Nitrogen removal by denitrification in the sediments of a shallow lake.

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Nitrogen removal by denitrification in the sediments of a shallow lake

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Stellingen

1 De gevolgen van vele jaren falend mestbeleid zijn terug te vinden in de eutrofe waterbodems van de Nederlandse plassen en meren. Herstel kan niet van vandaag op morgen worden verwacht.

2 De koppeling tussen nitrificatie en denitrificatie is niet afhankelijk van de nitraatconcentratie in het bovenstaande water.
dit proefschrift

3 Eutrofiëring is een zichzelf versnellend en handhavend proces zodra de nitrificatie geremd wordt door een zuurstoftekort.
*Kemp W.M., Sampou P., Caffrey J. and Mayer M. (1990) Limnol. Oceanogr. **35**: 1545-156*
Het optreden van gekoppelde denitrificatie kan, als de externe N- en P-belasting voldoende wordt gereduceerd, dit proces doorbreken en juist het herstel versnellen.
dit proefschrift

4 Hoewel het gecombineerde effect van temperatuur en afbreekbaar organisch materiaal belangrijk is voor bentische mineralisatieprocessen, wordt hiernaar weinig onderzoek verricht.
dit proefschrift

5 Holle vaten klinken het hardst; lekke vaten 'denitrificeren' het hardst.

6 Hades, de god van de Griekse onderwereld, bleek geen hulp bij het ontrafelen van de stikstofprocessen in eutrofe waterbodems.

7 "De blanke man behandelt zijn moeder, de aarde, en zijn broeder, de lucht, als koopwaar die hij kan uitbuiten en weer verkopen als goedkope bonte kralen. Zijn honger zal de aarde kaal vreten en slechts woestijn achterlaten".
Opperhoofd Seattle van de Duwamish

8 Indien politici meer naar de lange dan naar de korte termijn zouden kijken, kan het dreigend tekort aan zoet water door verslechtering van de waterkwaliteit en onzorgvuldig beheer wellicht worden voorkomen.

9 Het begrip 'duurzaam' is door veelvuldig misbruik een waarden-loos begrip geworden.

10 AIO's passen niet goed binnen de Westerse economie: op het moment dat ze na grote investering rendabel worden, worden velen afgeschreven waardoor veel kennis verder onbenut blijft.

11 Het is jammer dat de invoer van deeltijdwerk nog steeds zoveel weerstand ondervindt. De werkgelegenheid neemt immers toe, veel ongebruikte creativiteit en kennis wordt benut en de werkende krijgt meer tijd voor zichzelf.

12 Terwijl het aantal koeien in Nederland kleiner wordt, neemt de heilige koe nog steeds in aantal toe: slechts weinig mensen, ook 'milieudeskundigen', zijn in staat deze te laten staan.

13 De door Word Perfect voorgestelde correctie voor 'Veluwemeer', Vuilemmer, is achterhaald.
Word Perfect 5.1

14 Het oplossen van het stik-stofprobleem is een zaak van lange adem.

15 In een zwarte Schimmel zit muziek.

Stellingen behorende bij het proefschrift "Nitrogen removal by denitrification in the sediments of a shallow lake" van Francien van Luijn.

Wageningen, 15 januari 1997

CONTENTS

		page:
1.	Introduction.	1
2.	Comparison of denitrification rates in lake sediments obtained by the N_2 flux method, the ^{15}N isotope pairing technique and the mass balance approach.	13
3.	Why dinitrogen fluxes from freshwater sediments?	31
4.	Nitrogen fluxes and processes in sandy and muddy sediments from a shallow eutrophic lake.	37
5.	Variation in N fluxes from sediments of a freshwater lake.	55
6.	Influence of benthic diatoms on the nutrient release of shallow lakes recovering from eutrophication.	69
7.	Interpretation of experimental N losses to the field situation.	81
8.	General discussion	99
	References.	111
	Summary.	121
	Samenvatting.	125
	Dankwoord.	129
	Curriculum vitae.	130

Aan mijn ouders, Sjoerd en Harold.

Chapter 1
Introduction

GENERAL INTRODUCTION

One of the main problems in water management is eutrophication. Eutrophication is defined as the enrichment of surface waters with nutrients (Vollenweider, 1968). Of these nutrients nitrogen (N) and phosphorus (P) are important controlling factors for the primary production, in both marine and freshwater ecosystems (Keeney, 1973). The excessive anthropogenic loading of surface waters with N and P therefore often leads to an increased algal growth and enhanced standing crop. High concentrations of algae result in a decrease of the water transparency. Because of the decreased light intensity growth of water plants can decrease and the plants even may disappear. Further, this can result in a sudden depletion of dissolved oxygen in the water and fish kills can occur. Furthermore the algal species composition may change and some algae blooms can produce toxins (Rijkswaterstaat, 1989). The use of the surface water by humans for various functions like e.g. fishery, drinking water and recreation becomes difficult or impossible and the ecological value of the surface waters reduces. As good freshwater is an essential resource and its supply is limited, protection of good water systems and restoration of surface waters with a poor water quality are needed.
In the Netherlands most shallow freshwater lakes are highly eutrophicated. Since many years the most common strategy for restoration of these eutrophic surface waters is the reduction of the P loading (Rijkswaterstaat, 1989), because phosphate is considered to be the natural growth limiting factor in the temperate regions in freshwaters (Schindler, 1977). The success of the recovery of lakes after reduction of the phosphorus loading however is frequently limited. A major reason is the release of phosphorus from the sediments (Mortimer, 1941 and 1942; Van Liere and Gulati, 1992). Because of the unsatisfactory results of the P reduction and because in some lakes the phytoplankton growth is related to nitrogen availability during parts of the year (Boers and Van der Molen 1993) renewed interest is directed to the fate of nitrogen in surface waters. Nitrogen now is one of the main points of attention for water management in the Netherlands.
In marine ecosystems, including the North Sea, nitrogen is considered to be the controlling factor for algal growth (Ryther and Dunstan 1971). To diminish the N-

loading to the sea, the N-loading of the freshwaters draining into this sea has to be reduced. The international programs for the North Sea and the river Rhine therefore are another reason for the interest in nitrogen. In these programs attention is paid to the reduction of loadings by e.g. industries, urban runoff and diffuse sources. The purpose is to reach integrated protection and restoration of the aquatic ecosystems by considering and controlling all sources (Rijkswaterstaat, 1989). Reduced N-loadings however will not only effect the input into the sea. The N fluxes and mass balances in the freshwaters and its sediments also will change and the primary production may be limited as well. At reduced nitrogen concentrations in the overlying water cyanobacteria may form a complicating factor in the restoration of waters, as these bacteria can introduce nitrogen into the system by N_2 fixation (Boers and Zevenboom, 1992).
When the external N-loadings are reduced, the benthic processes (forming the internal loading) define more and more the N-loading and N concentrations of the overlying water. Most of the processes in the nitrogen cycle are performed by bacteria. The magnitude of these bacterial processes and the contribution of the various reactions to the total N flux are largely unknown and so are the effects of reduction of the N-loading on the Dutch surface waters. One of the questions to be answered is whether N reduction will lead to an improvement of the eutrophicated lakes or will the situation remain unchanged because nitrogen compounds are released by the sediments to the overlying water and consumed by the algae.
The objective of this thesis therefore is to investigate the important nitrogen processes, particularly the processes which can cause N losses from the sediment-water system and to quantify these processes and the related fluxes to the overlying water. Primarily this will be considered in laboratory experiments where no nutrients are available in the overlying water. This knowledge will give a deeper understanding of the possibilities of diminishing the eutrophication by reduction of the N-loading to the surface water.
This introduction gives furthermore a brief general review of the (microbial) processes of the nitrogen cycle in aquatic systems and a description of the studied area Lake Wolderwijd/Nuldernauw. The chapter concludes by an outline of the thesis.

THE N-CYCLE IN THE SEDIMENT-WATER SYSTEM.

In aquatic systems nitrogen is found in several forms. Most common are the inorganic compounds (NH_4^+, NO_3^- and NO_2^-), PON (particulate organic nitrogen), DON (dissolved organic nitrogen), N in the biota and N_2. These forms of nitrogen are interconnected by microbial processes in the nitrogen cycle.

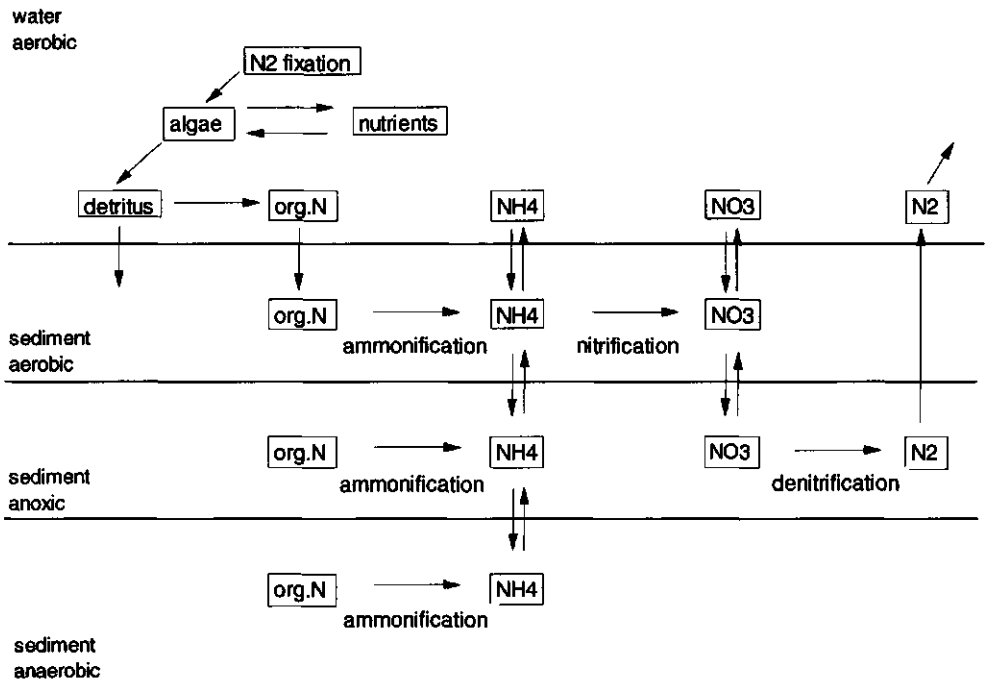

Fig 1. Simplified scheme of the various microbial N processes in a sediment-water system.

Some of these processes are performed by strictly aerobic bacteria (nitrification), others by strictly anaerobic bacteria (nitrate ammonification) and a number of processes can be performed by both, aerobic and anaerobic micro-organisms (ammonification, NH_4^+/NO_x^- assimilation, N_2 fixation). The aerobic and anaerobic processes take place in various parts of the sediments. Figure 1 is a simplified scheme of the N processes. It is assumed that the sediment can be divided in an aerobic layer, where O_2 is the final electron acceptor and an anaerobic layer with various electron acceptors like NO_3^-, Mn, Fe and SO_4^{2-}. *In situ* the relations are more complex as the redox gradients are not one-dimensional but often occur in a 3 dimensional matrix, e.g. because anoxic microsites are part of the oxic layer (Jahnke, 1985). Depending on the environmental conditions temperature, presence of electron donors and acceptors, pH and redox potential, various species of micro-organisms are active in the decay of organic N and further conversion to N_2, NH_4^+ and NO_x^- fluxes to the overlying water. In the water algae grow by assimilating these inorganic N compounds. After die-off and sedimentation of the produced biomass, the organic matter becomes available for the micro-organisms again. Additional extra nitrogen can enter the sediment-water N cycle by N_2 fixation. The main processes of the N cycle are described hereafter.

N_2 fixation

Several cyanobacteria (blue green algae) are capable to fix N_2. The most common species are the heterocystus genera *Aphanizomenon sp., Anabaena sp.* and *Nostoc sp.* (Schreurs, 1992; Paerl, 1990). By the fixation atmospheric nitrogen (N_2) is reduced to NH_4^+ with help of the enzyme nitrogenase and incorporated in organic matter (Kamp Nielsen and Andersen, 1977). As this requires much energy, uptake of inorganic N is preferred (Howard-Williams, 1985). It is therefore not surprising that in most studies dominant populations of N_2 fixing bacteria are found only when nitrogen is limiting (Schreurs, 1992). It remains however difficult to predict under what ammonium/nitrate concentrations N_2 fixation can be expected. It seems that besides the nitrogen concentrations in the overlying water, the availability of phosphorus is an important factor too (Pearl, 1988; 1990; De Nobel et al., 1995).

Algal growth

Nitrogen is one of the main nutrients needed for algal growth. Algae can grow on both organic and inorganic nitrogen (Forsberg, 1975; Keeney, 1973). From the inorganic nitrogen most algae prefer ammonium, as for the reduction of nitrate extra energy is needed. The nitrate has to be converted into ammonium before incorporation can take place. Photosynthesis generally is the major energy source, but heterotrophic growth also occurs (Keeney, 1973).

Ammonification

A large part of nitrogen in sediments is particulate organic nitrogen (PON). In the mineralization process the proteins of PON are split into peptides and amino acids. In the following step in the decay of the organic matter ammonium is set free. This process is called the ammonification. Larger organic molecules however are not always reduced to ammonium directly. Sometimes intermediates are formed. When these intermediates are not further reduced accumulation may occur. Accumulation of these intermediates happens more frequently under anaerobic than under aerobic conditions (Zehnder, 1988). Ammonification is performed by heterotrophic bacteria which use the organic matter as an energy source. Only when the organic matter contains more nitrogen then is needed for the bacterial growth, ammonium is released (Patrick, 1990). Ammonification starts already in the water phase during the sedimentation and proceeds within the sediments, under both aerobic and anaerobic conditions. Highest rates however are generally found at the sediment-water interface (Klump and Martens, 1983). Rates of ammonification are effected by temperature and by the sedimentation flux of organic matter.

Nitrification.

Nitrification is the biological oxidation of NH_4^+ to NO_2^- and NO_3^-. The nitrifiers can be divided into two groups, the autotrophs and the heterotrophs. The distinction is

made because of their manner of energy generation. Autotrophic nitrification occurs in two steps (Focht and Verstraete, 1977):

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O \quad (1)$$
$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \quad (2)$$

The oxidation of ammonium is generally performed by bacteria of the genus *Nitrosomonas*, whereas the second reaction is generally performed by bacteria of the genus *Nitrobacter*. Both bacterial groups are aerobic and chemo litho autotroph; the energy from the oxidation of the substrate (inorganic N compounds) is used for the assimilation of CO_2. Heterotrophic nitrifiers can oxidize organic as well as inorganic nitrogen compounds. The oxidation of nitrogen however is not the sole source of energy (Painter, 1970) and for growth an additional external organic energy source is needed (Van Niel, 1991). Although at low oxygen concentrations the heterotrophs grow better (Henriksen and Kemp, 1988), the autotrophic nitrification is often more important (Henriksen et al., 1981). Furthermore methanotrophs, which gain energy for growth from the oxidation of methane, are able to nitrify as well. Under certain, as yet unknown conditions they even may contribute substantially to the nitrification (Bédard and Knowles, 1989; Roy and Knowles, 1994). Methanotrophs are able to nitrify because they contain the enzyme monooxygenase. This enzyme catalyses the oxidation of ammonium in nitrifying bacteria. In methanotrophs monooxygenase is normally used for the oxidation of methane, but it can also be used for the oxidation of ammonium. The methanotrophs however do not gain energy from the latter reaction and attempts to grow methanotrophs on ammonium in the absence of methane have failed (Bédard and Knowles, 1989; Bosse et al., 1993).

Several factors effect the (autotrophic) nitrification:

- The optimal temperature for nitrification in suspensions ranges from 25-35°C, but growth occurs from 3-45°C (Focht and Verstraete, 1977). Henriksen and Kemp (1988) suggested that the nitrifying bacteria are able to adapt to low temperatures and that the optimum temperature and growth ranges for nitrification in sediments depends on the ambient temperature regimes.
- The oxygen concentration highly influences the nitrification. At the same time the nitrification itself has a significant effect on the total oxygen consumption and thus on the oxygen concentration (Klapwijk and Snodgrass, 1982). The oxygen dependency of the nitrification follows Michaelis-Menten kinetic. The Michaelis-Menten equation is the basic expression used to describe enzymatic reactions (Berner, 1980). In suspensions the K_m value (Michaelis constant; the substrate concentration at which the rate of the catalyzed reaction is half of the maximum rate) for *Nitrosomonas* is about 16 µM O_2 and for *Nitrobacter* about 62 µM O_2 (Focht and Verstraete, 1977). Heterotrophic nitrifying bacteria are better adapted

to low oxygen concentrations (K_m < 3µM O_2; Kuenen and Bos, 1989) and also methanotrophs are able to oxidize methane at lower oxygen concentrations. Therefore the autotrophic nitrifying bacteria may loose the competition for oxygen at the sediment-water interface by better adapted bacteria. The availability of oxygen further determines which products are formed. The bacteria of reaction (1) are better adapted to low oxygen concentrations than the bacteria of reaction (2), what can result in the accumulation of NO_2^- at low oxygen concentrations (Focht and Verstraete, 1977). Whereas generally reaction (1) is the rate limiting step and no accumulation of NO_2^- occurs (Henriksen and Kemp, 1988), at low oxygen concentrations the intermediates NH_2OH, NO and N_2O formed by the bacteria of reaction (1) will accumulate (Goreau, 1980). Several of these trace gases play a role in the depletion of stratospheric ozone. The production of these intermediates thus is a source of greenhouse gases (Rogers and Whitman, 1991).

- In general also for the substrate, NH_4^+ respectively NO_2^- the Michaelis Menten kinetic is followed. The K_m values however have a wide range (70-700 µM for pure cultures and sewage treatment systems; 0.1 µM for open oceans; 7-80 µM for estuarine sediments), probably because the substrate affinity depends largely on the ambient nutrient concentrations (Henriksen and Kemp, 1988).
- The optimal pH range is 7 - 8, whereas the limiting range is 6 - 9.5 (Focht and Verstraete, 1977). The upper limit is due to the concentration of free NH_3, which is toxic to the bacteria (Anthonisen et al., 1976).

Denitrification

Denitrification is the dissimilatory reduction of oxidized nitrogen into gaseous oxides or dinitrogen by facultative anaerobic organisms:

NO_3^- -> NO_2^- -> NO -> N_2O -> N_2.

Not all denitrifiers are capable of reducing nitrate to N_2. Some reduce nitrate to nitrite or N_2O only or are incapable to reduce nitrate, requiring nitrite for denitrification. Thus many various denitrifiers exist. Mostly these bacteria are heterotrophic and gain their energy from the oxidation of organic matter. The organisms prefer oxygen as an electron acceptor. This is not amazing because respiration of oxygen delivers 50% more energy than the respiration of nitrate (Wiltshire, 1992). If oxygen is available no NO_x^- (nitrate and nitrite) is used. If no oxygen is available enzymes for NO_x^- reduction are formed and a nitrogen oxide acts as the terminal electron acceptor for the respiration (Knowles, 1982). Robertson et al. (1988) worked with *Thiosphaera pantotropha*, a heterotrophic nitrifier and aerobic denitrifier. Under fully aerobic conditions ammonium is converted via NO_2^- to N_2. In these bacteria the enzymes for reduction of nitrogen oxides are active under both anaerobic and aerobic conditions. If the oxygen

concentration is 30-80% saturation both oxygen and nitrogen oxides are reduced. When the oxygen concentration drops below 30% of air saturation the nitrogen oxides are preferred. These heterotrophs are favoured by fluctuating or limiting oxygen concentrations (Kuenen and Robertson, 1988).
In general however, denitrification occurs under anaerobic conditions if enough organic matter is available. Depending on the end product of the denitrification, denitrification can also act as a source for "greenhouse" gases. Which end products are formed depends among others on the following environmental factors:

- Denitrification occurs between 2 - 60°C. The optimal temperature is 20-25°C (Jones, 1985) but in aquatic sediments the denitrification rates vary only slightly between 5 - 23°C (Knowles, 1982). At lower temperatures the end product is often N_2O (Keeney, 1973).
- The optimum pH for denitrification ranges from 7-8, with higher rates at higher pH (Knowles, 1982). At lower pH the enzymes for N_2O reduction are suppressed hence more N_2O is formed, being the sole end product at pH 4.0 (Knowles, 1982). Moreover N is lost at low pH due to the abiotic reaction of NO_2^- to NO, N_2O, N_2 and N_2O_4 (Knowles, 1982).
- At low concentrations the denitrification rate depends on the NO_3^- concentration, following first order kinetics (Knowles, 1982). At higher concentrations, however, the rate becomes independent of the nitrate concentration.

Nitrate reduction to ammonium

Another dissimilatory reaction is performed by some obligate anaerobic bacteria. These fermentative bacteria reduce NO_3^- to NH_4^+ with the intermediate NO_2^- (Koike and Sørensen, 1988). This process occurs under similar conditions as the denitrification and therefore competition for nitrate and organic matter takes place. In most sediments the denitrification is favoured (Tiedje et al., 1982). The contribution of the nitrate reduction to ammonium on the total nitrate reduction, however, is largely unknown as only a few studies on this process have been carried out.

TRANSPORT PROCESSES

Besides the various reactions transport is an important factor too. The various biotic and abiotic reactions in the sediments result in steep concentration gradients of dissolved components near the sediment-water interface. The concentration gradients are the driving force for the diffusion of the dissolved species involved. Diffusive and advective transport processes generate fluxes of dissolved species across the sediment-water interface. In general advective transports like compaction, (groundwater) infiltration and seepage are of minor importance in the

sediment-water system as compared to the diffusive flux (Berner, 1980). In models simulating the fluxes between sediment and overlying water the advective transport therefore is frequently omitted. However, in the shallow Dutch freshwaters infiltration and/or seepage can be important. The dimensionless Peclet number (D_m = L*U, with D_m: molecular diffusion coefficient, L: average distance of migration and U: interstitial water flow velocity) is a simple criterion to estimate whether or not advective transport is important in relation to the molecular diffusion. If $D_m >> L*U$, diffusion is the major transport process (Berner, 1980).
The diffusion rates in the interstitial and overlying water respectively differ markedly (Davison, 1985). In the interstitial water and in the diffusive boundary layer the transport occurs mainly by molecular diffusion, whereas in the overlying water the much greater turbulent Eddy diffusivity is dominant. The diffusive boundary layer is a thin layer of water adjacent to the sediment. It is formed due to the decrease and approach to zero of the velocity fluctuations towards the sediment surface (Berner, 1980). The exchange rate of dissolved species then depends on the thickness of the diffusive boundary layer (Berner, 1980; Revsbech et al., 1986). The thickness of this layer is influenced by the turbulence of the overlying water (Jørgensen and Revsbech, 1985). The diffusion of dissolved components in the diffusive boundary layer can be described by analogy of the diffusion in free water according to the second law of Fick (Berner, 1980):

$$\frac{\delta C}{\delta t} = D_m * \frac{\delta^2 C}{\delta z^2}$$

C	concentration
t	time
D_m	molecular diffusion coefficient
z	depth coordinate

In the interstitial water of the sediments the diffusion path for the molecules is lengthened because of the sediment particles. This effects the diffusion rates in the interstitial pore water. A measure for the length of the effective path of diffusion compared with the direct path of diffusion is tortuosity. Tortuosity is difficult to measure, but it can be derived indirectly from the electrical resistivity factor, which on its turn is related to porosity (ϕ). The apparent diffusion coefficient (D_s) for the interstitial water then can be estimated with:

$$D_s = \phi^n * D_m$$

with *n* a number between 1 and 2; lower for sand and higher for porous mud (Ullman and Aller, 1982).
So the fluxes at the sediment-water interface result from interaction of biological, chemical and physical processes.

STUDY AREA

The lakes studied were lake Wolderwijd and Nuldernauw. These lakes were created in 1968 when the polder Flevoland was made and separate the 'old' land and this new polder (Figure 2). The surface of the lakes is 2670 ha: 1800 ha for Lake Wolderwijd, 870 ha for Lake Nuldernauw. The lakes are shallow (mean depth 1.60 m) and are traversed by a shipping canal (about 200 m across and 4.0 m deep). One year after the lakes were formed the transparency was good and macrophytes were abundant (Meijer et al., 1994). In the period 1970-1981 the water quality became very poor. The transparency became low, the submerged vegetation disappeared and during this time the water became dominated by blue-green algae (Meijer, 1989).

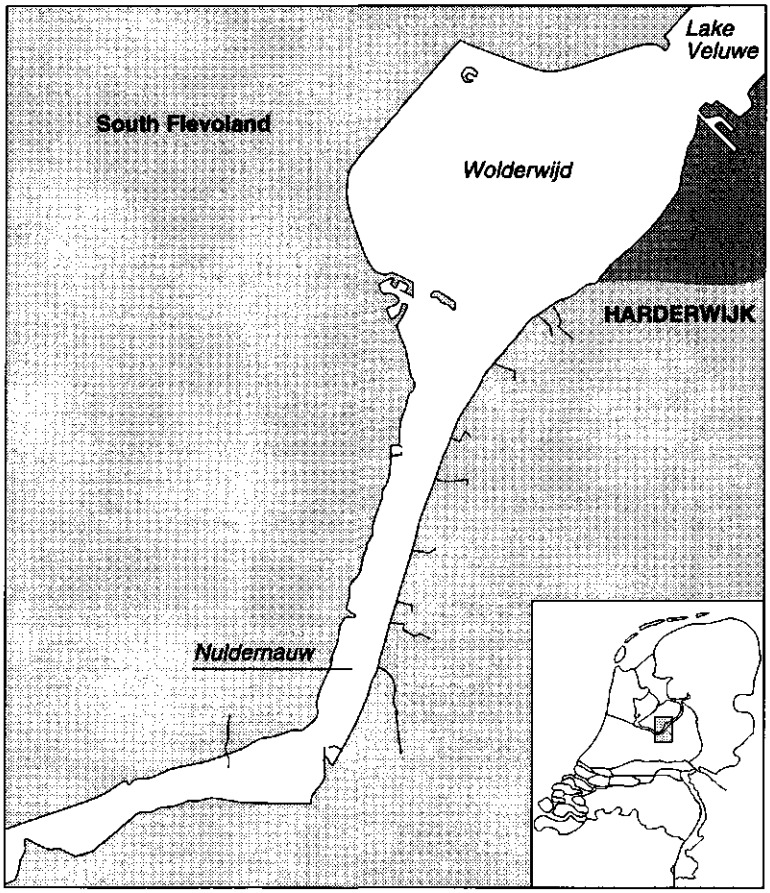

Fig. 2. Sketch of Lake Wolderwijd/Nuldernauw .

In the eighties the nutrient loading was reduced and the lake was flushed with water poor in phosphorus. From 1982 onwards the water quality improved. Parts of the year green algae and diatoms appeared and chlorophyll-a decreased significantly (Meijer, 1989). The lake however remained quite turbid and additional measures were taken. In 1990 a biomanipulation project was started; the fish stock was reduced substantially (Van Nes *et al.*, 1992). Just after the fish stock reduction the transparency of the water improved enormously and it was expected that the water quality would improve definitely. In advance of and during this biomanipulation project many parameters were monitored intensively, in order to enhance insight in the functioning of the ecosystem. As furthermore the nutrient loadings of the lakes were low, the present study was performed in the shallow freshwater lakes Wolderwijd and Nuldernauw.

The two lakes have a different nutrient loading. For Lake Wolderwijd seepage and rainfall are the most important nutrient input terms whereas for Lake Nuldernauw these are rather the small streams and a polder outlet. For the nitrogen loading in summer dry deposition is important too.The total N-loading on lake Wolderwijd is 8 g N m^{-2} y^{-1} in average if no flushing occurs. With flushing the loading nearly doubles due to the higher nitrate concentrations in the flushing water. The total N-loading on lake Nuldernauw is higher and more variable (about 25 g N m^{-2} y^{-1}). The influence of flushing is less clear (Meijer and Hosper, 1994).

The loadings result in a total nitrogen concentration of 2 g N m^{-3} in average (Van Ballegooijen and Van der Molen, 1994). In this nitrogen concentration a seasonal pattern can be observed. The ammonium concentration is always low (0 - 0.3 g N m^{-3}), with higher values in winter. Nitrate concentrations range from 0 g N m^{-3} in summer to 1.5 g N m^{-3} in average in winter. The total nitrogen concentration remains quite stable as the sudden decrease in nitrate in spring is compensated by an increase in organic N (algae). The phosphorus loading on lake Wolderwijd is 0.2-0.5 g P m^{-2} y^{-1} and also higher when flushing takes place. The phosphorus loading on lake Nuldernauw is 2.5 g P m^{-2} y^{-1}. The total P concentration in the lakes is 0.1 g P m^{-3} in average (Van Ballegooijen and Van der Molen, 1994).

OUTLINE OF THIS THESIS

In Chapter 2 three methods used in this thesis for the measurement of the denitrification (the N_2 flux method, the mass balance approach and the ^{15}N isotope pairing technique) are described and compared.

In Chapter 3 the possible sources of anoxic N_2 fluxes observed with the N_2 flux method and the possible mechanisms and consequences of these N_2 fluxes are discussed.

With the N_2 flux method furthermore the influence of temperature, organic matter content and season on the N fluxes from muddy and sandy sediments was examined (Chapter 4).
Chapter 5 describes the spatial variability of the nitrogen fluxes throughout the lake and the possible relations with sediment characteristics. In the shallow parts of the lake benthic algae occur.
Chapter 6 deals with the influence of these benthic algae on the release of nutrients, especially of nitrogen.
In Chapter 7 the results of the laboratory experiments are translated to the field situation, with special attention for the N losses due to the coupled denitrification.
Chapter 8 compares the results with literature data and discusses the presence of microsites and the dependence of the coupled denitrification from the nitrate concentration in the overlying water. Also model requirements for the analysis and interpretation of laboratory observations and for a predictive simulation tool for the field situation are addressed in this chapter and it ends with the conclusions.

Chapter 2
Comparison of denitrification rates in lake sediments obtained by the N_2 flux method, the ^{15}N isotope pairing technique and the mass balance approach.

ABSTRACT

Coupled nitrification-denitrification rates in sediments of the shallow lake Nuldernauw, The Netherlands, were measured using the N_2 flux method and the ^{15}N isotope pairing technique. It was clarified by various checks that the coupled nitrification-denitrification can be measured with the N_2 flux method after a pre-incubation time of about 10 days. These checks included tests on contamination by atmospheric nitrogen, the incubation of irradiated sediments with and without addition of $HgCl_2$ and addition of nitrate.

The results of the two methods were different. In general the coupled nitrification-denitrification rates measured with the ^{15}N isotope pairing technique were lower than measured with the N_2 flux method. With the ^{15}N isotope pairing technique the denitrification rates of muddy sediments seemed to depend on the sampling date: spring sediments gave much higher denitrification rates than sediments collected in winter.

In comparison with the denitrification calculated from a mass balance of the lake the ^{15}N method appears to underestimate the denitrification, whereas results of the N_2 flux method were in agreement with mass balance data. The following explanation for this difference was suggested. The ^{15}N isotope pairing technique is based on the idea that the various nitrate species ($^{14}NO_3^-$ and $^{15}NO_3^-$) are uniformly mixed and that the sediment is divided in aerobic and anaerobic layers. If however microsites exist, probably uniform mixing of the nitrate species cannot be assumed because of the very tight coupling between nitrification and denitrification in these sites. The coupled nitrification-denitrification will be underestimated.

