treatment in comparison to the NT treatment with 9.9 and 9.5, respectively (Table 3.5). In 0-5 cm and 25-40 cm soil depths no further statistical differences between the tillage treatments were found.

The C_{org}/N_{tot} ratio of the micro-aggregates in 0-5 cm soil depth was significantly higher for the MT treatment than for the CT treatment, with 9.7 and 9.4, respectively. No significant differences were detectable in the deeper soil layers.

Table 3.5: Average macro-aggregate (>250 µm) contents, organic carbon (C_{org}) contents in macro-aggregates, relative C_{org} contribution in macro-aggregates to whole C_{org} content and C_{org}/total N (N_{tot}) ratios for macro- and micro-aggregates, calculated from mean values of the three pseudoreplicates per site; standard deviation in brackets (n = 4).

Depth (cm)	Treatment[a]	Aggregate content >250 µm (g kg^{-1} soil)	C_{org} content in aggregates >250 µm (g kg^{-1} aggregates)	C_{org} content in aggregates >250 µm (% of C_{org} bulk soil)	C_{org}/N_{tot} ratio in aggregates >250 µm	C_{org}/N_{tot} ratio in aggregates <250 µm
0-5	CT	518 (76.5) b	13 (2) b	60 (6) b	10.0 (0.7)	9.4 (0.4) b
	MT	666 (79.2) a	19 (3) a	75 (13) a	10.4 (0.7)	9.7 (0.3) a
	NT	711 (59.1) a	20 (3) a	81 (4) a	10.5 (0.3)	9.5 (0.5) ab
5-25	CT	606 (86.1)	12 (3)	69 (10)	9.9 (0.8) a	9.6 (0.4)
	MT	636 (94.7)	14 (2)	71 (10)	9.7 (0.7) ab	9.3 (0.7)
	NT	639 (88.8)	12 (2)	72 (8)	9.5 (0.6) b	9.1 (0.8)
25-40	CT	533 (105.4)	10 (3) a	64 (7)	9.5 (1.2)	9.4 (0.6)
	MT	530 (108.3)	9 (3) ab	60 (14)	9.3 (1.0)	9.7 (1.3)
	NT	563 (101.8)	7 (2) b	66 (7)	9.2 (0.9)	8.7 (1.0)

[a]CT: conventional tillage, MT: mulch tillage, NT: no-tillage

For each parameter, different letters indicate significant differences between treatment means within one depth ($p \leq 0.05$)

3.3.4 Correlation between organic carbon, macro-aggregates and light fraction

Across all sites and treatments the macro-aggregate and C_{org} contents showed a positive linear correlation. The degrees of determination decreased with depth from 0.58 to 0.13. While the macro-aggregate content was less influenced by depth, the C_{org} content decreased with increasing depth. This resulted in a declining slope of the linear model with depth when C_{org} was plotted against macro-aggregate content (Fig. 3.1). In 0-5 cm soil depth the CT treatment was separated from the MT and NT treatments due to lower C_{org} and macro-aggregate contents. This was also valid for the correlation of the macro-aggregate content against the oLF content and against the fLF content, respectively. However, in 5-25 cm soil depth the tillage treatments had no further effect on these relationships. Macro-aggregate content was distributed equally among the tillage treatments, but the higher oLF and fLF contents under CT and MT led to a separation on the y-axis in 5-25 cm soil depth, reflecting the different amounts of incorporated crop residues.

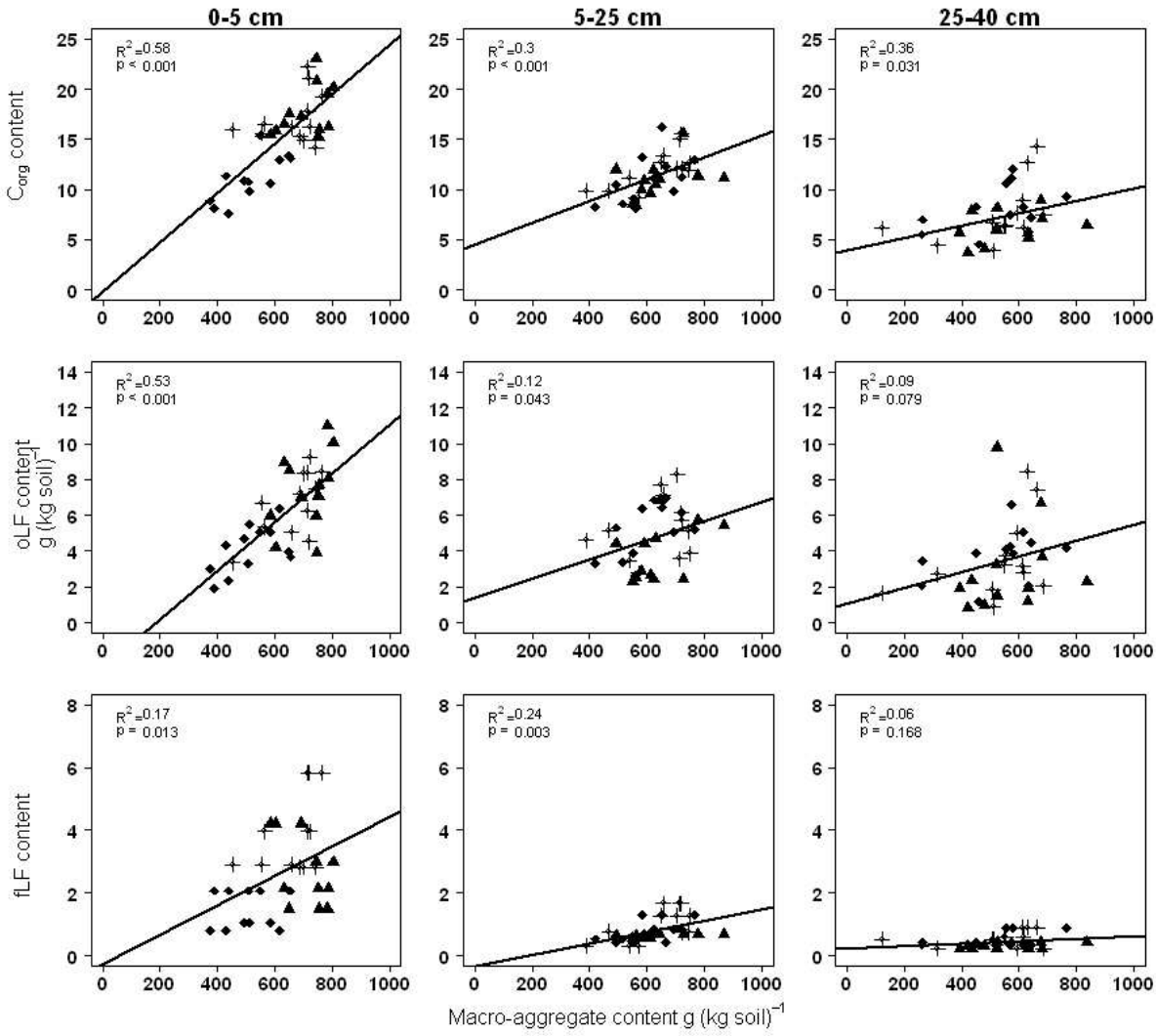

Fig. 3.1: Correlation of the macro-aggregate (>250µm) content with the contents of organic carbon (C_{org}), free light fraction (fLF), and occluded light fraction (oLF) for different soil depths. Tillage treatments are marked as follows (♦ = conventional tillage, + = mulch tillage, ▲ = no-tillage).

Stepwise multiple linear regression analysis, including all treatments and depths, indicated that the C_{org} (positively related), fLF and silt contents (negatively related) were important variables for the prediction of macro-aggregate content (Table 3.6) and the linear regression of the estimated against the measured macro-aggregate contents predicted 51% of the variability in the macro-aggregate content (Fig. 3.2).

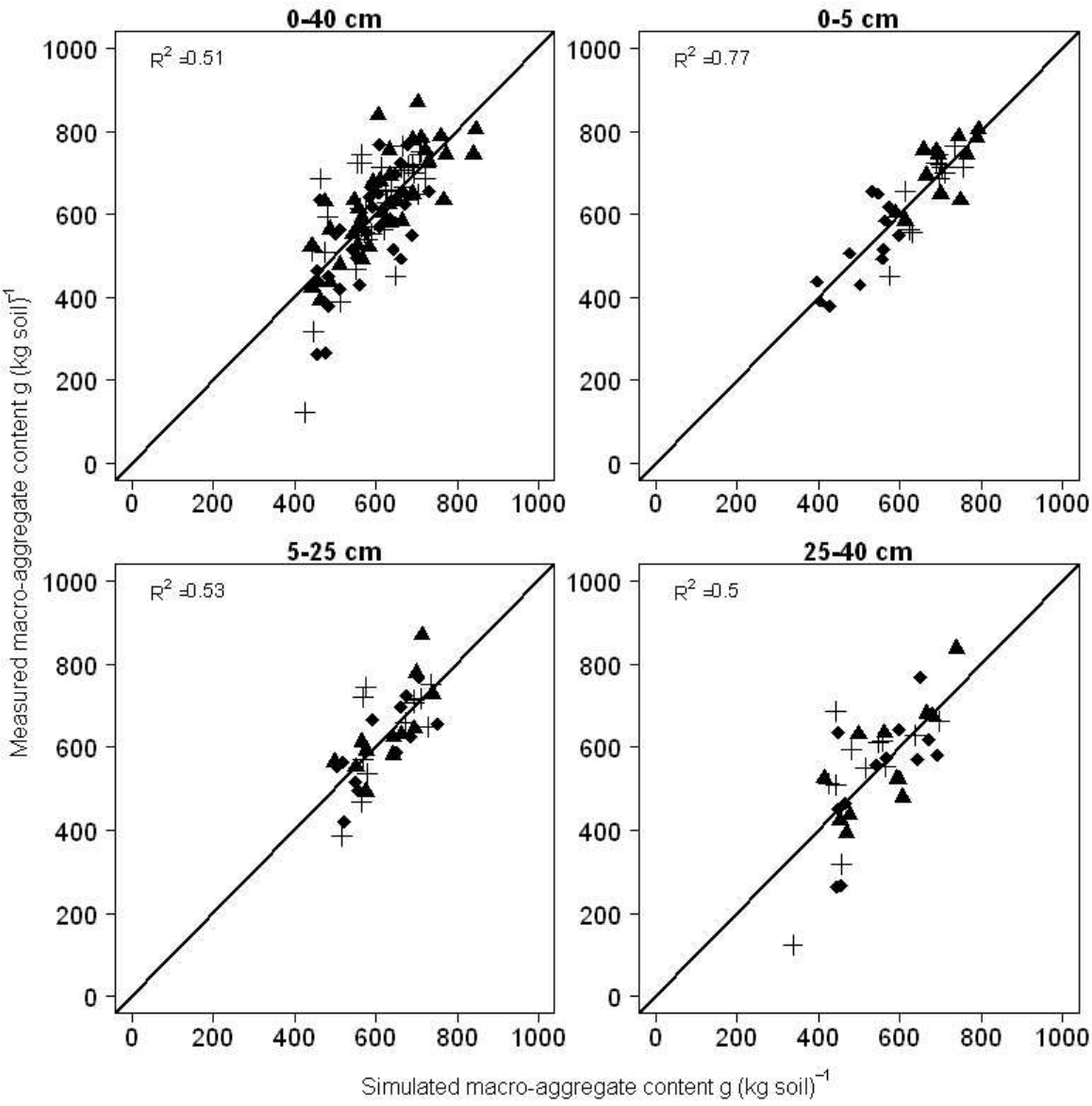

Fig. 3.2: Estimated against measured macro-aggregate (>250 µm) contents for different sampling depths. Tillage treatments are marked as follows (♦ = conventional tillage, + = mulch tillage, ▲ = no-tillage).

Thus, C_{org} and silt contents were important parameters for the prediction of macro-aggregate contents, independent of sampling depth or tillage treatment (Table 3.6). The fLF as well as the silt content always showed negative regression coefficients. The soils with the highest C_{org} contents, such as 0-5 cm sampling depth and MT treatment, revealed the oLF content as a positive variable determining the macro-aggregate content (Table 3.6).

Table 3.6: Stepwise multiple linear regression for macro-aggregate content (g (kg soil^{-1})) including the contents of organic carbon (C_{org}), free light fraction (fLF), occluded light fraction (oLF), silt, clay and water extractable organic carbon (WEOC)[b].

Aggregate content >250 µm g (kg soil)$^{-1}$	Intercept g (kg soil)$^{-1}$	C_{org} content g (kg soil)$^{-1}$	fLF content g (kg soil)$^{-1}$	oLF content g (kg soil)$^{-1}$	silt content g (kg soil)$^{-1}$	clay content g (kg soil)$^{-1}$	R^2
0-40 cm	772.5	22.0	-25.2		-0.6		0.51
0-5 cm	446.5	19.5		18.1	-0.2	-0.3	0.77
5-25 cm	769.3	21.5			-0.6		0.53
25-40 cm	871.5	31.8	-177.1		-0.9	0.5	0.5
CT[a]	816.4	23.3	-48.2		-0.7		0.5
MT[a]	659.1	12.3		14.7	-0.4		0.49
NT[a]	837.1	19.5	-33.7		-0.6		0.59

[a]CT: conventional tillage, MT: mulch tillage, NT: no-tillage

[b]The WEOC content was considered in the stepwise multiple regression, but it did not improve the regression.

3.4 Discussion

3.4.1 Crop yields, organic carbon contents and stocks under different tillage treatments

The higher crop yields for the CT and MT treatments than for the NT treatment were probably due to an increased penetration resistance and dry bulk density, and diminished air filled pore volume in the topsoil of the NT treatment (Koch et al., 2009) and also likely due to the high amounts of plant residue remaining on the surface of NT soils. This led to lower germination and therefore lower plant densities of sugar beet and winter wheat in 1995 to 2005 (Dieckmann et al., 2006) and the subsequent years.

The crop residues accumulated on the soil surface under MT and NT due to the reduced litter incorporation. This resulted in significantly increased C_{org} contents in the top 5 cm of the MT and NT treatments in comparison with the CT treatment. However, the differences between the tillage treatments became smaller with increasing soil depth and in 25-40 cm soil depth, the CT treatment even showed a significantly higher C_{org} content (Table 3.3). Similarly, Hermle et al. (2008) found in an Orthic Luvisol after 19 years of different tillage treatments increased C_{org} contents in 0-10 cm of NT and MT soils in comparison with CT soils and also in 20-30 cm soil depth increased C_{org} contents under CT.

The C_{org} stocks, based on the equivalent soil mass approach, in the 0 to 5-6 cm soil layer decreased in the order NT > MT > CT. These findings are in accordance with several studies that concluded that reduced tillage is able to sequester C in arable soils (Balesdent et al., 2000; Cambardella and Elliott, 1993; Mikha and Rice, 2004; Six et al., 2000a; Tisdall and Oades, 1982; Zotarelli et al., 2007).

The C sequestration rates of the NT and MT treatments in the 0-5 (MT) and 0-6 (NT) cm soil layers in our study, calculated with an average duration of the four long-term experiments of 18-25 years, in comparison with the CT treatment are 23 g C m^{-2} yr^{-1} and 21 g C m^{-2} yr^{-1}, respectively. West and Post (2002) calculated, based on a global dataset of 67 long-term trials, an average C sequestration rate of 57±14 g C m^{-2} yr^{-1} for conversion from CT to NT for the 0-30 cm layer.

