NITROGEN FIXATION (ACETYLENE REDUCTION) IN THE SEDIMENTS OF THE PLUSS-SEE

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NITROGEN FIXATION (ACETYLENE REDUCTION) IN THE SEDIMENTS OF THE PLUSS-SEE

With special attention to the role of sedimentation

PROEFSCHRIFT

ter verkrijging van de graad van
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STELLINGEN.

1. Het vóórkomen van stikstofbinding in sediment met hoge ammonium-concentraties kan verklaard worden door de aanwezigheid van micro-milieus met lage concentraties opgelost ammonium.

2. De mate waarin de stikstofbinding in het sediment van belang is voor de stikstofhuishouding van een meer hangt direct samen met de efficiëntie van dit proces.

3. Als men geïnteresseerd is in natuurlijke omzetsnelheden van suikers in sediment, moeten volgens Fleischer methoden waarbij radioactieve tracers worden toegevoegd aan verdunde en geroerde sedimentmonsters met de nodige reserve beoordeeld worden. Evenveel reserve is nodig, als sedimentmonsters niet verdund en geroerd worden.

   Fleischer, S. (1975): Sugar turnover in lake water and sediment. -Verh. Int. Ver. Limnol. **19**: 2627-2635.

4. Het feit dat permanente accumulatie van sediment niet alleen optreedt op het diepste punt van een meer toont aan, dat bij de sedimentatie van gesuspendeerd materiaal in meren het "Trichter-effekt" als zodanig niet optreedt.

   Ohle, W. (1962): Der Stoffhaushalt der Seen als Grundlage einer allgemeinen Stoffwechseldynamik der Gewässer. - Kieler Meeresforschungen **18**: 107-120.
   Ohle, W. (1984): Measurement and comparative values of the Short Circuit Metabolism (SCM) of lakes by POC relationship of primary production of phytoplankton and settling matter. -Arch. Hydrobiol. Beih. Ergebn. Limnol. **19**: 163-174.

5. Het berekenen van de afbraak van organische stof uit verschillen in het organische-stofgehalte van gesuspendeerd materiaal en sediment kan leiden tot een aanzienlijke overschatting van de

accumulatie van permanent sediment in meren, indien geen rekening gehouden wordt met de reductie van de totale hoeveelheid droge stof ten gevolge van de afbraak.

> Kimmel, B.L. & Goldman, C.R. (1977): Production, sedimentation and accumulation of particulate carbon and nitrogen in a sheltered subalpine lake. -In: Interactions between sediments and fresh water, pp. 148-155, Ed. H.L. Golterman - Junk Publishers, Den Haag.

6. In het kader van de ontwikkeling van integraal waterbeheer verdient het aanbeveling in navolging van de normdoelstelling basiskwaliteit te komen tot een normdoelstelling basiskwantiteit.

7. Daar zoute kwel in het algemeen geen natuurlijk verschijnsel is, kan de natuurlijke verscheidenheid van soorten organismen en ecosystemen geen uitgangspunt zijn bij het formuleren van ecologische normdoelstellingen voor het brakke binnenwater.

8. Het is de vraag of de Voor-Delta zich zal ontwikkelen tot een waardevol natuurgebied, als dit niet samengaat met een drastische verbetering van de waterkwaliteit van de Schelde.

9. Het feit dat ook bij een kortere arbeidstijd volledige deskundigheid vereist is stelt grenzen aan de toepassing van arbeidstijdverkorting.

10. Gebrek aan effectiviteit van het ambtelijk apparaat wordt niet veroorzaakt door de luiheid van de ambtenaar, hoewel sommigen daar gemakshalve van uitgaan.

11. Om te voorkomen, dat bedrijven te afhankelijk worden van militaire produktie, dient de overheid bedrijven, waarbij militaire orders geplaatst worden of waaraan een vergunning tot export van militaire produkten wordt verleend, te verplichten een bepaald bedrag te besteden aan onderzoek naar produktiemogelijkheden in de civiele sector.

12. Om in sprookjes de gruwelijkheid van de boze wolf te benadrukken, wordt veelal verteld, dat hij in één hap zijn slachtoffers (grootmoeder, Roodkapje, geitjes, eend, Duimeling) naar binnen schrokt. Veel gruwelijker is het echter dit in meer dan één hap te doen.

Proefschrift van Tj.S. Blauw
Nitrogen fixation (acetylene reduction) in the sediments of the Pluss-See -with special attention to the role of sedimentation.
Wageningen, 5 juni 1987.

voor Heit en Mem
Marijke, Hylke Merijn,
Jurre en Sanne Lise

CONTENTS

1. Introduction.

In the present investigation the significance of heterotrophic nitrogen fixation in the sediments for the nitrogen economy of the Pluss-See has been studied, as well as the factors controlling this process *in situ*. Special attention has been paid to the role of organic matter supply and ammonium.

During the last two decades biological nitrogen fixation has been the subject of intensive scientific research, especially after the development of the acetylene reduction assay (STEWART *et al.*, 1967). Since then this process has been studied in almost all types of ecosystems (cf. BURNS and HARDY, 1975; QUISPEL, 1974; STEWART, 1969, 1973). Studies of nitrogen fixation in aquatic ecosystems were, at least in the beginning, mainly focused on the water itself, especially on the role of nitrogen-fixing blue-green algae (e.g. GRANHALL and LUNDGREN, 1971; HORNE and FOGG, 1970; HORN and GOLDMAN, 1972; RUSNESS and BURRIS, 1970; STEWART *et al.*, 1968; STEWART, 1973). To a minor degree attention has been paid to nitrogen fixation in the sediments of aquatic ecosystems. The early studies concerned mainly the distribution and enumeration of nitrogen-fixing microorganisms (e.g. KUZNETSOV, 1970; NIEWOLAK, 1970) and the rate of nitrogen fixation (BREZONIK and HARPER, 1969; BROOKS *et al.*, 1971; KEIRN and BREZONIK, 1971; Mc GREGOR *et al.*, 1973). Several authors studied the effect of adding various compounds to the sediments on the acetylene-reducing activity (HERBERT, 1975; KEIRN and BREZONIK, 1971; PATRIQUIN and KNOWLES, 1975; HANSON, 1977; SYLVESTER-BRADLEY, 1976). Less attention has been paid to the factors that control heterotrophic nitrogen fixation in the sediments under natural conditions (JAEGER and WERNER, 1977; OLAH *et al.*, 1983; MACKENZIE, 1984).

Nitrogen fixation is a highly endergonic process. In contrast with photosynthetic nitrogen-fixing microorganisms, heterotrophic nitrogen fixers depend on the availability of exogenous organic substrate such as carbohydrates, alcohols and organic acids. Heterotrophic microorganisms need relatively large quantities of organic substrate for the nitrogen-fixing process. The efficiency of anaerobic bacteria of the *Clostridium pasteurianum* type is maximally 10 mg N fixed per g of sugar consumed but mostly less (MULDER and BROTONEGORO, 1974). Under

micro-aerophilic conditions the efficiency of *Azotobacter chroococcum* may amount to 46.5 mg N_2 fixed per g of glucose consumed (MULDER, 1966). An inverse relationship between the efficiency of nitrogen fixation and the concentration of glucose added to anaerobic soil systems has been observed by O'TOOLE and KNOWLES (1973). They suggested that efficiency in natural anaerobic systems may be very high, because under natural conditions carbohydrates become continuously available in small quantities. So far no attempts have been made to measure nitrogen fixation efficiency under natural conditions.

The nitrogen fixation by free-living heterotrophic microorganisms in soil systems is supposed to be limited by the availability of organic substrate (STEWART, 1969). This assumption is supported by the observation of high numbers of nitrogen fixing microorganisms and high rates of nitrogen fixation in the rhizosphere of higher plants in terrestrial and aquatic habitats where root excretions are available (BRISTOW, 1974; DOMMERGUES *et al.*, 1973; PATRIQUIN and KNOWLES, 1972; DÖBEREINER, 1968; YOSHIDA and ANCAJAS, 1973) and by the stimulation of nitrogen fixation by the addition of carbohydrates (HANSON, 1977; KNOWLES and DENIKE, 1974; O'TOOLE and KNOWLES, 1973; PATRIQUIN and KNOWLES, 1975; SYLVESTER-BRADLEY, 1976).

