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Phosphorus dynamics in the sediment of a eutrophic lake.

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Phosphorus dynamics in the sediment of a eutrophic lake.

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Front page: Aerial view of Lake Loosdrecht with Kievitsarea in front.
Photograph by Studio Koppelman, Maarssen, The Netherlands.

STELLINGEN

1 Het meten van fosfaatafgifte in sediment kolommen geeft uitsluitend kwalitatieve informatie over de betrokken processen.
Dit proefschrift.

2 Het feit dat King en co-auteurs dezelfde foutieve verwijzing naar het artikel van Harrits and Hanson hebben als Lidstrom and Somers, laat zien hoe makkelijk onwaarheden in de wetenschappelijke wereld voor vaststaand worden aangenomen.
Harrits S.M. and Hanson R.S. (1980) Limnol. Oceanogr. 25: 412-421.
King G.M. (1990) FEMS Microbiology Ecology 74: 309-324.
King G.M., Roslev P. and Skovgaard H. (1990) Appl. Environ. Microbiol. 56: 2902-2911.
Lidstrom and Somers (1984) Appl. Environ. Microbiol. 47: 1255-1260.

3 Mensen die stellen dat eutrofiering een achterhaald onderzoeks topic is, vissen in troebel water.

4 De door het RIVO getrokken konklusie met betrekking tot de relatie tussen het afnemend fosfaatgehalte in de Rijn en de daling van de visstand in de Noordzee, is vergelijkbaar met konklusies over de dalende trend in de ooievaarsstand en het aantal geboren baby's.

5 Het verpakken van paprika chips in blauw gekleurde zakken en van naturel chips in rood gekleurde zakken, draagt niet bij aan het snel en economisch winkelen.

6 In aanvulling op de door Blake beschreven soorten Siren sirena, S. indica en S. erythraea, moet de, in de Zeeuwse wateren waargenomen zeemeermin, beschouwd worden als een nieuwe soort: Siren zeelandica.
Blake K. (1990) Limnol. Oceanogr. 35: 148-153.

7 Het in toenemende mate aanbrengen van geluidswallen en overkappingen langs snelwegen kan gezien worden als de eerste aanzet tot het ondergronds maken van het Nederlandse wegennet.

8 In het kader van de verkeersveiligheid verdient het aanbeveling om buiten de spits de meerderheid van de verkeerslichten buiten werking te stellen.

9 Het handgebaar dat sommige mensen maken wanneer zij over een filmkamera spreken -alsof zij aan een wieltje draaien dat zich schuin voor hun gezicht bevindt- moet feitelijk tot de industriële archeologie gerekend worden.

10 Discussies in Nederland over de schrijfwijze van "ecologie" (oecologie) dragen niet bij tot de samenhang van het onderzoek en gaan voorbij aan de internationalisering van de wetenschap.

11 De hetze tegen niet-inheemse boomsoorten kan niet los gezien worden van het weer sterker wordend nationalisme.

12 De traditie dat stellingen bij een proefschrift geen politieke lading mogen hebben, is een uitvloeisel van de illusie dat wetenschap "waardevrij" is.

13 De Nederlandse benaming "proefschrift" doet vermoeden dat er ook nog een echt schrift verschijnt.

Stellingen behorende bij het proefschrift "Phosphorus dynamics in the sediment of a eutrophic lake".
Anja J.C. Sinke, Wageningen, 6 november 1992.

voor Laurus en Truida

CONTENTS

Chapter 1

Introduction

Since the sixties eutrophication has been recognized to affect the quality of surface waters. The prolonged loading with nutrients has led to increased algal growth. A series of management measures have been carried out to combat eutrophication in the Netherlands. However, despite all efforts, the majority of the dutch surface waters is still considered to be eutrophic. The disappointing results are often attributed to processes in the sediment that can delay the improvement of the water quality. This thesis deals with the phosphorus dynamics in the sediment of a eutrophic lake. In contrast to most studies which are chemically orientated, attention here is mainly given to the role of microbial processes in the phosphorus cycle.

The introduction gives a brief review of eutrophication of lakes, and depicts the processes that influence the phosphorus dynamics in the sediment and the exchange between sediment and water. Finally, the "Water Quality Research Loosdrecht Lakes" programme, of which this study has been a part, is described.

EUTROPHICATION

Eutrophication is defined as the process of excessive addition of inorganic nutrients, organic matter and/or silt to lakes, leading to increased biological production and a decrease in lake volume (Cooke et al 1986). The primary biological production can reach the point of nuisance proportions such as surface scum and unpleasant odours. The high concentration of algae results in a decrease in transparency of the water and the development of tedious animal populations e.g. bream ("verbraseming"). Fish kills can be caused by the sudden depletion of dissolved oxygen in the water. The production of toxins by blooms of algae has been recognized in coastal waters (Rijkswaterstaat 1989). All these factors can significantly reduce the ecological and recreational value of the waterbody. Eutrophied lakes and reservoirs also loose their usefulness as domestic or industrial water supplies. Protection of water systems of good quality and restoration of eutrophic water bodies is greatly needed because the supply of fresh, unpolluted surface water is limited.

RESTORATION

External loading.

The first and most obvious step towards protection and restoration of waterbodies is to reduce the nutrient load. Efforts have concentrated on the control of phosphorus inputs. Phosphorus is generally recognized as the major factor determining phytoplankton biomass in freshwater in the temperate regions (Schindler 1977). Phosphorus is commonly derived from controllable point sources such as sewage, stormwater discharges and industrial effluent. The importance of diffuse sources such as leaching from surface soils can be expected to increase in the future (Van der Zee 1988). Also the atmospheric input of phosphorus that is

carried along with wind and rain, can be important in some lakes (Cole et al 1990). To reduce the nutrient loading to surface water, stringent limits have to be imposed on the phosphorus concentration in sewage effluent. In 1972, Sweden was the first European country requirering a maximum level of 0.5 mg $P.l^{-1}$ in effluents of sewage plants in urban areas. A comparable decree passed in the Netherlands in 1990 (Surface Water Pollution Act, Kroes 1992).

Internal loading.

The rate at which the phosphorus concentration in the water decreases after diversion of the nutrient loading, is influenced by hydraulic properties such as residence time and seepage and by the phosphorus flux over the sediment-water interface. Even the complete removal of the external phosphorus input may be insufficient to produce immediate and long-lasting effects, due to the phosphorus release from the sediment. The release from the sediment is generally referred to as internal loading. Over an annual cycle, lake sediments typically retain more phosphorus than they release due to the sedimentation of particulate matter. However, when the concentration of phosphorus in the lake water is reduced, a net release from the sediment can occur until a new steady state is established. Especially in shallow lakes, internal loading can be a serious drawback for lake recovery. Some of the best described examples are Lake Trummen (Bengtson et al 1975) and Lake Søbygard (Søndergaard 1987). In these lakes the reduction of the external phosphorus loading did not result in any apparent recovery of the water quality within the period of observation.

The knowledge of the processes in the sediment that influence the internal loading is scarce and there are no reliable models that predict its magnitude and time lapse.

Prediction of effectiveness of restoration measures.

The development of eutrophication policy requires reliable predictive models to evaluate the response of waterbodies to changes in nutrient supply. In 1973 the OECD (Qrganization for Economic Cooperation and Development) started an international cooperative programme to develop a sound database on the eutrophication and restoration of inland waterbodies. The data were evaluated by Vollenweider and Kerekes (1982) who used statistical methods to determine the relation between the phosphorus input, its concentration in the lake and the biomass. The regression equation that described the relation between the phosphorus loading and the phosphorus concentration in the lake water, is given by:

$$P_w = 1.55(P_i/(1+\sqrt{\tau}))^{0.82}$$

with: P_w = the annual mean total phosphorus concentration of the waterbody, P_i = the annual mean total phosphorus concentration of the inflowing water and τ = the mean residence time. This equation would predict a decrease in the phosphorus concentration of the water when the total phosphorus loading is reduced (fig. 1.1). However the OECD/Vollenweider model assumes a steady state situation and is not able to predict the transient phase of recovery of the water quality after the application of restoration measures. Several authors have identified feedback loops in eutrophic systems that can maintain the eutrophic state long after the external loading has been diverted. These include internal loading (Marsden 1989, Boström et al. 1982), physiological adaptations of the phytoplankton to changes in the phosphorus concentration (Marsden 1989, Van Liere 1990), and resistance of the food-web to the altered conditions (Van Donk and Gulati 1989).

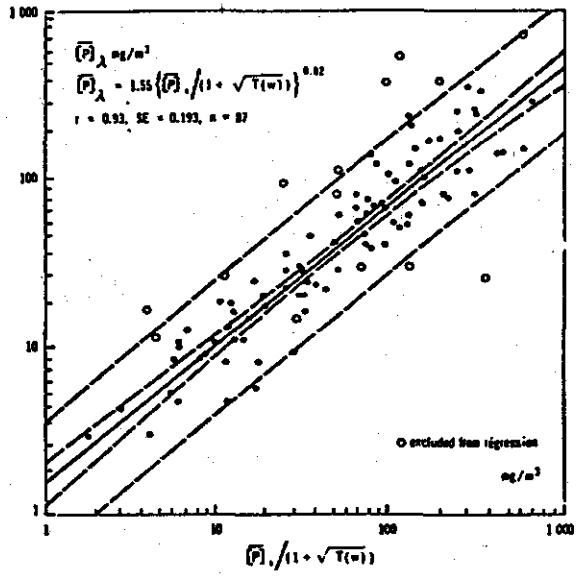
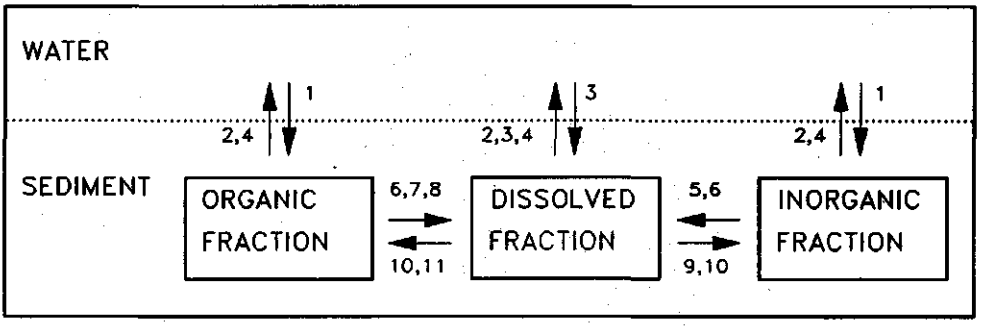


Figure 1.1: Relation between rescaled inflow concentration of phosphorus and inlake concentration, found in OECD survey. Reproduced from Vollenweider and Kerekes 1982.

PHOSPHORUS DYNAMICS IN THE SEDIMENT.

Distribution of phosphorus in the sediment.

The phosphorus in the sediment can be divided into three fractions; a dissolved fraction, and a solid organic and inorganic fraction (fig. 1.2). The dissolved fraction can be easily seperated from the two solid fractions by centrifugation, filtration or dialysis (Enell and Löfgren 1988). Generally the dissolved fraction is refered to as "phosphate". However, the phosphates in this fraction are often bound to organic or inorganic compounds. It is almost impossible to distinguish between the organic and inorganic solid fraction. Several multistep extraction schemes have been proposed which theoretically remove one component after another (Hieltjes and Lijklema 1980, Psenner 1985). In general 4 extraction steps are carried out; the first extraction with water (Psenner et al 1984) or a salts solution (1M NH_4Cl; Hieltjes and Lijkema 1980) removes the dissolved and the loosely sorbed phosphorus, the second extraction with a strong reductant (dithionite; Psenner et al 1984) removes the iron-bound phosphorus, the third extraction with NaOH removes the aluminum-bound phosphorus (Psenner et al 1984), and the last extraction with acid (0.5 M HCl; Hieltjes and Lijklema 1980, Psenner et al 1984) removes the calcium-bound phosphorus. The residual fraction which is recalcitrant to all extractants, includes the organic phosphorus. Although every step extracts part of the total phosphorus, the definition of the fractions is purely operational and, according to literature, each sediment type requires its own special treatment.



TRANSPORT PROCESSES
1. sedimentation
2. resuspension
3. diffusion
4. bioturbation

TRANSFER PROCESSES (solid --> dissolved)
5. dissolution
6. desorption
7. biological release
8. mineralization

TRANSFER PROCESSES (dissolved --> solid)
9. precipitation
10. adsorption
11. biological uptake

Figure 1.2: Scheme of phosphorus dynamics in the sediment.

Transfer reactions between the dissolved and solid phosphorus fractions.
The transfer between dissolved and solid fractions is mediated by chemical and biological processes. In most studies the bacterial activity is suppressed in order to quantify the influence of the seperate processes. Commonly used methods are addition of poisons (formalin: Premazzi and Provini 1985) or antibiotics (Kamp-Nielsen 1974, Bates and Neafus 1980). However, results obtained with these methods have only limited meaning as not all microbial processes are stopped and as the chemical environment is changed as well.

Mineralization of organic matter results in a liberation of phosphate from the solid organic fraction (Berner 1980). Mineralization of algae and detritus has been studied in detail in batch experiments (Fallon and Brock 1979, Jewell and McCarty 1971, Otten and Gons 1991, Ulen 1978b). The mineralization rate and the amount of phosphate that is mobilized from the decomposing algae was reported to be dependent on temperature (Jewell and McCarty 1971), the redox conditions (Fallon and Brock 1979) and the physiological conditions of the algae (Jewell and McCarty 1971, Otten and Gons 1991, Ulen 1978b). Several autors suggested that mineralization contributes to the actual phosphate release from the sediment (Kamp-Nielsen 1974, Lee et al 1977).

Dissolution of authigenic minerals results in a liberation of phosphate from the inorganic fraction. The dissolution and precipitation of minerals that contain phosphate such as vivianite ($Fe_3(PO_4)_2.8H_2O$) and apatite ($Ca_{10}(PO_4)_6.(H_2O)_2$), is governed by the solubility products of these minerals. When the pore water concentrations exceed the solubility product, a precipitation can be expected. Calculations on the ion activity product of Fe^{2+} and PO_4^{2-} in interstitial water of Lake Greifensee indicated the formation of vivianite (Emerson and Widmer 1978). This conclusion was further confirmed using X-ray diffraction and scanning electron microscopy (Emerson and Widmer 1978).

The adsorption - desorption equilibrium between the solid phosphorus fraction and dissolved phosphate is mainly determined by the redox conditions in the sediment. The redox conditions are strongly affected by microbial processes. During mineralization O_2, NO_3^-, Fe^{3+} and SO_4^{2-} are consumed and CO_2, Fe^{2+}, S^{2-} and CH_4 are produced (table 1.1). In the sediment the different mineralization processes succeed one another and generally a sequence can be observed with sediment depth of oxygen respiration, denitrification, iron reduction, sulfate reduction and methanogenesis. CO_2 is produced during all mineralization processes. The concomitant release of protons -due to dissolution of CO_2 in water-, may result in the dissolution of iron- and calcium-bearing carbonates, a pH buffering reaction.

The importance of this process in sediments has been demonstrated by Matisoff et al (1981).

The removal of phosphate from the dissolved fraction can be substantial when the redox potential is high at the sediment surface (Einsele 1936, Mortimer 1941, 1942, Stumm and Morgan 1981). Most authors ascribe this to the adsorption of phosphate onto the inorganic solid fraction. In sediments with an oxidized surface layer, upwards migrating iron(II) is rapidly oxidized and precipitated within the oxygenated zone as hydrous iron(III). Thus a micro-layer is formed on top of the sediment which has a high sorptive capacity for phosphate and limits its diffusive release into the water column (fig. 1.3A). If the sediment interface becomes depleted of oxygen, the thickness of the oxidized layer decreases and the amount of iron (III) diminishes. The iron(III) in the formerly aerobic zone is reduced which results in a desorption of phosphate from the iron complex and the mobilization of Fe(II) and phosphate (fig. 1.3B). The adsorption capacity of the aerobic surface layer is dependent of the stoichiometry of iron and phosphate. Phosphate is succesfully immobilized only when the ratio of iron to phosphate is greater than 1.8 (Holdren and Armstrong 1986) (fig. 1.3C). The capacity of iron(III) to adsorb phosphate decreases at high pH (Stumm and Morgan 1981). In shallow eutrophic lakes an increase in pH can be induced by high rates of photosynthesis. In laboratory experiments an increased phosphate release was observed when the pH was artificially increased (Andersen 1975, Boers 1991, Rippey 1977). However, the importance of the pH effect may be overestimated when the pH is controlled by NaOH additions instead of CO_2 removal as occurs in nature (Andersen 1975, Boers 1991).

Nitrate is a strong oxidizing agent that can maintain a high redox potential at the sediment-water interface and can thus prevent a release of iron bound phosphate. Andersen (1982) demonstrated that no phosphate release occurred in shallow lakes when the NO_3^- concentration exceeded 36 μmol.l^{-1} (0.5 mg N.l^{-1}). Several authors advized the addition of nitrate to lakes in order restore an oxidized state of the sediment and thus improve the water quality (Cooke et al 1986, Petterson 1984). However, nitrate can be used by denitrifying bacteria as an electron acceptor. Once all nitrate is consumed, the reduced conditions are re-established. Jansson (1987) reported a deterious effect of nitrate additions on the water quality as the bacterial population switched from nitrate to iron(III) as an electron acceptor after exhaustion of the nitrate.

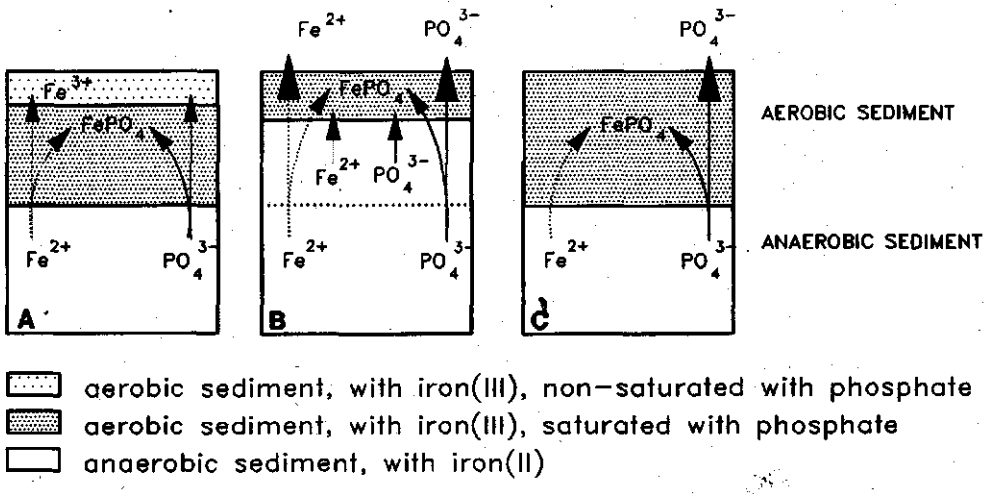


Figure 1.3: Phosphate adsorption in the surface layer of the sediment.
A) aerobic conditions, high atomic ratio of Fe:P.
B) as A, after a decrease of the oxygen supply to the sediment.
C) aerobic conditions, low atomic ratio of Fe:P.
(A and C redrawn after Löfgren and Boström 1989).

The redox couple iron(II)/iron(III) influences the phosphorus dynamics in the sediment directly as especially iron(III) complexes are able to retain phosphate. Iron(III) can be reduced chemically by sulfide and pyrite (Stumm and Morgan 1981) or by easily oxidizable organics e.g. thiols and weak organic acids (Suter et al 1991) and biologically by iron reducing bacteria (Lovley 1991). Iron reducing bacteria use iron(III) as an electron acceptor thereby decreasing the amount of iron(III) hydroxides. However, a clear distinction between chemical and microbial reduction is often difficult to make.

The redox couple sulfate/sulfide influences the phosphorus dynamics indirectly. Sulfide produced by sulfate reduction scavenges iron(II) and forms iron sulfide (FeS). The solubility of iron sulfide is much lower than that of iron(II) phosphate minerals. As a result of iron sulfide formation the equilibrium concentration of iron is lowered and iron(II) phosphate minerals dissolve. The release of phosphate causes an increase of its pore water concentration. In the aerobic zone, the number of adsorption sites for phosphate diminishes as the immobilization of iron as iron sulfide and eventually pyrite, prevents the upward flux of iron(II) and its oxidation to iron(III). In systems where the phosphate and iron dynamics are intermingled, the shift in equilibrium of iron solublity will lead to the release of phosphate by the sediment upon sulfate addition.

The redox couple CO_2/CH_4 does not seem to influence the phosphorus cycle

directly. However, the oxidation of upwards diffusing methane might induce an increased oxygen consumption in the sediment surface layer.

Recently it has been suggested that the redox conditions influence also the transfer between dissolved and organic fraction as they influence the uptake and release of phosphate by bacteria in the sediment surface layer (Fleischer 1983, Gächter et al 1988). Gächter and coworkers suggested that the redox dependent phosphate exchange in eutrophic Lake Sempach could be attributed partly to physiological changes of the bacterial population. They speculated that processes in the sediment could be similar to those occuring in activated sludge type sewage treatment plants designed for biological phosphate removal. In these plants phosphate is taken up during the oxic period and is subsequently released in the absence of oxygen (Wentzel et al 1985). Acinetobacter appeared to be the dominant genus storing phosphate intracellulary as polyphosphates (Fuhs and Chen 1975). Although results reported by Fleischer (1983) and Gächter and coworkers (1988) indicated active mediation by bacteria in the phosphate flux over the sediment-water interface, neither could quantify the importance of bacterial uptake compared to chemical adsorption because methods were lacking to measure the reversibly immobilized bacterial phosphate.

THE FLUX OF PHOSPHORUS OVER THE SEDIMENT-WATER INTERFACE.

Sedimentation of particles from the waterphase results in a phosphorus flux towards the sediment. The magnitude of this flux can be measured easily in deep, stratified lakes by using sedimentation traps. Sedimentation traps are often positioned at different depth in the lake and intercept the particles that sink from the upper water layers. In shallow lakes this method is not reliable due to turbulence. In these lakes the wind and wave action result in a resuspension of catched material from the traps and in the whirling of sediment particles into the traps.

Resuspension of the sediment can result in a substantial phosphorus release from the sediment. Although this is not easily quantified for in situ conditions, experimental results demonstrated that the release from sediment cores during resuspension in Lake Arresø, was 30-40 times higher than under undisturbed conditions (Søndergaard et al in press). In Lake Uttran the variation between years in total phosphorus and chlorophyll-a concentrations in the water were also related to the windforce and direction (Ryding and Forsberg 1977). In Lake Loosdrecht the resuspended material appeared to be a source of nutrients for phytoplankton growth in the lake (Rijkeboer et al 1991).

The concentration of dissolved phosphate is generally much higher in the porewater of the sediment than in the lake water (Enell and Löfgren 1988). This concentration

gradient results in a diffusive release of phosphate from the sediment into the water. The diffusion in enhanced by an increase in temperature.
Bioturbation of the sediment by benthic organisms (oligochaete worms, insect larvae, crustaceans, bivalve molluscs) and the fish consuming these organisms can also enhance the phosphorus release (e.g. Gallepp et al 1978).

Table 1.1: Mineralization processes in sediment with substrates and products.

electron acceptor	electron donor	mineralization process	products
O_2	organic matter	oxygen respiration	CO_2 + biomass + NH_4^+ + PO_4^{3-}
NO_3^-	organic matter	denitrification	CO_2 + N_2 + biomass + NH_4^+ + PO_4^{3-}
Fe^{3+}	organic matter	iron reduction	CO_2 + Fe^{2+} + biomass + NH_4^+ + PO_4^{3-}
SO_4^{2-}	organic matter	sulfate reduction	CO_2 + S^{2-} + biomass + NH_4^+ + PO_4^{3-}
CO_2	organic matter	methanogenesis	CO_2 + CH_4 + biomass + NH_4^+ + PO_4^{3-}

WATER QUALITY RESEARCH PROGRAMME LOOSDRECHT LAKES.

The Loosdrecht lakes ecosystem is representative for the majority of dutch lakes in the northern and western part of The Netherlands. This type of ecosystem has some very characteristic features: it is shallow, less than 3 meters deep, the water is brownish-yellow, indicating the presence of fulvic and humic acids, and the sediment consists of old peat. The pH of the water is neutral or alkaline which is caused by the fact that most of these lakes receive (ground) water with a relatively high alkalinity. The peaty sediment has a very high organic matter content, sometimes exceeding 50 % on a dry weight basis.

Table 1.2: General characteristics of Lake Loosdrecht.

Hydraulic properties		
Surface area[a]	12.2	km^2
Average depth[c]	1.8	m
Water residence time[c]	0.6	yr
Average external phosphorus load[d]	0.03	$mmol.m^{-2}.yr^{-1}$
Lake water (1990) (n=13)		
Total phosphorus[b]	2.4 ± 0.7	$\mu mol.l^{-1}$
Dissolved reactive phosphate[b]	0.06 ± 0.10	$\mu mol.l^{-1}$
Chlorophyll-a[b]	93 ± 18	$\mu g.l^{-1}$
pH[b]	8.4 ± 0.3	-
Lake sediment 0-10 cm (1989) (n=8)		
Dry weight[b]	9.0 ± 1.0	%
Organic carbon content[b]	53.6 ± 3.0	% of dry weight
Iron (total)[b]	0.36 ± 0.04	$mol.kg^{-1}$
Iron (acid extractable)[b]	0.20 ± 0.03	$mol.kg^{-1}$
Phosphorus (total)[b]	0.022 ± 0.003	$mol.kg^{-1}$
Phosphorus (acid extractable)[b]	0.005 ± 0.001	$mol.kg^{-1}$
Calcium (total)[b]	1.0 ± 0.1	$mol.kg^{-1}$
$CaCO_3$[b]	0.46 ± 0.14	$mol.kg^{-1}$

[a] Kievits area included, Kal et al 1984, [b] Keizer and Sinke 1992, [c] Van Liere et al 1990, [d] Van Liere et al 1991.

Some general characteristics of Lake Loosdrecht are given in table 1.2. The ecosystem has become severely eutrophied the past decades. The summer average of the chlorophyll-a concentration is 130 $\mu g.l^{-1}$ (1986; Van Liere et al 1990a) and a maximal value of 306 $\mu g.l^{-1}$ has been registrated in 1983 (Ebert and Van Liere, 1992). The average total phosphorus concentration of the water is 2.4 $\mu mol.l^{-1}$ (75 $\mu g.l^{-1}$, Keizer and Sinke 1992) but exceeds sometimes 4.8 $\mu mol.l^{-1}$ (150 $\mu g.l^{-1}$, Van Liere et al 1990).

In 1979 the working group "Water Quality research Loosdrecht Lakes" was founded with the objective to define and quantify the effects of a reduction of the external phosphorus loading on the structural and functional aspects of a shallow lake ecosystem (Van Liere 1986).

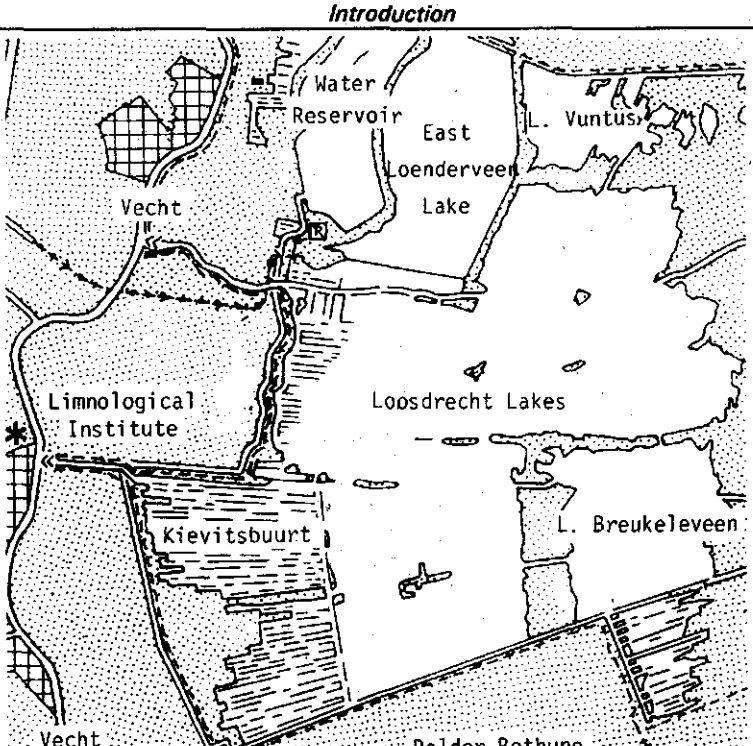


Figure 1.4: The Loosdrecht lakes and surroundings.

Loosdrecht lakes ecosystem: origin

The Loosdrecht Lakes ecosystem consists of a series of shallow interconnected lakes (fig. 1.4). The surface area of Lake Loosdrecht, including Kievitsbuurt, is 12.2 km^2 (Kal et al 1984). Lake Breukeleveen and Lake Vuntus are smaller, 1.8 and 0.9 km^2 (Kal et al 1984) respectively. The lakes were partly artificially created in the 17th century when the 7000 years old Sphagnum peat was excavated (Van Liere 1986). Long strips of peat removed from the cuts ("trekgaten") were deposited on the adjacent bank ("legakkers") to dry. The dried peat was used for fuel. Industrial peat mining was formalized in 1633 and the dimensions of the cuts and banks were strictly defined (Gulati et al 1991). However, the cuts were dredged deeper and the banks were made smaller than specified which led to a considerable erosion that was further enhanced by wind action. The continuous crumbling of the banks resulted in the formation of a shallow lake ecosystem. Even at present the alternating system of cuts and banks is still preserved in the Kievits area (front page).

Loosdrecht lakes ecosystem: eutrophication.

In the 19th century the reclamation of the adjacent, low-lying polders Bethune, Mijdrecht and Horstermeer, induced an increased seepage from the lake ecosystem (Engelen et al 1992). The water level in the lakes was controlled by suppletion with water from River Vecht (Van Liere 1986). The input of the polluted river water together with the increased habitation of the area, resulted in a heavily eutrophication of the ecosystem. From 1945 to 1983 the external phosphorus load averaged 35-40 $mmol.m^{-2}.y^{-1}$ (1.1-1.2 $g.m^{-2}.y^{-1}$) (Van Liere et al 1990).

Loosdrecht lakes ecosystem: restoration of water quality ?

The first attemts to improve the water quality were made in the seventies when the sewage of the villages surrounding the lakes was diverted to sewage plants (Van Liere 1986). The most important management measure was taken in 1984 when the inlet water from River Vecht was replaced by water from the Amsterdam-Rhine canal. This water is led to the lakes via a pipe-line. Phosphorus is precipitated using ferric chloride ($FeCl_3.6H_2O$) before the water enters the lake. These efforts resulted in a reduction of the external phosphorus loading to about 10.3-15.2 $mmol.m^{-2}.y^{-1}$ (0.32-0.47 $g.m^{-2}.y^{-1}$) (Van Liere et al 1991). At the same time the total phosphorus concentration of the water decreased with 0.4 $\mu mol.l^{-1}.y^{-1}$ (12 $\mu g.l^{-1}.y^{-1}$) (Van Liere et al 1990a). No improvement of the chlorophyll-a concentration could be detected thusfar, despite the encouraging trend in the phosphorus concentrations (Van Liere et al 1990b). Apparently feed-back mechanisms within the ecosystem hinder the restoration into the state with clear water and water plants that existed from 1930-1950.

Outline of this thesis:

The scope of this thesis is to investigate the role of microbial processes in the phosphorus flux over the sediment-water interface in Lake Loosdrecht. The importance of microbial processes for the phosphate release from aerobic sediment was investigated using temperature experiments (chapter 2). Experiments were performed with control sediment and sediment that was sterilized by γ-irradiation. Descriptive statistical models gave some insight in the impact of mineralization processes on the pore water chemistry and phosphate flux over the sediment-water interface (chapter 3). As the importance of the different mineralization processes varied during the year, a more detailed study was carried out on sulfate reduction and methanogenesis (chapter 4).

To examine the direct role of bacteria in the uptake and release of phosphate, a new method had to be developed to quantify the amount of bacterial bound phosphate (chapter 5). This method enabled the measurement of phosphate uptake by methanotrophs (chapter 6). The effect of methane oxidation on the thickness of the oxidized sediment surface and on the phosphate flux was investigated. Finally the results, suggestions for further research and implications for management measures are briefly discussed (chapter 7).

Chapter 2

Influence of bacterial processes on the phosphorus release from sediments in the eutrophic Loosdrecht Lakes, The Netherlands.

Anja J.C. Sinke and Thomas E. Cappenberg.

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ABSTRACT

Microbial reactions are an important factor for sediment phosphorus release. In this paper the effect of temperature experiments on phosphorus release was investigated, both in sterilized and non-sterilized cores. Cores were sterilized by γ-irradiation (25 kGy). The combination of temperature and γ-irradiation experiments made it possible to distinguish between bacterial and physico-chemical processes. In temperature experiments performed with cores sampled in autumn and winter, differences in phosphorus release patterns were found. Phosphorus release from sterilized cores closely followed the Arrhenius equation. A decrease in phosphorus release with time was found in cores in which bacterial processes were excluded. The hypothesis is brought up that microbial processes influence sediment phosphorus release in two manners: 1) a direct temperature dependent reaction, 2) an indirect effect acting on a long term basis, governed by mineralization.

INTRODUCTION

Phosphorus is generally considered to be one of the main controlling factors for primary production in lake ecosystems. To improve water quality of eutrophic lakes, restoration measures are often focussed on the reduction of the external phosphorus load. However, recovery may be retarded by phosphorus release from the sediments. This release is a complex function of physical, chemical and biological processes (for a review see e.g. Boström et al. 1982). The transport of phosphorus in the sediment and its release rate to the overlying water is mainly controlled by physico chemical processes such as diffusion and adsorption. The driving force behind these processes might well be the rate at which soluble phosphorus is generated by mineralization. Generally, aerobic conditions are considered to promote phosphorus sorption by the sediments while anoxic conditions favour release. However, a number of mass balance and laboratory studies deal with the occurence of phosphorus release to well aerated water (Lee et al. 1977, Bates and Neafus 1980, Boers et al. 1984, Boers 1986). It is suggested that this release under aerobic conditions is related primarily to mineralization reactions of organic phosphorus compounds (Lee et al. 1977).

