

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

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Abstract The objectives were to investigate (1) to which extent water-stable macro- and microaggregates sequester organic matter (OM) in a minimum tillage (MT) system compared to a conventional tillage (CT) system and (2) if the content of biochemically stabilized OM differs between both tillage systems, and (3) to study the temporal dynamics of the distribution of aggregate size classes and of storage of OM within aggregates in the field. Surface soils (0–5 cm) and subsoils (10–20 cm) were sampled after fallow (March 2007) and directly after tillage (November 2007) from a long-term experimental field near Göttingen, Germany. Macroaggregates (>0.25 mm) were in general less abundant after fallow than directly after tillage. In March, only 21% (CT) and 45% (MT) of C_{org} was stored within macroaggregates in the surface soil, whereas in November, the percentages increased to 58% and 73%, respectively. CT and MT soils of both depths were incubated as bulk soil (CT_{bulk} , MT_{bulk}) and with macroaggregates disrupted (<0.25 mm) (CT_{md} , MT_{md}) for 28 days at 22°C and water content of 50% of the maximum water holding capacity. For the MT_{bulk} and MT_{md} surface soils, C mineralization was significantly higher compared to the CT soils. Incubation of md soils did not generally result in a

significantly higher C mineralization compared to the respective bulk soils, except for the MT_{md} subsoil. Acid hydrolysis showed that the proportion of biochemically stabilized, nonhydrolysable, C_{org} to total C_{org} was lower in the MT than in the CT soils. Overall, the data indicate that the effect of physical stabilization of OM stored in the macroaggregates may not be a mechanism protecting very labile C with a turnover time of weeks, but that longer preservation likely occurs after macroaggregate transformation into microaggregates, and the surplus of OM found in the surface soil of MT does not only depend on the biochemically stabilized OM. Finally, our data suggest that the temporal variability of distribution of aggregate size classes in the field is large, but spatial and operator variability also contributed to the observed differences.

Keywords Acid hydrolysis · Incubation · Macroaggregates · Potential mineralization · Water-stable aggregates

Introduction

Sustaining the fertility of arable soils and reducing CO₂ emissions from soil require an understanding of mechanisms leading to a sustainable sequestration of organic matter (OM). It was shown for various soil types and climatic regions that tillage systems that minimize soil disturbance (conservation tillage, minimum tillage [MT], reduced tillage, and no tillage [NT]) generally increase the storage of soil OM compared to conventionally tilled (CT) soils (Paustian et al. 2000; Six et al. 2000a, b; Kushwaha et al. 2001; Jacobs et al. 2009). However, the processes underlying this increased OM sequestration are not completely understood.

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In general, the stabilization of OM in soils, i.e., the protection of OM against microbial decomposition, may be mainly attributed to three mechanisms, namely, (1) spatial inaccessibility, (2) biochemical recalcitrance, and (3) organo-mineral association (von Lützow et al. 2006). However, the importance of each of these three mechanisms for OM stabilization can differ for each soil horizon and depends on several factors such as soil type and texture, mineralogical composition, and land use.

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

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

In order to investigate the role of macroaggregates for protection of OM against microbial decomposition, several incubation experiments have been carried out using soils from CT and NT experiments with contrasting results about the increase in the OM mineralization after the disruption of macroaggregates (Beare et al. 1994; Oorts et al. 2006). An increasing size of a recalcitrant OM pool (isolated by acid hydrolysis) under NT compared to CT plots was reported by Collins et al. (2000), and it was attributed mainly to increasing soil organic carbon stocks. However, the contribution of recalcitrant to total C_{org} (% of C_{org}) remained similar or even slightly decreased in the NT compared to CT plots (Collins et al. 2000).

Jacobs et al. (2009) suggested that long-term MT resulted in an enhanced physical protection of OM accounting for the higher concentrations of C_{org} and N in MT soils. However, density fractionation of the surface soil did not reveal more particulate OM occluded within

aggregates but rather higher C_{org} and N concentrations in the mineral fractions of MT than of CT soils (Jacobs et al. 2009). These findings were supported by the results of Jastrow (1996) and Zotarelli et al. (2007) who suggested that most of OM is sequestered as a mineral-associated fraction that is further occluded into aggregates.

Overall, several mechanisms that possibly contribute to an increased OM sequestration were suggested by various studies and are still discussed controversially. Furthermore, information on MT systems is scarce, since mainly NT systems have been investigated. We hypothesize that physical protection, and not biochemical stabilization, is the main reason for an enhanced preservation of OM in MT systems compared to CT systems, and that surplus OM is mainly located in water-stable microaggregates.

The objectives of our study were to investigate (1) to which extent water-stable macro- and microaggregates sequester OM in an MT system compared to a CT system and (2) if the content of biochemically stabilized OM differs between the two tillage systems, and (3) to study the temporal dynamics of the distribution of aggregate size classes among tillage systems in the field.

