

## Denitrification in a sandy loam soil as influenced by climatic and soil conditions

*Denitrifikationens afhængighed af jordklimaet i en sandblandet lerjord*

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### Summary

The production of gaseous nitrogen from spring barley grown in sandy loam soil was estimated over two seasons. The  $N_2O$ -flux was measured in the field by a soil canopy method, while the  $N_2$ -flux was determined on incubated soil with the acetylene inhibition method. When soil water content was near field capacity, the N-loss was 45 and 71 kg N/ha during April–November after application of 40 and 160 kg  $NH_4NO_3$ -N/ha respectively. 30 and 70 kg were lost after application of 25 and 100 t pig slurry/ha. With lower water contents, the corresponding figures were 15, 12, 14 and 69 kg N/ha respectively.

The  $N_2O$ -flux depends on the production rate of  $N_2O$  and on the gas movement within the soil.  $N_2O$ -flux was found to depend on mass transport and on diffusion. At a certain  $N_2O$  increase in soil air the increase in  $N_2O$ -flux at the soil surface was 4 times greater in dry soil ( $>20$  kPa) compared to wet soil ( $>20$  kPa).

The  $N_2:N_2O$ -ratios averaged 2.9 following periods with wet soil conditions ( $<20$  kPa) compared to 0.8 under drier conditions ( $>20$  kPa). With some modification, the soil incubation technique could quantify the size of the production of gas-N in the field.

A multiplicative model indicates that soil moisture, soil nitrate and breakdown of organic matter during autumn and winter all are significant in determining the denitrification.

**Key words:** Denitrification,  $N_2O$ -loss, N-loss, soil moisture, manure, fertilizer.

### Resumé

Denitrifikationen i en sandblandet lerjord dyrket med vårbyg blev bestemt i to vækstsæsoner.  $N_2O$ -produktion blev bestemt i marken ved måling af  $N_2O$  i en luftstrøm gennem omvendte kasser på jordoverfladen.  $N_2$ -produktionen bestemtes ved inkubation af jord i laboratoriet ved acetyleninhiberingsmetoden. I jord nær markkapacitet var kvælstoftabet fra april til november 45 og 71 kg N/ha efter tilførsel af henholdsvis 40 og 160 kg N/ha i kalkammonsalpeter, mens 30 og 70 kg N/ha blev tabt efter tilførsel af 25 og 100 t svinegylle/ha. Ved lavere vandindhold var de tilsvarende tab henholdsvis 15, 12, 14 og 69 kg N/ha. I vandmættet jord målte den største denitrifikationsaktivitet om foråret.

$N_2O$ -fluxen fra jorden er bestemt af produktionen i jorden og transporten i jordluften ved gasdiffusion og ændringer i lufttryk. I en våd jord vil  $N_2O$ -koncentrationen i jordluften være højere end i mere tør jord ved samme  $N_2O$ -flux.  $N_2$ -produktionen var i gennemsnit 2,9 gange større end  $N_2O$ -produktionen i jord nær markkapacitet (<20 kPa) sammenlignet med en faktor 0,8 i mere tør jord (>20 kPa).

En model til beregning af denitrifikationsraten har lav præcision, men underbygger at jordens fugtighed og nitratindhold har stor betydning.

Derudover understøtter modellen betydningen af sæsonvariationen i letomsætteligt organisk stof på denitrifikationsraten.

**Nøgleord:** Denitrifikation,  $N_2O$ -flux, kvælstoftab, vandindhold, gylle, handelsgødning.

## Introduction

Gaseous nitrogen is formed in the soil by several microbial reactions. Denitrification is commonly regarded as the most important process in wet agricultural soils, but  $N_2O$ -formation during nitrification is found to be a main N-gas forming reaction under dry conditions (5, 27).  $N_2O$  is formed by several soil microorganisms during the conversion of  $NO_3^-$  or  $NH_4^+$  (41, 37, 8).

A direct quantification of denitrification involves the measurement of  $N_2$  and  $N_2O$  produced in the field. This can be done for  $N_2O$ , but not for  $N_2$  because of its high ambient concentration. Practicable ways to quantify denitrification are indirect methods of calculating denitrification as the difference between all other N-transforming processes and gaschromatographic procedures eventually coupled with N-labelling. A denitrification measure based on the difference method will be very inaccurate because it contains all the uncertainties in determining the other N-reactions (21). An improvement in this approach is to label the added fertilizer with N-15 and ascribe the N-15 loss to denitrification (6).

Labelling procedures and gaschromatography have been used to measure N-15 labelled  $N_2$  directly in the field (30), and have been refined to measure small  $N_2$ -fluxes (34). Large  $^{15}NO_3^-$  additions are necessary to get detectable N-15 amounts in the gas phase. As opposed to this, the high specific activity of N-13 facilitated measurement of gas-production at small  $^{13}NO_3^-$  additions (38).

There are two gaschromatographic procedures without use of N-labelling: By removing  $N_2$  from

the soil system a small  $N_2$ -production can be measured. This procedure requires an air-tight incubation system. Closed systems (18, 25) and air-percolated systems (40) have also been used. The last mentioned procedure is based on the finding that acetylene inhibits  $N_2$ -formation in pure cultures of denitrifying bacteria, leaving  $N_2O$  as the end product (2). The technique has been validated with N-13 (36) and was found to give a good estimate of denitrification in incubations not exceeding a few hours.

The purpose of this work was to estimate the denitrification in an agricultural soil, and to estimate the total N losses by denitrification by modelling the environmental factors.

The  $N_2O$ -formation was measured directly in the field while  $N_2$ -formation was estimated from soil incubated with and without acetylene.

## Materials and methods

### *Experimental site*

A sandy loam soil at Roskilde Research Station was used in this study; physical and chemical characteristics are listed in Table 1. 8 plots of 25 m<sup>2</sup> were laid out. Two amounts of limed  $NH_4NO_3$  and two amounts of pig slurry, in two replications, were added in April 1983 and 1984 (Table 2). Immediately after the application, the soil was rotavated and spring barley was sown. After the harvest in August, the area was harrowed. The soil was ploughed late in the autumn both years.