The C_{org} stocks in the entire sampled soil layer were significantly higher for the MT treatment than for the NT and CT treatments. The combination of higher yields and reduced tillage intensity likely led to the increased C_{org} stocks under MT (Table 3.3). The calculated C sequestration rate for the MT treatment in comparison with the CT treatment is 31 g C m^{-2} yr^{-1}, while there was no C sequestration under NT, presumably due to decreased aboveground and root biomass input because of lower yields. These results are in contrast with the findings from 0 to 5 cm soil depth and are contradictory to the calculated C sequestration rate by West and Post (2002). The differences may be explained by the shallow sampling depth of those studies. None of the treatments of the dataset with tillage as a variable was sampled below 30 cm soil depth (Baker et al., 2007). In contrast, our results are comparable to surveys that also included deeper soil sampling or subsoil. For example, Luo et al. (2010) found in their meta-analysis of a global data set from 69 paired tillage experiments no significant differences between C_{org} stocks of CT and NT soils. They especially focused on studies with soil sampling deeper than 40 cm and revealed that conversion from CT to NT significantly increased the C_{org} stocks in 0-10 cm depth, whereas the C_{org} stocks between 10 and 40 cm depth were significantly lower. Also Embacher et al. (2007) showed an increased C_{org} content in the upper cm of an Eutric Cambisol after 10 years of NT located in Bavaria, Germany. On the contrary, an Eutric Luvisol under conventional tillage located in North Rhine-Westphalia, Germany, showed similar C_{org} contents with depth.

3.4.2 Water extractable organic carbon contents under different tillage treatments

The WEOC contents increased with decreasing tillage intensity from CT > MT > NT in the 0–5 cm soil layer (Table 3.3). With increasing depth the WEOC contents decreased under MT and NT and increased under CT. Reports on the effects of reduced tillage techniques on WEOC are contradictory in the literature. The results of our study are in accordance with Coppens et al. (2006a) who measured increased WEOC contents in the upper 2 cm of soil (silt loam) columns with ^{13}C and ^{15}N labelled oilseed rape residues which were applied on the soil surface. The soil columns in this trial with incorporated marked oilseed rape litter had the highest WEOC contents in 10 cm depth. Similar results were found by Linn and Doran (1984) who measured WEOC in 0-7.5 cm depth and 7.5-15 cm depths at four different tillage experiments across the United States on a clay loam, two silt loams and a silty clay loam. The tillage experiments were conducted for 5-11 years at time of sampling. They found trends for increased WEOC contents under NT in the top 7.5 cm and under CT in 7.5-15 cm soil depth. However, Soon et al. (2007) did not find different WEOC contents between CT and NT treatments in the top 15 cm of a sandy loam (Grey Luvisol) after 12 years of different tillage treatments.

One reason for increased C_{org} and WEOC contents in the top 5 cm of the NT and MT treatments could be the remaining crop residues on the soil surface (Coppens et al., 2006a; Oorts et al., 2007b; Wright and Hons, 2005). Additionally, higher C_{org} and WEOC contents under CT with increasing soil depths could be caused by enhanced root growth due to soil loosening (Dwyer et al., 1996; Pietola, 2005). These assumptions are also in line with the increased yields under CT in this study (Table 3.2). The belowground C_{org} input via roots and root exudates might even be 2.4 times higher than the aboveground litter input (Rasse et al., 2005), leading to higher C_{org} and WEOC contents in higher depths of the CT treatments.

3.4.3 Density fractions

In 0–5 cm soil depth the fLF content was significantly higher for the MT treatment than for the CT treatment, with the NT treatment being in between (Table 3.4). With increasing soil depths the NT treatment showed always the lowest fLF contents. The fLF distribution likely reflected the differences in litter input by tillage into the different soil layers (Coppens et al., 2006a; Lenz and Eisenbeis, 1998; Oorts et al., 2007b),

suggesting that the fLF contents can be taken as an indicator for the amount of incorporated litter.

The oLF content was significantly higher for the NT and MT treatments than for the CT treatment in the top 5 cm. As with the fLF contents, the oLF contents under NT were always lower in higher soil depths, however only in 5–25 cm depth were the differences between the tillage treatments significant (Table 3.4), which is in line with the findings of Yoo and Wander (2008) and can be attributed to the missing ability of NT systems in litter incorporation.

The increased litter incorporation under CT and MT may have led to higher microbial activity (Denef and Six, 2005) and therefore incorporation of LF into macro-aggregates (Helfrich et al., 2008). Similarly, Mikha et al. (2010) reported for a silt loam located in the Great Plains that after 15 years of different tillage treatments the LF contents were increased with decreasing tillage intensities in 0-5 cm soil depth. However, in 5-15 cm soil depth no significant differences were detectable in the present study.

The C_{org} content of both density fractions showed only little effect of tillage treatment and depth (Table 3.4). This result is in line with Oorts et al. (2007a), who found increased contributions of LF on C_{org} contents of the bulk soil in the top 5 cm of a Haplic Luvisol, but concluded that the difference is mainly due to increased LF contents of NT soils rather than due to increased C_{org} contents of the LF. However, this is in contrast to Zotarelli et al. (2007) who reported increased C_{org} contents for fLF and oLF under NT in the top 5 cm of an Oxisol. The average proportion of C_{org} provided by the density fractions of the total C_{org} was 2.5 and 12.2% for the fLF and oLF, respectively (data not shown). This is in accordance with other findings. For example Sequeira et al. (2011) showed in an arable soil under different tillage treatments that the C_{org} content of the fLF (sodiumpolytungstate solution, 1.6 g cm^{-3}) and oLF (sodiumpolytungstate solution, 1.8 g cm^{-3}) averaged 5.5 and 5.2% of total C_{org}, respectively. Wander and Bidart (2000) found in arable soils under NT and CT that the percentage of the fLF and the oLF (sodiumpolytungstate solution, 1.6 g cm^{-3}) of the total C_{org} ranged from 2.5 to 6.5 and 12.2-22.6%, respectively.

3.4.4 Distribution of water-stable macro-aggregates and correlation between organic carbon, macro-aggregates and light fraction

The amount of the different aggregate fractions found in soils highly depends on whether the contents were obtained by wet sieving procedures after slaking or dry sieving procedures (Ashman et al., 2003). Therefore we only compared our findings with results provided by methods, where wet sieving was applied during the aggregate fractionation procedure.

The higher macro-aggregate contents and C_{org} contents within macro-aggregates in the top 5 cm for MT and NT soils are in line with the findings of Oorts et al. (2007a) on a Haplic Luvisol developed on loess. They stated that the additional C_{org} in NT soils is mainly due to higher macro-aggregate contents. However, this effect was limited to the top 5 cm of the soil. Additionally, they suggested that the higher bacterial and fungal activity, rather than the slower macro-aggregate turnover in 0-5 cm soil depth of NT soils, was the reason for higher macro-aggregate contents. Similar results were found by Alvaro-Fuentes et al. (2009) in a Mediterranean semiarid agroecosystem, with the contradictory conclusion in comparison with Oorts et al. (2007a) of reduced aggregate turnover under NT as reason for higher macro-aggregate contents.

The correlation of the macro-aggregate content with the soil C_{org} content, the oLF content and the fLF content showed only in the top 5 cm a separation between the NT treatment and the MT and CT treatments (Fig. 3.1). This is in contrast to the hypothesis of physical macro-aggregate destruction by tillage (Mikha and Rice, 2004; Six et al., 2000b; Tan et al., 2007; Tisdall and Oades, 1982). However, in 5-25 cm soil depth, the increased contents of C_{org} for the MT treatment and oLF for the MT and CT treatment against the NT treatment, respectively, led to a vertical differentiation of the correlation. Below 5 cm soil depth there was no effect of the tillage treatments on macro-aggregate and C_{org} contents. Therefore, we assume that the increased litter input and distribution under MT and CT was the main driving factor behind macro-aggregate content rather than the physical tillage impact.

Using stepwise multiple linear regression the C_{org} (positively related), fLF and silt contents (negatively related) were identified as important factors determining macro-aggregate content (Table 3.6). In the surface (0-5 cm) soil, clay was not positively correlated with the macro-aggregate content as indicated by its negative coefficient. At the 25–40 cm depth, a positive coefficient suggested the importance of

clay for the aggregate content. The latter is in line with the findings of de Gryze et al. (2006a) and Kiem and Kandeler (1997) who found significant correlations of the clay content with the macro-aggregate content. However, the clay content is more seen as a factor determining the aggregate stabilization than the aggregate formation (De Gryze et al., 2006a). Therefore, the clay content in our study does not positively correlate with the aggregate content in soil depths with presumably higher aggregate turnover rates like in 0-5 cm and 5-25 cm. Due to the fact that the experimental setup of this study consists of four different commercially used fields with differing site specific soil properties and therefore high scattering of the data, it might have been difficult to clearly identify the factors for aggregate formation with the stepwise multiple linear regression analysis.

The findings of this study are supported by Helfrich et al. (2008) who showed that litter incorporation into the soil fosters the macro-aggregate formation in an incubation experiment. However, after less than two months of incubation the macro-aggregates disintegrated and broke down into smaller aggregate size fractions and macro-aggregates failed as long-term C sink (Helfrich et al., 2008). This breakdown, due to missing litter input, could be the reason why the soils of the reduced tillage treatments in our study did not reveal higher macro-aggregate and C_{org} contents in macro-aggregates than the soils of CT below the top 5 cm. The physical tillage impact might have been compensated by macro-aggregate formation due to the more homogenous litter incorporation of the CT treatment.

Because the fLF content is related to biomass input we compared our results with studies that investigated the relevance of biomass input in context of soil aggregation. For example, Wright and Hons (2005) found on a silty clay loam that the tillage effect on soil aggregation in 0-5 cm depth was only visible in sorghum monoculture but not at high intensity crop rotation (sorghum, wheat and soybean) with elevated biomass input. Due to plant litter incorporation with tillage, no effect on soil aggregates could be found in higher depths (5-15 cm), which is in line with our findings.

3.5 Conclusion

We found the same trend in C_{org} storage for the different tillage treatments in all four different study sites differing in texture and climatic conditions. Twenty years of NT treatment showed no significant effect on C_{org} stocks in 0-40 cm and therefore on

C sequestration in comparison with CT treatment. The correlation of the LF with macro-aggregates and stepwise multiple linear regression suggest that the LF contents in the different soil depths were the driving factors affecting macro-aggregate formation rather than the physical impact of the tillage equipment. Higher litter input and effective litter distribution under CT and MT, which was shown by density fractionation, compensated the higher physical impact by tillage in comparison with NT soils. However, the combination of high crop yields, reduced physical impact and litter incorporation under MT led to increased C_{org} stocks and a C sequestration rate of 31 g C m^{-2} yr^{-1}. Our study showed higher effects of the different tillage treatments on the density fraction contents than on their C_{org} contents and also emphasized the importance of the amount of available biomass on macro-aggregate content.

4 Effect of long-term tillage treatments on the temporal dynamics of water-stable aggregates and on macro-aggregate turnover at three German sites

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Abstract. The protection of organic material within aggregates against microbial decomposition is regarded as an important process in soil organic carbon stabilization, however detailed knowledge about the processes is still lacking. The objective was to determine the longer and short-term impacts of three different tillage treatments (conventional tillage, mulch tillage and no-tillage) on water-stable aggregate size distribution. Soils from three long-term tillage trials on loess soils in Germany, planted with sugar beet followed by two years of winter wheat, were sampled in 0-5 cm, 5-25 cm and 25-40 cm depth in April 2010 (wheat stand), September 2011 (after harvest or sugar beet stand), November 2011 (after tillage) and April 2012 (bare soil or wheat stand). Generally, the soils under no tillage and mulch tillage showed higher yields of macro-aggregates and carbon contents within macro-aggregates in 0-5 cm soil depth than under conventional tillage for all sampling dates, probably mainly due to litter accumulation in the topsoil under reduced tillage treatments. Tillage in November 2011 showed no effect on macro-aggregate yield in comparison to earlier sampling in September 2011. This suggests that either the physical impact of the mouldboard plough did not markedly affect macro-aggregate dynamics or that high macro-aggregate rebuilding rates due to litter incorporation and soil mixing under conventional tillage counterbalanced the physical impact. In 0-5 cm soil depth the carbon content of the micro-aggregates within macro-aggregates was higher under reduced tillage treatments, indicating accelerated macro-aggregate turnover under conventional tillage. In contrast, it was lower in 5-25 cm under no tillage and 25-40 cm under mulch tillage and no tillage than under conventional tillage. Overall, the pattern of yields of macro-aggregates

and carbon contents within macro-aggregates over time and depth suggest that the interaction of soil disturbance and litter incorporation of the different tillage treatments created a steady state in terms of macro-aggregate turnover within the different tillage treatments.

4.1 Introduction

Increased C sequestration in soils may not only slightly reduce global warming (Powlson et al., 2011b), but higher carbon contents also provide several other ecosystem services like retention of nutrients and water, prevention of erosion and increased soil biodiversity (Christensen, 2001; Janzen, 2004; Lal and Kimble, 1997).

Aggregate formation in soils is considered as an important process in soil organic carbon (C_{org}) stabilization (Alvaro-Fuentes et al., 2009; Tisdall and Oades, 1982; Von Lützow et al., 2006) and can be influenced in agricultural soils by litter input, tillage and crop rotation (Balesdent et al., 2000; Grandy and Robertson, 2007; Malhi et al., 2008). Tisdall and Oades (1982) described in their hierarchical theory of aggregate formation that micro-aggregates (53-250 µm) are formed by primary particles being bound together by persistent organic binding agents. Micro-aggregates, more resistant against management influences than macro-aggregates, are bound together by temporary and transient organic binding agents and form macro-aggregates (>250 µm), rich in C_{org}. Several researchers concluded that declining C_{org} contents and yields of macro-aggregates in arable soils are caused by physical disruption of carbon-rich macro-aggregates due to tillage and the exposure of previously protected organic material to microbial decomposition processes (Balesdent et al., 2000; Cambardella and Elliott, 1993; Mikha and Rice, 2004; Six et al., 2000a; Tisdall and Oades, 1982; Zotarelli et al., 2007).

Indications for macro-aggregate disruption due to tillage were increased yields of macro-aggregates under no-tillage (NT) treatments in comparison to conventional tillage (CT) treatments (Alvaro-Fuentes et al., 2008a; Oorts et al., 2007a; Tan et al., 2007; Zotarelli et al., 2007). Further, increased CO_2-evolution rates were measured after tillage events *in situ* (Alvaro-Fuentes et al., 2007; La Scala et al., 2008; Morell et al., 2010) as well as under simulated conditions during incubation studies (De Gryze et al., 2006a; Plante and McGill, 2002b). This provided some evidence for physical disruption of macro-aggregate structures by tillage and increased decomposition rates of former incorporated organic material. However, despite aggregate disruption,

tillage also influences aggregate size distribution in arable soils by several interacting factors. For instance, CT influences the gravimetric moisture content, which leads to decreased soil moisture after tillage (Hermawan and Bomke, 1997). Further, tillage management directly affects the soil mixing and litter distribution within the soil layer. The litter may be mainly concentrated on the soil surface under NT or distributed relatively evenly within the plough layer under CT (Luo et al., 2010; Morell et al., 2010), which influences the decomposition rates (Coppens et al., 2006a; Oorts et al., 2007b) and microbial activity (Bossuyt et al., 2002) in different soil depths. Moreover, the mulch layer under NT offers some protection against rainfall (Alvaro-Fuentes et al., 2008b) and freeze/thaw cycles during winter (Layton et al., 1993) and the vegetation period influences the aggregation by root growth (Alvaro-Fuentes et al., 2008b; Denef et al., 2002; Plante and McGill, 2002a).