Materials and methods

Sampling site and sample processing

Our study was conducted on a long-term experimental site near Göttingen, Germany (Garte-Süd). The mean annual precipitation and temperature are 645 mm and 8.7°C, respectively. The soil type is a Haplic Luvisol (WRB) derived from loess (15.1% clay, 72.7% silt, 12.2% sand in 0–30 cm, Ehlers et al. 2000). In 1970, two different tillage treatments were established with four field-replicates for each treatment: conventional tillage (CT) by mouldboard ploughing to 25-cm depth and MT using a rotary harrow to 5- to 8-cm depth. Before 1970, the field had been mouldboard ploughed. The crops grown from 2003 to 2005 were winter wheat (*Triticum aestivum* L.) in 2003, pea (*Pisum sativum* L.) in 2004, and winter wheat (*T. aestivum* L.)/mustard (*Sinapis alba* L.) in 2005. All crop residues were incorporated by the respective tillage operations. In 2006, forage maize (*Zea mays* L.) was grown until September, where aboveground parts were harvested. The maize stubble was not incorporated, and the fields were bare fallows until the end of March 2007. The tillage operations were then carried out, and field bean (*Vicia faba* L.) was sown on the 29th of March 2007. Harvest took place on the 29th of August. Tillage operations were carried out on the 1st (CT) and 2nd (MT) of November 2007. Further site characteristics are given by Ehlers et al. (2000) and Jacobs et al. (2009).

Samples were taken in March 2007 after fallow (and before the tillage at the end of March) and on the 7th of

November 2007, 5 (MT) and 6 (CT) days after tillage. In these 6 days, daily mean soil temperature was constant at 12°C (detected at 5-, 10-, and 20-cm depth). From each field replicate, a composite sample out of three subplots was taken from 0- to 5-cm (surface soil) and 10- to 20-cm (subsoil) depth. Directly after sampling, samples were stored at 4°C. Samples were carefully broken apart and sieved (<10 mm) at room temperature as soon as possible. Directly after sieving, samples were dried at 40°C for 48 h. In the following days, fractionation was carried out as described below.

Contents of C_{org} and N for the surface soils (0–5 cm) and subsoils (10–20 cm) are shown in Table 1. The sampling depths 0 to 5 and 10 to 20 cm were chosen in order to distinguish accurately between those layers that were affected by MT and those that were not. However, one has to keep in mind that the 10- to 20-cm depth was not entirely representative of the plough layer of the CT treatment. At the sampling date of November, an accumulation of the harvest residues was visible in the lower third of the Ap horizon of the CT treatment.

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

The temporal dynamics of water-stable aggregates was studied by sampling soils in March 2007 (after fallow, Jacobs et al. 2009) and directly after tillage in November 2007. We used the method developed by Cambardella and Elliott (1993) and modified by Six et al. (1998). Briefly, 100 g (LP6200S; Sartorius, Göttingen, Germany) of dry soil (<10 mm) was placed on a 2-mm sieve and submerged into distilled water for 10 min to allow slaking. Thereafter, the sieve was moved up and down into the water with 50 repetitions. Water-stable aggregates remaining on the mesh (large macroaggregates: 2–10 mm) were collected, vacuum filtered (<0.45 μ m) to remove water, dried at 40°C for 48 h on the filter, and weighed (LP3200D, Sartorius). Aggregates that passed the 2-mm mesh were poured onto the next smaller mesh size, and the fractionation procedure was

continued as described above. Mesh sizes used were 1 mm for medium macroaggregates, 0.25 mm for small macroaggregates, and 0.053 mm for microaggregates. Finally, the supernatant (silt and clay together with finest microaggregates <0.053 mm) was precipitated with 0.5 M $AlCl_3$ (5 mL on 2 L of supernatant). To recover the <0.053-mm fraction after precipitation, the water was siphoned off, then the deposit was dried at 40°C for 48 h and weighed (LP3200D, Sartorius). All fractions were ball milled (Retsch, Haan, Germany), and C_{org} and N concentrations were determined in all water-stable aggregate size classes obtained and in the bulk soil. Carbonates were destroyed by 10% HCl prior to analysis (Elementar Vario El; Heraeus, Hanau, Germany).

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

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

Incubation experiment

The incubation was carried out with the surface soils and subsoils taken in November 2007 (sieved <10 mm and dried at 40°C for 48 h) in the following treatments: (1) CT, bulk soil <10 mm (CT_{bulk}), (2) CT, macroaggregates destroyed (CT_{md}), (3) MT, bulk soil <10 mm (MT_{bulk}), (4) MT, macroaggregates destroyed (MT_{md}). For the CT_{md} and MT_{md} treatments, all naturally occurring macroaggregates were gently destroyed by a mortar, and the soil was passed through a 0.25-mm mesh prior to incubation in order to guarantee complete macroaggregate disruption.