### *Climatic recordings*

Air temperature data (2 m elevation) is given as the weekly mean of daily observations of

**Table 1.** Soil description.  
*Jordbeskrivelse.*

Organic carbon	1.5	% of
total nitrogen	0.12	dry matter
Clay	10.4	
Silt	23.4	% of inorganic
Fine sand	59.2	fraction
Coarse sand	7.0	
pH (CaCl <sub>2</sub> )	5.3	
Phosphorus	15.3	mg/100 g
K	12.9	soil

**Table 2.** Added N amounts and N yield in harvested barley (kg N/ha).

<i>Tilført N og N-udbytte i byg (kg N/ha).</i>					
N-source <sup>1)</sup>	1 N	4 N	1 S	4 S	
<i>N added (12 April)</i>					
1983	NH <sub>4</sub> <sup>+</sup> -N	20	80	80	320
	NO <sub>3</sub> <sup>-</sup> -N	20	80	0	0
	Org.-N	-	-	28	110
<i>N yield</i> <sup>2)</sup>	84	116	93	124	
<i>N added (10 April)</i>					
1984	NH <sub>4</sub> <sup>+</sup> -N	20	80	82	326
	NO <sub>3</sub> <sup>-</sup> -N	20	80	0	0
	Org.-N	-	-	23	92
<i>N yield</i>	83	157	114	152	

<sup>1)</sup> 1 N and 4 N corresponds to 40 and 160 kg N/ha respectively in limed NH<sub>4</sub>NO<sub>3</sub>. 1 S and 4 S corresponds to 25 and 100 t manure/ha respectively in pig slurry.

<sup>2)</sup> N content in barley grain + straw at harvest.

maximum and minimum temperature at the experimental station. Soil tensiometers were placed in the field at 15 cm depth with 4 replicates in the area. Soil moisture tension was recorded when measurements of denitrification were made. Soil temperature at hourly intervals throughout the day of measurements was obtained from a nearby experimental farm (Højbakkegård). The air pressure data shown is the mean observations at three meteorological stations in the region (Tune, Kastrop and Værløse).

#### *N<sub>2</sub>O-concentration in soil air*

Soil air was sampled from probes placed at 15 cm

depth in the soil. Samples were collected from the probes with a gas pipette and an aliquot was analyzed for N<sub>2</sub>O-concentration (12). There were two (1983) or four (1984) probes in each plot, giving four or eight replicates from each treatment, respectively.

#### *Surface flux of N<sub>2</sub>O*

The flux of N<sub>2</sub>O from the soil surface was measured in air tight boxes, placed on frames and open toward the soil surface. The N<sub>2</sub>O-concentration in air constantly flowing through the boxes was measured at hourly intervals throughout a 24 hour period by automatic gas chromatography (9). There was one frame and box in each plot, giving two replicate measurements per treatment. The data presented is the daily sum of hourly measurements of N<sub>2</sub>O-flux.

#### *N<sub>2</sub>O-production with and without acetylene*

Soil was sampled from the plots at intervals (1983) or every time N<sub>2</sub>O-flux was measured (1984). In 1983 the incubation was performed in 500 ml flasks with 200 g fresh soil. The flasks were closed with a septum cap. In 1984, incubation was carried out in the 3 cm Ø × 30 cm tubes in which the soil was sampled. By pushing the tapered tubes into the soil, 140–180 g soil was collected. The soil in the tubes was perforated with a 4 mm hole along the length to improve gas exchange between the soil and headspace. The tubes were sealed with a silicon stopper in both ends at incubation. Six replicate samples from each plot were sealed in flasks or tubes. To half of the flasks or tubes 10% acetylene was added after a similar amount of air was removed. In the tubes air removal and acetylene addition occurred at opposite ends to obtain an even distribution. The distance of diffusion for acetylene through the soil is thereby reduced to 10–15 mm. All tubes or flasks were incubated aerobic for 2–4 hours at room temperature. The incubations were carried out within one day after sampling. During the incubation, the N<sub>2</sub>O-concentration in the headspace was determined three times on a gas chromatograph.

A  $^{63}\text{Ni}$  EC-detector was operated at  $400^\circ\text{C}$  and separation was done on a Poropak N + Poropak Q column system. Both columns were  $60\text{ cm} \times 2\text{ mm}$ . This combined column allows separation of  $\text{O}_2$ ,  $\text{CO}_2$  and  $\text{N}_2\text{O}$  (Por Q) and a delay of the acetylene peak relative to the  $\text{N}_2\text{O}$ -peak (Por N). If the increase in  $\text{N}_2\text{O}$ -concentration was not linear, the results were discarded. At the end of incubation the acetylene concentration was controlled. The production of  $\text{N}_2\text{O}$  in flasks and tubes was calculated on a per g soil per hour basis, taking the  $\text{N}_2\text{O}$  dissolved in soil water into consideration (39). The  $\text{N}_2\text{O}$ -production without acetylene  $[\text{N}_2\text{O}]$  was then subtracted from the  $\text{N}_2\text{O}$ -production with acetylene ( $\text{N}_2\text{O}$ ) and divided by the  $\text{N}_2\text{O}$ -production without acetylene to give an estimate of the ratio of  $\text{N}_2:\text{N}_2\text{O}$ -production:

$$\text{N}_2:\text{N}_2\text{O} = \frac{(\text{N}_2\text{O}) - [\text{N}_2\text{O}]}{[\text{N}_2\text{O}]}$$

For comparison of field and laboratory determinations of  $\text{N}_2\text{O}$ -production, the  $\text{N}_2\text{O}$ -production rate without acetylene was recalculated to an area basis, assuming a 20 cm production layer and a vol. weight of 1.5.

#### *Soil nitrate*

Samples for determination of nitrate in the plots were obtained parallel with the samples for incubation. The samples were frozen until analysis. The  $\text{NO}_3^-$ -concent was measured colourmetrically on an autoanalyzer (Technicon).

#### *Calculation of gaseous N-loss*

The total N-loss as  $\text{N}_2\text{O}$  and  $\text{N}_2$  in the field was calculated on basis of  $\text{N}_2\text{O}$ -flux measured in the field and the  $\text{N}_2:\text{N}_2\text{O}$ -ratio determined in laboratory incubations. The  $\text{N}_2\text{O}$ -flux determined during one measuring day was taken as representative for a period of equal length before and after the flux measurement. The  $\text{N}_2\text{O}$ -flux for the period was then multiplied by the  $\text{N}_2:\text{N}_2\text{O}$ -ratio + 1 to give the loss of  $\text{N}_2\text{O} + \text{N}_2$  in the field.

## **Results**

### *Soil characteristics*

In the two years of investigation the air temperature increased from  $5\text{--}6^\circ\text{C}$  in the middle of April to about  $17\text{--}18^\circ\text{C}$  in August and decreased again to  $6\text{--}7^\circ\text{C}$  in November (Fig. 1).

The soil moisture tension was  $6\text{--}11\text{ kPa}$  corresponding to field capacity until the start of June 1983 (Fig. 1) From the middle of June until October soil water content was low with tensions above  $40\text{ kPa}$ . During October – November the soil was wet ( $7\text{--}16\text{ kPa}$ ). In 1984 the soil water content had already decreased to below field capacity at the beginning of May. From the beginning of June precipitation gave a water content at field capacity until July. The water content was low from July until the middle of September, followed by moisture contents at field capacity during the autumn.