Investigations on the influence of bacterial processes on sediment phosphorus release, have been performed in several ways. In most cases sterilized sediment is compared with untreated sediment. Antibiotics (Kamp-Nielsen 1974) and formalin (Premazzi and Provini 1985) are used as sterilizing agents in intact cores. It is assumed that these compounds penetrate only just below sediment-water interface. Thus, the influence of anaerobic mineralization processes, which usually take place in the deeper layers, cannot be neglected. Bates and Neafus (1980) used antibiotics in sediment slurries. However, by mixing the sediment, physical and chemical

conditions are severely changed and results cannot be easily compared to the actual situation. Using γ-irradiation could help to overcome these problems as this method makes it possible to sterilize intact sediment cores.

A different method to distinguish biological from physico-chemical processes is to establish the temperature dependence of a reaction. Biologically mediated reactions display a temperature optimum while the rate of physico-chemical reactions increases with temperature according to the Arrhenius equation. This method has been succesfully used for oxidation processes in lake water (Brock 1978, Tipping 1984).

The purpose of the present study is to investigate the influence of bacterial processes on the aerobic phosphorus release. This was done by combining temperature and irradiation experiments and following the phosphorus release rate at different temperatures in irradiated and non-irradiated cores. The study is carried out in the eutrophic Loosdrecht Lakes. In these aerobic lakes Boers et al. (1984) found variable phosphorus release with a high peak in spring time. As in the sediment up to 73% of the total phosphorus is bound to organic matter, involvement of bacterial processes can be expected.

METHODS

The Loosdrecht Lakes are situated in the central part of the Netherlands. Originally the lakes were man made by peat excavation and the sediment contains up to 80 % organic matter. In 1984 the external phosphorus loading was reduced from 1.1 to 0.3 $g.m^{-2}.yr^{-1}$ (Kal et al. 1984). As a result of the large lake area of 18.2 km^2 and the low uniform depth of 1.8 m, the surface layer of the sediments remains aerobic during the year. The sediment phosphorus content is up to 1 $mg.g^{-1}$ on a dry weight base, and phosphorus is mainly bound to humic acids and other organic compounds (Boers et al. 1984). For further description of the Loosdrecht Lakes restoration and the hydrological and chemical data see Van Liere et al. 1984 and Kal et al. 1984.

In October 1985 and in January 1986 undisturbed sediment cores were obtained with a hand driven steel core sampler, containing a perspex inner core of 40 cm long and of an internal diameter of 5 cm. Out of each core three or four mini cores (8 cm long with an internal diameter of 1.5 cm) were taken in the laboratory. The overlying water was siphoned off as completely as possible and replaced by 6 ml Artifical Lake Water, ALW, with an ionic composition of: Cl^- 2.3 mM; HCO_3^- 0.8 mM; SO_4^{2-} 0.2 mM; NO_3^- 0.1 mM; Na^+ 1.1 mM; Ca^{2+} 1.0 mM; Mg^{2+} 0.2 mM; K^+ 0.1 mM; Fe^{3+} 2 μM; citrate 2 μM. The pH of the ALW in the mini cores was within the range usually found in the lakes (pH = 8-9). Half of the cores were sterilized by γ-irridiation for 7 hours at 10^6 Curie (25 KGy) (Gammaster, Ede, The Netherlands). Duplicate cores were kept in darkness at 4 °C. Prior to irradiation the mini

cores were closed with rubber stoppers perforated with two pipettes filled with cotton wool as to allow for sterile air supply during the experiment (fig. 2.1). After irradiation the cores were kept overnight in darkness at 4 °C to equilibrate. To investigate the direct effects of irradiation, overlying water was siphoned off for analysis after 17 h of equilibration and replaced with autoclaved ALW. All manipulations were performed in a Laminair Cross Flow Bench (Clean Air Techniek, Woerden, The Netherlands) in order to prevent microbial contamination.

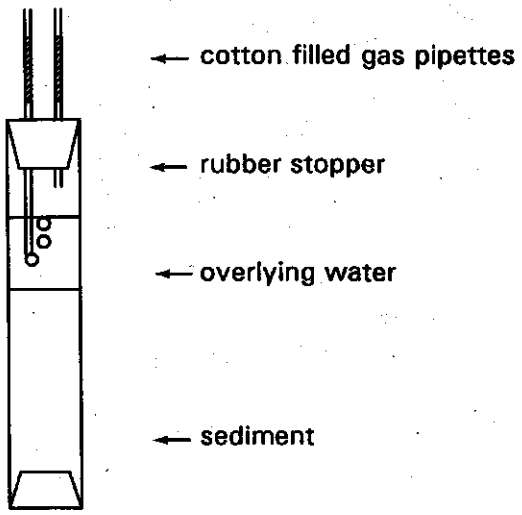

Figure 2.1: Scheme of the used mini cores.

For the temperature experiment both γ-irradiated and non-irradiated cores were incubated at different thermostated temperatures. During the experiment air was bubbled through the overlying water to maintain oxygen saturation levels. During the experiment overlying water was siphoned off several times and replaced. The sampled water was filtered through 0.45 μm Millipore HA filters and analysed for molybdate reactive phosphorus (Murphy and Riley 1962) with a Skalar Continuous Flow Analyser. After destruction with a persulfate-sulfuric acid mixture (modified after Eisenreich et al. 1975) soluble total phosphorus and soluble total iron (spectrophotometrically with TPTZ) were determined.

To determine the effect of γ-irradiation on the redox conditions, complete cores with septa at intervals of 2 cm, were irradiated. Redox potential was measured with a reference calomel electrode in the waterphase and platinum wire that was plugged through the septa. The system was left undisturbed for 10 minutes to

equilibrate before millivolt measurements.
Occasionally after the experiment colony forming units were counted on a modified Sepers medium (Sepers 1982) with bactocasiton. Plates were aerobically incubated for 10 days at 25 °C. The same medium was used to check sterility of the irradiated cores.

RESULTS AND DISCUSSION

Effects of γ-irradiation.

γ-irradiation effectively inactivated all bacteria as in undiluted sediment no colony forming units were counted even after incubation of the cores during a week. The irradiation treatment resulted in an iron and phosphorus release to the overlying water. The amounts of released iron and phosphorus were not correlated which makes the solubilization of iron phosphates as a main process not feasible. The iron release was 9.5 ± 6.0 $mg.m^{-2}$. The phosphorus release was higher and more variable in autumn (16.5 ± 25.7 $mg.m^{-2}$) than in winter (3.5 ± 1.2 $mg.m^{-2}$). The differences in the effect of irradiation between autumn and winter probably can be explained by the differences in total amount of biomasss which is present in or at the surface of the sediment and which is subjected to lysis. Exact data on the biomass content during the year of the sediment are not available. A comparison can be made with the sestonic biomass in the waterphase which is main source of algal biomass at the sediment surface. Gons et al. (1986) who measured sestonic biomass (size fraction <150 μm) in the Loosdrecht Lakes, observed in autumn a biomass of about 6 g carbon m^{-3} and during winter the biomass was at least twice as low. Although it is difficult to compare sediment with soil, the phosphorus release on a dry weight base (0.5 - 5 $\mu g.g^{-1}$) seems to be in the same range as the values found by Bowen and Cawse (1964) for soil, irradiated with 50 kGy (0.6 - 6 $\mu g.g^{-1}$). They ascribed nutrient release mainly to lysis of biota.
The redox potential in cores prior to irradiation in most cases did not fall below 70 mV. The effect of γ-irradiation on the redox conditions over the first 10 cm depth in the cores, which mainly consists of very lose brown mud, fell within the variation due to sediment heterogeneity and the used method (50 mV). The redox conditions in non-irradiated and irradiated cores thus are camparable. However, the underlying peat was found to be strongly reduced as a result of irradiation. No explanation for this phenomenon can be given. Most authors (Bowen and Cawse 1964, McLaren et al. 1962) presume that humic compounds are not affected by irradiation as the temperature rise during the treatment is low, approximately 7 - 8 °C (McLaren et al. 1962).

Influence of temperature on phosphorus release.

In irradiated cores, sampled in autumn, the release rates during the inital incubation

period of two hours at different temperatures were high and varied strongly. This variation presumably is caused by differences in soluble phosphorus content due to lysis, as already indicated by the above mentioned large standard deviation. In γ-irradiated cores which were sampled in winter, the phosphorus release rate increased exponentially with increasing temperature, being at 80 °C 6 times as high as at 10 °C (fig. 2.2). The Q_{10}, calculated over the temperature range 10 - 50 °C, is 1.4. This low value, caused by purely physico-chemical processes, is within the range expected for desorption reactions.

In non-irradiated cores, sampled in autumn, phosphorus release rates followed a comparable exponential increase with temperature. Superimposed on this exponential relation, a clear tendency for a temperature optimum at about 40 °C was found (fig. 2.3A). This optimum was found in both soluble reactive and soluble total phosphorus release. On the contrary, in winter no temperature optimum was found (fig. 2.3B). These results suggest that in autumn a biological process is involved in phosphorus release which is directly influenced by temperature. The Q_{10} of the phosphorus release rate in autumn, calculated over the range where both physico chemical and biological processes occur (10 - 40 °C), varies between 1.1 and 3.0 for soluble total and 2.7 and 4.8 for soluble reactive phosphorus. These values are comparable with the Q_{10} value of 3 found by Boers (1986) for the Loosdrecht Lakes and the value of 2.3 found by Ulen (1978) for aerobic phosphorus release in Lake Norvikken.

Kamp-Nielsen (1975) found a comparable exponential relation between temperature and phosphorus release for the aerobic Esrom sediment (Denmark). He ascribed this phenomenon to the increased oxygen consumption by the sediment and the concomitant breakdown of the oxidized microlayer on top of the sediment which normally serves as a diffusion barrier. The effects of an increase in chemical oxygen demand and diffusion rate with temperature could explain the increasing phosphorus release rates of both control and γ-irradiated cores. This implies that the trigger mechanism for phosphorus release under aerobic conditions is a purely physico-chemical process. The extra release at the lower temperatures in the non-irradiated cores in autumn which culminates in the optimum release rate at 40 °C is caused by microbial processes. Tessenow (1972) already showed that stimulation of biological activity by the input of easily degradable substrate, led to an increase in phosphorus release from the sediment. Increasing temperature can have a comparable effect on the activity of bacteria and thus be an explanation for the temperature optimum found in autumn. The higher release rates induced by bacterial activity could be caused by an increased biological oxygen demand and thus proceed basically by the same mechanism as the chemical process described above.

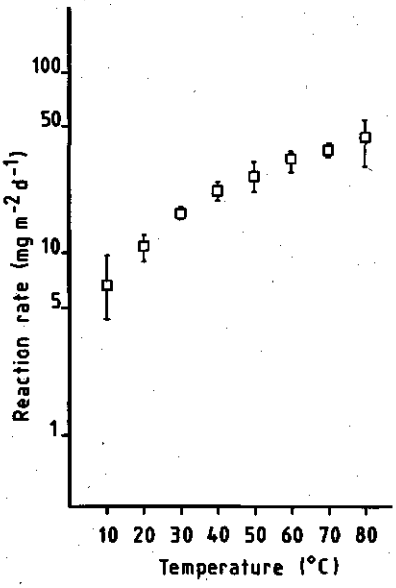

Figure 2.2: Soluble phosphorus release rate from γ-irratiated cores incubated for 3 hours at different temperatures (means of triplicates).

In winter microbial processes seem to play only a minor role. The difference between autumn and winter may be caused by changes in bacterial number, different composition of bacterial population or limitation of available substrate. A difference in concentration of terminal electron acceptors which eventually can play a role in the actual field situation, can be ruled out as a possibility as in both experiments the same conditions are maintained. In agreement with these results are the changes in phosphorus release rate found in Loosdrecht by Boers (1986). He varied the temperature in phosphorus release experiments with Continuous Flow Release Reactors and found that increasing temperature had a stronger influence on cores sampled in summer than on those sampled in winter.
Repeated analyses throughout the year are necessary to reveal the differences in the role of bacterial processes in phosphorus release.

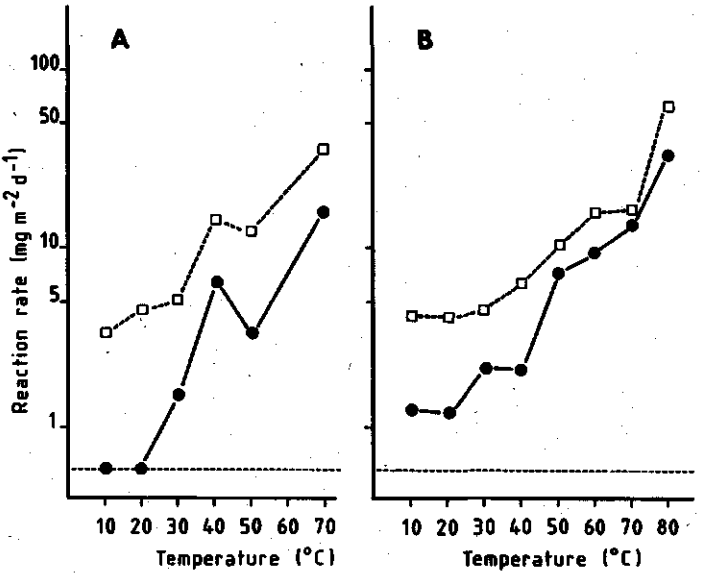

Figure 2.3: Soluble reactive (●) and soluble total (□) phosphorus rate from cores incubated at different temperatures, mind logarithmic scale. Broken line represents detection limit. A) sampled in autumn incubated for 2 hours (means of duplicates) B) sampled in winter incubated for 3 hours (means of triplicates).

Phosphorus release with time.

In cores sampled in autumn, phosphorus release with time was followed. In γ-irradiated cores the phosphorus release rate decreased with time at all incubation temperatures (fig. 2.4A).In the non-irradiated cores a same decrease in reaction rate was found at 10, 20, 30 and 70 °C. However, in the cores incubated at 40 and 50 °C, a sharp increase in reaction rate was found after an initial drop (fig. 2.4B). Plate counts after the experiment made clear that in the sediment incubated at 50 °C a higher number of colony forming units was present (table 2.1).

The explanation for the decrease in phosphorus release rate in cores in which no bacterial activity is expected, e.g. the irradiated or heated cores, can be the exhaustion of the phosphorus pool in the sediment. It is stated by several authors (Lijklema 1985, Boers 1986) that generation of soluble phosphorus by mineralization can maintain a phosphorus pool in the sediment which acts as a driving force for release. By the exhaustion of this pool the concentration gradient between porewater and overlying water gradually disappears and, consequently, release rates diminish. The relatively low release rates at high temperatures in the irradiated cores (fig. 2.2), which cause a significant deviation from a straight line ($P < 0.01$), are in accordance with this hypothesis.

The increase in phosphorus release rate at 40 and 50 °C in the control cores probably is a result of the activation and growth of the bacterial population in the sediment. The influence of this reaction at the low actual temperature (range 0 - 22°C) of the Loosdrecht Lakes can only be of minor importance. Surprisingly a constant phosphorus release rate with time, which could be expected at an incubation temperature close to the situ temperature, was not found.

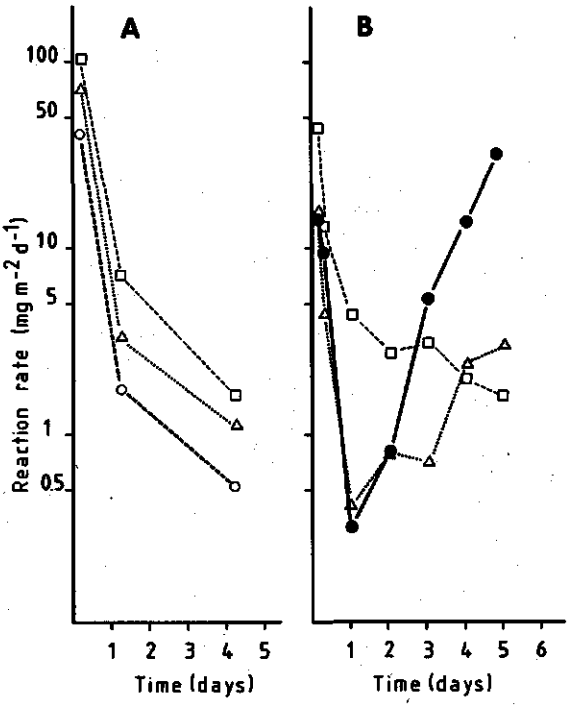

Figure 2.4: Soluble total phosphorus release rate with time from cores incubated at different temperatures 20 (○), 40 (Δ), 50 (●) or 70 (□) °C. A) γ-irradiated cores B) non-irradiated cores (means of duplicate cores, mind logarithmic scale).

Table 2.1: Numer of colony forming units per gram dry sediment in cores incubated for 6 days at different temperatures.

Temperature (°C)	Number of colony forming units
10	$3.1 \pm 0.7 * 10^6$
20	$5.1 \pm 0.9 * 10^6$
30	$6.0 \pm 1.4 * 10^6$
40	$4.4 \pm 0.7 * 10^6$
50	$17.8 \pm 1.5 * 10^6$
70	$< 1 * 10^4$

SUMMARY

The aim of this study was to get an insight in the influence of bacterial processes on the phosphorus release from sediments. The study was carried out in the eutrophic Loosdrecht Lakes (The Netherlands) in which the sediment surface stays aerobic throughout the year. Undisturbed sediment cores were sampled in autumn and winter.

The effect of temperature on the sediment phosphorus release was investigated in sterilized and non-sterilized cores in order to distinguish between biological and physico-chemical processes. Cores were effectivily sterilized with γ-irradiation (25 kGy). The treatment results in low releases of phosphorus, probably caused by lysis of biota. The amount of released phosphorus was clearly influenced by the sampling season and was higher and more variable in autumn (16.5 ± 25.7 mg.m^{-2}) than in winter (3.5 ± 1.2 mg.m^{-2}). The effect of irradiation on the redox conditions seemed to be dependent of the sediment composition at the different depths but did not interfere with these experiments.

The phosphorus release rate in γ-irradiated cores incubated at different temperatures approached the Arrhenius equation (fig. 2.2). In non-irradiated cores a comparable exponential increase with temperature was found (fig. 2.3). However, in cores sampled in autumn a clear tendency for a temperature optimum at 40 °C was found to be superimposed on this exponential function (fig. 2.3A).

The phosphorus release rate decreased with time in all cores in which bacterial processes were excluded by γ-irradiation or heat (fig. 2.4). In non-sterilized cores incubated at 40 and 50 °C a strong increase of the phosphorus release rate with time was found after an initial drop (fig. 2.4B). It was made plausible that this increase was due to bacterial activity.

It is brought up as a hypothesis that the phosphorus release rate is influenced by

two different microbial processes; a direct reaction influenced by temperature and a more less long term effect governed by mineralization. The importance of these two processes seems to vary during the year.

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Chapter 3

Mineralization, pore water chemistry and phosphorus release from peaty sediments in the eutrophic Loosdrecht lakes, The Netherlands

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SUMMARY

1) Pore water chemistry in peaty sediment was monitored for a year at two representative locations of the eutrophic shallow Loosdrecht lakes, The Netherlands. Phosphorus fluxes over the sediment-water interface were calculated using measured concentration gradients in the pore water and compared to fluxes measured under laboratory conditions. Results were analysed with Redundancy Analysis to detect patterns of variation in pore water chemistry and in measured and calculated fluxes, that could be ascribed to environmental variables.
2) It was demonstrated that phosphorus fluxes measured in long-term laboratory incubations were not correlated to any of the pore water characteristics.
3) Initial phosphorus fluxes measured in sediment columns, which varied between -7.7 and 1330 μmol.m^{-2}.d^{-1}, were correlated significantly to the calculated phosphorus flux over the sediment-water interface.
4) The high correlation between calculated fluxes of ammonia, phosphorus and methane and measured initial flux of phosphorus, conclusively pointed to mineralization of organic matter as driving force for phosphorus release from the sediment.
5) Redundancy Analysis demonstrated that the rates of mineralization and phosphorus release were only weakly related to temperature. They appeared to be especially stimulated by the autumnal decrease in temperature which was probably related to an extra input of organic matter.

INTRODUCTION

To improve water quality of eutrophic lakes, water management measures have often been focused on the reduction of the external phosphorus load. However, an internal feedback loop as for instance phosphorus release from the sediment, may retard a long-term improvement of the lake's trophic state. Especially in shallow lakes, phosphorus release from the sediment can be an important drawback for lake recovery (Ryding and Forsberg 1977). Phosphorus release depends on a great variety of physical, chemical and biological processes. Knowledge of the importance of the various processes contributing to phosphorus release rates is necessary to judge the utility of supplementary management measures.

The effect of several environmental variables on phosphorus release from sediment has been studied in detail. Ever since the pioneering work of Einsele (1936) and Mortimer (1941, 1942), a decrease in redox potential has been recognized to stimulate phosphorus release due to the chemical reduction of Fe(III) which results in a mobilization of Fe(II) and phosphates. The importance of this process for aerobic systems has been demonstrated by Tessenow (1972) who manipulated the redox potential by stimulating microbial activity. Kamp-Nielsen (1975), Holdren and

Armstrong (1980) and Sinke and Cappenberg (chapter 2) demonstrated that phosphorus release rates increased with increasing temperature. This temperature dependence of phosphorus release may be very important in shallow temperate lakes where annual temperature ranges frequently span 20 °C. An increase in pH in eutrophic lakes directly caused by the high primary productivity in summer, can be an important stimulus to phosphorus release (Rippey 1977). Also other environmental variables e.g. bioturbation (Holdren and Armstrong 1980) and nitrate concentration (Jansson 1987), can play a role in controlling the mobilization of phosphorus from the sediment. In most of these studies though, the effect of a single environmental variable has been investigated in sediment columns under well defined laboratory conditions. The extrapolation of these results to field conditions is severely complicated as the effects of the different environmental variables in situ may be counteracting or synergistic.

In situ measurements of the phosphorus flux in bell-jars probably mimic best the natural phosphorus release which is the resultant of all contributing processes in the field. However, to elucidate the seasonal impact of the various processes, tedious measurements have to be repeated throughout a year. In several studies carried out with bell-jars, independently determined pore water concentration gradients have been used succesfully to calculate the flux of phosphorus across the sediment-water interface (Callender and McHammond 1982, McCaffrey et al. 1980). So, regular measurements of pore water concentrations might be a useful approach to gain insight in seasonal processes affecting phosphorus release.

Phosphorus in the pore water of recent sediments is primarily regenerated as phosphate by microbial mediated decomposition of organic matter (Berner 1974). Once regenerated, phosphates can diffuse, adsorb, complexate with (in)organic cations and precipitate as authigenic minerals. Especially in organic rich peaty sediments, it might be expected that mineralization plays an important role in phosphorus chemistry.

The aim of this study is to relate the seasonal variation in pore water chemistry to environmental variables and thus gain insight in the phosphorus regeneration in peaty sediments. For comparison, phosphorus release experiments were carried out under standardized laboratory conditions.

MATERIALS AND METHODS

Sampling area.

The research was carried out in the Loosdrecht lakes, a series of interconnected lakes which are partly man made through peat mining in the seventeenth century (Van Liere 1986). Due to eutrophication, water quality of the system severely diminished in the last decades. From 1970 to 1984 water management measures were taken to reduce external phosphorus loading (Van Liere 1986). As a result of

the morphometry (area = 14.5 km^2, mean depth = 1.8 m) and continuous wind-induced turbulence, the surface layer of the sediment remains aerobic throughout the year. The sediment phosphorus content is up to 1 $mg.g^{-1}$ of dry weight and we have evidence that phosphorus is mainly bound to humic acids and other organic compounds (Boers et al. 1984). The sediment pore water is slightly alkaline. The pH decreases from pH = 8.3 in the surface layer to pH = 7.3 at greater depths. Samples were collected in 1988 every fourth week at two representative locations in the Loosdrecht lakes. One sampling station -Marcus Pos- is situated in the open lake area and the other -Kievitsbuurt- in a more or less separated part sheltered from wind influence. Temperature of the lake water was measured just above the sediment surface. Total phosphorus in the lake water was measured as molybdate reactive phosphorus (Murphy and Riley 1962) after destruction of the samples for 2 hours at 125°C with a persulfate-sulfuric acid mixture (after Eisenreich Bannerman and Armstrong 1975). For pH and chlorophyll-a values, the routine measurements of the "Water Quality Research Loosdrecht Lakes" programme, as described by Van Liere (1986) and Gons, Gulati and Van Liere (1986) were used. At both locations the sediment consisted of relatively loose organic debris. The percentage dry weight of the sediment increased with depth. At Marcus Pos from 11% at the sediment-water interface to 14 % at 10 cm depth and in Kievitsbuurt from 6% to 11%. The organic matter content was fairly constant with depth and was slightly lower at Marcus Pos than at Kievitsbuurt: 55 versus 65%.
Sediment columns were obtained using a hand-driven stainless-steel corer containing a perspex inner core of 40 cm long with an internal diameter of 5 cm. The sharpened edges of the inner core extended well below the metal parts to minimize disturbance of the sediment.

Pore water measurements.

Two duplicate columns were placed in a nitrogen filled glove box (Coy Laboratory Products Inc., Michigan) and 0.5 or 1.0 cm thick layers were scooped off. Interstitial water was pressed out over 0.45 μm filters using a Reeburgh type squeezer (Reeburgh 1967) at a maximal N_2 pressure of 10^5 Pascal. Part of the pore water was acidified to pH 1 with 3 M H_2SO_4 to prevent precipitation of iron phosphates. The following ions were measured spectrophotometrically: dissolved reactive phosphorus (DRP), ammonia (NH_4^+) after reaction with salicylate and dichloro-isocyanurate (Krom 1980), ferrous and ferric iron (Fe^{2+}, Fe^{3+}) after reaction with tripyridyltriazine (Fries and Getrost 1977), calcium (Ca^{2+}) after reaction with cresolphthalein, and dissolved organic carbon (DOC) after photochemical (UV) oxidation using persulfate (Schreurs 1978). Sulfate (SO_4^{2-}) was measured with HPLC according to Hordijk, Hagenaars and Cappenberg (1985). Dissolved total phosphorus (DTP) was measured as DRP after destruction of the

samples. Dissolved organic phosphorus (DOP) was calculated as the difference of DTP and DRP.

For methane measurements two columns were sliced at 1 cm intervals and subsamples were put in bottles immediately. Methane was measured in the headspace after vigorously shaking. Time series made clear that the loss of methane during handling was approximately 10% and all data were adjusted. A Packard model 428 gas chromatograph equipped with a flame ionization detector (FID) was used for methane measurements.

Diffusive fluxes.

The flux of phosphorus under laboratory conditions was measured in two duplicate columns. The overlying water was carefully siphoned off within two hours after sampling and replaced with filtered (0.2 μm) lake water. The columns were incubated in dark at in situ temperature. The overlying water was gently aerated to achieve mixing and to keep the system aerobic. During the first hours of the incubation, the sediment columns were treated as a batch and after 4 hours the initial phosphorus release to the overlying water was measured. Afterwards, filtered lake water was continuously added and removed to obtain a residence time of the overlying water of 1.6 days. Phosphorus fluxes were followed for 21 days and the average release rate was calculated (Boers et al. 1984).

Measured pore water gradients were used to calculate diffusive fluxes of NH_4^+, DRP and CH_4 towards the sediment surface according to Fick's first law of diffusion.

$$J_i = \frac{\phi}{\Theta^2} * D_{oi} * \frac{dC_i}{dz}$$

with J_i = flux of i (μmol.cm^{-2}.sec^{-1}), i = either NH_4^+, DRP or CH_4, D_{oi} = molecular diffusion coefficient for i (cm^2.sec^{-1}), ϕ = porosity (dimensionless), Θ = tortuosity (dimensionless), and dC_i/dz = linear concentration gradient of i in the pore water (μmol.cm^{-4}).

D_o is the diffusion coefficient in water at infinite dilution. D_o for NH_4+ (16.8 x10^{-6} cm^2.sec^{-1}, at 18 °C) and $H_2PO_4^-$ (7.15 x10^{-6} cm^2.sec^{-1}, at 18 °C) were taken from Li and Gregory (1974) and D_o for CH_4 (15.3 x10^{-6} cm^2.sec^{-1}, at 15 °C) was taken from Broecker and Peng (1974). The rate of sulfate reduction was calculated from the measured pore water profiles according to Berner (1964). The diffusion coefficient for sulfate (8.9 x10^{-6} cm^2.sec^{-1}, at 18 °C) was taken from Li and Gregory (1974). D_o values were corrected for the prevailing in situ temperature. Porosity was taken as 0.95 and the square of tortuosity as 1.40 (J.-P. Sweerts

pers.comm.).

Statistical analyses.

Redundancy Analysis (RDA) was applied to analyse pore water data and both measured and calculated fluxes. A recent version of programme CANOCO (Ter Braak 1987a) was used. RDA (Rao 1964) is a technique which relates a set of multivariate data to explanatory variables. RDA combines the features of Principal Component Analysis, summarizing multivariate data, and multiple regression analysis, relating data (pore water constituents or fluxes) to environmental variables. RDA selects successive linear combinations of environmental variables that give the smallest residual sum of squares. The environmental variables in the analysis were depth below sediment-water interface (DEPTH), temperature (TEMP), chlorophyll-a concentration, total phosphorus concentration and pH in the lake water (CHLA, TPWAT, pH) and the over all change in temperature between successive sampling dates (δTEMP). The interactions between these main variables were considered (e.g. δTEMP*CHLA).

Environmental variables were selected stepwise, maximizing the explained sum of squares with the minimum number of environmental variables. However, interactions between variables were only introduced after the main effects. The results are presented as correlation biplots (Gabriel 1971, Ter Braak 1987b). In these biplots the cosine of the angle between the vectors of two variables is an indication of the correlation between them.

To get an overall insight in the response of the sediment, the data sets of the two sampling locations were lumped. To compensate for the major differences between the two locations, the location itself was introduced as a 0/1 explanatory variable. The measured pore water chemistry variables were standardized to zero mean and unit variance to perform the analysis on a correlation matrix. A log transformation was applied to sulfate data before calculations because the sulfate values were approximately log normally distributed. Due to missing values in the data set, especially for iron, the RDA was run twice, once with only those sediment columns in which iron was measured (80% of all measured columns) and once with all sediment columns, leaving iron out of the analyses. The outcome for the other measured ions hardly differed in these two analyses, so results are focussed on the RDA including iron.

RDA was applied to both calculated and measured fluxes, using mean values for each sampling date and location: methanogenesis (CH_4FX), sulfate reduction (SO_4FX), NH_4^+ and DRP flux in the anaerobic sediment (NH_4FX, DRPFX), DRP flux across the sediment-water interface (DRPIF) and measured initial and average DTP release (INIT, AVG). Two sets were left out of the analysis as they appeared to be clear outliers. RDA was run twice, the first time sulfate reduction was left out as

especially in spring at location Marcus Pos, sulfate concentration at 2 cm depth was higher than at the interface and sulfate reduction rates could not be determined. The overall relation between the fluxes and the environmental variables showed only minor differences and results are focussed on the RDA including sulfate reduction.

RESULTS

Pore water chemistry.

The ionic composition of the interstitial water of the two locations differed markedly (table 3.1). The concentrations of measured ions were significantly higher in Kievitsbuurt except for dissolved organic phosphorus. In most columns concentrations of NH_4^+, DRP, Ca^{2+}, Fe^{2+} and CH_4 increased significantly with depth. Sulfate concentrations decreased exponentially with depth in the top 3 or 4 cm and remained at a constant low level (2-11 μM) at greater depths (fig. 3.1). The pore water mostly had a yellowish colour. Dissolved organic carbon concentrations did not change with depth but dissolved organic phosphorus had a tendency to be higher at greater depths.

The mean concentrations of especially NH_4^+, DRP, and CH_4 and the slope of the concentration gradients in the sediment varied strongly during the year and were highest in September and October and lowest in January and February (fig. 3.1). The interstitial NH_4^+ and DRP concentrations were strongly correlated in all sediment columns (table 3.2). The correlation matrix clearly indicates that concentrations of NH_4^+, DRP, Ca^{2+}, CH_4 and probably Fe^{2+} react in a comparable way. The independent variables which sequentially entered the redundancy analysis and the proportion of variance in the dependent variables which can be additionally explained are listed in table 3.3. The value of 0.14 for DEPTH for additional variance explained, implies that 14 % of the variance in standardized pore water concentrations can be explained by depth.

The total percentage of the variance in the measured pore water characteristics which can be explained by all independent variables is 55 %. The major fraction of the total explained variance is described by the first two axes (29.3 on the first and 8.9 % on the second axis), on the third and fourth axis 5.5 and 2.7 % is described. The residual fraction of 8.6 % is described by subsequent axes with a higher dimension.

Examination of the correlation matrix between the environmental variables (table 3.4) reveals that several significant relationships exist between the variables e.g. temperature, pH, TP and chlorophyll-a in the lake water. After the addition of temperature to the RDA, the contributions of TPwat, pH and CHLa to the explained variance are only of minor importance.