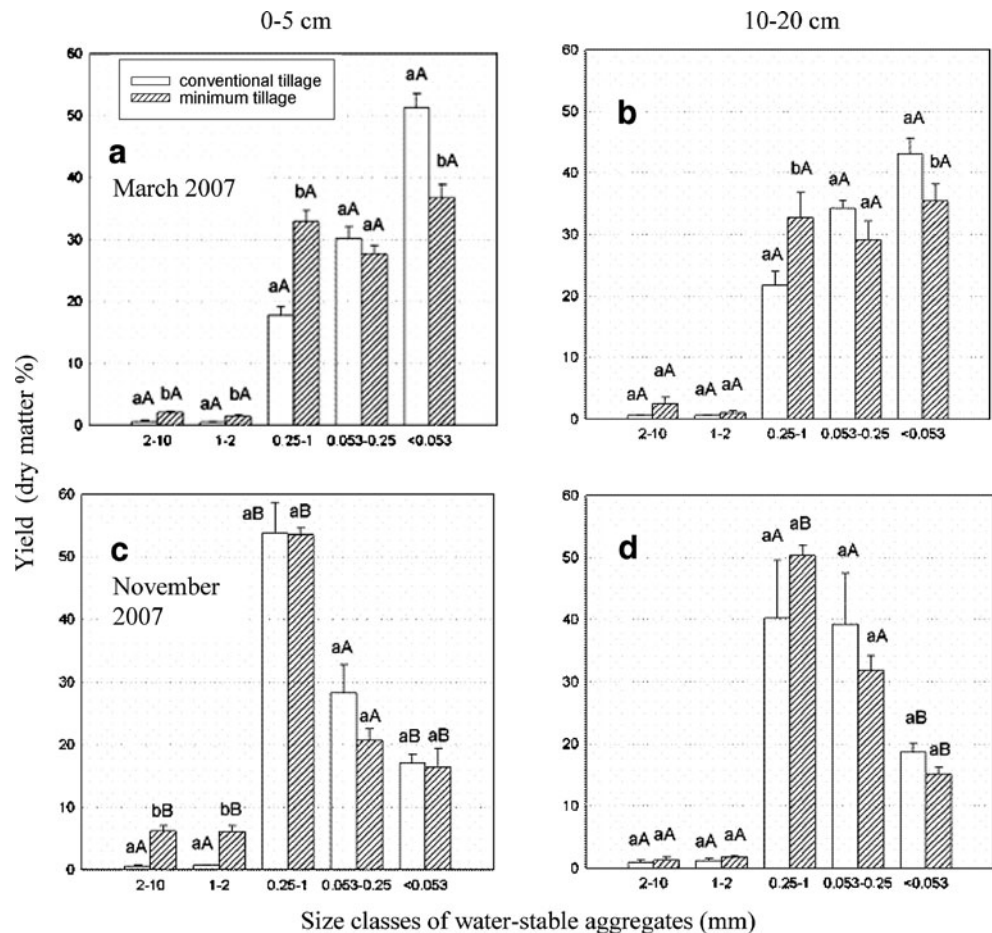
For each treatment, 40 g of soil (either md or bulk soil) were incubated in glass jars at 50% of the maximum water holding capacity and 22°C for 28 days in the dark. Soil

Table 1 Site properties (bulk soil <2 mm) at November 2007 for different tillage systems and sampling depths (means and standard errors, $n=4$)

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

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

Fig. 1 Total yield of water-stable aggregate size classes (means and standard errors, $n=4$) among tillage systems (CT and MT) and sampling depths in March 2007 (Jacobs et al. 2009; subfigure a, b) and November 2007 (subfigure c, d). Lowercase letters indicate significant differences ($p \leq 0.05$) among tillage systems. Uppercase letters indicate significant differences ($p \leq 0.05$) between sampling dates



moisture was controlled regularly by weighing and corrected if necessary by adding distilled water.

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

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

Basal respiration (Heinze et al. 2009) of soil was determined by trapping CO_2 with 5-mL 0.5 M NaOH, which was replaced after 1, 3, 7, 10, 14, 21, and 28 days.

The trapped CO_2 was precipitated as $BaCO_3$ by 5 mL of saturated $BaCl_2$ solution, and the residual NaOH was titrated to pH 8.3 by 0.5 M HCl. The amount of CO_2 evolved ($\mu g (g \text{ soil})^{-1} \text{ day}^{-1}$) was calculated as follows:

$$CO_2\text{-C emission} = [(B - S) \times M - E/DW - 1, 000] / \text{days of incubation} \quad (1)$$

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

Acid hydrolysis

In order to determine the biochemically stabilized OM pool, the samples taken in November 2007 were subjected to acid hydrolysis as described by Plante et al. (2006), but slightly modified. Briefly, 0.5 g of sample was treated with 25-mL 6 M HCl for 16 h under reflux at 100°C. Thereafter, samples were washed three times with 40-mL distilled H_2O , the supernatant was discarded after centrifugation at 5,200 g for 15 min, and the soil was freeze-dried. The remaining,

nonhydrolysable, C_{org} and N fractions, which are regarded as biochemically stabilized, recalcitrant soil organic matter (SOM) pools (Paul et al. 2006), were determined by dry combustion (Vario Max, Elementar, Hanau, Germany).

