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Soil Biology & Biochemistry 34 (2002) 1797–1806

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Low temperature control of soil denitrifying communities: kinetics of N_2O production and reduction

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Received 23 November 2001; received in revised form 16 July 2002; accepted 19 August 2002

Abstract

To explore the reason for reported high field fluxes of nitrous oxide (N_2O) from temperate soils in winter and early spring, we investigated the temperature response of denitrifier N_2O production and reduction in soil from three arable field sites along a temperature transect reaching from Finland over Sweden to Germany. Process rates were determined in anaerobic slurries with or without added NO_3^-, N_2O and C_2H_2 at 0, 5, 10, 15, and 20 °C (and 30 °C in one experiment). The experiments were conducted immediately after the soils had become anaerobic, and after a long (48 h) anaerobic pre-incubation with excess of carbon and electron acceptors. All denitrifying enzymes were found to be active in the soil at onset of anaerobiosis. Significant levels of N_2O production and reduction occurred at 0 °C, both at onset of anaerobiosis and after the 2 days anaerobic pre-incubation. Temperature response of N_2O production and reduction could be fitted to an Arrhenius function in the range 5–20 °C, yielding apparent activation energies between 28 and 76 kJ mol^{-1}. The estimated activation energy of the N_2O reduction was found to be similar or lower than that for N_2O production. High field N_2O fluxes in winter and early spring could thus not be explained by the temperature sensitivity of the two processes. However, major deviations from the regular Arrhenius response were found for two soils at near freezing temperature. The rates measured at 0 °C were much lower than those predicted by the Arrhenius function based on data in the temperature range 5–20 °C. Low temperature may thus exert a particular challenge to denitrifying communities for some reason, and the effect was found to be most severe for the N_2O reduction process. When such a breakdown affects N_2O reductase to a greater extent than the N_2O producing enzymes (NO_3^-, NO_2^-, and NO reductase), as was found in our soils, it will result in high N_2O fluxes at low temperature. The temperature response of the estimated net N_2O emission potential (based on measured N_2O production and reduction rates) differed significantly between the three sites, indicating inherent differences between their microbial communities.

Keywords: Denitrification; Temperature response; Activation energy; Nitrous oxide emission; Soil microbial community

1. Introduction

Nitrous oxide (N_2O) is a greenhouse gas and a potential destroyer of the stratospheric ozone layer. The microbial processes nitrification and denitrification are the main contributors of N_2O to the atmosphere, and the relative importance of these processes in soils varies with local environmental conditions. The present study focuses on the sequential reduction of NO_3^- by soil denitrifying communities during anaerobic conditions; i. e. $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. Most denitrification studies reported in the literature concentrate on the process of NO_3^- removal and/or N_2O production. To evaluate a soil's propensity to emit N_2O, it is, however, also crucial to study the relation between N_2O production and reduction.

Biological processes respond positively to temperature upshift within a range in which the enzymes are stable and retain full activity. Several studies have confirmed that this is true also for the denitrification process (Firestone, 1982). In experiments addressing both N_2O production and reduction to N_2, the product ratio (N_2O/N_2) has been found to increase with decreasing temperature (Bailey and Beauchamp, 1973; Keeney et al., 1979; Avalakki et al., 1995). One possible explanation for this is that N_2O reduction has a higher activation energy than N_2O production.

Denitrification response to temperatures prevailing in temperate and high-latitude soils have not been studied in detail, and temperatures above 5 °C are often considered to

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0038-0717/02/$ - see front matter
PII: S0038-0717(02)00169-4

be required for a significant denitrification rate (Granli and Bøckman, 1994). However, N_2O production in soil in the presence of C_2H_2 has been observed at −2 °C (Dorland and Beauchamp, 1991) and removal of NO_3^- from soil at −4 °C has been reported (Malhi et al., 1990). To the best of our knowledge, N_2O reduction at temperatures ≤0 °C has not been studied so far, despite the fact that field measurement have revealed excessive N_2O emissions from temperate soils during winter and early spring (Christensen and Tiedje, 1990; Flessa et al., 1995). High winter emissions have been previously attributed to specific freeze/thaw phenomena such as physical disruption of soil organic matter, release of readily degradable carbon compounds from deceased soil microbes (Christensen and Tiedje, 1990) or liberation of N_2O previously 'entrapped' in frozen soils (Burton and Beauchamp, 1994). Our hypothesis was that high N_2O emissions at low temperatures might be additionally ascribed to a specifically higher activation energy (or Q_{10}) of N_2O reduction as compared to N_2O production, and/or the existence of a critical temperature threshold below which N_2O reductase activity is arrested.

The present study was a part of the EU-project 'Greenhouse Gas Emission from Farmed Organic Soils' (GEFOS), in which whole-year-round field fluxes of N_2O were monitored throughout a 2 year period in Finland, Sweden, and Germany (Klemedtsson et al., 1999). In a recent study, we explored the possible intrinsic differences between denitrifying communities by assessing the kinetics of N_2O production and reduction at 20 °C in soil from these three sites (Holtan-Hartwig et al., 2000). The results suggested that intrinsic differences exist, with consequences for the N_2O emission potential. In the present study, we aimed to further investigate this matter by assessing the temperature response of N_2O production and reduction within a range from 0 to 20 °C. We determined apparent activation energies of the process rates in laboratory studies, both immediately after the onset of anaerobiosis and after a 48 h anaerobic pre-incubation at 10 °C with excess of carbon and electron acceptor. In the latter experiment, we additionally estimated the apparent growth rates based on the observed increases in process rates.

