Nitrate reduction in the subsoil

III. Nitrate reduction experiments with subsoil samples

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Summary

This paper deals with anaerobic nitrate reduction in the subsoil. The conditions required for nitrate reduction to proceed are reviewed.

Nitrate reduction experiments have been performed with subsoil samples from three borings (depth 0–20 m). The nitrate reduction ability of the subsoils is evaluated based on measurements of the gaseous reduction products evolved by storing under anaerobic conditions: nitrous oxide and elementary nitrogen.

On the basis of the results from the experiments referred, the nitrate reduction possibilities of the three boring profiles are discussed.

Introduction

In a previous paper (*Lind* and *Pedersen,* 1976), the sampling and chemical analyses of subsoil cores from some boring profiles were described. The relationship between the content of nitrate and ferrous iron throughout a profile has been evaluated, and informations on the variation of other important chemical properties of the profiles have been obtained.

Besides being valuable as analytical material, the soil samples from such a boring must be very valuable as sample material for laboratory experiments, in order to detect whether the subsoil is able to reduce nitrate under conditions similar to those in the field.

The ability of the subsoil to reduce nitrate would be very important for keeping the ground water free of nitrate.

As an introduction, partly based on literature, the nitrate reduction process and the conditions being favorable for its proceeding are described.

The nitrate reduction process

A brutto scheme for the stepwise nitrate reduction process can be written as follows:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow N_2O \longrightarrow N_2$$

nitrate　　nitrite　nitrous oxide　nitrogen
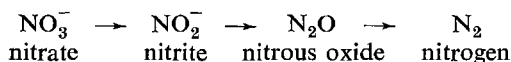
Intermediates other than the above mentioned may exist, but they are not determined with certainty in soil experiments, and their stability would probably be less than the stability of N_2O and N_2.

The foremost condition for the nitrate reduction to occur is that oxygen is lacking or only present in very small amounts. The reduction involves an electron absorption by the nitrate. Consequently, there must be one or more substances present, which are able to act as electron donors.

In the biological nitrate reduction, commonly

called denitrification, a group of microorganisms are able to transport electrons to nitrate, or said otherwise, these microorganisms are able to use the oxygen content in the nitrate instead of free oxygen as they normally do (*Woldendorp*, 1963).

Chemical compounds in soil layers of low redox level, e.g. ferrous iron and manganous compounds, are also able to supply the electrons necessary for the reduction. Since the amount of manganous compounds normally is much smaller than the amount of ferrous iron, the latter must be the most important potential reduction agent for the nitrate in the soil layers below the oxidation limit. The oxidation limit separates the oxidized or weathered soil layers from the reduced or unweathered soil layers (*Lind* and *Pedersen,* 1976). *Chao* and *Kroontje* (1966) have examined the thermodynamic possibility for the nitrate reduction with ferrous iron, and they concluded that this process may play an important role in the soil.

Within soil research, the denitrification or biological nitrate reduction is the most throughly investigated and best described subject (*Wijler* and *Delwiche,* 1954; *Cooper* and *Smith,* 1963; *Myers & McGarity,* 1972; *Woldendorp,* 1963), and it has been proved that this process occurs in the upper soil layers under certain circumstances.

It is, however, our opinion, that the reduction of nitrate being leached through the soil profiles under the root zone must be primarily chemical, since the number of ordinary denitrifying microorganisms are assumed to be very small in these dephts (*Lindgreen* and *Jensen,* 1973).

It should be possible to make such a chemical reduction of nitrate by means of laboratory experiments, if the special environmental conditions of the soil profiles can be recreated.

Literature on nitrate reduction experiments
In the following sections some investigations on denitrification in soil are described. These investigations have to some extent been useful as technical experience.

Several investigations are based on a sort of incubation technique with soil, in closed systems under anaerobic and varying environmental conditions (*Burford* and *Stefanson,* 1973; *Cady* and *Bartholemew,* 1960; *Cooper* and *Smith,* 1963).

Stefanson (1972) has made extensive investigations on soil denitrification in sealed soil-plant systems. He used undisturbed soil as experimental material, but this did not influence the denitrification.

The investigations mentioned above involve analyses of the evolved gaseous nitrogen compounds, nitrous oxide and elementary nitrogen. *Cady* and *Bartholemew* (1960) also found nitrogen oxide, NO.

Myers and *McGarity* (1972) used undisturbed soil cores for denitrification experiments. They used the evolved amount of nitrous oxide as an index for the denitrification.

Burford and *Millington* (1968) measured nitrous oxide in the soil air down to a depth of 1 meter, and they found that the nitrous oxide content fluctuated according to the season and to the soil water content.

