Fertilizer quality and labile soil organic matter fractions are vital for organic carbon sequestration in temperate arable soils within a long-term trial in Switzerland

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ABSTRACT

Agricultural management of soils has led to severe losses of soil organic matter (SOM), accompanied by an increased release of CO_2 into the atmosphere and a reduction of soil fertility. Especially under the aspect of global warming and the increasing demand for food, there is a need for sustainable management options increasing soil organic carbon (SOC) storage in agricultural soils, but knowledge gaps exist regarding C persistence in, and its transfer between functional SOC pools, within different farming systems. Here we report on impacts of different farming systems on the temporal dynamics of SOM fractions within the DOK long-term trial (Switzerland), from 1982 to 2017. A purely minerally (CONMIN), a minerally and organically (CONFYM), and a purely organically fertilized farming system (BIODYN) were compared with an unfertilized control (NOFERT). We separated archived soils from the Haplic Luvisol (0–20 cm depth) into particulate (POM) and mineral-associated OM (MAOM) fractions, via physical fractionation, and analyzed the chemical composition of selected fractions via solid-state ^{13}C CPMAS-NMR spectroscopy. We demonstrate that under none of the analyzed farming systems, additional SOC was sequestered in the clay-sized MAOM fraction (<6.3 μm) over a period of 36 years. In all fertilized systems, the amount of SOC in this pool did not change, but strongly decreased in NOFERT (-27%). Bulk SOC increased in BIODYN (+13%) and CONFYM (+5%), but decreased in CONMIN (-8%) and NOFERT (-20%). As no additional SOC accumulated in the clay-sized MAOM fraction, this implies that bulk SOC increases were solely stored within labile POM fractions. NMR spectra showed comparable POM chemical compositions between different systems. Differences in fertilizer quality (BIODYN = composted farmyard manure vs CONFYM = stacked farmyard manure + mineral fertilizer) and the omission of pesticides resulted in better conditions for POM stabilization and consequently significantly higher C contents of occluded POM (oPOM) within aggregates, in BIODYN. However, this labile fraction is at high risk of being lost within a few days, as illustrated by the strong annual oPOM-C content fluctuations depending on the timing of soil sampling after harvest. The highest post-harvest oPOM-C losses in BIODYN indicate the higher dynamics compared to CONFYM. It is anticipated that only sustainable fertilization methods with continuous application of solely organic fertilizers in the long-run can maintain SOC in the labile POM fractions at elevated levels, thereby ensuring soil fertility. It also illustrates the need for prevention of major losses by careful management of the labile POM fractions, as this OM could associate with fine mineral particles at a later stage and thus contribute to OC sequestration in the stable SOC pool. Overall, the potential of arable soils to accumulate stable OC for long-term sequestration is questioned.

1. Introduction

The potential of agricultural soils to serve as a long-term carbon (C) sink is currently subject of intense debate at political and economic levels (Minasny et al., 2017; Rumpel et al., 2020) as a measure to take action against climate change and counteract soil degradation. However, land-use change to and intensive use of arable land has led to considerable depletion of soil organic matter (SOM), which plays a key

role in soil structure formation, plant nutrition and soil fertility (Büne-mann et al., 2018; Dignac et al., 2017).

Soils act as C sink when organic C inputs are bigger than the losses by decomposition. Sequestration of such added C is achieved when it is stabilized and thus stored in the soil for a long time. "Climate-smart" agricultural management not only offers the opportunity to sequester atmospheric CO_2, it helps fostering key soil functions and therefore improving soil health (Lehmann et al., 2020; Paustian et al., 2016; Smith, 2012; Smith, 2016). A variety of management options has been established aiming to enhance C inputs to agricultural soils. Organic matter (OM) input via manure (Gross and Glaser, 2021), improved crop rotations with legumes (Kumar et al., 2018), cover crops (McDaniel et al., 2014; Poeplau and Don, 2015), biochar application (Smith, 2016), agroforestry (De Stefano and Jacobson, 2018) and organic farming with grass-clover in the rotation (Jarvis et al., 2017) are prominent examples.

In the soil, any OM is subject to decomposition and subsequent mineralization, stabilization through physical protection in aggregates or association with mineral soil particles (Kleber et al., 2015; Schmidt et al., 2011; Wiesmeier et al., 2019). During decomposition, its chemical composition is altered. Therefore SOM quality spans all levels of decomposition from fresh, undecomposed and structurally complex plant litter to highly processed organic molecules (Kögel-Knabner, 2002). These forms are characterized by variable turnover rates (Christensen, 2001; Kölbl and Kögel-Knabner, 2004).

Particulate OM (POM) and mineral-associated OM (MAOM) are generally accepted as important functional SOM fractions, that enable the prediction of SOM dynamics (Lavallee et al., 2020). POM is mainly of plant origin, with wide C to N ratios (Gregorich et al., 2006) and predominantly consists of OM in early stages of decomposition. It can be further divided into free POM (fPOM) outside of aggregates (i.e. unprotected) and occluded POM (oPOM), the latter being physically protected from decomposition in aggregates (Six et al., 2002). C inputs, e.g. via organic fertilizers, are essential for the accumulation of OM, which positively influences soil aggregation and aggregate stability (Karami et al., 2012; Kong et al., 2005), with POM and microbially secreted substances serving as major binding agents (Costa et al., 2018; Golchin et al., 1994; Oades and Waters, 1991). All POM forms represent a dynamic, labile SOM pool that is distinctly affected by agricultural management practices (Poeplau and Don, 2013). The stability of oPOM varies widely with turnover times from years to decades depending on aggregate turnover (Lavallee et al., 2020; Liao et al., 2006). Large aggregates have a shorter turnover time, contain younger and less decomposed SOM (Puget et al., 2000), whereas smaller aggregates are more stable and contain older and more processed SOM (Six et al., 2000; Steffens et al., 2011).

In contrast, MAOM is further decomposed, microbially transformed and stabilized through sorption onto surfaces of fine-sized mineral particles (Basile-Doelsch et al., 2015; Cotrufo et al., 2019; Kleber et al., 2015; Newcomb et al., 2017). This stabilization mechanism increases SOM turnover rates to ranges of decades up to centuries (Kleber et al., 2015; Kögel-Knabner et al., 2008). Thus, MAOM represents a stable SOM pool, enriched with chemical components of microbial origin and low C to N ratios (Courtier-Murias et al., 2013). The extent of microbially transformed SOM from OM inputs is largely constrained by stoichiometric imbalances between OM inputs and microbial communities, i.e. elemental plant resource composition and nutrient requirements of microbial biomass (Mooshammer et al., 2014). The input of supplementary nutrients (e.g. via mineral fertilizers) might alleviate these constraints and increase the rate to which OM inputs are transferred into the stable SOM pool (Kirkby et al., 2016; Kirkby et al., 2014).

However, Steffens et al. (2009) claimed that this fraction, which is considered stable, can be mobilized and mineralized when land management and climatic conditions are changing. With advancing global warming, substantial losses of SOM from agricultural soils are projected (Wiesmeier et al., 2016), posing a challenge to efforts to increase or even maintain current soil C stocks through C inputs (Riggers et al., 2021).

Given these premises, it is essential to understand how long it takes until C inputs to arable land are transferred to different functional SOM pools and for how long the sequestered C is stabilized (Schmidt et al., 2011; Smith, 2005).

The objective of this study was to investigate the temporal dynamics of quantity and quality of SOM fractions in different farming systems in the DOK long-term experiment (Therwil, CH) (Mäder et al., 2002). Our research focused on four farming systems: two conventional systems, one with the exclusive use of mineral fertilizer (CONMIN), and another with the use of mineral fertilizer and farmyard manure (CONFYM), an organic farming system with only organic manure (BIODYN) and an unfertilized control (NOFERT).

There is an extensive body of literature covering different perspectives of farming system impacts on soil properties in the DOK experiment. However, only two studies analyzed C dynamics (Flieβbach et al., 2007; Leifeld et al., 2009), none of which considered the stable clay-sized MAOM fraction for testing of climate-friendly soil management. We hypothesized, that the POM fractions would reflect the continuous OM inputs, and thus farming systems receiving organic fertilizers (CONFYM and BIODYN) would accumulate significantly more labile POM than systems without organic fertilization (NOFERT and CONMIN). For the MAOM fraction, we hypothesized a steady decline towards a new steady-state equilibrium at a lower level, where we expected the dynamics of C incorporation into this fraction to differ between the analyzed systems and to be most efficient in BIODYN and in CONFYM due to the higher microbial activity in these plots. We aimed to answer, if farming systems differing in form and amount of added fertilizers have measurable effects on functional SOM fractions, and if recommendations for climate-friendly soil management can be drawn from long-term experiments.

2. Materials and methods

2.1. Experimental setup and sampling

The DOK field experiment is a long-term farming system comparison trial, located in Therwil, Canton of Basel Landschaft, Switzerland (7°32′ E, 47°30′ N), which started in 1978. The soil type is a Haplic Luvisol (WRB, 2015), developed on deep deposits of alluvial loess. The mean annual temperature is 10.5 °C (1.5 °C increase since 1978) with a mean annual precipitation of 840 mm (Krause et al., 2020). In the DOK trial, two organic and two conventional farming systems are compared with an unfertilized control (Mäder et al., 2002). They have the same crop rotation within seven-year crop rotation periods (CRP) with two years of grass-clover ley (*Supplementary Table S1*), but receive different types of fertilizers. Each farming system is replicated four times (columns), and the crop rotation is running temporally shifted in three subplots. This sums up to 96 parcels (5 × 20 m each), arranged in a randomized spilt-split-block design. All farming systems have the same type and frequency of tillage but receive different types of plant protection (Table 1).