Profundal lake sediments are often rich in organic matter, but this organic matter consists partly of refractory substances. No relation has been found between acetylene-reducing activity and the total organic matter content of the lake sediments (OLAH *et al.*, 1983). So far no attention has been paid to the relation between the readily decomposable part of sedimentary organic matter and the acetylene-reducing activity in lake sediments.

The ammonium concentration of the interstitial water of lake sediments is rather high. Ammonium is known to repress the synthesis of nitrogenase (BROTONEGORO, 1974; DAESCH and MORTESON, 1972). The simultaneous occurrence of relatively high rates of acetylene reduction and high concentrations of ammonium in lake sediments is still not clarified. BROOKS *et al.*, (1971) and KEIRN and BREZONIK (1971) suggested, that a large part of the ammonium is immobilized by adsorption to sediment particles. This is supported by the observation of KNOWLES and DENIKE (1974) that the ammonium concentration below which nitrogenase is derepressed depends on the organic matter

content of the system. Ammonium has been shown to be adsorbed preferentially by organic matter (ROSENFELD, 1979). In contrast to the supposed repressing effect of ammonium, JAEGER and WERNER (1977) observed a positive correlation between the ammonium content and the acetylene-reducing activity of the sediments of Harkortsee (FRG).

No measurements of the nitrogen fixation rates in the Pluss-See have been performed so far. HÄLKE (1971) studied the distribution of *Azotobacter* species in the lake. From the low numbers in the pelagic region of the lake he concluded that nitrogen input due to nitrogen fixation is mainly mediated by blue-green algae. So far the role and the activity of anaerobic heterotrophic nitrogen fixers have not been studied.

In chapter 3 the application of the acetylene reduction assay to lake sediments is evaluated. Special attention is paid to the saturation of nitrogenase with acetylene. In chapter 4 the effect of the addition of carbohydrates and ammonium to sediment samples on the acetylene-reducing activity is discussed. In chapter 5 the relation between temperature and acetylene reduction in sediments is described.

In chapter 6 the seasonal and spatial fluctuation of the acetylene-reducing activity in the sediments are discussed in relation to several sediment characteristics. In chapter 7 attention is paid to the relation between acetylene reduction and the uptake and consumption of glucose by the microbial population in the sediments.

In chapter 8 the processes that control the supply of organic matter to the sediments and their relation to the acetylene-reducing activity and the sediment composition are described. In chapter 9 an estimation of the ammonium transport through the sediment-water interface is given. In chapter 10 the quantitative role of heterotrophic nitrogen fixation for the nitrogen economy of the sediments and the whole lake is discussed.

Finally in chapter 11 some general conclusions of this investigation are discussed.

2. Material and methods.

2.1. Description of the study area.

The investigations of the nitrogen fixation in the sediments were focussed on the Pluss-See, a small kettle lake 5 km north of the town of Plön (FRG). The surface area is 142905 m^2, the maximum depth (z_m) of the lake is 29 m and the mean depth 9.42 m. The shore line development of the lake is 1.05, pointing to the almost circular form of the lake surface. The relative depth (z_r), i.e. the maximum depth as a percentage of the mean diameter, is 6.8, which means that the surface area is relatively small compared to the maximum depth of the basin. The Pluss-See is surrounded by forested hills (mainly beech). The influence of the wind is therefore relatively small. The lake is dimictic with the thermocline situated not far below the surface (about 4-5 m).

The lake is eutrophic. OHLE (1962) measured in the period from May till December 1960 a mean primary production of 1.05 $gC.m^{-2}.day^{-1}$. STABEL (unpublished results) measured in the period 1977-1978 an annual primary production of 208 $gC.m^{-2}$. In 1981 the annual primary production amounted to 187 $gC.m^{-2}$ (MEFFERT and OVERBECK, 1985b). Because of the anaerobic conditions in the hypolimnion during varying parts of the stratification period no macrofaunic species can be observed in the profundal sediments (ALSTERBERG, 1925).

UNGEMACH (1960) points to the special position of the Pluss-See sediments because of the low calcium sedimentation in the lake. Neither biogenic decalcification (important for gyttja sediments) nor sedimentation of calcium complexes with humic acids (important for dy sediments) is finding place. The sediments have dy properties (low calcium content and high organic matter content) as well as gyttja properties (low humic acid content and high nitrogen content). KOPPE (1924) classifies the sediments as a link between the calcium-rich, highly productive lakes and the dystrophic lakes.

The Pluss-See was formed as a kettle lake during the last glacial period of Würm, about 10,000 years ago. In the course of its existence a sediment layer of about 11 m thickness has been formed at the deepest part of the basin. AVERDIECK (1983) distinguishes several periods in the history of the lake; the last period from 1500 till

the present with a sediment growth of 2.34 mm/year. OHLE (1962) measured a relatively low sedimentation pointing to the very efficient decomposition of organic matter in the water column of the lake ("kurzgeschlossener Kreislauf"; OHLE, 1984).
Several aspects of the lake have been investigated during the past years. For detailed information the relevant publications should be consulted (e.g. ALBRECHT, 1973; GOLACHOWSKA, 1979; KRAMBECK, 1974; MEFFERT and OVERBECK, 1985 a + b; MUNSTER, 1985; OHLE, 1960, 1964, 1965, 1976, 1984; OVERBECK, 1971, 1972, 1975, 1982; SCHMIDT, 1977; STABEL and MUNSTER, 1977; UNGEMACH, 1960).

2.2. Sampling.

2.2.1. Sampling stations.

Apart from some incidental sampling elsewhere in the lake all samples have been taken at three locations in the lake (see figure 1):

a. in the littoral at 5 m water depth;
b. in the profundal at 15 m water depth, where the lake bottom shows a large inclination;
c. in the profundal at the deepest part of the lake at 29 m water depth.

2.2.2. Sampling of sediments and water.

Sediment core samples were taken with a Züllich sediment corer. The samples were immediately split up into fractions in order to prevent disturbance by gas bubbles developing in the sample as a result of the decreased hydrostatic pressure. After a subsample was taken from the water just above the sediment in the tube a sediment fraction of desired thickness was pushed out of the tube into another one placed above it. The two tubes were then separated by a thin steel plate and the sediment subsample was transferred to a plastic vial with screw cap as quickly as possible. The vials were filled completely with the sediments. The whole procedure was carried out as quickly as possible in order to avoid unnecessary contact of the sediments with the atmosphere. Transport of the sediment samples into the laboratory was carried out under ice.
Other water samples than those from the sediment core were taken by a Ruttner sampler and transported into the laboratory under ice in

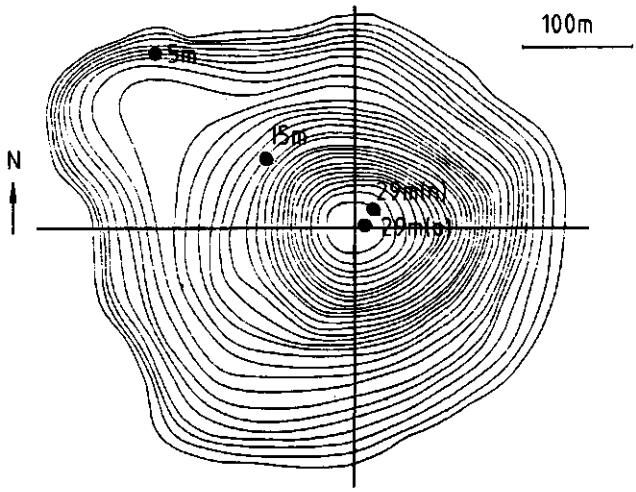

Figure 1. Bathymetric map of the Pluss-See (from KRAMBECK, 1974) with isobathes from 0 to 28 m and with the location of the sampling stations.

polyethylene bottles of appropriate volume.