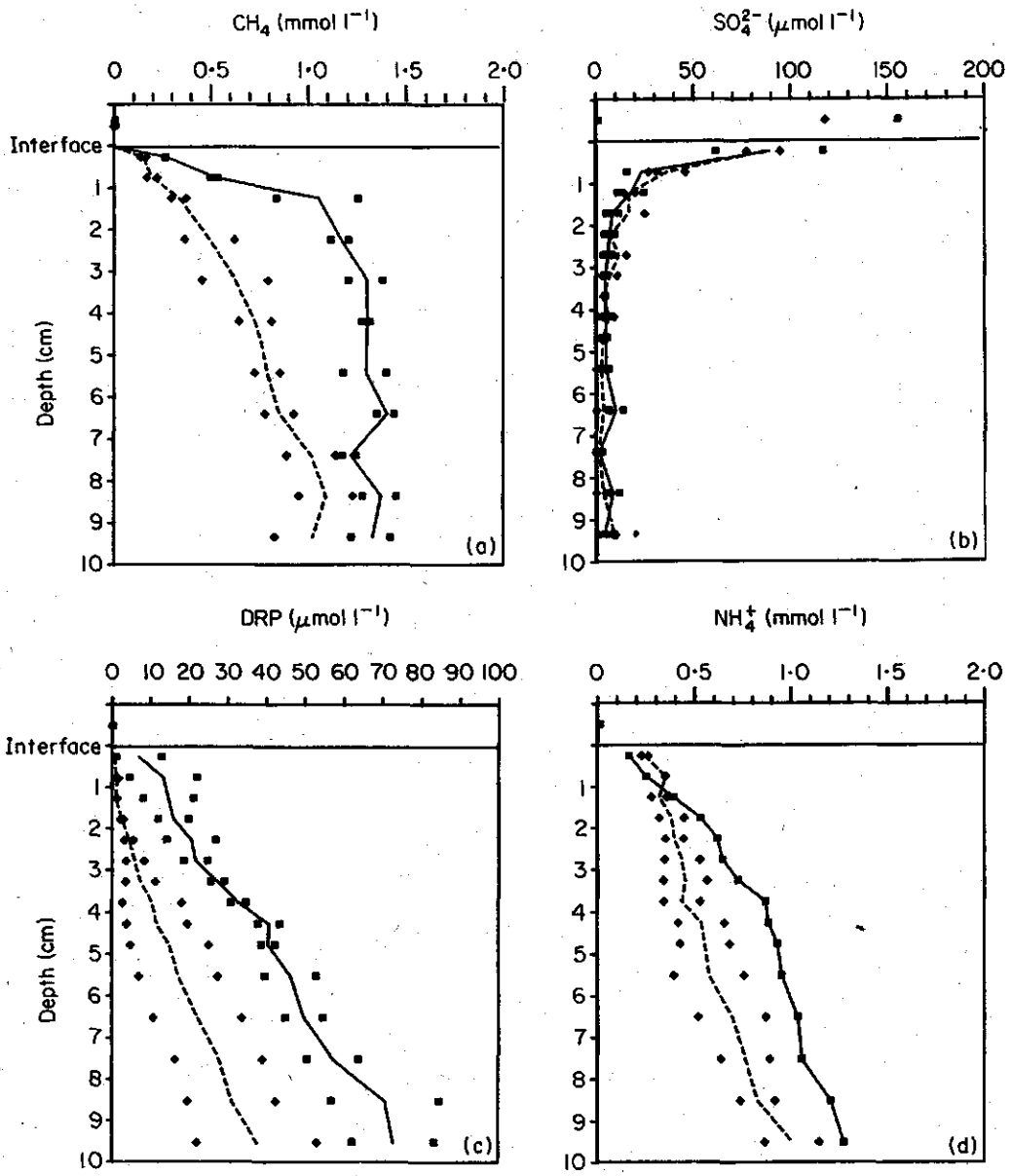

Figure 3.1: Concentrations of methane (A), sulfate (B), dissolved reactive phosphorus (C), and ammonium (D) in pore water of Kievitsbuurt sediment in January 1989 (-------) and October 1989 (—————)

Table 3.1: Differences between the two sampling locations in DTP release ($\mu mol.m^{-2}.d^{-1}$), sulfate reduction ($\mu mol.m^{-2}.h^{-1}$), methanogenesis ($\mu mol.m^{-2}.h^{-1}$) and concentrations of ions in the pore water ($\mu mol.l^{-1}$). Minimum, maximum and median values are given.

	Marcus Pos			Kievitsbuurt		
	min.	max.	median	min.	max.	median
DTP release (AVG)	-55.5	2.8	-1.6	-8.1	9.1	-0.7
DTP release (INIT)	-0.2	1200	110	-7.7	1300	150
Sulfate reduction	7.2	45	35	13	51	39
Methanogenesis	7.7	135	47.1	52.8	274	69
NH_4^+	43	1080	237	107	1590	489
DRP	0.2	30.2	10	0.5	62.4	11.1
DOP	0	50	7.5	0	55	7.2
Fe^{2+}	1.6	54.2	6.4	3.5	87.5	12.6
Fe^{3+}	0	32	3.4	0	50	7.6
DOC	1020	4800	1970	1130	5960	2280
Ca^{2+}	1020	2490	1330	770	3780	1750

Table 3.2: Correlation matrix of pore water characteristics of the Loosdrecht Lakes.

	NH_4^+	DRP	DOP	Fe^{2+}	Fe^{3+}	DOC	Ca^{2+}	CH_4
NH_4^+		0.74***	0.04	0.43**	0.19	0.19	0.64***	0.59***
DRP			-0.02	0.24	-0.13	0.33*	0.36*	0.52***
DOP				0.06	0.08	0.13	-0.03	0.01
Fe^{2+}					0.25	-0.07	0.44**	0.35*
Fe^{3+}						-0.13	0.32*	0.09
DOC							0.07	0.03
Ca^{2+}								0.50***

n = 42 sediment columns, with each fifteen measurments (NH_4^+, DRP, DOP, Fe^{2+}, Fe^{3+}, DOC and Ca^{2+}) or twelve measurements (CH_4). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 3.3: Sequence and additional proportion of variance explained of the environmental variables used in the redundancy analysis of the pore water variables.

	additional variance explained	cumulative variance explained R^2
Depth in the sediment (DEPTH)	0.14	0.14
Location (Marcus Pos)	0.10	0.24
Temperature (TEMP)	0.07	0.31
Change in temperature (δTEMP)	0.06	0.37
Total Phosphorus in the lake water (TPWAT)	0.03	0.40
Chlorophyll-*a* in the lake water (CHLA)	0.01	0.41
CHLA * δTEMP	0.03	0.44
pH of the lake water (pH)	0.02	0.47
CHLA * TEMP	0.02	0.49
all other interactions between variables (none significant)	0.06	0.55

Table 3.4: Correlation matrix of environmental variables measured in the Loosdrecht Lakes; TEMP is temperature, CHLa is the chlorophyll-a concentration, TPwat is the phosphorus concentration of the lake water and δTEMP is the overall change in temperature between two successive sampling dates.

	TEMP	CHLa	TPwat	pH	δTEMP
TEMP		0.53*	0.42	0.79***	0.23
CHLa			0.65**	0.36	-0.07
TPwat				0.32	0.09
pH					0.21

n=15, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

The resulting correlation biplot of the first two axes of the measured variables points out high correlations between NH_4^+, DRP, Fe^{2+} and CH_4 concentrations as those vectors roughly point towards the same direction (fig. 3.2). Concentrations of all measured variables, except sulfate and dissolved organic carbon, are lower at location Marcus Pos than at Kievitsbuurt, as can be seen from the location of the centroid of Marcus Pos samples opposite to the vectors of DRP, CH_4, Ca^{2+},NH_4^+, Fe^{2+}, Fe^{3+} and DOP. Comparison with the environmental variables, demonstrates that with increasing depth in the sediment (DEPTH) and with decreasing temperature difference (δTEMP), the concentrations of NH_4^+, DRP, CH_4 and Ca^{2+} and, less pronounced, Fe^{2+} increase. The sulfate concentration gradient is opposite to the gradients of the other ions as sulfate decreases with depth. The concentrations of Fe^{3+} and dissolved

organic phosphorus increase slightly with depth and tend to be higher at low temperature (TEMP). The concentrations of NH_4^+, Ca^{2+}, DRP, Fe^{2+} and CH_4 hardly react on temperature as indicated by the 90° angle between these vectors and the temperature vector. Concentrations of dissolved organic carbon, however, strongly increased with temperature. The biplot scores for chlorophyll-a, total phosphorus and pH in the lakewater are close to the biplot scores of temperature, due to their strong correlation.

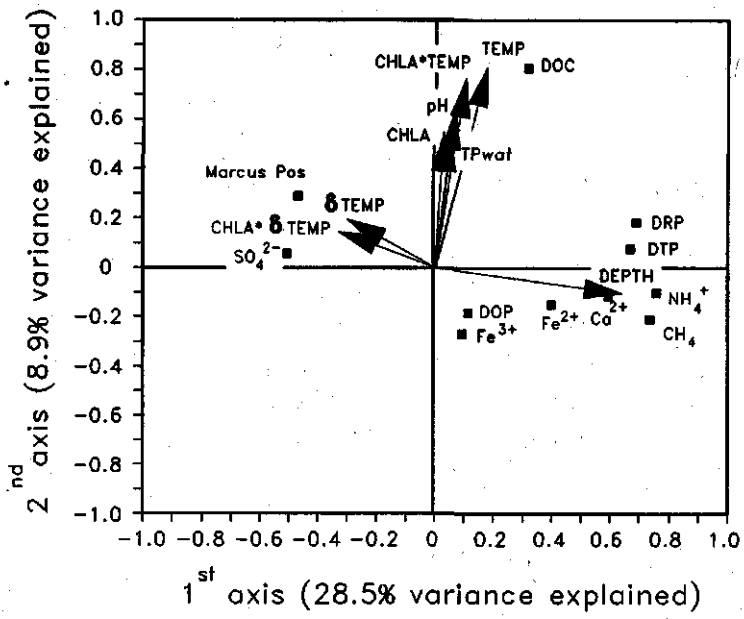

Figure 3.2: RDA correlation biplot of pore water chemistry variables. Environmental variables (see table 3.3) are represented by arrows.

Diffusive fluxes.

In the release experiments DRP concentrations were very low and below or only just above detection limit (0.05 μmol.l^{-1}). In those samples where both DRP and DTP could be determined, a significant correlation was calculated ($p < 0.0001$). DTP concentrations were 1.17 times higher than DRP concentrations. This relation was similar at both sampling locations. Release rates were calculated based on the DTP concentrations. At Kievitsbuurt the average DTP release rate was positive in summer only and very low (fig. 3.3A). The initial release rate was positive during most of the year and reached high values in autumn (fig. 3.3B). The release rates at Marcus Pos followed the same trends at a lower level (table 3.1).

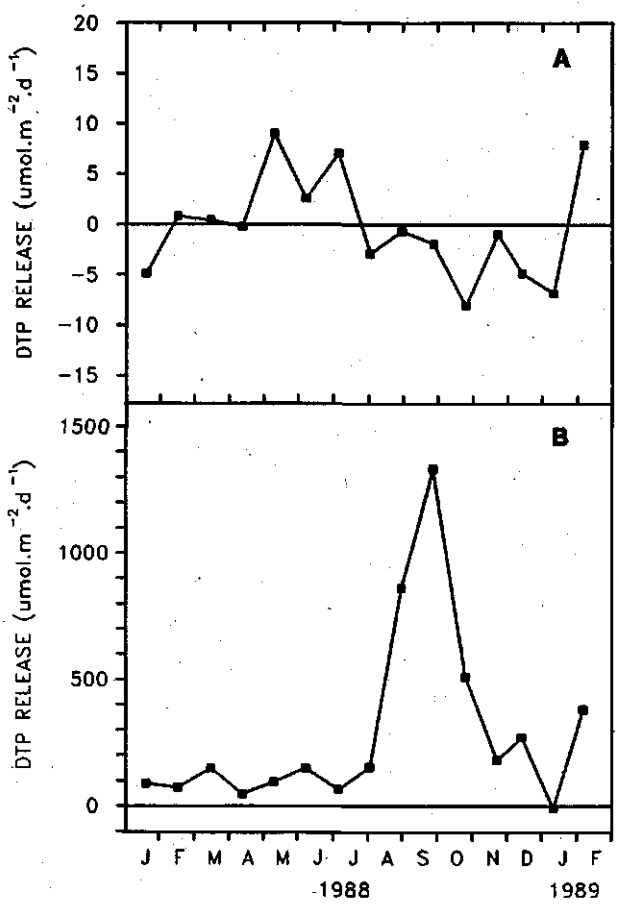

Figure 3.3: Dissolved total phosphorus release in laboratory systems from sediment columns retrieved from Kievitsbuurt at 4-week intervals. **A:** average release rate, **B:** initial release rate (note different scaling).

Sulfate reduction rates ranged from 7.2 to 51 μmol.m^{-2}.h^{-1} and did not differ at the two sampling locations. The calculated CH_4 flux ranged from 7.7 to 274 μmol.m^{-2}.h^{-1} and was lower at Marcus Pos than at Kievitsbuurt. Diffusive fluxes for NH_4^+, DRP and CH_4 were calculated based on the concentration gradients in the sediment pore water itself. Thus the fluxes represent a flux in the anaerobic sediment towards the sediment top-layer. The NH_4^+ flux ranged from 0-46.7 μmol.m^{-2}.h^{-1} and the DRP flux ranged from 0-1.0 μmol.m^{-2}.h^{-1}. In all sediment columns the calculated fluxes of CH_4, NH_4^+ and DRP were highly correlated (fig. 3.4).

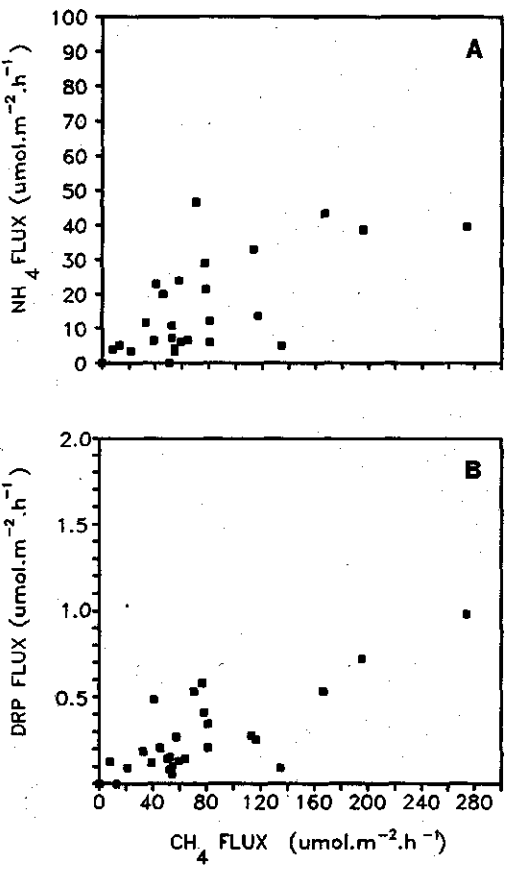

Figure 3.4: Relation between calculated methane and ammonium flux (A) and methane and phosphorus flux (B) in sediment columns of the Loosdrecht Lakes (n=25).

Often a very steep concentration gradient, especially for phosphates, was found between the pore water concentration in the uppermost sediment layer and the overlying lake water. Diffusive fluxes of DRP from the uppermost sediment layer to the overlying water were calculated from this concentration gradient. These fluxes were generally much higher than the DRP fluxes in the anaerobic sediment and ranged from 0 to 14.6 μmol.m^{-2}.h^{-1}.

The percentage of variance described in the RDA by all independent variables was 46 % and 31.4 % was accounted for by the first two axes. Location (0.18), δTEMP (0.10) and TEMP (0.06) were the most important environmental variables which contributed to the variance explained. The correlation biplot of the RDA points out that methanogenesis, NH_4^+ and DRP flux were closely correlated (fig. 3.5). The calculated DRP flux over the sediment water interface is related to the flux of CH_4 and, less pronounced, to the fluxes of DRP and

NH_4^+ in the anaerobic sediment. The measured initial DTP flux apparently reacts in a similar way on the environmental variables as the calculated DRP flux over the sediment water interface.

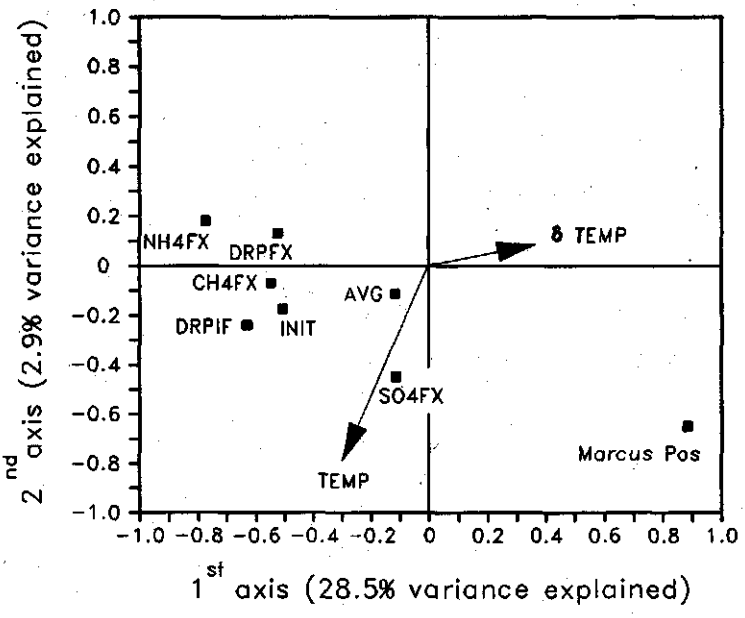

Figure 3.5: RDA correlation biplot of calculated fluxes in the anaerobic sediment of methane (CH_4FX), sulfate (SO_4FX), ammonium (NH_4FX), phosphate (DRPFX), calculated flux of phosphate over the sediment-water interface (DRPIF) and measured average and initial fluxes of phosphate (AVG, INIT). Environmental variables (see table 3.3) are represented by arrows.

The measured average phosphorus release rates were hardly related to any of the other fluxes and only slightly influenced by the environmental variables as can be inferred from the length of the vector for the average release rate. All fluxes, except sulfate reduction, were lower at Marcus Pos than at Kievitsbuurt. Sulfate reduction increased with increasing temperature. The fluxes of NH_4^+, methane and the calculated and measured initial fluxes of phosphates, apparently increased with a decrease in temperature since the vector of δTEMP is pointing to the opposite direction.

DISCUSSION

Our results provide strong evidence that mineralization is the driving process determining pore water chemistry in peaty sediments. The concentrations of CH_4, DRP, NH_4^+, Ca^{2+} and Fe^{2+} are highly correlated and increase with depth in the sediment.

At the two sampling locations, with a calculated median methane production of 47.1 μmol.m^{-2}.h^{-1} for Marcus Pos and 69.2 μmol.m^{-2}.h^{-1} for Kievitsbuurt, methanogenesis differed markedly. This difference can not be explained by the higher organic matter content of the Kievitsbuurt as on a volume base, calculated by multiplying the mean dry weight and the organic matter content, the organic matter content at Marcus Pos is 69 kg.m^{-3} and at Kievitsbuurt 55 kg.m^{-3}. Probably the biodegradability of the organic matter is higher in the Kievitsbuurt. As the more pronounced wind exposure of Marcus Pos can contribute to a continuous sweeping away of the loose top layer of the sediment -indicated by the higher % dry weight- this could diminish the input to the sediment of easily degradable sedimenting algae.

Methanogenesis was higher than usually measured in deep freshwater systems during the non-stratified period. Kuivila et al. (1989) reported values of 16 μmol.m^{-2}.h^{-1} in mesotrophic Lake Washington and Rudd and Hamilton (1978) mentioned 33.3 μmol.m^{-2}.h^{-1} for aerobic epilimnetic sediments of eutrophic Lake 227. Probably in those systems the input of organic matter onto the sediment is low as the main part is mineralized in the water phase already. Methane production in shallow holomictic eutrophic lakes is hardly investigated. However, the methane production rates for acid swamps and wetlands, recently reported by Svensson (1986), are of the same order of magnitude as our findings al though reported maximal values are somewhat higher.

Sulfate reduction rates, 40 μmol.m^{-2}.h^{-1}, were comparable at both locations but lower than sulfate reduction rates reported for other freshwater systems. King and Klug (1982) measured sulfate reduction rates of 240-400 μmol.m^{-2}.h^{-1} in eutrophic lake Wintergreen. In an acid cedar swamp sulfate reduction rates varied from 70-260 μmol.m^{-2}.h^{-1} (Spratt, Morgan and Good 1987). However in these systems the pool of dissolved sulfate in the pore water was much higher than found in the Loosdrecht lakes. King and Klug (1982) reported a concentration of 23 μmol.l^{-1} at 10 cm depth and Spratt et al. (1987) measured sulfate concentrations ranging from 20 to over 500 μmol.l^{-1} at 7.5-10 cm depth. In the Loosdrecht lakes sulfate concentrations at that depth were always very low (2-11 μmol.l^{-1}) and probably limit sulfate reduction. This agrees well with the observation that sulfate reduction rate is mainly influenced by temperature as at higher temperature diffusion of sulfate into the sediment is accelerated.

Dissolved organic carbon in the pore water reaches very high values (2000

μmol.l^{-1}) and probably consists for a major part of humic acids deriving from the peat. Apparently the concentration of dissolved organic carbon is not influenced by methanogenesis. The seasonal trend of dissolved organic carbon in the pore water with high values in summer, that coincide with high chlorophyll-a concentrations and high pH in the lake water, has not been described before and needs further investigation.

As in several studies on freshwater sediments, phosphate concentrations are demonstrated to be determined by the formation of iron-phosphate authigenic minerals, the occurrence of precipitation reactions has to be considered. In Lake Greifensee (Switzerland) the formation of vivianite was measured (Emerson and Widmer 1978) and also in the hypereutrophic Lake Stone and Lake Charles, the phosphate concentration in the pore water appeared to be iron controlled (Theis and McCabe 1978). In the Loosdrecht lakes DRP concentrations are comparable to those of Theis and McCabe (1978) for Lake Charles (0.3-65 μmol.l^{-1}) but lower than those reported for Lake Greifensee (10-170 μmol.l^{-1} in the top 10 cm, Emerson and Widmer 1978) and for Lake Stone (16-160 μmol.l^{-1}, Theis and McCabe 1978). The iron concentration in the pore water was lower than found for Lake Charles (20-210 μmol.l^{-1}, Theis and McCabe 1978), although the total iron content of the sediment was comparable, 18.6 mg.g^{-1} on a dry weight base for Lake Charles and 15 mg.g^{-1} for the Loosdrecht lakes (Boers et al. 1984). Based on the measured pore water concentrations of Fe^{2+} and DRP and a pH of 7.3, in several columns a slight oversaturation is calculated with respect to vivianite. Probably the high amount of dissolved organic carbon in the pore water acts as a ligand and thus prevents precipitation reactions. Jones, Salonen and De Haan (1988) showed that humic acids can prevent precipitation of iron(III) phosphates to a large extent. This humic acid-iron-phosphate complex is not necessarily measured as dissolved organic phosphorus as the bond might be susceptible to the used acid molybdenum method (Tarapchak et al. 1982, Jones et al. 1988).

To deduce the elemental C:N:P ratio of the decomposing organic material from the pore water concentrations, corrections have to be made for differential diffusion and adsorption (Berner 1977).

$$A = \frac{dCCH_4}{dC_i} * L * \frac{D_{CH_4} * k/w^2+(1+K_{CH_4})}{D_i * k/w^2+(1+K_i)}$$

with: A = average ratio of C:N or C:P in the decomposing organic matter (dimensionless), i = dissolved species i (NH_4^+ ,DRP), C_i = concentration of i (μmol.cm^{-3}), L = number of CH_4 produced for each atom organic carbon oxidized

(L = 0.5, dimensionless), w = sediment burial ($cm.sec^{-1}$), D_i = pore water molecular diffusion coefficient for i ($cm^2.sec^{-1}$), k = first order rate constant for decomposition (sec^{-1}), K_i = adsorption constant for i (dimensionless).
In the Loosdrecht lakes, where decomposition proceeds at a high rate, probably $D_{NH4} * k/w^2 >> 1+K_{NH4}$ and then adsorption of NH_4^+ can be neglected. As in the sediment of the Loosdrecht lakes a linear increase in concentration of NH_4^+, DRP and CH_4 with depth is found, we can directly compare the calculated fluxes (fig. 3.4). This comparison results in a C:N:P ratio of 106:14.1:0.19. Depending on the measure of adsorption of DRP and NH_4^+, lower C:P and C:N ratios would be calculated. The calculated C:N:P ratio deviates significantly from the Redfield ratio of 106:16:1 which is the average composition of algae. Apparently, decomposable organic matter in the sediment is slightly deprived of nitrogen and very low in phosphorus. For accurate calculation of the C:N:P ratio of the decomposing organic matter, the adsorption of phosphates and ammonia in peaty sediments has to be determined.
The results suggest that both calcium and, to a certain extent, iron(II) concentrations are influenced by mineralization as well. As calcium and iron are present in organic matter in only minor amounts, a direct effect of mineralization is not likely. However, methanogenesis and the concomitant production of CO_2 can seriously influence the environmental conditions in the sediment. As a result of CO_2 production and the proton production -due to dissolution of CO_2 in water- a dissolution of calcium- and iron bearing carbonates may occur as pH buffering reactions. The importance of this process has been demonstrated by Matisoff, Fisher and McCall (1981) who worked in batches with Lake Erie sediment. They observed a strong correlation between the production of HCO_3^- and the release of calcium and calculated that for each mole of HCO_3^- produced, 0.6 mole $CaCO_3$ was dissolved.
Average phosphorus release rates were very low and in most cases a phosphorus uptake occurred. This release was not related to any of the pore water characteristics nor to any of the environmental variables. Therefore, it can be doubted if the measured average fluxes are representative for the fluxes occurring in situ. Measurements on phosphorus fluxes from the sediment are often performed in experiments which last several days or even weeks (Kamp-Nielsen 1975, Tessenow 1972, Holdren and Armstrong 1980). However in peaty sediments where phosphorus release is mainly driven by biological reactions, the changes during a long term experiment can be too large to allow for correct release measurements. Because sedimentation stops during an experiment, mineralization and substance fluxs are likely to be underestimated. There is also an indirect effect on the sediment cores, since the absence of an input of organic matter probably leads to a diminished oxygen consumption rate and an

increase in the oxygen penetration depth. An increasing oxygen penetration depth, which can be as little as 1.6 mm in summer in Kievitsbuurt (Sweerts and Cappenberg 1988), is expected to restrain the phosphorus release (Mortimer 1941, 1942, Einsele 1936, Tessenow 1972).

The initial phosphorus release rate was positive in most cases and reached relatively high values of over 1000 $\mu mol.m^{-2}.d^{-1}$, which is more than 30 times higher than the external phosphorus loading (Van Liere 1986). The correlation between the initial phosphorus release and the calculated diffusive DRP flux over the sediment-water interface suggests that indeed the measured phosphorus gradient in the sediment pore water has a predictive value for phosphorus release. The calculated phosphorus flux was of the same order of magnitude as the measured flux. However to allow for reliable predictions, a more precise sampling of the pore water at millimetre intervals is necessary as the concentration gradient over the sediment-water interface generally rose so steeply that our sampling procedure was rather insufficient for accurately determining the slope.

Methane production and ammonium and phosphate fluxes are only slightly stimulated at a high temperature but increased mainly with a decreasing temperature (δTEMP). This response is probably due to an increase in sedimentation rate at the end of the growing season. The sedimentation rate itself can hardly be measured directly in shallow systems.

The correlation between the fluxes of phosphate, ammonium and methane in the anaerobic sediment on the one hand and the phosphate flux over the sediment water interface and measured initial phosphate flux on the other hand, conclusively points to mineralization as driving force for the phosphorus release from peaty sediments. The question whether the high mineralization and phosphorus release by the sediment in autumn concerns only the sedimenting algae -and thus be no serious threat to lake recovery- or involves an enhanced mineralization of the autochtonous peat, needs further investigation.

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Chapter 4

Seasonal variation in sulfate reduction and methanogenesis in peaty sediments of eutrophic Lake Loosdrecht, The Netherlands.

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ABSTRACT

During one year, concentration profiles of sulfate and methane were measured in sediment cores of eutrophic Lake Loosdrecht. Sulfate concentrations decreased exponentially with depth towards a constant threshold value of 7.6 ± 6.1 μM. Concentration profiles were used to calculate fluxes of sulfate and methane and to estimate the anaerobic mineralization rate. Anaerobic mineralization was highest in autumn which was probably due to an increased sedimentation of easily degradable organic carbon. At high rates (> 600 μmol organic carbon.m^{-2}.h^{-1}), sulfate reduction appeared to be limited by sulfate and methanogenesis accounted for over 80 % of the anaerobic mineralization. At low anaerobic mineralization rates, measured in winter and spring, sulfate reduction was predominant.

There was little methanogenesis below 5 cm depth in the sediment which indicated a rapid decrease of degradable organic matter with depth. There was a remarkable difference, especially in winter, between methane fluxes which were measured in batch experiments and those calculated from the concentration profiles in the sediment. These differences may be due to methane diffusing upward from deep layers.

INTRODUCTION

Sulfate reduction and methanogenesis are regarded as alternative degradation reactions that compete for common substrates such as acetate (Winfrey and Zeikus 1977) and hydrogen (Lovley et al. 1982, Robinson and Tiedje 1984). The extent to which sulfate reducers can outcompete methanogens depends on the availability of sulfate. In marine and salt marsh systems with sulfate concentrations higher than 1 mmol.l^{-1}, sulfate reduction accounts for the majority of anaerobic respiration (Capone and Kiene 1988). However, in fresh water systems sulfate concentrations in the overlying water are much lower, typically between 0.01 and 0.2 mmol.l^{-1}, which results in a very steep gradient of sulfate in the sediment (Winfrey and Zeikus 1979, Lovley and Klug 1983, Cappenberg et al. 1984, Adams and Van Eck 1988). As sulfate reduction in freshwater sediments was demonstrated to be sulfate-limited at concentrations below 0.1 mmol.l^{-1} (Ingvorsen et al. 1981, Lovley and Klug 1983), this process is probably limited by the availability of sulfate during most of the year. As a consequence, most of the anaerobic mineralization is channeled via methanogenesis (Kelly et al. 1982, Cappenberg et al. 1984, Adams and Van Eck 1988).

The availability of sedimenting organic matter can be expected be an important factor in determining the pathway of the terminal carbon metabolism, especially in fresh water ecosystems. Most studies on the role of sulfate reducers and methanogens have been carried out in deep (> 10 m) lakes (Winfrey and Zeikus 1977, 1979, Lovley et al. 1982, Lovley and Klug 1983, Cappenberg et al. 1984,

Kuivila et al. 1989) in which most of the organic matter from the primary production probably is degraded in the water column. However, in shallow lakes, processes in the water and in the sediment may interfere more intensely. So, in shallow eutrophic lakes, the large seasonal fluctuations in primary production and temperature may affect the role of sulfate reduction and methanogenesis in the terminal carbon flow during the year. The present study was carried out to elucidate the seasonal and spatial variation in the importance of sulfate reduction versus methanogenesis in peaty sediments in a shallow eutrophic lake.

METHODS

Sampling.

The research was carried out in eutrophic Lake Loosdrecht. This lake was made artificially by peat mining in the 17th century when the 7000 years old Sphagnum peat was excavated, dried and used as fuel (Van Liere 1986). Primary production in the lake, which is 30-45 mol carbon.m^{-2}.y^{-1} on a yearly basis, sometimes exceeds 400 mmol carbon.m^{-2}.d^{-1} during summertime (Van Liere et al. 1986). As a result of the morphometry (area = 14.5 km^2, mean depth = 1.8 m) and continuous wind-induced turbulence, the surface layer of the sediment remains aerobic throughout the year. The oxygen penetration depth into the sediment is as shallow as 1.6 mm in summer and increases to 5.4 mm in winter (Sweerts and Cappenberg 1988). The sediment pore water is slightly alkaline. The pH decreases from pH = 8.3 in the surface layer to pH = 7.3 at greater depths.

Sediment cores were obtained using a hand-driven stainless steel corer containing a 40 cm long inner core of PMMA (polymethylmetacrylate) with an internal diameter of 5 cm. The sharpened edges of the inner core extended well below the metal parts to minimize disturbance of the sediment. Samples were collected in 1988 every fourth week at two representative locations. One sampling station is situated in the open lake and the other, Kievitsarea, is more or less sheltered from wind influence. The sediment at both stations consists of relatively loose organic debris and the organic matter content is very high, 55% - 65 % on a dry weight basis (chapter 3). Temperature was measured just above the sediment surface. Chlorophyll-a and primary production in the lake water were measured by the routine programme of the "Water Quality Research Loosdrecht Lakes" (Van Liere 1986 and Van Liere et al. 1986).

All sediment cores were placed in a nitrogen-filled glove box (Coy Laboratory Products Inc. Michigan). Two duplicate cores were used for pore water extraction. Half or 1.0 cm thick layers were scooped off and interstitial water was pressed out over 0.45 μm filters using a Reeburgh type squeezer (Reeburgh 1967) at a maximal N_2 pressure of 10^5 Pascal. Two cores were sliced at one cm intervals for methane measurements and subsamples were put in 30 ml serum bottles immediately.

During processing of the sediment cores some methane-loss occurred. Time series extracting subsamples from one layer every 10 seconds, demonstrated that the methane-loss during handling was approximatly 10 % and data were adjusted for these losses. Methane was measured in the headspace after vigorous shaking. The bottles containing sediment used for methane measurements were incubated in dark at in situ temperature. From the third week on, the accumulation of methane in the headspace was measured every second week for a period of 6-8 weeks. The detection limit for methane production measured this way was 1 $nmol.g^{-1}.d^{-1}$.

Analytical procedures.
Sulfate was measured with HPLC (Hordijk et al. 1985). Methane was measured using a Packard model 428 gas chromatograph equiped with a Porapak Q column and flame ionization detector (at 160 °C). The carrier gas was helium at a flow rate of 20 $ml.min^{-1}$.

