

Soil organic matter pools and crop yields as affected by the rate of farmyard manure and use of biodynamic preparations in a sandy soil

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Abstract One aim of organic and biodynamic agriculture is to improve soil fertility. Our objectives are to (1) explain previously reported differences in the soil organic matter levels between soils receiving farmyard manure (FYM) with or without biodynamic preparations (BD), (2) quantify the effect of three levels of FYM applications on microbial biomass and soil organic matter (SOM) pools with different stability, and (3) relate SOM pools to crop yields in a long-term experiment on a sandy soil at Darmstadt, Germany. Soils of the BD-FYM treatments had significantly higher C_{org} contents compared to soils of the FYM treatments. However, soil fractionation indicated that there was a greater storage of C_{org} in the intermediate and passive pools of the BD-FYM treatments, and the

temporal course of C_{org} contents suggested a slow convergence of C_{org} stocks between FYM and BD-FYM with time. Thus, the observed differences between BD-FYM and FYM treatments were likely to have existed since the beginning of the experiment. Contents of labile C (70–114 g $[kg\ C_{org}]^{-1}$, turnover time 462 days) and labile N (35–49 g $[kg\ N_t]^{-1}$, turnover time of 153 days) were strongly related to the application rate and also to crop yields. Yield of potatoes, winter rye, and clover significantly increased in proportion to the application rate of FYM, while BD had no effect. Overall, the study showed that increasing rates of FYM increased C and N availability independent of the use of BD. Nevertheless, efficiency of C sequestration in a more stable form (intermediate pool) decreased with increasing rate.

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Introduction

Fertilization with farmyard manure (FYM) has many beneficial effects on soil. For instance, fertilization with FYM leads generally to higher levels of organic carbon (C_{org}) and total nitrogen (N_t) than the application of mineral fertilizers (Christensen 1996; Weigel et al. 1998). Similarly, increasing rates of FYM increase the stocks of C_{org} and N_t (Christensen 1996; Weigel et al. 1998), the content of microbial

biomass (Weigel et al. 1998), and the rate of net N mineralization (Whalen et al. 2001; Khorsandi and Nourbakhsh 2007). Less information is available about the impact of FYM with biodynamic (BD) preparations on the soil (Turinek et al. 2009).

Biodynamic preparations consist of plant parts or extracts treated with animal tissues, water, and/or soil (Koepf et al. 1996; Turinek et al. 2009). When added to composting dairy manure piles, it has been shown by Carpenter-Boggs et al. (2000a) that BD-treated compost piles maintained a higher temperature and had 65% more nitrate at the end of the composting process. Furthermore, the phospholipid fatty acid profile of the microbial community differed. Contrasting results exist on the effect of BD-FYM on soil properties: compared with fertilization with FYM, higher C_{org} contents and enzyme activities but similar microbial biomass contents were observed in a sandy soil fertilized with BD-FYM (Bachinger 1996; Raupp and Oltmanns 2006) at the field experiment of the current study (Darmstadt, Germany). In a loamy Fluvisol, treated for 9 years with FYM and BD-FYM, a faster decomposition and lower metabolic quotient were reported in the BD treatments, but contents of microbial biomass C (C_{mic}) did not differ (Zaller and Koepke 2004). In a loamy Haploxeroll, no significant effects on soil biological properties were reported after 2 years of application of FYM and BD-FYM (Carpenter-Boggs et al. 2000b).

Soil organic matter (SOM) is conceptually divided into different pools with distinct turnover times. For example, a classification into three SOM pools was proposed by von Lützow et al. (2008): an active (turnover time < 10 years), an intermediate (turnover time = 10–100 years), and a passive pool (turnover time > 100 years) were described. Since active and intermediate pools provide a supplement of nutrients in addition to direct fertilization, a special aim of BD agriculture is to achieve a high amount of SOM stored in these pools (Koepf et al. 1996). However, there is no standard method to quantify the conceptually defined pools.

For the determination of active C and N pools, potential mineralization of C or N in laboratory incubations has often been used in combination with the application of simple models (Benbi and Richter 2002). In many studies, two pools, each with its specific decay constant, were most useful to describe the mineralization data (Cabrera et al. 2005; Mallory

and Griffin 2007). Henceforward, we will use the terms “very labile” and “labile” for the pools obtained by applying curve fitting to mineralization data. Both are part of the active pool as defined by von Lützow et al. (2008).

For determination of the passive pool, Helfrich et al. (2007) evaluated several chemical fractionation methods. They concluded that fractionation with sodium peroxodisulfate ($Na_2S_2O_8$) mainly removed young C. The ^{14}C age of the remaining C fraction was more than 10,000 years and apparent turnover time (derived from ^{13}C measurements) was much more than 600 years. Furthermore, land-use and initial C content did not influence the C content of the remaining fraction. Thus, fractionation of SOM with $Na_2S_2O_8$ can be used as a measure for the passive pool.

Quantifying the partitioning of C and N into active, intermediate, and passive pools is important for our understanding of crop nutrition, soil fertility, and SOM stabilization. In a previous study, we compared the effect of mineral fertilizer and manure application (without BD preparations) on the distribution of C and N in several SOM pools in soils of the Darmstadt experiment (Heitkamp et al. 2009). Here, we report the effects of BD-FYM additions on crop yields and on total C and N stocks and different SOM pools. Until now, little information has been available on how the rate of BD-FYM application influences partitioning of organic matter into the abovementioned pools. Our objectives are to (1) explain previously reported (Bachinger 1996; Raupp and Oltmanns 2006) differences in the C_{org} content between manure and BD-manure treatments, (2) compare the effects of the application rates of both types of manure on microbial biomass and SOM pools, and (3) relate the storage of SOM in different pools to crop yields in a sandy soil at the long-term experiment site in Darmstadt, Germany.

