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Denitrification in the root zone

Denitrifikation i rodzonen

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Summary
This paper concerns investigations of denitrification in some Danish soils. Soil samples were incubated in flasks under anaerobic conditions. The nitrogeneous gases produced during thirty-day periods of incubation were determined with the purpose of estimating the denitrification capacity of clay soils and sandy soils in increasing depths within the root zone.

The top layer of the clay soils possessed about twice as much denitrification capacity as the top layers of the sandy soils. The denitrification capacity decreased to about one tenth from the top layers to the lower layers of a root zone profile. The denitrification capacity varied with the soil temperature, and was related to biological manifestations such as carbon dioxide production and number of denitrifying bacteria.

Key words: Denitrification, nitrous oxide, dinitrogen, denitrifying bacteria, soil depth, soil temperature.

Resumé
Beretningen omhandler undersøgelser over denitrifikation i nogle danske jorde. Forsøgene blev gennemført med jordprøver placeret i lukkede anaerobe kolber og omfattede bestemmelse af de luftformige nitrogenforbindelser, der er produceret i løbet af 30 dage. Ved denne metode er variationen i denitrifikationskapacitet med jordtype og jorddybde undersøgt. Det er fundet, at det øverste lag af de undersøgte lerjorde besidder dobbelt så stor denitrifikationskapacitet som det øverste lag af sandjordene, og at denitrifikationskapaciteten falder kraftigt fra øverste til nederste lag af en rodzone-profil. Variation i denitrifikationskapacitet med temperatur og tid er blevet undersøgt. Denitrifikationens afhængighed af biologiske egenskaber, såsom kuldioxidproduktion og antal denitrificerende bakterier, er undersøgt.

Nøgleord: Denitrifikation, dinitrogenoxid, dinitrogen, denitrificerende bakterier, jorddybde, jordtemperatur.

Introduction

Denitrification, which is the same as biological or dissimilative nitrate reduction, is defined as the microbiological reduction of nitrate, by which the end products are the gaseous nitrogen compounds, dinitrogen, N_2, and nitrous oxide, N_2O. This process can occur in the soil, if the amount of free oxygen is reduced. In case of lacking of atmospheric oxygen a group of soil bacteria (facultative anaerobic) can utilize the oxygen incorporated in the nitrate ion. Simultaneously the nitrate nitrogen is reduced in accordance with the reaction scheme shown in Fig. 1. A detailed description of the mechanisms in the individual steps of the process is given by *Payne* (1973).

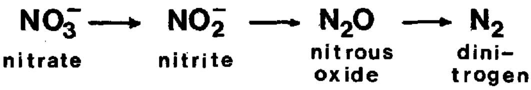

Fig. 1: The denitrification process
Denitrifikationsprocessen

The purpose of the investigations is to examine the importance of the denitrification process as a part of the nitrogen cycle in the soil. Fig. 2 shows what may happen to a nitrate ion in the root zone of a cultivated soil.

A better knowledge of the denitrification process is important, partly because the process could mean a loss of nitrogen, which otherwise should have been available for the plant growth, but also because it may contribute to a removal of a possible excess of nitrogen, which otherwise could be leached to under-lying soil layers.

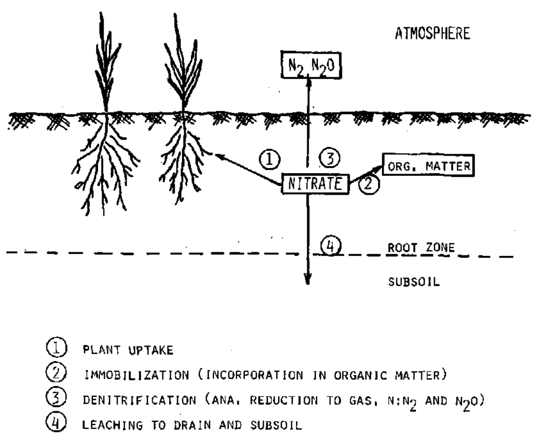

Fig. 2:
Nitrate transformations and movements in the root zone
Nitratomsætning og nitrattransport i jordens rodzone

Previous investigations

The denitrification process has been known during most of the last century, in pure bacteria cultures as well as in soil. Many methods of analysis have been used to estimate the extent of the denitrification: changes in contents of nitrate or total nitrogen, and gas evolution, but only in the last ten to twenty years have the analytical possibilities been improved, with the use among others of the stable nitrogen isotope, ^{15}N, and gas chromatographic methods.