Diagenetic model.
The theoretical model developed by Berner (1964 1980) is applied to describe both sulfate reduction and methanogenesis in the sediment. The model descibes changes in concentration of sulfate and methane with time as a function of diffusion, sedimentation and sulfate reduction or methanogenesis:

$$\delta C/\delta t = D_s \delta^2 C/\delta z^2 - w\delta C/\delta z - f(x)$$

with C: concentration of sulfate or methane at z cm depth ($nmol.cm^{-3}$), D_s: the whole sediment diffusion coefficient ($cm^2.s^{-1}$), w: sedimentation rate ($cm.s^{-1}$) and f(x) = sulfate reduction rate or methane production rate ($nmol.cm^{-3}.s^{-1}$).
The measured concentration profiles were fitted according to:

$$C = (C_o - C_\infty) \exp(a\ z) + C_\infty$$

with z: depth (cm), C_o: concentration sulfate or methane in overlying water ($nmol.cm^{-3}$), C_∞: concentration sulfate or methane at infinite depth ($nmol.cm^{-3}$) a: attenuation constant (cm^{-1}). The attenuation constant is defined as the ratio of the first order reaction rate constant for sulfate reduction or methanogenesis and the net sedimentation (Berner 1980). The measurements of duplicate cores were pooled. C_o, C_∞ and a could be estimated by fitting of the model to the data by a least squared residual minimization routine. For these calculations BMDPAR (BMDP Statistical Software inc. Los Angeles, USA) was used (Ralston 1988). The diffusive fluxes of SO_4^{2-} into the sediment and CH_4 towards the sediment surface, were calculated according to Fick's first law of diffusion using the tangents to the fitted

equations of the concentration gradients at the sediment-water interface:

$$J = \phi/\Theta^2 * D_s * \delta C/\delta z$$

with J: flux of sulfate or methane (nmol.cm^{-2}.sec^{-1}), D_s: whole sediment diffusion coefficient for either sulfate or methane (cm^2.sec^{-1}), ϕ: porosity (dimensionless), Θ: tortuosity (dimensionless) and $\delta C/\delta z$ = concentration gradient of either sulfate or methane (nmol.cm^{-4}). D_s were calculated from the molecular diffusion coefficients (D_o sulfate: 8.9 x 10^{-6} cm^2.sec^{-1}, at 18 °C, Li and Gregory 1974, D_o methane: 15.3 x 10^{-6} cm^2.sec^{-1}, at 15 °C, Broecker and Peng 1974) that were corrected for in situ temperature and sediment characteristics. Porosity of the sediment was taken as 0.95 and the square of tortuosity as 1.40 (J.-P. Sweerts pers.comm.).
The relative importance of sulfate reduction and methanogenesis in the anaerobic mineralization was determined on the basis of the calculated fluxes. The total anaerobic mineralization was approximated as twice the sum of sulfate reduction and methanogenesis.

Statistical analyses.
A recent version of CANOCO (Ter Braak 1987a) was used to analyse data with Redundancy Analysis (RDA). RDA (Rao 1964) is a technique which relates a set of multivariate data to explanatory variables. RDA combines the features of Principal Component Analysis, summarizing multivariate data, and multiple regression analysis, relating data (fluxes and rates) to environmental variables. RDA selects successive linear combinations of environmental variables that give the smallest residual sum of squares. The environmental variables used in the analysis were temperature (TEMP), chlorophyll-a concentration and primary production rate in the lake water (CHLA, PROD) and the overall change in temperature and primary production rate between two successive sampling dates (δTEMP, δPROD). Environmental variables were selected stepwise, maximizing the explained sum of squares with the minimum number of environmental variables. The results are presented as a correlation biplot (Gabriel 1971, Ter Braak 1987b). In a biplot the the length and the cosine of the angle between vectors of two variables is an indication of the correlation between them. The variables which were analysed were: total anaerobic mineralization rate (TOTAL), methane flux and sulfate reduction rate calulated from the concentration profiles (CH_4, SO_4^{2-}), the relative importance of sulfate reduction expressed as percentage of the total anaerobic mineralization (%SO_4) and the methane production in batches (BATCH). To get an overall insight in the respons of the sediment, the data sets of the two sampling points were pooled. To compensate for the differences between the two sampling stations, the location itself was introduced as a 0/1 explanatory variable.

RESULTS

Sulfate reduction and methanogenesis in the sediment

Sulfate concentrations decreased exponentially with depth in the top 3 or 4 cm and remained at a constant low level ($SO_4\infty$) at greater depths (fig. 4.1A). The value for $CSO_4\infty$ fluctuated between 0 and 17.7 μM with an average value of 7.6 ± 6.1 μM. During most of the year the $CSO_4\infty$ was reached within 4 cm from the sediment-water interface. However, in winter and early spring sulfate penetrated as deep as 8 cm in some cases. Concentration profiles of sulfate could be well described by the models which were used, p being in most cases < 0.01 (table 4.1). The value for the attenuation constant for sulfate reduction showed large seasonal variations with high values in autumn and ranged from 0.07 to 2.88 cm^{-1}. Methane concentration increased with depth towards a constant level (fig. 4.1B). The average value of the methane concentrations at the sediment-water interface (CCH_4o) did not deviate significantly from zero, and calculations were repeated with $CCH_4o = 0$. The measured methane concentrations could be described reasonably by the model except for cores with low values for a, especially in winter and early spring. In these cores methane concentrations increased linearly with depth and could only be fitted when $CCH_4\infty$ was allowed to increase to extreme oversaturating values (table 4.1). The values for the attenuation constant for the methane profiles ranged from 0.01 to 0.85 cm^{-1} and were always lower than the values for the sulfate profiles.

Sulfate reduction rates ranged from 3.2 to 44.6 $\mu mol.m^{-2}.h^{-1}$ and were somewhat lower in winter and early spring. During the rest of the year, the sulfate reduction rates were relatively constant between 20-40 $\mu mol.m^{-2}.h^{-1}$. The calculated CH_4 flux towards the surface layer showed large variations and ranged from 5.5 to 342 $\mu mol.m^{-2}.h^{-1}$. In the open lake station a clear seasonal trend in the methane fluxes could be seen with high values in autumn and low values in winter and early spring (fig. 4.2). Values for Kievitsarea followed the same pattern (not shown).

The total anaerobic mineralization of organic carbon ranged from 0.7 $mol.m^{-2}.y^{-1}$ in the open lake to 1.2 $mol.m^{-2}.y^{-1}$ at Kievitsarea. At high total anaerobic mineralization rates, the contribution of methanogenesis became increasingly important (fig. 4.3). In autumn when anaerobic mineralization was at a maximum of 750 $\mu mol.m^{-2}.h^{-1}$, methanogenesis accounted for approximatly 80 % of the total anaerobic mineralization. Sulfate reduction rates levelled off at a constant rate of 40 $\mu mol.m^{-2}.h^{-1}$ (fig. 4.3). During this period the primary production rates decreased from 200 to 18 mmol carbon.$m^{-2}.d^{-1}$ (table 4.1). The chlorophyll-a content of the water also showed a decrease from 156 to 90 $\mu g.l^{-1}$ (table 4.1).

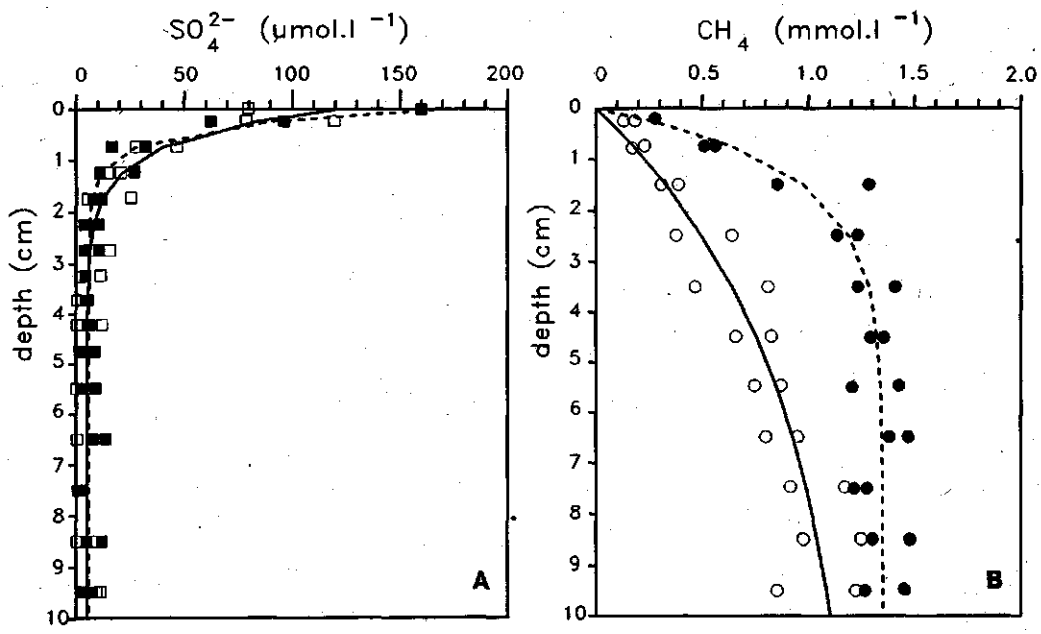

Figure 4.1: Concentration profiles of sulfate (A) and methane (B) in sediment cores of station Kievitsarea in September (closed symbols) and in February (open symbols). Lines represent fitted values (September - - - - -, February ————)

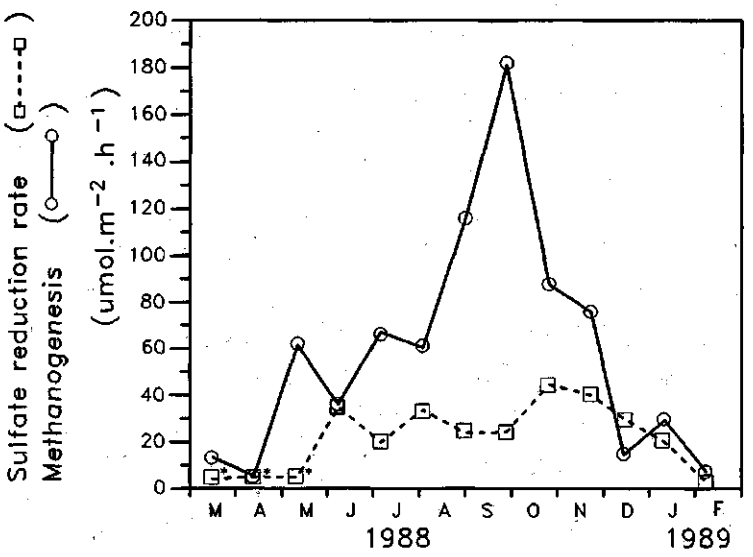

Figure 4.2: Seasonal variation in the open lake in sulfate reduction rate (□) and methanogenesis (○) calculated from the fitted concentration profiles. *represent unreliable results.

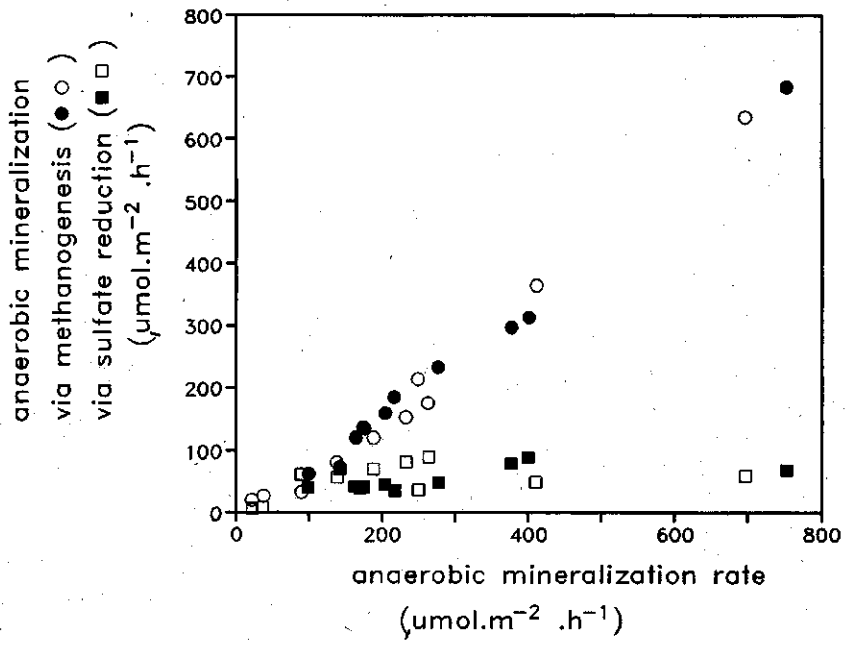

Figure 4.3: Sulfate reduction rates (□, ■) and methane fluxes (○, ●) in Lake Loosdrecht as a function of the total anaerobic mineralization. (open symbols: open lake, closed symbols: Kievitsarea).

Table 4.1: Temperature, chlorophyll-a and primary production rate in the water and fitted values for the attenuation constant (a) and concentrations at infinite depth (C∞) for the sulfate and methane concentration profiles in the open lake and Kievitsarea for each sampling date.

sampling	temp	chl-a	PP	OPEN LAKE				KIEVITS AREA			
				SO_4^{2-} profiles		CH_4 profiles		SO_4^{2-} profiles		CH_4 profiles	
date	°C	μg.l⁻¹	mmol.m⁻² d⁻¹	a	$CSO_4\infty$	a	$CCH_4\infty$	a	$CSO_4\infty$	a	$CCH_4\infty$
03.15.88	6	93	35	0.24	0.***	0.01	#4.20***	1.86	22.1***	0.19	1.23***
04.12.88	10	93	68	N.D.	N.D.	0.01	1.42**	0.07	0.	0.01	1.42***
05.10.88	16	63	98	0.93	5.3	0.24	0.69***	1.32	5.2***	N.D.	N.D.
06.07.88	16	156	124	0.61	0.**	0.04	#2.71***	1.75	17.8***	0.03	#3.53***
07.05.88	16	113	180	0.88	10.8***	0.30	0.62***	0.88	7.9**	0.08	#2.11**
08.02.88	19	156	200	1.10	17.9**	0.31	0.50***	0.72	0.7***	0.34	0.62**
08.30.88	18	108	172	0.83	5.7***	0.77	0.41**	0.95	4.7***	0.34	0.79**
09.27.88	14	98	88	1.38	8.0***	0.62	0.88***	1.08	6.1***	0.50	0.93**
10.25.88	12	103	50	2.88	6.3***	0.24	1.11***	2.71	5.8***	0.85	1.35**
11.22.88	5	92	24	2.45	17.7***	0.42	0.69***	2.37	14.2***	0.47	1.21**
12.13.88	5	90	18	1.70	7.9***	0.01	5.54***	1.88	5.6**	0.02	#6.04**
01.10.89	6	117	43	1.08	4.9***	0.05	2.11***	1.60	4.9***	0.20	1.28**

PP primary production, **p < 0.1, ***p < 0.01, #extreme oversaturation, N.D. not determined

Methane production in laboratory experiments.

The methane production rate in batches was linear with time. The production rate in the sediment decreased exponentially with depth. The highest values were measured in August (120 nmol.g^{-1}.d^{-1}). Production rates were high in late summer and at or below detection limit in winter (fig. 4.4). The methane production rate in batches was integrated over depth (table 4.2). The 0-0.5 cm layer was excluded from the integration to allow for a better comparison with the calculated fluxes. In situ, oxygen and sulfate are present in the surface layer and probably reduce methanogenesis strongly. The integrated methane production was dependent on temperature ($p<0.01$) with an average Q_{10} value in the range from 10 - 20 °C of 2.4. In general the integrated methane production in batches was lower than calculated fluxes (tabel 4.2). Especially at temperatures below 10 °C hardly any significant production was measured in batches while the fluxes that were calculated from the concentration gradients ranged from 5.5 - 91.6 μmol.m^{-2}.h^{-1}.

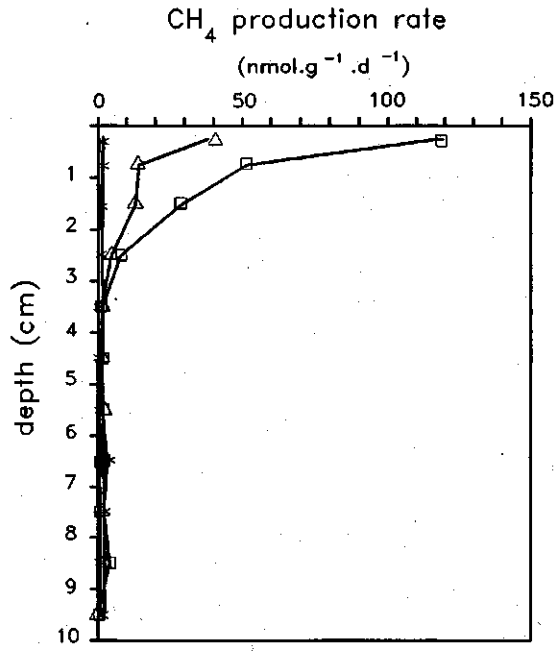

Figure 4.4: Methane production in batches of different sediment layers of sediment columns sampled in August (□), September (Δ), and December (*) in the open lake.

Table 4.2: Methane fluxes (μmol.m^{-2}.h^{-1}) towards the sediment-water interface, in situ values calculated from concentration profiles and integrated batch values, for the open lake and Kievitsarea for each sampling date.

sampling date	OPEN LAKE		KIEVITS AREA	
	in situ	batch	in situ	batch
03.15.88	13.3	1.0	64.6	4.0
04.12.88	N.D.	N.D.	5.5	13.5
05.10.88	61.6	16.4	N.D.	115
06.07.88	35.9	65.1	40.3	59.0
08.02.88	67.2	6.6	60.5	37.8
08.30.88	60.2	28.1	106	118
09.27.88	115	54.7	79	165
10.25.88	181	30.9	155	168
11.22.88	87.5	63	342	91.7
12.13.88	76.0	B.D.	149	B.D.

Statistical Analysis.

The independent variables which sequentially entered the redundancy analysis and the proportion of variance in the dependent variables which additionally could be explained are listed in table 4.3. The value of 0.21 for TEMP for additional variance explained, implies that 21 % of the variance in the complete data-set can be explained by temperature. The total percentage of variance explained was 54%. The major fraction of the total explained variance was described by the first two axes (35.7 % on the first and 8.2 % on the second axis). The residual fraction of 9.1 % was described by axes with a higher dimension. Addition of the primary production rate and chlorophyll-a content as explaining variables to the RDA gave no significant improvement of the explained variance. This is due to the high correlation of the primary production rate and chlorophyll-a content of the water with temperature (tabel 4.4).

Table 4.3: Sequence and additional proportion of variance explained of the environmental variables used in the Redundancy Analysis.

	VARIANCE EXPLAINED	
	additional	cumulative
temperature (TEMP)	0.21	0.21
change in primary production rate (δPROD)	0.15	0.36
station (OPEN LAKE vs. KIEVITS AREA)	0.10	0.46
all other variables (none significant)	0.08	0.54

The introduction of the change in primary production rate (δPROD) added 0.15 to the explained variance. δPROD is not correlated with temperature and in the correlation biplot the vectors of TEMP and δTEMP describe almost a 90° angle (fig. 4.5).

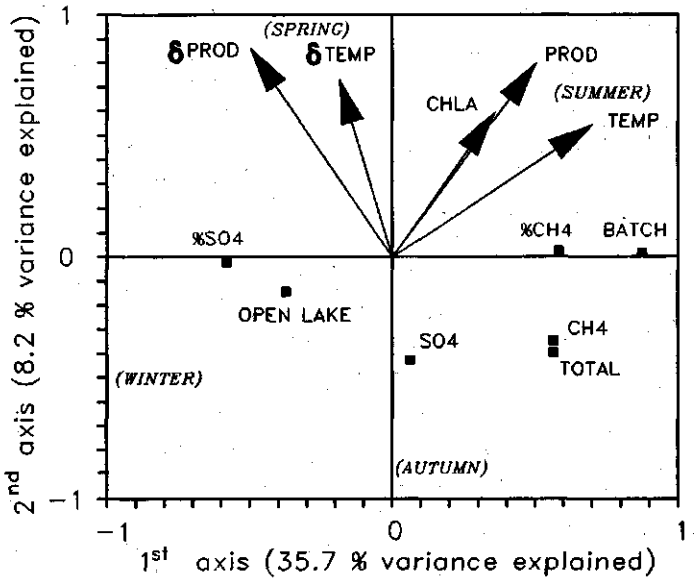

Figure 4.5: Correlation biplot of dependant variables. Environmental variables are represented by arrows (see Table 4.3). An approximate indication of the seasons is given in brackets.

The correlation biplot points out that the methane flux towards the sediment-water interface increased with a decrease in primary production rate and a decrease in temperature as can be seen from the opposite direction of the vectors for δPROD and δTEMP versus CH_4. Although temperature is an important variable in explaining the complete dataset, it contributed only slightly to the explained variance of the calculated in situ methane flux (≈ 60° angle between CH_4 and TEMP). Methane production in batches reacted more intensely on temperature. As the total anaerobic mineralization (TOTAL) is strongly determined by CH_4, these vectors are positioned closely together. Sulfate reduction was low in spring as can be inferred from the opposite direction of SO_4 and δTEMP and which is in accordance with fig. 4.2. The contribution of sulfate reduction to the total anaerobic mineralization (%SO4) increased at low temperatures and tended to be higher at positive δPROD in spring. Sulfate reduction appears to be more important in the open lake than at the Kievitsarea.

Table 4.4: Correlation matrix of environmental variables measured in Lake Loosdrecht; TEMP is temperature, CHLA is the chlorophyll-a concentration, PROD is the primary production rate, δPROD and δTEMP are the overall change in temperature and primary production between two successive sampling dates.

	TEMP	CHLA	PROD	δTEMP	δPROD
TEMP	-	0.56*	0.92***	0.35	0.09
CHLA		-	0.61*	0.22	0.32
PROD			-	0.39	0.32
δTEMP				-	0.68**

n=13, *p<0.05, **p<0.01, ***p<0.001

DISCUSSION

Calculated in situ fluxes of sulfate and methane.

The anaerobic mineralization rate in Lake Loosdrecht, was highest in autumn. Probably this can be ascribed to an increase in sedimenation rate at the end of the growing season. However in a shallow holomictic lake, it is difficult to distinguish net deposition from total deposition due to wind and wave influences (Håkanson and Jansson 1983). Changes in chlorophyll-a and primary production rate in the water might provide a rough indication of the sedimentation of easily degradable organic matter, since a decrease in chlorophyll-a and primary production rate is partly caused by decay and sedimentation of algae. The inverse relation between the decrease in primary production rate in the lake and the total anaerobic mineralization suggests, as expected, sedimentation as a driving force for the anaerobic mineralization rate. The impact of sedimentation in autumn is emphasized by the correlations of fluxes of methane, phosphates and ammonium in the anaerobic sediment which were reported earlier (chapter 3). On a yearly basis only 1.5-4 % of the primary production in the lake (30-45 mol organic carbon.m^{-2}.y^{-1}, Van Liere 1986), is channeled via anaerobic processes. A comparable low value (3 %) has been found for shallow Lake De Grote Rug which has a primary production of 25 mol.m^{-2}.y^{-1} (Adams and Van Eck 1988). Temperature which is often of major importance in determining the anaerobic mineralization rate in deep systems (Klump and Martens 1989, Kelly and Chynoweth 1981) appears to play only a minor role in shallow Lake Loosdrecht.

There was a large seasonal variation in the relative importance of sulfate reduction and methanogenesis. The relative importance of sulfate reduction in the anaerobic mineralization varied during the year and appeared to be highest in winter and early spring when the total anaerobic mineralization rates were low, 20-100 μmol carbon.m^{-2}.h^{-1}. During this period sulfate reduction was dominant in the bulk of the sediment and penetrated as deep as 8 cm in some cores. Sulfate reduction appared

to outcompete methanogenesis, which was very low, succesfully. In autumn, at high total anaerobic mineralization rates of 600 μmol organic carbon.$m^{-2}.h^{-1}$, methanogenesis accounted for over 80 % of the anaerobic mineralization. Sulfate reduction levelled off at 40 μmol.$m^{-2}.h^{-1}$ indicating an overall sulfate limitation. Diffusion of sulfate from the overlying water probably could not keep up with the sulfate reduction rate which was also indicated by the sulfate penetration depth which extended no further than 3.5 cm. The results suggest that in the sediment of this shallow eutrophic lake, the anaerobic mineralization is determined by a seasonal shift from a limitation by sulfate in summer and autumn towards a limitation by easily degradable organic matter in winter and early spring. In deep lakes the relative importance of sulfate reduction seems to be less liable to seasonal variation and can often be quantified as a percentage of total anaerobic mineralization as for instance 13 % in Wintergreen Lake (Lovley et al 1982) and 37 % in Lake Washington (Kuivila et al 1989).

At greater depths in the sediment an average $CSO_4\infty$ concentration of sulfate of 7.6 ± 6.1 μM was found. This threshold concentration below which no sulfate reduction occured, is in the same range as values reported for Lawrence Lake (30-40 μM, Lovley and Klug 1983), Lake Washington (18 μM, Kuivila et al. 1989) and Lake Gerritsfles (0-20 μM, Feijtel et al. 1989). Pooling all profiles from summer and winter, Feijtel et al. (1989) calculated a higher average threshold in summer (10 μM) than in winter (4 μM). In Lake Loosdrecht no such temperature effect could be detected but the threshold concentration tended to decrease with decreasing values for the attenuation constant for sulfate reduction. So, generally, at low sulfate reduction rates low threshold values were calculated. The relation between threshold concentration and sulfate reduction rate has hardly been investigated for field situations but might be influenced by changing physiological conditions of the sulfate reducing population. Ingvorsen et al. (1984) reported that the sulfate threshold concentration of cell suspensions of chemostat cultures of Desulfobacter postgatei decreased after prolonged energy starvation.

The values for $CCH_4\infty$ were in most cores below the saturation values for methane in water. This same phenomenon was also observed for this sediment by Sweerts et al (in press). During most of the year the CCH_{4o} did not deviate significantly from zero, this can be caused by the rapid diffusion into the overlying water or by the oxidation of methane in the aerobic surface layer of the sediment. Sweerts et al. (in press) could not detect any methane release from sediment cores of Lake Loosdrecht which were incubated under standardized conditions, suggesting that the entire methane flux from the anaerobic sediment was oxidized in the small surface layer. Based on the oxygen consumption rates determined by Sweerts and coworkers (1988, in press), the oxygen consumption rate for our sediment cores sampled in October is estimated at 0.67 mmol.$m^{-2}.h^{-1}$. Assuming a stoichiometry

of 1.5 moles O_2 per mole CH_4 (Joergensen and Deng 1983), the complete oxidation of all methane produced (0.34 mmol.m^{-2}.h^{-1}) would account for 76 % of the total oxygen consumption in the sediment. The rest of the oxygen consumption can be ascribed to aerobic mineralization and, to a lesser extent, to nitrification and the oxidation of iron and sulfide. Hardly any results are reported in literature on the seasonal competition between all these oxygen consuming processes.

Calculated methane production versus methane production in batches.
The methane production measured in batches depicted an exponential decrease with depth. The major part of the methane was produced in the top layers (0 - 4 cm) of the sediment just as found by Kelly and Chynoweth (1981) for Frain's Lake and Third Sister Lake. At greater depths in the sediment extremely low rates of methanogenesis (< 1 nmol.g^{-1}.d^{-1} below 5 cm depth) were determined. This steep exponential decrease in methane production could be caused by a limitation of degradable organic material thus suggesting a low net sedimentation rate and a high breakdown of organic matter. Loss of methane due to anaerobic methane oxidation is probably only a minor fraction of all methane produced (Zehnder and Brock 1980).
The integrated methane production rates measured in batches and those calculated from the profiles showed large differences, especially in winter and early spring. The methane production rates which were calculated using the concentration profiles, were usually higher that the batch values. Apparently, the escape of methane from the sediment by bubbles which was reported to be important in Cape Lookout Bight (Martens and Klump 1984), is only of minor importance in Lake Loosdrecht. Recent measurements during a warm (26 °C) summer's day did not indicate any methane above the sediment-surface (unpublished results). During integration of the methane production in batches the toplayer of the sediment was left out. This might have resulted in an underestimation of methanogenesis if methanogenesis and sulfate ruduction occured simultaneously in the toplayer. Winfrey and Zeikus (1977) reported that addition of non-limiting concentrations of acetate reversed the inhibition of methanogenesis by sulfate. So, especially in autumn at a high supply of easily degradable organic matter, the co-occurance of sulfate reduction and methanogenesis is likely to occur in the top layer of the sediment. This could contribute to the observed discrepancy between batch and profile measurements in autumn. The windsheltered situation of Kievitsarea where a more pronounced sedimentation is expected, might contribute to the discrepancy. In winter hardly any methane production could be detected in batches though the calculations from the concentration profiles indicated methane fluxes of 5.5 - 91.6 μmol.m^{-2}.h^{-1}. We suggest that in winter hardly any methane production occurs and that the calculated methane flux in the sediment cores originates from a methane

pool at greater depth in the sediment. Upward diffusion of fossil methane from older geological layers has been described for Lake Lungern by Bossard and Gächter (1981). Comparing methane fluxes in hydroxide poisoned and non-poisoned bell-jars, these authors concluded that 41.7 $\mu mol.m^{-2}.h^{-1}$ originated from deeper layers. ^{14}C radiocarbon measurements confirmed that the methane was from older deposits (Bossard and Gächter 1981).

Diagenetic model.

Berner's diagenetic model is generally used to describe sulfate and carbon dioxide profiles in marine or brackish sediments where sulfate reduction prevails (Berner 1980, Klump and Martens 1989). In Lake Loosdrecht the model was also applied to describe methane profiles but appeared to be reliable only when methanogenesis was predominant in the anaerobic mineralization process. In winter the measured methane flux resulted mainly from upward diffusion and the model could not be applied.

The values for the attenuation constant for sulfate reduction with an average of 1.32 ± 0.75 cm^{-1} and a maximal value of 2.88 cm^{-1}, are much higher than the values found for Cape Lookout Bight (0.54 cm^{-1}, Klump and Martens 1989) and Lake Gerritsfles (0.33-0.46 cm^{-1}, Feijtel et al. 1989). High values for the attenuation constant, a, can be caused by high first order constants for the degradation of organic matter, by a low sedimentation rate or by a combination of both. As the highest values for a were calculated in autumn when sedimentation can be expected, we suggest that variations are mainly due to changes in the rate constant caused by the supply of easily degradable organic matter. As the sedimentation rate is independant of depth (Berner 1980), the difference between the attenuation constants for sulfate reduction and methanogenesis, can be ascribed exclusively to differences in the first order constants for the degradation of organic matter. Our results from the batch experiments indicate that the amount of degradable organic matter decreases exponentially with depth which can be an explanation for the observed lower values of the attenuation constant for methanogenesis. These results are conform the diagenetic model, which also predicts an exponential decrease of degradable organic matter with depth (Berner 1980). In earlier observations on phosphatase activity and chlorophyll-a content in the sediment of Lake Loosdrecht, an exponential decrease with depth of both parameters was reported and suggested to be a reflection of the amount of degradable organic matter (Sinke et al. 1991).

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Chapter 5

A new method to determine the contribution of bacteria to the phosphate uptake by aerobic freshwater sediment.

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ABSTRACT

An extraction method is proposed to determine the contribution of bacterial processes to the phosphate uptake of aerobic freshwater sediment. The method was tested on iron hydroxyphosphate that was either synthesized or formed under in situ conditions, and a pure culture of Acinetobacter 210 A. Using a mild acid extraction we could distinguish between chemical and biological phosphate uptake. This method allowed the solubilization of the entire iron hydroxyphosphate fraction but did not extract bacterial phosphate.

The new method was applied to determine the contribution of bacterial processes to the phosphate uptake of the surface sediment in eutrophic Lake Loosdrecht. Phosphate uptake of randomly sampled surface layers of the sediment was considerable and ranged from 12 to 138 μmol.g^{-1} on a dry weight basis. Phosphate uptake was correlated positively with the amount of extractable iron and phosphate and negatively with dry weight. The contribution of bacterial processes ranged from 12 to 32 %. Addition of an easily degradable substrate, such as acetate, to the sediment stimulated the uptake of phosphate and augmented the biologically bound phosphate fraction.

INTRODUCTION

Sediments of aquatic ecosystems play an important role in controlling the phosphorus content of the water column. Over an annual cycle sediments act as a net sink for phosphorus due to sedimentation of particulate matter (Boström et al 1982). After the application of restoration measures that lead to a decrease in the phosphate concentration in the water, the sediment may act as a transient source of phosphate, thus retarding the improvement of the water quality. The flux of phosphate across the sediment-water interface depends on physical factors as temperature (Holdren and Armstrong 1980, Kamp-Nielsen 1975, Chapter 2) and resuspension (Boström et al 1982, Søndergaard in press), on chemical factors as redox potential (Mortimer 1941, 1942) and pH (Andersen 1975), and on biological factors as bioturbation (Holdren and Armstrong 1980). Generally, a decrease in redox potential is considered to be the most important stimulus for phosphate release from sediments (Mortimer 1941, 1942). Under aerobic conditions phosphate adsorbs to iron(III) to form solid iron hydroxide-phosphate complexes. When the redox potential decreases, iron is reduced and phosphate is released from the complex. According to this classical model, microorganisms only influence the phosphorus cycle indirectly by affecting redox conditions. Recently it has been suggested that bacteria also play a direct role in uptake and release of phosphate by sediments (Fleischer 1983, Gächter et al 1988). Gächter and coworkers suggested that the redox dependent phosphate exchange in eutrophic Lake Sempach could be attributed partly to physiological changes of the bacterial

population. They speculated that processes in sediment could be similar to those occuring in activated sludge type sewage treatment plants designed for biological phosphate removal. In these plants phosphate is taken up during the oxic period and is subsequently released in the absence of oxygen (Wentzel et al 1985). Acinetobacter appeared to be the dominant genus storing phosphates intracellulary as polyphosphates (Fuhs and Chen 1975). Although results reported by Fleischer (1983) and Gächter and coworkers (1988) indicated active mediation by bacteria in the phosphate flux over the sediment-water interface, neither could quantify the importance of bacterial uptake compared to chemical adsorption because methods were lacking to measure the reversibly immobilized bacterial phosphorus.
To unravel the chemical speciation of phosphate in sediments several multistep extraction schemes have been proposed. Theoretically these extractions remove sequentially loosely sorbed phosphate, iron- and aluminum bound phosphate and calcium bound phosphate (Hieltjes and Lijklema 1980, Psenner et al 1984). However, the extraction of bacterial phosphate has not been considered in these schemes. The objective of the present study was to develop a method to quantify the contribution of bacteria to the phosphate uptake by aerobic sediment. We used a mild acid extraction to distinguish between aerobic phosphate uptake by bacteria and adsorption onto iron complexes. During the extraction biota remained intact and no phosphate was extracted from a pure culture of Acinetobacter, able to accumulate polyphosphate, while phosphate adsorbed onto iron hydroxide complexes was dissolved almost completely.

