

## Improvement of soil structure through organic crop management, conservation tillage and grass-clover ley

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### ABSTRACT

Conventional intensive tillage is a widespread soil management practice that controls weeds and promotes nutrient mineralization at the expense of a degraded soil structure and soil carbon (C) loss. Alternative soil management practices such as no tillage, reduced tillage and temporary leys, however, can minimize the negative effects of intensive tillage on soil structure. To improve understanding of these management practices on soil structure, we sampled a field trial combining organic and conventional crop management with different intensity levels of tillage, resulting in four cropping systems: conventional intensive tillage (C-IT), conventional no tillage (C-NT), organic intensive tillage (O-IT) and organic reduced tillage (O-RT). A ley period was added following a 4-year arable crop rotation. We measured mean weight diameter (MWD), total C and total nitrogen (N) in whole unfractionated soil and water-stable aggregate fractions after the 4-year arable crop rotation and again after a following 2-year grass-clover ley period, to assess the impact of the cropping system and the ley, respectively. Results showed that 4 years of organic crop management including the application of cattle manure slurry combined with reduced tillage led to significantly better soil structure (i.e. aggregate MWD) at the 0–6 cm soil depth, compared to the other cropping systems tested. After the ley period, the proportion of large macroaggregates increased by 65% for C-IT and 47% for O-IT at 0–6 cm depth. Total C increased significantly for only O-IT and O-RT after the ley, both of which also showed a high C stratification between 0–6 cm and 6–20 cm depth. In conclusion, soil structure is most improved during continuous cropping when combining organic crop management with reduced tillage, while a ley period was effective in improving the soil structure in intensive tillage plots to the level of O-RT.

### 1. Introduction

The impacts of tillage and crop management type extend well beyond crop productivity; they influence soil microbial activity (Lori et al., 2017), greenhouse gas emissions (Stavi and Lal, 2013), soil structure and C sequestration (Gattinger et al., 2012; Guo and Gifford, 2002). The widespread and long-term use of intensive tillage has led to significant soil degradation under a number of soil types, including soil compaction and soil erosion, with a concomitant loss of soil organic carbon (SOC).

Sustainable agricultural intensification and ecological intensification are examples of different initiatives which aim to reconcile agricultural productivity with long-term environmental sustainability

(Bommarco et al., 2013; Govers et al., 2017). Both initiatives advocate for supporting soil functionality and improving nutrient recycling. Practices that allow for this include conservation tillage, organic crop management and the use of leys.

Conservation tillage spans tillage variations that leave at least 30% of crop residue on the surface (Soil Science Society of America, 2008). These types of conservation tillage including no tillage, reduced, ridge, chisel, disk, sweep tillage, alone or combined, have shown to be effective in reducing soil erosion (Lynch, 2012). No tillage covers about 4% and 25% of arable land in the EU and USA, respectively (EUROSTAT, 2013; U. S. Department of Agriculture, 2014), while other forms of conservation tillage are practiced in almost 20% of arable land in the EU and 27% in the U.S. Organic crop management has been shown to

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benefit biodiversity and increase soil organic matter (SOM) content in comparison with conventional crop management (Gomiero et al., 2011). One drawback, however, is that it usually relies on intensive tillage for mechanical weed control since synthetic herbicides are not permitted. Leys are temporary grasses or grass-forb mixtures grown during arable crop rotations that can serve to facilitate weed control and produce forage.

Soil structure affects the movement of water, solutes, microorganisms, gases and plant roots, which influence soil functions (Bronick and Lal, 2005; Carter, 2002; Nicolodi and Gianello, 2014). Soil aggregation is often used as a measure for soil structure (Six et al., 2000) despite ongoing difficulties in defining critical limits (Carter, 2002). The formation of aggregates, i.e., water-stable soil size classes with intrinsic varying physical and chemical characteristics (Elliott, 1986), leads to the physical protection of C from mineralization by microorganisms (Balesdent et al., 2000; Schimel and Schaeffer, 2012). During the formation of aggregates, inter-aggregate organic matter is incorporated (Six et al., 2004a). Considering that physical access to occluded substrates is a limiting factor for organic matter breakdown in mineral soils (Schimel and Schaeffer, 2012; Tisdall and Oades, 1982) this incorporation of organic matter into aggregates can contribute to longer-term soil C sequestration (Kong et al., 2005). Aggregate stability is often lower under intensive tillage (Elliott, 1986; Kravchenko et al., 2011), encouraging a loss of C-rich macroaggregates ( $> 250 \mu\text{m}$ ) while increasing C-poor microaggregates ( $250\text{--}53 \mu\text{m}$ ) (Six et al., 2000). Increasing SOM is sought, since it improves soil quality through altering nutrient availability, water holding capacity, soil porosity, cation exchange capacity and soil aggregation (Bronick and Lal, 2005; Kaiser et al., 2008; Mangrich et al., 2015). Given the advantages of good soil structure in supporting soil processes, practices that foster aggregate stability should be encouraged.

In this study, we used a field experiment to quantify the effect of four different cropping systems resulting of the combination of organic or conventional crop management with different levels of tillage, i.e. conventional intensive tillage, conventional no tillage, organic intensive tillage and organic reduced tillage, as well as a ley period on soil aggregate stability and aggregate-associated C and N storage. Measurements were taken after a 4-year arable crop rotation to evaluate the effects of the different cropping systems, and repeated following a 2-year grass-clover ley period to assess the effect of ley. We hypothesized that reduced tillage and no tillage would improve soil structure by reducing soil physical disturbance in comparison with intensive tillage, resulting in a higher mean weight diameter and total carbon (TC) content in comparison to intensive tillage. Similarly, we hypothesized that organic crop management would improve soil structure compared to conventional crop management due to the additional C input from the cattle slurry fertilizer. Finally, we hypothesized that the minimal physical disruption and particle binding action of plant roots and their exudates during the ley period would result in an increase in C content and improvement in soil structure across all cropping systems.