Equivalent soil mass approach

For the surface soils of the CT and MT systems, we calculated the C mass per unit area in an equivalent soil mass as described by Ellert et al. (2001), and data are given in kg C m^{-2} in 68-kg soil (Table 2). For the calculations, the depths considered ranged from 0–5 to 0–5.4 cm, depending on the bulk densities (Table 2), and we assumed that the bulk densities measured in the 0- to 5-cm depths were also approximately valid down to 5.4 cm.

Statistical analysis

Means and standard errors were calculated for each parameter detected within each sampling depth and for each treatment. For the investigation of differences between water-stable aggregate size classes and of the proportion of biochemically stabilized OM, both tillage systems were evaluated by a *t*-test. A paired *t*-test was performed for each tillage system and sampling depth to evaluate the temporal variation of water-stable aggregates and C_{org} stored within the aggregate size classes between the two sampling dates. We carried out an analysis of variance analysis followed by a Student–Newman–Keuls test including tillage system and aggregate treatment as fixed factors. Each of the sampling depths was investigated separately. The level of significance was fixed at $\alpha=0.05$. All statistical analyses were carried out using GLM of Statistica 7, Statsoft (Tulsa, USA).

Results and discussion

Total contents of C_{org} and N in soils

Thirty-seven years of two different tillage systems resulted in significant differences in C_{org} and N contents in the surface soil: $15.8 \text{ g kg}^{-1} C_{\text{org}}$ were present after tillage in November 2007 in the 0- to 5-cm depth of the MT system

compared to 9.3 g kg^{-1} of the CT system (Table 1). This difference is more pronounced than in March 2007, where the values were 16.3 (MT) and 13.3 (CT) g kg^{-1} (Jacobs et al. 2009). The same pattern as for the C_{org} contents was observed for the N contents in the surface soils. However, in the subsoils, no significant differences between tillage systems were observed for C_{org} and N for either sampling date. The higher C_{org} content in the surface soil in March than November 2007 probably depended on the fact that maize stubble was not incorporated after the maize harvest in September 2006.

In November 2007, 5 (MT) to 6 days (CT) after tillage, an intensive turnover of C_{org} in the soil was likely due to the incorporation of harvest residues and due to a possible breakup of aggregates by tillage. Thus, one sampling date with such intensive turnover may create artefacts in evaluating long-term effects of tillage systems on C_{org} storage. In our study, spatial variability of C_{org} contents in November was small (Table 1), but no attempt was made in this study to sample the plots in exactly the same spots on both sampling dates by using a global positioning system. Thus, the variance given in Table 1 is likely not a true measure of the spatial variability.

A calculation of the C_{org} stocks in the surface soils using the equivalent soil mass approach suggests that between March and November 2007, 0.27 (CT) and 0.04 (MT) kg C m^{-2} in 68-kg soil was lost in the surface soil (Table 2). The large C loss as suggested for the CT treatment in 8 months is unlikely for two reasons. First, steady-state systems show much smaller inputs and outputs than such a C loss. For instance, Ludwig et al. (2007) reported C inputs to the Ap horizon of 0.09 to 0.24 kg C m^{-2} for winter wheat and 0.06 to 0.12 kg C m^{-2} for spring barley in the Bad Lauchstädt trial, and since stocks were approximately constant (with a large spatial variability) in the fertilization treatments, outputs were probably of the same order (Ludwig et al. 2007). Second, tillage was carried out twice between the two sampling dates, resulting in a slight (MT: down to 5–8 cm) or intensive mixing (CT: down to 25 cm) of the soil, which makes an interpretation difficult for the surface soil of the CT system. Spatial heterogeneity and mixing during tillage were probably the main reason for the large deviation in C_{org} stocks between sampling dates.

