

Emission rates of N₂O and CO₂ from soils with different organic matter content from three long-term fertilization experiments—a laboratory study

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Abstract Increasing organic matter stocks in soils reduce atmospheric carbon dioxide (CO₂), but they may also promote emissions of nitrous oxide (N₂O) by providing substrates for nitrification and denitrification and by increasing microbial O₂ consumption. The objectives of this study were to determine the effects of fertilization history, which had resulted in different soil organic matter stocks on (1) the emission rates of N₂O and CO₂ at a constant soil moisture content of 60% water-holding capacity, (2) the short-term fluxes of N₂O and CO₂ following the application of different fertilizers (KNO₃ vs. farmyard manure from cattle) and (3) the response to a simulated heavy rainfall event, which increased soil moisture to field capacity. Soil samples from different treatments of three long-term fertilization experiments in Germany (Methau, Spröda and

Bad Lauchstädt) were incubated in a laboratory experiment with continuous determination of N₂O and CO₂ emissions and a monitoring of soil mineral N. The long-term fertilization treatments included application of mineral N (Methau and Spröda), farmyard manure + mineral N (Methau and Spröda), farmyard manure deposition in excess (Bad Lauchstädt) and nil fertilization (Bad Lauchstädt). Long-term addition of farmyard manure increased the soil organic C (SOC) content by 55% at Methau (silt loam), by 17% at Spröda (sandy loam) and by 88% at Bad Lauchstädt (silt loam; extreme treatment which does not represent common agricultural management). Increased soil organic matter stocks induced by long-term application of farmyard manure at Methau and Spröda resulted in slightly increased N₂O emissions at a soil moisture content of 60% water-holding capacity. However, the effect of fertilization history and SOC content on N₂O emissions was small compared to the short-term effects induced by the current fertilizer application. At Bad Lauchstädt, high N₂O emissions from the treatment without fertilization for 25 years indicate the importance of a sustainable soil organic matter management to maintain soil structure and soil aeration. Emissions of N₂O following the application of nitrate and farmyard manure differed because of their specific effects on soil nitrate availability and microbial oxygen consumption. At a soil moisture content of 60% water-holding capacity, fertilizer-induced emissions were higher for farmyard manure than for nitrate. At field capacity, nitrate application induced the highest emissions. Our results indicate that feedback mechanisms of soil C sequestration on N₂O emissions have to be considered when discussing options to increase soil C stocks.

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Introduction

Carbon sequestration in soils reduces atmospheric CO₂, but it may result in increased N₂O emissions. The global data analysis on N₂O emission from agricultural fields by Stehfest and Bouwman (2006) suggest that N₂O emissions increase with increasing soil organic C content. Furthermore, it was shown that organic matter accumulation in topsoils by no-till farming can stimulate N₂O emission and that this effect occurs in particular in fine-grained, poorly aerated soils (Smith and Conen 2004; Rochette 2008). The long-term application of organic fertilizers may not only improve soil quality but also contribute to climate protection by increasing C sequestration in soils (Powelson et al. 1998; Lal 2004; Blair et al. 2006; Janzen et al. 2006). However, increased contents of organic C and total N (N_t) in arable soils by regular application of organic fertilizers may also promote emissions of N₂O. Thus, from the view of global warming, it is crucial to assess the impacts of C sequestration strategies not only for CO₂ but also for the greenhouse gas N₂O (Qiu et al. 2009).

Several authors have reported increased N₂O emissions directly after the application of organic fertilizers (Chang et al. 1998; Petersen 1999; Flessa and Beese 2000; Van Groenigen et al. 2004). In particular, easily available organic matter fractions were found to trigger N₂O emissions since they promote the formation of anoxic microsites in soils (Parkin 1987; Flessa and Beese 1995) and because they provide an easily available substrate for nitrification and denitrification (Velthof et al. 2003; Chang et al. 1998). However, the significance of long-term effects of SOC accumulation in agricultural soils on N₂O emissions is less well understood. The few experimental results on long-term effects of increased organic matter stocks do not provide a consistent picture (Chang et al. 1998; Kilian et al. 1998; De Wever et al. 2002; Meng et al. 2005) even though it is often assumed that increased C sequestration results in increased N₂O emissions. Kilian et al. (1998) reported a promoting effect of C and N enrichment in arable soils on N₂O release, whereas Meng et al. (2005) found that long-term application of manure on a sandy loam did not result in greater N₂O emissions than those observed for the application of mineral fertilizer, despite higher C and N contents in the manured soil. Model results suggest that N₂O emissions probably increase with increasing C sequestration in soils (Li et al. 2005; Qiu et al. 2009). The inconsistent picture with regard to the long-term effects of C sequestration on N₂O emissions might be partly due to the different experimental conditions and site-dependent differences in the extent of organic matter accumulation.

We hypothesize that the long-term application of farmyard manure and the associated increase in SOC and N_t stocks increase N₂O emissions from soils and that it

affects the emissions following fertilizer application and rainfall events. The objectives of this study were to determine the effects of long-term fertilization of differently textured soils with either mineral fertilizer or farmyard manure, which result in different soil organic C (SOC) and N_t stocks, on (1) the emission rates of N₂O and CO₂ at a constant soil moisture content of 60% water-holding capacity, (2) the short-term fluxes of N₂O and CO₂ following the application of different fertilizers (KNO₃ vs. farmyard manure from cattle) and (3) the response to a simulated heavy rainfall event, which increased soil moisture to field capacity.

Materials and methods

Study site and soil sampling

In October 2008, soil samples from the Ap horizon (0–25 cm) were collected from three German long-term fertilization experiments in Spröda, Methau and Bad Lauchstädt. Samples were taken 12 months (Bad Lauchstädt) and 7 months (Spröda and Methau) after the last fertilizer application at all sites (except for the unfertilized treatment at Bad Lauchstädt).