2. Materials and methods

2.1. Soil

Topsoil (0–30 cm) was collected (10–12 soil cores of 7 cm diameter were bulked) from the arable field research sites of the GEFOS-project, in Germany (48°40′N, 11°04′E), Sweden (58°20′N, 13°30′E), and Finland (62°55′N, 29°30′E). Soil and climatic properties are given in Table 1. The soil was sieved (4 mm) and stored for 4–12 months at field moisture and 4 °C in polyethylene bags which allow for gas exchange.

2.2. Washing of soil to reduce the amount of NO_3^- (Fig. 1, A)

Soils were washed in order to reduce the amount of NO_3^- in the soil solution before the start of the experiments. Soil (36 g fresh weight) was placed in centrifuge buckets with a perforated screen 35 cm above the removable bottom. A 1 mM glutamic acid solution was added on the top of the soil and centrifuged through the soil to the bottom compartment. This washing was repeated three times. The procedure has been described previously in detail (Holtan-Hartwig et al., 2000). The soil washing procedure did not influence potential denitrification in the soil, nor did we find measurable activity in the filters or the effluent (data not shown).

2.3. Temperature response at onset of anaerobic conditions (Fig. 1, BI)

Washed soil corresponding to ca. 4 g dry weight was transferred to 120 ml glass bottles with 16 ml 1 mM glutamic acid solution. The bottles were capped and the slurries made anaerobic by repeated evacuation and flushing with He, as described by Holtan-Hartwig et al. (2000). The bottles were stirred (magnetic stirring 600 rpm) and kept at the experimental temperatures (0, 5, 10, 20, and 30 °C) during the flushing by means of a water bath. After having established anoxic conditions, the residual over-pressure was released through a 0.5 mm (id) canulla which was pierced through the rubber septum. To prevent O_2 from entering during venting, the canulla was mounted on a 5 ml plastic syringe (without piston) filled with 0.5 ml distilled water. Half of the bottles were provided with 12 ml 100% C_2H_2 to block N_2O reduction (Balderstone et al., 1976; Yoshinari et al., 1977) before releasing the over-pressure. The bottles were incubated in a temperature adjusted water bath shaker (stroke 40 mm, 200 rpm) for 5 h. During the incubation, the change in N_2O concentration in the bottles was followed by withdrawing gas samples (0.8 ml) from the headspace of the bottles. The samples were injected into He-filled (1 atm) 11.6 ml vials which were then analysed for N_2O by GC as described previously (Holtan-Hartwig et al., 2000). The experiments were run at 0, 5, 10, 20, and 30 °C.

2.4. Temperature response after 48 h anaerobic pre-incubation (Fig. 1, BIIa–f)

In order to determine the 'inherent' temperature response of denitrifying communities from the three sites we provided optimal conditions (no oxygen, excess of electron acceptors and carbon) with respect to N_2O production and reduction for 48 h before the kinetic measurements were started. Previous experiments (Holtan-Hartwig et al., 2000) have indicated that this period is sufficiently long to ensure high activity of all denitrification enzymes in the investigated soils. These pre-incubations differed with respect to the electron acceptors provided:

Table 1
Soil properties

	C (%)	pH (H_2O)	Average ground water depth (cm)	Bulk density (kg m^{-3})	Mean temperature (°C)	Annual precipitation (mm)
Finnish	23	6.1	106	320	2.2	612
German	35	5.5	67	360	7.1	579
Swedish	24	7.2	60	270	4.9	645

(i) Washed soil designated to the measurement of N_2O production was dispersed in a medium containing 1 mM KNO_3 and 1 mM glutamic acid (144 g soil in 230 ml) in 500 ml Duran bottles. The bottles were capped with Teflon screw caps equipped with butyl rubber septa, and made anaerobic by eight cycles of evacuation and He-flushing (called PROD pre-treatment).

(ii) Washed soil designated to the measurement of N_2O reduction did not receive NO_3^-. Instead, 50 ml 100% N_2O was injected after the system had been made anaerobic. Residual NO_3^- present after washing (0.5–2.5 μg NO_3^--N g dry weight^{-1}) was reduced rapidly so that N_2O was the main electron acceptor in this pre-treatment (called RED pre-treatment).

KNO_3 and N_2O were provided to stimulate metabolism and growth for the PROD and RED assays, respectively. The concentration levels of electron acceptors were chosen on the basis of data from pre-experiments (not shown), ensuring ample supply throughout the whole pre-incubation period. The reasons for using N_2O rather than NO_3^- in the RED pre-treatment was that the RED assay required absence of NO_3^- during measurement of N_2O reduction activity. If NO_3^- was used, the absence of nitrate could only be obtained by complete depletion of nitrate prior to measurements. The depletion of electron acceptors, however, may impose immediate energy starvation on denitrifying bacteria (Hojberg et al., 1997), and could result in a severe reduction in activity. It would, thus, have been necessary to ensure that the measurements took place after NO_3^- depletion, but before N_2O depletion, which was difficult to achieve. We are aware that the use of different electron acceptors in PROD- and RED-pre-treatments may have caused differences in the species composition of the denitrifying community by the time of the enzyme assay, thus limiting comparability of temperature response of N_2O production and reduction.