The soil nitrate contents were nearly the same in all plots before nitrogen application in spring 1984, ( $4\text{ mg NO}_3^- \text{-N per kg soil}$ ). The slurrytreated soil showed an increase in nitrate content during the weeks following the application as opposed to soil receiving inorganic fertilizer. In both years the low inorganic or organic nitrogen applications had similar nitrate contents compared to the high dressing. The nitrate content of all treatments was low during the growth period (June to August), followed by an increase during the autumn. Heavy precipitation between 4 and 7 June 1984 resulted in a drastic decrease in nitrate content in the soil of all treatments (Fig. 1). The gradual increase in nitrate content observed in the weeks after water saturation of the soil can be a result of nitrification.

### *$\text{N}_2\text{O}$ -flux in the field*

The  $\text{N}_2\text{O}$ -flux from the soil surface, measured in the field and the  $\text{N}_2\text{O}$ -production in laboratory incubations is shown in Fig. 2. The  $\text{N}_2\text{O}$ -flux followed a distinct seasonal pattern correlated with soil moisture (Fig. 1).

The  $\text{N}_2\text{O}$ -flux was at maximum in the spring and autumn at high water content and at minimum during summer at low water content. A

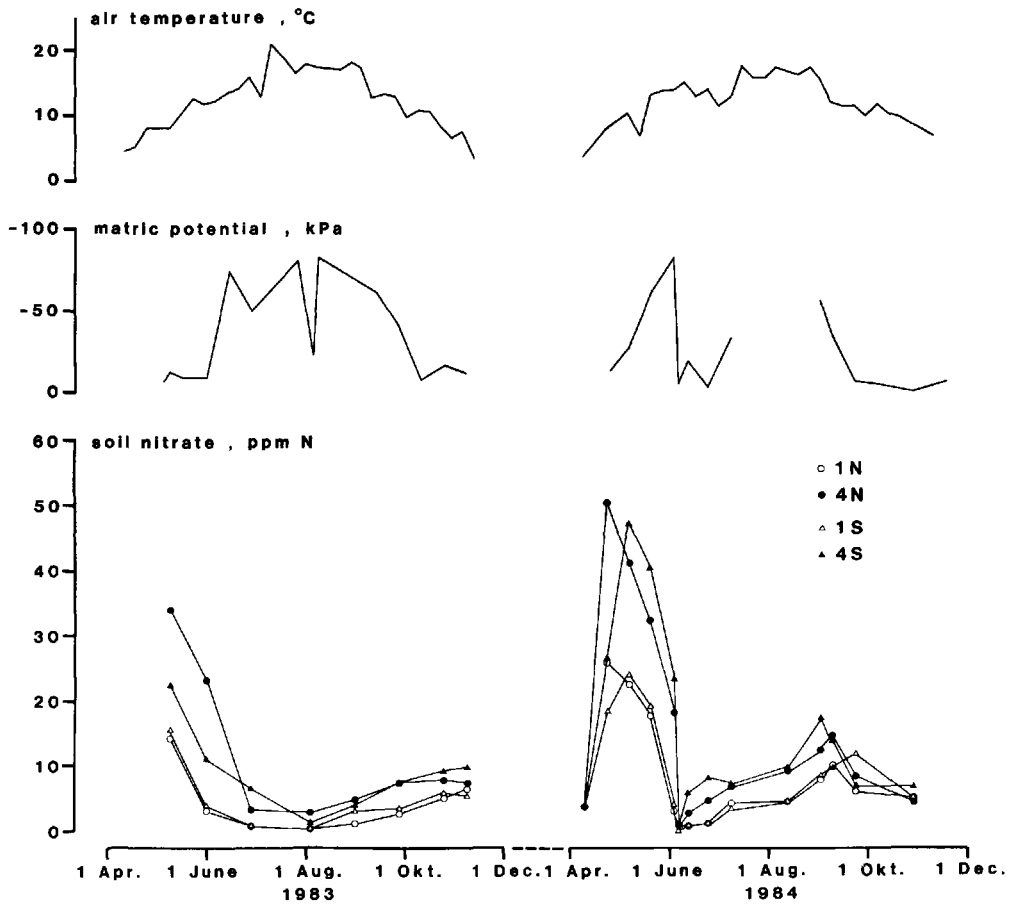


Fig. 1. Air temperature, (2 m elevation) soil water potential (15 cm depth) and soil nitrate (0–20 cm) throughout 1983 and 1984, ○, ●, △, and ▲ represents 1 N, 4 N, 1 S and 4 S respectively.

Lufttemperatur (2 m højde), jordens vandpotential (15 cm dybde) og jordens nitrat 0–20 cm i 1983–1984. ○, ●, △ og ▲ repræsenterer henholdsvis 1 N, 4 N, 1 S og 4 S.

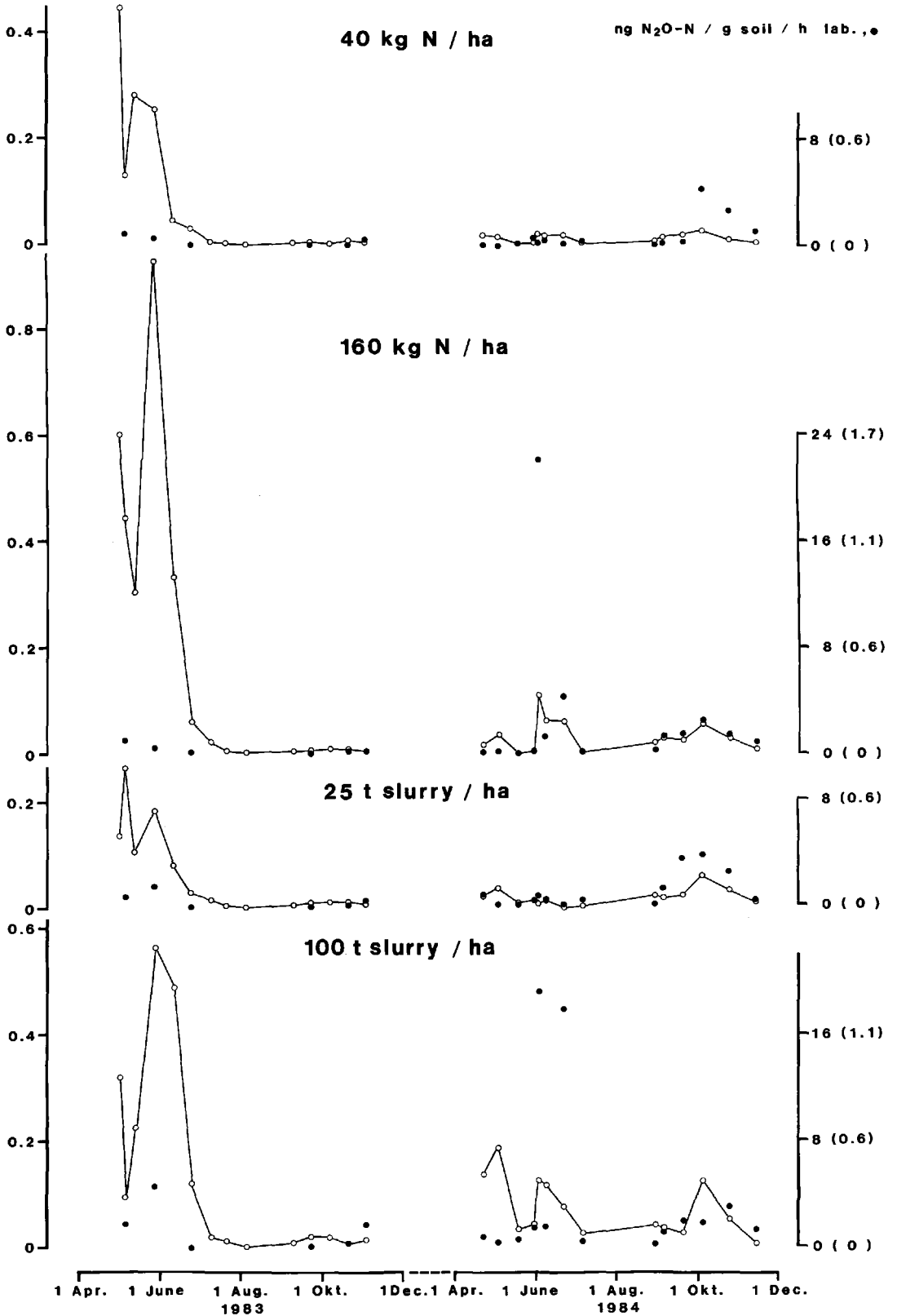
temporary increase in moisture in June 1984 resulted in an increased  $N_2O$ -flux. In periods of low water content and an elevated content of nitrate, the  $N_2O$ -flux was low as exemplified by September 1983 and May–June 1984. The temperature (Fig. 1) did not follow the variations in  $N_2O$ -flux. At low temperatures in the spring high fluxes were found while low fluxes occurred during summer at high temperatures.