In the following some of the most important contributions to the clearing up of the extent of the denitrification process in soil are mentioned. *Woldendorp* (1963) studied the influence of plant growth on denitrification in pot experiments with the use of ^{15}N. Calculations of the single elements of the total nitrogen balance showed that the plant growth appeared to have a strongly stimulating effect on the denitrification. Furthermore the dependence of the denitrification on hydrogen donors and microorganisms has been examined.

In addition to this, *Woldendorp* (1968) has written a review concerning losses of nitrogen, including the influences of oxygen contents, hydrogen donors, plant growth, pH and temperature on the denitrification, and a description of the microorganisms involved in the soil denitrification.

Another investigation on denitrification in soil has been made by *Nõmmik* (1976), who also used ^{15}N as a mean to quantify the denitrification. These investigations comprised the dependence of the denitrification on soil type, time (2–12 days), oxygen partial pressure, size of soil aggregates, nitrate concentration, temperature and pH.

The denitrification in different soil depths has not been investigated to a great extent. *Jones* (1974) has made experiments with small soil columns in soil depth 0–17 cm, divided into 7 different layers. He found the greatest denitrifying activity in the lowest 4 layers and no activity in the upper 3 layers. The conditions were 90 pct. water saturation in the soil and ordinary atmosphere. *Stefanson* (1976) has measured denitrification from some reconstructed soil columns of a length of 30 cm placed in sealed growth chambers, and with nitrate placed in various depths: 5, 15 and 25 cm. The atmosphere was 80 pct. argon and 20 pct. oxygen, and the water content was field capacity. The actual position of nitrate fertilizer did not influence the denitrification. Cropping the soil, however, resulted in twice as much volatilization from the soil. *Stefanson* (1972) also made comprehensive denitrification investigations in sealed soil-plant systems with a possibility for controlling the atmosphere. These investigations have among others included the dependence of the denitrification on water content, amount of organic matter, plant growth and type of nitrogen fertilizer applied.

Series of papers concerning the latest investigations on denitrification and other parts of the nitrogen cycle are collected in the paper »Nitrogen in the Environment«, ed. *Nielsen* and *MacDonald* (1978).

Materials

For the experiments undried soil samples from 6 different sites (Governmental Exp. Stations) were used, taken from different depth intervals in the root zone down to approximately 1 meter. The soil was sampled from depth intervals corresponding to a natural stratification of the soil profiles. For some of the experiments, the soil samples have, still in undried condition, been stored in polyethylene boxes at a temperature of 5°C. The physical properties of the soil samples and

Table 1. Physical and chemical properties of soil used for denitrification experiments
Fysiske og kemiske egenskaber for jorde anvendt til denitrifikationsforsøg

Site[1])	No.	Depth cm	pH (H_2O)	Pct. clay <0.002 mm	Pct. silt 0.002–0.02 mm	Pct. fine sand 0.02–0.2 mm	Pct. coarse sand 0.2–2.0 mm	humus	Pct. N
Virumgård	1 A	0– 20	6.6	10.5	15.8	49.2	22.6	1.9	0.120
	1 B	20– 40	6.2	9.8	13.5	51.3	23.7	1.7	0.107
	1 C	40– 60	6.3	9.8	16.3	46.0	26.7	1.2	0.073
Askov	2 A	0– 23	6.5	13.0	8.7	43.9	30.6	3.8	0.186
	2 B	23– 50	6.3	19.8	8.8	25.4	44.6	1.4	0.061
	2 C	50–100	5.2	18.9	10.5	39.5	31.4	0.7	0.032
Lundgård	3 A	0– 25	5.9	4.3	3.3	20.0	70.5	1.9	0.086
	3 B	25– 55	5.8	4.0	1.9	9.5	83.4	1.2	0.040
	3 C	55–100	5.2	1.5	0.8	5.7	91.7	0.3	0.008
Borris	4 A	0– 40	6.5	5.5	7.2	48.2	37.6	1.5	0.086
	4 B	40– 90	6.2	4.4	4.4	45.2	45.6	0.4	0.021
	4 C	90–120	5.1	5.9	3.3	39.4	51.2	0.2	0.008
Roskilde	5 A	0– 27	7.2	12.6	28.7	46.4	9.9	2.4	0.160
	5 B	27– 50	6.4	19.7	25.8	49.1	4.4	1.0	0.070
	5 C	50– 80	5.0	30.3	24.2	39.1	5.8	0.6	0.047
	5 D	80–100	5.1	10.7	5.7	45.9	37.5	0.2	0.016
Ødum	6 A	0– 40	6.8	12.3	12.7	51.5	21.6	1.9	0.137
	6 B	40– 65	6.9	13.1	13.1	53.5	19.6	0.7	0.053
	6 C	65–100	7.0	16.5	11.6	47.9	22.1	1.9	0.025

