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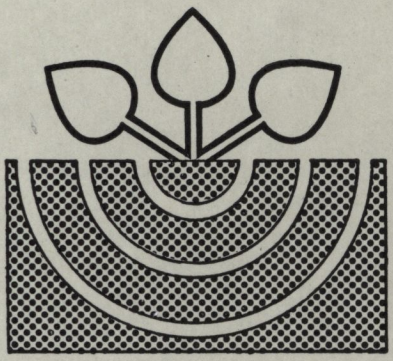

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Giessen, FRG, March 17-19, 1989

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To achieve this aim, CTA commissions and publishes studies; organizes and supports conferences, workshops and courses; supports publications and translations; and offers an extensive information service.

Preface

Microbial denitrification has become a key process in nitrogen losses from topsoils as well as in the elimination of nitrate from aquifer, sewage and drinking water. As a global process its extent has obtained additional attention due to the possible role of N_2O in the destruction of the ozone layer in the stratosphere. Although denitrification was recognized as a microbial "fermentation" process by Gayon and Dupetit back in 1882, real progress in the understanding of the ecophysiological prerequisites and conditions of this energy conserving reaction has accelerated only during the last two decades. Today, ecophysiological rather than purely physiological views and interpretations prevail more and more. Not until recently the dominating role of the amount of easily decomposable organic matter for denitrification, particularly under *aerobic* conditions, has been recognized as the *essential* triggering mechanism. In the field, however, the conditions determing the onset of relevant denitrification - locally and periodically - are highly complex.In fact soil moisture regime and nitrate diffusion rate rather than available carbon seem to play an important role.

The **International Workshop on Denitrification in Soil, Rhizophere and Aquifer** held in Giessen (FRG) on March 17 -19, 1989, was intended to assess the state of art, particularly with respect to

- methods and techniques to evaluate denitrification *in situ*

- sink and source mechanisms of denitrification products in soils

- direct and indirect effects of plants on denitrification

- denitrification losses caused by manuring and fertilization

- ecological prerequisites and mechanisms of denitrification in subsoil and aquifer

- ecophysiology and kinetics of denitrification

- organism-specific denitrification products

- modeling of denitrification.

The major part of the contributions presented at the Workshop in form of key notes, original papers, panel discussion (see "future research needs in denitrification") as well as of posters has been compiled in this volume. Both for participants and other colleagues this brief information may be valuable in providing an overview of the scientific progress in the field of denitrification and a useful base for interdisciplinary contacts.

The Organizing Committee of the Workshop wishes to thank all those who made the Congress and this volume possible. It is particularly grateful for the support provided in different ways by scientific and commercial organizations.

J.C.G. Ottow

Chairman of the Commission for Soil Biology,
International Society of Soil Science

President of the Organizing Committee

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Denitrification losses as a result of manuring and fertilization

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Modeling of denitrification

Mitteilungen der Deutschen Bodenkundlichen Gesellschaft 60, 13-18 (1990)

FIELD MEASUREMENT OF DENITRIFICATION

by

A. Mosier[+], O. Heinemeyer, and
K. Haider[++]

INTRODUCTION

Until 1979 methods to directly measure denitrification in the field were limited (Ryden et al., 1979). Denitrification was measured indirectly by ^{15}N balance where the difference between the amount of ^{15}N recovered in plants and soil, plus N leached and N in water run-off and the amount of ^{15}N added was assigned to denitrification losses (Hauck, 1981). Using this technique, denitrification is considered responsible for the volatile loss of 20-70% of the fertilizer N applied to a variety of agricultural systems. In systems, such as rice fields, direct ammonia flux measurements coupled with ^{15}N balance suggest that denitrification losses in flooded soils vary from 10 to 70% of the fertilizer N applied (Buresh and De Datta, 1989; Freney et al., 1989).

Although field estimates of the amount of N denitrified have been made in a variety of natural and agronomic sites, measurements which directly compare gas flux with ^{15}N balance measurements are rare. In many instances where these comparisons were made, the directly measured denitrification losses were less than those measured by N-balance (Buresh and Austin, 1988; Mosier et al., 1988; Mosier et al., 1985). At the present time we do not know if there are problems with either or both direct or N-balance measurements that account for the frequent lack of agreement of total N loss between methods. Techniques developed since the late 1970's are now used routinely to measure denitrification in the field. This paper describes the basic field gas collection methods, quantification techniques and briefly relates problems associated with them.

GAS COLLECTION TECHNIQUES

To estimate the amount of N_2 and N_2O emissions from the soil to the atmosphere, in most field situations, we must use some sort of soil surface gas concentration device. Some version of such devices, chambers, have been used to estimate the fluxes of a number of gases, including N_2O and N_2, from soil. Both of the common chamber types enclose a distinct volume of air above a known area of soil and prevent or control emanating gas from mixing with the external atmosphere. The concentration of N_2O, for example, beneath the cover will increase or decrease whenever there is a positive or negative flux out of the

[+]USDA-ARS, P.O. Box E, Fort Collins, Colorado 80522, USA

[++]Institute for Soil Biology and Institute for Plant Nutrition and Soil Science, Federal Center for Agricultural Research, Braunschweig, FRG.

soil. The two chamber types used are those with forced flow-through air circulation designated as "open soil covers" and those with closed-loop air circulation or no forced air circulation designated as "closed soil covers".

Closed Chambers. Gas flux from the soil using closed chambers is determined by periodically collecting gas samples from the chamber and measuring the change in concentration of a gas with time during the period of linear concentration change. When nonlinear increases in gas concentration occur flux can be calculated as described by Hutchinson and Mosier (1981). Reasons for selecting the closed cover method are typically: 1) very small fluxes can be measured, 2) no extra equipment requiring electrical supply is needed, 3) there is diminished disturbance of the site due to the short time a cover has to be in place for each gas flux estimate, 4) the chambers are simple to construct, 5) they are easy to install and to remove thus giving the opportunity to measure different locations at different times with the same equipment, and 6) they are relatively inexpensive to prepare.

Problems generally attributed to closed soil covers include: 1) concentrations of gas in the enclosure atmosphere can build up to levels where they inhibit the normal emission rate. This problem can be limited by using short collection periods and correction equations (Jury et al., 1982; Hutchinson and Mosier, 1981). 2) closed covers either eliminate or alter the atmospheric pressure fluctuations which normally are found at the soil surface due to the natural turbulence of air movement. These fluctuations can pose a "pumping action" on the surface layer of soil which increases soil air movement, thus a totally closed cover may underestimate the flux that would have occurred without the cover in place. An appropriately designed vent does, however, allow pressure equilibration in and outside the chamber (Hutchinson and Mosier, 1981). 3) Pressure changes in the soil can be caused by inserting the chamber into the soil. This problem may be overcome by installing collars in the soil that are normally open to the atmosphere and to seal the cover to the collar when the chamber is used (Seiler and Conrad, 1981; Duxbury et al., 1982). Alternatively, after initially inserting the chambers into the soil the chambers may be removed for a brief time to allow dissipation of any N_2O released during the disturbance and then replaced (Livingston et al., 1988). 4) temperature changes in the soil and atmosphere under the chamber can occur. Temperature differences within and outside the chamber can be reduced by insulating the chamber and covering it with reflective material.

Open Chambers. Open soil covers used by Ryden et al., (1979) and Denmead (1979) are coupled to the atmosphere via an air inlet through which outside air is continuously drawn into the cover and forced to flow over the enclosed soil surface. The gas flux from the soil surface can be calculated from concentration difference, flow rate and area covered by the open soil cover. The main advantage of an open cover is that they maintain environmental conditions close to those of the uncovered field. Open chambers are, however, sensitive to pressure deficits inside the chamber caused by the induced air flow which may cause artificially high fluxes. If even small pressure deficits

occur, induced mass flow of the gas into the cover will lead to overestimation of the gas flux. This can be readily overcome by insuring that the size of the inlet gas orifices are large compared to size of outlet (Denmead, 1979). An additional consideration in open cover systems is the time required for gas concentration in the soil and chamber air to adjust to new equilibrium values. Measurements assume an equilibrium flux between soil atmosphere and chamber atmosphere, so estimates will be erroneous during the time of equilibration.

METHODS FOR QUANTIFYING DENITRIFICATION IN THE FIELD

During the past 10 years denitrification has been estimated in the field utilizing the acetylene inhibition technique (Benckiser et al., 1985; Mosier et al., 1985; Ryden et al., 1979; Ryden and Dawson, 1982) and by ^{15}N techniques (Buresh and Austin, 1988; Craswell, et al., 1985; Mosier et al., 1986; Mulvaney and Vanden Heuvel; Rolston et al., 1982; Siegel et al., 1982). We'll briefly describe the techniques and refer you to Mosier and Heinemeyer, 1985, for more detailed discussion.

Acetylene Inhibition. The discovery that acetylene blocks the reduction of N_2O to N_2 (Fedorova et al., 1973) led to development of techniques to directly measure denitrification in the field (Ryden et al., 1979). The method is applicable in both fertilized and unfertilized field sites and is adaptable to both in situ and core collection methods. The measurement of N_2 and N_2O separately involves allocating two "identical" soil samples and treating the atmosphere of one soil with 0.1 to 10% acetylene and adding no acetylene to the atmosphere of the second soil. The soils are incubated for a few hours under identical conditions and the N_2O produced from each soil is analyzed by gas chromatography. The difference in N_2O produced between the acetylene-treated soil and the untreated soil represents the amount of N_2 produced during denitrification. If only total $N_2 + N_2O$ is required then the nonacetylene-treated soil can be eliminated.

There are at least three variations of this basic technique that have been applied to field estimates of denitrification. Ryden et al., (1979) and Ryden and Dawson (1982) use the method to make in situ measurements by employing a dual flow through (open) soil cover technique where they subjected the soil under one chamber to a flow of acetylene and compared the amount of N_2O collected on molecular sieve from acetylene-treated plot to the untreated plot. Denmead (1979) described an open chamber method where he continuously monitored N_2O evolution from the soil using infrared gas analysis.

Another approach to estimating denitrification in field soils with acetylene inhibition is demonstrated by Aulakh et al., (1982). They collected soil cores in perforated aluminum cylinders and placed the cores into jars, injected 5 atmosphere per cent acetylene, incubated the soils at field temperatures for 24 hrs, then analyzed the jar head space for N_2O. Gas flux rates were calculated based on soil surface area and time. Parkin et al., (1984) and Rice and Smith (1982) collected intact soil cores from the field and took them to the laboratory where the cores were exposed to acetylene by first

irrigating the core with acetylene-saturated water then flowing acetylene-containing air over the soil core surface. Increase in headspace N_2O concentration were measured by GC.

Side Reactions of C_2H_2 in Soil Systems. Acetylene affects other process in the soil as well as nitrous oxide reduction. Some of the common reactions affected by the gas are: 1) inhibition of methane oxidation, 2) microbial utilization of C_2H_2 as a carbon source, 3) inhibition of nitrification, and 4) acceleration of soil carbon mineralization resulting in an increased NO_3^- reduction rate. Most of these potential problems can be overcome with appropriate experimental design (Ryden and Dawson, 1982; Ryden et al., 1987). Diffusion of C_2H_2 throughout the soil profile can also be a problem in quantifying total denitrification that is exacerbated by increasing soil water content (Chalamet et al., 1989).

^{15}N Techniques. Denitrification can also be estimated in N-fertilized fields utilizing ^{15}N. The methods involve applying highly enriched ^{15}N (> 20 atom % ^{15}N) to a specified plot of soil and using a closed soil cover method to confine the gases evolved from the soil during a prescribed time period. When N_2 formed from denitrification of ^{15}N-labeled nitrate is evolved into this confined atmosphere that already contains air (78% N_2), the ^{14}N and ^{15}N atoms in the entire mixture are not distributed randomly among the N_2 molecules. Hauck and Bouldin (1961) used this non-equilibrium condition to calculate the amount of N_2 evolved from the soil. Their technique permits a quantitative estimate of the relative contributions to the gas evolved from the soil into the chamber by both the added ^{15}N-labeled fertilizer and from native soil N. (Mulvaney and Vanden Heuvel, 1988; Mosier et al., 1986). The evolution of N_2 from the applied fertilizer can be measured by analyzing the chamber head space gas to determine an atom % ^{15}N (Rolston et al., 1982).

Since the ^{15}N in the N_2 is not randomly distributed it is necessary to determine the amount of masses 28, 29, and 30 in the sample. If a triple collector mass spectrometer is not available, the isotopic arrangement of the N_2 can be randomized by an arcing method described by Craswell et al., (1985) that has been used in field studies (Buresh and Austin, 1986).

Problems associated with ^{15}N methods. Measuring denitrification using ^{15}N is expensive as ^{15}N-labeled chemicals cost about $125-250/g of ^{15}N and require a mass spectrometer for analysis. The denitrification method described by Hauck and Bouldin (1961) and updated by Siegel et al., (1982) theoretically requires that the nitrate pool undergoing denitrification be uniform with respect to ^{15}N distribution. It is highly unlikely that a uniform distribution of $^{15}NO_3^-$ could exist in a soil where mineralization, immobilization and plant N uptake of nitrogen occurs. Mulvaney and Vanden Heuvel (1988) show, however, that "appreciable error did not necessarily arise when the method was used to measure denitrification of nitrate that was not isotopically uniform."

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Methods to separate N_2O produced from denitrification and nitrification

by

Klemedtsson, L. and Hansson, G.I. +)

INTRODUCTION

Since the early 1970s there has been concern about the adverse environmental effects of N_2O. The release of N_2O has a major effect on the atmospheric ozone layer which protects living organisms from harmful ultraviolet radiation (Crutzen, 1981). N_2O also has a significant effect on the earth's thermal balance through the absorption of reradiated longwave infrared radiation, the "greenhouse effect" (Lacis et al., 1981). The yearly increase of N_2O in the atmosphere is presently about 0.2 % (Weiss, 1981).

The production as well as the consumption of N_2O in the biosphere is dominated by biological processes. The gas can be produced during denitrification (Knowles 1982), nitrate-respiration by non-denitrifying bacteria (Smith& Zimmerman, 1981), autotrophic nitrification (Poth&Focht, 1985), heterotrophic nitrification (Hynes&Knowles, 1982), and assimilation of nitrate by yeasts and other fungi (Bleakley&Tiedje, 1982). Only during denitrification can significant amounts of N_2O be consumed.

In agro-ecosystems, denitrification and nitrification are assumed to dominate N_2O production. The importance of heterotrophic nitrification in agricultural soils has been assumed to be low (Focht&Verstrate 1977), but Kuenen et al.,(1988) show that some heterotrophic nitrifiers can denitrify nitrite under aerobic conditions. In ecosystems where the content of organic material is high, nitrate-respiring, non-denitrifying bacteria may produce significant amounts of N_2O under anaerobic conditions (Tiedje et al.,1982). In forest ecosystems, fungi may contribute to N_2O production (Robertson&Tiedje 1987).

The N_2O production from nitrification and denitrification in agricultural soils is highest when the oxygen level of the soil is low (Klemedtsson et al., 1988). The organic content of soil is also important for the ratio of N_2O/N_2 from denitrification; a higher organic content gives a higher proportion of N_2. In order to predict the N_2O emission from soils, it is necessary to be able to distinguish between nitrification and denitrification. There are several chemicals available that inhibit autotrophic nitrification but do not prevent denitrification.

An expedient inhibitor of nitrification should diffuse rapidly into soil and give a rapid inhibition of ammonia oxidation. It should also permit a rapid diffusion in soil of the N_2O produced. It should not affect other soil processes to any great extent; soil respiration in particular must remain unaffected by the inhibitor, since the O_2 level affects the ratio of N_2O/N_2 produced during denitrification. The inhibitor should also be easy to apply. We will discuss two of the most commonly used nitrification inhibitors: acetylene at low partial pressures and nitrapyrin, and their use in agricultural soils. Acetylene does not inhibit N_2O production from heterotrophic nitrifiers of the species Arthrobacter, and nitrapyrin does not inhibit heterotrophic nitrification (Keeney, 1988); the N_2O produced by heterotrophic nitrifiers will probably be included in the estimation of N_2O production from denitrification. Both acetylene and nitrapyrin will thus separate autotrophic N_2O-production from heterotrophic. Methods in which inhibitors of soil processes are used always involve problems, and we will also give a brief outline of the advantages and disadvantages of the two inhibitors.

+) Department of Microbiology, Swedish University of Agricultural Sciences, Box 7025, S-750 07 Uppsala, Sweden

THE PPM METHOD

The inhibitory effect of acetylene on biological processes in soil differs with different partial pressures of the compound. The reduction of N_2O to N_2 by denitrifiers is inhibited at partial pressures above 100 Pa. At partial pressures above 1 Pa, autotrophic nitrification is inhibited. If N_2O production is determined at different partial pressures of acetylene, and in the absence of acetylene, the contribution to the total N_2O-production from heterotrophic and autotrophic processes can be calculated, as well as the total N_2-production from denitrification.

Acetylene was used to inhibit autotrophic nitrification in the following studies to determine the effect of: (1) plants on N_2O production in soil cores from experimental field plots (Svensson et al., 1985); (2) water on different N2O-producing processes in soil (Klemedtsson et al., 1988a); (3) earthworm casts on N_2O-losses from soil. Davidson et al. (1986) also used the method to determine the relative importance of autotrophic nitrification and denitrification in a forest soil.

1. Inhibition of nitrification

Acetylene inhibits nitrification both in pure cultures of Nitrosomonas europaea (Hynes&Knowles, 1978), and in soil (Berg et al., 1982). Acetylene probably binds covalently to the enzyme that catalyzes the conversion of ammonia to hydroxylamine, ammonia monooxygenase (Campbell&Aleem, 1965). Hynes&Knowles (1982) followed the course of the inhibition through measurements of the oxygen taken up by cell suspensions, since oxygen uptake is stochiometrically related to ammonia oxidation. The inhibition was complete within 10-15 minutes. Inhibition is probably instantaneous as the time-lag represented the time needed for the acetylene to diffuse through the medium. A partial pressure of 1 Pa acetylene gave strong inhibition of the ammonia oxidation. Berg et al. (1982) found that nitrification was totally inhibited by between 1 and 10 Pa acetylene in soil, and partially inhibited by 0.5 Pa. Both N_2O and NO are produced during ammonia oxidation (Poth&Focht, 1985), and since acetylene inhibits the first step of ammonia oxidation, the production of both compounds will be inhibited by acetylene.

If acetylene, or any other nitrification inhibitor, is used in long-term experiments, the soil will eventually become depleted of nitrate, since no nitrate production occurs when nitrification is inhibited. Consequently, the denitrification rate will gradually decrease. The duration of an experiment where acetylene is used to inhibit nitrification must be adjusted to the nitrate content of the soil and the initial denitrification activity.

2. Diffusion of acetylene and N2O in soil

When acetylene is used in soil, there will be a time lag before the inhibition occurs, while the gas is diffusing through the soil. In a sandy loam at 95 % of waterfilled porosity, inhibition was complete after 3 hours (Figure 1). The detection of the inhibition is of course similarly delayed by the diffusion time of the produced gases from the soil. The emission rate of N_2O from a soil is theoretically the difference between the production rate and the consumption rate. In reality, the emission rate is influenced by the diffusion of N_2O in soil, which in turn is determined by the water content and the porosity of the soil. The transport of N_2O from soil occurs through airfilled pores by diffusion/advection/convection, and by vascular transport within plants. The most important limitations of the PPM-method are connected with the diffusion of acetylene in soil, caused by variations of soil water content. The diffusion rate of O_2, for example, is about 10,000 times slower in water than in air. Even though acetylene diffuses rapidly in soil, there will be a time lag before the inhibition of nitrification takes place, since the gas has to diffuse to the active site of nitrification. This applies to acetylene inhibition of the reduction of N_2O to N_2 by denitrifying bacteria as well. The diffusion constraints are presumably even greater in soil where the denitrification activity is high, since a high water content stimulates

denitrification. The relatively rapid diffusion of acetylene may also be a problem, since the acetylene can be lost from soil through diffusion.

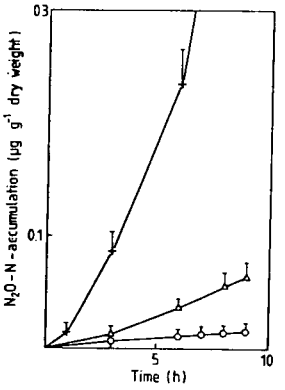

Figure 1. N_2O production from a sandy loam (40 ug NO_3^--N g^{-1} dw) +:10 kPa acetylene, Δ :2.5 Pa acetylene, o :control. (I:standard deviation, n=5)

3.Loss of inhibition due to consumption af acetylene

Acetylene is consumed by microorganisms, both in pure culture and in soil, and under both aerobic and anaerobic conditions (Watanabe&de Guzman, 1980; Yeomans&Beauchamp, 1982; Haider et al., 1983; Adkins and Knowles, 1984; Terry&Duxbury, 1985). A population of acetylene-consuming bacteria may develop both during long-term experiments and repeated short-term experiments in the same soil ; Nocardia spp. and Arthrobacter spp. are examples of microorganisms which consume acetylene. These organisms may also stimulate denitrification by excretion of ethanol, acetate and acetaldehyde.

The soil used in Klemedtsson et al. (1988b) showed only a minor reduction of acetylene concentration during 10 days of incubation. Since easily available carbon compounds stimulate the consumption of acetylene in soil, we conducted an experiment to study the effect of acetylene consumption on the inhibition of nitrification (Klemedtsson et al., 1988b). Glucose (1 mg/g soil, d.w.) and nitrate (40 mg/g soil, d.w.) were added to a sandy loam soil and the mixtures were incubated. The inhibition of N_2O production from autotrophic nitrification lasted for 2 days at 2.5 Pa acetylene and for 5 days at 5 Pa. Even after glucose had been added, acetylene consumption was very low in this soil during short-term incubation.

4. Effects of low acetylene concentrations on N_2O reduction to N_2 by denitrifying bacteria

Acetylene inhibits the reduction of N_2O to N_2 by denitrifiers in pure culture by about 10 % at a partial pressure of 1 Pa (Yoshinari&Knowles, 1976). This is also the partial pressure at which nitrification is inhibited (Berg et al. 1982). We did not, however, find any significant effect of 1 Pa acetylene on N_2O production from a sandy loam soil at 100 % and 90 % water filled pore

space during the first 6 days of incubation (Klemedtsson et al., 1988b). Because of the diffusion constraints that exist in soils at high water contents, the partial pressure of acetylene has to be higher than 1 Pa during short-term incubations. Davidson et al., (1986) found that 10 Pa acetylene had no significant effect on the reduction of N_2O in a forest soil during 8 hours of incubation when N_2O was added to the soil after it had been depleted of NO_3^-. The effect of low partial pressures of acetylene on the reduction of N_2O to N_2 should not be studied in soils that have been depleted of NO_3^-, since it has been shown that acetylene inhibition of N_2O reduction by denitrification is more effective in the presence of nitrate (Firestone et al.,1979; Smith&Tiedje 1979). In Klemedtsson et al. (1988b) denitrification potentials were determined with the soil slurry method. Soil samples were incubated anaerobically after addition of nitrate and 0.5 g glucose per gram soil (Smith&Tiedje, 1979). The partial pressures of acetylene used were 0, 3 and 10,000 Pa. N_2O production from the soil was not significantly affected by 3 Pa acetylene during the first 6-8 hours of incubation, but the production was subsequently stimulated by 10 %. The reduction of produced N_2O to N_2 after NO_3^- had been depleted occurred at about the same rate in the samples without acetylene as in the samples containing 3 Pa acetylene. In order to test the method under conditions more similar to natural conditions, an experiment similar to Davidson's et al (1986) was conducted. The soil was amended with nitrate and glucose to stimulate denitrification and incubated with an air-filled headspace at 100 % of WHC. The production of N_2O did not increase at a partial pressure of 2.5 Pa, as compared with the control, during 15 hours of incubation. N_2O production did not increase significantly at 5 Pa during the first 9 hours of incubation, but then became higher than in the control. This shows that 3 Pa acetylene can be used during short-term experiments, even when the NO_3^- level is high.

5. Nitrapyrin as an inhibitor of autotrophic nitrification

Nitrapyrin is only moderately volatile, and will diffuse rather slowly through air-filled pores in the soil; its water solubility is low (0.04 g/kg H_2O at 25°C), and it will move slowly through the soil water. The most convenient way of adding nitrapyrin to soil is to dissolve it in an organic solvent, disperse the solution in water and mix soil and the nitrapyrin emulsion. This means that the O_2 status of the soil, which regulates production rates of N_2O for both nitrification and denitrification, will be affected. The extent of the disturbance decreases over time, and will be negligible in long-term experiments. Another disadvantage of nitrapyrin is that its inhibiting effect varies depending on the strain of nitrifiers present in the soil that is treated (Belser&Schmidt, 1981), which means that a selection of more nitrapyrin tolerant nitrifiers may take place during long-term experiments. The inhibitory concentration of nitrapyrin also varies between different soil types. Organic content and pH level influence the inhibition (Goring, 1962; Hendrickson&Keeney, 1979). The degradation rate of nitrapyrin is strongly influenced by soil temperature; the half-life of nitrapyrin may be up to 80 % lower at 20°C than at 10°C (Herlihy&Quirke, 1975).

Respiration was more strongly increased during long-term incubations with nitrapyrin than with 1 Pa acetylene, and nitrapyrin stimulated N_2O production from denitrification (Klemedtsson et al., 1988b). The increased respiration may have stimulated denitrification, and may have been caused by the solvent, xylene, rather than by nitrapyrin. Notton et al. (1979) found, however, that nitrapyrin in itself gave rise to an increase in nitrate reduction in soil.

In most soils, acetylene is to be preferred to nitrapyrin, since it causes fewer disturbances of soil processes. In soils where gas diffusion is very low nitrapyrin might be more effective, although N_2O production is probably difficult to measure with any method in such soils; it is likely that one measures the diffusion rate of N_2O from the soil, rather than the production rate. Both inhibitors may have undesired effects during long-term experiments, and short-term experiments are probably to be preferred when

inhibitors are used to distinguish between N_2O production from nitrification and denitrification.

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RATES OF DENITRIFICATION UNDER FIELD CONDITIONS AS INDICATED BY THE ACETYLENE INHIBITION TECHNIQUE - A CRITICAL REVIEW -

von

K.-W. BECKER, H. HÖPER and B. MEYER*

1. THE ACETYLENE INHIBITION TECHNIQUE (AIT): HISTORY AND APPLICATION

In 1973 FEDOROVA et al. discovered the inhibiting effect of acetylene on the microbial N_2O-reduction and proposed to make use of it for the quantification of denitrification. This has been studied in the following years by experiments with cell suspensions of denitrifiers (BALDERSTON et al., 1976; YOSHINARI et al., 1976) or with mixed soil samples (YOSHINARI et al., 1977; KLEMEDTSSON et al., 1977; RYDEN et al., 1979a). It could be demonstrated that the inhibition of N_2O-reduction in the presence of acetylene is complete and instantaneous while nitrate and nitrite are still quantitatively reduced to N_2O. Inhibitory effects of acetylene on the rate of denitrification and on soil respiration were generally not observed. Thus, the results of laboratory experiments indicate that the AIT is a useful method for measuring denitrification, the more as it permits direct determination of denitrification rates.

Attention has to be paid to the complete inhibition of nitrification (WALTER et al., 1979) and to a possible acetylene-metabolism caused by adaptation of soil microflore to acetylene after a certain lag-time (YEOMANS et al., 1978). Therefore acetylene incubation of soil samples should not last longer than 5 - 7 days.

RYDEN et al. (1979b) were the first who used the acetylene inhibition technique in field measurements. Since then preference has been given to this method in measuring actual soil denitrification.

But there still remain doubts which concern the reliability of this method in connection with undisturbed soils. Compared with ^{15}N-balance studies the acetylene inhibition technique generally leads to lower denitrification losses as shown in fig.1.

The problem is still unsolved which of the competing methods is the most reliable.

One of the reasons for the discrepancy between both methods is probably due to their different nature. The ^{15}N-balance technique allows long-term studies while the AIT is only able to measure short-term denitrification rates (hours,

*Institut für Bodenwissenschaften, von Siebold Str. 4, D-3400 Göttingen

days) which need extrapolation in order to obtain data on long-period denitrification.

Comparing the possible errors of AIT and ^{15}N-balance techniques for denitrification measurements this report aims to elucidate only the problem areas of AIT. A critical review of ^{15}N-balances is given by BECKER (1989).

The main point of criticism of the AIT has to take gas diffusion phenomena into consideration.

2. THE GAS DIFFUSION PROBLEM

The actual denitrification in soils as well as its measurement by the AIT depend on the gas diffusion conditions of the soil. According to Fick's first law the gas flow by diffusion in soils can be described by the following equation:

(1) $$J = - Da * dC/dX$$

The diffusion flow (J) is proportional to the apparent diffusion coefficient (Da) and to the concentration gradient (dC) as well as inversely proportional to the diffusion distance (dX).

The apparent diffusion coefficient (Da) of the gas is proportional to the diffusion coefficient in air (Do), the volumetric air content (a) and the continuity of the pore system (1/tortuosity):

(2) $$Da = Do * a * 1/\tau$$

Denitrification is mainly controlled by oxygen partial pressure (pO_2) within soil atmosphere. Oxygen represses the activity of the denitrification enzymes. pO_2 of the soil atmosphere results from a steady state between the O_2-consumption rate in the soil and the rate of O_2-influx by diffusion. According to equation (2) the oxygen diffusion into soil decreases by the reduction of the volumetric air content (a).

Consequently, the worse the gas diffusion conditions the higher the rates of denitrification.

But the same conditions enhancing the rate of denitrification impede its measurement by AIT due to restriction of gas exchange. Acetylene as the inhibiting gas has to diffuse to the microsites of denitrification and N_2O as the product has to diffuse out of the soil.

To assure quantitative inhibition of N_2O-reduction a minimum C_2H_2-concentration of 0,1 Vol-% has to be built up in the sites of denitrification. Under restricted gas diffusion conditions in soil the diffusion time of acetylene into the

soil can be reduced by increasing gas pressure whereas the reduction of diffusion distance induces new sources of errors like enhanced O_2-diffusion.

Assuming that the infiltration of acetylene does not act as a limiting factor for the complete inhibition of the N_2O-reduction by denitrification, the diffusion of N_2O out of the soil plays the most important role for the reliability of AIT. In AIT the flow rate of N_2O leaving the soil is measured assuming that it corresponds to the denitrification rate in the soil. Two critical remarks are to be made about this assumption:

1.) The N_2O gas flow is generated by a concentration gradient and thus is not allways directed towards the soil surface.

2.) During a certain lag-time after the beginning of the acetylene incubation the rate of N_2O-flow passing the soil surface is not in equilibrium with the rate of denitrification in the soil.

ad 1: The concentration gradient determines the direction of gas flow. Gas diffusion always takes place from locations of higher partial pressure to those of lower one. In open field measurements gas diffusion in the soil takes place from the center of gas production into all directions and not only upwards to the free atmosphere. This is an important disturbing factor for AIT recording the N_2O flow into collection chambers on top of the soil.

ad 2: In a non-acetylene affected soil N_2O presents only the smaller part of the entire gas production (N_2O + N_2). An equilibrium is being built up between the rate of this natural N_2O-production and the N_2O-flux leaving the soil, corresponding to steady state concentrations of N_2O in soil atmosphere and in soil solution.

When the soil is incubated with acetylene, N_2O becomes the single denitrification product. The rate of N_2O production increases. So do the N_2O concentrations in soil atmosphere and in soil solution until a new steady state concentration has been build up. During this lag-time most of the produced N_2O is consumed by this equilibration process. Thus, the N_2O flux from the soil surface cannot be used as an indicator for denitrification rates within a certain transition phase.

The length of lag-time between the first injection of acetylene and the adjustment of flow equilibrium depend on the rate of denitrification in the microsites and the diffusion conditions in the pore space. Depending on the size of the incubated systems lag-times up to some days have to be expected. Measurements can only start when both constancy of N_2O concentration inside soil and linearity of N_2O outflow indicate that the new flow equilibrium has adjusted.

During the lag-time the incubation conditions (temperature, humidity) have to be maintained constant in order to avoid undesired influences on the N_2O outflow.

3. APPLICATION OF THE AIT TO UNDISTURBED SOIL SAMPLES

3.1. OPEN-FIELD MEASUREMENTS

In field measurements open soil volumes are incubated with acetylene and the N_2O flow leaving the soil is trapped in collection chambers on top of the soil.

The first problem of this approach consists in the diverging directions of gas flow. Not all N_2O produced in the soil will reach the collection chambers. BENCKISER et al. (1986) for example found a maximum N_2O concentration in a depth of 20 - 30 cm. Gas diffuses from that zone into all directions, upwards to the collection chamber as well as downward into the subsoil. This causes an underestimate of the rate of denitrification even when lateral diffusion is minimized by incubation of larger soil areas.

Uncontrolled lag-time is the second methodological source of errors. According to JURY et al. (1982) and our own experiments the lag-time lasts up to two and more days. Especially under conditions most favourable for denitrification, i.e. high water saturation, very long lag-times have to be expected. The increase of incubation time renders it more difficult to maintain constant experimental conditions in the field.

From these observations and arguments it can be concluded, that the AIT in open-field measurements mostly leads to an underestimate of the true rate of denitrification and therefore is not reliable.

3.2. MEASUREMENTS ON EXTRACTED SOIL CORES

In contrast to field methods, incubation of fully enclosed soil cores in phytotrons excludes N_2O-losses by lateral and downward diffusion. It permits to maintain constant experimental conditions over a long period, until the rate of N_2O-flow leaving the soil sample is equilibrated with the rate of denitrification.

The duration of lag-time and the reduction potential are highly influenced by the size of the soil cores. Small cores such as usually sampled artificially enhance the entrance of O_2 into the soil by reducing the diffusion distance.

Maximum denitrification in the field can be expected in the lower zone of the plough layer where O_2-consumption due to microbial activity is high while O_2-diffusion is restricted. Consequently to represent natural conditions soil cores should be 30 - 35 cm in height.

4. CONCLUSIONS

- Denitrification as well as its measurement by AIT depend on the gas diffusion conditions of the soil.
- The more restricted the gas diffusion, the higher the denitrification rate, the longer the incubation time necessary for AIT.
- The application of the AIT in open-field measurements is not a reliable approach.
- Measurements with extracted soil cores will allow to estimate short-term denitrification rates in soils, if the size of the soil cores is sufficient to represent the natural O_2-diffusion conditions of the undisturbed soil.
- Long-term data for denitrification from AIT can only be obtained by modelling.

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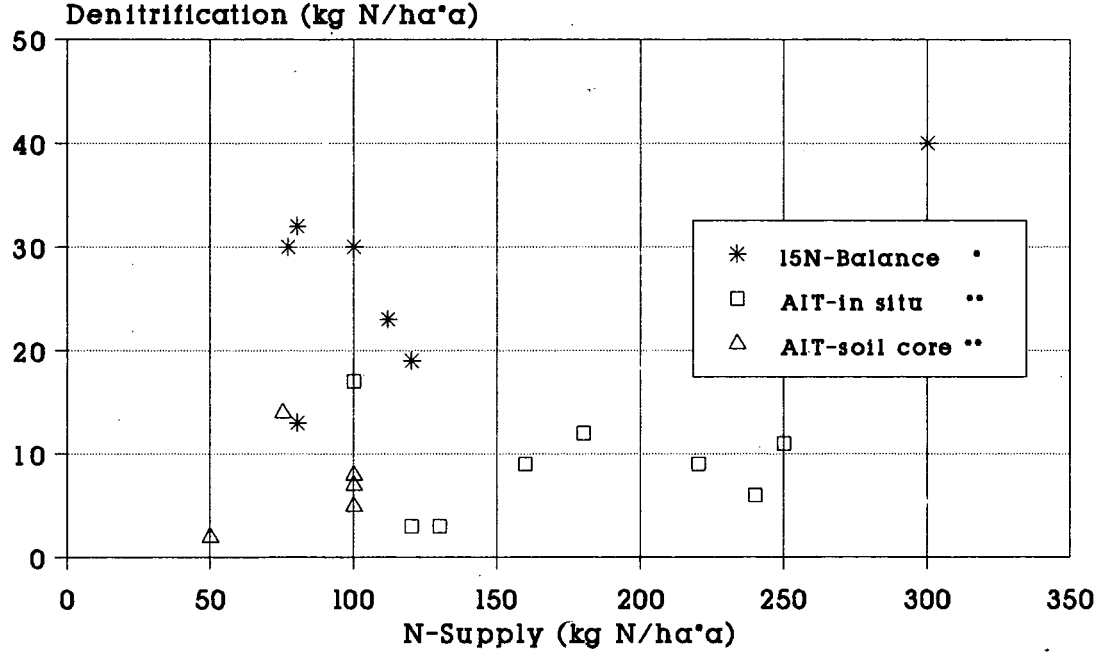
Fig.I: Denitrification in the Field in Function of the N-Supply

-Comparison between 15N-Balance and AIT-



* only 15N-gas losses
** total N-gas losses

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RATES OF DENITRIFICATION AS INDICATED BY ^{15}N LABELLED SOIL NITROGEN BALANCE EXPERIMENTS IN GERMANY - A CRITICAL REVIEW

K.-W. Becker*

TYPES OF EXPERIMENTS

In the FRG several studies on transformation of soil and fertilizer nitrogen and its denitrification have been carried out by means of ^{15}N. These experiments have demonstrated a competition between high rates of uptake by plants and high rates of denitrification of the labelled N-fertilizer added.

^{15}N balance studies frequently favoured for denitrification measurements offer a general problem. They require high analytical precision for quantitative detection of the ^{15}N-isotopes remaining in the soil plant system and consider the difference between ^{15}N added and recovered as loss by denitrification.

Except the experiments of THIES and in part of DRESSEL conducted on sand most of ^{15}N-experiments in the FRG have been run on loess borne silt loam soils. Pots of 25 to 100 cm in diameter were filled with soil material by casting, underlain by a layer of gravel or coarse sand. The length of the soil column varied between 25 and 100 cm. Measurements started after a short time of consolidation of the soil. DRESSEL's pots had been installed long before in 1927 and since that time been covered by rotating field crops.

A second type of lysimeters was constructed by surrounding naturally layered soil monoliths with plastic films or by driving in pipes of steel or synthetic material. Because of the undisturbed layering and connection with the subsoil it was not possible to collect the drainage water from these pots. But in loess soils the small annual rate of leaching creates no problems in balancing ^{15}N, if the soil is become analyzed down to a depth of 1 m. The lysimeters of THIES were prepared by connecting natural layered upper parts of the soil with bottom parts, which have been filled with sand by casting. He installed a permanent level of ground water at a depth of 2 m.

The main crops fertilized with ^{15}N in the FRG were spring grain such as wheat, barley and oats or in some cases winter wheat (NIEDER) or sugar beets (VILSMEYER). After harvest green rape was sawn by DRESSEL and later incorporated to the soil.

Two different ways for the application of ^{15}N-fertilizer have been chosen: Mixing with the soil or application as a nutrient solution. The fertilization was carried out at one, two or three times. Most of the experiments were finished at harvest. In some cases the analyses for ^{15}N were continued within a subsequent

* Institut für Bodenwissenschaft, von Siebold-Str. 4, D 3400 Göttingen

year of plant growth without isotope fertilization. DRESSEL fertilized his plants with ^{15}N isotopes during a sequence of 6 years.

The transformation of nitrogen in the soil during the vegetation period was investigated by taking soil samples with an auger and sealing the holes by suspended soil material or wooden sticks. In some experiments quite a number of lysimeters had been installed. At certain varying times several pots were dissected, and the whole soil content was devided into different layers, each layer becoming thoroughly mixed.

HEINEMEYER et al. used a closed phytotron for studying nitrogen transformations. Corn was planted and kept under controlled conditions. During 79 days gaseous nitrogen emissions were measured inclusively nitrous oxide.

A general problem of all experiments with ^{15}N consists in the high costs for isotopes and for conducting the work. Thus most of the investigators choose small experimental areas. This disadvantage was compensated by a sufficient number of replicates, three or four for each sampling date.

ANALYTICAL ERRORS

A much greater problem concerning transferability and reliability rises from the analytical procedures. In FRG soil nitrogen and its fractions become analyzed by the fundamental technics of BREMMNER. FLEIGE et al. tested these methods and made good use of it.

There are still two special difficulties concerning the reduction of nitrate into ammonium, which often led to an underestimation of the true nitrogen content. One reason for this is the insufficient determination of nitrate in the total N-analysis by the KJELDAHL procedure. By the simple KJELDAHL digest with sulfuric acid, selen, and potassium sulfate we could not exclude the nitrate quantitatively. Otherwise salicylic sulfuric acid and sodium thiosulfate could only reduce 70 to 95 % of the added ^{15}N-nitrate. Van Slyke processes can cause gaseous N-losses, thus faking natural denitrification.

The other problem is the incomplete reduction of dissolved nitrate by DEVARDA's alloy, which leads to an underestimation of ^{15}N-nitrate remaining in the soil depending strongly on the kind of extractant (BECKER). Using 2m potassium chloride we recovered markedly less nitrate. Results are more reliable using 0,01 m $CaCl_2$ for the extraction. The same phenomenon occurred while reducing nitrate in lysimeter water containing much organic or silicic substance. In several samples more than 90 % of the total nitrate remained undetected.

Beside us other investigators were also affected by these methodical faults. For example one day after application NIEDER recovered only 81 to 88 % of his ^{15}N-nitrate by KJELDAHL analysis. He reported a deficit of anions in the yellow to brown colored leaching water of his lysimeters, probably due to an incomplete reduction of nitrate by DEVARDA's alloy. THIES interpreted the undetected ^{15}N-nitrate as losses by denitrification taking not into account that leaching losses of nitrate could have been undetected.

Wherever high amounts of nitrate isotopes are present at the time of sampling, misinterpretations of the experiments leading to an overestimation of denitrification have to be taken into account originating from analytical errors. Under covered fields these conditions are realized only within a few weeks after application of fertilizer before plant uptake of the nitrate.

Thus the analytical defects are negligible at harvesting time, when the ^{15}N-balance studies usually are carried out. In fallow soils fertilizer nitrate can be conserved over a period of several months and cause analytical problems. Retrospectively due to this observation the rate of denitrification seems often being overestimated by BREMNER's method in case of fallow fields, fields early in the period of vegetation and of sandy soils in general, when leaching of nitrate is going on.

EXPERIMENTAL ERRORS

The experimental conditions offer another problem for the comparison of denitrification rates to be measured at various places. Main factors controlling denitrification are the content of easily decomposable organic matter and the oxygen content reverse water content of the soil.

Consequently, the interpretation of data from lysimeters filled by casting meets difficulties, because after their preparation the organic matter is not in an environmental equilibrium state. The results of VÖMEL and of VILSMEYER have shown, that mineralization and leaching of nitrate can be enhanced for several years. These effects can be neglected in the case of DRESSEL's lysimeters, which had been installed in 1927.

The water content of filled lysimeters is another disturbing factor in the comparison of denitrification rates. Especially in pots of small depth, water contents higher than in naturally layered soils are the rule. HEINEMEYER et al. installed ceramic pipes in their pot to regulate the water content by suction.

COMPREHENSIVE EVALUATION

Taking the possible methodical and analytical errors into account, the following summarizing results of all ^{15}N trials in W.-Germany are allowed, despite their distribution over different years and climatic regions: The uptake of nitrate by the plants is the main control of ^{15}N-transformation in the soil-plant system. It varies between 24 and 81 % of the nitrogen added. High plant uptake percentage is found especially , when the application is subdivided into two or three additions. Also mixing of the nitrogen with soil material results in higher plant uptake. In contrast monolith experiments and those with only one fertilizer addition show a low plant availability of the labelled nitrogen.

The few experiments studying the second year uptake of remaining ^{15}N by plants have shown only very little rates of uptake, proved by DRESSEL's lysimeters, which were fertilized during 6 subsequent years and which also stated the low availability of ^{15}N remaining from the years before.

The immobilized nitrogen at harvest time has to be looked at as absorbed

by roots or incorporated on this pathway into postmortal organic soil matter. In model experiments AMBERGER and VILSMEYER have shown, that the nitrogen of plant roots becomes mineralized again, but only at low rates. Even under most favorable conditions of incubation, 10 months at 25°C, less than 39 % of the total root nitrogen are converted into nitrate.

Without very few exceptions the immobilization effect in all experiments covers between 25 and 35 % of the fertilizer-N, and with respect to this fact the results of all trials are in good accordance.

If leaching losses are negligible the ^{15}N not recovered can be accounted to denitrification or, more exactly spoken, to gaseous losses of N_2, NO_x and NH_3.

Considering the rates of organic immobilization as nearly constant as mentioned above the rate of denitrification of soil nitrate has to be looked as strongly competitive to nitrate uptake by the plants.

The higher the rate of nitrate uptake the lower the rate of denitrification - demonstrated by the experiments with subdivided fertilizer addition, break crops and unusual high rooting in small pots.

One problem remains, leading possibly to a severe underestimation of natural denitrification by tracer studies because they are only tagging the fate of the fertilizer nitrogen. But presumably soil born nitrate will behave in the same way as fertilizer nitrogen present at the same time.

Due to the wide range of soil and fertilizer nitrate in the soil and its change by time it is impossible to calculate the total denitrification losses from those experiments which only use one single time mark of tagged fertilizer injection.

A calculation of the total denitrification losses from the soils based on the ratio of fertilizer and soil born nitrogen in the plants at harvest indicates losses two to four times as high as losses only related to the labelled fertilizer-N. But this overall calculation includes to much assumptions and can only mark targets.

One way for solving the problems by ^{15}N tracer experiments in the field could consist in tracing the soil at different stages of nitrogen turnover and nitrate formation and catching gaseous losses in short term intervals under measuring their ^{15}N content.

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Fig. 1: Distribution of ^{15}N-Fertilizer on Plant Uptake, Immobilization and Gaseous Losses (%)

Reference	Fertilization N-form	Number of treatments	Plant uptake Year 1	Year 2	Immobilization	Gaseous losses	Leaching
Strebel et al.[4]	NO_3	3 x	81	-	19	0	0
Vilsmeyer[1]	NH_4NO_3	2 x	75	-	-	-	0
Heinemeyer+al.[1,2,5]	NO_3	1 x	73	-	20	5	0
Dressel+Jung[1,5]	NH_4	1 x	53-65	-	29-35	7-16	0
Vömel[1]	NH_4NO_3	1 x	46-53	2-3	24-33	9-21	4-10
Vömel+Döll[1]	NH_4NO_3	1 x	42-63	-	21-51	12-16	0
Dressel[3]	NO_3	2 x	49-58	<2	26-42	8-11	1-6
Dressel[3]	NH_4	2 x	38-45	<2	28-54	8-16	0-2
Capelle+Baeumer[4]	NH_4	1 x	41-44	-	24-28	32-33	0
Capelle+Baeumer[4]	NH_4	1 x	32-36	-	21-24	40-48	0
Thies[4]	NO_3	1 x	24	2	47	27	2
Thies[4]	NH_4	1 x	38	3	38	24	0
Mba-Chibogou+al.[4]	NO_3	1 x	36-40	-	23	37-41	0
Mba-Chibogou+al.[4]	NH_4	1 x	33-35	-	13-22	43-54	0
Fleige+Capelle[4]	NO_3	1 x	41	-	8	51	0
Nieder[4,6]	NO_3	1 x	31-37	-	30-32	31-39	0

[1]: Filled pots / lysimeters; [2]: Phytotron 79 days; [3]: lysimeters 55 years old; [4]: naturally layered soils; [5]: fertilizer mixed with the soil; [6]: results were corrected, the fertilizer ^{15}N set 100 %, not the ^{15}N recovered

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PRELIMINARY RESULTS BY COMPARING GASEOUS DENITRIFICATION LOSSES WITH ^{15}N-BALANCE LOSSES IN A WHEAT AND A BARLEY CROPPED FIELD

U. Schneider[1], K. Haider[1] and T. Mahmood[2]

Introduction

Denitrification losses during a vegetation period in arable fields or grasslands are still a matter of contradiction. In a recent survey by HAUCK and WEAVER (1986) fertilizer N losses ranging in between 20 to 40% of applied fertilizer N were indicated. Even essentially higher N losses were reported by RYDEN and LUND (1980), which amounted 200 kg N ha^{-1} and year. The latter authors, however, found such high losses in intensively fertilized, irrigated vegetable fields with normally high soil temperatures. On the other hand quantification of gaseous losses measured by GERMON (1985), BENCKISER et al. (1986) as well as by MOSIER et al. (1986) and several others in arable fields of the temperate region ranged only from 3 to 10 kg N ha^{-1} and vegetation period. These gaseous losses were either obtained by the acetylene inhibition method or by measuring ^{15}N labeled gases evolving from plots fertilized with highly enriched ^{15}N-nitrate. Usually, losses measured by the ^{15}N-balance method are higher than the reported gaseous losses (CAPELLE und BAEUMER, 1985). Both methods, however, sometimes agree well as indicated by MOSIER at al. (1986).

The aim of our study was to compare the N losses obtained by different analytical methods in an agricultural used water catchment area south of Braunschweig. This soil, a silty loam overlaying Emscher-marl, was cropped to wheat. A former experiment was made on a field located in the FAL. The sandy silt loam soil was cropped to summer barley. Since 1983 N_2O-evolution from these cultivated fields in both areas were monitored after acetylene inhibition by the open cover method by BENCKISER et al. (1986, 1987) and averaged from about 3 - 8 - 15 kg N ha^{-1} during a vegetation period.

In the present experiments N_2O- evolution measured by the open cover method (RYDEN et al., 1978, BENCKISER et al., 1986) was com-

[1] Inst. Pflanzenern. u. Bodenkde., Bundesforschungsanst. f. Landw. (FAL), Bundesallee 50, D-3300 Braunschweig, FRG
[2] Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan

pared with N_2O-N losses after C_2H_2-treatment of undisturbed soil cores (RICE and SMITH, 1982) obtained from 0 to 10 cm and 10 to 20 cm depth.

The undisturbed soil cores were inserted in closed gastight glass cylinders. The field-moist soil was immediately incubated after sampling for 24h at field temperature conditions. Before incubation the gas atmosphere was supplied with 1% acetylene.

In addition ^{15}N-balance losses were quantified. Steel cylinders (65 cm length, 30 cm diameter) were driven into the soil of a field planted by winter wheat at the early spring before fertilization. Forty percent ^{15}N enriched $(NH_4)_2SO_4$ was applied on the soil surface in the cylinders at the same amounts and times as the field was normally fertilized. Three parallel cylinders were removed at four different stages of plant maturity. Two more cylinders were used as lysimeters to control possible ^{15}N leaching losses during and after vegetation. After harvest plants and roots were separated from the soil. The soil cores were divided into layers of 10 cm depth, which were separately analyzed for total N and ^{15}N contents as well as for mineral N. Precipitation, soil temperature and mineral N-contents were monitored independently throughout the year.

The gaseous losses were measured from April to August and are presented together with precipitation, % water filled porosity and soil temperature in Fig. 1. The gaseous losses measured by the open cover method are not shown here. They averaged from 3 to 6 kg N ha^{-1} and agreed well with those formerly reported by BENCKISER et al. (1986, 1987). The losses determined from soil cores from 0-10cm soil depth averaged from 9 to 15 kg N ha^{-1} and vegetation period. Each measurement was calculated as a mean of 15 independent samples obtained from a 100 m^2 plot. Due to high spatial variability they show a high standard variation. They are nearly three times higher than the losses obtained by the open cover method. The course of the dayly N_2O-release rates by both methods correlate well with the precipitation rates and with the respective percentages of water filled soil porosity. From April to mid of May only little N_2O evolved from soil due to low precepitation and little soil NO_3-N contents, although soil temperatures were mostly above 10°C. After high precipitation (30 mm) at the end of May and begin of June together with an additional fertilization of 70 kg N ha^{-1}, gaseous losses increased strongly and reached a maximum at the end of June. Then a second and third period of increasing N_2O losses followed during high precipitations in July and August and after addition of 40 kg fertilizer N at the ear filling stage.

The ^{15}N balances were made at four different stages of plant developement. The next two figures (2 and 3) show the results at shooting and at harvest. At shooting stage most of the fertilizer-N remaining in soil (35%) is still located in the upper 10 cm layer and is equally distributed between the inorganic and organic N-fraction. It decreases significantly with soil depth and less than 1% can be found in the layers from 40 to 65 cm. Insignificant amounts are in the soil below the cylinders. Nearly 56% of the fertilizer N was incorporated into the plants. The sum of fertilizer N in soil and in plants amounted 91 ± 3.3 % and results in a loss of 6.3 ± 2.3 kg fertilizer N ha^{-1}. This loss agrees well with the very small gaseous loss until this time.

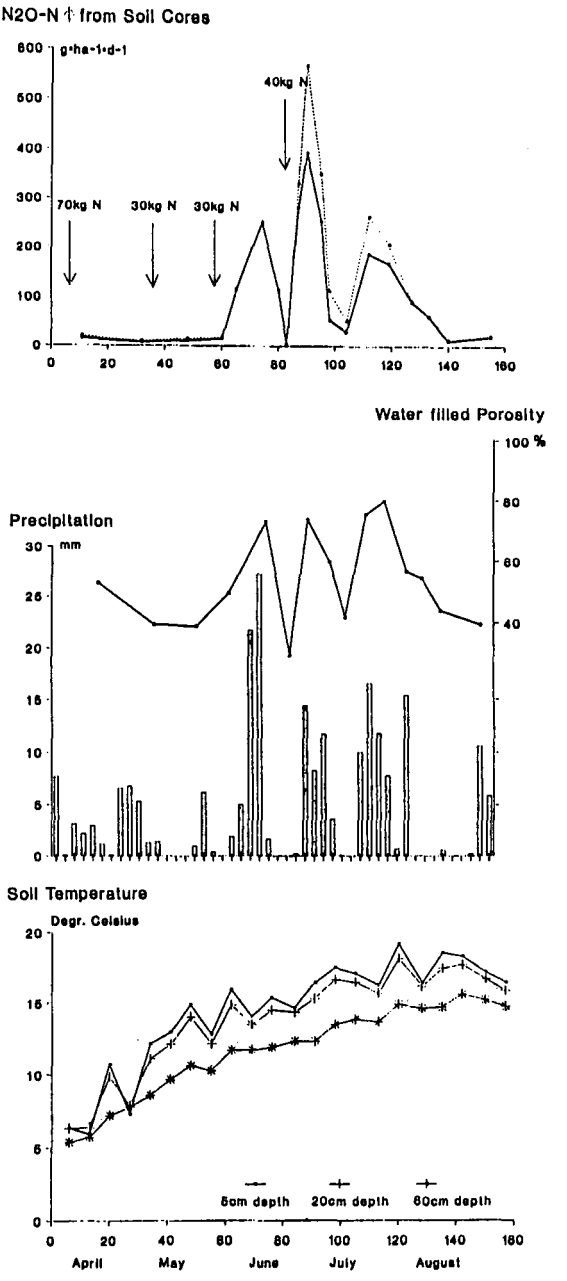

Figure 1: N_2O-N losses and environmental conditiones in the wheat field in 1988

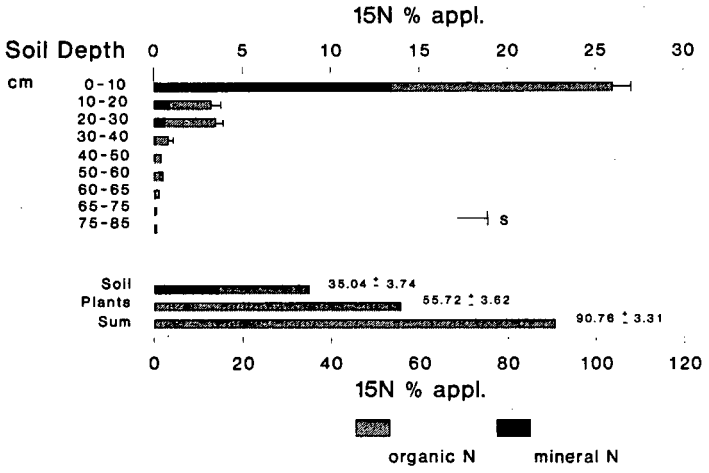

Figure 2: ^{15}N balance in plants and soil in Mai 1988 at shooting stage (bar length = Σ mineral N + organic N)

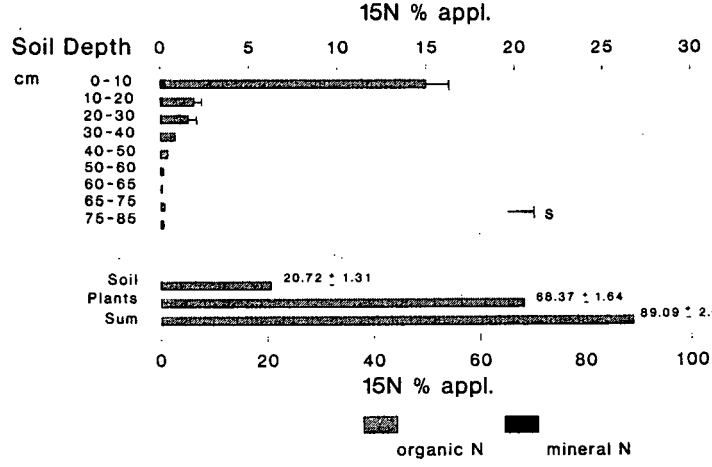

Figure 3: ^{15}N balance in plants and soil in August 1988 at harvest (bar length = Σ mineral N + organic N)

Results of ^{15}N balance analyses at harvest are shown in Fig.3. Again most of the ^{15}N from fertilizer remaining in soil is still concentrated in the surface layer with a small portion of mineral N. Less than 1% is located in the layers from 40 to 85 cm with a negligible ^{15}N enrichment in the layer below the cylinders. At

harvest nearly 70% of fertilizer N was concentrated in the plants and mostly in the ears (50 to 60%). The total plant nitrogen consisted, however, only to 50% of fertilizer N while 50% was contributed from the soil N. Despite this high contribution of soil N, mineralized soil N should not essentially participate in denitrification, because of generally small nitrate contents and their little dilution by soil N.

Table 1: ^{15}N balance in plant and soil, and fertilizer losses at different stages (N applied per hectare = 100%)

Days	Experiment in the barley plot (sandy loam) ^{15}N appl. (%)				in the wheat plot (silty loam) ^{15}N appl. (%)			
	Plants	Soil	Sum	Loss[1] (kg/ha)	Plants	Soil	Sum	Loss[2] (kg/ha)
30	38 ±1.0	62 ±4.1	100 ±4.3	0.0 ±5.4	56 ±3.6	35 ±3.7	91 ±3.3	6.3 ±2.3
60	38 ±2.2	48 ±4.9	86 ±5.4	17.6 ±7.9	57 ±2.6	26 ±0.6	83 ±2.4	22.1 ±3.1
90	37 ±2.3	51 ±1.1	88 ±2.5	15.1 ±2.9	66 ±3.2	30 ±3.4	96 ±3.6	6.8 ±6.1
120					68 ±1.6	21 ±1.3	89 ±2.1	18.8 ±3.6

1) 125 kg N ha applied at the beginning of the experiment.
2) 70 kg N ha applied at the beginning, 60 kg N ha at day 30 and 40 kg N ha more at day 90

A comparison of the losses determined by the ^{15}N-balance method at different stages of vegetation of wheat or barley is shown in table 1. The experiment in the barley field was conducted in 1987 where barley was grown on a sandy loam soil in the FAL. Here only two cylinders were harvested at each period after 30, 60 and 90 days. ^{15}N fertilizer was applied only at the beginning of the experiment in one dose of 125 kg N ha^{-1} in June. The conditions did not correspond to usual agricultural practice, therefore additional high mineralisation of soil N led to a relatively high uptake of this unlabeled mineral N by the plants. This is indicated by the rather constant amounts of fertilizer N in the plants. The sum of ^{15}N in plants and soil averaged nearly 100% at day 30 and about 88% at day 90. Losses increased from about 0 at day 30 to 18 kg N ha^{-1} at day 90 during the vegetation period with relatively high standard deviations of the ^{15}N-analyses from different soil layers.

The fertilizer losses determined on the silty loam soil cropped to wheat are in the same range as those determined from the barley field. The total recovery of the applied fertilizer ranged from 85 to 91%. Based on the splitted application of fertilizer N of 70, 60 (30+30) and 40 kg N ha^{-1} the total N losses amounted 9, 16 and

11% of the applied fertilizer N at day 30, 60 and 120, respectively. Although 6 different layers were separately analyzed and the average data from 3 replicates were calculated, the standard deviations are relatively small and amounted from 2 to 3 % of the mean value. The losses of fertilizer N never reached 20 or even 50% as they were sometimes reported in the literature. Due to the high enrichment of ^{15}N in the applied fertilizer and the analyses by a highly sensitive mass spectrometer the detection limits of fertilizer N in the soil equaled about 0.5 ppm.

The gaseous losses detected from the soil cores and measured throughout the vegetation period averaged from 9 to 15 kg ha^{-1}. They are only slightly less than the losses determined by the ^{15}N balance. They, however, exceeded the losses of 3 to 8 kg N ha^{-1} measured by the open cover method by two to three times.

From this comparison it could be concluded that the open cover method somewhat underestimates the N losses. On the other hand it seems reasonable that the ^{15}N balance losses overestimate denitrification since they do not consider losses during nitrification, those of NO and NH_3, and also possible volatile losses by the vegetative parts of the plants (FARQUHAR et al., 1979; WEILAND et al., 1979).

The results of the lysimeter experiments indicated that even shortly after fertilizer addition the mineral N content was small due to plant uptake and immobilisation in the soil organic fraction. Even after high precipitation during the vegetation never any leaching losses occurred. After harvest, at fall and during winter, however, nitrate from mineralized soil N was leached down and appeared in the water collection bottles of the installed lysimeters. The contribution of fertilizer N to the leached nitrate was small and it therefore consisted predominately from mineralized soil N. In future experiments it is planned to follow the remineralisation of immobilized N during fall, winter and spring more detailed, to get information about the contribution of fertilizer and soil N to the nitrate pool and its leaching during that period.

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Denitrification characteristics of a compacted pasture soil

by Butler, A.R. and W.A. Adams +)

A fall in herbage production has been observed on Denbigh series soils in Wales after several years in permanent pasture. In practice this is shown as a poor response to fertiliser N. Under intensive grazing systems a zone of compaction develops at a depth of 8-12 cm, leading to shallow rooting and the appearence of stagnogley features in the surface soil. Previous work has demonstrated that substantial increases in herbage production and N off-take can be acheived on such soils by using a simple mechanical soil aeration technique(1). It was felt that N loss through denitrification may be a factor responsible for limiting the growth and N uptake of herbage on these compacted soils as surface wetness is coupled with high levels of root derived organic matter. The aims of the work were to: i) quantify the compaction problem; ii) establish if and where denitrification occurs in the profile and to examine the effect of shallow rooting, and iii) quantify denitrification to establish if this could account for the differences in N off-take between aerated and non-aerated swards reported by Davies et al.(1).

MATERIALS AND METHODS

Site : Experiments were carried out on a Denbigh series soil (typic brown earth or typic dystochrept) located at Aberystwyth, Wales (SN 595823), which had been under permanent pasture for >20 years and intensively grazed by dairy cattle for 10 years. Soils of this series have a silty clay loam or clay loam particle size class and a moderate content of medium and small stones.

Soil physical analyses : Bulk density (D_b) was measured by sand replacement at 2 cm intervals to a depth of 16 cm. Measurements of stone content, allowing calculation of fine earth bulk density (D_{bf}), and organic matter, allowing calculation of particle density (2) and thus total porosity (E_t), were also made.

+) Soil Science Unit, U.C.W., Aberystwyth, Dyfed, U.K. SY23 3DE.

Denitrification potential : Samples were taken at 2 cm intervals to a depth of 16 cm and from 25 cm and 50 cm. Soils were air dried sufficiently to pass through a 2 mm sieve and stored moist at 4°C. A slurry was made with 20 ml of KNO_3 solution (150 ug ml^{-1} N) and 10g soil (oven dry weight) in 150 ml serum bottles. Soils were incubated anearobically under a N_2 headspace at 25°C for a total of 130 hours. Acetylene was introduced to give a headspace concentration of 1%. Gas samples (1 ml) were analysed for N_2O on a Pye 104 chromatograph fitted with a Porapak Q column and a ^{63}Ni electron capture detector.

Measurement of soil organic fractions : Total organic matter was estimated by LOI at 400°C. Soil polysaccharides were measured on 1.5M H_2SO_4 hydrolysates using a method described by Brink et al.(3). Sugars extracted by 0.01M $CaCl_2$ at 100°C for 1 hour as described by Stanford et al.(4) were also measured colormetrically(3). Results are expressed as glucose equivalents.

Denitrification from undisturbed cores : A core incubation system similar to that described by Ryden et al.(5) was chosen to measure denitrification in the field and laboratory. Only when cores were retained in their sampling rings, to minimise exposure of the sampled soil faces to air, did measured rates of denitrification approach those obtained using an accepted cover technique described by Colbourn et al.(6) (unpublished data). In the field, 5.3 cm x 8.0 cm deep cores were used, but for laboratory experiments, the same sampling rings were used to collect 5.3 cm x 6.0 cm cores. This allowed ponding of differing NO_3^- solutions on the soil surface which leached down to impose a range of NO_3^- contents representative of field conditions. Moisture status was regulated using tension tables. Data is presented for samples equilibrated to 1kPa matric potential at the centre of the core with mean air-filled porosities of 0-5%. All pre-treatments and incubations were carried out at either 5°C or 10°C. Soil cores were then sealed at their bases with polythene film and placed in 1 L fruit preserving jars fitted with gas tight lids and gas sampling ports. Acetylene (50 ml) was injected directly into the samples before the jars were sealed. A pre-incubation of 1.5 hours was suffi-

cient to inhibit N_2O-reductase. The change in concentration of N_2O in the jar head-space was measured over a further 1.5 hour period to determine denitrification rate. Jars were then fumigated using chloroform [5] to prevent further N loss and cores sectioned and analysed for moisture content and NO_3^- using a 1M KCl extraction.

RESULTS AND DISCUSSION

The measurement of soil physical properties (Tab. 1) showed the presence of a zone of high density, high stone content and low porosity. This layer is restrictive to root growth and water infiltration.

Denitrification potential (DP) showed an exponential decay with depth (Fig. 1), and little activity below 8 cm depth. These results followed a similar trend with depth as the various organic fractions measured (Tab. 2). Soil polysaccharides and in particular extracted sugars showed a clear cut-off point at 8 cm depth. Regression analysis of the data (Tab. 3) identified highly significant correlations between DP and all forms of organic matter.

TABLE 1. Soil physical properties of a compacted Denbigh soil.

Soil depth (cm)	D_b (g cm^{-3})	D_{bf} (g cm^{-3})	Stone content (%)	E_t (%)
0-2	0.46	0.46	0.0	63
2-4	0.80	0.80	0.0	56
4-6	1.10	1.07	7.4	47
6-8	1.40	1.17	29.8	36
8-10	1.55	1.18	44.6	35
10-12	1.83	1.56	39.6	22
12-14	1.54	1.34	27.1	33
14-16	1.40	1.12	35.1	39

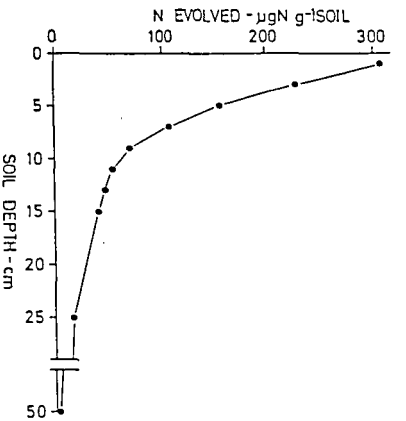

Fig. 1. Changes in denitrification potential (N evolved after 130 hours) with depth.

TABLE 2. Changes in the amounts of various organic fractions with depth in a Denbigh soil.

Soil depth (cm)	LOI (%)	Polysaccharides mg g^{-1} soil	Sugars $\mu g\ g^{-1}$ soil
0-2	20.7	21.5	2660
2-4	19.1	18.1	2270
4-6	16.3	13.6	1480
6-8	12.8	11.3	1030
8-10	10.0	7.6	591
10-12	9.1	6.5	596
12-14	9.3	6.4	537
14-16	9.0	6.0	518
24-26	5.4	3.4	202
49-51	2.5	1.4	67

but the closest correlation was with extracted sugars. This fraction, which comprised 0.3%-1.3% of the total organic matter, was presumably the most available to denitrifying organisms. It is well recognised that availability of readily metabolisable carbon is a major factor limiting a soil's capacity for denitrification (4,7). It appears that the build up of organic matter in the surface soil as a result of shallow rooting enhanced this soil's ability to denitrify in a zone which is likely to experience low air-filled porosities and high concentrations of NO_3^-.

TABLE 3. Regression of denitrification potential (Y) on LOI, soil polysaccharides and extractable sugars.

Independent variable (X)	Regression equation	R^2 %
LOI %	Y = -9.00 + 0.113X	90.7 ***
Polysaccharides mg g^{-1}	Y = -83.1 + 16.3X	97.6 ***
sugars $\mu g\ g^{-1}$	Y = -42.2 + 15.2X	98.9 ***

Core sampling and incubation for denitrification measurements at 5°C was carried out between January and March 1988. The data indicated that soil NO_3^- became non-limiting at 40 ug N g^{-1} when the denitrification rate was around

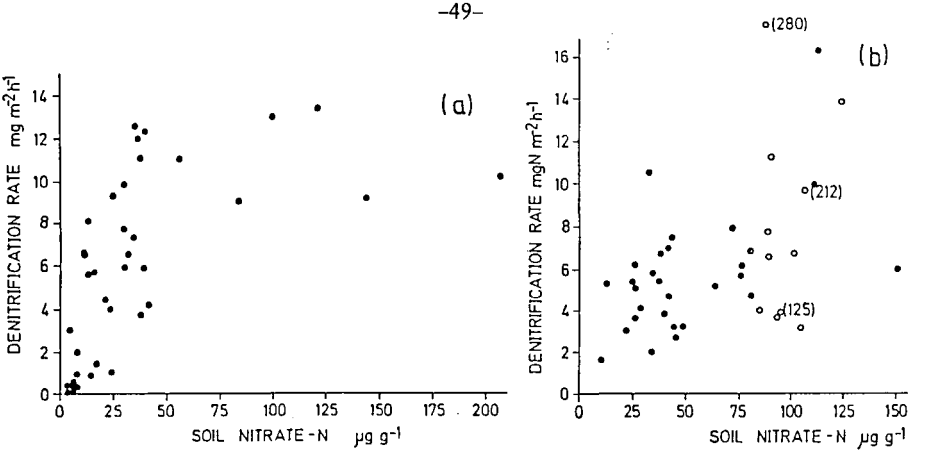

Fig.2. Effect of NO_3^- on denitrification rate from cores sampled in: (a) 1988, incubated at 5°C and (b) 1989, incubated at either 10°C (•) or 5°C (o). Numbers in brackets refer to levels of anthrone reactive carbohydrate for selected samples (µg g^{-1} soil).

11 mg N m^{-2} hr^{-1} (Fig. 2). Although the points showed a degree of scatter, it was hoped that a series of such graphs from experiments at different temperatures and air-filled porosities would enable predictions of denitrification rate under a relevant range of field conditions. However, results obtained from cores incubated at 10°C taken between January and March 1989 showed more variability and little trend (Fig. 2). With more data, a plateau in the rate of denitrification may have been found at 6-7 mg N m^{-2} hr^{-1}. This was below the maximum seen at 5°C and contrary to the result expected. It was thought that a change in the availability of carbon between sampling occasions might have caused this anomaly. If a shift in available carbon had occurred (perhaps due to the unusually mild winter in 1989), another incubation at 5°C should have shown a lower rate of denitrification than that observed in 1988. The results (plotted in Fig. 2) followed a similar pattern as those obtained at 10°C, although over a limited range of soil NO_3^- levels. There was however a large variation in the measured rate of denitrification. In order to try to

explain this, anthrone reactive carbohydrate[3] was determined on selected KCl extracts. These results are shown in brackets next to the samples they represent (Fig.2). Although not conclusive, they suggest a trend between denitrification rate and available carbon. The similarity of results for incubations at 5°C and 10°C on samples collected in 1989, suggests a temporal change in the amount of available carbon in the soil between sampling periods and the spatial distribution of this fraction probably accounts for much of the variability in denitrification rates observed. The effect of temporal and spatial variability in available carbon has serious implications for the measurement and interpretation of denitrification rates in the field and laboratory and has been used to explain the "hot-spot" phenomenon[8,9].

The core incubation technique proved to be versatile for use in the field and laboratory, but variability made it difficult to make a precise assessment of the extent of N loss through denitrification likely to occur in the field. Nonetheless, denitrification rates in excess of 3 Kg ha^{-1} day^{-1} were measured, but such rates required conditions close to water saturation and soil NO_3^- - N levels > 40 μg g^{-1}. These conditions have only been observed in wet weather after application of NH_4NO_3 fertiliser and persist for no more than a few days. It is concluded that while the presence of high amounts of organic matter may promote denitrification, N loss by this process is not the sole cause of low herbage productivity and N recovery on these compacted soils.

ACKNOWLEDGEMENTS

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DENITRIFYING ACTIVITY MEASUREMENT BY SOIL CORE METHOD. EFFECT OF DEPTH AND CHARACTERISATION OF N_2O/N_2 RATIO IN DIFFERENT SOILS.

J.C. GERMON - D. JACQUES
Laboratoire de Microbiologie des sols
INRA, 17 rue Sully, 21034 Dijon-Cédex, France

Summary

Validity of soil core method was tested to quantify N_2O natural production and denitrification rate using acetylene inhibition technique. Log normal distribution of N_2O production rate , in absence or presence of acetylene, is confirmed.

Denitrifying activity in a soil profile appears particularly developped between 20 and 30 cm depths. Denitrification rates in 20 cm top soil allow to take into account only 31 % of total activity and 94 % of total activity is localised in the first 40 cm in the profile. Denitrification rate appears correlated with bulk density only.

Data on N_2O production measured in absence and in presence of acetylene in 16 different soils, from a same pedological unit, indicates a constant N_2O /N_2 ratio during denitrification, despite very different denitrification rates and pH.

Introduction

Denitrification losses can be appreciated by direct *in situ* measurements using acetylene inhibition properties as suggested by RYDEN et al. (1979), RYDEN and DAWSON (1982), ROLSTON et al. (1982), GERMON et al. (1985), TERRY et al. (1986). However that method has several limitations (HAUCK, 1986) and is not really suitable for a more analytical approach. The gas-flow soil core method developped by PARKIN et al. (1984) allows to avoid constraints due to C_2H_2 and N_2O diffusion at least partially. (LETEY et al., 1980 ; MYROLD and TIEDJE,1985). Such a method can be used to study the effect of different parameters on denitrification : soil depth, pedological origin, humidity, temperature and so on. It can be an interesting tool to develop a modeling approach derived from ROLSTON et al. (1984) in order to forecast denitrification losses .

In this part we studied the opportunities presented by that method : kinetics of N_2O production without and with acetylene and statistical distribution with soil samples from a same parcel. The gas flow core method was applied to measure denitrification in a soil profile and to study the relationships between natural N_2O production and total denitrification in different soils from a same pedological unit.

Material and method

Incubation system is presented in figure 1. Soil samples were collected in field wet enough to push gently PVC cylinders in the soil. Denitrification kinetics and statistical distributions were studied with samples 20 cm high. Then 10 cm cores were collected in order to avoid soil compaction as far as possible.

All soils indicated in table 1 were sampled from a same pedological unit of a brown loamy soil of Dijon area (CHRETIEN, 1976). N_2O production kinetics without and with acetylene were studied on 33 samples of 20 cm top soil number 2 collected at random on 400 m^2 area.

Samples from the same soil were collected from 10 to 10 cm until 70 cm with 7 replicates from a pedological trench in order to study the depth effect.

Denitrification rates and ratios between N_2O and total denitrified nitrogen were compared from 16 different top soils indicated numbers 147 to 811 collected at the same date in february 1984 with 6 replicates.

Soil samples were incubated the days following sampling or were kept at 4°C and reestablished at ambient temperature 3 days before measurement. Incubations were realised at 20° C and at field capacity in the following conditions : water was added to attain saturation whereas water could flow freely at bottom of cylinders ; 24 hours later each core received 20 ml nitrate solution corresponding to 300 kg N per ha and is maintained in wet atmosphere at 20° C during 2 extra days.

Then cylinders were airtightly closed and 5 ml Krypton were added as an internal standard. Gas atmosphere was periodically recirculated with airtight pumps allowing a 1200 ml/min flow rate. After preliminary trials gas was recirculated during 3 min each hour and 3 ml gas were sampled immediatly with Venoject tubes to analyses. After a first incubation time to measure natural N_2O production, 2 % acetylene were added in order to appreciate total denitrification.

N_2O production without and with acetylene was measured during 9 and 12 hours respectively for the statistical approach on 33 cores ; measurement times were only 6 hours for each phase with samples from different depths and 2 hours for the comparison of 16 different soils.

N_2O, C_2H_2 an Kr were analysed with Girdel 3000 gas chromatograph in accordance with GERMON (1980).

Distribution normality and log-normality were tested with Kolmogorov and Smirnov method better adapted than Pearson X^2 for low populations (DAGNELIE, 1975). In log normal distribution means and confidence intervals were calculated in accordance with the method proposed by TIEDJE group (PARKIN et al., 1985).

$$\bar{x} = e^{(\bar{y} + \sigma_y^2/2)} = e^{\bar{y}} . e^{\sigma_y^2/2}$$

$$\bar{x} . e^{\frac{-t\sigma_y}{\sqrt{n-1}}} < \bar{x} < \bar{x} . e^{\frac{+t\sigma_y}{\sqrt{n-1}}}$$

where $\bar{x}$ is the mean estimated from the initial data (x) and $\bar{y}$ is the arithmetical mean of transformed data ($y = \text{Log } x$).

Results and discussion

In experimental conditions N_2O production in absence or presence of acetylene is a very linear kinetics (figure 2) indicating a rapid effect of acetylene on N_2O reduction when atmosphere gas is homogenized. This suggests a constant ratio between N_2O and total denitrified nitrogen during biological denitrification and is in accordance with some other observations (PARKIN et al. 1984). However that kinetics does not indicate a growing phase or enzyme synthesis as suggested by SMITH and TIEDJE (1979) : but such a phase could be developped during the 2 days preceding measurements. Extrapollated denitrification rates is attaining 480 g N/ha/day whereas N_2O production alone is 320 g N/ha/day.

Statistical distribution of denitrification rate among 33 cores from a small area is very expended (figure 3). KOLMOGOROV and SMIRNOV test does not allow to accept a normal distribution hypothesis but a log normal one (Table 2), so confirming the log normal distribution of many biological processes in soil (FOCHT et al., 1979 ; ROBERTSON and VITOUSEK, 1981 ; FOLORUNSO and ROLSTON, 1984 ;

PARKIN et al. 1985). Log normality involves a dissymetric confidence interval of denitrification rates but does not change the mean values significantly (Table 3)

Denitrification rate at different depths in a soil profile in the experimental conditions is the highest between 20 and 30 cm depths (Figure 4a). This horizon presents simultaneously a high level of carbon (Figure 4b) and a low air porosity (Figure 4c), and is a convenient medium for denitrification. Measurements in the first 20 cm take into account only 31 % of total denitrification in the profile and it is necessary to summarize denitrification rates on 40 cm depth to attain 94 % of total nitrogen losses. That suggests denitrification is probably very important in such a soil in natural conditions between 20 and 30 cm corresponding to a compacted level due to ploughing, provided that nitrate is available. So it points out the difficulty to measure correctly denitrification *in situ* when a dense horizon exists in a profile and can involve gas leakages in depth. Such nitrogen losses due to denitrification are considered scarcely (ROLSTON et al., 1978).

Comparison of natural N_2O production and denitrification rates in 16 different parcels from a same pedological unit indicates a great variation. Statistical analysis allows to point out only one significant relationship between denitrification rate and soil core bulk density. In these conditions physical properties are more relevant to determine denitrification rates than chemical parameters such as carbon availability. However the most astonishing result is the very closed relationship between N_2O production in absence and presence of acetylene (Figure 5) indicating a natural N_2O production equivalent to 77 % of total denitrified nitrogen. That is an unusual value excepted in acid soils, N_2O production being considered as a pH dependent process (GARCIA, 1973 ; FIRESTONE et al., 1980 ; KOSKINEN and KEENEY, 1982). In the present situation pH is varying from 5.2 to 7.2 (table 1) ; the results suggest that in a homogenous pedological area denitrifying microflora could behave similarly and natural N_2O production could be a constant proportion of denitrified nitrogen in despite of different pH. These loamy soils were naturally acidic and pH has been increased by cultural techniques. This important N_2O production could be a consequence of indigenous microflora undisturbed by agricultural conditions. A constant ratio between N_2O and denitrified nitrogen was previously observed with a waterlogged soil in different conditions by LENSI and CHALAMET (1979). However it is necessary to check if such a behaviour is not dependent of our experimental conditions and can be observed with other soil types.

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Soil Number	*Clay %*	*Total C %*	*Kjel.N %*	*pH Water*	*Relat.humid. 1000 g, %*	*Bulk density*
2*(0-20)	17.3	0.86	1.05	7.9	18.5	1.33
2*(30-40)	26.4	0.41	-	8.2	21.7	1.37
2*(60-70)	40.3	0.20	-	8.1	29.4	1.27
147	15.0	0.76	0.78	5.9	19.4	1.52
160	12.6	0.88	0.95	5.9	17.7	1.46
193	14.0	1.36	1.52	6.2	21.5	1.39
194	16.7	1.17	1.22	5.6	20.0	1.49
197	10.0	0.95	1.05	5.9	16.7	1.41
297	21.2	1.25	1.23	5.2	24.4	1.36
302	15.1	0.82	0.89	6.4	19.1	1.47
328	14.9	1.15	1.25	7.1	19.8	1.43
353	24.8	1.17	1.27	6.1	24.2	1.40
363	15.9	0.91	1.08	6.6	19.1	1.34
367	10.3	0.69	0.75	7.2	16.9	1.48
369	12.4	0.88	0.93	5.3	20.0	1.41
377	13.8	0.83	0.89	5.7	18.8	1.44
390	10.1	0.58	0.68	5.5	16.0	1.53
810	13.9	1.83	1.81	6.5	24.2	1.26
811	13.6	0.96	0.85	6.1	20.3	1.38

Table 1 : Main analytical data of soils used : * sample depths in brackets.

		(1)*	*(2*)*	*N(x)*	*U*	*F(x)*	*N(x)-F(x)*	*Normality hypothesis*
N_2O production without acetylene	measured	15	27	0.818	0.160	0.564	0.254	Rejected
	transformed	3.75	29	0.878	1.693	0.955	0.077	Accepted
N_2O production with acetylene	measured	20	23	0.697	-0.001	0.500	0.197	Rejected
	transformed	1.875	5	0.151	-0.749	0.227	0.076	Accepted

Limit value for N (x) - F (x) at 5 % : 0.151

Table 2 : Application of Kolmogorov and Smirnov's test for N_2O production rates and for logarithmic transformed values.
Results are given for the data groups with the highest differences N(x) - F (x)
(1) Limit value of the data class ; (2*) Concerned cumulative population*

Hours	*Normal distribution*	*Log-normal distribution*
1	14.4	14
4	53.4	52.9
8	100.6	101.2
9	124.9	126.5
13	192.2	192.1
17	270.4	270.1
21	351	353.4

Table 3 : Comparison of mean values of N_2O production (g N/ha) calculated in accordance with normal distribution and log normal distribution.

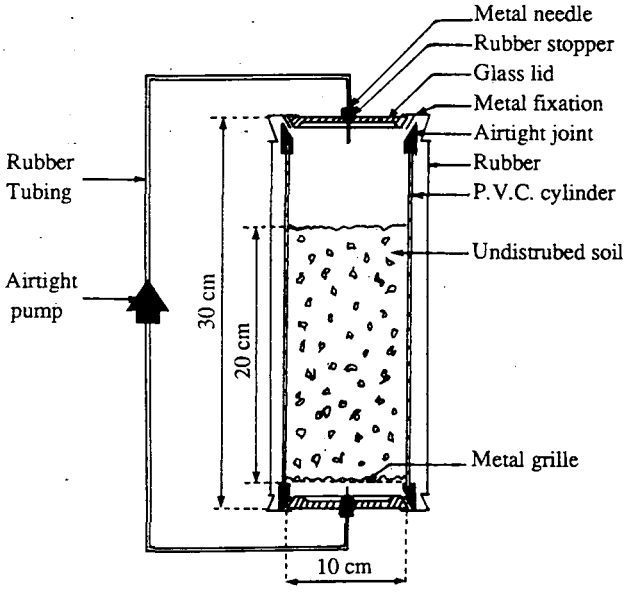


Figure 1 : Incubating system with gas flow

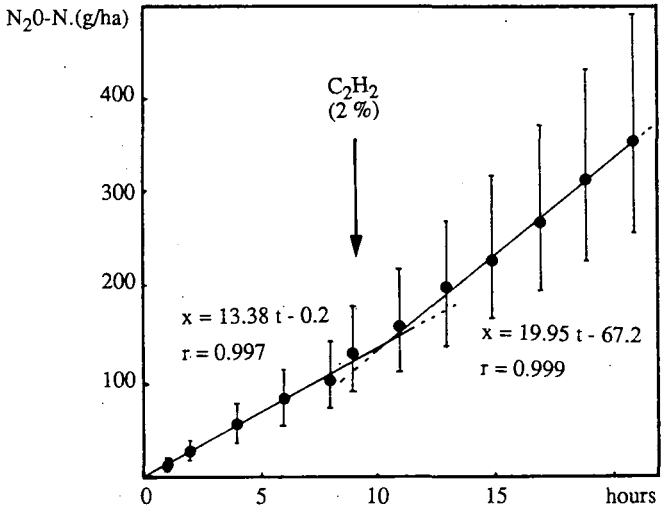


Figure 2 : N_2O production from 33 soil cores without and with 2 % acetylene. Means and 5 % confidence intervals being calculated from a log-normal distribution

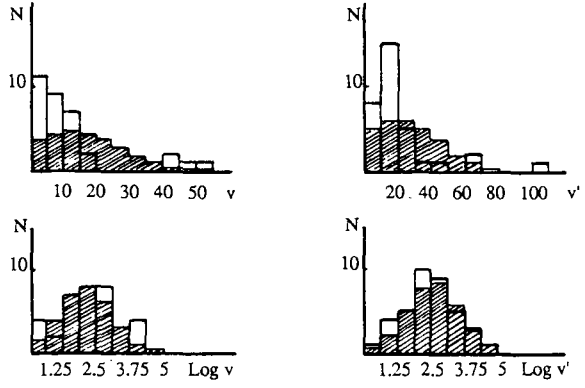


Figure 3 : Statistical distribution of N_2O production rates (v and v') and Log-transformed values (LN v and LN v') without (v) and with acetylene (v'). Hachured area are theorical distributions of normal populations with the same means and standard deviations.

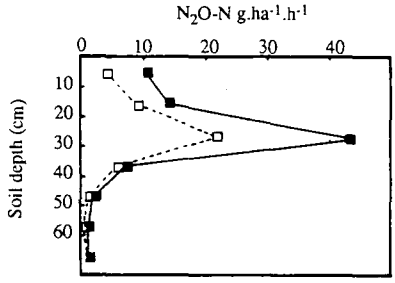


Figure 4a : Effect of depth on N_2O production in undisturbed soil cores in presence of normal (□) or 2 % C_2H_2 enriched atmosphere (■).