The flux of  $N_2O$  from a plot supplied with 100 t slurry/ha throughout a two day period in October is shown in Fig. 3. The fluctuations in air pressure and soil temperature are also shown. The  $N_2O$ -flux increased over 9 hours, reaching a peak twice as large as the initial flux. Simultaneously a minimum in air pressure was recorded. The highest hourly  $N_2O$ -flux was up to 2–3 times larger than the lowest hourly  $N_2O$ -flux within the

kg N<sub>2</sub>O-N / ha / day field, ○

40 kg N / ha

ng N<sub>2</sub>O-N / g soil / h lab., ●



single measuring days throughout the two seasons.

Fig. 4 shows the  $N_2O$ -flux from the soil surface, plotted against the  $N_2O$ -concentration in the soil air throughout the seasons. The soil air data is the mean  $N_2O$  concentration in the treatments measured at the same time as flux data was

collected. The  $N_2O$ -concent in the soil air was correlated with the  $N_2O$ -flux.  $N_2O$ -concentrations above the ambient concentration (0.3 ppm) were frequently observed in wet soil and tended to occur at relatively low  $N_2O$ -fluxes as compared to the fluxes from drier soil (Fig. 4).

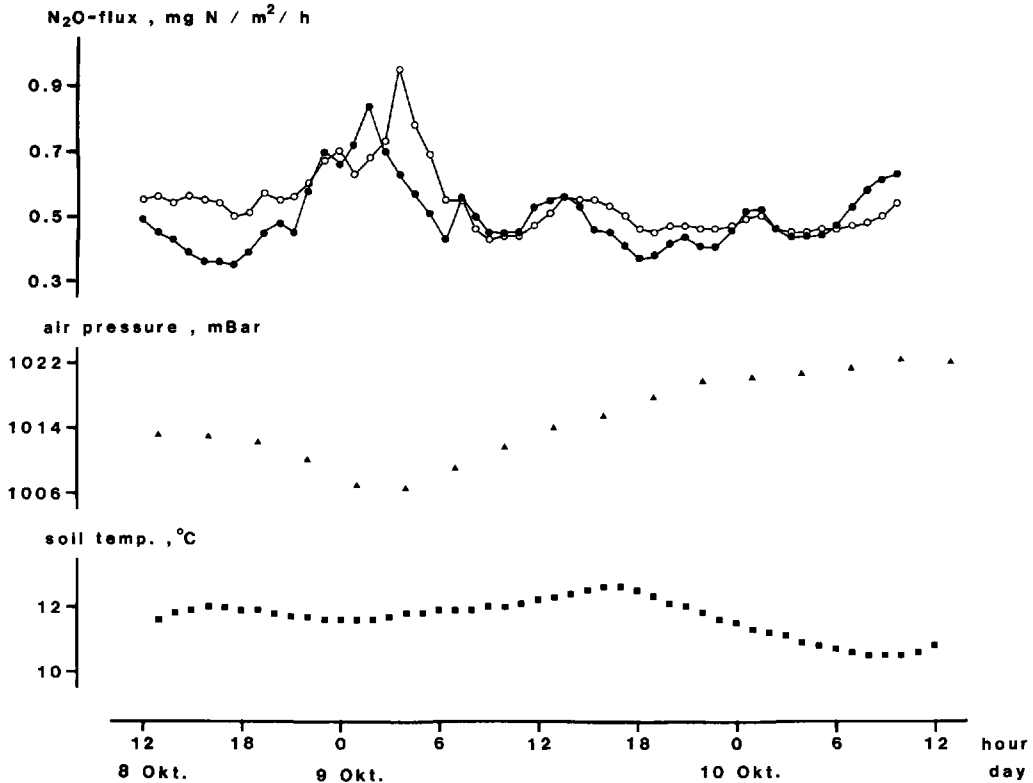


Fig. 3.  $N_2O$ -flux from the two 4 S plots, air pressure and soil temperature (10 cm depth) throughout 2 days (8–10 October 1984). Air pressure data is the mean of observations at Tune, Værløse and Kastrup meteorological stations.

*$N_2O$ -flux fra de 4 S parceller, lufttryk og jordtemperatur gennem 2 døgn (8.–10. oktober 1984). Lufttrykket er gennemsnit af målinger ved Tune, Værløse og Kastrup.*

← Fig. 2.  $N_2O$ -flux measured in the field (kg  $N_2O$ -N/ha/day, ○), and  $N_2O$ -production in soil incubated in the laboratory without acetylene (ng  $N_2O$ -N/g soil/h, ●). Figures in parenthesis are  $N_2O$  production in incubated soil recalculated to an area basis assuming  $N_2O$ -production to occur in the 0–20 cm layer with a volume weight of 1.5 g/cm<sup>3</sup>:  
 Field flux = 1.4 × lab. production + 65 ( $r^2 = 0.22$ ); flasks 1983.  
 Field flux = 0.072 × lab. production + 26 ( $r^2 = 0.22$ ); tubes 1984.