Material and methods

Site description

The long-term experiment is situated near Darmstadt, Germany (49°50' N, 8°34' E, mean annual temperature 9.5°C, mean precipitation 590 mm) and focuses on organic, mineral, and biodynamic treatments. The soil is

a Haplic Cambisol with sandy texture (5% clay, 10% silt, and 85% sand). Historical maps show that the experimental area was converted from a mixed forest stand to arable land between 1904 and 1920. The experiment started in 1980 with nine treatments. Crop rotation (since 1985) has consisted of spring wheat (*Triticum aestivum* L.), potatoes (*Solanum tuberosum* L.), winter rye (*Secale cereale* L.) and legumes (clover, *Trifolium* spp. or Lucerne, *Medicago sativa* L.). Yields are given in Table 1.

Soil (A horizon, 0–25 cm) was sampled from plots (25 m²) receiving BD field sprays plus BD-treated manure at three different rates. Application rates (see below for details) were high (BD-FYM_H), medium (BD-FYM_M), and low (BD-FYM_L). Biodynamic compost preparations (herbal extracts, 502–507) were applied at rates of 2–4 mg kg⁻¹ to FYM. Preparations 500 (horn manure) and 501 (horn silica) were sprayed on the field at rates of 200–300 and 4 g ha⁻¹, respectively. The preparations were used with the intention to optimize nutrient cycling and plant health. More information about biodynamic preparations can be found in the literature (Koepf et al. 1996; Carpenter-Boggs et al. 2000b; Zaller and Koepke, 2004; Turinek et al. 2009). Three treatments using unprepared FYM at corresponding rates served as control (FYM_H, FYM_M, FYM_L). Since 1985, the FYM and BD-FYM applications have corresponded

for one crop rotation (4 years) on average to 108, 75 or 43 kg N_t ha⁻¹ year⁻¹. Legumes were not fertilized; application rates for the other crops are as follows for the applications at high, medium, and low rates, respectively:

High	27 t fresh weight ha ⁻¹ (150 kg N _t ha ⁻¹) to potatoes or 16 t fresh weight plus 40 kg N _t ha ⁻¹ as urine to cereals (140 kg N _t ha ⁻¹)
Medium	18 t fresh weight ha ⁻¹ to potatoes or 12 t fresh weight ha ⁻¹ plus 20 kg N _t ha ⁻¹ with urine to cereals (both 100 kg N _t ha ⁻¹)
Low	9 t fresh weight ha ⁻¹ to potatoes (50 kg N _t ha ⁻¹) or cereals (60 kg N _t ha ⁻¹)

The fresh weight additions given above (27, 16, 18, 12, and 9 tha⁻¹) are mean values; the actual applications on each date depended on the moisture and N_t contents of the farmyard manure and were scaled to match the N_t additions given above. Farmyard manure was rotted for 3 to 6 months before application. All management actions (e.g., tillage, irrigation, weeding, sowing, or harvesting) were the same apart from the application rate and use of preparations. In 1980, at the commencement of the field study, baseline soil data were only collected on a single bulked sample (C_{org} 10.9 g kg⁻¹, N_t 0.8 g kg⁻¹). Since 1982, all treatments were analyzed for C_{org} and N_t, but for the period prior to 1988, only mean values

Table 1 Yields (t ha⁻¹) of the main products (grain, tubers, forage) of the crops at the Darmstadt long-term experiment (2003–2006)

Treatment	Spring wheat	Potatoes	Winter rye	Clover forage
BD-FYM _H	3.2 (0.3)	21.7 (2.4) ^a	4.3 (0.3) ^a	4.8 (0.6) ^a
BD-FYM _M	3.1 (0.2)	19.6 (2.4) ^{ab}	3.8 (0.4) ^{ab}	4.1 (0.4) ^b
BD-FYM _L	2.7 (0.3)	17.6 (0.9) ^{bc}	3.2 (0.7) ^{bc}	3.5 (0.5) ^c
FYM _H	3.4 (0.2)	21.3 (1.0) ^a	4.1 (0.2) ^a	5.1 (0.2) ^a
FYM _M	3.2 (0.1)	19.7 (1.6) ^{ab}	3.7 (0.2) ^{ab}	4.6 (0.0) ^b
FYM _L	2.8 (0.2)	17.8 (0.3) ^{bc}	3.3 (0.4) ^{bc}	3.3 (0.2) ^c
<i>p</i> -values				
BD-preparations	0.307	0.939	0.535	0.553
Rate	0.100	0.012	0.049	< 0.001
BD × Rate	0.992	0.585	0.048	0.063

Yield of grain contains 14% water, yield of tubers is given in fresh weight and yield of clover aboveground biomass is given in dry weight. Mean values with standard errors (*n*=4)

BD-FYM_H farmyard manure application plus biodynamic preparations at high rate, BD-FYM_M farmyard manure application plus biodynamic preparations at medium rate, BD-FYM_L farmyard manure application plus biodynamic preparations at low rate, FYM_H farmyard manure application at high rate, FYM_M farmyard manure application at medium rate, FYM_L farmyard manure application at low rate

without standard errors are available. A detailed description of the field experiment is given by Raupp and Oltmanns (2006).

C and N inputs

The C inputs with harvest residues and roots were estimated as a linear function of the crop yields (Table 1) by additionally accounting for rhizodeposition (Franko 1997; Ludwig et al. 2007; Eq. 1).

$$C_{\text{input}} = (K + \text{yield} \times F) \times R \quad (1)$$

C_{input} is the input of C by crop residues and roots (dt C ha^{-1}), yield refers to the crop yield (dt ha^{-1}), and K (intercept) and F (slope) are crop-specific constants. The values used for K (dt C ha^{-1}) were 23.9 (berseem clover, *Trifolium alexandrinum* L), 3.1 (spring wheat), 0.8 (potatoes), and 4.0 (winter rye) and for F 0.014 (berseem clover), 0.078 (spring wheat), and 0.080 (winter rye) $\text{dt C (dt dry matter yield)}^{-1}$ or 0.016 (potatoes) $\text{dt C (dt fresh matter yield)}^{-1}$ (Franko 1997). R accounts for the portion of the C input by rhizodeposition (potatoes 1.35, other crops 1.50) (Ludwig et al. 2007).