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NOMENCLATURE

C = N_2 concentration (mol m^{-3})
D_m = molecular diffusion coefficient (m^2 h^{-1})
D_s = apparent diffusion coefficient (m^2 h^{-1})
D14 = coupled nitrification-denitrification (µmol N m^{-2} h^{-1})
D15 = denitrification of nitrate from the overlying water (µmol N m^{-2} h^{-1})
z = depth coordinate

INTRODUCTION

In aquatic ecosystems, both marine and freshwater, N is an important controlling factor for algal growth (Ryther and Dunstan, 1971; Keeney, 1973). Various microbial N processes in the sediment like ammonification, nitrification and denitrification influence the availability of N for algal growth in the overlying water. Especially denitrification is an important process as it removes N from the system to the atmosphere as gaseous dinitrogen. The denitrifying bacteria in the sediments can reduce both nitrate diffusing from the overlying water as well as nitrate formed by nitrification within the sediment (Nielsen, 1992).
Several methods to measure denitrification rates have been used and reported. These include: N mass balance approach (Knowles, 1982), consumption of nitrate (Andersen, 1977), acetylene inhibition (Revsbech et al., 1988), ion selective micro-electrodes for NO_3^- (De Beer and Sweerts, 1989), ^{15}N tracer techniques (Goeyens et al., 1987; Nishio et al., 1983; Nielsen, 1992) and the direct measurement of the N_2 flux (Seitzinger et al., 1980; Devol, 1991). Only two of these techniques allow measurement of the coupled nitrification-denitrification, namely the N_2 flux method and the ^{15}N isotope pairing technique. With the N_2 flux method the increase in N_2 concentration due to denitrification is measured after sediment incubation in a gastight chamber. Before incubation the background N_2 in the headspace is lowered by purging with a $He/O_2/CO_2$ gas mixture (Seitzinger, 1980). With the ^{15}N isotope pairing technique both denitrification of nitrate from the overlying water and coupled nitrification-denitrification are measured. To this end $^{15}NO_3^-$ is added to the overlying water of sediment cores. It diffuses into the denitrification zone, meanwhile mixing with the natural $^{14}NO_3^-$. After a short incubation time $^{30}N_2$ and $^{29}N_2$ are measured on a ratio mass spectrometer and the various denitrification rates can be calculated (Nielsen, 1992; Rysgaard et al., 1993).
This study is part of a research on nitrogen exchange between sediment and water in the shallow lakes Wolderwijd/Nuldernauw. In this study the N_2 flux method and the ^{15}N isotope pairing technique are used. Purpose of the study presented here is to examine the potentials and limitations of the N_2 flux method in measuring coupled

nitrification-denitrification in aquatic sediments and to compare the results of this method with results of the ^{15}N isotope pairing technique and with denitrification rates estimated from the nitrogen mass balance of lake Nuldernauw. Further experiments with the N_2 flux method, dealing with several incubation temperatures, sediments sampled in various seasons and from several locations are in progress and will be published elsewhere.

MATERIALS AND METHODS

Study area and sediment collection

Intact sediment cores were collected between April 1992 and May 1994 from lake Nuldernauw, The Netherlands, a lake with a mean depth of 1.60 m and an area of 870 ha. One side is bordered on lake Wolderwijd, the other side is connected to an adjoining lake by a sluice. Inputs of water include exchange with lake Wolderwijd, a few small streams, rainfall and seepage. The outputs are the sluice, evaporation and infiltration. The bottom of the lake consists for 70 % of sandy sediments, occasionally covered with a layer of sandy clay (porosity 0.31). In the shipping channels (depth 2-4 m) the sediments are muddy and rich in freshly settled organic matter. The average porosity of the top cm was 0.87.

For comparison of both methods sediment cores from the shallower, sandy parts and from the muddy shipping channels were collected. For the testing and evaluation of the N_2 flux method only muddy sediments were used. Undisturbed sediment cores (inner diameter 56 mm) were sampled using a Beeker sampler (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). The cores were transported to the laboratory on ice to slow down the processes. In the laboratory they were prepared immediately for the various experiments.

Measurement of N_2 flux

Procedure. The coupled nitrification-denitrification was measured with a modification of the method of Seitzinger (1980). The upper 2-4 cm sediment of an intact core were pushed up into a glass incubation chamber with the same inner diameter (Figure 1), preventing as much as possible smearing and disturbance. The bottom of the chamber was closed by a flange and held together with a metal clamp. The sampling port at the top of the chamber, sealed with ground glass tap and screw cap with rubber septa, was used for flushing the headspace, replacing the water and taking gas- and water samples. After the sediment was transferred into the chamber, artificial nutrient free surface water (100-150 ml), purged for 30 min. with a gas mixture (21% O_2, 312 ppm CO_2 and He) was added carefully to the sediment until a headspace of 100 ml remained.

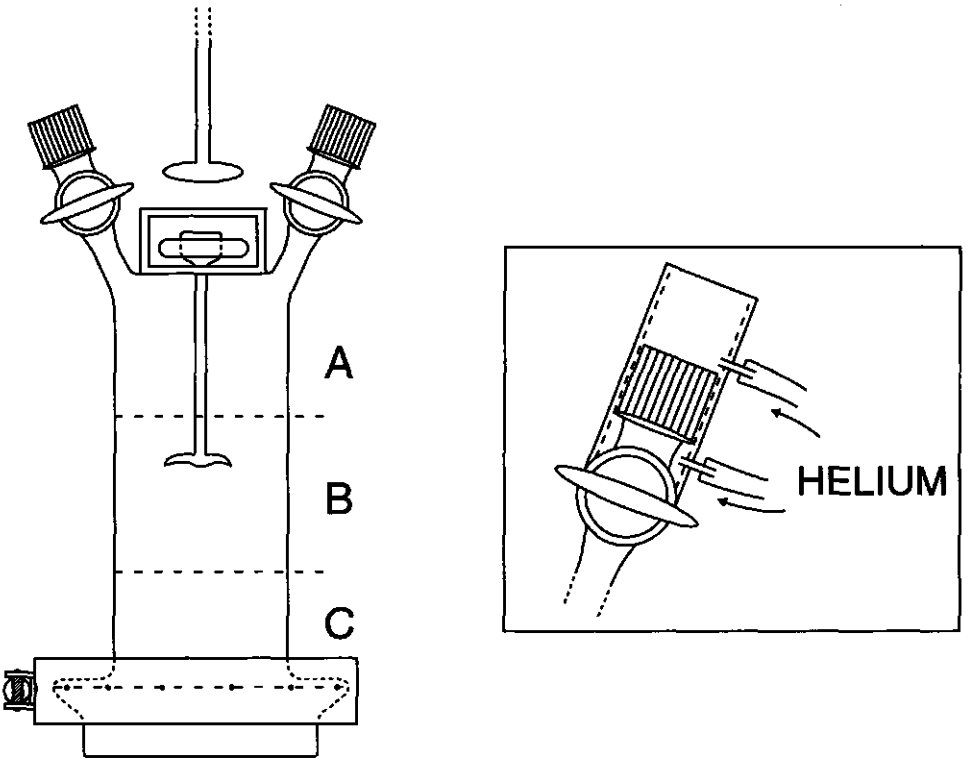

Fig. 1. *Incubation chamber.*
Insert: tap, screw cap and flushing system.

The water was replaced daily by freshly purged water and was stirred gently and continuously by a magnetic stirrer (25 rpm). After addition of the water the headspace was flushed for 1-2 hours with the same gas mixture through the sampling port using syringe needles. The N_2 concentration in the headspace was measured after the flushing and after an incubation period of about 20 hours. Hereto 4 gas samples were taken with a gastight sideport needle and analyzed immediately for N_2 on the gas chromatograph. The chamber was incubated at 20°C, in the dark. The N_2 flux from the sediment was calculated for each incubation period from the difference between the final and the initial N_2 concentrations. The influence of the incubation time between two successive measurements was checked by measuring the N_2 amount after 2, 6.5 and 22 hours. The amount of N_2 released from the sediment increased linearly with time. To avoid contamination by atmospheric nitrogen several parts of the incubation system were flushed vigorously with He before and during sampling of the

headspace and the water phase. Before opening the tap the gap between the screw cap and tap was flushed several minutes with the gas mixture (Figure 1).
While replacing the water, the headspace was flushed continuously with the gas mixture. Before sampling the headspace, the syringe was flushed with He six times and filled with He. Just above the sampling port the He was ejected to the air. After opening the tap a sample of 250 µl was taken. Before injection into the gas chromatograph 50 µl was ejected into the air. During sampling and analysis the needle, the septum on the sampling port and the injection port on the gas chromatograph were flushed continuously with He.
Before each experiment contamination of the incubation chamber by atmospheric nitrogen was checked. Chambers were used only if the background N_2 flux due to leakage was smaller than 16 µmol N m^{-2} h^{-1}.
Analyses. Gas samples were analyzed on a gas chromatograph (Hewlett Packard, 5890 series 2) equipped with a chrompack plot fused silica column (25m x 0.53mm, coating molsieve 5Å) and a thermal conductivity detector. The oven temperature was 35°C. A He flow of 50 ml/min was used. The detector was linear within the measuring range. A standard gas (21% O_2, 312 ppm CO_2, 1000 ppm N_2 and He) was used to assess the N_2 content corresponding to the measured peak areas. Spectrophotometry was used to analyze nitrate and ammonium concentrations in the overlying water on an auto-analyzer (Jagtman et al., 1992).
Experiments. With the above described method several experiments were carried out.
- Before sediments were incubated the contamination by atmospheric nitrogen was tested. Hereto the increase in N_2 concentration in the headspace (100 ml) of a chamber filled with water without N_2 was measured. The flushing gas was He instead of the gas mixture.
- To check whether the measured N_2 flux was due to denitrification, to contamination by atmospheric N_2 or to release of N_2 from the pore water, sterile sediment cores were used. The sediment was made sterile by irradiation (25 kGy). The overlying water was autoclaved and the flushing gas passed through a bacteria filter. When increasing N_2 fluxes showed that this precaution against microbial growth was insufficient, also $HgCl_2$ (1 ml of 5 mg ml^{-1}) was added. Addition of NaN_3 (final concentration 0.02%) for this purpose failed: the N_2 concentration still increased during the incubation (data not shown).
- As another check for contamination, also ensuring that carbon was not limiting the denitrification, in some experiments NO_3^- (final concentration 2.1 mg N l^{-1}) was added to the overlying water after 3 weeks of incubation. After this addition the water phase was not changed for several days. Water samples were taken and analyzed for NO_3^-. The decrease in NO_3^- concentration was compared with the increased production of N_2.

- The occurrence of coupled nitrification-denitrification was determined by adding N-serve (nitrapyrin= 2-chloro-6-trichloromethyl pyridine, final concentration 500 mg l^{-1}). N-serve inhibits nitrification, so that no NO_3^- is formed. Without nitrate in the overlying water and without nitrification there should be no N_2 production.
- In intact muddy and sandy sediment cores the N_2 fluxes were measured. As the overlying water did not contain nitrate or ammonium, only coupled nitrification-denitrification was measured. This coupled denitrification was measured in sandy and muddy sediments incubated at 2, 6, 12 and 23°C. Denitrification rates of these experiments were compared with results of the ^{15}N isotope pairing technique and with results of the mass balance of the lake.

Simulation and parameter estimation

To asses the pre-incubation period needed to degas the sediment, the diffusion of N_2 from the sediment pore water was simulated with a numerical mass balance model. Only the transport of N_2 present in the sediment to the overlying water was considered. The sediment column was conceived as a multi layered column. According to Fick's second law the diffusion of N_2 across the various sediment layers was described as:

$$\frac{dC}{dt} = D_s * \frac{\partial^2 C}{\partial z^2}$$

D_s: apparent diffusion coefficient
z : depth coordinate
C : N_2 concentration

Boundary and initial conditions were entered according to experimental conditions. The initial concentration (0.43 mol m^{-3}) in the sediment layers was based on the N_2 amount measured in the top 4 cm sediment layer (43 ± 3 µmol N_2). For this the N_2 concentration in the headspace was measured after the sediment was shaken with N_2 free water. This concentration corresponds to the concentration in equilibrium with the atmosphere. In the simulation the initial N_2 concentration in the flushed overlying water was set to zero. The thickness of each sediment layer was taken as 0.002 m and the total depth was 0.035 m. The apparent diffusion coefficient was estimated as D_m * porosity^2 (Berner, 1980). The molecular diffusion coefficient for N_2 at 20°C was set to 6.5 * 10^{-6} m^2 h^{-1} (Broecker and Peng, 1974). The porosity (0.87) was calculated from the loss of weight measured of a known volume of sediment dried for 24 hours at 105°C and assumed to be constant with depth. The fluxes between the sediment layers were calculated using a time step of one minute.

^{15}N isotope pairing technique.

Procedure. The coupled nitrification-denitrification was also measured using a modification of the method described by Nielsen (1992). In this method $^{15}NO_3^-$ is added to the overlying water. Diffusing into the sediment this nitrate will mix with

$^{14}NO_3^-$ formed in the sediment. Denitrification will form N_2 with mass 28, 29 and 30. Formation of the latter two can be measured by mass spectrometry, the formation of mass 28 can be calculated assuming random isotope pairing. The denitrification of nitrate from the overlying water (D15) and the coupled nitrification-denitrification (D14, as the overlying water did not contain $^{14}NO_3^-$) were calculated from the formed $^{14}N^{15}N$ and $^{15}N^{15}N$ (Nielsen, 1992; Rysgaard et al., 1993). For each experiment a series of cores (length 15 cm, inner diameter 56mm) was incubated. First the overlying water (about 300 ml) of the sampled sediment cores was replaced by artificial nutrient free surface water, not containing any nitrogen compounds. The series of cores was put in a larger box, where the cores were surrounded and overlaid with the same artificial surface water and incubated overnight. The water in the cores was stirred continuously by magnetic stirrers, driven by a master magnet. Next $^{15}NO_3^-$ was added to the surrounding water. After about 10 minutes of mixing, by moving the cores gently through the water in the box, the cores were closed with plastic stoppers and the incubation started. A sample of the surrounding water was taken as blank for the initial mass 29 and 30 ratio. Depending on the sediment oxygen demand and the total incubation time of the experiment the incubation of the individual cores was terminated with 20-60 min. intervals. Total incubation time of a series after nitrate addition was 1.5-7.5 h. At the end of the incubation of a sediment core, some drops of $ZnCl_2$ (concentration 1 g $ZnCl_2$ ml^{-1}) were added to reduce bacterial activity. Then the sediment and overlying water were shaken vigorously for several minutes. After settling of the sediment a water sample was taken by syringe for later analysis on single labelled ($^{14}N^{15}N$) and double labelled ($^{15}N^{15}N$) N_2 by mass spectrometry. The water samples were quickly transferred to tubes or bottles, which were opened just before and closed immediately after the transfer to prevent exchange with the atmosphere.

As our institute does not have a ratio mass spectrometer samples were send (about 10 ml water with 0.2 % $^v/_v$ $ZnCl_2$ solution) to the National Environmental Research Institute (NERI), Silkeborg, Denmark. Using the headspace technique mass 28, 29 and 30 of the water samples were measured on a isotopic ratio mass spectrometer (Europa Scientific Tracermass, UK). Samples (110 ml water with 0.2 % $^v/_v$ $ZnCl_2$ solution) were also send to the Institute for isotopic research (CIO), Groningen, The Netherlands. Here N_2 was withdrawn from the water using a cold trap. Mass 28, 29 and 30 were measured on a ratio mass spectrometer (VG-Isogas, SIRA 9).

Experiments ^{15}N experiments were carried out with sandy and muddy sediments, collected in various seasons. Several total incubation times and final $^{15}NO_3^-$ concentrations (40-120 µM) were used in separate experiments (Table 1).

Table 1. Denitrification rates (µmol N $m^{-2}h^{-1}$) obtained with the N_2-flux-method and ^{15}N isotope pairing technique. For the ^{15}N isotope pairing technique the various incubation times and final $^{15}NO_3^-$ concentrations used are listed too. D14: coupled nitrification-denitrification; D15: denitrification of nitrate from the overlying water.

		Denitrification rates				
sampling date	sediment type	N_2-flux	^{15}N-technique D14	^{15}N-technique D15	incubation time (min)	$[^{15}NO_3]$ (µM)
930921	sand[1]	42	19	35	100	40
930921	mud[1]		33	156	100	40
931102	sand[2]	92	29	29	150	40
931207	mud[2]	214	33	76	200	40
940124	sand[2]	68	23	36	510	40
940124	mud[2]		32	57	240	40
940124	sand[1]	68	17	39	510	40
940124	mud[1]		24	54	240	40
940418	mud[2]	140	57	146	190	40
940418	mud[2]		65	295	190	120
940524	mud[2]		116	537	180	60
940524	mud[2]		141	465	180	80
940524	sand[2]		18	53	540	60
940524	sand[2]		22	116	540	80

[1] measured at CIO: Institute for isotopic research, Groningen, The Netherlands
[2] measured at NERI: National Environmental Research Institute, Silkeborg, Denmark

Mass balance.

Nitrogen mass balances of lake Nuldernauw were performed in 1985, 1986 and 1987. The mass balances for water and species were described as: input-output-storage = residual. The input comprises rainfall, dry deposition, sluice leakage, a polder outlet, some small streams and seepage. The output contains the terms evaporation, infiltration, sluice and a polder inlet. Storage was calculated from the monthly watermark. Based upon an annual balance, storage is low in comparison with the other terms. First a water balance was made. The good agreement between measured and calculated chloride concentrations indicated the correctness of this balance. For the calculation of the total N mass balance the water flows were

multiplied with the concentrations in the various terms. Data on rainfall, dry deposition and evaporation were obtained from daily measurements of the KNMI (Royal Dutch Meteorological Institute). It was assumed that no nitrogen was lost by evaporation. Most flows were measured daily. Seepage and infiltration were based on a ground water model. The other terms of the in- and output were measured at least once a week.

Flows and concentrations at days between two measurements were estimated by linear interpolation. In winter the major part of the nitrogen input was due to the small streams. In summer dry deposition became more important and the contribution of the small streams reduced markedly. The residual term of the total N balance consists of the net amount of nitrogen removed from the water column (coupled nitrification-denitrification, denitrification of nitrate from the overlying water and burial) but also of the errors in the in- and output (Table 2). On an annual basis the error of the residual was estimated on 13%. The residual term of the total N mass balance forms the upper limit of the yearly average denitrification in the lake. Denitrification rates obtained with the mass balance approach do not give information on temporal and spatial variations. They are rather an indication of the year average denitrification (Seitzinger, 1988).

Table 2. Contribution and relative errors of the various input and output terms of the annual N mass balance in ton N y^{-1}.

balance terms	in/output	rel. error
rainfall	17.9	0.2
dry deposition	22.1	3.8
sluice leakage	2.9	0.6
polder outlet	42.3	2.1
seepage	0.7	0.1
streams	147.7	8.8
storage	-0.1	-
evaporation	-0.0	-
infiltration	-24.0	3.6
sluice leakage	-15.1	3.0
polder inlet	-0.2	-
sluice	-97.0	5.8
total	97.2	12.3*

* the error of the residual was estimated by the root of the sum of squares of the individual errors

RESULTS AND DISCUSSION

Measurement of N_2 flux

Contamination by atmospheric N_2 of water filled chambers was 16 µmol N m^{-2} h^{-1}, expressed per unit surface area of the sediment. The denitrification rates reported here are corrected for this background flux.

Several checks were carried out to ensure that the measured N_2 flux is due to denitrification and thus the coupled nitrification-denitrification can be measured. During the first days of the incubation period large N_2 fluxes were found (Figure 2). Seitzinger (1980) also observed large initial fluxes. Simulation of the diffusion of N_2 present initially in the sediment pore water also showed high initial fluxes (Figure 2). When the measured fluxes were corrected for leakage and the steady state denitrification rate after 10 days of incubation, no difference was found between simulated and measured N_2 fluxes (Figure 2). The results indicate that the large initial N_2 fluxes were due to the diffusion of N_2 present in the pore water. These fluxes initially shade the N_2 flux of the denitrification. Hence a pre-incubation period is needed before the denitrification flux can be measured. The length of this period is related to the sediment depth. With a total sediment depth of about 4 cm, the diffusion of N_2 from the pore water became negligible compared to the flux by denitrification after about 10 days.

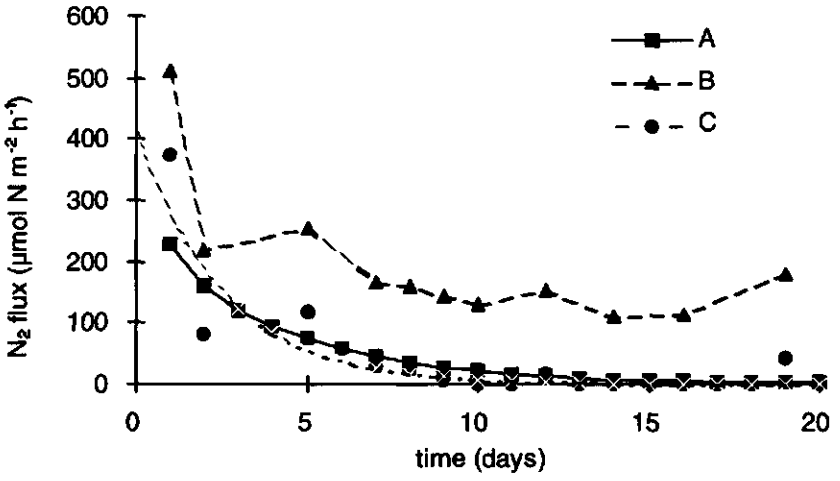

Fig. 2. Simulated and experimental measured N_2-fluxes.
A. Simulation of the N_2-flux by diffusion;
B. High measured N_2-fluxes during the first days of incubation;
C. Measured N_2 fluxes corrected for the mean denitrification after 10 days of incubation

Another check was the incubation of irradiated sediment. In the irradiated cores a flux of 46 ± 54 μmol N m^{-2} h^{-1} persisted after 10 days of incubation. Plating showed that the sediment was contaminated by bacteria again. Addition of $HgCl_2$ decreased the N_2 flux to 22 ± 14 μmol N m^{-2} h^{-1}. These fluxes are comparable to the background flux (16 μmol N m^{-2} h^{-1}) and are low compared to the flux of contamination (60-80 μmol N m^{-2} h^{-1}) observed by Yoon and Benner (1992). The checks described above demonstrate that in the experiments presented here, the N_2 flux after a pre-incubation period of about 10 days and corrected for leakage was caused by denitrification only.
After 3 weeks of incubation addition of nitrate to the overlying water resulted in an enhanced N_2 flux. Thus after this incubation time the bacteria still were active and organic C was not limiting. The increase in N_2 flux was compared to the NO_3^- flux into the sediment (Table 3). The mean N_2 flux before addition of NO_3^-, due to coupled denitrification was 118 μmol N m^{-2} h^{-1}. The first day after addition there is a large nitrate flux, due to the steep concentration gradient at the sediment-water interface. The final nitrate flux is low. At that time most of the added nitrate has already been denitrified and the concentration difference between water and sediment has become low. Because the NO_3^- has to diffuse to the denitrification zone and the N_2 formed in the sediment has to diffuse out of the sediment there is a time lag between the N_2- and NO_3^--flux. It is evident that the addition of nitrate caused the enhanced N_2 production. Over 90 % of the nitrate added is recovered in the increased N_2 flux. After the added nitrate had been consumed, the N_2 production returned to its initial rate (118 μmol N m^{-2} h^{-1}), because the coupled nitrification-denitrification went on.

Table 3. Denitrification rates estimated by N_2-production and nitrate decrease.

N_2-flux [1] (μmol N $m^{-2}h^{-1}$)	Δ N_2 [2] (μmol N $m^{-2}h^{-1}$)	Δ NO_3^- [3] (μmol N $m^{-2}h^{-1}$)	incubation time (hours)
118.0	-	-	
160.4	42.4	105.3	20.3
160.2	42.2	40.4	21.5
155.0	37.0	39.8	23.0
156.6	38.6	7.3	24.0
134.8	16.8	3.5	67.8
	total amount: 11.7 μmol	total amount: 10.8 μmol	

[1] measured N_2-flux due to total denitrification, [2] N_2-flux due to NO_3^- addition,
[3] N_2-flux calculated from the decrease in NO_3^- concentration

The tight coupling between nitrification and denitrification was further demonstrated in the experiment with addition of N-serve. N-serve inhibits the production of nitrate and thus the N_2 flux decreases. When nitrate and N-serve were added simultaneously denitrification could occur again (Figure 3). Without additions of nitrate and/or N-serve the N_2 fluxes measured after the pre-incubation period in muddy and sandy sediments gave, after correction for the leakage, denitrification rates of about 141 μmol N m^{-2} h^{-1} and 64 μmol N m^{-2} h^{-1} respectively (Table 4).

Two disadvantages were found using the N_2 flux method: the long pre-incubation period and the risk of contamination by atmospheric nitrogen. Although sedimentation of fresh organic matter stops when the sediment is removed from the lake, the long incubation period in this study did not seem to change the various processes of the N mineralization. As discussed before the addition of extra nitrate demonstrated that C was not limiting. Furthermore the N_2 flux did not decrease with time.

Vigorous flushing around the parts prone to contamination helped to eliminate contamination during sampling. Using the precautions to avoid contamination, a stable flux, due to denitrification can be measured after a pre-incubation period of about 10-13 days. The coupled nitrification-denitrification along with the reduction of nitrate from the overlying water can be distinguished and quantified.

Table 4. Coupled nitrification-denitrification rates (μmol N m^{-2} h^{-1}) at 20°C measured with the N_2 flux method, corrected for leakage.

muddy sediments		sandy sediments	
sampling date	denitrification rate	sampling date	denitrification rate
920408	132	930625	54
921013	130	930921	42
921123	110	931102	92
921123	120	940124	68
930427	154		
930503	102		
931207	214		
940418	140		
940524	170		

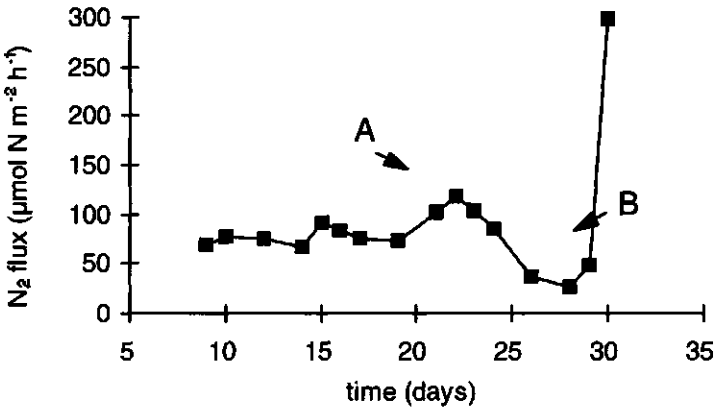

Fig. 3. Effect of addition of N-serve and nitrate on the N_2-flux.
A. start of N-serve additions, B. addition of NO_3 and N-serve

^{15}N isotope pairing technique

Results of the ^{15}N experiments are listed in Table 1. Rates for the coupled nitrification-denitrification (D14) were about 21 µmol N m^{-2} h^{-1} for sandy sediments. For muddy sediments the rate seemed to depend on the collecting date. It ranged from 24 µmol N m^{-2} h^{-1} in winter to 141 µmol N m^{-2} h^{-1} in late spring. Although measured at different institutes with various sample treatment and mass spectrometers, the differences between the measurements were only small (Table 1; September 1993 - January 1994). No clear difference for D14 was found between sandy and muddy sediments collected in winter. However, the denitrification rates of nitrate from the overlying water (D15) for the sandy sediments were much lower than for the muddy sediments. The incubation time did not seem to influence the formation of $^{29}N_2$ and $^{30}N_2$ (Figure 4). The $^{15}NO_3^-$ concentration added did also not influence the D14 significantly (Table 1).

Comparison of coupled nitrification-denitrification rates obtained with the N_2 flux method, the ^{15}N isotope pairing technique and the mass balance approach

When comparing the coupled nitrification-denitrification rates as measured with the two methods, a quite large difference was found. In general the rates obtained with the ^{15}N isotope pairing technique were lower than through the N_2 flux method (Table 1), especially with the muddy sediments. The expected difference between sandy and muddy sediments due to the organic matter content, was not always found with the ^{15}N isotope pairing technique, but clearly with the N_2 flux method. Because of the long pre-incubation period of the N_2 flux method even lower rates might be expected with the N_2 flux method than with the ^{15}N method, because some of the readily biodegradable organic matter could have been used already when the coupled nitrification-denitrification rate was measured after 10 days.

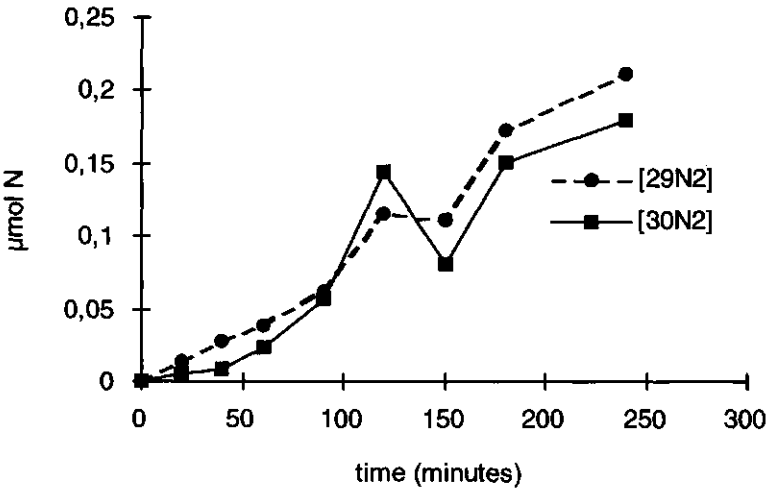

Fig. 4. Formed $^{29}N_2$ and $^{30}N_2$ against the incubation time.

As discussed above the rates obtained with the N_2 flux method were not due to contamination by atmospheric nitrogen. Also it was shown that addition of NO_3^- to the overlying water did not influence the coupled nitrification-denitrification process. The results from both methods were compared with the denitrification estimated from a mass balance of the lake. The yearly average denitrification rates of both, the performed laboratory experiments and the mass balance approach, yield indications rather than exact values of the denitrification in the field. Experiments with the N_2 flux method performed at several temperatures, covering the natural annual temperature range, showed a temperature influence for the N_2 flux (data not shown). With a temperature increase of 10°C, the N_2 flux increased with a factor 1.9. Corrected for this temperature effect and the percentage sandy (70 %) and muddy (30 %) sediments, 72 % of the residual term of the mass balance could be accounted for (Table 5). The NO_3^- loading of the lake was highest in winter and accounted for the increase of the concentration in the lake (maximum concentration 1 mg l^{-1}), so the microbial activity was low. The rest of the year the loading with nitrate was very low. In spring the nitrate concentration in the lake suddenly drops to zero. The removal of this nitrate accounts for 14 % of the residual term and thus may be caused by uncoupled denitrification and/or algae uptake. Some burial of organic N may occur, but this is considered to be of minor importance. The agreement between mass balance and N_2 flux method was therefore considered as quite satisfactory. As described before with the ^{15}N method a kind of seasonal effect was observed for the muddy sediments, although the incubation temperature was constant at 20°C (Table 1).