Table 2 Bulk densities (means and standard errors, $n=4$ in March and $n=2$ in November) and stocks of C_{org} (means and standard errors, $n=4$) on an equivalent soil mass approach for the surface soils of different tillage systems (CT and MT) and two sampling dates

Tillage system	Sampling date in 2007	Bulk density in 0–5cm (g cm^{-3})	Depth considered in the equivalent soil mass approach (cm)	C_{org} stock (kg C m^{-2} in 68-kg soil)
CT	March	1.36 (0.09)	0–5	0.90 (0.17)
	November	1.36 (0.19)	0–5	0.63 (0.09)
MT	March	1.25 (0.04)	0–5.4	1.11 (0.08)
	November	1.33 (0.09)	0–5.1	1.07 (0.08)

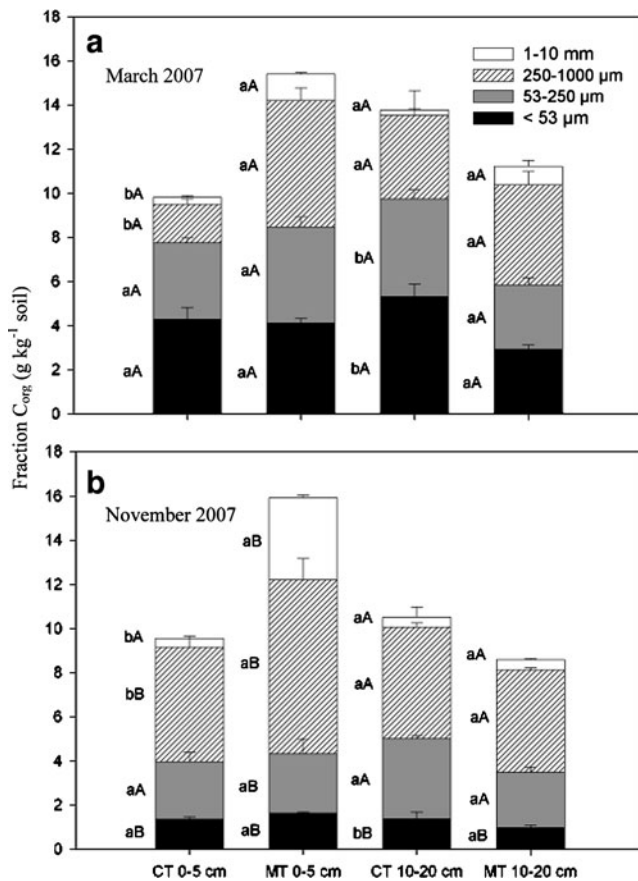


Fig. 2 C_{org} concentration of water-stable aggregate size classes (<10 mm) per kilogram soil (means and standard errors, $n=4$) among tillage systems (CT and MT) and sampling depths in March 2007 (modified after Jacobs et al. 2009; subfigure a) and November 2007 (subfigure b). Lowercase letters indicate significant differences ($p \leq 0.05$) among tillage systems. Uppercase letters indicate significant differences ($p=0.05$) between sampling dates

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

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

The separation of the soil samples taken in November 2007 into different size classes of water-stable aggregates showed a higher occurrence of macroaggregates (>0.25 mm) in the surface soil (0–5 cm) of MT than of CT (Fig. 1c). This was pronounced especially for the size classes 1 to 2 and 2 to 10 mm. The subsoil followed the same trend but with no significant differences between the tillage treatments (Fig. 1d), thus, confirming those detected in March 2007

for the same site (Fig. 1a, b; Jacobs et al. 2009) and also for a tropical agroecosystem (Kushwaha et al. 2001). Jacobs et al. (2009) outlined that a higher occurrence of water-stable macroaggregates under MT can be attributed (1) to a reduced physical impact by the machinery leading to less disruption of aggregates than under CT or (2) to a higher formation of macroaggregates due to a higher concentration of OM in the surface soil.

In November 2007, directly after tillage (Fig. 1c, d), macroaggregates, especially the size class 0.25 to 1 mm, were significantly more abundant under MT than in March 2007 after fallow (Jacobs et al. 2009; Fig. 1a, b). The increase of the contents of smaller macroaggregates (0.25–1 mm) with