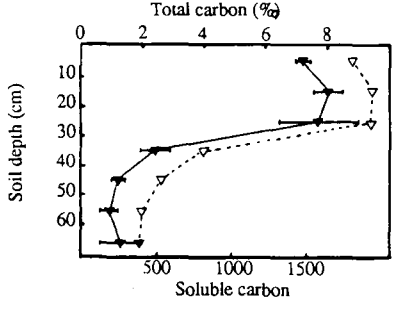


Figure 4b : Total carbon in percent of dry soil (▽) and soluble carbon at 120°C in mg COD per kg dry soil (▼) at different depths.

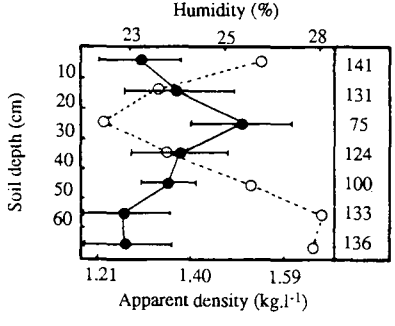
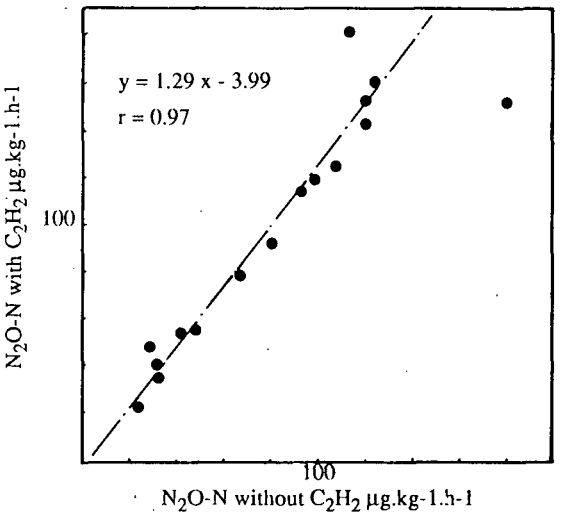


Figure 4c : Humidity (○) and apparent density (●) at different depths. Data in the column indicate ml air gas in 1l of soil at different depths.



Figures 5 : Relationship between N_2O production rates in absence and in presence of acetylene in 16 different soils from a same pedological unit.

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THE ^{15}N-BALANCE METHOD FOR CALCULATING DENITRIFICATION LOSSES IN ARABLE FIELDS AND ITS VERIFICATION BY DIRECT MEASUREMENT OF GASEOUS ^{15}N-LOSSES

by

BECKER, K.-W., E. JANSSEN u. B. MEYER *)

SCIENTIFIC PROBLEM

The problem of measuring denitrification rates of soils under field conditions cannot be looked at as being sufficiently answered. Between indirect measurements by isotope tracing technique of soil nitrate, the so-called ^{15}N-isotope-balancing (IB), and direct measurements by means of acetylene inhibition technique (AIT) are still heavy discrepancies existing - due to methodical errors. In this congress-volume critical reviews are given by BECKER for IB and by Becker et al. for AIT.

The experiments presented by the following paper (originally presented as a poster by JANSSEN) try to connect the IB-technique in the field, conducted under observation of the latest methodical standard, with the collection of $^{15}N_2$- and $^{15}N_2O$-gas formed by the process of denitrification.

AIM OF THE EXPERIMENTS

Aim of the experiments is to find out a) the errors connected with different modes of calculating long term field N_2-losses by denitrification from spot and moment measurements, b) reasons for the methodical differences between the isotope-balancing (IB) and the gas collection method (GC) applied at the same soil cylinders in the field.

METHODICAL CONCEPT

The methodical concept consists in labelling the nitrate content in the soil and its superficial horizons at a given time by injection of ^{15}N as nitrate. This procedure is being repeated weekly during the vegetation time.

The short term fate of the soil nitrate horizontally labeled by this injection is always studied 7 days after this procedure by analyzing the collected gas and by dissecting the tagged soil block.

*) Fachgebiet Bodenkunde, von Siebold-Str. 4, D 3400 Göttingen

OBJECT

The experiments were concentrated on farmers fields on Typudalfs derived from loess with the crop rotation sugarbeet-winterwheat-winterbarley and included a comparison between a pure cash crop farm with only mineral fertilization and a farm with cattle and mixed mineral and organic N-fertilization of soil. The results reported here comprehend a small section of these studies: The behavior of nitrogen turnover under nearly fallow conditions under young sugarbeet cultures before closing the rows, that means conditions without a marked N-uptake by plants.

The sites A and B compared demonstratively in this paper belong to one single sugarbeet field of the cattle farm: "A" being a stripe compressed by wheels, "B" a stripe with a normal soil structure.

METHOD

At sowing time in March many plastic cylinders, 30 cm in height, 25 cm in diameter, are driven into the soil down to the basis of the Ap-horizon. Between the begin of May and the middle of June every week soil columns of this series are treated with 23,5 mg ^{15}N per pot = 5 kg ^{15}N per ha by injection and evenly distribution of a $K^{15}NO_3$-solution with 65 AT %. According to fig. 1 this injection is performed by a syringe and a top plate with 19 jig holes. Six depths of injection at 2,5 cm distance by descending intrusion create 114 injection points in total per block.

During the 7 days period after injection the ^{15}N treated cylinders are closed by cover box overnight to collect the N_2- and N_2O-gas emitted by the soil (fig. 2). After stirring the air by a rotating magnetic fan in the morning 3 gas samples are taken by a syringe penetrating a silicon septum. The syringes are stored and transported under water.

At the same time the soil content of the cylinder and - according to rainfall and possible leaching of nitrate - also the subsoil down to a depth of 45 or 60 cm is cut into 15 cm thick slices which are thoroughly mixed. NO_3 and NH_4 are determined by steam distillation. The organic N-fractions are gaschromathographically measured after combustion at 1000 °C. The purified N_2-gas is trapped in evacuated glass tubes for measurement of the $^{15}N/^{14}N$ ratio by mass spectrometry.

CALCULATIONS

Because within the heptade of reaction not only the ^{14}N present as soil nitrate at the start but also ^{14}N produced by the permanent process of mineralization are participating in denitrification, calculation of total gaseous losses by using $^{15}N/^{14}N$ quotients has to take this fact into account. Modell calculations on basis

of soil conditions outside the cylinders as well as inside the cylinders have been developed.

RESULTS

Fig. 3: Because the cylinders were free of plants and nitrate ammonification was excluded by structure, besides the remaining tagged nitrate only 2 labelled new fractions resulted from microbial nitrate turnover: N in organic fraction and N in gaseous products going lost. Between 2 and 26 % of the injected ^{15}N are recovered as bound in the organic matter. The redistribution of labelled N shows strong variation with time and strong dependence on the difference in potential reduction at site A and B. While at site B 95 to 100 % of the added ^{15}N are recovered, the losses at site A sum up to 26 to 56 %.

Fig. 4: This strong local dispersion of more or less reductive stripes and spots within one single field is also demonstrated by the results of ^{15}N-gas measurements. The level of the daily denitrification rates at site A is more than 15 times higher than that of site B with maximum values above 5000 g N/ha·day, whereas at site B a rate of more than 500 g N occured only at one single day.

Fig. 5: A comparison of the results of the two methods used demonstrates the needs of further adaptation. The weekly sum of daily gaseous N-losses represented by the hatched columns, and the denitrification rate calculated on basis of ^{15}N-balance, represented by the white columns, show marked differences. The losses measured by gas analysis average only 48 % of the losses calculated by the ^{15}N-balance method.

Reasons for this discrepancy may be:

1 Overestimation of losses by the IB-technique due to incorrect $^{14}N/^{15}N$-factors derived from ^{14}N-contents of the soil outside the cylinders. This error seems small, compared with....

2 Underestimation of losses by the gas measurements due to

2a: gas diffusion into the subsoil,

2b: gas dissolution in water,

2c: reduced emittance of ^{15}N compared with ^{14}N by the edaphon,

2d: day and night oszillation,

2e: Unknown $^{15}N/^{14}N$-ratios in the precise subhorizons from which gaseous losses arise.

CONCLUSIONS

The cylinder technique offers the possibility to study the decrease of $^{15}NO_3$ in the soil by denitrification and the production of $^{15}N_2$ and $^{15}N_2O$ at the same spot.

The first way seems to have reached a higher degree of analytical maturity, and the higher values for denitrification rates seem to be more reliable, but suffer

from high variability of the horizontal and vertical nitrate distribution. The second way as the direct one offers chances, but the relativ low values found for denitrification rates still point out further needs for technical improvement and adaptation.

The high variability of denitrification rates in one single field supports the demand for measuring denitrification rates at the same spot with direct and indirect methods and gives rise for continuation of these experiments.

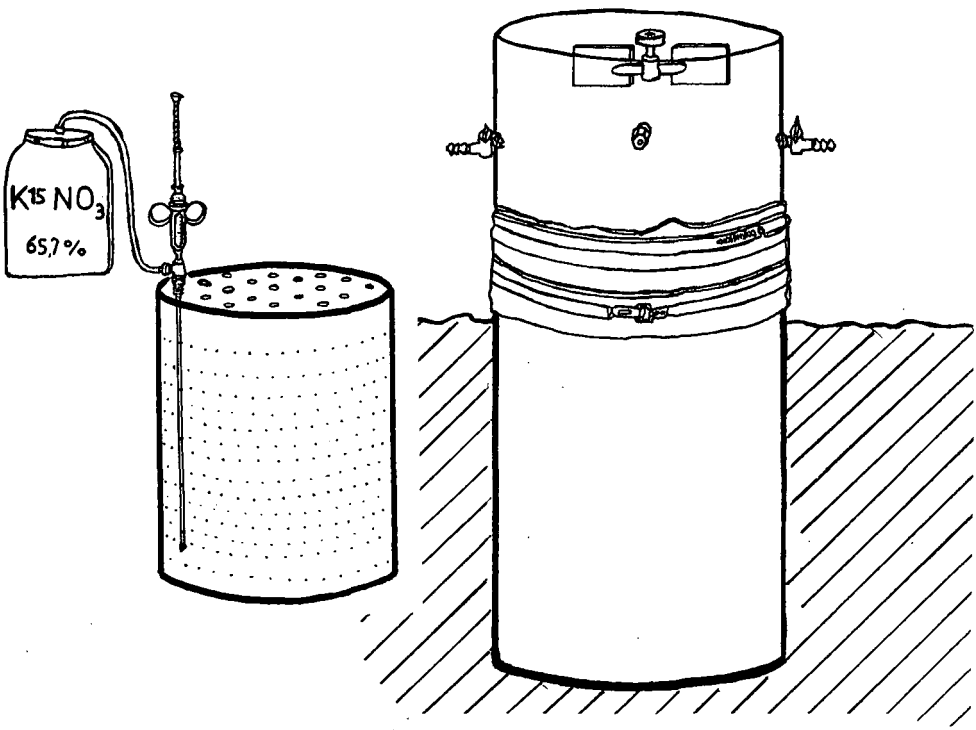

Fig.1 (left) and 2 (right) : Plastic cylinder driven into the soil down to the basis of plough layer about 30 cm deep.
Fig. 1 : State at the injection of ^{15}N .
Fig. 2 : Cylinder covered by translucent gas collection cover box with a propeller driven by rotating magnet , in- and outlets and a septum for syringe penetration.

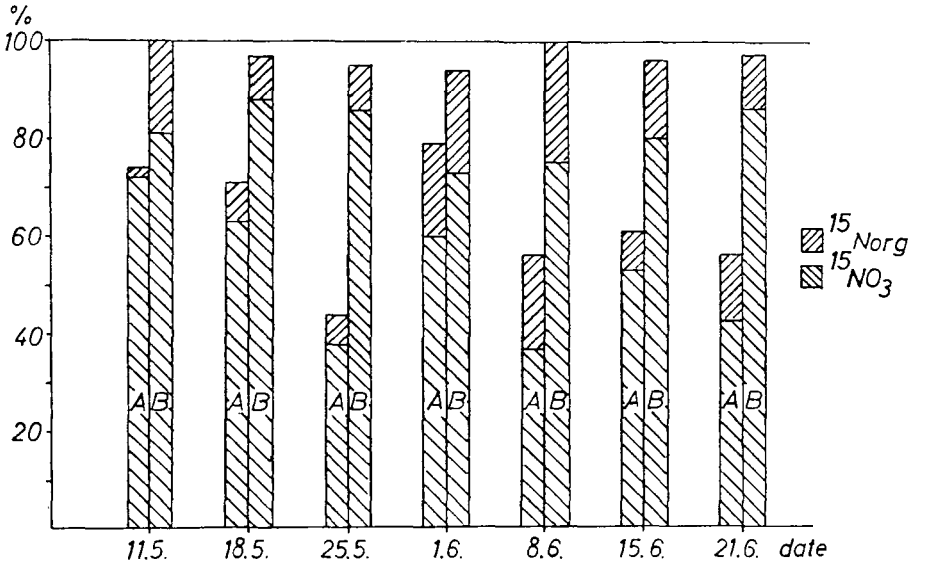

Fig. 3: Percentage of ^{15}N-recovery and nitrate-N redistribution at subsequent heptades. - Comparison between different sites A and B.

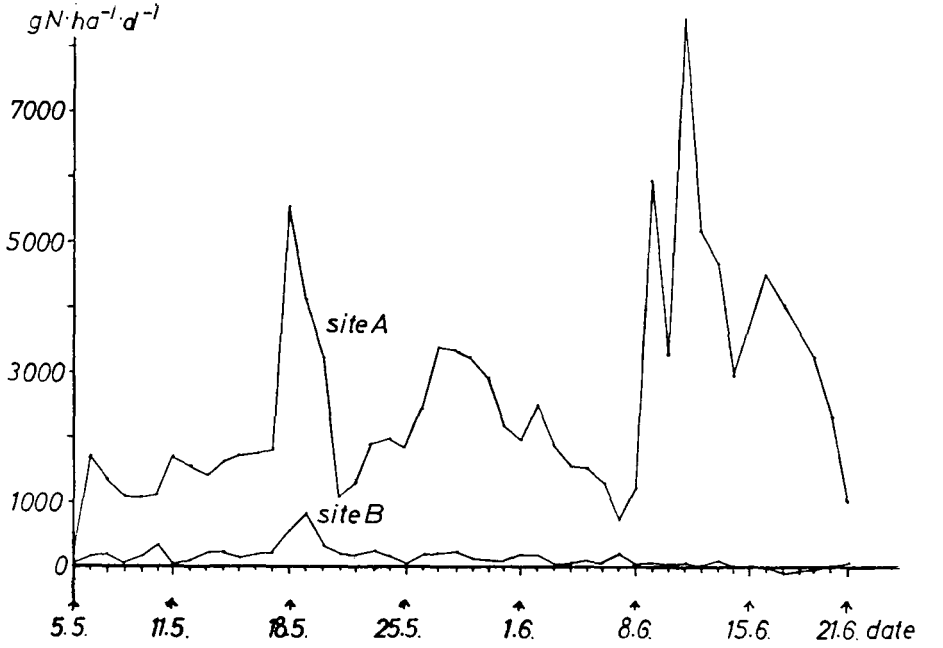

Fig. 4: Daily denitrification losses calculated by gas measurements. Comparison between site A (upper curve) and B (lower curve) Data on abszissa: Days of ^{15}N-Injection

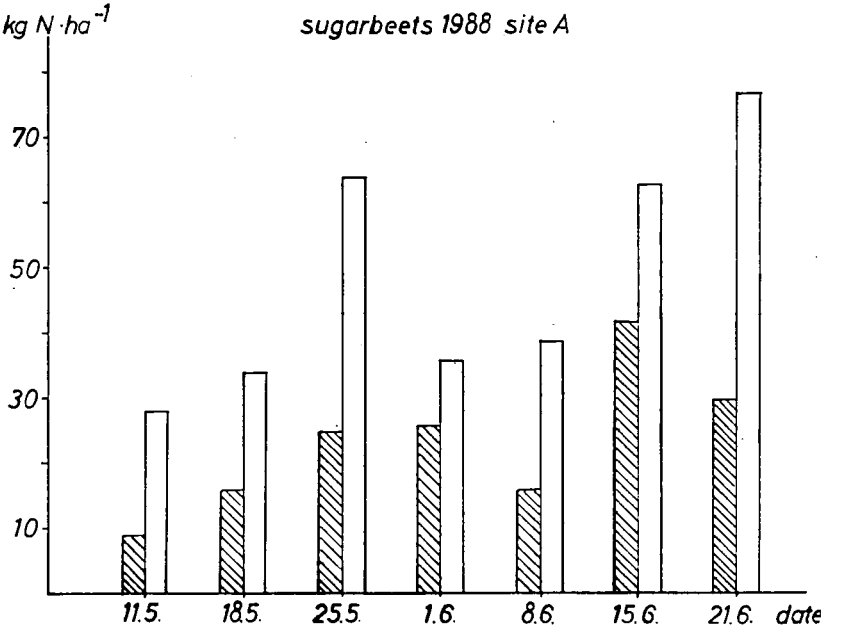

Fig 5: Denitrification losses by gas measurement (hatched columns) and by recovered ^{15}N (white colums) in the same soil cylinders in subsequent heptades

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SOURCE AND SINK MECHANISMS RELATED TO DENITRIFICATION MEASUREMENT

by

Chalamet A. *

Denitrification is characterized by two main features :

- The first one, denitrification represents a nitrogen output in the nitrogen cycle, at the end of a sequence of reactions. As a consequence the process intensity depends on the nitrogen and carbon fate through the system.

- The second one, denitrification is characterized by gas production : NO, N_2O, N_2. In soil, gas diffusion is an important transport mechanism and gas behaviour is related to gas properties in complex porous media.

Then, successively, sink mechanisms of N_2O, definition of denitrification sites and source and sink mechanisms of denitrification substrates will be envisaged.

Until now, the acetylen inhibition method, developped in the previous session, has been widely used for its simpleness, low cost and sensitivity (19, 25, 36, 38). One must be aware that other processes may be disturbed, such as nitrification (42).

Denitrification measurements are based on N_2O recovery in an enclosure at the soil surface. Generally, it's assumed that NO production is negligible. A question arises : does measured denitrification flux represent the actual denitrification ?

I - N_2O SINKS-

Actualy many mechanisms may act as N_2O sink (figure 1) :

N_2O may be reduced to N_2 by the N_2O reductase, fulfilling the last step of the denitrification reactions chain, if the acetylen blockage is not total (47). It is generally assumed that zero point one to one per cent of C_2H_2 in soil ensures a total blockage (38, 48). It has not yet been shown that such a rate is established in any denitrification site, within soil aggregates. Howewer, at a larger scale, in macroporosity, such a rate is established in one hour, in the top ten centimeters of soil (22).

Other sink mechanisms are abiotics :

- N_2O diffusion to the soil surface may be restricted which can limit quantitative N_2O recovery. The apparent diffusion of N_2O does not follow the often quoted diffusion models , such as PENMAN or MILLINGTON ones (22). Furthermore a downward diffusion of N_2O in deeper soil layers is often envisaged, but not estimated (29).

- N_2O water solubility is another sink. For example, N_2O concentration in a grassland soil solution varied from below ambient (that is about zero point three ppm) to thirteen times ambient (6). This N_2O pool can be calculated using the BUNSEN absorption coefficient (42) or it can be measured using an interfaced gas chromatograph-desorption apparatus (28).

- N_2O can be sorbed on dry soil clay (4). However N_2O clay sorption is negligible in denitrifying conditions compared to soil-water interactions (5).

Laboratoire de Microbiologie des Sols, URA CNRS 697 Ecologie Microbienne, Université C. Bernard Lyon I, 43, boulevard du 11 novembre 1918, F-69622 Villeurbanne Cedex, France.

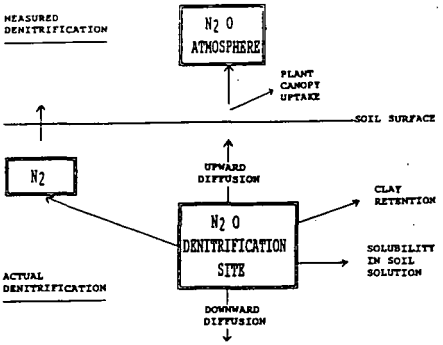

Figure 1 : Denitrification measurement by acetylene incubation method : N_2O sink mechanisms.

- The N_2O evolved at the soil surface can be taken by plants through foliar uptake. This path was shown in maize using N 15 (24). It was suspected in experiments with clover (27)and rice (29) This path has been rarely studied although it could be of importance as in the case of atmospheric ammonia. In the work by DENMEAD et al (8) ammonia was completely absorbed by a clover canopy . This process can restrict N_2O quantitative recovery in enclosures.

II - DEFINITION OF DENITRIFICATION SITES

In field studies, denitrifcation measurements were characterized by a high variability from hundred sixty per cent to five hundred, for example (10). This is connected to the problem of definition of denitrification sites in relation with substrates sources and O_2 concentration. These high variabilities could well be related to the concept of "hot spots" of denitrification advanced by PARKIN (32), that is : limited sites where decaying organic matter generates anaerobic microsites. Such a site could be represented for example by a plant debris-clay aggregate around a bacteria cluster (12).
This high variability could well be related too, to the variability of O_2 diffusion coefficients which is very large, according to environnmental conditions (table 1).

Table 1 : O_2 diffusion coefficients

D $cm^2.\ sec^{-1}$	Media
$1.8\ \ 10^{-1}$	Air
10^{-3}	Gas phase of soil
10^{-5}	Free water and mud
$8.5\ \ 10^{-6}$	Inter aggregates
10^{-7}	Sediments

The concept of anaerobic site setting up, based on the oxygen diffusion along the distance, applies to various systems :

- in flooded soils or sediments, nitrification occurs in the surface layer, denitrification in deeper layers (33).
- in planted soils, depending on root environnement two situations are encountered :
  - in flooded soil, nitrification and denitrification occur in a sequence and the root is an O_2 path as shown in the case of rice (33).
  - in aerated soil, the situation is reversed, the root acting as an O2 sink through respiration.

- In soil aggregates, O_2 concentration were measured, showing anaerobic sites varying with microaggreagtes size (41). In these individual aggregates, the denitrification measurement is correlated with the presence of anaerobic site . Oxygen is a major regulator of denitrification activity. A large part of aggregate is below one per cent O_2 which corresponds to a high denitrification rate as it was demonstrated by Lensi et al (26).

However, to be noticed in the work by SEXTONE (41) is the fact that not all aggregates with anaerobic site denitrified. This limitation can be due to :

- first, absence of denitrifiers inside the aggregates. Presence of denitrifiers inside aggregates has not yet been proved but bacteria can colonize two micrometer diameter pores inside a thirty five micrometer diameter microaggregate (18).

- second, absence of substrates, NO_3^- and /or carbon.

NO_3^- diffusion models proposed by MYROLD AND TIEDJE (31) revealed that aggregates could be nitrate diffusion limited but that carbon rather than nitrate was limiting in their experimental denitrification results.

Soil moisture may serve both : to increase anaerobic volume and to redistribute soluble carbon and nitrate. These observations show the importance of the water soluble organic carbon which can be a good index of available carbon related to denitrification measurements (23).

In denitrification studies, N_2O measurements must be completed by measurements of the main factors available carbon, nitrate concentration, O_2 concentration or water content which could explain the measured denitrification.

Then, the third question is relative to substrates availability.

III - SUBSTRATES AVAILABILITY

Figure 2 is an out line of the processes involved in the build-up of carbon and nitrate pools, processes which are coupled with O2 sinks, essentially, respiration and nitrification. I'll developp successively the two path-ways relative to carbon and nitrogen.

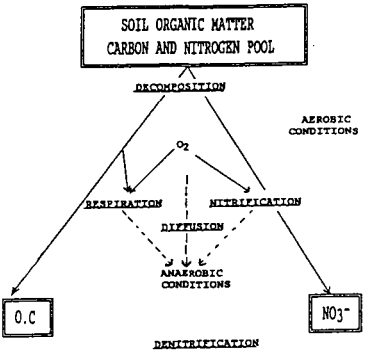

Figure 2 : Main processes involved in the build-up of carbon and nitrate pools and coupled with O_2 sink.

- Carbon availability

The carbon main roles (energetic source, electron deonnor, bacteria growth substrate) are presented in figure 3. Emphasize is given to the similitude of respiration in aerobic conditions and denitrification in anaerobic conditions with interaction between the two processes.

The soil organic carbon pool is large : in DAKOTA soil for example, twelve to sixteen kilogramme per cubic meter (1). Through out the literature the water soluble carbon amounts to only zero point seven to four per cent of the total organic carbon pool. Measurements of this carbon form, extracted by cold water, are realized by various methods : the technicon method (7), Walkley-Black method (17), anthrone method, the results corresponding to the glucose equivalent (23).

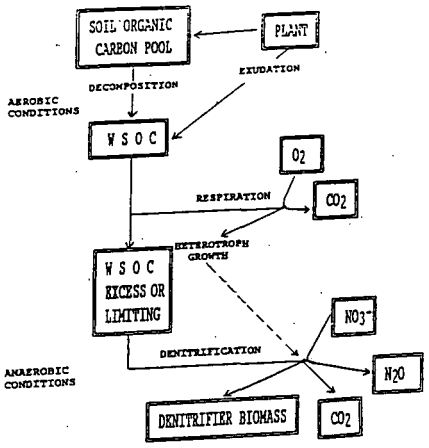

Figure 3 : Water soluble carbon path-way from organic carbon to denitrification.

Little is known on the nature of the carbon compounds supporting denitrification. The work by KATZ et al (17) showed that fulvic acids correspond to water soluble organic carbon mineralized during denitrification.
Works by TROLLDENIER's group suggest amino acids play a key role in rhizosphere (20, 44).

In aerobic conditions, carbon is consummed by respiration and simultaneous growth of heterotrophic bacteria including denitrifiers. The correlative O_2 consumption largely contributes to create anaerobic conditions in aggregates or in the rhizosphere.

A range of partial pressure O_2 values between six and thirteen hecto pascal, where aerobic respiration and denitrification occured simultaneously, is demonstrated by KROEKEL and STOLP (21).
So it appears clearly that denitrification rate is controlled by the rate at which carbon is made available to the denitrifiers.
The rate of denitrification, coupled with organic carbon mineralization in an anaerobic soil, was described as a combined first order process (33, 35) :

$$\frac{dN}{dt} = - k_N \; C \; N$$

C = carbon concentration ($\mu g\ C\ ml^{-1}$)
N = nitrate concentration ($\mu g\ N\ ml^{-1}$)
k_N= denitrification constant ($\mu g\ C\ ml\text{-}1$)

The coefficient k_N was essentially constant for nine mineral soils and it was used in a denitrification field simulation model by ROLSTON's group (14, 37).
In anaerobic conditions the carbon main role is source of available electron for denitrification. This availability concept explains in part DE CATANZARO and BEAUCHAMP results (7).

In that case the electron availability was around one millimole electron for sucrose, glucose and mannitol. It is not surprising to observe the same CO_2 production during denitrification measurement.
However CO_2 production observed with glycerol was not in agreement with the electron availability. The CO_2 production was only half, although the same quantity of N_2O was producted and the electron availability was higher (1,12 mmol e^-). During denitrification glycerol metabolism other than oxydation into CO_2 can be suspected.
The carbon partition between electron availability and denitrifier biosynthesis is illustrated by the work of HALLER and STOLP (16) in rhizosphere where seventy per cent of carbon exsuded is used to reduce NO_3^- and thirty per cent is used to build up denitrifier biomass. This electron availability represents eight per cent of photosynthates.

- Nitrate availability

The nitrogen way from organic pool to nitrate is summarized in the figure 4.
The soil organic nitrogen pool represents for example around one point five kilo gramme per cubic meter in Dakota soil (1). The inorganic nitrogen form is usually low, for example around ten ppm ammonium in grassland soil (45).
As a consequence denitrification rate strongly depends upon mineralisation-nitrifcation kinetics.
In well aerated soil nitrification is not a limiting factor and denitrification can occur vhen anaerobic conditions are established. For example after irrigation or rainfall, a pulse of N_2O production is observed (36, 40).

The NO_3^- production sequence mechanisms (figure 4) can be stopped at various steps due to inhibitory mechanisms or sink mechanisms. I want to point out that chemodenitrification of nitrite (2, 3, 30) is a by-pass as it leads to N_2O production measured as biological denitrification in enclosure..

The last step, nitrification generates two self-regulation mechanisms : decreasing of O_2 concentration, an oxygen sink and decreasing of pH.

Although only one O_2 originates from molecular oxygen during the nitrification (in 9) the O_2 consumption by nitrifiers, in limiting O_2 diffusion conditions, may well help creating anaerobic conditions (39). This process plus nitrate production through nitrification are leading to favourable denitrification condition thus thitghening the coupling of the two processes.

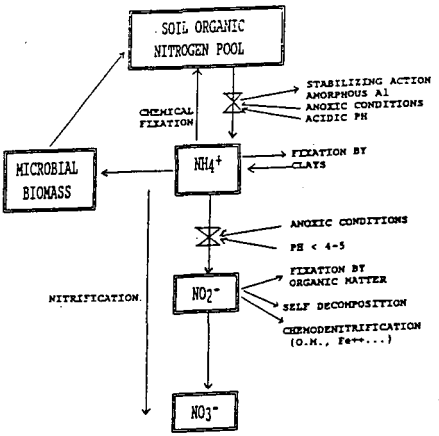

Figure 4 : Nitrogen path-way from organic nitrogen to nitrate pool.

What are the other sinks for nitrate other than denitrification ? There are summarized in figure 5.

Plant uptake is an important sink for nitrate. The complex role of plant will be discussed in an other session.
The role of carbon depending on aeration conditions can explain the observed denitrification measurement.
In aerobic conditions at the begining of incubation, nitrogen immobilization revealed to be a competitive process for denitrification when material is added. For example, straw or cellulose enhanced nitrate immobilization and decreased denitrification (13). Carbon is used essentially to heterotrophic bacteria growth and nitrate is assimilated.
In recent anaerobic conditions and moderate carbon availability, denitrification is the prevailing process. About seventy percent of carbon is used as electron donnor to nitrate reduction and thirty percent to denitrifiers biosynthesis (16).

Dissimilatory nitrate reduction to ammonium is well suited to permanent anaerobic environnement (42).

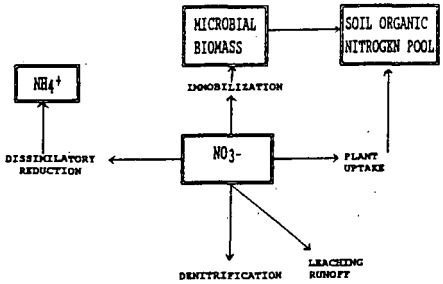

Figure 5 : Nitrate sinks in soils.

In soil, the environment conditions seem to be more favourable to denitrification than dissimilatory reduction, which represents only one per cent, except when the ratio available carbon - to - electron acceptor is higher (43). For example when a carbon source is added to soil, the essential role of this carbon seem to be electron donnor.

CONCLUSION

What are the limiting knowledges to the improvement of denitrification measurements ? There are essentially :

- N_2O foliar absorption and N_2O downward diffusion for methodological aspects.
- nature and production kinetic of carbon compounds involved in the process.

The present issue for denitrification measurements is the choice of a measurement scale.
On the one hand, at the microsite scale, the oxygen measurement is realistic. But NO_3^- and carbon concentration measurements haven't been yet achieved : this is a challenge to be taken up.
Moreover in the determination of oxygen threshold for denitrfication, TIEDJE (42) selected data in which oxygen concentration near the denitrifier cell surface could be assured. This is possible in liquid media, but such a possibility is precluded in soil.

On the other hand, at the field scale, what method could take these "hot spots" of denitrification into account? Are geostatistics methods used by ROLSTON's group (11, 15) feasible and realistic at smaller scales (46) ? What is the right spacing between sampling sites : few centimeters or few meters ? Understanding of soil biological properties spatial variability is another challenge to take up.

A new strategy of denitrification measurement is perhaps necessary.

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Investigations on Adsorption and Diffusion of Nitrous Oxide in Soil Matrices

D. Stegemann, H.K. Cammenga*

Special Collaborative Program 179
Project C4

Abstract

Investigations on the adsorption behaviour of N_2O at room temperature were made at soil samples with a wide range of clay content. It was found that oven dried soil is a strong sorbing medium for N_2O. The sorbed mass of gas is due to the clay content. If the soil samples are air-dry (watercontent 1-3%) adsorption can no longer be observed, taking into account that the error of the method lies between 8 and 10%.

Simultaneous measurements of the diffusion coefficient of N_2O were carried out by a method using equations, which take into consideration the adsorption and solubility of the gas in soil. The results for the soil samples lie within the data predicted by empirical relations. The MILLINGTON equation tends to overestimate the N_2O diffusion coefficient in dry soil. When comparing the different results by the ratio D_a/D_o it must be taken into account that the value for D_o varies from 0.121 to 0.162 cm^2/s depending on its kind of determination.

Introduction

Some phenomena related to denitrification measurements lead to the assumption, that there might exist a sink mechanism when N_2O is transported from

*Institut für Physikalische und Theoretische Chemie der TU, Hans-Sommer-Strasse.10, 3300 Braunschweig, F.R.G.

the soil to the surface. The often observed differences between the N_2O concentrations in the soil air and the measured fluxes from the soil surface [1] could as well be explained by physical adsorption of N_2O at the soil matrix as by uptake of N_2O by soils involving its reduction to N_2 by microbial processes [2,3]. Furthermore extremly low diffusion coefficients should be discussed when concerning with the problem mentioned above. Physical adsorption might also be responsible for the effect of spontaneous release of N_2O when dry surface soil, often found at warm and dry summer days, is wetted.

Investigations have therfore been made on the (physical) adsorption behaviour of N_2O at dry and air-dry soils and at very low partial pressures of N_2O as they are often found under natural conditions. The method used to investigate the adsorption behaviour is very similar to the process of N_2O release and diffusion in the field.

To control the transport behaviour, the diffusion coefficients were measured simultanously by using the same vessel.

Materials and Methods

Surface soil samples (0-30 cm) of clay content between 8 and 30 % were taken from an experimental field at *NEUENKIRCHEN* (Lower Saxony, FRG). To avoid N_2O-production during the measurements, the soil was steam-sterilized. All experiments were carried out at packed soil samples with a particle size not greater than 0.5 cm in order to get a maximum of surface and better reproducible properties of the material.

The adsorption isotherms were obtained by adding different amounts of N_2O $(5 - 50\mu l)$ into a closed vessel (fig.1) of known volume, containing the packed soil sample. After 3 h, when the equilibrium is reached, the N_2O concentration within the vessel is determined by gas chromatography and compared with the value, which is expected if no adsorption would occur.

The diffusion coefficients were measured by using the same closed vessel shown in fig.1. The method is based on the stepwise iteration of a simulated concentration-time curve to the n measured points $C_i^m(t)$ by varying the soil diffusion coefficient D_s in equations 1-3 until

$$\frac{1}{n}\sum_{i=1}^{n}(C_i^m - C_i^c)^2 = minimum$$

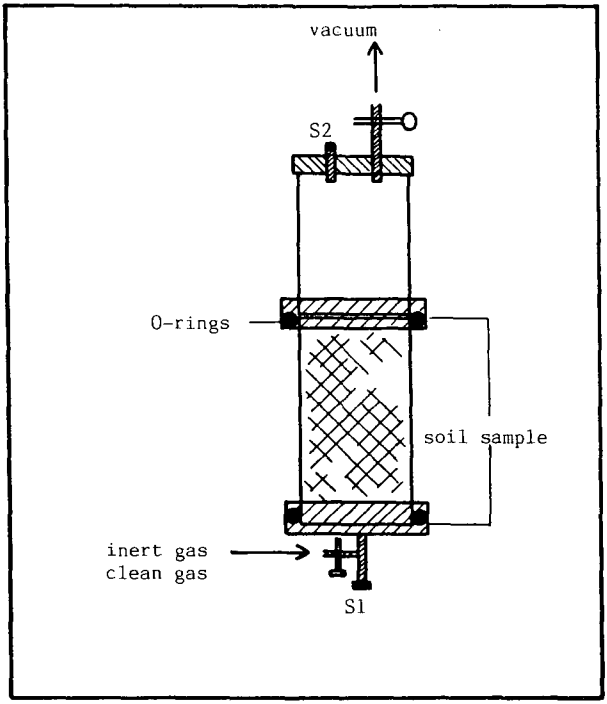

Figure 1: Vessel used for the determination of the adsorption- isotherms and diffusion coefficients

where C_i^c are the computed concentrations at time t. The equations used for the determination of C_i^c are:

- for dry, non-sorbing porous media

$$\epsilon \frac{\partial C}{\partial t} = \frac{\partial}{\partial z}\left(\epsilon D_s \frac{\partial C}{\partial z}\right) \tag{1}$$

- for dry, sorbing porous media using a linear adsorption-isotherm $S = kC$

$$\epsilon \frac{\partial C}{\partial t} + \rho \frac{\partial S}{\partial z} = \frac{\partial}{\partial z}\left(\epsilon D_s \frac{\partial C}{\partial z}\right) \tag{2}$$

- for wet porous media, where the solubility of the gas in soil water must be taken into consideration

$$H_s^i \frac{\partial P}{\partial t} = \frac{\partial}{\partial z}\left(H_s^i D_s \frac{\partial P}{\partial z}\right) \tag{3}$$

$$H_s^i = H_g^i \epsilon + H_l^i \theta$$

$C_i^m(t)$ is obtained by analyzing the headspace N_2O concentration (through $S2$) in the vessel at different times t, after the known volume of N_2O was added through $S1$ and before the equilibrium is reached [4].

Results and Discussion

Table 1 summarizes some results for two samples P4 and P5. For oven dry soil it can be seen that N_2O is strongly sorbed by the soil matrix. The calculations indicate further that the adsorption isotherms are linear in the investigated concentration range (fig.2) and the adsorbed volume of gas is due to the clay content (fig.3). Similar results have been obtained by *RUNKLES et al.* [5] for oxygen and by *CHALAMET* [6] for N_2O, who used a gaschromatographic technique. A possible explanation may be given by an activation of the clay minerals within the soil, when surface bound water is removed by heating. This assumption is supported by the linear dependence of the adsorbed N_2O volume on the clay content and the non sorbing behaviour of quartzsand under the same conditions.

When the soil is air-dry, most of the active places are occupied with water molecules and therefore no adsorption can be observed[1] as Tab.1 shows.

21°C						
air dry soil samples						
sample	clay %	moisture %	C^c	C^m	C^c	C^m
sand	-	-	11.35	11.3 ± 0.9	2.82	2.8 ± 0.6
P4	29.9	2.1	25.47	25.3 ± 1.5	2.77	2.5 ± 0.6
P5	27.3	1.8	11.35	11.3 ± 0.9	2.82	2.8 ± 0.6
oven dry soil samples						
sand	-	-	11.10	11.2 ± 1	2.70	2.5 ± 0.6
P4	29.9	-	24.99	14.5 ± 1.2	4.86	2.7 ± 0.6
P5	27.3	-	24.50	18.5 ± 1.2	4.76	3.7 ± 0.8

Table 1: Comparison between measured C^m and calculated C^c N_2O-concentrations in the closed vessel after equilibrium was reached. C in $ppmv/v$.

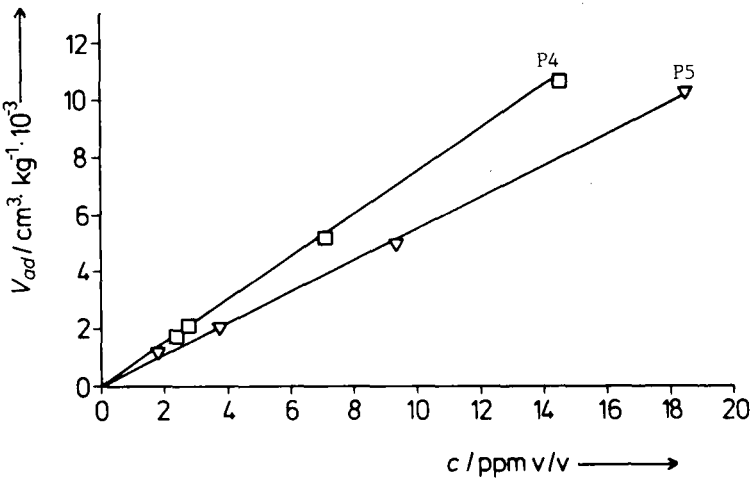

Figure 2: Adsorbed volume of N_2O as a function of the equilibrium partial pressure for oven dry soils

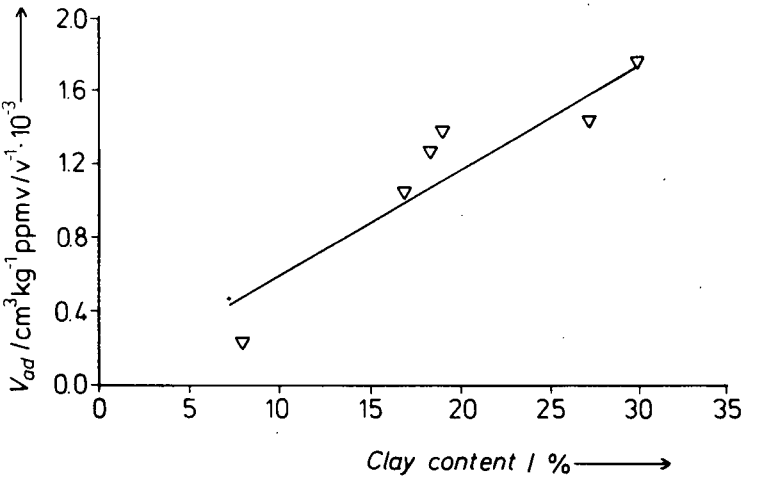

Figure 3: Adsorbed N_2O volume as a function of the clay content of oven dry soil.

21°C							
ε	this work			FLÜHLER	CURRIE	PENMAN	MILLINGTON
	$\frac{D_a}{D_o^1}$	$\frac{D_a}{D_o^2}$	$\frac{D_a}{D_o^3}$	[8]	[7]	[9]	[10]
quartzsand							
0.4	0.39	0.30	0.22	0.20	0.25	0.26	0.29
0.35	0.26	0.23	0.19	–	0.21	0.23	0.24
sample P5 air dry							
0.51	0.39	0.31	0.23	0.22 (2)	0.36	0.33	0.40
sample P4 air dry							
0.52	0.34	0.27	0.20	0.21 (2)	0.37	0.34	0.41

Table 2: Comparison between measured and predicted data for the diffusion coefficient of N_2O in dry soil samples.
D_o^1= 0.121 [$\frac{cm^2}{s}$] [11]; with data given by GILLILAND [12]
D_o^2= 0.136 [$\frac{cm^2}{s}$]; [13]
D_o^3= 0.162 [$\frac{cm^2}{s}$]; [14]

The results for the diffusion coefficients are given in table 2. The problem when comparing the gas-independent ratio D_a/D_o for N_2O is indicated in the columns 2-4. Depending on the diffusion coefficient used for N_2O in free air, which has been obtained by different methods, the given ratio varies up to 65%.

In spite of this deviation one can conclude that the measured data for dry soil lie within the distribution of the predicted values given by the empirical relations of CURRIE and PENMAN, and the experimental data from FLÜHLER in clayey sand with similar porosity and moisture content. [2]

The MILLINGTON relation, derived from consideration of the planar distribution of spherical pores and the interaction of two adjacent planes, tends to overestimate the N_2O diffusion coefficient in packed soil samples, whereas the data for quartzsand are closer to the calculated values. The assumption of spherical pores may have better been realized in quartzsand because of a narrower particle size distribution and a homogenous particle shape.

List of Symbols

Symbol	Unit	Meaning
C_i^m	mol/cm^3	measured concentration
C_i^c	mol/cm^3	calculated concentration
D_a	cm^2/s	apparent diffusion coefficient
D_s	cm^2/s	effective diffusion coefficient
D_o	cm^2/s	diffusion coefficient in air
H_g^i	mol/cm^3Pa	HENRY coefficient in the gas phase of gas i
H_l^i	mol/cm^3Pa	HENRY coefficient in the liquid phase of gas i
P	Pa	partial pressure of gas i in the soil atmosphere
S	cm^3/kg	Sorbed volume gas per kg soil
t	s	time
z	cm	vertical length of the soil column

[1] Further measurements, carried out by another method with a much higher resolution, indicate that, assuming *HENRY's* law to be valid for soil bound water, the amount of adsorbed N_2O lies between 5-7% of the added volume. These are preliminary results and will be discussed in a later paper dealing with the N_2O-solubility in soil water.

[2] Natural soil samples WINZELERBODEN from B-horizon (clayey sand);70-80 cm depth.

ε	—	air filled porosity
θ	—	water filled porosity
ρ	g/cm^3	bulk density of the soil sample

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Denitrification with dry- and wet-sieved soil aggregates

by

E.G. Beauchamp and A.G. Seech[+)]

Denitrification rate has been shown to decrease with increasing soil aggregate size (1). Thus spatial variability of denitrification in the field is related to the distribution of aggregates of different sizes. Seech and Beauchamp (1) also showed that the aggregate size effect was related to substrate C availability. The study by Seech and Beauchamp was done with dry-sieved aggregates from undried (field-moist) soil from the field. A study by Elliott (2) in which wet-sieved aggregates were used, suggested that substrate C availability increased with increasing aggregate size. This study was done to determine the effect of dry- and wet-sieving on denitrification in different size aggregates.

Materials and Methods

Soil samples were obtained from 3 sites (replicates) 10 m apart on Conestogo silt loam (Typic Hapludalf) near Guelph on October 30, 1988, following maize harvest. Soil was removed from the upper 15 cm at each site ($1m^2$) with a spade and stored at 4°C until sieving operations and incubations were done.

Dry-sieving was accomplished by gently shaking field moist soil (130 g H_2O kg^{-1} OD soil) through a nest of sieves (20, 10, 5, 2, 1, 0.5, 0.25, 0.15 and 0.05 mm diameter holes). Water-sieving was accomplished with a Cenco water sieving apparatus using a nest of sieves (4.7, 2, 1, 0.5, 0.25 and 0.1

[+)] Department of Land Resource Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

mm diam. holes) with a 30 min shaking time (4). Water-sieved aggregates were either used without further treatment or oven dried (105°C, 30 min). Elliott (2) dried water-sieved aggregates at 60°C whereas Patton et al. (3) showed that oven drying increased denitrification rate.

Aggregates (20 g OD soil) were incubated in 125 mL erlenmeyer flasks following addition of 600 g H_2O kg^{-1} and 150 mg NO_3^--N kg^{-1}. Nitrous oxide emission was monitored, with C_2H_2 (10% v/v) in in the headspace (5), at 72, 144 and 216 h. Mineralizable C was determined by CO_2 production under aerobic conditions. Soluble C was determined by autoanalyzer following extraction with 0.5 M K_2SO_4 solution. Soil particle size determination was done on water-sieved aggregates (6).

Results

There was a tendency for N_2O production to decrease with increasing size of dry-sieved aggregates (Fig. 1) in agreement with a previous study (1). Nitrous oxide production in water-sieved aggregates generally increased with increasing aggregate size (Fig. 2) in marked contrast to dry-sieved aggregates. Oven drying of the water-sieved aggregates enhanced N_2O production but the same pattern occurred as with undried aggregate sizes (Fig. 2).

In contrast to previous findings (1), neither mineralizable C nor extractable C provided any explanation for differences in N_2O production amongst dry- or water-sieved aggregates of different sizes.

It was evident during the water-sieving operation that some aggregate disintegration occurred. About 43 per cent of the soil was "lost" during the water-sieving process. There was generally no difference in particle size composition in aggregates of different sizes except for the 0.25-0.5 mm size fraction in which greater sand but less silt occurred as a result of

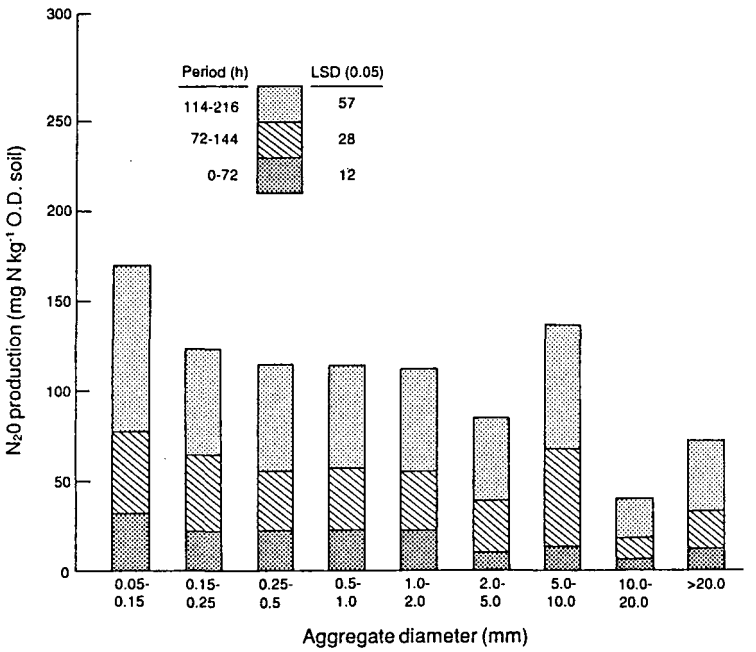

Figure 1: Cumulative nitrous oxide production by dry-sieved soil aggregates of different diameters at 72, 144 and 216 h. LSD (0.05) values pertain to cumulated values at each time.

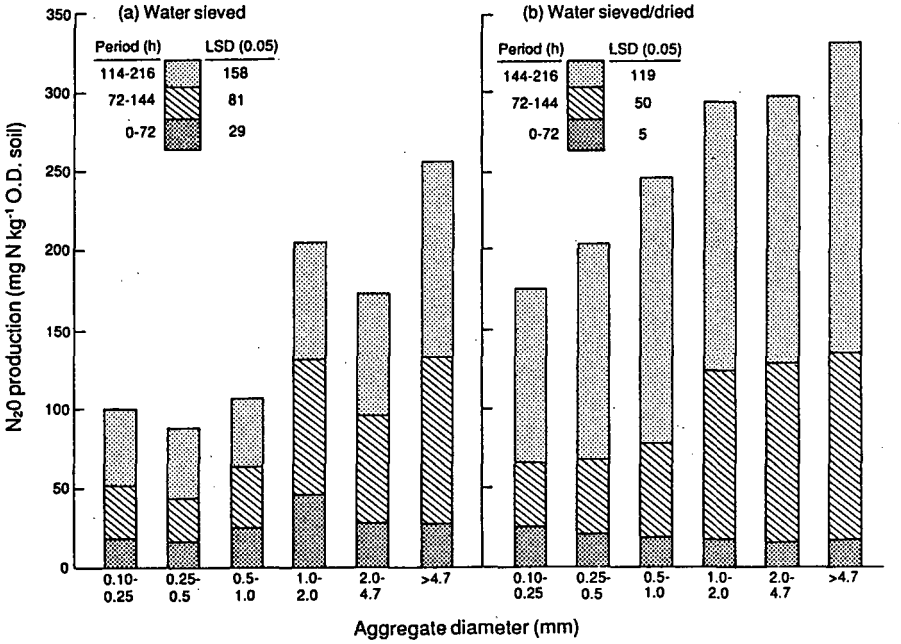

Figure 2: Cumulative nitrous oxide production by wet-sieved soil aggregates of different diameters (a) without or (b) with drying at 105°C for 30 min. at 72, 144, and 216 h. LSD (0.05) values pertain to cumulated values at each time.

water sieving. The lowest N_2O production occurred with this fraction of the water-sieved aggregates reflecting the higher sand content (Fig. 1,a). Dormaar (7) observed that the hydrolysed monosaccharide content of water-stable aggregates increased with size. It is proposed that microbial polysaccharide content increased with water-sieved aggregate size in this study and that this is associated with greater denitrification.

Although Parkin (8) demonstrated the occurrence of "hot spots" in soil in association with plant residue fragments, it appears that there may also be "warm" spots in association with water stable aggregates depending on size providing NO_3^- is present and suitable conditions for denitrification prevail. This research has shown a contrasting relationship between aggregate size and denitrification depending on dry- or wet-sieving preparation procedures.

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Effect of soil temperature on the ratio between N_2 and N_2O produced during the denitrification process.

by

Vinther, F. P.*

Introduction.

In some methods the total denitrification is estimated by multiplying field emission of N_2O with the N_2/N_2O-ratio, measured in the laboratory (Christensen, 1985). In periods of the year, where the soil temperature is low, e.g. early spring, it is important to know the relationship between the temperature and the ratio between N_2 and N_2O produced during the denitrification process, in order to get the best estimate of the total denitrification.

A number of different factors is known to affect the N_2/N_2O-ratio, either directly or indirectly (Focht, 1974; Blackmer and Bremner, 1978 and 1979; Bremner and Blackmer, 1979; Keeney et al., 1979; Breitenbeck et al., 1980; Vinther, 1984; a.o.). Focht(1974) found that the percentage of N_2O with relation to N_2 not was greatly affected by temperature changes. Aeration and pH was the two factors causing the greatest variability in N_2O production. On the other hand Keeney et al.(1979) clearly found an effect of temperature on the N_2/N_2O-ratio. Of other factors which have been found to affect the N_2/N_2O-ratio can be mentioned

* Dept. of Soil Biology and Chemistry, State Laboratory for Soil and Crop Research, Lottenborgvej 24, DK-2800 Lyngby, Denmark.

nitrate concentration, which have a direct effect, and plant growth, which have an indirect effect by consuming oxygen and water.

The purpose of the present investigation was to evaluate the effect of temperature in combination with soil moisture content on the N_2/N_2O-ratio.

Materials and methods.

A sandy loam soil (pH(H_2O) 6.2, 0.12 % total N, 1.4 % org. C) was collected in the month of April from an unfertilized field. At the time of sampling the soil contained 8 ppm NO_3-N and 4 ppm NH_4-N. The soil was sieved through a 5 mm sieve, and stored at 2 ^oC until use.

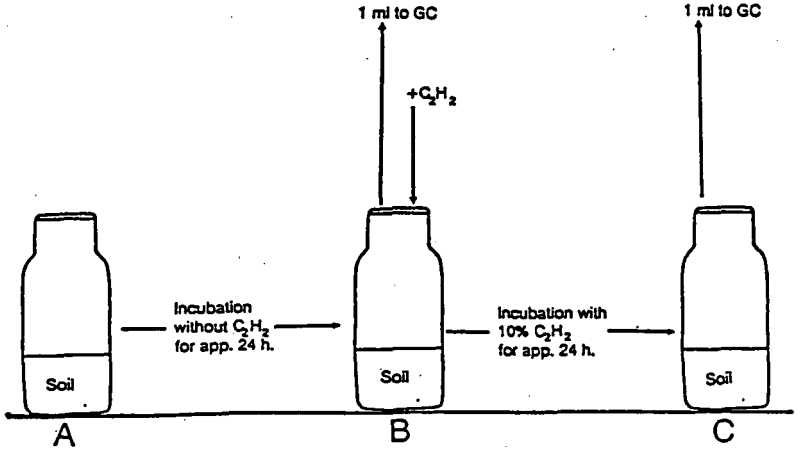

Fig. 1. Diagram showing how the measurements were carried out. GC = gaschromatograph.

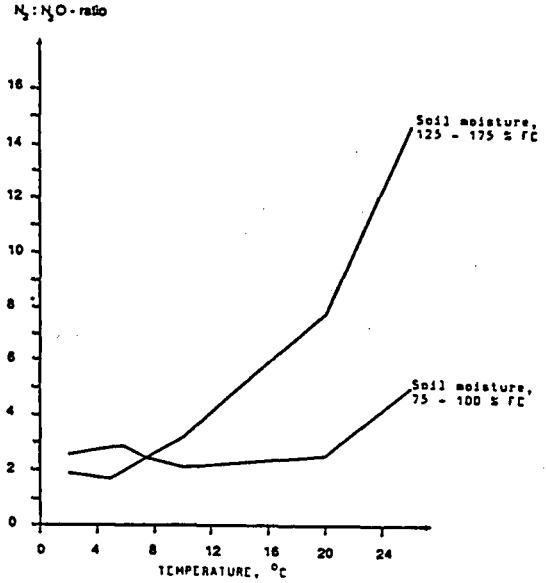

Fig. 2. Effect of temperature on the N_2/N_2O-ratio.

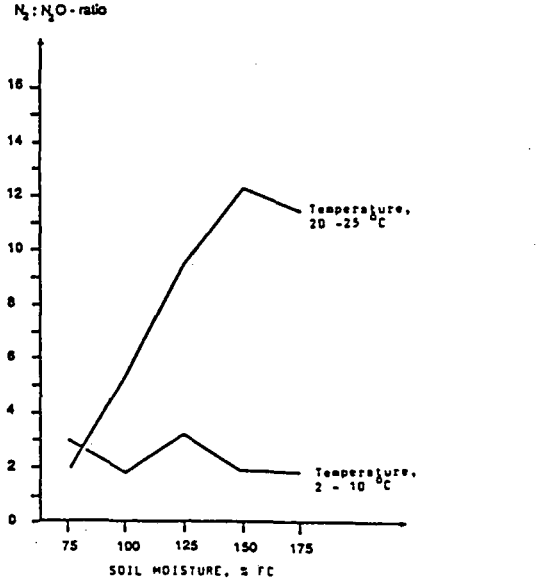

Fig. 3. Effect of soil moisture on the N_2/N_2O-ratio.

Experiments were made at 75, 100, 125, 150 and 175 % field capacity (FC). In this soil 100 % FC correspond to a soil moisture content of app. 25 % (Hansen, 1976). The soil was incubated at 2, 5, 10, 20 and 25 °C respectively. Each combination of soil moisture and temperature was measured with 10 replicates.

The soil (40 g dw) was placed in 100 ml serum bottles, water was added to get the wanted soil moisture content, and the bottles were incubated without acetylene for 24 h before the first gas analysis was made. After the gas analysis, 10 vol. % acetylene was injected, and the bottles were incubated for another 24 h before the second analysis was made. Assuming that the ambient concentration of N_2O was 0.3 ppm (A), the N_2/N_2O-ratio was calculated as follows (letters are refering to Fig. 1):

$$N_2/N_2O\text{-ratio} = \frac{(N_2O(C)-N_2O(B)) - (N_2O(B)-N_2O(A))}{(N_2O(B)-N_2O(A))}$$

Results.

Table 1. The N_2/N_2O-ratio at different combinations of temperature and soil moisture. Numbers in brackets represent the variation coefficient (standard variation * 100/mean).

Soil moisture, % FC	Temperature, °C 2	5	10	20	25
75	3.5(254)	4.2(216)	1.4(121)	1.3(138)	2.7(63)
100	1.6(213)	1.3(192)	2.6(238)	3.8(184)	7.2(26)
125	2.9(197)	2.5(192)	3.9(133)	7.2(94)	11.7(90)
150	1.6(275)	1.1(500)	3.1(281)	10.7(117)	14.2(111)
175	1.5(85)	1.0(175)	2.6(138)	5.8(31)	16.9(63)

Conclusions.

Based on the results shown above, the following conclusions is drawn:

- at low soil moisture contents (< 100 % FC), the ratio between N_2 and N_2O was not affected significantly by changes in the temperature in the range from 2 to 25 ^{o}C.
- at higher soil moisture contents (> 100 % FC), the ratio between N_2 and N_2O increased with increasing temperatures.
- at low temperatures (2 - 10 ^{o}C), the ratio between N_2 and N_2O did not seem to be affected by soil moisture contents.
- at higher temperatures (20 - 25 ^{o}C) the N_2/N_2O-ratio increased with increasing soil moisture contents.

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WARNING ! ! !

If you want to get statistical significant differences between treatments, or want to avoid the words "seems to" or "tendency to", then choose another field of interest than denitrification.

Finn P. Vinther, 1989.

Mitteilungen der Deutschen Bodenkundlichen Gesellschaft 60, 95-100 (1990)

Effects of plants on denitrification

Søren Christensen*

The effects of plants on denitrification can be analyzed by looking at soil NO_3^-, organic C and anaerobity as influenced by the presence of plants (Figure 1).

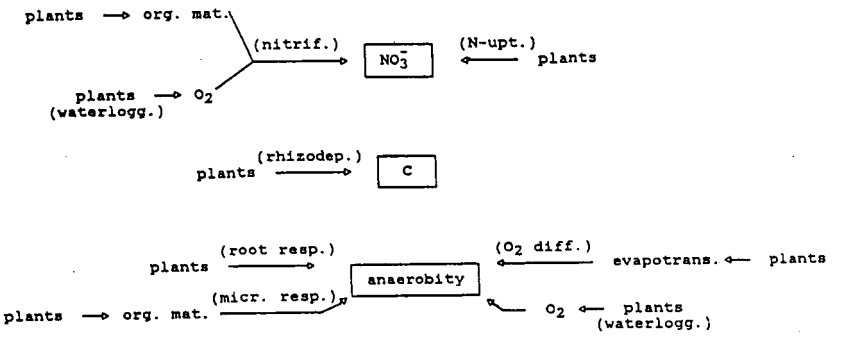

Figure 1. Theoretical examination of mechanisms whereby plants influence the primary determinants of soil denitrification. Plant effects increasing denitrification to the left, negative effects to the right.

Starting with nitrate, the direct effect is through NO_3^- uptake whereby it is made unavailable to denitrification. An indirect effect could be supply of organic matter of root origin; mineralization and nitrification of this substance can mean more NO_3^- for denitrification. Another indirect effect is supply of O_2 to waterlogged root systems promoting NO_3^- formation via nitrification. Moving to carbon, the main mechanism is provision of C substrate by rhizodeposition. Presumably, however, it is only the soluble fraction (5-10 % of the total rhizodeposition) that can be directly utilized by denitrify-

*) Institute of Population Biology, Copenhagen Univ., Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark.

ing bacteria. These organisms are mostly unable to decompose the complex carbohydrates in the root cells. Finishing with anaerobity, the utilization of O_2 for plant respiration is probably of minor importance, since root respiration is less intense compared to microbial respiration. Microbial degradation of root derived organic matter is presumably more important in reducing the O_2 tension in the rhizosphere. The opposite effect can be caused by plants increasing evapotranspiration and soil gas exchange, thereby increasing soil oxygen. The inhibitory effect of roots supplying O_2 to the rhizosphere of waterlogged soils is suggested to be of minor importance compared to the positive effect of this extra O_2, stimulating nitrification and aerobic decomposition of complex organic molecules in this environment.

The most important plant effects on denitrification in arable soils are: Negative; plants decreasing soil water content and/or removing nitrate from the soil solution, Positive; rhizodeposition giving organic carbon to the rhizosphere microbes thereby decreasing the oxygen tension during decomposition.

Extreme heterogeneity of the plant soil system.

Many of the abovementioned mechanisms operate simultaneously in the rhizosphere and some factors show substantial interaction. Regarding the plant soil system as a black box will therefore often give contradictory results. More understanding of the mechanisms involved can probably be gained by addressing the spatial heterogeneity of the plant-soil system.

Microbial density and activity normally increase by more than an order of magnitude when moving from the bulk soil through the rhizosphere towards the root surface. The composition of the microbial population also show dramatic changes over these mm of soil. Moreover, decomposition of recalcitrant soil organic matter is often increased in the rhizosphere. Root age also influ-

ence quantity and quality of rhizodeposition which adds a temporal component to the heterogeneity. Cumulating evidence points to that microfauna grazing on soil microbes is greatly increased in the rhizosphere. It is evident that denitrifying bacteria as members of the rhizosphere community will be affected dramatically in this environment.

Investigations described below use two different approaches to describe the heterogenous occurrence of denitrification in the plant-soil system: (i) Mapping of denitrification hot spots in the field and (ii) controlling simulated microniches in the laboratory.

Mapping of plant-induced denitrification hot spots.

A sandy loam soil of pH 3.8 at Michigan State University Farms only produced N_2O when denitrifying. Denitrification was determined as N_2O flux from 30 permanent points in a 5 by 6 grid with 1 m spacing (Christensen et.al. 1). Measurements were made at conditions of no NO_3^- limitation and with soil water contents at field capacity or above.

Figure 2a shows that mean denitrification in the area increased during a week following 1-2 days of heavy thunderstorms (130 mm rain). The distribution of the replicates during this event (Figure 2b) revealed that four points in the field were responsible for much of the increase in the mean rates and that these hot spots had been active for several days. Following the heavy rain, plants started to decompose in three spots in the field. These three spots were all among the four denitrification hot spot cores. In two of the four hot spot cores, the whole denitrification activity could be traced to occur within 4-8 % of the core volume when transferred to the laboratory and segmented (Christensen et.al. 1).

Trying to follow the events occurring during the formation of denitrification hot spots will be easier if

the timing is controlled, i.e. experiments with artificial hot spots could be advantageous.

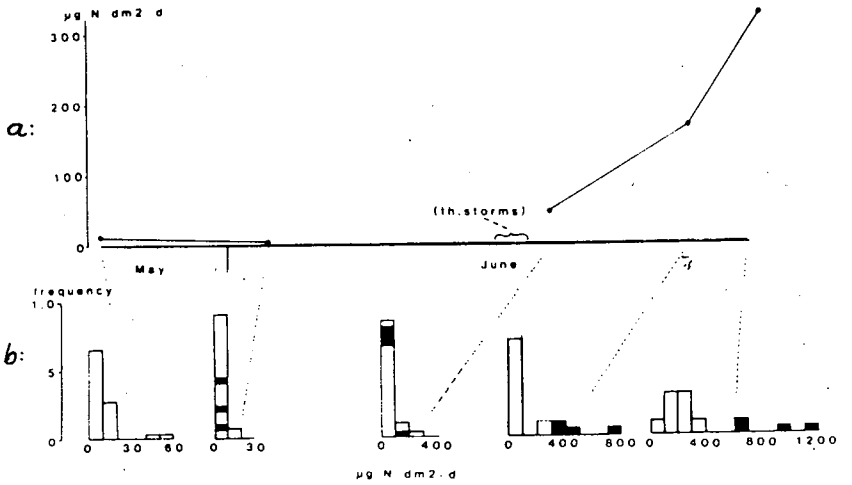

Figure 2. Denitrification as affected by heavy thunderstorms. a: Mean rates of N_2O production. b: frequency plots of the mean rates in a:. The occations marked black are the cores with highest rates at the last measurement. These are shown at the relevant position in the frequency columns at the preceeding days. (Christensen et.al. 1).

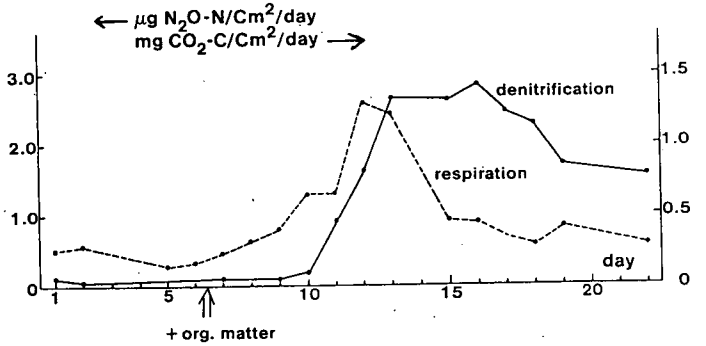

Figure 3. Respiration and denitrification in a soil core in the laboratory following injection of organic matter, (Christensen et.al. 2).

Figure 3 shows the changes in respiration and denitrification occurring in one soil core with the same soil as above following injection of 9 ml dead bacterial cells to a depth of 4 cm (Christensen et. al. 2). The increase in respiration occurs a few days before a rise in denitrification. Adding the same carbon source to anaerobic soil resulted in an immediate increase in denitrification. The native denitrifying enzymes in this soil were thus able to respond to the addition given the right environment. The lag observed in the cores with atmospheric air (Figure 3) must therefore be due to the time required to create an anaerobic zone at the point of injection of organic matter.

Controlling the microniches

To get further information on limiting factors for denitrification in the hot spots it will not be sufficient to measure limiting factors in these locations. We have

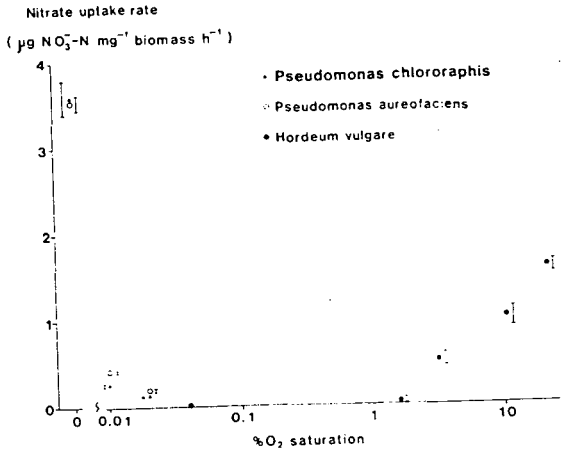

Figure 4. Plant uptake and denitrification at different O_2 saturations in liquid culture. Bacteria are kept without growth (chloramphenicol added), (Christensen and Tiedje 1988).

to make experiments where these factors can be controlled. To determine the influence of O_2 on denitrification and plant uptake of NO_3^-, a chemostat jar with strict control of O_2 saturation and -homogeniety was used. With Barley and two denitrifying bacteria lacking N_2O reductase as test organisms, an O_2 interval of almost two orders of magnitude existed where neither plant-, nor denitrifyer NO_3^- uptake occurred (Figure 4). Therefore, plants and denitrifying bacteria will not take up NO_3^- simultaneoulsy in the single microniche.

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Direct and Indirect Effects of Plants on Denitrification

Haider, K. [+] O. Heinemeyer [+] und A. R. Mosier [++]

INTRODUCTION

For denitrification to occur in soil the microorganisms involved need anaerobic conditions, nitrate and suitable carbon sources. These suitable carbon sources consist of aliphatic or sugar components, that are normally available only at low concentrations in soils (Burford & Bremner, 1975; Stanford et al., 1975). Additional easily available carbon compounds are excreted by plant roots and consist of aliphatic acids, sugars, low molecular weight polysaccharides and amino compounds (Warembourg & Morall, 1978).

In addition to excretion high rhizodeposits are reported (Martin & Puckridge, 1982; Lynch, 1983), attributable to sloughed off root hair and other root debris. Generally more photosynthetates are transported to the roots than can be found in the root biomass at harvest. Ratios of 1 to 2 for root carbon to total carbon flux into the roots were reported by Sauerbeck (1983) and Martin & Puckridge (1982). But depending on plant species or age sometimes 3 to 4 times more carbon can be released from the rhizosphere than found in the root-biomass at harvest. The proportion of total rhizosphere CO_2 originating from root respiration or from microbial comsumption of root deposits is not yet quite clear; ratios of 1:1, (Warembourg & Billes, 1979), 2:3 (Martin, 1977) or even 1:4 (Sauerbeck & Johnen, 1977) are reported. Even though large amounts of rhizodeposits are found, their availability for denitrifying microorganisms under normal growth conditions is not clear. Woldendorp (1962), Stefanson (1972), Rheinbaben & Trolldenier (1984) and Prade & Trolldenier (1988) found enhanced denitrification in the root zone, which they attributed to rhizodeposits, more microorganisms in the rootzone and lowered oxygen tension by O_2 uptake of the roots. Others, (Guenzi et al., 1978; Kovalenko & Cameron, 1978; Mosier et al., 1986) found decreased denitrification losses in planted soils due to plant competition for available NO_3^- and to possibly dryer conditions in a rooted soil.

[+] Bundesforschungsanstalt für Landwirtschaft (FAL), Bundesallee 50, D-3300 Braunschweig, FRG

[++] USDA-ARS, P.O. Box E, Fort Collins, Colorado, 80522, USA

MATERIAL AND METHODS

In attempt to better understand the relationship between growing plants and denitrification we made several phytotron experiments under carefully controlled conditions. In these experiments, the N- and C-turnover during a growth period of corn or wheat were investigated. Pots, containing 17 kg of dry soil (typic Normudalf silt loam, Bodenstedt, West Germany) mixed with ^{15}N labeled fertilizer as indicated in the tables were used. N-losses were measured by the ^{15}N method and either gaseous or balance losses were determined or compared as described (Haider et al., 1985, 1987; Heinemeyer et al., 1985, 1988a, 1988b). The water regime was kept constant at -33 kPa and lowered to -5 kPa on occasion.

RESULTS AND DISCUSSION

In the first set of experiments ^{15}N labelled ammonia fertilizer was added in a single dose at the beginning. The contents of ^{14}C and ^{15}N during the vegetation period are shown in table 1.

Table 1 Experiment with corn: ^{14}C and ^{15}N remaining in plants and soil

Plant Age Days	Root ^{14}C g/Pot	$^{14}CO_2$-C g/Pot	Soil ^{14}C g/Pot	% Appl. $^{15}NO_3$ Planted	% Appl. $^{15}NO_3$ Unplant.	% ^{15}N-Soil Planted	% ^{15}N-Soil Unplant.	Σ % ^{15}N in Plant + Soil
0	-	-	-	(100)	(100)	100	100	100
37[1)]			0.11	66	84			
39	0.36	0.58	0.13	64	80	78	100	98
52[1)]			0.24	30	75			
54	0.91	1.33	0.27	28	74	36	84	94
73[1)]			0.26	26	63			
75	1.10	2.54	0.36	23	60	30	71	82
86[1)]			0.52	15	52			
88	0.94	3.36	0.53	12	51	19	67	82

Each pot contained 17 kg of dry soil mixed with 1 620 mg N_{min} as NH_4^+ and NO_3^- (7.5 atom % ^{15}N). 2 corn plants per pot.

1) The water content was kept at 33 kPa, then increased at the distinct day to 5 kPa and decreased again to 33 kPa before the pot was harvested.

The added ammonia fertilizer was completely converted into nitrate within the first two weeks. Naturally, the nitrate and the total ^{15}N content in planted soils decreased due to plant uptake much more rapidly than in the unplanted soil. At the end of the experiment, the unplanted soil contained more than 50% of applied ^{15}N in the form of nitrate and nearly 20% in the organic soil N. If total N in the unplanted soil is, however, compared to the sum of ^{15}N in plants and soil more ^{15}N remained in the planted system. The unplanted soil lost more than 30% while the planted system lost only 18% of the applied fertilizer. Similar results were obtained from the experiment with wheat.
Obviously the nitrate content of unplanted soils remained higher than in planted soils. A model developed from field data by Mo-

sier & Parton (1985) indicated that the amount of volatile N lost from soil increases in direct proportion to soil nitrate concentration. Furthermore, Smith & Tiedje (1979) reported that only at concentrations above 0.1g NO_3^--N per kg of soil denitrification was higher in planted versus unplanted soil. A decrease of denitrification in a planted soil was observed at nitrate contents below 0.1g NO_3^--N per kg of soil.

Because of this possible effect of soil nitrate concentrations we followed N-losses in further experiments, where soil nitrate levels were kept similar in planted and unplanted soil (Haider et al., 1987).

Table 2 Corn experiment: Plants supplied with splitted N-doses as ^{15}N-NO_3^-

Plant Age Days	Added ^{15}N plant.	Added ^{15}N unplant.	Soil NO_3^- ^{15}N plant.	Soil NO_3^- ^{15}N unplant.	Recovered ^{15}N plant.	Recovered ^{15}N unplant.	Denitrified ^{15}N plant.	Denitrified ^{15}N unplant.
0	610	610	610	610	610	610	0	0
26[1)] 28	610	610	198	465	583	606	27	4
54[1)] 57	1870	610	464	322	1763	450	107	160
68[1)] 71	2340	800	587	362	2136	482	204	318
82[1)] 85	2530	800	336	282	1895	462	635	338

All numbers except days represent mg/pot with 16.9 kg dry soil, each.

1) The water content in the soil was kept at 33 kPa (60 % WHC), then increased at the distinct day to 5 kPa and decreased again to 33 kPa after 1.5 days when the pot was harvested.

A total of 2.53 g and 0.80 g of ^{15}N-labelled nitrate was added to planted and unplanted replicates, respectively. Until day 71 the recovery was always above 91% in the planted soil, while in the unplanted replicates the recovery decreased to 60%. About 230 mg N disappeared from the planted and 320 mg N from the unplanted pots. Between days 71 and 85 an additional large quantity of N was, however, lost from the planted soil. This suggests that the large N-loss during these days was caused by additional carbon sources released from plant roots. During this time the root biomass decreased from 3.4 g at day 71 to 1.7 g at day 81 per pot. We therefore conclude that only during maturity of the corn plants and senescense of the roots, denitrification loss due to plantation was significantly increased, while actively growing plants did not enhance the loss of fertilizer N.

Data in table 2 show the absolute amount of added fertilizer N not recovered in both systems. Comparable N_2O losses could be measured from unplanted and planted replicates until day 57, after that day N_2O losses from planted pots were greater. Most of the losses occurred from planted pots during senescens of the root system. The measured N_2O losses do not include losses in the form of dinitrogen. Furthermore, the possibility exists that

plantation affects N_2O to N_2 ratio differently than in unplanted soil.
In a further experiment (Heinemeyer et al., 1988a) we directly measured N_2-N and N_2O-N losses and compared them to the losses estimated by the ^{15}N balance. The soil was fertilized with a 46 atom% ^{15}N enriched KNO_3. This allowed us to estimate N_2 losses according to a ^{15}N method described by Siegel et al., (1982). The N_2O efflux from the pots was continuously measured by an exact determination of flow rates and GC measurement of N_2O in the in- and outflowing air.

The soil water tension was normally maintained at -33kPa and was lowered to -5 kPa for 2 days, three times during the experiment. At normal water tension, N_2 and N_2O release of both volatile N-species were low. During the periods of increased water content a high evolution with maxima of 150 µg N_2O-N h^{-1} and 140 µg N_2-N h^{-1} was detected . The high coincidence of N_2 and N_2O efflux makes evident that both gases are released simultaneously.

Table 3 N-losses estimated by ^{15}N balance or by direct N_2O and N_2 flux measurements

Time after initiation (days)	Fertilizer-N in plants	Fertilizer-N in soil	Fertilizer-N loss estimated by	
			^{15}N balance	N gas evolved
	% ^{15}N applied			
20	14.9	84.5	0.6	0.19
34	19.0	80.3	0.7	0.58
49	67.0	28.4	4.6	0.88
79	74.0	21.3	4.7	1.08
LSD 0.05	6.0	2.2	2.7	0.29

each pot contained 17 kg dry soil mixed with 2700 mg N as nitrate with 46% ^{15}N.

About 99% of the applied fertilizer N could be recovered until day 34. These recoveries decreased then more steadily and amounted about 95 % at the end of the study.

Until day 34 the ^{15}N balance losses agree within the limits of error rather well with those measured directly by the evolved gases. However, N-losses determined by the ^{15}N balance are nearly 4 times higher compared to the gaseous losses at the end of the experiment. Total N-losses for the entire experiment if calculated on a hectar base are rather low, they amounted about 7 ± 1 kg N ha^{-1} as gaseous losses and 30 ± 6 kg N ha^{-1} as ^{15}N balance losses. Considering the rather favourable conditions for denitrification, with a constant soil temperature of 23°C, high nitrate and temporary high water contents, these N-losses are relatively small. Compared with denitrification losses of 10 to 20% from applied fertilizer as reported by Frede et al. (1975), Hauck (1981) or even higher as reported by Capelle & Bäumer (1985) the losses shown here amount only approximately 5% for the balance losses and 1% for the gaseous losses from the applied fertilizer. Similar differences between ^{15}N balance and gaseous losses were reported by Ryden & Lund (1980), Germon (1985) or Parkin et al., (1985). Better agreements between both were reported by Mosier et al., (1986). It can yet not be excluded that the observed differences in our experiments between direct and balance losses could be partly caused by additional NO losses (Tortoso et al., 1987) or by N-volatilization from the leaves of plants (Farquhar et al., 1979).

CONCLUSIONS

In our ^{14}C labelling experiment we always found that the ^{14}C contents analyzed in the soil samples, after careful removal of the root biomass, amounted only about 30 mg C kg^{-1} soil. This ^{14}C present in the soil was not readily extractable with water or 1 M Na_2SO_4. A comparison of the denitrification capacity from planted and unplanted soil, revealed this root derived carbon not to lead to increased gaseous N losses or nitrate consumption. Similarly, Shamoot et al., (1968) found that rhizodeposited organic material was not easily decomposable. Furthermore, Smith & Tiedje (1979), reported that only soil in direct contact with the root surface of corn plants showed an enhanced denitrification capacity, while it decreased rapidly in the first few millimeters away from the roots.

They also indicated that nitrate concentration affected the denitrification rates of planted and unplanted soils. When the NO_3^--supply was limited, denitrification was actually lower in planted than in unplanted soil. This observation agrees well with our findings: In our first experiments where fertilizer N was applied only at the beginning and nitrate concentration in soil decreased rapidly due to plant uptake, denitrification losses were smaller than in unplanted soil. In a second series of experiments where fertilizer N was applied in splitted doses to keep nitrate levels high, denitrification losses from planted soil exceeded that of the unplanted soil only during ripening when roots became senescent. Here, obviously an additional supply of carbon available for denitrifying organisms caused the increased denitrification.

Bakken (1988) recently reported that soil water tension is a critical parameter on denitrification in planted versus unplanted soils. Only when plants were kept for extended time under flooded conditions a significant increase of denitrification due to plantation in the presence of sufficient nitrate was observed. The stimulation of denitrification by roots was practically eliminated at water tensions above - 3 KPa.

These prolonged times at low water tensions can lead to anaerobic conditions, which we did, however, not observe in our experiments, where we monitored continuously the redox potential at different depth (Heinemeyer et al., 1988b). Conditions of anaerobiosis favour root exudation (Grineva, 1962), therefore results of Haller & Stolp (1985) and also those of Bakken (1988) under low water tension can be assigned to a stimulation of root excretions during anaerobiosis. Prade & Trolldenier (1988) examined the effect of wheat roots on denitrification at varying soil air filled porosities. Their results showed that a rhizosphere effect on denitrification was confined on an air filled porosity below 10-12% and became significantly greater with increasing soil organic C contents. In our experiments even during periods of low water tension at -5 kPa, the air filled porosity was never below 14%.

In arable soils airfilled porosities will only rarely fall below 14%. We conclude that plants increase denitrification only during longer periods of water saturation that are coupled with high nitrate contents.

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Labelled dinitrogen emission from flooded rice fertilized with ^{15}N-urea

by

Freney, J.R.[+], A.R. Mosier[++] and S.L. Chapman[+]

Abstract. This paper reports a study on the distribution of dinitrogen (N_2) between the atmosphere, floodwater and porewater of the soil in a flooded rice field after addition of ^{15}N-labelled urea into the floodwater.

Of the urea N added, 0.02% was lost to the atmosphere as N_2O, 0.9% was lost by ammonia (NH_3) volatilization, and 3.6% was lost as N_2 gas during the 7 days of measurement. At the end of this period 0.028% and 0.002% of the added N was retained as N_2 gas in the floodwater and soil porewater, respectively. Recovery of the ^{15}N applied as N gases, plant uptake, and soil and floodwater constituents totaled about 94% of the N added.

Introduction

In Asia and Australia urea is the main fertilizer used to supply N to flooded rice, but it is not used efficiently (DeDatta 1981). While leaching and runoff losses occur in some situations, it seems that the main cause of fertilizer inefficiency for flooded rice is gaseous emission of NH_3 and N_2 to the atmosphere (Simpson and Freney 1988).

Methods now exist for the direct determination of NH_3 emission in the field, but there is no accepted method for the direct determination of losses by denitrification. Currently most estimates of denitrification loss from flooded rice are obtained by calculating the difference between total N loss and NH_3 loss. Mosier *et al.* (1986), utilizing the ideas of Siegal *et al.* (1982) directly quantified N_2 emission from corn and barley crops during a growing season using a soil cover and highly enriched ^{15}N labelled fertilizer.

+Division of Plant Industry, CSIRO, GPO Box 1600, Canberra, ACT 2601.

++United States Department of Agriculture, Fort Collins, Colorado, 80522.

As this technique seemed promising, and is direct and relatively simple, we used it to determine the N_2 emitted from a flooded rice field immediately after the addition of urea.

Experimental

The experiment was conducted in 1987, on a grey clay soil (Pelloxerert, SMSS 1983; Ug 5.25, Northcote 1971) near Griffith, N.S.W., within an established crop of water-seeded rice (*Oryza sativa* L. cv. M7), at the panicle initiation stage. The surface soil had a pH of 8.2, a total N concentration of 0.07%, a cation exchange capacity of 36 cmol $[Na^+]$ kg^{-1}, and contained 55% clay.

Six microplots were established in a rice bay, by inserting stainless steel cylinders (0.33 m diameter x 0.60 m high) ~ 0.29 m into the soil. Four rice plants were confined within each cylinder. At the time the rice plants extended 0.65 m above the water surface, and the floodwater was 0.20 m deep. All of the microplots were fertilized with urea at the rate of 80 kg N ha^{-1}; three received unlabelled urea and three received labelled urea containing 79.25 atoms % ^{15}N. The unlabelled microplots were used to monitor floodwater ammoniacal N concentrations, pH and temperature for the determination of ammonia emission by the bulk aerodynamic method (DeDatta *et al.*, 1989), while the ^{15}N labelled plots were used to determine the recovery of ^{15}N in the soil, plants and water, and to assess N_2 and N_2O emission and total N loss Mosier *et al.* 1989).

Results and Discussion

Wind speeds during the study ranged from 0.4 to 4.2 m s^{-1}, floodwater temperatures 17°C to 24°C, and the floodwater pH values from 7.0 to 7.6. There was very little diurnal variation in pH values on most days because the large leaf area reduced light intensity at the water surface, so that algal photosynthesis was restricted.

The NH_3 flux densities above the microplots were low throughout the study and never exceeded 0.35 μg N $m^{-2}s^{-1}$. This was a direct consequence of

the low floodwater pH values and temperature and the low wind speeds. The NH_3 emission rates are presented in Fig. 1. From these it was calculated that the total loss by NH_3 volatilization was 0.72 kg N ha^{-1} (Table 1).

Table 1. Distribution of fertilizer N in a flooded rice field seven days after urea application (% of applied N)

Organic nitrogen	Soil	16.8 ± 2.59[a]
	Plant	19.3 ± 4.48
	Floodwater	3.6 ± 2.48
Ammoniacal nitrogen	Soil	18.2 ± 1.83
	Floodwater	30.9 ± 0.87
Nitrate nitrogen	Floodwater	0.4 ± 0.14
Dinitrogen gas	Porewater	0.002 ± 0.002
	Floodwater	0.028 ± 0.02
	Lost	3.6 ± 1.18
Nitrous oxide lost		0.02
Ammonia lost		0.9
Total		93.75 ± 0.17
Nitrogen unaccounted for		6

[a]Mean ± standard deviation

Nitrous oxide represented only a small proportion of the N gases evolved from the floodwater; the amount lost was 0.02% of the N applied.

Dinitrogen flux densities were not affected by the period of enclosure or the time of measurement. The dinitrogen flux measurements indicated that denitrification occurred soon after urea was added. The rate of N_2 emission was largest (> 700 g N $ha^{-1}d^{-1}$) about 48 h after fertilizer application and this had decreased to <130 g N $ha^{-1}d^{-1}$ by day 7 (Fig. 1); 2.87 kg N ha^{-1}, or 3.6% of the applied N, was lost as N_2 during the study.