Table 5. Comparison of denitrification rates found experimentally with denitrification rates of the mass balance approach for lake Nuldernauw.

method	denitrification rate ton N y^{-1}
mass balance	97.2
N_2 flux, temp. range	69.9
N_2 flux, 20°C	93.2
^{15}N, 20°C	36.1

The denitrification based on data of the ^{15}N method corrected for the sediment type and the seasonal effect but at an incubation temperature of 20°C only, resulted in a much lower value (Table 5). The denitrification based on the N_2 flux method and an incubation temperature of 20°C was about 2.7 times higher than the denitrification based on the D14. An even larger difference would be found between the results of the mass balance and the ^{15}N method if the latter were corrected for temperature.
The good agreement of the mass balance data with the results of the N_2 flux method supports the conclusion that the N_2 flux method gives a realistic value for the coupled nitrification-denitrification. The large difference between these data and the ^{15}N isotope pairing technique suggests that the ^{15}N method underestimates the coupled nitrification-denitrification. Unfortunately specific research as to the cause for the difference between the two methods was beyond the scope of this investigation. However, some possible explanations are tentatively discussed.
The ^{15}N isotope pairing technique is based on the assumption that there is uniform mixing between the added $^{15}NO_3^-$ and the $^{14}NO_3^-$ formed in the sediment. Several reasons like aerobic denitrification, transport of nitrate to denitrification zones and anaerobic microsites can cause these assumption to be invalid. Robertson and Kuenen (1984, 1990) showed that bacteria exist which are capable of simultaneous heterotrophic nitrification and aerobic denitrification with NO_2^- as intermediate. When this aerobic denitrification occurs in aquatic sediments, the ^{15}N isotope pairing technique will underestimate the coupled nitrification-denitrification as the $^{28}N_2$ formed by the aerobic denitrification is not taken into account. For the same reason also in sediments containing or producing high concentrations of $^{14}NO_2^-$ the coupled nitrification-denitrification will be underestimated. In the sediments used here no free $^{14}NO_2^-$ could be detected.
The incubation time may be too short to reach steady state $^{15}NO_3^-$ transport and thus complete mixing between the nitrate species. Simulation of the $^{15}NO_3^-$ diffusing from the overlying water to the denitrification zone showed that the denitrification zone was reached by the first ions after several minutes, but it took about 2 hours to reach

steady state (data not shown). Rysgaard et al. (1993) did experiments in a continuous flow system in the dark after pre-incubation in diurnal dark/light cycles. After the light was switched off and benthic photosynthesis ceased, still a few hours of darkness were needed to reach steady state for oxygen. Since the diffusion coefficient of nitrate is very close to that of oxygen it can be questioned whether the $^{15}NO_3^-$ added in our experiments was mixed well enough with the endogenous nitrate to measure coupled nitrification-denitrification within these short incubation times. However, longer incubation times did not result in an increase in $^{29}N_2$ or $^{30}N_2$ (Figure 4). Further prolongation of incubation is not feasible because the oxygen concentration would drop too far. Perhaps the non-uniform mixing because steady state was not yet reached, could also explain the higher denitrification rates found in the spring experiments with the ^{15}N technique. When the sediment oxygen demand is higher (in the field in summer, at higher temperatures and higher organic matter input), the aerobic layer is thinner and the added $^{15}NO_3^-$ can reach the denitrification zone faster. Then steady state is obtained faster and the nitrate species will be mixed better. The coupled nitrification-denitrification would be less underestimated. In this study $^{30}N_2$ and $^{29}N_2$ indeed increased strongly in spring for the muddy sediments (Table 1), although all incubation conditions were the same. The sediment oxygen demand of the sandy sediments did not change, so the oxygen penetration depth did not change either and no higher denitrification rates were found. In the muddy spring sediments perhaps also more bacteria were active, which also would result in higher denitrification rates. But the denitrification rates of the winter sediments still were lower than the denitrification rates measured at 2°C with the N_2 flux method.

Even when the incubation time is not limiting for uniform mixing, the assumption of uniform mixing still can be the problem. Nielsen (1992) warns for underestimation of the coupled nitrification-denitrification because of the heterogeneity of the sediment. He suggested that addition of higher $^{15}NO_3^-$ concentrations reduces underestimation because more $^{14}NO_3^-$ is trapped and measured as $^{14}N^{15}N$. The correctness of the calculation of D14 therefore would increase. Of course the probability to trap $^{14}NO_3^-$ will increase. However, around anaerobic and aerobic microsites the nitrifying and denitrifying bacteria are optimal positioned and the nitrification and denitrification will be very tight coupled. It was therefore suggested that in and around these microsites the condition of uniform mixing of the two isotopes still may not be fulfilled. The production of the homogeneous isotope pairs $^{14}N^{14}N$ and $^{15}N^{15}N$ remains relatively high and the coupled nitrification-denitrification will be underestimated. In this study higher $^{15}NO_3^-$ concentrations did not result in significant higher D14 values (Table 1). As discussed this is no reason to exclude the existence of microsites and the coupled nitrification-denitrification still can be underestimated.

CONCLUSION

In this study with the isotope pairing technique lower values for the coupled nitrification-denitrification were obtained compared to the N_2 flux method and the N mass balance of the lake. It was suggested that the ^{15}N isotope pairing technique probably underestimates the coupled nitrification-denitrification. An explanation could be that the assumption of uniform mixing of the nitrate species is not valid, because of the tight coupling between nitrification and denitrification in the microsites. Although the N_2 flux method is very laborious, the method appears to produce realistic coupled nitrification-denitrification rates, as compared with mass balances of the lake. It was furthermore demonstrated that temperature corrections are very important for the interpretation of laboratory results to field conditions. Studies of temperature influence and seasonal variations of N-processes in lake Wolderwijd/Nuldernauw are in progress and will be published elsewhere (see Chapter 4).

Chapter 3
Why dinitrogen fluxes from freshwater sediments under anoxic conditions?

ABSTRACT

From anoxic incubated freshwater sediments, initially without inorganic nitrogen compounds in the overlying water, remarkable and unexpected N_2 fluxes were estimated. N_2 leakage from the atmosphere and oxygen leakage followed by nitrification and denitrification, two possible causes, were thoroughly examined but found to be incorrect. From these investigations it was furthermore concluded that the N_2 fluxes are real. The fluxes appear only under anoxic conditions and at least one step in the forming has to be biological. How these N_2 fluxes were formed remained unclear as alternative pathways for the anoxic forming of N_2 found in literature did not give an explanation to the source and/or mechanism leading to the N_2 fluxes either.

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INTRODUCTION

A method to quantify directly the denitrification in sediments, without addition of inhibitors or extra inorganic N species, is the N_2 flux method (Seitzinger et al., 1980). By this method the N_2 flux due to denitrification is estimated from the increase in N_2 after a 10 days pre-incubation of an intact sediment core with overlying water in a gas tight chamber. Before each incubation the background N_2 in the headspace is removed by purging with a $He/O_2/CO_2$ gas mixture. When instead of this gas mixture only He is used, anoxic incubations can be performed. In anoxic incubations without inorganic nitrogen compounds in the overlying water not only ammonium fluxes, but surprisingly also remarkable N_2 effluxes were found. In the absence of oxygen this is considered to be quite impossible. In this note the possible causes and consequences of these N_2 fluxes are investigated.

METHODS

Undisturbed sediment cores were collected between September 1993 and December 1994 from two sampling sites in lake Nuldernauw, The Netherlands, with a Beeker sampler (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). Sandy sediments were collected from a depth of about one meter, muddy sediments from about 2 meter depth. In the laboratory NO_x^- (nitrate and nitrite), NH_4^+ and N_2 fluxes from the sediments were quantified using the modified N_2 flux method, which is described in detail elsewhere (Van Luijn et al., 1996). After the top 4 cm of the undisturbed sediment was transferred into the incubation chambers artificial surface water, which was flushed with He, was carefully added to the sediment until a headspace of 100 ml remained. The artificial water was made from de-ionized water and had an ion composition similar to that of the lake water, with exception of the nutrients phosphor and nitrogen which were absent. The water phase was changed and sampled for N analysis daily, except when $HgCl_2$ was added. From the concentrations in this water phase at two successive sampling times the NH_4^+ and NO_x^- fluxes to the overlying water were calculated using mass balances according to Boers and Van Hese (1988). The headspace was flushed daily for at least one hour. After the flushing with He the headspace was sampled and analyzed for N_2 and O_2 on a gas chromatograph. From these measurements the N_2 flux from the sediment was calculated. Sandy and muddy sediments were incubated under anoxic conditions at 2, 12 and 23°C. To investigate the possible source of the N_2 flux, in some experiments the overlying water was replaced with artificial surface water without nutrients but containing the nitrification inhibitor N-serve (nitrapyrin = 2-chloro-6-trichloromethyl pyridine; 500 mg l^{-1}) or $HgCl_2$ (up to 2.7 g l^{-1}).

RESULTS

The measurements showed that oxygen was not present in the incubation chambers. For both sediment types no NO_x^- fluxes could be determined after the first day. The NH_4^+ fluxes were quite variable during the incubation, especially at 23°C. The average NH_4^+ fluxes varied with incubation temperature and sediment type (Table 1), with higher rates at higher temperatures and for the muddy sediments. Variation was also found in the NH_4^+ fluxes from the various sampling months. Because of the large variation of these fluxes within an incubation (13-20%), the differences between the various sampling months were not significant. Furthermore N_2 fluxes were detected (Table 1). The background N_2 flux due to contamination was estimated in water filled vessels and was 16 ± 4 μmol N m^{-2} h^{-1} (n = 5 chambers). When the detected N_2 flux was corrected for this background flux usually a positive N_2 flux remained. For the muddy sediments these N_2 fluxes usually were substantial: 23-71% of the total N flux, although 0 and 5 % were estimated as well. (Table 1). For the sandy sediments a much smaller N_2 flux was found. The difference with the background flux was less clear, but the corrected N_2 flux still formed 0-39% of the total N flux (Table 1).

Table 1. Anoxic NH_4^+ and uncorrected N_2 fluxes (μmol N m^{-2} h^{-1}). The background flux is 16 μmol N m^{-2} h^{-1}. Temperature is in °C.

	NH_4^+ flux			N_2 flux**			N_2 as % of Ntot#		
date	2°	12°	23°	2°	12°	23°	2°	12°	23°
sand									
93-11	NA	59	71	NA	33 ± 12	30 ± 12	NA	22	16
94-01	10	35	52	22 ± 7	37 ± 4	28 ± 3	36	36	17
94-05	12	26	69	24 ± 6	16 ± 4	40 ± 13	39	0	26
94-08	39	95	150	18 ± 3	37 ± 17	23 ± 7	5	16	5
mud									
93-12	32	48	243	59 ± 12*	47 ± 14	92 ± 22	57	44	23
94-03	10	37	114	56 ± 11	80 ± 22	81 ± 20	71	66	37
94-04	56	68	144	42 ± 18	61 ± 15	16 ± 8	31	43	0
94-06	21	37	187	55 ± 12	65 ± 12	NA	65	65	NA
94-09	34	73	157	35 ± 7	45 ± 12	25 ± 9	49	28	5
94-11	25	51	58	32 ± 4	41 ± 8	34 ± 5	51	25	24

* 6°C; # N_2 and Ntot, both corrected for the background flux; NA = not available

** the mean N_2 flux and standard deviation over the total incubation period

DISCUSSION

N_2 fluxes were not expected in these anoxic experiments, as NO_x^- was not available and without oxygen NO_x^- could not be produced by nitrification either. Therefore several possibilities as to the source of this N_2 will be discussed. Detailed checks to exclude that the measured N_2 was due to contamination with atmospheric dinitrogen or to N_2 diffusion from the sediment pore water were performed by Van Luijn et al. (1996). A simulation of the diffusion of N_2 initially present in the pore water demonstrated that the N_2 flux due to degassing of the pore water became negligible indeed after about 10 days. The simulated fluxes were comparable with measured N_2 fluxes corrected for leakage and the steady state denitrification in oxic incubations and therefore assumed to be correct. Furthermore it was demonstrated that the N_2 flux from irradiated muddy sediments and addition of $HgCl_2$ was comparable to the background flux of water filled chambers. Nowicki (1994) did not find a N_2 background flux in empty He filled chambers. After 10 days of anoxic incubation however, she still found a N_2 flux of 20-68 µmol N m^{-2} h^{-1}, which she assumed to be the background flux. Meanwhile the predicted N_2 flux of her simulation model was only 32 µmol N m^{-2} h^{-1}. From her figures followed furthermore that her observed N_2 fluxes during anoxic incubations were about 15 µmol N m^{-2} h^{-1} higher than the predicted N_2 fluxes. This flux is comparable with our remaining N_2 fluxes after correction for the background flux for our sandy sediments. It might thus be possible that (part of) her background N_2 flux after about 10 days of incubation, had another source too.

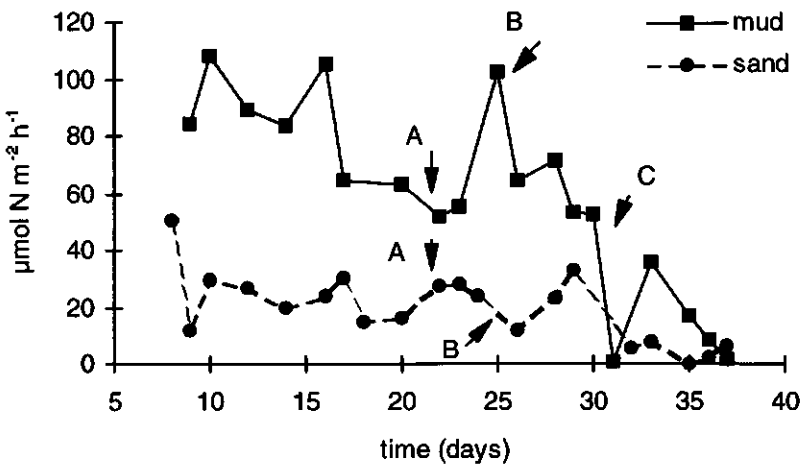

Fig. 1. Effect of addition of N-serve and $HgCl_2$ on anoxic N_2 fluxes.
A. start of the N-serve additions, B. $HgCl_2$ addition, C. excess of $HgCl_2$ addition.

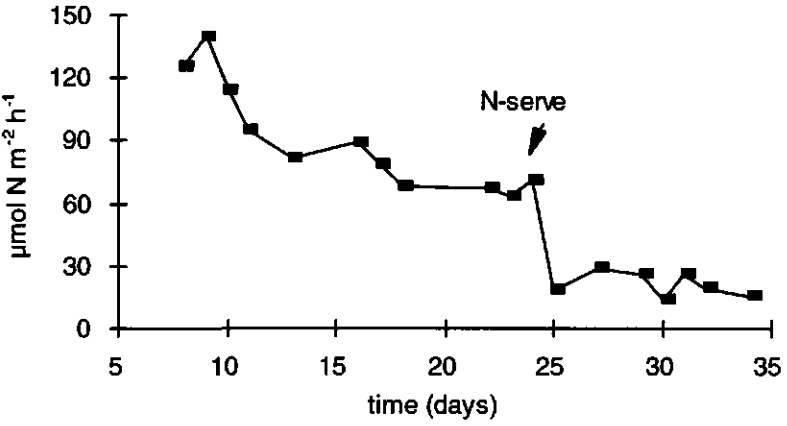

Fig. 2. Effect of N-serve addition on oxic N_2 fluxes.

The N_2 fluxes also were not due to oxygen leakage and simultaneous nitrification (and further denitrification): NO_x^- was not detectable in the overlying water and addition of N-serve did not result in lower N_2 fluxes (Figure 1). The N-serve worked well, as in oxic incubations the N-serve immediately caused lower N_2 fluxes (Figure 2). As the N_2 flux under oxic conditions decreased to the background flux, it is not likely that the N_2 flux obtained under oxic conditions contains other N_2 than produced by the denitrification process. The unexpected N_2 flux thus only appears under anoxic conditions. Addition of $HgCl_2$ in the anoxic incubations decreased the N_2 flux in muddy sediments and reduced this flux in sandy sediments to the level of the background flux (Figure 1). From this it was concluded that at least one step in the generation of N_2 has to be biological. Moreover, far higher oxygen leakage is needed to obtain the measured N_2 fluxes, than can be expected from the N_2 leakage of 16 µmol N $m^{-2}h^{-1}$. Based upon this background flux 4 µmol O m^{-2} h^{-1} can leak into the incubation chamber. When this whole flux is used for nitrification, an extra N_2 flux of only 1.3 µmol N $m^{-2}h^{-1}$ would be found. The N_2 fluxes detected under anoxic conditions thus seem to be real and not due to experimental artefacts, but it is unclear how the N_2 was formed.

Is it possible to form N_2 under anoxic conditions? The most common mineralization path is from organic nitrogen by ammonification to NH_4^+, subsequent nitrification to NO_x^- and denitrification to N_2. In these reactions several intermediates are formed (Knowles, 1982; Robertson and Kuenen, 1988) and probably more pathways are possible as well. Maybe oxidized nitrogen compounds are formed directly in the mineralization of the organic matter. Barnes (1980) suggested an organic N -> N_2 reaction which is not linked stoichiometrically to the organic C diagenesis. A possible intermediate could be hydroxylamine (NH_2OH). Hydroxylamine can be converted into

N_2O by chemical decomposition (Bremner et al., 1980), whereafter N_2O can be readily reduced to N_2 by denitrification. N_2O or NH_2OH was not measured. For denitrification in literature only reactions of nitrogen oxides to N_2 have been described (Kuenen and Robertson, 1988). Recently Van de Graaf et al. (1995) demonstrated that biological conversion of ammonium to N_2 under anoxic conditions is possible. This was however in the presence of nitrate which was not available in our system. Thermodynamically it is just possible to convert ammonium with iron or manganese to N_2, but this has not been verified experimentally (Barnes, 1980). This possibility was checked by addition of iron oxide in one experiment. An increase in N_2 could not be detected, maybe because the iron oxide was not mixed with the sediment.

In conclusion, the N_2 fluxes from anoxic incubated sediments were rather variable and not always detectable. Based upon the various checks discussed above however, it was concluded that the N_2 fluxes are real, although an explanation for the source of and/or the mechanism leading to these N_2 fluxes cannot be given.

It was recommended to estimate the denitrification rates in oxic incubations and by correction for the background flux as measured in water filled control vessels. Subtraction of the anoxic N_2 fluxes from the oxic N_2 fluxes namely can underestimate the denitrification. Further research to the mechanism, the production of hydroxylamine and the ammonium oxidation by Fe and Mn seems worthwhile, as this could be another path to reduce the internal N-loading.

Chapter 4
Nitrogen fluxes and processes in sandy and muddy sediments from a shallow eutrophic lake.

ABSTRACT

Nitrogen fluxes and processes were estimated in sandy and muddy sediments from the shallow eutrophic lake Nuldernauw, The Netherlands. N_2, NH_4^+, NO_x^- and CH_4 fluxes were measured from sediment samples collected throughout the year and incubated under both oxic and anoxic conditions at 2°, 12° and 23°C.

Fluxes increased with temperature with a mean temperature factor of 1.9 ± 0.3 for a 10°C increase for both sediment types.

At the same temperature the total N fluxes (N_2 + NO_x^-+ NH_4^+) from the muddy sediments were generally larger than from the sandy sediments. These differences are related to the relatively high availability of decomposable organic matter in the muddy sediments compared to the sandy sediments. Especially the denitrification was influenced by the organic matter content: 75-90% of the total N flux was denitrified by the muddy sediment, whereas only 45-65% was denitrified by the sandy sediments.

NH_4^+ fluxes were much higher and NO_x^- fluxes were much lower in cores collected just after spring bloom of phytoplankton, compared to cores collected during other periods. This effect was most pronounced at the high incubation temperature. The freshly settled and easily degradable organic matter at the top of the sediment appeared to be of great influence. At higher concentrations of easily degradable organic matter more oxygen was consumed by the aerobic mineralization and the CH_4 oxidation and no or less oxygen was available for the oxidation of nitrogen. Consequently no or less coupled denitrification could occur. Although high temperatures are not often found in the Dutch surface waters, these conditions can occur in spring and summer. Then nitrogen will not be removed from the sediment-water system by the coupled nitrification-denitrification and ammonium will be released to the overlying water where it can be consumed by algae.

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INTRODUCTION

Due to anthropogenic activities most surface waters are enriched with nutrients. This can lead to algal blooms and can cause a poor water quality. To diminish eutrophication often the nutrient loadings to the surface waters are reduced. When the nitrogen loading on surface waters is reduced, nitrogen can become a limiting growth factor and the benthic nitrogen regeneration will become important. Regeneration is brought about by bacteria, which perform various reactions of the N cycle like ammonification, nitrification and denitrification. Numerous studies have been carried out to quantify these various N-processes in sediments, although most were performed in estuarine and marine waters (Gardner et al., 1987; Rysgaard et al., 1993; reviews in Koike and Sørensen, 1988; Seitzinger, 1988). In most studies varying fluxes throughout the year were found. These variations are often ascribed to changing temperatures. Although temperature is an important regulating factor and it is changing with the season, it is not the only fluctuating factor. The organic input (e.g. detritus of algae) may change as well. Temperature and the organic matter content of the sediment will influence the oxygen concentration and hence the other electron acceptors (Klump and Martens, 1983; Kemp et al., 1990) and therefore may have a significant effect on the rate and timing of the various N-processes.
In this study results of experiments with sandy and muddy freshwater sediments from lake Nuldernauw are described. The experiments were carried out under oxic and anoxic conditions at three temperatures to search the influence on the various N-processes of temperature and of the organic matter content of the sediment. Also the influence of the season during an annual cycle is investigated. Furthermore the contribution of the N- and C-oxidation to the sediment oxygen demand (SOD) is estimated.

MATERIAL AND METHODS

Undisturbed muddy and sandy sediment cores were sampled with a Beeker sampler (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) from lake Nuldernauw. Lake Nuldernauw is a shallow lake (mean depth 1.6 m) in the centre of The Netherlands and has a surface of 870 ha. The residence time was 0.4-0.6 year with a longer detention in summer than in winter. Because of the flushing (especially in winter) since 1990 the residence time decreased to 0.2 year. The total N-loading on the lake is about 25 g N m^{-2} y^{-1}, resulting in a total N concentration of 2 g N m^{-3} in average. Ammonium concentrations in the water column are always low (0 - 0.3 g N m^{-3}). Nitrate concentrations range from 0 g N m^{-3} in summer to 1.5 g N m^{-3} in average in winter. After return in the laboratory the SOD was measured. This was done at 20°C, in the dark. The oxygen concentration in stoppered cores without headspace

was continuously measured and recorded. The water column was continuously stirred without disturbing the sediment surface. The SOD was estimated from the slope of the initial linear decrease of the oxygen concentration. Furthermore the N_2 and CH_4 fluxes from the sediment were measured in 6 other cores using the N_2 flux method (Seitzinger, 1980). A modification of the N_2 flux method described in detail by Van Luijn et al. (1996) was used. Here only the outlines are given. The upper 2-4 cm of a sediment core were, as undisturbed as possible, transferred into a gastight chamber and overlaid with artificial, nutrient free surface water (100-150 ml) until a headspace of 100 ml remained. Before the water was added to the sediment, it was purged with a $He/O_2/CO_2$ gas mixture. 60 ml of the water phase was replaced daily. While replacing the water, the headspace was flushed continuously with the gas mixture and after addition of the water the headspace was flushed with this gas mixture for about 1 hour. For anaerobic incubations He instead of the gas mixture was used. The N_2 and the CH_4 concentrations in the headspace were measured immediately after flushing and after an incubation period of about 20 hours by gas chromatography (Hewlett Packard, 5890 series 2 with chrompack plot fused silica column (25m x 0.53mm, molsieve 5Å) and thermal conductivity detector). After a pre-incubation of about 10 days the N_2 initially present in the pore water was almost completely removed and the denitrification rates could be calculated from the increase in N_2 concentration after correction for the background flux due to small leakage (Van Luijn et al., 1996). For detection of CH_4 a minimal concentration of 0.65 µmol CH_4 l^{-1} is needed. This corresponds to a flux rate of 1.3 µmol CH_4 m^{-2} h^{-1} at a 20 hours incubation time. Unfortunately the O_2 uptake in the aerobic incubations could not be estimated. With the used settings of the gas chromatograph (optimal for the detection of nitrogen) it was not possible to integrate the peaks for the oxygen concentration accurately. The overlying water was replaced and sampled daily and thus additionally the NO_x^- (nitrate and nitrite) and NH_4^+ fluxes could be estimated. The replaced water was analysed for NH_4^+ and NO_x^- and using the mass balance and the equation given by Boers and Van Hese (1988) the NH_4^+ and NO_x^- fluxes were calculated.

To examine the influence of organic matter, sediment samples from both a muddy and a sandy site were taken. The seasonal effect was searched by collecting the samples in several seasons, covering a whole year period (Table 1+2).

To investigate the effect of temperature the cores were incubated at 2°, 12° and 23°C, with exception of the cores of November and December 1993, which were incubated at 6°, 10° and 23°C. At each temperature an oxic and an anoxic core was incubated. Anoxic conditions were not observed in the field but anoxic cores were incubated in order to examine one of the N processes, the ammonification. In the anoxic cores furthermore the CH_4 production could be measured.

Table 1. SOD values and N-fluxes on the various sampling dates, **mud.**

date	SOD	temp.	NO_x^-	NH_4^+	N_2	Ntot
	g O_2 $m^{-2}d^{-1}$	°C		µmol N m^{-2} h^{-1}		
931207	1.1	23	66	4	205	275
		10	27	8	109	144
		6	17	4	67	88
940308	1.3	23	32	3	188	223
		12	26	4	83	113
		2	12	2	42	56
940418	1.2	23	22	4	248	274
		12	20	16	80	116
		2	13	4	44	61
940628	1.3	23	22	17	252	291
		12	11	6	65	82
		2	8	2	28	38
940921	1.7	23	4	130		130
		12	1	4	65	70
		2	5	25	37	67
941110	3.6	23	25	15	115	154
		12	14	2	80	95
		2	7	1	20	28
mean	1.3 ± 0.2, without November					

As the overlying water of the sediment was replaced by artificial surface water without nutrients, it was assumed that the following N processes could be quantified.

aerobic: ammonification = N_2 flux + NO_x^- flux + NH_4^+ flux = total N flux
nitrification = N_2 flux + NO_x^- flux
denitrification = N_2 flux
anaerobic: ammonification = NH_4^+ flux

This is a simplification as nitrate assimilation and nitrate reduction to NH_4^+ were assumed to be negligible. With the method used in this study these reactions cannot be measured.

Table 2. SOD values and N fluxes on the various sampling dates, **sand.**

date	SOD	temp.	NO_x^-	NH_4^+	N_2	Ntot
	g O_2 $m^{-2}d^{-1}$	°C		µmol N m^{-2} h^{-1}		
931102	0.9	23	50	4	92	146
		10	32	4	60	96
		6	26	4	55	85
940124	0.9	23	45	3	64	112
		12	27	3	28	58
		2	10	2	16	28
940427	1.2	23	41	12	64	117
		12	28	5	94	127
		2	0	2	0	2
940525	1.5	23	45	3	102	150
		12	14	30	16	60
		2	12	8	32	52
940818	NA	23	3	105	8	116
		12	2	63	3	68
		2	10	11	13	34
mean	1.1 ± 0.3					

RESULTS

SOD measurements

With exception of November (3.6 g O_2 m^{-2} d^{-1}) the sediment oxygen demand (SOD) was quite stable throughout the year (1.2 ± 0.3 g O_2 $m^{-2}d^{-1}$) and almost no difference was found between sandy and muddy sediments (Table 1+2).

Oxic incubations

The results obtained from the various sampling months could for both sediment types be divided into 3 periods: November to April, April to June and August/September.

November-April. In the experiments of this period substantial NO_x^- and very low NH_4^+ fluxes were found from the beginning of the experiment, although in some experiments at 23°C the first 5 days of the incubation higher NH_4^+ and lower NO_x^- fluxes were found. The N_2 flux was high initially, due to the N_2 which was already present in the pore water, but after about 10 days a N_2 flux due to denitrification remained. This pattern will be referred to as 'equilibrium' pattern.

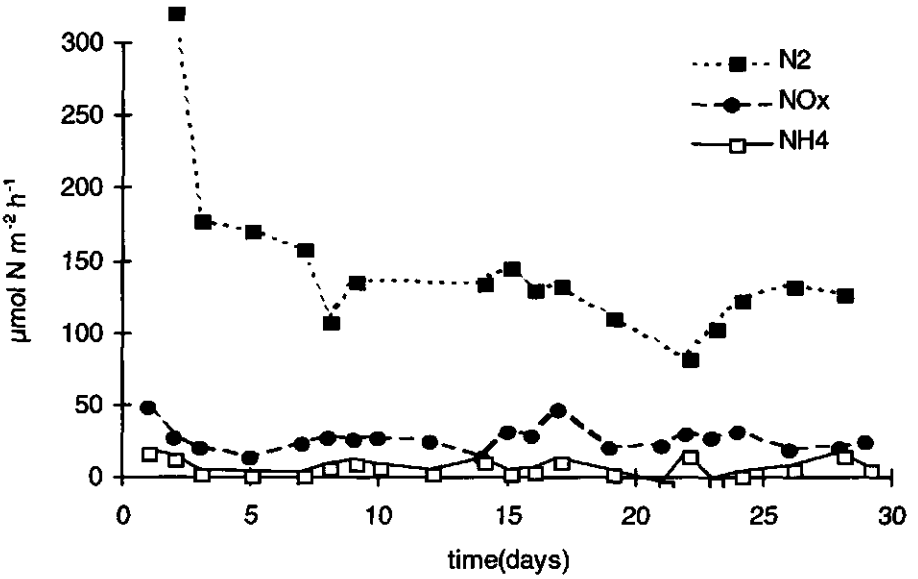


Fig. 1. Example of the 'equilibrium' pattern of the N fluxes in the experiments from November until April (mud, 10°C, December 1993).

In Figure 1 an example of this 'equilibrium' pattern is given for a muddy sediment incubated at 10°C.

April-June. From April onwards the N fluxes of the 23°C and 12°C incubated cores deviated from this pattern. In the beginning of the 23°C experiments high NH_4^+ fluxes (as high as in anoxic incubations; see later on) and very low NO_3^- fluxes were found. After about 10 days a switch to the 'equilibrium' pattern of NH_4^+ and NO_x^- fluxes took place (Figure 2). In the April and May experiments also a high NO_2^- flux was detected just before the NO_3^- flux increased. Because the N_2 flux due to denitrification can only be measured after 10 days (before this the back ground is too high), it is not known if the N_2 flux switched too. During the first part of these incubations in the oxic 23°C experiments also CH_4 fluxes were detected. In the sandy sediments these fluxes were very low (less than 2 µmol CH_4 m^{-2} h^{-1}) and only detectable once or twice during an incubation period. After the switch when the 'equilibrium' pattern was reached no methane fluxes could be detected. In the muddy sediments distinct CH_4 fluxes were found (10-25 µmol CH_4 m^{-2} h^{-1}) in the initial phase of the incubation (about 10 days) and low CH_4 fluxes (2-9 µmol CH_4 m^{-2} h^{-1}) remained when the 'equilibrium' pattern was reached. At 12° the various N fluxes changed too. In the sandy sediments collected in April after the initially high NH_4^+ and low NO_x^- fluxes a switch was found as well. In the other 12°C experiments of the period April-June the NO_x^- fluxes were lower and the NH_4^+ fluxes were higher than during the 'equilibrium' pattern and both fluxes were within the same range during the whole incubation period.

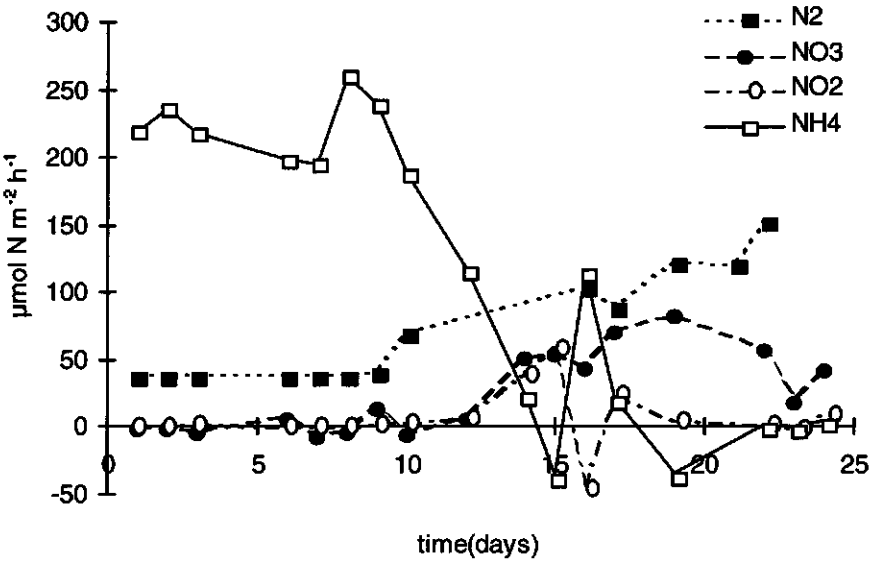


Fig. 2. Example of pattern with 'switch' of the N fluxes in the experiments from April to June (sand, 23°C, May 1994).