## 2. Methods

### 2.1. Field site and experimental design

The Swiss Farming System and Tillage experiment (FAST), described in detail by Wittwer et al. (2017), compares conventional and organic crop management with different tillage intensities in 6-year rotations. The field experiment is located at the Swiss federal research station Agroscope, Reckenholz near Zurich, Switzerland ( $47^{\circ}26'20''\text{N}$ ,  $8^{\circ}31'40''\text{E}$ ). The soil is a Cambisol on glacially deposited Pleistocene sediments containing 23% clay, 34% silt and 43% sand (IUSS Working Group, WRB, 2014). Mean annual temperature is  $9.4^{\circ}\text{C}$  (Swissmeteo), while annual precipitation averages  $1054 \text{ mm}$  (1981–2010 data).

The crops rotating in the first 4 years are representative of local

Swiss farming practices. The rotation started with winter wheat (*Triticum aestivum* L. cv. Titlis), followed by maize (*Zea mays* cv. Padrino), field bean (*Phaseolus vulgaris* cv. Fuego) and winter wheat (*Triticum aestivum* L. cv. Titlis). Finally, a grass-clover mixture (UFA 330) was sowed for a 2-year period. The experiment started in August 2009 and was set-up as a randomized complete block design replicated four times, where each plot measured  $6 \text{ m} \times 30 \text{ m}$ .

The management practices tested are crop management (i.e., conventional and organic) and tillage type (i.e., intensive tillage, reduced tillage and no tillage). The combination of both factors resulted in four cropping systems: conventional intensive tillage (C-IT), conventional no tillage (C-NT), organic intensive tillage (O-IT), and organic reduced tillage (O-RT). Intensive tillage was applied to  $0.2 \text{ m}$  depth using a moldboard plow (Menzi B. Schnyder, Brütten, Switzerland) followed by a rotary harrow at  $0.05 \text{ m}$  depth (Amazone, H. Dreyer GmbH, Germany) for both conventional and organic crop management. Conservation tillage consisted of no tillage and direct seeding under conventional crop management, whereas reduced tillage was applied under organic crop management, with a superficial tillage at  $0.05 \text{ m}$  depth using a disk harrow in the first year and a rotary harrow thereafter.

Both O-IT and O-RT were fertilized with cattle manure slurry whereof on the average 40% of the total N content was in the form of  $\text{NH}_4\text{-N}$ , and the rest organic N. This was distributed among the wheat crops ( $107 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in two applications), maize ( $137 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in two applications), and the grass-clover ley ( $205 \text{ kg N ha}^{-1} \text{ year}^{-1}$  in four applications). Fertilization in C-IT and C-NT consisted of ammonium-nitrate applications with an input of  $110 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  for the wheat crops,  $90 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  for maize, and for the grass-clover ley  $130 \text{ kg N ha}^{-1}$  the first year and  $100 \text{ kg N ha}^{-1}$  the second year. The grass-clover was harvested five times the first year and four times the second year, with fertilizer applied in equal splits after each cut.

### 2.2. Soil sampling

Soil was sampled at the end of the fourth growing season (August 2013, after wheat harvest), as well as immediately following two years of grass-clover ley (August 2015). Four intact soil cores ( $5.5 \text{ cm} \times 20 \text{ cm}$ ) were taken at  $3 \text{ m}$  intervals from the center of each replicate plot using a Giddings hand sampler (Giddings Machinery Co, Windsor, Colorado, USA). Each  $20 \text{ cm}$ -length core was manually cut at  $6 \text{ cm}$ , separating the top  $0\text{--}6 \text{ cm}$  from the bottom  $6\text{--}20 \text{ cm}$ . Field-moist cores were sieved at  $8 \text{ mm}$  by manually crumbling along natural fracture lines in order to minimize aggregate disruption. The four cores from each plot were combined and each composite sample was air-dried and stored at room temperature.

### 2.3. Physical fractionation of soil aggregates

Air-dried soil was wet-sieved following Elliott (1986) to separate four aggregate size classes: large macroaggregates (LM;  $> 2000 \mu\text{m}$ ), small macroaggregates (SM;  $2000\text{--}250 \mu\text{m}$ ), microaggregates (mi;  $250\text{--}53 \mu\text{m}$ ) and silt and clay (S + C;  $< 53 \mu\text{m}$ ). To accomplish this, eighty grams of air-dried soil was distributed evenly on a  $2000 \mu\text{m}$  sieve and for  $5 \text{ min}$  submerged in deionized water for slaking. Then, the sieve was manually raised and lowered rhythmically 50 times over the course of two minutes taking care to maintain an even force throughout the process (Elliott, 1986). The water-stable aggregates on the sieve (i.e. macroaggregates) were backwashed into a tin and oven dried at  $60^{\circ}\text{C}$ . The remaining soil-water mix was poured over the next smaller sieve and the procedure was repeated with the  $250 \mu\text{m}$  and  $53 \mu\text{m}$  sieves to isolate the remaining size classes. Mean weight diameter (MWD), used as a measure of soil structure, was calculated using the proportional abundance of each aggregate fraction and the mean diameter of each size class as defined in Eq. (1).