[1]) Governmental Exp. St.

their contents of total N and total C are shown in Table 1. There were 4 sites of a clayey type, (no. 1, 2, 5 and 6), and two of a sandy type, (no. 3 and 4).

Methods

The following chemical and physical analyses were made on the soil samples in accordance with »Fælles arbejdsmetoder for jordbundsanalyser« (1972): particle size distribution, total N and organic C.

Exchangeable ammonium and nitrate were determined just after sampling the soils, and with the set up of denitrification experiments. As extractant 0.1 M $CaCl_2$ was used, and the filtered extract has been steam distilled according to the procedure described by *Bremner* (1965). For liberation of ammonia MgO has been used, and Devarda's alloy for the reduction of nitrate. The choice of extractant is of importance concerning how much of the exchangeable amount of ammonium in the soil will be extracted. 0.1 M $CaCl_2$ was found to be more efficient than 2 N KCl, but do not release so much ammonium as the mixture of 2 M KCl and 1 N HCl.

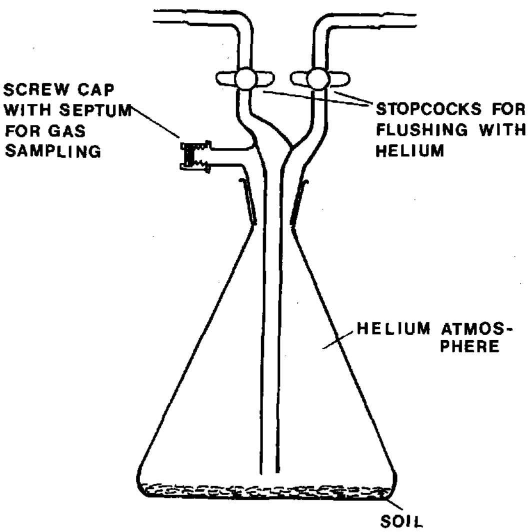

Fig. 3: Equipment for denitrification experiments
Udstyr til denitrifikationsforsøg

Nitrite was determined in the extract from the denitrification experiment in accordance to a modified Griess-Ilosvays method *(Barnes,* 1951).

The amount of denitrifying bacteria has been estimated by the Most Probable Number Method, with 10 tubes for each dilution. The substrate was Nitrate Broth and Bray's powder was used for detecting nitrite/nitrate. The method is based on *Focht* and *Joseph* (1973), and the statistical tables is in accordance to *deMan* (1975).

The equipment used for denitrification experiments shown in Fig. 3 was to some extent especially adapted for these experiments.

The soil sample to be examined, normally 50 g fresh soil, was placed in the bottom of the flask, and 5 ml solution with a known amount of nitrate (5 mg KNO_3-N) was added to the soil. The atmosphere in the flask replaced with an inert gas, normally helium, by evacuating and filling with helium three times, and thereafter flushing with helium for 30 minutes. After a period in a thermostate room, the atmosphere in the flask contained the gases evolved from the soil: nitrous oxide, N_2O, dinitrogen, N_2, and carbon dioxide, CO_2. These gases were analyzed on a gas chromatograph: a sample was withdrawn from the flask through the fitting containing a double septum, one of silicone, and the other of neoprene rubber, which is very resistant to diffusion. It is very important for such experiments to choose material, such as septa and grease for stopcooks, which are as tight as possible against diffusion of dinitrogen in particular.