On day 7 the assessment of gaseous emission rates was stopped so that the distribution of N_2 in the floodwater and soil could be determined. At this time 30.9% of the applied N was in the floodwater as ammoniacal N, and 18.2% of

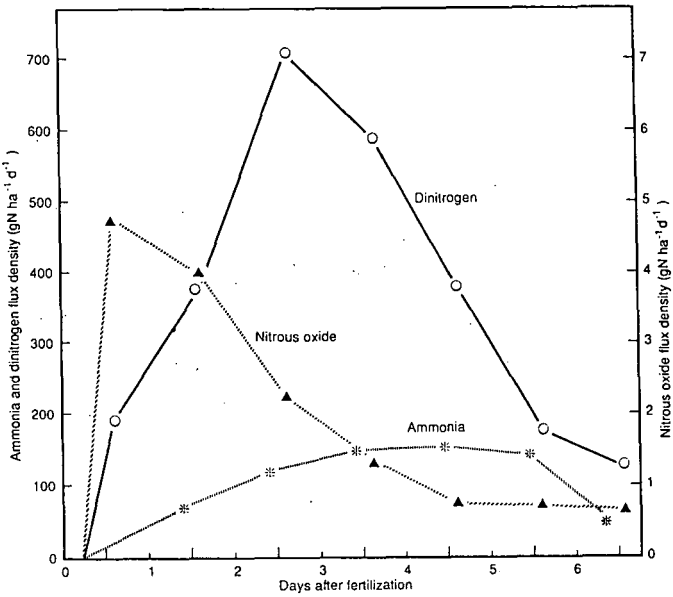

Fig. 1. Dinitrogen, nitrous oxide and ammonia flux densities over microplots in a flooded rice field after urea application.

the applied N had been adsorbed as NH_4^+ on the cation exchange complex. In addition, 16.8% of the added N had been immobilized into soil organic matter, 19.3% had been assimilated by the rice plants and 3.6% taken up by microorganisms in the floodwater (Table 1). Very little nitrate or nitrite N was detected in the soil or floodwater.

Appreciable N_2 was dissolved in the floodwater (477 mg N $plot^{-1}$, but of this only 147 μg N $plot^{-1}$, amounting to 0.028% of the applied N, was derived from the fertilizer. Much less N_2 was held in the soil porewater (27 mg N $plot^{-1}$ and only 9 μg N $plot^{-1}$, amounting to 0.002% of the applied N, was derived from the fertilizer.

As there is no standard method for assessing N_2 loss from flooded soils we cannot determine by comparison if our results are correct. However, some confidence in the results would be obtained if we could achieve a nitrogen

balance from the known inputs of labelled urea, the measured amounts of labelled N retained in the soil-plant-floodwater system, and the measured or inferred gaseous losses; runoff from the microplots was prevented by the steel enclosures and leaching losses from this soil appear to be negligible (Simpson *et al.* 1984). The results in Table 1 show that ~94% of the applied N was accounted for in the water, soil and plants and N lost to the atmosphere.

Other work (Lindau *et al.* 1988) in a flooded soil without rice plants indicated that considerable N_2 derived from the added fertilizer was retained in the soil-water system during denitrification. Buresh and Austin (1988) confirmed this observation in the absence of plants and concluded that the low N_2 and N_2O emissions from a flooded rice field were due to retention of the N_2 and N_2O in the soil-water system. However, their N balance was not complete as NH_3 loss was not determined, and they determined N_2 and N_2O loss in chambers placed between the rice plants. The work of Mosier *et al.* (1988) shows that emission rates were considerably larger when the chambers were placed over the plants rather than between the plants.

It was demonstrated recently that rice plants serve as a conduit to transport CH_4 from the flooded soil to the atmosphere (Cicerone and Shetter 1981). Our results, obtained in the presence of rice plants, show that very little of the added N was retained in the porewater of the soil or the floodwater in the field. These results confirm the CH_4 findings in that there are mechanisms in the field for the rapid transfer of gases from the soil to the atmosphere.

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INFLUENCE OF RICE PLANTS ON THE EVOLUTION OF N_2 AND N_2O FROM THE SOIL TO THE ATMOSPHERE

by

A.R. Mosier[+], S.K. Mohanty, A. Bhadrachalam and S.P. Chakravorti[++]

INTRODUCTION

Recovery of fertilizer N in plant and soil applied at transplanting to flooded rice (Oryza sativa L.) rarely exceeds 50% (De Datta, 1981). The inefficiency of N use is generally considered due to gaseous N volatilization. Gaseous N loss processes of major importance in N-fertilized rice production are ammonia volatilization and N_2 emission resulting from the nitrification-denitrification process. Ammonia loss after application of urea to flooded rice varies widely in importance, depending on conditions in the floodwater and on windspeeds (Simpson and Freney, 1988).

The N_2 loss from denitrification can occur concurrently with NH_3 loss over 7-10 days following urea application (Fillery, et al., 1986; Simpson and Freney, 1988; Simpson et al., 1985). The amount of denitrification loss varies with site and soil conditions, but the process appears to be less susceptible to variations in ambient conditions and application methods than NH_3 loss.

Denitrification N losses in the studies cited above were determined from the difference between fertilizer N additions, N uptake by crops, N leaching, residual soil N and NH_3 volatilization (Fillery et al., 1984; Fillery, et al., 1986) and were not directly measured. Only a few studies to directly quantify denitrification in rice fields have been attempted. Direct measurement of N_2 and N_2O production has been achieved in nonflooded systems using gas chromatographic and ^{15}N techniques (Rolston et al., 1982; Siegel et al., 1982; Mosier et al., 1986; Mulvaney and Vanden Heuvel, 1988).

When direct measurement of N_2 and N_2O from flooded rice was attempted (Fillery et al., 1986; Buresh and Austin, 1988) the amount of N gas loss measured directly was very small compared to the amount of N lost when ^{15}N balance was calculated on the soil-plant system. These poor recoveries were attributed to entrapment of denitrification gases in the flooded soil. Lindau et al., (1988) and Holt et al., (1988) show that denitrification gases are entrapped in flooded soils and can be quickly released only by physical disturbance. Both of these laboratory studies and the field study of Buresh and Austin (1988) did not, however, include plants either in the laboratory incubation vessels or the field gas collection chambers.

[+]USDA-ARS, P.O. Box E, Ft. Collins, CO, 80522, USA.
[++]Central Rice Research Institute, Cuttack, India.

There is reason to believe that the rice plants themselves serve as a conduit to transport the N gases produced during denitrification from the flooded soil into the atmosphere above the floodwater. Seiler et al., (1984) show that methane produced in a rice field soil is transported from the soil to the atmosphere almost exclusively by movement through the rice. Since the physical processes that control CH_4 and N_2 movement in a saturated soil are the same, it seems reasonable to think that N gases produced during denitrification (N_2 primarily) should not remain entrapped in a soil where rice is growing.

In this paper we report the results of a field and a greenhouse study where we investigated the effect of young rice plants on the movement of N_2 and N_2O from a saturated soil to the atmosphere. The field study was conducted at the Central Rice Research Institute in Cuttack, India. We report here our initial efforts to determine if rice plants need to be included in gas collection chambers when field N-gas flux measurements are made. A ^{15}N method was used to quantify the amount of N denitrified in a urea-fertilized, intermittently flooded rice field. We later conducted a greenhouse experiment in Ft. Collins to determine if growing rice plants affected release of N_2 and N_2O from a urea-fertilized flooded soil. We also measured the effect of the plants on the amount of these N gases entrapped in the soil.

MATERIALS AND METHODS

1. Field Experiment.

Inside a 5 m X 20 m field plot, a 0.96 m^2 plot was isolated by driving a rectangular steel confinement 30 cm into the soil. This small plot was covered when urea (100 kg N ha^{-1}) was surface applied to the entire plot. Four-week old rice seedlings were hand planted (10 cm apart with 20 cm row spacing). Inside the small plot an equivalent amount of urea-N (99 atom % ^{15}N) was uniformly surface applied and rice seedlings were hand planted into the soil slurry. The standing water was about 3 cm deep at planting. The soil was an Aeric Fluvaquept which is only slowly permeable to vertical water movement.

Measurements of N gas flux (N_2 and N_2O) began about 4-h after urea application. The gases were sampled using rectangular chambers, 14 cm wide X 40 cm long and 12 cm high, which were made from plate glass glued together. The joints were sealed with silicone caulking. Two holes were made in the top of each chamber to accommodate rubber septa through which a needle could be inserted to withdraw a gas sample. The serum stoppers were left out while the chambers were installed to alleviate a pressure build up. During early evening each day, chambers were installed in the plots by inserting the chambers into the soil so that a 9 cm gas headspace existed. Two chambers were installed between the rice row and two in the rice row, enclosing three rice plants. Gas samples from these chambers were withdrawn after 0, 4 and 15-h by syringe. The gas was immediately injected into an evacuated glass tube closed with a rubber septum which was quickly coated with silicone sealant to prevent leakage.

The gas samples were shipped to Ft. Collins where the collected gas was

analyzed for N_2O by gas chromatography (Mosier and Mack, 1980) and for $N_2 + N_2O$ by isotope ratio mass spectrometry (IRMS) (Siegel et al., 1982; Mosier et al., 1986).

2. Greenhouse Experiment.

A set of small plots were prepared from polyvinyl chloride pipe (30 cm high X 10 cm diameter) by sealing a plastic plate onto one end of the pipe. A 12 cm depth of a clay loam soil (612 g) was added to each cylinder and the soils were flooded (3 cm deep) with distilled water 30 d before 4 wk old rice seedlings were transplanted into half of the plots. One week after rice transplanting 60 mg urea-N (75 atom % ^{15}N) was added into the floodwater of each plot. At each sampling time 3 planted and 3 unplanted plots were taken.

The efflux of N_2 and N_2O from the floodwater was monitored daily beginning the day that urea was added. Gas sampling was accomplished by sealing each of 3 planted and 3 unplanted plots with a rubber stopper that was fitted with a gas-tight chromatographic septum. The stoppers were installed each evening (plants were folded over and maintained in the plot) and gas headspace was withdrawn by syringe, 12-15 h later. Gas samples were either analyzed during the same day or transferred into evacuated gas-tight tubes for later analysis. Samples analyzed immediately and duplicate samples stored and analyzed later were not different in N_2 or N_2O content. Gas analyses and flux calculations were performed as described for the field study.

At weekly intervals, after headspace gas samples were withdrawn, three replicate planted and three unplanted plots were vigorously shaken on a reciprocal shaker for 15 min. After shaking another gas sample was taken from each plot. The difference in the amount of N_2 and N_2O or ^{15}N in the gas head space before and after shaking is considered to be the amount of N gases entrapped in the soil-water system.

After the gas samples were withdrawn, slurry samples were quickly taken in triplicate for total N analysis by Kjeldahl digestion, or mixed 1:1 by weight with 2 N KCl and shaken so that NO_3 and NH_4 content could be determined. Water content was measured gravimetrically. The remaining soil was air dried, then ground to pass through a 150 um sieve. Samples of the finely ground material were analyzed for total N and ^{15}N by automated combustion-IRMS (a VG-903 IRMS coupled by a Europa Scientific Interface to a Carlo Erba C/N analyzer).

Whole plants (roots and shoots together) were removed from the slurry, washed with distilled water, then dried at 60 C. The dried plant material was ground to pass through a 150 um sieve then analyzed for total N and ^{15}N by automated combustion-IRMS.

RESULTS AND DISCUSSION

1. Field Experiment

The rice plants were 10-15 cm tall at transplanting. Even though the plants were small, they appeared to increase the flux of $N_2 + N_2O$ from the soil to the atmosphere (Table 1). Fifteen d after transplanting, the plants were 20-30 cm tall and began to show physical damage from being covered. Because of

this the plants were not enclosed after d-15. By that time, about 90% of the measured $N_2 + N_2O$ emitted during the growing season had evolved. The presence of the young rice plants inside the chambers increased measured $N_2 + N_2O$ by an average of 1.29 times (Table 1). The effect of the plants is small in this case because the plants were young and the floodwater was allowed to recede so that the soil surface cracked before floodwater was returned.

2. Greenhouse Experiment.

In this study, young rice plants facilitated the efflux of N_2O from the soil-water system (Table 2). In the planted plots little N_2O evolved after the first week following urea application. In the unplanted plots, N_2O efflux was

Table 1. $N_2 + N_2O$ gas flux, measured overnight in an intermittently flooded rice field fertilized with urea.

Time After Transplanting	$N_2 + N_2O$ Flux			
	No Plants In Chamber		Plants In Chamber	
	mean	S.D.	mean	S.D.
days	g N ha^{-1} d^{-1}			
1	14	7	14	6
2	19	2	21	1
3	35	2	39	13
4	51	9	68	20
5	110	26	130	52
6	210	40	280	93
7	310	35	510	95
8	150	25	250	190
9	750	71	980	110
10	500	85	580	11
11	410	28	390	1
12	270	5	410	36
13	190	1	270	53
14	160	34	220	33
15	240	11	270	14

greatest 16-d after fertilization but was less than half the maximum flux rate observed on d 6 in the planted system. Total N_2O release was < 2 % of the total $N_2O + N_2$ production.

$N_2O + N_2$ efflux was greater in the planted (5.6 mg N) than in the unplanted plots (4.8 mg N). N gas release was also faster in the planted system, as about 50% of the total occurred during the first week after planting. When we include the amount of N gas entrapped in the soil, however, more denitrification occurred in the unplanted systems (Table 2). A total of 7.2 mg N gas was produced in the unplanted soil compared to 5.6 mg N in the planted soil.

Table 2. Recovery of added ^{15}N from rice-planted and unplanted flooded soils

Treatment	Time After Fertilizing (weeks)				
	0	1	2	3	4
	% of Initial ^{15}N				
^{15}N Recovery Before Drying Soil					
Unplanted					
Soil	100	77.5	54.1	53.5	44.4
N gas emitted	0	1.8	5.4	7.0	7.7
Gas entrapped	0	0.5	4.1	6.4	8.4
Total	100	79.8	63.6	66.9	60.5
Planted					
Soil	100	74.9	36.2	16.2	15.6
Plant	0	16.8	35.9	49.3	62.7
N gas emitted	0	5.2	7.8	8.6	8.6
N gas entrapped	0	0.9	1.1	1.2	1.2
Total	100	97.8	81.0	75.3	88.1
^{15}N Recovery After Drying Soil					
Unplanted					
Soil	100	63.6	45.1	43.5	39.4
N gas emitted	0	1.8	5.4	7.0	7.7
N gas entrapped	0	0.5	3.0	5.9	8.4
Total	100	65.9	53.5	56.4	56.5
Planted					
Soil	100	57.2	38.6	15.4	16.8
Plant	0	16.8	35.9	49.3	62.7
N gas emitted	0	5.2	7.8	8.6	8.6
N gas entrapped	0	0.9	1.1	1.2	1.2
Total	100	80.1	83.4	74.5	89.3

In Table 2 are the data which show the fraction of added urea that was held in the soil-water, the plant, N gases evolved, and N gases entrapped in the soil-water at each weekly interval. Recovery of added urea was always >75% in the planted system but only 60% in the unplanted soil. Ammonia volatilization was not measured but we suspect that our lack of quantitative recovery of added urea was due to this process. From the N-balance data (Table 2), the soil NH_4^+ and floodwater pH we expect that NH_3 evolved from the soil during the course of the experiment and during sample processing. Total N analyses were conducted on the soils both before and after they were air dried (Table 2). In both planted and unplanted soils total ^{15}N recovery was highest at week one. When the analysis was performed before the sample was dried, about 15% higher N recovery was found in each case. Thereafter, in the planted soil the recovery of ^{15}N is about the same in samples analyzed before and after drying. In the unplanted soil, however, ^{15}N recovery remains higher in the samples analyzed before drying the soil.

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Denitrification in the rhizosphere of rice and wheat seedlings as influenced by the K status of plants

by

K. Prade*) and G. Trolldenier**)

A series of studies was devoted to the effect of plant nutrition and soil factors on denitrification in the rhizosphere. In the course of previous experiments summarized by Trolldenier (1989) it had been found that K deficiency had stimulated denitrification on wheat roots. Later, a technique was developed allowing measurements of denitrification on roots grown at a whole range of different soil air-filled porosities (Wollersheim et al. 1987, Prade and Trolldenier 1988). The present study was aimed at investigating the effect of K supply to plants at certain soil air-filled porosities and the interference of organic matter as well as the attack of root pathogens on denitrification.

Material and methods

Pure nutrient-free quartz silt of particle size distribution ranging from 630 to < 2 µm was used as substrate. The medium was amended with major and minor nutrients, including different amounts of K. After being supplemented with nutrients the quartz silt was moistened to 16.6 % (w/w) with distilled water, mixed thoroughly and compacted in PVC cylinders to bulk densities of 1.4-1.5 g/cm^3. Thereafter the quartz bearing cylinders were mounted on ceramic plates of a water supply system (Wollersheim et al. 1987). The technique allowed maintenance of certain air-filled porosities by continuous water supply during seedling growth. In one experiment using

*) Institut f. Umwelt-, Energie- u. Geotechnik GmbH, Willhelm-Will-Straße 7, D-6330 Wetzlar 21, FRG

**) Landwirtschaftliche Forschungsanstalt Büntehof, Bünteweg 8, D-3000 Hannover 71, FRG

wheat as test plant the germinating seedlings were inoculated with the take-all fungus Gaeumannomyces graminis var. tritici (Ggt). Agar discs of 0.5 cm in diameter, colonized with the fungus, were placed under the pregerminated seedlings. Discs from agar plates with killed mycelium obtained by heat treatment at 95°C for 30 minutes were used in the control pots. Ten wheat or 11 rice seedlings were grown per cylinder in a growth chamber and subjected to the acetylene inhibition assay for denitrification measurements after 14 and 21 days, respectively (Wollersheim et al. 1987). During growth the plants were additionally topdressed as described elsewhere (Prade and Trolldenier 1990). Immediately before the acetylene inhibition assay all pots received 25 mg N as $NaNO_3$ to ensure an unlimited nitrate concentration. The total amount of N_2O production expressed in nmol/mg dry matter (DM) was ascribed to rhizospheric denitrification, since the quartz substrate was free of organic substances.

Results and discussion

Decreasing air-filled porosity of the quartz substrate increased rhizospheric denitrification both in the experiment with wheat and rice which is in agreement with earlier findings using soil substrate (Prade and Trolldenier 1988). It was confirmed that below a threshold of 10-12 % denitrification increased exponentially. With wheat the increase was considerably higher in K deficient plants. This finding is concomitant with earlier experiments in which other procedures had been applied (Trolldenier 1971, von Rheinbaben and Trolldenier 1984). Increased carbon exudation as well as accelerated root collapse is supposed to be responsible for this K deficiency effect.

The lower K nutrition reduced plant growth to approximately one half. The K content of these plants was low (1 mg K/g DM), while in high K individuals it was in the range normally found in young growing plants (43 mg/g DM).

Roots of plants growing under natural conditions are usually colonized not only by saprophytic organisms but also by minor

and major root pathogens. A common root pathogen of wheat is Ggt. Roots attacked by this fungus are more densely populated by rhizosphere microorganisms (Bednarova et al. 1979) probably due to the greater leakage of damaged roots. Inoculation with Ggt in fact increased denitrification (Table 1).

Tab. 1: Incidence of the root pathogen Gaeumannomyces graminis in relation to denitrification in the rhizosphere of wheat (Prade and Trolldenier 1990b)

K level	Incidence of G. graminis	Denitrification[1)]	n
high K	+	120.8 b[2)]	7
	-	59.5 c	7
low K	+	167.8 a	7
	-	128.5 b	9
$HSD_{0.05}$		37.9	

1) in nmol $N_2O \cdot (48\ h \cdot mg\ DM)^{-1}$, age of plants: 14 days
2) Differences with same indices are not specifically different at the 5 % level

On plants well supplied with K the incidence of Ggt doubled denitrification. Again, K deficiency was found to have increased denitrification. Highest N_2O production was observed on diseased plants low in K. From the results it may be deduced that laboratory experiments with young healthy plants obviously underestimated the rhizosphere effect on denitrification. In the field, however, roots are generally attacked to a varying degree by pathogens increasing indirectly the number of rhizosphere microorganisms.

Though it was also observed with rice that a decrease in soil air-filled porosity increased denitrification, the level was considerably lower than with wheat. While the highest

rates in the rice experiment were only 80 nmol N_2O/mg DM, up to 200 nmol N_2O were found with wheat. The higher rates on wheat roots were found in spite of the younger plant age and lower temperatures. Thus, it seems not unlikely that plants possessing a well organized aerenchyma, which improves the oxygen status of the root surface, support denitrification less.

As already observed with wheat, lower K supply reduced root and shoot dry weight as well as the K content in the experiment with rice. However, it remains uncertain, if K deficiency is affecting denitrification. Data of lower air-filled porosities were not available for the low K treatments. Above 5 % soil air-filled porosity essentially no difference in denitrification occured between the K treatments in rice. The difference to wheat may be explained by the lack of substantial root collapse as evidenced by microscopic observation.

In soil, denitrification in the rhizosphere is favoured by soil organic matter acting as additional oxygen sink during decomposition (Prade and Trolldenier 1988). In order to simulate these conditions cellulose was mixed to the quartz silt as a slow release carbon source. As the experiment did not include uncropped treatments, denitrification was calculated as nmol N_2O per cm^3 of substrate. The enhanced denitrification at the higher cellulose level at sufficient K supply suggests that a strong oxygen sink of the bulk substrate may counteract eventual oxygen release from roots. This effect was not found at low K supply. It is conceivable that K deficiency not only reduced plant growth but also limited microbial activity. The rather low K contents of deficient rice plants (4 mg/g DM) support this hypothesis. Compared to wheat roots the rice roots of plants subjected to K deficiency maintained their structure and did not collapse. However, as observed with wheat the formation of adventitious roots was severely inhibited.

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Deutschen Bodenkundlichen Gesellsch.

ce of plants on denitrification in pot exper.. with soils

by
W. v. Rheinbaben*)

ing vegetation 10 to 30 % of the photosynthetic products pro-.ced in the shoots are translocated to the roots. From there a .arge part of these products is lost in the form of the socalled rhizosdeposition. This rich and easily mineralizable C supply leads to a high microbial population density in the rhizosphere. Due to the high C turnover the microflora, the major proportion of which is capable of denitrification, has a high demand for oxygen in addition to the oxygen requirements for root respiration. C supply together with oxygen deficiency which may easily occur under these circumstances contribute to a situation in which a high denitrification was expected and also confirmed (v. RHEINBABEN and TROLLDENIER 1984). Due to this rhizosphere effect laboratory experiments frequently reveal higher denitrification in rooted than in uncropped soil (inter alia BAKKEN 1988, PRADE and TROLLDENIER 1988). Other experiments, on the contrary, had shown that denitrification was reduced in the presence of plants (HAIDER et al. 1985). Our own study was intended to clarify this contradiction.

Methods

The investigations were carried out with 9 soils of different origin (v. RHEINBABEN 1988). After having received a basal dressing of N, P, K, Mg and minor elements the soils were filled into PVC pots (1.8 l in volume), seeded to wheat and cultivated for 2 to 5 weeks at 60 % of the water holding capacity (WHC) (v. RHEINBABEN and TROLLDENIER 1984). At the start of denitrification measurements the soils were given 50 mg NO_3^--N pot^{-1} and the

*)Landwirtschaftliche Forschungsanstalt Büntehof, Bünteweg 8, D-3000 Hannover 71, FRG

..reased to 95 and 100 % WHC,
..n pot was covered with a plastic b
..e volume. The air in the bags was replaced
- 90 % air and 10 % acetylene. Acetylene at this c
..ion is known to inhibit the conversion of N_2O to N_2. Gas
ples were taken after 24 h and analysed by gas chromatogra
for N_2O (v. RHEINBABEN and TROLLDENIER 1983).

Results and discussion

Two and three weeks after sowing of wheat the N_2O production measured in the sandy soil Berkhof was 1.8 and 1.7 µM N_2O pot^{-1}, respectively (Table 1) and was thus in the same order as the rates measured in the uncropped soil (Table 2). Obviously the growing plants had had no effect on denitrification at this early stage, whereas four to five weeks after sowing a remarkable increase in denitrification on cropped soil could be observed. At non-limited NO_3^- supply and high soil moisture denitrification had consequently increased with greater root density.

Table 1: Root and shoot dry matter as well as N_2O production in soil Berkhof cropped to spring wheat (At start of measurement: 95 % WHC)

Weeks after sowing	Root DM g pot^{-1}	Shoot DM g pot^{-1}	N_2O production µM N_2O pot^{-1} 24 h^{-1}
2	0.53	0.74	1.8
3	0.64	1.81	1.7
4	1.21	4.21	15.3
5	1.82	7.14	31.3

Another experiment was made with various soils to test if denitrification was always increased in the presence of plant roots. Denitrification was measured on 9 soils of different ..rigin and varying C contents in the presence and absence of ..ants (Table 2). On uncropped soils denitrification was tend.. ..lly increased with increasing C content of the soils, exc..

for the forested soil Berkhof. In view of the high C content of this soil denitrification was relatively low due to the small degree of humus degradation. The presence of 4-week-old wheat plants significantly increased denitrification on 7 soils. On 2 soils, which in the uncropped state exhibited already a high rate of denitrification due to their relatively high C contents, the presence of plants had only tendentially increased denitrification. It may therefore be concluded that at non-limited NO_3^- supply and high soil moisture the presence of plants will only increase denitrification, when the amount of soil organic substances providing a suitable carbon source for denitrifiers is low.

Table 2: C content of soils and N_2O production in cropped and uncropped soils. (Period of vegetation: 4 weeks; at start of measurement: 50 mg NO_3^--N pot^{-1} and 95 % WHC)

Origin	% C	µM N_2O pot^{-1} $24h^{-1}$ uncropped soil	cropped	HSD 5 %
B 2 Büntehof	0.65	n.d.	4.4	---
Havixbeck	1.08	n.d.	1.9	---
Grohnde	1.09	0.3	2.7	1.3
B 5 Büntehof	1.14	0.2	3.4	1.4
Aligse	1.19	0.8	12.3	5.3
Reeken	1.24	5.6	24.1	9.3
Pattensen	1.46	9.5	14.9	11.7
Berkhof	3.22	1.9	10.9	7.3
Garbsen	3.57	24.7	29.7	8.9

n.d.: not detected

The increase in denitrification due to rhizodeposition is possibly counteracted by the reduction of N losses because of decreased soil moisture and lower nitrate concentrations as a consequence of plant uptake. The influence of nitrate supply

was demonstrated in an experiment with wheat plants grown with complete and K deficient nutrition. As the control exhibited approximately the same level of denitrification after 2 and 3 weeks (Table 3), it was assumed that NO_3^- supply had become limited. When additional N (50 mg NO_3^- pot^{-1}) was given at the start of the 3rd measurement, denitrification increased significantly. Until the last measurement this additional nitrogen had obviously been taken up until the last measurement, so that no denitrification could be detected. In pots without K additions, however, sufficient NO_3^- was obviously still present in the soil due to lower N uptake. Another reason for the high rate of denitrification was the lower water uptake of K deficient plants, so that high soil moisture was maintained throughout the experimental period.

Table 3: Influence of complete nutrition versus K deficiency on N_2O production in soil Berkhof cropped to spring wheat (At start of measurment: 100 % WHC)

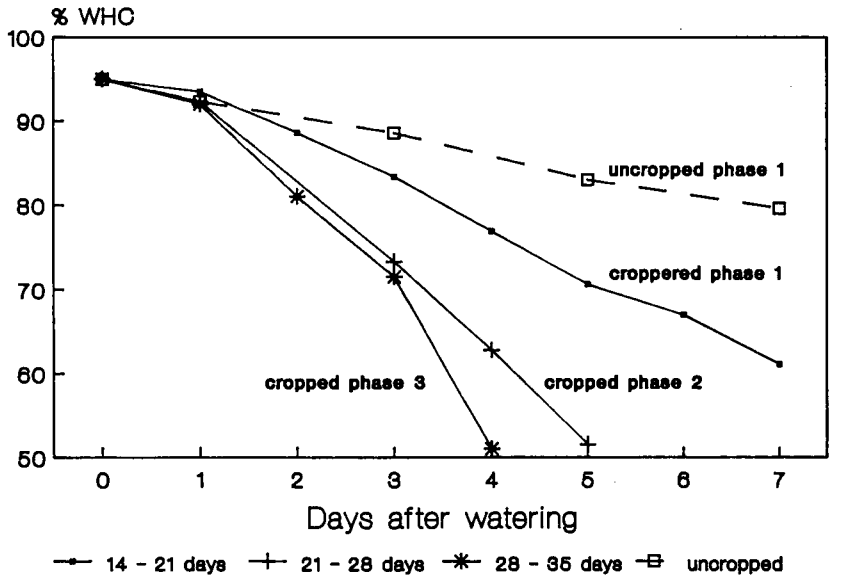
Weeks after sowing	µM N_2O pot^{-1} 24 h^{-1} Control	-K	HSD 5 %
2	4.9	8.5	3.2
3	5.0	25.8	11.5
4*	11.7	44.6	8.5
5	n.d.	27.2	---
HSD 5 %:	4.5	15.5	

* 50 mg NO_3^--N per pot added before measurement

The influence of the soil water content could be demonstrated in an experiment in which denitrification was measured in the period of 2 to 5 weeks after sowing (Table 1), the water status having been raised from 60 to 95 % WHC at each start of the measurements (Figure 1). During the vegetation phase between the 14th and 21st day soil moisture declined due to water uptake of plants from 95 to about 60 % WHC within a period of 7 days. Thereafter WHC decreased more rapidly, namely within a period of 4.5 days between the 21st and 28th day and in about 3.5 days in the last phase. In this soil no denitrification had occurred, when the water content

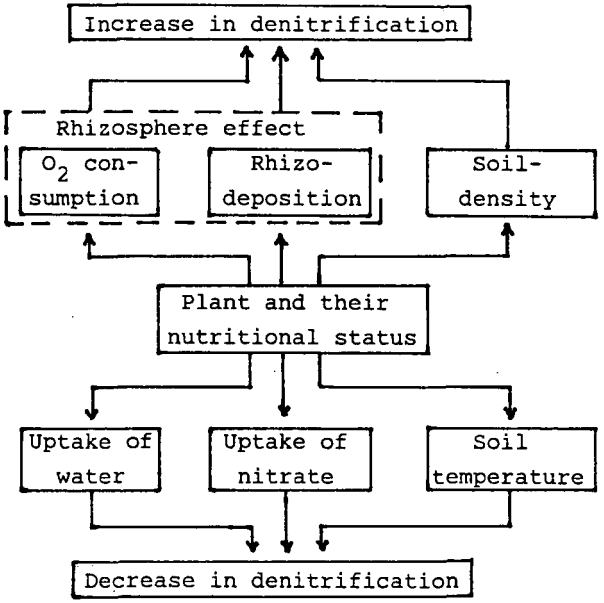
declined to 60 % WHC. In contrast, soil moisture decline in the absence of plants did not go further than to 80 % WHC in all phases of observation. This shows that the soil water decline in its dependence on dry matter production is greater in the presence of plants with the consequence that denitrification is reduced or does not occur at all.

Figure 1: Water content of cropped and uncropped soil Berkhof



The schematic representation (Figure 2) shows on the one hand that plants are well able to provide conditions that may result in enhanced denitrification due to their production of organic substances, the increased O_2 consumption by respiration of roots and rhizosphere microflora as well as due to greater soil density. On the other hand, compared with uncropped soil, the risk of denitrification in cropped soil is reduced because of decreased soil moisture, higher nitrate uptake and lower soil temperature. Consequently, the combination of parameters decides, whether the presence of plants will increase or decrease denitrification.

Figure 2: Influence of plants and their nutritional status on denitrification in comparison with uncropped soil



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Seasonal variation in denitrification in a clay soil under a cultivated crop and a permanent pasture

by

K.L. Weier[1] amd I.C. MacRae[2]

ABSTRACT

Biological denitrification in a clay soil was measured by sampling the soil atmosphere beneath black gram (Vigna mungo) and green panic (Panicum maximum) prior to the application of water, water plus acetylene, and water plus acetylene plus glucose treatments and again 24 h later.

Before the application of the treatments, production of N_2O from crop and pasture was greatest in spring and summer with approximately 75% of the total amount being produced. Under black gram, higher concentrations of N_2O occurred below 50 cm.

Soil moisture was correlated with N_2O evolution beneath black gram ($r = 0.87$, $df = 6$, $P<0.01$) and CO_2 production under green panic ($r = 0.69$, $df = 6$, $P<0.05$). Multiple regression analysis showed that 73.9% of the variation in N_2O production from beneath black gram was associated with monthly rainfall, nitrate concentration and soluble carbon.

Gas samples collected 24 hr after the application of treatments showed no significant difference between treatments in N_2O and CO_2 production.

INTRODUCTION

When fertile clay soils of the brigalow lands of eastern Australia are cultivated and cropped, large quantities of nitrate accumulate below the root zone (Catchpoole, 1981). This nitrate may then be susceptible to loss by denitrification or leaching during prolonged wet conditions. Thus, V.R. Catchpoole (pers. comm.) observed that soil nitrate (275 kg N ha^{-1}), accumulated in the subsoil of a cultivated clay soil, was lost from the system during a

[1] CSIRO, 306 Carmody Road, St Lucia, Brisbane, Queensland, Australia.

[2] Dept of Microbiology, University of Queensland, St Lucia, Brisbane, Queensland,

wet winter. One possible pathway for such a loss is biological denitrification where both N_2O and N_2 are produced by microbial reduction of nitrate.

Losses of N from the subsoil have been observed elsewhere (Burford and Stefanson, 1973; Gambrell et al, 1975) while a preliminary study on the above soil (Weier and Doran, 1987) revealed that these soils had the potential to denitrify but this was limited by soil water content and available carbon. Measurement of these losses was usually made over a set period with no assessment taken of the seasonal variation in denitrification in the field. The first seasonal estimation of N_2O evolution was reported by Bremner et al (1980) where they found that 67% of the N_2O produced was evolved in summer with little production occurring in winter.

In the work reported here, endogenous N_2O production and total (N_2O + N_2) gas production, using C_2H_2 inhibition, were measured beneath a cultivated food legume and a perennial grass pasture to establish if biological denitrification was responsible for the loss of accumulated nitrate and to discover if gas production was affected by seasonal variation.

MATERIALS AND METHODS

The experimental plots of black gram (Vigna mungo) and green panic (Panicum maximum var. trichoglume) were part of an experiment established in 1975 at the CSIRO Narayen Research Station (25°41'S, 150°52'E). The area has a sub-humid, subtropical continental climate with a mean annual rainfall of 710 mm. The soil is a black earth of principal profile form Ug5.13 (Northcote, 1965) characteristics of which are given in Table 1.

For the collection of the soil atmosphere samples, cylindrical brass probes were installed to depths of 7.5, 22.5, 52.5 and 112.5 cm beneath each plot. The probes were 6.35 mm in diameter (4.6 mm I.D.) containing 32 perforations (1.5 mm diameter) evenly spaced over a distance of 40 mm from the closed (lower) end of the probe. Three soil treatments were established by delivering down the probe to the soil at each depth 2 ml of: (1) water only (2) water saturated with C_2H_2 and (3) water saturated with C_2H_2 and containing 800 μg glucose. Each probe was then sealed with a rubber septum and a volume of gas equivalent to 125% of the dead space volume of the probe withdrawn and discarded.

TABLE 1. Characteristics of the soil underlying the experimental plots.

PLOT	DEPTH (cm)	pH	TOTAL N (%)	TOTAL C (%)	MINERAL-N* ($\mu g\ g^{-1}$ soil) NO_3^--N	NH_4^+-N	BULK DENSITY ($Mg\ m^{-3}$)	TEXTURAL ANALYSIS (%) SAND	SILT	CLAY
BLACK GRAM	5-10	8.2	.22	0.75	21.8	3.5	1.07	19.7	17.4	58.4
	20-25	8.3	.12	1.65	20.9	3.2	1.37	17.7	15.9	63.6
	50-55	8.7	.06	1.73	11.9	2.7	1.50	15.7	15.5	60.8
	110-115	9.3	.02	2.29	19.7	2.3	1.45	19.8	19.5	41.0
GREEN PANIC	5-10	8.3	.28	3.28	0.9	3.7	1.11	15.0	18.2	59.7
	20-25	8.6	.14	1.73	0.6	2.8	1.37	14.9	18.8	63.1
	50-55	8.8	.05	1.19	0.7	3.1	1.43	12.7	17.3	64.5
	110-115	8.5	.04	0.97	0.6	2.3	1.61	13.4	11.9	65.4

* *Values are the means of 8 sampling periods.*

The soil atmosphere at each depth was sampled prior to and 24 hr after the application of the treatments using evacuated venoject tubes (12.8 cm^3) and double ended needles. N_2O, CO_2 and O_2 were measured by gas chromatography.

Total N was estimated by Kjeldahl digestion using the modification of Dalal et al (1984) while total C and soluble C were measured using a modification of the procedures of Carr (1973) and Heanes (1984) respectively. Mineral N was extracted with 2M KCl (Catchpoole and Weier, 1980), and the NO_3^--N and NH_4^+N measured colorimetrically (Henzell et al, 1968).

RESULTS

Unamended soil

The total quantities of N_2O evolved from the profiles at a single sampling were greater from beneath black gram (1.7-26 g N_2O-N ha^{-1}) than from beneath green panic (0.7-19 g N_2O-N ha^{-1}). N_2O concentrations were highest in spring and summer (September to February) and lowest in autumn and winter (March to August). Distribution of N_2O evolution over the season was estimated to be approx. 75% in spring-summer for both crop and pasture, with almost equal amounts in spring and summer under black gram but 54% in spring and 24% in summer under green panic. N_2O concentration under black gram tended to increase with depth with the greater production occurring below 50 cm.

The greatest proportion of CO_2 were evolved in the summer months (December to February) (42% for black gram and 46% for green panic) with similar proportions being evolved from both profiles during the other seasons of the year. Beneath black gram, evolution of CO_2 was greater from depths below 50 cm, where 73% of CO_2

was produced. Variations in CO_2 production beneath green panic showed no change or a slight decrease with depth.

There was little difference in NH_4^+-N values between plots but black gram had approx. 20 times more nitrate present in its profile than green panic (Table 1).

Results for soluble C were similar for the first three depths beneath both crop and pasture but there was a significantly higher concentration of C present at the 110-115 cm depth beneath black gram than under green panic (mean values of 33.1 and 18.5 μg C g^{-1} soil respectively).

N_2O production from the black gram plot was closely related to % WFPS ($r = 0.87$, $P<0.01$, Figure 1). No such relationship was found for green panic but in terms

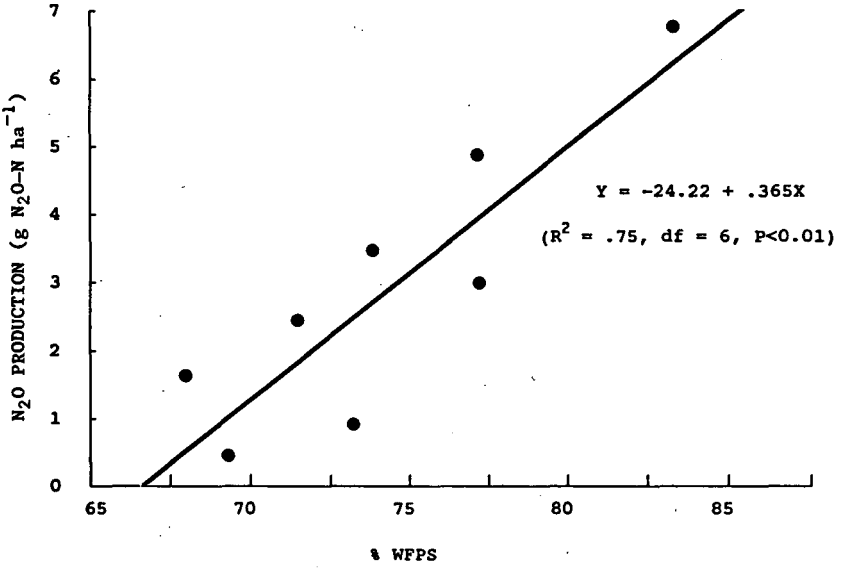

Figure 1. The relationship between N_2O production from unamended soil in the black gram plot and % WFPS ($r = 0.87$).

of water relationships, a significant correlation was observed between monthly rainfall and CO_2 production beneath green panic ($r = 0.69$, $P<0.05$, Figure 2).

Amended soil

There was no significant difference between treatments, in either N_2O or CO_2 concentration, 24 hr after they were applied. N_2O evolution was again higher from beneath black gram (3.3-31.8 g N_2O-N ha^{-1}) than from beneath green panic (0.07-19.4 g N_2O-N ha^{-1}). The pattern of N_2O production alluded to at the 0 hr sampling

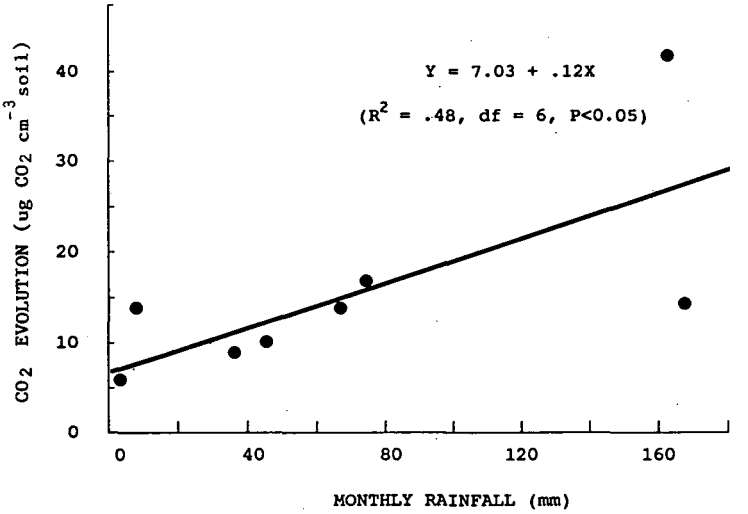

Figure 2. The relationship between CO_2 evolution from unamended soil in the green panic pasture and monthly rainfall (r = 0.69).

for black gram was again followed but greater quantities of gas were evolved after the treatments were applied. This was not the case for green panic, where there was a decrease in N_2O production associated with the treatments. Values obtained for CO_2 production after 24 hr were very similar to the 0 hr values for both black gram and green panic.

DISCUSSION

Differences in the N_2O evolved between crop and pasture before the application of the treatments may have reflected differences in nitrate concentrations in the respective soil profiles. The smaller concentrations of N_2O detected beneath the pasture may have been a result of greater N_2 evolution because of lower nitrate concentrations (Limmer *et al*, 1982) but this was not confirmed on the measurement of total gas production.

The seasonal pattern in N_2O production observed beneath black gram was similar to that found by Bremner *et al* (1980) for soybean in Iowa soils. Winter production was much smaller than observed at Narayen and was attributed to the lower temperatures. N_2O evolution beneath black gram was closely related to soil moisture with no relationship being found with soil temperature.

The increase in N_2O production with depth beneath black gram indicated that nitrate may have been lost from the subsoil through denitrification. The presence of large quantities of soluble C at 110-115 cm beneath black gram and greater production of CO_2 below 50 cm may mean that there is sufficient substrate for the microbial population. Thus, percolation of soluble organic C to the lower depths after heavy rainfall may provide the energy source for microbial reduction of nitrate.

A multiple regression analysis of the data from the black gram plot relating N_2O production to monthly rainfall, nitrate concentration and soluble C gave the following equation:

$$y = 0.39x_1 - 0.39x_2 + 0.24x_3$$

where y = N_2O production from the soil profile x_1 = monthly rainfall

x_2 = nitrate concentration and x_3 = soluble carbon.

The multiple regression coefficient was highly significant and accounted for 73.9% of the variation in N_2O production ($r = 0.86$).

The absence of any differences between the 3 treatments in N_2O and CO_2 production may mean that either the conditions established on application of the treatments did not vary greatly from the conditions that already existed beneath both systems or that the evolution of dinitrogen from the soil profile was not large enough to influence the results. The failure of glucose to increase gas production implies that the available C present at 0 hr was sufficient for the needs of the microbial population. The absence of an increase in respiration of C, 24 hr after treatment application, seems to verify the observation.

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Characterization of denitrifying bacterial communities with distinct trophic requirements from soil under various agricultural use

by

Weissenhorn,I., Munch,J.C. and Fischer,W.R+)

SUMMARY

Total and denitrifying bacterial counts as well as the spectrum of denitrifiers and their specific denitrification behaviour of 15 soil samples (from 5 plots at 3 dates) were investigated by using 3 different nutrient media (copiotrophic, oligotrophic, minimal).

The results showed that copiotrophic nutritional conditions support microbial communities in which, under anaerobic conditions, fermentation predominates over denitrification. On the other hand, the oligotrophic and the minimal medium selected obligately respiratory organisms with intensive denitrification activity in case of oxygen-deficiency. This shows that both type and concentration of organic substrate are important for the composition of the denitrifying communities as well as for the denitrification potential of a soil. It also underlines the necessity of using a range of various culture media for the characterization of such a heterogeneous and variable group of soil microorganisms as denitrifiers are.

INTRODUCTION

Denitrification is an ubiquitous, facultative microbiological metabolic process carried out by a wide range of physiologically and taxonomically different types of bacteria (FOCHT & VERSTRAETE, 1977; KNOWLES, 1981,1982; PAYNE, 1981,1985; SCHMIDER, 1985). For the investigation of denitrifying bacterial communities as present in complex habitats like soil, (waste-)water, etc. it seems therefore not to be adequate to use only a single, often copiotrophic standard-medium as usually is practised. The intention of the present work was to improve the method for characterization of the heterogeneous denitrifying microflora exemplarily for an agriculturally used soil. For that purpose, the number and spectrum of denitrifying bacteria were determined in a range of samples taken from soil under various agricultural use during three months, with media varying in nutritional composition. The denitrification behavior of the isolated pure denitrifying strains was examined in model experiments.

+) *Inst. f. Bodenkunde & Standortslehre, Univ.Hohenheim, D-7000 Stuttgart 70*

MATERIALS AND METHODS

Soil samples were taken from the A_p horizon (0-30 cm) of an alfisol from 5 plots (tab.1) of an agricultural field experiment varied in N-supply, crop and tillage after harvest. Samples were taken at three dates: before harvesting (september), soon after harvest (october) and in winter (december).

TAB.1: Description of field samples

plot	N-supply	crop	tillage
1	none	fallow	cultivator
2	biolog.N_2-fixation	*vicia faba*	cultivator
3	$Ca(NO_3)_2$+NH_4NO_3	*zea mays*	none
4	liquid organic manure	*zea mays*	none
5	like plot 4 + Didin[1)]	*zea mays*	none

1) nitrification inhibitor: dicyandiamide

Determination of total and denitrifying bacterial counts was performed by the MPN-method: 20 g of soil were, immediately after sampling, suspended in 180 ml of physiological salt solution and serially diluted (10^{-4} - 10^{-8}) for inoculation of the different liquid nutrient media (5 test tubes per both dilution and medium: tab.2; 1 ml inoculum per tube). Incubation was performed at corresponding field temperatures (15, 9 and 6°C, respectively) to avoid additional selective effects; Durham fermentation tubes were used to indicate production of gas. Most Probable Numbers were obtained from de MAN's table (1983).

Denitrifiers were **isolated** from 5 tubes with denitrification (gas bubbles) of the highest decimal dilutions from the MPN-determination test from each medium (tab.2). After two-fold subcultivation in the corresponding liquid medium (enrichment culture) the bacteria were streaked on similiar solid medium. Macroscopically different colonies were subcultivated on agar plates until yielding pure cultures for which denitrifying ability was checked by reinoculation of liquid media containing Durham fermentation tubes. Only gas producing strains were cultivated furthermore and **differentiated** by evaluating the following characteristics: colony and cell morphology, mobility, Gram staining, formation of endospores, catalase reaction, oxidase reaction (KOVACS, 1956), oxidative/fer-mentative glucose catabolism (HUGH & LEIFSON, 1953). The different isolates were **classified** according to BERGEY's Manual of Determinative Bacteriology (KRIEG & HOLT, 1984).

The denitrification behavior of the isolated pure strains was examined by gaschromatographic determination of produced CO_2, N_2O and N_2. For that, each isolate was incubated (30°C) in a 50 ml tube with 25 ml of liquid nutrient medium and 100% Helium atmosphere, closed gastight with a septum. Every 6 - 10

hours 0,5 ml of the tube atmosphere were taken by a syringe for analysis.

TAB.2: Culture media for characterization of denitrifying bacteria

Name Characterization	KNO_3 (g/l)	Tot.org.matter (g/l)	Na-citrate (g/l)	Vitamines
MM[1] minimal	3	9,5	8,5	-
CME[2] complex-eutrophic	3	13,0	3,0	+
CMO[2] complex-oligotr.	3	0,3	0,1	+

[1] after BOLLAG et al.(1970)
[2] after TOMLINSON & HOCHSTEIN (1972)

RESULTS

1. Absolute and relative counts of denitrifying bacteria

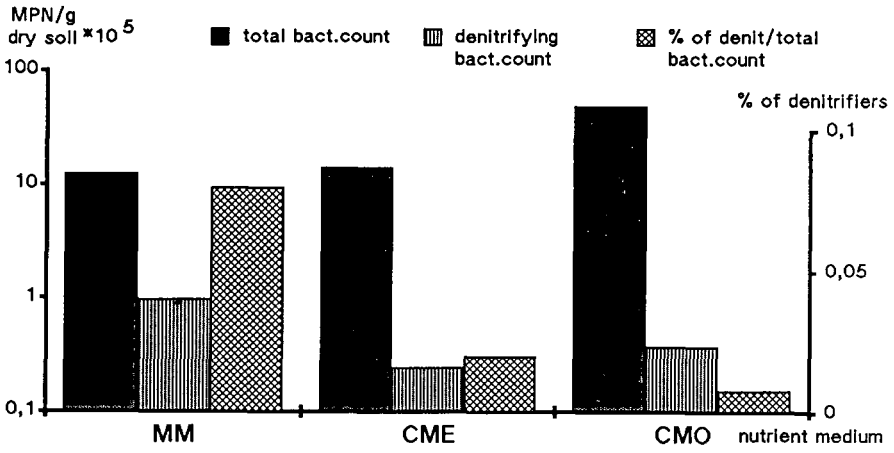

FIG.1: Logarithmic plot of the absolute and relative counts of denitrifiers (average of all 15 soil samples) determined by using 3 different nutrient media

The total and denitrifying bacterial counts are shown in fig.2 and fig.3. Both total and denitrifying bacterial counts depended on the used culture medium (fig.2) as well as on the date of sampling (fig.3). With the oligotrophic medium (CMO), much higher total numbers of bacteria were determined than with the eutrophic media (CME, MM). On the other hand, the highest counts of denitrifiers were found using the minimal medium. Soon after harvest, remarkably more bacteria were counted than before harvest and in winter. Against that, the percentage of denitrifiers was much lower at that second date of sampling.

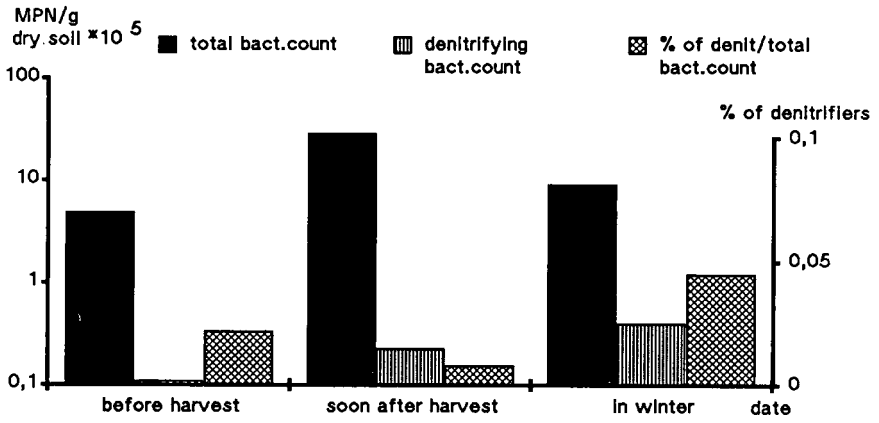

FIG.2: Logarithmic plot of the absolute and relative counts of denitrifiers (average of the 5 field variants) at 3 different dates determined with medium CME

2. Spectrum of denitrifying bacterial species

TAB.3: Classification of the denitrifying bacterial strains isolated on the different culture media

isolation medium	N*	main taxonomic** groups	metabolism	trophic/ecolog. characterization
CME complex eutrophic	16	*Vibrionaceae Enterobacteriaceae*	facultatively fermentative	auxotrophic zymogenous
CMO complex oligotrophic	4	*(Pseudomonas) (Alcaligenes) Coryneform bacteria*	obligately respiratory	partially prototrophic autochthonous
MM minimal	18	*Pseudomonas Alcaligenes Coryneform bacteria*	obligately respiratory	prototrophic autochthonous

* number of differentiated strain-types
** () = not predominant

The classification of denitrifying bacteria from the 15 soil samples (from 5 plots at 3 dates) is shown in tab.3. There was only little overlapping between the strain types isolated by the three different nutrient media: one strain appeared on both the oligotrophic and the minimal medium, another one on the minimal medium as well as on the copiotrophic medium.

3. Denitification behaviour of the isolated pure denitrifying strains

Though each of the investigated strains showed a specific composition of the produced gas (CO_2, N_2O and N_2), it could be attached to one of three types of

metabolism (types I, IIa and IIb; fig.3):
Most of the strains isolated with the copiotrophic CME-medium (*Enterobacteriaceae, Vibrionaceae*) showed a high CO_2-production, while only less gaseous N-compounds were formed from nitrate. Fermentation predominated over denitrification (Type I).
All strains obtained with oligotrophic (CMO) or minimal (MM) medium (*Alcaligenes, Pseudomonas, Coryneform bacteria*) produced remarkably more gaseous nitrogen than CO_2 which could be attached to a respiratory metabolism. Type II a produced N_2O only at the beginning and it has been completely reduced to N_2 at the end of measurements. Type II b exclusively formed N_2O which obviously could not be reduced to N_2, at least under the given conditions.

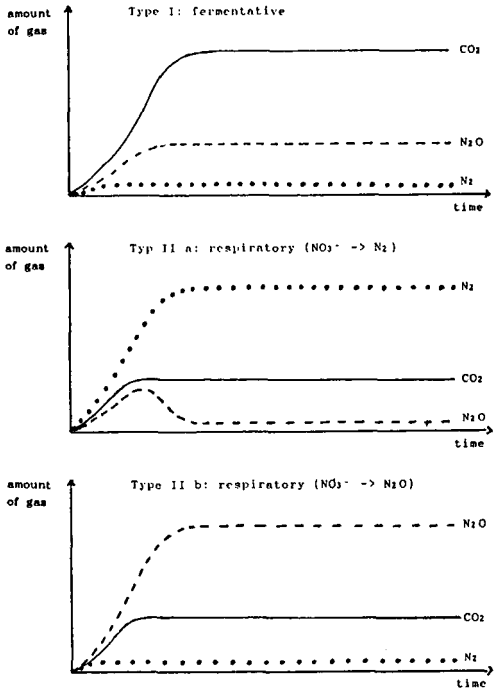

FIG.3: Schematic diagram of the three metabolic types which the isolated strains could be attached to by their CO_2-, N_2O-and N_2-production

DISCUSSION

The various nutrient media selected different communities of the soil microflora, each with a characteristic percentage of denitrifiers which show a distinct taxonomical and physiological composition. The amount of these different communities in the whole soil microflora varied depending on the date of sampling. Copiotrophic nutritional conditions (as given by medium CME and - in

situ - soon after harvest by crop residues) supported the denitrifying microbes less than a poorer ("minimal") supply did (MM-medium and, in situ, before harvest and in winter) and selected mainly facultatively fermentative bacteria where fermentation predominated over denitrification. On oligotrophic and minimal media, on the other hand, exclusively obligately respiratory microbes were isolated that showed an intensive denitrification activity under anaerobic conditions.

These results elucidate that both kind and amount of available organic matter in soil are very important for the composition of the denitrifying communities (DAVIES & TOERIEN, 1971; FABIG & OTTOW,1979; BENCKISER, 1980; NURSE, 1980; SCHMIDER, 1985) and - because of the specific denitrifying activity of the different strains (BURTH-GEBAUER, 1983; MUNCH & OTTOW, 1986) - also for the denitrifying potential of an ecosystem. Only the simultaneous use of various nutrient media allows it to estimate the heterogeneity and variability of the denitrifying communities in complex habitats like agricultural soils.

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Denitrification in the rooting zone of cropped soils with regard to methodology and climate

by

G. Schollmayer +) and R. Nieder + +)

Introduction

Estimated losses of nitrogen through denitrification vary greatly not only among different agricultural ecosystems but also within one crop production unit. These variations are due in part to differences in methodology as well as to the inherently high temporal and especially spatial variability of denitrification in the field. Presently available denitrification data do not permit precise global extrapolations and many of the nitrogen budgets published reflect values assigned to denitrification that were forced in order to "balance the nitrogen cycle". That means, values were often assigned equal to the discrepancy in all other inputs and outputs.

It is argued that denitrification is still gaining significance due to increases in the combined nitrogen inputs from either biological or industrial N_2-fixation. The rapid increase in application of N-fertilizers is being followed by an appreciable rise in the nitrate contents of surface and underground waters as well as by rising atmospheric pollution by oxides of nitrogen responsible for the destruction of the life preserving ozone layer. A better understanding of soil and environmental controls combined with better quantitative estimates of denitrification are increasingly required to control this process before management practices for a reduction in pollution can be developed. Research techniques developed recently enable us to quantify more precisely the nitrogen transformations involved in the denitrification process under field conditions.

Methods of measurement

Until now most studies to estimate denitrificative N-losses have used indirect methods. Techniques for direct measurement of gaseous nitrogen products have become available during the last decade. Actually, total denitrification losses can be determined by three methods.

a) Nonrecovery of ^{15}N-labeled compounds

This method, which allows the quantification of long term gaseous nitrogen losses is available since the early fifties. Denitrification losses are deduced from the balance of a nitrogen budget having accounted for transformations in soil, plant uptake and leaching losses at the end of a vegetation period. The procedure demands labeled fertilizer nitrogen (^{15}N). One disadvantage of this method is that it does not account for denitrification of the native soil nitrogen and moreover, this approach does not identify the mechanisms responsible for N loss. But the major factor limiting the precision of the denitrification estimate is the determination of total nitrogen in the system which is the base for reliable mass spectrometric determination of ^{15}N abundance in the nitrogen pools of soil and plant material.

b) In situ field measurements of $^{15}N_2$ production

For this procedure again the isotope ^{15}N is needed. Fluxes of labeled gases ($^{15}N_2O$ and $^{15}N_2$) emitted by denitrification are currently measured by mass spectrometric detection after collecting them with in situ enclosures. The method has the same disadvantage as the ^{15}N balance approach since it does not include gaseous nitrogen evolved by the native soil N pool.

c) Acetylene inhibition technique

Since the late seventies procedures were developed which base on the inhibition of bacterial N_2O reduction to N_2 in presence of acetylene (C_2H_2). The product (nitrous oxide) can be easily measured by gas chromatography. The advantage of this method is that it determines the denitrification of all nitrate nitrogen irrespective of its source. The experimental set up in the field consists essentially of three components: a chamber to measure the surface N_2O flux, a system to take gas samples of the soil atmosphere in function of depth and a system to inject C_2H_2 into the soil *(in situ treatment)*. Indeed, this treatment involves minimal physical disturbance of the soil. But the fundamental problem of this procedure in the field refers to the question whether the measurement of the surface N_2O-flux reflects the total N-loss by denitri-fication i.e. N_2O surface flux corresponds to N_2O produced. The main difficulty associated with this approach may be the restricted diffusion of C_2H_2 or N_2O in wet, compacted soils with low air filled porosities. The second problem is that long term exposure of acetylene to soil can affect the mineralization of soil

+) Geologisches Landesamt NW, De-Greiff-Str. 195, 4150 Krefeld

+ +) Inst. f. Geographie, Langer Kamp 19c, 3300 Braunschweig

organic matter or inhibit the oxidation of ammonia to nitrate. The effect of nitrification is of particular importance in assessment of denitrification in farming systems where nitrogen, returned to the soil in manure or semi-liquid manure, is present initially as organic or ammonium-N.
Recently, a variation of the *in situ treatment* has been developed which seems to overcome many of the constraints of enclosure systems: the *incubation of soil cores*. Using this procedure, acetylene amended air is recirculated through intact soil cores with about 2-5 cm in diameter. In contrast to the *in situ treatment* with enclosures, the incubation method achieves rapid diffusion of C_2H_2 across the small radius of soil cores and is therefore considered to provides a reliable estimate of the denitrification rate even in poorly drained soils. A further advantage is the relatively short term exposure of soil to acetylene which overcomes the problem of inhibited nitrification and reducing the rate of denitrification.

Denitrification on arable land

Estimation of N-losses from arable land

Magnitudes of denitrification rates on arable land obtained by the ^{15}N-balance technique or the direct measurement of evolved $^{15}N_2$ can be drawn from table 1, which also contains informations about the experimental sites and cultivation. Regarding the estimated gaseous N-losses, the different experimental periods (last column) have to be taken into account.
Much more data on ^{15}N-balance studies are available (reviewed e.g. by Fleige and Capelle 1974; Capelle and Baeumer 1985), but not all of them can be cited here. Most of the ^{15}N-balance investigations reflect estimated N-losses in the vegetation period of annual crops as being part of investigations on N-fertilizer efficiency. The amount of fertilizer-N not recovered in the soil plant system at the end of the investigation period varies in the range of 20-50 % of the applied nitrogen (= 10-70 kg N ha^{-1}). As the determination of total nitrogen still limits the precision of any estimate of denitrification, results obtained by the ^{15}N-balance procedure have to be handled with great care. Moreover, the evolution of native soil nitrogen cannot be measured by this way.
For assaying reliable estimates of denitrification rates, sensitive and precise measurements of total N_2 and N_2O evolved from the soil is the necessary basis. The acetylene inhibition technique is the most recent advance for quantifying directly the denitrification flux. Losses of nitrogen from arable land measured by the C_2H_2 inhibition technique can be drawn from table 2. As most of the direct field measurements in the temperate regions show, only up to 20 - 30 kg N ha^{-1} are lost via denitrification during the growth period of annual crops. Comparison of the results obtained by the acetylene inhibition technique with those estimated by the nonrecovery of ^{15}N-labeled compounds (see table 1) shows that the former only accounts for about one half or one third of the N-loss recorded by the latter. Similar discrepancies in the determination of N-losses are reported from simultaneous direct gas flux measurements and ^{15}N-balance estimates in the field (Germon 1985; Parkin et al.1985; Heinemeyer et al.1988). On the one hand these discrepancies may be caused by an overestimation of ^{15}N-loss due to errors in determination of total nitrogen by the Kjeldahl procedure (Knowles 1981). On the other hand there are indications for a possible underestimation of denitrificative N-loss by the acetylene blockage method: one reason may be that N-losses by diffusion into deeper soil horizons were not taken into account. A further reason for underevaluating the denitrification flux may be the limited diffusion of C_2H_2 in relation to the time needed for the required C_2H_2-concentration, especially in poorly structured soils.
This aspect is particularly significant for acetylene treatments with enclosures on, e.g. clay soils, that require several days to achieve a quasi-homogeneous distribution of C_2H_2 sufficient to inhibit N_2O reduction. Because of this problem, nitrogen losses observed on gleyic soils or clay soils by the enclosure technique may be too low in relation to the actual N_2O-production (see table 2). This hypothesis is confirmed by experiments of Ryden et al. (1987) who found disagreements of measuring the N-flux between the enclosure technique and the soil core method applied to poorly drained soils, with higher rates of N-flux using the latter technique. In contrast, there was good agreement between the two measuring methods on well-drained soils over a wide range of denitrification fluxes. In view of the factors listed above, the rates of denitrification measured on drained soils (e.g. xerosols, chernozems, luvisols) by the enclosure procedure seem to represent almost realistic results (see table 2).

Factors affecting field denitrification

Most of the direct field measurements of the denitrification flux reflect maximum rates after rainfall or irrigation events. The temporal response of nitrate reduction after wetting depends greatly on soil structure. Sextone et al.(1985) compared the response of field denitrification to increased soil moisture in a non-aggregated sandy loam with an aggregated clay loam. The nitrate reduction rate

in the sandy loam increased immediately after water addition, reached a maximum within 3-5 *h* and returned to preirrigation levels within 12 *h*. A slower denitrification response to irrigation occured in the clay loam soil, requiring 8-12 *h* before a maximum flux was observed and 48-60 *h* before the original background rate was restored. The longer periods prior to increased denitrification flux are probably due to the development of three dimensional mosaics of anaerobic microzones which exist particularly in fine textured soils with a well developed system of aggregates (Smith 1977). Aggregates can remain partly water saturated even though most of the larger pores and fissures between them have been drained. Anaerobic microsites within aggregates can be considered as an important cause for continuation of nitrate reduction even under apparently aerobic conditions (Eh values between 400 and 650 *mV*) in the soil (Ryden and Lund 1980). In contrast to this, experiments on sandy soils reflect significant gaseous N-losses only during a short period after precipitation events. It is likely that in a sandy soil a sufficient diffusion barrier for gaseous oxygen only occurs for brief periods following wetting, as water percolates through the soil macropores.

The above presented relations could give one cause to think that cumulative N-losses from fine-textured aggregated and frequently wet soils (mainly clay soils) may be greater than those from coarse-textured ones. However, direct field measurements (enclosure technique as well as soil core method, see table 2) do not confirm this. The N-evolution rates on gleysoils with high clay contents are about of the same order of magnitude as nitrogen losses on coarser substrates (see especially Parkin et al.1985 and Sextone et al.1985). An explanation for these unexpected findings may be the depletion of available carbon sources necessary to drive the respiratory oxygen and nitrate consumption. For that reason Rolston et al.(1982) as well as Sextone et al. (1985) observed after initial irrigation vigorously decreasing N fluxes following subsequent irrigations in a loam soil as well as in a clay loam soil. Moreover, it has to be considered that in well aerated coarse-textured soils "aerobic denitrification" may take place in special situations (Ottow and Fabig 1985). Gaseous N-fluxes from an aerobic and microbiologically active soil to which organic manure or easily decomposable plant residues have been applied can be distinctly larger than those from wet, poorly structured and compact sites with relatively low contents of available carbon.

On the other hand, field experiments on one and the same soil with 3 % organic C compacted by tractor traffic reflect accumulated values of denitrification with four to five times higher rates of N-flux for the compacted as compared to the uncompacted substrate (Bakken et al.1987, see table 2), presupposed wet soil conditions.

Greater rates of denitrification are usually observed in zero tillage compared to ploughed soils. Total annual N-losses are generally two to three times higher when the soil is not tilled (Aulakh et al.1984/I/II, see table 2). This increase in N-evolution is related to increased soil organic matter and greater available C-amounts in the upper part of the top soil as well as to greater soil densities and decreased soil aeration (Myrold 1988).

The effect of growing roots on denitrification is presently discussed controversialy. It has often been observed that living roots have a stimulating effect on the N-evolution flux (e.g. Rheinbaben and Trolldenier 1984; Klemedtsson et al.1987), which is attributed to the stimulation of bacterial respiration by exudation as well as to the reduction of $p\ O_2$ by root respiration. Other studies have shown that plant roots have negative effects on denitrification (e.g. Aulakh et al.1982, see table 2). This may be due to nitrate depletion by root uptake and reduction of soil moisture content by transpiration (Bakken 1988). However, conflicting reports particularly as to the influence of root exudation on denitrification continue to appear in the present literature (reviewed by Bakken 1988).

Indeed, each of the factors cited above undoubtedly exerts some influence on the denitrification dynamics. But with exception of special situations (e.g. organic manure applications, high mineral fertilizer input) in which the N-flux cannot be satisfactory quantified, annual gaseous N-losses do not exeed the order of about 30 kg ha^{-1} in classical crop production systems of the temperate zone.

The extent of denitrification losses observed on intensively cultivated soils of the subtropics (e.g. mediterranean climate of California with dry, hot summers and mild, wet winters) can be considerably greater than in those of the temperate regions (Ryden et al.1979; Ryden and Lund 1980, see table 2). The large N-losses (possibly underestimated because measured by in situ treatment with enclosures) most probably reflect the impact of frequent wetting cycles -especially in the summer months- necessary for high vegetable crop production in this region. Furthermore, denitrification flux can be maintained at a relatively high level throughout the year due in part to the mild winters characteristic for the coastal valley of California. Nitrate reduction on vegetable production units is forced also by the plowdown of large quantities of crop residues.

The conditions for high denitrification rates in the subtropics obviously are not fullfilled in case of deficient summer precipitation or lack of irrigation. In rye grass and winter wheat cropping systems

of Western Oregon (mediterranean climate), Myrold (1988, see table 2) measured denitrification rates using the soil core method of about only 1-2 kg ha^{-1} in spite of high fertilizer-N-inputs and high temperatures throughout the year. The same author even found a negative correlation of denitrification with temperature which was probably caused by the strong negative correlation of temperature with soil water content.

Conclusions

All of the results listed above suggest that only soils receiving adequate inputs of C and N combined with frequent wetting and drying cycles and high tem-peratures should be susceptible to extensive denitrification losses. However, further work is required to assess first of all the accuracy of the new methods for direct measuring of N-loss on more sites and in special management situa-tions in order to a better control of the denitrification process.

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Table 1. Losses of fertilizer nitrogen from arable land estimated by the nonrecovery of ^{15}N-labeled compounds [a] and in situ field measurements of $^{15}N_2$ productions [b]

Author	Location	Soil Type (FAO) and (-Texture)	Soil Cultivation	Land Use	N-Fertilizer [kg*ha^{-1}] and Fertilizing Substances	Denitrificative N-Losses [kg*ha^{-1}]	Experimental Period
Rauhe and Borchak (1968) [a]	Seehausen/GDR [c]	Orthic Luvisol (silty loam)	"conventional"	winter rye [e]	40 sulphate of ammonia	10	growth period of winter rye
-"-	-"-	-"-	-"-	-"-	80 -"-	28	-"-
Pleige et al. (1971) [a]	Einbeck/FRG [c]	Orthic Luvisol (silty loam)	"conventional"	oats [e]	80 sulphate of ammonia	40	growth period of oats
Pleige and Capelle (1974) [a]	Einbeck/FRG [c]	Orthic Luvisol (silty loam)	"conventional"	summer barley [e]	80 potassium nitrate	41	end of april - end of july
Knappe et al. (1974) [a]	Halle/GDR [c]	Cambisol (sandy loam)	"conventional"	winter rye [e]	80 urea	30	growth period of winter rye
-"-	-"-	-"-	-"-	-"-	120 -"-	50	-"-
-"-	-"-	-"-	-"-	-"-	160 -"-	70	-"-
Pleige and Capelle (1975) [a]	Göttingen/FRG [c]	Luvisol (silty loam)	"conventional"	oats [e]	80 sulphate of ammonia	26	end of april - mid august
Focht and Stolzy (1978) [b]	San Emigdio/USA [d]	? (sandy loam)	"conventional"	maize/barley [e]	< 440 sulphate of ammonia	< 5	1 week in summer
Kowalenko (1978) [a]	Caldwell/Canada [c]	Humic Gleysol (clay loam)	"conventional"	fallow land [e]	152 sulphate of ammonia	60	end of may - beginning of september
Limmer et al. (1982) [b]	Hamilton/New Zealand [c]	Andosol (silty loam)	"conventional"	fallow land [e]	? ?	23	november
Sotiriou et al. (1983) a	Freising/FRG [c]	Luvisol (silty loam)	"conventional"	potatoes [e]	50 urea	23	growth period of potatoes
-"-	-"-	-"-	-"-	-"-	100 urea	46	-"-
Colbourn et al. (1984/1) [b]	Denworth/GB [c]	Dystric Gleysol (45 % clay)	"conventional"	winter wheat [e]	53 ammonium nitrate	30	may/june
Capelle and Baeumer (1985) [a]	Göttingen/FRG [c]	Luvisol (silty loam)	"conventional"	oats [e]	80 sulphate of ammonia	47	mid april - mid october
-"-	-"-	-"-	"zero tillage"	-"-	80 -"-	40	-"-
Mosier et al.(1986) [b]	Fort Collins/USA [c]	Vertic Castanozem (36 % clay)	"conventional"	maize/barley [e]	200 sulphate of ammonia	5	beginning of may - end of august

[a] *fertilizer-N-losses estimated by the ^{15}N balance method*
[b] *$^{15}N_2$ and $^{15}N_2O$ evolved measured (only fertilizer-N)*
[c] *temperate climate*
[d] *subtropic climate*
[e] *not irrigated*

Table 2. Total losses of nitrogen from arable land measured by the acetylene inhibition technique

Author	Location	Soil Type (FAO) and (-Texture)	Soil Cultivation	Land Use	N-Fertilizer [kg*ha-1] Fertilizing Substances	Denitrificative N-Losses [kg*ha^{-1}]	Experimental Period
Rydea et al. (1979) [a]	Simas/USA [d]	Salic Xerosol (loam)	"conventional"	celery [f]	335 different substances	51	123 days (in summer months)
Rydea and Lund (1980) [a]	Simas/USA [d]	Salic Xerosol	"conventional"	intens. vegetable cultures [f]	290-665 ?	95-233	1 year
Aulakh et al.(1982) [b]	Saskatoon/Canada [c]	Orthic Chernozem (clay loam)	"conventional"	summer wheat [e]	100 urea	< 3	beginning of may - end of november
-"-	-"-	-"-	"conventional"	fallow land	-"-	9	-"-
Aulakh et al. (1984/I) [b]	Saskatoon/Canada [c]	Orthic Chernozem (clay loam)	"conventional"	summer wheat [e]	45-75 urea	3-7	1 year
-"-	-"-	-"-	"zero tillage"	-"-	-"-	12-16	1 year
Aulakh et al. (1984/II) [b]	Saskatoon/Canada [c]	Orthic Chernozem (clay loam)	"conv. - straw"	summer wheat [e]	100 sulphate of ammonia	4	beginning of june - mid september
-"-	-"-	-"-	"conv. + straw"	-"-	-"-	7	-"-
-"-	-"-	-"-	"zero till. - straw"	-"-	-"-	12	-"-
-"-	-"-	-"-	"zero till. + straw"	-"-	-"-	23	-"-
Rheinbabea and Trolldenier (1984) [a]	Hannover/FRG [c]	Fluvic Gleysol (36 % clay)	"conventional"	fallow land [e]	160-320 calcium ammonium nitrate	10	beginning of may - end of october
Germon (1985) [a]	Dijon/France [c]	Different Soils	"conventional"	cereals [e]	100-200 different substances	about 10	1 year
Parkin et al. (1985) [b]	East Lansing/USA [c]	Stagno-Dyst. Gleysol (34 % clay)	"conventional"	fallow land [e]	4,4 potassium nitrate	10	may/june
-"-	-"-	Salic Luvisol (sandy loam)	-"-	-"-	14,2 ammonium nitrate	5	-"-
Sexstone et al. (1985) [b]	East Lansing/USA [c]	Stagno-Dyst. Gleysol (34 % clay)	"conventional"	fallow land [e]	4,4 potassium nitrate	12	30 days in summer
-"-	-"-	Salic Luvisol (sandy loam)	-"-	-"-	14,2 ammonium nitrate	6	-"-
Benckiser et al. (1986) [a]	Braunschweig/FRG [c]	Orthic Luvisol (silty loam)	"conventional"	sugar beets [e]	180 ?	12	beginning of april - end of october
Bakken et al. (1987) [a]	As/Norway [c]	Dystric Gleysol (26 % clay)	"conv., not compacted"	cereals [e]	about 100 composite fertilizer	3-5	end of may - beginning of august
-"-	-"-	-"-	"conv., compacted"	-"-	-"-	15-20	-"-
Colbourn and Harper (1987) [a]	Deachworth/GB [c]	Dystric Gleysol (45 % clay)	"zero till., drained"	winter wheat [e]	about 150 ammonium nitrate	9	beginning of november
-"-	-"-	-"-	"zero till., undrained"	-"-	-"- ammonium nitrate + calcium nitrate	14	- beginning of june
Myrold (1988) [b]	Willamette/USA [d]	Gleyic Luvisol (silty loam)	"conventional"	annual ryegrass [e]	107 ?	1,7	1 year
-"-	-"-	Gleyic Castanozem (silty loam)	-"-	winter wheat	203 ?	0,7	1 year

[a] *total N-losses after C_2H_2 inhibition technique (in situ treatment with enclosures)*
[b] *total N-losses after C_2H_2 inhibition technique (soil core method)*
[c] *temperate climate*
[d] *subtropic climate*
[e] *not irrigated*
[f] *irrigated*

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Denitrification losses from manured and fertilised soils, and the problems of measurement

by

Smith, K. A. +)

Introduction

In non-flooded soils, denitrification occurs in anaerobic zones or microsites within a generally aerated matrix (Fig. 1). The process shows great spatial and temporal variability, due mainly to factors such as structure, air-filled porosity, soil water content and temperature, and to the distribution of decomposable organic compounds. Estimation of the extent of N losses by denitrification is made even more difficult by problems of measurement.

Nonetheless, some important features have been established. For example, there is clear evidence that nitrate N promotes denitrification, and that in many arable and grassland soils typically 10-30 per cent of the N applied may be lost in this way. For example, estimated losses of the 335 kg/ha applied to intensively farmed land in California amounted to 15 per cent over a 4-month period, and 25 per cent over a year (Ryden et al., 1979). Ammonium-N has been shown to give lower losses than nitrate, because nitrification has to take place before denitrification becomes possible.

Animal manures also promote denitrification in warm conditions, when mineralisation and nitrification are rapid, but in very cool conditions there may be little loss of N compared with that from mineral nitrate (Egginton and Smith, 1986).

Flooded soils, e.g. rice paddies, have a continuous anaerobic

+) The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG, Scotland, U.K.

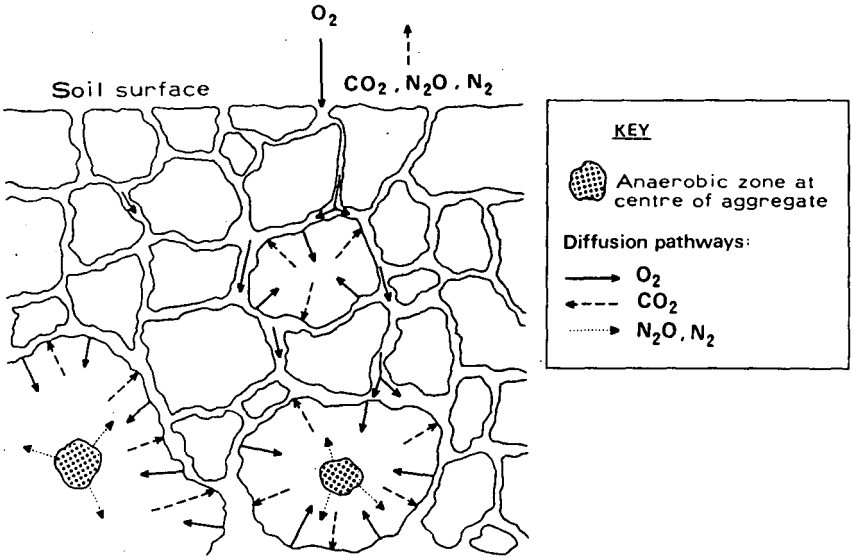

Figure 1 Denitrification in anaerobic zones in soil aggregates.

soil layer underlying a thin aerobic surface layer. In warm conditions large losses by denitrification can occur, even from ammonium fertilisers, if they are surface-applied, because of rapid nitrification and subsequent downward diffusion of nitrate into the anaerobic layer.

Problems of Measurement

The available data on denitrification losses from fertilised soils are as good, or bad, as the methods of measurement allow. The most widely used method for measuring denitrification in the field in recent years has been the "cover box" method, in which the soil profile is perfused with acetylene, to prevent any reduction of N_2O to N_2, and the N_2O released from the soil is trapped under an inverted box cover and measured by gas chromatography. Studies using this method in which high denitrification losses have been found have tended to be on soils which are by no means the most

impermeable (e.g. Ryden et al., 1979). In contrast, in poorly drained heavy clays or glacial tills, in which it might be expected that denitrification would be a major source of loss of N, measured losses have tended to be small (Colbourn et al., 1984a).

It has now been established that the cover box method may fail completely in coarsely structured clayey soils, because the acetylene cannot diffuse to the sites of anaerobic activity within a reasonable time (Arah, 1988). This effect leads to the highest observed, as opposed to real, denitrification fluxes occurring in soils of intermediate texture, coarseness of structure, and/or drainage status (Table 1). Such soils are most likely to have microsites of anaerobic activity within aggregates small enough to allow the acetylene inhibition to take place.

Table 1. Observed rates of denitrification at sites on Edinburgh School of Agriculture farm, using cover box method.

Site/Soil/Land Use	Denitrification rate (g N/ha/d)	Reference
1. Macmerry SCL; grassland, reasonably structured	up to 2500	Egginton & Smith, 1986
2. Macmerry/Winton SCL/CL; continuous arable, bad structure	< 5	Arah, 1988
3. Alluvial loam	up to 600	Smith et al., 1989
4. Darvel coarse sandy soil	up to 180	Arah, 1988

The very low values for denitrification flux from the poorly drained soil at site 2 (Table 1) were measured in the spring and early summer of 1986. They contradicted strong evidence of considerable N loss by denitrification at this site: the direct evidence of high N_2O concentrations in the soil profile (Fig. 2), and the indirect evidence of a very poor recovery of labelled nitrate fertiliser in this particular season. In view of the

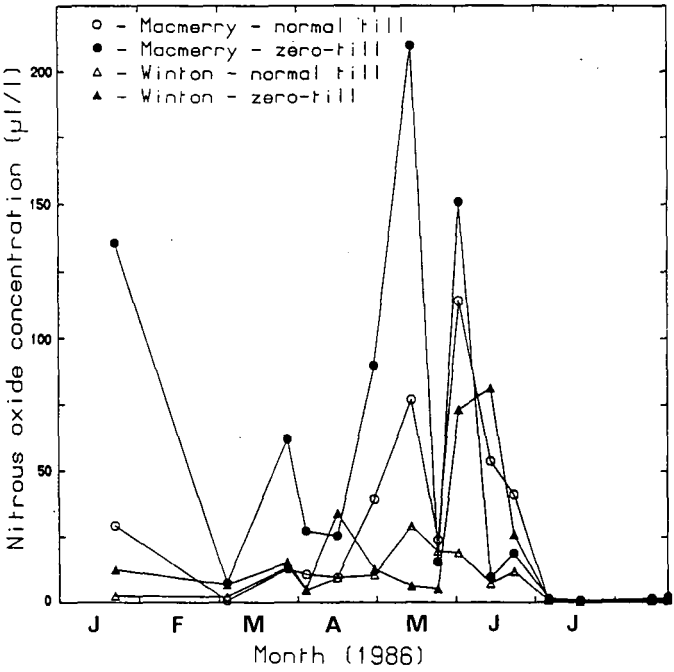

Figure 2 Nitrous oxide concentrations in soil profile, site 2.

failure of acetylene to diffuse more than a few cm into the soil at site 2 even after 10 h it must be concluded that virtually all of the gaseous product of denitrification at this site was N_2. The presence of less N_2O in the Winton soil than in the somewhat better drained Macmerry (Fig. 2) also points in this direction.

Supporting evidence for this hypothesis can be found in the data of Colbourn et al. (1984a,b), who measured fluxes from a heavy clay soil by the cover box (acetylene) method and by N-15/mass spectrometry. The latter method gave maximum values an order of magnitude higher than the former, and much higher estimates of total losses over a period.

Attempts have been made to adapt the acetylene inhibition method to heavy soils by taking soil cores, incubating them in

sealed jars in the presence of acetylene, and measuring the evolved N_2O (Ryden et al., 1987). This reduces the diffusion path length for acetylene, but also drastically alters the aeration condition of the core, particularly its outer part, compared with the conditions prevailing in the soil profile. This is likely to result in anaerobic zones ceasing to be anaerobic, and thus ceasing to denitrify.

Evidence in support of this hypothesis was obtained by incubating cores from sites 2 and 3 (Table 1) at oxygen concentrations down to 5 per cent (Fig. 3). For cores from site 2 (Winton soil), the denitrification rate was more than an order of magnitude higher than with the cover box method, even at 20 per cent oxygen, but it increased by a further order of magnitude when the oxygen was reduced to 5 per cent. The rates for the cores from site 3, however, were of the same order as those

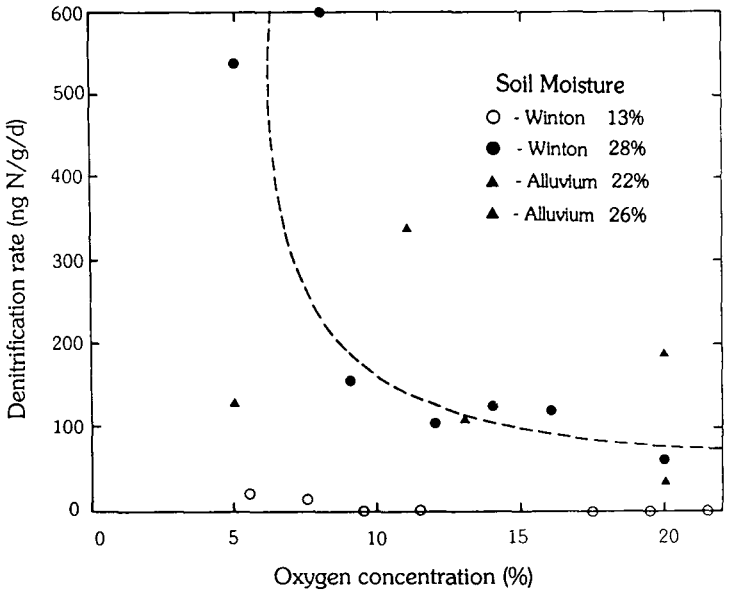

Figure 3 Denitrification in soil cores from sites 2 and 3, incubated in different oxygen concentrations.

measured by the cover box method (Table 1).

The relationships between denitrification rate and oxygen concentrstion shown in Fig. 3 are qualitatively similar to those of Parkin and Tiedje (1984), but the steep increase in denitrification rate in their work occurred at a much lower oxygen content, presumably reflecting a much finer soil structure.

Measurements of the composition of the soil atmosphere at site 2 at the time at which the cores were taken showed oxygen concentrations in the macropores down to 3 per cent. It is concluded that the core method can be used to measure denitrification in heavy soils, provided that the atmosphere round the cores is representative of the gaseous environment in the soil profile; only by introducing this extra complexity is one likely to make measurements of the right order of magnitude.

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Denitrification Losses From Puddled Rice Soil in the Tropics

R.J. Buresh[+] and S.K. De Datta[++]

Abstract

Direct field measurements of $(N_2 + N_2O)$-^{15}N flux from ^{15}N-labeled urea were conducted from 1986 to 1988 at three locations in the Philippines to assess the significance of denitrification loss in puddled rice soils. Direct recovery of $(N_2 + N_2O)$-^{15}N in closed chambers, that were placed periodically over the soil and floodwater during the 17-20 days following N application, was consistently 1% or less of the applied urea-N. Total gaseous N loss estimated by the ^{15}N balance technique was greater, ranging from 26 to 56% of the applied N. Denitrification loss appeared to be limited by nitrate supply rather than by available carbon.