Increased C_{org} contents in surface soils under NT systems may not only be due to higher yields of carbon-rich macro-aggregates, but also due to reduced rates of macro-aggregate turnover under NT systems compared to CT systems (Alvaro-Fuentes et al., 2009; Plante and McGill, 2002b; Six et al., 2000a; Zotarelli et al., 2007). Six et al. (2000a) suggested that slower macro-aggregate turnover may promote the formation of stable micro-aggregates within macro-aggregates, which leads to a long-term C_{org} stabilization. The micro-aggregate associated C_{org} content occluded within macro-aggregates may serve as an indicator for management-induced changes in macro-aggregate turnover and C stabilization (Denef et al., 2004; Kong et al., 2005).

Up to now, insufficient field data are available that quantify the direct impact of tillage both on macro-aggregate yield directly after tillage and on macro-aggregate turnover. An understanding of these effects is essential for questions regarding the relationship between aggregation and soil C_{org} stabilization (Alvaro-Fuentes et al., 2009; Denef et al., 2007).

The objective was to investigate the effects of CT in relation to reduced (mulch tillage, MT) and NT treatments on temporal changes in water-stable aggregate distribution and on macro-aggregate turnover. To exclude site specific tillage effects (Alvarez, 2005) and include higher soil depths, soil samples from three different long-term tillage trials with almost identical experimental setups were sampled in three different depths for this study.

4.2 Material and Methods

4.2.1 Experimental sites

In the 1990s three commercial fields, cultivated by the agricultural division of the Südzucker AG Mannheim/Ochsenfurt, were selected to establish a series of long-term soil tillage experiments. The sites, namely Friemar and Luettewitz (both established in 1992/93) and Zschortau (established in 1997/98), are located in arable loess regions of eastern Germany. Annual precipitation ranges from 512 to 572 mm and the mean annual temperature from 7.8 to 8.8℃ (Table 4.1). The soils are a Phaeozem (Friemar) and Luvisols (Luettewitz and Zschortau). Silt is the dominant size fraction in the soils and decreases in the order Luettewitz (78%) > Friemar (65%) > Zschortau (56%) (Table 4.1). Additional information on the sites and the experimental setup are given in Koch et al. (2009).

During the study period from 2010 to 2012, the gravimetric moisture contents (averaged for the four sampling dates, in %, mean values of the three sites and standard errors in brackets) of the different tillage treatments ranged from 13 (2) to 20 (2) under CT, from 15 (2) to 24 (4) under MT and from 15 (1) to 24 (2) under NT. The bulk densities (averaged for the four sampling dates, in g cm^{-3}, mean values of the three sites and standard errors in brackets) of the different tillage treatments ranged from 1.1 (0.0) to 1.4 (0.0) under CT, from 1.0 (0.1) to 1.5 (0.0) under MT and from 1.0 (0.1) to 1.5 (0.0) under NT.

Table 4.1: Site characteristics, pH (CaCl2) and texture are mean values of the three treatments per site with standard error in brackets (n=3), data refer to the 0–25 cm depth.

Site	Year trial started	Soil type	Mean annual temperature (℃)	Annual precipitation (mm)	pH ($CaCl_2$)	Sand (%)	Silt (%)	Clay (%)
Friemar	1992/93	Haplic Phaeozem	7.8	517	7.1 (0.14)	5 (1)	65 (3)	31 (3)
Luettewitz	1992/93	Haplic Luvisol	8.6	572	6.7 (0.18)	12 (1)	78 (2)	14 (2)
Zschortau	1997/98	Gleyic Luvisol	8.8	512	7.1 (0.1)	28 (2)	56 (1)	16 (1)

4.2.2 Crop rotation and management

At each site the same crop rotations were set up, which consisted of sugar beet (*Beta vulgaris* L.) - winter wheat (*Triticum aestivum* L.) - winter wheat. The crop rotations for the three sites from 2010 to 2012 were: (i) Friemar and (ii) Luettewitz:

winter wheat - winter wheat - sugar beet, (iii) and Zschortau: winter wheat - sugar beet - winter wheat.

One large field per site was divided into three different tillage-plots with the following tillage treatments per plot:

(i) CT with annual mouldboard plowing to 25-30 cm,

(ii) MT with a cultivator or disc harrow 10-15 cm deep, and

(iii) NT with direct drilling.

Due to higher demands in seedbed preparation in the NT treatment, 3-5 cm deep cultivation before sugar beet sowing was carried out.

In all treatments, the crop residues remained on the field and the crop management, including the use of pesticides, was carried out following the standards of agricultural practice. The different treatments at each site were fertilized equally but the nitrogen fertilization varied between the sites.

4.2.3 Soil sampling

Sampling took place on four different dates from April 2010 to April 2012:

(1) April 2010 (wheat stand on all three sites),

(2) September 2011 (before tillage, after wheat harvest in Friemar and in the sugar beet stand in Luettewitz and Zschortau),

(3) November 2011 (after tillage) and

(4) April 2012 (bare soil before sugar beet planting in Friemar and in the wheat stand in Luettewitz and Zschortau). The four sampling dates allowed us to evaluate both longer-term (April 2010 and 2012) and short-term (Sept 2011, before tillage vs. Nov 2011, after tillage) effects of tillage on aggregation dynamics.

Three subsamples were taken from every plot per site. Each subsample consisted of a composite sample from three soil cores, taken with a core sampler of 8 cm in diameter. Samples were taken from 0-5 cm, 5-25 cm and 25-40 cm soil depths.

A representative subsample was sieved to pass a 2 mm sieve for gravimetric moisture content (dried for 48 h at 40℃), C_{org} and total N (N_{tot}) analysis. The C_{org} and N_{tot} content of the aggregate fractions was determined by dry combustion (Elementar Vario El, Heraeus, Hanau, Germany). In accordance with the soil sampling, undisturbed soil cores (100 cm^3) were collected at each sub-sampling point in the corresponding depths. The bulk density was then determined by oven drying at 105℃ for 24 h.

4.2.4 Fractionation of water-stable aggregates

For the water-stable aggregate fractionation the method of John et al. (2005) was adapted and the amount of soil used was reduced. Briefly, 30 g of gently sieved (≤ 10 mm) and oven dried soil (48 h at 40°C) was placed on a 250 µm sieve and submerged into distilled water to allow slaking for 10 min. After removing and dipping the sieve back into the water for 50 times the water-stable aggregates remaining on the sieve (>250 µm) were collected, vacuum filtered and dried for at least 48 h at 40°C. Aggregates passing the sieve were poured onto the next smaller mesh size (53 µm) and the procedure was repeated as described above. The material passing the 53 µm sieve was also vacuum filtered and dried for 48 h at 40°C. The three aggregate size fractions >250 µm, 250-53 µm and <53 µm are termed macro-aggregates, micro-aggregates and silt and clay sized fraction, respectively. To exclude site specific and seasonal effects on temporal changes, yields of macro-aggregates and carbon contents within macro-aggregates were also calculated on a relative basis for every sampling date with CT set as 100%.

Determination of the sand content within the macro- and the micro-aggregates was performed following the DIN ISO 11277 (2002) method. Approximately 10 g of a sub-sample was oxidized with H_2O_2 (30%) and dispersed with 0.4 M sodium hexametaphosphate by shaking for 16 h on an orbital shaker. The C_{org} and N_{tot} content of the aggregate fractions was determined by dry combustion as described for the bulk soil above.

Carbon content within the macro- and micro-aggregate fractions (in g kg^{-1} fraction) are shown after correction for sand content (Fig. 4.4). Sand corrected carbon content within the aggregate size fractions (C_{org}-$fraction_{sand\text{-}free}$) was calculated as follows (John et al., 2005):

$$C_{org}\text{-}fraction_{sand\text{-}free} = C_{org}\text{-}fraction/(1 - \%\ sand\ content\text{-}fraction) \quad (Eq.\ 1)$$

where C_{org}-fraction was the C_{org} content (%) in the respective aggregate size fraction and sand content-fraction was the relative proportion of sand in the aggregate fraction.

Yields of macro- and micro-aggregate fractions (in g kg^{-1} soil) are presented without correction for sand content (Fig. 4.1; Fig. 4.2), due to findings of John et al.

(2005) and Yang and Wander (1998) indicating that sand-sized particles were included in such aggregate fractions.

4.2.5 Fractionation of macro-aggregate-occluded micro-aggregates

The content of macro-aggregate-occluded micro-aggregates was determined according to Six et al. (2000a) and Gulde et al. (2008). A subsample of approximately 10 g dried macro-aggregates was placed on a 250 µm sieve, submerged in deionized water for 10 min and then gently shaken with 50 glass beads (5 mm in diameter) until all macro-aggregates were destroyed. A continuous and steady stream of deionized water flushed the material <250 µm on a 53 µm sieve to avoid the disruption by glass beads. The material on the 53 µm sieve was then wet sieved, as described for the water-stable aggregate fractionation, to isolate the water-stable micro-aggregates within macro-aggregates. The fractions remaining on the 250 µm, 53 µm sieve and passing through the 53 µm sieve were collected, vacuum filtered and oven dried for at least 48 h at 40℃.

4.2.6 Statistical evaluation

All statistical evaluations were conducted with the statistic software R (R Development Core Team, 2010).

Mean yields and contents per site and treatment were calculated from the three subsamples per treatment and served as field-replicates; the mean values of these three field-replicates were then used for statistical evaluation. The analysis of variance was conducted with the nlme package (Pinheiro et al., 2011) on the basis of a linear mixed-effects model with the different sites and sampling dates as nested random and fixed effects, respectively.

Pairwise comparisons were performed with a Tukey-HSD test of the multcomp package (Hothorn et al., 2008). Effects were considered significant at $p < 0.05$.

4.3 Results

4.3.1 Water-stable aggregate size distribution

The yields of water-stable macro-aggregates (Fig. 4.1), micro-aggregates and clay- and silt-sized particles (not shown), calculated as the mean values of the three

subsamples per plot, followed the same pattern under the different tillage treatments at all three sites (Fig. 4.1).

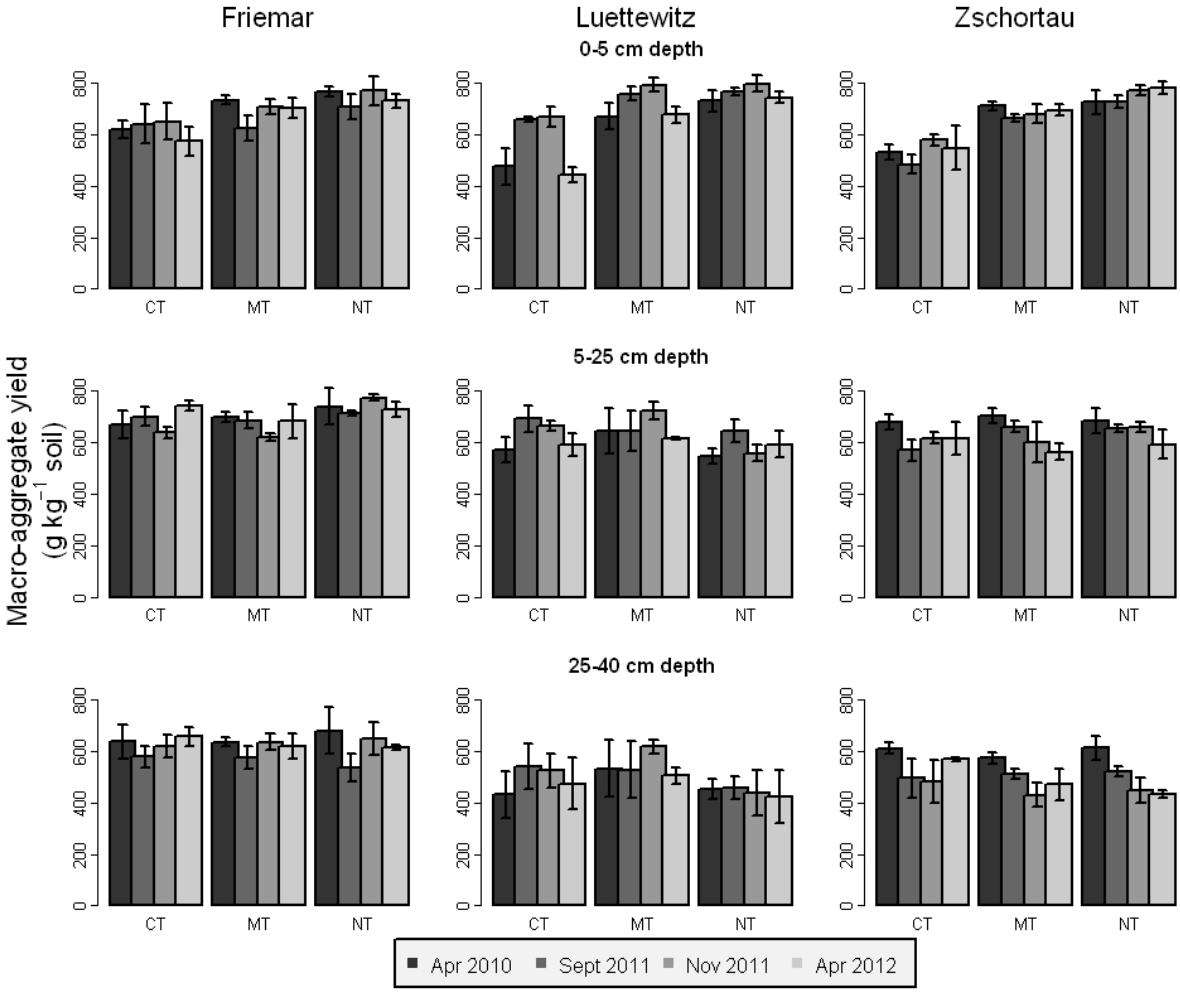

Fig. 4.1: Average dry matter yields of the macro-aggregate (>250 µm) fractions of the different tillage treatments, sampling depths and sampling dates per site, calculated from the three subsamples per site; error bars represent standard errors (n=3).

In the surface soil, the mean yields of water-stable macro-aggregates (calculated from the three field replicates) were significantly higher under MT (all four sampling dates) and NT (three sampling dates) than under CT treatment. Statistically significant differences below 5 cm were only found in 25-40 cm soil depth under NT in April 2012 (Fig. 4.2).

Temporal variations in yields of macro-aggregates within the different tillage treatments were mainly restricted to the 0-5 cm soil depth. Before tillage in September 2011 the MT and NT treatments showed only slightly higher yields of macro-aggregates in relation to CT. Although the soil was intensively mixed, there

was no visible tillage-induced decrease of the macro-aggregate yield under CT in November 2011 (Fig. 4.2).