**Table 1**

Distribution of soil aggregate size class fractions of whole soil after a 4-year crop rotation and following 2 years of grass-clover ley. LM: large macroaggregates, SM: small macroaggregates, mi: microaggregates, S + C: silt and clay.

Cropping system	Whole soil fraction (% dry soil)							
	LM (> 2000 μm)		SM (250-2000 μm)		mi (53-250 μm)		S + C (< 53 μm)	
	0-6 cm	6-20 cm	0-6 cm	6-20 cm	0-6 cm	6-20 cm	0-6 cm	6-20 cm
<b>After a 4-year crop rotation</b>								
Conventional intensive tillage	26 b	27 b	44 a	43 a	23 a	26 a	5 a	4 a
Conventional no tillage	34 b	34 ab	39 a	41 ab	21 a	20 ab	5 a	4 a
Organic intensive tillage	32 b D	47 a	42 a D	32 bc	21 a D	17 b	5 a	3 a
Organic reduced tillage	57 a D	46 a M	22 b D	31 c M	17 a	18 ab M	3 a	4 a
<b>Following 2 years ley</b>								
Conventional intensive tillage	43 a Y	43 a Y	27 a Y	25 a Y	23 a	24 a	7 a Y	7 a Y
Conventional no tillage	42 a	43 a	30 a Y	26 a Y	20 a	21 a	6 a Y	6 a Y
Organic intensive tillage	47 a Y	42 a	30 a Y	27 a	18 a	21 a	5 a	6 a Y
Organic reduced tillage	50 a	50 a	28 a	22 a Y	17 a	17 a	5 a Y	5 a Y
Source of variation								
Block	n.s.		n.s.		n.s.		*	
Cropping system	***		*		*		*	
Year	**		***		n		***	
Depth	n.s.		n.s.		n.s.		n.s.	
Cropping system*year	*		**		n.s.		n.s.	
Cropping system*depth	n.s.		n.s.		n.s.		n.s.	
Year*depth	n.s.		n.s.		n.s.		*	
Cropping system*year*depth	*		*		*		n.s.	

Statistical significance was tested at \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$  and \*  $p < 0.05$  for all parameters evaluated. Different letters represent significant  $p < 0.05$  differences between least-squares means of cropping systems within a depth and year. "D" represents the significant difference ( $p < 0.05$ ) between depths of a cropping system, within year, while "Y" denotes a significant difference ( $p < 0.05$ ) between years of a cropping system, within depth. Significant effects ( $p < 0.05$ ) across management types (organic vs. conventional), within depth and year tested with orthogonal contrasts are marked with "M".

$$\text{MWD} = \sum_{i=1}^n \chi_i \omega_i \quad (1)$$

Where  $\chi_i$  is the mean diameter of the particle range of each size class,  $\omega_i$  is the weighted abundance of each aggregate fraction in whole soil and  $n$  is the number of aggregate size classes used.

Occluded mi within macroaggregates (LM + SM) were separated from the 0–6 cm soil samples to understand macroaggregate composition changes, and possible re-allocation of carbon and nitrogen, according to Six et al. (2000). This layer was chosen considering its richer microbial composition is more responsive to change, and surface proximity increases the impact of management practices compared to those at 6–20 cm depth. A total of 15 g of large and small macroaggregates were mixed in similar proportion as their occurrence in whole soil, calculated using the percentage abundance of large macroaggregates and small macroaggregates recorded during the previous fractionation procedure. After slaking in deionized water for 20 min, they were shaken atop a 250 μm metal mesh on a reciprocal shaker at 150 rpm together with fifty 4 mm diameter metal balls under a constant flow of deionized water. A clear outflow stream indicated the disruption of all soil macroaggregates. The outflow tube was placed atop a 53 μm sieve positioned over a basin. Subsequent sieving was performed as described previously for separating the water-stable microaggregates from silt and clay. The mass of all soil fractions collected was recorded after oven drying at 60 °C in pre-weighed aluminum tins.

#### 2.4. Carbon and nitrogen quantification

For each whole soil and fraction sample, approximately 2 g of dry soil was finely ground and subsampled for total carbon (TC) and total nitrogen (TN) determination by combustion on an elemental analyzer (LECO Corporation, United States). TC and TN of the entire 0–20 cm soil profile was calculated using the weighted TC and TN contents of both depths (0–6 cm and 6–20 cm). Annual C accumulation rate during the grass-clover ley period at 0–6 cm and 6–20 cm depth was calculated as the difference in C concentration between the end and beginning of

this period divided by its duration, in years.

#### 2.5. Statistical analyses

All analyses and figures were performed in R 3.3.2 (R Development Core Team, 2017). Linear mixed effect models with restricted maximum likelihood were used to estimate differences in MWD, TC, TN and fraction proportions using R package lme4 (Bates et al., 2015). Block, cropping system, depth (when applicable) and year (i.e. before and after the 2-year grass-clover ley, when applicable) were set as fixed effects. The random effects of cropping system within block, and cropping system within block and year (when applicable) were accounted for with a varying intercept. The data were log-transformed when visual inspection of residual plots revealed deviations from the assumption of homoscedasticity or normality. To facilitate comparison, figure means and standard deviations present untransformed data. Type III ANOVA with Satterthwaite degrees of freedom approximation were followed by Tukey familywise adjustment of least squares means for post-hoc testing. Different lower case letters represent significant differences between cropping systems, within fraction. The letter "M" indicates significant differences between crop management type (organic vs. conventional) within the same depth, year and with both tillage types combined. "D" indicates differences between years, within the same cropping system and depth. "Y" indicates differences between years, within the same cropping system and depth. "D", "M" and "Y" were tested with custom contrasts using least squares means with Tukey multiple comparison correction. Statistical significance was tested at  $p < 0.05$ .