Additionally to the FYM-N_i input, we considered symbiotic N₂-fixation of berseem clover (Bachinger and Zander 2003):

$$N_{\text{fix}} = P_{\text{fix}} \times \text{yield} \times (N_{\text{yield}} + R_{\text{yr}} \times N_{\text{res}}) \times N_{\text{red}}, \quad (2)$$

where N_{fix} is the amount of fixed N ($\text{kg N ha}^{-1} \text{ year}^{-1}$), P_{fix} is the crop specific portion of N in the plant derived from fixation, yield refers to the yield of dry matter from three cuts ($\text{dt ha}^{-1} \text{ year}^{-1}$), N_{yield} is the N content of the yield (% N), R_{yr} is the ratio of yield and residues plus roots, N_{res} is the N content of residues and roots (% N), and N_{red} is a reduction factor depending on the N content of the residues from the previous crop. We set P_{fix} to 0.8 (Russelle and Birr 2004), N_{res} to 1.6, and N_{red} to 0.85 for rye as the preceding crop (Bachinger and Zander 2003). N_{yield} was measured (2.0 to 2.3%) and R_{yr} was calculated as:

$$R_{\text{yr}} = \frac{\text{yield} \times 0.45}{C_{\text{input}}}, \quad (3)$$

where yield is the dry matter yield (dt ha^{-1}), 0.45 is the relative C content of the yield, and C_{input} is the estimation for C input (dt ha^{-1}) of clover as revealed by (Eq. 1).

Yields, soil sampling, and characterization

Crops were harvested from the core of the plots (18.75 m^2), cleaned and weighed fresh (potatoes), air-dried (grains, 14% water content), or oven-dried (legumes, 105°C).

Soil samples were taken in February 2007 after berseem clover cultivation. Twenty-five soil samples (A-horizon, 0–25 cm) were bulked for each of the four field replicates for each treatment. Samples were sieved ($<2 \text{ mm}$) and dried (40°C) or stored moist at 4°C . The pH (soil/solution [w/v] ratio 1:2.5) was determined in 0.01 M CaCl_2 solution. Organic carbon and N_t were measured by dry combustion (Elementar Vario El, Heraeus, Hanau, Germany). If present, CaCO_3 was destroyed by pretreatment with HCl (10%). Contents of C_{mic} and microbial biomass N (N_{mic}) were analyzed by chloroform fumigation extraction (Vance et al. 1987) at 55% water holding capacity (WHC) with conversion factors of 0.45 for calculation of C_{mic} and 0.54 for N_{mic} (Brookes et al. 1985; Wu et al. 1990).

Incubation experiment

Since microbial activity responds to the effects of physical and chemical protection of SOM, incubation approaches are considered a good measure for active C and N in the soil (Khanna et al. 2001). For the mineralization experiment, we incubated 60 g of soil in leaching columns (three replicates per soil sample) at 10°C for 266 days at 55% WHC. Concentrations of CO_2 were measured with a photoacoustic measurement device (INNOVA 1312 AirTech Instruments, LumaSense Technologies AS, Ballerup, Denmark). Measurement intervals increased from weekly (until day 35) to biweekly (until day 91). Thereafter, CO_2 evolution was measured on days 91, 119, 147, and 266. Measurement of net N mineralization was conducted by leaching (Stanford and Smith 1972) on days 7, 14, 21, 35, 63, 91, 119, and 266. Greater intervals compared to CO_2 measurements were chosen to assure that N concentrations in the leachates were above detection limits. The volume of leaching solution

(10 mM CaCl₂) was 800 ml. Extracts were filtered (0.45 µm) and analyzed for NO₃[−] and NH₄⁺ with a continuous flow analyzer (Evolution II auto-analyzer, Alliance Instruments, Cergy-Pontoise, France).

Passive pool

To estimate the size of the passive pool (C_{passive} , N_{passive}), we carried out an oxidation with Na₂S₂O₈ (Helfrich et al. 2007). The residue after oxidation represents mineral protected and highly recalcitrant C (Cuyper et al. 2002) with apparent ¹⁴C ages of more than 10,000 years (Helfrich et al. 2007). Oxidation with Na₂S₂O₈ is therefore a good measure for SOM not participating actively in the C and N cycling. Briefly, 0.5 g soil was dispersed in 250 ml distilled water by ultrasound and 20 g NaS₂O₈ and 22 g NaHCO₃ were added. After stirring (48 h, 80°C), the soil was washed and carbonate traces removed with HCl. The remaining fraction was analyzed for C and N.

Data processing

Experimental results of incubation experiments are highly dependent on the incubation conditions, such as temperature, moisture, and especially duration (Böttcher 2004), and simple models are generally used to describe the mineralization kinetics and to determine potentially mineralizable pools (Stanford and Smith 1972; Whalen et al. 2001; Benbi and Richter 2002; Mallory and Griffin 2007). We fitted a two-pool double exponential model to the data of every plot in order to determine very labile and labile C and N pools in the soils of the different treatments. The equation is:

$$Y_{m(t)} = \sum_{i=1}^2 Y_i \times (1 - \exp(-k_i \times t)), \quad (4)$$

where $Y_{m(t)}$ is C or N mineralized (milligrams per kilogram) at time t (days), Y_i is the i th C or N pool (milligrams per kilogram), k_i the i th decay constant (per day).

Because of uncertainties in estimation of four unknown parameters (Böttcher 2004), we followed the approach of Mallory and Griffin (2007) and fitted (Eq. 4) to the combined dataset of all plots (treatments and replicates) and fixed the decay constants to the

obtained values. Briefly, decay constants were 0.0589 day^{−1} (very labile C pool), 0.0022 day^{−1} (labile C pool), 0.1075 day^{−1} (very labile N pool), and 0.0065 day^{−1} (labile N pool).

Intermediate pools ($C_{\text{intermediate}}$, $N_{\text{intermediate}}$) were calculated as the difference between C_{org} resp. N_t and the sum of the very labile, labile, and passive pools. The intermediate pool represents theoretically the part of SOM which is not directly involved in the active C and N cycling but may be influenced in time spans of decades by management (von Lützow et al. 2008). A more detailed description of the methodology can be found in Heitkamp et al. (2009).