The conditions of the gas chromatographic analysis were as follows: The columns were 1/8″ stainless steel, packed with molecular sieve no. 5 A. The detector was a thermal conductivity detector, working at a temperature of 300°C; the bridge current is 180 mA; carrier gas is helium at a flow rate of 60 ml/min. Instead of programming the temperature of the column oven from 70°C to 250°C as is usual for this gas mixture used together with molecular sieve no. 5 A, gas samples were injected twice, first at a temperature of 70°C for dinitrogen, N_2, and then once again at 250°C for nitrous oxide and carbon dioxide. This procedure resulted in a better stability, when the gas chromatograph was used at the highest sensitivity, and it was easier and faster to analyse samples and standards. Detection limits are 100 ppm for dinitrogen and 20 ppm for nitrous oxide.

For sampling the gas a 1 ml gas tight syringe is used, Hamilton or Precision Sampling Corporation, and for the separation and analysis of the gas mixture a Hewlett Packard gas chromatograph is used.

Results

The described denitrification experiments have been used for several investigations:

1. Standard denitrification experiments. For these experiments a specific temperature has been selected (25°C), and a fixed incubation period (30 days). This empirical method first of all results in some relative measurements for the denitrifying capacity of different soils and the variation of this capacity with the soil depth. The results of these experiments are shown in Table 2. The denitrifying capacity is stated as mg gaseous N per kg dry soil, evolved in a period of time of 30 days, i.e. the sum of both gaseous products: N_2 and N_2O.

Furthermore, the table shows how much of the initial application of nitrate there has been transformed into the denitrification products, N_2 and N_2O. Only in the top layer of the clay soils (1 A, 2 A and 5 A), near 100 pct. of the original nitrate has been transformed.

There is a good agreement between gaseous nitrogen produced and the decrease in soil con-

Table 2. Denitrification capacity of soil profiles
Denitrifikationskapacitet i jordprofiler

		Denitrification products (25°C, 30 days)			
Soil no.	Soil depth cm	mg N_2	mg N_2O-N	mg $N_2 + N_2O$-N per kg dry soil	mg $N_2 + N_2O$-N pct. of init. NO_3-N
1 A	0– 20	5.37	0	122	96
1 B	20– 40	0.95	0.86	41	34
1 C	40– 60	0.31	0.50	18	15
2 A	0– 23	2.50	1.61	99	97
2 B	23– 50	0.27	0.40	16	12
2 C	50–100	0.14	0.25	9	9
3 A	0– 25	1.25	2.04	74	58
3 B	25– 55	1.02	1.72	60	51
3 C	55–100	0.11	0.05	3	3
4 A	0– 40	1.15	0.80	44	37
4 B	40– 90	0.30	0.13	9	8
4 C	90–120	0.27	0	6	5
5 A	0– 27	3.43	1.21	116	96
5 B	27– 50	0.48	0.31	19	16
5 C	50– 80	0.45	0.36	20	16
5 D	80–100	0.24	0.02	6	5
6 A	0– 40	2.17	2.03	101	74
6 B	40– 65	0.45	0.38	19	15
6 C	65–100	0.35	0.19	12	10

tent of nitrate. Furthermore, to control the method with the gaseous products as a measure for denitrification, some of the soils have been used for similar experiments with addition of $K^{15}NO_3$. The ^{15}N-content of the gases produced has been measured by direct transfer of a part of the gas mixture into an ampoule for later measuring by emission spectrometry. The method is described by *Pheiffer Madsen* (1977).

Based on the rather limited number of soils used as experimental material by the present investigations, and the empirical methods mentioned, it is concluded, that the top layer of the clay soils examined possesses twice as much denitrification capacity as the sandy soils examined, and that the denitrification capacity of a soil profile in the root zone is decreasing to one tenth when moving from the top layer to the lowest layer.