### 3. Results

#### 3.1. Soil aggregation

Macroaggregates were the dominant aggregate fraction overall, accounting for between 68% and 79% of whole soil across both depths and years (Table 1). The proportions of LM and SM showed significant

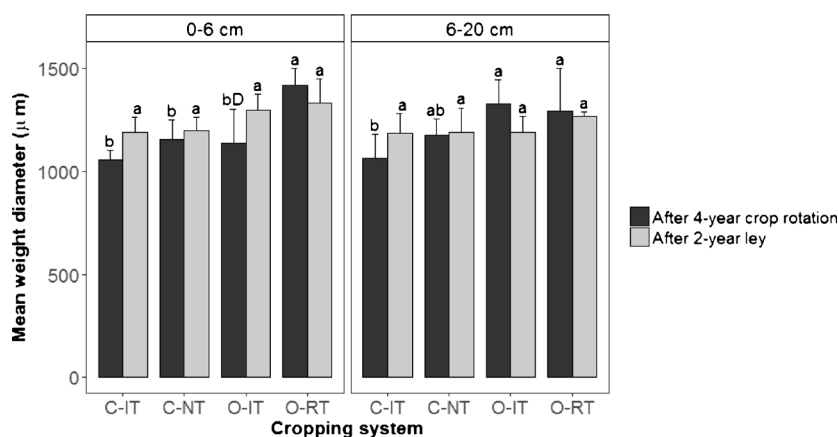


Fig. 1. Mean weight diameter (MWD) at 0–6 cm and 6–20 cm depth after a 4-year crop rotation and after a 2-year ley. Error bars represent standard deviation of the arithmetic mean. Different letters represent least-square means tested significant differences ( $p < 0.05$ ) between cropping systems, within year and depth. Differences ( $p < 0.05$ ) between years, within depth are identified with “Y”, while “D” identifies differences between depths within cropping system and year.

interactions between cropping system, year and depth (Table 1). After the crop rotation, O-RT had significantly more LM compared to the other cropping systems while the opposite was true for SM abundance at 0–6 cm depth (Table 1). MWD followed the same trend in LM at both depths: O-RT had a significantly greater MWD than all other cropping systems after the crop rotation (Fig. 1). In the top soil layer, the MWD decreased in the order: O-RT (1417  $\mu\text{m}$ ) > C-NT (1155  $\mu\text{m}$ ) > O-IT (1139  $\mu\text{m}$ ) > CIT (1053  $\mu\text{m}$ ). Orthogonal contrasts between crop management (O-RT + O-IT vs. C-NT + C-IT) indicated that O-IT and O-RT contained significantly more LM ( $p < 0.001$ ) while at the same time significantly less SM ( $p < 0.05$ ) than C-IT and C-NT in the 6–20 cm depth. Among all cropping systems, macroaggregates at 0–6 cm depth consisted predominantly of occluded microaggregates (58%–62%) and to a significantly lesser degree, occluded silt and clay (14%–18%). The proportion of these fractions was, however, unaffected by cropping system (data not shown).

The 2-year grass-clover ley eliminated differences in LM and SM proportion between cropping systems. This was mainly due to an increase of LM together with a decrease in SM, indicating that SM were incorporated into the newly formed LM in both C-IT and O-IT at 0–6 cm depth and C-IT at 6–20 cm depth. Before the ley, SM was the most abundant aggregate fraction in all cropping systems besides O-RT. However, afterwards it was LM, evidencing an effect of the grass-clover ley in changing aggregate distribution. The remaining mi and S + C aggregate fractions both followed a similar trend across cropping systems, depth and year: C-IT and C-NT had an increased proportion, significant at 6–20 cm depth, in comparison with O-RT and O-IT (Table 1). However, these differences in aggregate proportion did not translate into differences in MWD attributable to the ley, despite a trend of increase in O-IT and C-IT at 0–6 cm depth (Fig. 1).

### 3.2. Total C and total N concentrations

At the end of the crop rotation, TC was not different between cropping systems, at any depth (0–6 cm, 6–20 cm or 0–20 cm). This changed after the ley, where TC was significantly higher in O-RT (24.3  $\text{g C kg}^{-1}$  dry soil) than C-NT (17.9  $\text{g C kg}^{-1}$  dry soil, Table 2). TC depth stratification after the crop rotation persisted after the ley period: C-IT and O-RT showed higher TC at 0–6 cm than at 6–20 cm depth (Table 2). Within cropping system TC increased after the ley in both O-RT and O-IT at 0–6 cm depth. These differences in TC after the ley translated into a C-accrual of between  $-0.69$  and  $2.2 \text{ g C kg}^{-1}$  dry soil year $^{-1}$  across cropping systems and depths. Here, O-IT and O-RT gained significantly more C than C-IT and C-NT at 0–6 cm depth. Also, O-IT and O-RT had accrued significantly more C at 0–6 cm depth than at 6–20 cm depth, where there was even a net loss in C (Fig. 2). The higher TN after the crop rotation between depths of O-RT was maintained after the ley period. Likewise, O-IT and C-IT had significantly

higher TN at 0–6 cm compared to 6–20 cm depth after the ley period. As with TC between cropping systems at 0–6 cm after ley, TN was highest at O-RT compared to C-NT and C-IT.