The investigations mentioned above only utilized material for denitrification which have been taken from down to 1–2 meter soil depth. However, since the ground water quality is singled out as an important factor in the present investigations, the attention must be concentrated on the soil layers below the root zone. These subsoil layers do not give the usual possibilities for nitrogen removal or transformations by plant uptake, immobilization, and microbial denitrification. The chemical and physical properties of these subsoil layers determine whether the nitrate leached from the root zone is allowed to move down to the ground water, or whether the soil is able to reduce the nitrate resulting in gaseous nitrogen compounds.

Methods of the nitrate reduction experiments
The equipment used for reduction experiments consisted of a 500 ml conical flask with a top device for gas flushing and with a septum cap

for sampling the gas mixture in the flask with a gas tight syringe.

50 g wet soil was placed in the flask and 5 ml water containing 5 mg N as KNO_3 was added. The flask was immediately fitted with the top device, and the atmosphere was replaced by pure argon by pumping out and refilling with argon three times. This procedure has shown to be sufficient to ensure a content of oxygen and nitrogen below the detection limits of the analytic apparatus. The atmosphere replacement was repeated 1 or 2 days later to remove the initial carbon dioxide liberated from the soil sample.

The flasks were stored 40–50 days in thermostate at a temperature of 10 °C and 25 °C, respectively. 10 °C was chosen as the temperature close to the field temperature, and 25 °C was used in order to accelerate the reduction processes in the soil.

After undisturbed storing for 40 to 50 days, gas samples were taken out with a gas tight syringe for gas chromatographic analysis:

A Hewlett-Packard 5750 gas chromatograph with a thermal conductivity detector was used for the gas analysis. The columns were stainless steel, $1/8'' \times 6'$, packed with Molecular Sieve nr. 5 A. Carrier gas was helium at a flow rate of 50 ml per min. Instead of using temperature programming of column oven, the gas samples

were injected two times and analysed isothermically, at first at a temperature of 70°C for elementary nitrogen and then once again at 260°C for nitrous oxide. This procedure resulted in greater stability, when the gas chromatograph was used at the highest sensitivity, and it was easier faster to analyse the standards of the gases. The volume of the injected gas sample was 0.5 ml. Detection limits were 50 ppm for nitrogen and 5 ppm for nitrous oxide.

When the atmosphere of the flasks had been analysed for content of nitrogen and nitrous oxide, the soil-water medium was analysed for nitrite and nitrate using the methods described for the chemical analyses of the profile samples (*Lind* and *Pedersen, 1976*).

Material for the nitrate reduction experiments
Reduction experiments have been carried out with soil samples from 4 different depths of each boring profile. Some physical and chemical properties of the 12 samples are given in Table 1.

Results
The results of the reduction experiments for the Herlufmagle profile are seen in Table 2, for the Bramminge profile in Table 3, and for the Skælskør profile in Table 4. The Tables are separated in one part with analyses in the

Table 1. Some properties of the soil samples used for nitrate reduction experiments

Sample site	No.	depth m	pH (H$_2$O)	Fe++ ppm	CaCO$_3$ pct.	Physical profile characteristics
Herlufmagle	7	2.1	8.5	3	32	
	25	8.0	8.8	48	34	
	40	12.1	8.7	41	41	homogeneous clay
	54	17.6	8.8	66	28	
Bramminge	8	1.3	4.5	3	0.04	
	37	6.5	5.3	5	0.06	
	60	11.6	7.4	3	0.06	stratified sand
	79	15.9	7.7	8	0.12	
Skælskør	7	1.0	7.8	1	4	
	14	2.3	8.1	3	14	
	50	9.9	8.4	40	22	cracked sandy clay
	72	15.5	8.6	125	18	

reaction medium before and after storing, and in another part with the analyses of the flask atmosphere after storing.

Discussion of results

By a general evaluation of the results from the nitrate reduction experiments, the presence of nitrogen and nitrous oxide indicates that nearly all the soil samples under the described conditions have been able to reduce the nitrate added to the reaction medium. From all three Tables (2, 3 and 4), it appears that the reduction process is temperature dependent, since the amount of gaseous nitrogen compounds is greatest at 25°C.

The next step in the evaluation of the results

Table 2. The Herlufmagle profile. Nitrate reduction experiments.
Reaction time 45 days. The results are stated as mg N per flask.