PROD and RED pre-treatments were conducted uniformly for all temperature assays at 10 °C in a temperature controlled incubator provided with a roller table (16 rpm) for 46.5 h (Fig. 1,BIIb). The bottles were then evacuated to remove any remaining N_2O and flushed with He (eight cycles) (Fig. 1, BIIc). Portions of 20 ml slurry were transferred rapidly with an automate pipette to 120 ml glass bottles containing 0.1 M glutamic acid solution. To minimise the oxygen exposure, the 120 ml bottles were immediately capped with butyl rubber stoppers and flushed

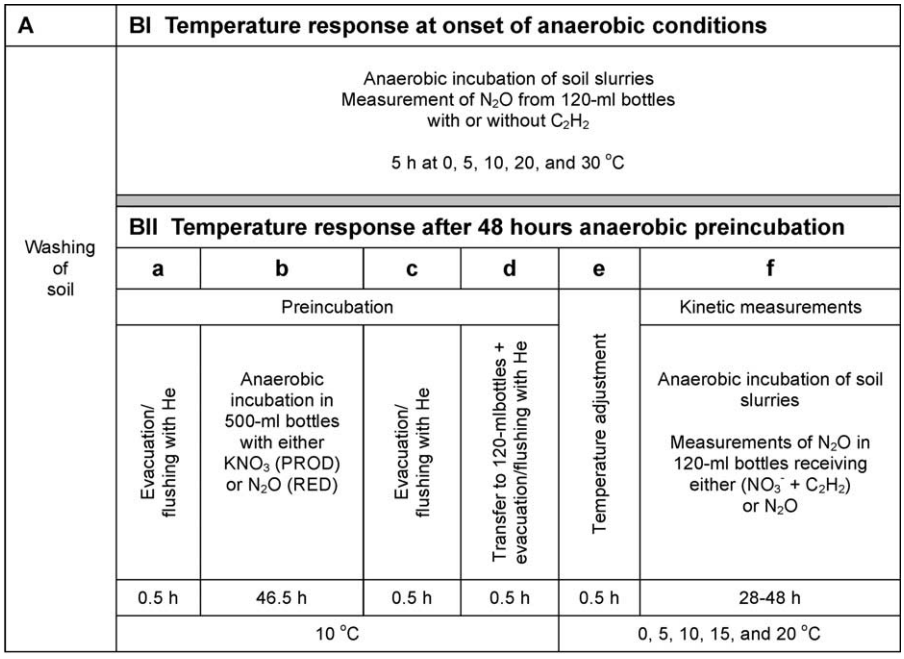

Fig. 1. Logistic design of experiments. See text for further explanation.

with He (Fig. 1, BIId). The pressure of He was ca. 1.5 atm after the evacuation/flushing procedure.

Three PROD-bottles and three RED-bottles were placed in incubators set at 0, 5, 10, 15, and 20 °C (Fig. 1, BIIe). Roller tables (25 rpm) were used to ensure continuous agitation of the soil slurry and equilibration with the gas phase. The bottles were left for 30 min in the incubators before starting the kinetic measurements to adjust the soil slurries to the incubator temperature. After temperature adjustment, residual over-pressure was released as described. The PROD-bottles were then loaded with 0.2 ml 0.1 M deoxygenated KNO_3 solution and 12 ml 100% C_2H_2 and the pressure was released again. The RED-bottles received 1 ml of a N_2O standard yielding a final concentration of about 100 μM in the soil slurry, and the pressure was released.

The moment of addition of C_2H_2 and N_2O was regarded as the start of incubation ($t = 0$). The bottles were incubated on the roller tables for 28–48 h at their respective temperatures. Gas samples (0.5 ml) for N_2O analyses were periodically withdrawn and injected into He-filled vials as described earlier. Pre-experiments were run to optimise the sampling times so as to avoid depletion of N_2O in RED-bottles.

Separate experiments were conducted to ensure that the rubber stoppers remained gas tight at low temperatures when pierced repeatedly. Bottles (containing 20 ml distilled water) capped with fresh septa and bottles with septa that had been pierced 30 times by a gas sampling needle received N_2O resulting at a concentration of 80 μM in the liquid phase. After keeping the bottles rolling at 0 °C for 3 days, no significant changes in the N_2O concentration in the bottles were found after 3 days ($p < 0.05$; data not shown).

All experiments were run in triplicate, and were conducted with soil from the three sites: Finnish, German, and Swedish.

2.5. Analysis and calculation

Concentration of N_2O in the 11.6 ml dilution vials was determined by gas chromatography as described in detail by Sitaula et al. (1992). The amount of N_2O in the bottles was calculated as described previously (Holtan-Hartwig et al., 2000). The solubility of N_2O in aqueous solutions was taken from Wilhelm et al. (1977).

For the experiments with soil slurries at onset of anaerobic conditions (Fig. 1, BI), the initial rates of N_2O production and reduction were calculated from the first near-linear parts of the progress curves. The reaction rates to be estimated were

$$NO_3^- \xrightarrow{V_1} N_2O \xrightarrow{V_2} N_2$$

The gross production of N_2O (V_1) was estimated by linear regression of data for bottles containing C_2H_2. The reduction rate of N_2O (V_2) was estimated as the difference between the estimated gross production (V_1) and the estimated net increase in N_2O in the bottles without C_2H_2.