The focus of this study was on four systems, which represent an input gradient of fertilizers in terms of quantity and quality of OM input (Table 1). NOFERT (NO FERTilization) is a control treatment that has received no fertilizers since 1978. CONMIN (CONventionally managed and MINeral fertilizer) receives only mineral fertilizer and was left unfertilized during the first CRP (1978–1984). CONFYM (CONventionally managed, mineral fertilizer and FarmYard Manure) is an integrated conventional system, that combines the use of mineral and organic fertilizers, and BIODYN (BIODYNamically managed) receives only organic fertilizers (Table 1), and is managed according to principles of biodynamic farming. Straw biomass was removed from the plots after harvesting. In CONFYM and BIODYN, manure amendment corresponds to 1.4 livestock units per hectare and year (Table 1). The amount of raw manure prior to treatment-specific processing, defines the amount of OM inputs via organic fertilizers in CONFYM and BIODYN. Aerobic composting of BIODYN manure results in higher C losses via microbial

Table 1

Overview of the four farming systems, their respective types of fertilization, pest and weed control, and average annual organic matter (OM) inputs via organic fertilizers between 1982 and 2017 in the analyzed subplot in the DOK system comparison trial. Modified from Krause et al. (2020). LU = livestock units fertilization equivalents. 1 LU approximately corresponds to 105 kg N, 15 kg P and 149 kg K (Richner and Sinaj, 2017).

farming system		LU [ha^{-1} yr^{-1}]	fertilizer	OM inputs via manure and slurry (1982–2017) [kg ha^{-1} yr^{-1}]	weed control	disease control	pest control	special treatments
NOFERT	control	–	–	–	mechanical	indirect	biocontrol	–
CONMIN	conventional	–	mineral N, P, K	–	mechanical, herbicides	fungicides (thresholds)	insecticides (thresholds)	plant growth regulators
CONFYM	conventional	1.4	mineral N, P, K, stacked manure and slurry	2508	mechanical, herbicides	fungicides (thresholds)	insecticides (thresholds)	plant growth regulators
BIODYN	organic	1.4	biodynamic composted manure and slurry	2000	mechanical	indirect	plant extracts, biocontrol	biodynamic preparations

decomposition, compared to manure stacking in CONFYM, manifested by 20% lower OM inputs in BIODYN (Table 1). Qualitatively, BIODYN compost is relatively enriched with more recalcitrant OM due to the higher losses of labile C compounds (Fließbach and Mäder, 2000). Slurry is added to supply nutrients according to plant needs, mainly N, of the given crop. Mineral nutrient inputs in CONMIN and CONFYM are given up to the limits of Swiss fertilization recommendations (Richner and Sinaj, 2017). Mineral fertilizers were added on top of manure in CONFYM to reach the recommended N level, and as N inputs from farmyard manure are not fully accounted for as readily bioavailable, CONFYM received higher N inputs than CONMIN (Table 1 & *Supplementary Table S2*).

Overall, we analyzed archived soil samples from 16 plots (four farming systems with four replicates each) taken every fifth year (1982, 1989, 1996, 2003, 2010 and 2017) within each of the six completed seven-year CRP (CRP 1 = 1978–1984, CRP 2 = 1985–1991, CRP 3 = 1992–1998, CRP 4 = 1999–2005, CRP 5 = 2006–2012, CRP 6 = 2013–2019) (*Supplementary Table S1*). This resulted in 96 bulk soil samples in total, each representing a composite of 15–20 randomly distributed samples, taken after harvest with a soil corer (0–20 cm depth, 3 cm diameter) in the inner part of the plots, leaving out border zones. Samples were transported to the lab, air-dried at 40 °C, until no further moisture loss was observed and subsequently sieved to 2 mm.

2.2. Soil characterization

We analyzed all 16 plots for their basic soil characteristics, to provide an assessment of the current state of the studied soils. Therefore, bulk SOC and total N were measured on the most recent archived samples from 2017, while texture and mineralogy were analyzed using the most recent soil samples from the latest DOK sampling campaign in 2019 (Table 2).

2.2.1. Bulk SOC and total N
Bulk SOC and total N content were determined in duplicate via dry combustion on a Vario EL cube elemental analyzer (Elementar Analysensysteme, Hanau, Germany). All bulk samples were free of carbonates so that the total C concentration equals the organic C (OC) concentration.

2.2.2. Texture
The texture of bulk soil samples was determined with the PARIO Plus Soil Particle Analyzer (METER Group, Germany/USA). Prior to measurement, H_2O_2 (30%) was added to 25–30 g of soil sample and heated in a water bath for OM oxidation (12 h). H_2O_2 was removed from the sample through subsequent repeated addition of distilled water, centrifuging and decanting. We repeatedly washed the samples until an electrical conductivity of <400 μS m^{-1} was reached, to ensure the absence of soluble salts. Sodium hexametaphosphate ($Na_6O_{18}P_6$) was used for sample dispersion.

2.2.3. Mineralogy
Mineralogy of the bulk soil and the clay fraction (<2 μm and <6.3 μm) was determined using powder X-ray diffraction (P-XRD, Cubix[3], Malvern Panalytical, Almelo, Netherlands). Prior to P-XRD analyses, the samples were treated with hydrogen chloride (HCl) to remove OM. P-XRD was performed on random powder samples and on oriented samples after saturation with Ca^{2+} and ethylene glycol (room temperature) and K$^+$ (room temperature and stepwise heated to 550 °C).

2.3. Physical fractionation

Following a modified fractionation scheme of Kölbl and Kögel-Knabner (2004) and Steffens et al. (2009), we separated POM and MAOM fractions from all 96 bulk soil samples (Fig. 1). Briefly, 250 ml of Na-polytungstate solution (ρ = 1.8 g cm^{-3}; TC-Tungsten Compounds, Grub am Forst, Germany) was added to 30 g of air-dried bulk soil (<2 mm) for capillary saturation and left settling overnight. The floating fPOM was aspirated with a vacuum pump. Using ultrasound for aggregate disruption, subsequent centrifugation and aspiration, the further decomposed POM, receiving physical protection through occlusion (oPOM) within macro-aggregates of the heavy fraction (>1.8 g cm^{-3}) was separated.

Prior, we tested a range of ultrasonic energies (50–450 J ml^{-1}) for optimal aggregate disruption, adjusted to the texture and C content of the samples (Griepentrog and Schmidt, 2013). 200 J ml^{-1} released the most oPOM-C and therefore disrupted macro-aggregates most efficiently, minimizing redistribution of POM into finer particle-size fractions (Oorts et al., 2005). The ultrasonic homogenizer (Sonopuls HD 2200.0, Bandelin, Berlin, Germany) operated at a constant output of 75 W. A calorimetric calibration (North, 1976) of the ultrasonic device was regularly conducted, due to considerable deviations between nominal and actual energy outputs of ultrasonifiers (Schmidt et al., 1999), caused by deterioration of the probe tip (Amelung and Zech, 1999; Mentler et al., 2017). The probe tip of the sonotrode (VS 70 T, 13 mm diameter, Bandelin, Berlin, Germany) was immersed 25 mm into the solution for complete turbation of the sample. We regulated the temperature of the solution (<30 °C) to prevent alteration of SOM quality. The obtained POM fractions were washed (<10 μS cm^{-1}) over a 20 μm sieve, freeze-dried, weighed and ground for further analyses. The remaining heavy fraction (>1.8 g cm^{-3}) was washed (<50 μS cm^{-1}) via centrifugation (15 min, 7000 rpm) and wet-sieved to separate the sand (2000–63 μm) and the coarse silt fraction (63–20 μm).