The field experiment at Spröda (51°32' latitude, 12°26' longitude), Saxony, was established in 1966. The annual rainfall is 540 mm and the mean annual temperature is 8.3°C. The soil is an albic Luvisol (WRB), and the soil texture consists of mainly sand and silt, whereas the clay content in the soil was only small (6%, Table 1). The crop rotation consisted of winter wheat (2005), sugar beet (2006), spring barley (2007) and potato (2008). Straw was removed from the field. Long-term fertilization consisted of 150 kg N ha⁻¹ year⁻¹ as calcium ammonium nitrate (Mineral N treatment) and additionally 2,100 kg Cha⁻¹ and 102 kg N ha⁻¹ as cattle farmyard manure every second year (Mineral N and manure treatment, total annual N input=201 kg N ha⁻¹; Albert and Lippold 2002).

The field experiment at Methau (51°04' latitude, 12°51' longitude), Saxony, was also established in 1966. The annual rainfall is 600 mm and the mean annual temperature is 8.0°C. The soil is gleyic Luvisol (WRB) with silt and clay contents of 80% and 15%, respectively. Crop rotation and long-term fertilization treatments were the same as at Spröda (Albert and Lippold 2002).

The experiment in Bad Lauchstädt (51°24' latitude, 11°53' longitude), Saxony-Anhalt, started in 1983. The annual rainfall is 484 mm and the mean annual temperature is 8.7°C.

The soil is a haplic Chernozem (WRB) with silt and clay contents of 68% and 24%, respectively. The crop rotation consisted of sugar beet (2006), silage maize (2007) and potato (2008). The organic fertilized field plots in Bad Lauchstädt were treated with cattle manure in excess

Table 1 Description of soil characteristics (0–25 cm) at the three sites with different long-term fertilization histories

Site	Long-term fertilization history	OC mg g ⁻¹	OC g vessel ⁻¹	N _t mg g ⁻¹	CEC mmol _c kg ⁻¹	pH	Sand %	Silt %	Clay %
Methau	Mineral N	9.9 a	11.9	1.02 a	107.2 a	6.4	5	80	15
	Mineral N and manure	15.3 b	18.4	1.48 b	125.7 b	6.4			
Spröda	Mineral N	7.1 a	11.4	0.65 a	45.4 a	5.6	63	31	6
	Mineral N and manure	8.3 b	13.3	0.75 b	48.8 a	5.3			
Lauchstädt	Control	21.6 a	25.9	1.79 a	275.8 a	7.2	8	68	24
	Excess manure	40.5 b	48.6	3.52 b	389.2 b	7.1			

For the contents of organic C (OC) and N_t and the CEC, means are shown ($n=4$). Values followed by different letters indicate significant differences between two fertilization treatments of the same site (Student t test: $p<0.05$)

(excess manure treatment). The application rate of 100 t manure ha⁻¹ year⁻¹ (dry matter content 24.4% with 424 g C_{org} kg⁻¹ dry matter, 28.5 gN_tkg⁻¹ dry matter calculated as mean over the last 4 years) greatly exceeded a common fertilization rate. It represents an extreme example of manure deposition. The second treatment at Bad Lauchstädt was not fertilized (control treatment) (Körschens et al. 1998).

Incubation experiments

Field-moist, sieved (<6 mm) soil was filled in cylindrical incubation vessels with a height of 10 cm and a diameter of 14.4 cm (volume of 1.65 l). The filling height was 7 cm for all samples. The soils were compacted to bulk densities which were typically observed in the surface horizons of these soils (1.1 gcm⁻³ for the silty samples from Methau and Bad Lauchstädt and 1.4 gcm⁻³ for the sandy soil from Spröda). The corresponding soil dry weight in each incubation vessel was 1.2 kg for the treatments from Methau and Bad Lauchstädt and 1.6 kg for the treatments from Spröda. Soil samples were adjusted to a soil moisture content of 60% water-holding capacity and incubated for 105 days at 12°C in darkness. The set soil moisture content was controlled gravimetrically and readjusted if necessary. The incubation experiment consisted of the consecutive periods:

Periods: (1) during the first 23 days, soil samples (eight replicates per treatment) were incubated at a soil moisture content of 60% water-holding capacity to determine N₂O and CO₂ fluxes under well-aerated conditions; (2) the second incubation period started with the application of fertilizer at day 24 and lasted for 28 days until day 51. Four replicates each were fertilized with KNO₃ (mineral N treatment) and cattle farmyard manure (manure treatment), respectively, to determine short-term effects of fertilizer addition. In the mineral N treatment, KNO₃ was applied superficially at a rate of 100 kg Nha⁻¹ (165 mg N per vessel) with 15 ml distilled water. The change in mean soil moisture due to fertilizer addition was small (less than +2%

gravimetric soil moisture). In the manure treatment, cattle manure was mixed with the upper 2 cm of the soil at the same rate (100 kg Nha⁻¹, 165 mg N and 2440 mg C per vessel); (3) at day 51, the third period started with the addition of water to determine the effect of a simulated rainfall and the associated increase of soil moisture to field capacity on emission rates of N₂O and CO₂. At day 52, we added water to each soil sample until 100% water-holding capacity was reached. Water was injected into the samples to ensure a homogeneous increase of soil moisture within the samples. The water-holding capacity of the treatments differed as a result of different soil texture and organic C content. The adjusted soil moisture during period 3 equalled 75% water-filled pore space (WFPS) at Methau, 50% WFPS in Spröda and 90% WFPS at Bad Lauchstädt.

Overall, 12 different treatments were performed which differed with respect to the sites, the fertilization history and the fertilization in period 2 of the incubation experiment (Table 2).

Automated CO₂ and N₂O flux measurements

The incubation vessels were sealed with a lid, which had an air inlet port and an air outlet port. The headspace of each incubation vessel was continuously flushed with 10 ml min⁻¹ of fresh air. Concentrations of CO₂ and N₂O in the fresh air input, in the exhaust air of each incubation vessel and in calibration gases were measured automatically every 4 h during the entire incubation period using an automated gas chromatographic system as described by Flessa and Beese (1995) and Loftfield et al. (1997). Gas flux rates were calculated from the air flow rate through the incubation vessels and the difference in the gas concentration between the input air and the exhaust air.