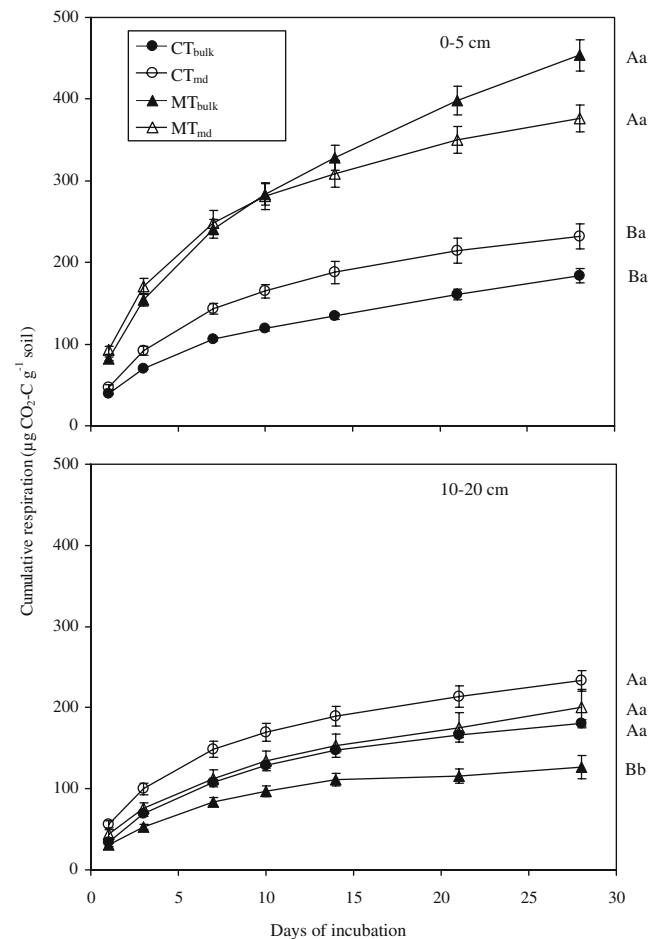


Fig. 3 Cumulative basal respiration during incubation among tillage systems (CT and MT), aggregate treatments, and sampling depths (means and standard errors, $n=4$). Capital letters indicate significant differences ($p \leq 0.05$) between tillage systems with the same aggregate treatment (CT_{bulk} vs. MT_{bulk}, CT_{md} vs. MT_{md}); lowercase letters indicate significant differences ($p \leq 0.05$) between aggregate treatments of the respective tillage treatment (CT_{bulk} vs. CT_{md}, MT_{bulk} vs. MT_{md}). Probability values were aggregate treatment 0.41, tillage system <0.01, aggregate treatment \times tillage system <0.01 at 0 to 5 cm; and aggregate treatment <0.01, tillage system 0.01, aggregate treatment \times tillage system 0.39 at 10 to 20 cm

respect to March was larger in CT than MT. This was pronounced in the surface soil, but the subsoil followed the same trend. However, spatial and operator variability have also contributed to the observed changes in the distribution of aggregate size classes. Additionally, one has to keep in mind that the method uses dried soil. Since drying of soil is known as an aggregate forming process, the results obtained by this method do not entirely reflect the field state aggregation.

The marked contents of smaller macroaggregates in the CT treatment after tillage suggest that physical disruption of aggregates by the plough may mainly affect the larger macroaggregates, resulting in small yields of large macroaggregates compared to MT as observed (Fig. 1). Moreover, the data suggest that during the cropping of field bean, differences in the aggregate distribution between the two tillage systems levelled off. The slightly larger contents of macroaggregates under MT suggest that more macroaggregates were formed directly after tillage due to the input of fresh plant residues. Daraghmeh et al. (2009) reported a significant temporal variation in wet aggregate stability (WAS) over the year for a Danish sandy loam under different tillage systems. They found that WAS was lowest in winter (December) and higher shortly after tillage (October) and in summer. The low WAS in winter was probably due to the interaction of freeze/thaw cycles with soil moisture, whereas the recovery of WAS from spring onwards probably depended on the increasing biological activity.

The calculation of the C_{org} concentration (expressed as $g\ kg^{-1}$ soil) in the size classes of water-stable aggregates indicated that the major part of C_{org} (58% and 73% for CT and MT surface soils, respectively, and 59% and 57% for CT and MT subsoils, respectively) was sequestered within macroaggregates ($>0.25\ mm$) in November 2007 (Fig. 2b). The same occurred for total N (data not shown). Thus, macroaggregates, especially the size class 0.25 to 1 mm, were the most important fraction sequestering of OM directly after tillage (Fig. 2b), but this OM sequestration is short-lived as discussed below. The comparison between the tillage systems revealed a significantly higher amount of OM within the macroaggregate fractions of MT than of CT surface soils (Fig. 2b). In a comparable tillage system, Kushwaha et al. (2001) also found that macroaggregates stored the surplus of OM in MT surface soils, and the same was reported by Beare et al. (1997) for an NT soil. In March 2007 after fallow, microaggregates and silt and clay particles were more abundant and more important in storing C_{org} (79% and 55% for CT and MT surface soils, respectively, and 72% and 53% for CT and MT subsoils, respectively) than macroaggregates (Figs. 1a,b and 2a). However, a comparison of CT with MT surface soils showed that the surplus of C_{org} stored under MT was located within the macroaggregate fractions.