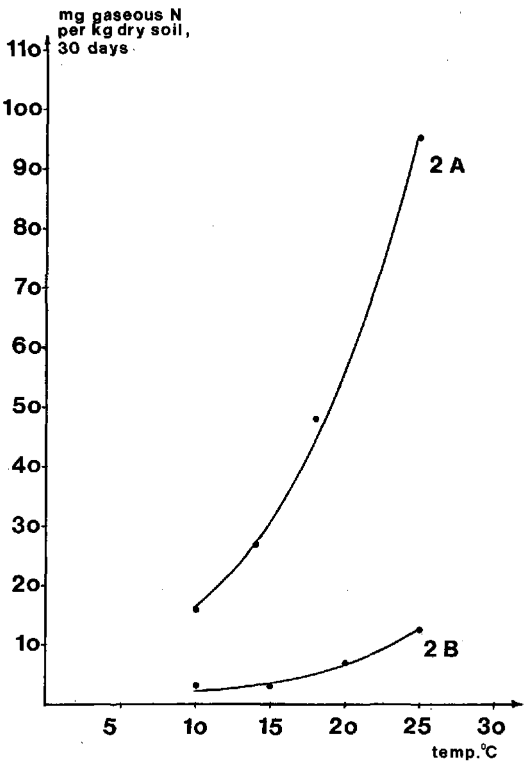

Fig. 4: Effects of temperature on denitrification capacity for soils no. 2A and 2B
Denitrifikationskapacitetens variation med temperaturen (Jord nr. 2A og 2B)

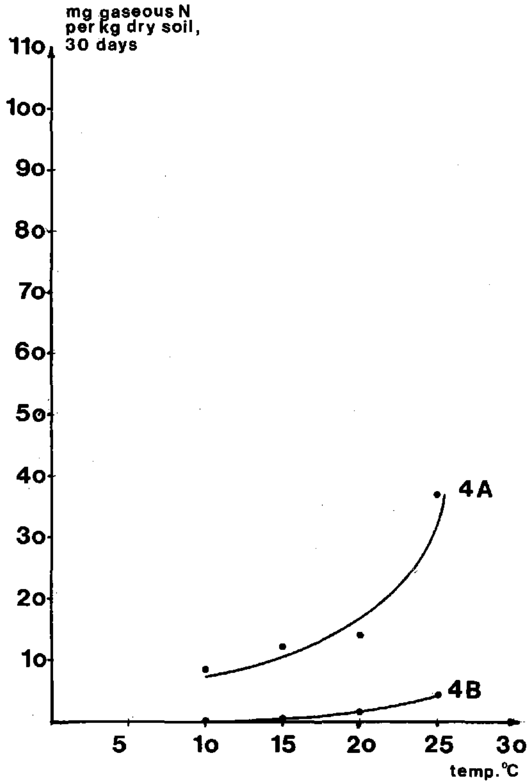

Fig. 5: Effect of temperature on denitrification capacity for soils no. 4A and 4B
Denitrifikationskapacitetens variation med temperaturen (Jord nr. 4A og 4B)

The reasons for the differences in the soil denitrifying capacity are illustrated by the other investigations or experiments described below.

2. *Experiments with varying temperature.* The standard denitrification experiments have been performed at a temperature higher than the temperature normally found in Danish field soils. Therefore, at the same experimental conditions as just described under 1., some of the soils have been investigated for denitrifying capacity at different temperatures below 25°C. The results from these experiments with 2 soils of 2 depths intervals are shown in Fig. 4 and 5. As an average, the

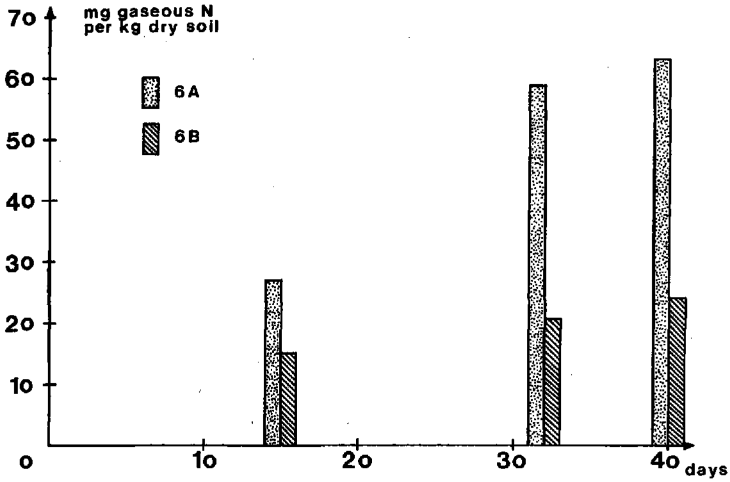

Fig. 6: Effect of incubation time on denitrification capacity for soils no. 6A and 6B
Dinitrifikationskapacitetens variation med inkuberingstid (Jord nr. 6A og 6B)

proportion of the denitrifying capacity at 25°C and 10°C is appoximately 4–5 for all the soil layers. It is more than the average figure for biological processes which normally are calculated with twice as much activity for each 10°C increase of the temperature.