Soil analyses

The organic C and the total N contents of all soil samples were determined after drying and grinding by an automated C and N analyzer (Heraeus Elementar Vario EL, Hanau,

Table 2 Emissions of CO₂ (means, $n=8$ for period 1 and $n=4$ for the other periods) from soils with different fertilization history during the incubation in the three consecutive periods

Site	Long-term fertilization history	Period 1	Treatment in incubation period	Period 2	Period 3	Cumulative
		CO ₂ emission rate mg C m ⁻² h ⁻¹	2	CO ₂ emission rate mg C m ⁻² h ⁻¹	CO ₂ emission rate mg C m ⁻² h ⁻¹	CO ₂ emission g C m ⁻²
Methau	Mineral N	11.1 a	Mineral N	14.0 aA	6.3 aA	23.4 aA
			Manure	41.2 aB	26.5 aB	66.3 aB
	Mineral N & manure	17.5 b	Mineral N	14.3 aA	6.4 aA	27.7 aA
			Manure	40.8 aA	26.6 aB	69.2 aB
Spröda	Mineral N	10.8 a	Mineral N	11.6 aA	6.8 aA	21.9 aA
			Manure	36.2 aB	25.0 aB	61.2 aB
	Mineral N & manure	13.4 b	Mineral N	11.4 aA	6.0 aA	22.7 aA
			Manure	23.5 bB	26.3 bB	55.3 aB
Bad Lauchstädt	Control	14.3 a	Mineral N	11.5 aA	9.0 aA	26.3 aA
			Manure	29.2 aB	27.3 aB	61.2 aB
	Excess manure	28.5 b	Mineral N	17.3 bA	13.0 bA	43.8 bA
			Manure	46.3 bB	33.8 bB	88.2 bB

Period 1 (constant soil moisture content of 60% water-holding capacity) lasted for 23 days, period 2 (application of mineral N or farmyard manure) for 29 days and period 3 (simulated heavy rainfall) for 53 days. Different lowercase letters indicate significant differences between the long-term fertilization treatments and different capital letters indicate significant differences between the fertilization treatments at the beginning of period 2 (Mann–Whitney U test, $p<0.05$)

Germany). Soil pH was measured in a 10⁻² M CaCl₂ solution with a soil/solution ratio 1:2.5. To quantify the effective cation exchange capacity (CEC), soil samples were leached with 100 ml 1 M ammonium chloride (NH₄Cl) for 4 h as described by König and Fortmann (1996). The concentrations of cations in the extract were measured by ICP-AES (Spectro, Kleve, Germany). The concentrations of extractable NH₄⁺ and NO₃⁻ were measured after extraction with 10⁻² M CaCl₂ solution. The soil/solution ratio during extraction was 1:2. The photometric analysis of mineral N was performed using a continuous flow analyzer (S/A 20/40 Skalar Analytical, Erkelenz, Germany). Soil samples for the extraction of mineral N were taken four times during the incubation period: on days 0, 24 (before fertilizer application), 68 (16 days after increasing soil moisture to field capacity) and 105 (at the end of the incubation).

The fractionation of water-stable soil aggregates was conducted after the incubation experiment with the four treatments from the site Bad Lauchstädt in order to obtain further insights into the causes for the unexpected emission patterns of these soils (discussed below). The fractionation scheme was modified from the method described by Elliott (1986) in accordance with Helfrich et al. (2008). Briefly, 100 g of dried soil were soaked in distilled water for 10 min to allow slaking. The mixture was poured into a 250 μ m sieve, which was moved up and down in water by approximately 3 cm 50 times. Aggregates >250 μ m (macroaggregate fraction) were collected, and sieving was repeated using a 53- μ m sieve. Aggregates (53–250 μ m; microaggregate fraction) were collected, and particles <53 μ m were precipitated with 0.5 M AlCl₃. All size fractions (macroaggregates=250–2,000 μ m, microaggre-

gates=53–250 μ m, fraction <53 μ m) were oven-dried at 40°C and weighed.

Statistical analyses

The Student t test ($p<0.05$) was used to test for the differences between the two soils of the same site with different fertilization history with regard to the content of SOC, total N (N_t) and CEC. The Mann–Whitney U test ($p<0.05$) was employed to test for the differences between mean emission rates of CO₂, N₂O and contents of NO₃⁻ measured in treatments with different long-term fertilization history and the differences between emissions following the fertilizer application in incubation period 2 because data were not normally distributed.

Results

Soil organic matter and total N

At all sites, the different fertilization history resulted in significantly higher SOC and N_t contents in the organic fertilized treatment than in the treatments with long-term mineral N application. This was especially important for the Bad Lauchstädt soil with the excessive manure fertilization (Table 1). Besides the annual quantity of added manure, the soil texture also affected the organic C stocks. The contents of SOC and N_t increased with increasing soil clay content in the order Spröda<Methau<Bad Lauchstädt. The increase in the SOC stocks induced by long-term application of farmyard manure (calculated as difference of the SOC

contents of manure and mineral N (Spröda, Methau) or nil fertilization treatments (Bad Lauchstädt) increased with increasing clay content (Spröda 1.2 mg kg⁻¹, Methau 5.4 mg kg⁻¹, Bad Lauchstädt 18.9 mg kg⁻¹). The same order was found for the effect of fertilization history on total soil N content (Table 1).