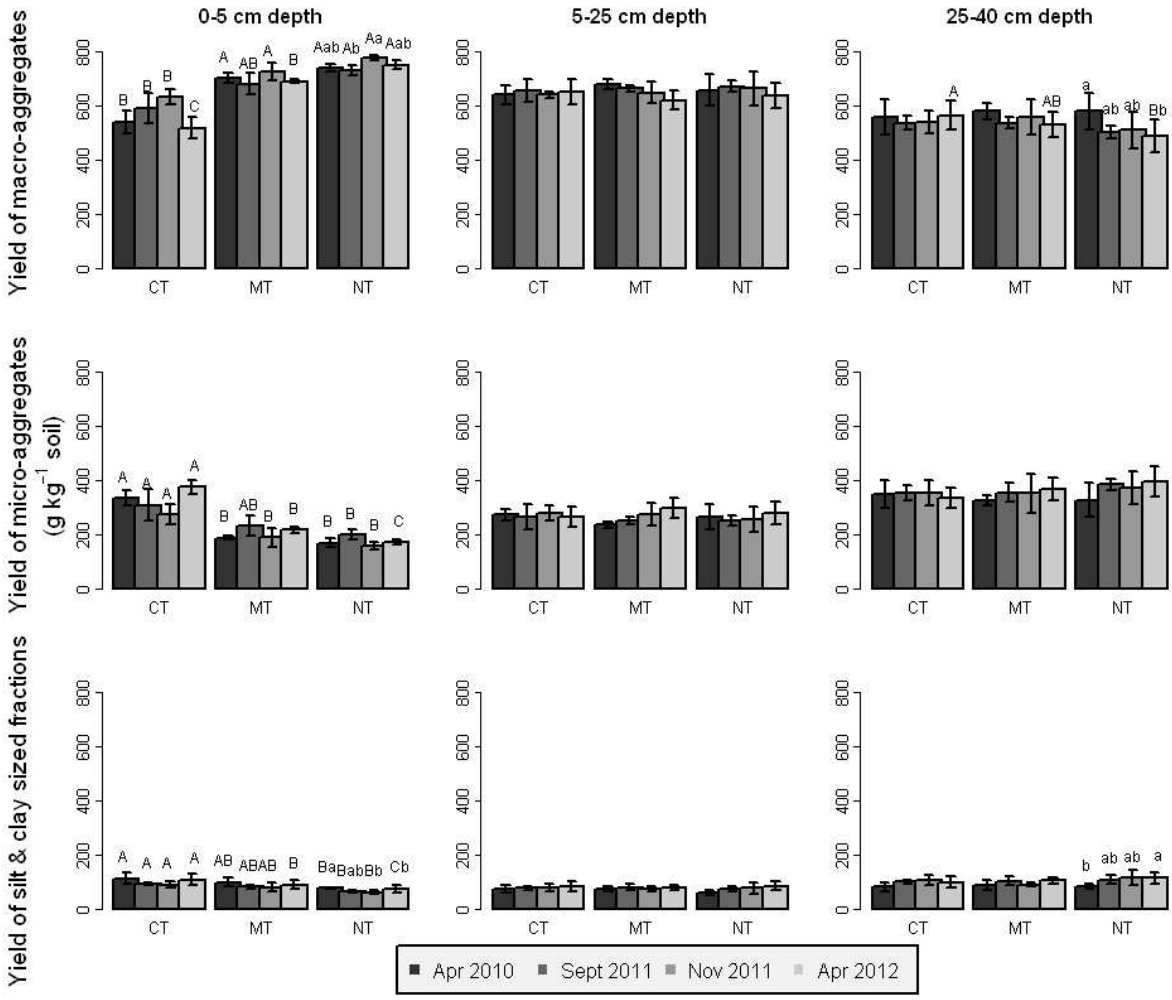

Fig. 4.2: Average dry matter yields of macro-aggregate (>250 µm) and micro-aggregate (250-53 µm) and of the silt- and clay-sized (<53 µm) fractions of the different tillage treatments, sampling depths and sampling dates, calculated from mean values of every site; error bars represent standard errors (n=3); significant differences between the respective sampling dates and tillage treatments are indicated with different lower case letters or capital letters, respectively ($p < 0.05$).

In higher soil depths at 5-25 and 25-40 cm no temporal variation of the aggregate dynamics as affected by different tillage treatments was detectable, with one exception under NT in 25-40 cm soil depth. In general, higher yields of macro-aggregates led to lower yields of micro-aggregates in the corresponding treatments and depths, since the yields of clay- and silt-sized particles remained approximately constant (Fig. 4.2).

The correlation between macro-aggregate yield and gravimetric moisture content was poor (r ranged from 0.07 to 0.23, Fig. 4.3). In contrast, bulk density showed a

marked correlation with macro-aggregation under MT (r = 0.55; p <0.05) and NT (r = 0.57; p <0.05), whereas under CT the correlation was low (r = 0.21; p <0.05).

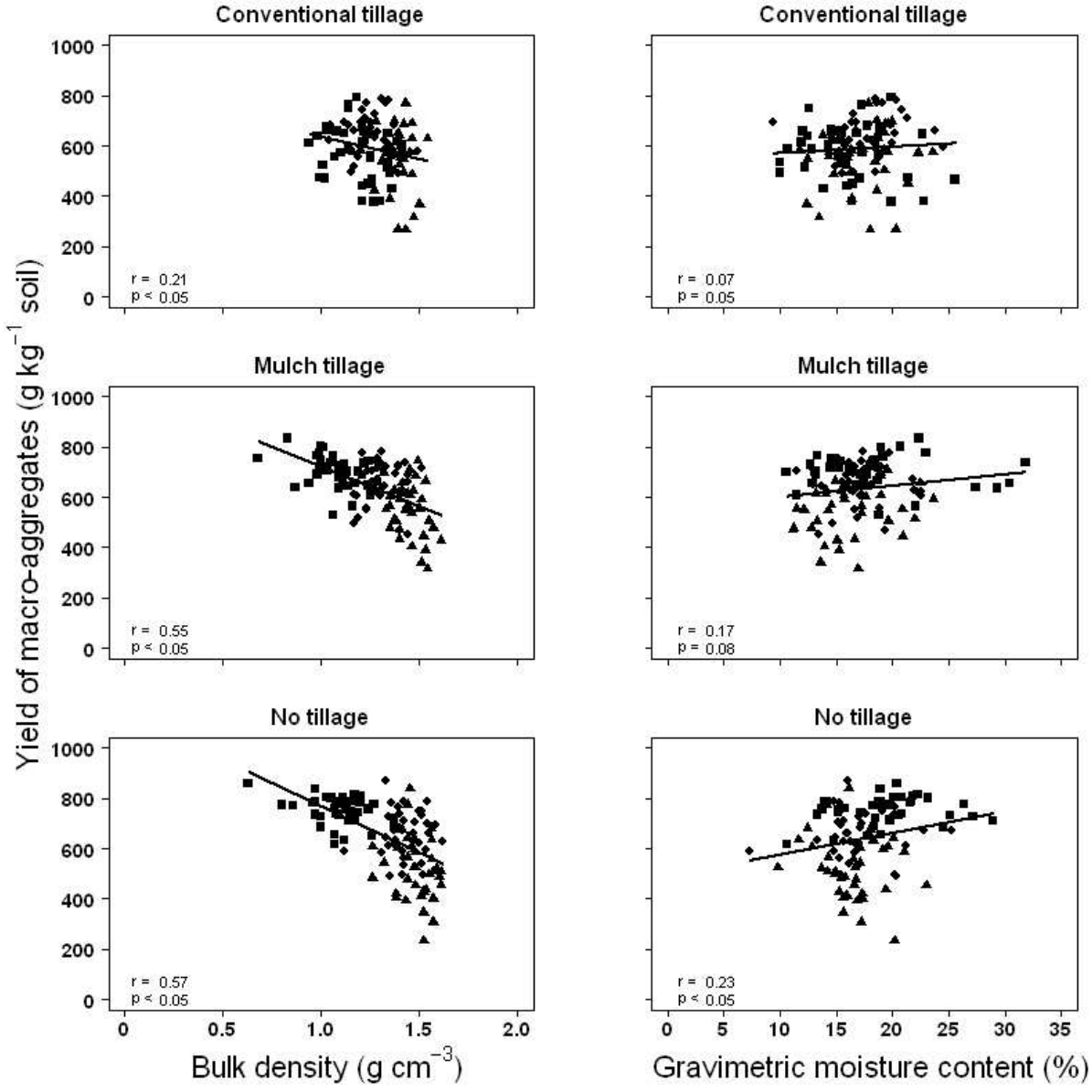

Fig. 4.3: Correlation of the water-stable macro-aggregate yield against the bulk density and gravimetric moisture content. Tillage treatments are marked as follows (■ = 0-5 cm, ● = 5-25 cm, ▲ = 25-40 cm)

4.3.2 Organic carbon contents within macro-aggregates

In comparison to the CT treatment, both treatments under reduced tillage had in general significantly higher C_{org} contents within macro-aggregates in 0-5 cm soil depth (Fig. 4.4). Due to decreasing C_{org} contents within macro-aggregates with depth under MT and NT, the differences in comparison to the CT treatment were less pronounced in 5-25 cm soil depth. In 25-40 cm soil depth the C_{org} content within

macro-aggregates was in general higher under CT than under MT and NT, with significant differences between CT and NT in April 2010 and November 2011. Over time the C_{org} content within macro-aggregates showed only significant variations under NT in 0-5 and 25-40 cm soil depth (Fig. 4.4).

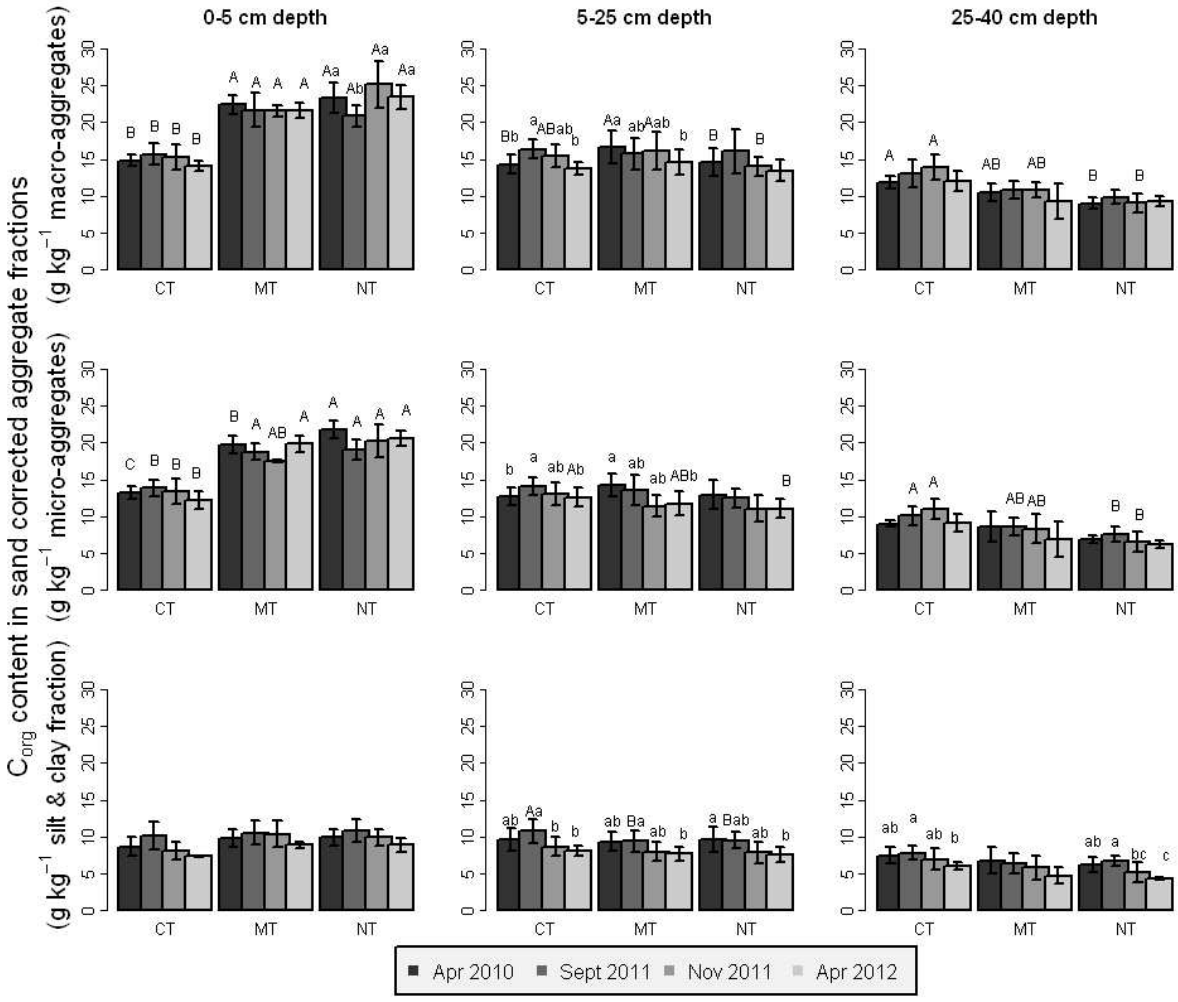

Fig. 4.4: Average C_{org} content in sand corrected macro-aggregate (>250 µm) and micro-aggregate (250-53 µm) fractions and of the silt and clay sized (<53 µm) fractions of the different tillage treatments, sampling depths and sampling dates, calculated from mean values of every site; error bars represent standard errors (n=3); significant differences between the respective sampling dates and tillage treatments are indicated with different lower case letters or capital letters, respectively ($p < 0.05$).

4.3.3 Carbon contents of macro-aggregate occluded micro-aggregates

The surface soil samples from April 2012 had a significantly higher micro-aggregate associated C_{org} content occluded within macro-aggregates under MT and NT in comparison with CT in 0-5 cm soil depth (Table 4.2). This suggests a significantly higher macro-aggregate turnover under CT treatment in this depth. In 5-25 and 25-40

cm soil depth, however, the micro-aggregate associated C_{org} content occluded within macro-aggregates was equal under MT or significantly lower under NT in comparison to CT.

The percentage of the total C_{org} difference between the tillage treatments explained by the C_{org} content of micro-aggregates within macro-aggregates was 58% (0-5 cm) and 75% (5-25 cm) between CT and MT, and 54% (0-5 cm) and 69% (5-25 cm) between CT and NT. At greater depths below 25 cm, however, the contribution was 25% between CT and MT and 43% between CT and NT (Table 4.2), indicating the decreasing importance of micro-aggregates in macro-aggregates for C sequestration in reduced tillage systems with depth. Compared to that, the carbon content within macro-aggregates (mean values of the three sites and standard errors in brackets, n=3) explained 105% (7), 107% (23) and 65% (23) between CT and MT, and 104% (3), 56% (6) and 88% (22) between CT and NT in 0-5, 5-25 and 25-40 cm soil depth, respectively (data not shown), which indicates that differences in C dynamics of different tillage systems are mainly explained by C stored in macro-aggregates.