Prior to further fractionation by sedimentation in Atterberg-cylinders, we conducted tests to choose between a particle size cut-off at <2 μm or <6.3 μm. Both fractions were separated from four replicates of one NOFERT and of one BIODYN plot, and ^{14}C age was determined (Mini Carbon Dating System, MICADAS, ETH Zürich, Switzerland). We found no significant differences between ^{14}C ages of the two fractions in neither of the two farming systems (data not shown). Thus we used a particle size cut-off at <6.3 μm, because of the

Table 2

Bulk soil characteristics (<2000 μm, 0–20 cm depth) of all 16 observed plots. Texture and mineralogy were analyzed with soil samples from 2019. C and N analyses were conducted with soil samples from 2017. Arithmetic means of farming systems are depicted in bold with standard deviations. Significant differences between systems at p <0.05 are indicated by post-hoc Tukey letters.

| farming system	plot	Texture			Main mineralogy					Clay mineralogy				OC	N$_{tot}$	C/N
		Sand (2000–63 μm)	Silt (63–2 μm)	Clay (<2 μm)	Quartz	Feldspars	Fe oxides / hydroxides	Carbonates	Phyllosilicates	Illite	Smectite	Chlorite	Kaolinite			
		[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[mg g^{-1}]	[mg g^{-1}]	
NOFERT	3	3.0	77.6	19.5	58.4	17.7	2.0	0.4	21.6	11.7	4.6	4.3	1.0	12.8	1.5	8.5
	37	3.1	81.5	15.5	61.1	18.8	1.5	0.7	18.0	10.9	2.7	3.5	0.9	10.1	1.2	8.3
	59	3.0	75.8	21.1	59.5	19.4	1.6	1.0	18.6	11.3	2.7	4.5	0.1	10.2	1.2	8.3
	93	1.8	73.5	24.8	56.8	18.1	2.1	1.3	21.7	8.6	7.3	2.7	3.0	12.2	1.5	8.3
	x̄	**2.7 ± 0.6**	**77.1 ± 3.4**	**20.2 ± 3.9**	**59.0 ± 1.8**	**18.5 ± 0.8**	**1.8 ± 0.3**	**0.9 ± 0.4**	**20.0 ± 2.0**	**10.6 ± 1.4**	**4.3 ± 2.2**	**3.8 ± 0.8**	**1.3 ± 1.2**	**11.3 ± 1.4**	**1.4 ± 0.2**	**8.4 ± 0.1**
		a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
CONMIN	4	2.9	75.2	21.9	60.1	16.8	1.7	0.6	20.8	12.9	3.1	2.9	1.9	14.4	1.6	8.8
	38	3.2	76.2	20.7	58.3	20.4	1.7	0.2	19.4	10.9	4.2	2.0	2.3	12.0	1.4	8.7
	60	2.7	77.4	19.9	62.5	19.3	1.8	0.7	15.7	8.0	4.1	1.8	1.9	12.5	1.5	8.6
	94	2.3	73.5	24.3	53.0	17.7	2.4	0.6	26.3	16.9	3.9	4.3	1.3	15.3	1.8	8.6
	x̄	**2.8 ± 0.4**	**75.6 ± 1.7**	**21.7 ± 1.9**	**58.5 ± 4.0**	**18.6 ± 1.6**	**1.9 ± 0.3**	**0.5 ± 0.2**	**20.6 ± 4.4**	**12.2 ± 3.7**	**3.8 ± 0.5**	**2.8 ± 1.1**	**1.9 ± 0.4**	**13.6 ± 1.6**	**1.6 ± 0.2**	**8.7 ± 0.1**
		a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
CONFYM	22	2.5	76.1	21.4	59.5	21.2	1.3	0.6	17.5	11.4	2.5	3.2	0.4	13.8	1.6	8.5
	32	3.1	80.4	16.5	58.2	18.6	1.6	0.6	21.0	14.7	2.6	3.1	0.6	16.3	1.8	8.9
	66	2.4	75.2	22.4	59.6	17.6	1.7	0.7	20.4	14.1	2.4	3.7	0.3	14.5	1.7	8.6
	76	3.4	75.4	21.2	56.2	20.1	1.7	0.6	21.5	14.4	2.6	3.4	1.1	13.9	1.6	8.8
	x̄	**2.9 ± 0.5**	**76.8 ± 2.4**	**20.4 ± 2.6**	**58.4 ± 1.6**	**19.4 ± 1.6**	**1.6 ± 0.2**	**0.6 ± 0.1**	**20.1 ± 1.8**	**13.7 ± 1.5**	**2.5 ± 0.1**	**3.4 ± 0.3**	**0.6 ± 0.4**	**14.6 ± 1.2**	**1.7 ± 0.1**	**8.7 ± 0.2**
		a	a	a	a	a	a	a	a	a	a	a	a	ab	ab	b
BIODYN	10	3.4	74.9	21.7	56.3	16.7	1.9	0.5	24.7	12.1	7.6	2.4	2.6	18.5	2.0	9.1
	44	2.2	76.2	21.7	59.5	20.7	1.6	0.7	17.5	11.5	2.1	2.0	1.9	17.0	1.9	9.0
	54	3.8	74.9	21.3	63.2	17.5	1.7	0.7	16.9	11.5	1.8	2.2	1.4	15.0	1.8	8.7
	88	1.5	69.2	29.3	49.7	16.3	1.6	0.9	31.5	21.0	4.2	2.6	3.7	21.0	2.4	8.7
	x̄	**2.7 ± 1.1**	**73.8 ± 3.1**	**23.5 ± 3.9**	**57.2 ± 5.7**	**17.8 ± 2.0**	**1.7 ± 0.1**	**0.7 ± 0.2**	**22.7 ± 6.9**	**14.0 ± 4.7**	**3.9 ± 2.7**	**2.3 ± 0.3**	**2.4 ± 1.0**	**17.9 ± 2.5**	**2.0 ± 0.3**	**8.9 ± 0.2**
		a	a	a	a	a	a	a	a	a	a	a	a	b	b	b
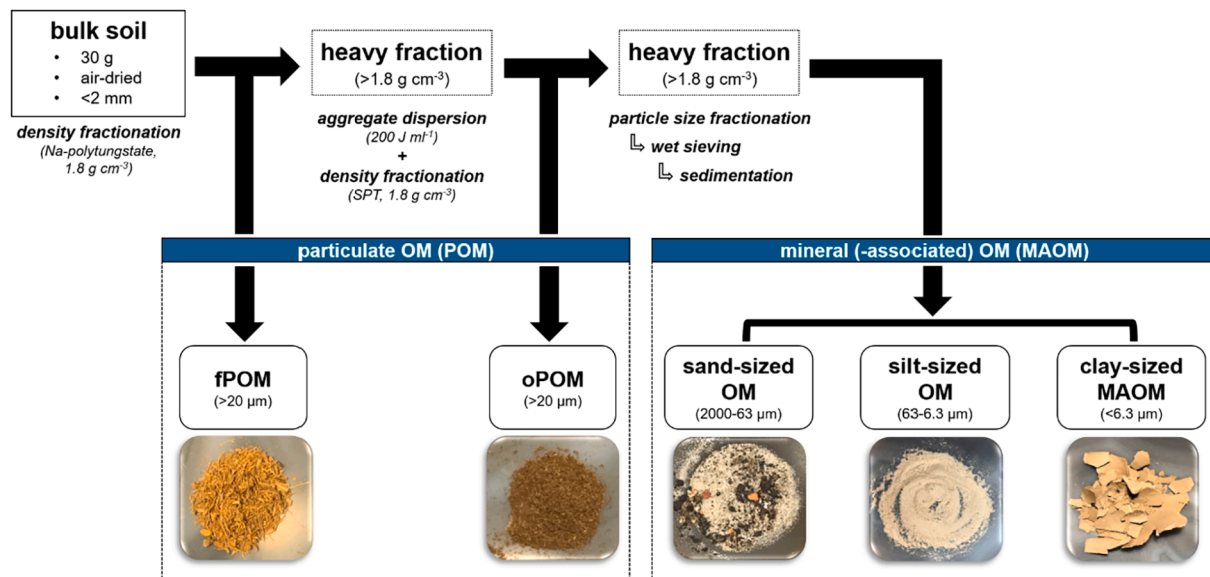
Fig. 1. Physical fractionation scheme and conducted measurements. Modified from Kölbl and Kögel-Knabner (2004) and Steffens et al. (2009). The coarse silt (63–20 μm) and medium silt fraction (20–6.3 μm) were put together and referred to as silt-sized OM (63–6.3 μm), and the fine silt + clay fraction is referred to as clay-sized MAOM fraction (<6.3 μm).

considerable increase in material and the reduction of sedimentation time. This fraction is denominated as clay-sized MAOM fraction (<6.3 μm).

Eventually, fPOM (>20 μm), oPOM (>20 μm), sand-sized OM (2000–63 μm), silt-sized OM (63–6.3 μm) and clay-sized MAOM (<6.3 μm) were separated. OM in the sand- and silt-sized fractions is probably not adsorbed to surfaces of these mineral particles, but rather originates as methodological artifact from fractionation procedures (e.g. wet-sieving) and still intact micro-aggregates (after ultrasonication). This is why only the clay-sized fraction (<6.3 μm) was assigned the term 'MAOM'. All obtained fractions were air-dried at 60 °C, weighed and ground for further analyses.

2.4. OC, total N, and calculation of mass and C recovery

We analyzed all archived bulk soil samples (n = 16 plots × 6 years = 96) and obtained SOM fractions from physical fractionation (n = 96 bulk soil samples × 5 fractions = 480) in duplicate for total C and N concentrations by dry combustion on a Vario EL cube elemental analyzer (Elementar Analysensysteme, Hanau, Germany). All bulk samples were free of carbonates so that the total C concentration equals the organic C (OC) concentration. The sum of the weights of all separated and air-dried fractions (n = 5) per bulk soil sample (30 g) gave the corresponding mass recovery. Measured OC concentration of each fraction was applied against the respective fraction weights, and the sum of all five fractions compared to the measured OC concentration of the bulk soil gave the C recovery.