Emissions of CO₂

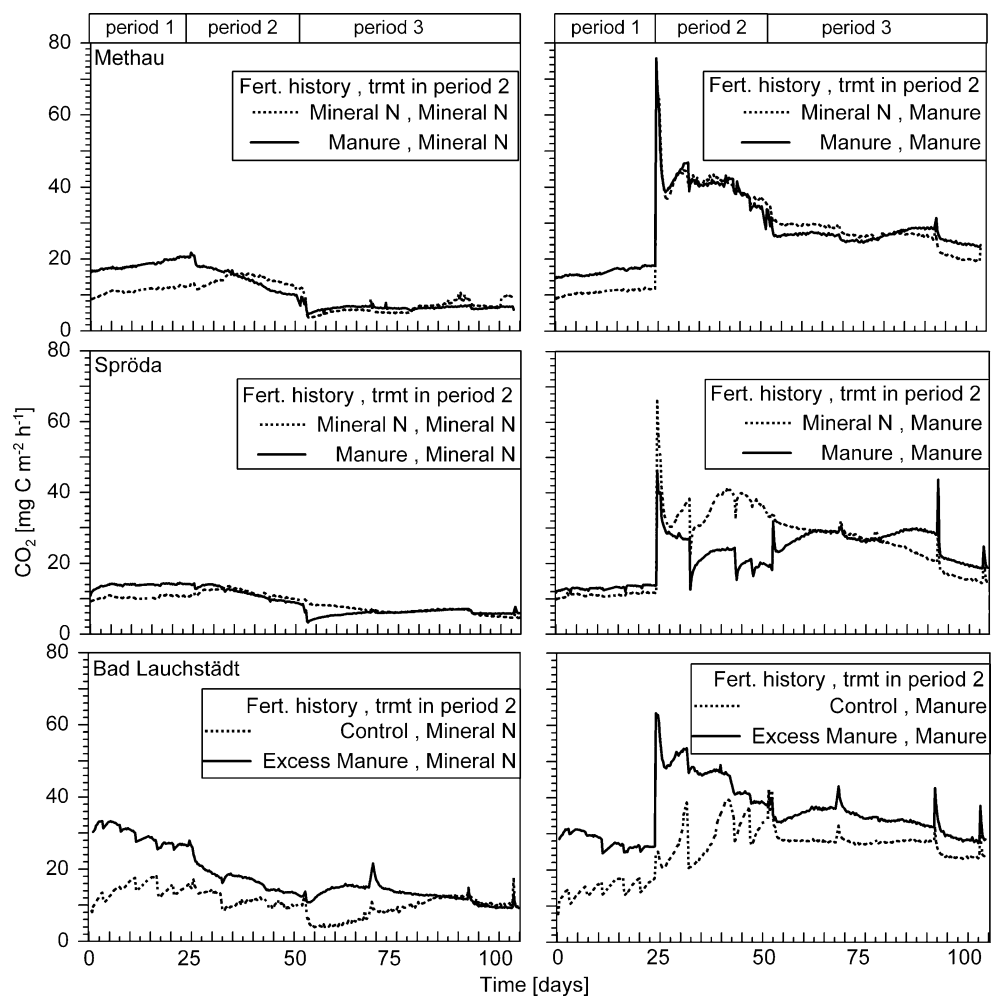
The emissions of CO₂ during period 1 (at constant soil moisture of 60% water-holding capacity) were 1.2 to 2.0 times higher for the treatments with long-term application of farmyard manure than those with mineral N or nil fertilization (Table 2, Fig. 1). As expected, the excess manure treatment at Bad Lauchstädt exhibited much higher soil respiration rates than all the other treatments. The specific SOC mineralization during period 1, which was calculated from the ratio (total CO₂-C emission during period 1)/(total SOC content) and which reflects the mean stability of SOC, decreased with increasing SOC content in the order Spröda > Methau > Bad Lauchstädt. The application of farmyard

manure for many years had no clear effect on the calculated specific mineralization of SOC during period 1.

The application of farmyard manure at the beginning of period 2 induced a rapid increase in CO₂ emissions from all soils and emissions remained on a higher level than the soil fertilized with mineral N until the end of the incubation experiment (Fig 1).

We estimated the quantity of the added farmyard manure, which was mineralised during period 2 (29 days), from the difference of the cumulative CO₂ emissions calculated for the treatments with and without farmyard manure addition. This approach assumes that manure application does not change mineralization of SOC (no priming effects). Using this simplified approach, estimated mineralization of the manure (added at the beginning of period 2) during incubation period 2 decreased in the order (site and fertilization history in parentheses) 13.5% (Bad Lauchstädt, excess manure) >12.6% (Methau, mineral N) >12.3% (Methau, mineral N and manure) >11.4% (Spröda, mineral N) >8.2% (Bad Lauchstädt, control) >5.6% (Spröda, mineral N and manure). Thus, a long-term history

Fig. 1 Mean emission rates of CO₂ from the three soils (Methau, Spröda, Bad Lauchstädt) each with different fertilization history (Fert. history: Manure/Excess Manure or mineral fertilizers/no fertilizer (Control)) at constant soil moisture of 60% water holding capacity (period1), following the application of KNO₃ (Mineral N) and farmyard manure (Manure; treatment in period 2), and after increasing soil moisture to field capacity (period 3)



of organic fertilization had no clear effect on the mineralization of farmyard manure in these soils. Carbon dioxide emissions of the treatments, which received no manure during the experiment, were generally lowest during period 3 when soil moisture was increased to field capacity (Table 2).

In addition to the general dynamics of CO₂ emission rates during periods 1 to 3, there were distinct short-term fluctuations of CO₂ emissions in each period. These fluctuations were due to the addition of small quantity of water to readjust soil moisture.

Emissions of Nitrous oxide and soil mineral N

Nitrous oxide emissions during period 1 (at constant soil moisture content of 60% water-holding capacity) were higher for treatments with long-term application of farmyard manure than mineral fertilizer at the sites Methau and Spröda (Table 3). At Methau, a 1.5 times higher SOC content in the mineral N and manure treatment was associated with a 2.5 times higher N₂O emission rate compared to the mineral N treatment. At Spröda, N₂O emission rates during period 1 were 9.0 times higher for mineral N and manure treatment than the mineral N treatment. However, the results from Bad Lauchstädt did not agree with the hypothesis that N₂O emissions increase with increasing content of SOC and N_t in soil. Surprisingly, the excess manure treatment showed a 2.3 times lower emission rate of N₂O during period 1 than the unfertilized treatment, which contained much less SOC and total N (Tables 1 and 3). The soil without fertilizer application for 25 years showed higher N₂O emission rates during period 1

than all the other soils, which were analyzed in our experiment.