*$N_2O$ -flux i marken (kg  $N_2O$ -N/ha/døgn, ○) og  $N_2O$ -produktion i inkuberet jord uden acetylen (ng  $N_2O$ -N/g jord/time, ●). Tallene i parentes er  $N_2O$ -produktionen i inkuberet jord omregnet til areal, forudsat et produktionslag på 0–20 cm med volumenvægt 1,5 g/cm<sup>3</sup>:*

*Mark flux = 1,4 × lab. produktion + 65 ( $r^2 = 0,22$ ); kolber 1983.*

*Mark flux = 0,072 × lab. produktion + 26 ( $r^2 = 0,22$ ); rør 1984.*

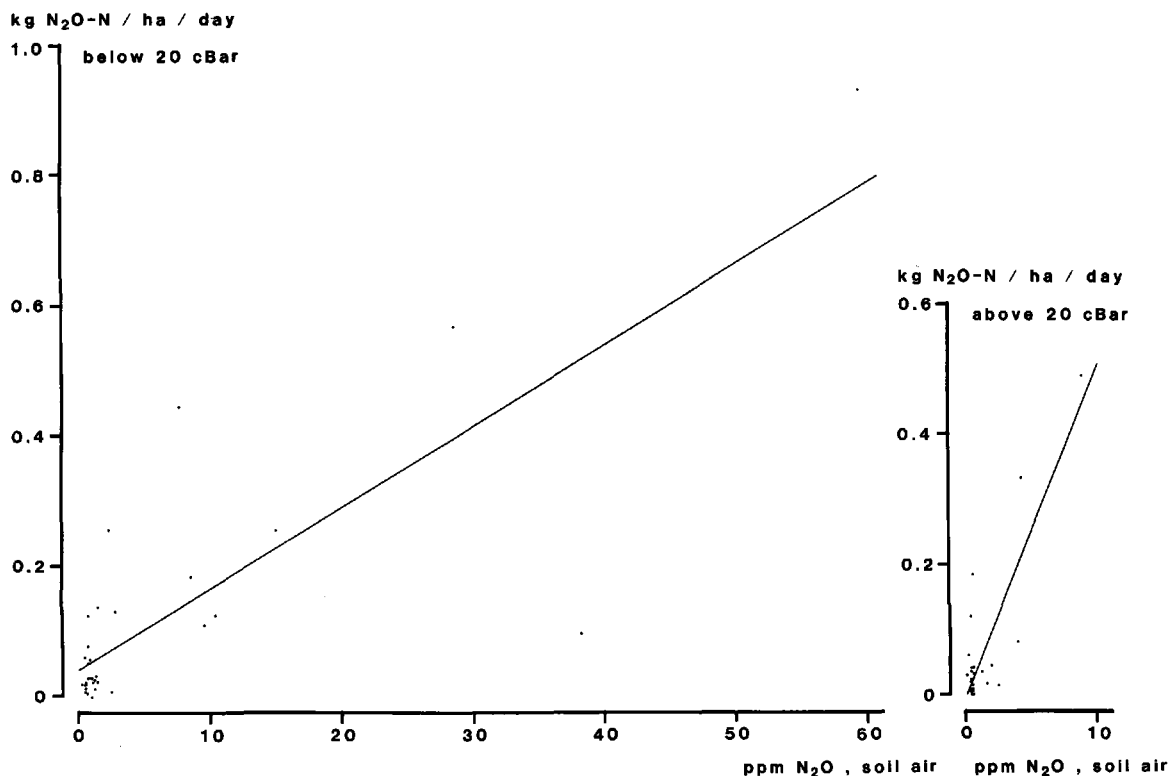


Fig. 4.  $N_2O$ -flux versus soil air  $N_2O$ -concentration throughout 1983 and 1984. The data is divided in moist (below 20 kPa) and dry (above 20 kPa) conditions.

*$N_2O$ -flux afbildet mod jordluftens  $N_2O$ -koncentration gennem 1983 og 1984. Data er opdelt i våde (under 20 kPa) og tørre (over 20 kPa) betingelser.*

#### *$N_2O$ -production during incubation*

The  $N_2O$  produced during incubation of soil in tubes and flasks without addition of acetylene is shown in Fig. 2. The  $N_2O$ -production during incubation increased over the same periods as the  $N_2O$ -flux measured in the field. At water saturated conditions in spring 1983 the field flux of  $N_2O$  was high while the  $N_2O$ -production in flask-incubated soil, increased only slightly. On the contrary,  $N_2O$ -production in soil incubated in tubes was up to 10 times the field measurement in June and late autumn 1984.

The ratio of  $N_2:N_2O$  produced during incubation is shown together with soil nitrate content and soil water content in Table 4. There was a tendency towards high ratios in the 20–40 kPa region and lower ratios at tensions higher and lower than 20–40 kPa. The  $N_2:N_2O$ -ratios were found to

**Table 4.**  $N_2:N_2O$ -ratios in relation to soil moisture and soil nitrate. Mean value, standard deviation and number of results.

*$N_2:N_2O$ -forholdet i forhold til jordfugtighed og jordens nitratinhold. Gennemsnit,  $N_2:N_2O$ -standardafvigelse og antal resultater er vist.*

Soil moisture kPa	0–10	10–20	20–40	40–80
Soil nitrate ppm N	$N_2:N_2O$			
0–10	2.2±2.9 (n=25)	2.5±2.2 (n=7)	3.6±7.0 (n=10)	0.7±1.1 (n=9)
10–20	0.7±1.0 (n=2)	2.9±2.1 (n=4)	3.0±4.2 (n=2)	2.2±2.9 (n=4)
20–30	1.1 (n=1)	2.2±1.9 (n=3)	6.7±1.9 (n=2)	0 (n=1)
30–40	(n=0)	0.6 (n=1)	(n=0)	0 (n=1)
40–50	(n=0)	1.3 (n=1)	4.7±0.9 (n=2)	0 (n=1)



**Table 3.** N<sub>2</sub>O-flux, N<sub>2</sub>:N<sub>2</sub>O-ratio and total N-loss (N<sub>2</sub>+N<sub>2</sub>O).  
N<sub>2</sub>O-flux, N<sub>2</sub>:N<sub>2</sub>O-forhold og total N-tab (N<sub>2</sub> + N<sub>2</sub>O).