For the experiments with soil slurries after 48 h anaerobic pre-incubation, the initial rates of N_2O production and reduction (V_0) and the specific growth rate constant (μ) were estimated from the experimental results by non-linear regression (JMP statistical software, SAS Institute Inc., 2000), using an equation for exponential growth with associated product formation (Stenström et al., 1991)

$$p = p_0 + (V_0/\mu)(e^{\mu t} - 1) \quad (1)$$

where p (μg N_2O-N g^{-1} dry soil) is the amount of product at the time t (h), p_0 (μg N_2O-N g dry $soil^{-1}$) the amount of product at $t = 0$, V_0 (μg N_2O-N g^{-1} dry soil h^{-1}) the initial rate of product formation and μ (h^{-1}) is the specific growth constant.

The apparent activation energies, E_a (kJ mol^{-1}), for N_2O production and reduction were determined by linearly regressing ln V_0 vs. $1/T$, assuming that the activities were Arrhenius functions of temperature

$$V_0 = A\, e^{-E_a/RT} \quad (2)$$

where A is a constant, R the universal gas constant (0.082057 l atm mol^{-1} K^{-1}), and T the absolute temperature (K).

Error estimates for the activation energies were derived from the standard error values of the x-coefficients (slopes) given by the 'least squares' method (linest-function in Excel 9.0) for linear regression of ln(V_0) vs. $1/T$.

3. Results

3.1. Temperature response at onset of anaerobic conditions

The investigation of the immediate response to anoxic conditions at different temperatures revealed that both N_2O production and reduction were detectable down to 0 °C, except for N_2O reduction in the Finnish soil at 0 °C. Here, the N_2O net curve measured without C_2H_2 was not significantly different from the N_2O gross curve measured with C_2H_2 (Fig. 2).

The estimated initial rates of N_2O production and reduction at onset of anaerobic conditions were exponential functions of temperature in the range 5–20 °C (Fig. 3). The apparent activation energy (E_a) for N_2O production in the Finnish soil was substantially higher than the E_a for N_2O reduction, while the activation energies were almost identical for the two processes in the German and Swedish soil (Table 2, 0 h).

The rates measured at 0 °C (and 30 °C) were much lower than those predicted by the Arrhenius function based on the temperature interval 5–20 °C (Fig. 3), at least for the Finnish and Swedish soils. The apparent activation energy for the 0–5 °C temperature interval would thus be much