The application of farmyard manure at the beginning of period 2 resulted in increased N₂O emission rates from all three soils (Fig. 2). The response to farmyard manure application was fast in all treatments. Increased emission rates were measured within the first four hours after manuring. The emissions which were induced by manure application were considerably higher than those occurring after the application of mineral fertilizer for three of the four treatments of the sites Methau and Spröda (Table 3, Fig. 2). At Spröda, emissions from soil with the fertilization history of mineral N and manure were higher than from the soil with the mineral N fertilization history when mineral N was applied in period 2 (Table 3).

The Bad Lauchstädt soils showed results which were very different from the Spröda and Methau soils: the unfertilized treatment had the highest N₂O emission rates after application of mineral N in period 2 (Fig. 2), and these emissions were much higher than those measured from all other soils and treatments. The extreme treatments at the Bad Lauchstädt site (nil fertilization and excess manure treatments) showed differences following application of KNO₃. Emission rates of N₂O from the nil fertilization treatment in period 2 were seven times higher than from the treatment with the excess manure history (Table 3, Fig. 2).

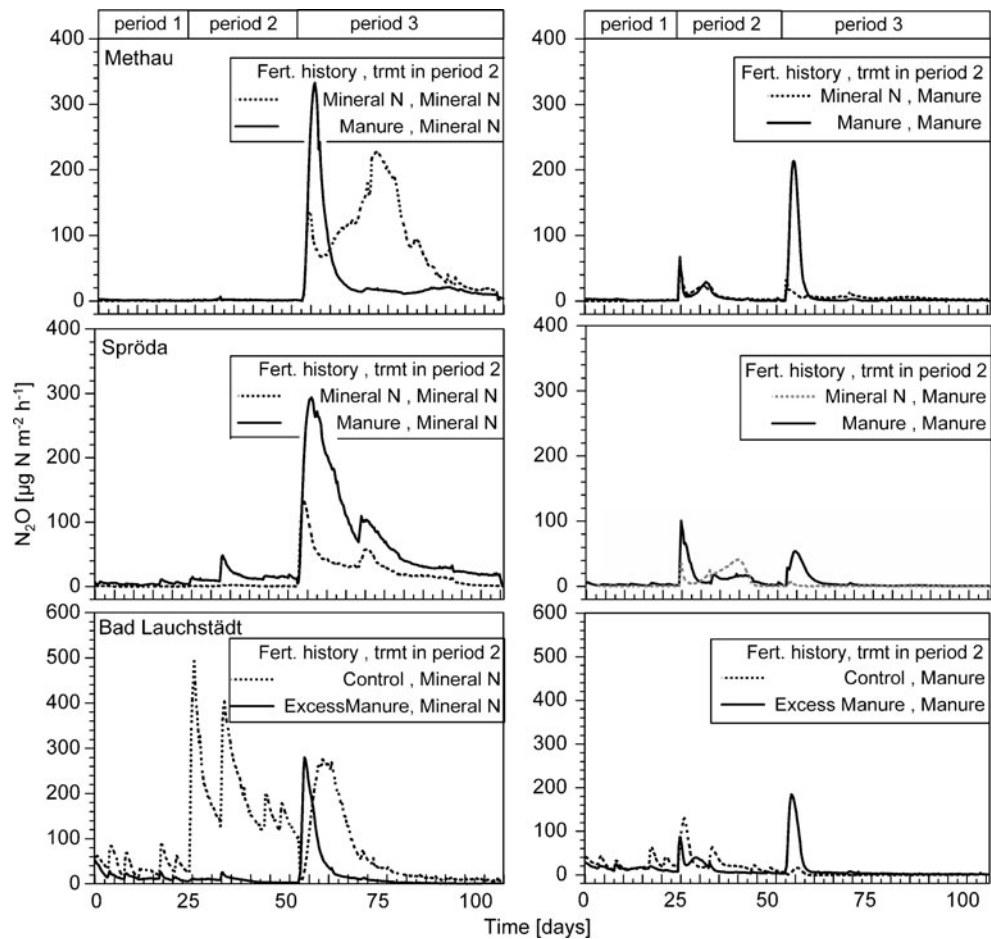
The increase in soil moisture to field capacity at the beginning of period 3 stimulated N₂O emissions at all treatments (Fig. 2), and the fast increase in N₂O emissions after the simulated heavy rainfall event was higher and

Table 3 Emissions of N₂O (means, $n=8$ for period 1 and $n=4$ for the other periods) from soils with different fertilization history during the incubation in the three consecutive periods

Site	Long-term fertilization history	Period 1	Treatment in incubation period 2	Period 2	Period 3	Cumulative N ₂ O emission
		N ₂ O emission rate $\mu\text{g N m}^{-2}\text{h}^{-1}$		N ₂ O emission rate $\mu\text{g N m}^{-2}\text{h}^{-1}$	N ₂ O emission rate $\mu\text{g N m}^{-2}\text{h}^{-1}$	
Methau	Mineral N	0.6 a	Mineral N	1.8 aA	90.1 aA	114.7 aA
			Manure	9.6 aB	6.0 aB	13.8 aB
	Mineral N & manure	1.5 b	Mineral N	1.6 aA	41.2 bA	51.6 bA
			Manure	8.5 aB	14.7 aB	22.5 aB
Spröda	Mineral N	0.3 a	Mineral N	1.0 aA	29.2 aA	36.5 aA
			Manure	15.3 aB	0.5 aB	10.6 aA
	Mineral N & manure	2.7 b	Mineral N	16.0 bA	83.5 aA	115.7 aA
			Manure	16.2 aA	6.4 bB	19.5 bA
Bad Lauchstädt	Control	34.6 a	Mineral N	211.2 aA	65.1 aA	240.4 aA
			Manure	30.5 aB	0.8 aB	34.3 aB
	Excess manure	15.0 b	Mineral N	8.2 bA	25.3 bA	43.7 bA
			Manure	15.9 aB	13.5 bA	30.8 aA