3. Experiments with varying incubation time. The standard denitrification experiments have resulted in an empirical value for the total denitrifying capacity of soil samples within the fixed time interval, 30 days. A denitrification rate would be a valuable supplement to this. Due to the time consuming and complicated techniques caused by the gas analysis, it has not been possible to carry out these analyses frequently enough (i.e. on a daily basis rather than a weekly one) to establish a real rate. The results from these analyses are for some of the soils shown in Fig. 6.

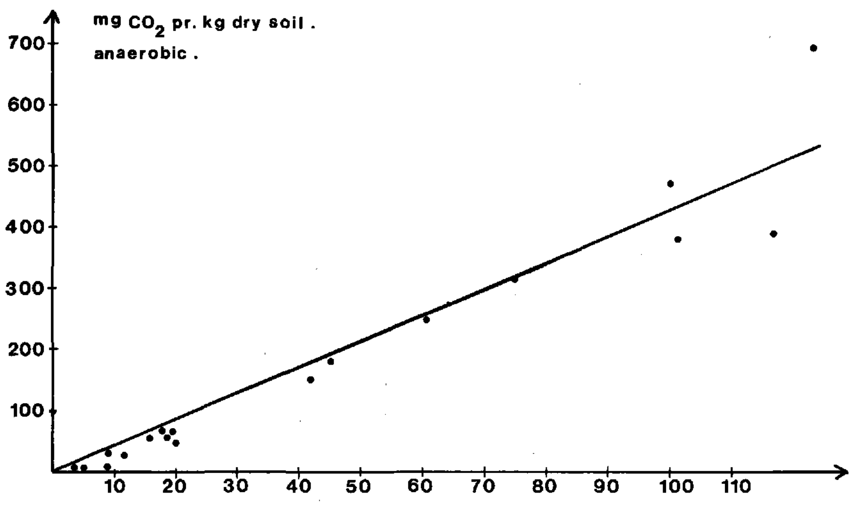

Fig. 7: Relationship between easily decomposable organic matter and denitrification capacity
Sammenhæng mellem let nedbrydeligt organisk stof og denitrifikationskapacitet

From the figure it appears that the denitrification rate is decreasing at an earlier time for the lower soil layer than for the upper soil layer. It is probably caused by a limitation of the amount of easily decomposable organic matter; for the A-layer this sets in after thirty days, while for the B-layer already after 10 days. The approximated denitrification rates are as follows:

6A:	The first 32 days	1.9 mg N/kg soil·day
	Thereafter	0.4 mg N/kg soil·day
6B:	The first 15 days	1.1 mg N/kg soil·day
	Thereafter	0.4 mg N/kg soil·day

4. Denitrification capacity and soil organic matter. The content of easily decomposable organic matter in the soil is estimated by aerobic incubation and determination of the CO_2 liberated, as also done by *Burford* and *Bremner* (1975).

The CO_2 liberated during anaerobic incubation shows a linear relationship with denitrifying capacity of soil (Fig. 7). The relationship between the CO_2 liberated during aerobic incubation shows not this linear relationship.

5. Denitrifying bacteria. Besides the nutrient conditions for the bacteria as the organic matter just described, the number of denitrifying bacteria in the soils may influence the denitrifying capacity. Would there always be sufficient denitrifying bacteria in the soils for the denitrification of nitrate, when partly or total anaerobic conditions arise?

The number of denitrifying bacteria in the soils was counted by means of a dilution technique described above. The result (Fig. 8 and 9) show a difference between clay soils (no. 1, 2, 5 and 6) and sandy soils (no. 3 and 4), especially in the top layer. The number of denitrifying bacteria decreases with the soil depth apart from the lowest layer of soil no. 6. This is also the case with other groups of microorganisms.