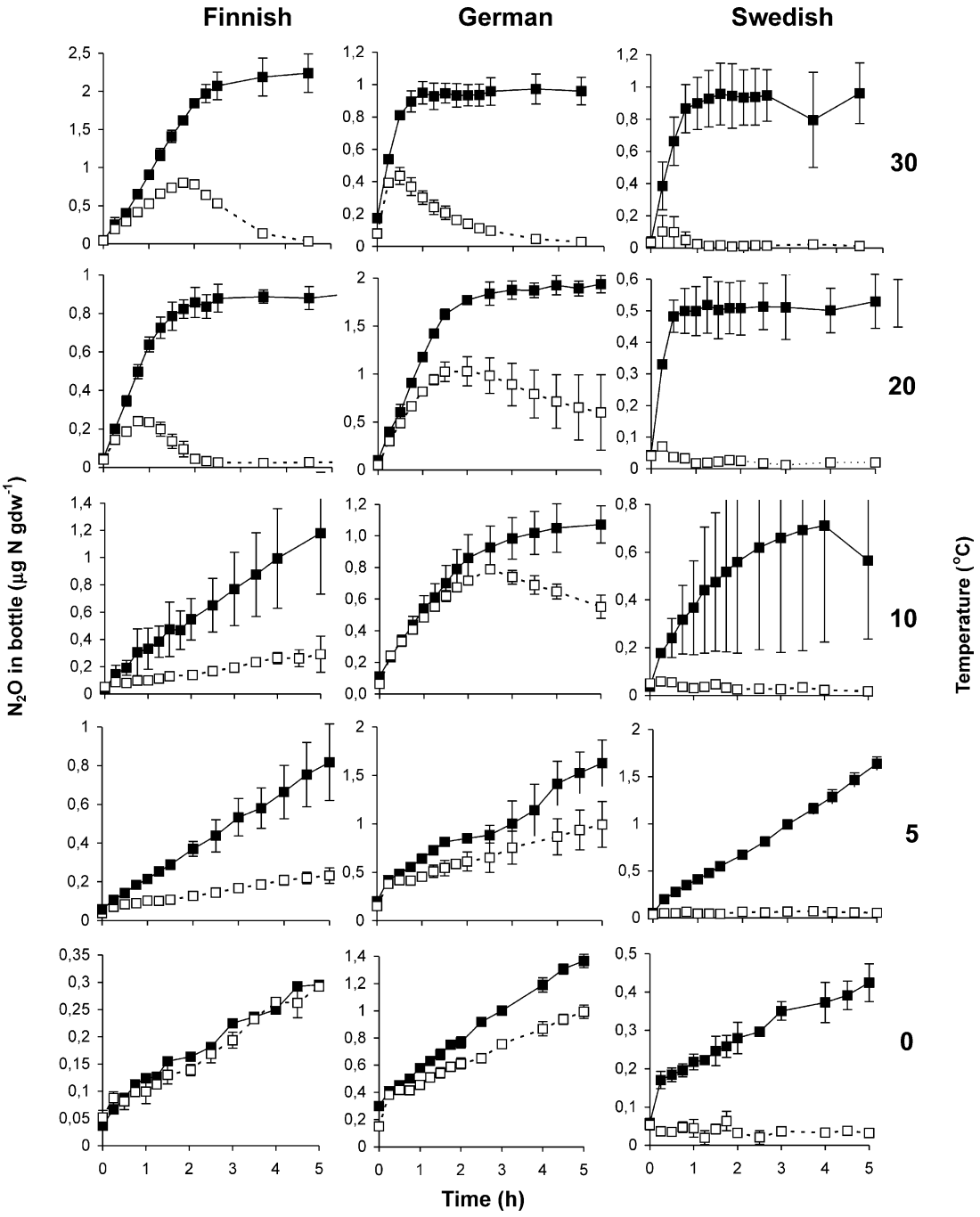

Fig. 2. Time course of N_2O concentration immediately after onset of anaerobic conditions in soil slurries at different temperatures. Samples were incubated anaerobically with (■) or without (□) addition of 10% C_2H_2. Bars indicate SD ($n = 3$).

higher, amounting to 147 and 500 kJ mol^{-1} for N_2O production and reduction, respectively, in Finnish soil. The equivalent values for the Swedish soil are 217 and 202 kJ mol^{-1}.

3.2. Temperature response after 48 h anaerobic pre-incubation

The apparent deviation from an Arrhenius function at 0 °C in Finnish and Swedish soil at onset of anaerobic conditions (Fig. 3) was not reproduced after the 48 h anaerobic pre-incubation (Fig. 4). Temperature relationships for V_0 followed an Arrhenius function over the entire temperature range (0–20 °C) (Fig. 4A and B). The N_2O reduction in the German soil was undetectable at 0 °C, and the temperature response for the higher temperatures appeared erratic and could not be fitted to an Arrhenius function.

After 48 h pre-incubation, the absolute values of the initial process rates (V_0) were quite different in the three

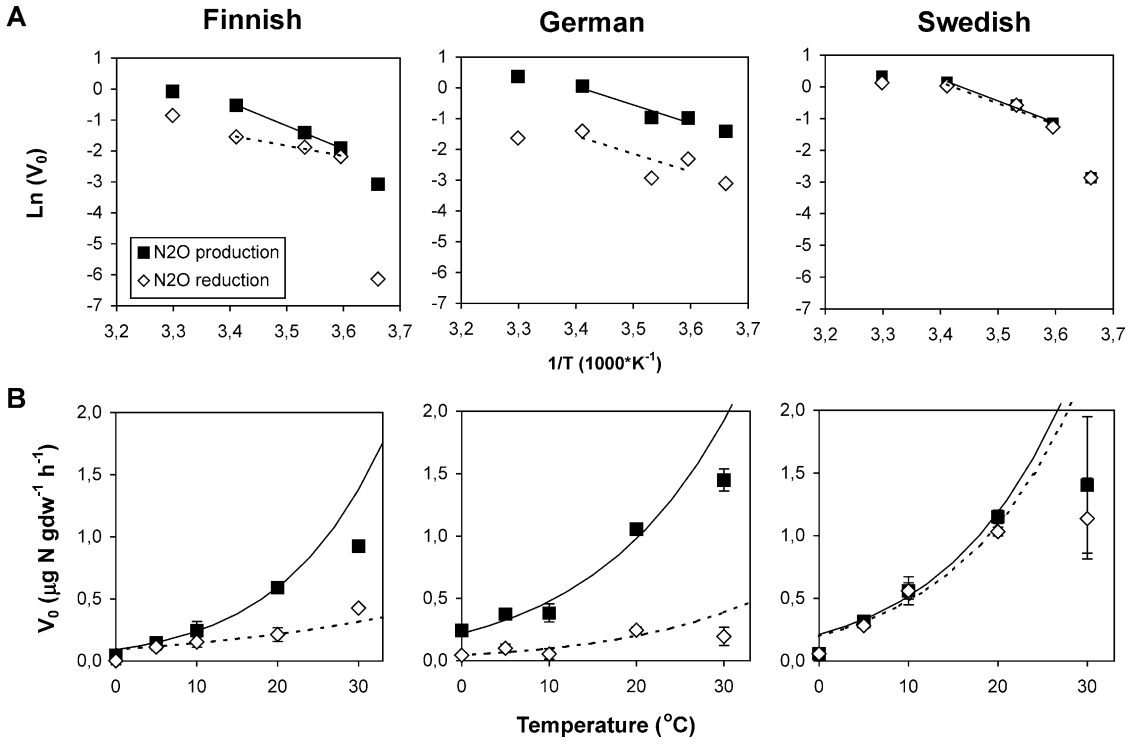

Fig. 3. Estimated initial process rates (V_0) of N_2O production and reduction at onset of anaerobic conditions in soil slurries at different temperatures. Bars indicate SD ($n = 3$). The plotted curves in B are Arrhenius functions estimated from the 5–20 °C range in A.

soils (Fig. 4B). The activity in the German soil was much lower than in the other soils (note different axis scaling, Fig. 4), both for N_2O production and reduction. Further, the German soil was extreme in having a much higher ratio (>1) between N_2O production and reduction than the Finnish and Swedish soil. This ratio was extremely low for the Swedish soil at all temperatures.