Statistical analysis was performed in SAS (SAS Institute Inc. Cary, USA) using a two-factorial (type and rate of manure) mixed model (Heitkamp et al. 2009). The experimental layout is a strip design with slightly restricted randomization. Therefore, the following mixed model was used to account for the restricted randomization structure:

$$Y_{ijkl} = \mu + r_k + c_l + rc_{kl} + t_i + a_j + ta_{ij} + rt_{ik} \\ + rca_{jkl} + e_{ijkl},$$

where:

μ	General mean value
r_k	Effect of the k th main row
c_l	Effect of the l th main column
rc_{kl}	Effect of the k th main row–main column combination
t_i	Effect of the i th fertilizer type
a_j	Effect of the j th fertilizer rate
ta_{ij}	Effect of the interaction of the i th fertilizer type and j th fertilizer rate
rt_{ik}	Error of the ik th fertilizer type–main row combination
rca_{jkl}	Error of the jkl th rate main column–main row combination
e_{ijkl}	Error of the $ijkl$ th plot

Effects were regarded as significant if $p \leq 0.05$. Differences between mean values were calculated as least significant differences (LSD). For analysis of the C_{org} time series, we used the repeated measurements function with “year” as a random effect. The given LSDs for type, rate, and year are only valid from 1989 onwards (no replicate data available before 1989).

Results

C and N inputs to the soil

The mean annual C inputs (measured C input by manure application and estimated C inputs by crop residues and roots, Fig. 1) between 2003 and 2006 increased in the order low rate ($2.1 \text{ t ha}^{-1} \text{ year}^{-1}$) < medium rate ($2.5 \text{ t ha}^{-1} \text{ year}^{-1}$) < high rate ($2.8 \text{ t ha}^{-1} \text{ year}^{-1}$). Estimates and measurements for BD-FYM and FYM treatments at same rates were identical, since yields were very similar (Table 1). Less than 50% of the total C input was derived from manure, and more than 30% was estimated to stem from clover (Fig. 1).

Estimated N fixation ranged from 70 to 105 kg ha^{-1} in the year of clover cultivation (Fig. 1). Because of higher yields, the estimated amount of fixed N increased with application rate. Therefore, between 20% (FYM_H and BD-FYM_H) and 30% (FYM_L and BD-FYM_L) of the mean annual N inputs (2003–2006) were derived from fixation.

Fig. 1 Measured (*manure*, *shaded*) and estimated (harvest residues and root C, symbiotically fixed N) C and N inputs of the last crop rotation period (2003–2006). For N fixation, a constant efficiency of 0.68 was assumed across treatments. For treatment abbreviations, see Table 1

Microbial biomass and activity

The C_{mic} contents ranged from 124 (BD-FYM_L) to $134 \text{ mg (kg soil)}^{-1}$ (BD-FYM_M) in the BD-FYM treatments and from 118 to $151 \text{ mg (kg soil)}^{-1}$ in the FYM treatments (Table 2). Only C_{mic} contents in soils of FYM_H and FYM_M treatments were significantly different.

The mean of cumulative C mineralization after 266 days (Table 2) was of similar magnitude in the soils of the BD-FYM ($395\text{--}543 \text{ mg (kg soil)}^{-1}$) and FYM ($336\text{--}527 \text{ mg (kg soil)}^{-1}$) treatments. Carbon mineralization was significantly affected by the rate but not by the type of fertilizer. Similarly, cumulative net N mineralization after 266 days (Table 2) increased significantly with application rate in BD-FYM and FYM treatments (Table 2).

C_{org} and N_t contents

The C_{org} contents in 2007 (Fig. 2, g (kg soil)^{-1}) decreased in the order BD-FYM_H (9.2) > BD-FYM_M

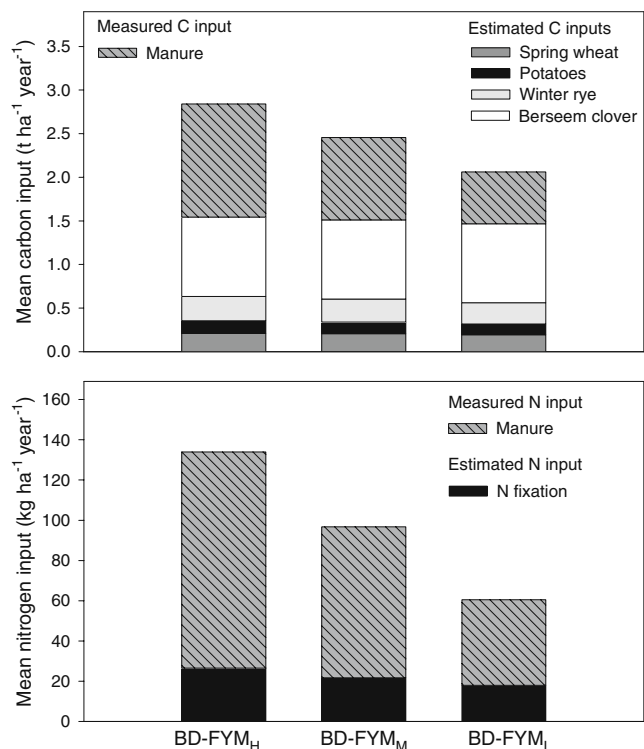


Table 2 Relative changes of the mean C_{org} contents (ΔC_{org}) between the cropping cycles 1982–1985 (first rotation period where individual treatments were sampled) and 2004–2007 (Fig. 2)

Treatment	ΔC_{org} (g kg ⁻¹)	ΔC_{org} (%)	C_{mic} (mg kg ⁻¹)	N_{mic} (mg kg ⁻¹)	Mineralized C (mg kg ⁻¹)	Net-mineralized N (mg kg ⁻¹)
BD-FYM _H	-2.2	-19	125 (6) ab	18 (2)	543 (32) a	38 (1) a
BD-FYM _M	-1.3	-12	134 (16) ab	20 (4)	529 (48) a	33 (1) b
BD-FYM _L	-1.2	-12	124 (14) ab	17 (2)	395 (17) b	29 (1) c
FYM _H	-1.0	-10	151 (9) a	20 (3)	527 (52) a	36 (1) a
FYM _M	-0.9	-9	118 (8) b	19 (3)	432 (56) a	33 (2) b
FYM _L	-1.2	-14	126 (22) ab	18 (5)	336 (28) b	28 (2) c
<i>p</i> Values						
BD preparations	nd	nd	0.790	0.733	0.557	0.739
Rate	nd	nd	0.601	0.847	<0.001	<0.001
BD × Rate	nd	nd	0.020	0.841	0.339	0.731