August + September. From the sandy sediments of August at all three temperatures almost no N_2 and NO_x^- and high NH_4^+ fluxes were found. The pattern and height of the NH_4^+ fluxes were comparable with the fluxes of the anoxic incubations. In these sandy sediments no CH_4 fluxes were found either. Unfortunately the 23°C chamber of the muddy incubations (September) was not gastight so no information on N_2 and CH_4 fluxes was available. But as almost no NO_x^- and quite high NH_4^+ fluxes were found, it was assumed that here also little or no N_2 was produced. At 2° and 12°C however, the N fluxes were quite normal.

In Table 1 and 2 the estimated NH_4^+, NO_x^- and N_2 fluxes are listed for all experiments at all incubation temperatures. The NH_4^+ and NO_x^- fluxes were averaged from the beginning of the experiment, the N_2 fluxes due to denitrification after about 10 days of incubation. When a switch was observed during the incubation, only the fluxes of the 'equilibrium' pattern were listed. The fluxes are means of the daily estimated fluxes during an experiment. The relative variation of the NH_4^+ fluxes was very large, due to concentrations around the detection limit. For the NO_x^- flux and N_2 flux the variation was about 32% and 23% respectively.

The effect of temperature is obvious. For all experiments at higher temperatures higher process rates were estimated. The temperature effect was expressed as a factor like Q10. This is not a Q10 value in the sense of a kinetic factor for one single

conversion as temperature not only affects the bacteria but also the transport, the oxygen penetration depth and therefore the redox conditions. This temperature factor was estimated from the N fluxes of the 'equilibrium' pattern by fitting e-functions through the calculated total N fluxes, which represent the ammonification rates, of each experiment (Figure 3). Nitrification and denitrification are closely coupled to the ammonification and for these processes the same influence of temperature can be expected. Nearly no difference in temperature effect was found between the aerobic sandy and muddy sediments (Table 3).

Furthermore the influence of the organic matter content of the sediment was demonstrated. The total N flux of the sandy sediments was much lower than the total N flux from the muddy sediments (Table 1+2; Figure 4). This difference was more distinct at higher temperatures, although the trends are the same for all temperatures. Therefore further only the results of the 23°C experiments were presented. In spite of the low total N flux of the sandy sediments, the NO_x^- fluxes from these sediments were slightly, but not significantly, higher than from the muddy sediments (Figure 4). This is due to the percentage denitrification (N_2), which was, affected by the amount of organic matter, much lower for the sandy sediments than for the muddy sediments. The percentage denitrification (N_2) for muddy sediments ranged from 75-90%, with the optimum in April. For the sandy sediments this range was 45-65%. However here the lowest percentage was found in April. The percentage nitrification ($NO_x^- + N_2$ flux) was high for both sediment types (about 96%) and remained quite constant throughout the year. Due to this high percentage of nitrification only low NH_4^+ fluxes were found from both sediment types.

As expected no NO_x^- fluxes were detected under anoxic conditions.

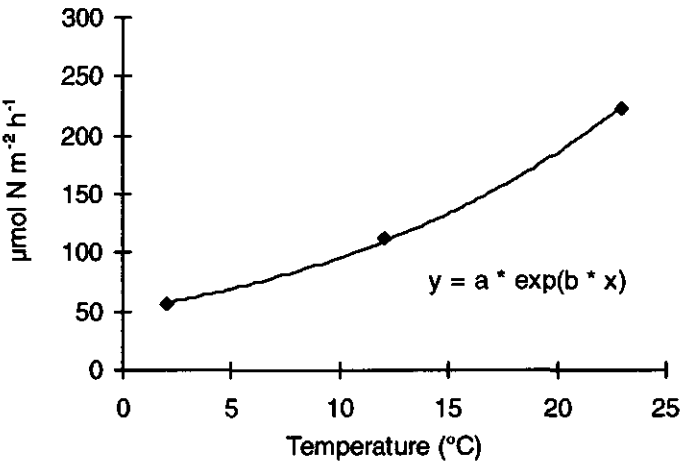


Fig. 3. Estimation of the temperature effect.

Table 3. Estimated temperature factors in oxic and anoxic experiments.

		temperature factor	
	date	oxic	anoxic
Mud	931207	2.1	3.3
	940308	2.0	3.2
	940418	2.1	1.6
	940628		2.4
	940921		2.1
	941110	2.3	1.5
	mean	2.1 ± 0.1	2.4 ± 0.7
Sand	931102	1.3	
	940124	1.9	2.2
	940525	1.8	2.3
	940818	1.8	1.9
	mean	1.7 ± 0.2	2.1 ± 0.2

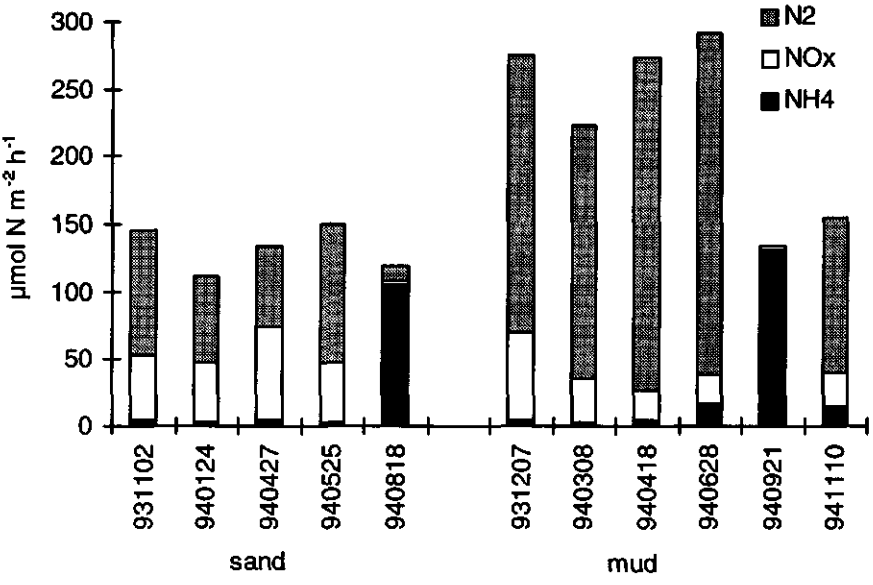


Fig. 4. Various N fluxes measured at 23°C. See the text for the period over which is averaged.

Anoxic incubations

The NH_4^+ fluxes of both sediment types in general first increased with time until a maximum was reached. Hereafter the NH_4^+ flux from the sandy sediments slightly decreased, whereas the NH_4^+ flux from the muddy sediments remained quite constant. From April to September, at 23°C, for both sediment types the initial NH_4^+ fluxes at 23°C were quite high. Just like in the oxic experiments, in the anoxic experiments also a positive temperature effect was found. The mean temperature factor for the oxic and anoxic incubations is for both sediments within the same range, although the anoxic muddy sediments showed a quite large variability (Table 3).

Surprisingly not only NH_4^+ fluxes but also N_2 fluxes were found. After correction for the background flux at 2°C 31-71% of the total N flux was N_2, at 12°C 16-66% and at 23 °C 5-37 %; with the lower values for the sandy sediments. These fluxes will be discussed elsewhere (Van Luijn et al., submitted). With increasing incubation time these fluxes then strongly decreased (Figure 5). At 23°C the NH_4^+ flux of the muddy sediments was always larger than the NH_4^+ flux of the sandy sediments. At lower temperatures this difference was less clear (Table 4).

Furthermore CH_4 fluxes were detected. The CH_4 fluxes also increased with time until a constant rate was reached. This rate depended on the sediment type, the incubation temperature and the month of core collecting (Table 4). At the lower incubation temperatures the production of CH_4 was below the detection limit. With increasing temperature the CH_4 flux increased strongly and the CH_4 production is far more influenced by temperature than the N processes.

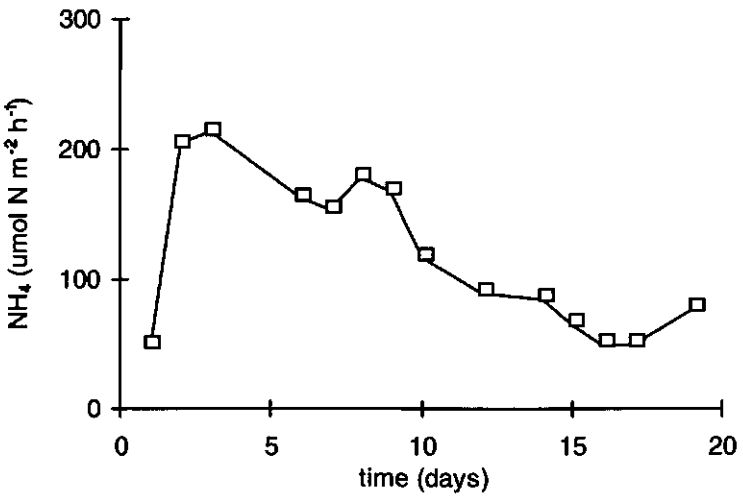


Fig. 5. Example of the decreasing NH_4^+ fluxes in the anoxic experiments from April to September (sand, 23°C, May 1994).

Table 4. Anoxic ammonium (in µmol N m^{-2} h^{-1}) and methane fluxes (in µmol CH_4 m^{-2} h^{-1}).

	2°C		12°C		23°C	
date	NH_4^+	CH_4	NH_4^+	CH_4	NH_4^+	CH_4
MUD						
931207	32	1.5	48	2.1	243	251
940308	10		37	4.3	114	114
940427	56		68	13.0	144	232
940628	21	0.9	37	29.0	187	337
940921	34		73	3.1	157	34
941110	25		51	5.2	58	101
SAND						
931102			59		71	4.2
940124	10		35		52	2.3
940525	12		26		69	26.0
940818	39		95		150	

The numbers in this table are means of the daily NH_4^+ and CH_4 fluxes when a quite constant rate was reached. During this period the variability was about 23%.

It was not possible however to estimate a temperature factor as CH_4 could not be detected at all temperatures. Visual observations and measurements furthermore showed that CH_4 bubbles were formed within the sediment and not only the production of CH_4 but also its ebullition is influenced by temperature. For the sandy sediments highest CH_4 fluxes were detected in May and August. For the muddy sediments at 12°C and 23°C highest CH_4 fluxes were found in April and June and at 23°C furthermore in December (Table 4).

DISCUSSION

Contribution of CH_4 and NH_4^+ oxidation to the SOD

Often only weak correlations are found between SOD and the organic matter content of the sediment. Di Toro et al. (1990) demonstrated that the SOD is related to the extent of oxidation of dissolved methane and ammonium produced in the anoxic zones of the sediment.

The contribution of the NH_4^+ and the CH_4 oxidation to the SOD at 23°C in this study was approximated on the basis of the following equations.

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + H_2O + 2\ H^+ \quad (1)$$

$$5\ CH_4 + 8\ O_2 \rightarrow 3\ CO_2 + 8\ H_2O + 2\ (CH_2O) \quad (2)$$

The detected CH_4 fluxes of the anoxic experiments were used as an estimate for the CH_4 production. For this it was assumed that the anoxic and oxic CH_4 production were the same and that the difference in CH_4 flux between oxic and anoxic incubations was due to CH_4 oxidation. The nitrogen oxidation was estimated from the sum of the NO_x^- and N_2 flux (nitrogen had to be oxidized before further reduction to N_2 could occur) in the oxic incubations. The contribution of the NH_4^+ and CH_4 oxidation to the SOD varied with the month of core sampling. Using equation 1, the NO_x^- and N_2 fluxes and the SOD values for sandy and muddy sediments from Table 1+2, the percentage of the SOD used for the oxidation of nitrogen was calculated to be about 33% for the muddy and about 20% for the sandy sediments. Zimmerman and Benner (1994) and Adams et al. (1992) found also that about 30% of the SOD was due to the oxidation of N, but in comparison with 7-12% found by Sweerts (1990) this is quite high. Throughout the year the contribution of the CH_4 oxidation to the SOD ranged from 11-32% for muddy and 0.6-2.0% for sandy sediments.

In sandy sediments the organic matter content is restricted to the top layers of the sediment. Due to organic matter limitation the methanogenic bacterial population will be small as well, resulting in a low CH_4 production. For the same reason the major part of the NH_4^+ in sandy sediments will be produced by aerobic mineralization and only a very small part by anaerobic mineralization (Figure 6).

With equation (1) and the equation for aerobic mineralization after Froehlich et al. (1979):

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 106\ O_2 \rightarrow 106\ CO_2 + 16\ NH_3 + H_3PO_4 + 106\ H_2O \quad (3)$$

it could be calculated that when 20% of the SOD was used for the oxidation of nitrogen, 66% of the SOD had to be used for the aerobic mineralization, to produce the equivalent amount of nitrogen. As not all the ammonium produced was oxidized this is a minimum value. It was therefore considered that, despite the fact that the 'steady state' from the lake was broken in the laboratory experiments, the SOD could be explained quite well. For muddy sediments also great influence of the easily degradable organic matter can be expected, although the situation is more complex. Oxygen now is used for the oxidation of NH_4^+ and CH_4 produced in the deeper anoxic sediment layers, for the aerobic mineralization and for the oxidation of NH_4^+ produced in the oxic zone (Figure 6).

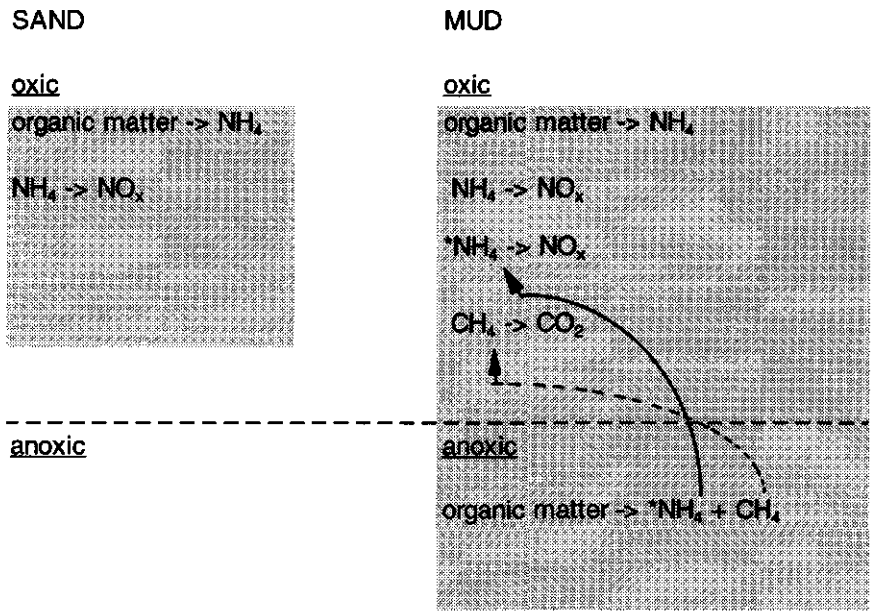


Fig. 6. Scheme of mineralization processes related to oxygen consumption in sandy and muddy sediments. The shaded area represents the area where organic matter is available.

Based upon the equations (1) and (3) the percentage SOD due to nitrogen oxidation (30%) seems overestimated; more N was oxidized than could be explained by the C:N ratio. As the SOD values seemed correct and the NH_4^+ flux of the anoxic incubations was large in comparison with the CH_4 flux it was supposed that the N mineralization is more complete or proceeds faster than the C mineralization.
The produced NH_4^+ and CH_4 not only affect the SOD. Oxygen itself is an important regulating factor for the various N processes in the sediment. The species composition of the total N flux during an annual cycle seems to be determined by oxygen. The high NH_4^+, low NO_x^- and the detectable CH_4 fluxes in spring suggest an O_2 deficiency. The detected NO_2^- flux during the switch further indicates that the sediments shift from anoxic to oxic conditions. In summer only NH_4^+ fluxes were found. During these months no nitrification and subsequent no denitrification occurred at all. In periods when almost no NO_x^- fluxes were found nearly all oxygen seemed to be used by the aerobic mineralization and the CH_4 oxidation and almost no oxygen was left for the oxidation of N. This is quite likely as the oxidation of CH_4 is thermodynamically favoured. Furthermore it is known that the Michaelis-Menten constant for oxygen for the methane oxidizers (<3 µM; Kuenen and Bos, 1989) is much lower than for the nitrifying bacteria (16-62 µM; Focht and Verstraete, 1977).

Therefore the methane oxidizing bacteria probably compete successfully with nitrifying bacteria for oxygen. This was also found by Sweerts (1991).

Influence of fresh and easily degradable organic matter

What can cause the lack of oxygen for nitrification in some of the experiments?

In the experiments, performed in various months, several important factors may have been changed. Temperature could have been strongly increased or decreased after sediment transfer from the field to the laboratory incubation conditions. During the year the changing field temperature could influence the development of the populations of bacterial species and furthermore the amount of fresh and easily degradable organic matter in the field will change during the year.

The sudden temperature increase from field to laboratory temperature could explain the lack of oxygen. Due to the higher temperature processes will go faster, more oxygen will be consumed, oxygen becomes limiting and therefore nitrification will not occur. This was also suggested by Hansen et al. (1981). The temperature rise was of influence but cannot be the whole explanation. In winter the temperature difference was even greater but then almost no limitation of nitrogen oxidation could be observed. In the winter experiments the bacterial populations could have changed too, due to the higher incubation temperature. It is however difficult to ascribe the changes in the N fluxes to changing bacterial populations. The effect of an increasing population could be overruled e.g. by the effect of the ceased input of fresh organic matter. The influence of fresh and easily degradable organic matter on the oxygen availability however, is evident.

The first experiments in which oxygen limitation occurred, were observed in samples collected in April shortly after an algal spring bloom. Therefore it was suggested that the availability of freshly settled and easily degradable organic matter was of major influence. When the top layer of the sediment contains high amounts of easily degradable organic matter, high mineralization rates can be expected. The fast decay of the easily degradable organic matter does not leave enough oxygen for the other oxygen consuming processes with as a result no nitrification or low rates. Consequently also no or less coupled denitrification can take place. This was already predicted by models of Blackburn (1990) and Blackburn and Blackburn (1993) and demonstrated by Sloth et al. (1995).

The assumption that the availability of fresh and easily degradable organic matter caused the oxygen deficiency would also explain the switch in the N fluxes of the spring experiments. During the first days the mineralization and CH_4 oxidation took away most of the oxygen and high NH_4^+ fluxes were found. By the time that the easily degradable organic matter was used oxygen became available for the nitrification and also coupled denitrification could occur. In August no switch occurred at all and denitrification was not found at the lower temperatures as well. Besides O_2 shortage, the nitrifying bacteria additionally may have been inhibited by e.g. H_2S (Henriksen and

Kemp, 1988). In relation to the high total microbial mineralization rates during this period, the total N fluxes were quite low. Sloth et al. (1995) however found that when easily degradable organic matter was available in the top layer of the sediment not only NH_4^+ but also dissolved organic N (DON) was released to the overlying water. Thus high mineralization can have taken place whereas lower NH_4^+ fluxes were found. Unfortunately we do not have data for DON.

Based upon our results it was suggested that due to the organic matter content of the deeper sediment layers a certain basic mineralization, and if the oxygen availability is sufficient, nitrification and denitrification takes place, whereas in the top sediment these processes and rates depend strongly on the freshly settled and easily degradable organic matter and the temperature. For the anoxic incubations the combined effect is shown in Figure 7.

With the equations derived from the temperature experiments, fluxes at in situ temperature were calculated. For both sediment types a distinct influence of the season was observed, with higher NH_4^+ fluxes in spring and summer and lower fluxes in winter. Although the temperature change between December and March was larger than between March and April, the NH_4^+ flux increased markedly in April, probably due to freshly settled and easily degradable organic matter. The NH_4^+ fluxes from the sandy and the muddy sediments were within the same range. The combined effect of sediment composition, availability of bacteria and temperature for the aerobic incubations is more difficult to demonstrate.

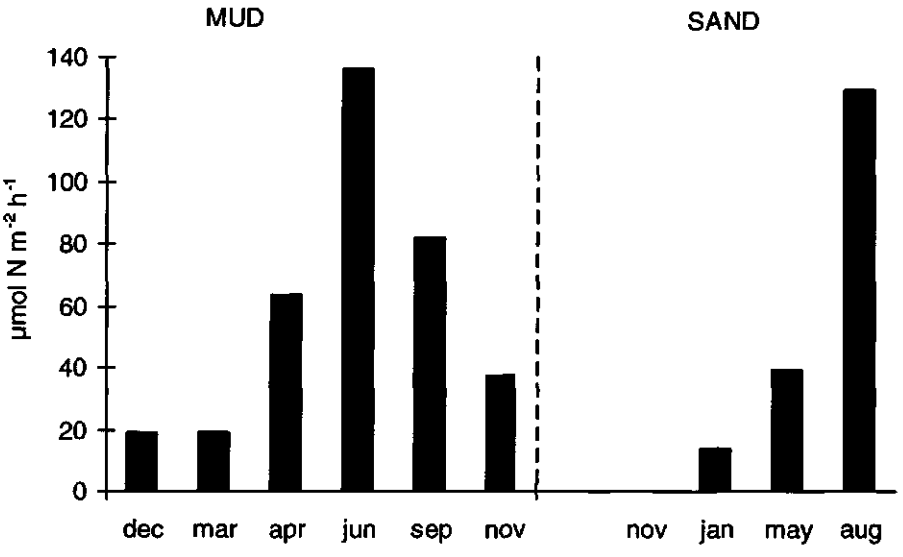


Fig. 7. Anoxic NH_4^+ fluxes at in situ temperature.

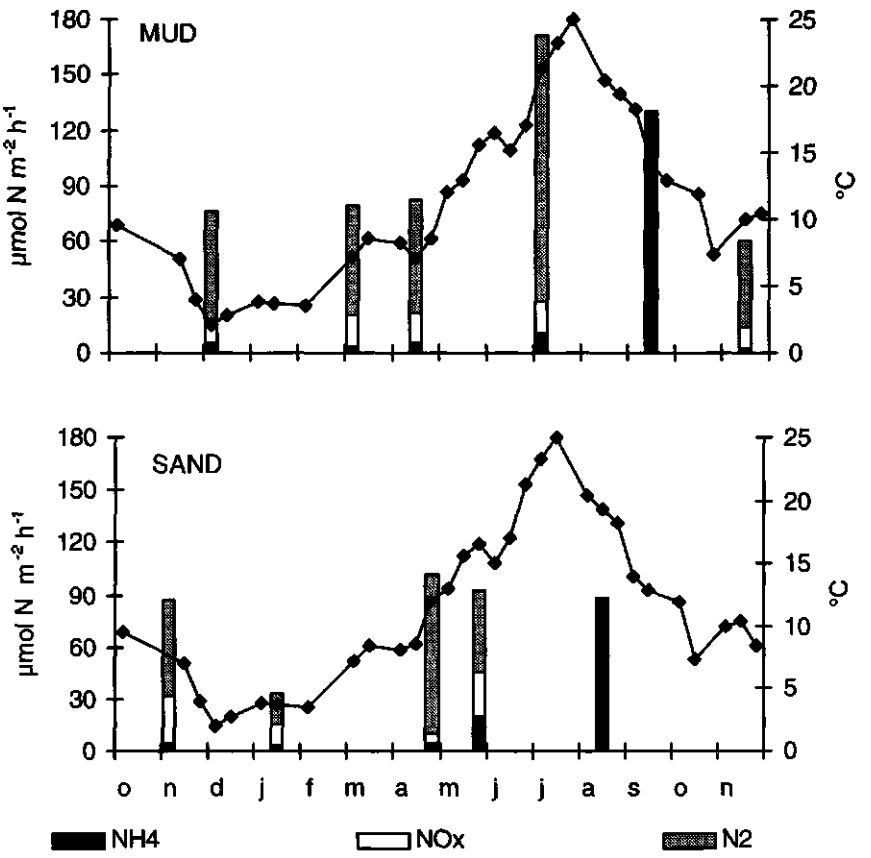


Fig. 8. N fluxes from oxic muddy and sandy sediments at in situ temperature.

As in the calculations for the in situ temperature only the 'equilibrium' fluxes could be used, only the effect of the bulk organic matter content combined with temperature could be shown (Figure 8).

The calculated total N fluxes of the sandy and muddy sediments are within the same range. Hence, although differences between sandy and muddy sediments were found (Table 1+2), the influence of temperature is much larger than the influence of the bulk organic matter content of the deeper sediment layers.

In conclusion, the results of this study demonstrated that the N flux from sandy and muddy sediments differs in height and species composition. The SOD is distributed among the various oxidation processes depending on the mineralization rate, which is regulated by the presence of freshly settled, easily degradable organic matter and temperature. It was demonstrated that at higher temperatures and with higher concentrations of easily degradable organic matter, nitrification and therefore the

coupled nitrification-denitrification are suppressed. This means that nitrogen will not be removed from the sediment-water system. Ammonium will be released to the overlying water where it can be consumed by algae. Although high temperatures are not often found in the Dutch surface waters, these conditions can occur in spring and summer and may contribute to algal blooms. Eutrophication then can become a self accelerating process.

Chapter 5
Variation in N fluxes from sediments of a fresh water lake.

ABSTRACT

In order to examine the spatial variability of N fluxes from lake sediments, muddy and sandy sediment cores were collected throughout lake Wolderwijd/Nuldernauw, The Netherlands. Sediment characteristics and nutrient fluxes measured in the laboratory showed little variation in a sediment type, whereas between sediment types these differences were significant. The variability of fluxes from lake sediments in comparison with terrestrial soils, however, is very small. It was hypothesized that this was due to the much larger influence of micro-sites in the soils.

Principal component analysis (PCA) performed on the fluxes, the sediment characteristics or the total set of variables distinguished the main sediment types mud, sand and peat each time. It was therefore concluded that the N fluxes depended on the sediment type, although an obvious and direct relationship between the measured sediment characteristics and the N fluxes was not found.

The conclusions mentioned above facilitate the estimation of the total N loss of a lake by denitrification. Only samples of the dominant sediment types in the lake have to be collected. Together with the corresponding areal coverage of these sediment types and the measured N_2 fluxes the whole lake fluxes can be calculated.

Francien van Luijn, Paul C.M. Boers and Lambertus Lijklema

INTRODUCTION

In many aquatic ecosystems the sediments are an important source of nitrogen for primary production because of nutrient cycling (Blackburn and Henriksen, 1983). A part of the mineralized nitrogen, however, is not returned to the overlying water. It is removed from the sediment-water system to the atmosphere as N_2 gas by denitrification. Denitrification therefore may be a regulating factor for phytoplankton and macrophyte production in natural systems. Similarly in systems receiving N from anthropogenic sources denitrification may help to control eutrophication by removing part of the load (Seitzinger, 1990).
The rates of the various N-processes in the sediments are, among others, influenced by oxygen availability, nitrate and ammonium concentrations in the overlying water, organic matter content of the sediment, organic matter settled on the sediment surface, pH, temperature, toxic compounds and benthic algae. These factors partly are interrelated. In previous work (Van Luijn et al., submitted) the influence of temperature and the organic matter content of the sediment was investigated on two sites in lake Wolderwijd/Nuldernauw. Both factors have an impact on the various N fluxes and especially their combined influence is large. To estimate the total N loss of a lake spatial variability furthermore might be an important factor. From terrestrial soil studies it is known that spatial variability of denitrification rates can be very large. It seems that the denitrification takes foremost place in so called hot spots: micro-sites with often high particulate organic matter contents and a patchy distribution in the soil (Parkin, 1987). Objective of this study was to examine the spatial variation of N fluxes from lake sediments. Furthermore it was determined if these fluxes were related to sediment type and if this was due to sediment characteristics.
The study on the contribution of the sediment in the N cycle was performed in the shallow freshwater lake, lake Wolderwijd/Nuldernauw, The Netherlands.

MATERIAL AND METHODS

Lake Wolderwijd/Nuldernauw has a surface of about 2670 ha and a mean depth of 1.6 m. In lake Wolderwijd most of the sediments consist of sand, although at some places the top of the sediment is formed by sandy clay or clay. In lake Nuldernauw more sandy clay is found and another area consists of peat. Furthermore, especially in the shipping canals, settled organic matter is found. To investigate the influence of the variability of the sediment in the lake on the nutrient fluxes, sediment was sampled on several locations throughout the lake (Figure 1).
In January 1995 cores were collected from lake Wolderwijd and in February and June 1995 from lake Nuldernauw. The sediment cores were collected with a Beeker sampler (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands).

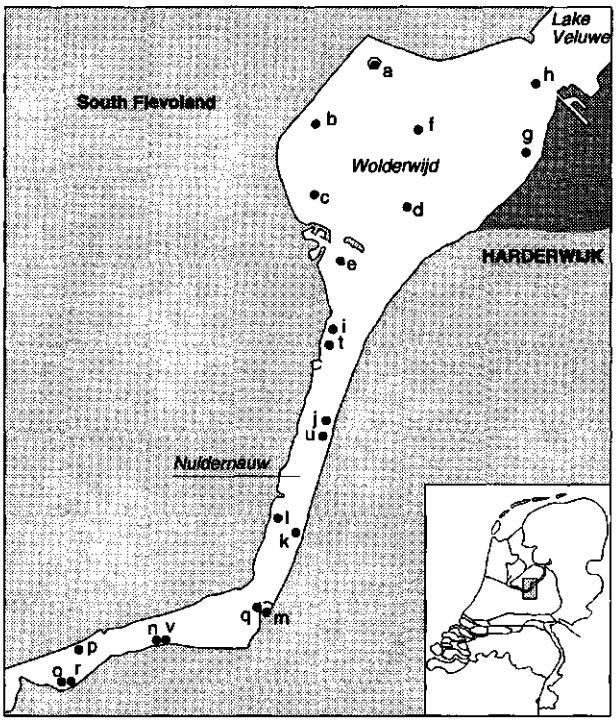


Fig. 1. Map of lake Wolderwijd/Nuldernauw, indicating the various sample locations.