The specific growth rates (μ, Fig. 4) estimated by non-linear regression seemed to exhibit a more or less exponential relationship with temperature for all soils. We calculated the apparent activation energies for the different soils (Table 3) as a way to quantify the obvious difference in temperature response for this parameter. The Swedish soil had much lower E_a values than the two other soils, both for the N_2O production and reduction.

Table 2
Apparent activation energies (E_a) of N_2O production and reduction (standard error in parenthesis) after 0 and 48 h anaerobic pre-incubation. The E_a values are based on the temperature range 5–20 °C (Figs. 3A and 4A)

Anaerobic pre-incubation	Soil	E_a (kJ mol^{-1})	
		N_2O production	N_2O reduction
0 h	Finnish	62 (1)	28 (4)
	German	50 (17)	49 (47)
	Swedish	57 (6)	57 (12)
48 h	Finnish	53 (7)	59 (14)
	German	30 (9)	nd
	Swedish	76 (5)	60 (8)

nd, not determined because the temperature response could not be fitted to the Arrhenius function.

4. Discussion

The experiment conducted at onset of anaerobic conditions revealed that the denitrifying enzymes were present or quickly induced at all experimental temperatures with one exception; N_2O reductase in Finnish soil at 0 °C (Figs. 2 and 3). This finding contrasts with the commonly noted delay of N_2O reductase activity after onset of anaerobic conditions (Firestone and Tiedje, 1979). After transition from aerobic to anaerobic conditions, Otte et al. (1996) found the activity of N_2O reductase to become measurable only after 27 h in continuous cultures of *Alcaligenes faecalis* TUD. Dendooven et al. (1996) did not find significant N_2O reductase activity during the first 15 h of anaerobiosis in soil slurries amended with KNO_3. On the background of these data, we were interested in whether low temperature would further delay the induction of N_2O reductase relatively to the NO_3^-, NO_2^-, and NO reductases. The apparent absence of a lag in N_2O reduction activity in our experiments might be due to the low, but realistic (for the field situation) concentration of electron acceptors left after washing the soils. While in most studies the initial concentrations of