Introduction

Denitrification has long been considered a mechanism of N loss in flooded soils (Shioiri, 1941). Ammoniacal-N can be converted to nitrate by nitrification in floodwater and oxidized soil zones. The nitrate can then move into reduced soil zones where it is readily denitrified to dinitrogen and nitrous oxide (Reddy and Patrick, 1986). Denitrification in flooded soils

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Joint contribution from the International Fertilizer Development Center (IFDC), P.O. Box 2040, Muscle Shoals, AL 35662, U.S.A., and the International Rice Research Institute (IRRI), P.O. Box 933, Manila, Philippines.

[+] Soil Scientist, IFDC and Visiting Scientist, IRRI.

[++] Principal Scientist and Head, Agronomy Department, IRRI.

has been the subject of much speculation and laboratory investigation. Quantification of denitrification loss in flooded rice fields, however, has been hindered by a lack of methodology.

In field measurements of denitrification in flooded rice soils, researchers have often used ^{15}N tracer techniques. In Japan (Ito and Iimura, 1986), the evolution of $^{15}N_2$ from ^{15}N-labeled ammonium chloride was measured using sealed chambers with an atmosphere of 80% He and 20% O_2. Buresh and Austin (1988) determined $(N_2 + N_2O)$-^{15}N collected in chambers that were placed periodically over the floodwater in microplots receiving ^{15}N-labeled urea.

This paper (i) summarizes recent research results on field measurement of denitrification loss from puddled rice soil in the tropics and (ii) highlights the current status of research methodology and methodological constraints in the field measurement of denitrification in flooded soils. The $^{15}N_2$ and $^{15}N_2O$ evolved from ^{15}N-labeled sources were not analyzed separately, and hence they are reported in this paper as $(N_2 + N_2O)$-^{15}N. All ^{15}N analyses of evolved N gases were conducted with the arc technique (Craswell et al., 1985).

Results and Discussion

Table 1 summarizes the cumulative recovery of $(N_2 + N_2O)$-^{15}N over either 17- or 20-day measurement periods following urea application by three different methods. In all cases, direct recovery of evolved $(N_2 + N_2O)$-^{15}N was very small, ranging from 0.1 to 1.1% of the applied ^{15}N. Total ^{15}N loss, determined from a ^{15}N balance in the microplots at the end of the 17- or 20-day measurement, was much larger ranging from 26 to 56% of the

Table 1. Summary of measured $(N_2 + N_2O)$-^{15}N evolution from urea-^{15}N applied to transplanted rice in the Philippines.

Urea application method	Water depth at N application (m)	Reference	Site	Measurement period (days)	Urea-N applied (kg ha^{-1})	Recovery of $(N_2 + N_2O)$-^{15}N (%)	Total ^{15}N loss[a] (%)
Basal incorporation	0.02	Buresh and Austin, 1988	Muñoz	17	58	1.1	40
Basal incorporation	0	Buresh and Austin, 1988	Muñoz	17	58	0.2	26
Broadcast at 10-15 DT[b]	0.05	Buresh and Austin, 1988	Muñoz	17	44	0.5	46
		De Datta et al., 1987	Calauan	20	80	0.1	52
		De Datta et al., 1988	Calauan	20	53	0.1	56
		John et al., 1989	Los Baños	20	29	0.5	41
		John et al., 1989	Los Baños	20	29[c]	0.5	40

[a]All measurements are the mean of 3 or 4 replications.

[b]DT designates days after transplanting.

[c]2.5 Mg ha^{-1} cowpea green manure (C/N=15) incorporated 15 days before transplanting.

applied ^{15}N. Total ^{15}N loss presumably represented only gaseous N loss by ammonia volatilization and denitrification since microplot borders prevented runoff loss and leaching loss was negligible on these puddled soils with a percolation rate of approximately 2 mm day^{-1}.

Results indicate that denitrification loss from urea was very small at all sites provided that the recovery of evolved $(N_2 + N_2O)$-^{15}N did not underestimate denitrification loss. An underestimation of denitrification might result from (i) entrapment of some $(N_2 + N_2O)$-^{15}N in the soil rather than quantitative evolution of the labeled N gases to the atmosphere above the floodwater, (ii) evolution of $(N_2 + N_2O)$-^{15}N through plants that were not covered by the gas collection chamber, (iii) an effect of the borders of the small 0.16-m^2 microplot on N loss, and (iv) an effect of the gas collection chamber itself on N losses.

The most probable factors limiting denitrification in flooded soils in the tropics are either available C for the heterotropic denitrifiers or supply of nitrate. Rapid disappearance of added nitrate and evolution of $(N_2 + N_2O)$-N from added nitrate at the study sites indicated that C did not limit denitrification. Moreover, in the study of John et al. (1989) in Los Baños, the water soluble soil organic C was 101 μg C g^{-1} in the top 0.15-m soil layer. The formation of nitrate by nitrification was the likely factor limiting denitrification.

Research results to date indicate that ammonia volatilization is more important than denitrification as a gaseous loss mechanism for fertilizer-N in puddled rice soils in the tropics. Even under conditions of alternate soil

drying and wetting following urea application to flooded or saturated soil, ammonia volatilization appears to be the main gaseous N loss mechanism of fertilizer-N. These findings raise doubts about the probable benefit of nitrification inhibitors to reduce fertilizer-N loss in puddled rice soils.

Future quantification of denitrification by the direct measurement of gaseous end products will require quantification of end products that are both evolved from or trapped within the soil/floodwater/plant system. Quantification of evolved gaseous end products must consider the transmission of gases through the rice plant.

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Use of Encapsulated Calcium Carbide to reduce denitrification losses in flooded rice from urea fertilizer as studied by direct N-15 measurements technique

K.S.Uppal, N.K.Banerjee and N.N.Goswami
Division of Soil Science and Agricultural Chemistry
Indian Agricultural Reasearch Institute, New Delhi, India

A.R.Mosier
USDA-ARS, Fort Collins, Colorado, U.S.A.

INTRODUCTION

Nitogen fertilizer use effilciency in flooded rice is very low. Nitrogen balance studies have shown that fertilizer nitrogen found in rice straw and grain usually totals only 20-30 % of the nitrogen applied while 20-30 % of the fertilizer nitrogen is immobilized in the soil. The remaining 30-60 % of fertilizer nitrogen is lost from the soil system. Of the potential loss mechanism in the typically impermeable soil used to grow flooded rice, nitrate leaching, surface runoff, ammonia volatilization and denitrification processes are considered the dominant loss mechanisms. Studies in India and China have shown ammonia volatilization losses of less than 10 % where as that in Phillipines it is 30-70 % of the applied fertilizer nitrogen. Because of the relatively low ammonia losses in the field studies in India, ammonia losses are considered less important than nitrogen emissions resulting from denitrification specially under low land conditions.

Direct measurement of denitrification loss as dinitrogen and nitrous oxide are extremely difficult to perform and very limited

literature is available. In soils where ammonia volatilization should be small (usually soils with pH below 8) the possibility of limiting denitrification and nitrate leaching with a nitrification inhibitor exists.

Unfortunately, the available commercial nitrification inhibitors are not effective in flooded soils. Coated Calcium Carbide (CCC) has been found to be potential nitrification inhibitor in Laboratory studies (Banerji and Mosier, 1988) and in the field studies (Mosier et al, 1988).

This paper describes the results of one field experiment where the efficiency of this newly developed nitrification inhibitor (NI) has been compared to a standard NI i.e. Dicyandiamide (DCD).

MATERIAL AND METHOD

Field Experiment

Field experiments were carried out in the Indian Agricultural Research Institute Farm, New Delhi during wet season (Kharif) of 1988, with rice under low land situation.

Three treatments namely, Urea alone at the rate of 120 Kg N/ha, Urea along with NI DCD and Urea along with NI under test i.e. CCC were taken in three replications following Randomised Block Design layout. Both the NI were applied as a physical mixture with prilled urea at the rate of 20 Kg/ha.

Each treatment plot was provided with 15 cm diameter

confined area for N-15 urea of 99 atom % excess application using PVC rigid pipe (30 cm length, 15 cm diameter) open from both ends and inserted to the depth of 20 cm in the soil. The treatment of these microplots were same to the plots in which they were installed. A specially designed enclosure of transparent acrylic pipe (45 cm length,7.5 cm diameter) was fabricated to cover the PVC pipe from time to time to collect gas flux samples. The assembly was made air tight by using rubber tubing grip around the rims of the two pipes (as shown in Photo plate 1 and 2).

The flood water inside and outside these PVC pipes was maintained at 5 cm to create low land conditions for rice plants.

Sample and Sampling procedure

Gas flux samples over each covered N-15 treated area was collected three times in a day viz: for 0 Hr, 2 Hr and 16 Hr period of time beginning in the morning. In between the above mentioned designated time the Chamber (cover) isolating the head space over N-15 treated soil was removed to allow plants to respire in open air.

The gas samples were collected by using 12 ml polypropylene syringe fitted with 2-way gas tight plastic stop cock which was fixed on the cover itself (Photo plate 2).

These samples were stored by injecting them into rubber stoppered evacuated glass tubes, Vacutainers (R)- a commercial product. These evacuated tubes were further sealed with silicone seal glue on the rubber stopper at the point puncture by syringe needle.

Photo Plate 1. PVC pipe inserted into flooded soil to demarcate N-15 microplot

Photo Plate 2. Device for collection of N2 + N2O gas flux smples

Measurement of N-15 ratio of gas samples

All the N-15 ratio measurements of these gas samples were done by VG ISOGAS MM 622 Mass Spectrometer using technique described by Mosier et al (1985) and Porter et al (1988).

This technique requires an additional inlet assembly to the mass spectrometer so that oxygen, carbon dioxide and water vapour are removed from the gas sample. The inlet system consists of the following components:

- 2 ml sample loop, which is shaped such that it can be immersed in cold trap.
- an oxygen scrubber. this scrubber was made by packing a tube with 10 gms of oxygen trapping material (obtained from L.C. Company, U.S.A.) and heating it with thermostatically controlled heaters at 300 degree centigrate.

  cold trap, which is a loop of copper tubing and can be immersed into a liquid nitrogen furnace.

Each of these components were connected by a gas tight valve to permit isolation at each portion of this inlet. All the fittings were done by using cajans seals and joints.

Once the measured volume of sample is given to the inlet sample loop, using a 2-way air tight plastic valve, it can be subjected if required to a cold trap before passing it to the oxygen trap, so that to remove Nitrous oxide from dinitrogen. In this experiment N2O and N2 both were taken into account for

determining ratio by mass spectrometer and thus no cold trap was used at this stage.When gas is allowed to pass into the oxygen scrubber portion, it traps all the oxygen and also converts all the nitrous oxide gas into dinitrogen, thus permitting N-15 ratio measurements of both N2O and N2 denitrified gas.

The remaining of the Carbondioxide and water vapours are trapped once they pass through cold trap of liquid nitrogen. Thus only dinitrogen gas is left as inlet sample for mass spectrometer.

The equipment VGISOGAS MM622 has got "JUMP Programme" which allows the measurement of 29/28 ratio,30/28+29 ratio and also oxygen present in each sample. This facilitates in keeping a check on efficiency of oxygen scrubber. On ocassioins when this scrubber becomes inefficient, it can be regenerated by passing Hydrogen gas slowly at 300 degree C for 2 hours .

Calculation of N2 +N2O gas flux

The calculation of N2 + N2O gas flux is based on following equations whose derivation is dealt in details by Siegel et al (1982), Mulvancy (1984) and Mulvancy and Boast (1986).

1. Δr = (29 N2/ 28 N2) sample - (29 N2/ 28 N2) reference

2. $\Delta r'$= (30 N2/ 28 N2 + 29 N2) sample

- (30 N2/ 28 N2 + 29 N2) reference

where: sample= air sample from collection chamber at some time t after installing chamber viz: 2 Hr and 16 Hr sample of this experiment

reference= air sample from field i.e. normal air sample taken from the chamber immediately

after installation viz: Ø Hr sample of this experiment

29/28 and 3Ø/28+29= are the ion current ratio determined by the mass spectrometer

3. $15\ XN = 2.Ø15\ (\Delta r'/\Delta r) / (1+ (2.Ø15\ (\Delta r'/\Delta r)))$

where: 15 XN= mole fraction of N-15 in the soil nitrate pool

4. $d = \Delta r' / (15\ XN)^2$

where: d= the fraction of total nitrogen gas in the gas collected chamber attributable to denitrification

5. $T = V \times d$

where: T= total nitrogen gas evolved from the soil into the collection chamber

V= total nitrogen in the chamber volume

6. $N2\ Flux = \Delta C / A \times t$

where: ΔC= the change in concentration of 3Ø N2 and 29 N2 in the chamber during time t

t= time that the chamber covered the soil

A= soil surface area covered by chamber

RESULTS AND DISCUSSION

The aim of the experiment was to compare the newly developed NI CCC with standard NI DCD. The yield data are presented in

Table 1. Grain and Straw yield as affected by application of nitrification inhibitor

Treatment	Yield data (q/ha)	
	Grain	Straw
Urea alone	46.6	47.9
Urea + DCD	53.6	53.Ø
Urea + CCC	61.3	63.3

Fig 1. DENITRIFICATION FLUX AS AFFECTED BY

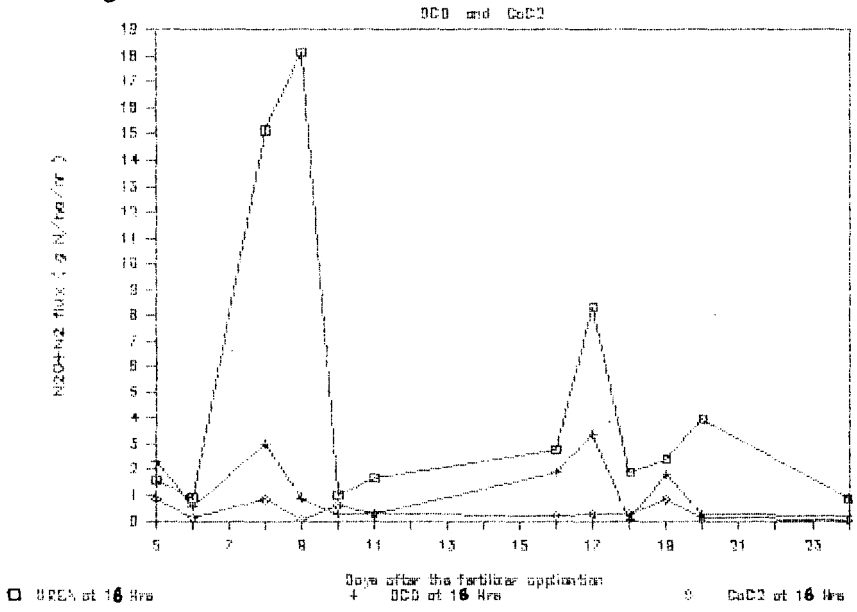

Fig 2. DENITRIFICATION FLUX AS AFFECTED BY

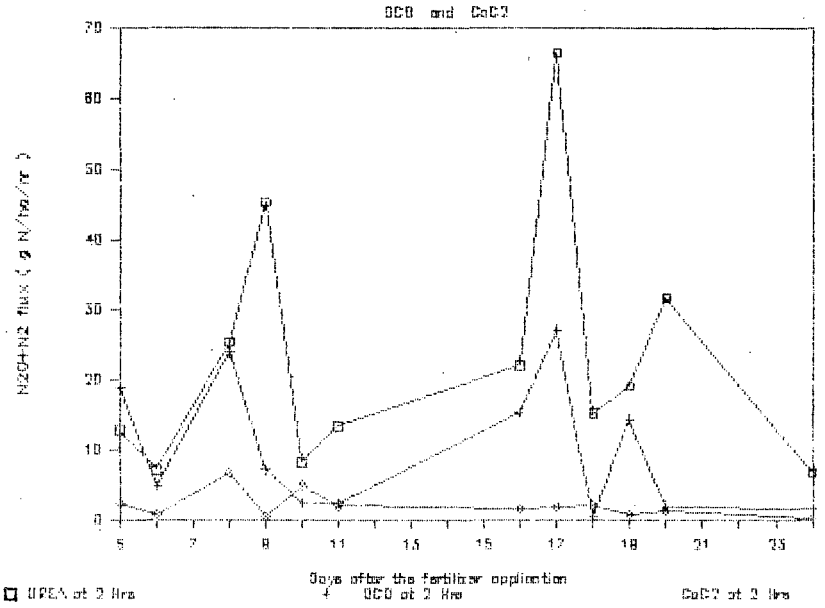

Table 1 and N2O + N2 gas flux measurements are shown in Fig 1 and 2.

The yield of grain and straw clearly shows that the nitrogen response and thus fertilizer nitrogen utilization has increased by about 30 % by using CCC as compared to only 15 % increase by using DCD along with urea over urea alone.

Similarly the N2O + N2 evolved from the flooded soil as shown in Fig 1 and 2 reveals that the use of CCC has checked the denitrification losses remarkably better as compared to that by DCD

Further more it is clear from the gas flux measurements that most of the denitrification losses take place in the first two weeks after the fertilizer application. The rate of loss of gases by denitrifiation is not uniform in all the days of plant growth and there are peaks in the gas flux which are the days of maximum loss

The results of this experiment clearly establish the efficacy of the newly developed NI CCC in improving the fertilizer use efficiency of urea in flooded rice system.

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Denitrification influenced by incorporated harvest residues

Van Cleemput O., Malkanti R.M., d'Ydewalle Y. and Baert L.*

Abstract

Several types of plant material (maize, barley, potato leaves, maize roots, barley roots and the commercial product humic acid) were added to a silt loamy soil (pH 6.8, 1.26 % C). Incubations were carried out under waterlogged conditions and at 2/3 field capacity and 100 mg NO_3^--N kg^{-1} was added. It was found that under waterlogged conditions the nitrate rapidly disappeared. The above-ground material had a more pronounced effect than the root material. Also at 2/3 field capacity, nitrate got reduced. It was clear that incorporation of readily available carbon into a soil promoted nitrate disappearance.

Introduction

It is common practice that of the grown plant material some part is incorporated into the soil after harvest. For potatoes and sugarbeets, leaves and top part are ploughed in again, while for cereals and maize only the stubble part is incorporated. Of course, root material is also subject to decomposition after harvest. Incorporation means a sudden increase of carbonaceous material and as such also the energy level. Increasing the energy for the microbial population might importantly influence their activity and results in a substantial change in rate of respiration and subsequent transformation processes (Andersen, 1985; Bremner & Shaw, 1958; El-Shinnawi & Eluaga, 1981; Guiraud & Berlier, 1969; Helal & Sauerbeck, 1986; Rolston et al., 1978; Ryden et al., 1979). Because of the differences of the different plant residues, their effect might also be different.

Especially the quantity and quality of the incorporated carbon is very important (Chalamet, 1985; Germon et al., 1981; Groffman et al., 1988; Kieft et al., 1987; Van Veen et al., 1985). Besides aeration and the nitrate level, the spatial position of the carbon should be considered as important parameters in the denitrification process (Seech & Beauchamp, 1988). The interaction with other parameters and C-influencing processes are shown in Fig. 1.

This paper deals with the influence of incorporated plant material on the behaviour of present nitrate nitrogen under two different moisture regimes.

*Faculty of Agricultural Sciences, University of Ghent, Coupure 653, 9000 Gent, Belgium

Materials and methods

For this study a silt loamy soil was used. This soil (13.8 % clay, 70.6 % silt, 15.6 % sand) had a pH-H_2O of 6.8 and 1.26 % C. To this soil the following types of finely ground plant material were added : maize straw, maize roots, barley straw, barley roots, potato leaves, sugarbeet leaves and a commercial product "humic acid". The characteristics of that material are given in

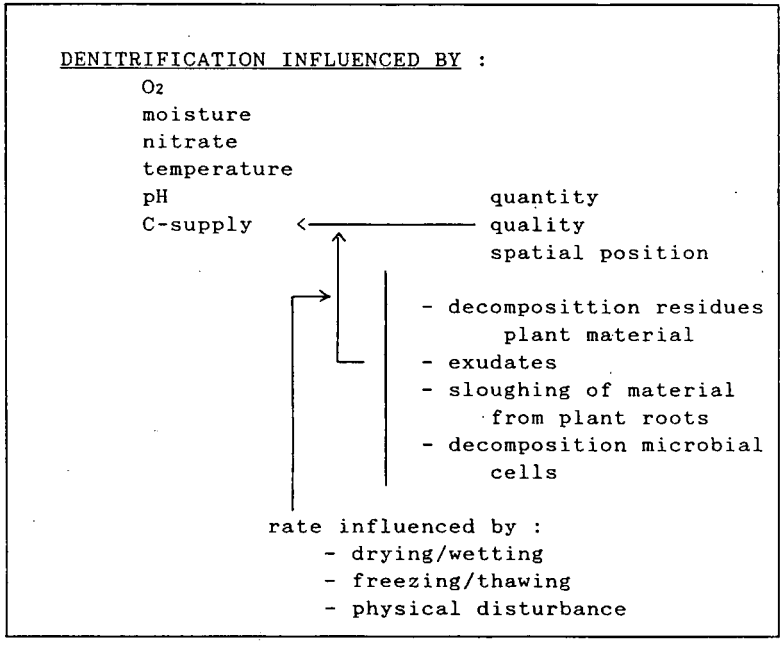

Fig. 1 Scheme of the parameters influencing the denitrification. Emphasis is put on carbon.

Table 1. Increasing amounts of the plant material were added : 0, 0.3, 0.6, 1 and 2 % on dry soil weight basis. Two moisture regimes were applied : waterlogged (30 g soil and 60 ml H_2O) and 2/3 field capacity (19 % moisture on dry soil weight basis). Incubations were carried out at 25°C and to all treatments 100 mg NO_3^--N was added. Separate flasks were analysed daily up to 5 days. Analysis was done for NH_4^+-N and NO_3^--N by steam distillation after extraction with 1N KCl.

Table 1 Characteristics of the used plant material

Type	% C	% N
Maize straw	28.7	1.05
Maize roots	26.4	0.63
Barley straw	27.0	0.91
Barley roots	30.4	1.04
potato leaves	26.4	3.79
Sugarbeet leaves	27.2	3.22
Humic acid	31.9	

Results and discussion

The evolution of the NO_3^--N as a function of time under waterlogged conditions in the presence of different types of harvested residues is given in Fig. 2. It is clear that the NO_3^--N rapidly disappeared under anaerobic conditions. Only for the lowest concentrations more than 3 days were necessary for a complete NO_3^--N removal. Some differences between the plant material were also noted. Maize straw, potato leaves and sugarbeet leaves have the most pronounced influence. The influence of barley straw was somewhat lower. It was, however, clear that addition of root material had less influence than addition of above-ground plant material. That material indeed contains less cellulose, hemicellulose and soluble carbohydrates than above-ground plant material. It should also be mentioned that some of the NO_3^--N could be immobilised. This should, however, be low because of the high nitrogen content of the plant material and the low C/N ratio.

Addition of an extra carbon source speeds up the respiration and, under waterlogged conditions, the O_2 supply cannot keep up with the O_2 consumption. Anaerobic conditions are rapidly created and denitrification started. The increased microbial activity by addition of plant material can also be seen out of the increased NH_4^+-N formation. In Fig. 3 the evolution of NH_4^+-N is given as a function of time for the different threatments. Here again, the different plant residues had a somewhat different influence. The differences were in the same order as for the disappearance of nitrate.

The commercial product "humic acid" behaved somewhat strange. It was not expected to have such an effect on the nitrate reduction. Most probably, next to the recalcitrant material, also readily available carbon was present in that product.

At 2/3 field capacity, the addition of plant material also promoted some nitrate disappearance. It was, however, at a much slower rate. Although that moisture content allows an overall aerobic condition, it was clear that through the stimulated

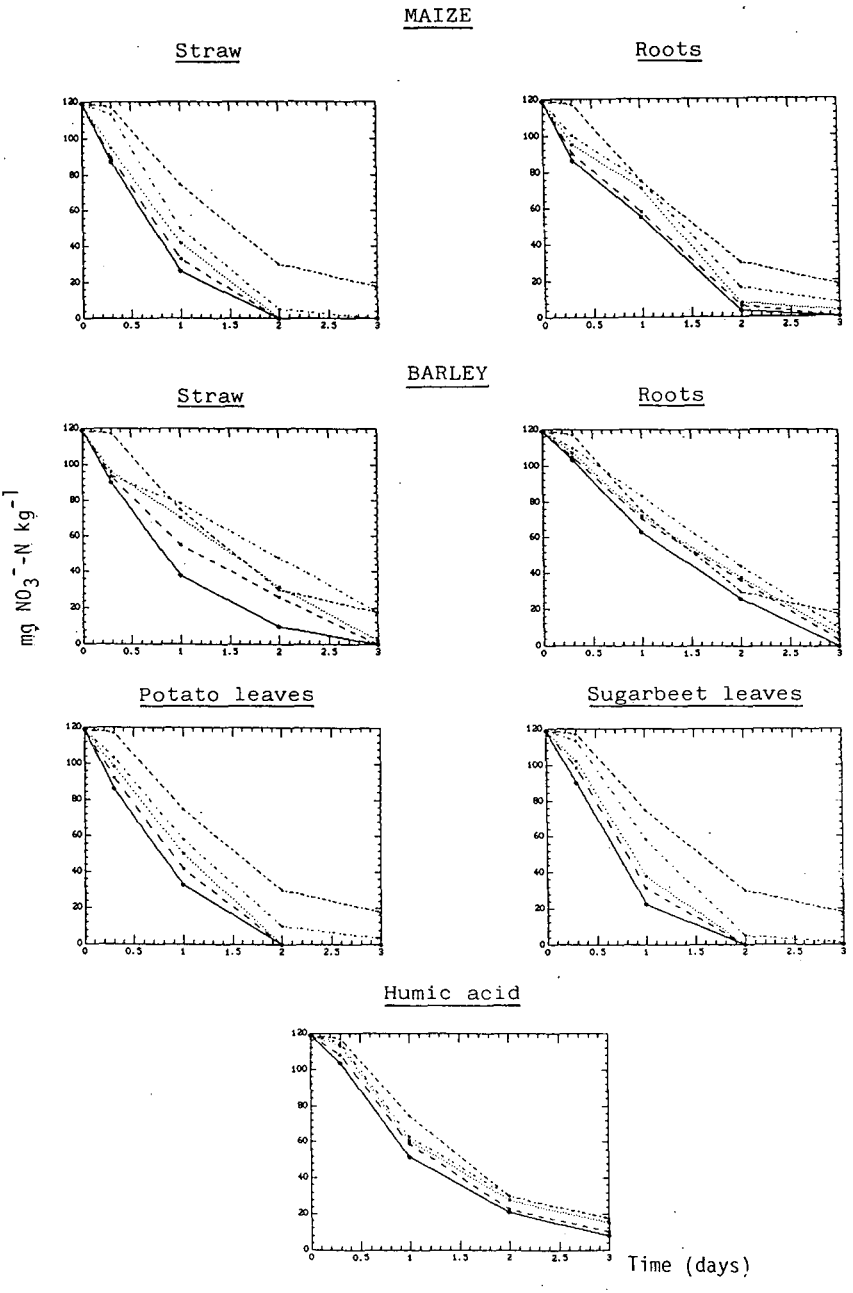

Fig. 2 Evolution of NO_3^--N under waterlogged conditions and addition of different amounts of organic matter (-·-·-0%; —·—0.3; ····· 0.6%; – – –1%; ——— 2%)

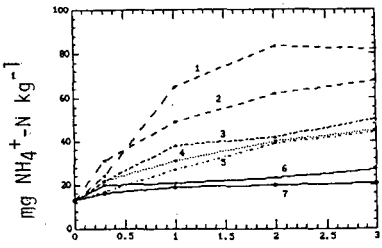

Fig. 3 Evolution of NH_4^+-N under waterlogged conditions and addition of 2% org. matter (1=maize straw; 2=sugarbeet leaves; 3=barley straw; 4=potato leaves; 5=barley roots; 6=maize roots; 7=humic acid)

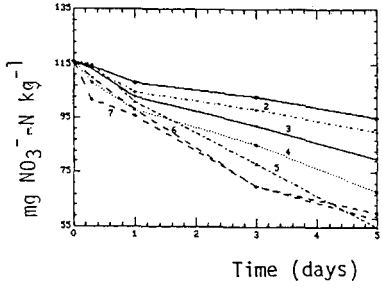

Fig. 4 Evolution of NO_3^--N at 2/3 field capacity (1=humic acid; 2=barley roots; 3=maize roots; 4=potato leaves; 5=barley straw; 6=sugarbeet leaves; 7= maize straw).

microbial activity, O_2 became limited in several places and nitrate started to be denitrified. Again the top part of the cereals, potato and sugarbeet leaves had a more pronounced influence than the root material.

It should be concluded that incorporation of amounts of harvested residues have an increasing effect on nitrate reduction. It was clear that the root material was of lower influence than the above-ground material. Maize, potato leaves and sugarbeet leaves had a somewhat more pronounced effect then barley straw. Finally, it was clear that incorporation of readily available carbon was detrimental for present nitrate.

Acknowledgement

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Denitrification in the top soil of production grassland

by

Corré, W J[1], W Dijkman[1] and W M Kieskamp[2]

INTRODUCTION

Modern west european dairy farming combines high inputs of nitrogen, in the form of fertilizers and purchased feeds, with relatively very low outputs of nitrogen in milk and meat. For a number of dutch intensive, well managed dairy farms a recovery of only 16 percent of the total nitrogen input was calculated, resulting in a nitrogen surplus of over 400 kgN/ha/yr (van der Meer et al 1986). This agricultural practice results in large environmental losses of nitrogen, and denitrification is one of the important pathways of loss.

This paper deals with denitrification as a loss of plant available nitrogen, and since the nitrate in deeper soil layers can be considered to be leached, our research was focussed on the aerobic top soil. Furthermore, denitrification in the top soil depends strongly on a locally and temporarily low oxygen pressure and on the presence of organic matter, and therefore it will be largely restricted to the rooting zone, which is rather shallow in highly fertilized grassland.

On this basis the measuring method described by Ryden et al (1987) was chosen. This method is based on acetylene inhibition in 15 cm soil cores incubated in glass jars in the field. In 1987 experiments were conducted on the effects of fertilizer nitrogen, cattle slurry, and dung and urine of grazing cattle in intensive perennial reygrass grasslands on well drained light clay soils.

1) University of Utrecht, Department of Plant Ecology
Lange Nieuwstraat 106, 3512PN Utrecht, The Netherlands
2) Netherlands Institute for Sea Research
P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands

RESULTS AND DISCUSSION

Fertilizer nitrogen

From March to October denitrification rates were measured weekly in a grassland with two silage cuts and six grazings at two nitrogen fertilizer (NH_4NO_3) levels: 250 and 600 kg of nitrogen per ha per year. Results are shown in figure 1.

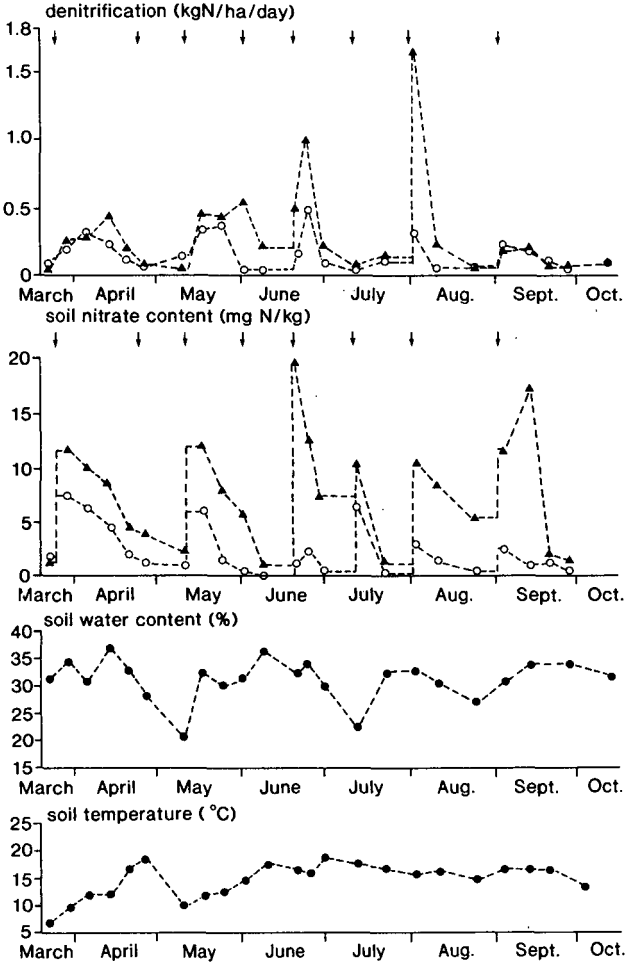

FIG. 1. Denitrification, soil nitrate content, soil water content and soil temperature at two nitrogen fertilizer levels. March to October 1987.
o: 250 kgN/ha/yr, ▲: 600 kgN/ha/yr, ↓: fertilizer application.

The figure shows the typical pattern of heavely changing denitrification rates, generally with a strong effect of fertilizer application. There are exceptions in this pattern; after the first fertilizer application in March the soil temperature was low and denitrification rates rose more slowly, and after fertilizer application on 10 July the soil was very dry and no reaction was found. Obviously, denitrification rates were related to soil nitrate contents, to soil water contents, and to soil temperatures, but the correlations were to weak to make a reliable prediction of the denitrification rate on the basis of these three parameters.
Integration of the curves gives the total nitrogen loss for the growing season, being 30 kg at the low nitrogen level, and 52 kg at the high nitrogen level. This is 12 and 8.5 percent of the fertilizer nitrogen applied, a considerable loss.

Cattle slurry

Another important nitrogen source in grassland is cattle slurry. In the same grassland, at a fertilizer level of 450 kg of nitrogen per ha per year, denitrification rates were measured for 17 days after application of potassium nitrate (62 kgN/ha) or cattle slurry (15 or 25 tons/ha, with a total nitrogen content of 5 kg per ton) on August 17. The results are shown in figure 2.
The difference is clear; immediatly after nitrate application a high denitrification rate was measured, but after slurry application the top was reached later, first nitrification had to provide sufficient nitrate. The effects do not last for a long time, probably due to drought, because after rain on August 25 the denitrification rates rose again. Total denitrification over the 17 days period did not differ significantly between treatments (5 to 6 kg of nitrogen per ha).

Dung and urine patches

The third and probably most problematic source of nitrogen in grassland are dung and urine patches. In these patches, much nitrogen is concentrated, comparable to fertilizer rates of circa 500 and 2000 kgN/ha, respectively for urine and dung patches (Lantinga et al 1987).
So measurements were made in dung and urine patches in a purely grazed grassland with a fertilizer (NH_4NO_3) level of 550 kgN/ha/yr during two regrowth periods immediatly after grazing in May and in the end of June. The results are shown in figure 3.

Overall denitrification rates were low during the first period and higher during the second, probably due to higher soil temperatures and higher soil water contents. During the first period the effects of dung and urine were marked, but did not last longer then ten days, despite the very high nitrate content of the urine patches. In the second period the effects of dung and urine was relatively small and lasted even shorter. The total denitrification losses are summarized in table 1. Because of the small area of the patches these losses did not make an important contribution to the denitrification and the extra loss did not account for more than one percent of the nitrogen input in the patches. So for the inevitable large nitrogen loss from dung and urine patches denitrification in the top soil cannot be hold responsible.

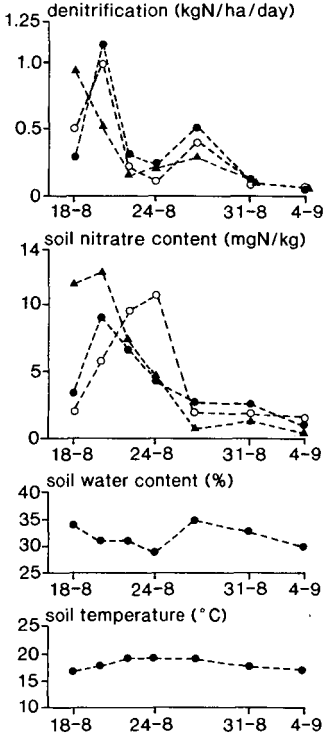

FIG. 2. Denitrification, soil nitrate content, soil water content and soil temperature after application of fertilizer nitrogen or cattle slurry. August to September 1987. ▲: KNO_3, 62.5 kgN/ha, o: slurry, 15 m^3/ha, ●: slurry, 25 m^3/ha.

Table 1. Denitrification losses (kgN/ha) in dung and urine patches

period	May (16 days)	June (7 days)
non affected areas	1.5	4
urine patches	5	7
dung patches	5	3

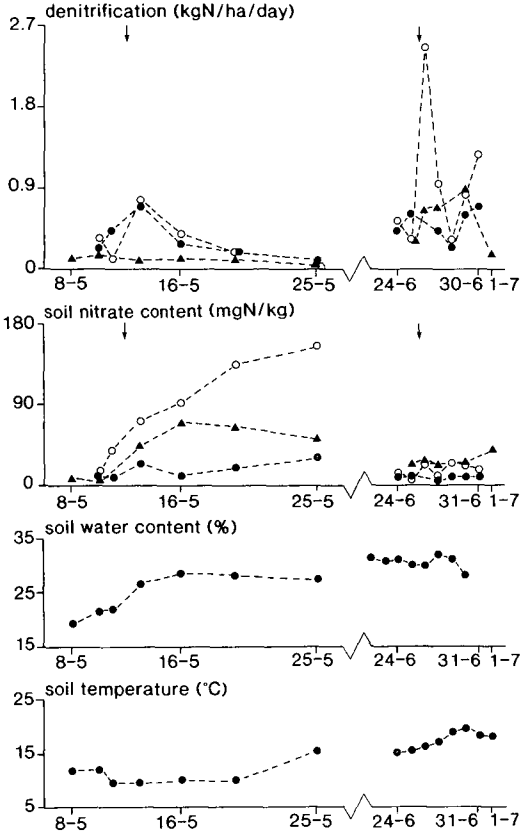

FIG. 3. Denitrification, soil nitrate content, soil water content and soil temperature in dung and urine patches and in unaffected areas in grazed grassland. May and June 1987. o: urine patches, ●: dung patches, ▲: unaffected areas, ↓: fertilizer application.

Additional measurements.

In both grasslands some incidental measurements were made concerning denitrification rates in deeper soil layers (15 to 90 cm) and concerning emission of nitrous oxide.

Generally more than 50 percent of the denitrification in the upper 90 cm soil appeared to be concentrated in the top 15 cm. On the one hand this means that the top soil is indeed the most active soil layer, but on the other hand this means also that the deeper soil layers are not unimportant and they should be taken into account, at least in this soil type.

From a number of measurements from incubations with and without acetylene an emission ratio of nitrous oxide to total denitrification nitrous oxide and dinitrogen of circa 20 percent was calculated. This percentage is not reliable as a mean value, but more systematic measurements of Ryden (1985) pointed to 21 percent. What part of the nitrous oxide was produced by denitrification and what part by nitrification cannot be concluded from these measurements.

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Field measured N_2O- and N_2-release relationships with carbon turnover, soil nitrate contents, water tensions and temperatures.

by
Benckiser, G.[+]
Gaus, G, K.M. Syring, K. Haider and D. Sauerbeck[++]

Mineral fertilizer rates exceed frequently the uptake of the growing crop and lead to eutrophication of lakes and rivers via erosion as well as to nitrate leaching into ground and drinking water. Additionally, increasing amounts of organics like sewage sludge, slurry or manure contribute to the overfertilization. Because 50 to 90% of the N in sludge is organic (Sommers, 1977), informations about the rates of N-transformation processes are essential to predict N availability during a cropping season. The formed nitrate is removed by the growing crop, by leaching or denitrification. Sludge application of about 12 metric tons ha^{-1} to a clay loam soil exhibited for example, that 194 mg l^{-1} of soluble nitrate are channeled through interconnected macropores into a soil depth of 120 cm during a period of 6 months (Sidle and Kardos, 1979). Next to nitrate leaching about 28-48% of the mineralized sewage sludge N might be lost via denitrification depending on soil type and amount of sewage sludge application (Lindemann and Cardenas, 1984).
The presented investigations compare soil variables important for denitrification with the N_2O+N_2 release measured over 3 years by the acetylene inhibition method in a variety of agricultural ecosystems amended with mineral fertilizer and/or sewage sludge in order to provide data for modelling gaseous N-losses.

Material and Methods

Experimental sites and field measurements: Employing the C_2H_2 inhibition technique measurements of N_2O+N_2 surface fluxes, N_2O+N_2 and CO_2 soil air concentrations as well as potential denitrification capacities, soil temperature, humidity and nitrate contents were started 1984 in a silty loam overlaying Emscher marl (Alfisol) in an agri-

[+]Institute of Microbiology, Justus-Liebig University, Senckenbergstr. 3, 6300 Giessen, FRG.

[++]Institute of Plant Nutrition and Soil Science, Federal Research Center of Agriculture (FAL), Bundesallee 50, 3300 Braunschweig, FRG.

cultural used water catchment area south of Braunschweig (Benckiser et al.,1986). The soil was planted with sugar beets. In order to study the role of available carbon, we continued with similar denitrification measurements 1985 in a loamy sand (Inceptisol), which was planted with spring wheat and amended either with mineral fertilizer or additionally with sewage sludge (300 m^3 ha^{-1}; Benckiser et al., 1987a). 1986 the studies were continued in an Inceptisol close to the 1985-field cropped to winter barley (Benckiser et al., 1987b). The N_2O+N_2 surface fluxes (4 replicates), the N_2O+N_2 and CO_2 concentrations of the soil air (3 replicates), the water and nitrate contents as well as the soil temperatures and potential denitrification capacities at different soil depths were measured as described by Benckiser et al.(1986 and 1987 a,b).

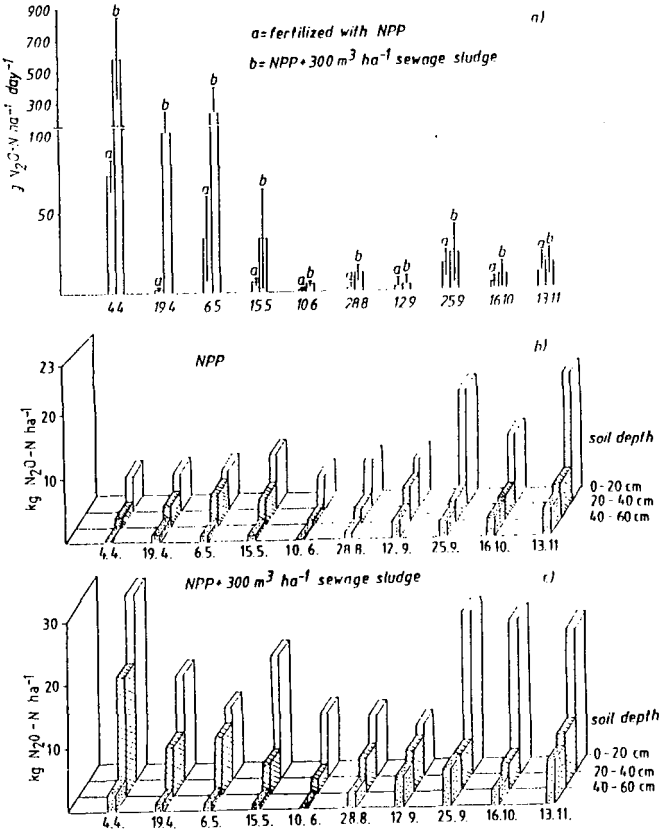

Fig. 1.: Daily N_2O+N_2 surface fluxes (a) as well as potential denitrification capacities of an Inceptisol field amended with mineral fertilizer (b) or additionally with sewage sludge (c) and cropped to spring wheat.

Results and Discussion

Comparing the actual denitrification losses of a minerally fertilized and/or sewage sludge amended loamy sand field (Inceptisol) calculated from the N_2O+N_2 surface fluxes (Fig. 1a) with the potential denitrification capacities (Fig. 1b,c), it became obvious that even the potential denitrification capacities in 40 - 60 cm depth of the only minerally fertilized Inceptisol surpassed the actual N_2O+N_2 release of both the only with mineral fertilizer-N applied and the sewage sludge amended fields. Similar results were obtained from the measurements in 1984 and 1986 (Benckiser et al., 1987b). These findings suggest that carbon is not limiting denitrification in agricultural ecosystems. A poor correlation of r = - 0.02 (n = 1085) between the log transformed N_2O+N_2- and CO_2-concentrations in the soil air of the studied field sites supports this finding. On the other side, sewage sludge amendment (300 m^3 ha^{-1}) over several years increased the actual as well as the potential dentrification losses by about 5

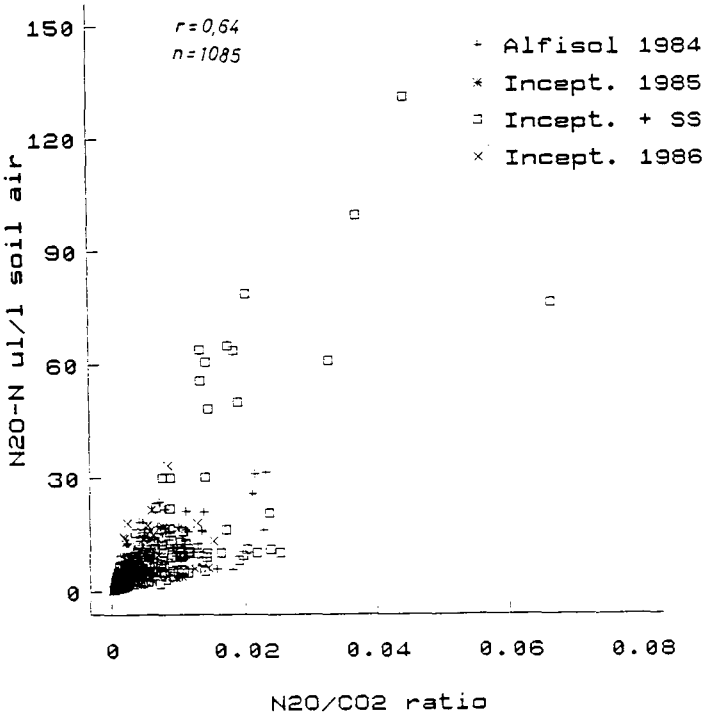

Fig. 2.: Relationship between N_2O+N_2 concentrations in soil air and the N_2O+N_2/CO_2 ratios of the Alfisol and Inceptisol fields (1984-1986)

times (Fig. 1). This in reverse pointed on a significant relationship between nitrate respiration and available carbon as mentioned in various laboratory experiments (e.g. Burford and Bremner, 1975; El Demerdash and Ottow, 1983). Plotting the N_2O+N_2 concentrations in the soil air against the ratio of released N_2O+N_2 and produced CO_2 (Fig. 2) the dependency of denitrification losses on carbon availability becomes obvious. The correlation coefficient of r = 0.64, however, depends strongly on the high denitrification rates (above 10 μg l^{-1} soil air). Evidently, only if the energy conserving processes in soils are dominated by nitrate respiration as in hot spots or on larger scale in carbon amended area a measurable relationship between N_2O+N_2 release and carbon turnover is given. Such events with high N_2O and CO_2 concentrations in the soil air were almost exclusively observed in the sewage sludge amended plots (Fig. 2).

Besides carbon availability sewage sludge improves the water and nitrogen budget of a soil (Benckiser et al., 1987a; Lindemann and Cardenas, 1984; Metzger et al., 1987). Considering all investigated soil depths and experimental sites the relationship between N_2O+N_2 soil air concentrations on the prevailing water regime is shown in Fig.3. Yet, the relationship is not significant.

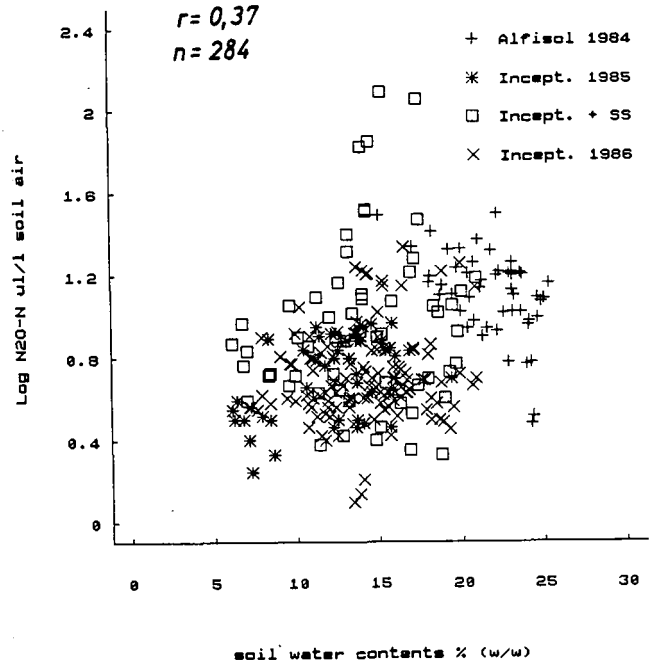

Fig. 3.: Relationship between the N_2O+N_2 concentrations in soil air and the water contents of the Alfisol and Inceptisol fields (1984-1986).

This poor correlation might be explained by only little fluctuations of the water contents of deeper soil layers (Benckiser et al, 1986 and 1987 a,b).

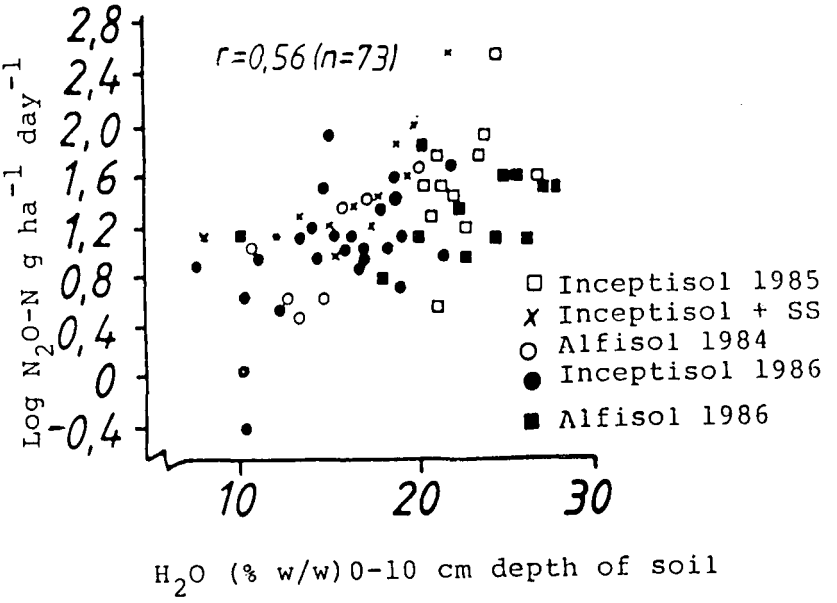

Fig. 4: Log-transformed N_2O+N_2 surface fluxes compared to soil moisture of the upper 10 cm measured in the Alfisol and Inceptisol fields (1984-1986).

Thus, it is not surprising that the correlation is significantly improved when the soil water contents of the upper 10 cm are correlate to the log transformed N_2O+N_2 surface fluxes (Fig. 4).Myrold (1988) observed a very similar correlation in cropping systems of Western Oregon illustrating the need for reduced oxygen partial pressures before higher denitrification losses can be registered.

Next to soil water and carbon nitrate contents are influencing denitrification rates as shown in Figure 5, which includes all data of the various agricultural sites. This is also by others (Burton and Beauchamp, 1985; Myrold, 1988). Again a rather poor correlation of r = 0.31 between denitrification and soil NO_3^- concentrations has been found. Yet, if the field sites as well as the different soil depths are considered

separately, correlations between both variables are improving (Benckiser and Syring, 1987; Benckiser et al., 1987 a,b).

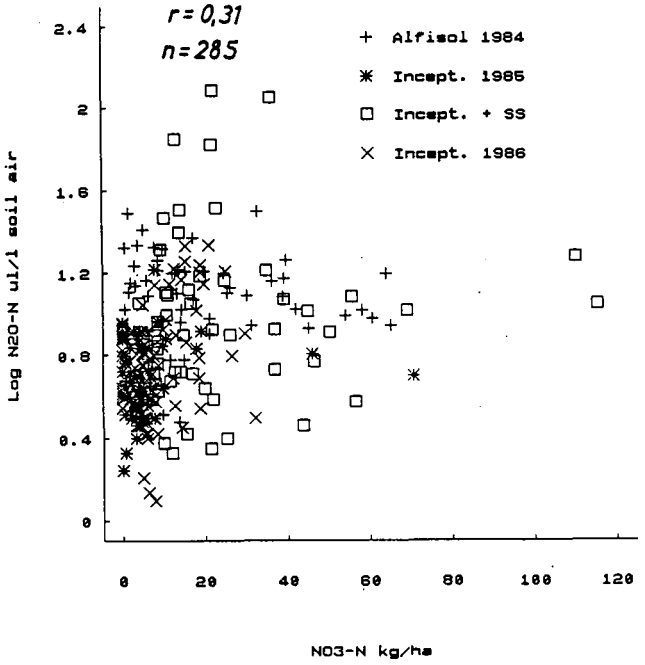

Fig. 5: Relationship between N_2O+N_2 in soil air and nitrate contents in the Alfisol and Inceptisol fields (1984-1986).

The multiple correlations between the N_2O+N_2 concentrations in the soil air up to 50 cm depth and soil parameters such as nitrate, potential denitrification capacities, ammonium, temperature and humidity (Benckiser and Syring, 1987; Benckiser et al., 1987 a,b) reveal further that the influence of soil temperature on denitrification is low under the prevailing climatic and field conditions of Germany. Although temperature undoubtedly effect the rate of denitrification, the negative correlation between soil temperature and water content (Benckiser and Syring, 1987; Benckiser et al., 1986 and 1987a,b) and the fact that soil microorganisms are well adapted to their temperature regimes (Powlson et al., 1988) are probably responsible for this nonsignificant relationship (Myrold, 1988).

Conclusions

- Compared to the potential denitrification capacities, which imply denitrification losses in the range of the applied N-fertilizer, actually measured denitrification losses of German agricultural ecosystems are relatively low (about 5 to 10% of applied N-fertilizer. Even excessive sewage sludge amendment resulting in high nitrate and carbon availibility throughout the year did not essentially increase denitrification under the temperate climate of Germany.
- Carbon, humidity, nitrate and to a lesser extent soil temperature affect denitrification in the field and restrict intensive nitrate respiration to relatively short periods during a year.
- Inspite of extended measurements of N2O+N2 surface fluxes, N2O+N2 soil air concentrations and soil variables important for denitrification, correlations are too poor to make possible predictions of denitrification losses by simple models. More sophisticated models as described by Rolston et al.(1984) and Benckiser and Syring (1985) seem to allow a better approach to the measured denitrification losses.
- Though the acetylene inhibition technique has some shortcomings, which might lead to an underestimation of the actual denitrification losses, this method enables to identify soil variables limiting nitrate respiration in the field and provide basical data for more economical and ecological fertilization.

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Distribution and Mineralizing Capacity of Nitrifying and Denitrifying Microorganisms in Forest Soils after Application of Various Liming Techniques

by

Fabig, W.[+)] and M. Meier[++)]

Introduction:

Anthropogenic immissions are responsible for considerably damaged forest ecosystems. Especially the input of nitrate and sulfate which generally reach the soil surface as free acids permanently affects the soil communities and the mineralizing capacity (FABIG, 1986). The resulting decrease of the pH-values cause an increasing imbalance of the cations' and anions' cycles.

In nearly all ecosystems nitrogen is the most important nutrition element. The ions of nitrogen, NH_4^+ and NO_3^-, play a predominant role in the ions' cycle "soil - plant". They further control the turnover of other important nutrition elements. Especially the internal microbial turnover of nitrogen and its consumption by plants decisively influence the soil compartment (BEESE, 1986). As the organically bound nitrogen is the dominating form of nitrogen in forest soils all impacts of the environment which influence the mineralization rates affect the ions' cycles and the stability of the ecosystem. Dependent on the actual soil pH the organically bound and the ammonia fractions of the nitrogen stock are transformed to nitrate to a higher or lower degree. In acid forest soils the turnover from NH_4^+ to NO_3^- is very low, and the nitrogen is stored as ammonia in the upper organic horizons. The negatively charged nitrate does not react with the ion exchange complex and is shifted into deeper horizons with the soil water.

For the compensation of the anthropogenic soil acidification different liming procedures were tested. Upon rapid increase of the pH in forest soils the mineralization processes of the humic

+) Fraunhofer-Institut für Umweltchemie und Ökotoxikologie, 5948 Schmallenberg

++) Institut für Chemische Verfahrenstechnik, Universität Stuttgart, Böblingerstr. 72, 7000 Stuttgart 1

fraction strongly increased. It is supposed that with increasing pH the nitrogen fraction of the humic substances is rapidly oxidized to nitrate which may finally lead to pollution of wells and ground water.

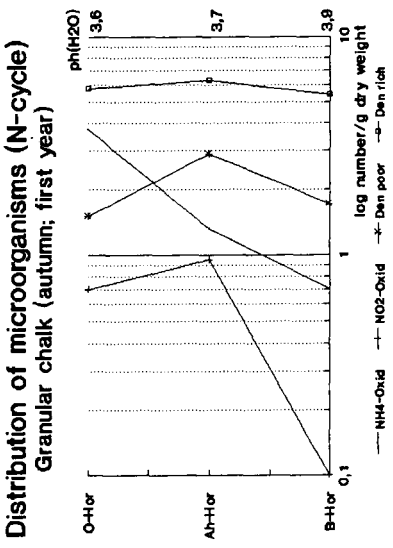
Materials and Methods:

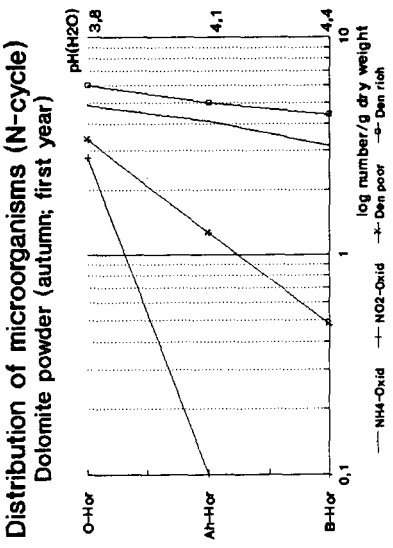
Acid "brown earth" experimental fields under spruce each with a plot size of 500 m^2 were limed with 1: 3 t/ha Dolomite powder and 2: 3 t/ha granular chalk with a particle size of up to 5 mm. A neighboring untreated field was chosen for control examinations. About 4 and 14 months after application of the lime in each field soil samples of the O-, A_h- and B-horizons were withdrawn from 10 randomly fixed places in each field. The soil samples collected from each horizon were mixed to a final soil mixture. Each soil mixture was used for the determination of the microbial communities of nitrifiers and denitrifiers as well as for the determination of the nitrification and the denitrification capacity. For the determination of the microbial populations MPN-procedures were used. Two groups were distinguished using selective media; nitrosomonas or nitrosomonas-like organisms using a medium according to MATULEVICH (1975); and nitrobacter like species using a medium according to ALLEEM and ALEXANDER (1958). The denitrifying organisms were again divided into 2 groups with organisms dependent on rich nutrition (mostly gram-positive organisms distributed in the bulk soil) and those growing also in a very poor medium (mostly gram-negative organisms domiciled in the rhizosphere). The compositions of the mediums were similar to FABIG (1979). Parallel with the determination of the population density the nitrification capacity (VD-LUFA) and the denitrification capacity (EL-DEMARDASH, 1981) were examined. With the obtained results the multiple correlation coefficients (SACHS, 1974) were calculated.

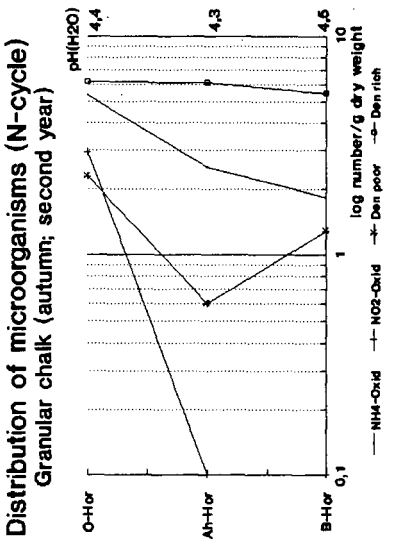
Results:

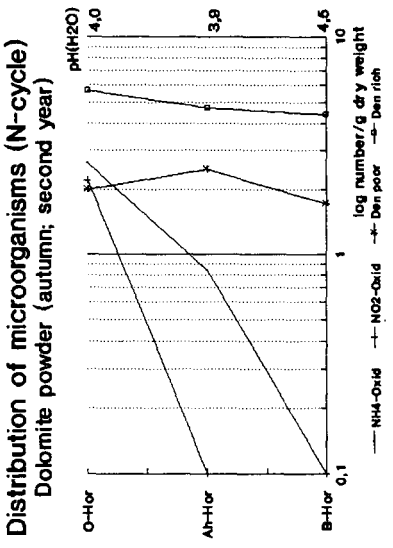
Figure 1 shows the distribution of microorganisms involved in the N-cycle. At all sites the number of microorganisms decreased with increasing depth. This is obviously due to the depletion of the organic matter in the deeper anorganic horizons. In the organic layer the number of organisms, especially of ammonia oxidizing organisms and denitrifiers which grow in rich medium increased with

Fig. 1: Distribution of microorganisms in the O-, A_h- and B-horizon in the control and the treated fields



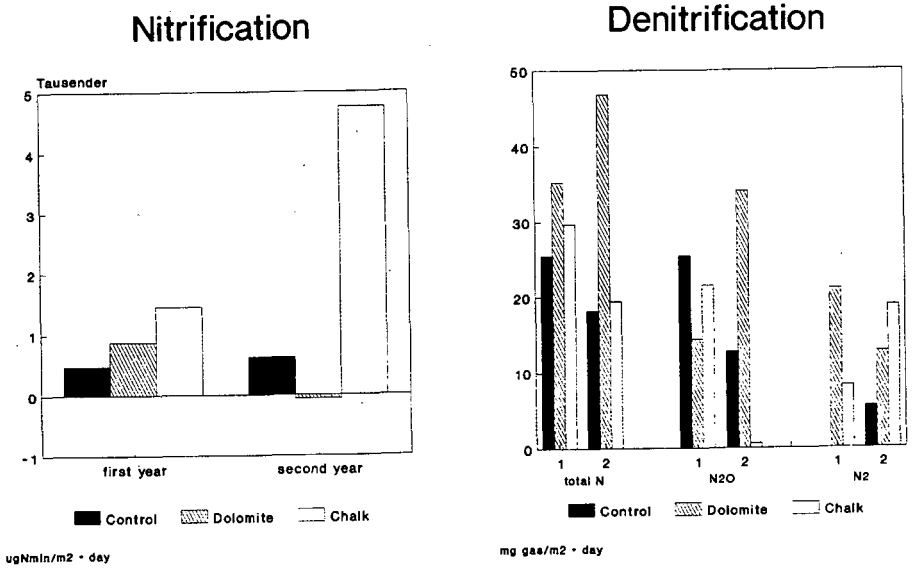






increasing pH-values. In the untreated control field the number of all groups of organisms generally was hundredfold lower. Some groups as e.g. the NO_2-oxidizing organisms only were found randomly. Parallel with the density of the microbial populations the nitrifying and denitrifying capacities were determined (Fig. 2). The mineralization of the nitrogen was more intensive in the limed fields than in the control field. The field which had been treated with granular chalk had the highest nitrification capacity. Similar results were obtained for the denitrifying capacity; the highest denitrifying rates were observed in the Dolomite field. It is noteworthy that in the untreated acid control field only small amounts of N_2 were produced in comparison with the limed fields. The N_2O production rates of the fields were vice versa.

Fig. 2: Nitrification and denitrification capacity of the control and the treated fields



In Table 1 the correlation coefficients between the population densities and the pH-values of the respective horizons are listed. Only for the organic horizon dependences between the populations

Tab. 1: Correlation coefficients between density of nitrifying and denitrifying organisms and the pH-value of each horizon

Groups of Organisms	O-horizon	A_h-horizon	B-horizon
NH_4-Ox - pH	0.466	0.359	0.445
NO_2-Ox - pH	**0.813**	0.518	0.265
NH_4-Ox - NO_2-Ox	0.002	**0.898**	0.166
Den poor - pH	0.048	0.447	0.362
Den rich - pH	**0.823**	0.166	0.190
Den poor - Den rich	0.294	**0.712**	0.202
Den poor - Nitrif.	**0.684**	0.461	0.459
Den rich - Nitrif.	**0.979**	0.514	0.258

and the pH-values were observed. In the group of nitrifying organisms dependence of the pH-value was found only for the nitrite-oxidizing microoganisms and for denitrifiers which grow in rich medium. For nitrite and ammonia oxidizing microorganisms however a very high correlation coefficient as compared to denitrifying organisms was determined. In the A_h-horizon no significant correlation between pH and the examined microorganisms was found. However nitrifiers as well as denitrifiers had similar densities.

In Table 2 the correlation coefficients between nitrification and denitrification capacity and the soil pH as well as the corresponding group of microorganisms are shown. For the nitrification capacity and nitrite oxidizing microorganisms no significant correlations exist. For the nitrification capacity and the population density of ammonia oxidizing organisms however a correlation coefficient of 0.795 was obtained. Upon additional consideration of the correlations and the pH the correlation coefficient did not change. This means that the nitrification capacity is mainly influenced by the population density of ammonia oxidizing microorganisms. Concerning the denitrification capacity it is noteworthy that the evolution of N_2 was strongly correlated with the pH-value (0.993) and the multiple correlation coefficient (including the population density of denitrifiers in rich medium) was 0.999. For the production of N_2O and the pH-value only a correlation coefficient of 0.346 was obtained.

Tab. 2: Multiple correlation coefficients between nitrification and denitrification capacity and pH-value as well as the corresponding microorganisms

Nitrification capacity (Coefficients)			
Corr. Param.		Corr. Param.	
nitrif. - pH	0.437	nitrif. - pH	0.437
nitrif. - NH_4-Ox	**0.795**	nitrif. - NO_2-Ox	0.320
NH_4-Ox - pH	0.534	NO_2-Ox - pH	0.280
multiple	**0.795**	multiple	0.480

Denitrification capacity (Coefficients)			
Corr. Param.	total N	N_2O	N_2
Denitrif. - pH	0.329	0.346	**0.993**
Denitrif. - D.rich	0.285	0.580	0.190
D.rich - pH	0.304	0.304	0.304
multiple	0.382	**0.606**	**0.999**
Denitrif. - D.poor	0.425	0.046	0.538
D.poor - pH	0.602	0.602	0.602
multiple	0.435	0.401	**0.993**

Discussion:

The increase of autotrophic nitrifying microorganisms with increasing pH-values was also observed at other forest sites (FABIG, 1987). It is obviously the reason for an increase of the nitrifying capacity (LANG, 1986). The highest increase of the pH values and of the nitrification capacity was found for the experimental field treated with granular chalk. Obviously the surface properties and the solubility of the used product were mainly responsible for this behaviour (GRUNWALDT, 1980). In contrast to the nitrification capacity the denitrification capacity (calculated as N) showed no significant correlation to the pH-values of the soil. These observations are in agreement with studies of DINTSCHEFF and BADJOFF (1977), which show that the denitrification capacity is influenced also by the organic fraction, temperature and moisture. The denitrification loss as N_2 was however strongly correlated with the pH-values. With increasing pH and increasing

populations a correlation coefficient of 0.999 was obtained. This supports the argument that denitrification losses as N_2 in forest soils are caused by both the pH-value and special denitrifying microorganisms, which was demonstrated for forest soils (KLEMEDTSSON and SVENSSON, 1988) and for agricultural soils by FOCHT (1974) as well as by BURTH and OTTOW (1979).

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N_2O PRODUCTION RATES AND DENITRIFICATION RATES IN SOIL AMENDED WITH PIG SLURRY

by

MAAG, M. *

Introduction

Particular attention is being given to the N content of pig slurry both because of the importance of crop responses to this element and of the risk of nitrate leaching to surface and ground waters if excessive amounts are used.

Among the processes leading to losses of nitrogen from the root zone, the denitrification process has been the most difficult to measure until the development of improved techniques (Ryden & Rolston, 1983). Measurements of denitrification losses under controlled conditions in the laboratory may result in a better understanding of the process and its influence on the total nitrogen balance in agricultural soils.

Denitrification in soil has been studied over a range of temperature and water contents (Bailey and Beauchamp 1973, Bakken 1988, Goodroad & Keeney 1984, Stanford et al. 1975 and Von Rheinhaben & Trolldenier 1984). Bailey & Beauchamp (1973) and Stanford et al. (1975) found that the denitrification increased with increasing temperature and water content,

* Dept. of Soil Biology and Chemistry, State Laboratory for Soil and Crop Research, Lottenborgvej 24, DK-2800 Lyngby, Denmark.

being near zero at 5°C. However, very little work has been done on the combined effect of water content and temperature on denitrification in soil amended with pig slurry.

The purpose of this study was to determine the relationship between temperature, water content, and the rates of denitrification and nitrous oxide production in a sandy loam soil.

Materials and method.

A sandy loam soil was amended with 20 g pig slurry/kg soil (50 Mg slurry/ha) and divided into two equal groups. After addition of slurry the soil contained 1.8 μg NO_3-N/g dw. soil and 6.5 μg NH_4-N/g dw. soil. The water content of each group was adjusted to 0.50 or 1.00 x field capacity (FC). 1 FC was equal to a soil moisture content of approx. 18 %.

The soil was packed into 10 cm long PVC tubes (Ø=28 mm) to in-situ density (1.54 g/cm^3). Each tube had a nylon net glued onto the bottom to prevent soil loss, and was placed into 1 l preservation jars, fitted with two septa.

Each group was divided into 4 equal subgroups, which were incubated at 5, 10, 15, or 20°C. At day 0, 7, 14 and 21, acetylene was added to 8 jars chosen randomly from each subgroup.

The 8 jars, incubated 7 days earlier, were removed on day 7, 14, 21 and 28, respectively, for soil chemical analysis. During the 7 days of incubation the jars were ventilated, when the concentration of N_2O became higher than 25 μl N_2O/l air, and the acetylene concentration was afterwards reestablished.

During the incubation time, the N_2O production rate (originating from nitrification and/or denitrification) and denitrification rate in the soil was determined several times. Determination of the rates involved 3 measurement of the increase in headspace concentration of N_2O in the jars, with or without acetylene, during 1-3 days. The 3 measurements from each jar were used to calculate the denitrification rate at the experimental temperature, using Bunsens coefficient of N_2O solubility in water (Tiedje 1982). A gaschromatograph equipped with a ^{63}Ni EC-detector was used.

Results and discussion.

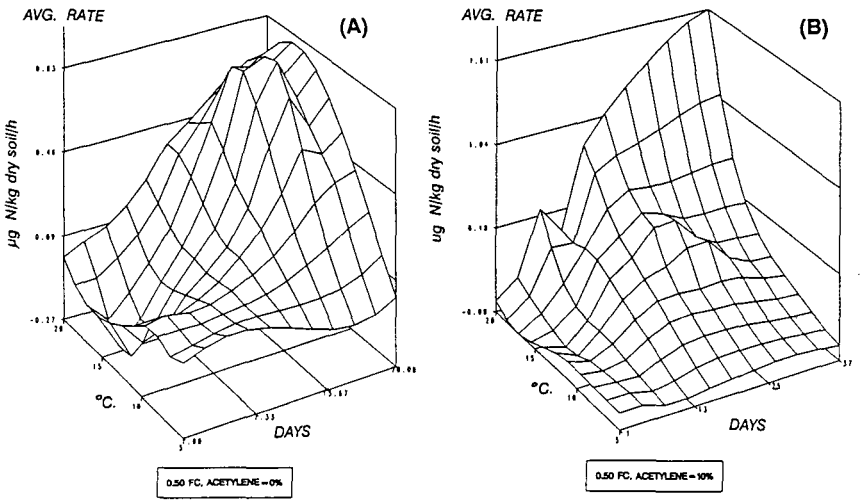

Fig. 1. (A) N_2O production rate, and (B) denitrification rate in a soil amended with 20 g pig slurry/kg soil on day zero, and incubated at 50% field capacity and 5, 10, 15, and 20° C.

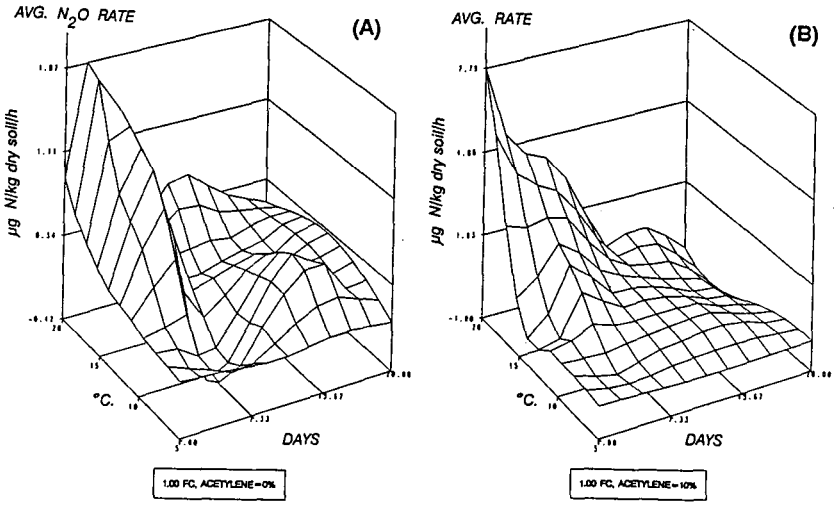

Fig. 2. (A) N_2O production rate, and (B) denitrification rate in a soil amended with 20 g pig slurry/kg soil on day zero, and incubated at 50% field capacity and 5, 10, 15, and 20° C.

The rate of N_2O production in the soil incubated at 0.5 x FC, increased steadily during 2 weeks of incubation, and reached a plateau after 3 weeks at temperatures > 10°C. The highest rate, 0.7 µg N/kg dw. soil, was observed in the soil after 3 weeks of incubation at 15-20°C (Fig. 1 A).

The denitrification rate under the same environmental conditions are shown in Fig. 1 B. At 5°C, no denitrification was observed. In the soil incubated at 20°C the denitrification rate increased to 1.6 µg/kg dw. soil during the incubation. At the temperatures 5-15°C, the soil reached its maximum rate 0.5 µg N/kg dw. soil/h, after approximately 3 weeks of incubation. After this maximum, the rate declined.

N_2O production and denitrification rates in the soil incubated at 1.0 x FC are shown in Fig. 2 A and 2 B, respectively. Denitrification and N_2O production were highest in the first week after amendment. During this period the denitrification rate was approximately 4 times higher than the N_2O production rate (approximately 1.8 µg N/kg dw. soil/h). This could be caused by mineralisation of easily decomposable organic matter with an increasing anaerobic volume in the soil as a consequence. During the next weeks the rates decreased to approximately 0.3-0.9 µg N/kg dw. soil/h.

Conclusion

Based on these result the following conclusion on N_2O production rates and denitrification rates in soil amended with pig slurry can be drawn:

- N_2O production and denitrification was very low at 5° C and at a water content of 0.5 or 1.0 FC.

- During the first week of incubation at a water content of 0.5 x FC or 1.0 x FC, N_2O production and denitrification seem to be unaffected by low temperatures (5-10° C).

- During the 2. and 3. week of incubation at 0.5 x FC, N_2O production and denitrification increased sharply with increasing temperatures.

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The Relative Importance of Denitrification and Ammonia Volatilization as Loss Processes in Flooded Rice in the Philippines

by

Freney, J.R.[+], A.C.F. Trevitt[++], S.K. De Datta[+++], W.N.Obcemea,[+++] and J.G. Real[+++]

Abstract. The relative importance of ammonia (NH_3) volatilization and denitrification as loss processes, when urea was applied to flooded rice, was assessed at four sites. The effect, on denitrification and total nitrogen (N) loss, of reducing NH_3 loss by incorporating the urea into soil was also studied.

The results show that reducing NH_3 loss by incorporating urea into soil does not necessarily result in reduced N loss, and suggest that efficiency of fertilizer N will be improved only when both N loss processes are controlled simultaneously.

Introduction

It appears that the main cause of fertilizer inefficiency in flooded rice, when N fertilizers are applied by farmer's traditional methods, is gaseous emission of dinitrogen and/or NH_3 to the atmosphere (Simpson and Freney 1988). Information on the relative importance of the two loss processes is available for very few sites in Asia (Fillery and Vlek 1986). Suitable management practices for reducing N loss and increasing the efficiency of fertilizer N can be developed only when the relative importance of these loss processes is known for a much greater range of rice growing areas and environments.

+Divison of Plant Industry, CSIRO, GPO Box 1600, Canberra, ACT 2601.

++Department of Forestry, ANU, GPO Box 4, Canberra, ACT 2601.

+++International Rice Research Institute, PO Box 933, Manila.

In this paper we report on the importance of volatilization as a loss process when urea was applied by broadcasting into the floodwater, the effect of incorporation treatments on NH_3 and total N loss, and the interdependence of NH_3 loss and denitrification loss at four sites in the Philippines.

Experimental

Table 1. Some characteristics of each site and cultural conditions for each experiment

Characteristic	Mabitac	Calauan	Aguilar	San Marcelino
Soil classification	Tropaquept	Tropaquept	Haplustoll	Ustifluvent
Soil texture	Silty clay	Clay	Silty loam	Loamy sand
pH	4.6	6.0	6.8	5.6
Total N ($g\ kg^{-1}$)	2.1	4.0	1.3	1.5
Organic matter ($g\ kg^{-1}$)	31.4	74.1	22.3	32.0
CEC [cmol (Na^+) kg^{-1}]	26	48	25	5

The experimental areas (~ 1.5 ha) were ploughed, flooded and harrowed to puddle and level the soil. After a second harrowing, 25-m radius circular areas were separated from the main fields by constructing earth banks 0.2 m high. The circular areas were used to measure ammonia volatilization directly, by a mass balance micrometeorological method (Cai *et al.* 1986), after urea was broadcast into the floodwater, and hence to formulate bulk aerodynamic relationships for each site. These were then used to assess ammonia loss from 4.8 m x 5.2 m plots subjected to different fertilizer and water management treatments. Full details of the methods for determining NH_3 loss, total N

loss, denitrification and floodwater parameters, and the fertilizer and water management treatments are given in De Datta *et al.* (1989).

A randomized complete block design with four replications was used. Urea was applied to all treatments 20 days after sowing. There was two rates of fertilizer application. Treatments 2, 3 and 4 received 53 kg N ha^{-1} and treatments 5, 6 and 7 received 80 kg N ha^{-1}. Treatment 1 was the control.

Results and Discussion

Ammonia loss for the various treatments varied from 4% to 56% of the applied N (Table 2). When urea was broadcast into 0.05 m deep floodwater, 10 days after transplanting (which is a traditional method of applying fertilizer for many Asian rice farmers), NH_3 losses for the four sites ranged from 10% to 56% of the applied N. The largest losses occurred at Mabitac and the smallest losses were observed at Aguilar. Consideration of the parameters affecting NH_3 loss suggests that the low values for NH_3 loss at Aguilar were probably due mainly to the low wind speeds at that site, while the high loss values at Mabitac were probably associated with the high wind speeds, pH values and temperatures at that location.

Incorporation of urea in the presence of floodwater was effective in reducing NH_3 loss at both rates of urea addition at one site only (Calauan, Table 2). The success of this treatment at Calauan seems to have been due to the high cation exchange capacity of the soil at that site; incorporation in the presence of floodwater markedly reduced the ammoniacal N concentration of the water at this site only.

Incorporation of the urea in the absence of floodwater (treatments 4 and 7) resulted in significantly lower NH_3 loss values at all sites. This effect presumably resulted because of the greater contact of the NH_4^+ ions, produced from the urea, with the cation exchange sites in the soils. This incorporation technique resulted in low ammoniacal N concentrations in the floodwater at all sites.

There was very little variation in total N loss values for the four sites when urea was broadcast into the floodwater 10 days after transplanting (treatments 2 and 5, Table 2). The effects of the incorporation treatments on total N loss varied from site to site. At Mabitac, incorporation in the presence of water did not reduce total N loss below the value obtained when urea was broadcast into the floodwater. Incorporation in the absence of water significantly reduced total N loss. These results are consistent with the observed effects of incorporation on NH_3 loss. However, the reduction in total N loss was not equal to the reduction in NH_3 loss.

Table 2. Effect of fertilizer and water management on NH_3, total N and denitrification (N_2) loss (% of applied nitrogen) from flooded rice

Treatment number	Urea applied (kg N ha^{-1})	Method of application	Mabitac			Calauan			Aguilar			San Marcelino		
			NH_3	N	N_2	NH_3	N	N_2	NH_3	N	N_2	NH_3	N	N_2
2	53	$B_{0.05}$[a]	48	60	12	27	59	32	14	60	46	26	65	39
3	53	$B_{0.05}$ & I[b]	42	54	12	12	53	41	10	48	38	30	80	50
4	53	B_0 & I[c]	11	33	22	11	62	51	4	44	40	14	76	62
5	80	$B_{0.05}$	56	59	3	23	65	42	10	60	50	36	71	35
6	80	$B_{0.05}$ & I	43	58	15	12	62	50	10	62	52	26	82	56
7	80	B_0 & I	7	32	25	16	55	39	5	50	45	10	80	70

[a]Basal dressing broadcast into 0.05 m floodwater 10 days after transplanting.

[b]Basal dressing broadcast into 0.05 m floodwater and incorporated into soil by rotary harrow.

[c]Basal dressing broadcast onto soil without surface water and incorporated into soil by rotary harrow; water returned to a depth of 0.05 m two days after urea application.

At Calauan there was no significant effect on total N loss of either incorporation treatment even though these treatments had resulted in reduced NH_3 losses. At Aguilar the results of the incorporation treatments were not consistent for the different rates of fertilizer application.

Both of the incorporation treatments at San Marcelino resulted in increased total N losses from the flooded soil at both rates of urea application (Table 2) even though incorporation in the absence of water had resulted in reduced NH_3 loss.

It is apparent from these studies that incorporation of urea affects other transformations and transfers of N apart from NH_3 loss.

When urea was broadcast into the floodwater at each site, 10 days after transplanting, it was found that denitrification losses varied widely (from 3% to 50% of the applied N). Loss by denitrification was lowest at Mabitac and highest at Aguilar. These results suggest that denitrification losses and NH_3 losses were complementary i.e. denitrification losses were low when NH_3 losses were high (Mabitac) and high when NH_3 losses were low (Aguilar). However, the results of the incorporation treatments did not always support this suggestion. For example, at Aguilar, reducing NH_3 loss by incorporation in the absence of floodwater had no effect on denitrification whereas at the other three sites this treatment increased denitrification when urea was applied at the low rate.

Results of experiments in China (Cai *et al.* 1986; Zhu *et al.* 1988), Philippines (Fillery and Vlek 1986) and Australia (Simpson *et al.* 1988) fail to resolve the question as to whether NH_3 losses and denitrification losses are independent or complementary. It is possible that the two loss processes are independent in some soils or treatments but complementary in others, and that the interaction depends on changes in the rate of nitrification of the NH_3 conserved and/or changes in availability of the organic matter as a result of the treatment. It is also possible that the methods of analysis used are not sufficiently accurate to provide the complete story.

The results show that reducing ammonia loss by treatments such as incorporation will not necessarily reduce total nitrogen loss from flooded rice.

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Measuring denitrification following application of pig slurry on a loamy soil.

by

Van den Abbeel, R., Paulus, D., De Ruysscher, C. and Vlassak K.

ABSTRACT

A field experiment commencing in winter (November) was conducted to determine the fate of nitrogen (N) following surface application of pig slurry to a bare loamy soil. Slurry was applied at 50 t ha^{-1}.

The total denitrification loss , measured using an adapted acetylene-inhibition technique, was 21 kg ha^{-1}. Losses through volatilization and leaching were 48 and 120 kg ha^{-1} respectively.

INTRODUCTION

Considering the density of pig and poultry farms in European countries, Belgium and the Netherlands are the most important (L. Van Acker et. al., 1987). To maintain the high levels of livestock production large quantities of nitrogen (N) as a fertilizer and imported animal feed are used.

Livestock production at an industrialised level is highly intensive and the surface to spread the waste products is often not available on the same farm.

Katholieke Universiteit Leuven, Fakulteit der Landbouwwetenschappen, Kardinaal Mercierlaan 92, B-3030 Leuven/Belgium

This leads to spreading of manure in the immediate surroundings of the production unit at high rates, thereby stimulating N losses from soil.

MATERIALS AND METHODS

Site Characteristics

The experiment was conducted at Ter Munck on a fallow plot. The soil was well drained and loamy with a Bt-horizon (Typic Hapludalf, USDA classification). Some soil properties can be found in Table 1.

Table 1. Selected soil properties of the Ter Munck soil (0-30 cm).

pH_{H2O}	7.5	Clay (%)	10.5
pH_{KCl}	6.6	Total N (%)	0.103
bulk density (g/cm^3)	1.35	Total C (%)	1.03

Slurry treatments

The slurry was applied at a rate of 50 tons ha^{-1}. Selected slurry properties are given in Table 2.

Table 2. Selected slurry properties.

	Composition	N applied (kg ha^{-1})
dry matter (%)	4.4	
pH	6.6	
Total nitrogen (%)	0.54	270
NH_4^+-N (mg/l)	4172	209
NO_3^--N (mg/l)	46	2.3

Design

The plot layout consisted of two rows of plots. One row of 4 plots for the surface application of manure, and a second row of 4 control plots.

Denitrification, ammonia volatilization and leaching losses

Measurements of denitrification were made at intervals of approximately one week using an adaptation of the acetylene inhibition technique similar to that described by Aulakh et al. (1982).

Loss of N through ammonia volatilization was determined using a system of wind tunnels. The system consisted of 12 wind tunnels through which an air flow was drawn (17,5 volumes/min), the air leaving the tunnels was bubbled through a 2% boric acid solution. The amount of NH_4^+ volatilized, was measured by titration of the acid solution.

Losses of NO_3^- through leaching were estimated on separate plots to which 200 kg ha^{-1} of KCl was added as a tracer. As Cl^- ions, in contrast with NO_3^- ions, are not subjected to any significant biological transformation, leaching can be estimated in this way. Every forthnight soil samples were taken to a depth of 90 cm and analysed for NO_3^-, NH_4^+ and Cl^-.

RESULTS

Denitrification

The addition of manure clearly stimulated the production of N2O through denitrification (Fig 1.). The loss of N was continuous for 4 months after the application of slurry until the end of Februari (Fig 2.).

Throughout this period the water content was always above field capacity (0.27 ml/cm^3 soil). Between the 7th of November and the 2nd of January, the rate of denitrification varied from 16 g ha^{-1} day^{-1} to 704 g ha^{-1} day^{-1} (Fig 2.). During the 4 months following application thirteen measurements were taken. The total denitrification loss (Fig 1.) from the manured plots was 21.3 kg N ha^{-1} and from the control plots 0.4 kg N ha^{-1}. The variation in denitrification rate reflected the variation in soil temperature, soil moisture and nitrate content (Fig.2). As an example the highest rate of denitrification at december 15 coincides with a rise in temperature (from 4.5 to 6.1 °C), a relatively high moisture content and a peak concentration of nitrate in soil. After this maximum rate the decrease in temperature is again reflected in a decreasing denitrification

rate. Despite low temperatures at february 6 denitrification continued equally 53 g N ha^{-1} day^{-1} at a temperature of 0.2 °C.