MATERIALS AND METHODS

This research was performed in Lake Loosdrecht which is a shallow (mean depth 1.8 m) eutrophic lake with an area of 14.5 km^2. The lake was created by peat excavation in the 17th century (Van Liere 1986) and the sediment consists of loose organic debris with an extremely high organic matter content of 55-65 % (chapter 3). As the lake receives water from the surrounding polders with a high alkalinity the sediment contains 3-6 % $CaCO_3$ (Keizer and Sinke 1992) and the pH of the interstitial water decreases from pH 8.3 in the surface layer to 7.5 at greater depth. Due to the continuous wind induced turbulence of the water, the sediment surface remains aerobic throughout the year with oxygen penetration depths of 1.6 mm in summer and 5.4 mm in winter (Sweerts and Cappenberg 1988).
Sediment columns were collected using a handdriven corer device (inner core 40 cm long, internal diameter 5 cm) in July and August 1991 at randomly choosen locations. One cm thick surface layers were sliced off just after sampling and kept refrigerated untill use. The surface sediment of each location was well mixed with a spoon and subsamples were taken for measurements of dry weight (105 °C, 24 h) and organic matter content (determined as loss on ignition 550 °C, 2h).

Phosphate uptake by surface sediment was determined using slurries of (1 to 100 w/w, wet sediment) in artificial lake water (ALW: 1 mM Ca^{2+}, 2 mM Na^{+}, 0.5 mM NH_4^{+}, 0.2 mM Mg^{2+}, 2.4 mM HCO_3^{-}, 2.1 mM Cl^{-}, 0.2 mM SO_4^{2-}, pH 8.3) enriched with 0.065 mM KH_2PO_4 (ALW + P). To ensure the maintenance of oxic conditions ALW was bubbled with air for two hours to ensure oxygen saturation. We used 500 ml polyethylene bottles that were opened daily for aeration of the headspace and were incubated at 22 ± 3 °C on an orbital shaker (150 rpm) in dark. Two series of controls were run, one with ALW + P without sediment and another with sediment in ALW without phosphate. Subsamples of the sediment slurries were taken at regular time intervals to determine the concentrations of dissolved and extractable phosphate and iron.

To investigate the chemical and biological phosphate uptake, we conducted experiments with iron hydroxide and a pure culture of Acinetobacter strain 210 A. Synthetic iron hydroxide was prepared by titrating 100 ml of $FeCl_3$ (0.2 M) in two hours to pH 7 with NaOH (0.6 M) while stirring continuously (Keizer and Sinke 1992). After a 1:10 dilution with demineralized water, a standard series of iron hydroxide in ALW + P was prepared within 15 minutes after synthesis. To investigate the effect of organic matter on the phosphate adsorption by iron hydroxide, a comparable standard series of synthetic iron hydroxide was added to sediment slurries in ALW + P. To compare the reactivity of synthetic iron hydroxide with in situ formed hydroxide, we carried out experiments with $FeCl_2$ (0.01 M). A standard series of $FeCl_2$ in sediment slurries in ALW + P was prepared, assuming that addition of iron(II) to oxidized sediment results in the formation of iron(III) complexes. Dissolved and extractable phosphate and iron were followed. To determine the final concentration of iron in the prepared iron hydroxide, a subsample was acidified to pH 1 with 3M H_2SO_4 and destructed with peroxidisulfate at 125 °C for 2 h.

Acinetobacter 210 A, isolated from sludge by Deinema et al 1985, is known to acculumate large amounts of polyphosphates. Strain 210 A was cultured on ALW + P to which sodiumacetate (2 mM) was added as a carbon source and 1 $ml.l^{-1}$ of a trace mineral solution (H_3BO_3 9.1 mM, $ZnSO_4.7H_2O$ 1.54 mM, $CoCl_2.6H_2O$ 0.85 mM, $MnCl_2.4H_2O$ 0.46 mM, $Na_2MoO_4.2H_2O$ 0.12 mM, $CuSO_4.5H_2O$ 0.08 mM). The medium was sterilized for 30 minutes at 120 °C under a CO_2 atmosphere to prevent precipitation of calciumcarbonate. The culture was incubated at 22 °C on a rotary shaker. Sterile subsamples were taken for measurement of dissolved and extractable phosphate and iron. At the end of the experiment bacterial dry weight was determined by centrifuging (10 min. 15,000 rmp) 160 ml sample of the culture, washing it with demineralized water, and centrifuging it for the second time (Deinema et al 1985). The pellet was dried at 95 °C for 24 h.

To determine concentrations of dissolved and extractable phosphate and iron 15

ml subsamples were taken at regular time intervals. 5 ml sample was filtered over 0.45 μm filters (Me mixed esters, Schleicher and Schuell, Dassel, Germany) that were prewashed with demineralized water. After filtration the samples were diluted 1:1 with 0.24 M H_2SO_4 and kept refrigerated at 4°C. 10 ml sample was used for extraction of acid soluble phosphate compounds. Extraction was carried out by diluting 1:1 with H_2SO_4 (0.24 M) and by vigourously shaking for 24 hours at 22°C. After the extraction, samples were filtered over 0.45 μm filters that were prewashed with 0.12 M H_2SO_4.

To compare the effects of the different treatments on the phosphate uptake, we calculated the recovery of phosphate originally added as ALW + P. In case of the sediment slurries a correction was made for the amount of phosphate extracted from the control slurry in ALW without phosphate.

Soluble reactive phosphate and ferrous iron were determined spectro-photometrically using a Skalar continuous flow analyser (Skalar, Breda, The Netherlands). Phosphate was measured according to Murphy and Riley (1962) and ferrous iron after reaction with tripyridyltriazine (Fries and Getrost 1977). Ferric iron was calculated as the difference between ferrous iron and total iron which was measured after reduction with hydroxylammoniumchloride.

RESULTS AND DISCUSSION

In a preliminary experiment, designed to appraise the phosphate uptake of our iron hydroxide and sediment slurries we noticed a striking difference in reaction rate (fig. 5.1). Phosphate adsorption onto iron hydroxide was almost instanteneous and saturation was reached within 5 hours. The uptake by sediment was much slower and even after 48 hours still uptake of phosphate could be measured. The slower uptake by the sediment slurry can have a twofold explanation; the reaction with iron hydroxide might be retarded as it is tied up in organic and inorganic complexes (Sojo and De Haan 1991) and the uptake by biological processes might impose a slow rate. In all succeeding experiments we took the measurement at 72 hours as the endpoint.

In experiments with standard addition of iron hydroxides to ALW + P the concentration of phosphate in solution decreased with increasing amounts of iron hydroxide (fig. 5.2A). Even in the blank ALW + P where no iron was added, filtration resulted in a loss of phosphate. This can probably be ascribed to the coprecipitation of phosphate with calcium carbonate which is enhanced by the high concentration of phosphate and the high pH (Otsuki and Wetzel 1972). No dissolved iron could be detected (fig. 5.2B). Extraction of the samples with 0.12 M H_2SO_4 resulted in a recovery of phosphate of 97.8 ± 2.0 % (fig. 5.2A). The recovery of iron was 93.1 ± 3.5 % (fig. 5.2B). From these results we calculated a ratio of iron to phosphate adsorption of 9:1 mol/mol.

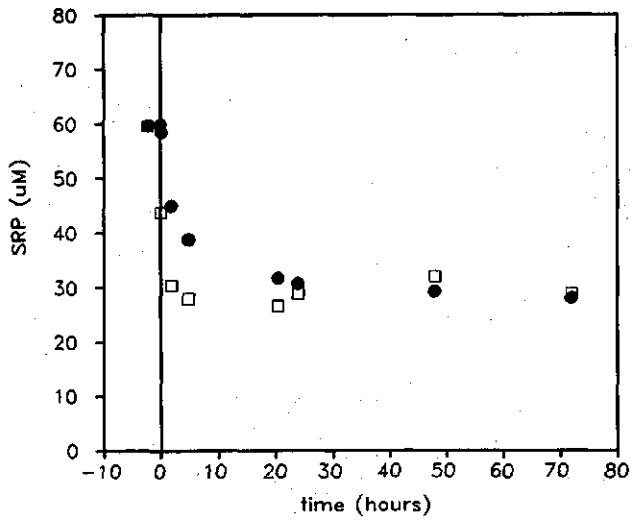

Figure 5.1: Uptake of phosphate by iron hydroxide (250 µM, □) and sediment slurry (1.8 g dry weight.l^{-1}, ●) in ALW + P. Iron hydroxide and sediment were added at time 0.

In experiments with standard additions of synthetic iron hydroxide to sediment slurries, the effects of low additions on the phosphate uptake were only minor (fig. 5.3A). The ratio of iron to phosphate was as high as 24.3 when 48 µM iron was added. At higher additions the adsorption of phosphate to iron increased, resulting in a gradual decrease of the ratio to 10.5 when 240 µM iron was added. Probably, competition with organic ligands and silica suppresses the phosphate adsorption by iron hydroxide (Stumm et al 1980, Sibanda and Young 1986). At high iron additions the relative importance of this competitive effect is only minor and the iron to phosphate ratio approaches the 9:1 mol/mol that is measured in ALW.

When iron(II) was added to sediment slurries and oxidized to iron(III) complexes, the phosphate adsorption capacity was drastically higher than for the synthetic iron hydroxide (fig. 5.3B). At iron additions above 48 µM, phosphate became exhausted and the amount of phosphate limited the adsorption. At an iron addition of 48 µM, a ratio of iron to phosphate adsorption of 1.8 was calculated.

Apparently, the conditions during formation of the iron hydroxide complexes determine the phosphate adsorption capacity. The synthesized iron hydroxide was formed in a mineral salts medium which enabled the polymerization and oxolation of the iron hydroxides thereby decreasing the number of surface hydroxylgroups. After formation the addition of organic substances compete with phosphate for the available hydroxylgroups (Stumm et al 1980, Sibanda and Young 1986). However, when humic acids are present during formation of iron hydroxide, they have been reported to decrease the polynuclear nature of the colloidal iron and thus increase

the number of adsorption sites (Young and Comstock 1986). It is difficult to assess whether the iron hydroxide formed by oxidation of Fe(II) is representative for in situ iron hydroxide. Tipping et al (1989) oxidized anoxic hypolimnetic water of Esthwaite Water and examined the formed products with electron microscopy. When oxidation experiments were carried out with unfiltered lake water, the formed iron hydroxide appeared to be comparable with ferrihydite found in the lake. They concluded that the presence of particulate organic matter was essential for the formation of large ferrihydite particles found in situ.

The recovery of added phosphate was comparable for both iron series (Fe(III): 97.2 ± 1.9 %, Fe(II): 96.5 ± 3.5 %). The recovery of phosphate in the unammended sediment was only 91 %. Apparently the competetive ability of the non-extractable fraction for phosphate is decreased when iron is added.

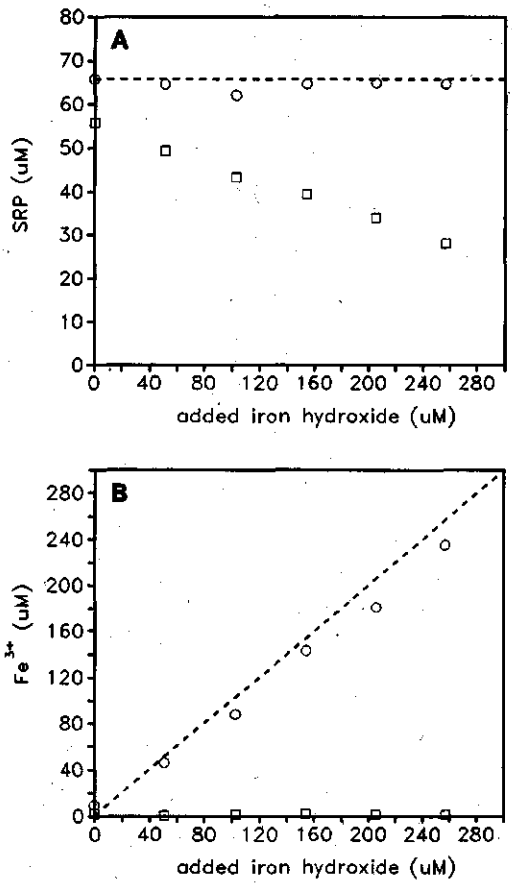

Figure 5.2: Dissolved (□) and acid extractable (○) phosphate (A) and iron (B) in ALW + P at increasing additions of iron hydroxide. Dashed lines represent 100 % recovery.

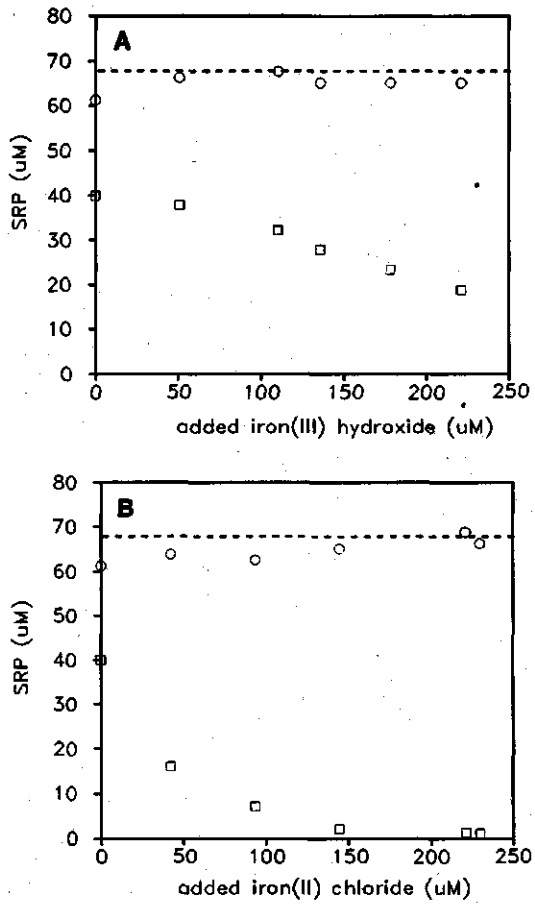

Figure 5.3: Dissolved (□) and acid extractable (○) phosphate in sediment slurries in ALW + P at increasing additions of iron(III) hydroxide (A) and iron(II) chloride (B). Dashed lines represent 100 % recovery for phosphate.

The yield of Acinetobacter 210 A on ALW + P with 2 mM acetate was 50 - 100 mg dw.l^{-1}. No phosphate could be extracted from the pure culture of Acinetobacter 210 A and 50 % of added phosphate was taken up by the culture (fig. 5.4). The phosphate uptake was estimated roughly between 0.5 and 1.0 mmol.g dw^{-1}. These values are rather low compared to values reported by Deinema et al (1985) who reported phosphate uptake of 1.5 - 3.5 mmol.g dw^{-1} in continuous culture at pH 7.5. The low phosphate uptake by Acinetobacter might be due to the growth conditions, as for instance the low concentration of applied acetate.

The phosphate uptake by the sediment slurry was 11.0 μmol.g^{-1} dry weight. Addition of sodium acetate (2 mM, 1.1 mmol.g^{-1} dry weight) increased the phosphate uptake to 14.8 μmol.g^{-1} dry weight. The recovery of phosphate in the sediment slurry was 82 % of which 41 % was left over in the dissolved fraction

and 41 % was extractable (fig. 5.4). When acetate was added to the sediment, 50 % of the phosphate that was taken up appeared to be non-extractable. The amount of acid extractable phosphate was 29 % which is less than in the unammended sediment slurry (41 %). This indicated that addition of acetate favours the competitive ability of the non-extractable fraction above the extractable fraction. From these results we concluded that the extraction procedure could be used to distinguish between iron hydroxyphosphate and bacterial phosphate, ascribing the non-extractable phosphate fraction to bacterial uptake and immobilization.

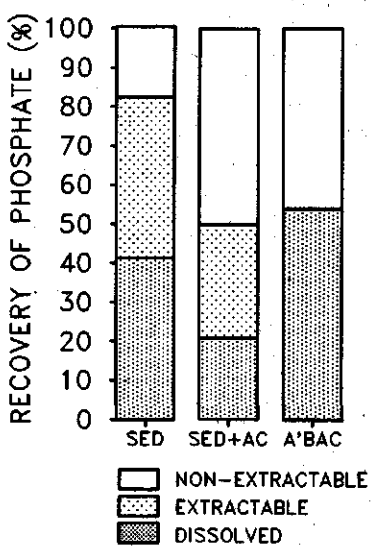

Figure 5.4: Recovery of phosphate as dissolved and extractable phosphate for different treatments in ALW + P. SED: sediment slurry (1.82 gram dry weight.l^{-1})
SED + Ac: sediment slurry with acetate (1.1 mmol.g^{-1} dw)
A'BAC: Acinetobacter 210 A (50 - 100 mg dw.l^{-1}).

In addition, the contribution of biological processes to the phosphate uptake in the surface sediment of Lake Loosdrecht was measured at different locations (tabel 5.1). The phosphate uptake differed markedly at the various locations and ranged from 12 to 138 μmol.g^{-1} on a dry weight basis. The phosphate uptake was positively correlated with the amount of extractable iron ($p < 0.01$) and phosphate ($p < 0.001$). A negative correlation was obtained between the phosphate uptake and the dry weight of the sediment ($p < 0.01$). The contribution of bacterial processes to the phosphate uptake ranged from 3.3 to 41.4 μg.g^{-1}dry weight but was rather constant 25.8 $\pm$ 6.0 % when expressed as percentage of total uptake. The ratio between extractable iron and phosphate was 4.0 which is intermediate

between the values found for high additions of synthetic iron hydroxide to sediment (Fe:P = 10.5) and iron(II) oxidized in slurries (Fe:P = 1.8). The ratio of iron to phosphate might be underestimated as manganese- and aluminum-components can be partly responsible for the phosphate uptake. Extraction of these components thus leads to an overestimation of the iron bound phosphate in the samples. To allow for more accurate estimates of the ratio of iron to phosphate adsorption under in situ conditions, the actual speciation of iron should be further investigated. The addition of 2 mM acetate (0.58 - 6.6 mmol.g^{-1} dry weight, table 5.1) increased the phosphate uptake significantly. The extra phosphate uptake was nonextractable and ascribed solely to bacterial processes. The amount of extractable iron was not influenced by the acetate addition (fig. 5.5A). The amount of extractable phosphate decreased slightly upon addition of acetate (fig. 5.5B) which might be contributed to an increased competition between chemical adsorption and biological uptake of added phosphate.

Table 5.1: Sediment characteristics of randomly sampled surface sediment in Lake Loosdrecht. DW: dry weight, OM: organic matter content, PUP: phosphate uptake, PEXT: extractable phosphate, PBAC: non-extractable (bacterial) phosphate, BAC: contribution of bacterial processes to PUP, FEXT: extractable iron, AC: added acetate.

SEDIMENT		INCUBATION IN ALW+P					INCUBATION IN ALW+P+Ac			
DW	OM	PUP	PEXT	PBAC	BAC	FEXT	Ac	PUP	PBAC	BAC
%	%	μmol.g^{-1}	μmol.g^{-1}	μmol.g^{-1}	%	μmol.g^{-1}	mmol.g^{-1}	μmol.g^{-1}	μmol.g^{-1}	%
3.0	62.6	121	94.7	26.3	22	448	6.64	164	82.7	51
3.1	61.6	114	85.5	28.5	25	210	6.49	137	43.6	32
3.8	54.2	138	96.6	41.4	30	405	5.24	160	56.0	35
4.3	49.1	85.2	64.8	20.4	24	168	4.65	107	40.8	38
5.0	51.0	82.5	63.5	19.0	23	209	3.98	94.3	30.2	32
6.3	66.0	54.3	37.1	17.2	32	190	3.15	78.6	49.1	63
6.6	56.8	70.4	62.2	8.2	12	441	3.01	86.3	37.5	44
13.4	37.1	39.9	28.5	11.6	29	125	1.50	43.8	19.3	44
18.2	23.2	11.0	7.7	3.3	30	32.5	1.10	14.8	9.3	63
34.8	9.0	12.0	8.3	3.7	31	17.6	0.58	14.8	5.8	39

The ratio between carbon uptake and the increase in phosphate uptake is an indication for the physiological conditions of the bacteria. There was a positive correlation between the amount of added acetate per gram dry sediment and the increase in phosphate uptake (fig. 5.6). Assuming that 40 % of the added carbon was converted to biomass, we estimated the carbon to phospate ratio at 80 (mol/-mol). This ratio is comparable to value of 50 reported by Fenchel and Blackburn (1979) for bacteria in sediment and suggests that growing bacteria largely contributed to the phosphate uptake. A major contribution of poly-phosphate is

unlikely because of the high carbon to phosphate ratio. As Lake Loosdrecht is a eutrophic lake with total phosphate concentrations of 2.4 μM in the water (Kelzer and Sinke 1992), the high and rapid phosphate uptake was unexpected. In situ the phosphate concentration in the surface layer generally does not exceed 10 μM (chapter 3). As we used higher phosphate concentrations, the measured chemical phosphate uptake probably overestimates the extent to which adsorption and desorption mechanisms buffer the phosphate concentration in the upper sediment layer under field conditions.

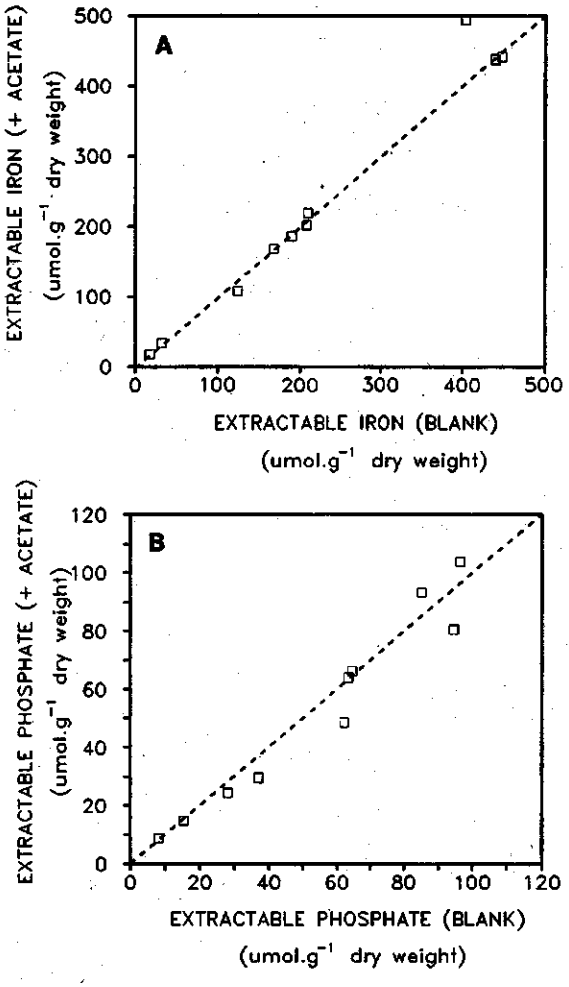

Figure 5.5: Amount of extractable iron (A) and phosphate (B) of acetate enriched sediment slurries plotted vs. the amounts extracted from untreated slurries. Dashed line represents 1:1 ratio.

More research is needed to determine the contribution of bacterial processes to the phosphate buffer capacity in the sediment under field conditions. So far, our results only give a rough indication about the relative importance of these processes. Biological uptake depends on the supply of organic carbon and varies between 12 and 63 % of the total phosphate uptake of the sediment. The measured bacterial uptake (20 - 40 μmol.g^{-1}) corresponds to a phosphate buffer capacity of 2 - 4 mmol.m^{-2} in the upper 2 mm of the sediment, which is in the same order of magnitude as the total amount of phosphate in the water column of Lake Loosdrecht. This rough estimate indicates that bacterial processes have the potential to regulate the seasonal dynamics of the phosphate concentration in the overlying water to a large extent. Although the applicability of the extraction procedure has to be investigated for other sediment types, it is a usefull tool to discriminate between bacterial phosphate uptake and adsorption in peaty sediments.

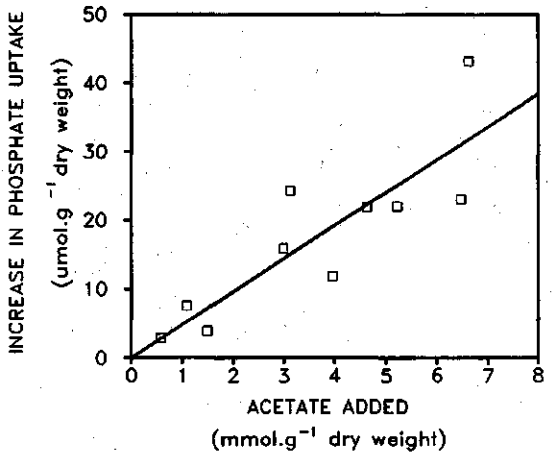

Figure 5.6: Increase in phosphate uptake by sediment slurries vs. amount of added acetate. Dashed line represents fitted linear regression ($y = 0.0048\ x$, $p < 0.000$).

Acknowledgments

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Chapter 6

Methane oxidation by methanotrophs and its effects on the phosphate flux over the sediment-water interface in a eutrophic lake.

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ABSTRACT.

The effect of methane oxidation in aerobic sediment on the oxygen consumption and phosphate flux was investigated in diffusion chambers. The diffusion chambers consisted of two compartments separated by a Teflon membrane. In the upper diffusion chamber a thin sediment layer was present and the lower chamber was continuously flushed with gas. The hydrophobic membrane allowed for diffusion of gasses from the lower chamber through the sediment layer towards the headspace of the upper chamber.

In experiments with a methane oxidation rate of 9.8 $mmol.m^{-2}.d^{-1}$, the oxygen consumption rate increased by a factor two compared to controls without methane oxidation (8.6 vs 17.7 $mmol.m^{-2}.d^{-1}$). The methane oxidation significantly decreased the oxygen penetration depth (2.5-4.0 vs 1.0-2.0 mm). However, despite the shrinkage of the oxidized microlayer, no differences were found in phosphate flux over the sediment water interface. Batch experiments with standard additions of methane revealed that the growth of methanotrophic bacteria contributes to the phosphate uptake of aerobic sediment. From the batches a molar ratio of carbon to phosphate of 45 mol:mol was calculated for the growth of methanotrophs.

Results suggest that a decrease in chemical phosphate adsorption caused by a decease in the oxygen penetration depth, could be compensated for entirely by the growth of methanotrophic bacteria.

INTRODUCTION

The magnitude of methane release from aquatic systems towards the atmosphere is a net result of the methane production rate and its consumption by oxidation processes. Under aerobic conditions the oxidation of methane can be carried out by methanotrophic bacteria. Methanotrophs are recognized by their ability to grow on compounds that contain no carbon carbon bonds such as methane, methanol and N-methyl-compounds (Bédard and Knowles 1989, Hanson 1980). Most data on the ecological significance of methanotrophs are derived from deep stratified lakes. In these lakes methanotrophs are confined to a narrow zone at the oxycline (Rudd and Taylor 1980, Harrits and Hanson 1980). More than 90 % of the diffusive methane flux from the anoxic hypolimnion was reported to be consumed (Rudd and Taylor 1980). The rate of methane oxidation was determined by concentration of dissolved methane, oxygen and nitrogen and by population dynamics of methane oxidizing bacteria (Harrits and Hanson 1980, Rudd and Taylor 1980).

Recently, it has been demonstrated that in sediments with an oxic surface layer, methane oxidation consumes the major fraction of the methane flux (Frenzel et al 1990, King 1990 a, 1990 b, King et al 1990, Sweerts et al in press). The methane oxidation rate was demonstrated to be dependant on the oxygen concentration in

the surface sediment (Frenzel et al 1990, King et al 1990). High oxygen concentrations as generated in actively photosynthesizing algal mats, could increase the methane oxidation by a factor 2 (King 1990 a, 1990 b).
The contribution of methanotrophs to the oxygen consumption of the sediment can be considerable in eutrophic lakes. In Lake Erie methane oxidation attributed 30% of the oxygen consumption (Adams et al 1982) and in Lake Vechten methane oxidation appeared to be the major oxygen consuming process in winter (Sweerts et al in press). The influence of methanotrophs on the oxygen consumption rate of the sediment and the thickness of the oxidized surface layer of the sediment has not been investigated experimentally thusfar. By affecting the oxygen consumption rate of sediments, methanotrophs might influence indirectly phosphate cycling in aquatic ecosystems. The presence of an oxidized microlayer at the surface of the sediment is thought to be one of the major factors retarding the phosphate release by the sediment (Boström et al 1982). In the oxidized surface sediment phosphate adsorbs onto iron(III) to form solid iron hydroxide phosphate complexes. When the redox potential is decreased, iron is reduced and phosphate is released from the iron complexes (Mortimer 1941, 1942). To get an insight in the impact of methane oxidation on the oxygen consumption and phosphate fluxes in freshwater sediments, we developed a diffusion chamber that permitted manipulation of the methane flux towards the sediment. By enhancing the methane oxidation, we could increase the oxygen consumption rate of the sediment. The expected decrease of the phosphate adsorption capacity, resulting from the shrinking of the oxidized surface layer, was balanced by an increased biological uptake of phosphate by methanotrophic bacteria.

MATERIALS AND METHODS

Sediment sampling.
Lake Loosdrecht is an eutrophic freshwater lake in the Netherlands. It was partly man-made through peat mining in the 17th century (Van Liere 1986). As a result of its morphometry (area 14.5 km^2, mean depth 1.8 m) and continuous wind induced turbulence, the surface layer of the sediment remains aerobic throughout the year. Previous studies of the lake include investigations of primary production (Van Liere et al 1986), porewater chemistry (chapter 3), phosphorus release from the sediment (Keizer et al 1990) and restoration perspectives (Keizer and Sinke 1992).
Sediment cores were obtained in january 1990, using a handdriven stainless steel corer containing a PMMA (polymethylmetacrylate) inner core of 40 cm long, with an internal diameter of 5 cm. The sharpened edges of the inner core extruded well below the metal parts to minimize disturbance of the sediment (chapter 3).
In the laboratory, the overlying water was carefully siphoned off and replaced by

200 ml filtered lake water (0.2 μm, polycarbonate filters, Schleicher & Schuell). The aerobic toplayer of the sediment was artificially saturated with phosphate. The overlying water of the sediment cores was spiked with 15 ml KH_2PO_4 (0.2 mM) to a final concentration of approximately 15 μM. During one week, the cores were maintained in dark as short-term microcosms in which the water was gently aerated. Prior to use in the diffusion chambers, the overlying water was carefully siphoned off and replaced by filtered lake water with a phosphate concentration of approximately 0.3 μM.

Diffusion chamber technique.

Flux experiments were conducted in diffusion chambers that permitted manipulation of the methane flux towards the sediment surface (fig. 6.1). Each experimental unit consisted of a 5-8 mm thick intact sediment layer isolated on a Teflon membrane (TE 36, pore size 0.45 μm, Schleicher & Schuell) that separated the upper compartment from the lower. The hydrophobic Teflon membrane only allowed exchange of gasses between the two compartments. The lower compartment was continuously flushed with pure methane (grade 2.5, Hoekloos Schiedam, The Netherlands) or nitrogen that was passed through a column filled with palladium catalyst (BASF, Arnhem, NL) to remove traces of oxygen. Gasses were saturated with water to prevent evaporation of water from the sediment towards the lower compartment. The upper compartment was closed with a rubber stopper providing a headspace of 95 cm^3 (0.075 $m^3.m^{-2}$) when 8 mm sediment and 5 mm water were used. The rubber stopper was perforated with two one-way valves (Unimed, Lausanne, Switzerland) which permitted sampling of either water or gasphase. The headspace was sampled at regular time intervals. During sampling, the headspace was replenished simultaneously with either air or N_2 to ensure that no pressure changes were induced in the upper chamber. A comparable procedure was followed during sampling of the water. Water samples were taken at the interface beteen water and air.

Methane fluxes and oxidation rates.

To determine the influence of methane oxidation on the oxygen consumption rate of the sediment four different treatments were applied in duplicate. In one series of diffusion chambers the headspace was kept aerobic. The lower compartment was either flushed with methane (AEROBIC/CH_4) or nitrogen (AEROBIC/N_2). In the treatment where methane was applied methane diffused upwards to the upper compartment and the activity of methanotrophic bacteria could continue. In the control, flushed with nitrogen, all oxygen consuming processes could proceed except for methane oxidation. In the other series anoxic conditions were established by flushing the headspace with nitrogen for approximately 30 min. In

the treatment where methane (ANAEROBIC/CH_4) was flushed through the lower compartment, methane could diffuse towards the headspace without being oxidized. The anaerobic treatment, flushed with nitrogen (ANAEROBIC/N_2) served as a control to check for methane production in the thin sediment layer. The water column above the sediment was adjusted to 5 mm in all diffusion chambers and left unstirred. The diffusion chambers were incubated in dark at 22 ± 2 °C in a thermostated waterbath. Concentrations of methane in the headspace were followed. Flux rates were calculated by linear regression analysis of methane accumulation in the headspace as a function of time.

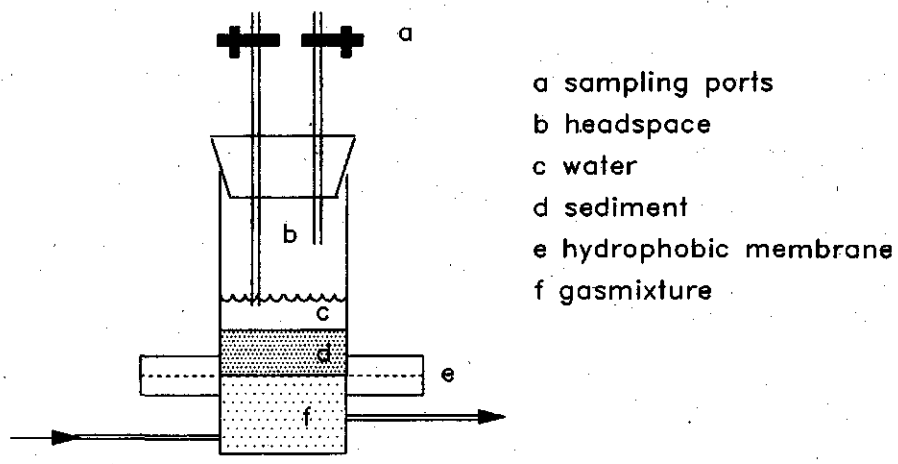

Figure 6.1: Scheme of the diffusion chamber.

Oxygen profiles and fluxes.