2.3. Sediment analysis.

The temperature of the water directly in contact with the sediment surface was measured in the sampling tube as quickly as possible after the core sample was taken. The temperature of the surficial sediments was assumed to be equal to the temperature of the water just above the sediments. The pH was measured by injecting the electrode directly into the sediment subsamples.

Dry matter content of the sediment as a percentage of the wet weight was calculated from the loss of weight after 24 hours drying at 105°C. The organic matter content as a percentage of the dry matter content was calculated from the loss of weight after heating dried sediments during three hours at 530°C. The carbon and nitrogen contents as a percentage of the dry matter content were determined in a Carlo Erba Elemental Analyzer. As shown by Ungemach (1960) the sediments of the Pluss-See are low in inorganic carbon. This was confirmed by preliminary investigations. Therefore carbon content values

can be considered as organic carbon content values.

Interstitial water was collected by centrifuging sediment samples for 30 minutes at 10,000 rpm in a Heraeus Cryofuge 20-3 centrifuge. The supernatant was then filtrated through 0.2-μ Sartorius membrane filters. The filtrate was used for the determination of the interstitial ammonium, Kjeldahl-nitrogen, orthophosphate, totalphosphate and nitrate concentrations with a Technicon Autoanalyzer following the procedures normally used at the Max-Planck-Institut für Limnologie (ALBRECHT, 1973).

The pellet was dried for 24 hours at 105°C and homogenized using mortar and pestle. The thus dried and homogenized sediments were used for the determination of the carbon and nitrogen contents in a Carlo Erba Elemental Analyzer and for the determination of the exchangeably adsorbed ammonium content. The exchangeably adsorbed ammonium content of the sediments was measured in the following way: A preweighed amount of dried sediment was suspended into 1N KCl and shaken for 1 hour. The suspension was centrifuged (30 min.; 10.000 rpm) and the supernatant was filtrated through 0.2-μ Sartorius membrane filters after which the ammonium concentration of the filtrate was determined with a Technicon Autoanalyzer following the procedures of the Max-Planck-Institut für Limnologie (ALBRECHT, 1973). The measured concentration was converted to mg NH_4^+-N per gram dry sediment. This method was compared with the steam distillation procedure of KEENEY and BREMNER (1966) and turned out to give identical results.

2.4. Sedimentation.

Sedimentation traps were made after ZEITSCHEL *et al.* (1978), however, without the automatic tube-changing mechanism. The traps consisted of a PVC funnel (upper diameter 40 cm, length 64 cm) which was covered by a PVC lid with an opening of 20 cm diameter, corresponding to a sampling area of 310 cm^2. The lid was vaulted by 10° to reduce turbulent mixing at the outer rim of the funnel. The opening in the lid contained a grid of PVC slats to prevent turbulent motion of water entering the interior of the funnel. The segments of the grid were 1 x 1 cm at the top and 4 cm deep (ratio 1:4). At the lower end of the funnel a replaceable 100 ml centrifuge glass was installed. The walls of the collecting funnel were steep (21° to the vertical) and

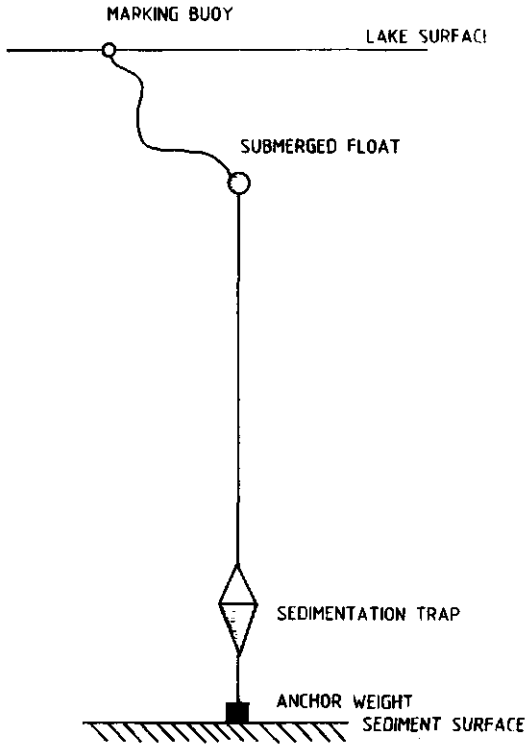

Figure 2. Installation of the sedimentation trap.

the inner walls were smooth to diminish the chance of particles adhering to the walls. A simple valve was built into the lower part of the funnel so that the water could escape from the inside of the funnel when the trap was pulled out of the water.

Traps were installed 1 m above the lake bottom by means of an anchor weight and a submerged float and were located by a marking buoy (see figure 2). Traps were exposed at 4 stations (see figure 1):

1. in the littoral where the water depth was 5 m;
2. in the profundal at the steep slope of the lake bottom where the water depth was 15 m;
3. in the profundal at the end of the steep slope where the water depth was 29 m (29N);
4. in the profundal 10 m separated from the end of the steep slope,

where the water depth was 29 m (290).

The traps were emptied every week except during a short period in the winter time when ice was covering the lake surface.

Dry matter content of the trapped suspension in the centrifuge glasses was determined by filtration of a known volume (2-4 ml) over a pre-heated (at 530°C) Whatman GF/C fibreglass filter and 24 hours of drying at 105°C. The organic matter content of the trapped material was measured by heating the dried filters during three hours at 530°C. The rest of the suspension was centrifuged, dried and used for the determination of the carbon and nitrogen contents (see 2.3). All values were converted to $g.m^{-2}.day^{-1}$ or $g.m^{-2}.year^{-1}$ of dry matter, carbon or nitrogen.

2.5. Acetylene reduction assay.

Sediment subsamples of 3.5 ml were brought into Hungate tubes (HUNGATE, 1969) (volume ca. 16 ml) by sterile plastic syringes. The exact weight of the subsample was determined by weighing the tube with and without the subsample. The volume of the head space of the tubes was measured by weighing the tube filled with water after termination of the assay. During all manipulations care was taken to avoid contact of the sediment with the air by gassing the tubes and the samples with oxygen-free helium gas. The tubes were closed with a septum and a screw cap. Through the septum an appropriate volume of C_2H_2 was brought into the tube by a syringe in order to establish the desired partial pressure. The tubes were shaken vigorously for 15 seconds after which the pressure inside the tubes was brought to equilibrium with the atmospheric pressure. After a pre-incubation period of 4 hours the C_2H_2 and the C_2H_4 concentrations were measured by injection of 100 µl from the tube head space into a Packard 427 gaschromatograph with FID-detection and a 160 cm glasscolumn filled with Porapak R. Carrier gas flow (N_2) was 30 ml/min., oven temperature 60°C, injection temperature 110°C and detection temperature 120°C. Under these conditions it could be shown that peak heights of ethylene and acetylene were linear with the concentration over at least 6 orders of magnitude.

As a standard procedure sediment subsamples were incubated at pC_2H_2 = 0.2 atm for 24 hours after a pre-incubation period of 4 hours.