Significant interactions were found between cropping system  $\times$  depth  $\times$  fraction, cropping system  $\times$  year  $\times$  fraction and cropping system  $\times$  year  $\times$  depth  $\times$  fraction for aggregate TC and TN contributions to whole soil ( $\text{g aggregate-C kg}^{-1}$  whole soil, Figs. 3 and 4). After the crop rotation, LM contributed significantly more TC and TN to whole soil in O-RT (11.5  $\text{g C kg}^{-1}$  and 1.3  $\text{g N kg}^{-1}$ ). In contrast, for the other cropping systems it was SM (between 7.1–8.5  $\text{g C kg}^{-1}$  and 0.8–0.9  $\text{g N kg}^{-1}$ ) or SM together with LM (between 5.3–6.2  $\text{g C kg}^{-1}$  and 0.5–0.8  $\text{g N kg}^{-1}$ ) at 0–6 cm depth (Figs. 3 and 4). Regarding macroaggregate occluded mi and S + C, all cropping systems had significantly higher TC and TN in the occluded mi than in the occluded S + C fraction at 0–6 cm depth after the crop rotation (Table 3). S + C occluded within macroaggregates contained 1.9% and 13.4% more TC than free S + C for C-IT and C-NT, respectively. However in O-IT and O-RT occluded S + C contained 14% and 5% less TC than free S + C, respectively.

The ley increased TC associated with LM in C-IT and O-IT, while TN increased for C-NT and O-RT. SM contribution to whole soil TC and TN decreased in all but the O-RT at 0–6 cm and O-IT at 6–20 cm depth (Fig. 3).

## 4. Discussion

The measurements after the crop rotation reflect the cropping system effect while the effect of the ley is indicated by the data collected following the ley period. The synergistic response found in combining organic crop management with reduced tillage for improving aggregate stability after the crop rotation is in agreement with results by Bottinelli et al. (2017). After seven years of a 4-year crop rotation under three tillage types (conventional tillage, surface tillage and no tillage) and two fertilization practices (organic and conventional) in a Humic Cambisol, they found the highest aggregate stability under no tillage with organic fertilizer (Bottinelli et al., 2017). Similarly, Bissonnette et al. (2001) and Whalen et al. (2003) reported greater aggregate stability when combining organic fertilization with conservation tillage (cattle slurry with chisel plowing, and composted cow manure with no tillage, respectively) in crop rotations.

Organic crop management can lead to higher microbial abundance and activity (Francioli et al., 2016; Lori et al., 2017). As a by-product of microbial activity, microbial exudates including polysaccharides increase aggregation by binding primary soil particles (Degens, 1997; Tisdall and Oades, 1982). Improvements in aggregate stability under organic crop management compared with conventional crop management have been documented previously (Kong et al., 2005). Abiven et al. (2009) reported in a literature review aggregate stability



**Table 2**

Total carbon (TC), total nitrogen (TN) and C:N of whole soil after the crop rotation, and following the grass-clover ley period at two soil depths and for the entire 0–20 cm profile.

Cropping system	Total C (g kg <sup>-1</sup> dry soil)			Total N (g kg <sup>-1</sup> dry soil)			C:N		
	0-6 cm	6-20 cm	0-20 cm	0-6 cm	6-20 cm	0-20 cm	0-6 cm	6-20 cm	0-20 cm
<b>After a 4-year crop rotation</b>									
Conventional intensive tillage	18.9 a D	15.6 a	16.6 a	2.0 a	1.9 a	1.9 a	9.5 a D	8.2 a	8.6 a
Conventional no tillage	16.7 a	15.9 a	16.1 a	2.0 a	1.9 a	1.9 a	8.5 a	8.2 a	8.3 a
Organic intensive tillage	17.8 a	16.6 a	16.9 a	2.1 a	2.1 a	2.1 a	8.6 a	7.9 a	8.1 a
Organic reduced tillage	19.8 a D	17.0 a	18.0 a	2.2 a D	2.0 a	2.1 a	8.8 a	8.5 a	8.6 a
		M							
<b>Following 2 years ley</b>									
Conventional intensive tillage	20.6 ab D	15.3 a	16.2 a	2.0 bc D	1.9 a	1.9 a	10.2 a	9.7 a	8.6 a
Conventional no tillage	17.9 b	14.4 a	16.6 a	1.9 c	1.8 a	1.9 a	9.1 a	8.7 a	8.8 a
Organic intensive tillage	22.3 ab D Y	14.3 a	17.8 a	2.4 ab D Y	1.9 a Y	2.0 a	9.4 a D	8.3 a	8.7 a
Organic reduced tillage	24.3 a D Y	15.5 a	18.2 a	2.5 a D Y	1.8 a Y	2.0 a	9.9 a D	8.5 a	9.1 a
	M			M					
Source of variation									
Block	n.s.	n.s.		n.s.	n.s.		n.s.		n.s.
Cropping system	n.s.	n.s.		n.s.	n.s.		n.s.		n.s.
Year	*	n.s.		n.s.	n.s.		**		n.s.
Depth	***	n.s.		***	n.s.		***		n.s.
Cropping system*year	n.s.	n.s.		n.s.	n.s.		n.s.		n.s.
Cropping system*depth	*	n.s.		**	n.s.		n.s.		n.s.
Depth*year	**	n.s.		***	n.s.		n.s.		n.s.
Cropping system*year*depth	n.s.	n.s.		*	n.s.		n.s.		n.s.

Statistical significance was tested at \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$  and \*  $p < 0.05$ . Lowercase letters represent significant ( $p < 0.05$ ) differences between least-squares means of cropping systems within depth and years. "D" represents differences ( $p < 0.05$ ) between depths within year, while "Y" denotes a significant difference ( $p < 0.05$ ) between years, within depth. Significant differences between management types (organic vs. conventional), within depth and year tested with orthogonal contrasts are marked with "M".