In the laboratory the various nutrient fluxes (NO_x^-, NH_4^+, N_2, ortho-P and Si) from the sediments were measured. The N_2 fluxes were estimated with the modified N_2 flux method which was described in detail by Van Luijn et al. (1996). Here only the outlines are given. The top 2-4 cm sediment, about 120 ml overlying water and a headspace of 100 ml were incubated in the dark, in gastight chambers (i.d. 56 mm). The air in this headspace was daily replaced with a gas mixture of He with 112 ppm CO_2 and 21% O_2 by flushing for about 1 hour with this gas mixture. The increase of N_2 in the headspace was measured daily after about 20 hours of incubation. The N_2 concentration was estimated by gas chromatography (Hewlett Packard, 5890 series 2 with chrompack plot fused silica column (25m x 0.53mm, molsieve 5Å) and thermal conductivity detector). Because we were interested in the N fluxes to the overlying water when no dissolved nitrogen compounds were available in this water, the water phase existed of nutrient free artificial surface water. The water phase was replaced daily by this artificial water, flushed with the gas mixture. Additionally, the nutrient fluxes NO_x^- (nitrate and nitrite), NH_4^+, ortho-P and Si could be calculated from the concentrations measured in the replaced overlying water at two successive sampling times (Boers and Van Hese, 1988). The nutrients were analyzed using standard

procedures on a SKALAR auto analyzer. During the first 10 days of the incubation N_2 gas, initially present in the pore water, gradually diffused from the sediment. Hereafter the measured increase in N_2 was due to denitrification (Van Luijn et al., in press).
A foregoing study demonstrated that the main variations in the rates of the various N processes between sediment types could be expected at higher temperatures (Van Luijn et al., submitted). Therefore the experiments were performed at 23°C. Furthermore the sediment characteristics Fe, Ca, % dry weight, % inorganic C, % organic C, P (total) and N (total) were measured in the top 5 cm of the sediment. Fe and Ca were measured by Atomic Emission Spectrometry after digestion with nitric acid and hydrochlorid acid. Total P was determined by photometric analysis on an auto analyzer after catalyzed (by mercury (II) oxide) destruction with concentrated sulphuric acid with potassium sulphate. Total N and total C were measured on a C/N analyzer. In a second sample the inorganic C was eliminated with hydrochlorid acid before the catalytic ignition, whereafter only organic C was measured. The inorganic C content then was calculated by subtracting the organic C content from the total C content. The percentage of dry weight was obtained by freeze drying during 7 days. The sediment oxygen demand (SOD) was estimated from the slope of the initial linear decrease of the oxygen concentration. The oxygen concentration was measured continuously in stoppered cores without headspace. The water column was continuously stirred without disturbing the sediment surface. These experiments were performed at 20°C, in the dark.
Multivariate analysis was performed to describe the differences between the sampling points and to determine which of the measured variables (sediment characteristics and nutrient fluxes) explained most of this variability between the sampling points. Furthermore it was searched if the fluxes were related to the sediment characteristics measured. The method applied was principle component analysis (PCA), as available in the statistical program SPSS (Norusis, 1993). This method first computes the correlation matrix for all variables in order to see if the variables are related. Hereafter the measure of sampling adequacy (MSA), an index which represents correlations between pairs of variables can be calculated. If this index for a specific variable is low, it can be eliminated from further analysis.
Next, the remaining set of variables is reduced to several uncorrelated factors which represent the data. These factors are a linear combination of the original variables. The coefficients which relate the variables to the factors (factor loadings) indicate how much weight is assigned to each factor. Factors with large coefficients for a variable are closely related to this variable. Next the factors were transformed by Varimax rotation with Kaiser normalization. This method minimizes the number of variables that have high loadings on a factor and makes the interpretation of the factors easier. High factor loadings for various variables on the same factor indicate that these variables may be correlated. If there is indeed a direct relation between such variables, this can be gathered from the square of the appropriate values in the

correlation matrix. PCA was performed on the total data set of variables (both the sediment characteristics and the nutrient fluxes, the SOD excluded; Table 1+2) and on the sediment characteristics and nutrient fluxes separately. With stepwise multiple regression analysis it was furthermore investigated if the observed fluxes were related to the sediment characteristics.

RESULTS

On the basis of visual judgement most locations could be classified as sandy or muddy sediments. Locations n and v were peaty sediments and the locations i and j were classified as intermediate between the sandy and muddy sediments.
In Table 1 the results of the sediment analyses are listed. Although the various variables of sandy and muddy sediments have a wide range, there is a clear difference between these two sediment types. In general the muddy sediments have a higher N, P, Ca and Fe content than the sandy sediments, a lower % of dry weight and a larger part of this sediment dry weight is formed by carbon (inorganic and organic C). The peaty sediment resembled in total Fe the sandy sediments, in Ca, N and % dry weight the muddy ones, while % inorganic C and especially % organic C were even higher than for the muddy sediments. The values found at location i were in between the values found in the sandy and the muddy sediments.
In Table 2 the nutrient fluxes for the various locations and SOD measurements are listed. Si and ortho-P fluxes were stable throughout the experiment and are presented as mean fluxes over the total incubation period. For the nitrogen species this was not always possible as on most locations the pattern of the N fluxes showed a switch: in the first days of the incubation high NH_4^+ and low NO_x^- fluxes were found, changing to very low NH_4^+ and higher NO_x^- fluxes after about 10 days. The N_2 flux due to denitrification was also influenced by this switch: higher N_2 fluxes at higher NO_x^- fluxes (Figure 2A). Van Luijn et al. (submitted) found a switch in the spring experiments of 1994 (data not shown) as well. They suggested that the sediment switched from anoxic to oxic conditions, due to the combined influence of temperature and the availability of freshly settled, easily degradable organic matter. Therefore initially high mineralization rates took place in the upper sediment layers. Most or all of the oxygen was used by the mineralization of organic matter and the oxidation of methane and little or no oxygen was left for the oxidation of ammonium.
After the easily degradable organic matter had been used, oxygen became available for the nitrification. Thus it was assumed that after the switch the fluxes were almost completely due to the bulk organic matter content and therefore only the mean fluxes of the latter part of the incubations are used in Table 2.

Table 1. Characteristics of the various sampling points.

code	date	dw*	inorg. C	org. C	N	P	Ca	Fe	
		%	%	%	g/kg	g/kg	g/kg	g/kg	
a	Jan	33	1.6	2.6	2.9	0.56	55.3	14.7	
b	Jan	38	1.1	2.0	2.1	0.53	40.5	12.7	
c	Jan	17	1.4	4.8	5.5	0.92	45.0	25.1	
l	Feb	21	0.7	5.3	5.7	0.98	32.7	25.2	
p	Feb	53	1.5	1.6	1.4	0.47	42.9	18.7	
o	Feb	53	0.8	1.5	1.7	0.28	17.8	9.3	
r	Jun	46	0.6	2.7	1.7	0.49	25.2	9.5	
q	Jun	33	0.6	4.1	4.0	0.15	11.2	14.5	mud
i	Feb	60	0.5	1.1	1.2	0.22	16.7	6.3	mud/
j	Feb	55	0	1.2	1.0	0.13	13.1	4.8	sand
d	Jan	70	0.3	0.6	0.5	0.20	6.6	3.8	
e	Jan	76	0.1	0.2	0.2	0.05	4.3	1.8	
f	Jan	75	0.2	0.2	0.1	0.03	10.3	1.6	
g	Jan	64	0.4	0.4	0.2	0.17	8.9	6.9	
h	Jan	80	0	0.2	0	0.09	1.0	1.7	
m	Feb	78	0	0.1	0.1	0.16	0.5	1.5	
k	Feb	80	0	0.3	0	0.10	3.8	1.3	
s	Jun	77	0	0	0	0.13	2.0	1.4	
t	Jun	75	0	0	0.2	0.10	1.7	1.6	
u	Jun	71	0.2	0.1	1.3	0.09	3.7	2.2	sand
n	Feb	32	1.8	7.6	3.2	0.21	44.0	3.9	
v	Jun	38	2.2	9.6	5.5	0.30	75.3	5.7	peat

* dw: dry weight

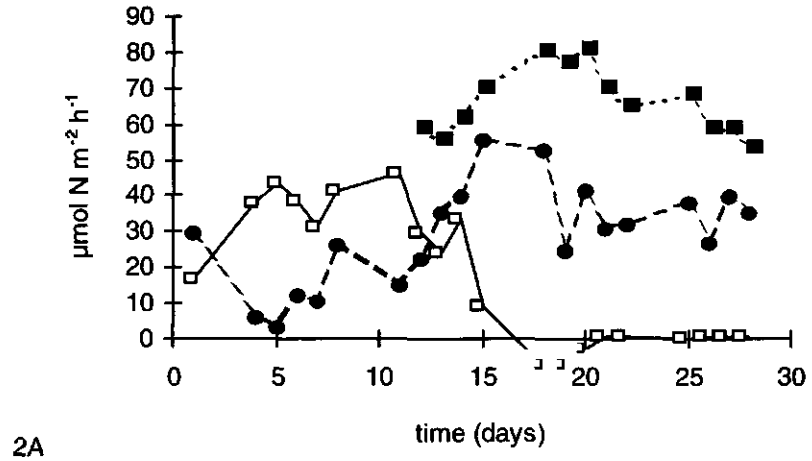
Between day 7-12 sporadic peaks of NH_4^+ together with low NO_x^- fluxes were found, whereas on location n the NH_4^+ flux was continuously higher than the NO_x^- flux. The pattern of when no switch occurred is shown in Figure 2B; the corresponding locations are marked in Table 2. In June the locations s, t, u and v showed a deviating pattern.

Table 2. Nutrient fluxes (in µmol N, P or Si m^{-2} h^{-1}; SOD in g O_2 m^{-2} d^{-1}) from the sediment on the various locations.

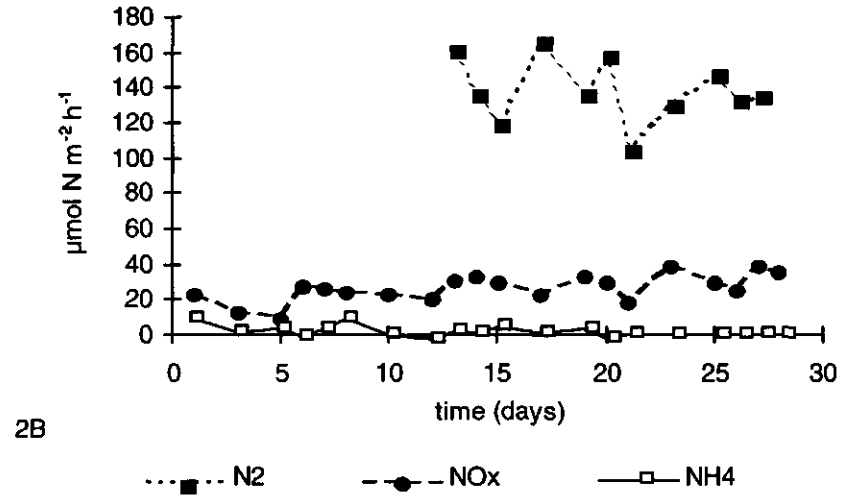
code	SOD	N_2	NO_x^-	NH_4^+	N_{tot}	o-P	Si	
a	1.8	147	40	8	189	0.1	170	
b	0.5	139	28	2	170	0.1	106	
c	NA**	137	38	8	175	0.2	137	
l*	1.5	199	40	2	250	3.5	195	
p*	1.1	144	27	2	158	2.3	129	
o	0.9	NA	38	3	NA	2.0	101	
r*	1.2	217	30	4	251	3.0	160	
q*	1.5	229	34	10	264	1.0	111	mud
i*	2.3	90	66	3	154	0.8	77	mud/
j*	1.5	91	36	6	134	1.2	127	sand
d	1.4	80	16	2	98	0.3	109	
e*	2.1	66	38	2	109	0.4	64	
f*	1.5	67	32	2	96	0.3	53	
g	1.1	73	42	2	112	0.4	47	
h	0.7	20	16	2	35	0.4	42	
m*	0.7	32	29	1	63	3.1	22	
k*	0.5	42	37	1	94	1.2	52	
s*	1.5	73	28	5	109	1.2	92	
t*	1.1	45	31	3	83	0.9	62	
u*	1.4		too variable			0.5	124	sand
n*	3.0	44	24	59	151	1.0	66	
v*	1.9	59	29	2	89	3.0	55	peat

* Experiments with switch: only the latter part of the incubation is used for the calculations; **NA: not available

Again the muddy locations were easily distinguished from the other locations. In general the N_2, N_{tot} and Si fluxes from the muddy sediments were higher than those from the sandy sediments. The ortho-P fluxes were very low for all sediment types. The NO_x^- and NH_4^+ fluxes from the various sediment types did not differ much.



2A



2B

Fig. 2. A. Example of switch in the pattern of N fluxes (mud, January 1995, location b);
B. Example of N fluxes without switch (sand, January 1995, location e).

The SOD ranged from 0.5 - 3.0 g O_2 $m^{-2}d^{-1}$ for all sediment types (Table 2). As the SOD was quite constant no relationships between the SOD and sediment type or SOD and nutrient fluxes could be established.

DISCUSSION AND CONCLUSIONS

The results showed that the mean ortho-P and NH_4^+ fluxes were quite low and did not vary much between sandy and muddy sediments. The variations of these fluxes were quite large, because the concentrations in the overlying water from which the fluxes were calculated, often were close to the detection limit. The relative standard deviation of the NO_x^- flux over all sampling points was only 30%. The two sample t-test furthermore did not discriminate between these fluxes of sandy and muddy sediments, suggesting that this flux is independent from the sediment type. Although the Si fluxes from sandy sediments had a large relative standard deviation (43%), these fluxes differed, according to the two sample t-test, significantly from the Si fluxes from the muddy sediments ($p << 0.01$; data not shown). The N_2 and N_{tot} fluxes of the muddy sediments were also significantly different ($p=0$) from the sandy sediments. The relative standard deviations of these fluxes in between a sediment type were for mud: 22 respectively 20%, and for sand: 35 respectively 31%. These standard deviations were much smaller than the differences in the fluxes of sandy and muddy sediments together (61% respectively 41% for N_2 and N_{tot} fluxes). The latter standard deviations in their turn were much smaller than those found in soils (114-355%; Christensen et al., 1990). Parkin (1987) found that an important cause of the variability is the patchy distribution of so called 'hot spots', i.e. relatively large micro-sites with a high particulate organic matter content, which have a high denitrification activity. At higher water contents of the soil, the denitrification was more evenly spread throughout the soil. Flooding of the soil inhibits O_2 diffusion into the soil and the variability of coupled nitrification-denitrification will decrease. Not only the micro-sites but the total of deeper soil parts becomes anoxic (Christensen et al., 1990). Lake sediments can be compared with flooded soils as the deeper parts of these sediments are anoxic too. The influence of micro-sites then also may be assumed of less importance. The relatively low variability in the N_2 fluxes of the lake sediments indeed seems to indicate that the occurrence of micro-sites in these sediments was less important.

The results of earlier experiments, however, demonstrated that the occurrence of micro-sites seems likely. Van Luijn et al. (1996) suggested that the occurrence of micro-sites would explain the differences observed in coupled denitrification rates as measured by the N_2 flux method and the ^{15}N isotope pairing technique. The latter technique is based on the assumption that $^{15}NO_3^-$ added to the overlying water is uniformly mixed with $^{14}NO_3^-$ produced by nitrification in the sediment. With the occurrence of micro-sites this assumption becomes invalid (Nielsen, 1992). The occurrence of micro-sites therefore can explain the underestimation of the coupled denitrification by the ^{15}N isotope pairing technique. Results of the N_2 flux method demonstrated that the coupled denitrification went on and remained unchanged after nitrate additions to the overlying water (Van Luijn et al., 1996). Also when the nitrate

concentration in the overlying water increased due to nitrification during prolonged incubation times, the coupled denitrification proceeded unchanged. This indicates that denitrification is independent of the nitrate gradient in the sediment and confirms the existence of micro-sites, where the nitrification and denitrification are coupled very tightly. In this we are not sure whether this coupling occurs through nitrate or through intermediates in the nitrification and denitrification process like N_2O and NO_2^-.
Based upon the above assumptions and results we hypothesize that in comparison with the size of the sampled core, the micro-sites in lake sediments are so small that the sediment sample gives a good average of the denitrification rates in the sediment. This makes it much easier to estimate the total denitrification rate of a lake. Only samples of the dominant sediment types in the lake have to be sampled. From the corresponding areal coverage and their measured N_2 fluxes the whole lake fluxes can be calculated.

Multivariate and regression analysis.
PCA was applied to ascertain that the various sampling points could be split into the 4 major groups (sand, mud, mud/sand and peat) reported before. Based on the very low values of the measure of sampling adequacy for ortho-P and NO_x^- these variables were eliminated from further analysis. For NH_4^+ this value was also low, but as this could be explained by one single extreme value in the data set (Table 2), this variable was not abandoned. PCA reduced the amount of variables to 2 factors, which explained 82.4 % of the total variability (Table 3; factor loadings $|< 0.3|$ are not shown).
Factor 1 comprises the N_2, N_{tot} and Si fluxes and the sediment characteristics Fe, P, N, Ca and % dry weight. High factor loadings on this factor were found for the fluxes N_2, N_{tot} and Si and the sediment characteristics Fe and P, whereas the correlation of this factor with N and Ca was much smaller. The % dry weight was negatively related to the other variables. Factor 2 comprises % dry weight, % inorganic C, % organic C, Ca, N, P and the NH_4^+ flux. Again there is a negative relation with the % dry weight. The correlation of P with factor 2 was much smaller than with factor 1. N and % dry weight had quite high factor loadings (absolute) on both factors. Interpretation of the factors is difficult. The negative relation of the % dry weight to the sediment characteristics in both factors may be explained by the fact that at higher % dry weight values the part of inert material increases. In factor 1 the strong correlation between N_2 and N_{tot} (R^2=90%) is easily explained as N_2 is the main constituent in the N_{tot} flux. N was strongly negatively related to % dry weight (R^2=78%), but no further distinct relation was obtained. The N content might be related to the N_{tot} flux as N fluxes cannot be produced if the sediment does not contain N. In factor 1 furthermore, Fe and P were strongly related to each other (R^2=83%). And although the relation with Fe and P is weak, it is not amazing to find Ca in this factor as well.

Table 3. Factors selected by PCA of the total set of variables minus ortho-P and NO_x^- after Varimax rotation with Kaiser normalization, with Eigenvalues > 1. Factor loadings < |0.3| are indicated by x. Factor 1 explains 62.4%, factor 2 20% of the total variability.

	Factor 1	Factor 2
% dry weight	-0.705	-0.678
% inorg. C	x	0.897
% org. C	x	0.915
Ca	0.357	0.840
N	0.592	0.723
P	0.819	0.338
Fe	0.888	x
N_2	0.934	x
NH_4^+	x	0.655
N_{tot}	0.874	x
Si	0.912	x

The quantity of P which can be bound in a sediment depends strongly on the available Fe and less on the amount of Ca. The ratio between total P and total Fe in the sediment is furthermore a good indicator of the potential internal P loading. When this ratio is below 0.08 g P/ g Fe little P release may be expected (Van der Molen and Boers, 1994). In our samples the P/Fe ratio was in general much lower (0.04 in average) and the ortho-P fluxes from our sediments were very low indeed. The N_2, N_{tot} and Si fluxes and the sediment characteristics Fe, P, N and Ca did not show a correlation although these variables were computed together in one factor. It might be that the relation is due to an underlying dimension like sedimentation. N, P and Fe are especially found in the finer fractions of the sediment, which are deposited in the deeper parts of the lake. In this material the mineralization rate often is also relatively high, resulting in e.g. N_2 and Si fluxes. Although the freshly settled and easily degradable organic matter is also of great influence on the nutrient fluxes (Van Luijn et al., submitted), the % organic C was not found in this factor. We assume this is due to the fact that the freshly settled and easily degradable organic matter was only a small part of the total organic matter content. This was confirmed by the peat samples. In factor 2 a strong relationship was found between % inorganic C and Ca (R^2=93%). This is explained by the fact that Ca is mostly bound to the inorganic C. Of course a correlation was also found between N and % organic C (R^2=78%). In Figure 3 the factor scores of all locations are plotted with respect to the 2 factors. Three major groups were distinguished: peaty, muddy and sandy locations.

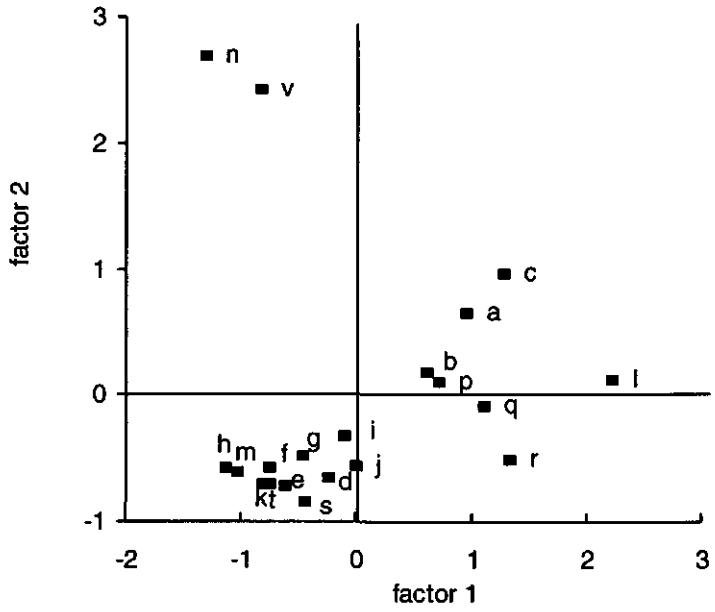


Fig. 3. Plot of the locations after PCA on the total set of variables.

The mud/sand locations (i and j) were classified nearby the sandy locations. This corresponded extremely well with the groups formed on visual basis (Table 1+2). When only the sediment characteristics were used for PCA (data not shown), the same main groups were found, whereby the mud/sand locations were part of the sandy group. An explanation could be that the sediment characteristics were dominated by the sand, whereas the mud had a higher contribution to the nutrient fluxes (mud was found in the top layer of these sediments). This was demonstrated by applying PCA to the fluxes. Two groups were found (Figure 4): one for sandy locations including a peat sample and one for muddy and mud/sand locations.

It was assumed that the nutrient fluxes would be related to % organic C and N. A direct correlation between the fluxes and the sediment characteristics, however, was not found, although Fe seemed to be an important variable for the N_2 and Si flux (R^2= 60%). In peat high organic C contents are combined with relatively low mineralization rates. The peaty samples of our study also had a relatively high % organic C and as a result of the low mineralization rates, relatively low nutrient fluxes. When the peaty sediments were excluded from the stepwise regression analysis of N flux and sediment characteristics, % organic C and N became important related variables. This indicates that the degradable organic C content would be a better sediment characteristic than the total % organic C. The degradable organic matter, however, was not measured.

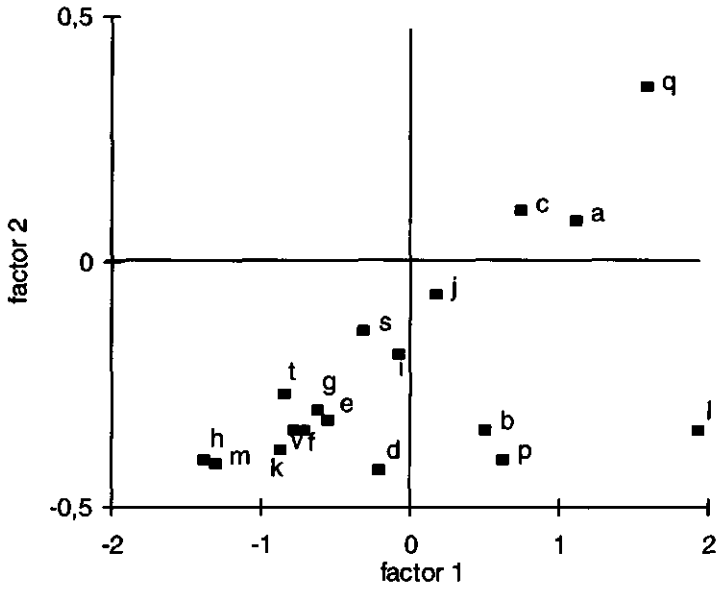


Fig. 4. Plot of the locations after PCA on the flux variables.

In conclusion, significant differences in the nutrient fluxes originating from the bulk organic matter content of muddy and sandy sediments were found and the various sediment types could be distinguished by the measured sediment characteristics. Almost the same distinction in sediment types was obtained when discriminating on basis of nutrient fluxes. Thus, although an obvious and direct relationship between the measured sediment characteristics and the N fluxes was not found, the flux depended on the sediment type. Therefore only samples of the dominant sediment types in a lake have to be sampled for the estimation of the denitrification rate of a lake. From the corresponding areal coverage of the sediment types and their measured N_2 fluxes the whole lake fluxes can easily be calculated.

Chapter 6
Influence of benthic diatoms on the nutrient release from sediments of shallow lakes recovering from eutrophication.

ABSTRACT

Measures taken to combat eutrophication resulted in a decrease in phytoplankton chlorophyll and an increase in transparency in the lakes studied. Because of the low nutrient concentrations in the overlying water, the increased light availability and the relatively nutrient rich sediments, a benthic algae community developed. In this study the interactions between the benthic algae and the nutrient release from the sediments is examined. In laboratory experiments it is demonstrated that benthic diatoms are able to grow on nutrients released from the sediments. The direct result is a decrease of the nutrient flux from the sediments by uptake by benthic diatoms. An indirect effect is an increased loss of nitrogen from the sediment-water system to the atmosphere by stimulation of the coupled nitrification-denitrification. This is caused by an increased O_2 penetration depth due to the photosynthesis of benthic diatoms. Based upon these laboratory results and additional calculations, it is concluded that the benthic diatoms in the field are able to reduce the nutrient release from the sediments and thus the availability for the phytoplankton. The benthic diatoms therefore may accelerate the process of recovery from eutrophication.

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INTRODUCTION

Most of the surface waters in the Netherlands are eutrophic and therefore have a poor water quality. In the last 20 years several measures were performed to improve water quality. Because of reduction of the external P loading, flushing and fish stock management the lakes studied here now seem to recover. The total phosphorus concentrations in the lakes decreased and P became the dominant growth limiting factor for phytoplankton. As a result the phytoplankton chlorophyll decreased significantly and the transparency increased (Jagtman et al., 1992; Meijer et al., 1994). In the lakes studied here the low nutrient concentrations in the overlying water, the relatively nutrient rich sediments and the increased light availability formed an ideal niche for benthic algae, so a benthic algae community dominated by diatoms developed (Van der Molen and Helmerhorst, 1991). Uptake of nutrients is a direct effect of the presence of benthic algae, but also indirect effects are observed. Due to the photosynthesis of the benthic algae oxygen is produced and the oxic surface layer of the sediment may increase (Revsbech and Jørgensen, 1986). As a consequence the microbial and chemical processes in the sediment may change as well, indirectly resulting in changes in the nutrient cycling.

The increase of the oxic layer can result in a higher phosphate adsorption capacity as ferric iron will be transformed to ferrous iron, which has a higher affinity for phosphate (Stumm and Morgan, 1981). The release of phosphate from the sediments therefore may be reduced by benthic algae production. An increased oxic layer gives also a longer diffusion path for NO_3^-. The denitrification of NO_3^- from the overlying water therefore decreases with an increasing oxic layer (Nielsen et al., 1990). The coupled nitrification-denitrification however increases with an increasing O_2 penetration depth, because the nitrification is positive correlated with the O_2 penetration depth and the coupled nitrification-denitrification depends on the intensity of the nitrification (Risgaard-Petersen et al., 1994; Jensen et al., 1994).

This study focuses on the interaction between benthic algae and nutrient release from the sediments, when the overlying water does not contain these nutrients. To what extent can sediments supply nutrients for the benthic algae and to what extent do the benthic algae influence the availability of nutrients for the phytoplankton? Can benthic algae accelerate the recovering of the lakes from eutrophication by reducing the internal nutrient loading? For this purpose the release rates of nitrogen, phosphorus and silicate compounds from intact sediment cores were measured. The experiments were performed under dark and light conditions to simulate the situation without and with benthic algae.

MATERIAL AND METHODS

Study area and sediment collection

Intact sediment cores were collected in April, July and September 1993, May, June, July and September 1994 from lake Wolderwijd-Nuldernauw, The Netherlands. The lake has a mean depth of 1.60 m and an area of 2670 ha. The sediments are mainly sandy (porosity 0.31), occasionally covered with a layer of sandy clay. The upper top mm of the sandy sediments have a porosity of 0.74. Undisturbed sandy sediment cores (inner diameter 56 mm) were sampled using a Beeker sampler (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). The sediments were collected at a depth of about 1 m. For each experiment samples from the same location were taken and the water temperature and transparency were measured.

Determination of benthic algae

The concentration of benthic algae and the algae species composition was estimated by analyzing the pigment composition of the sediments. All algae contain chlorophyll-a and the concentration of this pigment is often used to estimate the algal biomass. The concentrations of other pigments or pigment combinations are used to indicate the abundance of algal classes (Klein, 1989). As influence of light, and therefore difference in algae concentration between the light and dark incubated cores was expected, in the experiments of May and September 1994 sediment samples of the top 2 mm were taken from additional cores before incubation and from the incubated cores at the end of the experiment. Of each sediment core the same surface was sampled and freeze dried immediately. Pigments were measured on HPLC after destruction with acetone (Wright et al., 1991).

Oxygen measurements

To determine the influence of the benthic algae on the O_2 penetration, in the April 1993, and September 1994 experiments the O_2 penetration was measured at various times of the incubation. The O_2 penetration depth was measured using a Clark type O_2 micro-electrode (Diamond). During the measurement of the oxygen profiles the light conditions remained the same as during the incubation. With help of the model used by Epping and Buis (submitted) the production and respiration rates were estimated from the profiles of the September 1994 experiment.

Nutrient release experiments

In the laboratory first the overlying water of the cores was replaced by artificial nutrient free surface water. Then the cores were installed in a continuous flow system (Boers and Van Hese, 1988), where the same artificial water replaced the overlying water using a Gilson Minipuls 2 peristaltic pump. Residence time was about 2 days. The cores were continuously bubbled with air to aerate and mix the water column.

Incubation temperature was 20°C and held constant using a water bath. Half of the cores were incubated in the dark. For this purpose the cores were taped with black tape. The measured fluxes are an estimation of the potential release of the sediments in the absence of benthic algae. The other cores were continuously illuminated, so the effect of benthic algae on the nutrient fluxes could be measured. In the experiments of April 1993 until May 1994 the whole sediment core was illuminated. In the other experiments light was only allowed to reach the sediment surface. For this purpose the rest of the sediment was taped off. The light intensity was 85 $\mu Em^{-2}s^{-1}$. The experiments were conducted in triplicate.
During 3 weeks the overlying water was sampled daily and frozen for later analysis on NH_4^+, NO_3^-, PO_4^{3-} and SiO_2. These nutrients were analyzed using standard procedures on a SKALAR auto analyzer. The nutrient release rates in the cores were calculated daily from the nutrient concentrations in the overlying water at two successive sampling times. A mass balance of the nutrient was calculated with the equation used by Boers and Van Hese (1988).

Translation of laboratory results to the field

During the experiments in the laboratory the temperature and light conditions were optimal. In the field the light availability on the sediment surface depends on the depth of the lake and the extinction of the water. To translate the laboratory results to the field the following assumptions were made:
- the benthic diatoms are adapted to low light intensities and a steep linear relation between light intensity (I) and growth rate (G) is supposed (Figure 1);
- light availability in the field is calculated from irradiance at the water surface (I_0), corrected for the photosynthetic active radiation (45%) and the equation of Lambert Beer;

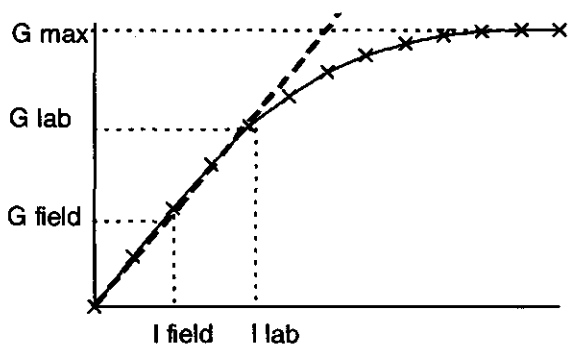


Fig. 1. Relation between light intensity and growth rate

The net growth (G) of the benthic algae can therefore be determined from the growth of the benthic algae under laboratory conditions with:

$$G_{field} = \frac{G_{lab} * I_0 * e^{-\varepsilon * h}}{I_{lab}} \quad (1)$$

G_{field} : net growth in the field
G_{lab} : net growth in the lab
I_0 : light intensity at the water surface
I_{lab} : light intensity in the lab
ε : extinction
h : water depth

RESULTS

Visual observations

In the illuminated cores of the May 1994 series not only algal growth was found on the sediment surface, but also on the whole direct illuminated side of the core. The sediments around the benthic algae were much more oxygenated then the other parts of the sediment core, which were more reduced and black. Therefore the sediment cores in the later experiments were taped off so that light could only reach the sediment surface. The nutrient fluxes of the total illuminated cores however did not differ from the later experiments in which only the sediment surface was illuminated, probably because all the released nutrients were consumed by the benthic algae.
In some illuminated cores of the September 1994 experiment snails (Potamopyrgus antipodarum) came out of the sediments during the incubation. Possibly they consumed the diatoms, as the O_2 penetration depth measured with the micro-electrodes increased only slightly.