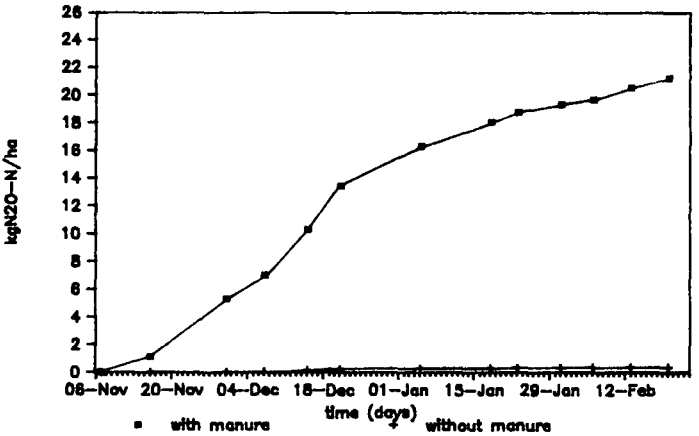

Fig. 1. Cumulative denitrification in time.

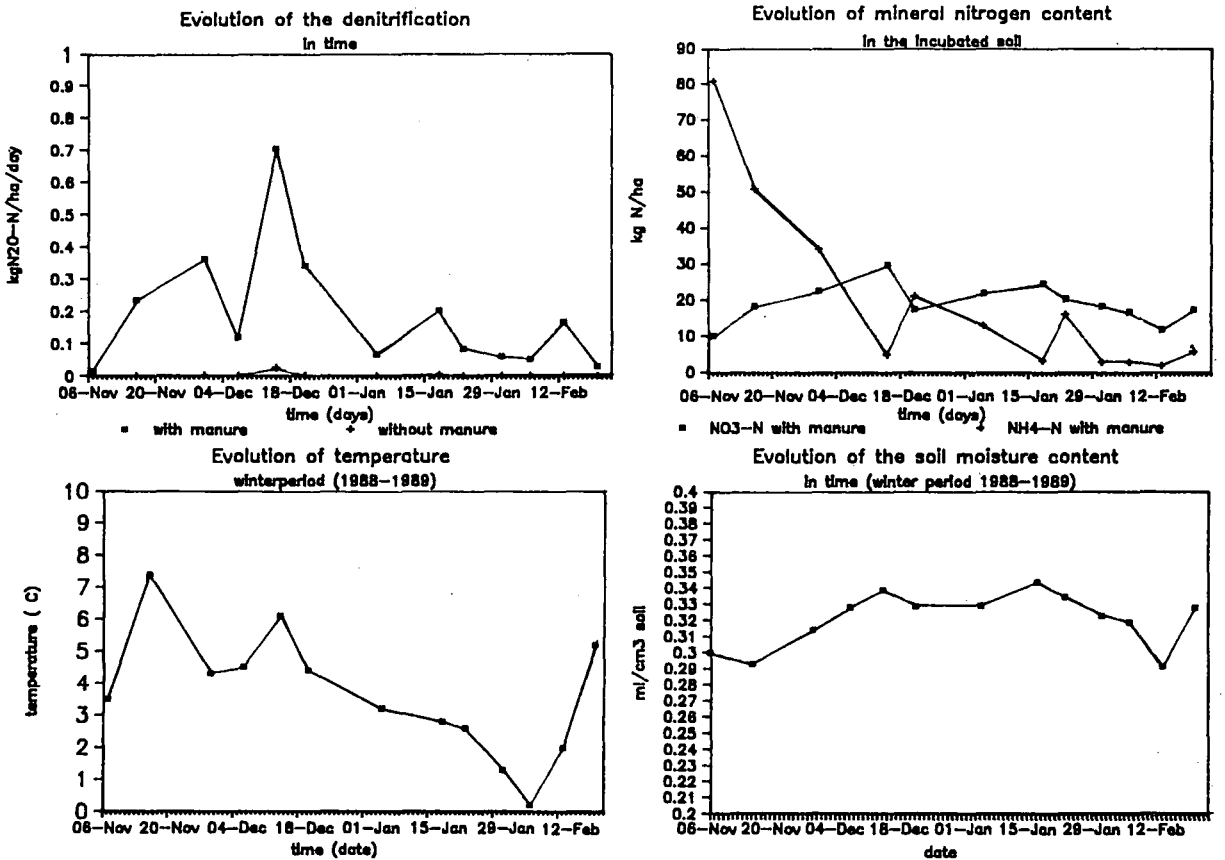

Fig. 2. Rates of denitrification, soil mineral contents, soil moisture contents and soil temperatures at 10 cm.

NH_3 volatilization and leaching

The total NH_3 loss following slurry application was 48.6 kg NH_3-N ha^{-1}. Variations in the rate of NH_3 volatilization were related to the air temperature, after 3 days the loss of NH_3 decreased substantially despite high temperatures and relatively constant windspeeds.

The amount of Cl^- leached out of the profile (0-90 cm) was 120 kg ha^{-1} within 4 months' time.

N balance

Table 3. Sinks for N following application of slurry.

Input (kg N ha^{-1})		Output (kg N ha^{-1})	
organic N	59	denitrification	21
mineral N	211	NH3 volatilization	48
		leaching	120
Total N	270	SUM	169

DISCUSSION

The denitrification loss from the manured plot was equivalent to 10% of the mineral N applied in the slurry. This loss was significantly different from the control plot and occurred even at the low soil temperatures.

This might be due to the presence of readily available carbon sources supplied through the manure (1.4 ton ha^{-1})(Burford and Bremner, 1975; Rice et al., 1988; Parkin, 1987).

The average coefficients of variation for the denitrification determination was 45 %, which was reduced to 39 % for fluxes larger than 0.1 kg N ha^{-1}. The variability of the denitrification measurements was high but within the range reported in other studies. Average coefficients of variation are frequently ranging between 50 and 100 % (Parkin et al., 1984;Thompson et al. 1987).

The loss of N through volatilization could be estimated to consist of 23% of the NH_4^+ initially present in the slurry . These losses are in the range reported in other studies (Thompson et al. 1987).

In contrast to realistic situations including the presence of field crops, leaching losses were considerable (44% of the N added). The data obtained agree with the findings of Van der Veen, 1984 and Steenvoorden, 1983 these authors find leaching losses after addition of manure in the range of 30 to 50 percent of the total N added, depending on climatic factors, amount of nutrient added, soil texture and structure.

The total quantity of N accounted for is 169 kg ha^{-1}, this means 63% of the total N added with the manure. The unrecovered N (100 kg ha^{-1}) is assumed to have contributed to the reserve of organic N in soil.

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Differentiation of N-losses *in situ* from a soil amended with ^{15}N-labelled green manure

by

v.Bischopinck,K.U.*; Munch,J.C.*; Chapot,J.Y.** and Heinemeyer,O.***

SUMMARY

In a lysimeter experiment to determine amounts and sources of N-losses occasioned by denitrification, a silty loamy soil from Alsace (France) was amended in 2 lysimeters with ^{15}N-labelled green manure (Sinapis arvensis L.). The manure was mixed into soil in December. Denitrification losses, plants, and percolated water were analysed up to August of the next year. The N-fluxes to the atmosphere were measured by use of the acetylene blockage method. The N_2O was collected in a chamber and trapped on a molecular sieve. ^{15}N was also trapped in chambers and collected in vacutainer tubes. Measurements of N_2O were performed then by gas chromatography (ECD), and of ^{15}N by mass spectrometry. At first time, measured N-losses originated from soil-N-compounds. At April, 6% of gaseous losses originated from green manure. This percentage rised only up to 50% in August. So, easily mineralizable organic matter will not immediatly be the major N-source for denitrification. The total amount of N losses from green manure was 0.6 kg N/ha from January to August.

* Institut für Bodenkunde und Standortslehre, Universität Hohenheim, D-7000 Stuttgart 70

** Station d'Agronomie (I.N.R.A.), F-6800 Colmar, France

*** Institut für Bodenbiologie, Bundesforschungsanstalt für Landwirtschaft, D-3300 Braunschweig

INTRODUCTION

Green manuring ist an essential agricultural practice in favour of more ecological kinds of cropping. Important goals of this farming are the amelioration of the soil texture and the conservation of mineral nitrogen in soil. The objective of field experiment presented here was primarily to establish what part of the nitrogen added to soil by green manuring will be lossed from soil in gaseous form in course of denitrification. On the one side addition of this easily metabolizable organic matter will possibly lead to nitrogen mineralization, nitrification and supply of plants. On the other hand, organic amendements enhance microbial activity and oxygen demand. In case of oxygen deficiency following an increased need in soil compartments, the alternative pathway for energy conservation of soil microorganisms will first be the denitrification as alternative anaerobic respiration. The consequence of this metabolism is a loss of nitrogen by the reduction of nitrate to gaseous nitrogen compounds (N_2O, N_2) emitted to the atmosphere.

MATERIAL AND METHODS

^{15}N-labelled mustard plants (Sinapis arvensis L.) were mixed at 86.12.23. into soil in lysimeters at the experimental station INRA (Institut National de la Recherche Agronomique) to Colmar (France). Table 1 give the N content and labelling proportions of mustard plant material, Table 2 give characteristics of soils used. Mustard plants were cultivated on an other soil with ^{15}N-labelled mineral nitrogen fertilizer as N source.The plants were harvested, cuted and mixed into the lysimeters soil. At spring, the soil of the lysimeters was planted with mais (Zea mais), and after harvest at autumn, mais plants analysed on total N and ^{15}N. Percolated water was collected and also analysed on N fractions. Total denitrification losses were measured by the acetylene inhibition technic (RYDEN et al. 1978). N_2O emitted from

Table 1:
Characteristics of plant material used as green manure (Sinapis arvénsis L.)

	Dry Matter	N	^{15}N
	t/ha	kg/ha	
green matter	4.7	53.0	6.0
litter	0.6	8.1	0.9
roots	0.5	1.6	0.2
Total	5.8	62.7	7.1

Isotopic enrichment: 11.32%

soil was trapped under soil chambers (0.1 x 0.5 m) and adsorbed on molecular sieve. N_2O was desorbed latter in the Lab at Stuttgart-Hohenheim and analysed by gas chromatography with electron capture detection. For analysis of gaseous N losses from green manure, ^{15}N-N_2 and ^{15}N-N_2O were capted under soil chambers and sampled at different times into vacutainer tubes. This samples were analysed on ^{15}N at the Lab of the Institute for Soil Biology at the Bundesforschungsanstalt für Landwirtschaft (FAL) at Braunschweig (FRG) by mass spectrometry. Amounts of gaseous N losses from green manure were calculated about the percentage of ^{15}N in the plants and the curves of ^{15}N accumulation under soil chambers (SIEGEL et al. 1982).

Table 2:
General soil characteristics

depth (cm)	0-30	30-60	60-90
clay (%)	23.4	23.0	17.4
silt (%)	67.8	69.6	71.9
sand (%)	6.8	6.3	10.2
C (%)	1.22	0.61	0.26
N (%)	1.56	0.76	0.31
pH (H_2O)	8.2	8.4	8.5
lime (%)	15.1	26.3	36.2

RESULTS

a. Leaching of nitrate:
Leaching of $N\text{-}NO_3$ was extremelly low in course of the experiment. In the winter 1986/1987, not nitrate was found in the percolated water, 0.6 kg $N\text{-}NO_3$/ha were measured in the spring 1987, and 0.7 kg $N\text{-}NO_3$/ha in the winter 1987/1988.

b. Denitrification losses:
Table 3 shows the total denitrification losses measured by the acetylene inhibition technic and the N-losses from the green manure measured and calculated by the ^{15}N isotope balancing methode. The comparison of both gaseous fraction shows an opposite development in course of plant growing season. Total denitrification losses were decreasing strongly from February (163 g N/ha/day to August (12.2 g N/ha/day). Gaseous losses from green manure increased clearly

Table 3:
Quantities of gaseous N lossed from lysimeter by denitrification

Date	Total	^{15}N	^{15}N % of total
	g N/ha/day		
02.01.87	0.0	0.0	
18.02.87	163.0	0.0	0.0
14.04.87	47.9	3.0	6.3
26.05.87	23.6	3.2	13.6
08.07.87	3.4	0.39	11.5
12.08.87	12.2	6.1	50.0

from February (0 g N/ha/day) to August (6.1 g N/ha/day). Also, relation of N-gases from green manure to total N-losses by denitrification showed the same development: an increase from 0 to 50% in course of growing season. Total N-losses do to denitrification were evaluated for the period from January to August on 10.1 kg N/ha. At the same time, the N-losses issue from the green manure from January to August are 0.6 kg N/ha.

c. Uptake by plants:

At the end of growing season, mais planted on the lysimeters had take up 160 kg N/ha, and 17.9 kg N/ha of this nitrogen originated from the green manure.

Table 4:
N-balance of green manure

	kg N/ha	%
applied	62.7	100
plant uptake	17.9	29
gaseous loss	0.5	1
leaching	0	0

DISCUSSION AND CONCLUSION

Organic matter of the green manure mixed into lysimeter soil was mineralized for a great part in the next summer. In course of the first growing season after manuring, the mobilized nitrogen was taken up for the greatest part (73 %) by plants. Only 1% of this organic N added to soil by green manure was lossed in gaseous form until August. This shows clearly that under the experimental conditions, green manuring leads to conserve nitrogen in soil. The organic matter as electron and proton donor for metabolism of soil microorganisms leaded not to notable denitrification of added nitrogen, obvioulsy half of denitrifyied nitrogen orginated from green manure. The dependence of denitrification intensity from amounts of easily mineralizable organic matter was often demonstrated in field and modell experiments (BURFORD and BREMNER, 1975; KNOWLES, 1982, DEMERDASH and OTTOW, 1983), so far nitrogen will not be limiting for metabolism (GÖK and OTTOW, 1987). However, field experiments without plantation done by LEHN-REISER et al. (1989) to study influence of green manure on denitrification intensity showed for the same locality only a little increase for manured plots (8.5 and 6.4 kg N/ha) in comparison to untreated plots (5.8 kg N/ha) for a periode from April to December. This additional experiments with ^{15}N-labelled plants presented here showed that green manure will rather contribute to nitrogen nutrition of the plants than to increased denitrification and gaseous losses of the added nitrogen.

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Field measured denitrification losses from a calcerous Inceptisol after green manuring

by
Lehn-Reiser, M.[1]
Munch, J.C.[2], Chapot, J.Y.[3] and J.C.G. Ottow [1]

Introduction

Prerequisites for heterotrophic denitrification are a relative high amount of easily decomposable organic matter, sufficient nitrate, and a reduced oxygen concentration or diffusion rate (Ottow and Fabig, 1985). Green manuring improves soil conditions and properties through an increased microbial activity. At the Institute National de la Recherche Agronomique (INRA) an interdisciplinary research project was established to study the effect of vetch (Vicia sativa) and rape (Brassica napus) incorporation on nitrogen-supply for the following crop (corn), N-turnover, NO_3^--leaching and denitrification. A calcerous soil close to Colmar/France received with 6.2 t ha^{-1} vetch and 7.1 t ha^{-1} rape biomass corresponding to 210 kg N ha^{-1} or 90 kg N ha^{-1}, respectively. In order to evaluate the efficiency of green manure-N for the following corn crop, the losses by denitrification during intensive mineralization processes should be quantified.
The denitrification losses were measured by using the acetylene inhibition technique and, up to a depth of 60 cm, nitrate, ammonium and watersoluble carbon contents as well as the population densities of bacteria were studied. The 336 N_2O-flux measurements were distributed throughout 7 measuring periods between April and October, 1986. Estimates of the denitrification losses were made by interpolating the unmeasured periods with the 4-day measurements at the soil surface.

Material and Methods

The experimental site is located at the Institute National de la Recherche Agronomique (INRA), Colmar, France. The soil is classified as calcerous Inceptisol.

1 Institut für Mikrobiologie und Landeskultur Justus Liebig Universität, Senckenbergstr. 3, D-6300 Giessen, FRG
2 Institut für Bodenkunde und Standortslehre, Emil Wolf Str.27, D-7000 Stuttgart-Hohenheim 70, FRG
3 Institute National de la Recherche Agronomique (INRA) Colmar, France

The main properties (Schlichting and Blume, 1966) after green manure incorporation are listed in Table 1. Further physicochemical analyses the untreated control (0-30 cm) exhibited the following characteristics: pH [H_2O]: 8,1; P_2O_5: 0,05%; cation exchange capacity, exchangeable K^+, exchangeable Mg^{++} were 127, 8 and 11,2 meq 100 g^{-1} dry soil, respectively. Max. water holding capacity reached 75 ml 100 g^{-1} dry soil. Clay, silt and sand were 25,1, 67,1 and 7,8 [% w/w], respectively. Denitrification measurements by C_2H_2-inhibition technique

Table 1: Some soil properties of the experimental sites

green manured plots	soil depth [cm]	Soil properties				
		C_t [%]	Humus [%]	N_t [%]	C/N [%]	$CaCO_3$ [%]
Vetch	0-20	1,22	2,1	0,14	8,9	12,9
	20-40	0,88	1,51	0,1	8,5	15,6
	40-60	0,62	1,06	0,07	8,7	25,4
Rape	0-20	1,25	2,15	0,14	9,1	10,1
	20-40	0,88	1,51	0,09	8,9	19,6
	40-60	0,55	0,94	0,061	9,1	30,7
Control	0-30	1,1	1,89	0,12	8,8	13,2
	30-50	0,74	1,27	0,08	8,8	20,0

were made at three different green manured (vetch, rape, control) plots (12 X 32 m). The field equipment (4 replicates) and operational procedures were those described by Ryden, (1983) and Benckiser et al. (1986). Briefly, C_2H_2-concentrations of 0,1-1% [v/v] were established in the soil air by the aid of six probes inserted into a depth of 60 cm (C_2H_2-supply three hours without pressure). The probes surrounded an open PVC-cover (50 X 10 X 15 cm). After an equilibration time of 1 hour the N_2O released was collected by a continous air stream (30 l h^{-1}) into molecular sieve (35 g, 2 mm Pellets) traps (5 A) for three hours. In order to avoid long term side effects the sites were changed daily. N_2O adsorbed by the molecular sieves was released in the laboratory by addition of water to an evacuated Erlenmeyer flask and analyzed gaschromatographically (GC Sigma 300 with ECD, Perkin Elmer, Porapak Q and R with Methan/Argon gas [20 ml^{-1}] as carrier; detector: 200°C; injector: 50°C; column: 54°C). For measuring N_2O concentrations in the soil atmosphere PVC-gas sampling tubes closed with a septum seal were installed at a depth of 20, 40 and 60 cm. With the aid of vacutainer glass tubes (5 ml) soil air was sampled and N_2O analyzed gaschromatographically.

From each soil samples were collected at frequent intervals and analyzed for nitrate and ammonium colometrically (Schlichting and Blume, 1966; DEV, 1983), as well as watersoluble and total carbon (Burford and Bremner; 1976). All results were related to dry weight base.

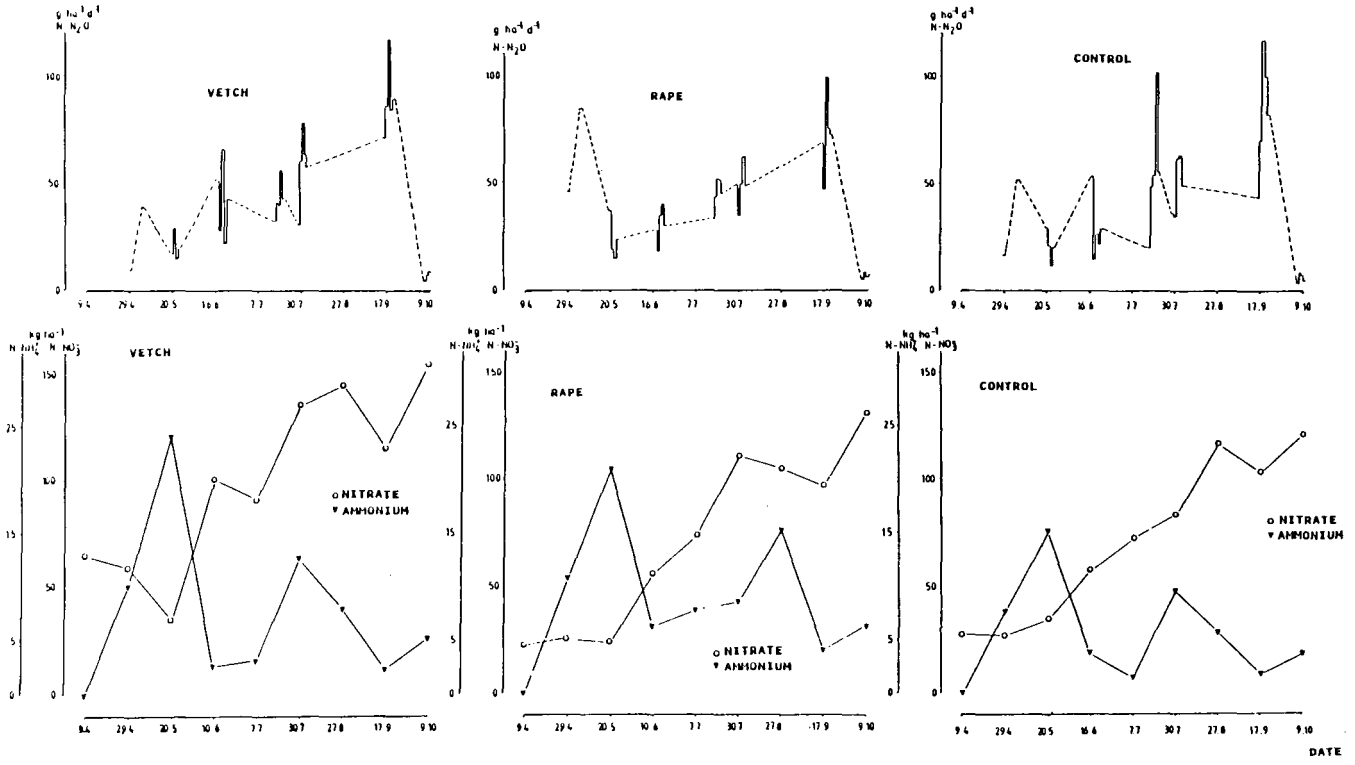

Figure 1: Daily N_2O-fluxes from plots amended with green manures in comparison to soil ammonium and nitrate contents (0-40 cm)

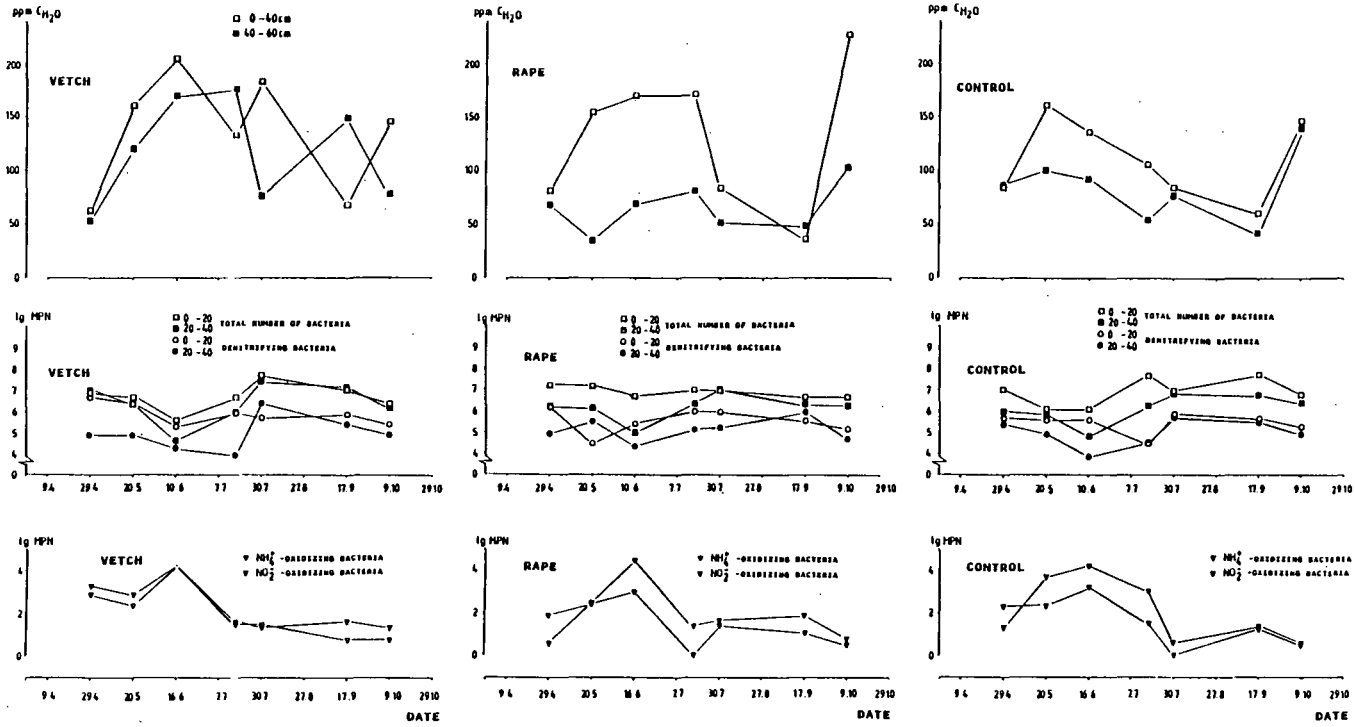

Figure 2: Available carbon, total number of bacteria and denitrifying and nitrifying bacteria at different soil depth during April-October, 1986

For soil microbiological analysis, soil samples were taken and frozen until analysis. Samples of 20 g were shaken for 1 hour in 180 ml Ringer-solution with 0,18% sodiumpolyphosphate, serially diluted and 1 ml samples used to inoculate different liquid media in order to enumerate the total number of bacteria, the population of denitrifying (Schmider and Ottow, 1984), as well as nitrifying bacteria (Parkinson et al., 1971). Population densities were expressed in MPN g^{-1} dry soil (De Man, 1983).

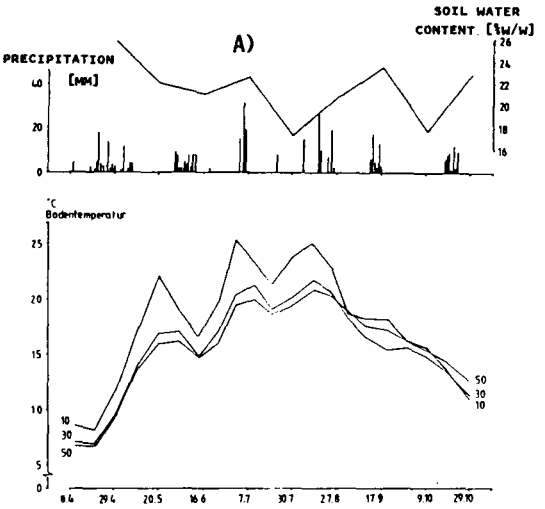

Figure 3: Soil temperature at different soil depths as well as precipitation and soil water content during April-October 1986

Results and discussion

In Fig.1 the rate of denitrification in control and vetch or rape amended plots are compared with developments in soil nitrate and ammonium contents. The N_2O-surface fluxes of all plots show similar trends. Yet, the vetch and rape plots surpassed the control by 47% and 10%, respectively. The NO_3^--content increased continiously in all 3 plots. The increase was highest in the sites amended with vetch, which surpassed the control by 33% and rape by 37%. Generally, NO_3^--contents correlated positively with the N_2O-surface fluxes. However, during the period of September 17 to 20 (1986), nitrate content decreased (vetch: 18%; rape: 14%) while Nitrous oxide surface fluxes were enhanced (vetch: 53%; rape: 47%) This increase in denitrification may be ascribed to several rainfall events (12.-17.9: 43 mm)(Fig.2). Correspondingly, the soil water contents of the upper 20 cm of the soil increased from 18% to 24% (w/w)(Fig.2). In spite of high nitrate contents (Fig.1), the low denitrification rates in October should be explained by very low water contents (16-18% w/w). Furthermore in Fig.2 soil temperatures at different depths are shown. If the denitrification rates at similar water regimes are compared with soil temperatures (0-10 cm), a significant correlation ($r=0,98$) is obtained. Due to the mineralization processes highest NO_3^--contents and denitrification rates

were found during autumn (Fig.1) which has been observed before in agricultural ecosystems (Ryden, 1983, Benckiser et al., 1987). Water-soluble carbon, total number of bacteria, denitrifyers and nitrifyers for the three variants are given in Fig.3. As recently shown by Paul and Beauchamp (1989) for leguminose green manure, available C (0-40 cm) was highest in the vetch plot (29% above control) followed by the rape plot (22% above control). These results corresponds well with the different denitrification losses (Fig.1). The N_2O-surface fluxes from April to October may account for 5.8 (control), 6.4 (rape) and 8.5 kg N ha^{-1} (vetch), respectively. Changes in total number of bacteria and denitrifyers densitiy were not always conform with the changes in N_2O emissions. However, denitrification rates were generally well correlated with total number of bacteria, particulary in the control plots.

Conclusion

Based on the results obtained under the conditions given green manuring seem to enhance denitrification losses. This may be explained by an increased soil nitrate content and subsequently nitrate respiration. This may be ascribed to an increased demand for electron acceptors caused by intensive mineralization processes. The most pronounced effect of soil conditions on denitrification was shown by soil temperature. The relationship between the total number of bacteria (MPN g^{-1} dry soil) in topsoil and gaseous N-losses support the explanation given above. Final conclusions, however, must await further results from field experiments.

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Estimation of denitrification losses by the acetylene inhibition method from a ryegrass field (Lolium perenne) as effected by mineral fertilization or animal slurry

M. Kapp[1], J. Schwarz[1], , G. Benckiser[1], P. Daniel[2], W. Opitz von Boberfeld[2] and J.C.G. Ottow[1].

About 200 million tons of animal slurry corresponding to 600 000 tons of NH_4^+-N are annually produced in the Federal Republic of Germany. In order to avoid nitrogen impacts on rivers, lakes, groundwater and atmosphere controlled slurry recycling especially in areas with high-density livestock numbers is a public necessity. In soil nitrogen becomes rapidly nitrified and denitrification is likely to occur in the microbiologically active root zone of the grass. So far little is known about total denitrification losses (N_2, N_2O, NO) from highly productive ryegrass fields treated with animal slurry as a nitrogen source.

Material and Methods

From October 1988 on denitrification measurements employing the C_2H_2 - inhibition technique were carried out to compare unfertilized ryegrass fields with plots that received mineral-N or organic-N as pig-cattle slurry. The field was designed as a Latin square (4 variants with 4 replicates, 4.5 * 14 m for each plot). The loamy soil of the upper 20 cm (sand 35.8%, silt 49.1%, clay 15.2%) is characterized by a pH($CaCl_2$) of 5.7, and a C_t- as well as N_t-content of 1.65% and 0.15%, respectively. The subsoil below 40 cm increased in clay content (sand 12.2, silt 32.3, clay 54.0). The long term mean of precipitation for the experimental site of the Department of Grünlandwirtschaft und Futterbau amounts 610 mm per year.

Fertilization started on 5th of July 1988 with calcium ammonium nitrate (KAS; 150 kg N ha^{-1}) and pig-cattle slurry (150 kg N ha^{-1}) alone or mixed with the nitrification inhibitor Didin (Dicyandiamide; 30 kg ha^{-1}).The treatments were repeated on August 22, 1988 and on April 4, 1989 and May 29, 1989. The application technique used allows a homogeneous distribution and is described by Vetter et al. (1981). To keep NH_3-losses as low as possible the plots were irrigated by a simulated rainfall (20 mm during two days) immediatly after slurry spreading.

1) Institut für Mikrobiologie und Landeskultur, Universität Giessen, Senckenbergstr. 3, 6300 Giessen, FRG

2) Institut für Pflanzenbau und Pflanzenzüchtung II - Grünlandwirtschaft und Futterbau - , Universität Giessen, Ludwigstr. 23, 6300 Giessen, FRG

The field units (open chambers 500*100*150 mm, molecular sieve (5Å) traps, C_2H_2-supply probes) for measuring N_2O surface fluxes after C_2H_2 application were similar as described by Benckiser et al. (1986). A departure from this technique was the use of open chambers equipped with a removable plexiglas lid to allow the entering of light during the measuring period. Furthermore, the $CaCl_2$-soda lime columns were replaced by a third molecular sieve trap. The probes for collecting soil air were constructed according to Benckiser et al.(1986) and Greilich et al.(1975). The day before each period of measuring the soil was flushed for 5 h with 50 l C_2H_2. On the following 4 days 10 l C_2H_2 per chamber were injected over 2 hours into 50 cm depth of soil. Afterwards the experimental sites were changed.

The evolved N_2O was trapped every day for 8 hours in molecular sieve and recovered in the laboratory by transferring the molecular sieve pellets into evacuated 1 l flasks filled with 150 ml H_2O. Nitrous oxide was analysed together with the N_2O, CO_2 and C_2H_2 from soil air samples by gaschromatograph (GC Sigma 300 Perkin Elmer, oven 50°C, ECD 300°C , GC 8510 Perkin Elmer ,oven 40°C, ECD 300°C or with a GC 8410 Perkin Elmer, oven 50°C, TCD 150°C; all columns Poropak Q).

Soil water contents were determined gravimetrically and nitrate concentration was analysed according to Scharpf and Wehrmann (1976).

Results and Discussion

The effect of fertilization on the denitrification rates during the first measuring period (October 20 to 26, 1988) are shown in Figure 1. Only mineral fertilization increased the N_2O efflux compared with the untreated plots.

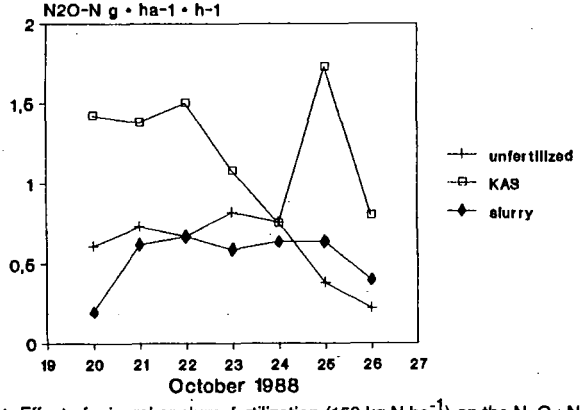

Fig.1: Effect of mineral or slurry fertilization (150 kg N ha^{-1}) on the N_2O+N_2 surface fluxes during October 20 to 26, 1988 in a ryegrass field. Slurry and mineral fertilizer was applied on July 5 and August 22, 1988, respectively.

In Figure 2 the N_2O surface fluxes (C_2H_2-treated) of the unfertilized ryegrass plots are compared with the soil nitrate and water content during the period of April to June 1989.

It may be concluded that denitrification losses from the untreated control are most intensive at periods of relative high water content. The nitrate concentrations in both top- and subsoil remained relatively low throughout the entire period. This may have restricted denitrification activity.

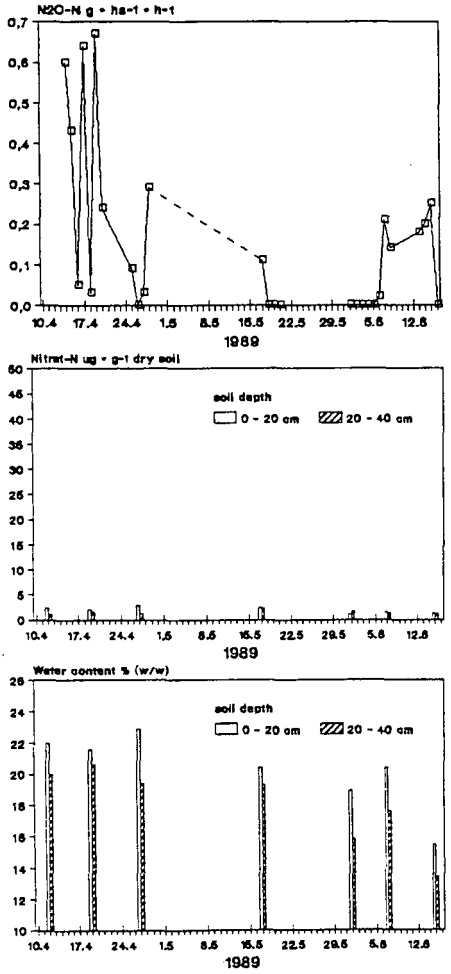

Fig.2: N_2O and N_2 surface fluxes ,soil nitrate and soil water content in an unfertilized ryegrass field (control) during April to June, 1989.

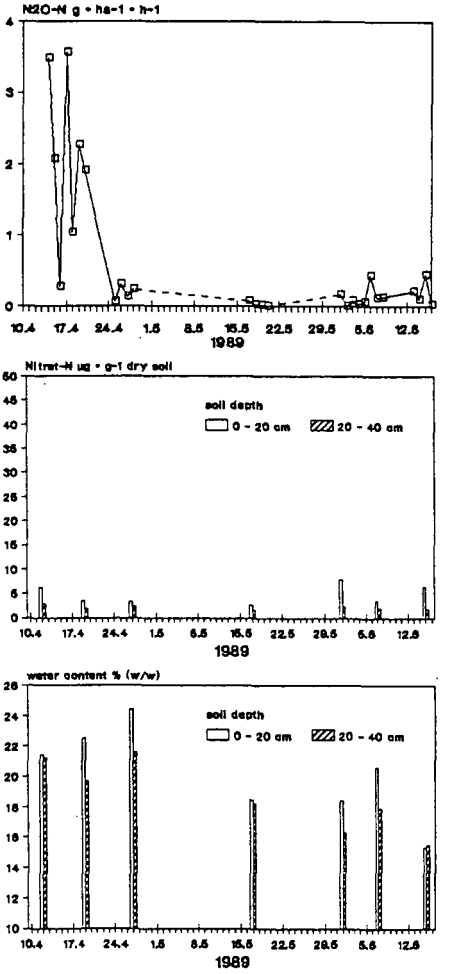

Fig.3: Effect of animal slurry (150 kg N ha^{-1}) on N_2O+N_2 surface fluxes, soil nitrate and soil water content during April to June (1989) in a ryegrassfield. Slurry was applied on April 4 and May 29, 1989, respectively.

In Fig. 3 the effect of slurry application on total denitrification (in the presence of acetylene) is presented in relation to the nitrate and water content.
Compared to the unfertilized control (Fig. 1), slurry amendments increased denitrification by

500% (April 14 to 28, 1989),
0 % (May 17 to 20, 1989) and
188% (June 1 to 16, 1989),

respectively. During the first 14 days after slurry application soil nitrate concentrations (in topsoil) were nearly twice as high as in the control (Fig.1). It seems as if nitrate is governing the rate of denitrification in April and June, 1989.
In Figure 4 the effect of mineral-N (KAS at 150 kg N * ha^{-1}) on the denitrification losses from the ryegrass field are given in relation to nitrate and water content. As the result of mineral N fertilization (ammonium and nitrate), the high nitrate concentration (in top- and subsoil) increased denitrification considerably. If compared to the control, the N_2O-N losses increased by

3350% (April 14 to 28, 1989),
185% (May 17 to 20, 1989) and
925% (June 1 to 16, 1989),

respectively.

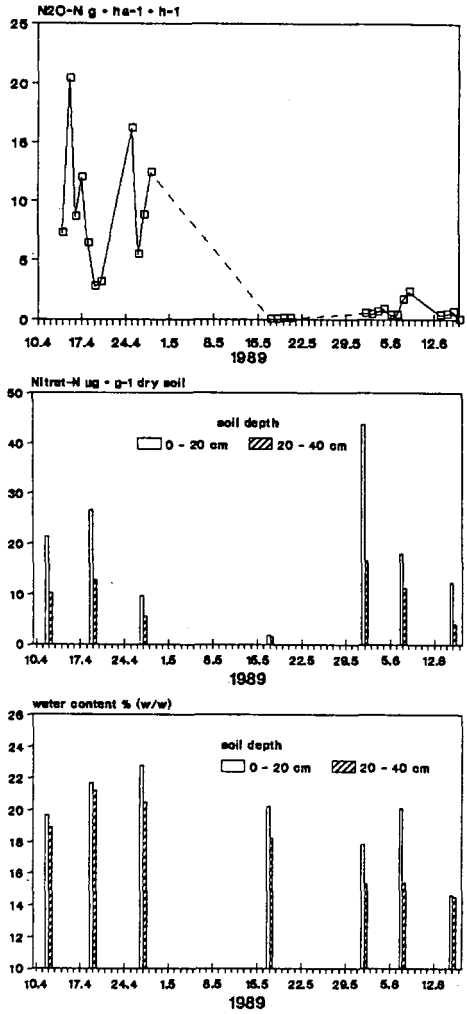

Fig. 4: Effect of mineral-N fertilization (KAS; 150 kg N ha^{-1}) on N_2O+N_2 surface fluxes, soil nitrate and soil water content during April to June (1989) in a ryegrassfield. Mineral fertilizer was applied on April 4 and May 29, 1989, respectively.

Since water contents and soil temperatures were similar in all plots, the increased denitrification losses should be ascribed to the enhanced availibility of soil nitrate. Carbon availability should not be considered as the denitrification restricting factor, because measurements of potential denitrification (not reported here) under optimal conditions in the laboratory are significantly higher than the field data. The differences between the denitrification rates of the plots fertilized with mineral N or organic N may be explained by volatillization losses of N as ammonia. Relatively high N losses via NH_3 after slurry application has been reported before (Ball and Ryden, 1984).

Tab. 1: Denitrification losses in control, slurry amended and minerally N fertilized ryegrass fields, respectively.[1)]

Treatments	measuring periods			
	Oct.,1988 (6 days)	April,1989 (16 days)	May,1989 (4 days)	June,1989 (12 days)
	g N * ha^{-1}			
unfertilized	101	101	3	31
slurry	91	500	3	58
slurry+Didin[2)]	74	457	7	58
KAS	208	3387	5	284

1) before each measuring period except May 150 kg N ha^{-1} were applied in the slurry and mineral fertilizer plots.
2) the nitrification inhibitor Didin was applied at rates of 30 kg ha^{-1}

In Table 1 the overall denitrification losses of the different treatments are compared. Dicyandiamide seems to have only a little effect on denitrification losses. The results are in good agreement with denitrification losses from grasslands reported by Christensen (1983) and Corré et al. (1989). Final results, however, must await repeated experiments.

Acknowledgement:
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Use of Encapsulated Calcium Carbide to Reduce Denitrification Losses from Urea-Fertilized Flooded Rice

by

N.K. Banerjee[+], A.R.Mosier[++], K.S. Uppal and N.N Goswami[+]

INTRODUCTION

Poor nitrogen fertilizer use efficiency in urea-fertilized flooded rice is a common problem. Nitrogen balance studies have shown that fertilizer N found in rice plant straw and grain usually totals only 20-40% of the N applied, while 20-30% remain in the soil and 30-60% is lost from the soil-plant system. In impermeable soils typically used to grow flooded rice, ammonia volatilization and denitrification are thought to be the dominant N loss mechanisms (Buresh and De Datta, 1989). In soils where ammonia volatilization is small it may be possible to limit denitrification with a nitrification inhibitor to increase N use efficiency.

A number of nitrification inhibitors have been and are being used, but commercially available nitrification inhibitors are not effective in flooded soils (Keeney, 1986). Sahrawat et al., (1986) demonstrated that acetylene is an effective nitrification inhibitor in conditions where other inhibitors were not effective. Because acetylene is a gas the problem of maintaining a supply of the compound in the soil at the few ppm concentration necessary to stop nitrification (Berg et al., 1982) presented a problem. The recent development of a slow release acetylene source utilizing encapsulated CaC_2 (Banerjee and Mosier, 1988a & b) may provide a solution to this problem. Since the efficiency of the encapsulated CaC_2 as a nitrification inhibitor had not been thoroughly tested under flooded rice field conditions, a study was initiated at the Indian Agricultural Research Institute (IARI) farm in New Delhi, India. The purpose of this study was to determine if encapsulated CaC_2 reduced N loss by denitrification (N_2 + N_2O emissions) or affected rice yield. We report here only the preliminary results of this study, since ^{15}N analyses of soils and plants are not completed.

MATERIALS AND METHODS

Ten 30 m^2 field plots were established at the IARI farm. Accepted cultural practices were used to sustain viable rice plant growth in the plots from seedling transplanting through harvest. Each plot was used as a

[+]Division of Soil Science and Agricultural Chemistry, Indian Agricultural Research Institute, New Delhi, India

[++]USDA-ARS, Fort Collins, Colorado, USA

treatment area. One of three urea/nitrification inhibitor combinations was used. Each of the three plots was fertilized with the equivalent of 120 kg urea-N/ha by uniform hand application. One plot was used as a control and not amended further. The second plot was amended with 9.4 kg DCD/ha (dicyandiamide, marketed by SKW Trostberg AG, Trostberg, West Germany). The application to the third plot included 20 kg CCC/ha (coated calcium carbide; Banerjee and Mosier, 1988a). Four week old rice seedlings were transplanted into the plots and 3-6 cm floodwater was maintained.

Inside each main plot three microplots were established by inserting polyacrylic pipes (15 cm diameter x 30 cm long) 20 cm into the soil. The microplots were covered when the main plots were fertilized. The covers were removed and the microplots were fertilized at the same rate of urea/nitrification inhibitor and planted with two rice seedlings. To enable direct measurement of the flux of $N_2 + N_2O$, urea containing 99 atom % ^{15}N was applied to the microplots.

Gas flux measurements were made daily by enclosing the microplots, plants were included in the enclosure, with a chamber fitted with a gas sampling port (Banerjee and Mosier, 1988b). Microplots were enclosed for two hour periods during midafternoon and 16 hour periods overnight. Gas samples were collected from the enclosures by syringe and either analyzed within a few hours or stored in evacuated glass-gas tight tubes for subsequent analyses by isotope ratio mass spectrometry. The mass spectrometer was equipped with a gas sampling inlet (Mosier et al., 1986). Total $N_2 + N_2O$ gas flux was calculated as described by Siegel et al., (1982).

At the end of the growing season crop yield was determined by harvesting each main plot. Yield data, expressed as grain and straw production, represent the mean of the four replicate subplots.

RESULTS AND DISCUSSION

The use of nitrification inhibitors increased dry matter production (Table 1). Compared to the control plots (no nitrification inhibitor) grain yield was 1.15 and 1.32 times greater for the plots treated with DCD and CCC, respectively. Each inhibitor apparently increased straw dry matter production by about the same amount.

Denitrification was substantially reduced by the nitrification inhibitors. N gas flux for the five sampling days (Table 1) averaged 61.9, 13.3 and 4.1 g N ha^{-1} hr^{-1} from the urea alone, urea + DCD, and urea + CCC, microplots, respectively. These initial data indicate that both DCD and CCC decreased denitrification losses from the flooded rice soils. The CCC treatment appears to be more effective than the DCD.

Most important is the apparent increase in rice yield when the nitrification inhibitors were used. The 32% yield increase attributable to CCC may prove to be an important first step toward improving rice yield through increasing fertilizer N use efficiency in flooded rice.

Table 1. Effect of nitrification inhibitors on rice grain and straw production and $N_2 + N_2O$ flux.

Treatment	Rice Yield		$N_2 + N_2O$ Flux				
	Grain	Straw	Time After Fertilization (d) 2	4	6	8	10
	(q/ha)		($g\ N\ ha^{-1}\ hr^{-1}$)				
Urea alone	46.6	47.9	27.3	81.3	52.0	87.0	62.0
Urea + DCD	53.6	53.0	10.4	15.9	16.7	10.8	12.5
Urea + CCC	61.3	63.3	7.8	3.5	5.0	2.0	2.0

ACKNOWLEDGEMENTS

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Significance of anoxic reaction zones in an aquifer in the lower Rhine region

by

Obermann, P.+)

1. Introduction

The NO_3-content of groundwater in shallow aquifers was examined in the catchment areas of some selected waterworks in the lower Rhine region of the Federal Republic of Germany from 1974 to 1988, here demonstrated for the

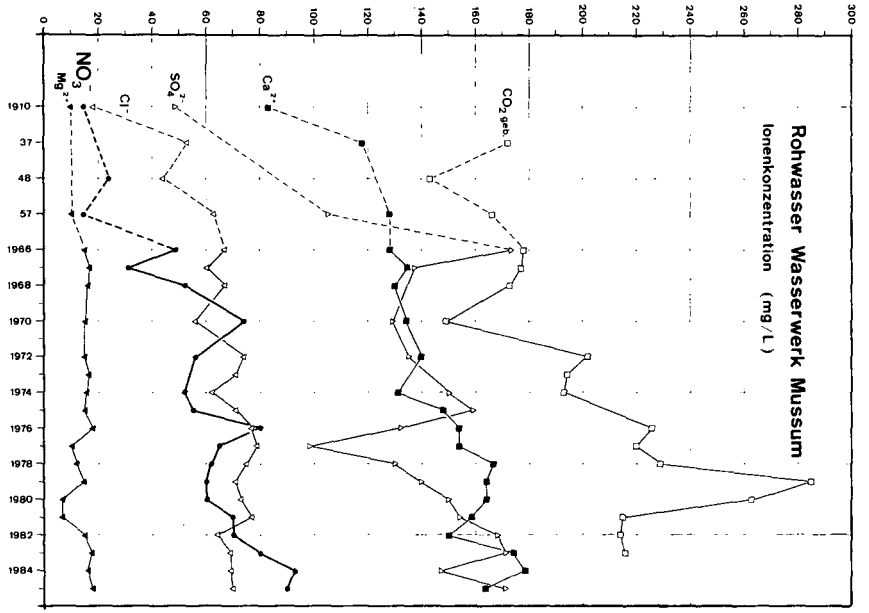

Fig. 1: Increasing ion concentrations in the groundwater of the waterwork Mussum

+) Institut für Geologie, Ruhr-Universität Bochum, Universitätsstr. 150, 4630 Bochum 1, FRG

- waterwork Mussum (= 67 ppm NO_3 in 1975),
- waterwork Gatzweiler (= 87 ppm NO_3 in 1979), and
- waterwork Liedern (= 6 ppm NO_3 in 1978 and 1984).

The oldest waterwork Mussum started to pump groundwater in 1910 with a natural NO_3-concentration of 15 ppm. In 1985 this concentration amounted to about 90 ppm (Fig. 1). Besides NO_3 most other ions show for these waterworks increased concentrations proportional to their supply mainly due to agricultural activities especially after the second World War.

2. Situation of Waterwork Mussum

The methods of investigation are described here for the waterwork Mussum. Streamlines of groundwater flow were determined in selected vertical sections through the aquifer by a mathematical method using "finite elements" and based on the "streamfunction theory" of potential flow. By integrating the flow velocity along each streamline one obtains the groundwater isochrones which represent the distribution of increasing water age below groundwater surface in the vertical section (Fig. 2).

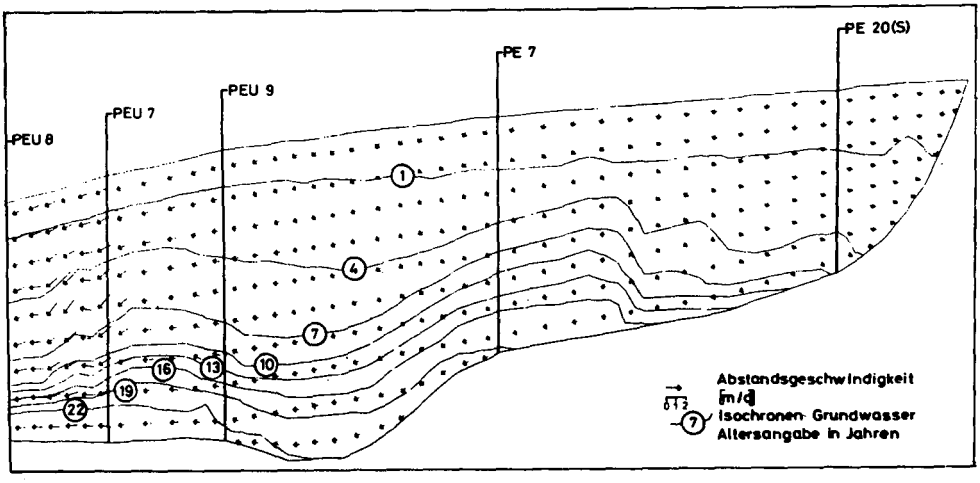

Fig. 2: Isochrones of groundwater showing convective transport of NO_3 and increasing groundwater age parallel to the groundwater surface

By applying the stratified sampling technique the following NO_3-concentrations in the groundwater surface have been determined in the catchment areas of the waterworks as a function of agricultural use (Fig. 3). The calculated mean values of nitrate in the groundwater surface exceed in all investigated cases the corresponding values in the water productionwells (Fig. 4).

Waterwork Year of measurement	WW M 1975	WW L 1978	WW G 1979	WW D 1979
	mg NO_3/l = ppm N, (kg N/ha/year)			
Forest	49=11,1 (33)	0=0 (0)	45=10,2 (25)	41=9,3 (23)
Greenland	71=16,0 (48)	1=0,2 (1)	106=23,9 (60)	53=12,0 (30)
Arable land - normally used	101=22,8 (68)	36=8,1 (24)	129=29,1 (73)	120=27,1 (68)
- intensively used	242=54,6 (164)			
Mean value - catchment area - waterwork	95 67	20 6	124 87	108 86

Fig. 3: Mean values of NO_3-concentrations for the groundwater surface in the catchment areas and in the wells of the waterworks Mussum (M), Liedern (L), and Gatzweiler (G)

The reasons for this may be

- the time laps between the growing NO_3-discharge into the aquifer and the resulting discharge into the wells or
- the microbiological nitrate reduction in the aquifer which normally causes NO_3-decrease in seepage- and groundwater.

Based on the orientation of isochrones parallel to the groundwater surface (Fig. 2) we compiled the concentrations measured by stratified sampling depth (reference level = phreatic groundwater surface) and computed for all samples the arithmetic mean value and the standard deviation (Fig. 5). The comparison between these values at groundwater surface and bottom of the aquifer shows with increasing depth and water age

- WW M: 0 m = 135 mg NO_3/l (no NO_3-reduction in seepage-water)
  8 m = 50 mg NO_3/l

  NO_3-decrease by reduction = 63 %
  by time-laps = 0 %

  total decrease = 63 %

- WW L: 0 m = 30 mg NO_3/l (full NO_3-reduction in seepage-water)
  10 m = 8 mg NO_3/l

  NO_3-decrease by reduction = 70 %
  by time-laps = 3 %

  total decrease = 73 %

- WW G: 0 m = 120 mg NO_3/l (little NO_3-reduction in seepage-water)
  9 m = 90 mg NO_3/l

  NO_3-decrease by reduction = 16 %
  by time-laps = 9 %

  total decrease = 25 %

Fig. 4: NO_3-decrease in groundwater caused by reduction and time laps in the catchment areas of the waterworks Mussum (M), Liedern (L), and Gatzweiler (G)

- a strong decrease of high mean values of NO_3-concentrations caused by a microbiological NO_3-reduction especially of high concentrations combined with
- a strong decrease of low O_2-concentrations and Eh-values,
- a weak decrease of high oxigen demand (here $KMnO_4$-consumption),
- and an increase of HCO_3- and pH-values.

The time laps has no influence in case of this aquifer because of constant Cl- and SO_4-concentrations.

Remarkable is the decrease of K-concentrations and the increase of Na-concentrations by anorganic cation exchange.

3. Situation of waterwork Gatzweiler

In a similar hydrogeological situation the aquifer of the waterwork Gatzweiler shows no influence of reduction of the high O_2- or NO_3-concentrations (Fig. 6). Some NO_3-decrease with increasing sampling depth is caused here by time laps combined with increasing fertilizer input. This is indicated by small and

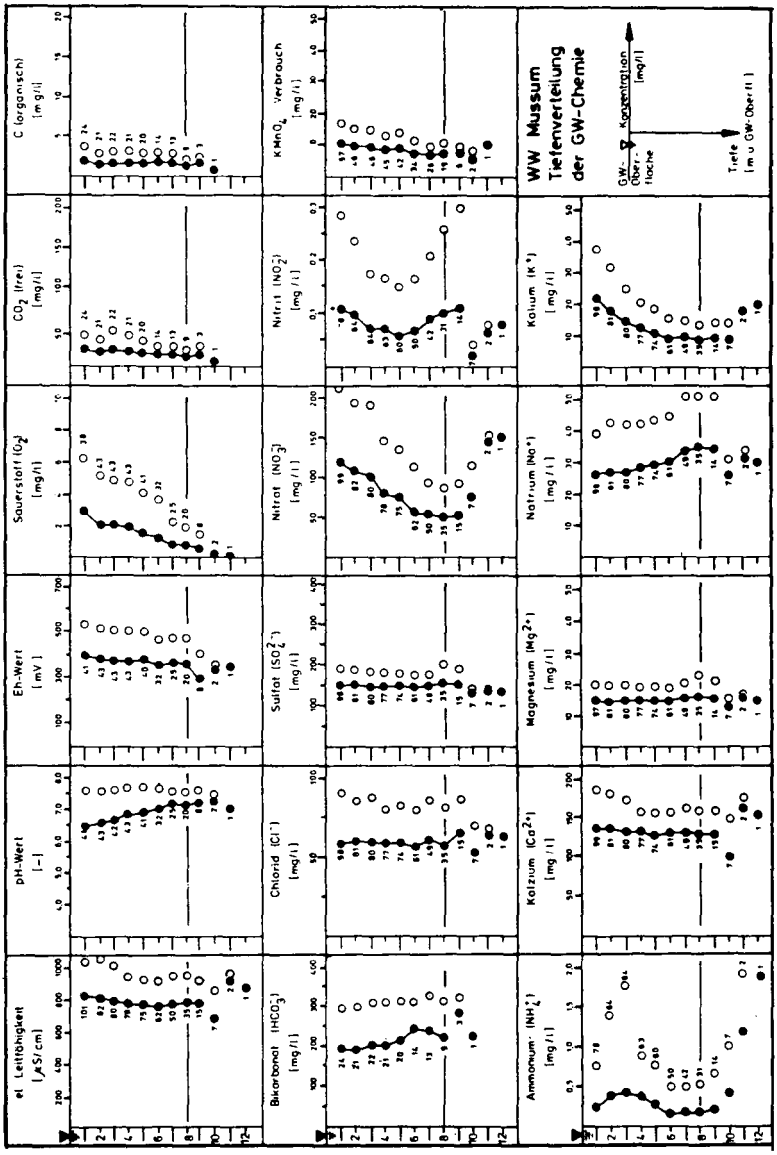

Fig. 5: Mean values (●) and standard deviation (O) of stratified groundwater sampling in the catchment area of the waterwork Mussum

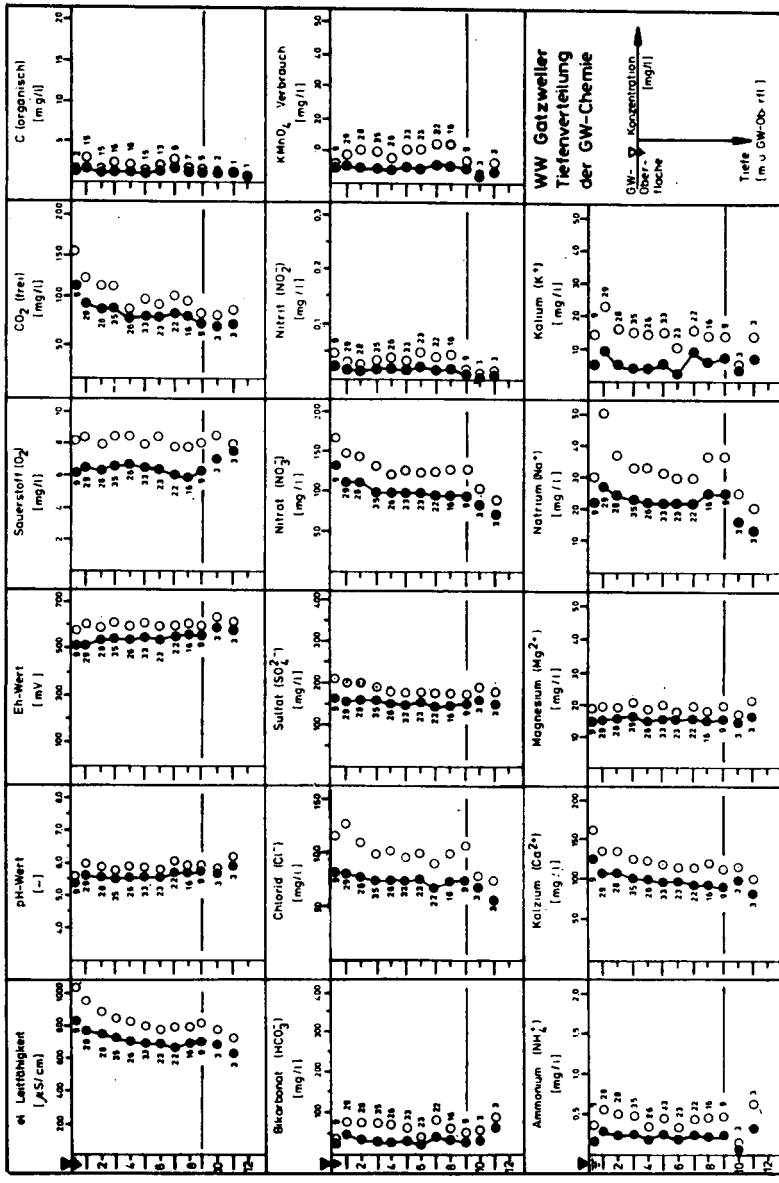

Fig. 6: Mean values (●) and standard deviation (O) of stratified groundwater sampling in the catchment area of the waterwork Gatzweiler

simultaneous NO_3- and Cl-decrease below groundwater surface. The reason for the observed NO_3-stability here are the low pH-values (= 5,5) and the low organic content of the groundwater and sediment. Our measurements indicate for the investigated waterworks the degree of NO_3-decrease between the sampling depths named in Figure 4.

4. Situation of waterwork Liedern

Bacteria utilize inorganic electron acceptors to oxidize organic carbon in a distinct succession. The most efficient metabolic process is the O_2-respiration. After oxygen has been diminished NO_3 can be used as the next efficient electron acceptor followed by SO_4-reduction and so on. In aquifers with downward groundwater movement and sufficient organic carbon content this reaction sequence should result in a vertical profile in which consumption of oxygen occurs at the groundwater surface and the reduction of the less efficient electron acceptors such as nitrate, manganese oxydes, iron hydroxide, sulfate, and hydrogen carbonate can be observed in success. The reduction of the less energy-yielding electron acceptors sulfate and hydrogen carbonate should therefore become more likely with increasing depth resulting in decreasing flow velocity and with increasing organic C-content.

To examine the above mentioned reaction sequence in the field we drilled some 50 meter deep boreholes near the waterwork Liedern where we assumed a total reduction of NO_3. The best example to demonstrate this was the multilevel well DFG 4 (drilled in 1984, Fig. 7). It showed downwards vertical zonation of microbiological

- O_2-reduction (up to 2 m below groundwater surface)
  combined with nitrification,
- followed by NO_3-reduction to N_2-gas (up to 18 m below groundwater surface)
  combined with Mn(+4)-reduction and $(Ca/Mn)CO_3$-precipitation,
- followed by SO_4-reduction to H_2S-gas (up to 43 m below groundwater surface)
  combined with Fe(+3)-reduction and $(Fe/Ca)CO_3$-precipitation.

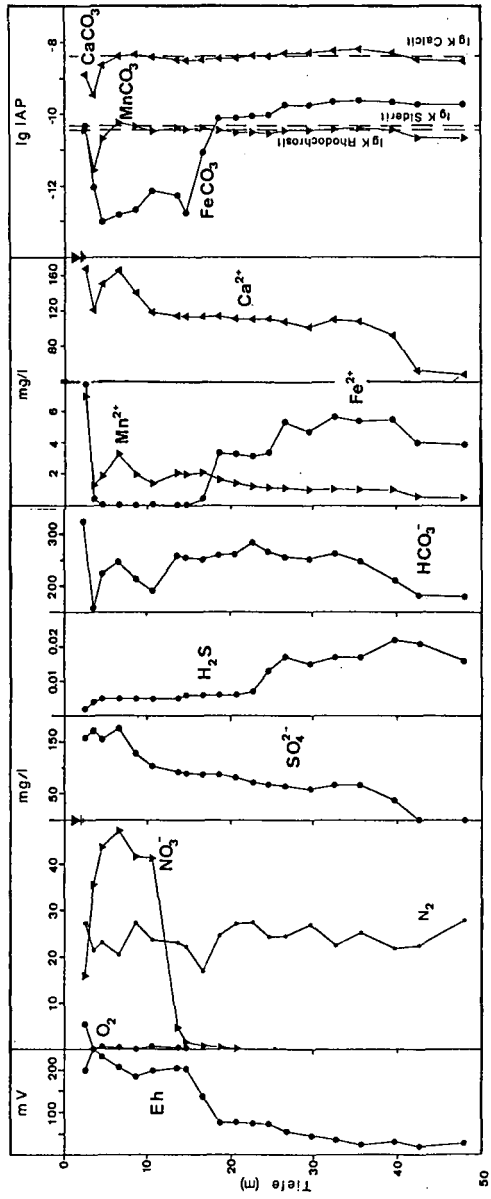

Fig. 7: Depth distribution of anion- and cation-concentrations on ion activity of Ca/Mn/Fe-carbonates in multilevel well DFG 4

5. Some important conclusions

Permanent reduction of the dissolved electron acceptors (mainly O_2, NO_3, and SO_4) will cause an irreversible consumption of most solid electron donators (mainly adsorbed organic matter; partially Fe-sulfides in the upper part of the aquifer). When all available organic matter will have reacted the capacity of the aquifer for "self-cleaning" will only depend on the amount of co-infiltrated dissolved organic carbon. However, in many aquifers this carbon source will only be sufficient for the reduction of considerably lower concentration of electron acceptors such as NO_3 compared with today's situation.

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DENITRIFICATION IN DUTCH AQUIFERS

Berg, R. van den, and L.J.M. Boumans +)

INTRODUCTION

Monitoring of the groundwater quality in the Netherlands has shown that nitrate concentrations in the groundwater in the south-eastern part of the Netherlands are increasing with time, especially in the phreatic aerobic aquifers in sandy areas. In spite of similar N-loads at the surface and similar leaching of nitrogen from the soil into the groundwater great differences have been observed in nitrate concentrations of the groundwater in the various areas. The anaerobic denitrification process is thought to be responsible for this nitrate removal in the aquifers.

In order to gain insight into the denitrification in the groundwater special attention is paid to this phenomenon as part of a larger research project on the occurrence and behaviour of nitrogen compounds in soil and groundwater.

The objective of the denitrification research is to determine why, where, at which rate and to what extent (finity) denitrification occurs in Dutch aquifers.

More specific it is tried to determine whether it is possible to predict denitrification from easy measurable soil and aquifer characteristics. Eventually, based on possible relations measures could be taken against important sources of nitrogen, e.g. manure application.

A derived objective was to develop methodology to measure denitrification in the laboratory as well as in the field.

The results of both field and laboratory studies are reported accompanied by rem with regard to relations between soil characteristics and the denitrification (rate) and comparison of field determined denitrification rates with those determined for field samples in the laboratory.

+) National Institute of Public Health and Environmental Protection (RIVM), Soil and Groundwater Research Laboratory, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

TEST METHODS

Both laboratory and field experiments have been carried out with the objective to compare the results and to try to calibrate the laboratory test method.

Laboratory test method - shaked incubation.

Denitrification in the laboratory was measured by incubation of 150 g of soil with 250 ml of nitrate solution (0.7-7.1 $mmol.l^{-1}$ NO_3^-) under a helium atmosphere on a shaker at 20 °C in the dark. Denitrification was determined as nitrate removal (measured by an auto-analyzer method). In course of time the method has been changed for improvements, e.g. the concurrent production of the nitrogen gases N_2 and N_2O (determined by gaschromatography equipped with thermal conductivity detector).

The soil samples for the laboratory studies (table 1) have been taken at a number of dairy farms, with known manure application, at the sites used for infiltration experiments and at a few additional sites. Generally the material was sampled in cores and if necessary kept at 4 °C.

Field infiltration experiments (Boumans et al, 1987).

Two bore holes penetrating into the saturated zone (groundwater table at 1 to 3 meters below land surface "m-ls") are equipped with screens at a depth of 5 to 6 m-ls. The wells are situated parallel to the hydraulic gradient at a distance of 1 to 2 m of each other. The natural groundwater is circulated, withdrawn downstream and injected upstream. Nitrate and a tracer, usually chloride, are added continuously to the recirculating flow and concentrations of tracer and nitrate in the injected and withdrawn water observed as a function of the time. Nitrate removal rates, considered to be denitrification rates, are calculated from the course of tracer and nitrate concentrations (determined by ion-chromatography).

A number of sites have been selected for performing the experiments. An important prerequisite was the anaerobicity of the site.

Comparison of the field method and the laboratory test method.

The laboratory test is considerd to give the potential denitrification rate, while the field test is expected to determine the actual denitrification. The

TABLE 1. RESULTS OF THE LABORATORY INCUBATION EXPERIMENTS.

sampling site	depth [meters below surface]	organic or total (#) carbon [percent.]	nitrate conc. [mmol/l]	decrease in nitrate concentr. [mmol/l] after 42 days	eval. of the denitr. 1)	denitrifi-cation rate [mmol/m3/h]	denitr. after C-add. 2)	pH	remarks
DAIRY FARMS									
Holten	1.2-7.0	0.09-1.15#	7.14	0.00 - 0.29	-		o	5.4-7.0	
Almen	1.0-7.8	0.11-2.16#	7.14	0.00 - 1.93	-		o	4.9-7.1	
Neede	3.0-6.0	0.02-0.96#	7.14	0.00 - 0.14	-		o	4.9-6.6	
	5.2	1.43	7.14	5.43 - 7.00	+	38.78 -50.00		7.4	
Moergestel	1.0-6.0	0.13-0.71#	1.4-7.14	0.00 - 0.64	-		o	4.7-5.4	
Wanroy	1.0-5.0	0.02-0.08#	1.4-7.14	0.00 - 0.29	-		o	4.8-7.1	
Sevenum	1.0-6.0	<0.01-1.52	1.4-7.14	0.00 - 0.86	-		++	4.5-6.8	
Dalen	1.2-7.0	<0.01-0.07	1.4-7.14	0.36 - 0.93	-				
Elp	2.5-7.0	<0.01-0.01	7.14	0.36 - 0.93	-				
Veendam	1.6-5.5	0.06-0.22#	1.43	0.00 - 0.44	-		o		
INFILTRATION CORES, FIELD EXPERIMENTAL SITES									
Wanroy	1.3-6.0	<0.1-0.3	1.43	0.00 - 0.24	-		+	5.9-7.1	
Neede	1.4-3.5	<0.1	1.43	0.29 - 0.34	o	0.94 - 1.11	o	5.6-7.9	
	3.6-5.0	<0.1-0.2	1.43	0.67 - 1.19	+ 3)	2.21 - 3.91			
Neede	1.5-5.2	<0.1	1.43	0.00 - 0.29	-		++	5.6-7.6	
	3.5-3.7	1.3	1.43	0.79 - 1.34	+ 3)	3.79 - 6.38			after 29 d
Nijkerk	0.9-4.9	<0.1	1.43	0.15 - 0.37	*	0.41 - 1.01		5.9-7.2	after 180 d
	1.5-2.0	0.1	1.43	1.19 - 1.22	+ 3)	5.70 - 5.83			after 29 d
Best	4.2-4.8	0.3	1.43	0.08 - 0.31	-			6.5-7.5	
	4.8-6.6	0.3-0.5	1.43	0.07 - 0.26	*	0.24 - 0.87			after 180 d
Wageningen	2.0		7.14	0.00 - 0.71	?	0.00 - 7.14		7.3-7.8	after 30 d
ADDITIONAL SITES									
Laag Soeren		10.21	7.14	2.79 - 3.21	+	19.90 -22.96			high org.C soil
Vierlingsbeek	35.0-35.4	0.20-0.32#	7.14	1.86 - 4.29	+	15.92 -36.73	++	5.6-6.5	pyrite
Vierlingsbeek	4.0-27.5	0.01-0.06#	1.43	0.00 - 0.33	-				pyrite 0.01-0.11 %
	34.9	0.30#	1.43	1.26 - 1.27	+	12.50 -12.57			pyrite 0.27-0.28 %

1) evaluation of the denitrification; by comparing nitrate decrease in samples and in references; scores:
+ = positive, - = negative, o = some indications, * = positive in longer incubated samples, ? = erratic.

2) denitrification observed after addition of sodium acetate or glucose after 2 months of incubation; scores:
o = positive, but not a complete nitrate removal, +/++ = positive, and a complete nitrate removal.

3) positive simultaneous nitrogen gas production determined.

laboratory test is a batch incubation under controlled conditions, using disturbed soil samples and heterogeneity has to be accounted for by testing a number of samples. The laboratory test is much less representative than the field test. In the field test the soil is undisturbed and heterogeneous and the conditions are uncontrolled. Nitrate is applied continuously in lower concentrations (0.14-0.7 in contrast to 0.7-7.1 mmol.l^{-1} NO_3^-) and about 10,000 kg soil is percolated. The field test has the disadvantage of being more labor- and costconsuming and is more sensitive to disturbances.

RESULTS AND DISCUSSION

The results of the laboratory denitrification experiments and additional information on the incubation are shown in table 1. The denitrification is evaluated from the nitrate removal after 6 weeks of incubation by comparison with blanks and in a few cases gaseous nitrogen production. Also a qualitative evaluation is given of the nitrate removal after addition of a carbon source.

Generally, no denitrification was observed in the laboratory experiments with the dairy farm samples, in contrast to samples from field experimental sites and other sites. Only in some samples of the dairy farm Neede, containing decomposing plant material, denitrification has been determined. Examination of the organic carbon content showed that almost all non-denitrifying soil samples contained less than one percent organic carbon. A number of samples taken at field experimental sites showed denitrification, but rates varied with depth. Denitrification was also found for samples containing pyrite (taken at Vierlingsbeek) or high organic carbon (Laag Soeren).

From the observation of denitrification subsequent to the addition of an easy degradable carbon source (glucose or sodium acetate) it is concluded that the denitrifying microorganisms were present and the proper conditions existed and therefore denitrification is not observed because of a lack of electron donors. The laboratory test method did not appear sensitive enough to measure the low denitrification rate which might occur at low organic carbon contents and combined with long residence times in the aquifer still might be relevant.

Table 2 presents data and results of the in situ experiments: some information on the incubation conditions, the nitrate removal rate and for comparison the rates determined for laboratory incubation of the field samples.

During the in situ experiments no denitrification was found in aerobic aquifers. In all five anaerobic sites denitrification was observed at a rate of 0.11 $mmol.m^{-3}.h^{-1}$ and higher. Denitrification occurred in the presence of organic substrates or pyrite (Wageningen). Remarkably, field denitrification was determined at Wanroy where no organic carbon was found.

Comparing the field test results with those of the laboratory studies (table 2) shows the qualitative agreement with exception of Wanroy. A possible explanation is thought to be the percolation of organic carbon containing layers at depths greater than sampled for in the laboratory test. Generally the denitrification rates in the laboratory experiments were about the same order or one order of magnitude higher than the in situ measurements, confirming a possible difference between potential (laboratory) and actual (field) rates.

CONCLUSIONS

In the laboratory tests denitrification was observed for only one of the dairy farms: Neede, in samples which samples contained decomposing plant material, and further in a number of samples from field experimental and other sites, for which occurrence and rates varied with depth.

No relation has yet been observed between availability of electron donors or conditions and the denitrification rate, probably because the denitrification rates at low organic carbon content are too low to be measured accurately.

No denitrification occurred in situ in aerobic aquifers, but in anaerobic aquifers denitrification occurred at a rate of 0.11 $mmol.m^{-3}.h^{-1}$ or higher.

Except for one site: Wanroy, in all cases of denitrification in the field test also denitrification in the laboratory test has been determined.

Laboratory tests provided equal or one order of magnitude higher rates than the in situ test, probably because it is a potential test using optimal conditions.

Calibration of the laboratory test method was not yet succesful.

These laboratory denitrification studies contribute to a better understanding of this process in aquifers, but suffer from the point of sensitivity. The in situ experiments described are more valuable, but more sensitive in performance and therefore limited in usefulness. A combined use of these two methods appears to offer the proper balance and desired information.

Pyrite as well as organic substrates were found to be suitable electron donors in the soils sampled. However, at organic carbon percentages below one percent denitrification was determined rarely.

Sites for further studies in determining the key factors of denitrification rate and capacity have to be selected either based on actually observed denitrification (also useful for validation of the field test method) or based on expected or predicted denitrification potential.

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TABLE 2. RESULTS OF THE IN SITU DENITRIFICATION EXPERIMENTS compared with the results of the laboratory studies.

sampling site	aerobicity/ presence Fe		presence of organic carbon	nitrate conc. [mmol/l]	in situ denitr. rate [mmol/m3/h]	laboratory denitrification qualitative evaluation	laboratory denitrification rate [mmol/m3/h]
Wanroy	an	+	-	1.79	4.36	-	0.00 - 0.78
Neede	an	+	+	0.18	>0.36	+	2.21 - 6.38
Nijkerk	an	+	+	0.18	>0.36	+	5.70 - 5.83
Best	an	+	+	0.64	0.13	+	0.24 - 0.87
Wageningen	an	+	+, pyrite	0.18	0.11	?	
Lips	ae	-	-	0.18	0.00		
Vredepeel	ae	-	-	0.18	?		

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Microbial denitrification in the groundwater of a sandy aquifer; kinetics and stream-tube model

by

Böttcher, J., O. Strebel and W.H.M. Duynisveld*)

INTRODUCTION

In the catchment area "Fuhrberger Feld", about 30 km north-east from Hannover (BRD), a high nitrate input into the groundwater from arable land poses a potential threat to the quality of the groundwater. High nitrate concentrations are measured, however, only within the top few meters of the aquifer. The reason for this is microbial denitrification with reduced sulfur compounds (sulfide) present in the aquifer material (KÖLLE et al. 1985). The reaction produces sulfate and consumes the limited reserve of sulfide. An essential prerequisite for developing a long-term prognosis for the groundwater quality and the depletion of the limited reserve of sulfide is the knowledge of the kinetics of this denitrification reaction under the actual conditions in the aquifer.
Objective of our paper is to determine the kinetics of the denitrification reaction and to integrate this into a hydraulically simplified simulation model for solute transport and transformation in groundwater stream-tubes to carry out case-study calculations.

STUDY AREA AND METHODS

The catchment area "Fuhrberger Feld" consists of an unconfined aquifer of about 20 - 30 m thick unconsolidated sands and gravelly sands, underlain by clays and clayey marls of cretaceous age. The

*) Bundesanstalt für Geowissenschaften und Rohstoffe
Stilleweg 2, D-3000 Hannover 51

quaternary sediments of the aquifer contain in uneven distribution small lignitic pebbles. Furthermore, reduced sulfur compounds are present in the sediment material in different amounts (STREBEL et al. 1985). The whole aquifer is oxygen-free, traces of oxygen ($\approx$0.1 mg O_2/l) can only be measured near the groundwater surface. In the recharge area of extraction well 1 of the "Fuhrberg" waterworks, defined from piezometer level contour maps and covering about 1000 ha, 13 multi-level samplers with 15 to 30 m depth were installed approximately following a horizontal groundwater flow line. These samplers are equipped with mini-screens and enable the determination of detailed depth functions of groundwater solutes with a depth resolution of mostly 1 m. Measured depth functions are analyzed on the basis of the cross-sectional flownet and isolines of groundwater age (BÖTTCHER et al. 1985).

RESULTS AND DISCUSSION

Kinetics of denitrification

In Fig.1, depth functions of nitrate concentrations at the multi-level samplers N5, N10, N12 and T1 are depicted. The four samplers are located in extensive areas of arable land. Fig.1 reveals a marked decrease of nitrate concentration in the upper groundwater portion down to about 7 m for each of the four samplers. This is due to microbial denitrification with reduced sulfur compounds as electron donor (KÖLLE et al. 1985). The decrease of nitrate concentration by denitrification is proven by isotope fractionation of nitrate-N and nitrate-O (BÖTTCHER et al. 1989a). According to the isolines of groundwater age (BÖTTCHER et al. 1985), the nitrate concentrations are shown in Fig.2 as a function of groundwater age. Nitrate concentrations decrease with increasing groundwater age. The dashed curve in Fig.2 was fitted to the data, according to a first-order decay process. The calculated reaction constant k_{NO3} is 0.557 a^{-1} (half-life $t_{0.5}$=1.2 a). This reaction constant is valid for the overall denitrification reaction without considering single, intermediate reaction steps, provided reduced sulfur compounds (sulfide) are available in non-limiting amounts. Taking into account the variability of the nitrate concentration data in Fig.2 we conclude, that the half-life of denitrification under the actual

conditions in the "Fuhrberg" aquifer is in the range of 1 to 2 years.

Model calculations

To carry out case-study calculations, a hydraulically simplified stream-tube model was developed. In the cross-sectional flownet, stream-tubes are oriented with respect to groundwater stream lines. This is schematically shown in Fig.3. If the horizontal length L of the aquifer is great in relation to its thickness H (for the "Fuhrberg" aquifer L≈6000 m, H≈20 - 30 m), a stream-tube can be approximated by an one-dimensional (1D) tube. The numerical model simulates nitrate and sulfate transport, denitrification, and sulfate production, as well as depletion of the reserve of reduced sulfur compounds along a 1D stream-tube. The governing equations are the convection-dispersion equation and a 1. order sink/source term. Furthermore, desulfurication and replenishment of the reserve of reduced sulfur compounds are involved in the model (BÖTTCHER et al. 1989b), but not described here, because this paper focuses on denitrification only.

In Fig.4, the results of a case-study calculation for a stream-tube of 2500 m length are plotted. The boundary and initial conditions for the calculation are:

$t \geq 0$, $x=0$, nitrate concentration = 130 mg/l,
sulfate concentration = 60 mg/l,
$t=0$, $x>0$, nitrate concentration = 0 mg/l,
sulfate concentration = 60 mg/l,
reserve of reduced
sulfur compounds = 200 g/m³ sediment;

groundwater flow velocities increase from 100 m/a at the left side of the stream-tube to 200 m/a at the right side; the reaction constant for denitrification is $k_{NO3}=0.557\ a^{-1}$. These data are congruent with realistic conditions in the "Fuhrberg" aquifer.

It can be seen in Fig.4, that after 60 years of nitrate input into the groundwater the reserve of reduced sulfur compounds (S_{re}) is already depleted in the first 300 m of the stream-tube. In the following denitrification zone (300 m up to about 1500 m), the nitrate concentration is decreased down to <1 mg NO_3/l, and the sulfate concentration is increased by denitrification with S_{re}. The small decrease of sulfate concentration and increase of S_{re} between 1500 m and 2500 m is due to desulfurication

(desulfurication zone). After 140 years (Fig.4), S_{red} is depleted up to 1000 m, nitrate concentration is >1 mg NO_3/l in the whole stream-tube, and the desulfurication zone has disappeared. After about 250 years, S_{red} will be completely exhausted, and the concentration of the nitrate output of the stream-tube will approach that of the input.
These model results are valid only for the boundary and initial conditions given above. However, it can be generally concluded, that long-term nitrate input into the groundwater leads to complete exhaustion of the reserve of reduced sulfur compounds.

A detailed description of the study will be given in BÖTTCHER et al. (1989b).

ACKNOWLEDGEMENTS

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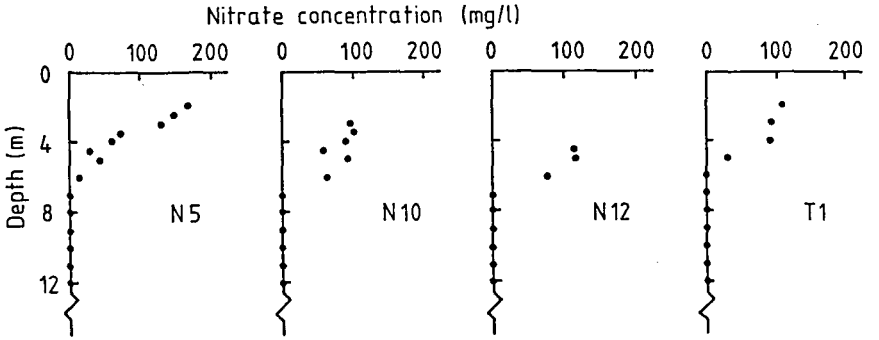

Fig. 1: Depth functions of nitrate concentration in the groundwater for four multi-level samplers on arable land

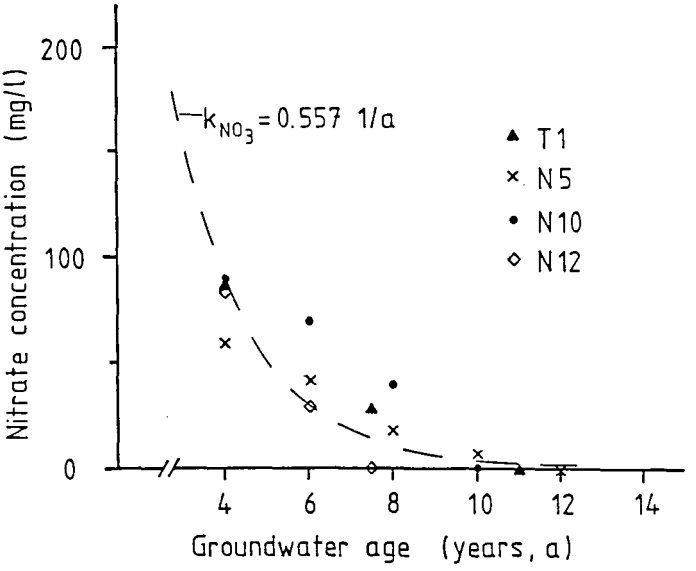

Fig. 2: Nitrate concentration in the groundwater as a function of groundwater age (dashed curve is valid for first order reaction kinetics)

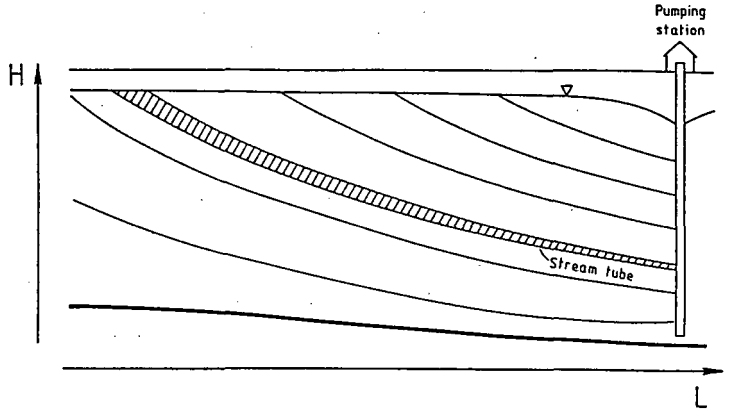

Fig. 3: Scheme of a two-dimensional vertical-plane groundwater flownet and a simplified model stream-tube

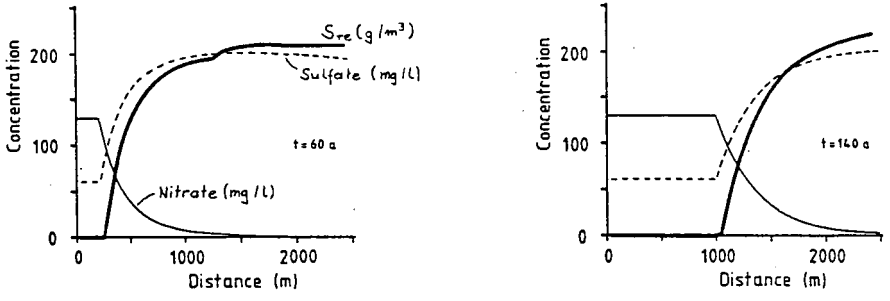

Fig. 4: Simulated concentrations of nitrate, sulfate and reduced sulfur compounds (S_{re}) as a function of stream tube length. State after 60 and 140 years, respectively

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Potentials for biological denitrification in the (un-)saturated zone with different soil managements

by

K. Isermann and G. Henjes*)

A) INTRODUCTION

Possible burdens of the ground water with NO_3 and therefrom arising other soluble N-forms (NH_4^+-N, organic N) are on the one hand the result of NO_3-leaching from the upper soil into the (un-)saturated underground and on the other hand of denitrification processes taking place there. Therefore potentials for biological denitrification (DP) were determined in the (un-)saturated zone down to 10 m below surface in cultivated soils mainly vulnerable to leaching (sandy soils) both in longterm experiments and in practice fields. The land use included forestry, horticulture and agriculture both with arable land and grazed grassland.