Hungate tubes were incubated at *in situ* temperature and at a standard temperature of 27°C in a horizontal position to maximize the gas exchange between the sediment and the overlying atmosphere. In the horizontal position the maximum thickness of the sediment in the tube was 2.5 mm. At the beginning and the end of the incubation period the ethylene and the acetylene concentrations were measured in the headspace of the tubes. Before measurement the tubes were shaken to allow the system to establish equilibrium of gas concentration between the sediment and the overlying atmosphere. It could be shown that the production of ethylene in the tubes was linear with time for at least 50 hours. Apparently nitrogen fixers were not affected by acetylene during this period, although a shorter incubation time is recommended for this assay (HARDY *et al.*, 1973; DE BONT and MULDER, 1974).
Aerobic incubations were carried out either by leaving out the anaerobic gas flow in the described procedure or by injecting appropriate amounts of oxygen gas through the septum into the (till then) anaerobic tube.
For the investigation of the effect of several substances on the acetylene-reducing activity of the sediments, appropriate amounts of anaerobic solutions were injected through the septum into the tubes.
All measurements were carried out in triplicate. A fourth tube was fixed with 0.2 ml 30% formaline as a blank. Peak height of ethylene was converted to concentration by comparing with the peak produced by 100 µl of a standard gas mixture of 100 vpm ethylene in nitrogen gas (Messer Griesheim, Lübeck) several times during the measurements.

2.6. Kinetic parameters for the uptake of glucose.

The method for measuring the uptake kinetics of glucose was essentially the same as the one described by WRIGHT and HOBBIE (1965), apart from the fact that the sediment was diluted and the incubation was performed anaerobically. The method is based on the assumption that the uptake of organic solutes by natural heterogeneous populations obeys Michaelis-Menten kinetics:

$$v = \frac{V_m \cdot S}{K_t + S} \qquad (1), \text{ where:}$$

v = uptake velocity at a given substrate concentration S

V_m = maximum uptake velocity

K_t = transport constant, by definition the substrate concentration when $v = \frac{1}{2} V_m$.

The uptake of organic compounds can be measured by employing labelled substrates (PARSON and STRICKLAND, 1962):

$$v = \frac{c.f. \ (S_n + A)}{C\mu t} \qquad (2), \text{ where:}$$

v = rate of uptake ($\mu gC.l^{-1}.hr^{-1}$)

c = radioactivity of organisms (cpm)

f = correction factor for isotopic discrimination (is neglected in the present study, as suggested by PARSON and STRICKLAND, 1962)

S_n = in situ substrate concentration ($\mu g.l^{-1}$)

A = added substrate concentration ($\mu g.l^{-1}$)

C = cpm from 1 μCi of ^{14}C -labelled substrate

μ = quantity of ^{14}C added to the sample (μC_i)

t = incubation time (hours).

Transforming (1) into the Lineweaver-Burk equation and combining with (2) gives (WRIGHT and HOBBIE, 1966):

$$\frac{C\mu t}{c} = \frac{K_t + S_n}{V_m} + \frac{A}{V_m} \qquad (3)$$

Using data from uptake measurements V_m and $K_t + S_n$ can be calculated by linear regression of $\frac{C\mu t}{c}$ on A. $\frac{K_t + S_n}{V_m}$ is equivalent to the turnover-time (T_t), i.e. the time required for complete removal of the natural substrate by the microflora.

Sediment subsamples were diluted fifty times with anaerobic water collected 1 m above the sediment at the sampling station and filtrated through a 0.2-μ Sartorius membrane filter. The dilution water was

kept or made anaerobic by bubbling oxygen-free nitrogen gas through it. During all manipulations the sediment suspension was kept anaerobic by means of bubbling with this gas. Preliminary investigations showed that aerobic conditions drastically lowered uptake of glucose. Five ml of the sediment suspension was dispensed in small glass vials (volume 10 ml), after which the vials were closed with Suba Seal stoppers. Through the stoppers ^{14}C-glucose (327 µCi/mmol) was added in appropriate amounts to make up final concentrations of 9.3, 18.6, 37.2, 74.4 and 148.7 µg/l glucose. Per concentration 3 parallel samples and one blank (+ 0.2 ml 30% formaline) were incubated in a shaken waterbath for 30 minutes at *in situ* temperature after which the uptake was terminated by adding 0.2 ml 30% formaline through the stopper. Then 2 ml of the suspension was filtrated over 0.2-µ Sartorius membrane filters which were rinsed twice with 2 ml demineralized water and dissolved in 10 ml Quickzint scintillator in scintillation vials, after which the bêta-activity was counted in a Betazint scintillation counter. After correction for quenching cpm's were converted to µg glucose.g (dry sediment)$^{-1}$.hour^{-1} or to µg glucose.g (sedimental carbon)$^{-1}$.hour^{-1}. After transformation of the data to a Lineweaver-Burk plot V_m, $K_t + S_n$ and T_t were calculated using linear regression analysis. Results with a correlation coefficient lower than 0.8 were discarded.

Corrections for $^{14}C\text{-}CO_2$ produced during the incubation (= mineralization corrections) were made following the method of HOBBIE and CRAWFORD (1969). For the absorption of the $^{14}C\text{-}CO_2$ 0.2 ml of ethanolamine was used. Determination of the mineralization was carried out with one glucose concentration (148.7 µg/l). Preliminary investigations showed no influence of the glucose concentration on the percentage of glucose mineralized. Also no difference in mineralization percentage could be shown between aerobic and anaerobic incubation.

2.7. Isolation and cultivation of nitrogen-fixing *Clostridium*-strains from the sediments of the Pluss-See.

For the isolation and cultivation of nitrogen-fixing *Clostridium* strains the following medium was used (adapted from PATRIQUIN and KNOWLES (1972) and SYLVESTER-BRADLEY (1976)): sucrose 10 g/l; K_2HPO_4 0.8 g/l; KH_2PO_4 0.4 g/l; $MgSO_4.7H_2O$ 0.2 g/l; $CaCl_2$ 0.02 g/l; $NaHCO_3$

0.1 g/l; ascorbic acid 0.1 g/l; sodium thioglycolate 0.2 g/l; biotin 5 µg/l; p-aminobenzoic acid 10 µg/l; $Na_2MoO_4 \cdot 2H_2O$ 5 mg/l; $FeSO_4.7H_2O$ 15 mg/l; yeast extract 40 mg/l. For solid media 1.5% Ionagar was added. The oxygen was removed by boiling and bubbling oxygen-free nitrogen gas through the medium. Then 5 ml of the medium was dispensed into Hungate tubes in an anaerobic way. The medium was then sterilized by fractionated sterilization (3 times 15 minutes at 100°C, every time interchanged alternately by incubation at 37°C for 24 hours). The solid medium was used for the preparation of roll tubes.

Sediment subsamples were taken by sterile syringes from the centre of a sediment core sample. This subsample was diluted 100 times with sterile anaerobic lake water. From this suspension dilutions of 1.000 and 10.000 times the original sediment were made. Roll tubes were inoculated with 0.5 ml of these dilutions. After preparation, the roll tubes were incubated at 27°C. By microscopic examination of the developed colonies *Clostridium*-like colonies were transferred to new roll tubes. This was repeated three times after which some *Clostridium*-like colonies were transferred into liquid medium. These cultures were checked for purity by microscopic examination. Checks for acetylene-reducing activity were made by transferring 1 ml in an anaerobic and sterile way into 4 ml Vacutainers and by assaying for acetylene reduction as described in 2.5.

3. The application of the acetylene reduction assay to the sediments of the Pluss-See.

The simultaneous discovery of DILWORTH (1966) and SCHOLLHORN and BURRIS (1967) that nitrogenase reduces acetylene exclusively to ethylene and inhibits the reduction of molecular nitrogen has revolutionized the research in the field of the biological nitrogen fixation. With the assay based on their discovery the activity of nitrogenase can be measured much more easily and sensitively than with the earlier usual methods, i.e. the measurement of the increase of total combined nitrogen and the measurement of ^{15}N-incorporation. Prior to the application of this assay to the Pluss-See sediments this technique had to be validated in a qualitative and quantitative way.

3.1. Qualitative aspects of the acetylene reduction assay.

The acetylene reduction assay is based on the assumption that the ethylene produced in this assay originates exclusively from the reduction of acetylene by nitrogenase and that during the incubation of the sample no ethylene disappears by ethylene-consuming reactions. Before using this assay for sediments of freshwater lakes it has to be established that:

1. the production of ethylene is a process associated with living organisms, i.e. there is no chemical production of ethylene;
2. the produced ethylene originates exclusively from the reduction of acetylene, i.e. there is no production of ethylene in samples without acetylene;
3. there are no ethylene-consuming reactions under the applied assay conditions.