The amount of denitrifying bacteria is not a limiting factor in the denitrification process. This will appear when it is stated, that the proportion between denitrifying bacteria, and the total amount of bacteria, estimated by the same proce-

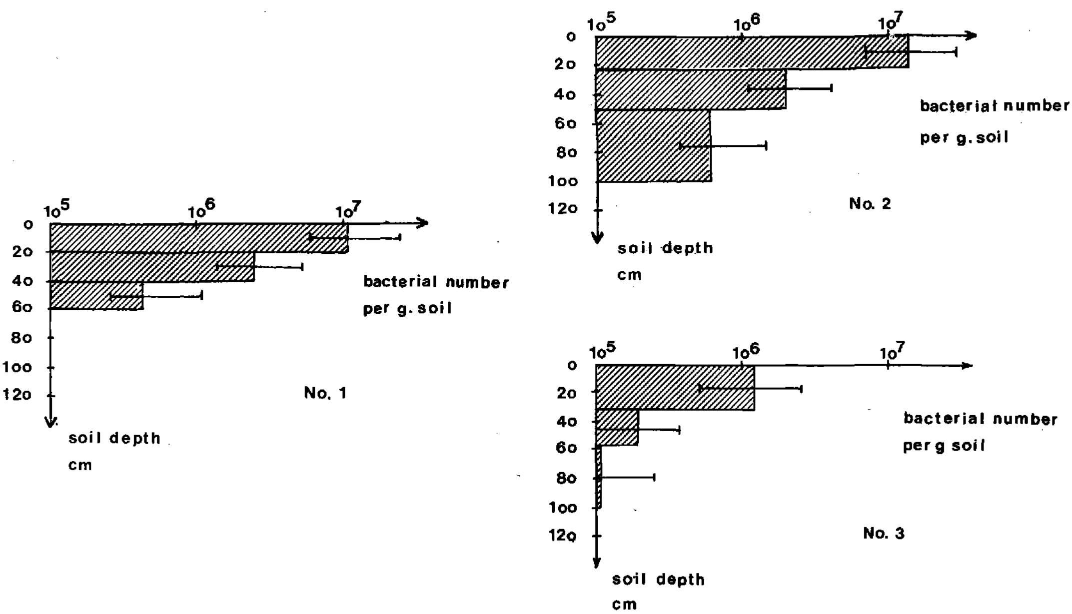

Fig. 8: Effect of soil depth on the number of denitrifying bacteria for soils no. 1, 2 and 3. The number is counted following the MPN-method and stated together with the 95 pct. confidence limits
Antal af denitrificerende bakterier i varierende jorddybde (Jord nr. 1, 2 og 3)

dure through growth in the culture tubes is of the order of magnitude 1:10. This is in accordance, too, with the results found by *Woldendorp* (1963). In other words, the group of bacteria, which by the optimum conditions, i.e. an excess of nitrate and nutrients as well as anaerobic conditions form a very great part of the total bacteria population in the soil.

Conclusions

The investigations have resulted in some relative evaluations of the denitrifying capacity for different soil types and different soil depth in a root zone profile. For the soils here investigated, the top layer of the clay soils have about twice as much denitrifying capacity as the top layer of the sandy soils, and the denitrifying capacity is decreasing considerably, when moving from the top layer to the lowest layer of the profile.

It is important to realize that the conclusions stated here are valid for optimum denitrification atmosphere, i.e. a total oxygen free atmosphere. By field conditions, the differences between clay and sandy soil and between upper and lower soil layers must be greater, since oxygen-limited conditions will be found more often in a clay soil than in a sandy one, and more often in deeper soil layer than in the upper.

As mentioned, results from laboratory experiments could not be directly transferred to field conditions. Therefore, these investigations will be continued as field investigations with sampling and analysing of soil air for content of oxygen, carbon dioxide and nitrous oxide in different soil depths. Furthermore the gaseous escape from the surface, especially of nitrous oxide and carbon dioxide will be investigated.

Aknowledgements

I should like to thank *Inger Andresen* and *Jytte Toft* for valuable technical assistance with the denitrification investigations.