Data of microbial biomass C, microbial biomass N, cumulative (266 days) mineralized C, and net mineralized N of the soils (0–25 cm) of the different fertilization treatments sampled in 2007. Mean values and standard errors in parentheses ($n=4$). Means followed by different letters are significantly different ($p \leq 0.05$). For treatment abbreviations, see Table 1; values of FYM treatments were recalculated from Heitkamp et al. (2009)

nd not determined because samples were bulked before 1988

(8.8) > BD-FYM_L (8.3) > FYM_M (8.2) > FYM_H (8.1) > FYM_L (7.5). The fertilizer type had a significant effect on the C_{org} contents. Furthermore, the C_{org} contents at low rate were significantly different from the contents at high and medium rates (Fig. 2). Total N contents showed a similar pattern (C/N ratio, 10). However, due

to the bulked soil sampling at the beginning of the experiment, initial values were not reported for individual treatments. Furthermore, Fig. 2 shows that there were already large differences in the C_{org} content between FYM types after 2 years, whereas differences between FYM rates were still small. Figure 2 indicates

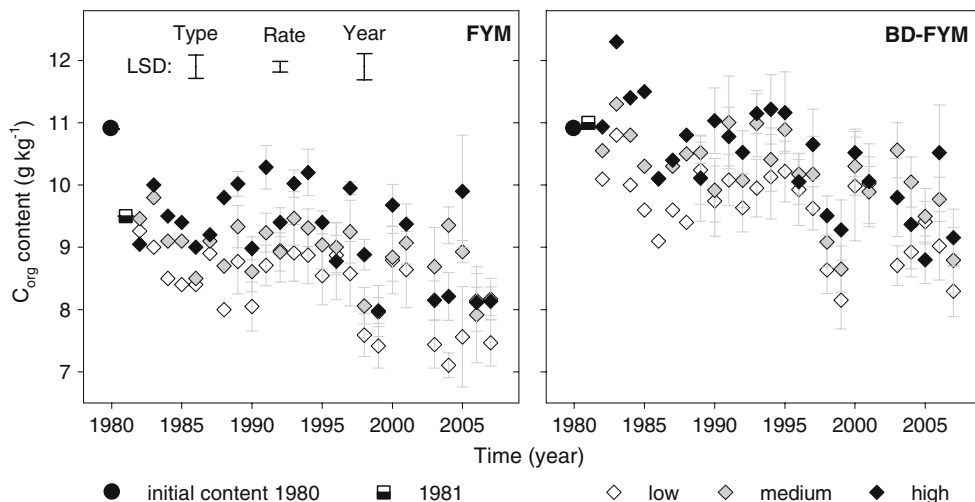


Fig. 2 Time course of C_{org} contents (0–25 cm) in soils of BD-FYM and FYM treatments. The initial value (1980) was measured for a bulked sample; measurements in 1981 were differentiated according to type but bulked across rate treatments. Until 1988, only means without errors were available, since then,

mean values ($n=4$) with standard errors. Values of FYM treatments were recalculated from Heitkamp et al. (2009). Least significant differences of means (LSD, $p \leq 0.05$) are given for the effects of type, rate and year. For treatment abbreviations, see Table 1

that spatial and temporal variation in the C_{org} contents is very high. To gain more confidence into the gradient in C_{org} contents between the beginning and the end of the experiment, we compared the 4-year means from 1982–1985 and 2004–2007. The C_{org} contents of the BD-FYM treatments decreased by 12–19% in the considered period and those of the FYM treatments by 9–14% (Table 2).

C and N pools

Regardless of fertilizer type and rate, approximately 300 g C (kg C_{org})⁻¹ and 25 to 70 g N (kg N_t)⁻¹ were stored in the passive pool (Fig. 3).

The relative pool size of $C_{intermediate}$ comprised 600 g (kg C_{org})⁻¹ and was not significantly influenced by the application rate (Table 3, Fig. 3) or fertilizer type. Similarly, the pool size of $N_{intermediate}$ (880–930 g [kg N_t]⁻¹) was unaffected by both fertilizer type and rate. However, the absolute pool sizes (not shown) of $C_{intermediate}$ and $N_{intermediate}$ (g (kg soil)⁻¹) were

significantly higher ($p < 0.001$) in the BD-FYM (5.3 and 0.8 for C and N, respectively) compared to the FYM treatments (4.8 and 0.7 for C and N, respectively).

Between 70 and 114 g C (kg C_{org})⁻¹ was located in the labile pool (turnover time, 462 days; Fig. 3). Increasing proportions of labile C were significantly related to the amount of FYM- N_t input ($R^2 = 0.53$, Table 3) and cumulative input of 1 t N_t ha⁻¹ with FYM or BD-FYM (C/N ratio, 13) led to an increase of labile C by 21 g (kg C_{org})⁻¹ (Table 3). The proportion of labile N (turnover time 153 days) ranged from 35 to 49 g (kg N_t)⁻¹ and was related to the application rate only ($R^2 = 0.43$, Table 3, Fig. 3). Therefore, increasing FYM applications led to an accumulation of labile N in SOM. In our study, we found an increase of 5 g (kg N_t)⁻¹ per ton applied FYM- N_t in the labile N pool.