Date	Period	N <sub>2</sub> O-flux								N <sub>2</sub> :N <sub>2</sub> O				N-loss			
		g N/ha/day				kg N/ha/period				1N	4N	1S	4S	kg N/ha/period			
		1N	4N	1S	4S	1N	4N	1S	4S					1N	4N	1S	4S
<i>1983</i>																	
5 May	12 Apr.-6 May	446	603	137	321	11	15	3.4	8.0	0.9	0.6	2.3	2.9	22.1	27.5	15.5	33.1
9 May	7 May-11 May	130	444	254	95	0.65	2.2	1.3	0.48								
16 May	12 May-24 May	281	305	106	227	3.4	3.7	1.3	2.7	2.0	1.1	1.9	1.4	21.0	35.1	11.3	25.4
1 June	25 May-6 June	255	930	184	566	3.6	13	2.6	7.9								
15 June	7 June-21 June	46	334	81	489	0.64	5.3	1.1	6.8	0	0	0*	0*	1.1	6.5	1.7	8.8
28 June	22 June-5 July	30	61	28	120	0.42	0.85	0.39	1.7								
13 July	6 July-19 July	6	23	16	21	0.08	0.32	0.22	0.29	0	1.0	0*	0*	0.3	0.9	0.3	0.7
27 July	20 July-2 Aug.	3	7	5	13	0.04	0.10	0.07	0.18								
9 Aug.	3 Aug.-27 Aug.	1	3	1	2	0.03	0.08	0.03	0.05	0	1.0	0*	0*	0.3	0.9	0.3	0.7
14 Sep.	28 Aug.-20 Sep.	4	6	5	8	0.10	0.14	0.12	0.19								
27 Sep.	21 Sep.-4 Oct.	6	8	8	20	0.08	0.11	0.11	0.28	2.4	0.6	1.1	2.3	0.8	0.6	0.8	1.7
11 Oct.	5 Oct.-18 Oct.	2	10	9	18	0.03	0.14	0.13	0.25								
25 Oct.	19 Oct.-1 Nov.	9	10	11	7	0.13	0.14	0.15	0.10	2.4	0.6	1.1	2.3	0.8	0.6	0.8	1.7
8 Nov.	2 Nov.-15 Nov.	5	6	6	13	0.07	0.08	0.08	0.18								
Sum						20.3	41.2	11.0	29.1	1.23	0.71	1.65	1.40	45.3	70.6	29.6	69.7
<i>1984</i>																	
26 Apr.	10 Apr.-1 May	19	17	18	137	0.41	0.37	0.40	3.01	0*	1.3	5.9	3.7	0.4	0.9	2.8	14.1
7 May	2 May-14 May	16	36	34	185	0.21	0.47	0.44	2.41	5.3	5.3	8.0	4.0	1.3	3.0	4.0	12.1
21 May	15 May-29 May	3	0	6	33	0.04	0	0.09	0.50	0	0	0	0*	0	0	0.1	0.5
5 June	30 May-6 June	6	6	9	42	0.05	0.05	0.07	0.34	0.9	2.0	0.3	0*	0.1	0.2	0.1	0.3
7 June	7 June-10 June	22	110	7	125	0.09	0.44	0.03	0.50	2.0	0.5	9.5	2.3	0.3	0.7	0.3	1.7
12 June	11 June-19 June	18	63	12	117	0.16	0.57	0.11	1.05	4.5	0.4	6.5	2.3	0.9	0.8	0.8	3.5
26 June	20 June-2 July	19	61	-1	77	0.25	0.79	-0.01	1.00	1.0	1.1	8.2	2.1	0.5	1.7	0	3.1
9 July	3 July-1 Aug.	5	4	1	26	0.15	0.12	0.03	0.78	1.8	0*	3.6	6.5	0.4	0.1	0.1	5.9
3 Sep.	2 Aug.-6 Sep.	8	22	19	42	0.29	0.77	0.67	1.47	2.6	0	2.7	6.3	1.0	0.8	2.5	10.7
10 Sep.	7 Sep.-17 Sep.	17	30	5	36	0.19	0.33	0.17	0.40	22.5	0	0.8	5.9	4.5	0.3	0.3	2.8
24 Sep.	18 Sep.-1 Oct.	22	26	22	27	0.31	0.36	0.31	0.38	11.0	0.9	0	1.6	3.7	0.7	0.3	1.0
8 Oct.	2 Oct.-18 Oct.	28	56	57	124	0.48	0.95	0.97	2.11	1.1	0.4	0.4	4.3	1.0	1.3	1.4	11.2
29 Oct.	19 Oct.-5 Nov.	12	27	28	51	0.22	0.49	0.50	0.92	1.1	1.0	0.4	1.6	0.5	1.0	0.7	2.4
19 Nov.	6 Nov.-1 Dec.	6	9	7	5	0.16	0.24	0.19	0.14	0	0	1.2	0	0.2	0.2	0.4	0.1
Sum						3.0	6.0	4.0	15.0	3.93	0.95	2.45	3.63	14.8	11.7	13.8	69.4

vary between 0 and 22.5. The lowest ratios were obtained at the highest NO<sub>3</sub><sup>-</sup>-addition both years. The manured soil showed higher ratios than the fertilized soil apart from the low fertilizer level in 1984. The mean N<sub>2</sub>:N<sub>2</sub>O-ratios in all treatments were higher in 1984 than in 1983.

#### Estimated N-loss in the field

Table 3 shows the total N-loss as N<sub>2</sub>O and N<sub>2</sub>. In the NH<sub>4</sub>NO<sub>3</sub> treatments and in the low slurry treatment, 2-6 times more N was lost in 1983 than

in 1984. Similar amounts of N were lost at the high slurry addition in the two years. Small discrepancies between the results in Table 3 and an earlier presentation (11) is due to differences in the calculation procedure.

#### Discussion

##### Method to determine N-loss

The N-loss is estimated from two components: The N<sub>2</sub>O-loss measured in the field and the N<sub>2</sub>:N<sub>2</sub>O-ratio determined in the laboratory. Dur-

ing measurement of  $N_2O$ -flux in the field the soil system is disturbed less than during measurement of  $N_2O$ -production in incubated soil. The flux of  $N_2O$  is therefore believed to give a more realistic measure of  $N_2O$ -loss in the field than the incubation. With no method available to measure  $N_2$ -production directly in the field, the addition of acetylene to inhibit  $N_2O$ -reduction appears to be a good method (2) whereby the total denitrification is measured as  $N_2O$ -production. The method is validated with  $^{13}N$  and found to give true values for denitrification in incubation experiments not exceeding a few hours (36). Side effects of acetylene on the soil system are mostly observed during use of acetylene over long periods (23). The most direct approach is to add acetylene directly to the soil in the field (32). It seems difficult, however, to maintain a constant acetylene concentration in the soil air, this is more easily obtained during laboratory incubation.  $N_2O$ -production was therefore determined in laboratory incubated soil with and without acetylene to obtain a relative measure of  $N_2O$ -production. In the first year incubations were performed in flasks. The disruption of soil structure may result in a decreased denitrification compared with undisturbed soil (29). When incubating undisturbed samples in tubes, as done in the second year, the distribution of acetylene throughout the samples will be a more critical factor compared to incubation in flasks. A higher denitrification rate was measured when gas was circulated through the incubation system and this was ascribed to the problem of getting an even distribution of acetylene in the soil (29). It must be remembered, however, that while soil was loosened by flask incubation, soil sampling in tubes resulted in a certain compaction, up to about 5% in water-saturated soil. The influence of this loosening and compaction on  $N_2O$ -production is illustrated by the linear correlation between field and laboratory measurements (Fig. 2). While  $N_2O$ -production in flasks should be multiplied by 1.4,  $N_2O$ -production in tubes should be multiplied by 0.07 to give the field flux of  $N_2O$ . The  $N_2:N_2O$ -ratios will tend to be underestimated in flasks and

overestimated in tubes, regarding the influence of  $O_2$  on  $N_2:N_2O$  (19). This means that the N-loss will tend to be underestimated in the 1983 and overestimated in the 1984 investigation.