Table 4.2: Average contents of organic carbon (C_{org}), yields of micro-aggregates within macro-aggregates, carbon contents of micro-aggregates within macro-aggregates and the percentage of the total C_{org} difference between the tillage treatments explained by the difference in the respective C_{org} content of micro-aggregates occluded within macro-aggregates from three different soil depths (0-5, 5-25, 25-40 cm) under conventional (CT), mulch (MT) and no tillage (NT), sampled in April 2012; means are calculated from the field replicates; standard errors are given in brackets (n=3)

Sampling depth	C_{org} content (g kg^{-1} soil)			Yields of micro-aggregates within macro-aggregates (g kg^{-1} macro-aggregate)			Carbon contents of micro-aggregates within macro-aggregates (g kg^{-1} micro-aggregates within macro-aggregates)			Percentage of the total C_{org} difference explained by the C_{org} content of micro-aggregates within macro-aggregates (%)	
(cm)	CT	MT	NT	CT	MT	NT	CT	MT	NT	CT vs. MT	CT vs. NT
0-5	11.29 (2.4) C	17.41 (3.1) B	19.60 (1.1) A	459.3 (35.6) B	480.9 (32.0) B	514.1 (34.5) A	13.9 (1.3) B	20.9 (2.2) A	23.5 (0.9) A	58 (4)	69 (7)
5-25	11.96 (2.5) A	11.47 (2.6) AB	10.90 (2.4) C	487.9 (31.4)	491.4 (35.4)	492.1 (29.4)	14.0 (1.0) A	13.7 (1.4) AB	12.5 (1.5) B	75 (39)	54 (13)
25-40	9.36 (2.4) A	7.59 (4.4) AB	7.06 (2.4) C	475.2 (23.3)	486.1 (55.6)	470.1 (62.6)	11.5 (1.5)	9.1 (2.5)	8.8 (0.8)	25 (11)	43 (9)

Different capital letters indicate significant differences between the tillage treatments ($p < 0.05$).

4.4 Discussion

4.4.1 Water-stable aggregate size distribution

In general, CT resulted in decreased yields of macro-aggregates in comparison to MT and NT treatments in 0-5 cm soil depth (Fig. 4.2). It has been reported several times that mouldboard ploughing has a negative physical impact on yields of macro-aggregates, whereas under reduced tillage treatments the litter accumulation in the top 5 cm results in higher yields of macro-aggregates (Andruschkewitsch et al., 2013; Oorts et al., 2007b; Six et al., 1999; Yang and Wander, 1998).

In 5-25 and 25-40 cm soil depth, generally no effect by tillage was found (Fig. 4.2). The input of fresh organic material into greater soil depths by higher soil mixing and litter translocation under CT might lead to a flush of microbial activity, producing binding agents, and therefore serves as nucleation site for macro-aggregates. This probably counteracts the physical impact by tillage (Bossuyt et al., 2002; Six et al., 1999).

Temporal effects were more pronounced in 0-5 cm soil depth under CT than under reduced tillage (Fig. 4.2). This was presumably a result of higher protection by litter on the soil surface against freeze/thaw cycles and rain splash during winter time under MT and NT (Layton et al., 1993). The restriction of this temporal pattern to the top 5 cm further supports this assumption.

Approximately constant yields of macro-aggregates after tillage under CT (Fig. 4.2) might be due to low physical macro-aggregate disruption or high re-aggregation within a few days because of efficient soil mixing and litter distribution. Overall, we observed no net tillage effect on macro-aggregate yield under CT treatment over time, which was also reported by Plante and Mc Gilll (2002a) for two contrasting Canadian soils, namely an Orthic Black Chernozem and an Orthic Clay Luvisol with 36 and 22% of clay, respectively, during two growing seasons.

We hypothesized a dependency of the yields of macro-aggregates on the gravimetric moisture content due to increasing tensile strength with decreasing moisture content. However, a correlation between soil aggregation in dependency on gravimetric moisture content could not be identified in our study (Fig. 4.3).

Yields of macro-aggregates were negatively correlated with bulk density (Fig. 4.3). The relationship had a greater slope and correlation coefficient under MT (r = 0.55)

and NT (r = 0.57) than under CT (r = 0.21). With increasing bulk density, water movement and rooting depth of plants will be affected, which may also affect the aggregate dynamics. However, a likely explanation is that under reduced tillage (MT, NT) in greater soil depths, not only bulk density is slightly higher than under CT (Fig. 4.3), but also C input in greater depths is much smaller under reduced tillage. Thus, the observed correlation between yields of macro-aggregates against bulk density may be at least partly due to the uneven litter distribution in the reduced tillage systems. Moreover, one has to keep in mind that the seasonal variation of aggregate size distribution is a consequence of several interacting factors (Yang and Wander, 1998).

4.4.2 Organic carbon contents within macro-aggregates

The C_{org} content within macro-aggregates decreased markedly below the top 5 cm under both reduced tillage treatments. In 5-25 cm soil depth the C_{org} contents under MT and NT reached the same level as the CT treatment and were even lower in 25-40 cm (but significant differences were found only between NT and CT for two sampling dates, Fig. 4.4). Bossuyt et al. (2002) and Plaza-Bonilla et al. (2010) found that under NT treatment higher C_{org} contents within aggregates were also restricted to 0-5 cm soil depth. Plaza-Bonilla et al. (2010) even found increased C_{org} contents within aggregates below 20 cm soil depth under mouldboard ploughing. Our study supports the conclusion of Plaza-Bonilla et al. (2010) that fresh carbon input by CT in higher soil depths results in higher C_{org} incorporation within aggregates.

4.4.3 Carbon contents of micro-aggregates occluded in macro-aggregates

Differences found in this study in the micro-aggregate associated C_{org} content occluded within macro-aggregates between the tillage treatments suggest higher macro-aggregate turnover rates under CT in 0-5 cm soil than under MT and NT (Table 4.2). Similarly, Alvaro-Fuentes et al. (2009) found under semiarid conditions a higher micro-aggregate associated C_{org} content occluded within macro-aggregates, and therefore a higher stabilization of C_{org} in the micro-aggregates within macro-aggregates under NT in comparison with CT. Similar findings were also provided by Denef et al. (2007) in two highly weathered Brazilian soils under CT and NT treatment. However, in both studies the effect was restricted to 0-5 cm soil depth and no sampling took place below the plough layer.

In greater soil depths the CT treatments showed slightly higher micro-aggregate associated C_{org} contents occluded within macro-aggregates, which suggests slightly lower macro-aggregate turnover rates than under MT and NT (Table 4.2). However, this might be due to reduced input of fresh organic material below 5 cm soil depth under MT and NT (Andruschkewitsch et al., 2013; Yoo and Wander, 2008). Therefore, the regular input of organic material and the soil mixing within the distinct soil depths are reduced under MT and NT as a factor for macro-aggregate formation and turnover (Blair et al., 2005; Bossuyt et al., 2002; Puget et al., 2000; Six et al., 2000a; Verchot et al., 2011). This is supported by the findings of Plante and Mc Gill (2002b), who suggested that a certain amount of fresh organic material input and soil disturbance is needed to successfully occlude fresh organic material within macro-aggregates. Otherwise, the macro-aggregate turnover is too slow to protect the fresh organic material from microbial decomposition and it will be rapidly mineralized before occlusion within macro-aggregates.

The effects by tillage were less pronounced for yields of micro-aggregates within macro-aggregates in 0-5 cm soil depth and could not be found in higher soil depths (Table 4.2). This is in accordance with the findings of Denef et al. (2007), who stated that a difference in micro-aggregate associated C_{org} content occluded within macro-aggregates is not always accompanied by a parallel difference in the yields of micro-aggregates within macro-aggregates.

The contribution of the micro-aggregate associated C_{org} content occluded within macro-aggregates fraction on differences in total C_{org} between the and treatments (Table 4.2) is with 75-25% (CT-MT) and 69-43% (CT-NT) somewhat lower than the findings of Denef et al. (2004) and Denef et al. (2007), who calculated a relative contribution of 90% after 15 years and 60% after 4 years of different tillage treatments, respectively. However, both studies were conducted under warmer climatic conditions in comparison to this study. Therefore the higher contribution of the micro-aggregate associated C_{org} content occluded within macro-aggregates on the difference in soil C_{org} under different tillage treatments might be explained by higher turnover rates of the used soils by Denef et al. (2004) and Denef et al. (2007).

4.5 Conclusion

We found in general significantly higher yields of macro-aggregates and carbon contents within macro-aggregates in 0-5 cm soil depth under reduced tillage treat-

ments (NT, MT) in comparison with CT. We assume that this was probably mainly due to the reduced soil mixing and incorporation of organic material and therefore litter accumulation at the soil surface. Further, the carbon content of the micro-aggregates within macro-aggregates was higher under reduced tillage treatments, indicating increased macro-aggregate turnover under CT.

In contrast, in 5-25 and 25-40 cm soil depth no negative effect by CT was found on yields of macro-aggregates and carbon contents within macro-aggregates. We assume that the soil mixing and litter incorporation in higher soil depths by CT might lead to a flush of microbial activity, producing binding agents as nucleation sites for macro-aggregates, probably counteracting the physical impact of tillage.

Tillage in November 2011 showed no effect on macro-aggregate yield in comparison to earlier sampling in September 2011. This suggests that either high macro-aggregate rebuilding rates due to litter incorporation and soil mixing under conventional tillage counterbalanced the physical impact or the physical impact of the mouldboard plough did not markedly affect macro-aggregate dynamics.

Altogether these results indicate that the interaction of soil disturbance and litter incorporation of the different tillage treatments may create a steady state in terms of macro-aggregate turnover within the different tillage treatments. Therefore, no tillage-induced temporal net changes in macro-aggregation were detectable during the study period.

5 Rate of soil aggregate formation under different clay and organic matter amendments - a short-term incubation experiment

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Abstract. To improve soil structure and take advantage of several accompanying ecological benefits, it is necessary to understand the underlying processes of aggregate dynamics in soils. Our objective was to quantify macro-aggregate (>250 µm) rebuilding in soils from loess (Haplic Luvisol) with different initial soil organic C (SOC) contents and different amendments of organic matter (OM) and clay contents in a short-term incubation experiment. Two soils differing in C content and sampled in 0-5 and 5-25 cm soil depth were incubated after macro-aggregate destruction. The following treatments were applied: (i) control (without any addition), (ii) OM1 (addition of OM: pre-incubated wheat straw (<10 mm, C/N 40.6) at a rate of 4.1 g C kg^{-1} soil;), (iii) OM2 (same as (ii) at a rate of 8.2 g C kg^{-1} soil), and (iv) OM2_c (same (iii), whereat the clay content was increased to 25%). Evolution of CO2 released from the treatments was measured continuously and contents of different water-stable aggregate size classes (>250 µm, 250-53 µm, <53 µm), microbial biomass and ergosterol were determined after 7 and 28 days of incubation. Highest microbial activity was observed in the first three days after the OM application. With one exception, more than 50% of the rebuilt macro-aggregates were formed within the first 7 days after rewetting and addition of OM. However, the amount of organic C within the new macro-aggregates was about 2-3 fold higher than in the original soil. The process of aggregate formation was still proceeding after 7 days of incubation, however at a lower rate. The fast aggregate formation rate within the first few days of

incubation, the increased amount of microbial biomass (bacteria and fungi) and therefore the decreasing substrate availability might have led to a shortage of free organic material in the soils receiving organic carbon and the microbial biomass used organic carbon within the newly built macro-aggregates, resulting in decreased organic C contents within macro-aggregates. Different clay contents played only a minor role in aggregate formation. Overall, the results confirmed for all treatments that macro-aggregate formation is a rapid process and highly connected with the amount of OM added and microbial activity. However, the time of maximum aggregation after C addition depends on the soil and substrate investigated. Moreover, the results suggest that the primary macro-aggregates, formed within the first 7 days, are still unstable and oversaturated with OM and therefore act as C source for microbial decomposition processes.

5.1 Introduction

Soil aggregation is an important factor for many soil properties such as aeration, erodibility, soil fertility and protection of soil organic C (SOC) (Christensen, 2001). Further, it is often used as an indicator for soil structure (Six et al., 2000b) and the turnover of aggregates is linked with C dynamics (Balesdent et al., 2000). Following the hierarchical theory of aggregate formation, macro-aggregates (>250 µm) are formed of micro-aggregates (<250-53 µm) and organic binding agents (Tisdall and Oades, 1982). Micro-aggregates consist of silt and clay sized particles (<53 µm) and humified plant material, but are much less susceptible to external influences than macro-aggregates (Christensen, 2001; Oades, 1988; Six et al., 2000a). However, more research is needed in context of SOC dynamics in interaction with soil structure formation (De Jonge et al., 2009; Powlson et al., 2011a).

Several researchers found that the formation of macro-aggregates is coupled with the input of fresh organic material (Christensen, 2001; De Gryze et al., 2005). Generally, macro-aggregate formation is a rapid process, with reported maximum aggregation between 14 to 21 days after C input into soil, and depends mainly on increased microbial activity (Coppens et al., 2006b; Denef and Six, 2005; Denef et al., 2002; Mikha and Rice, 2004). For example, Denef et al. (2002) reported that the duration of macro-aggregate formation was coupled with the amount of organic C added, with maximum aggregation after 14 days of incubation with low organic matter input (3 g kg^{-1} soil) and still increasing aggregation after high input (20 g kg^{-1} soil) in

three different soils (loam, silty clay loam, clay). Also De Gryze et al. (2006b) found in an incubation experiment after macro-aggregate destruction and maize litter amendment (6.4 g C kg^{-1} soil) a significant increase of aggregation during incubation with highest respiration rates between days 3 and 6 of the incubation in a silt loam.

Aggregation in soils also depends on the texture. For example, Wagner et al. (2007) found in an Australian arable clay soil, after artificially altering the texture with air dried commercial bentonitic clay and industrial quartz sand amendments, that the formation of macro-aggregates depended more on soil texture than on management practices. Generally, soil texture is seen as significantly affecting aggregate stability (De Gryze et al., 2006a; Norton et al., 2006). For example Balesdent et al. (2000) summarized in their review that in soils with clay contents ranging from 2% to 71%, higher clay contents have higher potential of SOM protection within macro-aggregates.

The above findings indicate that though the major factors influencing aggregate formation in soils are known, quantitative information is scarce and it is not clear to which extent the findings can be generalized. Our objective was to quantify macro-aggregate (>250 µm) rebuilding in soils with different initial C content and different amendments of organic matter and clay contents in a short-term incubation experiment. Additionally, microbial activity and the contents of microbial biomass and fungi were analysed in order to better understand the processes of macro-aggregate formation.

5.2 Material and Methods

5.2.1 Site description

Soil was sampled from an arable field near Luettewitz, Saxonia (Germany), in a winter wheat stand in April 2010. The field is part of a long-term tillage trial by the Südzucker AG in cooperation with the Institute of Sugar Beet Research, Göttingen on commercially used fields. It has been cultivated with different tillage methods since 1992. The soil is a loess derived Haplic Luvisol with a texture of 20% clay, 78% silt and 2% sand and a pH ($CaCl_2$) of 6.9. The crop rotation consisted of sugar beet (*Beta vulgaris* L.) - winter wheat (*Triticum aestivum* L.) - winter wheat, and white mustard (*Sinapis alba* L.) as a catch crop (Koch et al., 2009). Samples were taken from no-tillage plots with direct drilling (soil A) and conventionally tilled plots with

annual mouldboard ploughing down to 30 cm (soil B). The plots with the different tillage treatments were divided into three subplots. From each subplot a composite sample of three cores was taken with a core sampler 10 cm in diameter. The samples were taken from 0-5 cm (top layer) and 5-25 cm (bottom layer) soil depth.