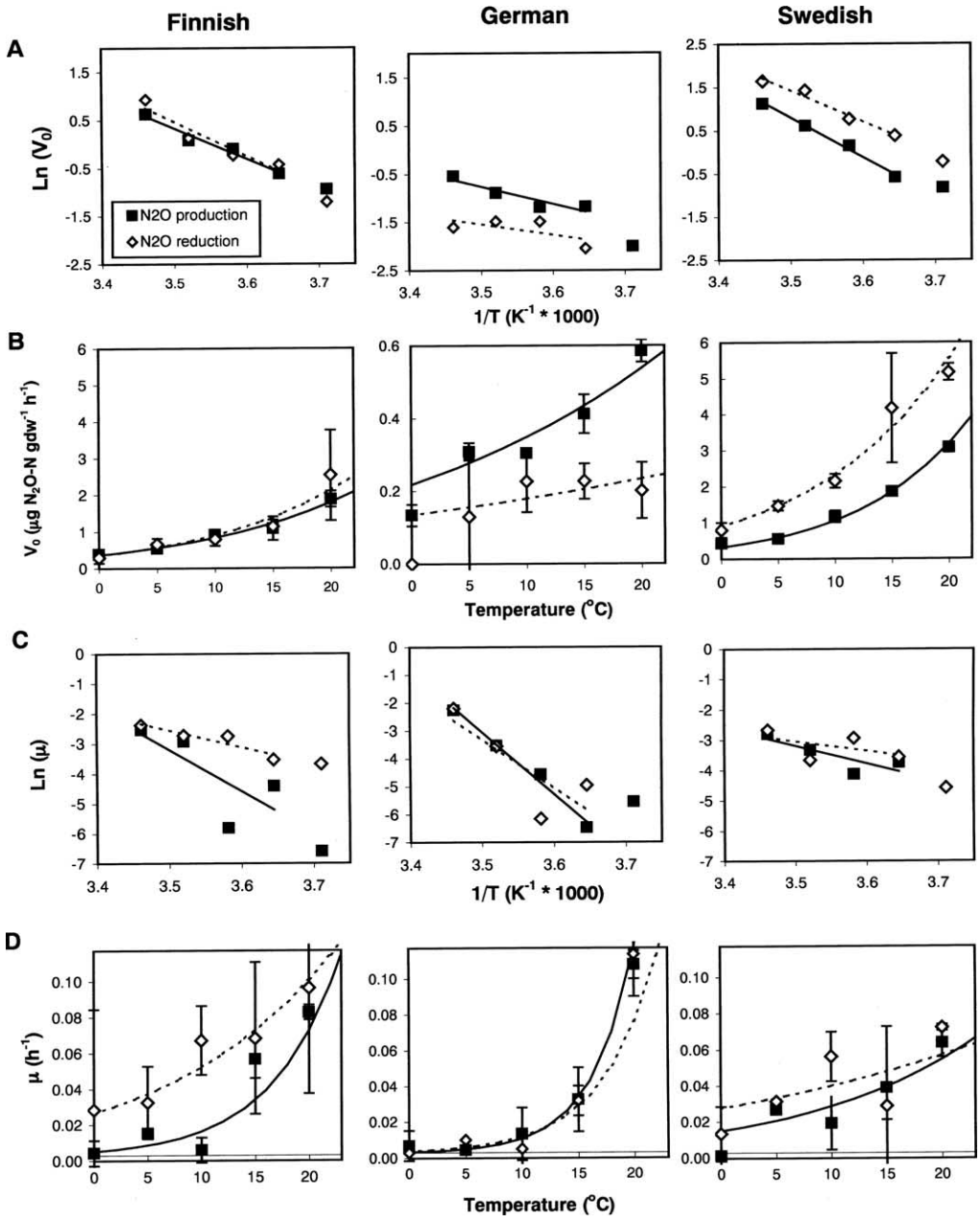

Fig. 4. Estimated initial process rates (V_0) and initial growth rates (μ) of N_2O production and reduction in soil slurries after 48 h anaerobic pre-incubation at different temperatures. Bars indicate SD ($n = 3$). The plotted curves in B and D are Arrhenius functions estimated from the 5–20 °C range in A and C, respectively.

NO_3^- are much higher, the denitrifying community in our set-up quickly ran short of NO_3^-, eventually forcing the reduction of N_2O to N_2. An alternative explanation could be that the organic soils used here preserved reduction enzymes during storage in anaerobic zones given by the amorphous structure of the soils and the high amount of particulate organic matter (Parkin, 1987). Therefore our results might not be valid for mineral soils.

Our temperature response curves for the 5–20 °C range (Table 2) are similar to those observed by others; the activation energy of NO_3^- disappearance by denitrification has been estimated to be 41 kJ mol^{-1} in a desert soil (Peterjohn, 1991), and for riparian soils values of 64.9 kJ mol^{-1} (Ambus, 1993) and 47–89 kJ mol^{-1} (Maag et al., 1997) are reported. Very similar apparent activation energies were estimated for N_2O production and reduction in our experiments (Table 2). An exception was the Finnish soil immediately after onset of anaerobic conditions, where the apparent activation energy of N_2O production was more than double of that for N_2O reduction. If differences in activation energies were to explain high N_2O emissions from soil during winter and early spring, one would expect

Table 3
Estimated apparent activation energies (E_a) and standard errors (in parenthesis) for the specific growth rate (μ) of the processes after 48 h anaerobic pre-incubation. The E_a values are based on the temperature range 5–20 °C (Fig. 4C)

Soil	E_a (kJ mol^{-1})	
	μ-N_2O production	μ-N_2O reduction
Finnish	114 (76)	47 (14)
German	185 (15)	146 (65)
Swedish	49 (24)	26 (13)

higher activation energies for N_2O reduction than for N_2O production. Since this was not the case, it seems that the high fluxes must be due to other factors rather than differences in E_a.

Our results indicate that the temperature response of the Finnish and Swedish soil at onset of anaerobic conditions does not follow a regular Arrhenius function down to 0 °C (Fig. 3). This low temperature seems to represent a particular challenge to the community for some reason. However, after 48 h anaerobic incubation at 10 °C, the temperature response became regular over the entire temperature range (0–20 °C) (Fig. 4A and B). This could be due to different physiological conditions of the communities of non-pre-incubated and anaerobically pre-incubated soil. Some induction might have to take place before the community is able to utilise the added glutamic acid and NO_3^- in the non-pre-incubated slurries. This induction might be more sensitive to 0 °C than the denitrification process itself. Further, the German soil was not able to reduce N_2O at 0 °C even after 48 h pre-incubation. The results strongly suggest the existence of a temperature limit for denitrification at or near 0 °C in this soil, below which one or several of the implied functions come to a complete arrest, or at least deviate significantly from the regular temperature response function. This could drastically increase the ratio between production and reduction of N_2O and we believe that such low-temperature perturbations can contribute to high emissions of N_2O in winter and early spring.

To our knowledge, the ability of denitrifying communities to reduce N_2O at 0 °C has not been previously addressed. Our findings of irregularities in the temperature response at onset of anaerobiosis were based on few observation points. Further studies within the temperature range from 0 to 5 °C could prompt more reliable conclusions.

The process rates of the German soil were remarkably low even after 48 h pre-incubation compared to the rates at onset of anaerobiosis. The pre-incubation treatment resulted in a 2–4-fold increase in reaction rates of the Finnish and Swedish soil (Figs. 3B and 4B), while a decrease of the reaction rates was found in the German soil. The apparent specific growth constant for the German soil at 10 °C (the pre-incubation temperature) was indeed low (Fig. 4D), but cannot explain the extraordinary low rates after 48 h of pre-incubation.