In this section the validity of these conditions are evaluated, assuming that, if the ethylene production is associated with living organisms and the ethylene is produced by the reduction of acetylene, acetylene reduction is mediated by the nitrogenase complex.

In samples treated with the fixative formaline (final concentration in the sediments 1.6%) no ethylene production could be detected. This means that after killing the living organisms the production of ethylene stops (= condition 1). Also the second condition for applying the assay holds for the sediments studied: no ethylene production

could be detected in samples incubated without acetylene. This means that the ethylene originates exclusively from the reduction of acetylene by the nitrogenase complex.

In sediment samples incubated anaerobically with low concentrations of ethylene (pC_2H_4 = 0.3 x 10^{-4} atm) no reduction of this concentration could be detected over an incubation period of 48 hours. There was, however, a considerable decrease of the ethylene concentration if the samples were incubated in the presence of oxygen (see figure 3). This decrease is probably due to the cooxidation of ethylene by methane-oxidizing bacteria (DE BONT and MULDER, 1974). Acetylene has been found to block the oxidation of methane to methanol resulting in the stop of the cooxidation of ethylene (DE BONT and MULDER, 1974). Indeed, in the presence of low acetylene concentrations (pC_2H_2 = 0.25 x 10^{-4} atm) no decrease of the ethylene concentration could be observed (figure 3). The conclusion can be drawn that neither under anaerobic nor under aerobic assay conditions there will be any ethylene-consuming reactions (= condition 3). Because of the inhibition of the oxidation of methane to methanol by

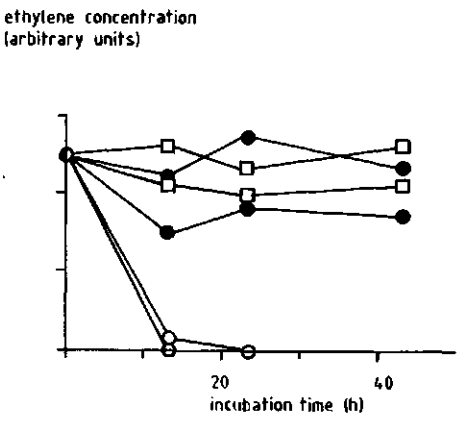

Figure 3. Absorption of ethylene by surficial sediments from 29 m water depth:
●: anaerobic incubation
○: aerobic incubation
□: aerobic incubation with 0.0025% acetylene.

acetylene, nitrogen fixation will be underestimated under aerobic conditions if nitrogen-fixing, methane-oxidizing microorganisms make up a significant part of the nitrogen-fixing population.

Summarizing, it can be concluded that the ethylene produced in the sediments under the assay conditions can be stoichiometrically compared with the acetylene reduced by the nitrogenase system.

In order to evaluate the nature of the living processes associated with the production of ethylene the influence of the antibiotic chloramphenicol on the ethylene production was studied in combination with varying glucose concentrations. Chloramphenicol is known to be

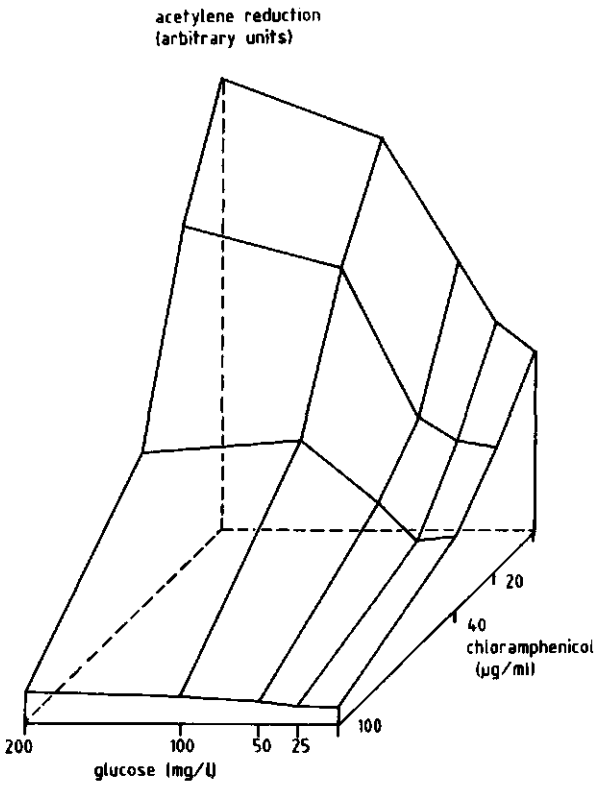

Figure 4. The combined effect of glucose and chloramphenicol on the acetylene-reducing capacity of the sediment.
Sediment: surficial sediment (0-5 cm) from 29 m water depth.
Incubation temperature: 27°C. Incubation time: 46 h.
pC_2H_2 0.2 atm.

an inhibitor of protein synthesis in procaryotic ribosomes by affecting chain elongation beyond the first peptide bond (HAHN, 1967). The results of the experiment show (see figure 4) that the acetylene reduction is strongly reduced at higher chloramphenicol concentrations. At the same time a positive influence of glucose on the acetylene reduction could be observed. From this it can be concluded that the acetylene reduction is associated with actively protein-synthesizing organisms and with a glucose (= energy)-demanding process.

BROTONEGORO (1974) observed a comparable effect of chloramphenicol on the acetylene-reducing activity of *Azotobacter chroococcum* cultures. He explained this inhibitory effect by assuming a competition between nitrogenase and chloramphenicol for reductants, as observed by O'BRIEN and MORRIS (1971). An additional explanation was thought to be the accumulation of soluble ammonium which may adversely affect the nitrogenase activity. He found that the presence of soluble ammonium enhanced the effect of the antibiotic, probably because of the competition for ATP of $NADPH_2$ between the assimilation of ammonium and the nitrogenase activity. The sediments of the Pluss-See contain high concentrations of ammonium. Therefore a pronounced effect of the antibiotic might be expected.

Acetylene might affect the anaerobic microorganisms in the sediments. BROUZES and KNOWLES (1971) found inhibition of growth of *Clostridium pasteurianum*. A short incubation time is recommended for the acetylene reduction assay (BROUZES and KNOWLES, 1971; HARDY *et al.*, 1973; DE BONT and MULDER, 1974). However, it could be shown (e.g. figure 9) that, after an initial lag phase, ethylene production was linear with time during at least 50 hours of incubation. This was also found by RICE and PAUL (1971). This linear ethylene production shows that inhibition is improbable under the assay conditions (24 hours of incubation).

OREMLAND and TAYLOR (1975) found that acetylene inhibited methanogenesis in sediments. This might effect the nitrogenase activity because methanogenic bacteria probably play a symbiotic role in anaerobic nitrogen-fixing communities. They state that it is not clear if this causes an over- or underestimate of sediment nitrogen fixation rates. Absolute values of nitrogen fixation should therefore be considered with care.