Therefore, our interpretation is that a considerable number of new macroaggregates were formed (as shown

Table 3 Microbial biomass and different indices of OM decomposition after 28 days of incubation (means and standard errors, $n=4$) for the incubation experiment with CT and MT bulk soils (CT_{bulk} , MT_{bulk}) and soils with destroyed macroaggregates (CT_{md} , MT_{md})

Sampling depth (cm)	Soil	C_{mic} ($\mu g\ g^{-1}$)	N_{mic} ($\mu g\ g^{-1}$)	Percentage of organic C present as C_{mic} (%)	Percentage of organic C evolved as $C-CO_2$ (%)	
0–5	CT_{bulk}	99 (12) ^{Aa}	17 (3) ^{Aa}	1.01 (0.09) ^{Aa}	2.03 (0.17) ^{Aa}	
	CT_{md}	56 (11) ^{Aa}	11 (2) ^{Aa}	0.57 (0.10) ^{Ab}	2.49 (0.21) ^{Aa}	
	MT_{bulk}	358 (27) ^{Bc}	44 (7) ^{Bb}	2.32 (0.15) ^{Bc}	2.85 (0.10) ^{Ba}	
	MT_{md}	222 (17) ^{Bb}	28 (6) ^{Aa}	1.49 (0.09) ^{Bd}	2.50 (0.13) ^{Aa}	
	Probability values					
	Aggregate treatment	<0.01	<0.05	<0.01	0.72	
	Tillage system	<0.01	<0.01	<0.01	0.02	
	Aggregate treatment \times tillage system	0.02	0.32	0.09	0.03	
10–20	CT_{bulk}	131 (4) ^{Aa}	16 (2) ^{Aa}	1.38 (0.07) ^{Aa}	1.92 (0.07) ^{Aa}	
	CT_{md}	117 (15) ^{Aa}	11 (2) ^{Ab}	1.17 (0.14) ^{Aa}	2.32 (0.07) ^{Aa}	
	MT_{bulk}	118 (6) ^{Aa}	13 (1) ^{Ab}	1.38 (0.10) ^{Aa}	1.41 (0.22) ^{Ab}	
	MT_{md}	84 (6) ^{Bb}	8 (0) ^{Ab}	0.95 (0.05) ^{Ab}	2.32 (0.25) ^{Aa}	
	Probability values					
	Aggregate treatment	0.02	<0.01	<0.01	<0.01	
	Tillage system	0.03	0.08	0.26	0.16	
	Aggregate treatment \times tillage system	0.27	0.94	0.27	0.16	

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

for our sampling in November) directly after tillage (and thus after residue incorporation). With ongoing time after tillage (as observed for the sampling in March 2007, which was 10 months after the last tillage and residue incorporation), macroaggregates disrupt into microaggregates, the latter being assumed to provide longer-term protection for OM (Paustian et al. 2000). This is in accordance with the previous finding that the turnover of macroaggregates is rapid if fresh residue inputs are lacking (e.g., Helfrich et al. 2008). However, two sampling dates are not adequate to provide the basis for accurate quantitative estimates of aggregate turnover. For instance, Plante et al. (2002) reported that soil macroaggregate mean residence times in different soils ranged from 4 to 95 days, where aggregate dynamics were generally two to three times more rapid in a Gray Luvisol compared to a Black Chernozem. De Gryze et al. (2006) estimated turnover times for macroaggregates and microaggregates to be 30 and 88 days, respectively.

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

After 28 days of incubation at 22°C and a water content of 50% of the maximum water holding capacity, cumulative CO₂-C emission was significantly higher from the MT (MT_{bulk}, MT_{md}) than from the CT (CT_{bulk}, CT_{md}) surface soils (Fig. 3). Furthermore, the specific respiration (percentage of organic C evolved as C-CO₂) showed the highest value in MT_{bulk} surface soils (Table 3). Higher potential OM mineralization in MT or NT than CT surface soils was also found by Alvarez et al. (1995), Salinas-Garcia et al. (1997), Wright et al. (2005), and Oorts et al. (2006) and probably depended on the organic C contents. In the subsoil, the cumulative and the specific respiration of the MT system was lower than in the surface soil, while it generally remained at the same level for CT soils (Fig. 3, Table 3). Overall, C mineralization in the subsoils was higher from CT than from MT with significant differences between CT_{bulk} and MT_{bulk}. The main reason for this is that harvest residues are concentrated in the surface soil in the MT treatments, whereas in the CT treatment, an accumulation of the harvest residues mainly occurred in the lower third of the Ap horizon.

The incubation of md soils (MT_{md}, CT_{md}) did not generally result in a significantly higher C mineralization compared to the respective bulk soils, except for the MT_{md} subsoil (Fig. 3). Probably the physical stabilization of OM stored in the macroaggregates does not depend on a mechanism protecting very labile C with a turnover time of weeks but rather on the formation of new macroaggregates, which may be higher than the destruction of macroaggregates within a few days. For instance, Bossuyt

et al. (2001) found 25% of the bulk soil in new-built macroaggregates after 6 days of incubation. However, for the latter hypothesis, marked differences in the respiration would be expected in the initial phase of the incubation, which were not observed for the MT surface soil (Fig. 3).