Period 1 (constant soil moisture content of 60% water-holding capacity) lasted for 23 days, period 2 (application of mineral N or farmyard manure) for 29 days and period 3 (simulated heavy rainfall) for 53 days. Different lowercase letters indicate significant differences between the long-term fertilization treatments and different capital letters indicate significant differences between the fertilization treatments at the beginning of period 2 (Man-Whitney *U* test, $p<0.05$)

Fig. 2 Mean emission rates of N₂O from the three soils (Methau, Spröda, Bad Lauchstädt) each with different fertilization history (Fert. history: Manure/Excess Manure or mineral fertilizers/no fertilizer (Control)) at constant soil moisture of 60% water holding capacity (period1), following the application of KNO₃ (Mineral N) and farmyard manure (Manure; treatment in period 2), and after increasing soil moisture to field capacity (period 3)



prolonged for those treatments which were fertilized with mineral N at the beginning of period 2 than for those amended with farmyard manure: N₂O emissions rates ($\mu\text{g N m}^{-2} \text{ h}^{-1}$) in period 3 decreased in the order (site, fertilization history and fertilization at the beginning of incubation period 2 in parentheses) 90.1 (Methau, mineral N, mineral N) $>$ 83.5 (Spröda, manure, mineral N) $>$ 65.1 (Bad Lauchstädt, control, mineral N) $>$ 41.2 (Methau, manure, mineral N) $>$ 29.2 (Spröda, mineral N, mineral N) $>$ 25.3 (Bad Lauchstädt, excess manure, mineral N, Table 3). In contrast, the other treatments with manure application at the beginning of incubation period 2 exhibited N₂O emission rates in a range of 0.5 to 14.7 $\mu\text{g N m}^{-2} \text{ h}^{-1}$ in period 3 (Table 3).

The initial contents of nitrate in the studied soils were similar with the exception of the Bad Lauchstädt soil with long-term application of excess manure (Fig. 3). In all soils, ammonium contents were always lower than 0.01 mg NH₄⁺-N kg⁻¹ (data not shown). Nitrate contents in the incubation periods 2 and 3 depended largely on the fertilization performed at the beginning of period 2. The availability of soil nitrate was increased in treatments with

KNO₃ addition, whereas it slightly decreased after application of farmyard manure (Fig. 3).

Soil aggregate stability of the treatments from Bad Lauchstädt

The two treatments at the Bad Lauchstädt site largely differed in soil structure. This difference was obvious by visual observation because the long-term deposition of manure (excess manure) led to a friable soil structure, whereas the treatments without any fertilization exhibited a sticky structure. To detect these differences and to elucidate the unexpected observation that long-term deposition of manure (excess manure) resulted in lower emissions of N₂O than the unfertilized soil, the distribution of water-stable aggregate fractions (Table 4) was determined for the treatments of the Bad Lauchstädt site. The proportion of megaaggregates ($>2,000 \mu\text{m}$) and macroaggregates (2,000–250 μm) were significantly lower in the unfertilized soil than in the treatment that received excess manure. No significant differences between the treatments were found for the fractions $<250 \mu\text{m}$, which made up 82% to 90% of the total soil mass.

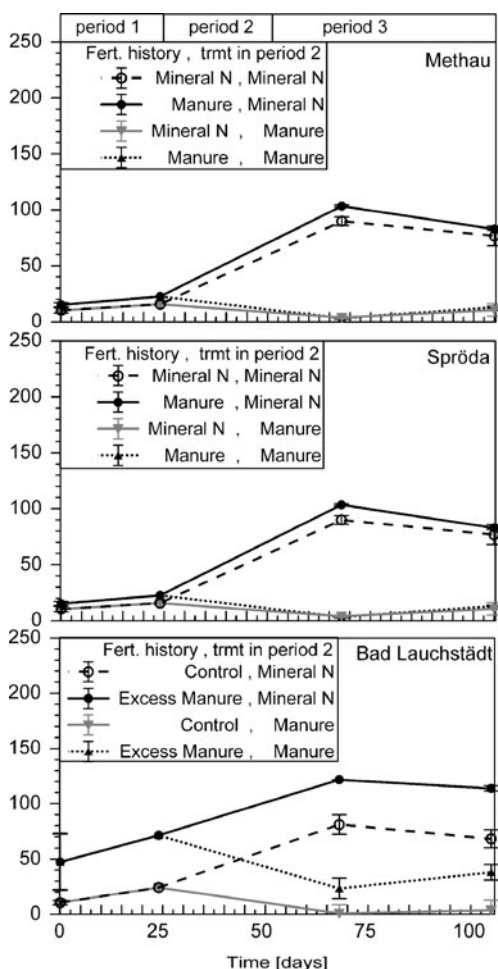


Fig. 3 Nitrate contents (means±standard error) measured in the three soils (Methau, Spröda, Bad Lauchstädt) each with different fertilization history (Fert. history: Manure/Excess Manure or mineral fertilizers/no fertilizer (Control)) at constant soil moisture of 60% water holding capacity (period1), following the application of KNO₃ (Mineral N) and farmyard manure (Manure; treatment in period 2), and after increasing soil moisture to field capacity (period 3)

Discussion

Soil organic matter and total N

Our data indicate that annual C inputs as manure as well as the clay contents of the soils are important determinants for C sequestration. For all three sites studied, the last

fertilization had been performed a year (Bad Lauchstädt) and 7 months (Methau, Spröda) before sampling. Thus, observed differences are not due to short-term management effects but are the result of the fertilization history. The increase in the SOC stocks in the treatments with application of farmyard manure reflects the potential of regular manure application for C sequestration and confirms the results of other long-term fertilization experiments (Edmeades 2003; Freibauer et al. 2004; Powlson et al. 1998).