B) MATERIAL AND METHODS

The soil samples were taken from the field down to 10 m in 1 m sections with help of a boring hammer, frozen immediately, taken into a N_2 atmosphere and later on - after thawing and homogenising - analysed in fresh condition. - NO_3-, NH_4- and the total soluble N were extracted with a 1 % K_2SO_4 solution, while the amount of soluble organic N was calculated from the total soluble N minus NO_3- and NH_4-N. - The dissolvable organic carbon (DOC) was extracted from the soil with dest. H_2O and analysed with a Technicon Autoanalizer II. 1 kg DOC corresponds now to a denitrification potential of 0.93 kg NO_3-N.

C) RESULTS

Table 1 gives an overview about the amounts of soluble nitrogen, the denitrification potential (DP) and the relation of denitrification potential to nitrate N in the (un-)saturated underground (1. - max. 10. m) with different types of soil cultivation (forest, horticulture, agriculture) and treatments on difficult locations within the Federal Republic of Germany.

*) Landwirtschaftliche Versuchsstation der BASF Aktiengesellschaft, Postfach 2 20, D-6703 Limburgerhof

Table 1

An overview about the amount of soluble (1 % K_2SO_4) nitrogen, the denitrification potential (DP) and the relation of DP/NO_3-N in the (un-)saturated underground (1. – max. 10. m) with different types of soil cultivation (forest, horticulture, agriculture) and treatments

Cultivation / Treatment	Location	Sampling depth (m)[1]	Soluble (1 % K_2SO_4) nitrogen (kg/ha)				Denitrification potential (DP) (kg NO_3-N/ha)	Relation DP / NO_3-N
			NO_3-N	NH_4-N	org. N	Total-N		
1. Mixed Forest	Limburgerhof (Palatinate)	9 (3-4)	120	43	>1	164	5703	47.5
2. Horticulture								
2.1 Fields								
2.1.1 Longterm exp. 24 a								
2.1.1.1 200 kg N/ha.a – without irrigation		10 (4-5)	>1617	>360	>22	>1999	>1443	0.9
2.1.1.2 120 kg N/ha.a – without irrigation		10 (4-5)	>1496	>278	>20	>1794	>1491	1.0
2.1.1.3 200 kg N/ha.a – with irrigation		10 (4-5)	>1333	>361	>54	>1748	>1463	1.1
2.1.2 Field 23 a		9 (4-5)	778	187	32	997	1260	1.6
2.2 Glass-houses								
2.2.1 I		8 (4-5)	597	178	119	894	1510	2.5
2.2.2 II		9 (4-5)	534	72	67	679	1190	2.2
3. Agriculture								
3.1 Arable fields								
3.1.1 Longterm exp. 12 a/b								
3.1.1.1 180 kg N/ha.a – without sprinkler irrig.		10 (4-5)	602	241	161	1004	>1561	>2.6
3.1.1.2 180 kg N/ha.a – with sprinkler irrig.		10 (4-5)	233	144	192	569	1540	6.6
3.1.2 D005								
3.1.2.1 Landau	(Palatinate)	10 (5-6)	61	242	18	321	1478	24.2
3.1.2.2 Ammendorf	(Lower Saxonia)	9 (5-6)	63	223	-	286	1866	29.6
3.1.2.3 Sick	(Schl.-Holstein)	10	82	68	26	176	1401	17.1
3.1.3 D008	Harber (Lower Saxonia)	6	75	239	74	388	694	9.2
3.1.4 Conventional/biol.-dyn.								
3.1.4.1 conventional (Gronauer Hof)	(Hessia)	10	109	2041	436	2586	2054	18.8
3.1.4.2 biological-dynamic (Dottenfelder Hof)	(Hessia)	10	113	1770	584	2467	1686	14.9
3.1.5 Wingst area								
3.1.5.1 cereals after newly broken cutted grassland	(Lower Saxonia)	10 (2-3)	305	500	256	1061	2811	9.2
3.1.5.2 cereals/root-crops (fym + min. N)		10 (3-4)	473	407	157	1037	1077	2.3
3.1.5.3 cereals/potatoes (slurry + min. N)		10 (4-5)	704	215	131	1050	841	1.2
3.2 Grazed grassland (min. N + slurry)	Wingst (Lower Saxonia)	10 (2-3)	797	397	228	1422	1952	2.4

[1] in brackets ( ): Beginning of the saturated zone

sel.225

1. Mixed Forest (Limburgerhof): It shows in the underground (1. - 9. m) only 120 kg/ha NO_3N and only traces of NH_4-N and organic N, but the highest DP (5703 kg NO_3-N/ha) ever found, increasing with soil depth. Therefore the relation of DP/NO_3-N also comes up to a maximum of 47.5.

2. Horticulture (Limburgerhof): ... is an extreme compared with the situation under the above mentioned mixed forest.

2.1 Fields

2.1.1 Longterm experiment 24 a: Irrespective of individual treatments there is > 1748 - 1999 kg soluble nitrogen in the underground, mainly (ca. 80 %) NO_3-N, but in the saturated zone also appreciable amounts of NH_4-N. The DP is with > 1443 - 1491 kg NO_3-N/ha only one fourth of that under the mixed forest; reaches more or less the amount of nitrate N. Therefore the relation of DP/NO_3-N is only between 0.9 and 1.6. Lowering the fertilizer N level from 200 to 120 kg N/ha.a without sprinkler irrigation or sprinkler irrigation on the N level of 200 kg/ha.a reduces the NO_3-N amounts to a small (7 % resp. 18 %) extent and has no influence on the DP. In all three treatments a rest of the DP peak at the depth of 7 - 8 m of the nearby mixed forest can be recognized.

2.1.2 Field 23 a: On a lower N level the situation is similar to the above mentioned longterm experiment 24 a.

2.2 Glass houses I + II: Perhaps due to a better water management the amount of soluble N - especially NO_3-N - is even lower than in nearby horticulture fields but the DP is similar and decreases with depth. Nevertheless the NO_3 leaching into the upper ground water floor is critically.

3. Agriculture

3.1 Arable fields

3.1.1 Longterm experiment 12 a/b: (details of the experiment are shown by Isermann, 1988a).

3.1.1.1 Without sprinkler irrigation: On a fertilizer N level of 180 kg/ha.a the amount of NO_3-N is considerable and reaches the situation as in nearby horticulture.

3.1.1.2 With sprinkler irrigation: But with sprinkler irrigation these amounts of soluble N in the underground can be (due to a higher yield) reduced to about 50 % with no influence on the DP. Therefore the DP/NO_3-N relation increases from > 2.6 to 6.6. The DP has in both treatments the similar extent (> 1561 resp. 1540 kg NO_3-N/ha) as under horticulture.

3.1.2 Practice field D005

3.1.2.1 Landau - Palatinate
3.1.2.2 Ammendorf - Lower Saxonia
3.1.2.3 Sick - Schleswig-Holstein and

3.1.3 D008 - Harber, Lower Saxonia: On all 4 locations the amount of total soluble N in the underground is quite low ("normal"), but the 3 locations Landau, Ammendorf and Harber show high amounts of NH_4-N in the saturated zone. In contrast location Sick has only traces of NH_4-N in the very dry underground. With exception of location Harber, the DP of the other three locations is in the above mentioned range (1401 - 1866 kg NO_3-N). Therefore the relation of DP/NO_3-N is quite high (17.1 - 29.6).

3.1.4 Conventional/biological-dynamic management: On both locations there are very high and similar amounts of soluble N (2586 resp. 2467 kg/ha) in the wet underground with the highest amounts of NH_4-N (2041 resp. 1770 kg/ha) ever noticed. The DP is also relatively high - especially in the deeper underground; therefore resulting in high DP/NO_3-N relations of 18.8 resp. 14.9.

3.1.5 Winqst area (Lower Saxonia): (details of these locations see Isermann, 1988b).

3.1.5.1 Cereals after newly broken cutted grassland with mineral N fertilizer

3.1.5.2 Cereals/root-crops with farmyard manure and mineral N fertilizer

3.1.5.3 Cereals/potatoes with slurry and mineral N fertilizer: All three locations don't differ in their total amount of soluble N in the underground (ca. 1050 kg/ha) but in the DP: Cereals after newly broken cutted grassland show the highest amount of DP with a peak in a depth of 4 - 5 m and therefore a high DP/NO_3-N relation. In contrast to this, the DP in the underground of location 3.1.5.2 and 3.1.5.3 is quite low (1077 resp. 841 kg NO_3-N/ha) and is decreasing with depth. Therefore the DP/NO_3- N relation on this both locations is extremely low. Nevertheless on all 3 locations the DP enables high amounts of NH_4-N in the saturated zone (215 to 500 kg/ha).

3.2 Intensively grazed grassland with 300 kg mineral N and 100 kg slurry N/ha.a in Westerberg (Lower Saxonia): The amount of total soluble N in the (un-)saturated underground is with 1422 kg/ha one third higher than under the nearby arable fields (ca. 1050 kg/ha). The DP is with 1952 kg NO_3-N/ha relatively high, with a peak at a depth of 7 - 8 m, but according to the high amounts of NO_3-N found there, the DP/NO_3-N relation is with 2.4 as low as under the nearby arable fields (without broken grassland).

D) DISCUSSION / CONCLUSIONS

Under arable land, grazed grassland and horticultural land (practice fields and glass-houses) significant amounts of DOC can mainly be found to a soil depth of 2 - 3 meters (50 - 90 % of total DOC to a soil depth of 10 m). The origin is the organic matter of the subsoil inclusive new growth and root excretions (rhizosphere). Therefore normally under these types of land use there exits mainly a significant potential for biological denitrification to a soil depth of 2 - 3 m (± hydraulic watershed).

Summarized from the 1st to the 10th meter the amount of DP in the (un-)saturated underground ranges under these types of land use from 700 - 2000 kg NO_3-N/ha as can be seen from table 1. Only under forest land and newly broken grassland higher amounts of fossil resp. recent DOC can also be found to the soil depth of 10 meters, corresponding to a DP of 5700 and 2800 kg NO_3-N/ha. These high amount of DP under the forest land on the location Limburgerhof was long lasting diminished by horticulture and agriculture to one fourth of its original value (see Table 1). This statement is in accordance with another investigation made by Isermann (1987a) nearby Limburgerhof at Bruchsal. If DP is related here also to the amounts of NO_3-N determined in the (un-)saturated underground (DP/NO_3-N) from the 1st to the 10th meter, this relation reaches values (Table 1)

- in horticulture from < 1 to 2.6
- in agriculture from > 1 to 30
- in forestry 48.

This high DP/NO_3-N relation under forestry comes from both high DP and low NO_3-N values. In contrast the generally low DP/NO_3-N relation in horticulture and in certain cases in agriculture is less the result of the low DP but more that of too high nitrate amounts. Higher or lower amounts of nitrate in the underground for instance by lowering the amount of N fertilizer or by sprinkler irrigation) have no effects on DP.

Is the term "denitrification potential" choosen here correctly? Apart from the fact that DP, calculated from DOC, is only a potential for denitrification, it seems that nitrate ammonification in the saturated zone and in wet microsites of the unsaturated zone is more important than denitrification. Therefore in that cases under arable fields (3.1.1.2 to 3.1.5.1, except 3.1.2.3) were DP/NO_3-N is high (6.6 to 29.6) also high amounts of soluble NH_4-N are found in the underground with the extremes of Gronauer Hof and Dottenfelder Hof (3.1.4.1 and 3.1.4.2).

Taking also into account that DOC is additionally involved in other catabolic (dissimilatoric) reduction processes (like respiration in well areated zones or sulphate respiration in the unsaturated zones the better term will be "catabolic or dissimilatoric reduction potential" (CRP or DRP in kg DOC/ha. depth). Considering the fact that we (Isermann, 1988 a+b)

found nearly all the atmospheric sulphur inputs of this century in the (un-) saturated underground, the investigation of sulphur processes in the underground and their interrelations with inorganic nitrate reduction processes (sulphate respiration resp. denitrification with pyrite, elemental or organic sulphur) is as important as the knowledge about dissimilatoric nitrate reduction itself (Isermann, 1987b). - Finally for land management it must be concluded: Denitrification is an important process within the nitrogen cycle. But taken into account all the other dissimilatoric and inorganic nitrate (and sulphate) processes in the (un-)saturated underground, they don't solve our problems with nitrogen (or sulphur) but increase them in material, space and time. The best we can do - both from economic and ecological point of view - is to minimize all the nitrogen losses simultaneously, nitrate leaching and denitrification included.

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TEMPERATURE DEPENDENCE ON THE DENITRIFICATION ACTIVITY OF BACTERIA IN THE ROTATING-DRUM BIOREACTOR

M. Luther, A. Lange, N. Salhani, C.J. Soeder

Institute of Biotechnology III, KFA-Jülich, 5170 Jülich

(This paper is dedicated to Prof.Dr. Heinz Bernhardt on the occasion of his 60th birthday)

INTRODUCTION:

Most water works in Western Germany process groundwater to drinking water. Since the concentration of nitrate has recently been restricted to 50 mg NO_3^-/l (TVO 1986) a number of water works are now forced to reduce the nitrate concentration to below this limit.

One of the biotechnological devices that have been developed to achieve this goal is the elimination of nitrate in a rotating-drum bioreactor (Overath et al., 1988; Fig. 1). In this process the denitrifying potential of an undefined heterotrophic bacterial population is used. The bacteria are attached to granular carrier particles. Ethanol serves as the biochemical reductant. One advantage of the rotating-drum bioreactor is the continuous removal of denitrification gas and surplus biomass by shearing forces. This prevents clogging of the moving fixed bed. Back washing is unnecessary.

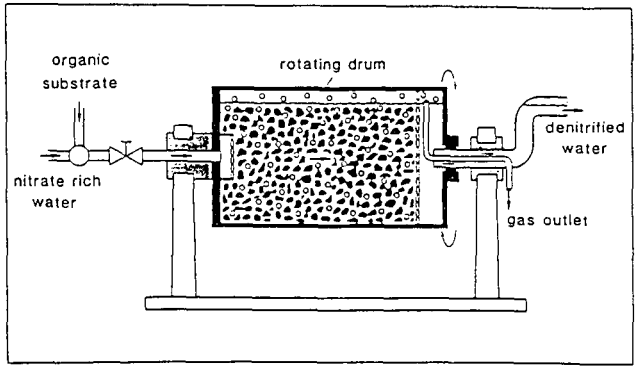

Fig. 1: Scheme of the rotating drum bioreactor

In previous runs with a pilot plant bioreactor (V = 1.7 m³, daily flow up to 100 m³) the nitrate concentration in the effluent could not be kept constant at 5 mg/l, although the inflowing nitrate load and the proportional ethanol supply were constant. One of the

possible reasons for fluctuating nitrate concentrations in the effluent was considered to be the diurnal temperature changes of about ± 1° C in the influent groundwater drawn from an overground storage tank. Moreover, maximal influent temperatures in summer reached 14° C, and winter minima were about 9° C.

Data gathered from a literature study revealed a fairly wide range of denitrification activities measured under various cultivation conditions . Data on denitrification in fixed bed reactors are scarce, and the temperature dependence on the denitrifying activity appeared to be quite low (Murphy and Sutton, 1975; Harremoës and Riemer, 1975). Up to now only few investigators have studied the relationship between short term and long term temperature response (Gujer, 1976; Andersen and Poulsen, 1976). This particular aspect was therefore of special interest in the studies reported here.

METHODS:

Long-term temperature adaptation experiments:

For the long-term temperature adaptation of the bacteria a laboratory reactor with a volume of 15 liters was used. Unchlorinated tap water with 31 mg NO_3^-/l was continuously enriched to 65 mg NO_3^-/l and 0.6 mg PO_4^{3-}/l; the flow rate of this medium was kept constant at 15 ± 0.2 l per hour which gave a mean hydraulic residence time of 25 min. By means of a thermostat it was possible to adjust the temperature with a precision of ± 0.1° C to any value between 5° C and 20° C. In contrast to the pilot plant we removed the oxygen from the influent by gassing with nitrogen in order to obtain strictly anoxic conditions. The nitrate removal rates were determined after about 3 weeks adaptation to the experimental temperatures.

Short term experiments:

For the short-term experiments we used temperature adapted biomass removed mechanically from the fixed bed. Continously shaken suspensions of this biomass were incubated in closed glass vessels (volume 50ml) for 3 hours at temperatures between 5° C and 20° C in the presence of acetylene, in order to inhibit the nitrous oxide reductase. The amount of nitrous oxide at the end of the incubation time was determined in a gaschromatograph and related to the protein concentration of aliquot samples measured by a modified Biuret reaction (Markwell, 1978). The denitrifying activity was calculated as µg N per day and mg protein.

Calculation of the K_t values:

Up to the temperature optimum, the influence of temperature on biological reactions can be approximated by an Arrhenius equation (1) (Precht et al. 1973):

$$K = A\, e^{-E/RT} \quad (1)$$

K = velocity of the reaction
A = activity coefficient
E = activation energy
T = temperature in Kelvin
R = gas constant

For denitrification Hultmann (1971) modified this common Arrhenius equation for the range 5 - 20° C. The size of the exponent K_T reflects the degree of temperature sensitivity of denitrification (2).

$$RD = RD_{20} \cdot 10^{K_t (t-20)} \quad (2)$$

RD = rate of denitrification
RD_{20} = denitrification rate at 20° C
K_t = temperature coefficient
t = temperature in Celsius

RESULTS:

In the long-term experiments at four different temperatures, steady states were reached after about 3 weeks of adaptation. Shortly after temperature change a certain amount of biomass was washed out in all cases. This gave us the impression that a population shift had taken place. If so, it might explain why it lasted up to 3 weeks to reach a new steady state.

Fig. 2 shows the denitrification activity at the individual temperatures.

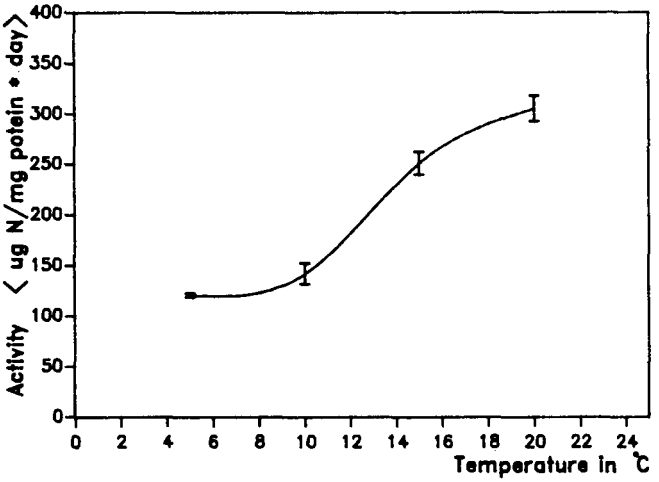

Fig. 2: Denitrification activity in the long term experiment as a function of temperature

The most drastic increase in activity was found between 10° C and 15° C (Fig. 2, Tab.1).

Temperature in °C	K_T-value C^{-1}
5-10	0.0145
10-15	0.0496
15-20	0.0170
5-20	0.0270

Tab.1: Temperature coefficients (K_T) for the long-term experiments

For the whole temperature range between 5° C and 20° C the nitrate removal rate increased 2.7-times. According to the Hultman equation the overall K_T-value for the long-term adaptation was about K_T = 0.027.

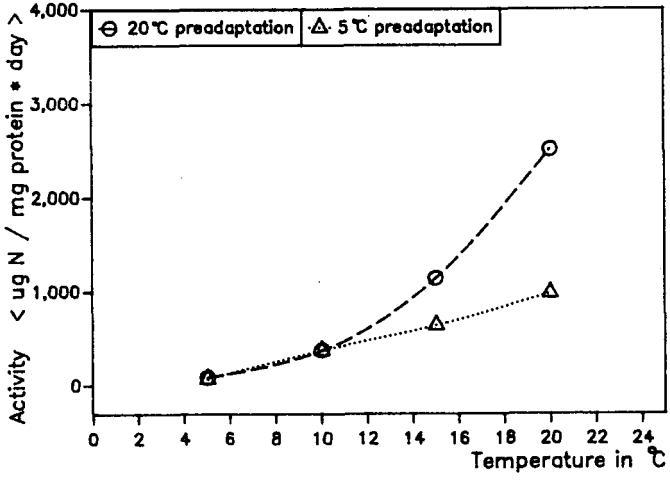

Fig. 3: Denitrifying activity of bacteria in short-term experiments after preadaptation at 5° C and 20° C

In contrast to the long term experiments, the total activity measured in the short-term tests at 20° C was up to 9-times higher (Fig. 3), and the sensitivity of the bacteria towards temperature changes increased (Fig. 4). After preadaptation at 20 °C and a temperature decrease down to 5° C the K_T value was K_T = 0.098. For preadaptation at 5° C and a temperature increase to 20° C the resulting K_T was 0.076.

The results of the short-term experiments also differed from those of the long-term experiments by a exponential activity-temperature relationship (Fig. 4).

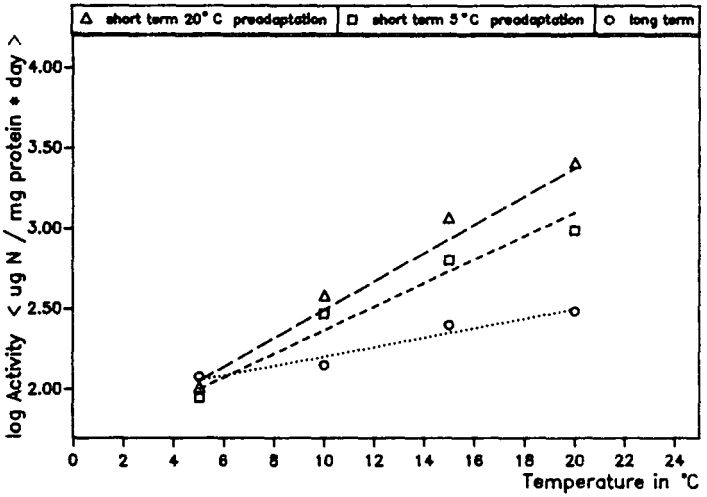

Fig. 4: Semi-logarithmic plot of the denitrification activity in long-term and short-term experiments.

The slopes of the regression lines obtained from the long term experiment were lower than those obtained in the short term experiments. Obvious again was the difference between 5° C preadaptation and 20° C preadaptation.

CONCLUSIONS:

Contrary to the situation in most topsoils, the temperature of the groundwater is normally quite constant. But if one draws water from an overground storage tank exposed to seasonal changes of temperature these will affect the technical denitrification. This demands a sound knowledge of the denitrification-temperature relationship and of the temperature sensitivity in the sense of Hultmann (1971) as one of the design criteria for technical denitrification plants. Of greatest practical importance is the temperature behaviour in the range 5 - 15° C.

In long-term experiments the bacteria were able to adapt physiologically and biocoenotically to the different temperatures employed. The temperature sensitivity calculated from the data in Fig. 1 was quite low (K_T = 0.027). This agrees well with the data reported for denitrification in fixed bed bioreactors (K_T = 0.02 in Harremoës et al., 1975; K_T = 0.03 in Murphy and Sutton, 1975).

In the short-term experiments, however, the bacteria were unable to adapt their physiological capacity within the incubation time of 3 hours to an altered temperature. Hence, their temperature sensitivity appeared to be much higher (K_T up to 0.098), reaching the same level as reported by Barnard (1974) for the endogenous respiration of activated sludge (K_T = 0.08).

Furthermore, when comparing the results of our short-term experiments with those of the long-term tests, it should be realised that we were dealing in the first case with a continuously mixed suspension and in the second case with the same bacteria embedded in a biofilm. Besides temperature, additional limiting factors e.g. diffusion, substrate limitation etc. must affect the denitrification activity. They also may responsible for the small K_T-value (0.027) encountered in our long-term experiments.

From the results of our long-term experiments it can furthermore be concluded that a stable temperature difference of 1° C will result in a change of approximately 6% of denitrification rate.

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Screening, Constructing and Testing of Denitrifying Bacteria for their Use as Starter Cultures in a New Nitrate Elimination Process for Drinking Water with Poly-β-Hydroxy Butyric Acid (PHB) in Solid Phase Reactors

by

Biehler, M. J.*) and Süßmuth, R.)**

A b s t r a c t : To eliminate nitrate out of drinking water a new biotechnological process was developed. In a solid phase reactor, filled with poly-β-hydroxy butyric acid (PHB) in form of granules, nitrate at least qualitatively was reduced to N_2 without dosing of any supplementing substances, because PHB served as adhesion material and carbon source at the same time.

To heighten the efficiency of the denitrification in the bioreactors with PHB-granules special bacterial strains were developed by means of a screening procedure on PHB-hydrolysis and denitrification followed by an optimalization program with induced chemical mutagenesis and anaerobic continuous cultures.

A first test of the resulting best adapted strain - *St. 2* - showed in comparison to an undefined mixed culture a much more constant denitrification velocity and a stable biofilm on the PHB-granules.

*) Institut für Siedlungswasserbau, Universität Stuttgart, Bandtäle 1, D - 7000 Stuttgart 80;
**) Institut für Mikrobiologie, Universität Hohenheim, Garbenstr. 30, D - 7000 Stuttgart 70;

1. Introduction

Since the limiting value of nitrate in drinking water has been lowered down in Germany from 90 mg NO_3^- /l to the EC-limit of 50 mg, for example in Baden-Württemberg 5,2 % of the drinking water resources had to be closed because of their nitrate concentrations.

The national activities to minimize the nitrate in the drinking water will have no regardable effects during the next decades, because the fertilizing of soil with N is hardly to control and impact everywhere. Therefore especially the at least 300,000 small water procuring works, which are situated mostly in agrarian regions, are threatened from closing.

Because of their difficult handling and high installations costs the already existing nitrate elimination processes are not suitable for these small water sources. Therefore a new biotechnological process was developed especially in regard to that problem: In solid phase reactors poly-β-hydroxy butyric acid (PHB) in form of granules was used as adhesion material for a bacterial biofilm and as carbon source at the same time. By this way it is not necessary to measure out any other supplementing substances to receive denitrification down to N_2. Furthermore the control and handling of this process is very simple because of its self-regulation.

To heighten the denitrification velocity and to shorten the lag-phase in the bioreactors as well as for the formation of a defined

and stable biofilm on the PHB-granules, the first time in biotechnological processing of drinking water especially adapted high efficiency bacterial strains should be developed for their use as starter cultures.

2. Screening Procedures for PHB-Hydrolysing Denitrifiers

a) Isolation of separated bacterial cultures

The single strains were isolated on petri dishes with PHB- as well as NO_3^--minimal media out of PHB-bioreactors with a mixed microbial population resulting from the influx.

b) Prescreening of the isolated strains

--> petri dish test on hydrolysis of biotechnological modified PHB by using a PHB-emulsion of the same content like the granules used in the bioreactors as only carbon source.

--> petri dish test on denitrification : *The colonies of the isolated strains were stratified, after their anaerobic growth in denitrification media, with semi-solid agar containing Pseudomonas aeruginosa as denitrifying indicator strain but no energy source.*

In both cases the diameters of the zones around the colonies without turbidity served as measure for the PHB-hydrolysis respectively denitrification capacity of the isolated strains.

c) Quantitative screening of the PHB-hydrolysing denitrifiers

--> Turbidometrical measurement of the PHB-hydrolysis in fluid media : *The decrease of the optical density in the medium because of the PHB-hydrolysis is overlapped by the increase of the O. D. resulting out of the bacterial growth. Therefore it is necessary to separate the cells from the medium by extraction with propylencarbonat.*

--> Denitrification in fluid media : *Nitrate (after its reduction to nitrite) and nitrite were determined photometrically by the conversion of sulfanilyc acid and 1-naphthylamin. NO, N_2O and N_2 were determined gaschromatographically.*

d) Results of the screening program :

It was possible to isolate two different PHB-hydrolysing denitrifying cultures: one single strain called *St. 2* and a mixed culture out of denitrifying *B. gelb* and PHB-hydrolysing *B. weiß*.

3. Optimalizing of the Obtained PHB-Hydrolysing Denitrifiers

a) Induced chemical mutagenesis :

The negative properties of the screened strains for their use as starter cultures in PHB-nitrate-elimination-reactors (*no constitutive PHB-hydrolysis, strong slime excretion*) should be converted by means of chemical mutagenesis :

--> N-Methyl-N'-N-nitro-N-nitrosoguanidin (MNNG)-Mutagenesis for the generation of constitutive PHB-degrading mutants : *After incubation in a MNNG-solution for 2,5 h the positive mutants were determined by means of the petri dish test on PHB-hydrolysis (compare 2.b) after several transfers onto complex media.*

--> MNNG-Mutagenesis in connection with the penicillin enrichment procedure by *Lederberg* and *Davis* for the generation of non-slime expressing mutants: With $CHCl_3$ as selective substance for growth inhibition of the rough mutants and penicillin to kill the smooth wild types, the positive mutants were enriched after their generation in a MNNG-solution for 2,5 h. Their detection took place by an Enzyme Linked Immunosorbent Assay (ELISA) with antibodies against the slime fraction of the wild type : the non-slime mutants showed no coloring in the immunoassay.

b) Continuous culture :

--> Use of the continuous culture for the adaptation of *St. 2* and *B. weiß/ B. gelb* to the milieu in a PHB-bioreactor : *With an especially constructed chemostat, placed in a gas-impermeable tent under denitrifying conditions, the adaptation of the isolates to different parameters in a PHB-bioreactor was performanced by laying on distinct gradients :*

I. Adaptation to the drinking water temperatures of 8 - 12 °C : *By means of a kryomat the temperature was lowered down from 20 °C to 8 °C.*

II. Adaptation to low nitrate and high nitrite concentrations : *By means of a gradient mixing apparatus the nitrate concentration was lowered down from 50 to 5 mg NO_3^- /l as well as upped from 2 to 75 mg NO_2^- /l.*

III. Adaptation to extremely oscillating nitrate concentrations : *The wavering nitrate values in the media were again regulated ba a kryomat.*

IV. Adaptation to low and high pH-values : *The pH-value was lowered down from 7,0 to 5,5 in a citrate buffer with a constantly sinking gradient as well as upped from 7,0 to 8,5 in a P-buffer with constantly increasing gradients.*

c) Results

With the induced chemical mutagenesis it was possible to generate and select two stable denitrifying mutants which hydrolysed PHB constitutivelly.

The adaptation of these strains to PHB as only carbon source as well as to the temperature of drinking water and to extremely oscillating nitrate concentrations by means of the continuous culture was successful. Especially *St. 2* showed a great flexibility. In contrary to those parameters the adaptation to very low nitrate and to high nitrite concentrations as to extreme pH-values was only possible to some extent. But for the expected conditions in a PHB-bioreactor the adaptation capacity of the tested strains - especially of *St. 2* again - was more than sufficient.

4. Use of the Constructed Strains in Small PHB-Bioreactors

In small reactors, filled with 0,5 kg of PHB-granules, with a flow through of 60 - 80 l /h and a recirculation rate of about 1 : 100, the especially developed strains of *St. 2* and *B. weiß /gelb* were used as starter cultures. In comparison to a reactor without inoculated bacteria the denitrification velocity of the three reactors was measured as main reference. Furthermore the TOC of the efflux and the removal by other bacteria were compared.

Results : Both of the two reactors with the different starter cultures showed a more stable denitrification in comparison to the reactor without any bacterial inoculation. But only *St. 2* denitrified with a constant denitrification velocity of 12 mg NO_3^-/(l*h) during six months. Moreover the TOC of this column in the efflux was nearly not increased in comparison to the influx and no metabolites of the nitrate reduction could be detected in the water.

5. Discussion

By means of chemical induced mutagenesis and continuous cultures it was possible to receive and modify two PHB-hydrolysing denitrifiers. The fittest isolate - *St. 2* - after all optimating processes - *St. 2* - showed a constant denitrification velocity without the excretion of any toxic metabolites and a stable biofilm on the PHB-granules. For really satisfying results it could be necessary to increase the denitrification velocity of *St.2*.

After finishing of the development of starter cultures and of the process technique nitrate elimination with PHB has to be tested now in columns of technical dimensions. Then it will be obvious, whether this process will be an alternative for processing especially smaller quantities of drinking water.

6.Literature

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Aerobic denitrification in sediments and bacterial suspensions studies by membrane inlet mass spectrometry

by

D. Lloyd,+) K. J. P. Davies++) and L. Boddy+)

Denitrification, defined as the reduction of NO_3^- or NO_2^- to gaseous end product(s) (NO, N_2O or N_2), is commonly regarded as primarily an anaerobic process, because (a) NO_3^- and O_2 "compete" as alternative terminal electron acceptors, (b) the more positive redox potential of O_2 makes its utilization energetically advantageous, (c) O_2 represses the syntheses of NO_3^--reducing enzymes, and (d) O_2 inhibits the activities of some of these enzymes. However, frequent observations of denitrification proceeding in the presence of O_2 suggests that the O_2 sensitivity of the process is variable (in different organisms and under different conditions, Table 1) and that "aerobic denitrificaion" may be commonplace in soils, sediments and some aquatic environments. By comparison, N_2 fixation is much more O_2-sensitive (Smith et al., 1988). Most early investigations, and many of the more recent ones, have omitted measurements of O_2: it should be stressed that it is dissolved O_2 is the crucial variable, as this measures its chemical activity which the organism senses, and to which it responds. Gaseous partial pressures are a step removed and, at high O_2 demand in heavy microbial populations, may be orders of magnitude higher than those of the liquid phase. Notably the contributions from Skerman's group used the polarographic technique to quantify concentrations of O_2 simultaneously with measurements of denitrification.

A further complication arises because some types of O_2 electrode operating at certain values of polarizing voltage may respond to oxides of nitrogen N_2O (Albery, 1979) or NO (André, this volume). These considerations led us to

+) Microbiology Group (PABIO), University of Wales College of Cardiff, Newport Road, Cardiff CF2 1TA, Wales, U.K.
++) Present address: Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-3300 Braunschweig, FRG.

reinvestigate the phenomenon of aerobic denitrification using the novel technique of membrane inlet mass spectrometry. The usefulness of this method lies in the fact that the formation of N_2O and N_2 can be directly and specifically measured alongside O_2 and CO_2 (either in solution or in the gas phase) without using isotopic labelling. The use of $^{15}NO_3^-$ also enables quantification of NO (Cox et al., this volume). We have used an inlet system consisting of a long steel capillary with a membrane-covered orifice at one end, for direct measurement of these gases at high spatial resolution in sediments, soils and aquatic environments and continuously over extended times (weeks) (Lloyd et al., 1987; Boddy & Lloyd, 1989). We have demonstrated both in sediments and in pure cultures (Davies & Lloyd, 1984, Davies et al., 1989) that denitrification occurs in the presence of O_2. Whereas in sediments it is possible that denitrification is confined to anaerobic microniches, homogeneously-stirred populations give unequivocal confirmation of the simultaneous utilization of O_2 and NO_3^- (respiration and aerobic denitrification).

METHODS

The practical details necessary for recording gas concentrations by mass spectrometric measurements have been described elsewhere (Lloyd & Scott, 1983, 1985; Lloyd et al., 1985; Lloyd et al., 1986; Boddy & Lloyd, 1989). In outline, gases diffusing continuously through a gas-permeable membrane (Teflon, polypropylene or silicone rubber) into the high vacuum (10^{-6}torr) of the mass spectrometer are ionized by electron detachment to give positive ions in an ion source. Analysis by separation of trajectories through a quadrupole filter is followed by amplification and detection of the consequent ion-current. Rapid scanning of mass spectra (typically 500amu.s^{-1}) enables selected peaks (i.e. mass/charge values) to be sequentially sampled and recorded in digital or analogue form. Thus time-dependent changes in peak heights may be converted into concentration changes provided that, where necessary, correction for overlapping contributions of various ions is made. In the present work,

provided that H_2S is absent, m/z = 32 is specific for O_2^+. N_2^+ at m/z = 28 has a small contribution from N_2O, but with suitable correction can be used for measurement of N_2. All oxides of nitrogen contribute to m/z = 30, but in these experiments (as is often the case) N_2O was the sole contributor (as shown by undetectability of NO or NO_2 by gas chromatography). Measurement at m/z = 44 has contributions from N_2O and CO_2. Therefore information at m/z = 30 and 44 is sufficient to enable precise quantification of both species. Calibration for conversion to gas partial pressures necessitates standard gas mixture(s), and for concentrations, saturated solutions and the use of solubility tables (Wilhelm et al., 1977).

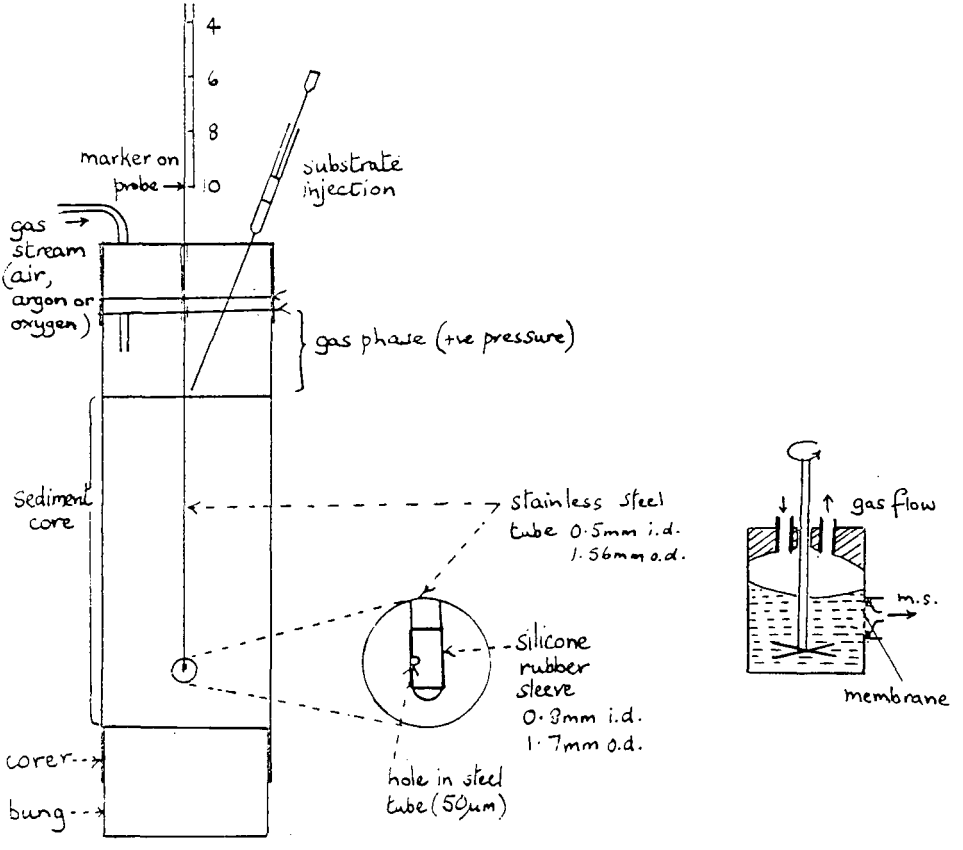

Fig. 1

Fig. 1a shows the equipment used for monitoring gases in a sediment core. The stainless steel probe is inserted to a measured depth and allowed to equilibrate until the mass spectrometer output reaches a steady value (about 20 min). Continuous monitoring then indicates changes (e.g. on addition of organic carbon sources, NO_3^-, O_2 or inhibitors to the surface or on changing the composition of the mobile gas phase etc.). Alternatively a depth profile may be obtained: at each insertion reequilibration is necessary. The same probe may be used to follow changes in headspace partial pressures. Multiplexed gasflow lines are now commercially available for use in the fermentation industry, and could possibly be used for multiple site sampling to a single mass spectrometric detector. Multiple probes for liquid phase measurements require an ancillary high vacuum system so that the off-line probes can be maintained in the evacuated state (Lloyd et al., 1985). Equipment is portable and has been used for ecological gas measurements in situ, e.g. in freshwater sediments using a generator to supply power (Boddy & Lloyd, 1989).

Paracoccus denitrificans NC1B8944 and Pseudomonas aeruginosa PA0129 were maintained and grown as described previously (Davies et al., 1989) either on defined medium containing 100 mM KNO_3 or on nitrate broth (Difco) which contains 10 mM KNO_3. Force aerated cultures were checked for the presence of dissolved O_2 just before harvesting: anaerobic cultures were harvested under an atmosphere of argon. Both types of culture were harvested, washed, resuspended in 20 mM K phosphate buffer at pH 7.2 and tested experimentally as quickly as possible (total processing time about 40 min).

Work with washed cell suspensions used a thermostatted stainless steel reaction vessel (5 ml working volume) stirred at fixed speed (1800 rev. min^{-1}) with a cross-shaped impellor (Fig. 1b). Gas flow over the stirred vortex was at 50 ml min^{-1} from a gas mixer. The PTFE membrane (2 layers, 2.5μm thick) were held on the probe with an "O"-ring held against a port below the liquid level. For further constructional details, see Lloyd & Scott, 1985.

RESULTS

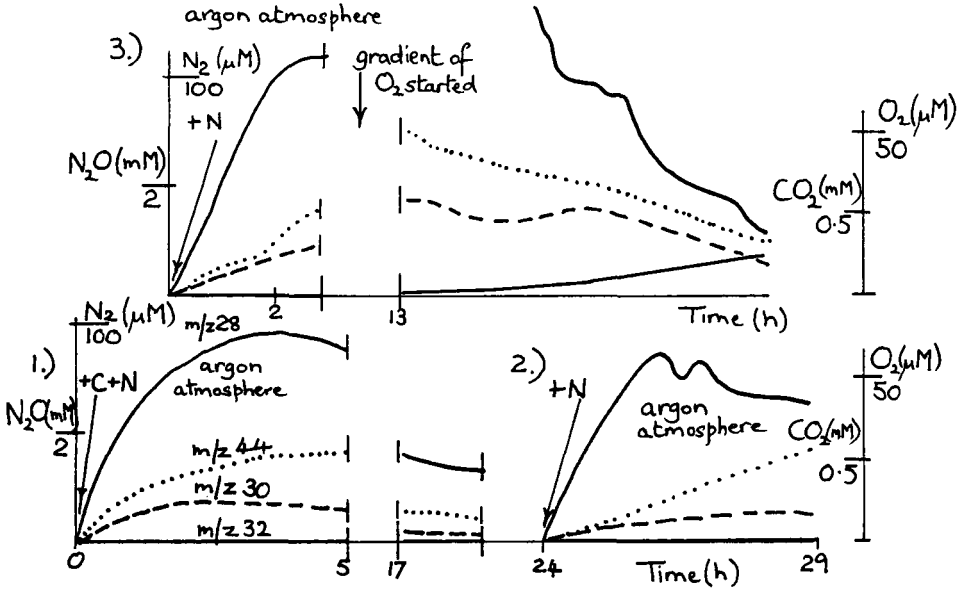

Fig. 2 shows the changes of dissolved gases at fixed depth (0.5 cm) in an estuarine sediment core. Addition of available carbon (5ml 0.5M Na acetate + 5 ml of 0.5M Na succinate) together with 5 ml of 0.5M $NaNO_3$ to the surface of the core gave evolution of N_2 and N_2O as well as CO_2. After 24 h when gas production had decreased to a low steady state level the addition of a further 5 ml of 1M $NaNO_3$ gave another phase of stimulated denitrification. This indicates that the process in this sediment was NO_3^--limited. A temporal gradient of O_2 introduced into the mobile gas phase of the headspace gave progressive inhibition of denitrification, but this was still incomplete during the period of aerobic respiration.

Evidence for aerobic denitrification was obtained in washed non-proliferating cell suspensions of P. denitrificans and Ps. aeruginosa. In the former organism the presence of O_2 led to a decrease of N_2 production and an increase of N_2, whereas in the latter both gaseous products increased aerobically. Disappearance of nitrate was monitored in anaerobically and aerobically grown cells which were maintained either anaerobically or aerobically; the rate and

extent of nitrate dissimilation in both species depended on these conditions and was most rapid under completely anaerobic conditions (Davies et al., 1989).

Lowest rates were when cells were grown anaerobically and maintained aerobically. After aerobic growth in the presence of nitrate, washed suspensions of both species converted over 87% of the added nitrate to N_2 + N_2O under both anaerobic and aerobic maintenance conditions (Davies et al., 1989).

DISCUSSION

The main conclusions from this work are as follows:-

1. A quadrupole mass spectrometer with membrane inlet provides an invaluable method for continuous direct monitoring of N_2, N_2O and CO_2 simultaneously with O_2 in the gaseous phase or (more usefully) in the liquid phase of cell suspensions, sediments and soils.
2. This device overcomes the problems associated with electrodes (fragility, long-term unreliability, incomplete specificity, e.g. for O_2, N_2O and NO).
3. Consumption of gas by the measuring device can be made very small and steel probes less than 1 mm diameter are easily constructed: the method may be regarded as minimally-perturbing and measurements can be made continuously over long periods (months and possibly years).
4. In an estuarine sediment O_2 consumption proceeds simultaneously with denitrification ("aerobic" denitrification). Anaerobic microzones may however still persist under a headspace containing O_2.
5. In washed cell suspensions of P. denitrificans and Ps. aeruginosa aerobic denitrification and nitrogen balances were quantitated at 50 μM-O_2 for comparison with the anaerobic process.
6. Care must be taken to define the term "aerobic" (Lloyd & Coombs, 1989). In any experiment where O_2 is provided to a homogeneously-stirred cell suspension the conditions are aerobic, even if dissolved O_2 is below the level of detectability (0.1-0.3 μM with a membrane-covered electrode or membrane inlet mass spectrometer): other methods are available for

measurement of lower oxygen concentrations (Lloyd, 1985; Lloyd et al, 1981). In the experiments described here aerobic denitrification proceeded at much higher dissolved O_2 (50 μM).

This work was carried out during the tenure of a NERC postgraduate studentship (KJPD).

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Table 1: A Chronology of "Aerobic" Denitrification

SACKS & BARKER	1949	J. Bact. 58, 11.
SKERMAN et al.	1951	Aust. J. Sci. Res. B4, 511.
MARSHALL et al.	1953	J. Bact. 66, 254.
SKERMAN & MACRAE	1957	Can. J. Microbiol. 3, 505.
KEFAUVER & ALLISON	1957	J. Bact. 73, 8.
SKERMAN et al.	1958	Can. J. Microbiol. 4, 243.
FEWSON & NICHOLAS	1961	Biochim. biophys. Acta. 49, 335.
CHANG & MORRIS	1962	G. Gen. Microbiol. 29, 301.
SCHMIDT & KAMPF	1962	Arch. Hyg. Bakt. 146, 171.
MECHSNER & WUHRMANN	1963	Pathol. Microbiol. 26, 579.
GILMOUR et al.	1964	Nature 203, 55.
WUHRMANN & MECHSNER	1965	Pathol. Microbiol. 28, 99.
DOWNEY et al.	1969	J. Bact. 98, 1056.
DOWNEY & KISZKISS	1969	Microbiol. 2, 145.
SINCLAIR & WHITE	1970	J. Bact. 101, 365.
SCHULP & STOUTHAMER	1970	J. Gen. Microbiol. 64, 195.
VOETS et al.	1975	J. Water Pollut. Cont. Fed. 47, 394.
KRUL	1976	J. Appl. Bact. 40, 245.
KRUL & VEENINGEN	1977	Water Res. 11, 39.
MEIBERG et al.	1980	J. Gen. Microbiol. 120, 453.
DUNN et al.	1980	J. Gen. Microbiol. 112, 379.
OTTOW & FABIG	1983	Proc. VI Int. Symp. Env. Biochem. N. Mexico p.61.
WATAHIKI et al.	1983	Agric. Biol. Chem. 47, 1991.
BAZYLINSKY & BLAKEMORE	1983	Appl. Environ. Microbiol. 46, 1118
NAKAJIMA et al.	1984	Water Res. 18, 339.
ROBERTSON & KUENEN	1984	Anton van Leewenhoek 50, 525
DAVIES & LLOYD	1984	Gas Enzymology Meeting, Odense, Denmark, Poster.
OTTOW & FABIG	1985	Planetry Ecol. 427-40 Caldwell et al, Vn Reinhold
TREVORS	1985	Appl. Microbiol. Biotechnol. 23, 152.
LLOYD et al.	1986	FEMS Microbiol Ecol. 38, 11.

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Ecophysiology and Kinetics of Denitrification in Soils

by

John M. Duxbury*

The purposes of this paper are to summarize the effects of environmental variables on denitrification in soils and to consider how the kinetics of denitrification in natural habitats can be described. Terms used to describe denitrification are not always used in the same manner so are briefly defined as:

Reaction kinetics	- Apply to individual reactions
Potential activity of *in situ* enzymes	- Reaction rates under ideal conditions
Denitrification capacity	- Amount of N denitrified without C addition
Denitrification rate	- Σ Reaction kinetics and controlling variables
Product flux	- Measured flux of N_2 and/or N_2O

Measurements of the potential activity of denitrifying enzymes are made under anaerobiosis with addition of C and N substrate and with inhibition of enzyme synthesis (Smith and Tiedje, 1979), i.e. they reflect instantaneous *in situ* enzyme levels. Measurements that can be made easily are N_2O generation from NO_3^- and reduction of N_2O to N_2; the former is made in the presence of C_2H_2 to inhibit reduction of N_2O and represents the combined activities of several enzymes and the latter is a direct measure of nitrous oxide reductase activity. Data gathered from different environments (table 1) show that, in many cases, large amounts of N could be denitrified without enzyme synthesis. The data also suggest that potential activities are higher in flooded soils and sediments than in upland soils and higher in no-tilled than tilled mineral soils. The potential of nitrous oxide reductase was found to be on the order of 50 kg N ha^{-1} day^{-1} in soils which contained <2 µg NO_3-N g^{-1} soil but net generation of N_2O, rather than reduction, was found in an alfalfa field where soil nitrate levels (5-8 µg N g^{-1} soil) may have been high enough to influence N_2O reduction (Erich et al., 1984).

*Department of Agronomy, Cornell University, Ithaca, NY 14853, USA

The persistence of denitrifying enzymes in aerobic environments appears to be variable. Smith and Parsons (1985) found that the potential activities of enzymes involved in N_2O generation were little affected by air drying and storage for 1 week and were still at 25% of the original value after 9 weeks of air-dry storage. In contrast, denitrifying enzymes did not survive a 1 week air-dry treatment when cultured denitrifiers were added to autoclaved soil, indicating that there may be some protective processes operative for organisms and/or enzymes developed *in situ*. Further, Erich et al. (1984) found that nitrous oxide reductase activity in fresh soil samples was essentially destroyed by 20h of air exposure, which led to the conclusion that anaerobic microsites must be more or less continuously present in nominally aerobic soils. There is little information, however, on the temporal variability of denitrifying enzyme levels in field environments. Rice and Smith (1982) found a 4-fold fluctuation in the potential for N_2O generation over a 9-month period in soil cropped to maize (*Zea mays*) with somewhat greater potential activities in a no-till than a conventional-till cropping system.

Table 1: Potential Activities of Denitrifying Enzymes in Surface Soils/Sediments from Various Habitats[a]

Soil/Sediment Habitat	----------N_2O Generation[b]----------		N_2O Reduction
	n mol N_2O g^{-1} h^{-1}	..... kg N ha^{-1} day^{-1} [c].....	
Temperate Forest (pH 5-6.4)	3-19	4-25	43-55
Tropical Forest (pH 4-4.4)	8-14	11-19	
Arable agriculture	<1-26	<1-13	-5-49
Pasture	10-20	13-26	30-63
Cropped organic soils	2-40	3-54	
Wetlands	<1-65	<1-87	
Stream/Lake sediments	75-230	101-309	

[a] data from Tiedje et al., 1982; Rice and Smith, 1982; Erich et al., 1984; Smith and Parsons, 1985; Parkin et al., 1985.
[b] measured using C_2H_2 inhibition of N_2O reduction.
[c] calculated for 15 cm soil depth with a bulk density of 1.33g cm^{-3}; N_2O reduction data at 30°C and N_2O generation at 20-30°C (not specified in most studies).

Many environmental factors affect both the expression of denitrifying enzyme activities and synthesis of denitrifying enzymes in soils. The primary regulator of enzyme activity and synthesis is O_2. Culture studies have shown that the threshold level below which denitrification occurs is about 10 m mol O_2 L^{-1} and that the activities of NO_3^-, NO_2^-, and N_2O reductase are derepressed sequentially as O_2 levels decline. The synthesis of nitrate and nitrite reductase occurs at higher O_2 levels than 10 mmol L^{-1}, but there is variability amongst organisms (Tiedje, 1988).

Laboratory denitrification studies under anaerobic conditions have shown strong correlations between the production of CO_2 and either NO_3^- disappearance (Reddy et al., 1982) or production of gaseous denitrification products (Patten et al, 1980). Such relationships are expected as all these parameters reflect electron flow through denitrifying organisms. Good correlations also often exist between indices of available carbon and flux of denitrification products where NO_3^- supply is adequate (eg. Burford and Bremner, 1975). Further, the denitrification capacity measurement is essentially a measure of the amount of available carbon in soils. While either carbon or nitrate availability can limit denitrification in anaerobic soils, the former is more likely to be the limiting factor in agricultural soils and the latter in unfertilized soils.

The effect of pH on denitrification is unclear. An early study with an acid soil from the Park Grass experiment at Rothamsted showed no denitrification at its native pH (4.5) but increasing denitrification as pH was increased (Bremner and Shaw, 1958). Although investigation into pH effects is complicated by abiological reactions at low pH, subsequent studies have often shown a positive relationship between pH and denitrification rate (e.g. Muller et al., 1980; Waring and Gilliam, 1983). However, Waring and Gilliam (1983) found that a major part of the pH effect was due to changes in carbon availability and Koskinen and Keeney (1982) found that denitrification product flux was more related to carbon availability than pH in soils from a long-term lime experiment. These results suggest a need to study the effect of pH on denitrification when other potential constraints are removed. Adaptation of organisms/enzymes to soil pH is suggested by the work of Parkin et al. (1985) and an effect of pH on the balance between denitrification and dissimilatory nitrate reduction is suggested by the work of Waring and Gilliam (1983). Both of these issues merit further research.

Diffusion of substrates is a major factor controlling denitrification rates in natural habitats and is likely the major reason that a 1st order kinetic model often describes denitrification reasonably well. Perhaps the most dramatic illustration of diffusion effects is the increase in denitrification product flux of up to 300-fold when shaken anaerobic slurries were compared with anaerobic cores (Myrold and Tiedje, 1985). Another good example of diffusion limitation is the reduced rate of nitrate loss from flooded soil as floodwater depth increases and diffusion of NO_3^- from the floodwater to anaerobic soil is slowed (Reddy et al., 1978). Diffusion constraints are probably also responsible for the finding that Km values for NO_3^- reduction vary considerably and are up to two orders of magnitude higher in soils compared to cultures (fig. 1)

Denitrification rates in soils reflect the combination of kinetics of several reaction steps, electron donor and acceptor levels and availability, and environmental variables which exert control on enzyme synthesis and activity. As shown in figure 1, the distribution of all of these materials and properties is generally heterogeneous in soils although distinct patterns may exist in flooded soils and in the rhizosphere. For example, flooded soils will have a thin aerobic layer at the soil-water interface, and patterns with respect to O_2, pH and substrates occur in the rhizosphere of both flooded and upland soils. It is also important to recognize that diffusion affects both substrate and product fluxes so that in most cases, product flux is not equal to *in situ* denitrification rate, a fact often ignored when interpreting denitrification measurements in kinetic terms.

Culture	Soil	Flooded Soil Sediment
Organisms, N and C substrates, O_2, and pH all homogeneous	Organisms, N and C substrates heterogeneous O_2, pH heterogeneous	Some pattern to O_2, pH
No diffusion limitations	Severe diffusion limitations (tortuosity, continuity)	moderate diffusion limitations
Product Flux = generation	Product flux < to << generation	Product Flux decoupled from generation (gas bubbles)
Km (NO_3) < 15μM	_____	Km (NO_3) 0.1 - 5 mM _____

Figure 1. Features of different denitrifying environments.

Denitrification in soils has been found to fit zero order (see Focht, 1974), 1st order (see Reddy et al. 1978), and Michaelis-Menten kinetics (Bowman and Focht, 1974). Any kinetic fit, if not quite fortuitous, is however, empirical rather than mechanistic (Kohl et al., 1976). Betlach and Tiedje (1981) developed a sequential reduction model, based on Michaelis-Menten kinetics, to describe the production and consumption of denitrification intermediates in pure cultures. An empirical inhibition term (P) scaled from zero to one, was also included for each reaction step in order to accommodate the negative effects of O_2, limited electron donor supply, reduced temperature and pH, and toxic chemicals, i.e.

for substrate i, $$v_i = P V_{max} \frac{S_i}{K_{mi} + S_i}$$

This model demonstrated that differences in reduction rates of the various N species were sufficient to explain observed patterns of accumulation of intermediates. A dual substrate Michaelis-Menten equation describing the availability of both C and N was used by Focht et al. (1974) and Reddy et al. (1982) to describe denitrification in soils under laboratory conditions., i.e.

$$V = V_{max} \frac{S_C S_N}{(S_C + K_C)(S_N + K_N)}$$

and could be coupled with one or more inhibition terms.

Kinetic description of denitrification in natural habitats is greatly complicated by the heterogeneous and dynamic nature of the real world. In recognition of the dynamic nature of denitrification, McConnaughey and Bouldin, (1985a and b) used a system of transient reaction-diffusion equations for O_2 and the various N species to describe denitrification at the soil aggregate level. Of four reaction terms considered, the sequential Michaelis-Menten reduction model of Betlach and Tiedje (1981) gave the best fit with experimental results (McConnaughey et al. 1985). The need for better knowledge of kinetic parameters was stressed, however, and spatial heterogeneity was not considered in this approach. While, it is clear that our understanding of the denitrification process will continue to be advanced by studies under defined conditions, application of this information to naturally heterogeneous environments will be a more difficult and challenging task.

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Kinetics of denitrification in *Paracoccus denitrificans*: Measurements using ^{15}N-nitrate and a mass spectrometer with a permeable membrane inlet

by

R.P. Cox, T. Geest and J.K. Thomsen +)

Denitrification involves the production of the gases dinitrogen and nitrous oxide from nitrate and nitrite. A measuring technique which allows on-line measurements of the concentration of these two gases is of obvious value in studies of the reaction kinetics of the process. Such a technique has been available for many years (1), and has recently been applied to studies of denitrification (2,3). It involves the use of small portable mass spectrometers together with a specially constructed inlet which allows the mass spectrometer vacuum to be separated from the aqueous sample by a membrane which is much more permeable to gases than to water or ions. All the dissolved gases in the solution will diffuse through the membrane and contribute to the mass spectrum of the sample.

Interpretation of the results is much simpler if individual peaks in the mass spectrum (ions of particular mass-to-charge ratios) can be unambigously assigned to particular products of microbial activity. This is however a problem in denitrification studies, since N_2O has a mass of 44, the same as CO_2. CO_2 also contributes to the spectrum at m/z=28 where N_2 is most readily measured, due to the CO^+ ion produced by fragmentation. Although it is possible to make measurements at a number of m/z ratios and solve the resulting simultaneous equations, it is much simpler to avoid the problem altogether and make measurements using the stable nitrogen isotope ^{15}N.

This allows N_2O to be determined unambigously at m/z=46, and N_2 to be measured at m/z=30 with only a correction for N_2O. Use of ^{15}N

+) Biokemisk Institut, Odense Universitet, Campusvej 55, DK-5230 Odense M, Denmark.

also allows CO_2 to be followed as an indicator of bacterial metabolism, and allows measurements of dinitrogen production against a low background. If the production of $^{14}N_2$ is measured, the background will be high unless atmospheric gases are removed by sparging, and even if this is done it is difficult to avoid a background due to atmospheric gases leaking into the vacuum of the mass spectrometer from points other than the membrane inlet.

We describe here the use of this approach in studies of denitrification kinetics in pure cultures of *Paracoccus denitrificans*.

Methods

Paracoccus denitrificans DSM 413 was grown in a nitrate-limited chemostat at a dilution rate of 0.2 h^{-1} on a Tris-buffered mineral salts medium with succinate as the carbon and energy source and ammonium as nitrogen source. Cells were removed from the bioreactor, washed by centrifugation, and resuspended in a medium containing buffer adjusted to different pH values with KOH, 10 mM sodium succinate and 1 mM $MgCl_2$.

Dissolved gases were measured using a Spectramass Dataquad quadrupole mass spectrometer connected to an Acorn BBC microcomputer. The inlet to the mass spectrometer consisted of two 0.4 mm diameter circular holes covered by silicone rubber 0.25 mm thick. The holes were bored near the closed end of a stainless steel tube and covered by a length of silicone rubber tubing. The reaction vessel had an adjustable stopper through which the mass spectrometer inlet probe was inserted. The stopper had a conical bottom with a capillary tube at the apex, to provide a closed reaction system of variable volume from which bubbles could be removed whilst allowing reagents to be added and samples to be taken. The measuring apparatus and reaction vessel are described in more detail by Jensen and Cox (4).

Nitrate and nitrite were determined colorimetrically using an autoanalyser. Samples were removed from the reaction vessel, quenched by diluting into ice-cold water followed by rapid

freezing, and the cells removed by centrifugation at 4°C immediately after thawing.

Results

Fig. 1 shows the results of an experiment in which a small volume of concentrated cell suspension was added to air-saturated succinate-containing medium at pH 8.5, the same as that in the chemostat. After the bacteria had consumed the dissolved oxygen by respiration, $Na^{15}NO_3$ was added, and changes in nitrate, nitrite, nitrous oxide and dinitrogen followed. At this pH value, the consumption of nitrate occurred at the same rate as the production of N_2. Nitrite rose rapidly to a steady-state concentration of about 100 µM, and remained close to that value until the nitrate was exhausted, whilst N_2O was hardly detectable.

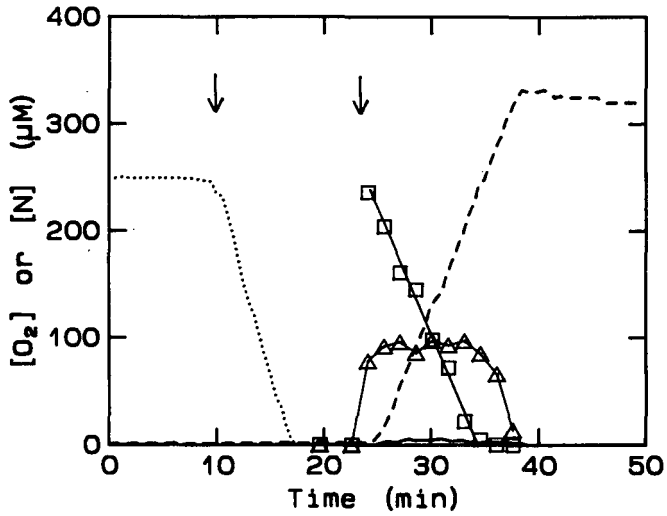

Figure 1. Changes in the concentrations of dissolved gases, nitrate and nitrite in washed cell suspensions of *Paracoccus denitrificans* in medium at pH 8.5 (Taps buffer). The mass spectrometer was used to measure dissolved O_2 (m/z=32,), $^{15}N_2O$ (m/z=46, ———) and $^{15}N_2$ (m/z=30, corrected for $^{15}N_2O$, - - - -). Nitrate (□) and nitrite (Δ) were measured in samples removed from the reaction chamber. At the beginning of the experiment the vessel contained air-saturated medium and the reaction was started by adding cells at the point indicated by the first arrow (final concentration 106 mg cell carbon/l). 300 µM $Na^{15}NO_3$ was added at the point indicated by the second arrow.

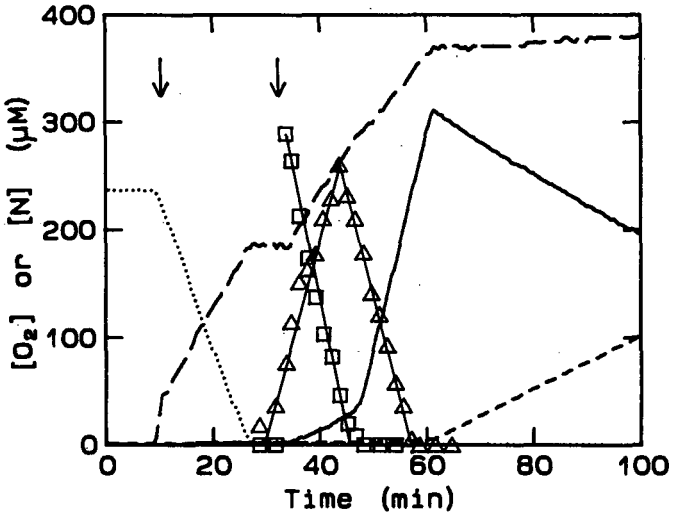

Figure 2. Changes in the concentrations of dissolved gases, nitrate and nitrite in washed cell suspensions of *Paracoccus denitrificans* in succinate-buffered medium at pH 5.5. The mass spectrometer was used to measure dissolved O_2 (m/z=32, ·····), $^{15}N_2O$ (m/z=46, ———), $^{15}N_2$ (m/z=30, corrected for $^{15}N_2O$, - - - -), and CO_2 (m/z= 44,). Nitrate (□) and nitrite ( ) were measured in samples removed from the reaction chamber. At the beginning of the experiment the vessel contained air-saturated medium and the reaction was started by adding cells at the point indicated by the first arrow (final concentration 106 mg cell carbon/l). 300 μM $Na^{15}NO_3$ was added at the point indicated by the second arrow.

When similar experiments were repeated at a series of pH values, strikingly different kinetic patterns were observed. At pH 5.5 (Fig. 2), there was essentially a quantitative conversion of nitrate to nitrite, and N_2O production only began once the nitrate was exhausted. Then nitrite was converted quantitatively to N_2O. Only when the nitrite was exhausted did the final step, the reduction of N_2O to N_2, commence. The very low rate of this reaction at pH 5.5 provides a kinetic explanation for the accumulation of N_2O.

The almost quantitative conversion of nitrate to nitrite was only observed at pH 5.5, nitrite accumulation being less at pH 6.0 and

above. Quantitative accumulation of N_2O was observed at pH 6.5 and below, but was much less at pH 7.0. The accumulation of N_2O at acid pH values by *Paracoccus denitrificans* was previously reported by Kucera et al. (5). Enhanced production of N_2O at acid pH seems to be a widespread phenomenon (6).

At low pH values it is also possible to measure changes in the dissolved CO_2 concentration as a measure of succinate metabolism. Since CO_2 but not bicarbonate ion can cross the silicone membrane at an appreciable rate, the signal observed depends on the difference between the medium pH and the pK_a for bicarbonate (about 6.4). Thus at alkaline pH values the signal is small and very affected by small changes in pH (up to 26% for 0.1 pH unit), while at a pH value of 5.5 nearly 90% of the maximum possible signal is obtained and buffering is much less critical.

Changes in dissolved CO_2 are also shown in Fig. 2; these show that the rate of succinate metabolism is greatest with O_2 or nitrate as electron acceptor, decreases slightly with nitrite, and is very much slower when N_2O is being reduced.

Conclusions

The results presented here demonstrate that the use of ^{15}N-labelled substrates together with membrane-inlet mass spectrometry is a potentially valuable approach to studying the kinetics of denitrification. The experiments reported here are laboratory measurements with pure cultures of vigourous denitrifiers, but there is no reason why the same approach cannot be applied to experimental systems which are more directly relevant to the situation in natural or agricultural environments, such as mixed cultures or soil or sediment slurries.

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Effect of oxygen on soil denitrifying expression and potential

by

Lescure+, C., P. Gamard+, A. Pidello++ and R. Lensi+

The ability to denitrify can be regulated at three levels by (SIMPKIN and BOYLE, 1988) :

- variations on the number of microorganisms able to denitrify
- synthesis of the enzymes involved in the denitrification reactions
- expression of these enzymes

Oxygen is one of the most important regulator of denitrification and it may act at each of these levels. Its influence on soil denitrifying expression has been studied by several authors (OTTOW and El-DEMERDASH, 1983 ; PARKIN and TIEDJE, 1984 ; KROECKEL and STOLP, 1985 ; LENSI et al., 1986). It is generally held that denitrifying enzymes are inducible and that their synthesis only occurs when oxygen is absent (KNOWLES, 1982 ; FIRESTONE, 1983). Still few works have been carried out on the repression of the synthesis of denitrifying reductases by oxygen in soil.

The aim of the presented work was to study the effect of different oxygen concentrations on (i) soil denitrifying expression and (ii) soil denitrifying potential.

The potential was interpreted using an hypothesis based on variations of the number of denitrifying microorganisms and the level of synthesis of reductases.

MATERIAL AND METHODS

Soil : The soil used was a sandy clay loam with the following characteristics : pH : 6.7 ; organic C : 3% ; total N 0.28% ; NH_4^+-N : 3µg.g^{-1} ; NO_3^--N : 16 µg.g^{-1}.

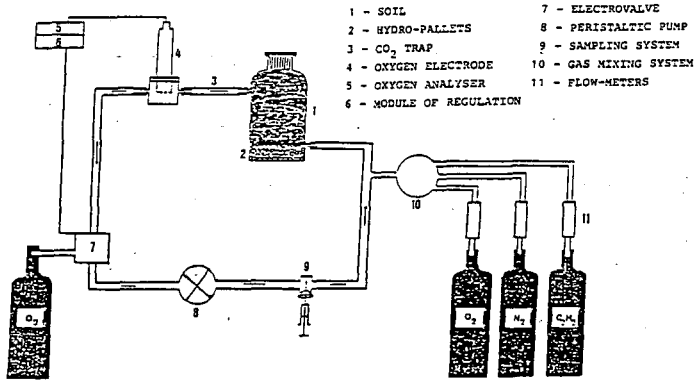
Experimental device used to monitor the soil oxygen partial pressure : The gas-flow system used in this study (fig. 1) was partially described previously (LENSI et al., 1986). Briefly, a known gas mixture (N_2+O_2+C_2H_2 or N_2+O_2) was prepared using pure gases and precise gas flow meters and recirculated through the soil sample. The choosen pO_2 was then maintained constant using a module of regulation connected to an oxymeter. An electrovalve monitored by the module automatically introduced the required quantity of oxygen in the circulating system to compensate for soil respiration.

+ Lab. Ecol. Microbienne, UA CNRS 697, Univ. Lyon I; 4" Bd. du 11 Novembre 1918, 69622 - Villeurbanne Cedex, France.

++ Lab. Quimica biologica I, Fac. Ciencias veterinarias, Univ. Nac. Rosario, Bv. O. Lagos y Ruta 33, 2170 - Casilda, Argentina.

The advantages of the gas-flow system are (i) a rapid homogenization of the gases and (ii) the possibility to maintain constant the soil oxygen concentration.

Fig. 1: EXPERIMENTAL DEVICE TO CONTROL OXYGEN IN SOIL



Experiment I was designed to study the effect of pO_2 on the expression of soil denitrifying activity (this part was published in LENSI et al. (1986)). Immediately after addition of an excess of nitrate, the soil samples were plugged to the gas-flow system and a flow containing 10 kPa C_2H_2 and various pO_2 was applied. After a period of equilibration, the one hour nitrous oxide accumulation was determined by gas chromatography.

Experiment II was carried out to evaluate the influence of oxygen on the soil denitrifying potential. Soil samples were subjected to various oxygen concentrations (0, 0.5, 5, 21 and 34 kPa) during 24 h using the gas-flow system described above. Each soil sample was then transferred into a plasma-flask and the Denitrifier Enzyme Concentration measurement was performed according to TIEDJE (1982)

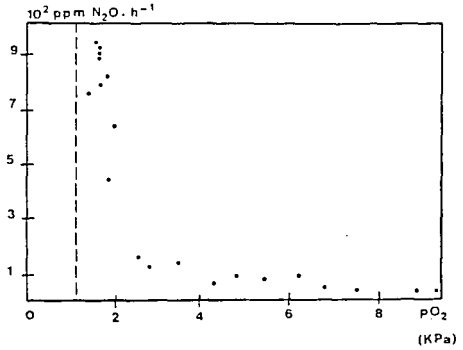
Experiment III was conducted to evaluate the influence of an anaerobic period following a 24 h aerobic incubation on the soil denitrifying potential. After a first experiment at 5 or 21 kPa O_2 as described in the paragraph above, a second incubation at 0 kPa O_2 has been carried out. The length of this anaerobic incubation was variable : 1, 2, 4, 6, 8 and 10 hours. After these two consecutive incubations, the soil denitrifying potential was revealed by the Denitrifier Enzyme Concentration.

RESULTS AND DISCUSSION

Experiment I : To evaluate the influence of oxygen on the expression of denitrification, the soil denitrifying potential must be assumed equal for all the soil samples. This is the reason why we used air-dried homogenized soil and performed short-term incubations (1 hour).

Fig. 2 :

INFLUENCE OF PARTIAL PRESSURE OF OXYGEN ON THE EXPRESSION OF THE SOIL DENITRIFYING ACTIVITY.



Accumulated N_2O was expressed against the pO_2 (fig. 2). The denitrifying activity vis-à-vis the pO_2 was a classical negative relation. This result was expected and two points must be be emphasized :

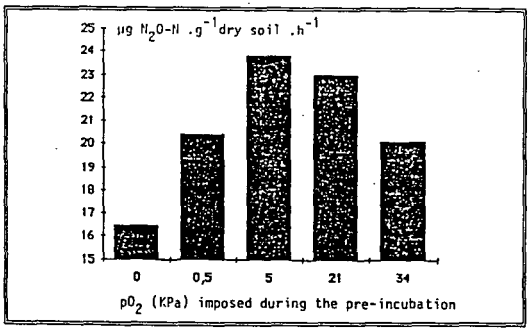
- The expression of denitrification strongly increased below 2 - 2.5 kPa O_2 which appeared as a critical value. This result is in agreement with PARKIN and TIEDJE (1984) which have shown a zone of high sensitivity at 3 kPa O_2 using a similar experimental device.
- A slow but detectable accumulation of N_2O occurred even at relatively high pO_2 demonstrating that, in the experimental conditions described, oxygen and nitrate could be simultaneously used as terminal electron acceptors (KROECKEL and STOLP, 1985).

Experiment II : The potential of denitrification (evaluated by the Denitrifier Enzyme Concentration measurement) against oxygen concentration applied during a preincubation was shown on figure 3. It appeared that only 24 h at different oxygen concentrations were sufficient to strongly modify the soil denitrifying potential. The lowest accumulation of N_2O was obtained after total anaerobiosis. It was increased by an incubation at 0.5 kPa. The optimal potentials were obtained after

incubations at 5 and 21 kPa O_2 and the potential were decreasing at higher pO_2 (34 kPa).

Fig. 3 :

INFLUENCE OF PARTIAL PRESSURE OF OXYGEN ON THE POTENTIAL OF DENITRIFICATION.



In a study on the sequence of phases of denitrification following oxygen depletion in soil, SMITH and TIEDJE (1979) interpreted the phase I (not affected by chloramphenicol) as the activity of preexisting enzymes and the phase II (inhibited by chloramphenicol) as the derepression of denitrifying enzyme synthesis. In our experiment, two points were basically different : (i) various pO_2 were imposed to the soil and (ii) the denitrifying measurements were performed under optimal conditions of expression.

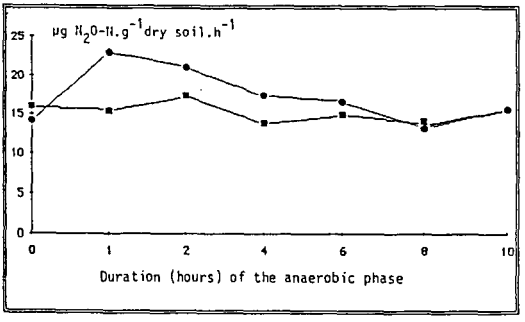
The hypothesis presented to explain our results included variations of the number of microorganisms (numerical component) and of the level of synthesis of the denitrifying reductases (physiological component). In the experimental conditions, 5 and 21 kPa O_2 concentrations appeared to ensure the best equilibrium between the two components. The 0 and 0.5 kPa O_2 were more favourable for enzyme synthesis but relatively unfavourable for bacterial growth, including growth of denitrifiers which are using preferentially oxygen as terminal electron acceptor. In this case, the numerical component limited the potential of denitrification. Conversely, for superior pO_2 values eg 34 kPa, the enzyme synthesis was too strongly inhibited by oxygen and, even if this condition favoured bacterial growth, this physiological component limited the potential of denitrification. Incubations at 5 and 21 kPa O_2 induced the highest denitrifying response; moreover, no significant difference ($p < 0.05$) appeared between the two treatments. Experiment III was performed to

investigate if such similarity signified an identical status for the denitrifying microflora in these two treatments.

Experiment III : Anaerobiosis did not induce the same response when the preliminary 24 h- aerobic incubations were conducted at 5 or 21 kPa O_2 (fig. 4).

Fig. 4:

EFFECT OF ALTERNATE AEROBIC - ANAEROBIC PHASES ON THE SOIL DENITRIFIER ENZYME CONCENTRATION MEASUREMENT



Legend : Pre-incubation : 5 KPa O_2 : ■——■
Pre-incubation : 21 KPa O_2 : ●——●

After an incubation at 5 kPa O_2, the soil denitrifying potential has not been modified by the anaerobic incubation, whatever its duration, suggesting that an oxygen partial pressure of 5 kPa allowed the reductase synthesis at an almost optimal level. On the other hand, following a 21 kPa O_2 incubation, only one hour of anaerobiosis greatly increased the soil denitrifying potential. This result suggested that, under 21 kPa O_2, bacterial growth occurred at a higher rate than under 5 kPa O_2. However, the level of reductases synthesis was not optimal. The subsequent drop of denitrifying potential for anaerobic period longer than one hour remained unclear.

CONCLUSION

Using the Denitrifier Enzyme Concentration measurement as defined by TIEDJE (1982), expression and potential of the process of denitrification can be distinguished. The study of the influence of oxygen on both types of regulation was made possible by the use of a gas- flow experimental system. The influence of oxygen on the potential of denitrifying activity was explained by an hypothesis involving a numerical and a physiological component. To study with accurately this influence on the relation number / physiology of denitrifiers in soil, enumeration of

denitrifiers by an m.p.n. procedure is not precise enough. The appropriate precision of counts can be obtained only by simplifying the experimental system, for example by working with a single known denitrifying organism inoculated into a sterilized soil. Then reliable and precise counts based on immunological techniques would be applied.

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Effect of the initial oxygen level on denitrification process, nitrogen and carbon balance in a water-sediment system of Ismailia region (Egypt).

by

Mehana, T.*), M. Ragab **) and R. Aldag*).

Introduction: The problems of nitrate pollution and eutrophication of surface waters are of increasing concern. The NO_3^--N increase has been attributed to a number of sources including mainly the use of N-fertilizer for field crops and surface disposal of N-containing wastes. The biological reduction of NO_3^- to gaseous nitrogen is a mechanism by which nitrate pollution is greatly reduced and the reduction is accentuated where there is an ample supply of organic detritus or energy sources in the mud of bacterial metabolism (ENGLER and PATRICK, 1974). Natural waters generally contain dissolved oxygen in such a concentration that denitrification in the water phase is practically impossible (VAN KESSEL 1976). On the contrary, aerobic denitrification was claimed by ABOU SEADA and OTTOW, (1985). Therefore natural sediment with overlying water was chosen as a model system for laboratory experiments to determine denitrification capacity, total mineralized C and oxygen consumption by an aquatic sediment incubated under initially aerobic and anaerobic conditions.

Materials and Methods: Sandy textured sediment was collected from the bottom surface layer of Ismailia drain canal (Egypt) which has a salinity of 0,04 S m^{-1}. The sediment was air-dried and screened through a 2 mm sieve before use. Organic N of this sediment was 125 ppm and total C was 1450 ppm. The native NH_4^+-N and NO_3^--N levels were 3.2 and 0.80 ppm, respectively. The pH value was 7.86. Fourty grams of the air-dried sediment and 40 ml of distilled water were placed into 165 ml glass tubes to form a 3.1 cm sediment layer and a 3.5 cm overlying water layer. All tubes were preincubated non stoppered in the dark for 21 days at 30°C. The overlying water lost by evaporation was replaced with distilled water every 2 days. At the end of this period, additional two tubes were used for determination of all parameters which are given as initial values for the experiments.

*) Institut für Geowissenschaften, Abteilung Agrarökologie, Universität Bayreuth, Universitätsstrasse 30, D-8580 Bayreuth, FRG.
**) Faculty of agriculture, Soil and Water Department, Suez Canal University, Ismailia, Egypt.

Thus, 20 ml of the overlying water from each tube were discarded and 0.20 g pure cellulose containing 42.3 % C was mixed carefully with the sediment. Twenty ml of neutral aqueous solution containing 200 mg NO_3^--N as KNO_3 plus 20 mg N-Serve per litre were also added to each tube. The tubes were evacuated and flushed several times with helium and corresponding volumes of helium inside the tubes were replaced by equal volumes of O_2 to forminitial O_2 concentrations of 0, 2,5, 10, 15 and 20 % O_2 and the final gas phase was thoroughly mixed. All tubes were incubated in the dark at 30°C for 26 days. Gas samples were removed at 0, 1, 2, 4, 7, 9, 12, 18 and 26 days and analyzed for N_2, N_2O, CO_2, O_2 and CH_4 using a gas chromatograph. The experiment was carried out in triplicate. Chemical analyses of the overlying water and sediment included total Kjeldahl-N using the procedure of BREMNER and MULVANEY (1982). Inorganic N was determined by steam distillation of a 2 M KCI extract according to KEENEY and NELSON (1982). Total organic N was obtained by subtracting the inorganic N from the total Kjeldahl-N. Total C was analyzed using a Carbon and Nitrogen Analyzer. Soluble organic C was determined by persulfate oxidizable C method as described by GILMOUR and GILMOUR (1985) and bicarbonate in the sediment extract and overlying water was determined by titration with dilute standard HCL to a pH of 4.5 according to GALE and GILMOUR (1988).

Results and Discussion:

During the 26 days incubation period, nitrification was inhibited due to the addition of N-Serve. At the end of incubation, almost all the applied NO_3^--N was transformed by denitrification and immobilization, (Table 1). Nitrite nitrogen was not detected in the system while NH_4^+-N decreased from 4.9 ppm at the beginning of experiment to about 2 ppm at the end (Table 1, Cols. 3 and 7). This decrease may be attributed to the immobilization process. Nitrous oxide was not detected throughout the incubation period. BROADBENT and CLARK (1965) stated that N_2O is rather water soluble and its production is inhibited above pH 7. The pH of the sediment used here was 7.86. The initial O_2 levels used were significantly reversible correlated with denitrification capacity ($r=-0.813^*$). Maximum denitrification loss was obtained between zero and 2.5% O_2 where it accounted for 74.7 and 84.7% of the total nitrate reduced, respectively (Table 1, Col. 11) These results may be attributed to the inhibitory effect of O_2 on the induction and formation of denitrifying enzymes as well as the drop in redox potential to levels required for nitrate respiration (DELWICHE and BRYAN, 1976). Further increases of O_2 level enhanced carbon mineralization (Table 2), whereas denitrification occurred at relatively low rate ranging from 46.2 to 55.1% of the total nitrate reduced (Table 1. Col. II). The aerobic denitrification can be explained by the increased demand for electron acceptors needed during intensive mineralization of organic matter (ABOU SEADA and OTTOW, 1985).

Table 1. Nitrogen balance in the water - sediment systems

(Figures as ppm)

Initial O_2 (V/V %)	Total N at the beginning of experiment as:				Total N at the end of experiment as:					Denitrification loss % of NO_3^--N reduced	N Immobilized	N (not accounted for)
	NO_3^--N *	NH_4^+-N	Organic-N	Total-N	NO_3^--N	NH_4^+-N	Organic-N	N_2-N	Total N			
Col. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
0	104.1	4.9	111.6	220.6	3.5	1.7	130.4	75.1	210.7	74.7	18.8 (8.5)**	9.9 (4.5)**
2.5	"	"	"	"	3.0	1.8	129.2	85.6	219.6	84.7	17.6 (8.0)	1.0 (0.5)
5.0	"	"	"	"	2.5	2.3	124.2	56.0	185.0	55.1	12.6 (5.7)	35.6 (16.10)
10.0	"	"	"	"	1.5	1.8	126.3	56.5	186.1	55.1	14.7 (6.7)	34.5 (15.6)
15.0	"	"	"	"	1.2	1.8	123.0	47.5	173.5	46.2	11.4 (5.2)	47.1 (21.4)
20.0	"	"	"	"	2.1	1.7	118.9	49.6	172.3	48.6	7.3 (3.3)	48.3 (21.9)

* Initial nitrate - N in sediment plus nitrate - N added (100 ppm).

** In percentage of original total N .

Table 2. Carbon balance in the water-sediment systems. (Figures as ppm)

Initial O_2 (v/v%)	Initial Total C	Total C at the end of the experiment as:					Total C loss as % of initial C (Col.2)	pH (overlying water)
		Total C in the sediment	Water soluble C		CO_2-C	CH_4-C		
			Organic	HCO_3^-				
Col.(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
0	3450*	2816	310.8	186.8	23.9	30.2	18.38**	7.40 ***
2.5		2703	257.1	232.6	56.6	29.0	21.65	6.86
5.0		2633	307.0	173.4	71.9	26.8	23.68	6.92
10		2600	303.8	164.8	75.6	24.9	24.64	7.24
15		2583	307.6	151.1	98.9	20.3	25.13	7.02
20		2500	321.0	204.4	101.4	17.5	27.54	6.91

* Original total C plus carbon added as cellulose (2122 µg C g^{-1} dry sediment).

** In percentage of initial total C.

*** Initial pH value of the overlying water = 7.64.

In all O_2 levels, organic N content of the sediment increased during the incubation period showing a net immobilization of applied N. The immobilized N highly significantly decreased with increasing O_2 level ($r=-0.919^{**}$), constituting amounts ranged from 7.3 to 18.8 ppm at 20 and zero % O_2, respectively (Table I, Col. 12). These results may be due to the addition of cellulose which increased the C/N ratio.

Oxygen depletion occurred after 4 to 18 days from the beginning of experiment in all O_2 level treatments and most of the added NO_3^- also disappeared during this period. These results indicate that the conditions in all O_2 treatments changed to anaerobic conditions. According to GALE and GILMOUR (1988), the mineralized C is best described as the sum of CO_2, CH_4 evolved plus the water soluble organic C produced. Organic C degradation gradually increased as a result of increasing initial O_2 level (Table 2, Col. 8). These results are supported by CO_2-C production which increased from 23.9 to 101.4 ppm with increasing initial O_2 level from 0 to 20% O_2, respectively (Table 2, Col.6). Also the results were confirmed by calculating the total mineralized C determined. From the correlation and regression analyses, it has been found that the initial O_2 levels used were significantly correlated with total mineralized C ($r=0.889^{*}$) and were highly significantly correlated with CO_2-C production ($r=0,919^{**}$). This reflects the effect of O_2 which greatly accelerated the overall mineralization of organic matter. After 18 days from the beginning of experiment, O_2 was not any more detected in all treatments and the overlying sediment retained a dark colour. As a result of these conditions, relatively small amounts of CH_4 were detected (Table 2, Col. 7) and as expected its formation was relatively rapid under anaerobic more than under aerobic conditions.