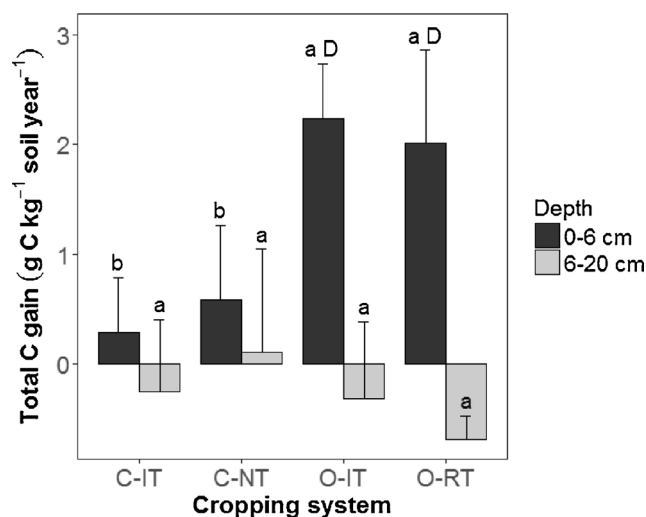


Fig. 2. Total C gain in each cropping systems and depth after ley period. Different letters indicate significant ( $p < 0.05$ ) differences between cropping systems within depth, while differences between depths of a cropping systems are noted with "D".

improvement with organic matter input, along with temporal variation depending on the type of the organic input. However, they found no trend relating to the rate of organic inputs and soil C and clay contents. Since it is possible that the type of C input significantly affects C turnover and consequently microbial response (Berti et al., 2016), there is a need for trials under diverse soil types and conditions to capture site-specific responses and interactions.

The types of conservation tillage used (i.e., no tillage and reduced tillage) were expected to increase aggregate stability following reports under different conditions: at the soil surface in a long-term Typic Kanhapludalf (Devine et al., 2014), in boreal soils under spring barley and spring wheat (Sheehy et al., 2015), in a Kenyan Ferrasol under a soy maize rotation (Paul et al., 2013) and in a long-term study in Typic Hapludalfs in Michigan, U.S. (Kravchenko et al., 2011). Bronick and Lal (2005) analyzed the relationship between soil structure and crop

management in a literature review, making a case for encouraging practices that decrease soil disruption and thereby increase aggregate stability. Both direct and indirect effects are most likely responsible for this increase, for example, a reduction in physical disruption directly preserves macroaggregates and reduces their turnover (Fiedler et al., 2016; Six et al., 1999). An increased microbial activity in these less disrupted systems may also increase aggregation indirectly through an increased production of microbial-derived binding agents (Nivelle et al., 2016; Zuber and Villamil, 2016). Improvements in soil structure can benefit water fluxes (Horn et al., 1994), reduce susceptibility to erosion (Barthès and Roose, 2002) and enhance biodiversity (Vos et al., 2013). Value in adopting conservation tillage and organic crop management can reach beyond soil-related aspects and include reduced management costs. At the same site used in this study, Wittwer et al. (2017) calculated an average management score considering energy use, N supply and pesticide use per cropping system. They found O-RT had the lowest intensity score; 36%, 109% and 118% lower than O-IT, C-NT and C-IT, respectively.

Although we found improved soil aggregate stability after ley under C-IT and O-IT at 0–6 cm depth, as well as at 6–20 cm depth for C-IT, there are contrasting results reported on the use of leys together with arable cropping in relation to soil structure (Haynes et al., 1991; Panettieri et al., 2017; Studdert et al., 1997). Variation between studies can be attributed to differences in experiment duration, plant composition and soil texture, e.g. higher clay contents can allow for higher C stabilization (von Lützw et al., 2006). Extended season rhizodeposition during the ley period (Jones and Donnelly, 2004) and resulting increased biological activity as opposed to under bare fallow may support aggregate-forming mycorrhizae and also increase soil C input (Rillig and Mummey, 2006). Different plant compositions can differ in quality. For example, a grass-clover ley may have a lower C:N compared to prairie grasses, allowing more plant residue decomposition and higher diversity of root exudates during the ley which provide additional C and result in a soil binding effect. An example is Zhang et al. (2016), who reported an increase in macroaggregates in Anthrosols under natural vegetation succession but not under bare fallow. The combination of this effect with the lack of physical disruption may have