In addition, we calculated the maximum potential C saturation of mineral particles <20 μm according to the empirical formula of (Hassink, 1997) and its adjustments made by (Wiesmeier et al., 2015) to consider texture differences between the analyzed plots:

$$C_{pot} = a + b * particles_{<20\mu m}$$

C_{pot} is the maximum potential C saturation of mineral particles <20 μm [mg g^{-1}], a and b are empirically determined constants ($a_{Hassink} = 4.09$ and $b_{Hassink} = 0.37$; $a_{Wiesmeier} = -0.23$ and $b_{Wiesmeier} = 0.36$). *particles$_{<20}$ $_{\mu m}$* is the concentration of mineral particles <20 μm [%], determined by texture analysis. Further, we calculated the current C contents (C_{cur}) [mg g^{-1}] of this fraction for 1982 and 2017, using its measured OC concentrations and fraction mass proportions determined by texture

analysis. C saturation (C_{sat}) is the percentage of C_{cur} from C_{pot}, and the C saturation deficit (C_{def}) is calculated as $C_{pot} - C_{cur}$.

2.5. Solid-state ^{13}C CPMAS-NMR spectroscopy

Selected fPOM (n = 2–3 plot replicates per farming system), oPOM (n = 2–4 plot replicates per farming system) and clay-sized MAOM fractions (n = 4 NOFERT and 4 BIODYN plot replicates) from 1982 and 2017 were analyzed by solid-state ^{13}C cross-polarization magic-angle spinning nuclear magnetic resonance (CPMAS-NMR) spectroscopy (Bruker DSX 200 NMR spectrometer, Bruker, Karlsruhe, Germany), to determine the OM chemical compositions. Clay-sized MAOM fractions were treated with hydrofluoric acid (HF) prior to measurement, to reduce mineral particle contents and enrich ^{13}C. We conducted measurements in 7 mm zirconium dioxide rotors at a spinning speed of 6800 Hz and a pulse delay time of 0.4 s. A ramped ^{1}H pulse was applied during a contact time of 1 ms, to avoid Hartmann-Hahn mismatches. Depending on the C contents and available sample material, the number of obtained scans ranged between 3600 and 205,000, and a line broadening between 0 and 100 Hz was applied for the spectra. The ^{13}C chemical shifts were referenced to tetramethylsilane (0 ppm). We used the following chemical shift regions for integration: -10 to 45 ppm (alkyl C), 45 to 110 ppm (O/N alkyl C), 110–160 ppm (aromatic C) and 160 to 220 ppm (carbonyl/carboxyl C). The ratio of alkyl C to O/N alkyl C (A/O-A ratio) was used as sensitive indicator for the degree of OM decomposition (Baldock et al., 1997).

2.6. Statistical analyses

We applied a linear mixed effects model with repeated measurements to determine the impact of farming systems on SOM fractions as a function of sampling year, using R version 4.1.2 and RStudio (RStudio Team, 2021). In short, the lme function of the nlme package was used (Pinheiro et al., 2020) with farming system nested in subplot and column as repeated random factors to account for spatial heterogeneity within the experimental design of the DOK trial. A two-way ANOVA was then employed to determine the impact of farming system, sampling year and their interaction on SOC contents. Subsequently, a Tukey HSD test was applied to test for differences between the faming systems, in case of model significance. Differences in bulk soil characteristics and

chemical shift regions of obtained ^{13}C CPMAS-NMR spectra between the farming systems were tested for statistical significance by a one-way ANOVA. Residuals were tested for normal distribution using visual inspection of QQ-plots and the Shapiro-Wilk test, and homogeneity of variances was tested using Levene's test. Subsequently, a post-hoc Tukey HSD test was performed using a significance level of α = 0.05, for all tests.

3. Results

3.1. Bulk soil characteristics

All analyzed plots had a silty texture with 2.8 ± 0.6% sand, 75.8 ± 2.8% silt and 21.4 ± 3.2% clay (Table 2). Mean sand (NOFERT: 2.7 ± 0.6%, CONMIN: 2.8 ± 0.4%, CONFYM: 2.9 ± 0.5% and BIODYN: 2.7 ± 1.1%), silt (NOFERT: 77.1 ± 3.4%, CONMIN: 75.6 ± 1.7%, CONFYM: 76.8 ± 2.4% and BIODYN: 73.8 ± 3.1%) and clay contents (NOFERT: 20.2 ± 3.9%, CONMIN: 21.7 ± 1.9%, CONFYM: 20.4 ± 2.6% and BIO-DYN: 23.5 ± 3.9%) showed comparable values between the plots of the farming systems after harvest in 2019. All 16 plots showed similar mineralogies, with phyllosilicate contents around 20%, dominated by Illite, which was lowest under NOFERT (10.6 ± 1.4%) and highest under BIODYN (14.0 ± 4.7%) (Table 2). Mean SOC contents in 2017 were lowest in NOFERT (11.3 ± 1.4 mg g^{-1}), higher in CONMIN (13.6 ± 1.6 mg g^{-1}) and CONFYM (14.6 ± 1.2 mg g^{-1}), and highest in BIODYN (17.9 ± 2.5 mg g^{-1}) (Table 2), with significant differences to CONMIN and NOFERT (Fig. 2).

3.2. Physical fractionation

3.2.1. Bulk SOC development

Bulk SOC contents in the four different farming systems developed as follows from 1982 to 2017: in NOFERT, it decreased by 2.8 mg g^{-1} (-20%) and in CONMIN by 1.1 mg g^{-1} (-8%). In CONFYM, it slightly increased by 0.7 mg g^{-1} (+5%) and in BIODYN by 2.7 mg g^{-1} (+13%) (Fig. 2).

3.2.2. Distribution and development of C in POM and MAOM fractions

The averaged mass recovery over all farming systems and years after physical fractionation was 94 ± 1%, and SOC recovery averaged 73 ± 4%. Both recovery rates showed similar values between farming systems and years. Based on these recovery rates, on average 8.0% of SOC was
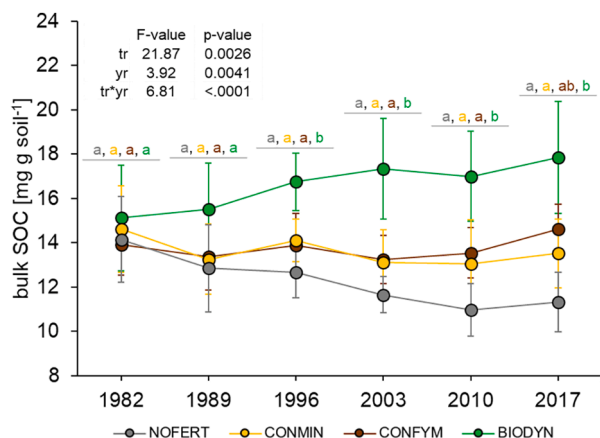

Fig. 2. Development of bulk SOC contents in the DOK trial (0–20 cm depth) under each farming system over a 36-year period (1982–2017). Data shows plot means (n = 4) per treatment and year. Error bars represent standard deviations between the four replicated plots per treatment. Results of a repeated two-way ANOVA (tr = treatment, yr = year) are depicted, using a linear mixed effect model that accounts for the spatial arrangement of plots within the experimental design. Post-hoc Tukey letters give significant differences at p <0.05.

stored in POM fractions (>20 μm) (3.3% fPOM, 4.7% oPOM), 1.8% in the sand-sized OM fraction (2000–63 μm), 20.8% in the silt-sized OM fraction (63–6.3 μm) and 69.4% in the clay-sized MAOM fraction (<6.3 μm). The absolute values of fraction-C contents per farming system for each analyzed year are presented in *Supplementary Table S3*.

C contents in the fPOM fraction were relatively stable over the whole period with minor increases in all treatments (Fig. 3a). Depending on farming system, oPOM-C contents showed major fluctuations. In NOFERT and CONMIN, they were relatively stable from 1982 to 2010. In 2017, oPOM-C increased in these systems by +161% and +87%, respectively, compared to 2010. In CONFYM and BIODYN, the development of oPOM-C contents was characterized by strong fluctuations between the analyzed years (Fig. 3b). From 1982 to 1989, oPOM-C in CONFYM and BIODYN increased by 106% and 145%, followed by a decrease of 66% and 50% from 1989 to 1996, respectively. The same but attenuated pattern was observed from 1996 to 2003, with oPOM-C increases of 56% and 70% and decreases of 58% and 48% from 2003 to 2010, respectively. In 2017, oPOM-C contents recorded their biggest increases of 289% in CONFYM and 184% in BIODYN. We found these oPOM-C fluctuations to strongly correlate with the elapsed time between harvest and sampling (R^2; BIODYN: 0.87; CONFYM: 0.96) each analyzed year, which ranged from 1 to 35 days (Fig. 4).

The sand-sized OM (2000–63 μm) fraction contributed the least to the total SOC content and slight fluctuations between the sampled years are assumed to be artefacts of methodical issues during fractionation, specifically wet-sieving. Therefore, the share of SOC in this fraction is rather negligible. In the silt-sized OM (63–6.3 μm) fraction, SOC contents decreased in all treatments from 1982 to 2017. In NOFERT 24%, in CONMIN 9%, in CONFYM 5% and in BIODYN 5% of SOC were lost (Fig. 5a).

From 1982 to 2017, SOC contents in the clay-sized MAOM (<6.3 μm) fraction decreased in NOFERT (-27%) and CONMIN (-14%), while in CONFYM (-3%) and in BIODYN (±0%) they remained stable (Fig. 5b). Between BIODYN and NOFERT, significant differences in clay-sized MAOM-C contents were detected from 2003 until 2017. CONFYM and CONMIN had similar SOC contents in the clay-sized MAOM fraction throughout the whole observation period and showed no significant differences to either NOFERT or BIODYN.