3.2. Quantitative aspects of the acetylene reduction assay.

3.2.1. Introduction.

Acetylene inhibits the fixation of molecular nitrogen in a competitive way. The affinity of the enzyme complex for acetylene is high compared with the affinity for molecular nitrogen. The mean of Michaelis constants K_m measured in a variety of systems is 0.006 atm (HARDY *et al.*, 1973); for nitrogen gas K_m ranges from 0.015 to 0.17 atm (HARDY *et al.*, 1968). The difference in K_m-values is mainly caused by differences in the solubility of the two gasses in water. Because of the high affinity of the enzyme for acetylene, low concentrations of the gas are sufficient for the practically complete inhibition of the nitrogen fixation. So the measured acetylene reduction rates are a proportional reflection of the nitrogen fixation rates. In many systems, therefore, elimination of the nitrogen gas from the sample is not necessary (AKKERMANS, 1971). In most systems the ratio between the moles acetylene reduced and the moles nitrogen fixed approaches the theoretical value of 3 (HARDY *et al.*, 1973). However, significant deviations from the theoretical ratio have been observed especially in waterlogged soils. In these systems slow gas diffusion into the soil sample are thought to cause this deviation (RICE and PAUL, 1971; LEE and WATANABE, 1977). The applied acetylene concentration has to be much higher to achieve saturation of the enzyme. MATSUGUCHI *et al.* (1978) found that the acetylene reduction rate depended strongly on the partial pressure of acetylene applied in the assay, even at relatively high concentrations, pointing to the non-saturation of nitrogenase in water-logged soil systems. Therefore care has to be taken in interpreting data on acetylene-reducing activity observed in these systems. The same phenomenon has been found in freshwater sediments (SYLVESTER-BRADLEY, 1976). She observed non-saturation of nitrogenase even in sediments exposed to an atmosphere completely consisting of acetylene.

In this part of the study attention has been paid to the question of whether comparable saturation problems complicate the measurement of the acetylene-reducing activity of Pluss-See sediments and what the causes and the consequences of this complication might be.

3.2.2. Results.

In a series of experiments acetylene-reducing activity of sediment samples from the Pluss-See was measured under partial pressures of acetylene varying from 0.15 to 1 atm. Figure 5 shows the result of one of these experiments. A strong relationship between the partial pressure of acetylene and the acetylene reduction of the sediments could be observed. All experiments gave comparable results, thus pointing to the non-saturation of the nitrogenase at C_2H_2-concentrations up to at least 0.4 atm. From the results as presented in figure 5 a half-saturation constant (K_s) for the sediment system under investigation can be calculated. This value varied from 0.18 to 0.58 atm for the different experiments, all considerably higher than the mean value of 0.006 atm for K_m given by HARDY *et al.* (1973).
For comparison, K_m-values were measured in nitrogen-fixing *Clostri-*

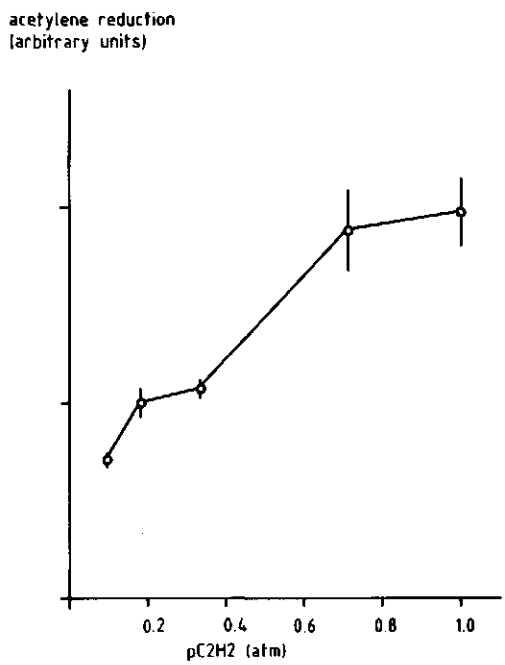

Figure 5. Influence of the partial pressure of acetylene on the acetylene-reducing activity of surficial sediments from 29 m water depth.
Vertical bars represent standard deviation.

dium cultures isolated from the Pluss-See sediments. K_m-values varied from 0.0049 to 0.013 atm, so comparable with the mean value given by HARDY *et al.* (1973). These latter results support the supposed universality of nitrogenase. Assuming that nitrogenase in Pluss-See sediments is indeed the same as the universal nitrogenase, the conclusion can be drawn that (1) either the acetylene concentration at the sites where nitrogenase is located in the sediments is significantly lower than the concentration that can be calculated from the solubility of the gas in water, (2) the acetylene reduction is inhibited in spite of the relatively high concentrations or (3) the phenomena are explained by a combination of these two possibilities. A potential inhibitor of the acetylene reduction is molecular nitrogen that might be still present in the sediments. To investigate this possibility the time during which the sediments are flushed with helium prior to the acetylene reduction assay was varied. The assay was performed at pC_2H_2= 0.2 atm and at pC_2H_2= 1.0 atm. Only in the first

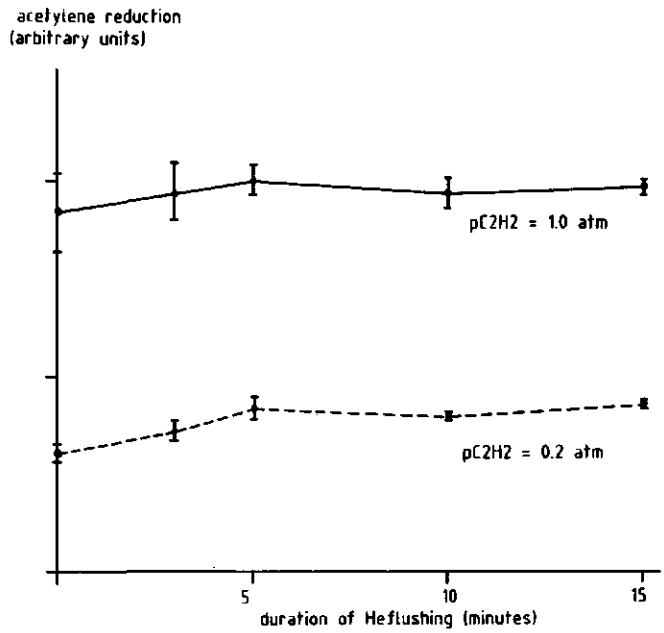

Figure 6. Effect of He-flushing on the acetylene reduction rate in Pluss-See sediment.

three minutes a relationship between flushing time and acetylene-reducing activity could be observed (see fig. 6). At flushing times longer than five minutes acetylene-reducing activity did no further increase. At the same time the difference between the acetylene-reducing activities at 0.2 and at 1.0 atm C_2H_2 is approximately constant. If inhibition by nitrogen gas was the reason for the non-saturation of nitrogenase this difference would decrease with increasing flushing time. For, as a consequence of flushing-out the nitrogen gas, nitrogenase would get more saturated with acetylene and the K_s-value would have approximated the mean K_m given in the literature. Consequently the acetylene-reducing activity at 0.2 atm acetylene would have approximated the activity at 1.0 atm in figure 6. The conclusion can be drawn that the possible presence of nitrogen gas cannot explain the non-saturation of nitrogenase in the sediments.

As a possible explanation SYLVESTER-BRADLEY (1976) mentioned denitrification in the sediments. The evolved molecular nitrogen would inhibit the acetylene reduction. This possibility, however, can be excluded for the Pluss-See sediments, because nitrate concentrations in the sediments are low, if not zero.

SYLVESTER-BRADLEY (1976) suggests also slow diffusion of acetylene into the sediments as a possible explanation for the non-saturation of nitrogenase, as did MATSUGUCHI *et al.* (1978) for water-logged soils. Considering the nature of the Pluss-See sediments and the applied assay technique this, however, does not seem to be a plausible explanation for the non-saturation found in this study. The mean dry matter content of the surficial sediments in the profundal was only 2% in the average. Maximal thickness of the sediment subsamples in the assay tubes was only 2.5 mm. RICE and PAUL (1971) found in water-logged soils problems with diffusion of gases into and out of the soil. From their figure 5 it can be seen, that this is hardly the case at 2.5 mm below the soil surface. Conditions in Pluss-See sediments are much more favourable because the porosity of these sediments is higher than the porosity of water-logged soils. In addition to this the assay tubes were shaken thoroughly prior to the pre-incubation period in order to establish equilibrium conditions between the sediments and the head space.