To summarize, the critical point in determination of the N-loss by the present method is determination of the  $N_2:N_2O$ -ratios. This means that the N-loss is presumably determined with greater accuracy the lower the  $N_2$ -formation is. The disturbance of the soil can be minimized by tube incubation compared to incubation in flasks. By decreasing the length in relation to the diameter of the core, soil compaction could be reduced. It is believed that this approach will make it possible to estimate N-loss in the field from laboratory incubations of soil with acetylene (29).

In addition, calculation of seasonal N-loss from measurements made at biweekly intervals can be critical. Especially in periods of high denitrification activity, changes in soil moisture content between measurements could give large variations, that will not be recorded, in the gaseous loss.

#### *$N_2O$ -flux in the field*

The daily  $N_2O$ -flux rate was closely dependent on soil moisture content. The diurnal variation in the  $N_2O$ -flux was sometimes correlated with changes in air pressure (Fig. 3). These variations in the  $N_2O$ -flux observed for cultivated soil are small compared to the variations from grass covered soil.

Here maximal  $N_2O$ -fluxes of up to 23 times larger than the minimal fluxes during the day were ascribed to changes in microbial activity in the rhizosphere coupled with photosynthesis (10).

The influence of soil moisture on the relation between soil air  $N_2O$ -concentration and the  $N_2O$ -flux is illustrated by the equation  $N_2O\text{-flux} = a \cdot N_2O\text{-concentration} + b$ : Considering all data, the slope (a) is 13.3; for wet soil 12.5 and for dry soil 50.6. This means that  $N_2O$ -flux increases four times faster with increasing  $N_2O$ -concentration in dry than in wet soil. Moreover the linear regression coefficient increases for both the dry and wet group ( $r^2 = 0.73$  and  $0.65$  respectively) compared

to when all data is taken together ( $r^2 = 0.62$ ). In a similar experiment,  $N_2O$ -flux was correlated with  $N_2O$  in soil air at 10 and 20 cm depth with  $r^2$  of 0.62 and 0.53 respectively (27). The influence of soil moisture on the relation between soil air  $N_2O$ -concentration and  $N_2O$ -flux rate illustrates the reduction in gaseous diffusion caused by increased water content. When soil moisture changes from air dry to field capacity, the diffusion coefficient decreases by 4 orders of magnitude (15, 16, 35). The  $N_2O$ -production is large at water contents from about field capacity. A small change in water content at this level results in a drastic change in the diffusion coefficient (17). The calculation of  $N_2O$ -flux on the basis of  $N_2O$ -content in the soil air and a moisture-dependent diffusion coefficient for  $N_2O$  (7) can therefore hardly be carried out with precision.

#### $N_2:N_2O$

In the period following nitrogen addition the  $N_2:N_2O$ -ratios depended on the amount of  $NO_3^-$  added: On an average lowest ratios were obtained at high  $NO_3^-$ -addition and manured soil showed higher ratios than fertilized soil.

Regarding the  $N_2:N_2O$ -ratios for single days throughout the seasons gives a more distorted picture: The observed maximum in unsaturated soil and the independency of  $NO_3^-$ -concentration does not agree with results stated in the literature. The  $N_2:N_2O$ -ratio decreased from above 99 without nitrate to 1 and 0.6 at 50 and 100 ppm  $NO_3^-$ -N respectively in the first half day of anaerobic incubation (4). During short time incubation of a soil slurry with  $^{13}N$ , the  $N_2:N_2O$ -ratio decreased from 20 to 6 when nitrate content increased from 0 to 20 ppm N (19). Furthermore, by increasing the  $O_2$  partial pressure from 0 to 0.05 atm, the  $N_2:N_2O$ -ratio decreased from 5.5 to 0.4–0.8.

The discrepancy between the present results and those from the literature may be explained by regarding the soil moisture in the period before the  $N_2:N_2O$ -ratio was measured rather than on the day of measurement: The  $N_2:N_2O$ -ratio was  $2.9 \pm 3.7$  ( $n = 56$ ) at water saturated conditions

compared to  $0.8 \pm 1.6$  ( $n = 20$ ) at unsaturated conditions in the week preceding the measurement. This agrees qualitatively with the above mentioned references and with the trend in  $N_2:N_2O$ -ratios depending on the duration of water saturation from other investigations: Incubating soil anaerobically with nitrate gave an increase in the  $N_2:N_2O$ -ratio from 0 to 10 during one week (24) and from 0.4 to 0.8 over two weeks in a sandy loam soil (22). During short-term incubation, the  $N_2:N_2O$ -ratio increased from 1 to 4 over 1–2 days (19). In field measurements following water saturation, the  $N_2:N_2O$ -ratio increased from about 3 to 23 in two weeks (31), or from 2 to 19 in one week (32).

The size of the  $N_2O$ -production with and without acetylene gives information about the amounts of  $N_2O$  derived from nitrification and denitrification respectively. When  $N_2O$ -formation with acetylene was lower than without, the  $N_2O$ -formation by nitrification exceeded the  $N_2 + N_2O$ -formation by denitrification (1). This condition was in some cases found in unsaturated soil (Table 3). The high  $N_2O$ -fluxes in June 1983 from slurry treated soil may not have been a result of nitrification alone, but could have been caused by liberation of  $N_2O$  accumulated in the soil during the preceding period of vigorous denitrification.

#### *N-loss in the treatments*

The loss of nitrogen was greatly stimulated by anaerobic soil conditions. Water saturated soil as well as unsaturated soil amended with large amounts of organic matter showed the highest denitrification.

The low N-loss in autumn 1983 compared to 1984 may be explained by water saturation occurring two weeks later in 1983 than in 1984 and that desiccated soil conditions lasting for three months in 1983 gave low numbers of denitrifying bacteria (see below).

The difference in N-loss in spring and autumn is remarkable. At comparable conditions of temperature, soil water content and nitrate, the N-loss in autumn 1984 was lower than in spring 1983. This apparent difference in denitrifica-

tion potential could have been caused by difference in amount of available organic matter. Most isolates of denitrifying bacteria in this soil belonged to genus *Pseudomonas*, mainly *P. fluorescens* and *P. putida*, (Christensen & Bonde in prep.). This group of bacteria seldom use organic compounds of high molecular weight (3). Therefore the organic matter input as root and stubble should be attacked by primary decomposers before being available to the denitrifying bacteria. This breakdown, followed by the disintegrating action of freezing and thawing on plant debris and on the primary decomposers, may be the reason for the higher denitrification potential in spring compared to autumn.