Determination of benthic algae

Specific pigments for diatoms are fucoxanthine, diadinoxanthine and chlorophyll-c_{1+2} (Klein, 1989). Analyses of the sediments showed that these were the dominant pigments in all sediment cores, so diatoms were the dominant algae species in the sediments (Table 1). After light incubation of the May and September 1994 sediments the chlorophyll-a concentration remained quite constant (0.19-0.47 g chl-a m^{-2}) and also no significant change in the diadinoxanthine concentration, chlorophyll-c_{1+2} and fucoxanthine was found. The relative standard deviation of all pigments was 20% in the May 1994 experiment and 10% in the September experiment. Apparently no change in algae composition occurred.

Table 1. Pigment concentrations before and after incubation

sampling date	pigment name	pigment concentration (g m^{-2})		
		before incubation	after light incubation	after dark incubation
94-05-17	chlorophyll-a	0.19	0.18	not available
	fucoxanthine	0.04	0.05	not available
	chlorophyll-c_{1+2}	0.03	0.04	not available
	diadinoxanthine	0.01	0.01	not available
94-09-21	chlorophyll-a	0.47	0.45	0.24
	fucoxanthine	0.09	0.09	0.05

In September 1994 also the pigments after dark incubations were measured. Here the chlorophyll-a and the specific diatom pigments decreased by about 50%. This indicates that due to light inhibition part of the benthic algae community had decayed. The biomass of diatoms of the two experiments was different. Because only data from these two experiments were available, no explanation can be given.

Oxygen measurements

During the first days of the incubation no difference could be found between the aerobic and anaerobic oxygen profiles. The O_2 penetration depth in the illuminated cores was even less than in the dark cores. At the end of the experiment there was production of oxygen by the benthic algae and the penetration depth of the illuminated cores was larger than of the dark incubated cores (Figure 2).

The average O_2 respiration in dark cores was estimated as 0.33 g O_2 $m^{-2}d^{-1}$. This is quite low, but the SOD measured at the beginning of the incubation was also low (0.35 g O_2 $m^{-2}d^{-1}$). In the illuminated cores the oxygen respiration estimated by the model ranged from 0.60-0.80 g O_2 $m^{-2}d^{-1}$ and the oxygen production from 0.80-0.95 g O_2 $m^{-2}d^{-1}$. Assuming no other losses, the net oxygen production as a mass for net growth is calculated by the difference between production and respiration. This resulted in a net oxygen production of 0.15-0.20 g O_2 $m^{-2}d^{-1}$.

Nutrient release

Generally, it took a few days before a relatively constant nutrient flux from the light incubated sediments was measured (Figure 3). The benthic algae needed some time to reach equilibrium between light and nutrient availability and assimilation/growth and decay. In the dark cores the nutrient fluxes decreased slightly after several days, perhaps because most of the easy biodegradable matter has been used by that time.

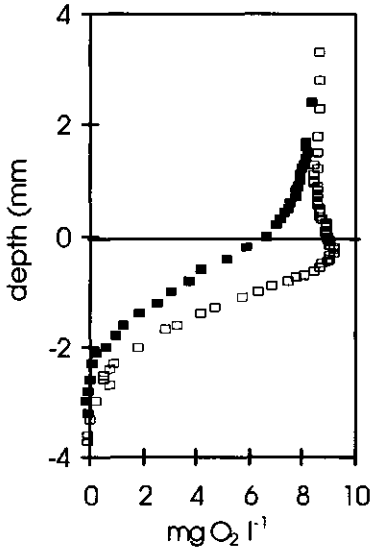


Fig. 2. Oxygen profiles in dark (solid) and light (open).

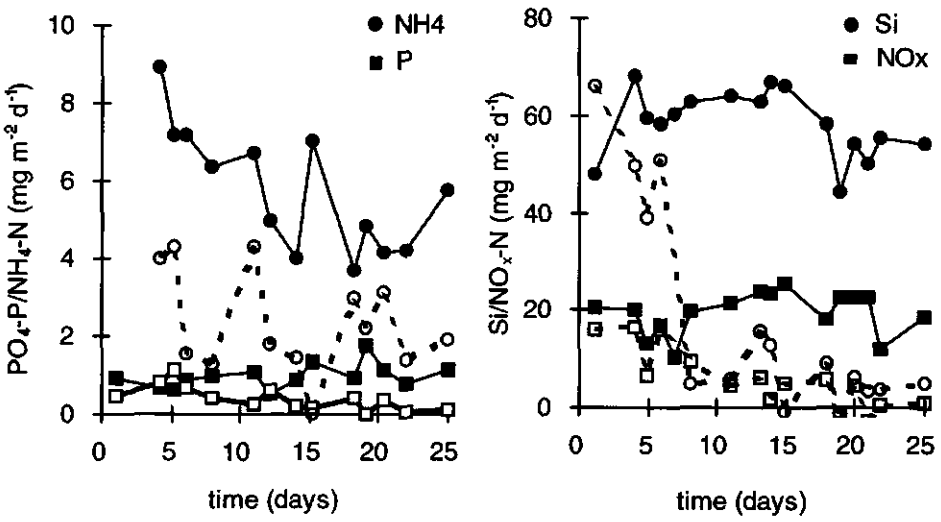


Fig. 3. Nutrient release fluxes in light (dashed lines) and in dark (solid lines).

Table 2. Field temperature, secchi depth, average nutrient fluxes (in mg $m^{-2}d^{-1}$) and one tailed p-values

sampling	field	secchi	Si		NO_x-N		NH_4-N		PO_4-P	
date	(°C)	(m)	dark	light	dark	light	dark	light	dark	light
930413	9.0	0.40	127.0	14.0*	0.30	2.6*	4.7	2.1**	0.8	0.2^{+}
930713	17.2	0.40	25.6	12.7***	15.4	0.6*	5.3	2.7^{+}	0.9	0.1*
930921	15.6	0.40	64.4	11.2*	21.7	3.4*	5.0	2.0*	1.1	0.2*
940517	15.8	0.35	61.7	4.2*	35.0	5.6*	2.6	1.8^{+}	1.9	0.4*
940614	17.7	0.35	42.0	10.3**	29.1	4.0*	3.7	2.2**	2.1	0.9***
940712	24.4	0.50	57.7	5.9*	21.0	2.3*	2.9	1.8^{+}	1.0	0.6***
940921	13.6	0.40	57.8	13.5*	17.6	7.2*	3.3	2.7^{+}	1.2	0.5*
mean			62.3	10.3	28.4	3.3	3.9	2.2	1.3	0.4

* $p = 0$; ** $p \leq 0.01$; *** $p \leq 0.1$; $^{+}$ $p > 0.1$

To compare the dark and illuminated cores of the various experiments, the mean nutrient fluxes after 10 days of incubation were used (Table 2). As expected higher net fluxes were found in the dark cores as the benthic algae in these cores were light limited and did not consume nutrients for their growth. The nutrients therefore were released by the sediments to the overlying water. In the illuminated cores the benthic algae took away the nutrients and no or only low net nutrient fluxes were found. The two sample t-test showed that almost all differences in fluxes between dark and light cores were significant (Table 2). No seasonal effects on the light and dark nutrient fluxes were found.

DISCUSSION AND CONCLUSIONS

Production of benthic algae

Among the nutrients measured here, Si is relatively inert. It is consumed mainly by diatoms and dissolution of Si from diatoms was found to be a slow non-enzymatic process (Golterman, 1960). Assuming only Si is consumed by benthic diatoms and the relation between Si consumption and benthic growth is linear, the growth of benthic diatoms follows from the difference between dark and light Si fluxes. The average of Si consumed by the diatoms was 52 mg Si $m^{-2}d^{-1}$. Si makes up 22% of the dry weight of diatoms (Bowie et al., 1985), so the average net production is 236 mg d.w. $m^{-2}d^{-1}$. Assuming the dry weight/C ratio of benthic diatoms is the same as for pelagic diatoms (3 mg/mg; Bowie et al., 1985), this results in a net growth of benthic

diatoms of 78.7 mg C $m^{-2}d^{-1}$. From the oxygen measurements a net oxygen production of 0.20 g O_2 $m^{-2}d^{-1}$ was estimated. Assuming a ratio $C:O_2$ = 1 mol mol^{-1} (Boers and Van Hese, 1988) the net production was 75 mg C $m^{-2}d^{-1}$. Despite the relatively low respiration and production rates, this corresponds well with the net growth based on the Si fluxes. The C:chl-a ratio is quite variable. De Jonge (1992) gives a ratio of 40:1 (mg/mg) for benthic diatoms measured in the field. With help of this ratio and the measured chlorophyll-a concentrations a biomass of 7.6-18.8 g Cm^{-2} is calculated. Together with the net production estimated from both the Si fluxes and the oxygen profiles a growth rate of 0.004-0.01 d^{-1} was calculated. This growth rate is low when compared to the maximum growth rate found in literature (Bowie et al., 1985). This was probably due to the used light intensity, which was closer to field intensities than to saturation.

The Si concentration in the field in spring decreases due to Si uptake by diatoms. Taking into account that in the field also diatoms of the phytoplankton are active and the temperature in spring (10°C) is below the laboratory conditions, the difference in Si flux between dark and light incubation compared quite well with the decrease of the Si concentration in the field when corrected for the external loading. This indicates that the released Si flux indeed can be consumed by benthic diatoms.

The P:N:Si ratios given for diatoms in literature are quite variable. Mostly a P:N:Si of 1:8:30 by weight (Brinkman and Van Raaphorst, 1984; Bowie et al., 1985) is assumed. The P:N:Si ratio of the difference in flux found between dark and light is totally different. The N:Si ratio found in the difference in flux between dark and light incubation ranged from 0.25 to 1.34 and was higher than 8:30. This indicates that consumption by benthic diatoms was not the only way by which N disappeared in the illuminated cores. The remaining part is probably denitrified to N_2 by the coupled nitrification-denitrification. Risgaard-Petersen et al. (1994) showed that this reaction rate can be doubled by a deeper O_2 penetration depth caused by benthic algae. When a N:Si ratio of 8:30 is assumed, an extra N_2 flux to the atmosphere of about 11 mg N m^{-2} d^{-1} can be calculated. Compared with coupled nitrification rates found in experiments with the N_2 flux method (average 21.5 mg N $m^{-2}d^{-1}$; Van Luijn et al., 1996) a stimulation of the coupled nitrification-denitrification of about 50% was found. The P:Si ratio found in the difference flux between dark and light incubation is quite variable and in average lower than 1:30. So no effect of extra adsorption due to an increased oxic layer was observed. The P release reduced less than was explained by the uptake by benthic diatoms. When the pH due to the activity of the benthic diatoms increased, some release of P could have occurred because of competition between OH^- and PO_4^{-3} (Lijklema, 1980). The P concentrations however were very low, resulting in quite substantial variations. Therefore it is not possible to discuss the effect of light on the P cycling.

Anyhow, the above results demonstrate that benthic algae are able to grow on nutrients released from the sediments. Their occurrence has not only an effect on the

direct nutrient release from the sediment to the overlying water, but also results in an extra N loss from the sediment-water system to the atmosphere by coupled nitrification-denitrification.

Translation of laboratory results to the field

Based on equation 1, G_{field} in the months May-June is about 30% of the net growth of benthic diatoms found in the experiments. This means that about 30% of the nutrient uptake measured in the experiments will be realized in the field in May-June, indicating that benthic diatom growth will significantly reduce the nutrient release in this period (Figure 4).

In periods with clear water and for shallow parts of the lake the production of benthic algae even is expected to dominate the production of phytoplankton. In earlier months almost no experiments were carried out. In these months the extinction of the water is lower than in May, so the percentage G_{field}/G_{lab} may increase. However, temperatures in the field in spring are also significant lower than the experimental temperature, so the uptake by benthic diatoms cannot directly be derived from the experiments. In summer less than 10% of the experimental nutrient uptake will be realized in the field, due to the high extinction of the water (2-3 m^{-1}).

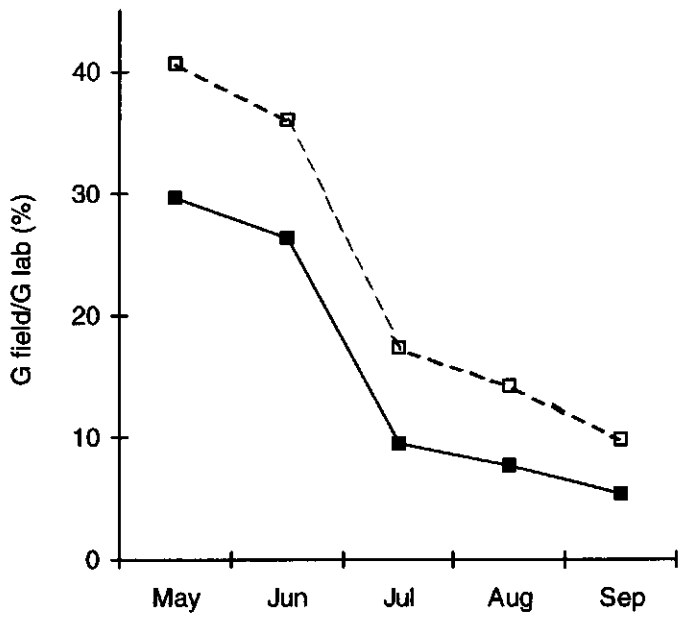


Fig. 4. Percentage of growth in the field to growth in the laboratory. Solid line: actual conditions; dashed line: by a 0.1 m increase of Secchi depth.

In Figure 4 also the effect of a 0.1 m increase in Secchi depth on the growth of benthic diatoms is depicted. The growth and consequently the effect on the release of nutrients, increases in May and even doubles in summer. This demonstrates that benthic diatoms at improving light conditions are able to decrease the nutrient release from the sediment to the overlying water and thus the availability of these nutrients for the phytoplankton. In this way benthic diatoms will accelerate the process of recovering from eutrophication. When in spring phytoplankton growth is minimized due to low nutrient concentrations, the extinction remains low, what profits the benthic diatoms. Furthermore extra N will disappear from the sediment-water system because of the indirect effects of the benthic diatoms.

Chapter 7
Interpretation of experimental N losses to the field situation.

ABSTRACT

The N losses from the sediment-water system estimated on the basis of laboratory experiments and from mass balance calculations of 1985-1992 for lake Wolderwijd/Nuldernauw are interpreted for the field situation. Based upon laboratory results a general concept, combining the effects of temperature and (easily degradable) organic matter on N loss due to the coupled denitrification, was postulated. The concept implies that the coupled denitrification initially increases with increasing contents of organic matter and/or temperature. A further increase of these factors, however, enhances the anoxic character of the sediment and the coupled denitrification decreases. The N losses measured in the laboratory at low temperatures, therefore, may be a correct estimation of the N_2 fluxes for the colder months in the field situation. If, however, the field temperature and/or the availability of easily degradable organic matter increase, the N losses based upon the laboratory experiments may no longer be representative for the N loss in the field.

From the comparison of the two estimates for the N loss, it was concluded that on an annual base about 60% of the total N input to the lake is removed, of which about 35% by the uncoupled and 65% by the coupled denitrification. This clearly demonstrates the importance of the coupled denitrification in the annual N loss of a lake. In individual months, however, storage of N in the sediment may be an important N loss as well. Each month about 60% of the total N input is removed, but the contribution of the various N loss terms is highly variable. Based upon the available data it is in general impossible to discriminate between the coupled denitrification and the storage of nitrogen in the sediment.

It was hypothesized that reduction of the nitrogen loading, along with a reduced phosphorus loading, may result in an improvement of the water quality. In systems with a high algal biomass production, however, a sufficient and immediate improvement of the water quality cannot be expected: initially the N loss will remain limited and therefore it may take some time before a significant reduction of organic matter and nitrogen is obtained. Once however the organic matter production decreases, the denitrification may increase and the improvement of the water quality will accelerate.

INTRODUCTION

Most surface waters in the Netherlands are eutrophicated due to anthropogenic loadings of N and P. To solve this problem in Dutch fresh waters for many years attention was mainly paid to phosphorus. Reduction of the P loading however did not always result in longterm improvements, due to the release of P stored in the foregoing years in the sediment (Mortimer, 1941, 1942; Marsden, 1989; Van der Molen and Boers, 1994). When nitrogen is not limiting either, algal growth can proceed. In recent years the opinion is growing that restoration of eutrophic waters can be achieved faster when the N loadings are reduced as well. It is however unknown if and how the various N transforming processes will respond to strongly reduced N loadings.
In order to obtain more insight in the N transformations in the sediments, the losses and the factors which are of influence, laboratory experiments were performed in which the overlying water was free from the nutrients N and P (Chapter 4, 5).
In this chapter the results obtained in the laboratory experiments will be interpreted for the field situation. The amount of N removed as dinitrogen (N_2) from the sediment-water system in the *in situ* situation will be estimated on the basis of results of several experiments described in foregoing chapters and compared with estimated N losses calculated from mass balances of lake Wolderwijd/Nuldernauw for 1985-1992. Furthermore the perspectives and difficulties of improving the water quality by diminishing the N loading are discussed.

LABORATORY EXPERIMENTS

The estimation of the amount of N_2 removed from the sediment-water system lake Wolderwijd/Nuldernauw by coupled denitrification is based on the results of the laboratory experiments described in the previous chapters.
Significant spatial differences were only found between sandy and muddy sediments, with higher fluxes for the muddy sediments (Chapter 5). For the estimation of the denitrification of the lake therefore only these two sediment types were distinguished.
The influence of temperature on the N_2 flux for the various sediment types can be described by e-functions (mean temperature factor = 1.9 for an increase of 10°C for both sediment types, Chapter 4). Using these e-functions and assuming that the temperature varied between months but not within a month, the N fluxes of both sediment types were estimated for the mean temperatures of the various months during the investigated years (1985-1992). Thus the monthly N losses from the lake could be calculated from the N fluxes of the two dominant sediment types at the prevailing temperatures by accounting for the areal percentage sandy (80%) and muddy (20%) sediments.

Temperature however not only influences the rates of the N transformations. Indirectly temperature influences also the availability of oxygen, as at higher temperatures more oxygen is consumed by the mineralization and CH_4 oxidation. Less oxygen remains available for the oxidation of nitrogen and therefore the species composition of the N_{tot} flux is influenced as well. In the oxic laboratory experiments from spring 1994 onwards 'anoxic' N fluxes (high NH_4^+ and almost no NO_x^- and N_2 fluxes) and CH_4 fluxes were observed at 23°C during the first 10-15 days of the incubation (Chapter 4). Due to high mineralization rates the oxygen availability was limited and the nitrification minor or absent. After these 10-15 days of incubation a sharp switch occurred and N_2 fluxes due to the coupled denitrification were observed. In the same months, at 12°C, in some experiments the NH_4^+ fluxes exceeded the NO_3^- fluxes, although a N_2 flux persisted. This indicates that the nitrification was partly limited. Although no differences in the total organic matter content of the sediments sampled in various months throughout the year were found and the fresh organic matter content of the sediment was not measured, the differences in the species composition of the N_{tot} flux in winter, spring and summer were assumed to be caused mainly by freshly settled and easily degradable organic matter (Chapter 4). In August and September 'anoxic' fluxes were even found at all three incubation temperatures (2°, 12° and 23°C) and these fluxes persisted throughout the whole incubation period of about 25 days (Chapter 4). At 2°C the NH_4^+ fluxes were higher than the NO_x^- fluxes, although the NO_x^- and N_2 fluxes did not differ from the other months.

In general, at 2°C the N_2 fluxes from the muddy sediments were only slightly higher than from the sandy sediments and for both sediment types no seasonal pattern in these fluxes was observed. It was therefore concluded that the N loss at 2°C only slightly increased with an increasing organic matter content.

Based on these results the qualitative scheme of Figure 1 serves as a framework for interpretation. The increase of the N loss with increasing organic matter content (the total of the bulk organic matter and the freshly settled and easily degradable organic matter) is based upon the results found in chapter 4 and 5. The initial organic matter content of the spring experiments is supposed to lie in range A. The observed increase of the 'anoxic' character of the sediment at 12°C is illustrated by a decreased N loss beyond a certain concentration of organic matter in the sediment. At 23°C the drop in the N loss represents the abrupt shift from the 'anoxic' N fluxes to the oxic N_2 fluxes. This shift was observed after 10-15 days, when most of the easily degradable organic matter was consumed (shift to the left on the x-axis). In August and September the initial organic matter content of the experiments is supposed to lie in range B. Here reduction of the organic matter content by mineralization during the incubation period did not yet result in a N loss. The organic matter content probably still was so large that at the high temperatures high mineralization rates limited the oxygen availability for the coupled denitrification.

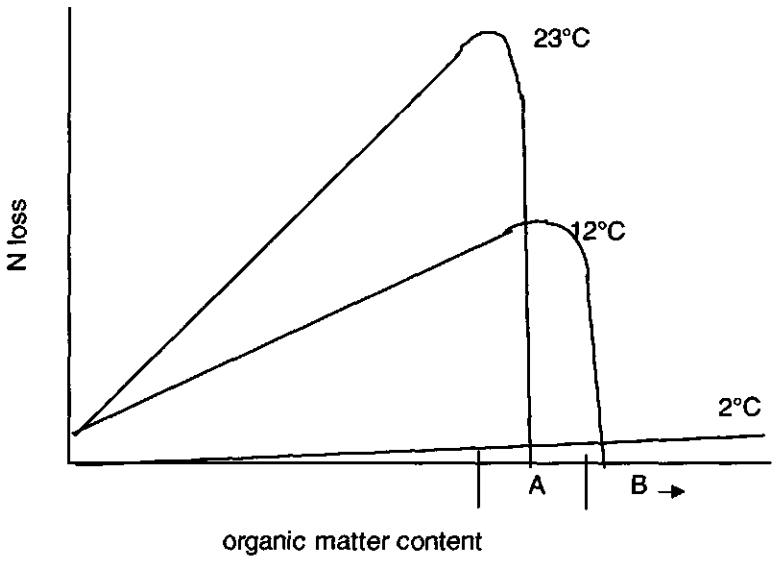


Fig. 1. Scheme of the qualitative influence of the organic matter content of the sediment on the N loss by denitrification.

Figure 1 shows that the effect of fresh organic matter settled on the sediment surface is two fold: due to an increased availability of both C and N, the coupled denitrification may be stimulated, but due to oxygen limitation caused by enhanced mineralization the coupled denitrification may be inhibited. This was also found by Billen (1982). The models of Blackburn (1990) and Blackburn and Blackburn (1993) show further that denitrification is controlled by the input of organic matter but that with increasing organic loadings the character of the sediment will become more and more anoxic, resulting in a decrease of the coupled denitrification. Figure 1 furthermore shows that mainly temperature will determine whether the coupled denitrification at a certain organic matter level will be stimulated or inhibited.

The combined effect of temperature and available (easily degradable) organic matter thus is very important. In the field not only temperature changes. Due to the primary production and decay of algae, the amount of fresh and easily degradable organic matter will change as well. In the calculation of the monthly N losses, however, the influence of the easily degradable organic matter was excluded (Chapter 4). Figure 1 therefore also is used for the interpretation of the calculated N losses over a year. The kind of N species that will be released to the overlying water will be determined by the temperature, the amount of fresh organic matter of the autumn algal bloom still available after winter (depending on the temperature in winter), the spatial (re)distribution of the organic matter settled and the amount of organic matter produced during the spring bloom. These factors control the (an)oxic character of the

sediment. The calculated N fluxes are assumed to be correct for the colder months, because at lower temperatures the bacterial activity is so low that the availability of fresh organic matter is of less importance (Figure 1, 2°C). Because the easily degradable organic matter content is unknown, it is difficult to predict what will happen when temperature increases. Based upon Figure 1 and the high field temperatures it is expected that in summer less nitrogen will be removed from the sediment-water system than calculated from the laboratory experiments: the coupled nitrification-denitrification cannot proceed at this level because, due to the fresh organic matter, the oxygen is limiting. Directly after the spring bloom oxygen was probably also limiting due to the very high organic loading. Hereafter the remaining easily degradable organic matter combined with the relatively low temperatures may stimulate the denitrification. The calculated N loss for the spring and summer months thus may not be comparable with the N loss in the field due to the influence of the freshly settled organic matter. Therefore the differences in N loss by denitrification between months also may be higher than calculated. This will be discussed in the paragraph 'N removal in the field situation'.

MASS BALANCES

Mass balances of lake Wolderwijd/Nuldernauw were described as:
input - output - storage = residual.
In Table 1 the input and output terms are listed. Data on rainfall, dry deposition and evaporation were obtained from daily measurements of the KNMI (Royal Dutch Meteorological Institute). It was assumed that no nitrogen was lost by evaporation. Seepage and infiltration rates were obtained from a ground water model.

Table 1. Input and output terms of the mass balance

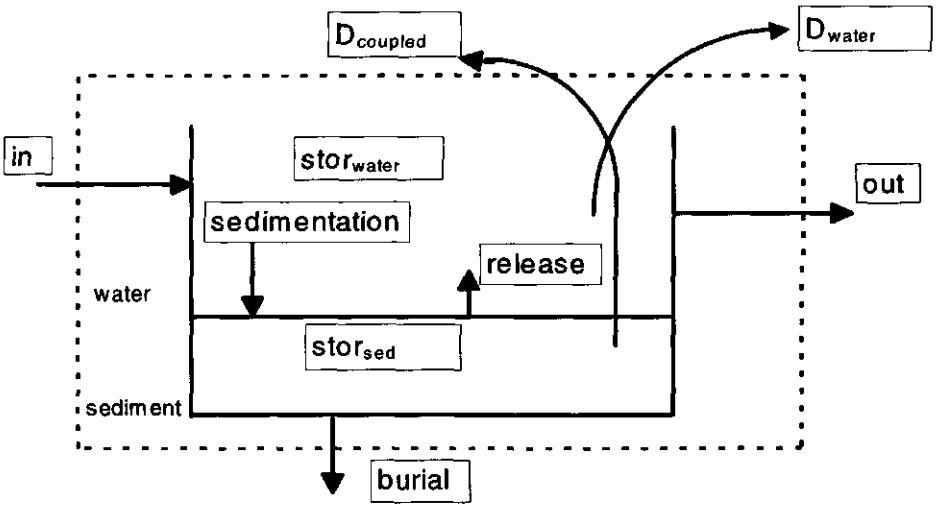
input	output
streams	polder inlet
polder outlet	infiltration
sluice leakage	sluice leakage
flushing	sluice
rainfall	evaporation
dry deposition	
seepage	

Flows and concentrations of the most important brooklet (Schuitenbeek) were measured weekly and analyzed for the various N species. The flows and concentrations of the other streams and the polder outlet were estimated by correlation with data from the main stream. At days between two measurements values were estimated by linear interpolation. The storage in the water column ($stor_{water}$) was estimated from concentration and water level measurements in the lake. The residual could be estimated with equation (1):

$$residual = in - out - stor_{water} = D_{water} + D_{coupled} + stor_{sed} + burial \quad (1)$$

D_{water}: the denitrification of nitrate from the overlying water
$D_{coupled}$: the coupled nitrification-denitrification
$stor_{sed}$: the storage in the sediment.

In Figure 2 the various terms for the N_{tot} mass balance over the sediment-water system are shown. The residual further includes the errors in the input and output terms and the $stor_{water}$. On a monthly base especially the errors caused by measurements of the water mark may be large. The estimated N losses on a monthly basis therefore are indications rather than exact numbers. On an annual base it is assumed that the nett storage in the sediment is zero.



water: in-out-$stor_{water}$= sedimentation - release + D_{water}.
sediment: sedimentation = release + $stor_{sed}$ + burial + $D_{coupled}$.
total: in-out-$stor_{water}$=D_{water} + $D_{coupled}$ + $stor_{sed}$ + burial

Fig. 2. Scheme of the various N loss terms of the N_{tot} mass balance.

Because burial is assumed to be of minor importance as well (Seitzinger, 1988), the residual consists of D_{water} and $D_{coupled}$ (1).

In Figure 3 the annual contribution of NO_3^- and N_{tot} in the input and output terms and the residual are shown for the years investigated (1985-1992). Several input terms were grouped in 3 clusters: one representing the input by the various streams and polder outlets (streams), another the sluice leakage and the input caused by the flushing (sluice) and the third the remaining terms (remaining). The input of the last cluster was mainly formed by nitrogen species other than nitrate and quite constant throughout the years investigated. The streams formed the largest nitrogen input and caused most of the variation between years. About 50% of this input consisted of nitrate. The share of nitrate in the total nitrogen input was about 30%. Since 1990 the lake is flushed with water from a nearby polder, which is poor in phosphorus and chlorophyll-a, but relatively rich in nitrate. This resulted both in an increased nitrogen loading and a stimulation of the denitrification of nitrate from the overlying water, as can be seen in the increased values of the residuals (Figure 3). Within a year the N_{tot} input is very variable (Figure 4; 1988). The highest inputs in general are found from November up to March and are caused for a very large part by the streams. In the other months the input is mainly due to the atmospheric deposition (dry and wet; rest of input terms). The residual estimated from the monthly N_{tot} mass balance varied as well (Table 2).

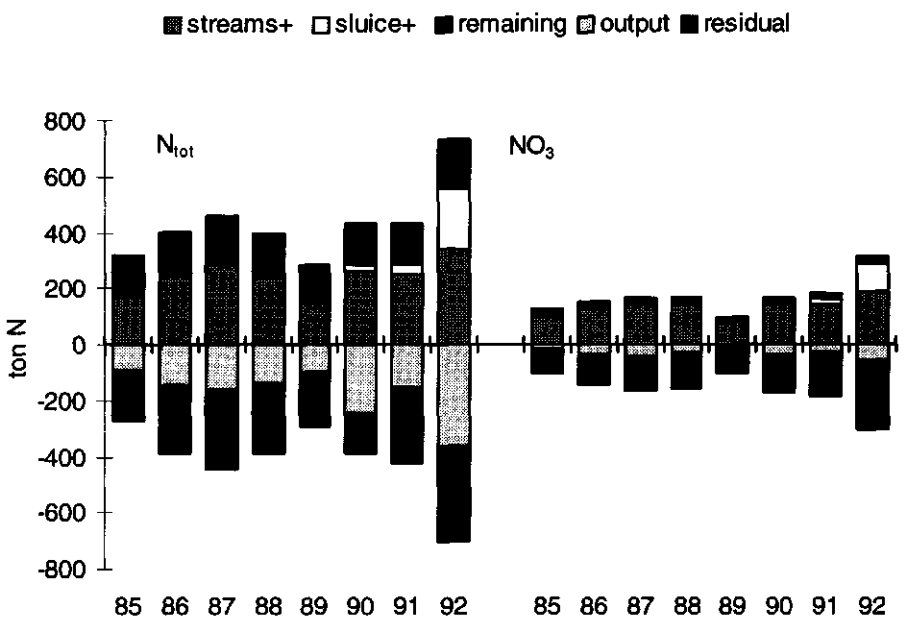


Fig. 3. Annual contribution of NO_3^- and N_{tot} in the various mass balance terms.