The oxygen profiles in the sediment were measured after one week incubation of the diffusion chambers. The aerobic diffusion chambers were transferred to the waterbath (22 °C) of the micro electrode equipement and left undisturbed for at least 16 hours. Oxygen concentration profiles were determined using microelectrode techniques previously described by others (Carlton and Wetzel 1988, Frenzel et al 1990, King 1990 b, King et al 1990, Revsbech et al 1980, Sweerts et al in press). Cathode type oxygen microelectrodes (tip diameter 3-10 μm, gold plated, cellulose acetate membrane, NIOO, Centre for Estuarine and Coastal Ecology, Yerseke, The Netherlands) were used in conjunction with a calomel reference electrode. The oxygen microelectrode was connected by a coaxial shielded cable to an ammeter (Keithly 480 digital picoammeter, polarization voltage -750 mV). The

microelectrode was mounted on a micro manipulator with a spatial resolution of 10 μm (Plato BV, Diemen, The Netherlands). Calibration was done by inserting the electrode tip approximately 20 μm below the air-water interface so that the current output corresponded to 100 % air saturation (Carlton and Wetzel 1988). The asymptotic minimum of the measured oxygen profiles was taken as 0 %. Fluxes of oxygen were calculated on basis of Fick's first law of diffusion by using the concentration gradients at the sediment-water interface. The molecular diffusion constant for oxygen was estimated from the data of Broeker and Peng (1974) at 1.88×10^{-4} $m^2.d^{-1}$ at 22 °C. Porosity of the sediment top layer was taken as 0.97 and the square of tortuosity as 1.2 (Sweerts et al in press).

Phosphate flux in diffusion chambers.
The phosphate flux over the sediment water interface in diffusion chambers was followed by daily sampling of the overlying water. Prior to exposion to the air, samples were acidified to pH 1 with 3 M H_2SO_4 to prevent precipitation of iron hydroxyphosphates.

Phosphate uptake by methanotrophic slurries.
The phosphate uptake by methanotrophs was examined in batch experiments using homogenized surface sediment (0 - 1 cm) which was diluted 1:100 (w/w) with artificial lake water (ALW: 1 mM Ca^{2+}, 2 mM Na^+, 0.5 mM NH_4^+, 0.2 mM Mg^{2+}, 2.4 mM HCO_3^-, 2.1 mM Cl^-, 0.2 mM SO_4^{2-}, pH = 8.3) enriched with 0.065 mM KH_2PO_4. 40 ml of slurry was put in serum bottles of different size (100 to 500 ml). Increasing amounts of methane were added resulting in a standard series of 1 to 14 μmol methane per gram dry sediment. As a result of the increasing size of the bottles used, the initial methane concentration was comparable in all bottles. By thus introducing a fixed methane to oxygen concentration in the headspace, an adequate supply of oxygen was ensured to enable methane oxidation. Slurries were continuously shaken. After one week the amount of methane remaining was measured. To determine the phosphate uptake, 5 ml samples were filtered over 0.45 μm membrane filters (mixed esters, Schleicher & Schuell) that were prewashed with demineralized water. After filtration, samples were acidified to pH 1 by diluting 1:1 with 0.24 M H_2SO_4. To determine the contribution of bacterial uptake to the total phosphate uptake by the slurry, an extraction procedure was used. 10 ml samples of the slurry were extracted with 10 ml 0.24 M H_2SO_4 during 24 hours at 22 ± 2 °C. During this extraction iron hydroxyphosphates are dissolved while bacterial phosphate remains intact (chapter 5).

Analytical procedures.
Methane was measured using a Packard model 428 gas chromatograph equipped

with a Haysep Q column (Chrompack, Middelburg, The Netherland) and a flame ionization detector (at 160 °C). The carrier gas was helium at a flowrate of 20 ml.min^{-1}. Phosphate was measured on a Skalar Continuous Flow Analyser (Breda, The Netherlands) according to Murphy and Riley (1962).

RESULTS AND DISCUSSION

When pure methane was used in the lower compartment and when the upper chamber was kept anaerobic, a methane flux towards the upper compartment of 14.4 ± 0.65 mmol.m^{-2}.d^{-1} was measured. Under the assumption of a steady state, the theoretical methane concentration profile in the diffusion chamber can be calculated according to (fig. 6.2):

$$F = - D_{CH4}/\Delta x * \phi/\Theta^2 * (C_1-C_2) = -D_{CH4}/\Delta z\ (C_2-C_3)$$

with: F = methane flux (mmol.m^{-2}.d^{-1}), D_{CH4} = molecular diffusion constant of methane (m^2.d^{-1}), Δx = thickness of the sediment layer (m), ϕ = porosity of the sediment (dimensionless), Θ^2 = squared tortuosity of the sediment (dimensionless), C_1 = methane concentration at surface of Teflon membrane (mol.m^{-3}), C_2 = methane concentration at sediment-water interface (mol.m^{-3}), C_3 = methane concentration in the upper diffusion chamber (mol.m^{-3}), Δz = thickness of water layer (m). The molecular diffusion constant of 1.64 x 10^{-4} m^2.d^{-1} at 22 °C was estimated from the data of Broeker and Peng (1974). The relatively low concentration of methane in the headspace of the upper diffusion chamber (C_3) was neglected.

With the measured flux of 14.4 mmol.m^{-2}.d^{-1}, ϕ = 0.97, Θ^2 = 1.2, Δx = 0.008 m and Δz = 0.005 m, the values for C_1 and C_2 can be calculated. The calculated value for the methane concentration at the upper side of the Teflon membrane (C_1) was 1.31 mol.m^{-3}, which is close to the saturation constant for methane in water at of 1.49 mol.m^{-3} (22 °C, Yamamoto et al 1976). Apparently the diffusive resistance of the Teflon membrane is low.

Methane fluxes and oxidation rates.

The methane accumulation rates were significantly higher under anaerobic conditions than under aerobic conditions (fig. 6.3). The calculated average fluxes were 14.4 ± 0.65 in the anaerobic diffusion chambers vs 4.59 ± 0.17 mmol.m^{-2}.d^{-1} in the aerobic chambers (table 1). Assuming that the difference between anaerobic and aerobic treatment was due to methane oxidation, 9.8 mmol.m^{-2}.d^{-1} was oxidized in the sediment surface layer. Comparable high methane oxidation rates were reported for Gator Pond peat where 5.5 - 13.6 mmol.m^{-2}.d^{-1} was oxidized depending on the location and oxygen supply (King et al 1990).

The applied methane flux of 14.4 mmol.m^{-2}.d^{-1} is considerably higher than in situ fluxes towards the sediment surface in Lake Loosdrecht. Maximal in situ methane fluxes in the anaerobic zone of 8.2 mmol.m^{-2}.d^{-1} were calculated using methane concentration gradients in autumn (chapter 4). In situ all upwards diffusing methane can probably be oxidized in the surface layer of the sediment. The same conclusion could also be drawn on base of mesocosm experiments (Sweert et al in press) and the measured in situ methane profiles (chapter 4).

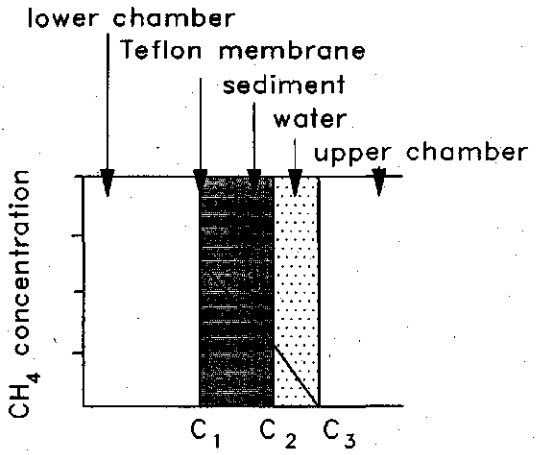

Figure 6.2: Scheme of theoretical methane concentration in diffusion chamber, with a sediment layer of 0.8 cm, a water layer of 0.5 cm. C_1 = CH_4 concentration at surface of Teflon membrane (mol.m^{-3}), C_2 = CH_4 concentration at sediment-water interface, C_3 = CH_4 concentration at the water-air interface (mol.m^{-3}).

In chambers with nitrogen in the lower compartment, methane fluxes were considerably lower. In the ANAEROBIC/N_2 diffusion chambers a flux 0.37 ± 0.08 mmol.m^{-2}.d^{-1} was measured. In the AEROBIC/N_2 chambers the concentration of methane was near the detection limit and the flux was estimated at 0.15 ± 0.04 mmol.m^{-2}.d^{-1}. Assuming a specific density of 1, the methane production in the top 8 mm of the sediment under anaerobic conditions is estimated at 1.9 nmol.cm^{-3}.h^{-1}. This value is rather low, considering the incubation temperature of 22 °C. Although part of the produced methane could have diffused into the lower chamber and be discharged by the continuous nitrogen flow, the results suggest that hardly any methane is produced in the upper 5 - 8 mm of the sediment. As the columns were sampled in spring time when overall methane production is low (chapter 4) and as in situ concentrations of sulfate were maintained, probably sulfate reduction is the dominant mineralization pathway in the few anaerobic millimeters of the sediment slices used.

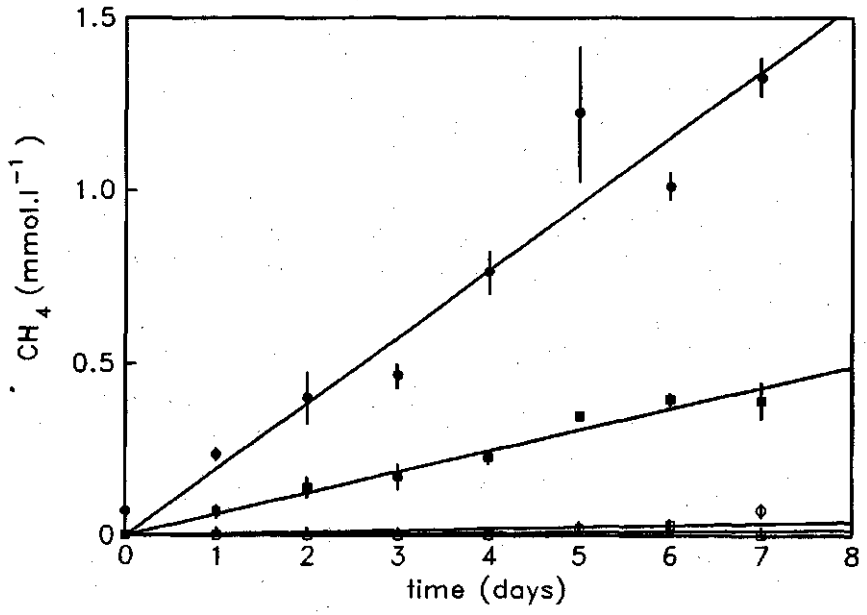

Figure 6.3: Methane accumulation in headspace of diffusion chambers. AEROBIC/N2 (□), AEROBIC/CH_4 (■), ANAEROBIC/N_2 (○), ANAEROBIC/CH_4 (●).

Oxygen profiles and fluxes.

After one week of incubation, depth profiles of oxygen were measured in the aerobic diffusion chambers. The oxygen profiles differed markedly between the two treatments (fig. 6.4). The profiles in the diffusion chambers where the lower compartment was flushed with nitrogen, closely resembled the oxygen profiles in whole columns just after sampling (results not shown). Due to millimeter scale irregularities of the sediment surface, the exact location of the sediment water interface was difficult to detect and could only be indicated roughly. The thickness of the diffusive boundary layer (DBL) was comparable in both treatments and was estimated at 1.5 - 2.5 mm. The oxygen concentration reached a constant value of 90 - 100 % in the uppermost millimeters of the water layer of both treatments. In a non-mixed water layer, a linearly decreasing oxygen profile would be expected in the waterphase. Apparently, the vibrations of the air and underground are sufficient to prevent the formation of a boundary layer through the complete water column. The thickness of the DBL probably fluctuated during the experiment due to the sampling of water and headspace. Extrapolation to in situ conditions is precarious as there is no information available on the in situ thickness of the DBL in Lake Loosdrecht. Probably in situ the thickness of the diffusive boundary layer will fluctuate with wind action and oxygen consumption rate comparable to results reported for the sea floor (Gunderson and Jørgensen 1990).

Table 6.1: Calculated oxygen and methane fluxes in diffusion chambers and measured oxygen penetration depth and phosphate uptake.

	O_2 flux	CH_4 flux	O_2 penetration	PO_4^{2-} flux
	$mmol.m^{-2}.d^{-1}$	$mmol.m^{-2}.d^{-1}$	depth in mm	$\mu mol.m^{-2}.d^{-1}$
AEROBIC/CH_4	17.7 ± 0.7	4.59 ± 0.17	1.0-2.0	-0.2 ± 0.1
ANAEROBIC/CH_4	--	14.4 ± 0.65	--	11.8 ± 0.7
AEROBIC/N_2	8.6 ± 0.9	0.15 ± 0.04	2.5-4.0	NS
ANAEROBIC/N_2	--	0.37 ± 0.08	--	9.6 ± 0.6

NS no significant flux occured.

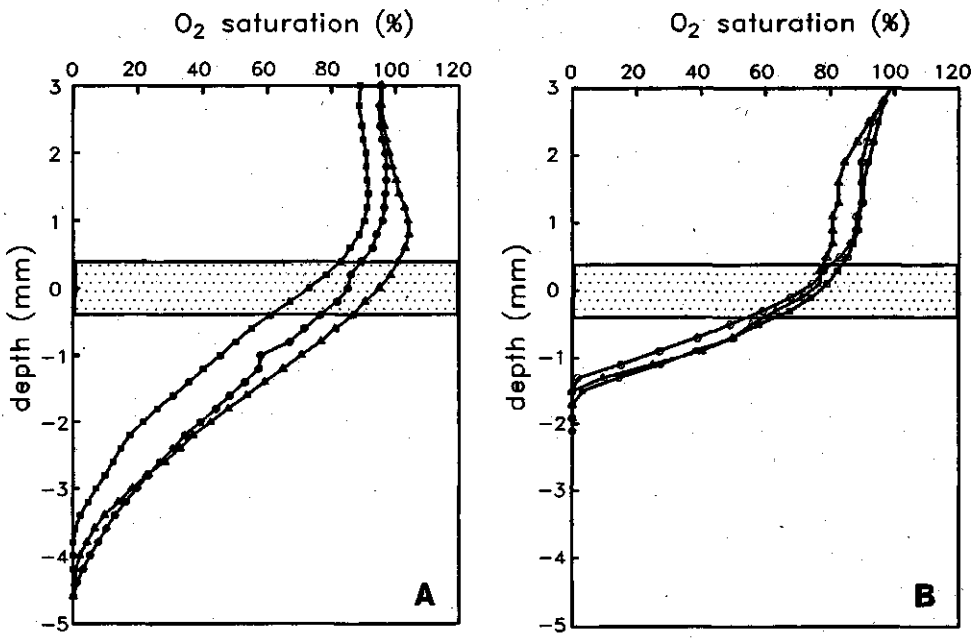

Figure 6.4: Oxygen profiles in the aerobic diffusion chambers (triplicates).
A: AEROBIC/N_2, B: AEROBIC/CH_4. Dotted area: sediment water interface.

There was an obvious difference in oxygen penetration depth between the two treatments. The oxygen penetration depth in the AEROBIC/CH_4 diffusion chamber was estimated at 1.0 - 2.0 mm. In the AEROBIC/N_2 diffusion chambers oxygen

penetrated approximately 2.5 - 4 mm. The oxygen profiles were also much steeper in the diffusion chambers where methane oxidation proceeded and the calculated oxygen consumption rate was twice as high under methanotrophic conditions as in controls (17.7 vs 8.6 mmol.m^{-2}.d^{-1}, table 1).
The oxygen requirement for methane oxidation was estimated by assuming that the difference in methane flux between aerobic and anaerobic conditions was due to methane oxidation and that 1.5 mol of oxygen was consumed per mol methane (Joergensen and Degn 1983). On basis of these assumptions, methane oxidation consumed 14.7 mmol oxygen.m^{-2}.d^{-1}, which accounts for 83 % of the total oxygen uptake. This value is comparable to calculations on in situ conditions in Lake Loosdrecht where in autumn at 12 °C 76 % of the oxygen consumption was attributed to methane oxidation at a methane flux of 8.2 mmol.m^{-2}.d^{-1} (chapter 4).

Phosphate flux in diffusion chambers.
There were large differences in the phosphate flux over the sediment-water interface of the diffusion chambers (fig. 6.5). Due to the extra phosphate loading, the aerobic surface layer of the sediment was saturated with phosphate and was expected to react rapidly on changes in redox conditions. In the anaerobically incubated diffusion chambers an obvious phosphate release occured. The observed releases of 9.6 ± 0.6 μmol.m^{-2}.d^{-1} for ANAEROBIC/N_2 and 11.8 ± 0.7 μmol.m^{-2}.d^{-1} for ANAEROBIC/CH_4 are somewhat lower than the value of 20 μmol.m^{-2}.d^{-1} reported for anaerobically incubated phosphate saturated whole columns (Keizer and Sinke 1992). Probably the difference is due to the fact that, in contrast with whole columns, in the diffusion chambers only the upper few millimeters can contribute to the phosphate release. No phosphate release occured in the aerobic diffusion chambers. Despite the observed shrinkage of the oxygen penetration depth with more than 50 %, the AEROBIC/CH_4 treatment did not release any phosphate. Instead a slight but significant uptake of phosphate of 0.21 μmol.m^{-2}.d^{-1} occured (table 1).
Generally, an increased phosphate release is observed when formerly aerobic sediment becomes reduced (Boström et al 1982, Mortimer 1941, 1942, Sundby et al 1986, Tessenow 1972). Also conditions that cause a partial breakdown of the oxidized microzone were reported to promote phosphate release (Carlton and Wetzel 1988, Tessenow 1972). All these authors ascribed the mobilization of phosphate to the dissolution of iron (hydr)oxyphosphate and several of them reported high correlations between iron and phosphate fluxes (Mortimer 1941, 1942, Sundby et al 1986, Tessenow 1972). The results in the AEROBIC/CH_4 diffusion chambers deviate from this general trend. Batch experiments were performed to elucidate if the growth of methanotrophic bacteria could be responsible for the observed phosphate retention.

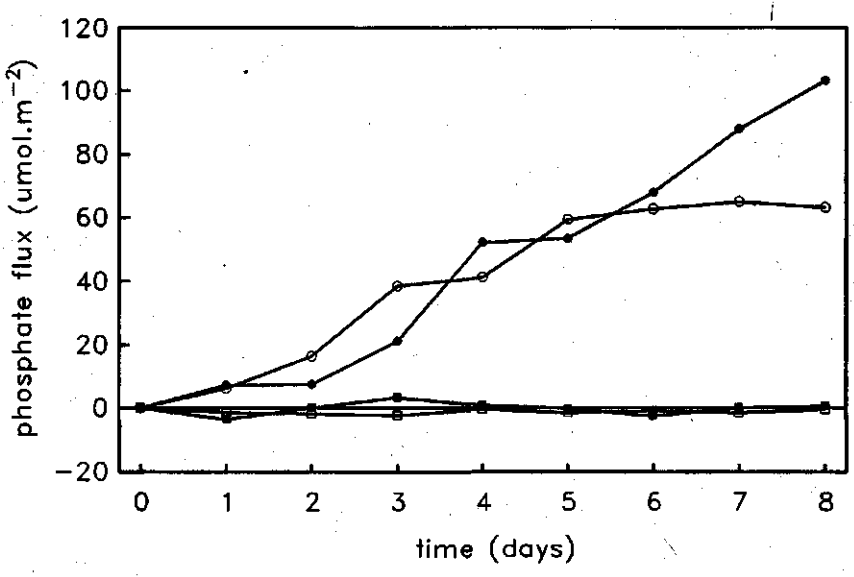

Figure 6.5: Phosphate flux over the sediment-water interface in diffusion chambers. AEROBIC/N2 (□), AEROBIC/CH_4 (■), ANAEROBIC/N_2 (○), ANAEROBIC/CH_4 (●).

Phosphate uptake by methanotrophic slurries.

The phosphate uptake of the sediment slurry increased linearly with increasing methane additions (fig. 6.6A). One clear outlyer could be ascribed to leakage of the used stopper, resulting in an overestimation of the methane consumption. The extra phosphate that was taken up upon methane addition appeared to be non-extractable (fig. 6.6B). The increase of the non-extractable fraction is an indication for biological uptake of phosphate (chapter 5). The amount of acid extractable, chemically bound phosphate tended to decrease slightly with increasing methane additions. This might be due to a small shift from chemical to biological phosphate retention. From the increase in phosphate of the non-extractable fraction, a carbon to phosphate ratio can be calculated for growth of methanotrophic bacteria. Assuming that the oxidized methane is converted into carbon dioxide and cell material in a ratio of 3.6:1 (Harrits and Hanson 1980), the carbon to phosphate ratio of the produced biomass is 46 mol:mol. This value is comparable to the value of 50 mol:mol reported by Fenchel and Blackburn (1979) but is considerably lower than earlier observed value of 80 mol:mol for bacteria that aerobically metabolize acetate in this sediment (chapter 5). When the calculated C:P ratio of 46 mol:mol is applied to the results of the diffusion chambers, where approximately 2 mmol carbon.m^{-2}.d^{-1} (9.8 mmol.m^{-2}.d^{-1} methane oxidized) can be turned into biomass, 43

μmol.m^{-2}.d^{-1} of phosphate could be immobilized by methanotrophs. Apparently this uptake rate is high enough to compensate for the decrease of the chemical adsorption capacity caused by the shrinkage of the oxidized microlayer. We suggest that the phosphate uptake by aerobic sediment is not an exclusively chemical process but can be ascribed to biological processes aswell.

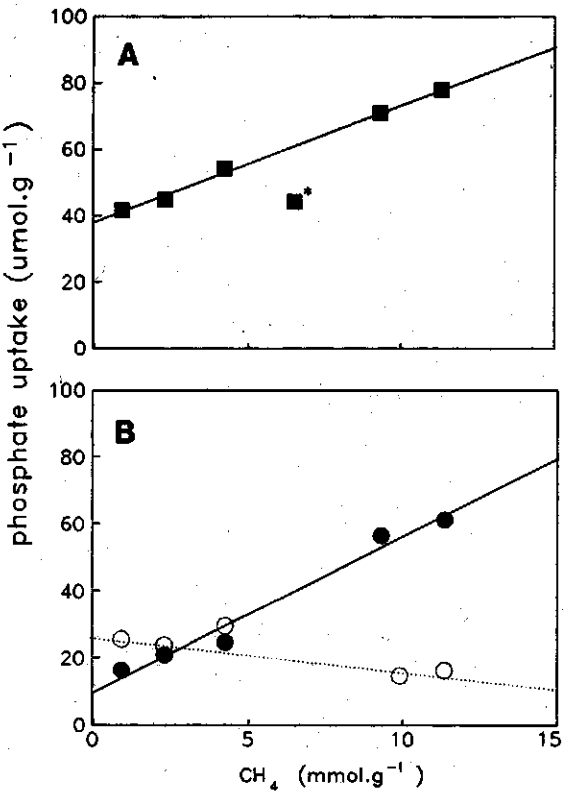

Figure 6.6: Phosphate uptake by sediment slurries as a function of methane consumption. **A)** total uptake, **B)** change in acid extractable fraction (o) and non-extractable fraction (●).
* unreliable result (see text). Lines represent linear regression of data.
A: total P uptake y = 38.1 + 3.5 x $p<0.0001$;
B: uptake in non-extractable fraction y = 8.5 + 4.7 x, $p<0.005$;
B: uptake in extractable fraction y = 26.7 - 1.04 x $p<0.05$ (dotted line).

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Chapter 7

Discussion, conclusions and perspectives

Here, results presented in this thesis are combined to demonstrate that microbial processes in the sediment influence the phosphorus cycle in eutrophic Lake Loosdrecht in two ways. Firstly, mineralization influences the distribution of phosphorus in the sediment and the mobilization of phosphate from the solid organic fraction into the dissolved fraction. Secondly, the uptake and release of phosphate by bacteria in the top layer of the sediment determines the phosphate flux over the sediment-water interface. The obtained results contributed to the development of a simple model that was presented previously (Keizer and Sinke 1992). In this chapter the model is used to evaluate lake management measures in Lake Loosdrecht. Finally some alternative management measures are proposed that may contribute to an improvement of the water quality in the lake.

PHOSPHORUS DYNAMICS IN THE SEDIMENT.

Distribution of phosphorus in the sediment.

The organic matter content of the sediment of Lake Loosdrecht is high 55-65 % on a dry weight basis (chapter 3). A large fraction of the organic matter is not susceptible to degradation by microorganisms. In the 0-0.5 cm top layer of the sediment only 5 % of the organic matter is degradable (Sinke unpublished results). The amount of degradable organic matter decreases exponentially with depth. Hardly any degradation takes place below 4 cm depth in the sediment (fig. 4.4). We suggest that the peat fraction is not susceptible to degradation and that the degradable organic fraction in the sediment consist of recently deposited algae. This is in accordance with earlier work where we demonstrated that the phosphatase activity and the chlorophyll-a content in the sediment decreased with depth (Sinke et al 1991, fig. 7.1).

The total phosphorus content of the sediment is 21.6 $mmol.kg^{-1}$ (Keizer and Sinke 1992). Approximately 80 % of the total phosphorus in the sediment is linked to organic matter and only a minor fraction is retained in autigenic minerals (Keizer and Sinke 1992). The phosphorus fraction that is linked to organic matter in the top layer of the sediment (16.8 $mmol.kg^{-1}$) can be mobilized rapidly. In laboratory experiments a mobilization rate of 80 $\mu mol.kg^{-1}.d^{-1}$ was determined at room temperature (Keizer and Sinke 1992). However, the phosphorus that is linked to the organic fraction in deeper layers cannot be mobilized, even when incubated aerobically (Keizer and Sinke 1992).

The average amount of phosphorus which is present in the pore water is 10 $\mu mol.l^{-1}$ (310 $\mu g.l^{-1}$) which is less than 0.005 % of the total amount present in the sediment (chapter 3). This pore water concentration is low compared to other eutrophic lakes where values have been reported of 39 to 410 $\mu mol.l^{-1}$ (1200 to 12700 $\mu g.l^{-1}$ (Boström et al 1982, Enell and Löfgren 1988). The major part of the dissolved fraction is reactive (DRP, dissolved reactive phosphorus). The exact speciation of

phosphorus in the DRP fraction is subject of discussion (Tarapchak et al 1982). Molecular sieve experiments made clear that phosphate is the major component of the DRP fraction in Lake Loosdrecht (Sinke and De Bles, unpublished results). Due to the concentration gradient between pore water and lake water, phosphate diffuses from the anaerobic zone towards the sediment surface at an average rate of 10 μmol.m^{-2}.d^{-1} (chapter 3). Approximatly 20 μmol.m^{-2}.d^{-1} is transported to deeper layers and the groundwater by seepage (Keizer and Sinke 1992).

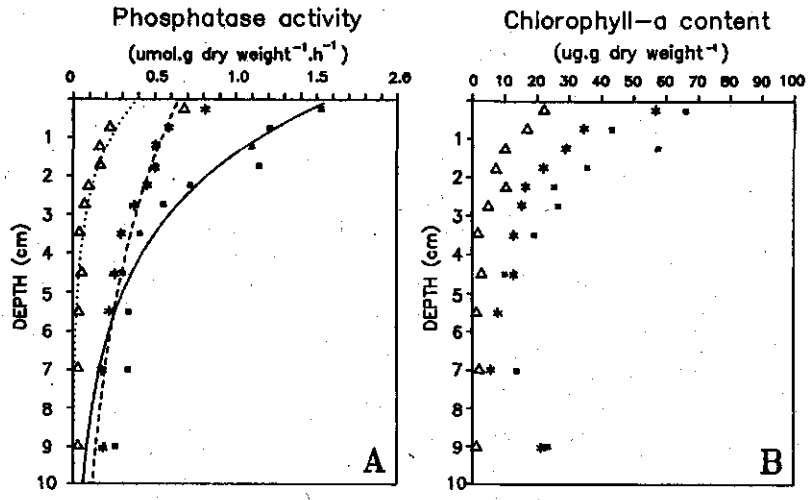


Figure 7.1: Phosphatase activity (A) and chlorophyll-a content (B) in the sediment of Lake Loosdrecht. Examples are given of a gytjja (•), a peat (*) and a sand (▲) column. Lines represent fitted functions: gytjja $y = 1.6\ e^{-0.39\,x}$, peat $y = 0.65\ e^{-0.17\,x}$ and sand $y = 0.40\ e^{-0.54\,x}$ (redrawn after Sinke et al 1991)

Transfer reactions between the dissolved and the solid phosphorus fractions.
The mobilization of phosphate from the organic and from the inorganic fraction in the sediment results in a continuous replenishment of the dissolved fraction in the pore water. The equilibrium between the mobilization of phosphate and its transport towards the overlying water, can be disturbed by a sudden increase of the diffusion rate. Diffusion is enhanced by a decrease of the phosphate concentration in the overlying water or by a sudden increase of the temperature. In experiments where sediment cores were incubated at elevated temperatures, the phosphate release rate was initially very high: 200 μmol.m^{-2}.d^{-1} at 40 °C (6 mg.m^{-2}.d^{-1}, fig. 2.3) but decreased rapidly (fig. 2.4). After a certain lag period the phosphate release resumed at the original level (fig. 2.4). This indicated that a new equilibrium was established and that the increased diffusion of phosphate was compensated by an increased mobilization. Inactivation of microorganisms by either heat or γ-irradiation

prevented this increase of mobilization, indicating the importance of microbial processes for long-term phosphate release.

Mineralization of organic matter results in a simultaneous mobilization of carbon, ammonium and phosphate (table 1.1). Matisoff et al (1981) incubated anaerobically sediment slurries from different depths in the sediment and observed a high correlation between the mobilization of ammonium and phosphate. Theoretically, the produced ammonium and phosphate are removed from the pore water at different rates by chemical and biological reactions (fig. 1.1). The stoichiometric adsorption of phosphate to the solid phase is generally higher than of ammonium (eg Berner 1974). Besides, phosphate is liable to precipitation reactions forming authigenic minerals such as vivianite and apatite. Ammonium and phosphate are both taken up by bacteria in the sediment. High correlations between the concentration of ammonium and phosphate are reported for some sediments (Berner 1977, Callender and Hammond 1982, McCaffrey et al 1980) which indicates the importance of mineralization in determining the pore water concentrations. In chapter 3 we reported that the concentrations of ammonium and phosphate were correlated in the sediment of Lake Loosdrecht. Both ions were also correlated with the methane concentration in the sediment (fig. 3.4). Methane and carbon dioxide are the major end products of carbon mineralization in Lake Loosdrecht as methanogenesis is dominant in the bulk of the sediment (chapter 4). The measured concentration profiles of methane, ammonium and phosphate were used to calculate a C:N:P ratio of the degradable organic matter. The calculated ratio of 106:14.1:0.19 deviated considerably from the Redfield ratio of 106:16:1 (Redfield 1958). The high C:N and C:P ratio indicated that the degradable organic matter was relatively poor in nitrogen and phosphorus. However, as the measured methane flux partly derived from geological older layers (chapter 4), the amount of mineralized carbon was overestimated. Correction of the C:N:P ratio for the upwards diffusing methane results in an estimated C:N:P ratio of 106:16.3:0.22. The magnitude of the mineralization rate cannot be determined directly in situ. Quantitative information on the in situ mineralization rates can be inferred from the oxygen consumption rate of the sediment and from calculated fluxes of methane, ammonium and phosphate. Based on a C:P ratio of 300 (Gulati et al 1991) and an oxygen consumption rate of 30 $mmol.m^{-2}.d^{-1}$ (Sweerts and Cappenberg 1988), the phosphate mineralization in the sediment of Lake Loosdrecht was estimated to reach 100 $\mu mol.m^{-2}.d^{-1}$ (3 $mg.m^{-2}.d^{-1}$) in summer and 50 $\mu mol.m^{-2}.d^{-1}$ (1.5 $mg.m^{-2}.d^{-1}$) on a yearly basis (Keizer and Sinke 1992). The latter value is comparable to the sum of the calculated average in situ phosphate fluxes towards the sediment surface (10 $\mu mol.m^{-2}.d^{-1}$, 0.3 $mg.m^{-2}.d^{-1}$, chapter 3) and towards the groundwater (20 $\mu mol.m^{-2}.d^{-1}$, 0.6 $mg.m^{-2}.d^{-1}$) (Keizer and Sinke 1992).

Phosphate adsorption to the sediment surface layer.
The aerobic toplayer of the sediment usually has a high affinity for phosphate. This is ascribed to the presence of iron(III) hydroxides which have a very high adsorption capacity for phosphate. Iron(III) hydroxides form solid complexes with phosphate. It has been demonstrated that an aerobic surface layer functions as lid on top of the anaerobic sediment and prevents the release of phosphate to the water column (Einsele 1936, Mortimer 1941, 1942). When the redox potential in the formerly aerobic sediment decreases, the iron(III) complexes are reduced and phosphate is released. The release of phosphate after the exhaustion of oxygen is an often observed phenomenon in deep lakes during stratification (Mortimer 1941, 1942). The effectiviness of the aerobic surface layer as a barrier is dependent on the ratio of iron to phosphate (Löfgren and Boström 1989, Jensen et al in press). Jenssen et al demonstrated that a ratio of iron to phosphate in the sediment of 8 mol:mol is sufficient to prevent phosphate release under aerobic conditions. In Lake Loosdrecht the iron to phosphate ratio is 21 mol:mol (Keizer and Sinke 1992) and the sediment surface is able to retain large amounts of phosphate.
Several authors suggested that also bacteria contribute to the phosphate adsorption of the sediment (Fleisher 1978, Gächter et al 1988, Gächter and Meyer in press). Gächter and coworkers (1988, in press) suggested that bacteria accumulate phosphate under aerobic conditions which is again released at the onset of anoxia. These authors suggested a parallel with sewage sludge plants where polyphosphate accumulating bacteria like Acinetobacter are present (Fuhs and Cheng 1975). Although their hypothesis is plausible, they were not able to detect polyphosphate. In Lake Loosdrecht a specific staining method on polyphosphate (Neissler staining, Gurr 1965) did not indicate the presence of polyphosphate accumulating bacteria (Deinema, pers. comm.). However, phosphate can also be taken up by growing bacteria without being stored as polyphosphates. In chapter 5 we demonstrated that the contribution of bacteria to the total phosphate uptake by aerobic Loosdrecht sediment was approximately 25 %. The uptake of phosphate by bacteria could be increased by adding extra organic substrate. In the presence of acetate, ammonium and phosphate, the biologically bound phosphate increased to 44 %. If either acetate or ammonium was omitted, the phosphate uptake was considerably lower (Sinke and Cottaar unpublished results). The actual phosphate uptake by bacteria will probably fluctuate seasonally as it is influenced by the supply of organic substrate. Regular sampling of the sediment surface can provide further information on the importance of bacterial phosphate uptake in Lake Loosdrecht. The mean residence time of the phosphate in bacteria remains to be determined. Preliminary experiments demonstrated that the major fraction of the phosphate is released rapidly under less favorable conditions such as the exhaustion of substrate (carbon or nitrogen) or anoxia (Cottaar unpublished results).