5.2.2 Soils and soil pretreatment

The soils A and B chosen differed in their contents of SOC with top layer A having significantly higher contents of SOC than the other three soils (Table 5.1). Macro-aggregate content was significantly higher in top layer A than in bottom layer A (Table 5.1).

Prior to incubation the soil samples were sieved (≤ 10 mm), oven dried (40℃) and carefully pestled to destroy all macro-aggregates. After macro-aggregate destruction the samples were sieved to pass a 250 µm sieve, then the fraction >250 µm, between 0.2 and 1.4%, discarded. After this soil pretreatment, yields of micro-aggregates were similar between the soils and the values (mean values ± standard errors, in g kg^{-1}) were 323 ± 6 (top layer A), 326 ± 16 (top layer B), 329 ± 8 (bottom layer A) and 280 ± 4 (bottom layer B).

Table 5.1: General soil characteristics of the soils used in the incubation experiment prior to macro-aggregate destruction (Andruschkewitsch et al., 2012). The C_{org}, macro-aggregate, C_{org} of the macro-aggregate and texture are mean values of the subplots with standard error in brackets (n=3).

Soil[a]	C_{org} content (g kg^{-1} soil)	Aggregate content >250 µm (g kg^{-1} soil)	C_{org} content (g kg^{-1} macro-aggregate)	Sand (%)	Silt (%)	Clay (%)
Top layer A	17.7 (1.0) a	729.5 (71.0) a	20.0 (1.0) a	2.5 (0.4)	77.7 (1.4)	19.9 (1.8)
Bottom layer A	10.4 (1.1) b	549.3 (51.1) b	13.4 (1.1) b			
Top layer B	11.0 (1.2) b	474.5 (125.8) ab	12.7 (0.8) b	1.5 (0.8)	78.1 (1.2)	20.4 (1.0)
Bottom layer B	10.6 (0.9) b	571.8 (87.3) ab	12.7 (1.2) b			

[a] Soil A: soil under no-tillage prior to incubation, soil B: soil under conventional tillage prior to incubation

For each parameter, different letters indicate significant differences between soils and depths ($p < 0.05$)

5.2.3 Incubation experiments - treatments and procedure

For the incubation experiment the following treatments were applied to soils A and B from two different soil depths (top layer: 0-5 cm and bottom layer: 5-25 cm):

(1) control (without any addition),

(2) OM_1 (addition of OM: pre-incubated wheat straw (described below) at a rate of 4.1 g C kg^{-1}),

(3) OM_2 (addition of pre-incubated wheat straw at a rate of 8.2 g C kg^{-1} soil),

(4) OM_{2_c} (addition of pre-incubated wheat straw at a rate of 8.2 g C kg^{-1} soil, whereat the clay content was increased to 25%).

Each 120 mL pot contained 60 g of the pretreated and manipulated soil, which was adjusted to 60% of its water holding capacity. The added material was mixed thoroughly with the soil to ensure a homogeneous distribution. Each sample was incubated in four replicates (n=4) per incubation treatment and sampling day (sub-samples). The two sub-samples for the destructive harvest after 7 and 28 days, respectively, were incubated together in one 1.5 L incubation jar. Water was added to the incubation jar to minimize evaporation from the soil samples.

Every sub-sample was analysed after harvest on water-stable aggregate content (>250 µm, 250-53 µm, <53 µm), microbial biomass C, ergosterol and SOC. Dry combustion (Elementar Vario El, Heraeus, Hanau, Germany) was used to determine total C and total N contents.

5.2.4 Incubation experiments - pre-treated wheat straw and increase of the clay content

The added organic material in the OM_1, OM_2 and OM_{2_c} treatments consisted of pre-incubated wheat straw (C content: 391.3 g kg^{-1} OM, C/N: 40.6). The chopped straw was incubated with soil from an arable field at about field capacity for 6 weeks at room temperature to get partly decomposed organic material (OM). The straw was placed in thin layers between the soil, separated by a mesh to more easily isolate the OM after pre-incubation from the soil. After the pre-incubation the OM was shredded <10 mm and oven dried at 40℃ prior to the incubati on experiment. The amount of 4.1 g C kg^{-1} soil, which corresponds to 1.05 g OM 100 g^{-1} soil, is the average above-ground C input (straw) at a grain yield of 5 t ha^{-1} on the surface area of the incubation pots.

An artificial soil with increased clay content in the OM_{2_c} treatment was created by thoroughly mixing a silty clay soil (soil C) from an arable field close to Göttingen, Lower Saxony (Germany) with the soils from Luettewitz at a ratio of 1:2 with a spatula in the incubation pots. It was chosen because of its high clay content of 47.2%, further the silt and the SOC content was 52.2% and 16.5 g kg^{-1} soil, respectively. Soil C was also treated with a mortar after oven drying at 40℃ and sieved <250 µm to destroy all macro-aggregates. In the OM_{2_c} treatments, clay content of the mixed soils was 25% for all four soils and their initial SOC contents (in g C kg^{-1} soil) were 20.8 (top layer A), 13.5 (bottom layer A), 13.9 (top layer B) and 13.5 (bottom layer B).

5.2.5 Determination of water-stable aggregates

After 7 and 28 days of incubation the water-stable aggregate-size fractions were separated in the sub-samples for the destructive harvest, according to the fractionation method described by John et al. (2005). Briefly, 30 g of gently sieved (≤10 mm) and oven dried soil (48 h at 40℃) was placed on a 250 µm sieve and submerged into deionized water to allow slaking for 10 min. After removing and dipping the sieve back into the water 50 times the water-stable aggregates remaining on the sieve (>250 µm) were collected, vacuum filtered and dried for at least 48 h at 40℃. Aggregates passing the sieve were poured onto the next smaller mesh size (53 µm) and the procedure was repeated as described above. The material passing the 53 µm sieve was also vacuum filtered and dried for 48 h at 40℃. The three aggregate size fractions obtained by this method are macro-aggregates (>250 µm), micro-aggregates (250-53 µm) and the silt and clay sized fraction (<53 µm). Adequate accuracy of the fractionation procedure and complete dryness of the aggregate fractions was controlled by mass recovery rates of the aggregate fractions between 99 and 101%.

The C_{org} and N_t contents of the aggregate fractions were determined by dry combustion as described for the bulk soil above.

5.2.6 Soil respiration rate

Soil respiration was measured by titration of the excess 1M NaOH with 1M HCl against phenolphthalein, after addition of 0.5 M $BaCl_2$ solution to precipitate CO_3^{2-} (Zibilske, 1992). Thereby a 60 mL beaker was placed in the incubation jars containing 10 mL 1M NaOH to capture respired CO_2. Sampling of the CO_2 traps took

place after 1, 3, 7, 14, 21 and 28 days of incubation. To avoid inhibitory effects due to CO_2 accumulation in the headspace, each jar was aerated for approximately 10 minutes after sampling the CO_2 traps.

5.2.7 Microbial biomass C content

Fumigation extraction was used to estimate microbial biomass C (Vance et al., 1987), 20 g of moist soil was divided into halves. One sub-sample was fumigated for 24 h at 25℃ with ethanol-free $CHCl_3$. Both sub-samples were shaken for 0.5 h at 175 rev. min^{-1} with 40 ml of 0.5 M K_2SO_4 and analysed for organic C by infrared detection of CO_2 (Dimatoc, Dimatec, Essen, Germany) after combustion at 850℃. Microbial biomass C was calculated as E_C/k_{EC} where E_C = (organic C from fumigated soils) - (organic C from non-fumigated soils) and k_{EC} = 0.45 (Wu et al., 1990).

5.2.8 Ergosterol content

Ergosterol is an important component of fungal cell membranes. It was extracted from 2 g of moist soil with 100 ml ethanol. After shaking the sample for half an hour at 250 rev. min^{-1} the suspension was filtered (Whatman GF/A) and evaporated in a vacuum rotary evaporator at 40℃. The residue was collected in methanol and after filtration the quantitative ergosterol determination was performed by reversed-phase HPLC analysis at 282 nm (Djajakirana et al., 1996).

5.2.9 Mineralogical composition of the soils

The mineralogical composition of the soils A and B and the silty clay soil C was determined by X-ray diffraction, using the following procedure: For separation of the clay fraction from the fine soil carbonates were eliminated with the acetate buffer at pH 3.5, organic matter by wet oxidation with H_2O_2 and iron oxides according to the dithionite-citrate-bicarbonate method. The clay fraction (<2 µm) was obtained by sedimentation and decantation. The resulting suspension was flocculated with NaCl, centrifuged and washed until salt-free. For analysis with X-ray diffraction, samples were saturated with Mg and K respectively and sedimented on glass slides to obtain oriented samples. Additionally the Mg-saturated samples were solvated with ethylene glycol and the K-saturated samples were heated for 2 h at 550℃. Samples were measured with a Siemens X-ray diffractometer D500 with Cu-Kα radiation in the range of 1-30 degrees (2Theta).

From the X-ray diffraction patterns it can be deduced that the clay fractions of soils A and B have a similar mineralogical composition, whereas marked differences exist compared with the patterns of soil C (data not shown).

Smectites, illite and kaolinite are observed in all three samples. The presence of smectites is indicated by reflections at low °2 The ta for Mg-saturated samples with ethylene glycol treatment. Reflections at d-values of 1.01 nm in the Mg and Mg/ethylene glycol saturated sample can be assigned to illite. These reflections do not appear separately but on the shoulder of the reflections of smectites.

Reflections of quartz and orthoclase are at the detection limit of the method (~3-5%) and can be observed in the clay fraction of soils A and B (typical d-values at 0.429 and 0.325 nm respectively). The presence of primary chlorite in soil C is indicated from reflections at 1.43 nm in the K-treated sample with 550°C treatment.

Considering the relative intensity of the reflections, illite and smectites are the most common mineral phases in all three clay fractions. 2:1 layer silicates are much more abundant than 1:1 layer silicates. The content of kaolinite, quartz and feldspar is at 20% in soils A and B and less than 10% in soil C. From the shape of the reflections of smectites it can be derived that smectite particles are most probably more fine-grained in soils A and B than in soil C and have in consequence a higher specific surface area. Together with the slightly lower content of illite and the presence of traces of quartz and feldspar it can be assumed, that the weathering degree of soils A and B is somewhat higher than that of soil C. All three clay fractions contain high amounts of permanent negatively charged clay minerals.

5.2.10 Statistical analyses

All statistical evaluations were conducted with the free available statistical software R (R Development Core Team, 2010). The data were analysed as a factorial, fully randomised design. For each soil, depth and treatment four replicates were used (n=4). Water-stable aggregate fraction, microbial biomass C, soil respiration and ergosterol contents were tested for significant differences ($p < 0.05$) with a one way ANOVA with the OM and texture treatment as the main factor, followed by a post-hoc Tukey-HSD test. Normality of the residuals and homogeneity of variances was tested graphically. If necessary, the data were Box-Cox transformed to obtain variance homogeneity and normal distribution.

5.3 Results

5.3.1 Formation of water-stable aggregates

After 7 and 28 days of incubation the yields of macro-aggregates were not significantly influenced by the different soils and sampling depths (Fig. 5.1). While the yields of macro-aggregates of soil A decreased with depth, soil B showed slightly increased macro-aggregate yields with depth. Increasing amounts of OM added led to significantly higher yields of macro-aggregates in comparison with the control soils, with one exception after 7 days in bottom layer B (Fig. 5.1). Time had a significant effect ($p < 0.05$) on the macro-aggregate yield, leading to a general increase in macro-aggregate yields from day 7 to day 28 of incubation. The increased clay content in the OM_{2_c} treatment showed no significant effect on macro-aggregate formation in comparison with the samples of the OM_2 treatment.

Increase in macro-aggregate yields after OM addition (OM_1, OM_2 and OM_{2_c} treatments) led to lower yields of micro-aggregates and of silt and clay sized fractions in the corresponding treatments (Fig. 5.1).

The SOC content within the macro-aggregates (in g kg^{-1} fraction) decreased over time (Fig. 5.2). After 7 days of incubation the addition of OM led to significantly higher SOC contents per kg macro-aggregate fraction in comparison with the control soil, however, the amount of OM added showed no effect. After 28 days the differences between the treatments and the control soil became smaller. The different soils and depths had no statistically significant influences on the SOC content of the macro-aggregate fractions.

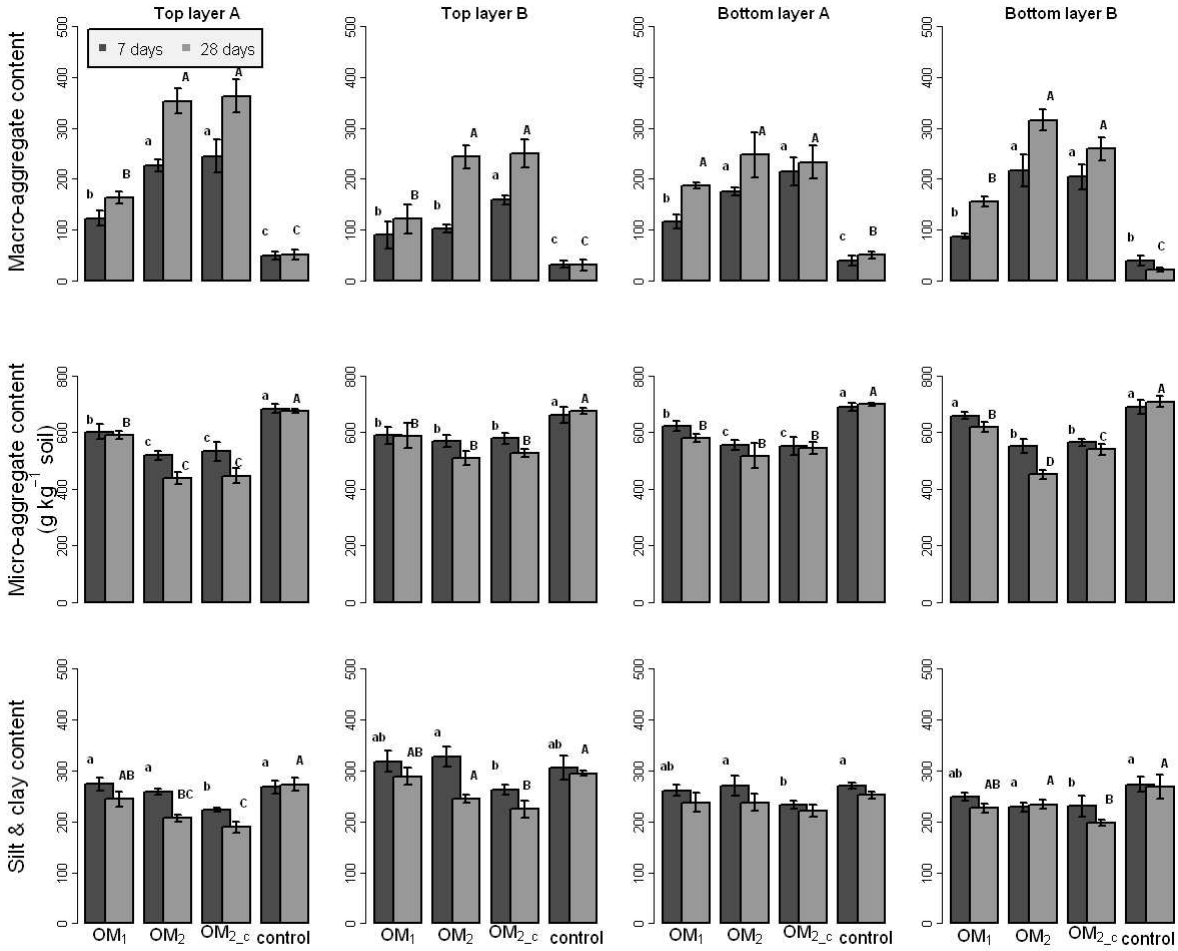

Fig. 5.1: Mean dry matter yields of macro-aggregate (>250 µm), micro-aggregate (250-53 µm) and silt & clay sized (<53 µm) fractions of the different soils, sampling depths (0-5 and 5-25 cm) and treatments (OM1: addition of OM: pre-incubated wheat straw at a rate of 4.1 g C kg^{-1} soil, OM2: addition of pre-incubated wheat straw at a rate of 8.2 g C kg^{-1} soil, OM2_c: addition of pre-incubated wheat straw at a rate of 8.2 g C kg^{-1} soil, whereat the clay content was increased to 25%, control: without any addition) after 7 and 28 days of incubation. Different lower case and capital letters indicate significant differences between the treatments after 7 and 28 days of incubation, respectively. Error bars show standard error of the replicates (n = 4).