Soil cores of 3.5 cm diameter from the top 5 cm layer were incubated at intervals throughout 1984. After the addition of acetylene, denitrification was measured as the N<sub>2</sub>O-accumulation from 2nd to 3rd day determined by a gas chromatograph with EC detector. (Marianne Kemner, pers. comm.). The estimated N-loss over the season was about 13, 70, 11 and 69 kg N/ha for the low and high NH<sub>4</sub>NO<sub>3</sub> and slurry additions, respectively. This N-loss is 6 times above the values found in this investigation for the high NH<sub>4</sub>NO<sub>3</sub> application while it was within 20% of this study for the other treatments. The large difference found between results with 160 kg NH<sub>4</sub>NO<sub>3</sub>-N/ha was for the major part caused by one of the replicates measured on 6 June.

The N-losses measured in this study are high compared to an earlier study with a similar soil kept without vegetation and fertilizer for more than 10 years. This soil treated in the same way only produced gaseous N in N<sub>2</sub>O amounting to 1.6, 0.4 and 0.5 kg N/ha for cow slurry, KNO<sub>3</sub> and unfertilized soil respectively in the period May–September (12). The N<sub>2</sub>O and N<sub>2</sub> + N<sub>2</sub>O-losses reported here are well above the values for drier conditions than the present (26, 27, 28), and also above values following nitrogen addition to a vigorously growing crop of grass or winter wheat (14). On the other hand, higher N-losses compared to this study were found from a fertilized and irrigated soil of heavier texture (33).

#### *N-loss and denitrifying bacteria*

The number of denitrifying bacteria in the soil was determined in 1983 in the 160 kg N/ha and the 100 t slurry/ha treatments (Christensen & Bonde in prep.). The most probable number (MPN) of N<sub>2</sub> and N<sub>2</sub> + N<sub>2</sub>O producing bacteria followed the variations in denitrification reported here; high numbers were found in April and May and low numbers in August. Bacterial isolates were grouped in vigorous N<sub>2</sub>O- and N<sub>2</sub>-formers and in NO<sub>2</sub>-formers with weak N<sub>2</sub>O-formation. The number of N<sub>2</sub>-forming isolates obtained at the samplings followed the variation in denitrification activity as opposed to the number of N<sub>2</sub>O-forming isolates. 84% of all isolates belonged to genus *Pseudomonas*. The influence of organic matter, pH (13) and O<sub>2</sub> on the isolates must be ruled out before the importance of the different groups of N-gas formers in soil denitrification can be determined.

#### *Denitrification model*

In this study, soil moisture (F), soil nitrate (N) and the time of the year (S) are assumed to be key variables responsible for the size of denitrification. The model is:

$$D = f(F)^a \times f(N)^b \times f(S)^c$$

D is the denitrification rate in kg N/ha/day calculated from Table 3. f(F) and f(N) is the dependency of denitrification on soil moisture and soil nitrate respectively. f(F) is determined in soil incubated in tubes as described above while f(N) is calculated from the data of Firestone *et al.* (20). f(S) express the effect of carbon mineralization during autumn and winter on denitrification. The three functions are listed in Table 5. a, b and c are constants. The equation was tested with a normalization constant (D<sub>0</sub>) on the right side. D<sub>0</sub> showed up to have numeric value 1 and was therefore omitted.

The data gives a = 0.59\*\*\* (p < 0.01%), b = 1.15\*\* (p < 0.9%) and c = 1.24\*\*\* (p < 0.01%), showing that all factors are highly significant in this model. The model predicts the denitrification

**Table 5.** Effect of soil moisture, soil nitrate and time of year on denitrification rate used in the model. All functions are linear between the shown values.

*Effekt af jordfugtighed, nitratindhold og tid på sæsonen på denitrifikationsraten anvendt i modellen. Alle funktioner er lineære mellem de viste værdier.*

Soil moisture (F) % of dry weight	Denitrification rate $\mu\text{g N/kg soil/h}$	$f(F)^{1)}$
15.0	0.082	0.03
17.5	0.069	0.03
20.0	1.32	0.46
22.5	2.38	1.00
Soil nitrate (N) ppm N	Denitrification rate <sup>2)</sup> $\mu\text{g N/g soil/h}$	$f(N)^{1)}$
0	0	0
0.5	0.54	0.47
2	0.73	0.63
20	1.15	1.00
Season (S) day of year		$f(S)$
60		1
360		0

<sup>1)</sup> The measured denitrification rate is normalized to give  $f(F)$  and  $f(N)$ .

<sup>2)</sup> Data from Firestone et al. (1979).

with a mean error of 0.47 kg N/ha/day and has a significance better than 99.99%.

A multiplicative model is used because the effect of one of the factors moisture, nitrate and organic matter is dependent on the state of the other factors. Temperature is not withdrawn as the data does not reveal any influence of this factor. The variation not accounted for is increased to 0.64 kg N/ha/day when the factor  $f(S)^c$  is omitted. This shows that although the denitrification predicted by the model is very inaccurate, it supports the seasonal fluctuation in easily available organic matter suggested above. The variation of 0.47 kg N/ha/day corresponds to 225% of the mean denitrification rate. This is of the same magnitude as the variation in the laboratory and field measurements used to estimate the denitrification. This shows that before more precise models of denitrification can be made, the precision of the measurement procedures should be improved.

## Conclusion

The size of denitrification is determined from the  $\text{N}_2\text{O}$ -flux measured in the field and the ratio of  $\text{N}_2$ - to  $\text{N}_2\text{O}$ -production determined on soil incubated in the laboratory. The  $\text{N}_2\text{O}$ -flux is regarded to be a good measure of the  $\text{N}_2\text{O}$ -production in the field. A comparison of field and laboratory measurements of  $\text{N}_2\text{O}$ -production shows that the technique of soil sampling has great influence on the denitrification activity. The size of  $\text{N}_2\text{O}$ -production in incubated soil differed markedly from the field  $\text{N}_2\text{O}$ -production. For this reason the estimate of denitrification is open to criticism. The field denitrification rate will be determined with lower precision the larger the  $\text{N}_2$ -production is. It is believed that sampling of soil in cores with a minimum of disturbance will give the most reliable measure of the ratio of  $\text{N}_2$ - to  $\text{N}_2\text{O}$ -production.

The denitrification rate was highest in water-saturated soil in periods without crop growth. Moreover the potential denitrification rate was larger in the spring than in the autumn. It is suggested that microbial breakdown of crop residues is followed by physical disruption of plant debris and microbes during the winter. This gives an increased amount of organic matter available to the denitrifying bacteria in the spring. A model to calculate the denitrification rate from factors in the soil environment had low predictive value ( $r^2 = 0.18$ ), but sustained the effect of seasonal variations in available organic matter as suggested above.

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