Methane-C formation was highly significantly decreased with increasing the initial O_2 level ($r=-0.995^{**}$). At the end of incubation period, water soluble C accumulated in relatively high amounts in all treatments (Table 2, Cols. 4 and 5). This may be due to the rapid depletion of O_2. Under these circumstances cellulose is expected rather to be fermentated to organic compounds than to end products of fermentation. GALE and GILMOUR (1988) reported high amounts of organic soluble C under strictly anaerobic conditions.

The pH values of the overlying water in all treatments slightly decreased during the incubation period (Table 2). This decrease may be attributed to the formation of organic acids during decomposition of organic matter. This is supported by accumulation of soluble organic C (Table 2). On the other hand, this decrease in pH values is not in agreement with the increased values of HCO_3^- in the system. GALE and GILMOUR (1988) reported that the increase in HCO_3^- may be partly due to titration of volatile fatty acids in solution and not HCO_3^- alone.From the results it can be seen that either total N or total C forms determined at the end of incubation period are smaller than their values at the beginning showing an incomplete balance. This may be attributed to trapped gaseous products of denitrification and carbon mineralization within the sediment or to ammonia volatilization. HOLT et al. (1988)

confirmed this assumption by detecting gaseous products of denitrification in a flooded soil system.

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A NEW METHOD FOR THE MEASUREMENT OF DENITRIFICATION ACTIVITY

by

Reinhard Schultz-Hock; Mohammad R. Hajirezaei

Kernforschungsanlage Jülich GmbH, IBT-III, Postfach 1913; 5170 Jülich

Keywords

Gas chromatography, denitrification activity, denitrification rate, acetylen inhibition technique

Summary

A new method for the measurement of denitrification activity is introduced coresponding to acetylen inhibition technique (AIT). A modified Warburg apparatus is therefore recomended with a gaschromatograph. The whole amount of nitrous oxide which is evolved during the short time test (90 min) is cryofocused and measured without losses in the gaschromatograph with thermal conductivity detector (TCD).

Introduction

The denitrification activity can be determined using the C_2H_2 inhibition method. The method is based on the principle that the denitrification rate is proportional to enzyme activity, if there are no other limiting factors /6/. Incubation is carried out anaerobically and sufficient NO_3 is added. The denitrification rate is expressed as the amount of N_2O formed per gram of soil per time, which reflects denitrification activity.The assay is analogous to phase I described by Smith & Tiedje (Fig.1) and may be related to field denitrification rates. Normally, a stirred slurry is incubated anaerobically and after the addition of NO_3 , chloramphenicol and C_2H_2, the evolved N_2O is measured by sampling the headspace every 10 to 15 minutes for 1 to 2 hours. In our method we use a Warburg apparatus which is combined with a gas chromatograph (GC). The Warburg apparatus was modified in such a way that insted of manometric measurement a specific gas analysis of the denitrification process was possible.

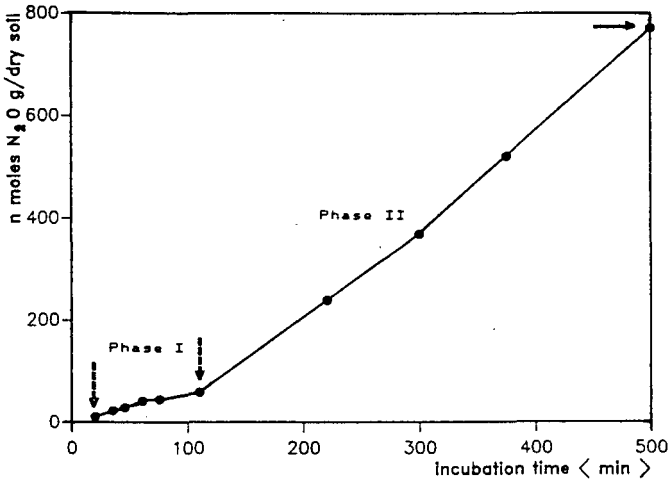

Fig. 1. : Evidence of two linear periods of denitrification, described as phase I and phase II (modified after Smith & Tiedje, 1978)

Material and methods

We used a Warburg apparatus from Braun/Melsungen connected to a gas chromatograph (Hewlett-Packard). The carrier gas flowed through the Warburg vessels (Fig. 2,3). The vessels were continously shaken and kept at a constant temperature in the water bath which additionly indicated leaks in the vessels. The vessels were modified so that an on-line connection was possible. We used 50 ml round-bottomed flasks with a glass neck fixed at the side. This neck was closed with a septum to make acetylene dosage possible. In order to resist the pressure of the carrier gas (2 bar) the round flasks were closed with a screw cap. The stopper included an inlet tube ending in sinter glass close above the bottom of the vessels through which the carrier gas streamed in. Stripped gases could leave the vessel by an outlet in the same stopper and were cryofocused in a U-shaped loop. The gases were trapped for 15 minutes and released by heating the U loop with hot water at 80°C for one minute. The concentration of N_2O was determined by a gas chromatograph.

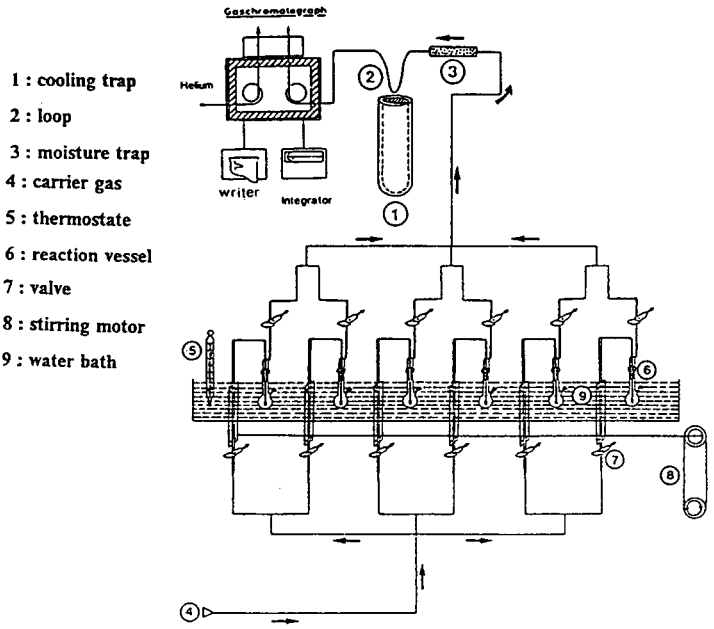

Fig. 2. : Schematic diagram of the modified Warburg apparatus

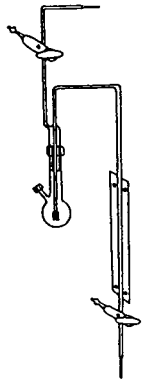

Fig. 3. : Schematic diagram of the assay vessel

Technical data of the GC

Material : V₄A; Porapack Q, 100 - 200 mesh; 2.5 m length; 1/8 inch in diameter; furnace temperature 25°C; He :14 ml/min; filamentous current :150 mA; TCD detector; Hitachi integrator (D-2000)

Calibration

For the calibration of N_2O we injected defined N_2O-saturated solutions into the Warburg vessels which containing 50 ml phosphate buffer (6.3 mM Na_2HPO_4 + 4.2 mM KH_2PO_4, pH 7.2) and 1.62 mM nitrate. Then the carrier stream was flushed through the liquid phase of the vessels for 15 minutes and the nitrous oxide was measured after the cryofucusing (see above). The detection limit was 2.5 ppb N_2O (Fig.5).

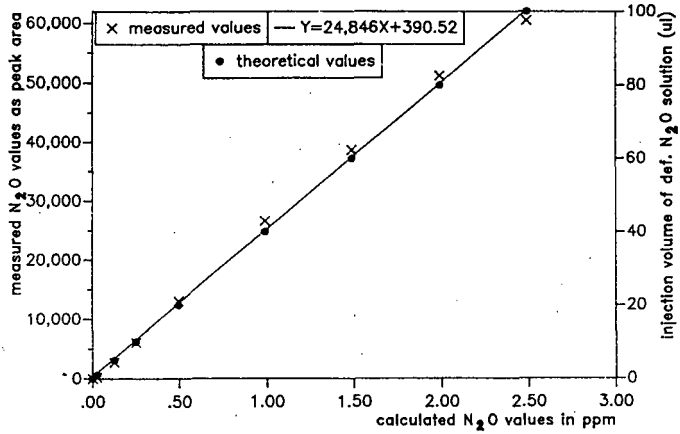

Fig. 4. : Calibration curve of nitrous oxide

Treatment of the samples

The samples were incubated in the vessels containing 50 ml buffer (see above) and nitrate (1.61 mM NO_3^-). Then the vessels were degased with helium for 45 minutes. A control measurement was made to ensure that the vessels were oxygen-free. 90 minutes after the acetylene blockage (0.46 mM C_2H_2) the nitrous oxide was measured /1/. We determined the dry weight of the samples and calculated the activity as μg N_2O/h × dry weight. For examples of use see Schultz-Hock & Hajirezaei /3,4/.

Discussion

The advantage of on-line measurement is that the anoxicity of the assay is controlled. Furthermore the whole amount of gases in equilibrium between solid, liquid and gaseous phases is stripped out and measured, and the detection limit is reduced efficiently (2.5 ppb N_2O, WLD detector). Therefore it is not necessery to calculate remaining N_2O in the liquid phase. In addition the adsorption effects of the solid phase are considered. Fur-

no syringes are needed for sampling so that sampling errors are avoided (untightness and adsorption effects on the syringe, loss during transport). Smith & Tiedje /5/ described two distinct phases of denitrification rate in examining various soils. An initial constant rate, termed phase I, lasted for 1-3 h. Phase I was attributed to the activity of pre-existing denitrifying enzymes in the soil microflora. After a second linear phase, termed phase II, was attained 4-8 h of anaerobic incubation. The linearity of this phase was attributed to the full derepression of denitrifying enzyme synthesis by the indigenous population and to the lack of significant denitrifier growth. Phase I rate was dependent on the initial or in situ aeration state of the soil sample; phase II was not. Therefore phase I may be more directly related to field denitrification rates. With our method we measure the denitrification rate in phase I (after 90 minutes) and the activity of the denitrifiers (or the denitrification rate) in the field. The method also can be used to determine denitrification potential (phase II), N_2O formation without acetylene dosage, acetylene, ethylene and CO_2 whereas the formation of CO_2 refers to the easily decomposable organic carbon.

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Mitteilungen der Deutschen Bodenkundlichen Gesellschaft 60, 331-338 (1990)

A New Technique for Measuring Denitrification Potentials in Soils

by

Birgit Hütsch, S. Heilenz, H. Schmeer and K. Mengel *

1. Introduction

The established method of soil management in our country is loosening and turning by plough, followed by shallow crumbling with harrows. During the last years, considerations have been discussed to assist pedogenic processes by using less intensive cultivation methods in order to favour and conserve a natural soil structure. In order to test long-term effects of different cultivation methods the Institute of Agricultural Engineering (Giessen) has set up experimental fields at different locations in Hesse where the following treatments are compared:

- plough (conventional tillage)
- heavy duty cultivator with double-heart share
- sweep share cultivator
- no-tillage
- others.

In the course of these experiments the Institute of Plant Nutrition in Giessen investigates the effect of different soil tillage practices on the nitrogen dynamics in the soil. Numerous observations suggest that there are significant differences in the N dynamics of the soil as a result of the various cultivation methods. Rates of mineralization and immobilization, leaching losses of nitrate, and also the denitrification potential can be influenced by tillage practices. As far as denitrification losses after ploughing and no-tillage are concerned there are controversial reports in the literature. Occasionally, higher gaseous N losses after no-tillage are observed but there are also examples without differences between the two treatments. In the following a model experiment is described which was designed to throw light on that issue.

* Institute for Plant Nutrition, Südanlage 6, 6300 Giessen, FRG

2. Materials and Methods

2.1 Soil and Tillage Treatments

Soil samples were collected from a luvisol from loess (21% clay), employing the following tillage treatments:

CT conventional tillage (plough),

soil was ploughed to a depth of 0.25 m and, in a second process, leveled and crumbled with a combination of harrows;

NT no-tillage,

origin soil structure was not disturbed; seeds were sown directly into slits drawn with a disk coulter (three disks ridge-drill).

The field experiments were set up in 1979 on 5 m wide long-plots with 3 replicates. In September 1987 after harvest of winter wheat and before soil cultivation soil samples for the model experiment were taken to a depth of 0.25 m. 4 independent replicates per treatment were analyzed in the experiment.

The fresh soil samples were air-dried, passed through a sieve mesh 5 mm and made homogeneous. Determination of max. water capacity, N_{min} and EUF-N contents followed.

2.2 Model Experiment for Measuring potential Denitrification (after SCHMEER, altered by HEILENZ and HÜTSCH)

2.2.1 Experimental Setup

For the measurement of potential denitrification a closed system (20 l laboratory exsiccators) was used which allowed gas sampling and pressure control. At the start of the experiment air was substituted by N_2-free Ar/O_2 atmosphere. An exactly defined gas mixture with 80% Ar and 20% O_2 was realized with a digital manometer.

The vacuum pump with connections to the exsiccators, manometer and gas supplies are shown in Fig. 1 . The temperature was kept constant at 25 ^{o}C by setting up the exsiccators in interconnected water-baths (Fig. 2).

2.2.2 Trial Procedure

12 kg of soil were filled into an exsiccator. In each exsiccator the soil was fertilized to a level of 480 mg N (as KNO_3) and watered to 100% max. water capacity. Each exsiccator was evacuated to sub-atmospheric pressure of 40 mbar and flushed with argon for 10 times. Then an artificial atmosphere of 80% Ar ($\approx$800 mbar) and 20% O_2 ($\approx$200 mbar) was established at an excess pressure of 10 mm Hg-column. This starting-pressure was marked at the manometers.

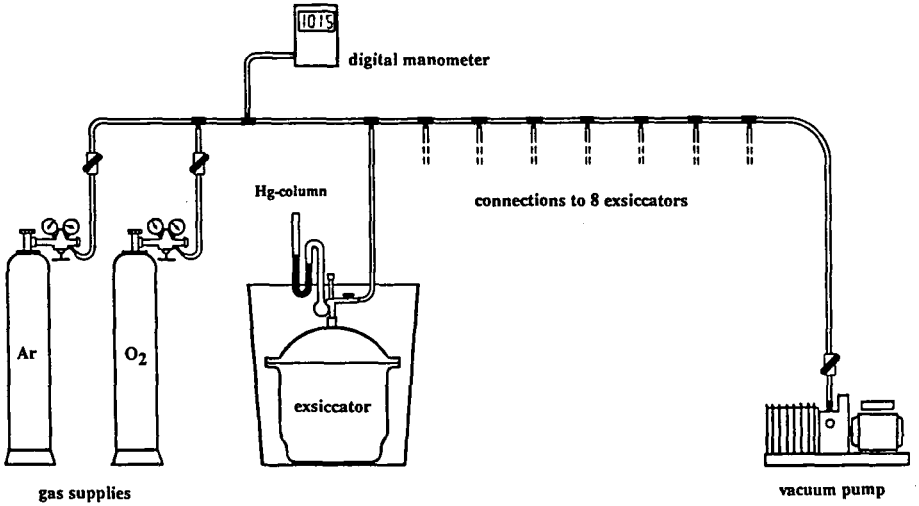

Fig. 1: Equipment for producing a vacuum in the exsiccators and for flushing with gas (Ar, O_2).

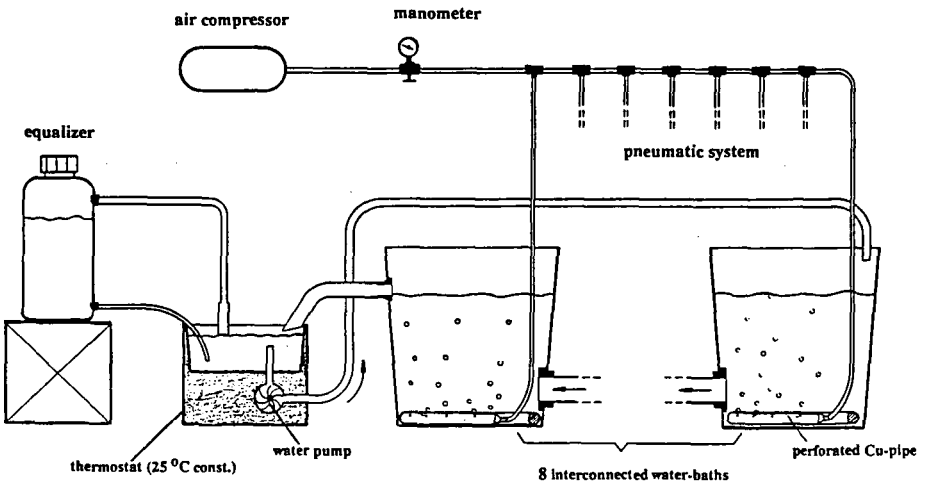

Fig. 2: Water circulation and pneumatic system

With the applied excess pressure, which was to prevent diffusion from atmospheric N_2 into the exsiccator, a frame for pressing the lid onto the exsiccator was necessary (Fig. 3).

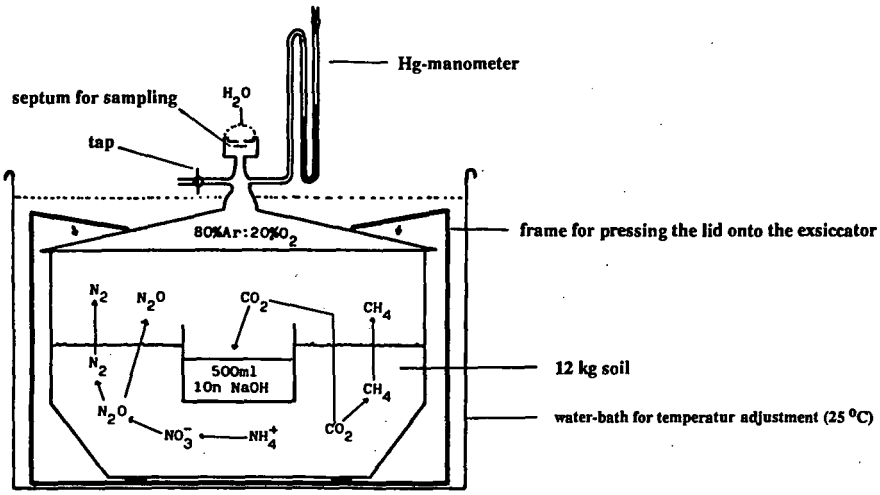

Fig. 3: Closed system (laboratory exsiccator) for measuring gaseous N losses (after SCHMEER, 1984).

A jar with 10 N NaOH was put into each exsiccator. CO_2 absorption in 10 N NaOH allowed to quantify carbon mineralization throughout the experiment. O_2 consumption by mineralization processes was determined indirectly due to decrease of pressure. The difference of pressure was measured at intervals of 2-3 days for each exsiccator and the original pressure was established by adding O_2. After about 80 days in some exsiccators pressure increased due to intensified CH_4 production. In these exsiccators the original pressure was adjusted by sucking off some of the gas.

The whole experiment lasted 152 days.

2.2.3 Gas-chromatographical Analysis

At distinct time intervals gas samples were drawn from the exsiccators by means of septa and the concentrations of N_2, N_2O, CO_2 and CH_4 were determined with a gas chromatograph ("Carlo Erba"). A hot-wire detector (HWD 430) was applied. For separation 2 packed glass columns, 2.5 m length, with the following filling-materials were used:

- Molsieve 5 Å, separation of N_2 and CH_4;
- Porapak Q, separation of CO_2 and N_2O.

Thus each sample had to be injected twice.

Helium was used as carrier gas with a flow rate of 30 ml / min.

For the calibration 1 l glass flasks were evacuated several times and filled with argon. Defined amounts of the gases to be analyzed were then injected through the septum located at one side of the flask. After attaining linearity of the calibration curves standardization was accomplished on a volumetric basis. During the experiment the calibration flasks were submerged. Successive analysis proved that the gas concentrations in these flasks remained constant throughout the experiment.

Gas sampling was realized with gas-tight Hamilton syringes (2.5 ml). These syringes were flushed with argon before use. Special care was necessary to avoid contamination with atmospheric N_2. Therefore, argon was used as safety gas and H_2O as a blocking fluid. Each component of a gas sample was analyzed at least in duplicate.

The measurements were made 1, 3, 6, 9, 15, 22, 36, 50, 68, 111, and 152 days after the start of the experiment.

After the last measurement exsiccators were opened and N_{min} contents were determined in the moist soil. An aliquot of the 10 N NaOH was gravimetrically analyzed for mineralized carbon.

3. Results and Discussion

3.1 N_2 Release

In Figure 4 N_2 release throughout the experiment is shown as mean values of 4 independent replicates. Up to 36 days there was no difference between the two treatments. Subsequently, the NT treatment first showed slightly higher values and was then (after day 80) inferior to the CT treatment; the difference was not significant.

3.2 CH_4 Release

CH_4 was first detectable in the exsiccator atmosphere after 3 weeks. After an initially weak rise the CH_4 concentration of the NT treatment increased rapidly after day 50. In the CT treatment, the CH_4 concentration remained at an almost constant low level (Fig. 5). At the end of the experiment the difference between CT and NT was significant at 0.05 level.

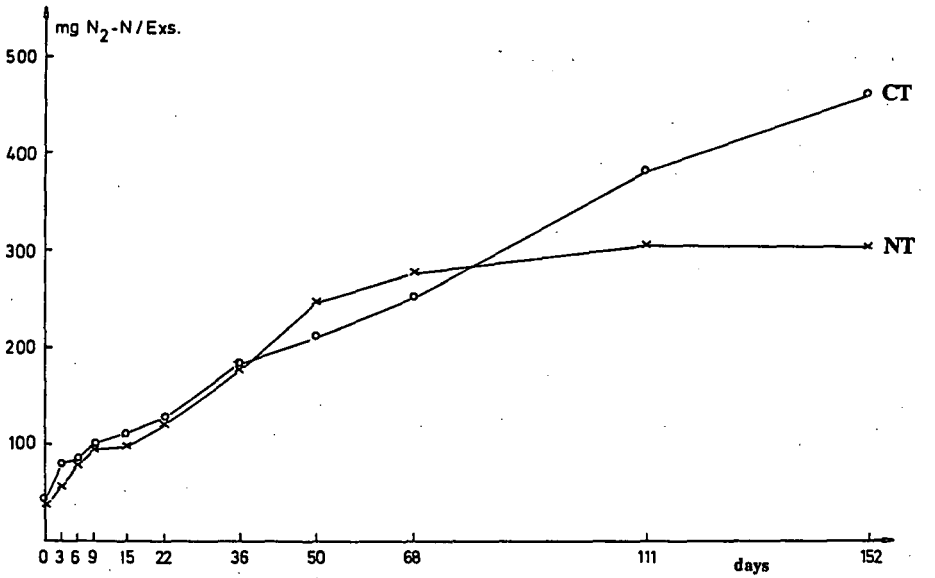

Fig. 4: N_2 release during the period of experiment (152 days), means of 4 independent replicates.

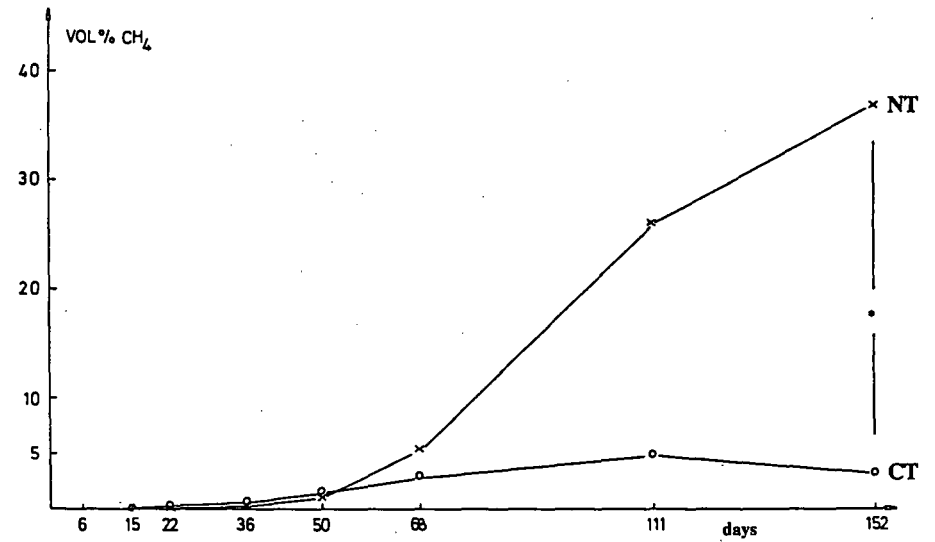

Fig. 5: CH_4 release during the period of experiment (152 days), means of 4 independent replicates. * significant difference between CT and NT at 0.05 level

3.3 O_2 Consumption

The course of O_2 requirements is presented at 10 days intervals in Figure 6. The NT treatment showed markedly lower O_2 requirements from the very beginning. After day 80 measurements were impossible due to strongly increasing CH_4 which caused excessive pressure in the exsiccators. In the CT treatment O_2 consumption increased continously during the experiment.

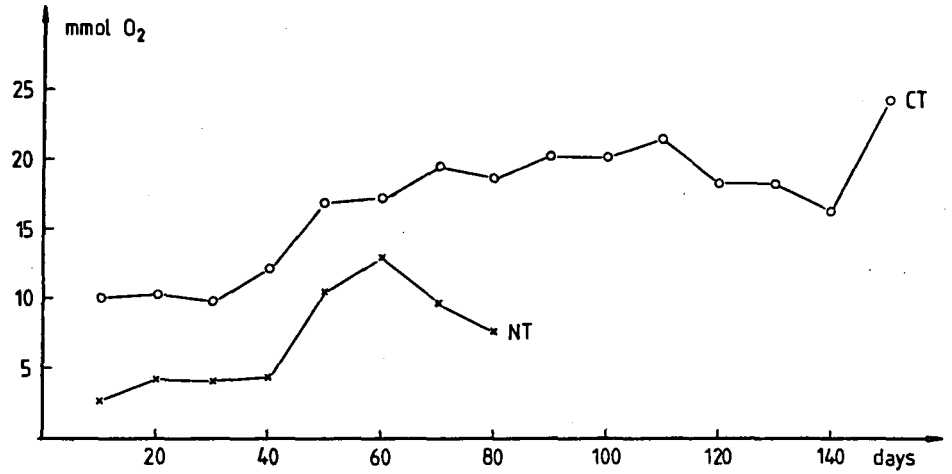

Fig. 6: O_2 consumption by mineralization processes, added up for 10 days each; CT = conventional tillage (n=4), NT = no-tillage (n=3).

3.4 N_2O and CO_2 Release

Only traces of N_2O were measured.

CO_2 concentrations in the exsiccator atmosphere remained at a low level: CT treatment 0.03 - 0.1 %, NT treatment 0.01 - 0.07 %. On the other hand, considerable amounts of CO_2 were bound by NaOH. Table 1 shows a comparison of C release in the CT and NT treatment, respectively. Similarly, values of N turnover are compared.

The CT treatment showed higher N_2 production and CO_2 evolution. However, the total release of carbon (CO_2 + CH_4) was 40 % higher in NT because in this treatment considerable amounts of CH_4 were produced.

The reason for strong CH_4 formation in the soil of the NT treatment is still unknown; possibly, the quality of organic matter in both treatments is different, favouring CH_4-forming bacteria in the soil of the NT treatment. To confirm the results obtained so far we plan to repeat this model experiment using samples of undisturbed soil (monoliths).

Table 1: C release and N turnover in the model experiment for conventional tillage (CT) and no-tillage (NT).

	tillage system			
	CT		NT	
Parameter				
C release (end of experiment)				
g CO_2-C / Exs.	2.3	(94%)	1.7	(49%)
g CH_4-C / Exs.	0.2	(6%)	1.8*	(51%)
total [g C / Exs.]	2.5	(100%)	3.5*	(100%)
N turnover				
EUF-N_{org}-contents [mg kg^{-1}] (start of experiment)	31.7		31.7	
mg N_2-N / Exs. (end of experiment)	460		304	
mg N_{min} / Exs. (end of experiment)	232		254	
percentage of NH_4-N_{min}	91%		91%	

* significant difference between CT and NT at 0.05 level
CT = conventional tillage
NT = no-tillage

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Potential denitrification in samples originated from irrigated plots.

by
Szili-Kovács, T. +)

Acetylene blockage method proved to be a suitable tool for denitrification studies in spite of side effect of acetylene (Yeomans and Beauchamp, 1978; and 1982; Ryden, 1982). Potential rate of denitrification (P.D.) of soil means (in this study) denitrification under oxygen free condition (at 28°C for 24 hours) involving two different kinetic phases (Smith and Tiedje, 1979) of this process. Potential denitrification (Phase 2) is closely related to the "available" soil carbon (Burford and Bremner, 1975; Stanford et al., 1975;).

The objective of this paper to study the effect of irrigation on potential denitrification and to identify affecting soil factors.

MATERIALS AND METHODS

Experiment was carried out in small plots, using winter wheat as an indicator plant. The soil was a loamy calcareous chernozem. The main properties of the soil are shown in Table 1.

Irrigation was carried out with a special sprinkling apparatus made of PVC tubes. The treatments were as follows: in 1986: 1. control, 2. 50 mm irrigation, 3. 5x10 mm irrigation (10 mm a day during 5d), 4. 150 mm irrigation, 5. 150 mm irrigation + 50 kg/ha N as NH_4NO_3); in 1987: 1. control, 2. 150 mm irrigation, 3. 150 mm irrigation + 100 kg/ha N as NH_4NO_3.

+) Res. Inst. for Soil Sci. and Agric. Chem. of the Hungarian Academy of Sciences, Herman O. u. 15., Budapest, H-1022.

Soil samples were collected weekly after irrigation.

Potential denitrification, the number of nitrifiers and soil moisture were determined on the day following the sampling day. Potential denitrification: Evolved N_2O-N [$\mu g\ g^{-1}$ soil day^{-1}]. Incubation was carried out at 28 °C in N_2 atmosphere with original nitrate, moisture and organic matter of the soil. Soil nitrate-N, ammonium-N and total organic-C were measured from air dried samples.

Table 1. The main properties of the soil.

Depth (cm)	organic C %	N %	$CaCO_3$ %	P_2O_5	K_2O	pH	sand/clay	bulk density
				$\mu g/g$				
0-25	1.8	0.23	5	250	200	7.6	1.78	-
25-50	1.5	-	12	200	100	-	-	1.31

RESULTS

Table 2. shows the distribution of potential denitrification according to soil depths. The highest amount of evolved nitrous oxide can be found in the upper 0-10 cm layer, while in case of the fifth sampling depth (50-75 cm) potential denitrification is below the detection limit. Maximum rate of P.D. could be observed on the sampling date two weeks after irrigation (06.03.).

Table 2. Result of ANOVA of P.D. values (1986)

	Sampling dates				
depths (cm)	05.08.	05.28.	06.03.	06.10.	06.17.
0-5	0.22	1.07	2.76	1.30	1.56
5-10	1.48	0.82	3.08	1.62	1.57
10-25	1.45	0.88	1.39	0.82	0.75
25-50	0.05	0.10	0.21	0.00	0.11
$LSD_{0.05}$	0.10	0.12	0.24	0.13	0.13

The distribution of P.D. and soil nitrate-N (Table 3., 4.)

show similar pattern in 1987 year experiment. There were no significant differences between treatments. The highest values of P.D. were at the first sampling date (one week) after irrigation.

Table 3-4. 2-way ANOVA of P.D. and nitrate-N values (1987)

	Sampling dates			Sampling dates		
treatments	05.26.	06.02.	06.08.	05.26.	06.02.	06.08.
1	8.37	2.22	3.00	12.30	3.80	2.98
2	8.55	2.11	2.65	14.28	5.56	6.08
3	8.00	2.28	2.79	14.23	7.48	5.05

$LSD_{0.05}$= 1.10 (between dates) $LSD_{0.05}$=6.92
$LSD_{0.05}$= 0.94 (between treatments) $LSD_{0.05}$= 4.73

Frequency distribution of data was calculated in the statistical analysis; frequency distribution histograms are suitable to establish whether the experimental data belong to one or more population.

Frequency distribution of P.D. was far from normal in both years, therefore results of linear regression analysis were neglected. The deepest soil layer (25-50 cm) appeared at the lowest P.D. category while the topsoil (0-5 and 5-10 cm) at the highest one (Fig.1.) in the 1986 year experiment. Frequency distribution of organic-C is shown in Fig.2. Potential denitrification of the first sampling date of the 1987 year experiment (Fig.3.) separated from the other ones and comparing them with the frequency distribution of nitrate-N values (Fig.4.) a similar tendency can be established.

Spearman's rank correlation analysis was applied to find out the main ecological factors responsible for affecting potential denitrification. Table 5. shows the result of rank correlation test of 1986 year experiment. The closest relationship with the organic-C can be pointed out, while nitrate-N was the main

Fig. 1. denitrification (P.D.)

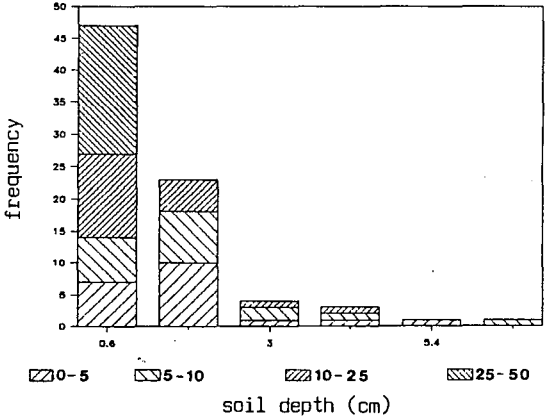


Fig. 2. organic-C (mg/g)

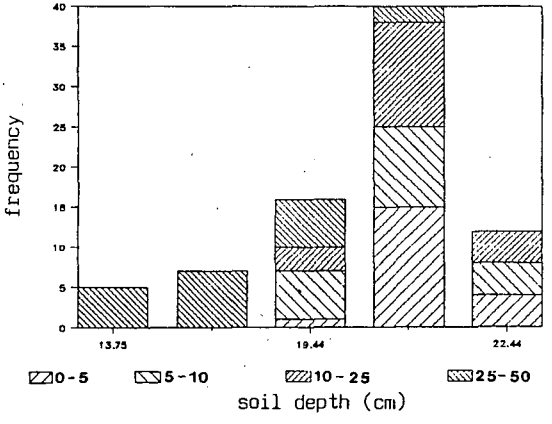


Fig. 3. denitrification (P.D.)

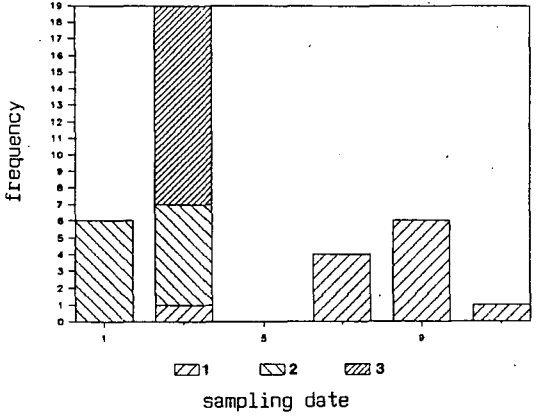
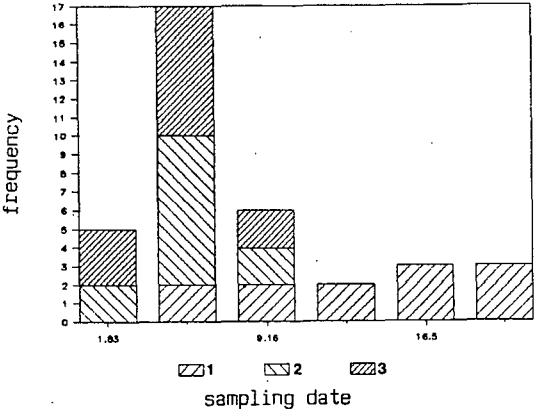


Fig. 4. nitrate-N (μg/g)



affecting factor in 1987 year experiment.

Soil moisture in the range occuring in these experiments had no effect on P.D.

Table 5. Spearman's rank correlation test

	1986		1987	
	r	s_2	r	s_2
P.D. - organic-C	0.451	0.0001	-	-
P.D. - nitrate-N	0.392	0.003	0.586	0.0001
P.D. - moisture	0.212	0.059	0.107	0.542
P.D. - ammonium-N	0.158	0.163	-0.221	0.197
P.D. - ammonia-oxidizers*	-	-	0.408	0.013
P.D - nitrit-oxidizers*	-	-	0.402	0.015

* values in log MPN s_2 two sided significance level

DISCUSSION

Irrigation and precipitation have been known to affect denitrification (Ryden and Lund, 1980; Sexstone et al. 1985). Enhanced soil moisture increases soil respiration and decreases oxygen diffusion and lead to greater anaerobic soil volume. Low partial pressure of oxygen (less than 2 kPa) is favourable to denitrification. Potential denitrification was slightly affected by soil moisture in this experiment perhaps due to the anaerobic incubation. On the other hand, since precipitation occured during experiment the range of soil moisture was relatively narrow (pF between 2.3-2.7).

P.D. showed the closest relation to soil organic-C in 1986 year experiment. In contrast Stanford et al.(1975) I can not find very close correlation between P.D. and organic-C, however I determined only the total organic-C which is known to be comparatively less suitable than various "available" forms of organic-C. Otherwise, only one type of soil was used without any manipulation (e.g. organic matter addition) and organic matter content of soil samples correlated with vertical distribution of organic-C and modified by soil heterogeneity.

Soil organic-C was not studied in 1987 experiment since all of the samples originated from the same layer. In this case nitrate-N was the main factor responsible for the measure of P.D. A relationship was found between P.D. and the number of nitrifying bacteria indicating the nitrate limitation under the given experimental conditions.

SUMMARY

The potential rate of denitrification and the number of nitrifying bacteria were studied under laboratory conditions in samples collected from small irrigated plots. Samples were collected weekly after the irrigation in 1986 and 1987, for four and three times respectively.

Concerning potential denitrification, ANOVA shows significant differences for soil depths and sampling date. Both the number of nitrifying bacteria and P.D. markedly decreased with soil depth. Irrigation had significant effect on P.D. in the first year but not in the second year.

There was no linear correlation between potential denitrification and investigated soil parameters. The main factors affecting P.D. were the total organic-C and nitrate-N.

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STRAW POLYSACCHARIDES AS SUBSTRATES FOR MIXED POPULATIONS OF DENITRIFYING MICROORGANISMS

by

R. Schultz-Hock; M.R. Hajirezaei

Kernforschungsanlage, Jülich GmbH, IBT-III; Postfach 1913, 5170 Jülich

Keywords

Modified Warburg apparatus, Gaschromatography, denitrification rate, denitrification activity, straw polysaccharides

Summary

Various straw polysaccharides were investigated to find out which substrates are suitable for the reconditioning of drinking water by straw percolators. It was found that the microorganisms used oat hulls and xylan as substrates most efficiently both aerobically and anoxically. Under aerobic conditions the denitrification rate with the oat hulls as substrate was approximately twice as high as under anoxic conditions.

Introduction

In a pilot plant at the Viersen waterworks nitratous groundwater is reconditioned in a 3-step process with the aid of phytogenic by-products, an artificial wetland unit and an underground passage (Deniplant, Fig.1).

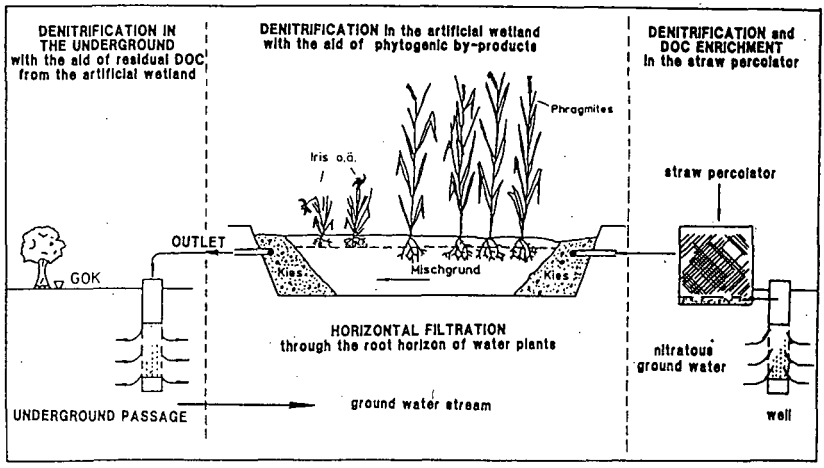

Fig. 1. : Schematic diagram of Deniplant by G. Plum (Viersen waterworks)

Nitratous water is filtered through a straw percolator in which it is partially denitrified and simultaneously enriched with washed-out organic matter. The water is pumped through the root horizon of an artificial wetland. Here the remaining nitrate is eliminated. Then the water is stabilized hygienically and residual organic matter and bacteria are removed during its passage (300 days) through a sandy-gravelly underground.

To optimize the straw percolators scientifical experiments were carried out in the Kernforschungsanlage, Jülich. One important question was which compounds of the straw were utilized favourably by microorganisms. The straw mainly consists of cellulose, hemicellulose and lignin /8/. As by-products starch and soluble phenolic compounds are mentioned. Here the denitrification experiments were carried out in batch cultures containing different straw polysaccharides.

Material and methods

Pure substances such as avicell-cellulose (Merck), starch (Merck), xylan (Fluka) and natural complex substances such as milled wheat straw and milled oat hulls were used as substrates. Before incubation the substrates were washed several times with distilled water and than centrifugated (10 min, 7000 rpm). All substrates (1%) were mixed with Fabig & Ottow mineral medium /1/. At the beginning the nitratous concentration was

2.3 g/l. For the inoculation the microorganisms taken from percolators were used /3/ and shaken with 0.18 % sodium pyrophosphate for one hour at 200 rpm. At the beginning the CFU (colony forming units) was 1.7×10^7 colonies/ml. Five aerobic (300 ml Erlenmeyer flasks) as well as five anoxic vessels (gas-tight flasks) were shaken continously for a week at 30°C and 100 rpm. Every second day a 20 ml sample was taken. Nitrate, nitrite (ion exchange chromatography), ammonium (flow injection analysis) and nitrous oxide with modified Warburg apparatus /7/ were analysed. The aerobic cultures were supplied with sterile air filters, the anoxic cultures with gas-tight covers. The air in the vessels was stripped out by helium for 30 min. To demonstrate anaerobiosis an indicator (disposable anaerobic indicator) which changes its colour in the presence of oxygen from white to blue was fixed into the gas space.

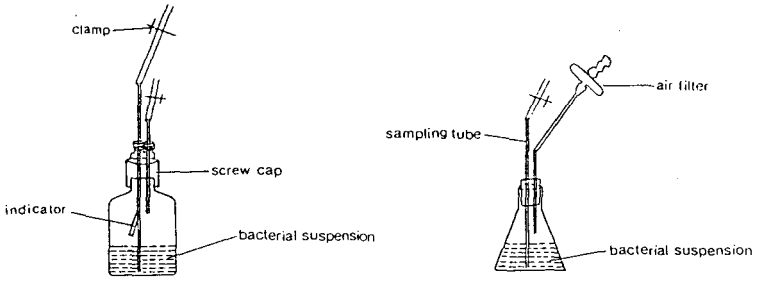

Fig. 2. : Schematic diagram of the assay vessels

Results

In Tables 1-4 the results describe both aerobic and anoxic cultures of the 3rd day or 5th day. With oat hulls as substrates the whole nitrate amount was used up both aerobically and anoxically after 3 days. Here the N_2O rate was the highest. After 5 days with xylan as substrate nitrate was hardly detectible. Under aerobic conditions the N_2O rate was higher in the cultures with oat hulls and xylan. With wheat straw the use of the nitrate and the formation of N_2O were higher under aerobic conditions whereas with starch and avicell-cellulose, more nitrate was used under anoxic conditions. There was no formation

of N_2O with avicell-cellulose. In the culture with starch, nitrite was formed under anoxic conditions.

Tab.1 : 3rd day, anoxic

substrate (mg/l)	NO_3^- (mg/l)	NO_2^- (mg/l)	NH_4^+ (mg/l)	N_2O rate (mg/l.h)	N_2O (mg/l)
oat hulls	6.18	0.72	5.9	0.75	-
xylan	1513.4	6.62	1.5	0.17	0.015
wheat straw	1949.6	0.36	1.3	0.007	-
soluble starch	1987.2	2.7	0.7	0.004	-
avicell cellulose	2089	0.62	0.9	-	-

Tab.2 : 5th day, anoxic

substrate (mg/l)	NO_3^- (mg/l)	NO_2^- (mg/l)	NH_4^+ (mg/l)	N_2O rate (mg/l.h)	N_2O (mg/l)
xylan	8.87	1.03	1.8	0.198	-
wheat straw	1769.7	0.77	0.5	0.009	-
soluble starch	1392	17.5	0.5	0.013	0.0057
avicell cellulose	2040	0.41	1.6	-	0.0062

Tab.1,2 : Influence of various substrates on denitrification under anoxic conditions

Tab.3 : 3rd day, aerobic

substrate (mg/l)	NO_3^- (mg/l)	NO_2^- (mg/l)	NH_4^+ (mg/l)	N_2O rate (mg/l.h)	N_2O (mg/l)
oat hulls	6.02	0.88	2.5	1.24	0.052
xylan	1758.6	1.39	1.2	0.047	0.068
wheat straw	1575.2	4.8	1.1	0.0028	0.14
soluble starch	1978.6	1.39	2.4	0.009	0.032
avicell cellulose	2139.4	0.46	1.4	-	0.038

Tab.4 : 5th day, aerobic

substrate (mg/l)	NO_3^- (mg/l)	NO_2^- (mg/l)	NH_4^+ (mg/l)	N_2O rate (mg/l.h)	N_2O (mg/l)
xylan	0.368	6.0	0.8	0.288	0.102
wheat staw	1657	2.79	0.2	0.04	0.13
soluble starch	1908	4.39	0.3	0.009	0.057
avicell cellulose	2470	-	0.4	-	-

Tab.3,4 : Influence of various substrates on denitrification under aerobic conditions

Discussion

It was found that the microorganisms used oat hulls and xylan (made from oat hulls) best. Starch and avicell-cellulose use was very poor. With avicell cellulose the formation of N_2O could only be measured after two incubation days. The wheat straw was decomposed fairly well. Therefore we assume that the hemicellulose as one main compound of the straw is specially suitable as a substrate for the denitrification. In our experiments lignin was not considered because it is well known that this compound cannot easily decomposed /6/. It was not possible to measure a denitrification rate for avicell

cellulose. Gök & Ottow /2/ showed that cellulose is suitable in principle as the sole C source for denitrification. With starch the denitrification seems to occur incompletely (nitrite accumulation). We demonstrate for quickly mineralizable compounds that aerobic denitrification occurs /4,5/ and proceeds even more intensively than under anoxic conditions. With substrates difficult to mineralize (starch and cellulose) it is the other way round.

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Denitrification in acid soils under forest

by

FAASSEN, H.G. VAN*

The denitrification process can only be understood within the context of the nitrogen cycle (Figure 1) and prediction of field rates requires an integration of soil biology and soil physics. Potential rates of N_2O production and N_2O reduction were determined for samples from the surface Lh and Ah layers of five Dutch soils under forest, with a pH (H_2O) between 2.9 and 4.2. Structurally intact soil cores were incubated at 20 °C in closed pots of which the gas phase was examined by gas chromatography for O_2, CO_2, N_2O and C_2H_2 (Figure 2). Peak N_2O production started when soil respiration had lowered pO_2 to less than 2 kPa. Upon prolonged incubation this was followed by less rapid N_2O reduction. Typical results of the measurements are shown in Figure 3.

PREDICTION OF FIELD RATES

Integration of soil biology and soil physics

Soil Biology	Soil Physics
Soil respiration → O_2 demand;	soil structure and soil moisture
nitrification → nitrate supply;	determine O_2 supply and N_2O emission
plant uptake of nitrate;	by gas phase diffusion
potential denitrification rate;	nitrate transport with soil water →
p.m. other N_2O sources and	possible nitrate leaching;
chemodenitrification.	

*Institute for Soil Fertility, P.O. Box 30003, 9750 RA Haren, The Netherlands

Importance

Denitrification counteracts acidification and decreases nitrate leaching; however, N_2O emission is unwanted.

Ecological prerequisites:

- presence of nitrate (or nitrite);
- presence of active microbial denitrifying enzyme complexes;
- presence of electron donors, often organic carbon compounds;
- absence of oxygen or low oxygen pressure;
- absence of inhibitory substances or conditions.

Reference

Kroeze, C, H.G. van Faassen and P.C. de Ruiter, 1989. Potential denitrification rates of acid soils under pine forest. Submitted to Neth. J. Agric. Sci.

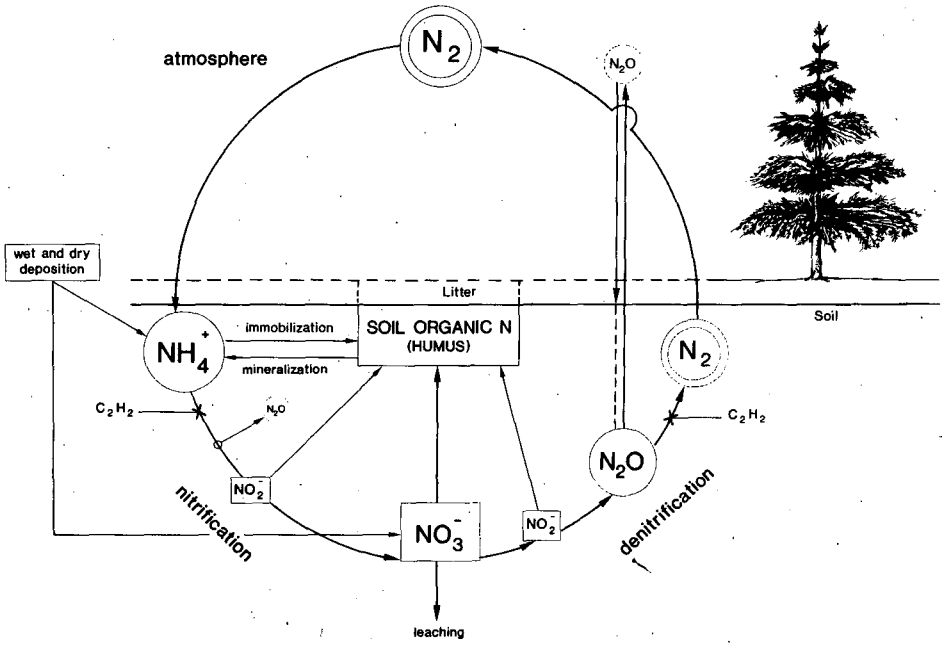

Figure 1. Denitrification is a process of the nitrogen cycle.

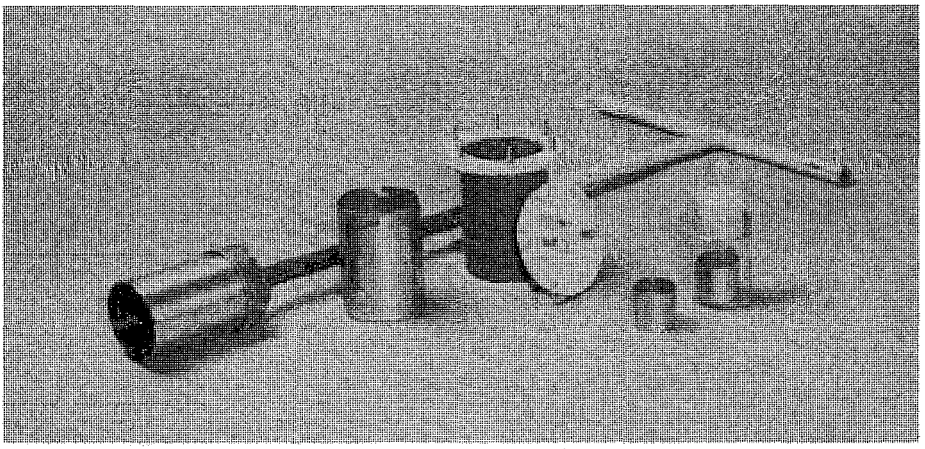

Figure 2. Structurally intact soil cores of different sizes were used to measure potential denitrification rates.

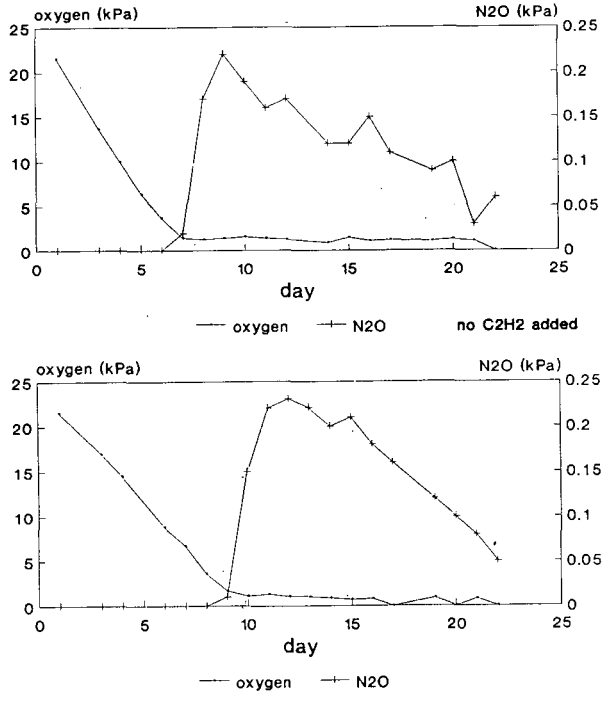

Figure 3. Typical results from the incubation of structurally intact cores (10cm diam.; 0-15 cm layer) in closed pots: respiration followed by denitrification. Top: Olterterp; bottom: Roden. Experimental period 11 May - 1 June.

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DENITRIFICATION KINETICS OF DIFFERENT STRAINS ISOLATED FROM THE ANOXIC HYPOLIMNION OF THE BIETRY BAY (EBRIE LAGOON, IVORY COAST)

by

Esnault, G., and J.L. Garcia *

Abstract

During an enrichment experiment from reduced hypolimnion of Bietry Bay, ten denitrifying strains were isolated. Their ecophysiological and kinetic characteristics suggest the presence of two different populations of denitrifying bacteria. Kinetics studies have shown the presence of high affinity systems for NO_3^- reduction.

Key Words: Denitrification; Kinetics studies; Pure cultures; Tropical lagoon.

Fig.1 represents the statement of the water column at time of sampling. The anoxic and reduced (due to high sulfide concentration) hypolimnion was overlied by an oxic epilimnion, and separated from this by a chimiocline characterized by a $NO_3^-+NO_2^-$ maximum. Sampling was performed at 8 meters depth, where $NO_3^-+NO_2^-$ concentration was 1.5 µM and the redox potential -350 mv. A true denitrification rate of 0.02 nmol. $N_2O.cm^{-3}.hr^{-1}$ was measured. 1 ml of water was inoculated in mineral anoxic media, reduced or not, containing nitrite as electron acceptor and various electron donors for enrichment. Five subcultures were made with enrichments showing growth, before isolation. Isolations were performed by dilutions series with same media amended with agar. Cultures issued from single colonies were tested for purity. Six strains were isolated in reduced condition. The others, isolated in anoxic non re-

*Laboratoire de microbiologie ORSTOM, Université de Provence, 13331 MARSEILLE cedex 03.

duced condition, failed to be enriched and to grow in reduced condition. Some ecophysiological characteristics of pure cultures are shown in table 1. It is notable that with lactate as electron donor, two different strains were isolated in reduced and non reduced conditions, called R-LAC and LAC respectively. Kinetics studies were performed with four of these strains (table 1). The acetylene block method was used. N_2O was determined by gas chromatography, with an electron capture detector. Fig.2 and Fig.3 represent kinetics of N_2O production for strains LAC and R-LAC respectively, with various nitrite concentrations (pic heights of chromatographic signals for N_2O are reported). Results for strains SUCC and R-BENZ are similar to those of LAC and R-LAC respectively. Such experiments allowed the determination of apparent Km values (table 1; fig.2 and fig.3), considered as half saturation constants for nitrite reduction, irrespective of the kinetics mechanisms. The apparent Km values determined for the strains isolated in reduced medium (3 µM) are lower than those of strains isolated in non reduced condition (10 µM). Thus on the basis of their physiological and kinetic properties, R-LAC and R-BENZ appeared to be more adapted than LAC and SUCC to the natural conditions of the hypolimnion of Bietry Bay. The latter strains are probably exported from overlying non reduced water containing higher nitrate and nitrite concentrations (Fig.1). Indeed, it was reported high flow of particulate material in this site, allowing the presence of a high percentage of heterotrophic aerobic bacteria in the hypolimnion (Guiral, 1984; Carmouze and Caumette, 1985). These results suggest the presence in the hypolimnion of that site of two denitrifying populations wich one is passing through during sedimentation and other is composed by sedentary bacteria able to develop in these conditions (Fig.4).

Acknowledgements: This work was supported by the centre de recherches océanographiques, B.P. V18, Abidjan, Cote d'Ivoire.

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Fig 1: IN SITU PHYSICO-CHEMICAL CHARACTERISTICS OF THE WATER COLUMN IN BIETRY BAY

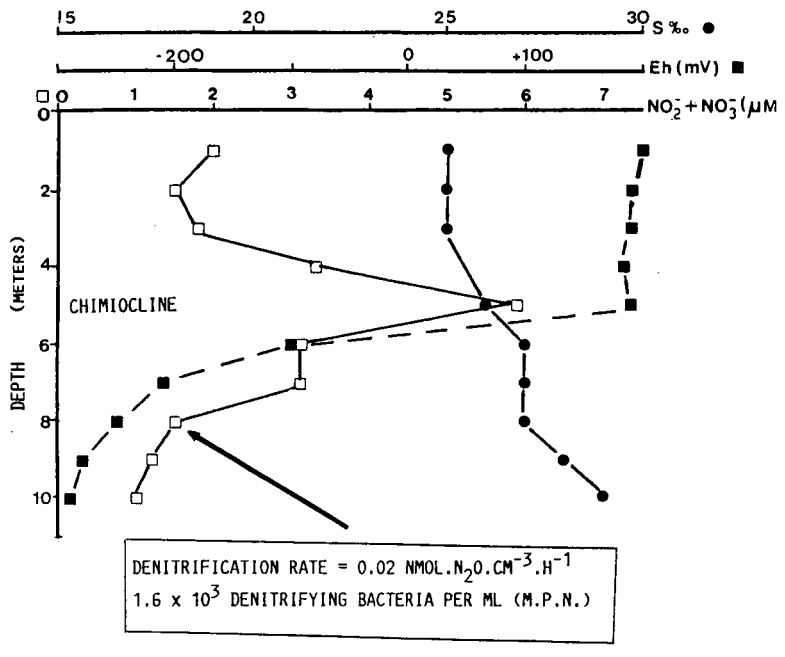
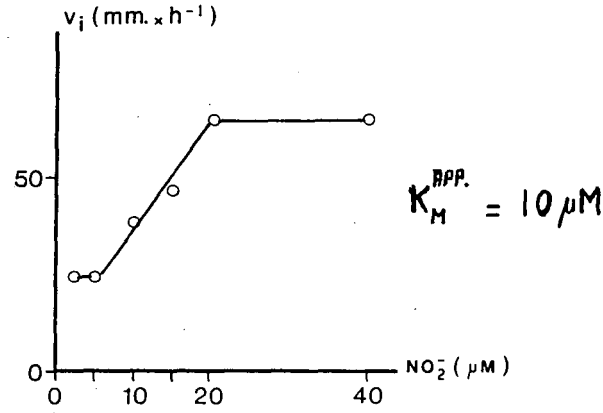


Fig 2: LAC. DENITRIFICATION KINETICS PERFORMED BY THE ACETYLENE BLOCK TECHNIQUE IN ANOXIC NON REDUCED CONDITIONS IN A MINERAL MEDIUM AMENDED WITH 1 mM LACTATE, VARIOUS NITRITE CONCENTRATIONS (0 TO 40 µM) AND 100 µG.ML^{-1} CHLORAMPHENICOL



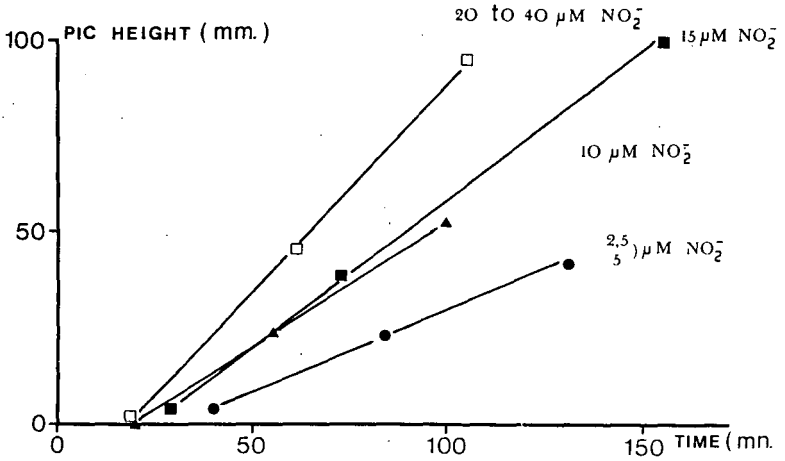


Fig 3: R-LAC. DENITRIFICATION KINETICS
PERFORMED AS THE LAC. DENITRIFICATION KINETICS

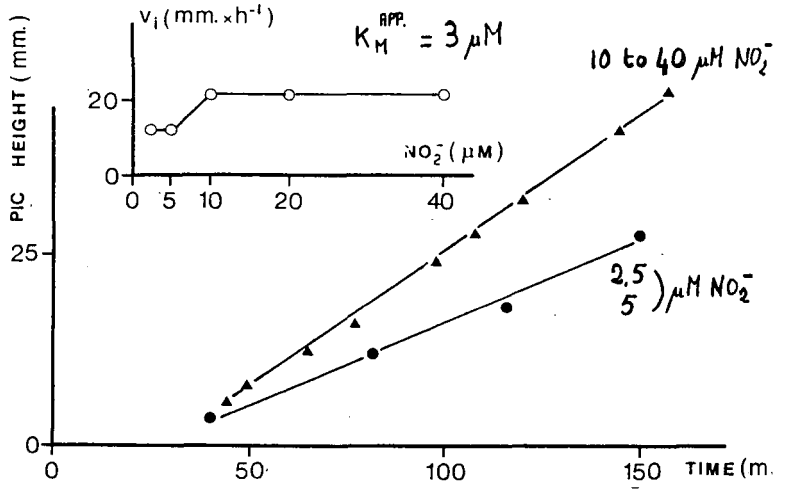


Fig 4: SCHEMATIC REPRESENTATION OF THE PRESENCE OF TWO DENITRIFYING POPULATIONS IN THE HYPOLIMNION

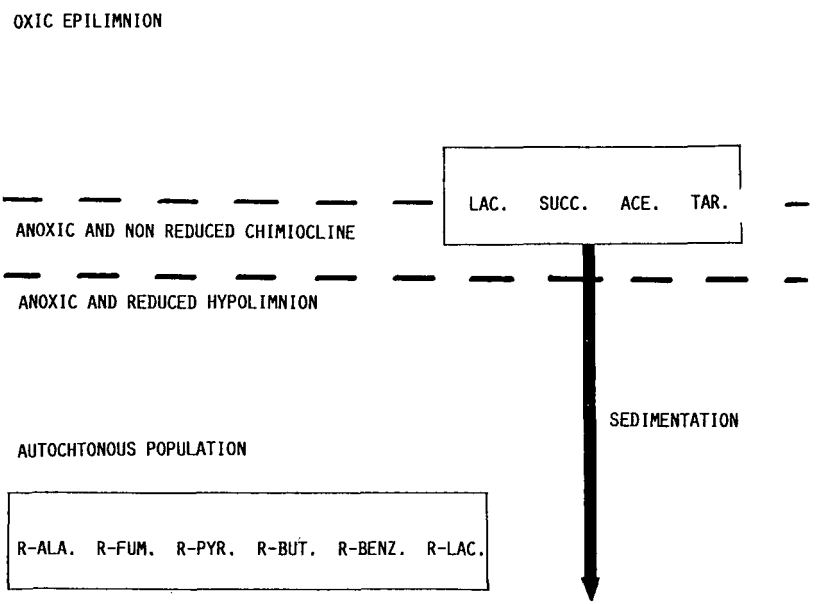


Table 1 ECOPHYSIOLOGICAL PROPERTIES OF THE TEN STRAINS OF DENITRIFYING BACTERIA ISOLATED FROM 8 M. DEPTH

STRAIN	R-ALA.	R-FUM.	R-PYR.	R-BUT.	R-BENZ.	R-LAC.	LAC.	SUCC.	ACE.	TAR.
EH OF ISOLATION MEDIUM (mV)	- 250[1]	- 250	- 250	- 250	- 250	- 250	0	0	0	0
EH REQUIRED FOR GROWTH (mV)	- 250 0	- 250 0	- 250 0	- 250 0	- 250 0	- 250 0	0	0	0	0
DENITRIFICATION PRODUCTS										
- IN REDUCED MEDIUM[1]	N_2O	N_2O	N_2O	N_2O	N_2O	N_2O	N.G.[2]	N.G.	N.G.	N.G.
- IN NON REDUCED MEDIUM	N_2	N_2	N_2	N_2	N_2	N_2	N_2	N_2	N_2	N_2
APPARENT K_M FOR NITRITE REDUCTION[3] (µM)	N.D.[4]	N.D.	N.D.	N.D.	3	3	10	10	N.D.	N.D.

1 : Na_2S AS REDUCING AGENT

2 : N. G. = NO GROWTH

3 : DETERMINED IN ANOXIC NON-REDUCED CONDITIONS

4 : N. D. = NOT DETERMINED

ABBREVIATIONS : R = REDUCED ; ALA = ALANINE ; FUM = FUMARATE ; PYR = PYRUVATE ; BUT = BUTYRATE ; BENZ = BENZOATE ; LAC = LACTATE SUCC = SUCCINATE ; ACE = ACETATE ; TAR = TARTRATE

Mitteilungen der Deutschen Bodenkundlichen Gesellschaft 60, 361-366 (1990)

Natural Oxygen and Nitrogen Isotope Abundances of Compounds Involved in Denitrification

Susanne Voerkelius* and H.-L. Schmidt*

Introduction

The natural isotope abundances of the N-compounds in the different pools of the nitrogen cycle are determined by
a) the isotope abundances of their primary N- and O-sources (N_2 and inorganic or organic N-compounds, respectively O_2 and H_2O),
b) isotope fractionations accompanying pool transfers and (bio)chemical reations,
c) oxygen exchange reactions of intermediates with water.
The small but distinct abundance differences are expressed in the δ-value scale, basing on the difference of the isotope ratios R (R = $(^{15}N)/(^{14}N)$ or R = $(^{18}O)/(^{16}O)$) to that of international standards.

$\delta^{15}N = (R_{sample}/ R_{standard} - 1)x1000$ (‰); standard= AIR (air N_2)
$\delta^{18}O = (R_{sample}/ R_{standard} - 1)x1000$ (‰); standard= SMOW (standard mean ocean water).

The $\delta^{15}N$-values of substances from the nitrogen cycle have often been used to identify their origin and their biological history (Hübner, 1986) while the importance and usefulness of the $\delta^{18}O$-values for that purpose has only recently been demonstrated (Amberger und Schmidt, 1987).

The base for a further development of the isotope method as a tool for the elucidation of correlations between the nitrogen pools and for the assignment of intermediates is the knowledge of the mechanisms and the isotope discriminations of the reactions implied. Mechanistic studies on distinct steps of the denitrification process are reported by Kim and Hollocher(1984) and Weeg-Aerssens et al.(1988). In our own laboratory we have recently determined the in vitro-isotope effects of the assimilatory nitrate reductase reaction (enzyme from Chlorella vulgaris):

*Lehrstuhl für Allgemeine Chemie und Biochemie der TUM
D-8050 Freising-Weihenstephan

k_{14}/k_{15}=1.030 and k_{16}/k_{18}=1.015 (Olleros, 1983). The intermolecular oxygen isotope effect is probably accompanied by an additional intramolecular one (preliminary value k_{16}/k_{18}=1.065). The isotope effects are depending on the actual NADH- concentration. In vivo-studies on denitrification, basing on the bacterial (dissimilatory) nitrate reductase reaction, may not attain identic values, however, it is to expect that the same correlation between N- and O-discrimination should be found.

Results

1. Isotopic Behaviour of Nitrate

Nitrate formed by nitrification can, depending on the nitrogen source, represent $\delta^{15}N$-values between -10 and +20‰. In agreement with the fact that the main source for the oxygen atoms is H_2O($\delta^{18}O$= -10‰), $\delta^{18}O$-values of the ion near 0‰ have been found(Amberger und Schmidt, 1987). Nitrate from industrial production showed $\delta^{15}N$-values close to 0‰ and $\delta^{18}O$-values around +20‰, as the nitrogen and oxygen sources are air (N_2, $\delta^{15}N$=0‰, O_2, $\delta^{18}O$=+23.5‰). A third source of NO_3^-, only found in water under forests, is rain. High ^{18}O-concentrations indicate an origin from atmospheric sources and processes which are still not completely investigated so far (Schmidt et al., in press). In all cases, a secondary ^{18}O and ^{15}N-enrichment is to be expected for remaining nitrate in the course of denitrification. The δ-values of nitrate from drinking water of known origin areas absolutely fitted in a $\delta^{15}N/\delta^{18}O$-value diagram (Amberger und Schmidt, 1987). Nitrate in water samples from different depth of drill holes under farming areas with different utilization but constant N-input, showed a typical correlation between the concentration of the remaining ion and its δ-values (Fig.1). The enrichment factors calculated from this result and from different other samples were ε=-16.0‰ for nitrogen and ε=-8.1‰ for oxygen ($\varepsilon = 1000x(k_{15}/k_{14}-1)$ or $\varepsilon=1000x(k_{18}/k_{16}-1)$). As predicted, the ε-values do not absolutely correspond to the kinetic isotope effects of the in vitro-reaction, however, the ratio of the fractionation is identic to that of the enzymatic process.

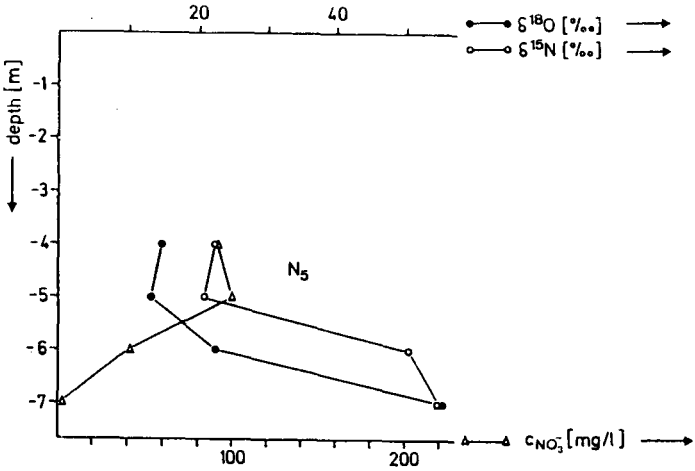


Fig. 1: δ-values of NO_3^- in water samples from different depths in a drill hole as a function of the NO_3^- concentration (Results from a cooperation with O.Strebel)

Another application was the proof of denitrification of NO_3^- in soil extracts under a farming area which had been fertilized with industrial nitrate for several years (Cooperation: R.Funk a. X.Maidl). The applied fertilizer had $\delta^{15}N$-values between +2 and +5‰ and $\delta^{18}O$-values near 20‰. The soil nitrate in the upper horizont showed similar δ-values as the fertilizer, but with increasing depth, parallel enrichments in the ^{15}N- and ^{18}O-concentrations, indicative for a partial denitrification were observed.

2.Isotope Abundances in Gaseous Products of Denitrification

In a given population of molecules submitted to a turnover the "light" molecules react slightly faster, hence the product will be relatively depleted, the remaining, non converted part of the substrate relatively enriched in heavy isotopes. As a matter of fact, in N_2-gas formed by denitrification in soil, a $\delta^{15}N$-shift of +18‰ relatively to that of the NO_3^--source was observed (Wellman et al. 1968, Amberger, 1987), and this in spite of a possible contamination by water dissolved air-N_2.

In order to check a similar enrichment of ^{15}N in the second product of denitrification, N_2O, we have incubated NO_3^- in soil in a gas-tight vessel under conditions of denitrification. The

N_2O isolated from the gas phase attained depletions in ^{15}N relative to the NO_3^- by -20 to -30‰, while simultaneously for the heavy oxygen isotope, an enrichment of 5‰ was measured in the gas.

Accompanying in vitro-experiments on the NO_2^--reduction to N_2O with Fusarium solani (strain and cultivation: P.Malinowsky a. J.C.G.Ottow) proved this result. A fractionation factor β=1.018 for nitrogen was found. The ^{15}N-depletion in the product N_2O was accompanied by a distinct ^{18}O-enrichment (about 9‰, Fig.2).

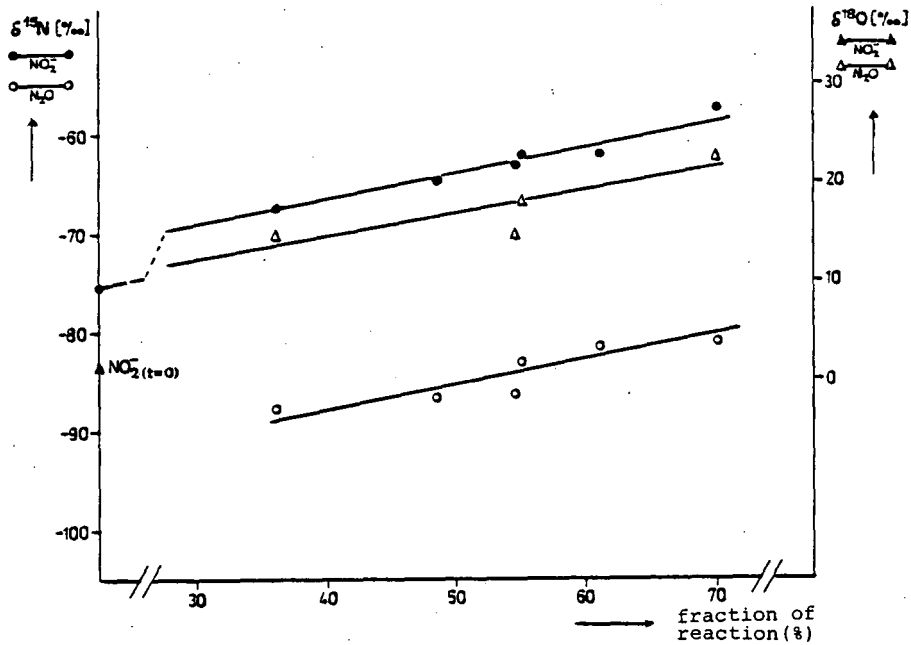


Fig.2: $\delta^{15}N$ and $\delta^{18}O$-values of NO_2^- and N_2O as a function of turnover in the reduction of NO_2^- by Fusarium solani. β-values see text.

According to the NO_3^--reduction mechanism accepted so far (Kim a. Hollocher,1984, Weeg-Aerssens et al.,1988) enzyme bound NO_2^- is split to NO^+ by the addition of H_2O ($NO_2^- + H_2O \rightarrow NO^+ + 2\ OH^-$) before further turnover (formation of NO or N_2O_3). This step must be accompanied by isotope effects for N and O, but in the case of oxygen an intermolecular and an intramolecular isotope effect

must be implied. We suppose that for oxygen the overlap of these two effects must be the cause for the ^{18}O-enrichment in N_2O observed.

In a further soil experiment the N_2O-formation from NO_3^- was pursued. The soil in a closed vessel, partially flooded with water, contained glucose as source for reduction equivalents, and the atmosphere was air. In a first period, a dramatic increase of the N_2O-concentration was observed, and the $\delta^{18}O$-value of the gas was more negative than that of the nitrate in the soil. In a second phase, the N_2O-concentration remained constant, but the $\delta^{18}O$-value rapidly increased. This result can be interpreted by the overlap of two influences: In the first phase, preferably "light" NO_3^- was reduced, leading to an enrichment of ^{18}O in the remaining nitrate (which was actually found), in the second phase, in addition to denitrification of "heavier" nitrate, a secondary reduction of N_2O to N_2 must have taken place, preferably with the ^{16}O-molecules, thus leading to the observed ^{18}O-enrichment in the rest-N_2O.

For a proof of this hypothesis, a soil experiment (gas phase: N_2) on the reduction of N_2O was performed, and the δ-values of both isotopes were measured in dependence of turnover in the remaining N_2O. In agreement with results from the literature (Yoshinari and Wahlen, 1985, Yoshida, 1988) the fractionation factor for oxygen (β=1.011) was higher than that for nitrogen (β=1.0043). In this case we have to expect - besides the intermolecular isotope effects for N and O - an additional intramolecular isotope effect for nitrogen, because the N_2O-molecule contains, like NO_2^- for oxygen, two atoms of the same element in the same molecule, which is in this case not only the prerequisite for different mesomer structures, but even for different isotopic configurations.

Conclusions

Isotope abundance measurements on substrates, intermediates and products of denitrification provide a very useful and powerful tool in natural tracerwork on the elucidation of this process, provided mechanisms and isotope effects of the single steps are known and well understood. The present study proves that the interpretation of isotope fractionations, on the background of

kinetic intermolecular and intramolecular isotope effects, is of great importance. As could be shown, the existence of this phenomenon does not only imply further complications, but also additional informations. This may be even more true with the determination of isotope distributions within a given population of molecules from the nitrogen cycle. It seems for example, that on this base it will be possible to distinguish whether N_2O in a certain surrounding is originating from a denitrification or a nitrification process (see also Yoshinari and Wahlen 1985, Yoshida, 1988).

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SPATIAL DISTRIBUTION OF DENITRIFICATION IN AN ARTIFICIAL WETLAND

by

R. Schultz-Hock; M.R. Hajirezaei

Kernforschungsanlage, Jülich GmbH, IBT-III; Postfach 1913, 5170 Jülich

Keywords

Modified Warburg apparatus, Gaschromatography, denitrification activity, artificial wetland

Summary

The spatial distribution of the denitrification rate was determined twice in autumn 1988 in the root horizon of an artificial wetland planted with Phragmites. It was found that relative to dry weight the highest activity appeared on or near the fine roots.

Introduction

As described by Schultz-Hock & Hajirezaei /7/ artificial wetlands are used for the re-conditioning of nitratous groundwater (Deniplant). The carbon-suppling sources for denitrification in this case are extracts of straw filters continuously eluted into the WBF and root exudates of Phragmites. In small artificial wetland units at the KFA Jülich, Raphael /6/ counted up to 10^8 colonies/g dry weight using the MPN test (most probable number) on the roots of helophytes. On the same system Stengel /9/ used methanol as an accessoric carbon source to maintain denitrification in winter down to 2°C. Furthermore using the AIT (acetylene inhibition technique) Stengel determined denitrification as the main nitrate elimination mechanism in this system.

Material and methods

The soil samples from the artificial wetland rhizosphere were taken by using a 3 l metal box (l = 10 cm, w = 10 cm, d = 30 cm). One hour after sampling the core was separated into 4 fractions : fine sediment, fine roots, rhizomes and gravel. The fine sediment was obtained by washing the stones with 2 l of KFA tap water and by sedimentation at 8000 r.p.m. for 45 minutes. 15 min. later the wet pellet was transferred into closed glass ves-

sels filled with phosphate buffer, pH 7.2 (6.3 mM Na_2HPO_4 + 4.2 mM KH_2PO_4) and 1.61 mM NO_3^-. The activity was measured by a gaschromatograph as released N_2O after acetylene inhibition. The device used for this determination was a modified Warburg apparatus described by Schultz-Hock & Hajirezaei /7/. The results were related to g dry weight/m² neglecting the gravel fraction. To cosider the stones we also related the denitrification rate to volume. The percentage distribution of the denitrification rates was calculated for the four different fractions.

Results

The distribution of the denitrification rate within samples of 0-10 cm depth is shown in Table 1. The highest denitrification rates were found on fine roots, followed by fine sediment when related to dry weight. Lower activity was measured on rhizomes and on the stones. The overall activity during the vegetation period (example 22.9.1988, Tab.1) was 5-fold higher than in winter (example 18.11.1988, Tab.1).

Table 1 : Denitrification rate of the 4 fractions in the root horizon of Phragmites

sample	denitrification rate in g N_2O/ (m²×d)		dry weight in g/m² (0-10 cm depth)	
	22.9.1988	18.11.1988	22.9.1988	18.11.1988
fine sediment	5.0	1.18	3535	5817
fine roots	3.36	0.49	865	229
gravel	0.838	0.155	-	-
rhizome	0.228	< 0.003	209	635
∑total	9.43	1.825	4609	6681

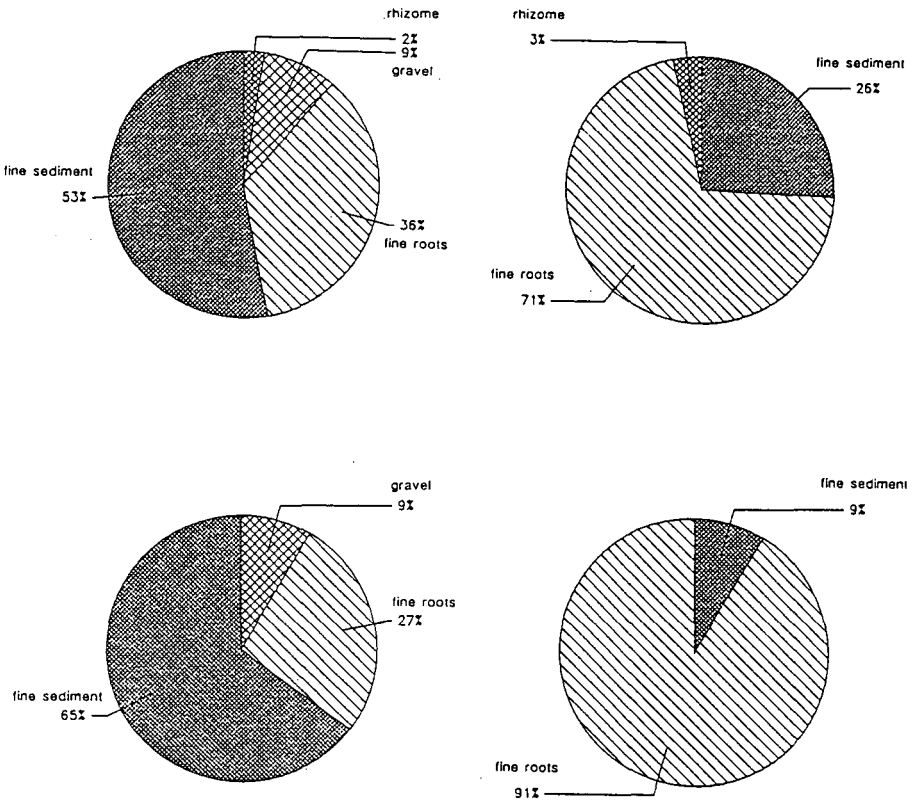

Fig.1 : Spatial distribution of the denitrification in the root horizon of an artificial wetland : above left : related to volume, above right : related to dry weight (22.9.1988), below left : related to volume, below right : related to dry weight (18.11.1988)

Discussion

For the spatial distribution of denitrification it can be concluded that the highest denitrification rates were obtained on fine roots or near the roots (rhizosphere). In winter there was low denitrification activity. Hence it follows that root exudates continuously emitted during the vegetation period are of great importance for effective denitrification. In agreement, Balandreau & Knowles /1/ concluded that plant roots influence rhizosphere directly by emitting soluble root exudates.

Furthermore Garcia /3/ ascertained that the microorganism population in rice rhizosphere was 514-fold greater than in the respective root free sediment. Although there is a significant agreement between the intensity of denitrification and the actual cell numbers of denitrifying bacteria these high densities do not involve high denitrification rates /2/. Intensity and products of denitrification depend, rather, on the amount of easy mineralizable organic matter /5,10/. The main factors influencing microorganism growth in rhizosphere are organic root exudates, decomposing root material and low oxygen concentrations (anoxicidity) as well as factors of minor importance such as the O_2/CO_2-quotient, pH, water supply and mineral nutrients. Woldendorp /11,12/ found that the oxygen consumption by living roots was 28-fold greater than by dead roots. 2/3 of the oxygen demand is based on root respiration and 1/3 on microflora demand. The stimulus for denitrification in the rhizosphere is oxygen consumption by plant roots and the release of organic H-donors and carbon through root surfaces. Therefore knowledge of the denitrification activity it is a good parameter for accessing spatial distribution of denitrification.

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Time course of gaseous N-losses from compacted soil cores

Walenzik, Gabriele +) and O. Heinemeyer +)

SUMMARY

The influence of varied soil densities on the loss of gaseous nitrogen has to be investigated in a field experiment. Special requirements for the *in situ* measurement technique were explored by laboratory experiments.
Time courses of gaseous N-losses from compacted soil cores were measured over a period of 40 days using the acetylen inhibition method. Soil was taken from the A_p-horizon of the experimental field.
Soil compaction effected time course and amount of gaseous N-losses from soil. From the results we conclude, that quantification of gaseous N-losses by the acetylen inhibition technique is not suitable for of our planned *in situ* field measurements.

The risk of irreversible damages to soil fertility due to the increasing use of heavy agricultural machines has become a matter of concern. In a cooperative research project different aspects of the effects of traffic with varied wheel-loads on the properties of soil are investigated. In this project an experimental field (4 ha) is tilled by applying 9 different wheel-load and traffic variations. Crops are rotated with winter wheat, winter barley and sugar beets and are fertilized and pest controlled following usual practices.
In this project we investigate the effect on soil microbial processes. Here we deal with efforts made to follow microbial denitrification, a process coupled to anaerobiosis, nitrate, and organic carbon sources.
Tillage may cause damages in soil structure which influence water conductivity, particularly when a plowlayer is formed (Ehlers, 1983). Denitrification rates generally increase with reduction of air-filled porosity (Wollersheim *et al.*, 1987; Linn and Doran,

+) Bundesforschungsanstalt für Landwirtschaft (FAL), Bundesallee 50, D-3300 Braunschweig, FRG

1984). Bakken *et al.* (1987) found that tractor traffic on wet soil caused reduced pore volume, effected soil structure and increased N-losses by denitrification.
Therefore, enhanced denitrification rates could be expected, if agricultural traffic with increased wheel-loads results in increasing soil compaction or damages of soil structure.
As a prerequisite to quantitatively confirm this presumption by field measurements we tested the acetylen blockage method for its suitability with compacted soils in a laboratory study.
Even though this method has several limitations (Rolston, 1986) its major advantage, to be inexpensive and simple compared to methods involving the use of ^{15}N (Duxbury, 1986), made it attractive for use in our field study. Furthermore, this method has been successfully used by several authors mentioned above.