A reduction in thickness of the aerobic sediment surface layer often results in a decrease of the phosphate adsorption capacity and a release of phosphate (Carlton and Wetzel 1988, Tessenow 1972). On a yearly scale, temperature seems to be the major factor determining the penetration depth of oxygen into the sediment (Sweerts 1990). On a short term scale, other factors may influence the oxygen penetration depth drastically. Tessenow (1972) demonstrated that the redox potential in the sediment decreased when easily degradable substrate was added to sediment cores. The presence of a photosynthesizing microbial mat can also result in considerable fluctuations of the oxygen penetration depth (Carlton and Wetzel 1988, King 1990a, King et al 1990). In Lake Loosdrecht the oxidation of methane contributes significantly to the oxygen consumption of the sediment (Sweerts et al in press, chapter 4). We performed experiments with diffusion chambers in which we artificially increased the methane flux towards the sediment surface. The increased methane oxidation resulted in a reduction of the oxygen penetration depth from 3.25 mm to 1.5 mm (table 6.1, fig. 6.4). However, the reduction of the thickness of the oxidized surface layer did not result in a release of phosphate. Even when phosphate saturated sediment was used, the expected phosphate release did not occur (fig. 6.5). The phosphate retention was due to the growth of methanotrophic bacteria (fig. 6.6). A prediction of the influence of microbial processes on the oxygen penetration depth and the phosphate uptake in situ is not easy to make. Regular measurements of the methane flux towards the sediment and of the amount of phosphate retained in biomass, could give information on the role of methanotrophic bacteria in the phosphate adsorption of the sediment.

THE PHOSPHORUS FLUX OVER THE SEDIMENT WATER INTERFACE.

Several authors have performed experiments with sediment cores under well defined laboratory conditions to quantify the release rate of dissolved phosphorus compounds (Bates and Naefus 1980, Boers 1986, Boers and Van Hese 1988, Kamp-Nielsen 1974, 1975, Lee et al 1977, Premazzi and Provini 1985, Søndergaard 1989, Tessenow 1972, Ulen 1977). Generally, the release rates are measured for several weeks (Bates and Naefus 1980, Boers 1986, Boers and Van Hese 1988, Kamp-Nielsen 1974, 1975, Premazzi and Provini 1985, Søndergaard 1989) or even months (Ulen 1977, Tessenow 1972). In experiments with Lake Loosdrecht sediment the initial release rates of the dissolved phosphorus compounds, measured over a period of several hours, sometimes exeeded 1000 μmol.m^{-2}.d^{-1} (30 mg.m^{-2}.d^{-1}) (fig. 3.3). However, during the incubation the release rates decreased rapidly (fig. 2.4) which often resulted in a net uptake when data were averaged over 3 weeks (fig. 3.3). Apparently the changes that occur during incubation are too large to allow for prolonged laboratory release experiments. The

suspended sedimentation of organic matter is probably the major factor that leads to the underestimation of the release rates.

Bell-jars that can be put onto the sediment, have been used to measure in situ fluxes of dissolved phosphorus compounds (Callender and McHammond 1982, McCaffrey et al 1980, Sundby et al 1986). Probably bell-jars mimic best the natural situation although corrections have to be made for the decreasing oxygen content of the water and the loss of turbulence. In Lake Loosdrecht the use of bell-jars was impossible as the equipement slowly sank away in the mud and was heavily disturbed by wave action (Van Brummelen 1988). Several authors compared fluxes that were measured in bell-jars with those calculated from concentration gradients in the pore water (Callender and McHammond 1982, McCaffrey et al. 1980). The fluxes that were measured with bell-jars were significantly higher than the calculated fluxes when macrofauna was present in the sediment (Callender and McHammond 1982). In Lake Loosdrecht the influence of macrofauna is probably only minor as the number of chironomids in the sediment is between 75-150 individuals per square meter (5-10 animals in the sediment slicing programme of 1988-1989, 52 columns of 12.6 cm^2, Sinke, Cornelese, Keizer, Van Tongeren and Cappenberg, unpublished results). The pore water concentration of phosphate was measured at two representative locations at four weeks intervals (chapter 3). The concentration profiles of phosphate were used to calculate fluxes towards the sediment surface and over the sediment-water interface. The calculated fluxes correlated well with the initial fluxes that were measured in core experiments (fig. 3.5). However, the calculated fluxes towards the sediment-water interface that ranged from 0 to 24 $\mu mol.m^{-2}.d^{-1}$ (0-0.7 $mg.m^{-2}.d^{-1}$) and over the interface of 0-350 $\mu mol.m^{-2}.d^{-1}$ (0-10.9 $mg.m^{-2}.d^{-1}$) differed significantly and were considerably lower than the measured initial fluxes that ranged from 0 to 1400 $\mu mol.m^{-2}.d^{-1}$ (0-43 $mg.m^{-2}.d^{-1}$). Probably the flux in the anaerobic zone that was calculated from the pore water profiles predicts best the average in situ release rate as it is the resultant of all phosphate mobilization processes in the sediment.

The calculated fluxes showed large seasonal fluctuations and were highest in autumn (fig. 3.5). This result cleary deviated from the general assumption that temperature is the steering variable determining the phosphate release (Boers and Van Hese 1988, Kamp-Nielsen 1975). The conclusions of these authors were based on laboratory release experiments. Extrapolation of those experiments to the field conditions does not take into account other in situ variables that may cause seasonal fluctuations in release rates. In chapter 3 we demonstrated that the high fluxes measured in autumn were related to increased sedimentation and mineralization of organic matter (fig. 3.5).

It can be concluded that results obtained with flux measurements in the field and in the laboratory, should be interpreted with care. Field measurements and flux

calculations based on in situ concentration profiles, probably represent best the combined effects of environmental variables (temperature, sedimentation) on the phosphate flux. Laboratory measurements might well be used to study the separate processes in more detail but they give no information on the actual impact of these processes on the in situ phosphate release rates.

IMPLICATIONS FOR LAKE MANAGEMENT.

In Lake Loosdrecht less than 2 % of the total phosphorus in the sediment and lake water takes part in the cycling within the system (Keizer and Sinke 1992). However, the processes keeping this small fraction cycling, are dynamic. Keizer and Sinke (1992) proposed a simple model to predict the effects of management measures on the total phosphorus concentration in the water (fig. 7.2). In this model three reactive fractions of phosphorus compounds are distinguished in the ecosystem: the phosphorus fraction in the water (PWAT) and an organic fraction (PORG) and inorganic fraction (PADS) in the sediment. The sediment is continuously replenished with organic material sinking from the overlying water (Psed). This sedimented organic matter (PORG) is mineralized (Pmin) which results in an increase of the pool-size of the inorganic phosphorus fraction in the sediment (PADS). Part of the dissolved fraction is transported to the groundwater by seepage (Pseep) and part of it diffuses towards the upper sediment layer. The internal loading of phosphorus (Pint) is the result of diffusion and of resuspension. Dissolved phosphate in the water is taken up by algae and detritus (PWAT). The phosphorus cycle in the system is fuelled by the external loading (Pext) and results in a continuous pollution of the ground water with phosphate (Pseep).

To achieve an improvement of the water quality, the pool size of phosphorus compounds in the water has to be reduced. Several management measures have been proposed to reach this goal:

A) Reduction of the external phosphorus loading (decrease in Pext).
B) Sediment dredging (partial removal of PORG and PADS).
C) Iron addition (increase in pool size of PADS).
D) Reduction of external phosphorus loading by a change of hydrological regime (decrease in Pext and Pseep).

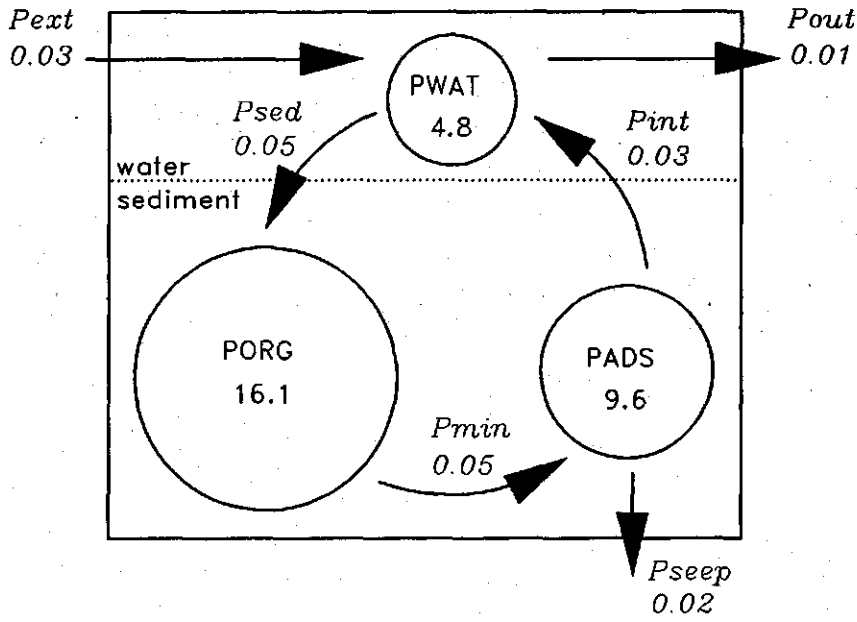


Figure 7.2: Simplified model of the phosphorus cycle in Lake Loosdrecht with pool sizes of phosphorus compounds (mmol.m^{-2}) in the water (PWAT), dissolved or adsorbed onto the inorganic fraction (PADS) and bound to the organic fraction (PORG). The processes (mmol.m^{-2}.d^{-1}) are given in italics: Pext = external phosphorus load; Psed = sedimentation; Pmin = mineralization; Pseep = seepage towards the ground water; Pint = internal loading and Pout = outflow of phosphorus from the system. (redrawn after Keizer and Sinke 1992).

A) Reduction of the phosphorus loading (decrease in Pext, figure 7.3 A)
A 50% reduction of the external phosphorus loading will force the system into a transient state in which all pool sizes and proces rates decrease. Simulations with the model indicate that a new steady state will be reached probably within a decade. In the new steady state the PWAT is substantially lower than it is now.

B) Sediment dredging (partial removal of PORG and PADS, figure 7.3 B).
By dredging the top layer of the sediment the pool sizes of PORG and PADS are decreased. Assuming an 80 % reduction of the PORG fraction in the sediment, the total phosphorus fraction in the water would decrease by 10 %. However, this improvement is only temporal as the phosphorus cycle is refuelled by the external phosphorus load. When the external phosphorus load is maintained at the original level the PWAT increases again and the pool sizes of PORG and PADS are replenished by sedimentation and mineralization.

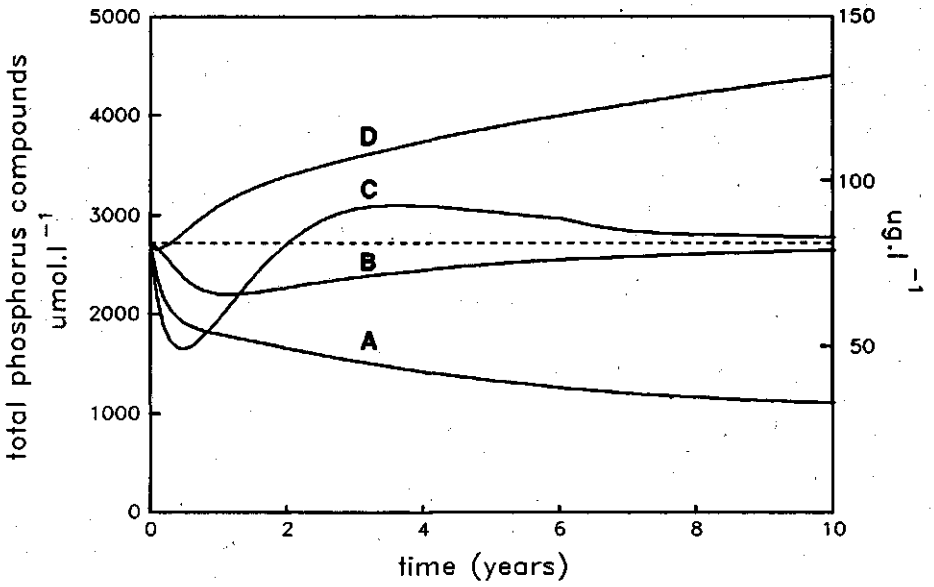


Figure 7.3: The concentration of total phosphorus compounds in Lake Loosdrecht after different management measures. **A**: 50% reduction of external phosphorus load, **B**: sediment dredging where 80 % of PORG is removed, **C**: addition of 1.8 mol.m^{-2} iron(III) chloride and **D**: change in hydrological regime where Pseep is stopped (A, B and C redrawn after Keizer and Sinke 1992).

C) Iron addition (increase in pool size of PADS, figure 7.3 C).

With the addition of ferric iron to the sediment, phosphate is scavenged and solid iron hydroxiphosphate is formed. Line C in fig. 7.3 was calculated assuming an addition of iron of 1.8 mol.m^{-2} (100 g.m^{-2}). This is about tha same amount as used in Lake Groot Vogelenzang (Boers et al 1992). The iron addition results in an increase of the pool size of PADS. The seepage of phosphate and its diffusion into the overlying lake water is prevented. However, the internal loading may be maintained at the original level due to resuspension of the top layer of the sediment. Whether algae are able to scavenge phosphate from the resuspended iron hydroxiphosphate as bacteria seem to do, needs further investigation (fig. 6.6). On the long-term all added iron is probably reduced by chemical and biological processes. This results in a mobilization of phosphate which might even be higher than the original level as during the initial stages of iron addition the removal of phosphate by Pseep was inhibited.

D) Change of hydrological regime (decrease in Pseep and Pext, figure 7.3 D).
In several nature conservation plans the inundation of the surrounding polders (e.g. Bethune polder) is suggested. Innundation of polders would reduce Pseep in Lake Loosdrecht and consequently the loss of phosphate into the groundwater. In this way the pollution of the groundwater is severely diminished. By reducing the Pseep, the deficit on the water balance of the Lake decreases automatically and the amount of water that is pumped into the lake can be reduced. However, only a minor part of the external phosphorus loading is introduced with this suppletion water (Engelen et al 1992). So, the external loading is not likely to decrease drastically as a result of such a hydrological operation. The reduction of Pseep and the maintainance of Pext results in an increase of the phosphorus compounds in the water which leads to a worsening of the water quality.

Restoration of Lake Loosdrecht ?
A possible option to decrease the amount of phosphorus compounds in the water is to construct dikes and isles and to extend the existing reed belts. The dikes and isles will diminish the surface of the lake that is vulnerable to wind influences. The reduction of resuspension has a positive effect on the turbulence (Gons et al 1986) and, consequently, decreases the internal phosphorus loading (Keizer and Sinke 1992). This management measure could be combined easily with extension of the existing reed belts. The amount of phosphorus that can be retained by 1 m^2 reed (Phragmites australis) in summer is 35-200 mmol (1.1-6.3 grams, Van Oorschot 1990). Part of the phosphorus can thus be withdrawn from the phosphorus cycle during the growth of the reed in spring and summer. Harvesting of the reed in autumn would result in a removal of phosphorus from the system.
In all management scenarios a drastic reduction of the external loading is prerequisite to decrease the amount of phosphorus compounds in the water. Whether such a decrease actually results in an improvement of the water quality depends on the ecological reaction of the system. Sas (1989) investigated the effects of a reduction of the external phosphorus load in 18 european lakes and concluded that a decrease in algal biomass can be expected only when the system was phosphate limited before the treatment. The growth of algae in Lake Loosdrecht seems to have shifted lately from phosphate limited towards light limited (Rijkeboer and Gons 1991). Van Liere et al (1991) demonstrated that the decrease in dissolved phosphorus compounds in the water of 0.4 $\mu mol.l^{-1}.y^{-1}$ (12 $\mu g.l^{-1}.y^{-1}$) during the period 1984-1988 did not result in any decrease of the chlorophyll concentration. Despite the low amount of dissolved phosphorus compounds in the lake water of 2.4 $\mu mol.l^{-1}$ (75 $\mu g.l^{-1}$) which is below the target value given by the Dutch governement for surface water (4.8 $\mu mol.l^{-1}$, 150 $\mu g.l^{-1}$,

Rijkswaterstaat 1989), the water quality is bad. The changes in the structure of the ecosystem that have been induced by the eutrophication might be irreversible. Whether Lake Loosdrecht can be restorated into a clear water system dominated by macrophyts depends on ecological interactions between bacteria, algae, zooplankton and fish in the ecosystem and is impossible to predict.

References

Adams D.D. and Van Eck G.T.M. (1988) Biogeochemical cycling of organic carbon in the sediments of the Grote Rug reservoir. Arch. Hydrobiol. Beih. Ergebn. Limnol. 31: 319-330.

Andersen J.M. (1975) Influence of pH on release of phosphorus from lake sediments. Arch. Hydrobiol. 76: 411-419.

Andersen J.M. (1982) Effect of nitrate concentration in lake water on phosphorus release from the sediment. Wat. Res. 16: 1119-1126.

Bates M.H. and Neafus N.J.E. (1980) Phosphorus release from sediments from Lake Carl Blackwell, Oklahoma. Wat. Res. 14: 1477-1481.

Bédard C. and Knowles R. (1989) Physiology, biochemistry and specific inhibitors of CH_4, NH_4^+, and CO oxidation by methanotrophs and nitrifiers. Microb. Reviews 53: 68-84.

Bengtsson L., Fleischer S., Lindmark G. and Ripl W. (1975) Lake Trummen restoration project. I Water and sediment chemistry. Verh. Int. Verein. Limnol. 19: 1080-1087.

Berner R.A. (1964) An idealized model of dissolved sulfate distribution in recent sediments. Geochim. Cosmochim. Acta 28: 1497-1503.

Berner R.A. (1974) Kinetic models for the early diagenesis of nitrogen, sulfur, phosphorus, and silicon in anoxic marine sediments. The Sea, Vol.5 Marine chemistry. Ed. E.D. Goldberg, pg 427-450. John Wiley & Sons, New York.

Berner R.A. (1977) Stoichiometric models for nutrient regeneration in anoxic sediments. Limnol. Oceanogr. 22: 781-786.

Berner R.A. (1980) Early diagenesis, a theoretical approach. Princeton University Press, New Jersey.

Boers P.C.M. (1986) Studying the phosphorus release from the Loosdrecht lakes sediments using a continuous flow system. Hydrobiol. Bull. 20: 5-7.

Boers P.C.M. (1991) The influence of pH on phosphate release from sediments. Wat Res. 25: 309-311.

Boers P.C.M., Bongers J.W.T., Wisselo A.G. and Cappenberg T.E. (1984) Loosdrecht Lakes Restoration Project: Sediment phosphorus distribution and release from the sediments. Verh. Int. Verein. Limnol. 22: 842-847.

Boers P.C.M., Van Der Does J., Quaak M., Van Der Vlugt J. and Walker P. (1992) Fixation of phosphorus in lake sediments using iron(III) chloride: experiences, expectations. Hydrobiologia 233: 211-212.

Boers P.C.M. and Van Hese (1991) Phosphorus release from the peaty sediments of the Loosdrecht Lakes (The Netherlands). Wat. Res. 22: 355-363.

Bossard P. and Gächter R. (1981) Methan- und Sauerstoffhaushalt im Mesotrophen Lungernsee. Schweiz. Z. Hydrol. 43: 219-252.

Boström B., Ahlgren I. and Bell R. (1985) Internal nutrient loading in a eutrophic lake, reflected in seasonal variations of some sediment parameters. Verh. Int.

Verein. Limnol. 22: 3335-3339.

Boström B., Jansson M. and Forsberg C. (1982) Phosphorus release from lake sediments. Arch. Hydrobiol. Beih. Ergebn. Limnol. 18: 5-59.

Bowen H.J.M. and Cawse P.A. (1964) Some effects of gamma irradiation on the composition of soil solution and soil organic matter. Soil Sci. 98: 358-361.

Brock T.D. (1978) The poisoned control in biogeochemical investigations In: Environmental biogeochemistry and geomicrobiology vol. 3 Ed. W.E. Krumbein. Ann Arbor Science.

Broecker W.S. and Peng T.H. (1974) Gas exchange rates between air and sea. Tellus 26: 21-35.

Callender E. and Hammond D.E. (1982) Nutrient exchange across the sediment--water interface in the Potomac River estuary. Estuarine Coastal and Shelf Science 15: 395-413.

Capone D.G. and Kiene R.P. (1988) Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic carbon catabolism. Limnol. Oceanogr. 33: 725-749.

Cappenberg T.E., Hordijk C.A. and Hagenaars C.P.M.M. (1984) A comparison of bacterial sulfate reduction and methanogenesis in the anaerobic sediments of a stratified lake ecosystem. Arch.Hydrobiol.Beih.Ergebn. Limnol. 19:191-199.

Carlton R.G. and Wetzel R.G. (1988) Phosphorus flux from lake sediments: Effect of epipelic algal oxygen production. Limnol. Oceanogr. 33: 562-570.

Cole J.J., Caraco N.F. and Likens G.E. (1990) Short-range atmospheric transport: A significant source of phosphorus to an oligotrophic lake. Limnol. Oceanogr. 35: 1230-1237.

Cooke G.D., Welch E.B., Peterson S.A. and Newroth P.R. (1986) Lake and Reservoir restoration. Butterworth, Boston. pp 392.

Deinema M.H., Van Loosdrecht M. and Scholten A. (1985) Some physiological characteristics of Acinetobacter spp. accumulating large amounts of phosphate. Wat. Sci. Tech. 17: 119-125.

Ebert J. and Van Liere L. (1992) Waterkwaliteits onderzoek Loosdrechtse Plassen, gegevens monitoring programma 1979-1990. WOL-rapport 1992-1 (in dutch).

Einsele W. (1936) Uber die Beziehungen des Eisenkreislaufs zum Phosphatkreislauf im eutrophen See. Arch. Hydrobiol. 29: 664-686.

Eisenreich S.J., Bannerman R.T. and Armstrong D.E. (1975) A simplified phosphorus analysis technique. Environ. Letters 9: 43-53.

Emerson S. and Widmer G. (1978) Early diagenesis in anaerobic lake sediments. II Thermodynamic and kinetic factors controlling the formation of iron phosphate. Geochim. Cosmochim. Acta 42: 1307-1316.

Enell M. and Löfgren S. (1988) Phosphorus in intersitial water: methods and dynamics. Hydrobiologia 170: 103-132.

Engelen G.B., Kal B.F.M., Buyse J.J. and Van Pruissen F.G.M. (1992) The hydrology of the Loosdrecht Lakes area. Hydrobiologia 233: 21-38.

Fallon R.D. and Brock T.D.(1978) Decomposition of blue-green algal (cyanobacterial) blooms in Lake Mendota. Appl. Environ. Microbiol. 37: 820-830.

Feijtel T.C., Salingar Y., Hordijk C.A., Sweerts J-P.R.A., Van Breemen N. and Cappenberg T.E. (1989) Sulfur cycling in a dutch moorland pool under elevated atmospheric S-deposition. Water, Air and Soil Poll. 44: 215-234.

Fenchel T. and Blackburn T.H. (1979) Bacteria and mineral cycling. Academic press, London.

Fleischer S. (1978) Evidence for the anaerobic release of phosphorus from lake sediments as a biological process. Naturwissenschaften 65: 109-110.

Fleischer S. (1983) Microbial phosphorus release during enhanced glycolysis. Naturwissenschaften 70: 415-415.

Frenzel P., Thebrath B. and Conrad R. (1989) Oxidation of methane in the oxic surface layer of a deep lake sediment (Lake Constance). FEMS Microbiology Ecology 73: 149-158.

Fries J. and Getrost H. (1977) Organische Reagenzien für die Spurenanalyse. E. Merck, Darmstadt.

Fuhs G.W. and Chen M. (1975) Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. Microb. Ecol. 2: 119-138.

Gabriel K.R. (1971) The biplot graphic display of matrices with application to principal component analysis. Biometrika 58: 453-467.

Gächter R. and Meyer J.S. (in press) The role of microorganisms in mobilization and fixation of phosphorus in sediments. Hydrobiologia.

Gächter R., Meyer J.S. and Maris A. (1988) Contribution of bacteria to release and fixation of phosphorus in lake sediments. Limnol. Oceanogr. 33: 1542-1558.

Gallepp G.W., Kitchell J.F. and Bartell S.M. (1978) Phosphorus release from lake sediments as affected by chrironomids. Verh. Internat. Verein. Limnol. 20: 458-465.

Gons H.J., Gulati R.D. and Van Liere L. (1986) The eutrophic Loosdrecht Lakes: current ecological research and restoration perspectives. Hydrobiol. Bull. 20: 61-75.

Gons H.J., Veeningen R. and Van Keulen R. (1986) Effects of wind on a shallow lake ecosystem: resuspension of particles in the Loosdrecht Lakes. Hydrobiol. Bull. 20: 109-120.

Gulati R.D., Van Liere L. and Siewertsen K. (1991) The Loosdrecht lake system; Man's role in its creation, perturbation and rehabilitation. In: Terrestrial and Aquatic ecosystems: Perturbation and Recovery. Ed. Ravera. pp 593-606.

Ellis Horwood limited.

Gunderson J.K. and Jørgensen B.B. (1990) Microstructure of diffusive boundary layers and the oxygen uptake of the sea floor. Nature 345: 604-607.

Håkanson L. and Jansson M. (1983) Principles of lake sedimentology. Springer-Verlag Berlin, New York.

Hanson R.S. (1980) Ecology and diversity of methylotrophic organisms. Advances in Applied Microbiology 26: 3-39.

Harrits S.M. and Hanson R.S. (1980) Stratification of aerobic methane-oxidizing organisms in Lake Mendota, Madison, Wisconsin. Limnol. Oceanogr. 25:412-421.

Hieltjes A.H.M. and Lijklema L. (1980) Fractionation of inorganic phosphates in calcareous sediments. J. Environ. Qual. 9: 405-407.

Holdren Jr G.C. and Armstrong D.E. (1980) Factors affecting phosphorus release from intact lake sediment cores. Environ. Sci. Techn. 14: 79-87.

Hordijk C.A., Hagenaars C.P.M.M. and Cappenberg T.E. (1985) Kinetic studies of bacterial sulfate reduction in freshwater sediments by High-pressure Liquid Chromatography and microdestillation. Appl. Environ. Microbiol. 49: 434-440.

Ingvorsen K., Zehnder A.J.B. and Jørgensen B.B. (1984) Kinetics of sulfate and acetate uptake by Desulfobacter postgatei. Appl. Environ. Microbiol. 47: 403-408.

Ingvorsen K., Zeikus J.G. and Brock T.D. (1981) Dynamics of bacterial sulfate reduction in a eutrophic lake. Appl. Environ. Microbiol. 42: 1029-1036.

Jansson M.(1987) Anaerobic dissolution of iron-phosphorus complexes in sediment due to the activity of nitrate-reducing bacteria. Microb. Ecol. 14: 81-89.

Jensen H.S., Kristensen P., Jeppesen E. and Skytthe A. (in press) Iron:phosphorus ratio in surface sediment as an indicator of phosphate release from aerobic sediments in shallow lakes. Hydrobiologia

Jewell W.J. and McCarty P.L. (1971) Aerobic decomposition of algae. Environ. Sci. Technol. 5: 1023-1031.

Joergensen L. and Degn H. (1983) Mass spectrometric measurements of methane and oxygen utilization by methanotrophic bacteria. FEMS Microbiology Letters 20: 331-335.

Jones R.I., Salonen K. and De Haan H. (1988) Phosphorus transformation in the epilimnion of humic lakes: abiotic interactions between dissolved humic materials and phosphate. Freshwater Biol. 19: 357-369.

Jørgensen B.B. (1983) Processes at the sediment-water interface. In: The major biogeochemial cycles and their interactions. Eds. B.Bolin and R.B. Cooke. pg 477-509. John Wiley & Sons, U.K.

Jørgensen B.B. and Revsbech N.P. (1985) Diffusive boundary layers and the oxygen uptake of sediments and detritus. Limnol. Oceanogr. 30: 111-122.

Kal B.F.M., Engelen G.B. and Cappenberg T.E. (1984) Loosdrecht lakes restoration project: Hydrology and physicochemical characteristics of the lakes. Verh. Int. Verein. Limnol. 22: 835-841.

Kamp-Nielsen L. (1974) Mud-water exchange of phosphate and other ions in undisturbed sediment cores and factors affecting the exchange rates. Arch. Hydrobiol. 73: 218-237.

Kamp-Nielsen L. (1975) A kinetic approach to the aerobic sediment-water exchange of phosphorus in Lake Esrom. Ecol. Modelling. 1: 153-160.

Keizer P., Buysman M. and Cappenberg T.E. (1991) Sorption and release of phosphorus in a peaty sediment. Verh. Internat. Verein. Limnol. 24: 722-725.

Keizer P. and Sinke A.J.C. (1992) Phosphorus in the sediment of the Loosdrecht lakes and its implications for lake restoration perspectives. Hydrobiologia 233: 39-50.

Kelly C.A. and Chynoweth D.P. (1981) The contributions of temperature and of the input of organic matter in controlling rates of sediment methanogenesis. Limnol. Oceanogr. 26: 891-897.

Kelly C.A., Rudd J.W.M., Cook R.B. and Schindler D.W. (1982) The potential importance of bacterial processes in regulating rate of lake acidification. Limnol. Oceanogr. 27: 868-882.

King G.M. and Klug M.J. (1982) Comparative aspects of sulfur mineralization in sediments of a eutrophic lake basin. Appl. Environ. Microbiol. 43: 1406-1412.

King G.M. (1990a) Regulation by light of methane emissions from a wetland. Nature 345: 513-515.

King G.M. (1990b) Dynamics and controls of methane oxidation in a Danish wetland sediment. FEMS Microbiology Ecology 74: 309-324.

King G.M., Roslev P. and Skovgaard H. (1990) Distribution and rate of methane oxidation in sediments of the Florida Everglades. Appl. Environ. Microbiol. 56: 2902-2911.

Klump J.V. and Martens C.S. (1989) The seasonality of nutrient regeneration in an organic-rich coastal sediment: Kinetic modeling of changing pore-water nutrient and sulfate distibutions. Limnol. Oceanogr. 34: 559-577.

Kuivila K.M., Murray J.W., Devol A.H. and Novelli P.C. (1989) Methane production, sulfate reduction and competition for substrates in the sediments of Lake Washington. Geochim. Cosmochim. Acta 53: 409-416.

Kroes H.W. (1992) The significance of the Loosdrecht Lakes research project for eutrophication policy in the Netherlands. Hydrobiologia 233: 165-170.

Krom M. (1980) Spectrophotometric determination of ammonia; A study of a modified Berthelot reaction using salicylate and dichloro-isocyanurate. The Analyst 105: 305-316.

Lee G.F., Sonzogni W.C. and Spear R.D. (1977) Significance of oxic vs anoxic

conditions for Lake Mendota sediment phosphorus release. In: Interactions between sediments and freshwater. Ed. H.L. Golterman. pg. 294-306.

Li Y-H. and Gregory S. (1974) Diffusion of ions in sea water and in deep sea sediments. Geochim. Cosmochim. Acta 38: 703- 714.

Lijklema L. (1980) Interaction of orthophosphate with iron(III) and aluminum hydroxides. Environ. Sci. Technol. 14: 537-541.

Lijklema L. (1985) Nutrient cycling in lakes. Proceedings of the Dutch-Hungarian Symposium on Eutrophication of Shallow Lakes, Siofok.

Löfgren S. and Boström B. (1989) Interstitial water concentrations of phosphorus, iron and manganese in a shallow, eutrophic swedish lake -imlications for phosphorus cycling. Wat. Res. 9: 1115-1125.

Lovley D.R. (1991). Dissimilatory Fe(III) and Mn(IV) reduction. Microbiol. Reviews 55: 259-287.

Lovley D.R., Dwyer D.F. and Klug M.J. (1982) Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. Appl. Environ. Microbiol. 43: 1373-1379.

Lovley D.R. and Klug M.J. (1983) Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations. Appl. Environ. Microbiol. 45: 187-192.

Marsden M.W. (1989) Lake restoration by reducing the external phosphorus loading: the influence of sediment phosphorus release. Freshwater Biol. 21: 139-162.

Martens C.S. and Klump J.V. (1984) Biogeochemical cycling in an organic-rich coastal marine basin. 4: An organic carbon budget for sediments dominated by sulftae reduction and methanogenesis. Geochim. Cosmochim. Acta 48: 1987-2004.

Matisoff G., Fisher J.B. and McCall P.L. (1981) Kinetics of nutrient and metal release from decomposing lake sediments. Geochim. Cosmochim. Acta 45: 2333-2347.

McCaffrey R.J., Myers A.C., Davey E., Morrison G., Bender M., Luedtke N., Cullen D., Froelich P. and Klinkhammer G. (1980) The relation between pore water chemistry and benthic fluxes of nutrients and manganese in Narragansett Bay, Rhode Island. Limnol. Oceanogr. 25: 31-44.

McLaren A.D., Luse R.A. and Skujins J.J. (1962) Sterilization of soil by irradiation and some further observations on soil enzyme activity. Soil Sci. Soc. Proc. 26: 371-377.

Mortimer C.H. (1941) The exchange of dissolved substances between mud and water in lakes I. Journal of Ecology 29: 280-329.

Mortimer C.H. (1942) The exchange of dissolved substances between mud and water in lakes II. Journal of Ecology 30: 147- 201.