To confirm the above assumption that thickness of the sediment sub-

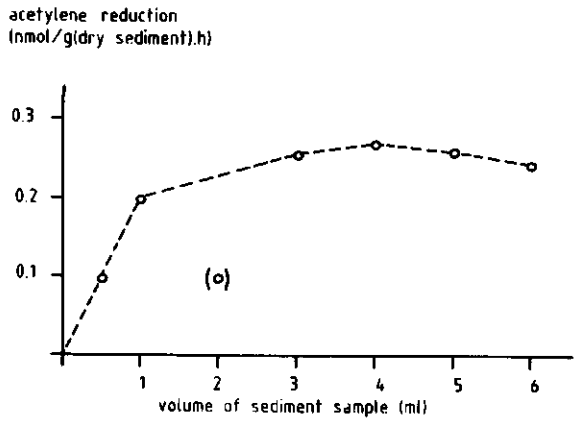

Figure 7. The influence of the volume of the incubated sediment sample on the acetylene reduction rate.

sample in the assay tube does not give rise to diffusion problems, the influence of this factor on the acetylene-reducing activity of the sediments was investigated. This was done by varying the volume of the sediment subsample in the tubes. The results of this experiment show that an effect of sediment thickness could not be detected below a volume of 5 ml (see figure 7). The normally applied volume in the assay was 3-3.5 ml. The decrease of the activity at volumes less than 2 ml, as shown in figure 7, can probably be ascribed to desiccation of the sediments during the incubation. This is relatively more important for small sediment volumes. From this experiment the conclusion can be drawn that the diffusion of acetylene from the overlying atmosphere into the sediment subsample does not limit the acetylene-reducing activity of these sediments. The acetylene concentration in the interstitial water is in equilibrium with the overlying atmosphere.

To support this conclusion the effect of dilution has been investigated. Assuming a higher porosity by diluting the sediments and hence a higher flux of acetylene into the sediments, the acetylene concentration at the nitrogen-fixing sites in the sediments and therefore the acetylene-reducing activity would increase if this flux is limiting the acetylene reduction in the sediments. The saturation of the nitrogenase at 0.2 atm C_2H_2 would therefore relatively increase by

dilution compared with the saturation at 1.0 atm. However, this was not the case: the ratio between the acetylene reduction at 0.2 atm and at 1.0 atm was not affected by the dilution of the sediment. This is in support of the above conclusion.

Summarizing, the following conclusions can be drawn from the previous results:

1. the acetylene concentration at the nitrogen-fixing sites in the sediments is significantly lower than the theoretical concentration based on the solubility of acetylene in water;
2. the transport of acetylene from the atmosphere into the sediment does not limit the acetylene reduction.

These conclusions are not contradictory if it is assumed that the nitrogen-fixing sites are not in direct contact with the interstitial water. In fact this means that the transport of acetylene from the interstitial water to the nitrogen-fixing sites limits the acetylene reduction. Obviously there is a barrier for the transport of acetylene on its way from the interstitial water to the nitrogen-fixing sites. This barrier is located within the mainly organic matrix of the sediment or within the nitrogen-fixing microorganisms themselves. In order to get information about the nature of this barrier, the influence of physical disturbance of the sediment structure on the acetylene-reducing activity was investigated. If the barrier is located in the sediment matrix, physical disturbance of its structure would bring the nitrogen-fixing organisms in more direct contact with the interstitial water. As a consequence nitrogenase would become more saturated with acetylene and the K_s value would decrease. Figure 8 shows the results of an experiment in which the effect of continuous shaking during the incubation on the acetylene reduction was investigated at different pC_2H_2. The half-saturation constant K_s of the shaken sediment is substantially lower than the K_s of the unshaken sediments, which means that the nitrogenase in the shaken sediments is more saturated with acetylene. Therefore the conclusion can be drawn that the structure of the sediments is at least one of the reasons for non-saturation of nitrogenase in the sediments of the Pluss-See. Apparently nitrogen-fixing microorganisms are located in such a manner inside the sediment structure that transport of acetylene from the interstitial water to these sites is strongly hindered.

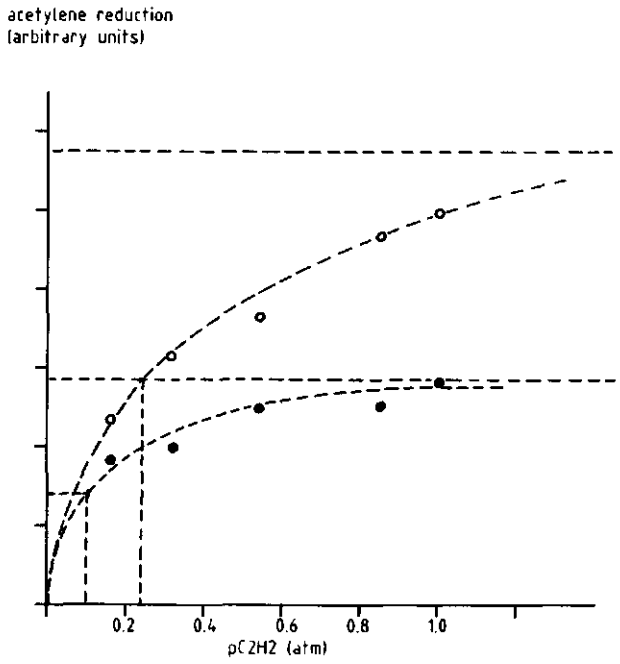

Figure 8. Influence of acetylene partial pressure on the acetylene reduction rate in the sediment.
● = shaken during incubation;
○ = unshaken during incubation.

However, figure 8 shows that shaking of the sediment substantially lowered the acetylene-reducing activity in spite of the better saturation of nitrogenase. The maximum acetylene reduction of the shaken sediments was only one half of the unshaken sediment. This result was confirmed by an experiment in which the acetylene-reducing activity in 10 shaken assay tubes was compared with the activity in 10 unshaken tubes. It was found that the acetylene-reducing activity in the shaken tubes was significantly ($p<0.005$) lower than the activity in the unshaken tubes. Apparently by bringing the nitrogen-fixing microorganisms in better contact with the interstitial water the acetylene reduction is negatively affected. The composition of the interstitial water, therefore, seems to be unfavourable for nitrogen fixation. This means that the conditions under which the ni-

trogen-fixing microorganisms are living in the sediments differ from the environmental conditions in the interstitial water. Nitrogen-fixing microorganisms seem to live in microsites in which the conditions for nitrogen fixation are more favourable than those in the interstitial water. At the same time this might mean that transport from the interstitial water to these sites is not only difficult for acetylene but also for other substances. One or more of these substances might negatively affect the functioning of the nitrogen-fixing process.

During the shaking of sediment samples a change in the consistency of the sediments could be observed. Normally the sediments of the Pluss-See have a pudding-like consistency, but after several hours of shaking they liquified. At the same time the colour of the interstitial water changed from light-yellow to dark-brown. These changes might have been caused by a change in the structure of the sediments, e.g. by the disturbance of the quaternary structure of macromolecules and their attachment to the particulate fraction. It is thinkable that the quaternary structure of macromolecules and their attachment to the particulate fraction of the sediments lead to a compartimentation of the sediments and consequently to the formation of micro-environments.

3.2.3. Discussion.

In the present study it has been shown that nitrogen-fixing microorganisms are located at sites in the sediments which have more favourable conditions for nitrogen fixation than the rest of the sediments. It is known that micro-organisms can be found in micro-environments, sometimes made by themselves from excretion products. Micro-environments are reported to exist for sulphate-reducing bacteria (JØRGENSEN, 1977), but also for caries-causing microorganisms (COSTERTON *et al.*, 1978). WEISE and RHEINHEIMER (1978) observed that colonization of marine sand grains by microorganisms takes place only at protected locations. The existence of micro-environments has many advantages for the microorganisms. Uptake and utilization of soluble degradation products, derived by extracellular enzymes from polymeric compounds, are facilitated. At the same time the microorganisms can be protected against inhibiting substances. Which of both advantages

might play a role in the nitrogen-fixing microorganisms in the Pluss-See sediments cannot be distinguished clearly. As shown later (see chapter 4) nitrogen fixation is affected by both substrate availability and ammonium concentration in the sediments. Hence probably both aspects (nutrient supply and protection) are important for this process.