Small differences between SOC contents were found between the treatments in the micro-aggregate and the silt and clay sized fraction (Fig. 5.2). The absence of changes in C content within the micro-aggregate and the silt and clay sized fraction over time might be due to missing aggregate formation processes in the fractions <250 µm. The C increase in the treatment OM_{2_c} was probably due to added micro-aggregates and silt and clay sized particles with the clay soil. This supports the thesis that the formation of aggregates and SOC occlusion within aggregates takes place in the fraction >250 µm and the smaller fractions serve as ‘material supplier’.

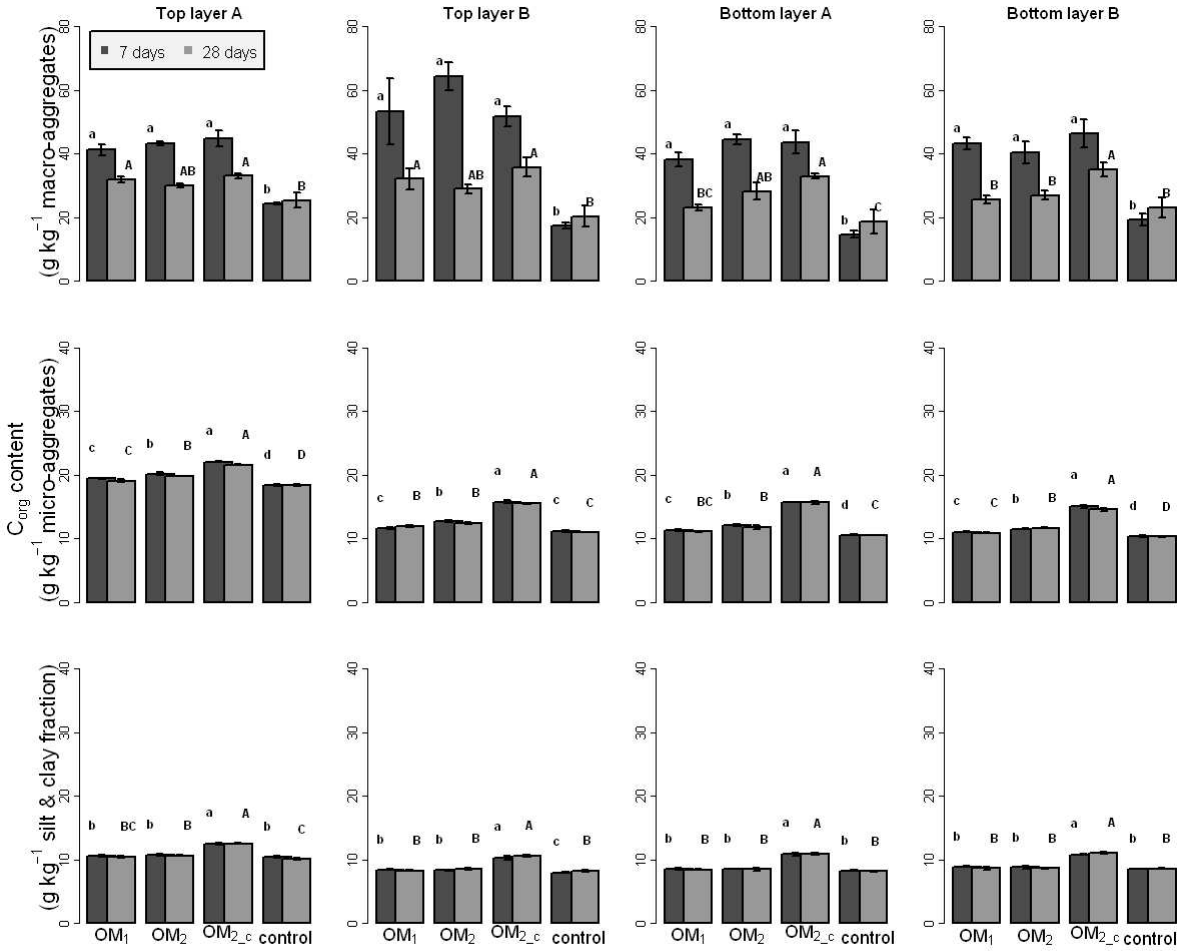

Fig. 5.2: Mean C_{org} content within macro-aggregate (>250 µm), micro-aggregate (250-53 µm) and silt & clay sized (<53 µm) fractions of the different soils, sampling depths (0-5 and 5-25 cm) and treatments (further information is given in caption of Fig. 5.1) after 7 and 28 days of incubation. Different lower case and capital letters indicate significant differences between the treatments after 7 and 28 days of incubation, respectively. Error bars show standard error of the replicates (n = 4).

5.3.2 Soil respiration and microbial parameters

Following aggregate disruption and rewetting, all soils and treatments showed strongly increasing CO_2 evolution rates within the first day (Fig. 5.3). However, the CO_2 evolution between the treatments differed: the CO_2 evolution of the control soils decreased steadily over time, while the CO_2 evolution of the soils from the other treatments peaked a second time between day 14 and 21, however this effect was more pronounced in the soils of the OM_2 treatment. For all treatments with OM addition, the CO_2 respiration rates were still higher than in the control soils after 28 days of incubation.

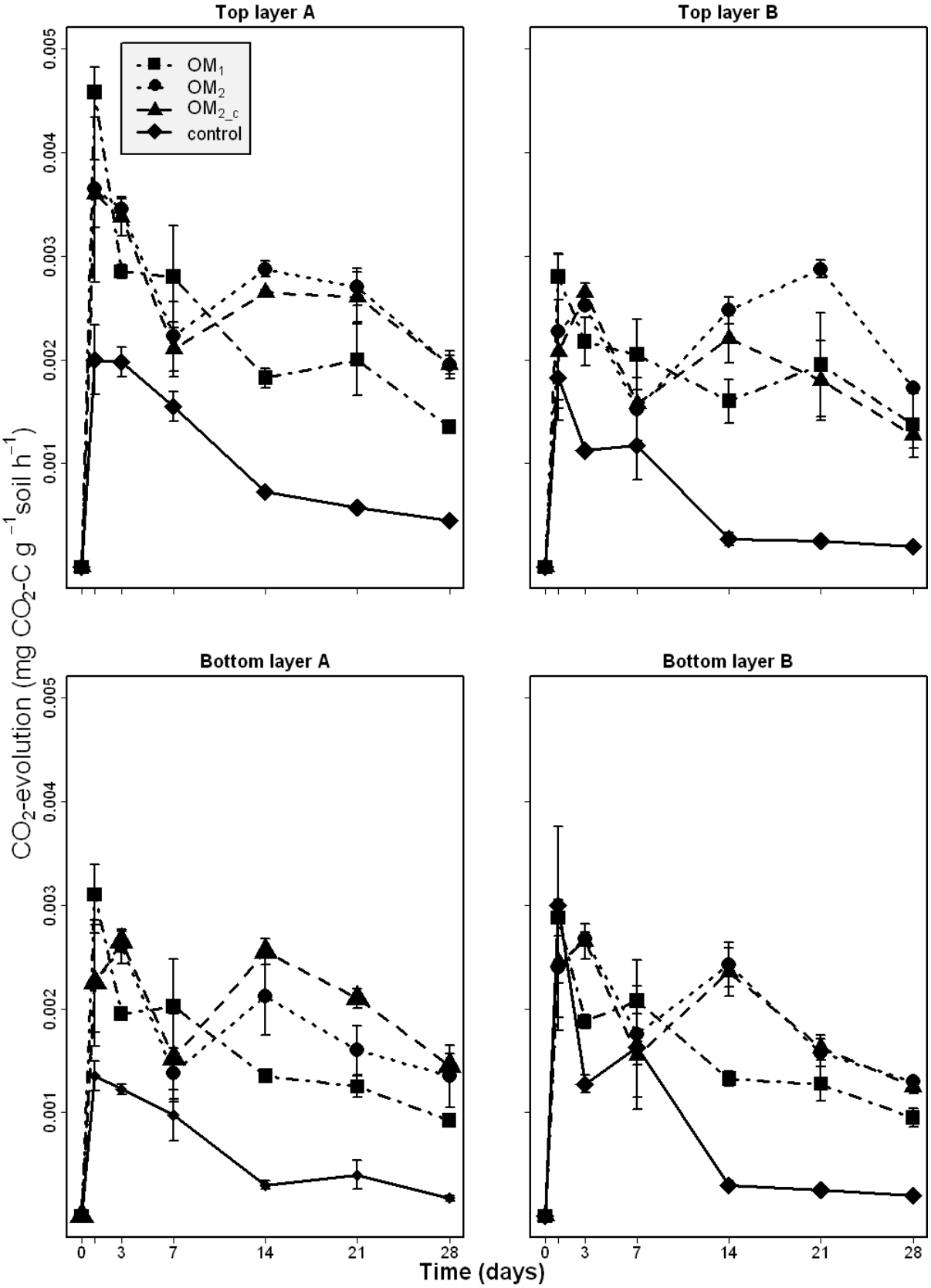

Fig. 5.3: Mean CO_2 evolution rates during the 28 days of incubation of the different soils, sampling depths (0-5 and 5-25 cm) and treatments (further information is given in caption of Fig. 5.1). Lines are to guide the eyes only. Error bars show standard errors of the replicates (n = 4)

Over all soils and depths the cumulative respiration after 7 and 28 days of incubation was significantly higher after the application of OM (OM_1, OM_2 and OM_{2_c} treatments) than in the control soils, respectively (Table 5.2), and positively correlated with the macro-aggregate content (0 to 7 days: r = 0.79; 7 to 28 days: r = 0.57; Fig. 5.4).

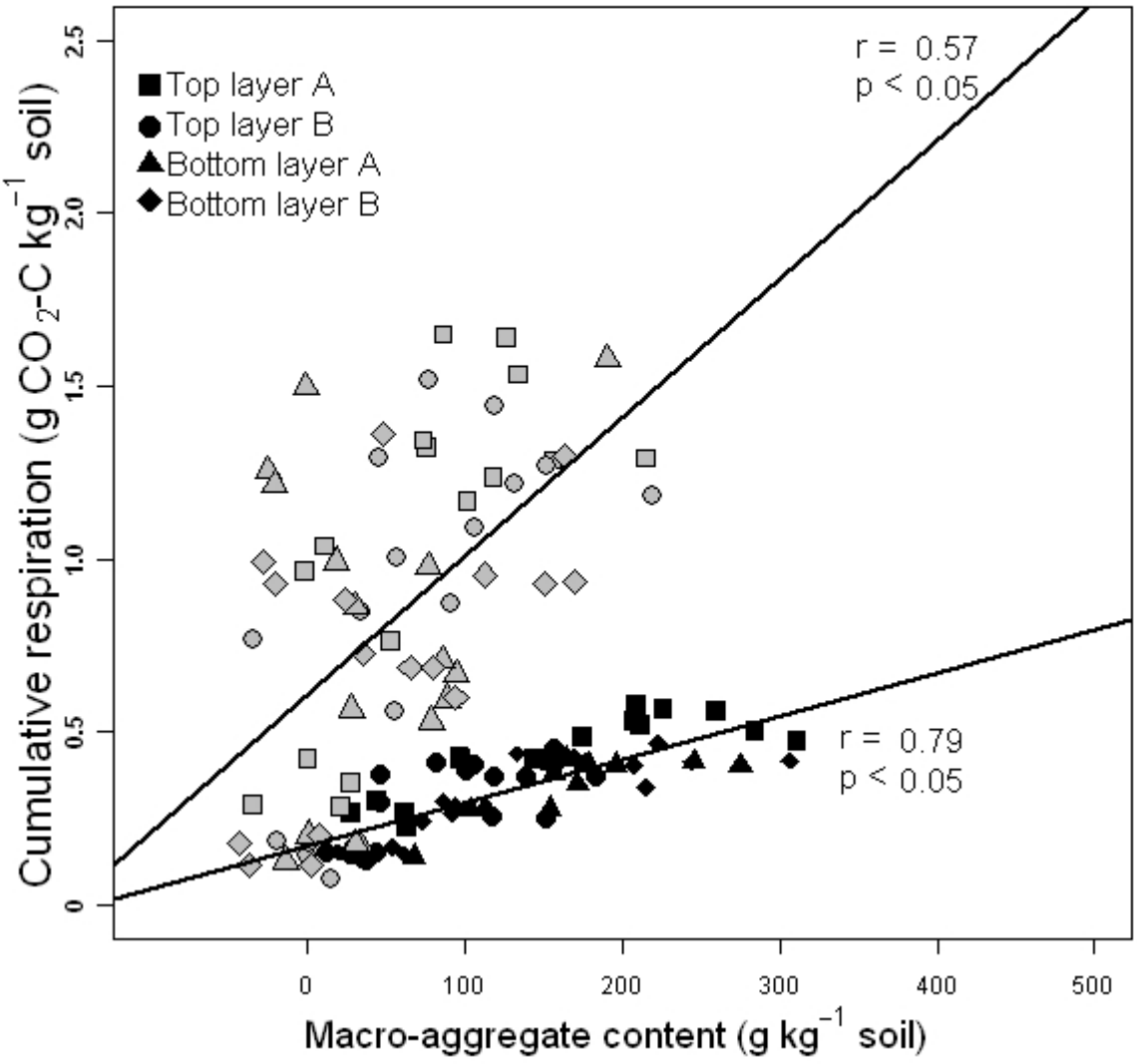

Fig. 5.4: Correlation of the macro-aggregate content against the cumulative CO_2 respiration of the different soils, sampling depths (0-5 and 5-25 cm) after 0-7 (black symbols) and 7-28 (grey symbols) days of incubation, respectively.