The very labile C pool (turnover time 17 days) comprised between 12 and 16 g (kg C_{org})⁻¹. Similarly, the very labile N pool (turnover time 9 days) comprised

Fig. 3 Proportion of carbon (a) and nitrogen (b) pools to C_{org} and total N contents of the soils (0–25 cm) of the different fertilization treatments. Mean values with standard errors ($n = 4$). Values of FYM treatments were recalculated from Heitkamp et al. (2009). For treatment abbreviations, see Table 1

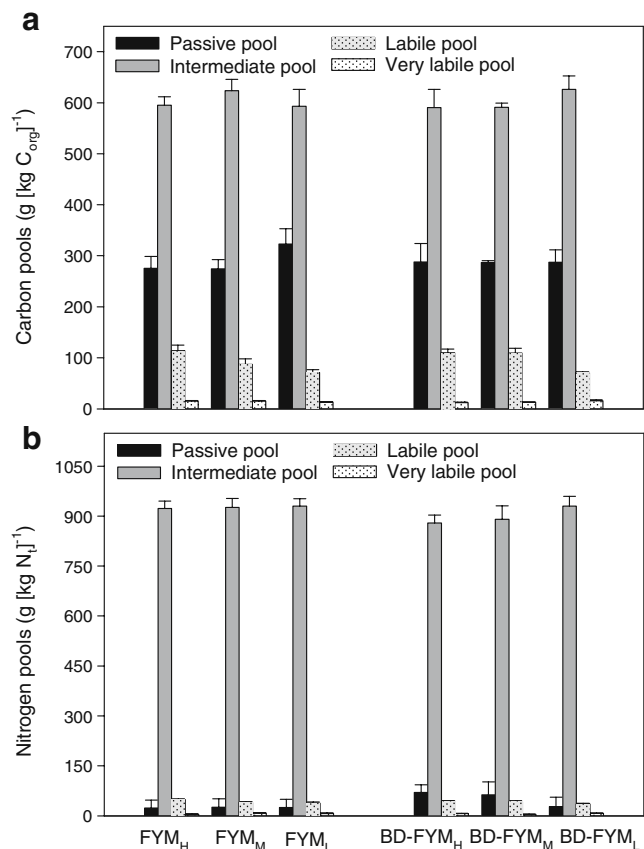


Table 3 Regression analysis of measured and modeled C and N pools of the soil (0–25 cm) between the rates and cumulative fertilizer N_t input (t ha^{-1}) from 1980 to 2007 ($n=24$).

Properties	Intercept ^a	Slope ^b	R^2	p Value
C_{org} (g kg^{-1})	7.5	0.4	0.13	0.086
C_{mic} (mg kg^{-1})	115	7	0.04	0.353
Very labile C ($\text{g [kg } C_{\text{org}}]^{-1}$)	15	0	0.02	0.546
Labile C ($\text{g [kg } C_{\text{org}}]^{-1}$)	47	21	0.53	< 0.001
$C_{\text{intermediate}}$ ($\text{g [kg } C_{\text{org}}]^{-1}$)	622	−9	0.02	0.492
C_{passive} ($\text{g [kg } C_{\text{org}}]^{-1}$)	316	−12	0.04	0.324
N_t (g kg^{-1})	0.75	0.05	0.14	0.082
N_{mic} (mg kg^{-1})	18	1	0.01	0.729
Very labile N ($\text{g [kg } N_t]^{-1}$)	8	−1	0.09	0.153
Labile N ($\text{g [kg } N_t]^{-1}$)	30	5	0.43	0.001
$N_{\text{intermediate}}$ ($\text{g [kg } N_t]^{-1}$)	946	−15	0.05	0.292
N_{passive} ($\text{g [kg } N_t]^{-1}$)	16	11	0.03	0.450

^a The unit is given within the first column

^b The unit is (unit of intercept) (t ha^{-1})^{−1}

between 5 and 7 $\text{g (kg } N_t)^{-1}$ and was not affected by treatment.

Crop yields

Yields for potatoes, winter rye, and clover increased significantly with the application rate but were unaffected by the application of BD preparations (Table 1). For spring wheat, we observed a trend ($p=0.10$) of an effect of the rate. A regression analysis (not shown) revealed an increase of yield by 20% (spring wheat, potatoes, clover) to 30% (winter rye) when N application with FYM or BD-FYM increased by 100%.

The yields of spring wheat, potatoes, and clover were significantly correlated with the labile N pool (Table 4). However, labile N and yields were both significantly related to the rates of farmyard manure (Table 3 and 4). Potato yields were also significantly correlated with N_t , N_{mic} , and $N_{\text{intermediate}}$. Correlations with C pools were similar (data not shown).

Table 4 Pearson correlation coefficients ($n=24$) of N pools with yields (t ha^{-1})

Properties	Spring wheat	Potatoes	Winter rye	Clover forage
N from manure ($\text{kg ha}^{-1} \text{ year}^{-1}$)	0.614**	0.637**	0.646**	0.834**
N_t (g kg^{-1})	0.274	0.722**	0.238	0.253
N_{mic} (mg kg^{-1})	−0.194	0.526**	−0.091	−0.077
Very labile N (g kg^{-1})	−0.134	−0.241	0.197	−0.020
Labile N (g kg^{-1})	0.565**	0.718**	0.344	0.564**
$N_{\text{intermediate}}$ (g kg^{-1})	0.258	0.640**	0.214	0.092
N_{passive} (g kg^{-1})	0.001	0.179	0.027	0.258

The correlation of crop yields with manure N was added for comparison

**($p \leq 0.05$)

Discussion

C and N inputs to the soil

The measured and estimated mean annual C inputs in the period from 2003 to 2006 increased in the order low rate < medium rate < high rate and ranged from 2.1 to 2.8 $\text{t C ha}^{-1} \text{ year}^{-1}$ and were identical for BD-FYM and FYM treatments. Our estimates of C inputs from roots and crop residues were within the range reported by others (van Veen et al. 1991; Aeschlimann et al. 2005).

The amount of N fixation estimated in our study ranged from 70 to 105 kg ha^{-1} in the year of clover cultivation and was well in the range (49–373 $\text{kg ha}^{-1} \text{ year}^{-1}$) reviewed by Ledgard and Steele (1992). However, our estimates of N fixation serve only as approximation, since increasing N availability with increasing FYM application may decrease the efficiency of N fixation. Consequently, the N fixation in treatments with high input could be overestimated.