Temp. °C	In the soil medium				Gaseous products		
	Initial NO_3^--N mg	Final NO_2^--N mg	Pct. NO_3^--N transformed	NO_2^--N formed mg	N_2 mg	N_2O-N mg	N_2+N_2O-N pct. of init. NO_2^--N
Sample no. 7.							
10	4.90	4.40	10	0.020	0.047	0.031	2
25	4.90	4.82	2	0.035	0.312	0.045	7
Sample no. 25.							
10	4.96	4.62	7	0.006	0.027	0.158	4
25	4.96	4.43	11	0.002	1.710	0.083	36
Sample no. 40.							
10	4.88	4.66	5	0.016	1.033	0.047	22
25	4.88	4.61	5	0.001	2.112	0.004	43
Sample no. 54.							
10	4.91	4.57	7	0.009	1.399	0.069	30
25	4.91	4.81	2	0.001	2.437	0.044	51

Table 3. The Bramminge profile. Nitrate reduction experiments.
Reaction time 45 days. The results are stated as mg N per flask.

Temp. °C	In the soil medium				Gaseous products		
	Initial NO_3^--N mg	Final NO_3^-N mg	Pct. NO_3^--N transformed	NO_2^--N formed mg	N_2 mg	N_2O-N mg	N_2+N_2O-N pct. of init. NO_2^--N
Sample no. 8.							
10	4.94	5.09	+	0.005	0.095	0	2
25	4.94	4.48	9	0.001	3.384	0	69
Sample no. 37.							
10	4.99	4.68	6	0.003	0.086	0	2
25	4.99	4.53	9	0.009	1.382	0.010	28
Sampe no. 60.							
10	4.67	4.56	2	0.005	0.098	0.003	2
25	4.67	4.70	+	0.005	2.644	0.002	57
Sample no. 79.							
10	4.94	4.74	4	0.003	0.108	0.003	2
25	4.94	4.72	4	0.002	2.395	0.008	49

Table 4. The Skælskør profile. Nitrate reduction experiments.
Reaction time 45 days. The results are stated as mg N per flask.

Temp. °C	In the soil medium				Gaseous products		
	Initial NO$_3^-$-N mg	Final NO$_3^-$-N mg	Pct. NO$_3^-$-N transformed	NO$_2^-$-N formed mg	N$_2$ mg	N$_2$O-N mg	N$_2$+N$_2$O-N pct. of init. NO$_2^-$-N
Sample no. 7.							
10	5.18	5.30	+	0.108	0.143	0.006	3
25	5.18	5.19	+	0.008	0.355	0.029	7
Sample no. 14.							
10	5.09	4.80	6	0.066	0.158	0.010	3
25	5.09	5.02	1	0.040	0.350	0.016	7
Sample no. 50.							
10	4.37	4.65	+	0.027	0.105	0.168	6
25	4.37	4.70	+	0.008	0.276	0.112	8
Sample no. 72.							
10	4.40	4.83	+	0.006	0.158	0.082	5
25	4.40	4.86	+	0.004	0.314	0.040	8

must be to point out the diversities between the three profiles caused by the varying chemical properties of the soil samples used.

An explanation of the analysis methods for the ferrous iron content in the soil samples is needed before a discussion of the results from the reduction experiments in detail. Any fraction of ferrous iron in the soil can, theoretically, be estimated by different empirical methods using extraction solutions of various type, ranging from the total amount to the easiest soluble fraction. We have selected an easily soluble fraction (extraction with 3 % aluminium chloride solution), because our reduction experiments are of rather short duration, seen in relation to the time avaible in the nature. Moreover, it is rather difficult to select an empirical method, when the conditions for the mobilization of the sligther soluble ferrous iron fractions are unknown.

Regarding the gas analyses, it should be noticed that the analyses do not comprise the gases dissolved in the aqueous reaction medium.

Herlufmagle
In the Herlufmagle profil (Table 2) the evolved amount of elementary nitrogen increases with increasing depth and increasing ferrous iron content. The nitrogen amount is greatest at 25°C.

The evolved nitrous oxide amount is also dependent on the temperature-ferrous iron content, but in quite a different way. With increasing ferrous iron content and increasing depth, the greatest amount of nitrous oxide was found at 10°C. The cause must be that nitrous oxide as an intermediate in the reduction process accumulates by the lower temperature, or that the entire process at the lower temperature is at an earlier stage.

Bramminge
In the Bramminge profile (Table 3) the evolved amount of nitrogen also increase with the temperature, but it does not seem to depend on the depth. The evolved amount of nitrogen is surprisingly great related to the very limited ferrous iron content throughout this profile. The amount is in fact of the same order of magnitude as that in Herlufmagle. There might be areas with higher ferrous iron content than the ferrous iron analysis shows, due to the stratification of the profile. During the experimental period, the soil may mobilize some fer-

rous iron reserves from sligther soluble compounds.