The comparison of the increase in SOC stocks as a result of long-term manure application between Spröda (1.2 mg kg⁻¹, clay content 6%) and Methau (5.4 mg kg⁻¹, clay content 15%) shows the importance of the fine soil particles for the increased C stabilization, as emphasised in several studies (Christensen 1992; Hassink 1997; Six et al. 2002; Jagadamma and Lal 2010). Our finding is also in line with the conceptual model proposed by von Lützwow et al. (2007), which considers organo-mineral associations as an important pool for C stabilization and also with the Rothamsted Carbon Model, which calculates a decreasing fraction of CO₂ produced to C sequestered in the pools microbial biomass and humified organic matter with increasing clay contents (Coleman and Jenkinson 1999). However, the site conditions in our study do not allow a detailed quantitative analysis because the two long-term experiments (which had the same crop rotations and manure applications) differed not only in the textures of the soils but also slightly in the annual precipitation (540 mm at Spröda, 600 mm at Methau) and soil types (albic and gleyic Luvisols).

Emissions of CO₂

In our study, long-term fertilization with manure resulted in a 1.6- to 2.0-fold increase of CO₂ emission rates during period 1 of the incubation (soil moisture content at 60% of the water-holding capacity). Increased emission rates of the manure-amended soils were expected because of the added organic materials. For instance, for long-term trials at Bad Lauchstädt and Darmstadt, Ludwig et al. (2010) reported that the manure C was mainly sequestered in the soils in an intermediate (turnover time estimated to

Table 4 Water-stable aggregate size fractions of differently fertilized soils from the site Bad Lauchstädt (n=4). Different letters indicate significant differences between the long-term fertilizer treatments Control and Excess Manure (Man–Whitney U test, p<0.05)

Long-term fertilization history	Treatment in incubation period 2	Aggregate size			
		>2,000 μm %	>250 μm %	>53 μm %	<53 μm %
Control	Mineral N	0.25 a	5.88 a	64.62 a	26.95 a
	Manure	0.67 a	9.24 a	62.89 a	26.50 a
Excess manure	Mineral N	0.55 b	10.68 b	61.27 a	24.95 a
	Manure	1.52 b	13.07 b	61.17 a	21.01 a

be in the range of 10 to 100 years) pool and to a smaller extent in a labile pool (turnover time estimated as <10 years), which are responsible for the increased CO₂ emissions. However, besides the manure additions, mineral fertilizations (due to increased plant growth) or straw applications also contribute to the soil respiration as demonstrated by Vanotti et al. (1997) and Jacinthe et al. (2002) in field experiments with different rates of mineral fertilizer or straw applications.

The specific mineralization (CO₂-C emission related to the SOC content) reflects the mean bioavailability of the soil organic matter. We observed a decrease in the specific mineralization with increasing clay content and SOC stock (Spröda>Methau>Bad Lauchstädt), which can be explained by the high capacity of the clay fraction for the stabilization of organic C (Baldock et al. 1997; Christensen 2001; von Lützow et al. 2007). Clay-sized particles provide a large surface area where soil organic matter can be sorbed by strong ligand exchange and polyvalent cation bridges (Sposito et al. 1999).

The long-term application of different fertilizers (mineral fertilizer versus farmyard manure) affected the mineralization rate as discussed above but had no definite effect on the specific mineralization rate. This absence of this effect suggests that the long-term partitioning of C inputs to labile SOC pools was similar in both fertilization treatments. However, since the last fertilization was performed a year (Bad Lauchstädt) or 7 months (Spröda, Methau) before the sampling, the turnover of any very labile compounds in the manure was probably no longer detectable.

In our study, the application of manure at the beginning of incubation period 2 resulted in a pronounced increase of CO₂ emission rates for all soils treated with manure, whereas mineral N fertilization did not affect CO₂ emissions markedly. Similar dynamics of CO₂ emissions following application of farmyard manure and other organic fertilizers (e.g. slurry) have been described in several studies (e.g. Merino et al. 2004; Säger et al. 2010). Moreover, it was shown that the high respiratory O₂ consumption during mineralization of the added substrate favours the formation of anoxic microsites and promotes the emissions of N₂O if nitrate is available (Flessa and Beese 1995, 2000).

During the incubation period 3, in which a heavy rainfall was simulated, CO₂ emission rates decreased in all treatments. Similarly, for incubation experiments with different irrigation patterns, Säger et al. (2010) reported that CO₂ emissions were negatively correlated with WFPS. Linn and Doran (1984) explained decreasing mineralization rates at high water saturation as limitation of O₂ diffusion through pore spaces and consequently limited microbial respiration.

Emissions of N₂O and soil mineral N

The results from the Methau and Spröda sites support our hypothesis that long-term application of farmyard manure and the associated increase in SOC and N_t stocks promote emissions of N₂O. The results are in agreement with observations by Kilian et al. (1998) and by Chang et al. (1998) that repeated application of organic fertilizers resulted in increased SOC contents and increased losses of N₂O. Several factors can contribute to enhanced emissions from arable soils with increased SOC stocks. The higher availability of organic C and N, and the greater microbial biomass promote the processes of N₂O formation (Granli and Bøckman 1994; Chang et al. 1998; Lal 2004). Furthermore, an increased microbial respiration, as was found in our study, can favour N₂O production due to its effect on soil aeration (Flessa and Beese 1995; Clemens and Huschka 2001; Smith et al. 2003; Russow et al. 2008). However, these findings should not be generalized because there are other studies which did not detect significant changes of N₂O emission as a result of organic matter accumulation in arable soils. For a sandy loam soil, Meng et al. (2005) found that long-term application of manure did not result in greater N₂O emissions than the mineral fertilized treatment, despite higher C and N contents in the manured soil. The lack of an unambiguous effect of SOC and N_t accumulation on N₂O emissions may be explained primarily by texture-dependent differences of soil aeration and by differences of the stability of the accumulated SOC. Fertilization systems can also affect N₂O emission by changing soil pH. Long-term pH differences were found to change total N₂O emission and the microbial processes producing N₂O (Baggs et al. 2010). However, soil pH cannot explain the differences between our long-term fertilization treatments (comparing treatments of the same site) because soil pH was not or only marginally influenced by the different fertilization history. In addition, the net effect of long-term application of organic fertilizers on N₂O emissions may also be influenced by changes in soil physical properties. An increase of soil porosity and aggregate stability, which is often observed in treatments with application of farmyard manure (Blair et al. 2006; Schjøning et al. 2007; Bronnick and Lal 2005), can improve soil structure and soil aeration. This might even result in a decrease in N₂O emissions.