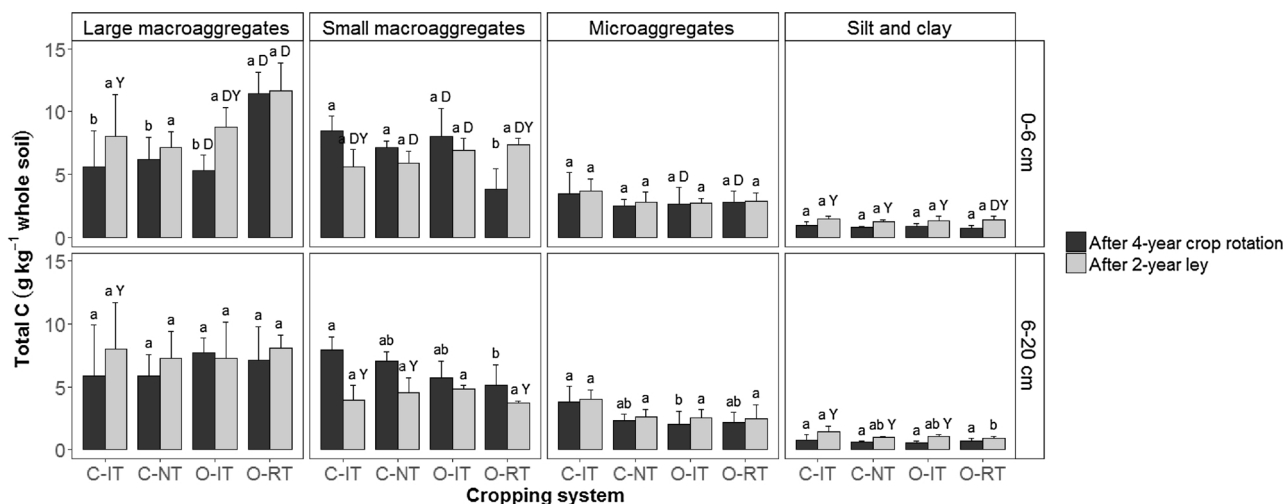


Fig. 3. Total C contribution to whole soil per aggregate fraction. Error bars represent standard deviation of the arithmetic mean. Different letters represent significant ( $p < 0.05$ ) differences between cropping systems within year, depth and fraction. Significant ( $p < 0.05$ ) differences between depths within cropping system and year are noted with “D”, while “Y” marks significant ( $p < 0.05$ ) differences between years, within cropping system, depth and fraction.

avored higher abundance of LM as measured in our study.

Higher contribution of LM to TC in whole soil of O-RT may reflect on longer-term SOC stabilization (von Lützw et al., 2006; Six et al., 2000). Beyond C and N, other dynamics such as organic phosphorus storage in LM may have been enhanced (Garland et al., 2017). Differently than Mikha and Rice (2004) who found an increase in aggregate TC and TN due to no tillage and manure application at 0–5 cm depth after 10 years of continuous corn in a Kennebec silt loam, in our study macroaggregates did not increase TC and TN under no tillage and organic crop management. The latter indicates no crop management or tillage effect in fraction TC enrichment in our study. The increase after ley in the amount of TC contributed by LM in whole soil for C-IT and O-IT was caused by an increase in LM abundance rather than an enrichment in LM-associated TC; again indicating that there was no macroaggregate enrichment of TC. Reallocation of TC within fractions can happen without significant changes in whole soil TC, such as those demonstrated by Sheehy et al. (2015) in a longer-term (9–11 years) study of no tillage, reduced tillage and conventional tillage in four boreal soils.

Balesdent et al. (2000), who summarized TC and TN for no tillage and conventionally tilled soil in a number of studies under different

Table 3

Amount of TC, TN and C:N in macroaggregate-occluded microaggregates (M-mi) and macroaggregate-occluded silt and clay (M-S + C) after a 4-year crop rotation at 0–6 cm, weighted per total proportion in macroaggregates.

Cropping system	Total C (g kg <sup>-1</sup> -1macros)	M-mi <sup>a</sup> Total N (g kg <sup>-1</sup> -1macros)	C:N	Total C (g kg <sup>-1</sup> macros)	M-S + C <sup>b</sup> Total N (g kg <sup>-1</sup> macros)	C:N
	C-IT	12.1 a	1.2 a	10.1 a	18.8 b	2.5 b
C-NT	11.1 a	1.2 a	9.1 ab	18.8 b	2.2 b	8.5 ab
O-IT	9.1 a	1.1 a	8.6 ab	16.5 b	2.3 b	7.2 ab
O-RT	11.5 a	1.2 a	9.8 ab	19.4 b	2.3 b	8.4 ab

Different letters represent significant differences between least-squares means ( $p < 0.05$ ) of cropping systems within fraction.

<sup>a</sup> Microaggregates within macroaggregates (pooled amount of large and small macroaggregates in similar proportions as present in whole soil).

<sup>b</sup> Silt and clay within macroaggregates (pooled amount of large and small macroaggregates in similar proportions as present in whole soil).

crop management histories, reported generally higher C levels under no tillage compared to conventional tillage. Similarly, in a meta-analysis Luo et al. (2010) found greater C stocks under no tillage compared to