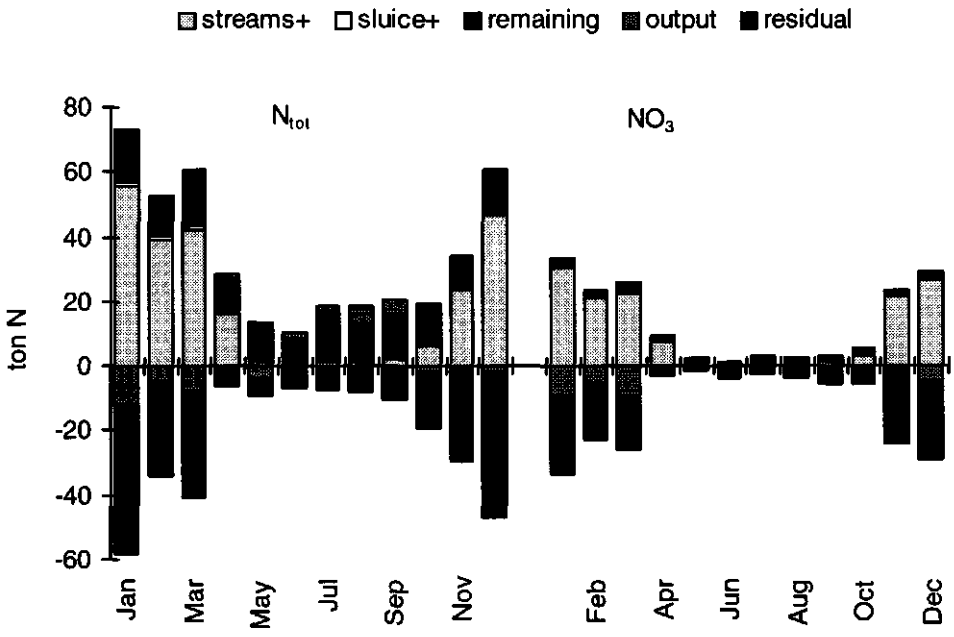


Fig. 4. Example of the monthly contribution of NO_3^- and N_{tot} to the various mass balance terms (1988).

From November up to March the residual was in average much larger than the rest of the year. As mentioned before the lake received during this period high N loadings. Because consequently the nitrate concentration in the lake increased, denitrification of nitrate from the overlying water may explain these higher residual values. According to the mass balance approach each month of the investigated period 61 ± 14 % of the N_{tot} loading of the lake was removed (data not shown). In the next paragraph it will be tried to specify the important processes causing this N loss in the various months.

N REMOVAL IN THE FIELD SITUATION

In Chapter 2 it was estimated that about 70% of the annual N_{tot} residual can be accounted for by the coupled nitrification-denitrification. In average over the period investigated in this study and without years with high flushing rates, the annual residual of the N_{tot} balance also was accounted for 70% by the coupled denitrification estimated from the laboratory experiments (Table 2). Monthly however, the differences between the two estimates of these N losses were high. The residual of the mass balances, representing the total N loss from the sediment-water system, was much higher in winter and lower in summer than the measured coupled denitrification, corrected for temperature (Table 2).

Table 2. Residuals of the N_{tot} mass balances of the various years in the various months, the mean residual over these years, the mean residual corrected for the uncoupled denitrification and the calculated N loss by the coupled denitrification based upon the laboratory experiments (all in ton N).

month	mass balance approach										laboratory
	85	86	87	88	89	90	91	92	mean	corrected	calculated
Jan	15.0	73.2	36.4	39.6	19.5	17.9	54.4	50.2	38.3	14.2	7.6
Feb	11.5	12.2	16.3	30.8	30.8	20.6	27.6	49.5	24.9	9.9	8.3
Mar	21.6	32.8	35.2	33.4	30.8	27.7	28.7	50.2	32.6	13.2	9.2
Apr	13.3	13.4	12.2	13.1	13.4	13.2	10.7	32.6	15.2	15.2	11.1
May	16.6	13.7	12.5	10.0	13.7	11.3	16.0	18.4	14.0	14.0	15.4
Jun	15.6	9.7	17.8	12.3	12.6	13.4	16.0	19.7	14.6	14.6	18.7
Jul	16.3	9.7	17.8	13.5	10.4	9.7	11.9	16.6	13.2	13.2	22.8
Aug	9.7	9.8	28.0	10.3	8.4	-11.2	13.2	19.8	11.0	12.4	19.4
Sep	9.7	10.5	15.5	10.6	8.4	-11.2	13.2	17.4	9.3	10.7	17.2
Oct	6.7	10.5	31.1	10.6	8.1	9.9	14.5	10.9	12.8	12.8	11.0
Nov	22.0	15.6	32.2	31.8	8.0	21.8	33.8	28.9	24.3	7.3	8.8
Dec	24.1	34.9	34.8	31.8	28.3	22.9	27.4	32.0	29.5	9.9	8.0
total	182.3	245.9	289.8	248.0	192.5	146.0	267.4	346.2	239.8	147.5	157.5
%*	86	64	54	64	82	108	59	45	66	107	

* part of the residual explained by the coupled denitrification calculated from the laboratory experiments.

To differentiate between the various N loss processes and to determine which processes are important in the field situation the year was divided into several periods (November-March, April, May-September, October). These periods were chosen because the N loading to the lake, the algal production and temperature in the field and/or the mineralization processes observed in the laboratory experiments typically varied between these seasons.

In November-March the temperature was relatively low and the organic matter content was below range A in Figure 1. The N loading was relatively high and the concentration inorganic nitrogen in the lake increased. The residual was larger than the coupled denitrification calculated from the laboratory experiments and it was assumed this was due to the denitrification of nitrate from the overlying water (D_{water}). According to the NO_3^- mass balance over the water:

$$\text{in - out - stor}_{water} = \text{residual} = \text{uptake (by phytoplankton) - water nitrification - release of } NO_3^- + D_{water} \quad (2)$$

and assuming that due to the low temperature in these months almost no nitrification takes place in the water column and that the uptake by algae is only minor, D_{water} will be equal to the sum of NO_3^- released from the sediment and the residual of the NO_3^- balance.

During November-March about 77% of the NO_3^- input appeared in the NO_3^- residual. The amount of NO_3^- released from the sediment was calculated from the results of the laboratory experiments in the same way as described above for the N_2 fluxes and was about 16% of the NO_3^- input. In the laboratory experiments however, the NO_3^- concentration in the overlying water was kept very low by the daily replacement of the overlying water with artificial water without nitrate. The NO_3^- release observed in these laboratory experiments therefore may have been overestimated with respect to the field. This is especially so because from November - March the NO_3^- concentration in the lake water was relatively high. Assuming D_{water} varies between 77% (value of the NO_3^- residual) and 93% (NO_3^- residual + release of NO_3^-) of the NO_3^- input and assuming that the latter value (93%) is overestimated, D_{water} was approximated to be 80% of the NO_3^- input. This is a high percentage, so the higher estimated N removal on the basis of the mass balance approach than on the basis of the laboratory experiments, in which only the coupled denitrification was measured, thus can mainly be explained by denitrification of nitrate from the overlying water (Table 2; corrected).

As stated before it was assumed that during the colder months the coupled denitrification could be calculated from the laboratory experiments. When burial furthermore was assumed to be of minor importance, the amount of the settled N which was temporary stored in the sediment can be estimated (equation 1; Table 3). During the period November-March a small monthly N loss due to storage in the sediment was found in average over the years investigated (Table 3).

Table 3. Contribution of the various N losses to the mean residual of the N_{tot} mass balance in the various months as estimated for lake Wolderwijd/Nuldernauw (in ton N).

month	residual	D_{water}*	$D_{coupled}$**	$stor_{sed}$	N_{tot} input
Jan	38.3	24.0	7.6	6.7	68.5
Feb	24.9	13.9	8.3	2.7	47.1
Mar	32.6	19.4	9.2	4.0	56.0
Apr	15.2	0.0	11.1	4.1	31.2
May	14.0	0.0	15.4	?	20.6
Jun	14.6	0.0	18.7	?	21.0
Jul	13.2	0.0	22.8	?	22.0
Aug	11.0	0.0	19.4	?	23.4
Sep	9.3	0.0	17.2	?	26.2
Oct	12.8	0.0	11.0	?	33.7
Nov	24.3	14.4	8.8	1.1	39.6
Dec	29.5	19.6	8.0	1.9	53.0
total	239.8	91.3	157.5		442.2

* estimated as 80% of the NO_3 input; ** calculated from the laboratory experiments

This N stored in the sediment will be mainly mineralized in another period, probably when the temperature increases. Due to the various errors accumulated in the residuals the estimation of the $stor_{sed}$ however is rather an indication than an exact value.

In April the external N loading decreased strongly. Enhanced temperatures and increasing irradiance induced an algal bloom as shown by higher chlorophyll-a concentrations in the lake. As the inorganic N concentration in the lake drops to zero, it is assumed that the available nitrogen (input and release) is immediately consumed by the phytoplankton and that therefore the denitrification of nitrate from the overlying water is negligible.The N_{tot} residual then is primarily due to $D_{coupled}$ and $stor_{sed}$. During and after the bloom the sedimentation rate will be high and the organic matter content reaches, due to the freshly settled easily degradable organic matter at least range A, Figure 1. As it was furthermore assumed that the temperature in the field during this month is still too low to cause oxygen depletion (Figure 1), the N loss by coupled denitrification calculated from the laboratory experiments is supposed to be correct. A positive storage of N in the sediment was expected and indeed calculated (Table 3). This quantity again is rather uncertain, because the combination of a sharp decrease of the inorganic N concentration in the lake and small errors in the water mark

Table 4. N loss terms (in ton N) for lake Wolderwijd/Nuldernauw in 1990.

	residual	D_{water}	$D_{coupled}$ [1]	$D_{coupled}$ [2]	$stor_{sed}$ [1]	$stor_{sed}$ [2]
Jan	17.9	10.9	7.6	7.6	-0.6	-0.6
Feb	20.6	11.9	8.3	8.3	0.4	0.4
Mar	27.7	24.8	9.2	9.2	-6.3	-6.3
Apr	13.2	0.0	11.1	0	2.1	13.2
May	11.3	0.0	15.4	0	-4.1	11.3
Jun	13.4	0.0	18.7	0	-5.3	13.4
Jul	9.7	0.0	22.8	0	-13.1	9.7
Aug	-11.2	0.0	19.4	0	-30.6	-11.2
Sep	-11.2	0.0	17.2	0	-28.4	-11.2
Oct	9.9	0.0	11.0	0	-1.1	9.9
Nov	21.8	14.1	8.8	8.8	-1.1	-1.1
Dec	22.9	13.9	8.0	8.0	1.0	1.0
tot	146.0	75.6	157.5	42.0	-87.1	28.5

[1] no inhibition of $D_{coupled}$; [2] $D_{coupled}$ partly inhibited (see text)

The total $stor_{sed}$ of a year is assumed to be about zero. In 1990 a total $stor_{sed}$ of zero was approximated better when total inhibition of the coupled nitrification-denitrification and therefore only release of ammonium was assumed to occur in spring and summer. Due to the lower N loss by the coupled denitrification (about 42 ton N instead of 157,5 ton N), the total N loss was small and the percentage of the N loss due to the coupled denitrification was only 29% and much lower than the average value (65%). So when during several months of a year $D_{coupled}$ or $stor_{sed}$ dominates, it may be possible to indicate whether nitrogen is foremost lost as N_2 or as ammonium. In the other years of the investigated period no distinct dominance of one of these processes was observed. The large inhibition of the coupled denitrification in 1990 is remarkable and could have been induced by the algal biomass in the foregoing year(s): the chlorophyll-a concentration in the lake increased since 1987 and reached in 1990 its maximum. Furthermore in 1989 a large autumn peak was observed (data not shown). For a good understanding, however, more research on phytoplankton growth/decay and organic matter contents and availability in the field situation during the year in relation to the nitrogen cycle is needed.

PERSPECTIVES AND DIFFICULTIES FOR LAKE RESTORATION

The foregoing paragraphs indicate that the N loss from the sediment-water system probably depends strongly on the combined influence of temperature and the available amount of fresh and easily degradable organic matter. This influence furthermore may vary during the year. Quantitative data concerning this influence, however, are not available. Therefore it is not yet possible to predict quantitatively which of the following mechanisms will occur:

- will denitrification regulate phytoplankton growth by removing most nitrogen from the system as N_2 or
- will most N still be released to the overlying water, either as nitrate or ammonium, where it can be consumed by e.g. the algae.

Hereafter some hypotheses concerning the improvement of the water quality after reduction of the external nitrogen loading are given. Improvement of the water quality after reduction of the external loading is assumed to depend largely on the initial conditions of the lake. When algal biomass concentrations are high, the sediments receive high loadings of fresh and easily degradable organic matter. Part of this degradable organic matter will be stored in the sediment until in the next growing season (spring/summer) the temperature increases. As discussed before higher temperatures together with the degradable organic matter which is still available in the sediment may cause limitation of the oxygen availability. Even when the external N loading is very low, the chances for phytoplankton to grow will be high, because the algae do not have to compete with denitrifying bacteria for nitrate as this will not be formed in the sediment. Ammonium, the preferable N species for algae, is released and immediately consumed by the phytoplankton and only little nitrogen will be removed from the system (Figure 6).

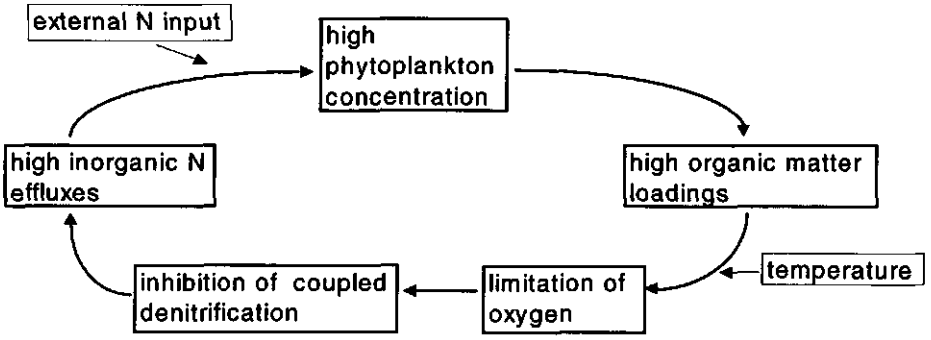


Fig. 6. Scheme of the combined influence of temperature and organic matter on the nitrogen cycling.

Although the N cycling is fast, the foregoing seems to demonstrate that once a certain algal biomass has developed in a system and enough P is available (due to external loading and/or sediment release) the eutrophic state can persist or even become more severe, depending on the external nutrient loadings and the hydrological conditions. Kemp et al. (1990) also concluded that eutrophication can become a self-accelerating process, once the nitrification is inhibited. Reduction of the N loading itself does not directly influence this self perpetuating cycle. But if the conditions in a growing season become favourable for the coupled denitrification, algal growth may become limited, especially when the nitrogen supply from external sources is small. An improvement of the water quality, however, requires a decrease in the organic matter content (and nitrogen stock) as well. For highly eutrophic systems this may take longer than one growing season.

Another problem is that in systems with a high internal or external P loading, reduction of the external N loading may be balanced by N_2 fixation. N_2 fixing bacteria have a lower affinity for P than non fixing algae (Suttle and Harrison, 1988). So, when enough P is available nitrogen fixing bacteria can bloom and introduce nitrogen to the system (Zevenboom and Mur, 1980). Schindler (1975) and Hellström (1996) concluded that N_2 fixation increases the N content until it balances the P content (stoechiometric ratio), which means that the algal biomass in fact is limited by P. Besides the external and internal N loading, the availability of ortho-P thus is very important for the phytoplankton production. As long as sufficient P is available nitrogen could be supplied and biomass formed by N_2 fixing cyanobacteria. This means that algal blooms still can develop when P is not limiting and the N loading is low and there is no standing crop of algae yet. Depending on the algal biomass developed, the sediment receives more or less fresh and easily degradable organic matter. Together with temperature this will determine if in the next growing season sufficient inorganic N is released to cause algal growth. If oxygen does not become limiting (due to a low temperature and/or because the degradable organic matter content was not sufficient), denitrification rates will be high, because the organic C concentrations will be relatively high. Due to the high denitrification rates less nitrate and ammonium will be released to the overlying water and as a consequence phytoplankton growth will be less. If ortho-P is not completely consumed additional N_2 fixation may occur. However, as long as NO_x^- and/or NH_4^+ is available in the water column, N_2 fixation will not take place (De Nobel, in prep.) and no extra algal biomass will be developed. In this situation more light can penetrate to the sediment surface so that benthic diatoms can develop. Once a benthic diatom population has developed, restoration will proceed faster. Because benthic diatoms not only use the released nitrogen but also the efflux of ortho-P, N_2 fixing bacteria have less opportunity for growth and the concomitant input of extra N will cease. Occurrence of benthic algae will furthermore result in increasing coupled denitrification rates: more N will be removed from the system and less nitrogen will be released to the overlying water (Chapter 6; Risgaard-

Petersen et al., 1994). However, the influence of benthic diatoms is not always positive. Due to the deeper oxic zone caused by the oxygen released during the photosynthesis of the benthic diatoms, the diffusion path for nitrate from the overlying water increases. Thus in systems with high nitrate concentrations due to external loading the overall effect of benthic diatoms could be reduction of the N loss, because the denitrification of nitrate from the overlying water in general exceeds the coupled denitrification over the diurnal cycle (Risgaard-Petersen et al., 1994). In systems without nitrate in the overlying water diatoms, however, will enhance the removal of N.
In conclusion, in general a large part of the nitrogen input can be removed by the coupled denitrification. N is not stored like P in inorganic form within the sediment, but mainly in the biomass of algae. Although this nitrogen of the organic matter can be recycled very efficiently, some nitrogen is always removed from the system during a year. Our hypothesis therefore is that if the external N-loading is reduced along with the reduction of phosphorus, the stock of nitrogen will decrease and cannot be filled up easily. The algal growth may decrease and the coupled denitrification may increase (Figure 6). Once this occurs, the improvement of the water quality will accelerate.
After reduction of the external N loading to systems with a high algal biomass, however, a significant improvement of the water quality N-cycles cannot be expected immediately: the results of 1990 indicate that the N loss by denitrification initially will remain limited. Therefore it may take some time before the stock of nitrogen in the organic matter is reduced sufficiently for an improvement of the water quality.

Chapter 8
General discussion

INTRODUCTION

The aim of this thesis was to investigate the important nitrogen processes and to quantify the process rates and the related fluxes to the overlying water. Special attention was paid to the coupled denitrification, because by this process N is lost from the sediment-water system. As we were in particular interested in the N effluxes after a decrease of the relatively high inorganic nitrogen concentration due to a reduction of the N loading towards the lake, the laboratory experiments were set up with a zero initial inorganic nitrogen concentration in the overlying water.
In this final chapter the main results of this research are presented. Three aspects are emphasised:
1. Results of denitrification measurements: denitrification rates obtained under various conditions and by various methods and studies are compared.
2. Oxic-anoxic interfaces and the coupling between nitrification and denitrification: the occurrence of microsites and the dependence of the coupled denitrification of the nitrate concentration in the overlying water are discussed.
3. Research by modelling: models may help to analyze the processes which control the N loss under various environmental conditions and may help to predict the field situation. The requirements of such models are indicated.

RESULTS OF DENITRIFICATION MEASUREMENTS

In the literature a broad range of denitrification rates and of the contribution of the denitrification to the N removal of the total N input is found. Part of this variation can be explained by the various methods that have been used to estimate the

denitrification (see review of Seitzinger, 1988; Chapter 2). Denitrification can be divided into the coupled nitrification-denitrification and the denitrification of nitrate from the overlying water. With the various methods often one of these sub-processes, or the total denitrification is measured. With the acetylene inhibition method e.g. only the denitrification of nitrate diffusing from the overlying water can be estimated, as the nitrification is inhibited as well (Rudolph et al., 1991). With the mass balance approach the nett N loss, of which the major part is due to denitrification, is calculated. The N_2 flux method measures the total: the coupled denitrification and the denitrification of nitrate from the overlying water is estimated (Chapter 2). For the ^{15}N isotope pairing technique the claim is that both the coupled and uncoupled denitrification can be estimated individually (Nielsen, 1992). Thus, depending on the restrictions of the method, various denitrification rates (coupled denitrification, denitrification of nitrate from the overlying water, total denitrification and nett N losses) or combinations of these rates are measured. It should furthermore be noticed that the incubation temperature, sediment type (organic matter content) and nitrate concentration in the overlying water differ for the various studies as well and this may also lead to a variation of the denitrification rates. Besides this, the studies are performed in marine and fresh water systems and for the latter in rivers as well as in lakes. These factors together make it difficult to compare the results of the various studies. Nevertheless, the denitrification rates of various studies often are compared regardless of the method and/or incubation conditions.

In Table 1 the results obtained in this research are listed together with denitrification rates of several freshwater lakes and marine systems estimated with various methods as found in literature. As expected in general higher denitrification rates were found at higher incubation temperatures. In the present study a clear influence of temperature, here on the coupled denitrification, was demonstrated as well (Chapter 4). The rates obtained with the mass balance approach are highly variable (Table 1), whereby the higher rates are found for lakes receiving high N loadings and with relatively high nitrate concentrations in the overlying water, indicating that a substantial fraction of the total N loss is due to denitrification of nitrate from the overlying water. Most denitrification rates obtained from mass balances are higher than those obtained from more direct measurements (Table 1). This is probably due to the fact that denitrification rates of the former method include the combined effect of coupled and uncoupled denitrification rates and other N removal processes, whereas only one of the two rates is measured by most of the more direct measurements.

The N_2 fluxes (due to the coupled denitrification) measured in this research are within the range of the N_2 flux measurements of other studies (Table 1). Within the same temperature range the results of the N_2 flux method are higher than the coupled denitrification rates found by the ^{15}N isotope pairing technique (Table 1;

Chapter 2). Several possible causes for the differences in denitrification rates obtained by these two methods were discussed in Chapter 2. It finally was suggested that the ^{15}N isotope pairing technique underestimates the coupled denitrification because, due to the tight coupling between nitrification and denitrification especially in microsites in the sediment, the requirement of uniform mixing of the nitrate species ($^{14}NO_3^-$ and $^{15}NO_3^-$) was not fulfilled. In the next paragraph the occurrence of microsites will be discussed.

Table 1. Denitrification rates (µmol N $m^{-2}h^{-1}$) of several freshwater lakes and marine systems measured at different incubation temperatures and with various methods.

system	method	temp	rates	ref.
freshwater lakes				
Danish lakes	NO_3 decrease	5-23	98-171	1
L. Vechten	NO_3 electrodes	7	11-55	2
L. Okeechobee	NO_3 profiles	-	31-108	3
L. Okeechobee	mass balance	-	8	3
Danish lakes	mass balance	-	245-383	4
Søbygård	mass balance	-	450-680/370	5
L. Wol-Nul.***	mass balance	-	51-106	6
L. Okeechobee	acetylene	-	2-11	7
Vilhelmsborgsø	acetylene	23	99*	8
Vilhelmsborgsø	^{15}N tracer	23	121-290*	8
Vilhelmsborgsø	^{15}N isotope	10	6-14**	9
Vilhelmsborgsø	^{15}N isotope	10	4-9**	10
Vilhelmsborgsø	N_2 flux	23	260; 395*	8
L. Vilhelmsø	^{15}N isotope	21	25-75**	11
Salten A	^{15}N isotope	13	23**	12
L. Wol-Nul.***	^{15}N isotope	23	17-141**	13
L. Wol-Nul.***	N_2 flux	23	64-252	14
L. Wol-Nul.***	N_2 flux	12	16-109	14
L. Wol-Nul.***	N_2 flux	2	13-68	14
Lake Michigan	N_2 flux	4-6	14-51	7
L.Pennsylvania	N_2 flux	-	50-56	15

system	method	temp	rates	ref.
marine waters				
Patuxent R. estuary	^{15}N tracer	-	77-89	16
Tama estuary	^{15}N tracer	9-23	13-39	17
Norsmindefjord	^{15}N isotope	10	28**	9
Norsmindefjord	^{15}N isotope	10	15-18**	10
Norsmindefjord	^{15}N isotope	-	4-8**	18
North Sea	^{15}N isotope	-	10-13**	19
North Sea	acetylene	-	0-8	20
Wadden Sea	acetylene	-	1-55	21
Lendrup Vig	acetylene	9-23	12-213	22
Texas estuary	N_2 flux	16-30	18-110	23
Texas estuary	N_2 flux	15-30	0-94	24
Narragansett Bay	N_2 flux	2-15	10-115	25
Narragansett Bay	N_2 flux	17	114-360	26

-: not available or variable; * NO_3^- enrichment; ** D14 (= coupled denitrification); *** abbreviation for lake Wolderwijd/Nuldernauw

1. Andersen, 1977; 2. Sweerts et al., 1989; 3. Messer and Brezonik, 1982; 4. Andersen, 1974; 5. Jensen et al., 1992; 6. Chapter 7; 7. Gardner et al., 1987; 8. Seitzinger et al., 1993; 9. Rysgaard et al., 1993; 10. Risgaard-Petersen et al., 1994); 11. Rysgaard et al., 1994; 12. Nielsen, 1992; 13. Chapter 2; 14. Chapter 4; 15. Seitzinger, 1988; 16. Jenkins and Kemp, 1984; 17. Nishio et al., 1983; 18. Nielsen et al., 1995; 19. Lohse et al. 1996; 20. Lohse et al., 1993; 21. Kieskamp et al., 1991; 22. Andersen et al., 1984; 23. Yoon and Benner, 1992; 24. Zimmerman and Benner, 1994; 25. Seitzinger et al., 1984; 26. Nowicki, 1994.

The underestimation of the coupled denitrification rates with the ^{15}N isotope pairing technique would also explain the low contribution of the coupled denitrification to the total denitrification (3-18%) as found with this method by Risgaard-Petersen et al. (1994) and Rysgaard et al. (1993). In the present study this contribution was estimated at 65% (Chapter 7). The various methods thus result in different denitrification rates and may explain the variable contributions of the denitrification in the total N removal as well. With results of more direct measurements which do not include the coupled denitrification 1-36% of the total N input was denitrified, with the mass balance approach this range was 0-62% (Seitzinger, 1988). In the present study the percentage of the total N input which was denitrified was 60%. Throughout the year the contribution of coupled and uncoupled denitrification to this N loss was variable, but on an average, annual base about 35% of the N loss was lost by the uncoupled denitrification and about 65% by the coupled denitrification

(Chapter 7). This clearly demonstrates the importance of the coupled nitrification-denitrification in the total N loss: the coupled denitrification accounts for about 39% of the total N input, the uncoupled denitrification for about 21%. The latter percentage fits rather well in the range for the direct measurements, which exclude the coupled denitrification. It indicates that the coupled denitrification must be included, otherwise the total N loss will be underestimated.
Seitzinger (1988) reported that the percentage N removed seemed unrelated to the rate of nitrogen loading or the extent of anoxic bottom waters. Based upon our conclusion that the availability of oxygen is very important for the coupled nitrification-denitrification (Chapter 4, 7) and the overall importance of coupled denitrification for the total N removal, this suggested lack of a relationship between the extent of anoxic bottom waters and the percentage N removed is very surprising.

OXIC-ANOXIC INTERFACES AND THE COUPLING BETWEEN NITRIFICATION AND DENITRIFICATION

In the introduction of this thesis a scheme of the important nitrogen transforming processes taking place in the sediment-water system was presented. This was a simplified scheme, dividing the sediment into an aerobic layer, where O_2 is the final electron acceptor and an anaerobic layer with various electron acceptors like NO_3^-, Mn^{4+}, Fe^{3+} and SO_4^{2-}. Furthermore only the nitrogen compounds organic N, ammonium, nitrate and dinitrogen were involved. Some results of the experiments of this thesis, however, cannot be explained by this simplified scheme. In this paragraph these results and some possible causes are discussed.
By the ^{15}N isotope pairing technique low values for the coupled denitrification were found in comparison with results of the N_2 flux method (Chapter 2). As discussed before, this difference can be explained by the occurrence of microsites (small anoxic locations surrounded by an oxic environment). Due to these sites locally a higher nitrification and coupled denitrification would occur.
In case of heterogeneity of the sediment underestimation of the coupled denitrification by the ^{15}N isotope pairing technique can occur: the assumption of uniform mixing of the nitrate isotopes, which underlies the ^{15}N isotope pairing technique, cannot be fulfilled (Nielsen, 1992; Boast et al., 1988). Nielsen (1992) suggested that this underestimation would depend on the $^{15}NO_3^-$ concentration applied: higher $^{15}NO_3^-$ concentrations would reduce underestimation of the coupled denitrification because more $^{14}NO_3^-$ would be trapped. If the coupled denitrification calculated by the ^{15}N isotope pairing technique remains unchanged under the various $^{15}NO_3^-$ concentrations applied, like in our research, this is no reason to exclude the existence of microsites. Due to an optimal positioning of the nitrifying

and denitrifying bacteria the uniform mixing in and around microsites still may not be fulfilled and we assume that underestimation of the coupled denitrification still is possible (Chapter 2).
The small black dots with a diameter in the range of 0.02-0.07 cm observed in the sandy sediments of this study may indicate as well that microsites occur in the sediments investigated. Although it was not proved, these sites probably contained more degradable organic matter and therefore had a higher microbial activity. This was clearly demonstrated for terrestrial systems (Parkin, 1987).
A very tight coupling between nitrification and denitrification is also required to explain the following results. In Chapter 2 it was shown that in a N_2 flux experiment addition of nitrate to the overlying water (increase of the nitrate concentration about 100%) resulted in an enhanced N_2 flux. Although the penetration depth and the gradient of nitrate in the sediment would have changed, the coupled denitrification went on at the same rate. During the weekend the nitrate concentration in the overlying water gradually increased due to the microbial processes in the sediment with 40-100%. The total N_2 flux now remained unchanged, so it was concluded that the coupled denitrification again remained at the same level. These experiments seem to indicate that the coupled denitrification is independent of the macro nitrate gradient (from the overlying water to the sediment) and of the nitrate concentration in the overlying water. Lohse et al. (1993), however, concluded from measured nitrate profiles that the nitrate concentration in the overlying water will effect the coupled denitrification. As they presumed a two-layer system, the influence of the tight coupling between nitrification-denitrification in potential microsites was excluded. We suppose that if the occurrence of microsites is included the effect of the nitrate concentration on the coupled denitrification may disappear. Our results then would indicate again that microsites do occur in the sediments investigated.
However, there is also another possible explanation. When the tight coupling between nitrification and denitrification occurs mainly via intermediates of the nitrification like N_2O and NO_2^- (the intemediates of the nitrification are used as substrate by denitrifying bacteria; see chapter 1), the coupled denitrification would also be independent of the nitrate gradient/concentration and the coupled denitrification would be underestimated by the ^{15}N isotope pairing technique as well. However, no other measurements were available to support this hypothesis and research is needed to answer the question.

For the N removal from the sediment water system the coupled nitrification-denitrification appears to be very important (Chapter 4, 7). Based upon the results discussed above it was concluded that this coupling between nitrification and denitrification is very tight. It is hypothesized that this tight coupling occurs especially in microsites or/and occurs mainly by intermediates of the nitrification. With the occurrence of microsites the redox gradients can no longer be

represented as in a two-layer system. However, the average N_2 fluxes obtained by the N_2 flux method still represent a good overall measure of the coupled denitrification, because the dimensions of the microsites are probably much smaller than the size of the sampling cores. This difference in size furthermore would explain the low spatial variability of the various N fluxes observed (Chapter 5).

MODELLING

The laboratory experiments presented in the previous chapters indicated that the combined effect of temperature and the availability of (fresh) organic matter is important for the N loss from the sediment-water system. For a good understanding and interpretation of the controlling factors and processes of the nitrogen cycle and for a good description of the field situation, however, more and for the latter purpose especially quantitative information is needed. This information may be deduced from model studies and/or from additional experiments and field measurements. We therefore tried to adapt an existing chemical model, which can describe dispersive and solid transport between sediment and water, by inclusion of the nitrogen transformations. After several model simulations serious errors in the equilibrium chemistry were detected. Thereupon, unfortunately, insufficient time remained to modify this part of the model and to make it operational. However, based upon the model practices and literature on modelling we may stipulate the requirements of

- a model that is suitable to simulate the laboratory experiments, to analyze the processes which control the N loss and to clarify the relationships between the microbial and transport processes as a function of environmental conditions.
- a model that is capable to simulate the actual field conditions and to predict the effects of e.g. reductions of the nitrogen loading.