MATERIAL AND METHODS

Experimental soil: Soil was taken from the A_p-horizon (0-30 cm) of the experimental field (Parabraunerde, derived from loess) sieved to 2mm and air-dried. After rewetting and storing at 15°C for 10 days, the soil contained 83 µg NO_3-N and 1 µg NH_4-N g^{-1} dry soil.
Soil compaction and water supply: Soil cores of 10 cm hight and 5 cm diameter, containing 47.5, 42.5 and 37.5 % (v/v) total pore space, were prepared using a hydraulic press. In order to achieve a homogeneous pore distribution, only the lower 7 cm part of the soil cores were used for further examinations. The experiment was initiated by carefully wetting 40 replicate cores to 35 % (v/v) water content which responds to an air filled pore space of 12.5, 7.5 and 2.5 % (v/v) respectively. Soil cores were kept at that moisture by daily weighing and watering.
Determination of acetylen diffusion rates: Soil cores were placed between empty tubes and 10 % of the headspace air was replaced by acetylen. Gas samples of 1 ml volume were taken from the space below the soil and acetylen concentration determined by GC-measurements, using a flame ionization detector.
Denitrification measurements: Soil cores were incubated at 15°C in the dark for a maximum of 40 days. During this period 40 replicates were used to determine N_2O and N_2O+N_2 release. Therefore, the entire core was enclosed in a glas vessel, acetylen (10 % v/v) added to a part of the replicates, N_2O-concentration increase fol-

lowed by GC-measurement (Heinemeyer *et al*,.1985), and vessels opend again. No acetylen-treated cores were used twice, later measurements were conducted using replicate cores.

Measurement of mineral nitrogen in the soil: The amount of mineral nitrogen (NO_3^-, NH_4^+) in soil cores prior and past the experiment was determined by extraction with 2 molar potassium chloride and colorimetry using a Techniccon AA II autoanalyzer.

RESULTS

Increase of bulk density effected the diffusion velocity of acetylen. At the lowest compaction level (47.5 % pore space) it took only two hours for establishing a sufficient acetylen concentration of > 0.1 % in the whole core. In the most compacted soil (37.5 % pore space) it required two days.

Therefore, gas samples for N_2O measurements from acetylen-treated cores were taken 48 and 72 hours after acetylen supply.

Over 40 days, soil cores with 42.5 and 37.5 % pore space emitted 1.1 µg and 4.8 µg N_2O-N g^{-1} soil with maximum emission rates after 3 and 5 days respectively (Tab. 1 and Fig 1).

Total volatile N-losses (N_2O + N_2) were found to be 2.4 µg and 25.0 µg N g^{-1} soil for cores of 42.5 and 37.5 % pore space respectively. Compared to the measured decreases in mineral nitrogen, these values are only slightly lower than expected (Tab. 1).

A time delay of about two days was observed for the delivery of maximum N_2O + N_2 emission rates between cores with 42.5 and 37.5 % pore space, the maximum rate for the latter being 15 times higher than for the first.

Tab. 1 Fluctuation of mineral N and amount of N_2O and N_2+N_2O emission after 40 days in compacted soil cores

Total pore space [vol%]	decrease mineral N [µg N g^{-1} dry soil]	emission N_2O	emission N_2+N_2O	N_2O/N_2
37.5	30.0	4.8	25.0	0.23
42.5	6.5	1.1	2.4	0.84
47.5	0.0	0.0	0.0	-

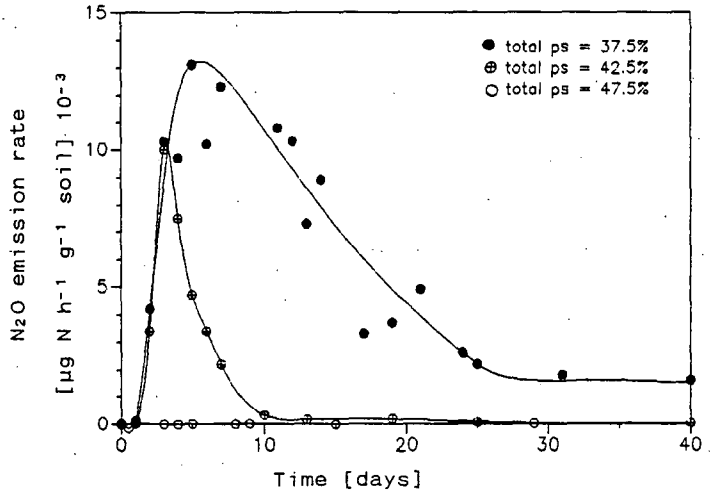

Fig. 1 N_2O emission rates from compacted soil cores

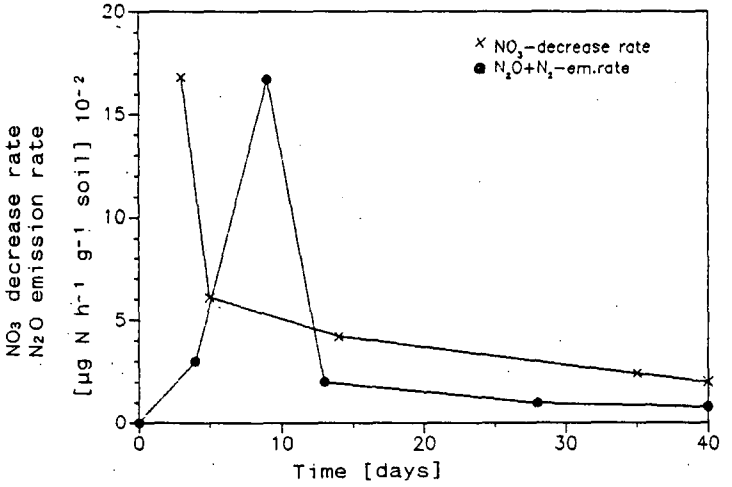

Fig. 2 N_2+N_2O emission rates and NO_3 decrease rates from soil cores of 37.5 % pore space

A time pattern of NO_3 disappearance in the most compacted cores (37.5 % pore space) revealed a clear delay of N-gas release from the soil surface. This must be attributed to restricted gas transport due to pore space reduction (Fig. 2).

No N-losses by denitrification were found for soil cores with 47.5 % pore space (Fig. 1, Tab. 1).

DISCUSSION

Gas transport for two kinds of gases (acetylen and N_2O) is involved, when using the acetylen-blockage technique in order to quantify denitrification losses of N_2 and N_2O from a soil surface correctly. Acetylen must be introduced to the soil and nitrous oxide has to leave the soil for the method to work. Two types of transports must be considered, mass flow and diffusion. In the soil mass flow normally does not or only very little contribute to gas transport, so precautions must be taken not to cause artificial massflow when introducing the acetylen.
Ryden *et al.* (1978) developed a technique to overcome this problem. If only gas diffusion occurs, it is driven by and along concentration gradients. The nature of the medium through which diffusion takes place and the nature of the gas determine the transport capacity and are described in Fick's law by the gas diffusion coefficient.
From this it can be derived that for the medium "soil", exhibiting a variety of structure elements like pore size distribution, soil density and water content, gas diffusion coefficients are only correct for a described set of these conditions. That makes it extremely difficult to predict gas concentrations at given times and for given places in the soil. It is, however, a prerequsite of the method to reach a concentration of > 0.1 % (v/v) acetylen at any location where denitrification occurs from the beginning of the N_2O-sampling period (Keeny, 1986).
We conclude from our results, that variations of soil density and air-filled porosity dramatically alter diffusive gas transport in soil. This would greatly complicate the use of the acetylen blockage method in a field experiment, where a number of soil structure influencing treatments are studied. We state therefore, that in case of our planned investigations the objective of study would directly corrupt the suitability of the method intended for. A suggestion to circumrent similar problems in a laboratory study by reducing the distances over which diffusion has to take place (Wollersheim *et al.*, 1987) cannot be applied for *in situ* field measurements.

Adding to this, a large spatial variability of N_2O-emission must be expected in the field generally (Folorunso and Rolston, 1985; Burton and Beauchamp, 1985; Benckiser *et al.*, 1986; Parkin, 1987). The use of ^{15}N method (Siegel *et al.*, 1982) might be a more promising outcome here.

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Composition of denitrification products as affected by ecological conditions and type of denitrifying microorganisms

by
Munch, J.C.[+)]

SUMMARY

Gaseous products of biological denitrification are characterized by variable composition. Relative amounts of N_2O, NO and N_2 in the denitrification gases emitted from soils are dependent *in situ* on ecological soil conditions that determine also intensity of denitrification. Among these factors are primarily amounts of easily mineralizable organic matter as electron donor, nitrate concentration, moisture content, and oxygen supply of microcompartments. As these ecological conditions determine also composition of microbial population, it was consistent to investigate effects of physical and chemical soil properties on gaseous products of denitrifying soil microorganisms. Model experiments conducted with strains of denitrifying soil bacteria, inoculated into sterilized soil samples and submitted to different incubation conditions (variation of nitrate amounts in soil and/or soil moisture content and/or aerobic or anaerobic atmosphere) showed that both N_2O and NO amounts depend on type of microorganism. Variation of ecological conditions resulted in reactions on N_2O and NO emission specific for organism. These results suggested that NO_x formation by denitrification in soils may be related directly to the composition of microbial flora, and only indirectly to physical and chemical soil properties.

INTRODUCTION

Since intensification of agricultural practices with increased use of nitrogen fertilizers and by increased soil fertility, N_2O concentrations in the atmosphere are rising continously with a rate of 0.2% pro year (WEISS, 1981). Sources of N_2O are at half technical systems and at half biological and chemical processes in all kinds of biotopes (WEISS, 1981). Total release of N_2O to the atmosphere is estimated actually up to 150 Tg N $year^{-1}$ (BOLLE et al., 1986). The interest in emission of nitrogen oxides to the atmosphere is considerable do to the concern over the reactions of this gases in the atmosphere: greenhouse effect in the troposphere, depletion of the tropospheric ozone by simultaneous reaction of chlorides, catalytic function by destruction of the stratospheric

[+)] *Inst. f. Bodenkunde & Standortslehre, Univ.Hohenheim, D-7000 Stuttgart 70*

ozone which is filtering UV-radiations from solar light (CRUTZEN, 1981; BOLLE et al., 1986). However, estimations of the gaseous nitrogen oxide losses (N_2O, NO) from soils and waters are few and fragmentary. All processes involving reduction or oxidation of nitrogen are possible sources for these gases. Especially denitrification is one of the most important processes of NO_x formation in natural sites. Less information is even available on N_2O emission and on the rate of N_2O reduction to N_2 by denitrification. Soil parameters that seem to influence the relative amounts of N_2O (and NO) in denitrification gases are the amount and type of electron and proton donors (generally organic matter), oxygen supply, nitrate concentration, pH, temperature, soil moisture, the type of plantation, and the interrelationships these factors (ROSSWALL, 1980; HAIDER et al., 1985; BAKKEN, 1988; PRADE and TROLLDENIER, 1988). The effect of these factors is often exceeded by the influence of the amount and of the type of organic matter in soil (DEMERDASH and OTTOW, 1983). Furthermore, since denitrification may be attributed to a great number of physiologically different microorganisms (KNOWLES, 1981), rate and gaseous products of denitrification also seem to depend on composition of active microbial population in biotopes, as shown by BURTH and OTTOW (1983) in model experiments with bacteria and fungi in nutrient solution. The aim of this study was to investigate the role of different microorganisms in production of gaseous NO_x by denitrification in soils, and the importance of variations in soil conditions such as nitrate amount and partial oxygen pressure on microbial gaseous metabolism. Model experiments were conducted with pure strains of denitrifying bacteria inoculated into sterilized soil.

MATERIALS AND METHODS

Soils, microorganisms, model experiments and methods used for this study are soon described (MUNCH and OTTOW, 1986; MUNCH, 1989a, 1989b).

Samples from 2 soils different in physical and chemical properties (sandy loam from an Udifluvent and clay from an Vertisol) were sterilized, inoculated with either their endogenous microflora or pures strains of denitrifiying soil bacteria (*Pseudomonas aeruginosa* S72, *Bacillus brevis* S19, *B.licheniformis* S187, *B.polymyxa* S55) and incubated bei 30°C in closed flasks unter defined conditions (soil moisture at 80% of WHC, nitrate addition of 50, 100 or 250 μg $N\text{-}KNO_3 \cdot g^{-1}$ dry soil, anaerobic helium or aerobic helium/oxygen atmosphere).

In constant intervals, atmosphere of flasks was analysed by GC with hot wire detection on N_2, N_2O, NO, O_2 and CO_2, and soil samples on nitrate and nitrite.

RESULTS

a. Influence of soil type on gaseous NOx formation by soil microorganisms:

Maxima of nitrite formed in soil samples and of N_2O accumulation (in case of *B.polymyxa*: + NO) in flask atmosphere as well as ratio of N_2O maxima to cor-

responding nitrate consumptions are given in Table 1 for endogenous soil populations and experimental bacteria strains incubated under anaerobic helium atmosphere in both soil (at 80% of WHC) supplied with 250 μg N-$KNO_3 \cdot g^{-1}$ dry soil.

Table 1: Formation of nitrite and N_2O (and NO) and ratio of N_2O maxima to nitrate consumption by the natural soil population of denitrifying bacteria or various denitrifying cultures in samples of 2 different soils (ls=loamy sand or clay) added with nitrate (100 μg N-$KNO_3 \cdot g^{-1}$ dry soil; 80% of WHC; anaerobic conditions; 30°C)

	soil					
Organisms	ls NO_2^--maximum[1]	clay NO_2^--maximum[1]	ls max.N_2O emission[1]	clay max.N_2O emission[1]	ls max.N_2O/NO_3^- decrease[2]	clay max.N_2O/NO_3^- decrease[2]
natural pop.	5	30	3	5	0.02	0.1
P.aeruginosa	25	12	4	1	0.02	0.04
B.brevis	13	3	260	272	1	1
B.licheniformis	16	115	149	224	0.6	0.8
B.polymyxa	47	3	100	234	0.6	0.8

[1] μg N-NO_3^-, N-NO_2^-, N-N_2O(+N-NO)$\cdot g^{-1}$ dry soil
[2] N-N_2O maxima/N-NO_3^- amounts respired at times of N_2O maxima
[3] including NO

Development of denitrification and growth parameters in the 10 experiments are soon published (MUNCH and OTTOW, 1986). Results from Table 1 show clearly that N_2O formation was dependend mainly of the type of active microflora and independent from the soil samples, from the high nitrate concentration and from the intermediate nitrite accumulation in soils. Because comparison of N_2O amounts is difficult in the experimental approach (in the closed system N_2O is reabsorbed by the soil and reduced to N_2), relations of N_2O maxima to nitrate decreases at corresponding times were calculated. This relations shows that N_2O formation from reduced nitrate depended directly on the type of organisms and only secondly on properties of soils in which organisms are denitrifying.

b. Influence of soil nitrate concentration on gaseous NOx formation by soil microorganisms

Summary of results obtained by the incubation of the cultures in soil samples (only loamy sand) supplied with nitrate at 2 different amounts are given in Table 2 (see also MUNCH, 1989a).

In comparison to a nitrate addition equivalent to a nitrogen amendement in the field, the high nitrate addition (250 μg N-$KNO_3 \cdot g^{-1}$ dry soil) leaded to an increase in the absolute NO_x release from soil. This NO_x formation was not in

Table 2: Formation of nitrite and N_2O (and NO) and ratio of N_2O maxima to nitrate consumption by the natural soil population of denitrifying bacteria or various denitrifying cultures as affected by the initial nitrate concentration of a loamy sandy soil (80% of WHC; anaerobic conditions; 30°C)

	nitrate addition[1]					
Organisms	50 NO_2^--maximum[1]	250	50 max.N_2O emission[1]	250	50 max.N_2O/NO_3^- decrease[2]	250
natural pop.	12	2	0.7	2.6	0.04	0.02
P.aeruginosa	19	25	-	4.0	-	0.02
B.brevis	11	13	58	260	0.8	1.0
B.licheniformis	49	16	14	149	0.2	0.6
B.polymyxa	46	47	58[3]	100[3]	0.8	0.6

[1] μg $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}N_2O(+N\text{-}NO) \cdot g^{-1}$ dry soil
[2] $N\text{-}N_2O$ maxima/$N\text{-}NO_3^-$ amounts respired at times of N_2O maxima
[3] including NO

accordance with the transient nitrite accumulations. The more expressive relative NO_x formation (NO_x/NO_3-decrease) shows that *B.licheniformis* and *B.polymyxa* released less gaseous nitrogen oxides to the atmosphere following the addition of the high nitrate amount.

c. Influence of aeration on gaseous NOx formation by soil microorganisms

Table 3: Formation of nitrite and N_2O (and NO) and ratio of N_2O maxima to nitrate consumption by the natural soil population of denitrifying bacteria or various denitrifying cultures in a loamy sandy soil (100 μg $N\text{-}KNO_3 \cdot g^{-1}$ dry soil; 80% of WHC; 30°C) as affected by the initial atmosphere in incubation flasks

	flask atmosphere					
Organisms	He NO_2^--maximum[1]	O_2/He	He max.N_2O emission[1]	O_2/He	He max.N_2O/NO_3^- decrease[2]	O_2/He
natural pop.	9	22	1,2	13	0.01	0.3
P.aeruginosa	9	21	-	43	-	0.4
B.brevis	37	9	60	81	1	1
B.licheniformis	85	119	100	86	0.9	0.7
B.polymyxa	66	27	95[3]	553)	0.7	0.6

[1] μg $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}N_2O(+N\text{-}NO) \cdot g^{-1}$ dry soil
[2] $N\text{-}N_2O$ maxima/$N\text{-}NO_3^-$ amounts respired at times of N_2O maxima
[3] including NO

Corresponding results of incubation of soil organisms in loamy sandy soil samples added with 100 μg $N\text{-}KNO_3 \cdot g^{-1}$ dry soil under anarobic helium and initally

aerobic He/O_2 (=98/4) atmosphere are given in Table 3. Development of denitrification and growth parameters of *P.aeruginosa* and *B.polymyxa* will be published (MUNCH, 1989b).
Oxygen leaded to increase in N_2O formation for the autochthonous soil microflora as well as for *P.aeruginosa* and *B.polymyxa*. In opposite to this generally accepted effect of oxygen, NO_x formation by *B.brevis*, *B.licheniformis* and *B.polymxa* was lowered by aeration of the jar atmosphere. Relative N_2O formation gives also the same results. Furthermore, oxygen had not the same effect on nitrite formation by the different cultures. This experiments showed also no correlation between nitrite and N_2O formation.

DISCUSSION

The results of the model experiments with ubiquitous denitrifying microorganisms in samples of 2 different soils have clearly demonstrated the specific effect of the type of organism on emission of gaseous nitrogen oxides from the soils. Production of the nitrogen oxides may not be related to the different soil properties. Furthermore, a high nitrate concentration does not lead automatically to relative increase (per unit of respired nitrate) of N_2O (and NO) emission by denitrification, as generally expected (NÖMMIK et al., 1984). At last, it was surprising that oxygen had also a specific effect on NO_x emission by the organisms and led to a decrease of N_2O formation by *B.licheniformis* and *B.polymyxa*. This result is to be completed by the oxygen effect in waterlogged soil samples (at 120% of WHC) (MUNCH, 1989b): the rate of denitrification was increased and the N_2O emission lowered by oxygen respiration in comparison to samples at 40% of WHC, where oxygen repressed the denitrification. The possibility of denitrification in the presence of oxygen has often be expected (ROBERTSON and KUENEN, 1984) and denitrification was observed in other model experiments in an aerated and homogenized chemostat (OTTOW and FABIG, 1985).
These considerations lead to conclude that specific metabolism of each of the active microorganisms in any biotope may be an essential factor for composition of denitrification gases. Therefore N_2O proportions in denitrification gases from a soil primarily depend on composition of its denitrifying microflora. Establishment of technical denitrification systems, e.g. for elimination of nitrate from water, may be performed under ecological conditions permitting the development of denitrifying organisms that release only N_2 and no N_2O to the atmosphere.

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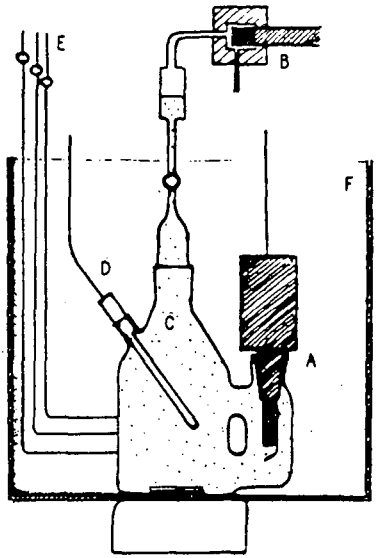
Direct and permanent measurement of dissolved nitric oxyde during microbiological processes of denitrification.

ANDRE J.P. (*)

Two methods are generally used for nitric oxyde analysis : the gas chromatography (1 - 2) and the chemiluminescence (3 - 4).

The relative lack of selectivity of the polarographic membrane detector for the measurement of dissolved oxygen (Clark's electrode principle) makes it able to detect other species, as dissolved nitric oxyde, allowing its direct and continuous detection.

This property was observed during denitrification processes in such an apparatus as on fig. 1. The detection of an other gas than oxygen was unexpected and, the first time, we sought long time in vain for an hypothetical entry of air !



(A) dissolved O_2 detector

(B) gaseous O_2 detector

(C) bacterial suspension

(D) pH meter

(E) carbon, nitrate, air, feeding

(F) thermostat.

fig 1

The detector is schematically designed on fig. 2 : the two electrodes are separated from the outer medium by a gas permeable membrane, and immersed in a thin layer of electrolyte (KCl solution). Only the dissolved gases of the outer medium enter the cell through the membrane and will be reduced (or not) on the cathode, according to the value of the electrode potential. In the best conditions, the measured intensity is proportional to the outer concentration of the dissolved gas.

* : Station d'Agronomie - I.N.R.A., 45, Bd du Cap - 06606 ANTIBES (France).

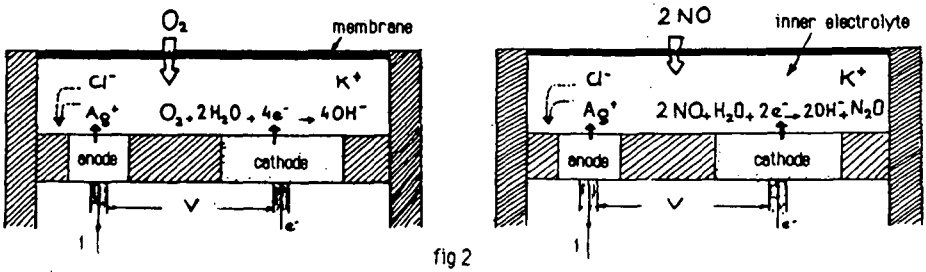


fig 2

For the same molar concentrations of O_2 and NO, the slope k theoretically is proportional to the number of electrons used for one reduced molecule : the theoretical value of k is the same for 1 dissolved mmole/l O_2 (32 ppm) and for 4 dissolved NO mmoles/l (120 ppm). Then, if calibrated with O_2 solutions, the detector indicates 1 ppm for 1 dissolved O_2 ppm and for 3,75 dissolved NO ppm (fig. 3). Practically, k also depends on physical and chemical properties of the membrane, the electrodes, etc.

measured intensity I = α reduction flux = β inner concentration = k outer concentration

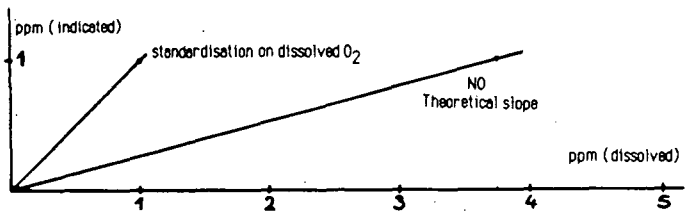


fig 3

We have used and compared two models of detectors made by the ORBISPHERE Corp, Vevey - Switzerland. The first (A1) that we commonly used for our experiments gives for NO a no linear response the mean slope of which is near the theoretical value, relatively to O_2 slope : this relative response is not really stable, when successive calibrations are compared. The second one (A2) which has been recently perfected by the maker, following our first observations, gives a stable and linear response, but with a lower slope than the theoretical one (fig. 4).

For these calibrations, gaseous NO was progressively dissolved in O_2 free water (apparatus of fig.1), or progressively displaced by pure N_2 bubbling : the increasing or decreasing concentration of dissolved NO was determined on samples, in the (NO_3^- + NO_2^-) forms, after H_2O_2 oxydation at pH 11.

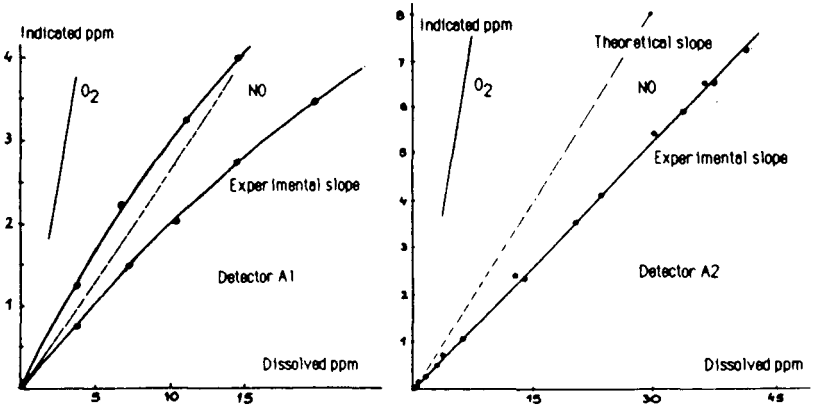


fig 4

The main object of our experiments was to study the occurence of aerobical denitrification in composts used as horticultural growing media. For that, the behaviour of the bacterial microflora of the compost was often studied in stirred liquid medium. We provided different levels of oxygen consumption and, for each one, we measured the rate of nitrate and nitrite reduction (5). The NO accumulation was only detected during the strict anaerobiosis steps (fig. 5).

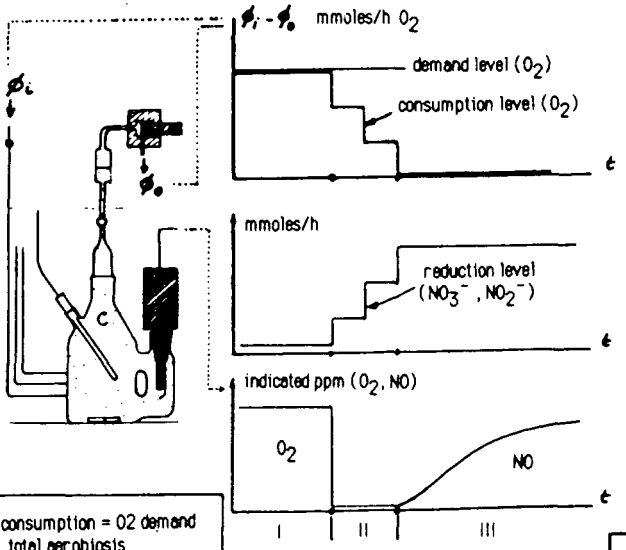


fig 5

I : 02 consumption = 02 demand
total aerobiosis

II : 02 consumption < 02 demand
aerobical denitrification

III : 02 consumption = 0
anaerobical denitrification

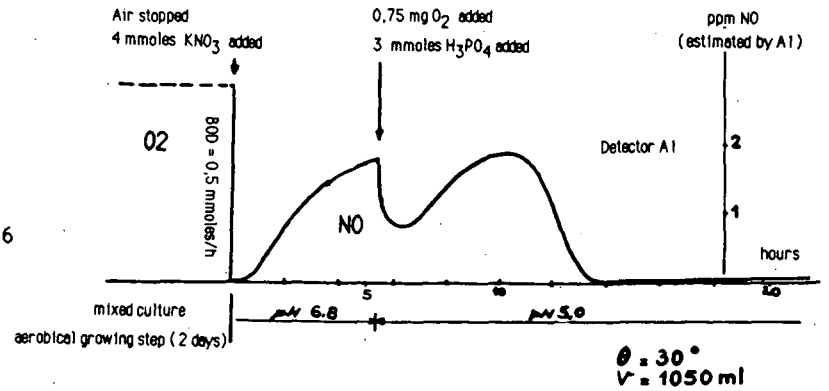
Experimental conditions

Inoculum : bacterial microflora of composts :
(a) mixed culture (non identified species)
(b) pure culture :
(b1) non identified species
(b2) *Enterobacter agglomerans sp*
(b3) *Pseudomonas aeruginosa sp*
Weakly buffered mineral medium (pH 5 to 7) Temperature : 30° ± 1° (generally)
Electron initial donor : glucose (for a b1 b2) acetate (for b3)
Electron final acceptor : NO3- (for a b2) NO2- (for b1, b3)

As examples, some typical results are now examined :

Example 1 (fig. 6) : After two days of aerobical growth of a mixed culture, the biological oxygen demand (BOD) being 0,5 mmole/h O_2, the air flow is stopped. At this time, 4 mmoles KNO_3 are added. A net production of NO is immediately observed and recorded. After 5 hours, a sample of 100 ml of the liquid suspension is removed and replaced, under N_2 and without stirring by an equal volume of with air equilibrated water ; the medium is acidified with 3 mmoles H_3PO_4 to pH 5. We observe, first, the effect of the chemical reaction between dissolved added O_2 and dissolved NO, much more important that the dilution effect, secondly that the accumulation of NO increases again, but not faster than for the previous pH. After 10 hours, the reduction of NO becames higher than the formation ; after 22 hours of anaerobiosis, followed by 6 h of aerobiosis and a second addition of KNO_3 we observed a second accumulation of NO at four fold higher intensity and longer duration than first (not represented).

fig 6



Examples 2 (fig.7) : When the aeration is stopped, the BOD of this pure culture is 2,1 mmole/h and the pH is 5,4. The addition of 1 mmole $NaNO_2$ provokes the accumulation of NO, which is now measured by the detectors A1 and A2, together assembled in the modified apparatus ; a second addition of $NaNO_2$ (after 150 mn) increases the rate of the accumulation. After 6 hours, a sample is removed and replaced as previously by an equal volume of water, apparently without effect on this rate at this point (dotted line). After 10 hours (point P) dissolved NO counted for **2,7** % of the added nitrogen.

fig 7

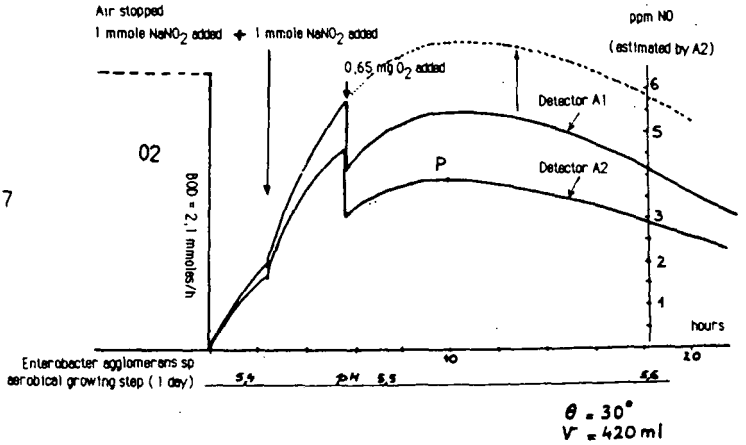
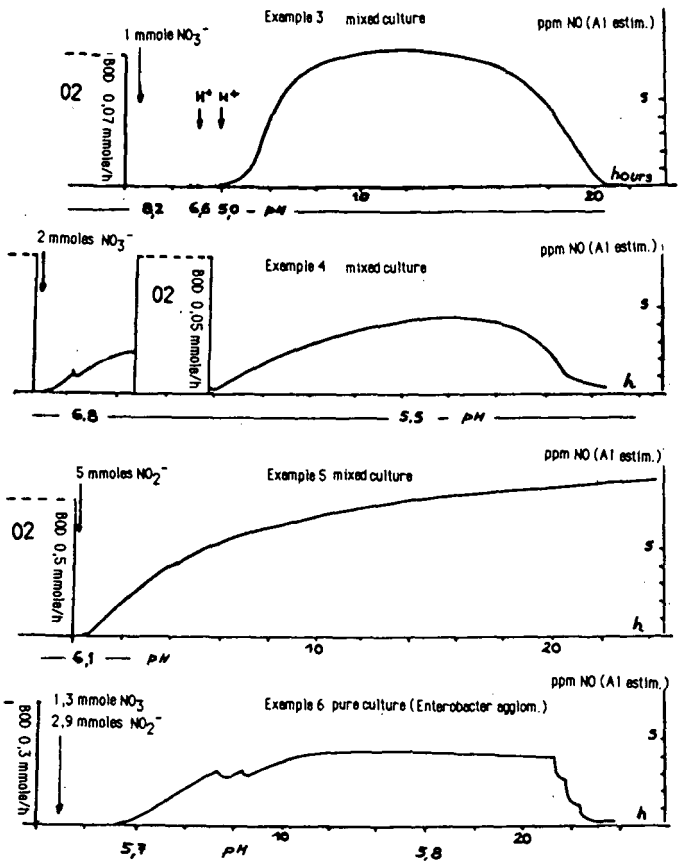

Air stopped
1 mmole $NaNO_2$ added
1 mmole $NaNO_2$ added
ppm NO
(estimated by A2)
0,65 mg O_2 added
Detector A1
Detector A2
P
02
BOD = 2,1 mmoles/h
hours
Enterobacter agglomerens sp
aerobical growing step (1 day)
pH 5,5
$\theta = 30°$
V = 420 ml

fig. 8.


Example 3 mixed culture
1 mmole NO_3^-
ppm NO (A1 estim.)
02
BOD 0,07 mmole/h
H^+ H^+
hours
8,2 6,6 5,0 - pH
2 mmoles NO_3^-
Example 4 mixed culture
BOD 0,05 mmole/h
6,8 5,5 - pH
Example 5 mixed culture
5 mmoles NO_2^-
BOD 0,5 mmole/h
6,1 pH
Example 6 pure culture (Enterobacter agglom.)
1,3 mmole NO_3^-
2,9 mmoles NO_2^-
BOD 0,3 mmole/h
5,7 pH 5,8

Four other examples are given on fig. 8.

If the growing pH is of the order of pH 6,0 - 7,0, the NO accumulation seems relatively independant of the anaerobiosis pH between 7,0 and 5,0 ; if the first is higher (example 3), it is necessary to acidify the anaerobical medium.

With mixed cultures, even extracted from the same compost, there is no relation between BOD values and the level and duration of NO accumulation (examples 1 to 6).

For some cases (examples 5 - 6), NO concentration reached a stable level and the bacterial respiration was suppressed (BOD = 0, after 25 hours of anaerobiosis).

The polarographic O_2 detector does not respond to CO_2 nor to N_2O, which is formed by reduction of NO (fig.2). At this stage, it enables to directly and continuously measure dissolved NO with an accuracy of 0,1 ppm.

These examples suggest the possibilities of application of this detector, particularly in the field of denitrification research.

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Reduction of nitrate to nitrous oxide or dinitrogen by Rhizobium leguminosarum.

by
Bertelsen, F.*

Abstract. Rhizobium leguminosarum biovar viceae strains, known to form N_2-fixing symbiosis with legumes, were grown under aerobic conditions in a chemically defined medium. Their ability to reduce nitrate to nitrous oxide (N_2O) or dinitrogen (N_2) under anoxic conditions were tested. Similar amounts of N_2O and N_2 were produced by the strains tested. No relationship between the denitrification potential and the nitrogen-fixing efficiency of the R. leguminosarum strains was observed. Denitrification potentials of the R. leguminosarum strains were in the magnitude of 0.01 to 0.08 mg N (g bacteria x h)$^{-1}$ compared to about 0.35 mg N (g bacteria x h)$^{-1}$ of a denitrifying Pseudomonas chlororaphis strain at identical conditions.

INTRODUCTION

In many agricultural systems symbiotic nitrogen fixation make an important contribution of the nitrogen input to soils. However, the root-nodule bacteria, Rhizobium spp, which form N_2-fixing symbiosis with legumes have been reported capable of anaerobic growth using nitrate as electron acceptor and thereby removing nitrogen from the ecosystem. Most work on rhizobial denitrification has been carried out on slow growing rhizobia (e.g. Bradyrhizobium japonicum). Only few strains of fast growing rhizobia (e.g. R. leguminosarum) have been tested and most of the strains were described as unable to denitrify (O'Hara and Daniel, 1984; Zablotowicz et al., 1978). However, Casella et al. (1984) reported denitrification by fast growing rhizobia when they where kept in a chemically defined minimal medium (MMD).

*Agricultural Research Department, Risø National Laboratory, DK-4000 Roskilde, Denmark.

The aim of this work was to study denitrification potentials of different R. leguminosarum biovar viceae (the symbiont on pea) strains in MMD to give an indication of the significance of nitrogen loss from agricultural systems caused by denitrification of R. leguminosarum biovar viceae.

MATERIALS AND METHODS

R. leguminosarum biovar viceae strains TOM and PF_2 were obtained from Dr T. A. Lie, Wageningen, The Netherlands. The other Rhizobium strains from Dr E. S. Jensen, Risø National Laboratory, Denmark, and the P. chlororaphis (formerly P. fluorescence biotype D) strain from Dr S. Christensen, University of Copenhagen, Denmark.

Strains of R. leguminosarum biovar viceae and a P. chlororaphis were grown aerobically at 27 °C in yeast-mannitol broth (YMB, Vincent, 1970). At the late exponential growth phase 1 ml culture was transferred to 125 ml serum flasks containing 50 ml MMD (Casella et al., 1984) and grown aerobically at 27 °C. In the early stationary growth phase the flasks were sealed with rubber stoppers and made anaerobic by flushing with N_2 for 3 minutes. Acetylene (10 ml) was added to half of the flasks. The flasks were incubated at 20 °C. Accumulation of nitrous oxide (N_2O) in the head space was measured on a gas chromatograph with electron capture detector. At the end of the incubation period bacterial dry weight was measured gravimetrically after filtration on a 0.2 µm filter.

RESULTS AND DISCUSSION

No relationship was observed between denitrification activity of the R. leguminosarum strains determined by this assay and the nitrogen fixation efficiency of the same strains as examined by Jensen (1987). Nitrous oxide production by two typical strains are shown in Fig. 1. Strain SV 10 (Fig. 1a) forms inefficient root nodules whereas strain Risø 1a (Fig. 1b) forms efficient root nodules on "Bodil" pea (Jensen, 1987).

The extent of N_2O production was similar in the two R. leguminosarum strains (Fig. 1). For both strains acetylene addition doubled the N_2O production rate which means that the bacteria produced N_2 and N_2O in similar amounts under the given conditions.

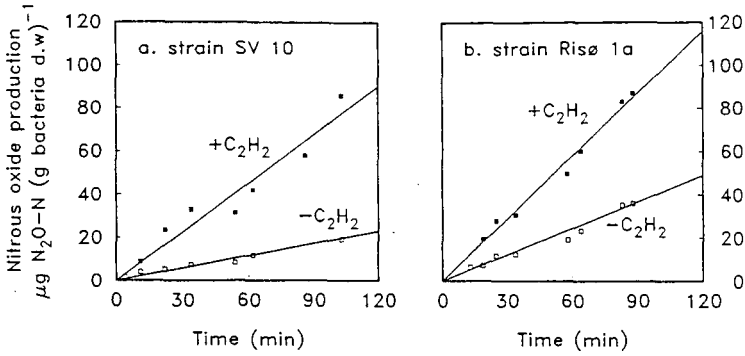

Fig. 1. Nitrous oxide production by two R. leguminosarum strains in presence ($+C_2H_2$) or absence ($-C_2H_2$) of acetylene. Strain SV 10 is inefficient and Risø 1a is efficient in fixing N_2 in symbiosis with "Bodil" pea.

Denitrification rates of representative strains are presented in table 1. It was possible to detect denitrification in all 15 strains investigated.

Strain	denitrification mg N (g bacteria d.w. x h)$^{-1}$
Risø 1a	0.06
SV 10	0.05
SV 15	0.04
A1	0.05
A6	0.07
A7	0.01
A8	0.01
A10	0.05
TOM	0.04
PF_2	0.01

TABLE 1. Denitrification of selected R. leguminosarum strains in presence of acetylene.

The rates are of similar magnitude as denitrification rates of R. trifolii, R. "hedysarum", and R. leguminosarum found by Casella et al. (1984). All strains produced N_2O in the absence of acetylene.

The P. chlororaphis strain showed about 10 times higher denitrification rates than the rhizobia, however (Fig.2).

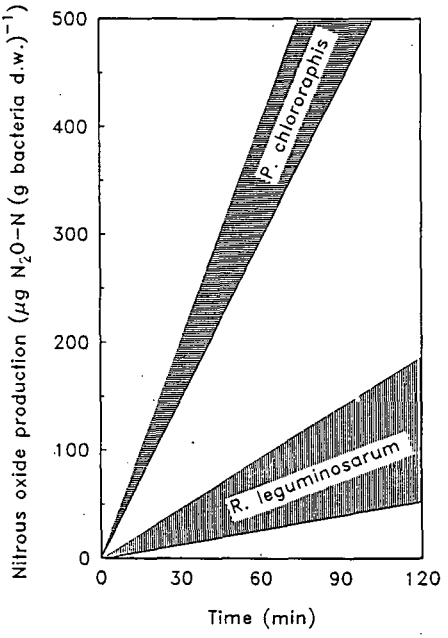

Fig. 2. Levels of nitrous oxide production by 15 different R. leguminosarum strains and a P. chlororaphis strain in presence of acetylene.

In most Danish agricultural soils the number of R. leguminosarum biovar viceae are between 10^3 and 10^4 rhizobia per gram soil (Engvild, 1989), but pea cultivation lead to a multiplication of the rhizobia in the soil. Engvild (1989) found up to 3 x 10^5 R. leguminosarum per gram of soil under a pea crop.

Compared to the number of denitrifying bacteria (3-42 x 10^6 per g soil, mostly pseudomonades) found by Christensen and Bonde (1985) in an adjacent soil, however, the number of R. leguminosarum is low.

Although the growth of pea crops lead to a multiplication of R. leguminosarum in the soil it is unlikely that these bacteria significantly enhance the loss of NO_3^- from the soil by denitrification.

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Modeling of denitrification: Approaches, successes and problems

by

Rolston, D.E.+

The purpose of this paper is to provide a review of approaches used in modeling denitrification, to present some examples where denitrification modeling has been reasonably successful for upland field situations, and to provide an evaluation of unsolved problems and challenges to more accurately predict denitrification rates in field environments.

MODELING APPROACHES

Modeling provides a means to help test hypotheses concerning the various physical, chemical, and biological mechanisms responsible for complicated processes. If the real processes can be accurately described by a model, it may also be possible to make predictions to other spatial locations or times. For the purposes of this paper, I elect to classify denitrification models to be either deterministic or stochastic. Within the deterministic group, models may be mechanistic, empirical, or some combination of the two. Deterministic models provide one value of denitrification rate or surface flux for the area of study, whereas stochastic models give probability distributions.

Deterministic Modeling

Mechanistic - Mechanistic approaches describe the physical, chemical, and biological processes by known laws or relationships. The geometry of the system and the initial and boundary conditions must be well defined. A mechanistic model for a whole soil system has been developed by van Veen and Frissel (1981). Models at the aggregate level have been given by Smith (1980), Leffelaar (1979, 1986), McConnaughy and Bouldin (1985), Myrold and Tiedje (1985) and Arah (1989).

+Land, Air & Water Resources, University of California, Davis, California 95616, USA

These models account for oxygen diffusion and respiration to calculate the amount of anaerobic volume within aggregates or the whole soil. In general, the rate coefficients and transport parameters are difficult to obtain experimentally. Only limited experimental verification has occurred in the field.

Empirical - Empirical models are generally not based on known laws, but are developed from regression relationships between denitrification and soil variables. Examples of such empirical models are Mosier et al. (1983), Benckiser et al. (1987), Burton and Beauchamp (1985), and Christensen (1985). Mosier et al. (1983) obtained a reasonably good correlation (r^2=0.5) between N_2O surface fluxes and water content, nitrate, and ammonium. Benckiser et al. (1987) using multiple regression found a good relationship between denitrification and several soil variables with the soil-water content being the dominant factor. Christensen (1985) found only a weak relationship between field denitrification rate and water content, nitrate, and carbon factors. Burton and Beauchamp (1985) also were not able to obtain very high correlations of denitrification rate from soil cores with water content, carbon, and nitrate because their soil cores were within a water content range not strongly affecting denitrification. These studies have shown that empirical models are partially successful in developing relationships between denitrification in field environments, but correlations are not very high in most cases. It is not known how well these relationships are transferable to other sites or soils.

Mechanistic/empirical combination - Within this group of models are those by Hagin et al. (1976), Mosier and Parton (1985), and Rolston et al. (1984). The Hagin et al. (1976) model was given by

$$F = k_T \, k_H \, k_{OM} \, k_O \, N$$

where F is denitrification rate, k_T is a temperature factor, k_H is a pH factor, k_{OM} is an organic matter factor, k_O is a factor for oxygen limitation in the soil water which is either 0 or 1, and N is nitrate concentration. The pH factor is a maximum for pH values between 6 and 8. The organic matter factor is proportional

to carbon decomposition and the rate of carbohydrates added. The model included equations for diffusion and respiration of oxygen within the soil profile.

Mosier and Parton (1985) expanded upon their earlier empirical model by inclusion of more mechanistic components into the model. Their model included the nitrous oxide flux from both denitrification and nitrification. The denitrification part of the model was given by

$$F = a\ E_\psi\ E_T\ N$$

where F is the N_2O surface flux, a is an empirical constant, E_ψ is an empirical water function, E_T is an empirical temperature function, and N is nitrate concentration. They found a reasonably good comparison (r^2=0.6) of model predictions with data collected over about a two year period. By fitting the constant a and the water and temperature functions to the data, the model was capable of reconstructing the temporal behavior of N_2O fluxes. Addition of carbon concentration (two different forms) did not greatly improve model predictions.

The model by Rolston et al. (1984) included water and solute transport as well as plant uptake and nitrogen transformations. The denitrification part of the model was described by

$$F = k_1\ \theta\ f_w\ f_T\ C_W\ N$$

where F is the denitrification rate, k_1 is a rate constant, θ is the volumetric water content, f_W is an empirical water function to account for the degree of anaerobic development, f_T is an empirical temperature function (Q_{10}=2), C_W is the water soluble organic carbon concentration, and N is nitrate concentration. The denitrification rate constant k_1 was fitted to field data in earlier research but for later research (Grundmann and Rolston, 1987), the value of k_1 was taken from Reddy et al. (1982) which indicated that k_1 was relatively constant for many different mineral soils within the U.S.A. The water soluble carbon was calculated from an empirical relationship $C_W = 24.5 + 0.0031\ C_s$ where C_s is total soil organic carbon and is considered to decay according to a first-order kinetic reaction. Plant or manure carbon could also be incorporated into the carbon pool.

Since soil-water content is generally the most important driving variable in denitrification, one of the features of both the Mosier et al. (1985) and Rolston

et al. (1984) models is an empirical water function which serves as an indirect measure of the degree of anaerobiosis in the soil. These functions are similar and show a very strong response to water in the range above 60% of water saturation. The original water function by Rolston et al. (1984) has been further evaluated and modified by Grundmann and Rolston (1987).

In the Rolston et al. (1984) model, the model was first fitted to one set of experiments in order to obtain the denitrification rate constant. In order to test the model, the same values for the rate constant were used in simulating denitrification for an independent experiment at several different irrigation frequencies. These results showed that the field-measured denitrification rates could be reasonably approximated with the simulation model.

Additional research by Grundmann and Rolston (1987) and Grundmann et al. (1988) evaluated the capability of the model in predicting denitrification at many spatial locations within a field soil. Denitrification flux, water content, nitrate concentration, and water soluble carbon concentration were measured at 64 locations along a transect. Comparisons of simulations and experimental data showed that this deterministic model could predict the overall behavior of denitrification along the transect but was not able to accurately predict the denitrification flux at specific spatial locations. A complicated interaction between water content and nitrate concentration in the field was also evident which was not accounted for in the present model. Such non-deterministic behavior may require a different approach, such as stochastic modeling.

Stochastic Modeling

Parkin and Robinson (1989) have proposed a stochastic model for denitrification dependent upon only two main driving variables, potential denitrification enzyme activity and carbon dioxide production. The enzyme activity was meant to represent the maximum possible denitrification which was modified by aeration status represented by carbon dioxide production rate. The stochastic nature of the enzyme activity and carbon dioxide production were given by measured probability density functions for a field site. These probability density

functions were then used as input to a Monte Carlo simulation model to calculate a probability density function for denitrification rate.

Using a different data set than that used to calibrate the model, the results showed that the model predicted the frequency distributions, the means, and the variances reasonably well. However, denitrification rates at a particular location were not predictable since the r^2 between calculated and measured rates was only 0.34. A stochastic approach offers increased possibility for improved understanding and description of natural spatial and temporal variability in field soils. Additional research is needed to evaluate if the fitted denitrification rate constant of this model can be used for different soils and times.

UNSOLVED PROBLEMS AND CHALLENGES

The generally high spatial and temporal variability in field soils indicates that denitrification is very sensitive to other variables exhibiting natural variability. Since denitrification is very sensitive to variables such as water content, accurate modeling of denitrification becomes quite difficult. Complex interactions between the various factors affecting denitrification occur in the field which are not yet well quantified or understood, and relationships to basic chemical, physical, and biological properties are not well defined.

The quantification of anaerobic microsites and their distribution within field soils remain a major challenge. The importance of organic carbon in development of anaerobic microsites is well recognized, yet little methodology exists for quantifying the effects of carbon on microsite development.

Methodology on how to "scale up" to larger measurement units is still lacking. Due to high spatial and temporal variability and the large amount of time and resources required to measure denitrification, most measurements of denitrification have been made on fairly small land areas. Information is needed on how to extrapolite denitrification measurements or predictions from the plot to the field to the soil mapping unit and eventually to the regional scale. Denitrification modeling offers another tool to help make such extrapolations.

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MODELING DENITRIFICATION LOSSES FROM ARABLE LAND

K.M. Syring[1] and G. Benckiser[2]

An attempt was made to validate a denitrification model with data obtained from field experiments. The model is designed to be a part of a larger model describing the water and nitrogen dynamics of the soil-plant system. While designing the description of the processes of gas and solute transport in the soil is rather straightforward, modeling the microbial processes during denitrification poses a major problem.

Denitrifiers are ubiquituous in soils (Gamble et al. 1976), but their kinetic properties are highly variable. There is a strong impact of soil properties like pH and clay content on the kinetic parameters. Furthermore, it is known that strictly anaerobic conditions are not required for the onset of denitrification (Ottow and Fabig 1985). All this makes it virtually impossible to apply kinetic parameters obtained from pure cultures to soil conditions.

A concept of competitive inhibition (Cho and Mills 1979; Benckiser and Syring 1985) was chosen to describe the biological processes. Electrons are delivered from mineralisation of soil organic matter at a defined rate and oxygen, nitrite and nitrous oxide are competing acceptors.

The model has been tested on data from field experiments on sandy soils, where denitrification rates were measured by the acetylene-inhibition method. Only measured N_2O fluxes were used because of the well known problems to relate N_2O concentrations in soil to N_2O fluxes at the soil surface. A data set obtained from an Incep-

[1] Institute of Plant Nutrition and Soil Science, Federal Research Center of Agriculture, Bundesallee 50, 3300 Braunschweig, FRG

[2] Institute of Microbiology, Justus-Liebig University, Senckenbergstr. 3, 6300 Giessen, FRG

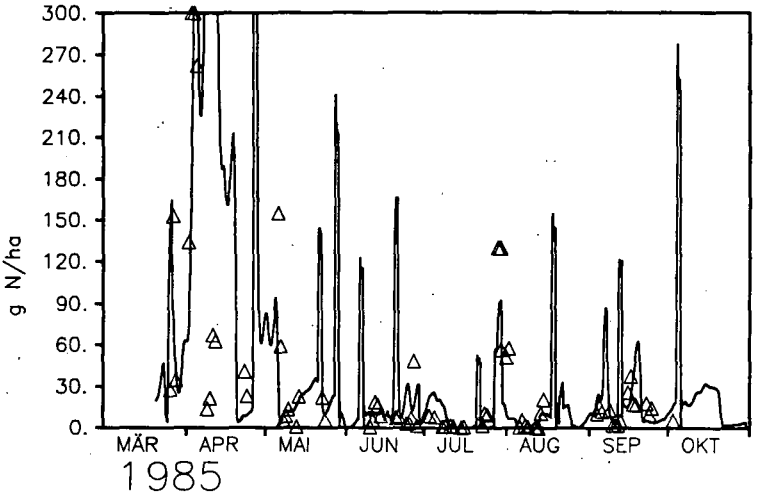

Fig. 1: Comparison of measured N_2O fluxes (Symbols) with model results (closed line). Soil: Inceptisol (total carbon 1.61%, total nitrogen 0.153%). These high carbon and nitrogen contents were obtained by long term application of sewage sludge (Benckiser et al., 1987)

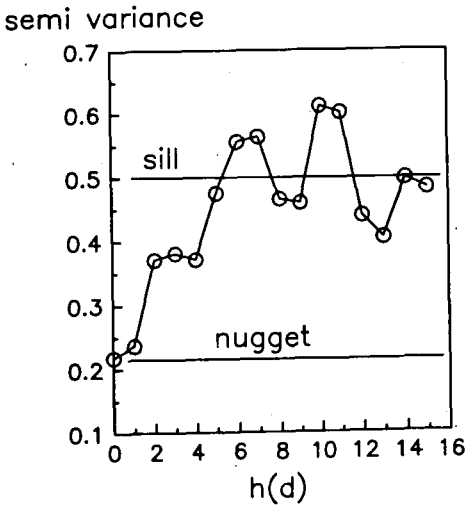

Fig.2: Semi-Variogram for log-transformed N_2O fluxes (n=285, same site as in Fig.1, h is the temporal distance between measurements). In this case, the random component of the semi-variance nugget accounts for 40 % of the maximum semi-variance sill

tisol was used to calibrate the model. In Fig. 1 the model results are compared with measured N_2O fluxes.

Problems are araising from the high variability of measured denitrification rates. By applying the semi-variogram plot known from geostatistics (Webster 1985) to the time series of measured N_2O fluxes (Fig. 2), it was estimated that from 40 to 100 % of the variance is due to random noise. Obviously, only the remaining variance can be explained by a deterministic model.

The model was used on other field plots. Only measured soil parameters (bulk density, carbon and nitrogen content) were changed in these model runs. Average denitrification losses were calculated successfully by the model. But validation with respect to single denitrification events is hampered by the statistical problems discussed. The model also suggests that under the prevailing soil conditions and climate in West Germany, denitrification losses are low (less than 20 kg N/ha/a) and N_2O is the dominating product of the denitrification process.

Further development of the model will incorporate the results from other work on modeling denitrification losses from arable land (Grundmann et al. 1988). A more explicit handling of soil structure as well as the transport of gases and solutes inside of soil aggregates without introducing to many model parameters would be an useful extension of the model. But an improvement of the model, with respect to the applications intended, is meaningful only if the signal to noise ratio in the measured data can be enhanced.

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Modeling of an in-situ remediation using denitrifying bacteria

by

Schäfer, W.[+], M. Finkel[++] and W. Kinzelbach[+]

Abstract: A coupled numerical model which describes transport and heterotrophic denitrification in the saturated subsoil is presented. The transport simulation is performed by a finite difference scheme. The biochemical model is based on Monod-type kinetics. Results for 1-D applications show the general functioning of the model. 2-D applications show possible advantages of using a coupled model in supporting in-situ remediation measures in the field.

1. Introduction

Most organic pollutants released into aquifers in accidental spills (e.g. benzene or fuel oil) are highly insoluble or strongly adsorbed to the aquifer matrix. Therefore mere flushing with water has a low purification effect.

Recently efforts were made to clean up polluted aquifers in-situ with the help of induced microbial activity. The microorganisms are supposed to use the rather immobile organic pollutant as a substrate and a mobile electron acceptor supplied by injection. Oxygen or nitrate could serve as electron acceptors, but the latter is preferred because it has a much higher solubility. The bacteria provided with nitrate then perform heterotrophic denitrification in the aquifer and thus degrade the organic pollutant. This paper presents an attempt to use a numerical model in simulating in-situ aquifer restoration.

2. Coupling of chemistry and transport

To calculate the concentration of chemically interactive species in the aquifer with a numerical model, coupling of terms describing chemical/biochemical transfers to those describing physical transfers by advection and diffusion-dispersion is necessary. The system of coupled transport equations is of the form:

$$\frac{\partial(\theta_i c_i)}{\partial t} = -\vec{\nabla}(\vec{v}_i \theta_i c_i) + \vec{\nabla}(D_i \theta_i \vec{\nabla} c_i) + \sum_{j=1}^{n} S_{ij}(c_1, ..., c_n) \quad i = 1, ..., n$$

v_i and D_i are zero for an immobile species.

The coupling terms S_{ij} are in general non-linear functions of the concentration. A standard method for the solution of the system is the split-operator method (e.g. Noye 1987). In the simplest form of application of this principle the full reactive transport is decomposed into a half-step of purely physical transport and a half step of chemical reaction only.

$$\frac{\partial c}{\partial t} + \vec{\nabla}(\vec{v} c) - \vec{\nabla}(D \vec{\nabla} c) = S(c)$$

$$\frac{1}{2}\frac{\partial c}{\partial t} + \vec{\nabla}(\vec{v} c) - \vec{\nabla}(D \vec{\nabla} c) = 0$$

$$\frac{1}{2}\frac{\partial c}{\partial t} = S(c)$$

The method can be improved by iterating between the two half-steps, the second step giving a predictive value for the chemical fluxes which can be incorporated in the overall mass balance of the first step in the following iteration. The solution method is described in more detail in Kinzelbach et al (1989). A documentation of the computer code is given in Herzer (1989).

+) Gh Kassel, FB 14, Moritzstr. 21, 3500 Kassel

++) Institut für Wasserbau, Universität Stuttgart, Pfaffenwaldring 61, 7000 Stuttgart 80

3. The biochemical model

Figs. 1 and 2 show the scheme of the biochemical model and the species and equations considered respectively. The bacteria are assumed to be attached to the aquifer matrix with dissolved organic carbon (DOC) as the only substrate at their disposal. To oxidize this substrate the optionally anaerobic bacteria (DVWK, 1988) use dissolved oxygen (DO) as long as it is available and then nitrate as electron acceptor. Bacterial growth is based on Monod-type kinetics and switching between aerobic and anaerobic growth is implemented by a DO dependent weight function (Kinzelbach et al, 1989).

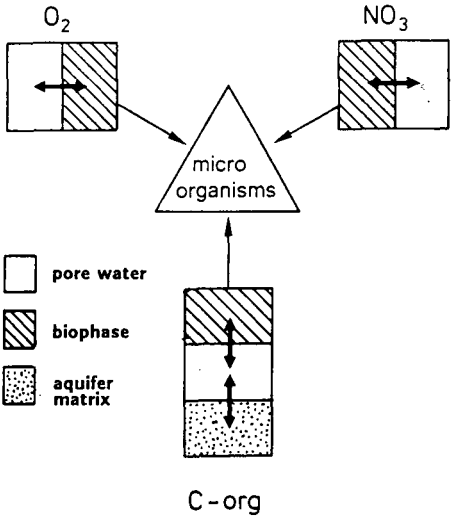

Fig. 1: Scheme of the biochemical model

While DO and nitrate are supplied to the aquifer by groundwater recharge or injection, for DOC further sources are considered: the additional release of organic material from a source in the aquifer itself (e.g. an immobile organic pollutant) and the utilizable portion of dead bacteria.

Chemical species not listed in fig. 2 are assumed to be available in sufficient quantities in the aquifer in order not to be limiting. Their impact is therefore neglected.

4. 1-D applications

Fig. 3 shows a sequence of concentration profiles for a numerical simulation in a hypothetic column. The length of the column is plotted along the horizontal axis and the normalized concentration C/C0 along the vertical axis. In these figures concentration profiles for nitrate, DOC, adsorbed carbon (in this case the pollutant, AC), bacterial mass and a conservative tracer are shown. For simplification DO is not regarded because in remediation nitrate is used in amounts which are so high that the additional oxygen can be neglected. The maximum value of the dissolved pollutant concentration is obtained from its solubility or - in the case of adsorption - from its adsorption isotherm. The organic spill is situated in the column interval from 1.5 to 4.5 m and the nitrate source is on the lefthand side of the column. As can be seen the modeled microorganism growth proceeds from left to right consuming nitrate and degrading the organic pollutant. After 200 days the organic pollutant is degraded to an amount that the DOC available now is no loger sufficient to support microbial activity. Therefore nitrate breaks through, much retarded in comparison to the conservative tracer. The numerical column studies showed that the availability of DOC is the crucial parameter governing both the breakthrough curves for nitrate and the success of the whole remediation action. This availability is controlled by the exchange between solid and liquid phase, which is a diffusion process, and the total amount of the pollutant.

8 Species: mobile: $O_{2\ mob}$, $NO_{3\ mob}$, $C_{org\ mob}$ in pore water

immobile: $O_{2\ im}$, $NO_{3\ im}$, $C_{org\ im}$ in bacterial phase, bacterial mass X, organic carbon in the aquifer matrix C_{mat}

8 Transport equations:

mobile:

$$\frac{\partial O_{2\ mob}}{\partial t} = L\,(O_{2\ mob}) + S_{O_{2}\ mob}$$

$$\frac{\partial NO_{3\ mob}}{\partial t} = L\,(NO_{3\ mob}) + S_{NO_{3}\ mob}$$

$$\frac{\partial C_{org\ mob}}{\partial t} = L\,(C_{org\ mob}) + S_{C_{org\ mob}}$$

immobile:

$$\frac{\partial O_{2\ im}}{\partial t} = S_{O_{2}\ im} \qquad \frac{\partial X}{\partial t} = S_{X}$$

$$\frac{\partial NO_{3\ im}}{\partial t} = S_{NO_{3}\ im} \qquad \frac{\partial C_{mat}}{\partial t} = S_{C_{mat}}$$

$$\frac{\partial C_{org\ im}}{\partial t} = S_{C_{org\ im}}$$

8 Explicit source/sink terms:

1.) $S_{O_{2}\ mob} = -\alpha\,(O_{2\ mob} - O_{2\ im})$

2.) $S_{NO_{3}\ mob} = -\alpha\,(NO_{3\ mob} - NO_{3\ im})$

3.) $S_{C_{org}\ mob} = -\alpha\,(C_{org\ mob} - C_{org\ im})$

4.) $S_{X} = \left[\frac{\partial X}{\partial t}\right]_{aer} + \left[\frac{\partial X}{\partial t}\right]_{den} - \left[\frac{\partial X}{\partial t}\right]_{dec}$

$$\left[\frac{\partial X}{\partial t}\right]_{aer} = v_{max}^{aer}\,(1 - F(O)) \cdot \frac{C_{org}}{K_{C}^{aer} + C_{org}} \cdot \frac{NO_{3}}{K_{N}^{aer} + NO_{3}} \cdot \frac{O_{2}}{K_{O}^{aer} + O_{2}} \cdot X$$

$$\left[\frac{\partial X}{\partial t}\right]_{den} = v_{max}^{den}\,F(O) \cdot \frac{C_{org}}{K_{C}^{den} + C_{org}} \cdot \frac{NO_{3}}{K_{N}^{den} + NO_{3}} \cdot X$$

$$\left[\frac{\partial X}{\partial t}\right]_{dec} = v^{dec} \cdot X$$

5.) $S_{C_{mat}} = -\beta\,(C_{sat} - C_{org\ mob})$

6.) $S_{O_{2}\ im} = -\frac{1}{Y_{O}} \cdot \left[\frac{\partial X}{\partial t}\right]_{aer} - S_{O_{2}\ mob}$

7.) $S_{NO_{3}\ im} = -\frac{1}{Y_{N}^{den}} \cdot \left[\frac{\partial X}{\partial t}\right]_{den} - \frac{1}{Y_{N}^{aer}} \cdot \left[\frac{\partial X}{\partial t}\right]_{aer} - S_{NO_{3}\ mob}$

8.) $S_{C_{org\ im}} = -\frac{1}{Y_{C}^{den}} \cdot \left[\frac{\partial X}{\partial t}\right]_{den} - \frac{1}{Y_{C}^{aer}} \cdot \left[\frac{\partial X}{\partial t}\right]_{aer} + f_{use} \cdot \left[\frac{\partial X}{\partial t}\right]_{dec} - S_{C_{org\ mob}} - S_{C_{mat}}$

Fig. 2: Species and equations of the nitrate system

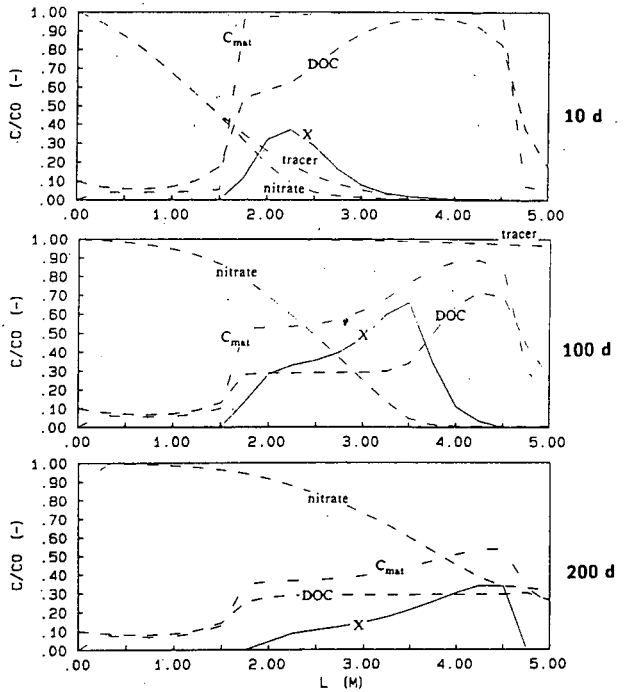

Fig. 3: Modelled concentration profiles for a 1-D application

5. 2-D applications

Fig. 4 shows the horizontal view of a field case of remediation (Battermann and Werner, 1984):

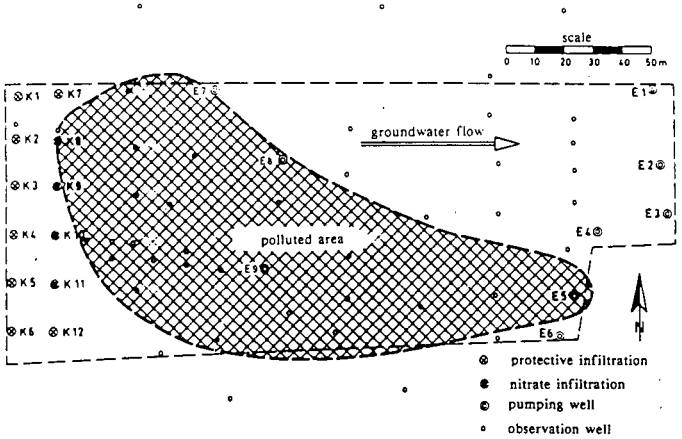

Fig. 4

An idealized but comparable situation with one infiltration well and one pumping well was modelled in two spatial dimensions. Figure 5 reflects the growth of bacteria in the polluted area between infiltration and pumping wells: A typical 2-D effect is the enhanced growth of bacteria at the edges of the spill due to nitrate supply by diffusion/dispersion.

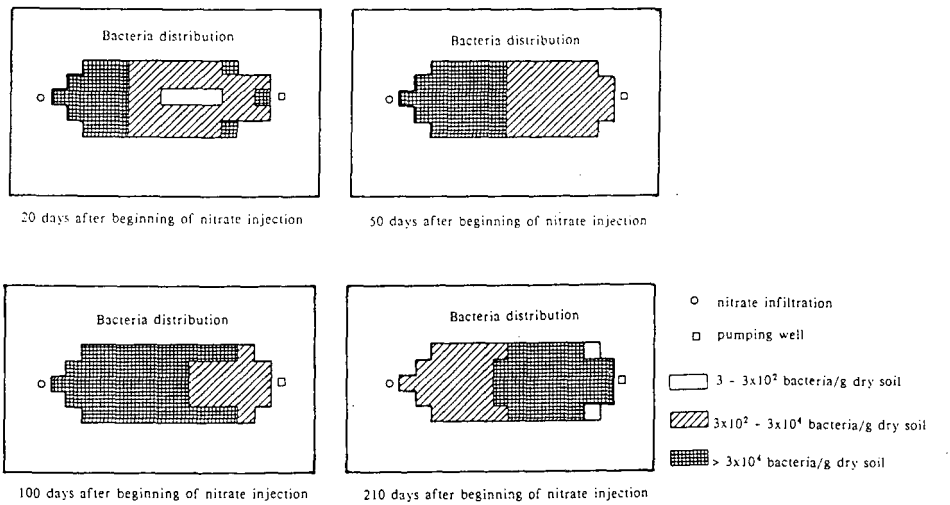

Fig. 5: Modelled growth of bacteria in 2 horizontal dimensions

A comparison of measured and calculated breakthrough curves for a tracer and nitrate in an observation well located in the middle of the spill is shown in fig. 6 (Finkel, 1988). The exchange coefficient governing the release of organic carbon into porewater again turned out to be the most sensitive model parameter.

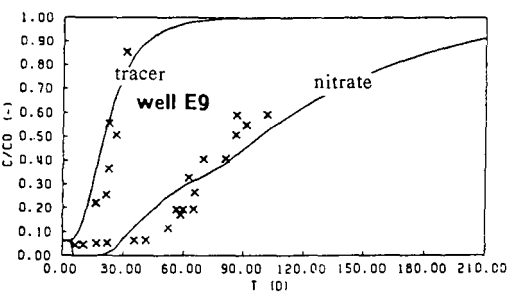

Fig. 6: Calculated and measured (x) breakthrough curves for a tracer and nitrate

One has to be aware that for field applications - in contrast to 1-D columns - there is a competition between transport and chemistry which can only be evaluated with horizontally 2-D or 3-D models: A breakthrough of nitrate in a well can be caused either by nitrate passing by the spill or by nitrate being no longer used up by microbial activity in the spill itself.

6. Conclusions

Mechanistic chemical models coupled to 1-D transport models can be helpful to interpret column experiments in the laboratory and to get ideas for further experiments.

In order to optimize remediation design in the field it is however necessary to couple chemical models to 2-D or even 3-D transport models. Then such coupled models allow testing of hypotheses and distinguishing between transport and chemical effects.

Because the model can help to predict microbial activity in the aquifer for various possible remediation designs, it can be useful to avoid injection of excessive amounts of nitrate on one hand and clogging of the aquifer on the other hand by appropriate choice of input concentrations and hydraulic conditions.

Appendix: Symbols

aer, den, dec	: aerobic, denitrifying, decay	[-]
C_{org}	: concentration of dissolved organic carbon in pore water	$[M/L^3]$
C_{sat}	: saturation concentration for C_{org} in porewater	$[M/L^3]$
D_i	: tensor of hydrodynamic dispersion of participant i	$[M/L^3]$
f_{use}	: utilizable portion of dead bacteria	[-]
K_O, K_N, K_C	: half velocity concnetration for DO, nitrate, DOC	$[M/L^3]$
mat, mob/im	: in matrix, mobile/immobile phase	[-]
t	: time	[T]
$\vec{v}$	: vector of average phase velocity	[L/T]
v_{max}	: maximum growth rate	[1/T]
Y_O, Y_N, Y_C	: yield factors (proportion of increase in bacterial mass to consumption)	[-]
α, β	: exchange coefficients	[-]
$\vec{\nabla}$	: nabla operator $(\partial/\partial x, \partial/\partial y)$	
θ_i	: volume fraction of phase containing participant i	[-]

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STEADY-STATE DENITRIFICATION: AN ADEQUATE APPROXIMATION?

by

Arah, J.R.M.[+)]

ABSTRACT

Most published attempts to model denitrification, whether at the scale of a microsite or that of a field, assume that the system being modelled is in a state of pseudo-equilibrium, or a "steady state". The *current* denitrification rate is a function of the *current* values of whatever parameters are invoked to account for denitrification. Denitrification may depart from a steady-state phenomenon in a number of ways. There may be a delay between a denitrification event at depth and its detection at the soil surface (this applies particularly to empirical field-scale models of denitrification); microbiological adjustments to altered conditions in the denitrifying microsites may take time; and the physical structure of the microsites themselves may retard their response. This paper examines the last problem, with particular reference to a model in which denitrification is taken to occur in microsites with a definite size and internal structure. It is concluded that in a soil with few large (greater than 10^{-2}m) aggregates a pseudo-equilibrium model of microsite denitrification is probably adequate. Where there are significant numbers of large aggregates, however, the steady-state assumption is less tenable.

NOTATION

c : concentration	C : external concentration	D : diffusion constant
I : inhibition function	J : denitrification rate	K : Michaelis constant
r : radius	R : microsite radius	t : time
V : reduction potential	x, y, z : controlling parameters	Δ : perturbation function
F : field	i : microsite	s : sample

INTRODUCTION

Any attempt to model denitrification involves the postulation or the empirical derivation of a relationship between denitrification rate J and a number of parameters x, y, z. These parameters may be properties of the whole soil, or more localised variables. They are generally treated as independent. In most cases it is assumed that the system being modelled is in a state of *pseudo-equilibrium*: there is a negligible time-lag between any change in x, y, z and the resulting change in J. The pseudo-equilibrium (or *steady-state*) assumption is rarely made explicit: it is the purpose of this paper to examine it.

+) The EdinburghSchool of Agriculture, West Mains Road, Edinburgh EH9 3JG, United Kingdom

Field-scale models of denitrification

Various attempts have been made to model denitrification at the scale of a field. Rolston *et al* (1984), for instance, obtained an empirical relationship between measured denitrification rate and soil nitrate concentration, "available" carbon concentration, soil moisture content, and soil temperature. Measurements were taken to be representative of the whole field, and the soil was assumed to be in a state of pseudo-equilibrium.

$$J_F(t) = f\,(\,x_F(t),\, y_F(t),\, z_F(t)\,) \qquad (1)$$

The transferability of such empirical models is always in doubt. Moreover, the great spatial variability encountered in any attempt to measure denitrification in the field (*eg* Myrold, 1988) is not addressed. Any model purporting to provide mechanistic insight into the denitrification process must take spatial variation into account.

Microsite models of denitrification

The concept of the denitrifying *microsite* or *hot-spot* has been increasingly employed to account for the spatial variability of denitrification. In the model of Parkin (1987) hot-spots are conceived of as featureless points, characterised only by their denitrification rates. Macroscopic sample denitrification rates are simulated by scattering these hot-spots around in the model soil.

$$J_S(t) = \Sigma\, J_i(t) \qquad (2)$$

Parkin and Robinson (1989) assumed that microsite denitrification rate is determined by two local (microsite) parameters, denitrification enzyme activity and carbon dioxide production rate, both of which are governed by (independent) log-normal probability distributions.

$$J_i(t) = f\,(\,x_i(t),\, y_i(t)\,) \qquad (3)$$

Arah (1988) and Arah and Smith (1989) modelled denitrifying microsites as finite volumes of soil (associated with aggregates where these are present). Microsite denitrification rate is determined by a number of microsite parameters (*cf* Equation 3); macroscopic rates are simulated using Equation 2. The model is discussed below. In all these models a steady state (pseudo-equilibrium) is assumed. Where microsites are attributed a finite volume and an internal structure it is possible to test the validity of the assumption.

THE MODEL

The spherical microsite model is discussed in detail elsewhere (Arah, 1988; Arah and Smith, 1989). The model soil consists of an assembly of independent microsites, each of which denitrifies at a rate (which may of course be zero) determined by its radius, diffusion constants, reduction potentials, and external concentrations of oxygen

and nitrate. These controlling parameters are governed by independent probability distributions, integration over which leads to a value for the denitrification rate of a *representative assembly* of soil microsites.

Microsite structure

Microsites have spherical symmetry. In the version of the model discussed here they are also physically homogeneous and the distribution of oxidisable organic matter is uniform. Thus the nitrate reduction potential in the anaerobic zone (if any) is proportional to the oxygen reduction potential in the aerobic region. Microbial reduction follows Michaelis-Menten kinetics.

Steady-state denitrification

The steady-state concentration profile c(r) of a substrate undergoing simultaneous diffusion and Michaelis-Menten reaction in a spherically symmetric environment of radius R, diffusion constant D, reduction potential V, and external concentration C is governed by Equation 4:

$$D\,[d^2c(r)/dr^2 + (2/r)\,dc(r)/dr] = I(r)\,V\,c(r)/[K+c(r)] \qquad (4)$$

with boundary conditions:

$$c(R) = C \text{ and } dc(0)/dr = 0 \qquad (4a)$$

where I(r) is a function (used in calculating nitrate concentration profiles) reflecting the inhibition of nitrate reduction by any oxygen present at radius r, and K is the relevant Michaelis constant. This equation may be solved using a numerical technique to calculate steady-state intra-microsite concentration profiles (first of oxygen, then of nitrate), which in turn may be employed to determine microsite reduction rates.

Non-steady-state denitrification

Equation 4 is a pseudo-equilibrium equation; if the system is not in a steady state (*ie* in general) a *transient* equation must be employed to calculate the time-dependent intra-microsite concentration profile c(r,t):

$$\partial c(r,t)/\partial t = D(t)\,[\partial^2 c(r,t)/\partial r^2 + (2/r)\partial c(r,t)/\partial r] - I(r,t)\,V(t)\,c(r,t)/[K+c(r,t)] \qquad (5)$$

with boundary conditions:

$$c(R,t) = C(t) \text{ and } \partial c(0,t)/\partial r = 0 \qquad (5a)$$

Once again, using a numerical technique, it is possible to solve this equation. Consider an individual microsite, initially in a state of pseudo-equilibrium, and subjected at time zero to a perturbation. Solution of Equation 5 allows us to follow the system as it moves towards a new steady state. Figure 1 illustrates this for a 2×10^{-2}m microsite; the normalised (*ie* divided by its external value) oxygen concentration is plotted against the normalised intra-microsite radius. Relevant parameters are given in Table 1; at time zero the gaseous (*ie* oxygen) diffusion constant is reduced by a factor of ten.

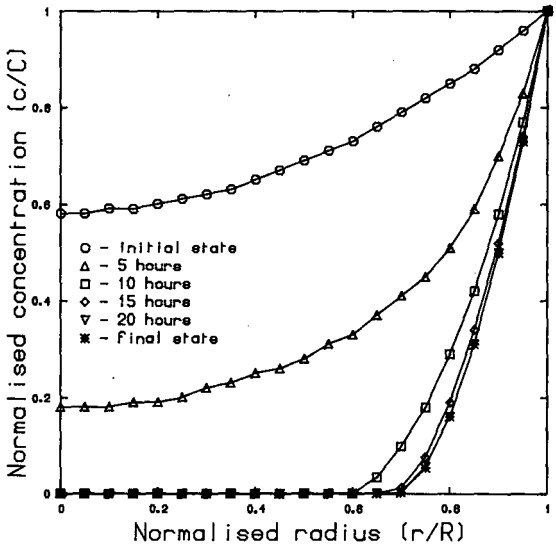

Figure 1. Normalised intra-microsite oxygen concentration profiles in a $2x10^{-2}$m microsite following an instantaneous tenfold reduction in gaseous diffusion constant at time zero.

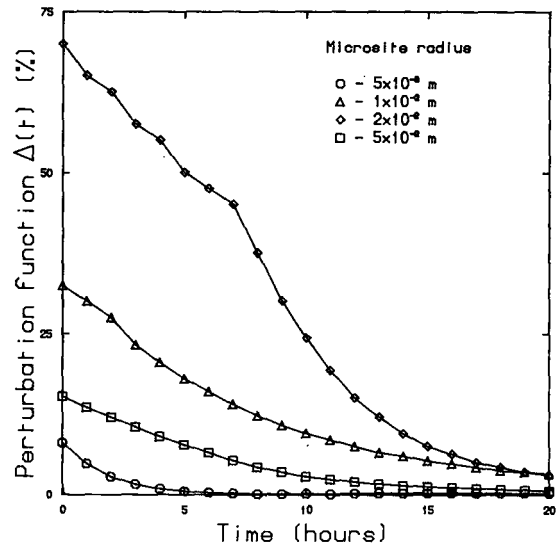

Figure 2. Perturbation function plotted against time for a series of microsites of different radius.

Table 1. Parameters governing concentration profiles shown in Figure 1.

Substrate	Michaelis constant mol m^{-3}	Reduction potential mol $m^{-3}s^{-1}$	External concentration mol m^{-3}	Diffusion constant m^2s^{-1}
Oxygen	10^{-3}	10^{-4}	4	$4x10^{-9}$ (time zero)
Nitrate	$2x10^{-1}$	$2x10^{-5}$	$4x10^{-2}$	$4x10^{-10}$

These values are typical of what might be expected in an aggregated arable soil (see Arah and Smith, 1989, for references). The inhibition function I(r), which describes oxygen inhibition of nitrate reduction, is given by:

$$I(r) = 1 - 100c_o(r);\ 0 \leq I(r) \lesssim 1 \qquad (6)$$

where $c_o(r)$ is the oxygen concentration at radius r.

Microsite relaxation times

It is apparent from Figure 1 that the oxygen concentration in the $2x10^{-2}$m microsite takes some twenty hours to adjust to the perturbation. But what about the nitrate concentration? And what about larger and smaller microsites? In order to be able to compare microsites it is useful to define a time-dependent *perturbation function* $\Delta(t)$ equal to the mean of the absolute values of the normalised deviations from the relevant steady-state solutions of the two concentration profiles (oxygen *and* nitrate) evaluated at the (twenty) points considered in the numerical simulation. $\Delta(t)$ is a measure of *how far from pseudo-equilibrium* the system remains at time t.

Figure 2 shows $\Delta(t)$ as a function of time for a number of microsites differing from that of Figure 1 only in their radius. In all cases the perturbation at time zero is a tenfold reduction in the oxygen diffusion constant. We may define the *microsite relaxation time* as that period within which $\Delta(t)$ falls to 5%. Figure 2 indicates relaxation times of around one hour for the $5x10^{-3}$m microsite, sixteen hours for the 10^{-2}m and $2x10^{-2}$m microsites, and eight hours for the $5x10^{-2}$m microsite. The largest microsite has a relatively low relaxation time because even in its initial state it contains a significant anaerobic fraction - anaerobism does not have to develop before denitrification can begin. It should be noted that these relaxation times probably represent upper limits; no perturbation in the field is likely to be as severe as that considered here.

DISCUSSION

Most published models of denitrification assume a state of pseudo-equilibrium in the soil. Departure from pseudo-equilibrium may arise from a number of causes.

1) The measured flux at the surface may not always be in step with the production rate at depth. This question, which bedevils any attempt at empirical field-scale modelling, is discussed by Jury *et al* (1982). Mechanistic

models focussing on microsite-scale phenomena are not affected, however, since the aim of such models is simply to predict $J_i(t)$ as a function of $x_i(t)$, $y_i(t)$ and $z_i(t)$: the relationship between $J_i(t)$ and the flux at the soil surface $J_F(t)$ would be a suitable topic for a secondary modelling exercise, but it is not really important so long as all the gaseous N evolved eventually reaches the atmosphere.

2) Microbiological adjustments take time. More work remains to be done to evaluate this: how rapidly do soil microbes respond? Evidence from laboratory incubations suggests that in many cases the transition from oxygen to nitrate reduction is rapid - the relevant enzymes are already present when the transition to anaerobic conditions occurs. Subsequent alterations in enzyme synthesis and microbial populations may be more gradual.

3) The physical structure of the microsites in which denitrification occurs may itself cause a significant delay. The work presented here suggests that the response of a spherical microsite smaller than 10^{-2}m in radius is in fact fairly rapid (under sixteen hours, even where the disturbance is as severe as an instantaneous tenfold reduction in the intra-microsite gaseous diffusion constant). It may therefore be concluded that a steady-state model of microsite denitrification is adequate provided no major microbiological changes occur, and the soil contains few large aggregates. Where soil structure is a major factor a transient model of denitrification (even at the microsite scale) would probably be more appropriate.

ACKNOWLEDGEMENT

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FUTURE RESEARCH NEEDS IN DENITRIFICATION

A.R. Mosier[+)]

Nitrogen is the element that limits primary production in many ecosystems. Consequently, inputs of available N are often necessary to attain desired levels of plant production. Nitrification of ammonium and denitrification of nitrate are important in both natural and agroecosystems, and can result in substantial production and loss of N through volatilization of NO, N_2O and N_2. Denitrification in the plant root zone of a soil is undesirable because it reduces N use efficiency. Once the nitrate moves out of the root zone then denitrification may be desirable in order to prevent NO_3^- contamination of water resources. Conditions may, however, be more favorable for denitrification in the root zone than deeper in the soil profile. Therefore NO_3^- leached below the root zone may contaminate ground and surface waters.

When denitrification does occur, the reaction sequence may not proceed to completion, ie. may not form only N_2. Varying amounts of NO and N_2O may also be produced and evolved from the soil to the atmosphere. The NO_x gases affect atmospheric chemistry while N_2O acts as both a greenhouse gas and plays a role in stratospheric ozone depletion. Because NO_x and N_2O are produced, it is necessary to know the ratios of production of these gases to N_2 as well as the total amount of N denitrified.

Denitrification is controlled by a number of physical and chemical factors such as temperature, pH, organic carbon, nitrate concentration and oxygen. The effect of each of these parameters on the process is well known. Whether or not denitrification is important in a particular system is determined by the interaction of the various factors. It is our lack of understanding of these complex interactions that limits our ability to effectively manage denitrification in soil systems.

The current state of knowledge about denitrification and where research on the topic needs to be directed can be discerned from our ability to answer the following basic questions:

1. Can we, for a specific agricultural or natural ecosystem, accurately predict how much of the N applied to that system, whether through atmospheric deposition, biological N fixation, or N fertilizer addition, will be denitrified?
2. For any selected ecosystem, can we say what the ratio of N_2O/N_2 will be when denitrification does occur?
3. Do we know the relationship of denitrification to N leaching and the rest of the N cycle?

+) USDA-ARS, P. O. Box E, Ft. Collins, CO 80522, USA

4. For natural ecosystems, can we predict the effects of high rates of N deposition on the cycling of nutrients (C,N,P,S...) within the ecosystem?
5. Do we know the effect of changing land use, particularly in the tropics, on denitrification and N_2O/N_2 ratios of the gases produced?
6. Most total denitrification and N_2O flux measurements that have been made are point source determinations. Can we quantitatively scale up to field, to landscape, to regional areas based upon these point source measurements?
7. Is sufficient field data available to permit development of quantitative, predictive models for denitrification?

The answer to all of these questions is an unequivocal no!

This is not to say that significant progress has not been made in some areas. We know, for example, the factors which can regulate denitrification, specific microorganisms which mediate the process, and microorganisms which produce N_2O or N_2 as an end product. Research technology is advancing so that new techniques can be applied to find answer for these questions.

Increasing quantities of mineral N compounds are being introduced into our environment through combustion, N-fixation, fertilizer production, and increased native soil N mineralization rates caused by changing land use (ie, conversion of tropical forests to pastures). These changes amplify the environmental pressures generated by increased NO_3^- leaching and NO and N_2O production. The urgency of problems associated with degradation of the quality of water supplies, increases in atmospheric ozone, global climate change and stratospheric ozone depletion necessitate an increased effort to understand denitrification in natural and agricultural ecosystems.