In the Bramminge profile there is a very small content of nitrous oxide in about half of the experiments, the other half contained no nitrous oxide. The reduction may have proceeded to the end, or part of the nitrogen content may originate from other processes in the soil than the nitrate reduction. However, according to current opinion this is not very probable.

Skælskør

In the Skælskør profile (Table 4) the content of nitrogen lies at a lower level than in the two other profiles. The same temperature effect for nitrogen as described in the other profiles was found, but the strongly increasing ferrous iron content does not seem to influence the evolved amount of nitrogen.

In the Skælskør profile, relatively large amounts of nitrous oxide were evolved, especially in the samples with high ferrous iron content. Here, the nitrous oxide content shows the same dependence on temperature-ferrous iron content as in the Herlufmagle profile. As mentioned above, the Skælskør profile is very inhomogenous, and the reduction process may be at an earlier stage than in the Herlufmagle profile. The Skælskør profile has in spite of the higher ferrous iron content below the oxidation limit a slower reduction rate of the nitrate than the Herlufmagle profile below the oxidation limit.

Nitrite content

In all the experiments the nitrite content shows a similar dependence on the temperature – ferrous iron content as does the content of nitrous oxide. The reason for this is obviously that nitrite also is an intermediate in the reduction process. In each profile and especially at low temperature, the nitrite content has a tendency to decrease with the depth and the increasing ferrous iron content. The higher ferrous iron content must have made the reduction process proceeding faster, since the intermediate, nitrite, is decreasing simultaneously with an increased content of the gaseous end products.

Irradiation of soil samples

To ensure that the nitrate reduction in these soils is a chemical process, some experiments with γ-irradiated soil samples were performed. This procedure for sterilization is commonly used in soil biology. But the irradiation dose required for sterilization (4 Mrad) strongly alter the proportion between ferrous and ferric iron in the soil. Therefore, this method for separating chemical from biological nitrate reduction can not be used, and the traditional methods for sterilization, such as autoclaving is obviously not usable for these undisturbed soil samples.

N-balance

A balance between the nitrate disappeared in the reaction medium and the gaseous nitrogen measured after the reaction period has not been found. A reasonable explanation might be that other nitrogen transformations than nitrate reduction may occur in the soil medium by the anaerobic experiment conditions. We are at the present time not certain about the possible nitrogen transformations, but an extension of the experiments by using ^{15}N-tagged nitrate or other nitrogen compounds may clear up some of the problems set forward.

Conclusion

The results from the nitrate reduction experiments have shown that nearly all the subsoil chemically have been able to reduce nitrate. Herlufmagle, one of the clayey soil profiles, offer the most favorable conditions for a nitrate reduction. The other clayey soil profile, Skælskør, shows also nitrate reduction ability, but at a slower reduction rate than Herlufmagle. Parts of Bramminge, the sandy soil profile, are able to reduce nitrate in spite of the low content of easily soluble ferrous iron.

The difference between the nitrate reduction ability of the three profiles is not so marked as it could be expected in view of their widely

different chemical properties. However, it should be taken into account that the reaction time used by these laboratory experiments is quite different from the time normally available in the nature.

The nitrate reduction ability of a soil profile can not be evaluated solely on the basis of its chemical properties. The time available for the nitrate to be in contact with the soil parts containing the ferrous iron is just as important. Therefore, the chemical description of such a profile must be combined with a physical and hydrological one in order to get a more complete survey of the risks of nitrate leaching into the ground water *(Pedersen & Lind,* 1976).

Sammendrag

Beretningen omhandler anaerob nitratreduktion i undergrundsjorde. Der gives en oversigt over de almene betingelser for, at en nitratreduktion kan finde sted.

Der er udført nitratreduktionsforsøg med jordprøver fra tre boringer (dybde 0–20 meter), der blev placeret på 3 arealer med varierende jordbundskemiske, jordbundsfysiske og geologiske egenskaber: 1. En homogen lerjordsprofil (Herlufmagle), 2. en lagdelt sandjordsprofil (Bramminge) og 3. en inhomogen lerjordsprofil (Skælskør). Jordprøvernes nitratreducerende evne vurderes ud fra målinger af de gasformige reduktionsprodukter: dinitrogenoxid, N_2O og elementært nitrogen, N_2, der udvikles ved henstand i et lukket system under anaerobe forhold.

På grundlag af resultaterne fra de beskrevne forsøg vurderes de tre undergrundsprofilers evne til at reducere nitrat således: Herlufmagle viser den bedste nitratreduktionsevne. Den anden lerjordsprofil, Skælskør, reducerer også nitrat, men processen forløber langsommere i denne inhomogene profil. Også sandjordsprofilen, Bramminge, er i nogle dybder i stand til at reducere nitrat.

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