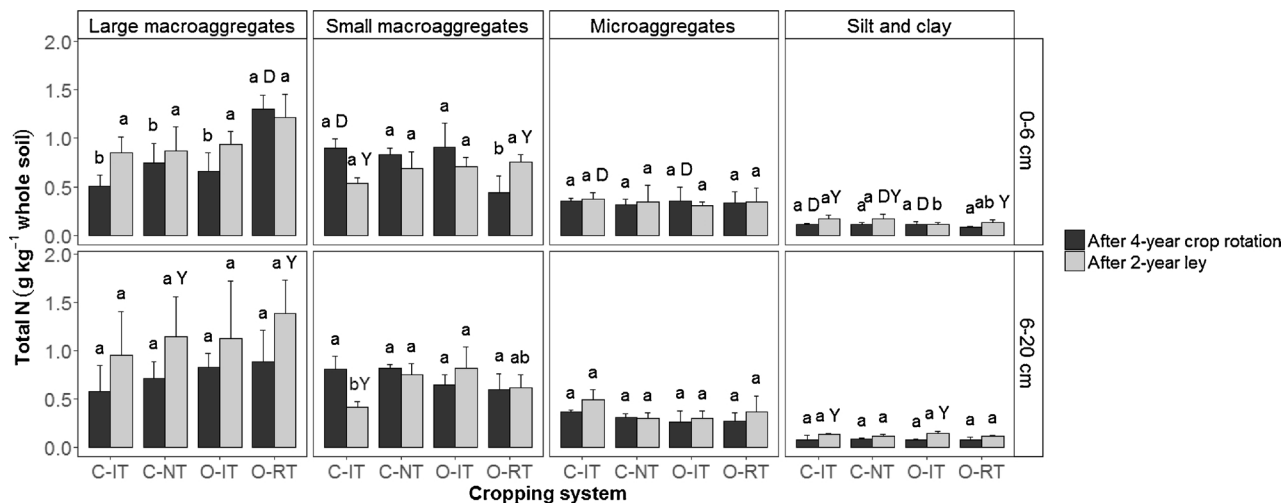


Fig. 4. Total N contribution to whole soil per aggregate fraction. Error bars represent standard deviation of the arithmetic mean. Different letters represent significant ( $p < 0.05$ ) differences between least-squares means of cropping systems tested within year, depth and fraction. Significant ( $p < 0.05$ ) differences of a cropping system between depth, within year and fraction are noted with “D”, while “Y” marks significant ( $p < 0.05$ ) differences between years, within depth and fraction.

conventional tillage. These and other similar reports of C accumulation in conservation tillage compared to conventional tillage, e.g. in the U.S. (Johnson et al., 2005; West and Marland, 2008), continental and maritime Mediterranean climates (Aguilera et al., 2013; González-Sánchez et al., 2012), tropical and temperate climates (Ogle et al., 2005; Six et al., 2004b; VandenBygaert et al., 2003), have led to the general belief that transitioning from conventional tillage to no tillage can lead to increased sequestered C. Consequently, the additional C sequestered in soil has been thought to contribute to mitigating climate change (Lal, 2010; Paustian et al., 1997). In contrast, we found no significant cropping system differences in whole soil TC values after the crop rotation although O-RT, and surprisingly C-IT, showed higher TC in 0–6 cm depth than in 6–20 cm depth. C sequestration rates can peak in 5 to 10 years (West and Post, 2002), therefore it is possible that at 4 years experiment start, the full potential of C sequestration had not been realized. However, any gains should be viewed cautiously, considering Powlson et al. (2014), who make a case that the apparent increases of organic carbon in soil under no tillage largely result from an altered depth distribution compared to conventional tillage, such that the quantity of additional carbon is relatively small.

TC depth stratification such as that found for O-RT, C-IT and O-IT has been reported before. Increased TC in the top 10 cm depth after conversion from conventional tillage to no tillage, coupled with decreased TC at 20–40 cm depth was reported by Luo et al. (2010) in an analysis of 69 paired conventional and no tillage experiments. Reduced soil disturbance together with organic matter inputs can lead to the stratification of soil C, which has been suggested as an indicator of soil quality (de Moraes Sá and Lal, 2009; Franzluebbers, 2002; Kay and VandenBygaert, 2002).

Compared to conventional mineral fertilizers, organic fertilizers such as the cattle slurry used in this study, include an array of C sources which can enhance microbe abundance and activity in organic farming (Lori et al., 2017). In a meta-analysis assessing 74 studies from pairwise comparisons of organic and conventional crop management systems of temperate zones, Gattinger et al. (2012) reported higher SOC concentrations in organic compared to conventional crop management. Observations from other studies have shown C gain in organically fertilized systems after conservation tillage. For example, Crittenden et al. (2015) reported a stronger tillage effect: after four years of crop rotations SOC was higher in reduced tillage compared to conventional tillage across both organic and conventional fertilization in a calcareous marine clay loam. Bottinelli et al. (2017) and Whalen et al. (2003) found aggregate stability related to TC. This increase may be explained by higher C stabilization in larger aggregates by physical protection and occlusion in microaggregates (Six et al., 2004a; von Lütow et al., 2006). However, we found higher TC under organic crop management compared to conventional crop management at 0–6 cm depth only after the ley period. Therefore, for our conditions, the combined effect of additional plant root deposition and no disturbance during the ley, with added C input from the organic fertilizer lead to increased TC.

A review on the short-term impacts of tillage found a 1–11 % of soil C loss after even one tillage event at depths of less than 30 cm. Losses were greater in the surface soil while less or not at all in the deeper profile (Conant et al., 2007). However, these losses may be ameliorated in the long-term. To avoid C losses, a one-time strategic tillage (every 10 or more years) has been suggested for homogenizing C stratification after no tillage without increased loss of labile SOC (Quincke et al., 2007). In loamy sandy soils of northern Germany, Linsler et al. (2013) demonstrated that occasional tillage of grassland had only short-term effects on C stocks. Although a single tillage event decreased C stocks and aggregate stability especially at 0–10 cm soil depth, the differences were no longer significant five years later (Linsler et al., 2013). Likewise, Kettler et al. (2000) reported that five years after a single tillage event for controlling a grass weed in winter wheat-fallow system of a silt loam soil under no tillage for over 20 years resulted in a decline in soil organic C at 0–7.5 cm depth, but increase at 7.5–15 cm depth.

Future studies on the impact of re-introducing intensive tillage after a ley period will clarify whether C content and aggregate stability is maintained long-term.

## 5. Conclusions

Organic crop management and reduced tillage significantly improved soil structure at 0–6 cm depth within a 4-year period of arable cropping only when applied together. Combining organic crop management with reduced tillage can be considered when aiming at improving soil structure.

Integrating a grass-clover ley into a crop rotation increased soil aggregate stability for C-IT and O-IT, suggesting short-term ley as a strategy to improve soil structure for cropping systems under intensive tillage. The ley also increased TC accumulation for O-IT and O-RT at 0–6 cm depth, showing that the use of organic crop management had a larger effect on short-term C-accrual than reduced tillage or no tillage. This highlights the potential of organic crop management for C accumulation.

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