The requirements are presented hereafter.

Model for the analysis of the microbial processes

The various nitrogen processes occur under different redox conditions and therefore in different parts of the sediment. This means that the integrated processes of the N cycle cannot take place at the same time in the same place. Such a complex system of nitrogen and related processes together with environmental conditions should be simulated by a dynamic model with a high spatial and temporal resolution.

The laboratory results indicate that the nitrification and denitrification occur at a short distance of each other and perhaps in microsites. In the literature two methods are applied to simulate the influence of microsites. To simulate measured porewater O_2 and NO_3^- profiles Brandes and Devol (1995) developed a two

dimensional model in which scattered, highly reactive discrete microsites were described. Blackburn et al. (1994) also needed for the reproduction of their experimental N fluxes a tight coupling between nitrification and denitrification. This was not achieved by spatial separation of the two processes, but the occurrence of microsites was simulated by allowing the denitrification to tolerate the presence of some oxygen. As both methods could reproduce the various fluxes and main gradients correctly, it was assumed that it is not necessary to simulate the microsites separately as such, when the main interest lies in the fluxes over the sediment-water interface. It therefore is suggested to allow the denitrification in the model to occur in the presence of low oxygen concentrations.

To obtain both spatial and temporal resolution a multi-layer system should be used. For the purpose of our research, the analysis of the nitrogen fluxes, the model should include the variables oxygen, ammonium, nitrate, dinitrogen, dissolved organic carbon (DOC), methane, particulate organic carbon (POC), carbon dioxide, dissolved organic nitrogen (DON), particulate organic nitrogen (PON) and furthermore porosity. With these state variables the following reactions can be simulated: hydrolysis of POC and PON, the mineralization of DOC into CH_4 and CO_2, the ammonification, nitrification, CH_4 oxidation and denitrification. Factors influencing the oxygen availability are demonstrated to be very important in controlling which processes will occur (Chapter 4). Most of the sediment oxygen demand, however, can be explained by the aerobic mineralization, the oxidation of methane and the oxidation of ammonium.

The variables mentioned above are assumed to be sufficient for the simulation of the nitrogen processes. Including more variables even may be undesirable: it will not affect the nitrogen processes, the model becomes more complex, the running time will become longer and the possibilities to identify and calibrate the model are not really improved further. Furthermore more process rates and concentrations of variables, from literature or measurements, are needed to calibrate the model.

Reactions between variables occur in one layer, transport between layers can occur by diffusion and most reactions can be described as first order. For the stimulation or inhibition of processes by oxygen, however, more complex functions are required. Furthermore temperature should control the various process rates, as it was demonstrated that temperature has a large influence (Chapter 4, 7). In the previous chapters estimates of the ammonification, nitrification, denitrification and CH_4 oxidation rates were presented. For the other processes no rates were measured and consequently these should be obtained from literature.

Blackburn (1990) and Blackburn and Blackburn (1992, 1993) developed dynamic models like the one described above, but temperature was not included. With one of their models they predicted initially enhanced denitrification rates with increasing organic loadings. With still higher organic loadings, however, the anoxic character

of the sediment increased and therefore the nitrification and consequently the (coupled) denitrification decreased (Blackburn and Blackburn, 1993). So, when the influence of temperature on the process rates is included as well in the model, it should be possible to simulate the laboratory experiments described in chapter 4. After calibration on the laboratory experiments such a model will be able to calculate the various nitrogen fluxes (dinitrogen, ammonium, nitrate) at various temperatures and/or POC(DOC) concentrations and more insight in the various processes will be obtained. When the POC/PON or DOC/DON concentrations on/in the sediment throughout the year are estimated it will furthermore be possible to indicate when and how much nitrogen is lost by the (coupled) denitrification and when and how much ammonium is released. This may improve the prediction and interpretation of the mass balances considerably.

Model for the field situation

Lake managers are in particular interested in predicting the water quality. The water quality of a lake is strongly related to the phytoplankton growth, which in turn depends largely on the temperature, light intensity, the availability of the nutrients nitrogen and phosphate and grazing pressure. With the kind of model described in the previous paragraph, the internal nitrogen fluxes, especially across the sediment-water interface, can be simulated. However, in order to describe the water quality in the field situation, more processes in the nitrogen cycle need simulation (e.g. uptake by phytoplankton, N_2 fixation). Furthermore a water balance and inputs and outputs of nutrients for the lake should be included. As seepage and infiltration cannot be neglected in Lake Wolderwijd/Nuldernauw, the advective transport must be included in the sediment compartments as well. An integrated description of nutrient flows and loadings (in this case of nitrogen) together with cycling in the water and the sediment-water interaction seem most suitable to predict the water quality.

The cycling in the water column should include the processes of phytoplankton growth, decay and sedimentation. These processes simulate the input of organic matter into the sediment. The production of phytoplankton can be formulated as N-limited, with a measured phosphate boundary concentration. Alternatively, the P cycle should be simulated as well. In the latter case more geochemistry is needed to describe the fate of phosphate in the sediment layers and more sediment characteristics must be available or measured for a proper representation.

With such an integrated model it should be possible to indicate the time after which the stock of nitrogen in the organic matter in a eutrophic phytoplankton dominated system is sufficiently reduced to expect an improvement of the water quality.

It must be noticed however, that the possible influences of N_2 fixing bacteria, benthic diatoms, zooplankton and aquatic plants has not yet been taken into

account. The benthic algae e.g. cannot only reduce the nutrient release but they also may influence the various N processes in the sediment (Chapter 6). For the system under investigation the influence of these processes has hardly been investigated yet.

CONCLUSIONS

The N_2 flux method proved to be a good method for the measurement of the coupled denitrification, while at the same time CH_4, NH_4^+ and NO_x^- fluxes can be quantified by this method as well. The ^{15}N isotope pairing technique, in contrary, seems to underestimate the coupled denitrification.
Disadvantage of the N_2 flux method is the long pre-incubation time before N_2 fluxes due to the denitrification can be measured. Furthermore it is quite impossible to perform additional measurements in the incubation chambers, like oxygen profiles.

With the N_2 flux method the influence of several factors on the nitrogen processes was demonstrated:
- Higher incubation temperatures result in higher process rates and a larger N loss
- Especially the denitrification is further stimulated by an increasing organic matter content of the sediment: the N_2 flux from muddy sediments is higher than the N_2 flux from sandy sediments.
- The combined effect of high temperatures and/or high contents of (easily degradable) organic matter in the sediment, however, can beyond a certain treshold result in a reduction of the coupled denitrification and therefore of the N loss. A direct proof and quantitative information on this combined effect requires further research.
- Factors influencing the oxygen availability are very important in controlling the various nitrogen processes.
- In anaerobic incubated sediments without nitrogen oxides in the overlying water also a N_2 flux has been observed.
- It was shown that the coupled denitrification is independent of the nitrate concentration in the overlying water. It is hypothesized that this is due to a very tight coupling between the nitrification and the denitrification: a tight coupling can occur in sediments with microsites and/or when the coupling between nitrification and denitrification occurs by intermediates of the nitrification.

To interpret the laboratory results for the field situation the variation of nitrogen fluxes throughout the lake can be important. Significant spatial variation in the nitrogen fluxes and sediment characteristics throughout the lake, however, was

only found between major sediment types. Within a sediment type the spatial variation was minor. So only samples of the dominant sediment types in the lake have to be collected. Together with the corresponding areal coverage of these sediment types and the measured N_2 fluxes the whole lake fluxes can be calculated. Furthermore the occurrence of benthic algae influences the nutrient processes. It was demonstrated that benthic algae are able to grow on nutrients released from the sediment, hereby reducing the nutrient flux from the sediment. Under light conditions the coupled denitrification increased, resulting in an enhanced N loss from the sediment-water system.

Based upon the available results it was calculated that in an average year 60% of the N_{tot} input to the lake is removed, of which about 65% by the coupled denitrification. The coupled nitrification-denitrification thus is important for the N-loss in the field situation.

Furthermore it was concluded that N is not stored like P in inorganic form within the sediment, but mainly in organic matter. Reduction of the N loading along with a reduced P loading may result in an improvement of the water quality. However, in systems with a high production of organic matter due to a high algal biomass, an immediate and significant improvement of the water quality due to reduction of the nitrogen loading cannot be expected: the N loss by denitrification initially will remain limited and so is the improvement of the water quality. However, after reduction of the nitrogen loading the stock of nitrogen and the organic matter will decrease. Once the organic matter production decreases, the denitrification will increase and the improvement of the the water quality will accelerate.

For a good interpretation of the laboratory results to field conditions or to other comparable systems, a modelling framework is very useful, especially when more quantitative data (e.g. various N fluxes at different temperatures and organic matter contents) become available.

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SUMMARY

Most surface waters in the Netherlands are highly eutrophicated due to high loadings with the nutrients nitrogen (N) and phosphorus (P). To improve the water quality of lakes often the phosphorus loading is reduced. Due to phosphorus release from the sediments the success of the recovery of these lakes, however, is limited. Therefore renewed interest is directed to the fate of nitrogen in surface waters: perhaps a reduction of the N loading may result in a better water quality. This interest is furthermore achieved by the international programs for protection and restoration of the North Sea and the river Rhine.
In the nitrogen cycle bacterial processes in the sediment are very important. When the external nitrogen loadings are reduced, these benthic processes (resulting in the internal loading) dominate more and more the nitrogen loading and the concentration in the overlying water. Little is known, however, about the magnitude of the various nitrogen process rates or about the effects of reduction of the nitrogen loading.
The aim of this study was therefore to investigate and quantify the important microbial N processes (ammonification, nitrification and denitrification) and the related fluxes to the overlying water, in order to obtain a better insight in the possibilities to diminish eutrophication by reduction of the N loading. Special attention was paid to the denitrification as by this process nitrogen can be removed from the sediment-water system as N_2 to the atmosphere. The experiments in this study were performed with sediments sampled from the shallow lake Wolderwijd/Nuldernauw, The Netherlands.

In the literature various methods to measure denitrification rates are described. In Chapter 2 the results of three of these methods, the N_2 flux method, the ^{15}N isotope pairing technique and the mass balance approach, are compared. Results obtained with the N_2 flux method were in agreement with mass balance data, but were higher than the results obtained with the ^{15}N isotope pairing technique. Various checks demonstrated that after a pre incubation period of about 10 days the coupled denitrification can be estimated very well with the N_2 flux method and that the N_2 flux is not due to leakage of atmospheric nitrogen. An important assumption for the calculation of the coupled denitrification by the ^{15}N isotope pairing is the uniform mixing of the nitrate species ($^{14}NO_3^-$ and $^{15}NO_3^-$). When micro-sites exist in the sediment, uniform mixing of the nitrate species probably cannot be assumed because of the very tight coupling between nitrification and denitrification at these sites. It is therefore suggested that the difference in the results is caused by an underestimation of the coupled denitrification by the ^{15}N isotope pairing technique, due to the occurrence of micro-sites. In the further research the N_2 flux method was used.

In order to estimate the ammonification rates beside the nitrification and the denitrification rates also anoxic cores were incubated. From sediments incubated under anoxic conditions and without nitrate and/or nitrite in the overlying water unexpected but remarkable N_2 fluxes were estimated (Chapter 3). Thorough investigations demonstrated that these fluxes were not due to N_2 leakage or oxygen leakage followed by nitrification and denitrification. Therefore it was concluded that the anoxic N_2 fluxes were real. No explanation, however, for the source and/or the mechanism leading to these N_2 fluxes could be given, although ammonium oxidation by Fe and Mn might be possible.

The influence of temperature and organic matter content on the various N fluxes was investigated by incubating muddy and sandy sediments sampled throughout the year at 2, 12 and 23°C (Chapter 4). As expected N_{tot} ($NH_4^+ + NO_x^- + N_2$) fluxes increased with increasing temperature. A temperature factor of 1.9 ± 0.3 for a 10°C increase was estimated for both sediment types. At the same temperature the N_{tot} fluxes from muddy sediments were generally higher than from the sandy sediments and the species composition of the N_{tot} flux differed with the sediment type. This was due to the higher organic matter content of the muddy sediments. In general especially the denitrification was influenced by the organic matter content: 75-90% of the N_{tot} flux was denitrified in the muddy and only 45-65% in the sandy sediments. However, in cores from both sediment types collected just after a spring bloom of phytoplankton, the contribution of the various N species to the N_{tot} flux was changed: much higher NH_4^+ and much lower NO_x^- and N_2 fluxes were calculated, especially at the high incubation temperature. It was assumed that this shift in species composition was mainly due to the freshly settled and easily degradable organic matter. At higher concentrations of easily degradable organic matter more oxygen is consumed by the oxic mineralization and CH_4 oxidation and no or less oxygen remains for the oxidation of nitrogen. Enhanced temperatures stimulate this further. A direct proof and quantitative data regarding the influence of easily degradable organic matter on the various nitrogen processes, however, are not given in this study.

Several factors like the O_2 availability and the (freshly settled) organic matter content may vary throughout the lake and can cause spatial variability in the nitrogen fluxes. Spatial variability might influence therefore the total N loss of a lake as well. The spatial variability of the N fluxes was investigated by collecting sandy and muddy sediment cores throughout lake Wolderwijd/Nuldernauw and measuring the sediment characteristics and nutrient fluxes (Chapter 5). Within the same sediment type the sediment characteristics and nutrient fluxes showed little variation, whereas between sediment types significant differences were found. In comparison with the spatial variability of terrestrial soils however, this difference is small. It was hypothesized that

this was due to the much smaller influence of micro-sites in lake sediments than in terrestrial soils.
By principal component analysis (PCA) performed on the sediment characteristics and/or the nutrient fluxes the main sediment types were readily distinguished. It was therefore concluded that the N fluxes depended on the sediment type, although no obvious and direct relationship between the sediment characteristics and the N fluxes measured was found.

Once enough light can penetrate to the sediment-water interface, benthic algae can develop. Their presence can influence the release of nutrients (Chapter 6). In laboratory release experiments, performed under dark and light conditions to simulate the situation without and with benthic algae, it was demonstrated that benthic algae were able to grow on nutrients released from the sediments. The flux of inorganic nitrogen to the overlying water thus decreased due to benthic algae. Due to an indirect effect furthermore the loss of nitrogen to the atmosphere increased. The photosynthesis of the benthic algae caused an increase of the O_2 penetration and therefore the coupled nitrification-denitrification was stimulated. In this study the coupled denitrification was stimulated by the benthic algae with about 50%. Hence, benthic algae can reduce the nutrient release from the sediment and may accelerate the rate of recovery from eutrophication.

In Chapter 7 the results of the laboratory experiments are interpreted for the field situation by comparing these results with data from mass balances of 1985-1992. Based upon the results from the experiments a general concept, showing the combined influence of temperature and (easily degradable) organic matter on the N loss due to coupled denitrification, is postulated. The concept implies that initially the coupled denitrification increases with increasing contents of organic matter and/or increasing temperature. A further increase however, changes the character of the sediment into more anoxic conditions and the coupled denitrification decreases. Therefore the N_2 fluxes measured in the laboratory at low temperatures may be a correct estimation for N_2 flux in the colder months in the field situation. If however the field temperature and/or the availability of easily degradable organic matter increase, the laboratory N loss may no longer be representative for the N loss in the field.
From the comparison of the two estimates it was concluded that in the field situation on average about 60% of the N_{tot} input is removed on an annual base, of which about 35% by the uncoupled and about 65% by the coupled denitrification. This clearly demonstrates the importance of the coupled denitrification in the annual N removal from a lake. In individual months however, storage in the sediment may be an important process in the total N loss. Although in each month also about 60% of the N_{tot} input was removed, the contribution of the various N loss terms is highly variable. Based upon the available data, it is in general impossible to discriminate between the

coupled denitrification and the storage of nitrogen in the sediments in spring and summer.
It was concluded that reduction of the N loading, along with a reduced P loading may result in an improvement of the water quality. For the perspectives for lake restoration by reduction of the N loading it was however hypothesized that in situations with a high algal biomass a significant improvement cannot be expected immediately: the N loss by denitrification initially will remain limited and no sufficient reduction of the stock of nitrogen is obtained. However, the stock of nitrogen will reduce and once the organic matter production decreases, the improvement of the water quality will accelerate.

In the last chapter the results obtained are compared with data from literature and the occurrence of micro-sites and the dependence of the coupled denitrification from the nitrate concentration in the overlying water are discussed. Furthermore the requirements of a model that is suitable to analyze the various processes occurring in a sediment column and of a model capable to predict the whole lake N cycle are discussed. Finally the main conclusions and recommendations are presented: several nitrogen transforming processes can be quantified and the influence of temperature, sediment type and benthic algae is clearly demonstrated. Furthermore several indications were found for the influence of fresh and easily degradable organic matter in the various nitrogen processes and for the importance of the coupled nitrification-denitrification in the total N loss in the field situation. For a good interpretation of the laboratory results to field conditions, however, more quantitative data are needed concerning the effect of the availability of easily degradable organic matter on the nitrogen processes.

SAMENVATTING

Stikstofverwijdering door denitrificatie in het sediment van een ondiep meer.

De meeste Nederlandse oppervlaktewateren zijn sterk eutroof, dat wil zeggen dat ze veel nutriënten (voedingsstoffen) bevatten. Door de hoge belasting met de nutriënten stikstof (N) en fosfaat (P) is de algengroei toegenomen en de waterkwaliteit verslechterd. Om de waterkwaliteit te verbeteren, wordt meestal de P-belasting verminderd. Door de jarenlange belasting is echter veel fosfaat opgeslagen in het sediment. Omdat na vermindering van de fosfaatbelasting dit opgeslagen fosfaat weer kan vrijkomen en door algen worden gebruikt voor groei, is het effect van reductie van de P-belasting meestal niet direct zichtbaar. De aandacht richt zich daarom nu op stikstof. Wellicht dat bij een verminderde stikstof belasting de algengroei vermindert en de waterkwaliteit verbetert. Hiernaast zijn de internationale programma's ter bescherming en herstel van de Noordzee en de Rijn aanleiding voor de interesse in stikstof.
Bacteriën in het sediment spelen een belangrijke rol in de stikstofcyclus. De verschillende stikstofprocessen hangen nauw met elkaar samen. Bij de mineralisatie (afbraak) van organisch materiaal (o.a. afgestorven algen) komt ammonium vrij (NH_4^+). Als er voldoende zuurstof aanwezig is wordt dit omgezet in nitraat (NO_3^-). Onder zuurstofloze omstandigheden wordt dit nitraat vervolgens door denitrificerende bacteriën omgezet in stikstofgas (N_2). Door deze denitrificatie kan stikstof uit het sediment-water systeem worden verwijderd. Wanneer en in welke mate dit proces precies optreedt en of het doorgaat na vermindering van de N-belasting is echter onvoldoende bekend. Het doel van dit onderzoek is daarom om de belangrijke microbiële N-processen (ammonificatie, nitrificatie en denitrificatie) en de bijbehorende fluxen (NH_4^+, NO_3^- en N_2) te bepalen en te kwantificeren. Hiertoe zijn laboratoriumexperimenten uitgevoerd met sediment uit het ondiepe Wolderwijd /Nuldernauw met hierboven een kunstmatig oppervlaktewater zonder nutriënten.

In de literatuur worden verschillende methoden beschreven om de denitrificatie te meten. In Hoofdstuk 2 worden drie van deze methoden, de N_2-flux methode, de ^{15}N isotope pairing techniek en de bepaling met behulp van een massabalans, met elkaar vergeleken. De resultaten van de N_2 flux methode zijn vergelijkbaar met data van massabalansen. Met de ^{15}N isotope pairing techniek werden veel lagere denitrificatiesnelheden gevonden. Verschillende controle experimenten toonden aan dat na een voorincubatie van ongeveer 10 dagen de gemeten N_2 flux kan worden toegeschreven aan de gekoppelde nitrificatie-denitrificatie en dat deze N_2 flux niet wordt veroorzaakt door lekkage van atmosferisch stikstof.
Een belangrijke aanname bij de berekening van de gekoppelde nitrificatie-denitrificatie met de ^{15}N isotope pairing techniek is dat het toegevoegde ^{15}N-nitraat

zich volledig mengt met in het sediment gevormd ^{14}N-nitraat. Als zich in het sediment echter micro niches bevinden, met lokaal sterk verhoogde microbiële activiteit, worden deze nitraatvormen door de sterke koppeling tussen de nitrificatie en denitrificatie in deze niches waarschijnlijk niet geheel gemengd en is de aanname niet geldig. Vermoedelijk is dit er de oorzaak van dat de gekoppelde denitrificatie met behulp van de ^{15}N isotope pairing techniek in deze studie is onderschat. In het verdere onderzoek is dan ook de N_2-flux methode gebruikt.

Om naast de nitrificatie en denitrificatie ook de ammonificatie te meten werden sedimenten anaeroob geïncubeerd. Van deze anaerobe sedimenten werden onverwachte (het bovenstaande water bevatte geen nitraat of nitriet), maar duidelijke N_2 fluxen gevonden (Hoofdstuk 3). Verschillende testen lieten zien dat deze fluxen niet werden veroorzaakt door lekkage van N_2 of van O_2 gevolgd door nitrificatie en denitrificatie. De conclusie is dat N_2 inderdaad werd gevormd en dat zeker één van de stappen een biologisch proces is. Een verklaring voor de oorzaak en/of het mechanisme dat hieraan ten grondslag ligt werd niet gevonden, maar oxidatie van ammonium door Fe of Mn gevolgd door denitrificatie lijkt mogelijk.

De invloed van temperatuur en organisch materiaal op de verschillende stikstoffluxen werd onderzocht door modder- en zandsedimenten bij 2, 12 en 23°C te incuberen. Om de temporele variatie te bestuderen werd het sediment op verschillende tijdstippen in het jaar bemonsterd (Hoofdstuk 4). Zoals verwacht werden bij hogere temperatuur hogere N_{tot} fluxen ($NH_4^+ + NO_3^- + N_2$) gevonden: bij een temperatuurstijging van 10°C werd voor beide sedimenttypes een versnellingsfactor van 1.9 ± 0.3 berekend. Door het hogere organisch stof gehalte van moddersedimenten was de N_{tot} flux van deze sedimenten, gemeten bij eenzelfde temperatuur, in het algemeen hoger dan van zandsediment. Bovendien was de samenstelling van de flux verschillend. Vooral de denitrificatie werd beïnvloed door het organisch stof gehalte: bij moddersediment bestond 75-90% van de N_{tot} flux uit N_2, bij zandsediment slechts 45-65%. De N_{tot} flux van zowel modder- als zandsediment bemonsterd na een voorjaarsbloei van algen bevatte echter veel meer NH_4^+ en veel minder NO_3^- en N_2, vooral bij hoge incubatietemperatuur. Verondersteld wordt dat deze verschuiving in de N_{tot} samenstelling wordt veroorzaakt door het verse en makkelijk afbreekbare organisch materiaal afkomstig van de afgestorven algen. Als er meer vers en makkelijk afbreekbaar organisch materiaal aanwezig is, wordt er meer zuurstof verbruikt voor de mineralisatie en voor de oxidatie van methaan. Er blijft dan geen of minder zuurstof over voor de oxidatie van stikstof en er wordt voornamelijk ammonium nageleverd. Bij hogere temperaturen wordt dit effect versterkt. Een direct bewijs voor de invloed van vers organisch materiaal op de verschillende stikstoffluxen werd in dit onderzoek echter niet geleverd: hiervoor waren onvoldoende kwantitatieve data aanwezig.

Verschillende factoren zoals onder andere de beschikbare hoeveelheden zuurstof en (makkelijk afbreekbaar) organisch materiaal kunnen ruimtelijk verschillen en leiden tot variatie in de stikstoffluxen. Ruimtelijke verschillen bepalen hierdoor mede het stikstofverlies van een meer. De ruimtelijke variatie is onderzocht door de N fluxen van sedimenten verzameld op verschillende plaatsen in het meer te meten (Hoofdstuk 5). Verder is bekeken of de fluxen afhankelijk zijn van het sedimenttype en of dit kan worden toegeschreven aan de gemeten sedimentkarakteristieken. Binnen een sedimenttype was de variatie in de sediment karakteristieken en de gemeten nutriëntfluxen klein. Tussen de sedimenttypen werden echter significante verschillen gevonden. In vergelijking met terrestrische bodems waren deze verschillen echter klein. Verondersteld wordt dat dit wordt veroorzaakt door de kleinere invloed van micro niches in sedimenten dan in terrestrische bodems. Ook met hoofdcomponentenanalyse (PCA) over de sedimentkarakteristieken en/of de nutriëntfluxen waren de verschillende sedimenttypen makkelijk te onderscheiden. Daarom is geconcludeerd dat, hoewel een duidelijke relatie tussen de sedimentkarakteristieken en de gemeten N-fluxen niet is gevonden, de N-fluxen afhankelijk zijn van het sedimenttype.

Als er voldoende licht tot het sediment doordringt, kunnen zich hier bodemalgen ontwikkelen. Deze bodemalgen kunnen de afgifte van nutriënten beïnvloeden (Hoofdstuk 6). Sedimentkolommen, geïncubeerd onder donkere en lichte omstandigheden om de situatie respectievelijk zonder en met bodemalgenactiviteit te simuleren, toonden aan dat bodemalgen kunnen groeien op nutriënten die worden nageleverd door het sediment. Hierdoor wordt de nutriëntenflux naar het bovenstaande water verlaagd. Hiernaast kan de hoeveelheid N die door denitrificatie wordt verwijderd toenemen: door de fotosynthese van de bodemalgen neemt de penetratiediepte van zuurstof in het sediment toe, waardoor de gekoppelde nitrificatie-denitrificatie en daarmee het stikstofverlies wordt gestimuleerd. In dit onderzoek nam de gekoppelde denitrificatie in aanwezigheid van fotosynthetiserende bodemalgen met ongeveer 50% toe. De aanwezigheid van bodemalgen vermindert dus de nutriënten nalevering en kan het herstelproces van meren versnellen.

In hoofdstuk 7 is vervolgens getracht de hoeveelheid N verwijderd door denitrificatie in de veldsituatie te schatten. Hiertoe zijn de resultaten van de massabalansen voor stikstof voor het Wolderwijd/Nuldernauw van 1985-1992 vergeleken met de stikstofverliezen berekend uit de resultaten van de laboratoriumexperimenten. Gebaseerd op de laboratorium experimenten is eerst een model gepresenteerd dat het gecombineerde effect van (vers en makkelijk afbreekbaar) organisch materiaal en temperatuur op de gekoppelde denitrificatie laat zien. In eerste instantie neemt de gekoppelde denitrificatie toe met stijgende temperatuur en/of organisch materiaal gehalte. Als de temperatuur en/of het organisch materiaal gehalte verder stijgen en

voorbij een bepaald optimum komen, wordt het sediment steeds verder anoxisch en neemt de gekoppelde denitrificatie steeds verder af. De berekende N_2 fluxen kunnen daarom alleen naar het veld worden vertaald bij de lagere temperaturen. Bij hogere temperatuur en beschikbaarheid van makkelijk afbreekbaar materiaal kan de in het laboratorium gemeten N_2 flux waarschijnlijk slecht worden vergeleken met het stikstofverlies in de veldsituatie. Uit de vergelijking van de resultaten van de twee bepalingen blijkt dat op jaarbasis ongeveer 60% van de totale stikstofaanvoer op het meer wordt verwijderd door denitrificatie. Hiervan wordt 35% door de ongekoppelde en 65% door de gekoppelde denitrificatie verwijderd. Hieruit blijkt duidelijk het belang van de gekoppelde denitrificatie op de jaarlijkse stikstofverwijdering. Maandelijks wordt ook ongeveer 60% van de totale stikstof input verwijderd. De bijdrage van de verschillende verliesposten voor stikstof (gekoppelde en ongekoppelde denitrificatie en opslag in het sediment) is echter variabel en met de beschikbare gegevens is het in het algemeen niet mogelijk om de gekoppelde denitrificatie en de opslag in het sediment in de verschillende maanden te onderscheiden.
Er wordt geconcludeerd dat door reductie van de stikstofbelasting, tezamen met een gereduceerde fosfaatbelasting, de waterkwaliteit kan verbeteren. Wat betreft de perspectieven voor de verbetering van de waterkwaliteit door reductie van de stikstofbelasting in situaties met een hoge algenbiomassa productie wordt echter verondersteld dat niet direct een verbetering kan worden verwacht. In deze systemen zal de stikstofverwijdering door de denitrificatie in eerste instantie beperkt blijven, waardoor herstel eveneens beperkt blijft. Als echter de voorraad stikstof in het systeem afneemt kan de produktie van organisch stof verminderen en de denitrificatie toenemen. Hierdoor zal het herstel van de waterkwaliteit versnellen.

In het laatste hoofdstuk worden de resultaten van deze studie vergeleken met data uit de literatuur en worden het voorkomen van micro niches en de afhankelijkheid van de gekoppelde denitrificatie van de nitraatconcentratie in het bovenstaande water bediscussieerd. Hiernaast worden de vereisten aangegeven voor een model dat de verschillende stikstofprocessen zoals in het laboratorium gemeten kan analyseren respectievelijk voor een model dat de situatie in het veld kan voorspellen. Tot slot worden de belangrijkste conclusies en aanbevelingen gepresenteerd: verschillende processen kunnen worden gekwantificeerd en het effect van de temperatuur, het sedimenttype en bodemalgen is duidelijk aangetoond. Bovendien zijn er duidelijke aanwijzingen gevonden voor het effect van de beschikbaarheid van makkelijk afbreekbaar organisch materiaal op de stikstofprocessen en voor het belang van de gekoppelde nitrificatie-denitrificatie bij de stikstofverwijdering in het veld. Voor een goede interpretatie van de laboratoriumexperimenten naar de veldsituatie is echter nog meer kwantitatieve informatie nodig over de invloed van vers organisch materiaal op de stikstofprocessen.

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CURRICULUM VITAE

Francien van Luijn werd geboren op 20 februari 1967 geboren te `s-Gravenhage. In 1985 behaalde zij het VWO diploma aan het Zandevelt College te `s-Gravenzande. In datzelfde jaar begon zij met haar studie Milieuhygiëne aan de Landbouwuniversiteit (toen nog Landbouwhogeschool geheten) te Wageningen. Binnen de specialisatie water koos zij zowel de richting waterzuivering als waterkwaliteitsbeheer en van deze laatste zowel de fysisch chemische als de ecologische kant. Dit komt tot uitdrukking in haar afstudeervakken (waterkwaliteitsbeheer, microbiologie en aquatische ecologie) en stages (waterzuivering; bij de "Eidgenossische Anstalt für Wasserversorgung und Abfallwasserbehandlung (EAWAG) in Dübendorf, Zwitserland en bij Anjou Recherche, Centre Général des Eaux in Parijs, Frankrijk). In juni 1991 behaalde zij het ingenieursdiploma.
In oktober 1991 kwam zij voor vier jaar als assistent in opleiding in dienst bij de toenmalige vakgroep Natuurbeheer, nu Waterkwaliteitsbeheer en Aquatische Ecologie. Het onderzoek dat tot dit proefschrift heeft geleid is echter geheel uitgevoerd bij het RIZA (Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling) te Lelystad, waar zij was gestationeerd. Hierna heeft zij als toegevoegd onderzoeker bij de vakgroep Waterkwaliteitsbeheer en Aquatische Ecologie gewerkt bij het RIZA: tot augustus 1996 aan een vervolg op het stikstofonderzoek, hierna aan de jaarlijkse voortgangsrapportage van de 3^{e} nota waterhuishouding.