This would mean that the differences in N input between the rates would be less distinct than presented in Fig. 1. Consequently, this would explain why there were not any significant differences in spring wheat yields (following clover cultivation) between the rates (Table 1). Nevertheless, Hatch et al. (2007) showed that direct application of uncomposted FYM (170 kg N ha⁻¹) to a new established red clover—grass sward decreased fixation efficiency by only 10% compared to an unfertilized sward. Therefore, other factors than different N fixation efficiency may also diminish rate effects on wheat yield. For instance, Raupp and Oltmanns (2006) reported that water availability restricted maximum wheat yields especially at high rates.

Microbial biomass and activity

Contents of C_{mic} were only low. These low contents may be explained by the sandy texture and low C_{org} content of the soil (Machulla et al. 2001). In the soil (sand-loam) of the long-term experiment in Skiernewiece, Poland, C_{mic} contents (70 to 150 mg (kg soil)⁻¹) reported by Weigel et al. (1998) were well comparable to the values obtained in this study. Other studies reported increasing contents of C_{mic} with increasing FYM additions for loamy and sandy soils (Dick 1992; Weigel et al. 1998); therefore, the lack of an increase with the rate in our study was unexpected. The last FYM applications occurred 29 months before sampling and the C input from berseem clover (previous crop) was estimated to be equal in all treatments (Fig. 1). This probably superimposed the effect of the application rate on microbial biomass in our study, suggesting that the reported response (Dick 1992; Weigel et al. 1998) may have been only short.

The absence of a significant effect of BD preparations on the content of C_{mic} or N_{mic} in our study is consistent with results from others (Carpenter-Boggs et al. 2000b; Zaller and Koepke 2004), indicating that BD preparations generally do not increase C or N incorporation by soil microbes.

The general increase of C and net N mineralization with the rate of FYM in our study is consistent with the results of others, reported for clayey loamy soils (Haploboroll, Haplargid) of temperate and subtropical climates (Whalen et al. 2001; Khorsandi and Nourbakhsh 2007). This suggests that increasing

FYM additions increase the potential of SOM for C and net N mineralization across various soil types, textures, and climates.

Total C_{org} and N_t contents

The effect of FYM to increase C_{org} levels (Table 3) with increasing rate has been reported for several soil types and textures (Christensen 1996; Weigel et al. 1998). Nevertheless, the quantitative effect of the FYM rates in this study was low (Table 3). A similar result was also reported by Christensen (1996) for a sand-loam. The less distinct rate effects on C_{org} levels of coarse textured soils indicate the importance of clay and silt particles for C stabilization in soils (von Lützow et al. 2008). Once the “capacity” of clay and silt particles approaches saturation, C input will be partitioned to a greater extent to a relatively labile pool (Stewart et al. 2008). These findings are supported by results of Heitkamp et al. (2011) from the field experiment of the current study. They showed that differences in mineral associated SOM were very small.

Since the initial C and N stocks in 1980 were not determined for individual treatments, there are uncertainties whether observed differences in the stocks of the different treatments in 2007 are entirely due to treatment effects. In the following paragraphs, arguments for two explanations of higher C_{org} contents in the BD-FYM treatments will be discussed:

1. The Darmstadt experiment was laid out as a strip design on alluvial sands. It has been shown by Heinze et al. (2009) that the pH value of the soil was specifically distributed with the highest values in the northern and the lowest values in the southern part. The pH value influenced some soil properties significantly (such as C_{org}) and Fig. 2 shows high spatial variation (standard errors) for the C_{org} contents. Therefore, explanation 1 for the higher C_{org} contents in the BD-FYM treatments is an unrecognized spatial heterogeneity since the beginning of the experiment and the differences in C_{org} were not induced by treatments.
2. In the DOK experiment, Switzerland, Fließbach et al. (2007) showed the soils of the BIOORG (comparable to FYM treatments in this study) treatment lost 6% of initial C_{org} , whereas BIO-DYN (comparable to BD-FYM treatments in this

study) treatments lost only 3% of C_{org} after 6 years in a loamy Luvisol. This shows that rapid effects of fertilization treatments might appear which was also supported by the high temporal variations of C_{org} contents in the sandy soil at Darmstadt (Fig. 2). Therefore, explanation 2 is an immediate effect of BD preparations on C and N storage and the preserving effect of BD-FYM treatments has lasted ever since.

The findings of Fließbach et al. (2007) apparently support explanation 2. However, the farmyard manure was left to rot in FYM as well as in the BD-FYM treatment in the Darmstadt experiment, whereas the farmyard manure in the DOK trial was left to rot in the BIOORG and was composted in the BIODYN treatment. Therefore, the use of BD preparations is not the only factor differing between treatments in the DOK trial. Furthermore, the difference of C_{org} in BIOORG and BIODYN in the DOK trial was diverging with time. This had to be expected due to the application of more stable compost in the BIODYN treatment. In the Darmstadt experiment, relative losses since the first 4-year period (1982–1985) were higher in the BD-FYMH and BD-FYMM treatments, compared to the corresponding FYM treatments (Table 2). This suggests a slow convergence between FYM and BD-FYM treatments, indicating that explanation 1 is more likely than explanation 2.

C and N pools

Our finding that the passive C and N pools were unaffected by fertilization treatments is consistent with the concept that the passive pool (assumed turnover time $\gg 600$ years) is not influenced by recent management (Helfrich et al. 2007). Although the values (approximately $300 \text{ g C (kg } C_{org})^{-1}$, Fig. 3) did not differ significantly between treatments, the amount of C in the passive pool (in $\text{g C (kg soil)}^{-1}$) was higher in the BD-FYM treatments with high and medium rate and accounted on average for 30% of the higher C_{org} values compared to the FYM treatments. Thus, at least 30% of the differences in C_{org} contents cannot be ascribed to treatment effects and are likely to have existed before the beginning of the experiment.

With increasing decomposition the C/N ratio generally declines (Swift et al. 1979). Thus, the C/N

ratio of the passive pool should be narrower than that of the bulk soil (Helfrich et al. 2007). This is, however, not the case for highly recalcitrant material such as charcoal or soot, which can have wide C/N ratios. The high C/N ratio (70) of passive SOM revealed in this study suggests the involvement of charred material in the formation of the passive pool.