4.1. Ecological implications

Temperature regulates soil denitrification both directly and indirectly, the latter by influencing the availability of O_2, NO_3^-, N_2O and C substrates. Our study focused on direct effects of temperature on the enzyme reactions, and we therefore measured process rates under complete anaerobiosis and in the presence of ample C-substrate. In situ enzyme activities in soils are likely to vary over a large scale, depending on recent exposures of the communities to aerobic or anaerobic conditions. A storage of the soils under aerobic conditions (at 4 °C for at least 4 months) is assumed to be sufficient to level out the effect of antecedent anaerobic events on denitrification enzyme status (Dendooven and Anderson, 1995). The conditions imposed in our experiment deviate significantly from field conditions, and the results cannot be extrapolated directly to field conditions. The results are, however, informative in order to evaluate the intrinsic capacity of the microbial communities to carry out a complete reduction of nitrogenous oxides to N_2, i.e. without losses of the greenhouse gas N_2O.

The potential for N_2O emission from the soils can be evaluated by comparing the estimated rate constants of N_2O production and reduction (Figs. 3B and 4B). When the rate of N_2O production exceeds the rate of N_2O reduction, the soil has a greater potential to emit N_2O. It can thus be predicted that immediately after onset of anaerobic conditions—caused by, for example, rainfall or irrigation—the Finnish and German soil will emit more N_2O than the Swedish soil. After a long lasting period with anaerobic conditions—due to for example, flooding (after heavy rainfall or snowmelt)—this potential will decrease. The Swedish soil, on the other hand, had a generally low intrinsic N_2O emission potential at all temperatures and irrespective of the duration of anaerobiosis. Such differences in potential net N_2O production under standardised conditions have been taken as evidence for differences in the species composition of denitrifying communities (Cavigelli and Robertson, 2000, 2001), with obvious consequences for the N_2O field emissions (Holtan-Hartwig et al., 2000). The community differences might, in turn, be due to differences in temperature conditions at each site. However, we did not find a pattern of activation energies for N_2O production and reduction consistent with the mean annual temperature. We would have expected lower activation energies in soils from colder sites in the measured temperature ranges due to cold adaptation of the denitrifying community. Probably, the long-term storage at 4 °C preceding the experiments had levelled out such in situ differences.

Laboratory studies with intact soil have indicated that the ratio N_2O/N_2 (corresponding to N_2O production/N_2O reduction) decreases as the temperature increases (Nõmmik, 1956; Bailey and Beauchamp, 1973; Avalakki et al., 1995).

Our results suggest that this temperature effect is not due to higher activation energies for N_2O reduction as compared to N_2O production, but might be possibly caused by anomalies at critically low temperatures (0 °C). An alternative explanation for the gradual increase of N_2O/N_2 product ratio with decreasing temperature in intact soils is the differences in temperature response of N_2O diffusion versus biological process rates. In an intact soil, the diffusion of N_2O from the active sites to air filled pores exerts a strong regulation on the product ratio, and the gas diffusion coefficient in water changes only slightly with decreasing temperature (approximately 20–25% decrease per 10 °C decrease in temperature). In contrast, the metabolic rates (both N_2O production and reduction) decrease drastically with decreasing temperature (60–70% decrease per 10 °C). As a result, the percentage of reduced N lost as N_2O to the atmosphere should increase with decreasing temperature.

The E_a values and the relation between E_a of N_2O production and reduction are not the only factors determining the reaction of the denitrifier community to low temperatures. The apparent growth rate will also be important. This parameter is not necessarily reflecting the average growth rate of the denitrifier community, but rather a combination of growth rates and the rates of induction of denitrifying enzymes. Nevertheless, it is a measure of the speed at which the denitrification processes increase under ideal (anaerobic) conditions, with implications for the emission of N_2O. For the Finnish soil, the apparent growth rate was much higher for N_2O reduction than for N_2O production. In contrast, the two other soils had very similar growth rates for the two processes.

The denitrifying communities appear to be different also in another respect. The apparent activation energies of the growth rates (Table 3) were much lower for the Swedish soil than for the two others (for both processes). This can also be seen by comparing the slopes of $\ln(\mu)$ vs. $1/T$ in Fig. 4. As for the growth rate, we are dealing with an 'operational' parameter which probably reflects various mechanisms by which temperature affects the process. The estimated values are primarily useful for parameterisation of models. In such cases, the temperature response is more commonly described by Q_{10} values. The E_a values can easily be translated to Q_{10} values, based on a chosen base temperature for the Q_{10} function. Thus, for a base temperature of 5 °C, the Q_{10} values for μ are around two for both processes in the Swedish soil (and for N_2O reduction in the Finnish soil) and above 10 for the others.

In the experiment conducted at onset of anaerobic conditions, the bacterial community was challenged by limited availability of NO_3^- and N_2O, while there was an excess of electron acceptors in the experiments conducted after 48 h of anaerobic pre-incubation. It might be argued that the amount of substrate has influenced the temperature response (Westermann et al., 1989; Nedwell, 1999). Unpublished experiments in our laboratory have shown that the apparent K_m of N_2O production and reduction of the Swedish soil is one order of magnitude lower than the K_m of the Finnish and German soil, but no severe changes of the apparent K_m were found with low temperatures. This suggests that the availability of NO_3^- and N_2O did not influence the temperature response in our experiments.

Acknowledgements

The EU-project GEFOS (Greenhouse Gas Emissions from Farmed Organic Soils) and the Research Council of Norway funded this work. Technical support of Hege Oustad is gratefully acknowledged.

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