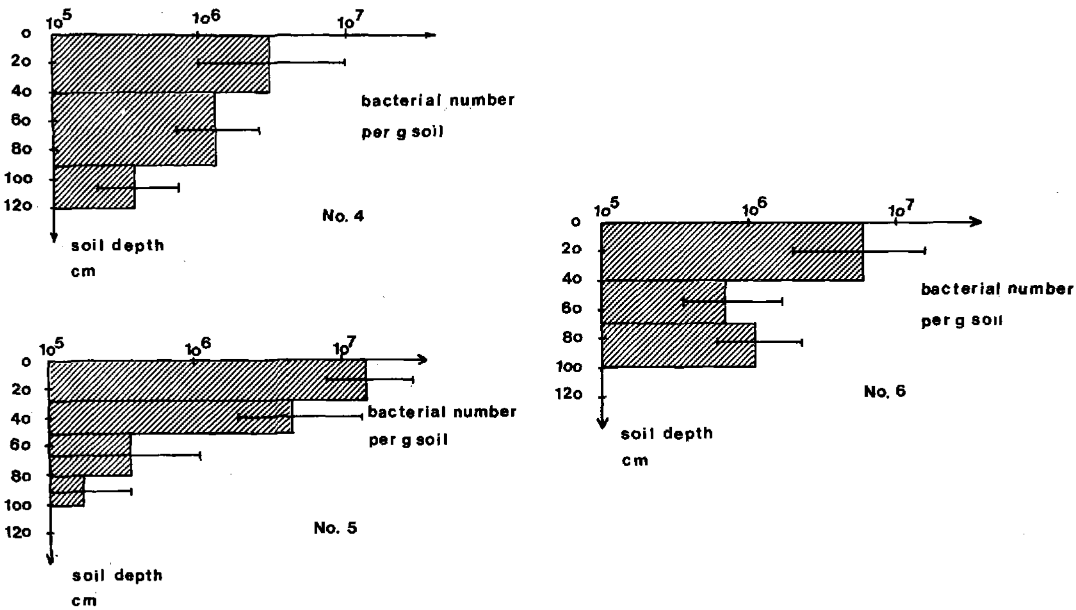

Fig. 9: Effect of soil depth on the number of denitrifying bacteria for soils no. 4, 5 and 6. The number is counted following the MPN-method and stated together with the 95 pct. confidence limits

Antal af denitrificerende bakterier i varierende jorddybde (Jord nr. 4, 5 og 6)

Litterature

Anonymous (1972): Fælles arbejdsmetoder ved jordbundsanalyser. The Danish Ministry of Agriculture, Copenhagen.

Barnes, H. & Folkard, A. R. (1951): The determination of nitrites. Analyst 76, 599–603.

Burford, J. R. & Bremner, J. M. (1975): Relationship between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. Soil Biol. Biochem. 7, 389–394.

Focht, D. D. & Joseph, H. (1973): An improved method for the enumeration of denitrifying bacteria. Soil Sci. Soc. Amer. Proc. 37, 698–699.

Jones, L. W. (1974): Effect of soil depth on denitrification. Soil Sci. 118, 280–281.

deMan, J. C. (1975): The probability of most probable numbers. European J. Appl. Microbiol. 1, 67–78.

Nielsen, R. D. & MacDonald, J. G. eds.: Nitrogen in the Environment. Academic Press. 1978.

Nõmmik, H. (1956): Investigations on denitrification in soil. Acta Agr. Scand. 6, 195–228.

Payne, W. J. (1973): Reduction of nitrogeneous oxides by microorganisms. Bacteriol. Rev. 37, 409–452.

Pheiffer Madsen, P. (1977): Analysis of gaseous nitrogen-15 by emission spectrometry in nitrate reduction experiments in soil. Stable Isotopes in the Life Science. IAEA, Vienna 1977, 171–177.

Stanford, G., Vander Pol, R. A. & Dzienia, S. (1975): Denitrification rates in relation to total and extractable soil carbon. Soil Sci. Soc. Amer. Proc. 39, 284–289.

Stefanson, R. C. (1972): Soil denitrification in sealed soil-plant systems L-III. Plant Soil 33, 113–149.

Stefanson, R. C. (1976): Denitrification from nitrogen fertilizer placed at various depths in the soil-plant system. Soil Sci. 121, 353–363.

Woldendorp, J. W. (1968): Losses of nitrogen. Stikstof, no. 12, 32–46.

Woldendorp, J. W. (1963): The influence of living plants on denitrification. Diss. Wageningen 1963.

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