The potential C saturation (C$_{pot}$) of the fine fraction (<20 μm), across all analyzed systems averaged 25.0 and 20.1 mg g^{-1}, according to the empirical formulas of Hassink (1997) and Wiesmeier et al. (2015) (*Supplementary Table S4*). C saturation (C$_{sat}$) of the fine fraction across all systems averaged 52.1% and 64.7% in 1982, and was lowest in NOFERT (Hassink: 49.4%, Wiesmeier: 61.5%) and highest in BIODYN (Hassink: 55.0%, Wiesmeier: 67.8%) (Fig. 6a). In 2017, C$_{sat}$ decreased in all systems, but remained the same in BIODYN (Hassink: 53.9%, Wiesmeier: 66.6%) (Fig. 6b). Concomitantly, C saturation deficits (C$_{def}$) increased in all systems from 1982 to 2017, but stayed the same in BIODYN (*Supplementary Table S4*).

In addition, the SOC to clay (SOC:clay) ratio, as an indicator for soil structural quality (Johannes et al., 2017), was <1:10 for all studied plots over the observed period from 1982 to 2017 (Fig. 6c & 6d). Averaged over all systems, we observed a linear correlation between 2:1 phyllosilicates (i.e. Illite and Smectite) and C contents in the clay-sized MAOM fraction (<6.3 μm) in all analyzed plots (1982: R^2 = 0.6493, 2017: R^2 = 0.5002) (Fig. 7).

3.2.3. Chemical composition of POM and MAOM fractions

Across all analyzed fractions, systems and years, NMR spectra were dominated by alkyl C and O/N-alkyl C (67.7 ± 2.4%) (*Supplementary Figure S1 & Table 3*). POM fractions showed lower shares of carboxyl C (8.86 ± 0.9%) and alkyl C (15.2 ± 1.9%) and higher shares of O/N-alkyl C (52.5 ± 2.8%) compared to the clay-sized MAOM fractions (carboxyl C: 13.26 ± 0.6%; alkyl C: 25.8 ± 1.0%; O/N-alkyl C: 42.0 ± 2.2%). This was reflected in the alkyl C to O/N-alkyl C ratios (A/O-A ratio; Baldock et al. (1997)), which increased in the order fPOM (0.27) < oPOM (0.31)
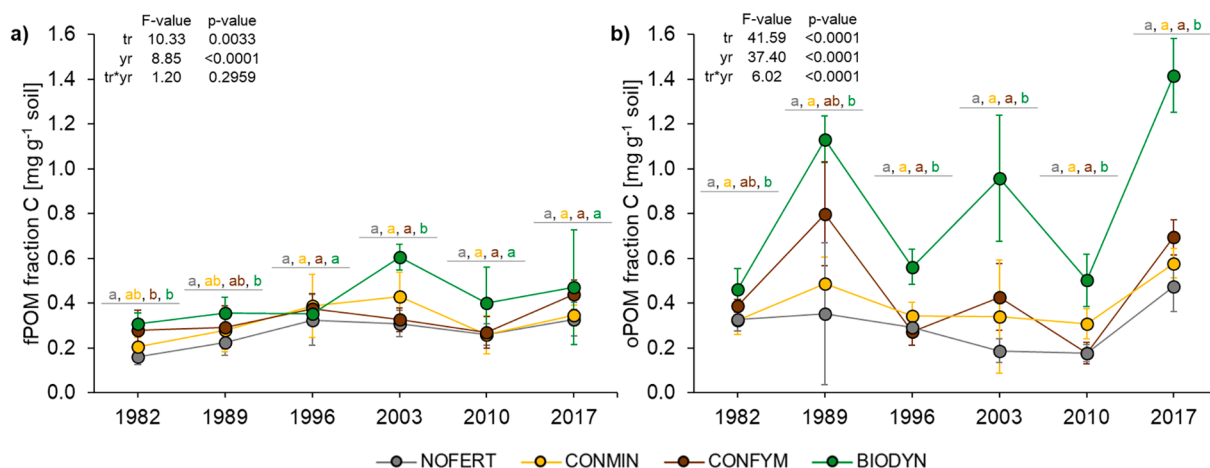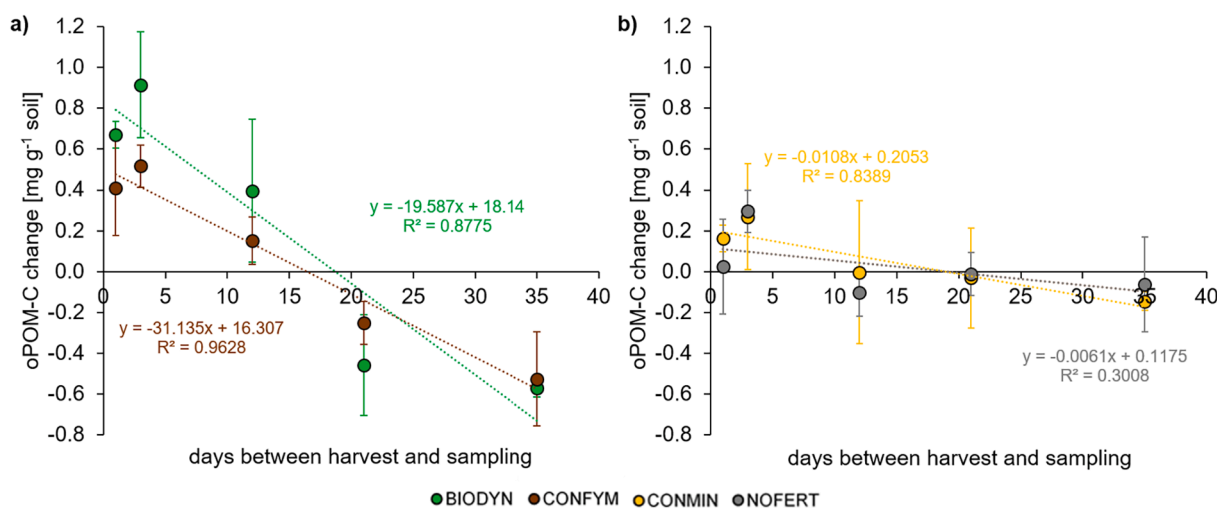
Fig. 3. Development of mean SOC contents in a) the fPOM (>20 μm) and b) the oPOM (>20 μm) fraction from 1982 to 2017 in all farming systems. Error bars represent standard deviations between the replicated plots (n = 4) per treatment. Results of a repeated two-way ANOVA (tr = treatment, yr = year) are depicted, using a linear mixed effect model that accounts for the spatial arrangement of plots within the experimental design. Post-hoc Tukey letters give significant differences at p <0.05.