Murphy J. and Riley J.P. (1962) A modified single solution method for the

determination of phosphate in natural waters. Anal. Chim. Acta 27: 31-36.

Otsuki A. and Wetzel R.G. (1972) Coprecipitation of phosphate with carbonates in a marl lake. Limnol. Oceanogr. 17: 763-766.

Otten J. and Gons H.J. (1991) Aerobic decomposition of settled algae in a laboratory system: the impact of resuspension on microbial activity. Verh. Internat. Verein. Limnol. 24: 734-737.

Petterson K. (1984) Transfers between forms of sedimentary phosphorus induced by nitrate treatment. Verh. Internat. Verein. Limnol. 22: 233-238.

Premazzi G. and Provinni A. (1985) Internal loading in lakes. A different approach to its evaluation. Hydrobiologia 120: 23-33.

Psenner R., Pucsko R. and Sagar M. (1984) Die Fraktionierung organischer und anorganischer Phosphatverbindungen von Sedimenten. Arch. Hydrobiol. /Suppl. 70: 111-155.

Ralston M. (1988) Derivative-Free non-linear regression-AR. In: BMDP Statistical Software Manual. Ed. W.J. Dixon. pg 389-417, University of California Press, Berkely, Los Angeles.

Rao C.R. (1964) The use and interpretation of principal component analysis in applied research. Sankhya A 26: 329-358.

Redfield A.C. (1958) The biological control of chemical factors in the environment. American Scientist 46: 205-222.

Redshaw C.J., Mason C.F., Hayes C.R. and Roberts R.D. (1990) Factors influencing phosphate exchange across the sediment-water interface of eutrophic reservoirs. Hydrobiologia 192: 233-245.

Reeburgh W.S. (1967) An improved interstitial water sampler. Limnol. Oceanogr. 12: 163-165.

Revsbech N.P., Sørensen J., Blackburn T.H. and Lomholt J.P. (1980) Distribution of oxygen in marine sediments measured with microelectrodes. Limnol. Oceanogr. 25: 403-411.

Rijkeboer M. and Gons H. (1991) Role of detritus in the phosphorus balance of Lake Loosdrecht water. Verh. Internat. Verein. Limnol. 24: 726-728.

Rijkeboer M. De Bles F. and Gons H.J (1992) Role of sestonic detritus as a P-buffer. Mem Ist. Ital. hydrobiol. 48: 251-260.

Rijkswaterstaat (1989) Derde Nota Waterhuishouding. SDU, Den Haag. (in dutch).

Rippey B. (1977) The behaviour of phosphourus and silicon in undisturbed cores of Lough Neagh sediments. In: Interactions between sediments and freshwater. Ed. H.L. Golterman. pp 348-353, W. Junk BV Publishers, The Hague.

Robinson J.A. and Tiedje J.M. (1984) Competition between sulfate reducing and *methanogenic bacteria for* H_2 *under resting and growing conditions*. Arch. Microbiol. 137: 26-32.

Rudd J.W.M. and Hamilton R.D. (1978) Methane cycling in a eutrophic shield lake

and its effects on whole lake metabolism. Limnol. Oceanogr. 23: 337-348.

Rudd J.W.M. and Taylor C.D. (1980) Methane cycling in aquatic environments. Advances in Aquatic Microbiology: 77-150.

Ryding S.O. and Forsberg C. (1977) Sediments as a nutrient source in shallow polluted lakes. In: Interactions between sediments and freshwater. Ed. H.L. Golterman pp 227-234, W. Junk BV Publishers, The Hague.

Sas H. (1989) Lake restoration by reduction of nutrient loading. Academia Verlag, Richarz.

Schindler D.W. (1977) Evolution of phosphorus limitation in lakes. Science 195: 260-262.

Schreurs W. (1978) An automated colorimetric method for the determination of dissolved organic carbon in sea water by U.V. destruction. Hydrobiol. Bull. 12: 137-142.

Sepers A.B.J. (1982) The enumeration of heterotrophic bacteria in aquatic environments on simple and complex media. Int. Rev. ges. Hydrobiol. 67: 567-573.

Sibanda H.M. and Young S.D. (1986) Competitive adsorption of humus acids and phosphate on goethite, gibbsite and two tropical soils. J.Soil Sci. 37:197-204.

Sinke A.J.C., Cornelese A.A. and Cappenberg T.E. (1991) Phosphatase activity in sediments of the Loosdrecht lakes. Verh.Internat.Verein.Limnol. 24: 719-721.

Sojo L.E. and De Haan H. (1991) Multicomponent kinetic analysis of iron speciation in humic Lake Tjeukemeer: Comparison of fulvic acid from the drainage basin and lake water samples. Environ. Sci. Technol. 22: 935-939.

Søndergaard M. (1988) Seasonal variations in the loosely sorbed fraction of the sediment of a shallow and hypereutrophic lake. Environ. Geol. Water Sci. 1: 115-121.

Søndergaard M. (1989) Phosphorus release from a hypertrophic lake sediment: Experiments with intact sediment cores in a continuous flow system. Arch. Hydrobiol. 116: 45-59.

Søndergaard M., Kristensen P. and Jeppesen E. (1992) Phosphorus release from resuspended sediment in the shallow and wind exposed Lake Arreso, Denmark. Hydrobiologia 228: 91-99.

Søndergaard M., Jeppesen E. and Sortkjaer O. (1987) Lake Søbygaard: A shallow lake in recovery after a reduction in phosphorus loading. GeoJournal 14: 381-384.

Spratt Jr H.G., Morgan M.D. and Good R.E. (1987) Sulfate reduction in peat from a New Jersey pinelands cedar swamp. Appl. Environ. Microbiol. 53: 1406-1411.

Stumm W., Kummert R. and Sigg L. (1980) A ligand exchange model for the

adsorption of inorganic and organic ligands at hydrous oxide interfaces. Croatica Chemica Acta 53: 291-312.

Stumm W. and Morgan J.J. (1981) Aquatic Chemistry, 2nd edition John Wiley & Sons inc., New York.

Sundby B., Anderson L.G., Hall P.O.J., Iverfeldt A., Rutgers van der Loeff M.M. and Westerlund S.F.G. (1986) The effect of oxygen on release and uptake of cobalt, manganese, iron and phosphate at the sediment-water interface. Geochim. Cosmochim. Acta 50: 1281-1288.

Suter D., Banwart S., Stumm W. (1991) Dissolution of hydrous iron (III) oxides by reductive mechanisms. Langmuir 7: 809-813.

Svensson B.H. (1986) Methane as part of the carbon mineralization in an acid tundra mire. In: Perspectives in Microbial Ecology, Proceedings IV. Ed. F. Megusar and M. Gantar. pp 611-616, Slovene Society for Microbiology.

Sweerts J-P.R.A. (1990) Oxygen consumption, mineralization and nitrogen cycling at the sediment-water interface of north temperate lakes. PhD thesis Rijksuniversiteit Groningen.

Sweerts J-P.R.A. and Cappenberg T.E. (1988) Use of microelectrodes for measuring oxygen profiles in lake sediments. Arch. Hydrobiol. Beih. 31: 365-371.

Sweerts J-P.R.A., Dekkers T.M.J. and Cappenberg T.E. (in press) Methane oxidation at the sediment-water interface of a shallow eutrophic lake (Lake Loosdrecht) and a deep meso-eutrophic lake (Lake Vechten). Arch.Hydrobiol.

Tarapchak S.J., Bigelow S.M. and Rubitschun C. (1982) Overestimation of orthophosphorus concentrations in surface waters of southern Lake Michigan: effects of acid and ammonium molybdate. Can.J.Fish.Aquat.Sci. 39:296-304.

Ter Braak C.J.F. (1987a) CANOCO- a FORTRAN program for canonical ordination by (partial) (detrended) (canonical) correspondence analysis, principal component analysis and redundancy analysis. TNO Institute of Applied Computer Sciences, Statistics Department, Wageningen.

Ter Braak (1987b) Ordination. In: Data Analysis in Community and Landscape Ecology. Eds. R.H. Jongman, C.J.F. Ter Braak and O.F.R. Van Tongeren. Pudoc, Wageningen.

Tessenow U. (1972) Solution, diffusion and sorption in the upper layer of lake sediments. Arch. Hydrobiol. 4 (Supp. 38): 353-398.

Theis T.L. and McCabe P.J. (1978) Phosphorus dynamics in hyper eutrophic lake sediments. Water Research 12: 677-685.

Tipping E. (1984) Temperature dependance of Mn(II) oxidation in lake waters: A test of biological involvement. Geochim. Cosmochim. Acta 48: 1353-1356.

Tipping E., Thompson D.W. and Woof C. (1989) Iron oxide particulates formed by the oxygenation of natural and model lakewaters containing Fe(II). Arch.

Hydrobiol. 115: 59-70.

Ulen B. (1978a) Seston and sediment in Lake Norvikken I: Seston composition and sedimentation. Schweiz. Z. Hydrobiol. 40: 262-286.

Ulen B. (1978b) Seston and sediment in Lake Norvikken II: Aerobic decomposition of algae. Schweiz. Z. Hydrobiol. 40: 104-118.

Ulen B. (1978c) Seston and sediment in Lake Norvikken III: Nutrient release from sediment Schweiz. Z. Hydrobiol. 40: 287-305.

Van Brummelen T.C. (1988) Fosfaatafgifte en bakteriële aktiviteit van sediment in de Loosdrechtse Plassen. Wol rapport 1988-5 (in dutch).

Van Der Zee S. (1988) Transport of reactive contaminants in heterogeneous soil systems. Thesis Wageningen Agricultural University.

Van Donk E. and Gulati R.D. (1988) Biomanipulation in the Netherlands: Applications in fresh-water ecosystems and etuarine waters. Hydrobiol.Bull.23:1-226.

Van Liere L. (1986) Loosdrecht lakes, origin, eutrophication, restoration and research programme. Hydrobiol. Bull. 20: 9-15.

Van Liere L. Parma S., Mur L.R., Leentvaar P. and Engelen G.B.(1984) Loosdrecht lakes restoration project, an introduction. Verh.Int.Verein.Limnol.22: 829-834.

Van Liere L., Van Ballegooijen L., De Kloet W.A., Siewertsen K., Kouwenhoven P. and Aldenberg T. (1986) Primary production in the various parts of Loosdrecht lakes. Hydrobiol. Bull. 20: 77-85.

Van Liere L., Gulati R.D., Wortelboer F.G. and Lammens E.H.R.R. (1990a) Phosphorus dynamics following restoration measures in the Loosdrecht Lakes, The Netherlands. Hydrobiologia 191: 87-95.

Van Liere L., Van Tongeren O.F.R., Breebaart L. and Kats W. (1990b) Trends in chlorophyll and total phosphorus in Loosdrecht lakes, The Netherlands. Verh. Internat. Verein. Limnol. 24: 707-710.

Van Oorschot M.M.P. (1990) the role of the vegetation in nutrient removal from water. The Utrecht Plant Ecology News Report 11: 64-85 (in dutch).

Vollenweider R.A. and Kerekes J. (1982) Eutrophication of waters, monitoring, assessment and control. OECD Paris.

Wentzel M.C., Dold P.L., Ekama G.A. and Marais, G.v.R. (1985) Kinetics of biological phosphorus release. Wat. Sci. Tech. 17: 57-71.

Winfrey M.R. and Zeikus J.G. (1977) Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. Appl. Environ. Microbiol. 33: 275-281.

Winfrey M.R. and Zeikus J.G. (1979) Anaerobic metabolism of immediate methane precursors in Lake Mendota. Appl. Environ. Microbiol. 37: 244-253.

Yamamoto S., Alcauskas J.B. and Crozier T.E. (1976) Solubility of methane in

distilled water and seawater. J. Chem. Eng. Data 21: 78-80.

Young T.C. and Comstock W.G. (1986) Direct effects and interactions involving iron and humic acid during formation of colloidal phosphorus. In: Sediment and Water Interactions Ed. P.G. Sly. pp 461-470, Springer Verlag, New York

Zehnder A.J.B. and Brock T.D. (1980) Anaerobic methane oxidation: occurence and ecology. Appl. Environ. Microbiol. 39: 194-204.

SAMENVATTING

De Loosdrechtse Plassen.

De Loosdrechtse Plassen vormen een groot plassen gebied vlakbij Utrecht. Vooral 's zomers is er veel rekreatie en kun je duizenden plezierjachten, zeilboten en surfplanken aantreffen. De plassen zijn voedselrijk en er groeien veel algen in. Het water is troebel; als je er je arm insteekt, kun je je hand niet meer zien. In de zeventiger jaren werd vastgesteld dat er beheersmaatregelen genomen moesten worden om de waterkwaliteit te verbeteren. Die maatregelen betroffen het aanleggen van riolering in de dorpen rond de plassen en het laten instromen van schoon water in de zomer. In 1979 werd de Werkgroep Waterkwaliteits Onderzoek Loosdrechtse plassen opgericht, kortweg WOL genoemd. WOL wilde de effekten van de beheersmaatregelen op de waterkwaliteit wetenschappelijk onderzoeken, zodat kennis en ervaring ook voor andere meren kon worden gebruikt.

Fosfaat.

De sterke algengroei is veroorzaakt door de enorme hoeveelheid fosfaat die de afgelopen decennia in het water terecht is gekomen. Een duur woord hiervoor is eutrofering. De fosfaten worden via de riolen op het Nederlandse oppervlaktewater geloosd. Ondanks het feit dat we in Nederland nu allemaal fosfaat-arme wasmiddelen gebruiken, blijft de waterkwaliteit van de meeste meren en sloten nog erbarmelijk slecht. Als fosfaat eenmaal in een meer aanwezig is, is het lastig om er weer vanaf te raken.

De onderwaterbodem.

De in de Loosdrechtse Plassen uitgevoerde beheersmaatregelen hebben ertoe geleid dat fosfaatbelasting is gedaald met een faktor 3 (van 1.1 tot 0.4 milligram per vierkante meter per jaar). Het water bleef echter troebel. De tegenvallende resultaten kunnen voor een deel het gevolg zijn van het feit dat fosfaten langzaam vrijkomen uit de onderwaterbodem. Een grote hoeveelheid fosfaat in de Loosdrechtse Plassen is gebonden in de onderwaterbodem: meer dan 95 % van de totale hoeveelheid. In principe is dit voldoende om het water nog vele jaren troebel te houden.

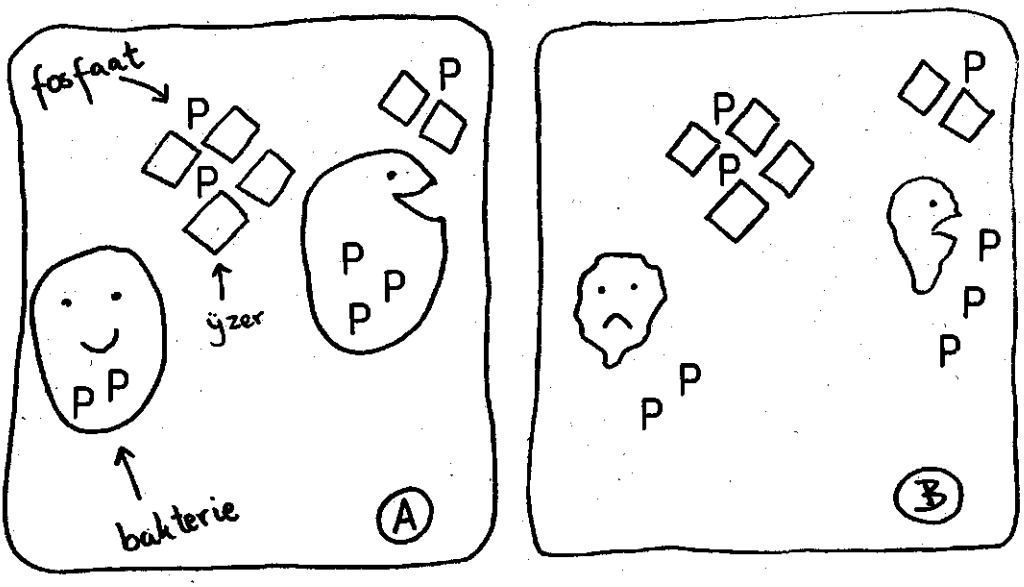
Het gedrag van fosfaat in de onderwaterbodem wordt bepaald door chemische en biologische processen. In het onderzoek van dit proefschrift is vooral gekeken naar het belang van bakteriën in de onderwaterbodem voor de fosfaatkringloop.

Bakteriën in de onderwaterbodem.

Bakteriën zijn belangrijk voor de fosfaatafgifte door de bodem maar ze kunnen ook een rol spelen bij het vasthouden van fosfaat. Dat vasthouden is, voorzover we

weten, alleen maar een tijdelijk proces.
Omdat bakteriën zo klein zijn - er kunnen er circa een miljard in een speldeknop -, worden de processen die ze uitvoeren, mikrobiologische processen genoemd. Het belang van mikrobiologische processen voor de afgifte van fosfaat is onderzocht door normale onderwaterbodemkolommen te vergelijken met kolommen waarin alle bakteriën gedood waren. De mikrobiologische processen bleken zowel op korte termijn als op lange termijn van groot belang voor de fosfaatafgifte door de onderwaterbodem.

Korte termijn opname en afgifte van fosfaat door bakteriën.
Op korte termijn bleken bakteriën de fosfaatafgifte door de onderwaterbodem drastisch te kunnen beïnvloeden. Dit werd in verband gebracht met de groei van bakteriën en de daardoor bewerkstelligde veranderingen in het chemische milieu. In het bovenste laagje van de onderwaterbodem, dat maar 1.5 tot 5 millimeter dik is, is zuurstof aanwezig. In dat laagje bevinden zich bakteriën die fosfaat kunnen opnemen (figuur 1A). In datzelfde laagje is ook ijzer aanwezig dat fosfaat kan binden.
Om verschil te kunnen zien tussen de fosfaatopname door bakteriën en door ijzer, werd een nieuwe methode ontwikkeld. Door kleine hoeveelheden van het bovenste laagje hard te schudden met een zure oplossing kon alle aan ijzer gebonden fosfaat worden opgelost. Het fosfaat in de bakteriën bleef echter vastzitten. De schudmethode werd getest met vers gemaakt ijzer-fosfaat en met een bepaalde bakterie soort: Acinetobacter 210 A. Uit onze proeven bleek dat de bakteriën en het ijzer met elkaar konkurreren om het fosfaat, dat opgelost in het water aanwezig is, op te kunnen nemen.
Vervolgens werden in de Loosdrechtse Plassen op verschillende punten monsters genomen van de onderwaterbodem en werd het bovenste laagje onderzocht. Het bleek dat door het bovenste laagje onderwaterbodem veel fosfaat uit het water kon worden verwijderd: 0.1-1.5 milligram fosfaat per gram droge bodem. De bijdrage van de bakteriën daaraan varieerde van 12 tot 30%, de rest zat aan het ijzer vast. De groei van de bakteriën konden we stimuleren door deze wat extra mest in de vorm van koolstof en stikstof te geven. Dat koolstof kon azijnzuur zijn maar bijvoorbeeld ook moerasgas (methaan). Als de omstandigheden verslechterden - zodat de bakteriën minder te eten kregen of er minder zuurstof aanwezig was - werd het fosfaat weer losgelaten (figuur 1B). Op de korte termijn zijn deze mikrobiologische fosfaatopname en -afgifte dus belangrijke processen die schommelingen kunnen veroorzaken in de hoeveelheid fosfaat die in het water is opgelost.



Figuur 1A: De vastlegging van fosfaten (P) aan ijzer en in bakteriën in het bovenste zuurstof-houdende laagje van de onderwaterbodem.
Figuur 1B: De afgifte van fosfaten door bakteriën bij het slechter worden van de omstandigheden.

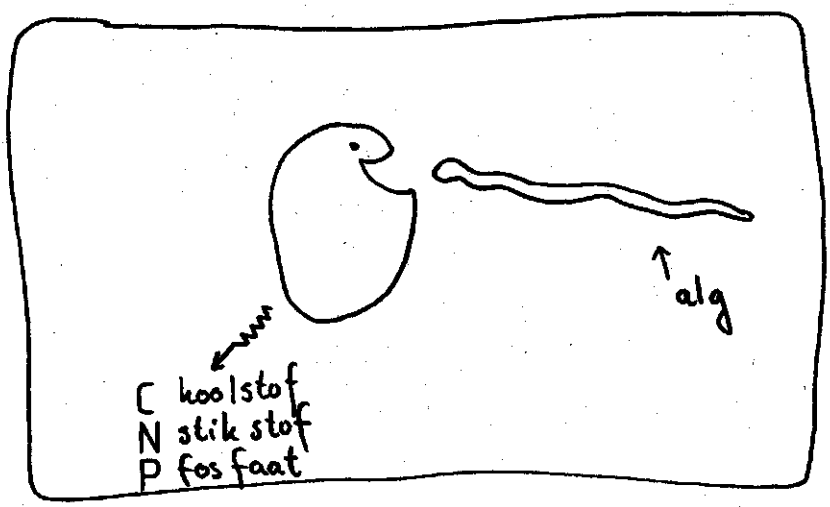
Lange termijn afgifte van fosfaat door bakteriën.

Bakteriën zijn ook in staat organisch materiaal, zoals dode algen, langzaam af te breken (figuur 2). Door dit mikrobiologisch proces kunnen fosfaten die eerst waren opgegeten door de algen en waren vastgelegd in de onderwaterbodem, weer vrijkomen. Omdat we vermoedden dat deze afbraak belangrijk was voor de fosfaatafgifte, moesten we meer in de onderwaterbodem gaan kijken. Uit de samenstelling van het bodemvocht valt, op een indirekte manier, veel informatie te halen over de processen die zich daar afspelen. Door op verschillende diepten in de onderwaterbodem de hoeveelheden opgelost fosfaat in het bodemvocht te meten, kon worden berekend hoeveel fosfaat er uit de onderwaterbodem naar het water kan worden afgevoerd.

In perioden dat er grote hoeveelheden fosfaat in het bodemvocht aanwezig waren, bleek dat ook veel stikstof (ammonium) en koolstof (methaan) te bevatten. Omdat deze stoffen allemaal worden vrijgemaakt bij de afbraak van organisch materiaal, duidde dit op het belang van mikrobiologische processen. Deze indruk werd versterkt toen bleek dat de hoeveelheden fosfaat, ammonium en methaan erg hoog waren in het najaar wanneer er veel algen uit het water op de onderwaterbodem bezinken.

Alleen de bovenste 4 centimeter van de onderwaterbodem speelde een rol in de

fosfaatkringloop. Op grotere diepten in de onderwaterbodem kon nauwelijks afbraak van het organisch materiaal worden gemeten. Dat komt vermoedelijk doordat het oorspronkelijke, dieper gelegen veen al circa 7000 jaar oud is.



Figuur 2: De afbraak van algen in de onderwaterbodem door bakteriën waarbij koolstof (C), stikstof (N) en fosfaten (P) vrijkomen.

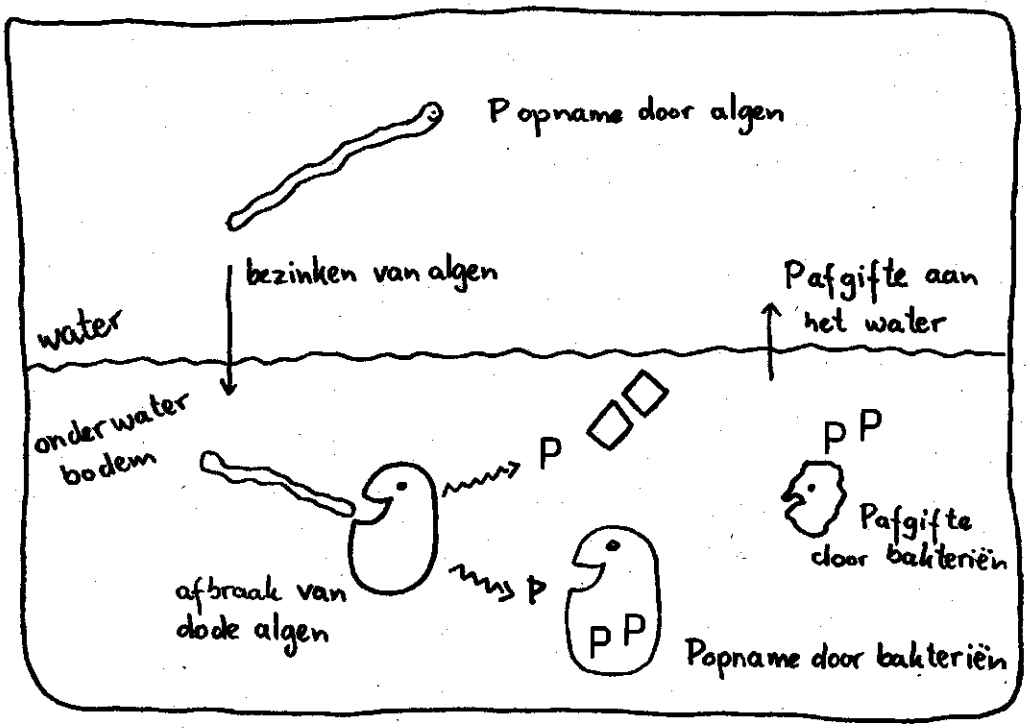
Worden de Loosdrechtse Plassen weer helder ?

Uit het onderzoek kwam uiteindelijk vast te staan dat een relatief kleine hoeveelheid fosfaten in een cirkel ronddraait (figuur 3): opname van fosfaten door algen, bezinken van die algen, afbraak ervan, tijdelijke vastlegging van fosfaten door bakteriën en tot slot weer fosfaatafgifte uit de onderwaterbodem. Die cirkel is vermoedelijk amper te doorbreken.

Om iets te kunnen zeggen over de toekomst van de Loosdrechtse Plassen werden de resultaten van dit onderzoek vergeleken met andere gegevens uit het totale WOL-projekt. Het is technisch goed mogelijk om het fosfaatgehalte in het water weer omlaag te krijgen. Als we de hoeveelheid fosfaat die er (nog steeds) instroomt halveren dan neemt ook de hoeveelheid fosfaat in het water af. Berekeningen geven aan dat dat proces ongeveer 10 jaar kost.

Echter, het blijkt dat door de eutrofiering allerlei veranderingen in de plassen zijn opgetreden. Zo groeiden er vroeger waterplanten in en waren er andere soorten algen aanwezig. Het is eigenlijk niet te voorspellen of de in de planten- en dierenwereld aangerichte schade nog valt te herstellen.

De eindkonklusie is dat het heel moeilijk zal zijn om de Loosdrechtse Plassen net zo helder te krijgen als ze in 1930 waren. Dat zal bovendien veel geld en tijd kosten.



Figuur 3: Een schematische voorstelling van de fosfaatkringloop in de Loosdrechtse Plassen.

SUMMARY

Since the sixties eutrophication has been recognized to affect the quality of surface waters. The prolonged loading with nutrients has led to high algal concentrations in the water and to concommitant environmental problems such as the depletion of oxygen, the production of toxins and the development of tedious animal populations e.g. bream. A series of management measures have been carried out to combat eutrophication in the Netherlands. However, despite all efforts, the majority of the dutch surface waters is still considered to be eutrophic. The disappointing results are often attributed to processes in the sediment that can delay the improvement of the water quality. This thesis deals with the phosphorus dynamics in the sediment of an eutrophic lake. In contrast to most studies which are chemically orientated, attention here is mainly given to the role of microbial processes in the phosphorus cycle. The research was performed in eutrophic Lake Loosdrecht where the external phosphorus loading was recently decreased from 35-40 to 10-15 $mmol.m^{-2}.y^{-1}$.

The importance of microbial processes for the release of phosphate by the sediment was investigated by comparing sterilized and non-sterilized columns (chapter 2). Columns were sterilized by γ-irradiation (25 kGy). The combination of temperature and γ-irradiation experiments made it possible to distinguish between microbial and physico-chemical processes. The release of dissolved phosphate from the sediment is controlled by microbial processes on a short-term and a long-term basis. Microbial mediated release responds directly on an increase in temperature. This is probably due to the induction of changes in the chemical environment such as a decrease in oxygen content of the surface layer. Mineralization of organic matter results in a mobilization of phosphate and is prerequisite to sustain the phosphate release on a long-term basis.

Phosphate fluxes over the sediment-water interface were calculated using measured concentration gradients in the pore water and were compared to fluxes measured under laboratory conditions (chapter 3). Results were analysed with a statistical method (Redundancy Analysis) to detect patterns of variation in pore water chemistry and in measured and calculated fluxes, that could be ascribed to environmental variables. Initial fluxes of phosphate measured in sediment columns, which varied between -7.7 and 1330 $\mu mol.m^{-2}.d^{-1}$, correlated significantly with the calculated fluxes over the sediment-water interface. The high correlation between calculated fluxes of ammonia, phosphate and methane and measured initial flux of phosphate, conclusively pointed to mineralization of organic matter as driving force for phosphate release from the sediment. Redundancy Analysis demonstrated that the rates of mineralization and of phosphate release are high in autumn. This was ascribed to an increased sedimentation at the end of the growing season.

The importance of anaerobic mineralization processes fluctuated seasonally (chapter

4). At high anaerobic mineralization rates (> 600 μmol organic carbon.m^{-2}.h^{-1}), sulfate reduction was limited by sulfate and methanogenesis accounted for over 80% of the total. At low anaerobic mineralization rates, measured in winter and spring, sulfate reduction was predominant. There was little methanogenesis below 5 cm depth in the sediment which indicated a rapid decrease of degradable organic matter with depth.

A new method was develloped to quantify the contribution of bacterial processes to the phosphate uptake of aerobic freshwater sediment (chapter 5). The method was tested on iron hydroxyphosphate that was either synthesized or formed under in situ conditions, and a pure culture of Acinetobacter 210 A. Using a mild acid extraction we could distinguish between chemical and biological phosphate uptake. This method allowed the solubilization of the entire iron hydroxyphosphate fraction but did not extract bacterial phosphate.

The method was applied to determine the contribution of bacterial processes to the phosphate uptake of the surface sediment. Phosphate uptake of randomly sampled surface layers of the sediment was considerable and ranged from 12 to 138 μmol.g^{-1} on a dry weight basis. Phosphate uptake was correlated positively with the amount of extractable iron and phosphate and negatively with dry weight. The contribution of bacterial processes ranged from 12 to 32 %. Addition of an easily degradable substrate, such as acetate, to the sediment stimulated the uptake of phosphate and augmented the biologically bound phosphate fraction.

A diffusion chamber was designed to investigate the effect of an enhanced oxygen consumption of the surface sediment on the phosphate flux (chapter 6). The diffusion chamber consisted of two compartments separated by a Teflon membrane. In the upper part a thin sediment layer was present and the lower part was continuously flushed with gas. The hydrophobic membrane allowed for diffusion of gasses from the lower part through the sediment layer towards the headspace of the upper part. In the diffusion chambers the methane oxidation was artifically increased to 9.8 mmol.m^{-2}.d^{-1}. This resulted in an increase of the oxygen consumption rate by a factor two compared to controls without methane oxidation (8.6 vs 17.7 mmol.m^{-2}.d^{-1}). The methane oxidation significantly decreased the oxygen penetration depth (2.5-4.0 vs 1.0-2.0 mm). However, despite the shrinkage of the oxidized microlayer, no differences were found in phosphate flux over the sediment water interface. Batch experiments with standard additions of methane revealed that the growth of methanotrophic bacteria contributes to the phosphate uptake of aerobic sediment. Results indicated that a decrease in chemical phosphate adsorption caused by a decease in the oxygen penetration depth, could be compensated for entirely by the growth of methanotrophic bacteria.

Finally it was concluded that the eutrophic conditions in Lake Loosdrecht are maintained by a relatively small but dynamic pool of phosphate (chapter 7).

Calculations with a simple model indicated that restoration measures such as dredging or addition of iron(III) compounds will not result in a long-term improvement of the water quality. The construction of isles and dikes might induce a decrease in turbulence and thus contribute to an improvement of the water quality. A further reduction of the external phosphorus loading is prerequisite to reduce the amount of phosphorus compounds in the water. However, the changes in the structure of the ecosystem that have been induced by the eutrophication might be irreversible. Whether Lake Loosdrecht can be restorated into a clear water system dominated by macrophyts depends on ecological interactions between bacteria, algae, zooplankton and fish in the ecosystem but is impossible to predict.

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Dit onderzoek is uitgevoerd op het Centre for Limnology van het Nederlands Instituut voor Oecologisch Onderzoek, wat toen nog gewoon Limnologisch Instituut heette. Begin 1985 ben ik daar als doktoraalstudent met een vak begonnen. Dit mondde uit in aanstellingen die werden betaald door de Europese Commissie (EG), Stichting voor de Technische Wetenschappen (STW), Provinciale Waterstaat Utrecht (PWU) en de Koninklijke Nederlandse Akademie van Wetenschappen (KNAW). Het aantal onderzoeksthema's en het aantal medische keuringen hield gelijke trend. In december 1987 belandde het onderzoek in wat veiliger vaarwater: één mooi kontrakt voor 2x2 jaar én een duidelijk omschreven onderzoeksplan. Het geheel werd gefinancieerd door de Programma Commissie Basisonderzoek Bodem (PCBB), die ik daarvoor zeer erkentelijk ben.

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CURRICULUM VITAE

Anja Sinke werd geboren op 6 juni 1960 te Middelburg en kreeg als officiële namen Jacoba Corstina mee. Na het doorlopen van het Montessori basisonderwijs bezocht ze van 1972 tot 1978 het Stedelijk Gymnasium te Utrecht. Vanaf 1978 studeerde ze biologie aan de Rijks Universiteit te Utrecht. Na het behalen van het kandidaats examen werd de studie voortgezet aan de Landbouw Hogeschool te Wageningen. Tijdens de doktoraalfase heeft ze onderzoekservaring opgedaan op de vakgebieden mikrobiologie, planten-ekologie en planten-fysiologie. Daarnaast behaalde ze haar onderwijsbevoegdheid. Na het afstuderen in 1985 startte ze als onderzoeker bij het Limnologisch Instituut te Nieuwersluis. In december 1987 werd ze aldaar aangesteld voor een vierjarig promotie onderzoek naar de rol van mikrobiologische processen in de fosfaat uitwisseling tussen water en bodem. De resultaten van dit onderzoek zijn beschreven in dit proefschrift.