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

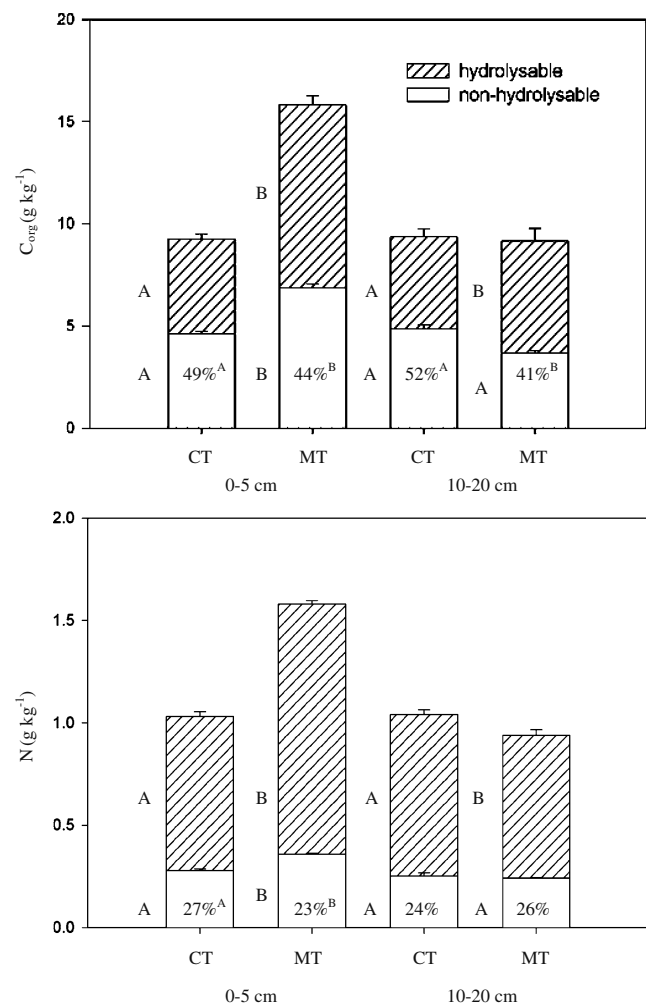


Fig. 4 Concentrations of hydrolysable and nonhydrolysable C_{org} and N among tillage systems (CT and MT) and depths (means and standard errors, *n*=4). Percentages give the contribution of non-hydrolysable C_{org} and N to total C_{org} and total N. Letters indicate significant differences (*p*≤0.05) among tillage systems

procedure in order to gain a better quantitative understanding of OM protection in soils.

The determination of the microbial biomass showed large differences between the tillage systems after 28 days of incubation. In the surface soil, C_{mic} was 3.6 and 4.0 times greater in the MT_{bulk} and MT_{md} treatments, respectively, compared to the respective CT treatments. In the subsoil, CT soil showed higher values, but differences between tillage treatments were much less pronounced than in the surface soil (Table 3). Furthermore, the percentage of C_{org} present as C_{mic} was significantly higher in MT_{bulk} and MT_{md} than in the respective CT surface soils, while no differences between tillage systems were found in the subsoil (Table 3). The differences found between MT and CT in the distribution of OM (Table 1) and of microbial biomass (Table 3) agree with findings of various other studies (e.g., Ahl et al. 1998; Kushwaha et al. 2001; Spedding et al. 2004) and depend on the difference in the depth of incorporation of plant residues in the two tillage systems (Kandeler et al. 1999; Stockfisch et al. 1999; Kushwaha et al. 2001; Jacobs et al. 2009).

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

In the surface soil, the content of nonhydrolysable C was slightly greater in the MT soil than in the CT soil (Fig. 4), whereas the difference in the content of hydrolysable C was more pronounced. The proportion of nonhydrolysable, C_{org} to total C_{org} , was even lower in the MT than in the CT soils (Fig. 4). Hydrolysable and nonhydrolysable N followed the same trend (Fig. 4). Overall, the data indicate that MT has a physical rather than a biochemical stabilization effect.

In the subsoil, the proportion of nonhydrolysable C_{org} to total C_{org} was significantly lower in MT than in CT, while no differences were detected for N. The lower contribution of nonhydrolysable, biochemical recalcitrant, C_{org} to total C_{org} of MT soils confirms that which was reported by Collins et al. (2000) and the hypothesis that the proportion of resistant SOM will increase with SOM depletion (e.g., Kiem et al. 2000; Plante et al. 2004).

Conclusions

This study indicates that a lower occurrence of water-stable macroaggregates in a CT soil compared to an MT soil cannot be mainly attributed to a physical disruption of aggregates by ploughing. Directly after tillage and residue incorporation, considerable amounts of new macroaggregates were formed, especially in the MT surface soil due to the high concentration of crop residues. In the short-term, macroaggregates can store the surplus of OM found under

MT compared to CT systems. Furthermore, it was shown that the surplus of OM in MT is stored in a biochemically degradable fraction, especially in the surface soil, and with ongoing time after tillage, macroaggregates disrupt into stabilized microaggregates, which provide longer preservation of OM.

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