Fig. 4. Correlation of oPOM-C changes and days between harvest and sampling under a) organically fertilized management (BIODYN and CONFYM) and b) under exclusion of organic fertilizers (CONMIN and NOFERT). Error bars represent standard deviations between the replicated plots (n = 4) per farming system.
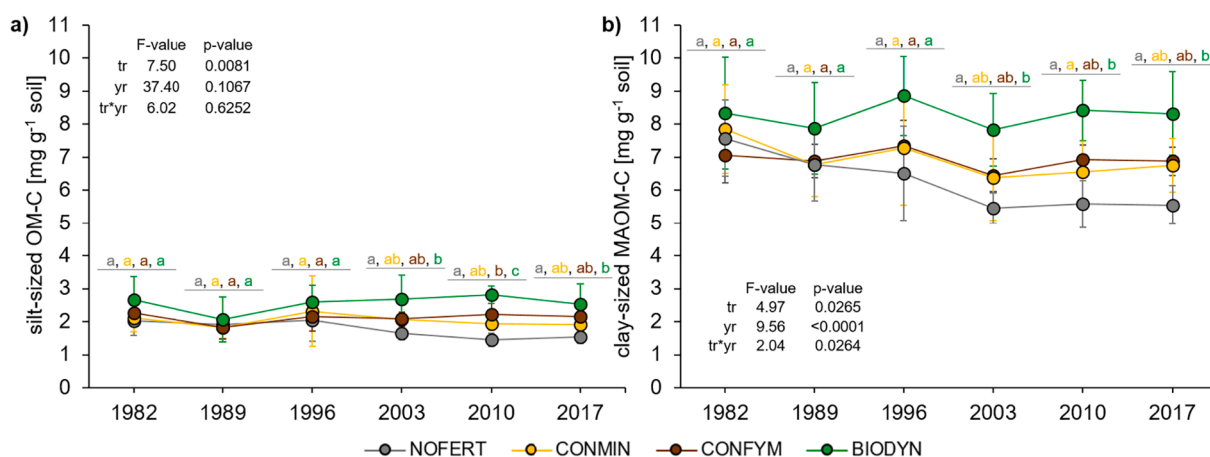
Fig. 5. Development of SOC contents in a) the silt-sized OM fraction (63–6.3 μm) and b) in the clay-sized MAOM fraction (<6.3 μm) from 1982 to 2017. Error bars represent standard deviations between the replicated plots (n = 4) per farming system. Results of a repeated two-way ANOVA (tr = treatment, yr = year) are depicted, using a linear mixed effect model that accounts for the spatial arrangement of plots within the experimental design. Posthoc Tukey letters give significant differences at p <0.05.
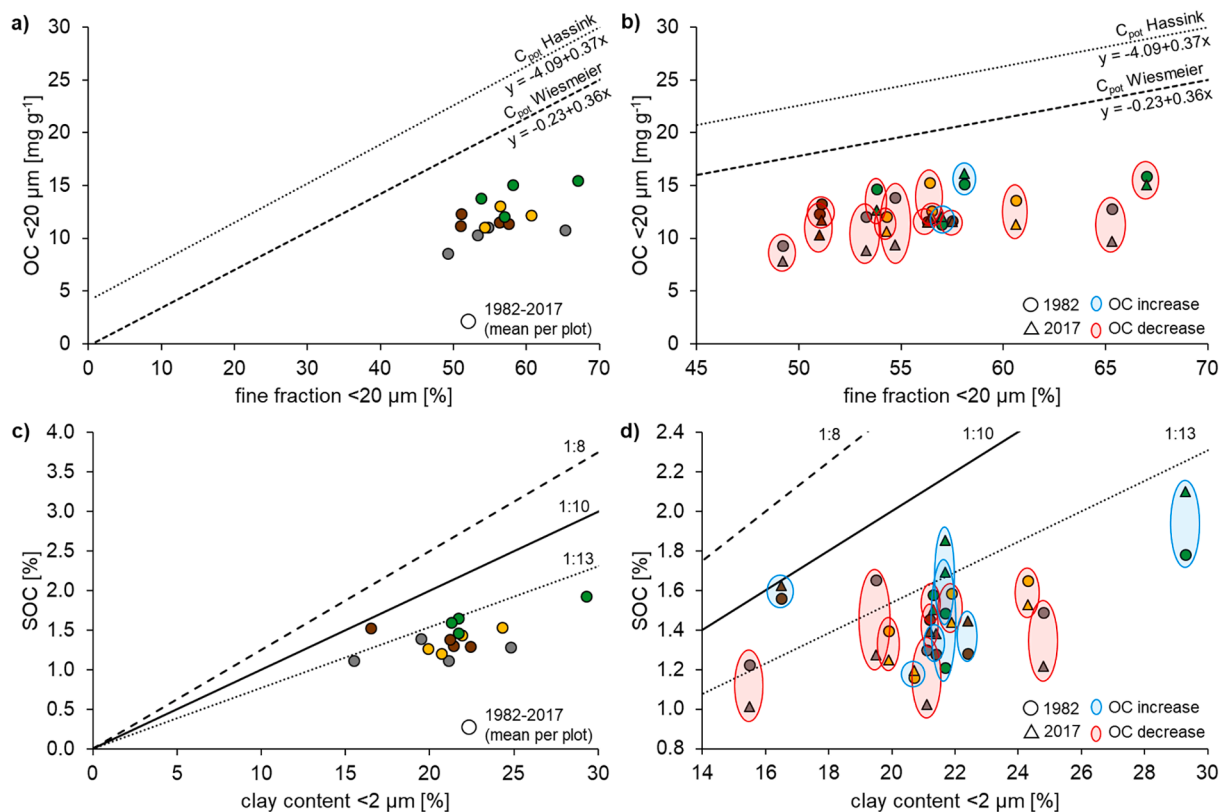
Fig. 6. Correlations of fine fraction (<20 μm) OC concentration with its mass proportion (i.e. C saturation, C_{sat}) compared to linear regressions according to Hassink (1997) and Wiesmeier et al. (2015) (C_{pot}) (upper two graphs). Correlations of clay content (<2 μm; %) with SOC (%) according to Johannes et al. (2017) (lower two graphs). The dashed, solid and dotted lines depict a SOC:clay ratio of 1:8, 1:10 and 1:13, respectively. A SOC:clay ratio of 1:10 is seen as reasonable goal for soil management, as its decrease leads to a lower soil structural quality. Overall, values are calculated with PARIO texture (2019) and C concentrations from elemental analysis for a) and c) each analyzed plot averaged over the observation period, and b) and d) "zoomed in" to follow the development per plot from the first (1982) to the last year of analysis (2017).
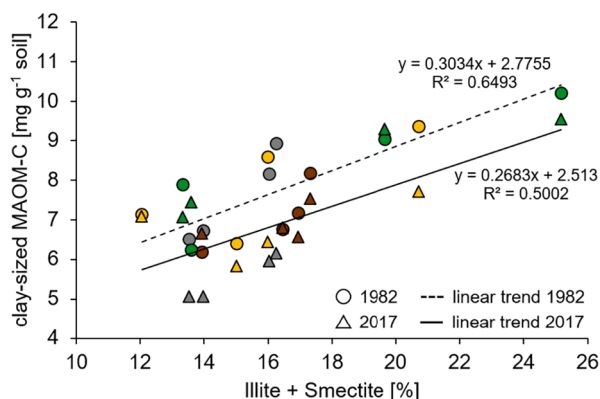

Fig. 7. Linear correlation between 2:1 phyllosilicates (i.e. Illite and Smectite) (%) as measured with XRD analysis on soil samples from 2019, and C contents in the clay-sized MAOM fraction (<6.3 μm) (mg g^{-1} soil) in all 16 analyzed plots. Circles depict values from 1982 and triangles from 2017. The dashed and solid lines represent the linear trends in 1982 and 2017, respectively.

< clay-sized MAOM (0.61). Within the four different systems, fPOM A/ O-A ratios were highest in BIODYN (0.28) compared to the other systems (0.23) in 2017. Other than that, no considerable differences regarding chemical composition of POM fractions were found between the systems. A/O-A ratios of NOFERT and BIODYN clay-sized MAOM fractions were the same in 1982 (0.65). In 2017 however, it strongly decreased to 0.55 in NOFERT, but remained constant in BIODYN (0.62).

4. Discussion

4.1. Fertilizer input quality rather than quantity is a prerequisite for POM accumulation

Despite different OM input quantities, all systems showed moderate increases in the fPOM (>20 μm) fraction between 1982 and 2017 (NOFERT: +0.17 mg g^{-1}; CONMIN: +0.14 mg g^{-1}; CONFYM: +0.16 mg g^{-1} and BIODYN: +0.16 mg g^{-1}) (Fig. 3a). Significant differences for fPOM-C were only found between NOFERT and BIODYN in 1982, 1989 and 2003. This implies that the higher OM inputs from organic manures had no beneficial effect on the accumulation of fPOM, compared to CONMIN. Qualitatively, BIODYN compost was relatively enriched with more recalcitrant OM due to the higher losses of labile C compounds (Fließbach and Mäder, 2000). NMR data confirmed the higher maturity of composted manure, as fPOM A/O-A ratios in soil were highest in BIODYN (0.28), indicating a higher degree of decomposition (Baldock et al., 1997) compared to the other systems (0.23) in 2017 (*Supplementary Figure S1* & Table 3). Nevertheless, these qualitative differences did not affect the fPOM accumulation in the DOK trial. We assume that the additional input of organic fertilizers enhanced microbial activity and soil aggregation, ensuring rapid incorporation of highly labile fPOM into aggregates. Consequently, a decisive factor for the amount of fPOM at the time of soil sampling is the elapsed time since the last fertilizer application, which was at least several months in the DOK trial. As soil aggregation and thus the conversion from fPOM to oPOM can take place within a month (Bucka et al., 2019), most of the POM is expected to be mineralized or occluded within aggregates at the time of sampling, and the measured fPOM represents only the biomass input from the crop.

Table 3

Integrated chemical shift regions of obtained ^{13}C CPMAS-NMR spectra of selected POM and MAOM fractions. Values are given as arithmetic means in bold with standard deviations of plot replicates, for POMs (n = 2–4) and clay-sized MAOM fractions (n = 4) (<6.3 μm), for 1982 and 2017. Post-hoc Tukey letters give significant differences between farming systems at p <0.05, for >3 plot replicates.