Such a positive effect of organic matter input on soil structure may have contributed to the surprising results of the two extreme treatments of the long-term experiment at Bad Lauchstädt, which do not represent usual agricultural management (without fertilization for 25 years and farmyard manure deposition in excess). Twenty-five years without fertilizer application resulted

in a lower yield of water-stable macroaggregates compared with the intensely manured soil. The poor soil structure may have decreased soil aeration and increased N_2O losses from the long-term unfertilized soil. Russow et al. (2008) determined N_2O emissions from nearby field plots with common fertilization rates but slightly different SOC contents (18 and 22 mg kg⁻¹ soil) which were a result of liquid manure fertilization at the site with enriched SOC. Emission rates in this field experiment reported by Russow et al. (2008) were much lower than those observed for the nil treatment of Bad Lauchstädt in our study, but the results agree with our findings for the treatments from Spröda and Methau where N_2O emissions were higher from soils with increased SOC contents. Furthermore, the results reported by Russow et al. (2008) suggest that increased SOC contents may also affect N_2O emissions following fertilizer application. They reported that emissions following nitrate application were higher for the treatment with higher SOC content and they identified denitrification as the main N_2O producing process. The results suggest that the unexpectedly high N_2O emissions from our control treatment of Bad Lauchstädt are exceptional for this site and that these emissions are probably a result of changes in soil structure, which were caused by 25 years of cropping without fertilization.

Nitrate application at the beginning of period 2 did not induce high N_2O emissions. This indicates that N_2O production was not restricted by nitrate availability, and it suggests that denitrification was restricted because of an adequate aeration of the soils at a soil moisture content of 60% water-holding capacity. However, there was one exception. Adding nitrate to the nil treatment of the Bad Lauchstädt site induced large and persistent N_2O emissions. This supports our assumption that soil structure and soil aeration were affected by the management without fertilization for 25 years. The emissions from the excess manure treatment at Bad Lauchstädt after nitrate application were much lower, even though the higher SOC content and the high nitrate availability provided more favourable conditions for N_2O production via denitrification. A nearly complete reduction of N_2O to N_2 in this treatment, which could explain the low N_2O emissions, seems to be unlikely because of the persistent high nitrate availability and the relatively low soil moisture content of 60% water-holding capacity (Weier et al. 1993; Swerts et al. 1996; Ruser et al. 2006). The results suggest that soil structure may be an underestimated control of N_2O emissions.

Overall, our results on N_2O emissions following the application of different fertilizer types (KNO_3 versus farmyard manure application at Spröda and Methau) show that the response of fertilizer-induced N_2O emissions to changes of soil moisture depends on the fertilizer type. Emissions were higher following application of farmyard

manure than KNO_3 at a soil moisture content of 60% water-holding capacity whereas increasing soil moisture to field capacity led to highest emissions from the treatments with nitrate application. The results can be explained by fertilizer-induced differences in nitrate availability and microbial oxygen consumption. At high soil moisture, N_2O emissions were strongly restricted by nitrate availability; thus, nitrate application resulted in highest emissions. At 60% water-holding capacity, oxygen availability appeared to be the most important factor limiting N_2O emissions. The local increase in C availability by the addition of farmyard manure triggered denitrification primarily because of the microbial O_2 consumption, which can result in the formation of anaerobic microenvironments (Flessa and Beese 1995; Clemens and Huschka 2001).

The simulation of heavy rain (increasing soil moisture to field capacity) increased N_2O emissions for all treatments indicating precipitation after N fertilization influences fertilizer-related N_2O emissions. The importance of soil moisture in fertilizer-induced N_2O emissions was also stressed by Dobbie et al. (1999), who found a strong positive correlation between the amount of rainfall during the first 4 weeks after N application and the cumulative N_2O emission in field measurements. Our results suggest that such rainfall-driven emissions following fertilization are probably higher for nitrate than farmyard manure addition.

Conclusions

We found significantly increased N_2O emissions from arable soils with increased SOC and N_t stocks, which were a result of the regular application of farmyard manure for many years. However, the effect of fertilization history and SOC content was small if compared to the short-term effects induced by the current fertilizer application. High N_2O emissions from the treatment without fertilization for 25 years indicate the importance of a sustainable organic matter and nutrient management for soil structure and N_2O emissions. Thus, increasing SOC stocks in arable soils can probably promote or lower N_2O emission, depending on the initial soil conditions and SOC stocks. Increasing SOC stocks by organic fertilizers includes the addition of N, which can induce high N_2O emissions. Application rates beyond the crop N demand have to be avoided even though they increase SOC stocks.

Emissions of N_2O following the application of nitrate and farmyard manure differed. At a soil moisture content of 60% water-holding capacity, fertilizer-induced emissions were higher for farmyard manure than nitrate. At field capacity, nitrate application induced the highest emissions. Our results are based on a laboratory incubation study

under controlled conditions and without plants, which makes it easier to identify significant effects of different fertilization (fertilization history and current fertilizer application) on N₂O emissions. It is still a challenge to determine whether these effects are significant under field condition where spatial and temporal variability of N₂O emissions are much higher.

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