The absence of a rate effect for both FYM types on storage of C or N in the intermediate pool (Fig. 3) indicated that the efficiency for C or N sequestration in a more stable form decreased with increasing rate. Instead, increasing FYM applications led to an accumulation of labile C and N in SOM. This shows that increasing input of organic matter may shift relative partitioning in favor of labile pools (Stewart et al. 2008). In our study, we found an increase of $5 \text{ g (kg } N_t)^{-1}$ per ton applied FYM- N_t (Table 3) in the labile N pool (corresponding to an increase of $6.0 \text{ mg (kg soil)}^{-1}$ (FYM- N_t) $^{-1}$). Increasing amounts of labile N with increasing FYM application rates have been reported for a loamy Chernozem by Whalen et al. (2001). They reported after 25 years of FYM applications (30 to $180 \text{ t FYM ha}^{-1}$, corresponding to ca. 320 to $1200 \text{ kg } N_t \text{ ha}^{-1}$) an increase (mg (kg soil)^{-1} ($\text{t FYM-}N_t$) $^{-1}$) of 2.2. Thus, the increase found in our study after two decades at comparably low rates of FYM application is relatively high. Two possible explanations for this finding exist. Firstly, at lower rates, such in the present study, more of the mineralized FYM-N will be taken up by plants, remain partly in the soil as crop residue and may build up the labile N pool (Fig. 4). With increasing rates of organic fertilizers, increasing N leaching occurs (Kirchmann and Bergström 2001) and the relative contribution to the buildup of labile N will be lower. Secondly, a smaller part of FYM-N may be stabilized in labile form and, due to higher physical and chemical protection from mineralization in loamy soils (von Lützow et al. 2008), a greater part in the intermediate pool in the loamy Chernozem studied by Whalen et al. (2001).

Crop yields

Compared to average yields of Germany, the yields are relatively low. For instance, mean yields in Germany (for years given in Table 1, ton per hectare) of spring wheat (5.3), potatoes (44.2), rye (5.1), and clover/grass-clover (8.4) (Statistisches Bundesamt

Deutschland 2009) were markedly higher than in the Darmstadt experiment. In Darmstadt, the crops yielded between 40% and 70% of the German average, due to the relatively dry site conditions and the low soil fertility of the sandy soil. Only few long-term experiments in Europe exist on low fertility sandy soils. In the fertilization experiment in Thyrow, Germany ($15 \text{ t FYM ha}^{-1} \text{ year}^{-1}$), potato yields were comparable (20 t ha^{-1}) with those of Darmstadt. The winter rye yield was remarkably higher than the yield at the Sandmarken site at Askov, Denmark (1.7 t ha^{-1}) (Edmeades 2003).

Crop yields of spring wheat, winter rye, and clover were not significantly related to the N_t content (Table 4). Similarly, Loveland and Webb (2003) reported that they did not find a critical SOM level below which crop yields would seriously decline, provided that crops were fertilized. The correlation of potato yields with N_t and $N_{\text{intermediate}}$ may be related to other beneficial effects of SOM, such as increased cation exchange capacity (Loveland and Webb 2003). Nitrogen supply from $N_{\text{intermediate}}$ is supposed to be very low, and this pool is more important in long-term storage (Benbi and Richter 2002) and slow replenishment of the labile pool (Fig. 4).

With the exception of winter rye, crop yields were positively related to the labile N pool (Table 4). This suggests that a relevant proportion of N uptake by plants was provided by N from SOM mineralization. In fact, Gardner and Drinkwater (2009) showed in a meta-analysis of ^{15}N -tracer studies that ca. 60% of N taken up by crops (mineral fertilization) was derived from SOM. Recovery of ^{15}N in cereals from organic fertilizers (straw or feces) was even lower and ranged from 6% to 17% in the first and from 3% to 6% in the second growing season (Christensen 2004). Therefore, a large proportion of organic fertilizers will remain in the SOM pool and may be mineralized subsequently. Consequently, no single conclusion can be drawn whether the crop yields were related to either FYM input or to labile N. More likely, with time a dynamic equilibrium (Fig. 4) will be established between FYM applications, buildup of labile N, mineralization of labile N, plant growth, and return of crop residue (Christensen 2004). This view is supported by inter-correlations between yields, labile N, and FYM rates (Table 4). However, correlations cannot provide mechanistic explanations and studies

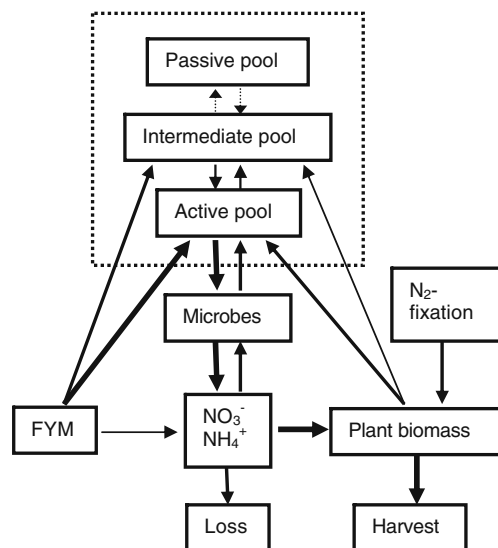


Fig. 4 Simplified scheme of the N flow through soil organic matter and crops in an agro-ecosystem. Adapted and modified according to Christensen (2004)

using ^{15}N -labeled organic fertilizers will be required to further deepen the understanding about interactions of SOM buildup and plant nutrition in organically managed agro-ecosystems.

Conclusions

The previously reported (Bachinger 1996; Raupp and Oltmanns 2006) differences of C_{org} contents between soils receiving FYM with and without BD preparations most likely existed before the commencement of the field experiment. At least 30% of the differences could not be ascribed to treatment effects due to storage in the passive pool. The absence of an effect of the FYM rates on the intermediate pool indicated that management of sandy soils via manure application rates does not offer the option of large sequestration of carbon and thus reduction of greenhouse gases.

The labile SOM pools were significantly related to the rates of farmyard manure (with or without BD preparations) and also to the crop yields. However, for a quantification and mechanistic explanation of these interrelations, long-term studies with isotopically labeled organic fertilizers will be required.

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