	Alkyl C		O/N-Alkyl C		Aryl C		Carboxyl C		Alkyl / O/N-alkyl ratio	
	1982	2017	1982	2017	1982	2017	1982	2017	1982	2017
	clay-sized MAOM (<6.3 μm) [%]									
NOFERT	**26.65**±0.88 a	**25.02**±0.99 a	**40.93**±0.63 a	**45.34**±1.73 a	**18.56**±0.41 a	**17.08**±0.19 a	**13.84**±0.13 a	**12.55**±1.05 a	**0.65**±0.03 a	**0.55**±0.04 a
BIODYN	**26.59**±0.82 a	**24.87**±1.36 a	**41.20**±1.12 a	**40.67**±3.02 b	**19.10**±1.37 a	**20.80**±1.94 b	**13.08**±1.22 a	**13.58**±0.82 a	**0.65**±0.03 a	**0.62**±0.08 a
	oPOM (>20 μm) [%]									
NOFERT	**13.92**±1.33	**16.96**±1.27	**50.86**±1.69	**53.40**±0.68	**25.79**±1.32	**21.37**±1.11	**8.71**±1.79	**8.06**±0.71	**0.27**±0.02	**0.32**±0.03
CONMIN	**17.14**±1.95	**14.67**±0.73	**47.47**±3.64	**53.41**±1.10	**24.20**±3.66	**23.17**±1.05	**10.74**±1.78	**8.69**±1.19	**0.36**±0.01	**0.27**±0.01
CONFYM	**16.62**±1.62	**15.29**±1.22	**51.03**±0.61	**54.66**±0.35	**23.55**±1.80	**21.97**±0.98	**9.07**±0.06	**7.98**±0.48	**0.33**±0.03	**0.28**±0.02
BIODYN	**17.59**±1.37	**17.00**±0.80	**50.23**±0.44	**51.18**±2.88	**22.76**±1.00	**22.61**±2.01	**9.03**±1.06	**8.98**±1.80	**0.35**±0.02	**0.33**±0.01
	fPOM (>20 μm) [%]									
NOFERT	**11.50**±3.26	**13.44**±0.19	**50.06**±5.75	**57.44**±7.11	**28.04**±2.59	**20.54**±4.13	**9.59**±0.39	**8.15**±2.22	**0.23**±0.09	**0.24**±0.03
CONMIN	**17.86**±5.25	**12.85**±0.78	**52.13**±2.70	**55.76**±4.98	**20.61**±2.51	**23.25**±4.18	**9.79**±4.67	**7.96**±1.13	**0.34**±0.08	**0.23**±0.01
CONFYM	**14.72**±0.81	**12.67**±1.91	**55.00**±5.27	**55.88**±4.89	**22.70**±4.73	**22.72**±3.55	**7.47**±0.40	**8.84**±3.26	**0.27**±0.04	**0.23**±0.01
BIODYN	**15.81**±0.13	**14.80**±4.29	**48.75**±3.48	**52.46**±0.49	**24.90**±2.19	**24.05**±0.75	**10.32**±0.53	**8.39**±2.65	**0.33**±0.03	**0.28**±0.08

In contrast to the fPOM, different OM input quantities (*Supplementary Table S2*) were manifested in the recovered oPOM-C contents. We can clearly separate systems with and without organic fertilization, as oPOM-C contents in BIODYN and CONYM were on average 46% higher than in CONMIN and NOFERT (Fig. 3b & *Supplementary Table S3*). OM inputs are incorporated as labile SOM fractions (Li et al., 2020; Yang et al., 2012; Zhang et al., 2021). These particles are proven to be hotspots of microbial activity (Francioli et al., 2016; Lazcano et al., 2012; Witzgall et al., 2021; Yan et al., 2007). Consequently, aggregate formation is enhanced (Cotrufo et al., 2013) e.g. through the production of extracellular polymeric substances during microbial decomposition (Costa et al., 2018), enhancing oPOM accumulation in BIODYN and CONFYM. If we compare systems without organic fertilization, we can attribute the higher oPOM-C contents in CONMIN compared to NOFERT to the mineral fertilization with the beginning of CRP 2, leading to higher biomass production and increased OM inputs from litter and crop residues. This input explains the 24% higher oPOM-C contents in CONMIN compared to NOFERT. Overall, higher A/O-A ratios of oPOM (0.31 ± 0.03) compared to fPOM (0.27 ± 0.05) (Table 3) confirm its higher degree of decomposition (Baldock et al., 1997).

Interestingly, the 20% higher OM inputs via stacked manure in CONFYM compared to BIODYN (Table 1) were not reflected in higher oPOM-C contents. On average, oPOM was even 45% higher in BIODYN, with significant differences from 1996 onwards (Fig. 3b). We assume that composted manure, also introduced considerable amounts of composting-borne microbial biomass and microbial decomposition products to the soil. Evidence can be drawn from Mäder et al. (2002) and Hartmann et al. (2015), who reported the highest microbial diversity in BIODYN plots, and Oehl et al. (2004), who found significantly higher arbuscular mycorrhizal fungi species diversity in the organic compared to the conventional farming systems of the DOK trial. In addition, Fließbach et al. (2007) reported a 25% higher microbial biomass in BIODYN compared to CONFYM plots.

Besides qualitative differences in organic manure inputs, two additional factors can contribute to the differences in oPOM-C contents between CONFYM and BIODYN: 1. the use of pesticides in CONFYM can constrain microbial decomposition; and 2. the additional application of mineral fertilizers in CONFYM (*Supplementary Table S2*) may have induced positive priming through accelerated initial microbial respiration of labile C compounds (Kuzyakov et al., 2000; Moran et al., 2005; Zhou et al., 2021), that were relatively enriched in the stacked manure, compared to the composted manure. This probably contributed to the inhibiting conditions for aggregate formation and accumulation of oPOM-C in CONFYM, compared to BIODYN.

Contrary to their previous trends from 1982 to 2010, there was a strong increase in oPOM-C contents in NOFERT (62%) and CONMIN (47%), in 2017 (Fig. 3b). At this time, soybean was present on the analyzed plots and green manure was incorporated into the soil in spring that year (*Supplementary Table S1*). We assume that the implementation of soybean into the crop rotation increases N availability in the soil through biological nitrogen fixation (Stagnari et al., 2017). Therefore, crop residues can be more efficiently transformed, and in turn enhance aggregate formation (Zhou et al., 2020) and oPOM accumulation. In addition, green manure plowed in during spring before soybean cultivation represents additional OM that can be stabilized in aggregates. The trends of oPOM-C in BIODYN and CONFYM showed a highly dynamic temporal behavior with high fluctuations in each year of analysis (Fig. 3b). We observed strong correlations between the extent of these fluctuations and the elapsed time between harvest and soil sampling, independent of the crop (Fig. 4b). Soil sampling shortly after harvest (i.e. <14 days) led to higher oPOM-C contents, while pronounced oPOM-C losses occurred when more time elapsed between harvest and soil sampling (21 to 35 days). Harvesting interrupts biomass production (above- and belowground), abruptly reducing the supply of fresh OM to the soil. In addition, soil disturbance induces the decomposition of labile SOM fractions (Bongiorno et al., 2019). Especially the harvest of root crops affects the soil structure through mechanical stress (Panagos et al., 2019). This leads to more atmosphere-connected soil pores (Kravchenko et al., 2015), accelerating the decomposition of previously occluded and protected SOM (Six et al., 2000). Lower OM inputs in NOFERT and CONMIN via crop residues only, led to reduced aggregate formation and thus less oPOM, and sampling timing after harvest influenced oPOM-C contents to lower degrees (Fig. 4a).

To summarize, neither quantity nor quality of organic fertilizer input affected the fPOM fraction over the 36 years of observation, which rejects our hypothesized accumulation of fPOM in the systems receiving organic fertilizers. This hypothesis applies for the oPOM fraction, where, however, large annual fluctuations of oPOM-C contents in BIODYN and CONFYM clearly demonstrate its high lability, and emphasize the importance of standardized sampling dates. We assume, the quality of OM inputs rather than the quantity applied is critical here. In contrast to stacked farmyard manure (CONFYM) did composted manure (BIODYN) increase microbial activity, promote aggregate formation and oPOM accumulation. However, the potential negative impacts of mineral fertilizer and pesticide application on labile SOM fractions in CONFYM should not be neglected.

4.2. No additional SOC accumulation in mineral-associated fractions - regardless of fertilizer inputs

The clay-sized MAOM-C fraction (<6.3 μm) accounted for by far the largest proportion (69.4%) of the SOC across all systems, which is generally in line with observations in temperate arable soils (Christensen, 2001). NOFERT and CONMIN showed severe SOC losses of 27% and 14% respectively, while it remained stable in CONFYM (-3%) and BIODYN (±0%) from 1982 to 2017 (Fig. 5b & *Supplementary Table S3*). In CONMIN, all SOC losses from the clay-sized MAOM fraction were recorded in CRP 1, when it served as an unfertilized control system. The insufficient supply with OM inputs from crop residues during that period led to SOC losses of 15% in CONMIN. Given the high standard deviations of the individual plot replicates (n = 4) (*Supplementary Table S3*), the SOC contents of the clay-sized MAOM fraction in all fertilized farming systems can be considered stable. The same holds true for the silt-sized OM fraction (63–6.3 μm), which accounted for 20.8% of total SOC averaged over all systems. Here, NOFERT showed severe SOC losses of 24%, while CONMIN, CONFYM and BIODYN remained relatively stable from 1982 to 2017 (Fig. 5a & *Supplementary Table S3*). Overall, the trends of all fertilized systems were uniform and SOC contents in the silt-sized OM and clay-sized MAOM fraction remained stable. Only with omission of fertilizers in NOFERT, large amounts of SOC from the mineral-associated SOC pool were lost, due to insufficient supplies with OM.

The unchanged SOC contents of the clay-sized MAOM fraction in all systems suggest that:

1.) the SOC storage capacity has reached saturation and/or that

2.) the observation period of 36 years was too short for new MAOM to form.