After 7 and 28 days, the content of microbial biomass C was in general significantly higher in the treatments receiving OM than in the control treatments, due to the presence of marked amounts of labile C. The highest microbial biomass C con-

tents in top layer A (Table 5.2) might be due to the high initial SOC content in this soil, which probably led to overall high microbial biomass C contents in top layer A and therefore a strong decrease with depth leading to a significant effect of the soil and the sampling depth.

The content of the fungal cell membrane component ergosterol was significantly influenced by the addition of OM after 7 and 28 days of incubation and in general, the contents decreased in the order OM_2 > OM_1 > control (Table 5.2). Ergosterol contents were not significantly different between different soils and sampling depths for both sampling times after 7 or 28 days of incubation.

Soil respiration and contents of microbial biomass C and ergosterol were not significantly different between the OM_2 and OM_{2_c} treatments, indicating that, in this study, an increased clay content did not markedly affect soil biological responses to OM additions.

Table 5.2: Average values for the cumulative respiration, microbial biomass carbon and ergosterol contents after 7 and 28 days of incubation. Standard error of the replicates in brackets (n=4).

Soil[a]	Treatment[b]	days 0-7			days 0-28		
		Cumulative respiration	Microbial biomass carbon content	Ergosterol content	Cumulative respiration	Microbial biomass carbon content	Ergosterol content
		(g CO_2-C kg^{-1} soil)	(mg kg^{-1} soil)	(g kg^{-1} soil)	(g CO_2-C kg^{-1} soil)	(mg kg^{-1} soil)	(g kg^{-1} soil)
Top layer A	OM_1	0.42 (0) b	419 (35) b	1.4 (0.3) ab	1.40 (0.09) b	440 (13) a	1.6 (0.2) b
	OM_2	0.54 (0.01) a	468 (39) ab	2.4 (0.6) a	1.99 (0.09) a	497 (15) a	2.8 (0.2) a
	OM_{2_c}	0.51 (0.02) a	552 (33) a	2.6 (0.3) a	1.89 (0.11) a	457 (61) a	3.0 (0.3) a
	control	0.26 (0.01) c	385 (40) b	0.7 (0.2) b	0.60 (0.04) c	260 (34) b	0.7 (0.1) c
Top layer B	OM_1	0.29 (0.03) b	263 (25) bc	1.3 (0) bc	1.20 (0.19) b	263 (20) b	1.1 (0.2) b
	OM_2	0.39 (0.01) a	318 (42) ab	2.7 (0.1) a	1.74 (0.07) a	368 (15) a	2.2 (0.4) a
	OM_{2_c}	0.40 (0.02) a	417 (44) a	1.8 (0.6) ab	1.40 (0.1) ab	350 (36) a	2.7 (0.2) a
	control	0.14 (0.01) c	191 (13) c	0.4 (0) c	0.28 (0.02) c	158 (13) c	0.3 (0.1) c
Bottom layer A	OM_1	0.28 (0) b	243 (70) ab	0.9 (0.3) ab	0.95 (0.07) b	195 (41) a	1.1 (0.2) a
	OM_2	0.38 (0.01) a	210 (26) ab	2.1 (0.6) a	1.40 (0.25) ab	217 (34) a	1.7 (0.3) a
	OM_{2_c}	0.41 (0) a	340 (12) a	1.9 (0.2) a	1.58 (0.12) a	295 (7) a	2.2 (0.6) a
	control	0.14 (0) c	148 (12) b	0.3 (0) b	0.31 (0.02) c	82 (14) b	0.2 (0) b
Bottom layer B	OM1	0.27 (0.01) b	222 (37) b	1.3 (0.1) a	0.94 (0.04) b	144 (55) b	1.1 (0.1) b
	OM_2	0.41 (0.01) a	338 (32) a	1.8 (0.5) a	1.45 (0.08) a	343 (42) a	2.7 (0.4) a
	OM_{2_c}	0.41 (0.03) a	321 (18) ab	2.4 (0.4) a	1.44 (0.13) a	336 (19) a	2.7 (0.2) a
	control	0.15 (0) c	129 (21) c	0.3 (0) b	0.30 (0.02) c	131 (14) b	0.2 (0.1) c
p-values	Soil	<0.05	<0.05	ns	ns	ns	ns
	Depth	<0.05	<0.05	ns	0.06	<0.05	ns
	Soil * Depth	<0.05	<0.05	ns	ns	<0.05	ns

[a]Soil A: soil under no-tillage prior to incubation, soil B: soil under conventional tillage prior to incubation; sampling depths: top layer: 0-5 cm, bottom layer: 5-25 cm.

[b]OM_1: addition of OM: pre-incubated wheat straw at a rate of 4.1 g C kg^{-1} soil, OM_2: addition of pre-incubated wheat straw at a rate of 8.2 g C kg^{-1} soil, OM_{2_c}: addition of pre-incubated wheat straw at a rate of 8.2 g C kg^{-1} soil, whereat the clay content was increased to 25%, control: without any addition.

For each parameter, different letters indicate significant differences between the treatments within the soils and depths ($p < 0.05$)

5.4 Discussion

5.4.1 Formation of water-stable aggregates

With one exception, more than 50% of the built macro-aggregates during the incubation experiment were formed within the first 7 days (Fig. 5.1). This is in line with the findings of De Gryze et al. (2006b), who incubated a silt loam (17 g SOC kg^{-1} soil) from a fertilised sheep meadow after macro-aggregate destruction with maize leaf residues at a rate of 6.4 g C kg^{-1} soil (C/N = 36.4) at 25℃ and 60% of water-filled pore space for 42 days. As in this study, the authors have shown that the largest aggregate formation took place within the first 3 to 7 days of incubation. Similar results in early stages of an incubation experiment were found by Helfrich et al. (2008), who investigated the aggregate formation in a silty loam from a long-term wheat cropping (12.6 g SOC kg^{-1} soil). The soil was incubated after macro-aggregate destruction for 84 days at 15℃ and with 60% of the water holding capacity. Single application of of maize leaves (2.1 g SOC kg^{-1} soil) led to a short-term effect on soil macro-aggregates with highest contents after 14 days of incubation. These findings suggest that the macro-aggregate formation is a rapid process and takes place within the first days of incubation.

After 28 days of incubation the fraction of rebuilt macro-aggregates (Fig. 5.1) of the initial yield of macro-aggregates prior to the destruction and incubation experiments (Tab. 5.1) was as follows: 22 to 34% (OM_1), 45 to 55% (OM_2), 42 to 53% (OM_{2_c}) and 4 to 9% (control). The rate of macro-aggregate formation was highest within the first 7 days of the incubation experiment but the process of aggregate formation was still proceeding, especially for the OM_2 and OM_{2_c} treatments in the top layers (Fig. 5.1). This is in contrast with the findings of De Gryze et al. (2006b), who found no significant changes of aggregate formation after day 17 of the incubation experiment. Contrary to the findings of this study and De Gryze et al. (2006b), Helfrich et al. (2008) determined a decreasing macro-aggregate content after two weeks of incubation. Even though the amount and quality of the organic material added to the soil varied between this study and the studies cited, higher C contents in soils and increasing amounts and qualities of litter input generally increased macro-aggregate formation rates and had a positive effect on C stabilisation within aggregates over time.

Highest yields of macro-aggregates were found in the OM_2 treatment in both soils after 7 and 28 days of incubation. The increased clay content from 20% to 25% in the OM_{2_c} treatment did not affect the formation of macro-aggregates significantly compared to the OM_2 treatment (Fig. 5.1), probably due to missing swelling and shrinking during the incubation experiment. The results of this study are in accordance with the findings of Denef et al. (2002) who found significantly increased aggregate formation after wheat residue (C/N = 96) application of 2 g 100 g^{-1} soil in comparison with 0.3 g 100 g^{-1} soil in three soils with differing texture (loam, silty clay loam and clay) and clay mineralogy (2:1, mixed, 1:1). However, despite an overall increase in aggregate formation in all soils, Denef et al. (2002) revealed differences between the soils varying in clay mineralogy and suggested an influence on aggregate formation by the same.

The initial C background of the top layers used in this study showed only a significant (<0.05) effect on macro-aggregate formation in the top layer after 7 days of incubation (Fig. 5.1). Due to only small differences in texture and the same origin of the soils, the differences in aggregate dynamics between the soils of the same treatments can be attributed mainly to different initial SOC contents of the soils.

5.4.2 Organic C contents of the aggregate size fractions, soil respiration and microbial parameters

Using the microbial activity (measured as soil respiration) as an indicator for aggregate formation rate (De Gryze et al., 2005; Denef et al., 2002; Helfrich et al., 2008), the first days after the organic material application were identified as the most active in macro-aggregate formation in this study (Fig. 5.1; Fig. 5.3). This is in accordance with the findings of several studies that measured aggregate formation and soil respiration over time (De Gryze et al., 2005; De Gryze et al., 2006b; Denef et al., 2002; Helfrich et al., 2008; Le Guillou et al., 2011). The input of recently available SOC led to higher microbial activity and therefore production of organic binding agents for macro-aggregate formation (Le Guillou et al., 2012; Martens, 2000).

Between day 14 and 21 a second peak in soil respiration evolved in the soils that received OM, with highest respiration rates after OM_2 addition (Fig. 5.3). This is in contrast to the findings of other incubation experiments, where, after an early peak within the first few days, the soil respiration steadily decreased over time during aggregate formation (Daraghmeh et al., 2009; De Gryze et al., 2005; De Gryze et al.,

2006b; Helfrich et al., 2008; Le Guillou et al., 2011). Le Guillou et al. (2012) presented a conceptual model where the early stage (weeks) of aggregate formation is controlled by the organic matter inputs. Whereas in the long-term (months) the macro-aggregate content is governed by N availability and related to the balance of microbial polysaccharides production and consumption. We suggest that the fast aggregate formation in this experiment within the first few days of incubation led to increased amounts of microbial biomass (Table 5.2) and hence possibly to decreasing substrate availability and N limiting conditions. Therefore the microbial community might have changed and the adapted microorganisms started mining for organic material within the macro-aggregates (Le Guillou et al., 2011).

The amount of C within the macro-aggregates was still markedly higher after 7 and 28 days of incubation (Fig. 5.2) than in the original soil prior to macro-aggregate disruption (Table 5.1). At early stages of formation the macro-aggregates are not yet resistant and can easily break up into micro-aggregates (Bossuyt et al., 2001; Helfrich et al., 2008). The fast aggregate formation rate within the first few days of incubation, the increased amount of microbial biomass (bacteria and fungi) and therefore the decreasing substrate availability might have led to a shortage of free organic material in the soils receiving organic C and the microbial biomass used organic C within the newly built macro-aggregates, resulting in decreased organic C contents within macro-aggregates (Fig. 5.2). These results suggest that the macro-aggregates after formation are oversaturated with SOC and only a smaller amount is stabilized for longer periods within macro-aggregates in the soil.

Cumulative respiration was highly correlated with the yields of water-stable macro-aggregates after 0 to 7 ($r=0.79$) and 7 to 28 ($r=0.57$) days of incubation for all soils, depths and treatments (Fig. 5.4). This finding confirms the results of previous studies (De Gryze et al., 2005; Kiem and Kandeler, 1997) which also underlined the dependency of aggregate formation on microbial activity.

Significant effects of the different soils on cumulative respiration and microbial biomass C content were accompanied by significant interaction between soil and sampling depth (Table 5.2). This might be due to highest initial SOC content prior to incubation in top layer 2 in comparison with the other soils (Table 5.1). However, in the bottom layers, containing about the same SOC contents, no differences were found in aggregate formation, respiration rate, microbial biomass C and ergosterol content between soil A and soil B. This is in line with findings from incubation

experiments (De Gryze et al., 2005) and field studies (Wright and Hons, 2005), suggesting that the input and distribution of organic material in soils is the main factor affecting macro-aggregate formation.

5.5 Conclusion

This study confirms that the input of organic matter is the main driving factor in macro-aggregate formation. Both higher input and higher initial content of soil organic C led to increased macro-aggregate formation. This process was positively correlated with soil respiration and accompanied by increased microbial biomass C and ergosterol contents.

Macro-aggregate formation was an ongoing process for the 28 days of incubation with highest rates within the first seven days of incubation. Between day 7 and 28 of incubation the macro-aggregate formation was still proceeding, however at a slower rate. At the same time the C content within macro-aggregates decreased, indicating that the microbial biomass started mining for organic C in the newly built C-rich macro-aggregates.

6 Synthesis and general conclusion

The evaluation of different functional pools applied to soil samples from long-term tillage trials from different sites increased the understanding of soil aggregation and C sequestration processes. Based on the research objectives, the following conclusions can be drawn.

(i) The four long-term tillage trials on plots differing in soil texture and climatic conditions revealed consistent results between them.

Density and aggregate fractionation showed that effective translocation in higher soil depths and higher litter input under CT and MT treatments compensated in the long-term the higher physical tillage impact by tillage equipment in comparison with NT treatments. Stepwise multiple linear regression and correlation of the LF yields with macro-aggregate yields revealed that LF yields within the different soil depths were the driving factors of macro-aggregate formation. However, the highest C stocks were found under MT due to a combination of high crop yields, reduced physical tillage impact and effective litter incorporation, resulting in a C sequestration rate of 31 g C^{-2} yr^{-1}.

(ii) Generally, higher macro-aggregate yields under MT and NT were restricted to the 0-5 cm sampling depth. However, temporal changes were only small and no tillage induced net effect on aggregate size distribution was detectable. This was probably due to low physical impact by tillage equipment or high re-aggregation, stimulated by higher soil mixing and effective litter translocation into higher soil depths under CT.

(iii) The incubation study showed that macro-aggregate yields increased with increasing rates of OM input, accompanied by higher contents of microbial biomass carbon and ergosterol. Macro-aggregate formation is a fast process, which was indicated by highest soil respiration rates after OM amendments within the first three days after rewetting of the soil and input of OM. Although, most of the macro-aggregates were formed within the first seven days of incubation in the soils receiving OM (42-75%), the process of macro-aggregate formation was ongoing throughout the 28 days of incubation, which was indicated

by higher soil respiration rates than in control soils. At the same time, decreasing carbon contents within macro-aggregates indicated that OM, occluded within the newly formed macro-aggregates, served as C source for microbial biomass. The different clay contents played only minor role in macro-aggregate formation under the particular conditions of this incubation study.

Overall, these results indicate that the due to the interaction of the respective soil disturbances and litter distributions and due to the ability of rapid macro-aggregate rebuilding, a distinct steady state per tillage treatment, in terms of macro-aggregate formation and turnover, has been established. Therefore no tillage induced net change on macro-aggregation could be identified in the short-term and no indications for an effective C sequestration under NT in comparison with CT were found in the long-term. However, a continuous application of MT, with a combination of reduced physical tillage impact and effective litter incorporation, may offer some potential in successfully improving the soil structure and therefore preventing incorporated LF from rapid decomposition, probably resulting in C sequestration in the long-term.

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