According to the empirical formula of Hassink (1997) and its adjustments made by Wiesmeier et al. (2015), C_{sat} of the fine fraction (<20 μm), across all analyzed DOK systems and years averaged 47.9% and 59.5%, respectively (Fig. 6a). This suggests that the fine mineral fraction of the studied plots still has high C sequestration potentials (*Supplementary Table S4*), and C sequestration deficits even increased from 1982 to 2017 in all but two BIODYN plots (Fig. 6b). This contradicts assumption 1.) that clay-sized MAOM-C did not increase due to a saturation of this fraction. However, the empirical formula relies on field observations and gives only rough estimates on C saturation levels of the <20 μm mineral fraction of DOK plots. Therefore, more detailed information on the C-loading of the clay-sized MAOM fraction in the DOK trial is required, to better assess the status of C saturation. In addition, Johannes et al. (2017) observed that a decrease of SOC associated with fine mineral particles (i.e. a decrease in SOC:clay ratio), would lead to a decrease in soil structural quality, and concluded that a SOC:clay ratio of 1:10 would be a reasonable goal for soil management. As all of the studied plots had a SOC:clay ratio <1:10 (Fig. 6c & 6d) which is in line with recent findings in arable soils (Prout et al., 2022), this confirms the high C sequestration potentials within the studied DOK plots. The differences in texture compared to other studies in the DOK trial are owed to sample preparation and the chosen particle size class limits (FAO) (e. g. Leifeld et al. (2009); USDA).

Confirmation for the fast incorporation of fresh OM in the MAOM fraction can be drawn from Vidal et al. (2021), who illustrated the formation of stabilized microbial-derived MAOM in micro-aggregates only 14 days after its addition to the soil. This is contradictory to assumption 2.) that OM inputs from fertilizers did not yet reach the clay-sized MAOM fraction. In addition, NMR spectra of the clay-sized MAOM-C fraction show decreasing A/O-A ratios in NOFERT from 1982 (0.65) to 2017 (0.55) (Table 3), expressing a lower degree of OM decomposition (Baldock et al., 1997). This is supported by the concomitant decrease of carboxyl C from 1982 (13.8 ± 0.1%) to 2017 (12.6 ± 1.1%), which has been observed to accumulate and stabilize in fine mineral fractions after decomposition of labile POM (Yu et al., 2015). We assume that younger (i.e. less decomposed) OM was incorporated in this fraction,

accompanied by losses of older (i.e. more decomposed) OM. In BIODYN, the A/O-A ratio (0.65 to 0.62), as well as carboxyl C remained similar between 1982 (13.1 ± 1.2%) and 2017 (13.6 ± 0.8%). This means that the degree of decomposition stayed the same in BIODYN, suggesting an adequate supply of decomposed OM into the clay-sized MAOM fraction.

Root-derived C inputs represent a large share of total C inputs to arable soils and can contribute more to stable SOC fractions than aboveground crop-derived C inputs (Ghafoor et al., 2017; Kätterer et al., 2011). However, MAOM-C may be susceptible to destabilization e.g. through priming via root exudation (Jilling et al., 2021; Keiluweit et al., 2015). In the DOK trial, Hirte et al. (2018a) & Hirte et al. (2018b) showed yield-independent belowground C inputs and found similar total root biomass and rhizodeposition between organic and conventional farming systems, under wheat, but significantly higher rhizodeposition in the organic system, under maize. This illustrates the complexity and system- and crop-dependency of root C dynamics and makes it difficult to draw accurate conclusions about the impact of belowground C inputs, especially since soil was sampled in two additional crops in this study (*Supplementary Table S1*). In NOFERT, however, the low aboveground C inputs mean a lack of OM replenishment, which cannot compensate for potential SOC losses from the clay-sized MAOM fraction, resulting in severe losses (Fig. 5b).

SOC contents of the clay-sized MAOM fraction ran on three different levels (Fig. 5b). BIODYN showed the highest, CONMIN and CONFYM a medium and NOFERT the lowest level of MAOM-C contents, with significant differences recorded only between BIODYN and NOFERT from 2003 onwards (Fig. 5b). We assume the different levels of SOC storage between the systems resulting from differences in clay mineralogy, which may quantitatively affect SOM stabilization. Soils of the DOK plots are dominated by 2:1 clay minerals (i.e. Illite and Smectite) (Table 2), that can store larger amounts of MAOM-C than 1:1 clay minerals (Barré et al., 2014; Feng et al., 2013; Six et al., 2002). The linear correlation between the proportion of Illite and Smectite and the amount of clay-sized MAOM-C within the studied plots emphasizes the importance of clay mineralogy for stabilization of OM inputs (Fig. 7).

The fact that bulk SOC increased in BIODYN (+13.1%) and CONFYM (+4.7%) from 1982 to 2017 (Fig. 2), but no additional SOC was sequestered within the clay-sized MAOM-C fraction, means that these increases were solely stored within the labile POM fractions. This result is in line with observations in fractionated topsoils from other temperate agricultural sites (Chung et al., 2008; Gulde et al., 2008) and grazed semi-arid steppe ecosystems (Steffens et al., 2011), where increased OM inputs solely led to increased SOC contents in POM fractions and macro-aggregates, while fine-sized mineral fractions showed no increase. We therefore suggest that the continuous application of composted manure in BIODYN creates best conditions for enhanced microbial activity and aggregate formation and can thereby maintain SOC in the oPOM fraction at elevated levels in the long run, ensuring soil fertility. This makes oPOM a highly labile precursor for SOC storage in fine mineral fractions, dependent on OM input quality, which is in line with recent findings (Cyle et al., 2016). It also emphasizes the need for protective measures of the labile POM fractions and questions the potential of agriculturally used soils, to serve as a stable long-term C sink. Overall, our hypothesis of a steadily declining clay-sized MAOM fraction towards a new steady-state equilibrium at a lower level is only true for NOFERT, whereas the highest efficiency of C incorporation into this fraction holds true for systems receiving organic fertilizers.

4.3. Implications and recommendations for SOC monitoring and management

The application of organic fertilizers in the DOK trial between 1982 and 2017 resulted in a net increase of bulk SOC in BIODYN and CONFYM. However, this bulk SOC increase was exclusively measured in the labile POM fractions. Especially the highly dynamic oPOM fraction is subject to severe SOM depletion within a few days after C input halt, and

sampling timing after harvest is critical for the proper measurement of the retained oPOM-C (Fig. 4). Based on this observation, we recommend standardizing sampling dates to a specific narrow interval after harvest every year. This would improve the validity of C model predictions, as information on precise C observations from field trials constitute a basis for estimations on SOC dynamics, and soil management strategies are often recommended based on such models. Inconsistencies in sampling timing after harvest cause losses of information and farming system significance, regarding climate friendly soil management.

CO_2 certificates are a promotional tool in the private industry sector for the build-up of SOC stocks, setting incentives for climate mitigation (Wiesmeier et al., 2020). The increased bulk SOC concentrations in this study under organic fertilization (Fig. 2) would have financially rewarded farmers in form of such certificates. However, dynamics of labile and stable SOC pools are not considered for its allocation, but only the development of bulk SOC. Based on the results of this study, a distinction between differently stable SOC pools is highly recommended, as none of the observed farming systems was sufficient to promote SOC build-up in the clay-sized MAOM-C pool. Counteracting this issue with more time-, labor- and consequently more cost-intensive laboratory analyses that account for SOM storage in differently stable SOC pools does not seem to be an adequate option.

With regards to global warming, severe SOC losses in temperate agricultural soils are projected (Wiesmeier et al., 2016), and unrealistically high amounts of additional future OM inputs and drastic management changes are needed as countermeasures (Riggers et al., 2021). POM stabilized within aggregates is the precursor for MAOM-C (Six and Paustian, 2014). Regarding the unchanged SOC contents of clay-sized MAOM fractions observed in this study (Fig. 5b), we propose that the reduction of high oPOM losses (Fig. 3b & Fig. 4a) would increase the amount of OM for possible long-term storage. Therefore, the focus should be on introducing management practices, preserving this highly labile SOM pool, as a guiding paradigm for farmers to make their soils more resilient to climate change (Berthelin et al., 2022). An important lever here is to create good living conditions for soil microbes under which the transformation of the OM inputs and aggregate formation is stimulated (Witzgall et al., 2021). The application of composted manure in the BIODYN farming system was most effective in this regard. In addition, management practices that reduce aggregate turnover (e.g. reduced or no-tillage (Six and Paustian, 2014)) and enhance aggregate stability (e.g. promotion of fungal activity (Hannula and Morriën, 2022; Six et al., 2006)) seem to be viable management options that would increase SOM persistence within agricultural soils.

5. Conclusions

Within this study, unfertilized and exclusively minerally fertilized farming systems showed substantial losses in bulk SOC, while the application of organic manure led to the build-up of bulk SOC, over a 36-year period. However, this build-up was solely recorded within the readily decomposable oPOM fraction and was mainly dependent on the quality of organic fertilizers (composted manure vs stacked manure). The use of composted manure without mineral fertilizer and pesticide application was most efficient. However, the oPOM fraction underlies heavy dynamics, and can be lost within a short time, consequently impeding its potential for long-term C sequestration. The fact, that no additional SOC was sequestered as MAOM from 1982 to 2017, raises concerns regarding the ability of soils to serve as a stable long-term C sink and the feasibility of climate-mitigating soil management strategies. It also emphasizes the need of continuous OM inputs to keep labile POM fractions at elevated levels, thereby maintaining soil fertility, crop performance and food security. With respect to global warming, it is likely that OM inputs would have to increase immensely to maintain current SOC stocks. In this context, it is of high importance to gain further information on the sorptive capacity and the current state of C-loading and stability of MAOM fractions, as well as turnover rates of OM

inputs, which would contribute to improve SOM models. This study shows that the potential of soils to mitigate climate change has to be carefully assessed and expectations of the effect of fertilization should be low.

Declaration of Competing Interest

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2022.116080.

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