Considering the observed high values for K_s (see figure 8), care has to be taken in interpreting the measured acetylene reduction rates. Applying 0.2 atm C_2H_2 to a sediment with a K_s ranging from 0.18 to 0.34 atm the maximum acetylene-reducing activity will be 2-3 times higher than the measured rates. By applying 1.0 atm acetylene to the sediments the maximum acetylene-reducing activity is approximated, but this high concentration excludes the possibility of aerobic incubation.

As shown above, shaking negatively influences the acetylene-reducing activity of the Pluss-See sediment. SYLVESTER-BRADLEY (1976) found a positive influence from shaking sediments during incubation. This contradiction might be explained by the lower porosity of the sediments studied by her. In the latter sediments, as in water-logged soils, the interstitial water is not in equilibrium with the gas phase. Shaking increases the interstitial acetylene concentration and therefore increases the acetylene-reducing activity of the studied systems.

From the present experiments it can be concluded that the transport of acetylene from the interstitial water to the nitrogen-fixing organisms is hindered. In relation to this, attention has to be paid to the availability of molecular nitrogen to these organisms. Considering the relatively low solubility in water much more serious complications might be expected for the nitrogen gas than for acetylene. No attempts have been made to measure concentrations of nitrogen gas in the sediments of the Pluss-See. The solubility of nitrogen gas in water varies from 23.3 $ml.l^{-1}$ at 0°C to 14.2 $ml.l^{-1}$ at 40°C (WEAST, 1971). REEBURGH (1969) measured nitrogen gas concentrations in the sediments of Chesapeake Bay. He found decreasing concentrations with depth into sediments. This decrease was thought to be caused by a stripping effect of methane bubbles. The concentrations in the surficial sediments were only slightly lower than in the overlying water

and ranged from 10 to 13 $ml.l^{-1}$. A comparable range was observed by MARTENS and BERNER (1977) in the surficial layers of anoxic Long Island Sound sediments and by EMERY and HOGGAN (1958) in the sediments of salt marshes and deep marine basins off southern California. The Michaelis constant (K_m) of nitrogenase for nitrogen gas is about 0.04 atm (HARDY *et al.*, 1968). This corresponds to 0.024 $mmol.l^{-1}$ or 0.53 $ml.l^{-1}$. It means that given a homogeneous distribution, nitrogen gas will not limit the nitrogen fixation in these sediments. If nitrogen gas concentrations are of the same range this nor will be the case in the Pluss-See sediments.

Annual nitrogen fixation at the deepest part of the Pluss-See has been estimated to be circa 1 g $N.m^{-2}$ (see 6.3.2). Most of this is fixed in the upper 5 cm of the sediments. Assuming a nitrogen gas concentration of 10 $ml.l^{-1}$ the total amount of nitrogen gas in this layer is 625 $mg.m^{-2}$. Therefore to realize an annual fixation of 1 g $N.m^{-2}$, nitrogen gas has to be transported from elsewhere to the surficial sediments. If the nitrogen gas concentration in the deeper regions is lower than in the surficial sediments (REEBURGH, 1969) nitrogen gas will be transported from the overlying water. Assuming a nitrogen gas concentration in this water of 10 $ml.l^{-1}$ the concentration gradient $(\frac{dc}{dx})$ needed for a transport $(\frac{du}{dt})$ of 1 g $N.m^{-2}.year^{-1}$ can be calculated as follows:

$$\frac{du}{dt} = -D.\Phi.\frac{dc}{dx} = 1g.m^{-2}.year^{-1}, \text{ where:}$$

Φ = porosity (assumed to be 1.0);

D = diffusioncoefficient (assumed to be $1.89.10^{-5}$ $cm^2.sec^{-1}$ = 0.0596 $m^2.year^{-1}$ (RICE and PAUL, 1971).

This gradient is 0.167 $mg.l^{-1}.cm^{-1}$. The concentration therefore has to decrease 0.84 $ml.l^{-1}$ over the upper 5 cm to sustain a nitrogen gas transport of 1 g $N.m^{-2}.year^{-1}$. If the value for D is realistic this concentration decrease will not complicate the saturation of the nitrogenase. Problems might be expected, however, in comparable systems with high nitrogen fixation rates. The conclusion may be drawn from this that the acetylene reduction assay underestimates the nitrogen fixation in the studied sediments. This conclusion is, however, in contrast with reports in the literature of C_2H_2/N_2 conversion factors

higher than the theoretical ratio in water-logged soils (RICE and PAUL, 1971; BROUZES *et al.*, 1971).

The problems associated with the acetylene reduction assay originated from the inhomogeneous distribution of acetylene in the sediments. The probability that acetylene under assay conditions is distributed in the same way as nitrogen gas under natural conditions in the lake sediments is not very high. Acetylene is added artificially from outside into the sediment. In a short time an equilibrium has to be established in order to saturate nitrogenase. If the enzyme is located in sites which are difficult to access by diffusion the external acetylene concentration has to be relatively high in order to establish a sufficient acetylene concentration at these sites within a period of a few hours. Nitrogen gas, however, is originally present at the nitrogen-fixing sites. The replenishment of the fixed nitrogen needs to be only a slow process if nitrogen fixation rates are relatively low, as shown above. Therefore a limitation of nitrogen fixation caused by the concentration of the nitrogen gas is not very probable under natural conditions in the sediments of the Pluss-see. Measurement of nitrogen fixation with $^{15}N_2$ also involves the artificial introduction of a gas ($^{15}N_2$) into the studied systems. As for acetylene, the distribution of $^{15}N_2$ throughout the sediments will not be identical to the distribution of the originally present N_2 gas. This will therefore also result in an underestimation of nitrogen fixation. This may be one of the reasons why SYLVERSTER-BRADLEY (1976) vainly tried to measure nitrogen fixation in comparable sediments with ^{15}N-nitrogen gas. It may also explain the high conversion factors in the literature, because they are mainly based on the comparison of acetylene reduction rates with $^{15}N_2$ -incorporation rates. Both methods may underestimate nitrogen fixation in sediment systems with low fixation rates. At higher rates diffusion of N_2 may complicate the saturation of nitrogenase under natural conditions. This may lead to an overestimation of nitrogen fixation by the acetylene reduction assay, as shown by RICE and PAUL (1971).

From the above discussion it can be concluded that conversion of acetylene reduction rates into nitrogen fixation rates is not only complicated by the structure of the studied system, but also by the height of nitrogen fixation rates in this system. Both over- and

underestimation are possible. Considering the low nitrogen fixation rates in the sediments of the Pluss-See and the conclusion that nitrogen fixers are situated in microsites within these sediments, underestimation of the actual nitrogen fixation rates is very probable. Nevertheless, estimating the quantitative significance of nitrogen fixation in the sediments, a wide range of conversion factors has been taken into consideration.

4. The influence of added inorganic combined nitrogen and organic substrate on the acetylene-reducing activity of the Pluss-See sediments.

4.1. Introduction.

Organic substrate is the energy source for the heterotrophic nitrogen fixers. Because of the much energy demanding character of the nitrogen-fixing process, STEWART (1969) hypothesized that the availability of the organic substrate will be the rate-limiting step for the free-living heterotrophic nitrogen fixers. Heterotrophic nitrogen-fixation is therefore strongly linked with the carbon-cycle of the lake.

Combined inorganic nitrogen and especially ammonium is known to have a negative influence on nitrogen fixation. In anaerobic nitrogen fixers (*Clostridium pasteurianum*) ammonium only represses the synthesis of nitrogenase. It does not affect the activity of the enzyme (DAESCH and MORTESON, 1972; KNOWLES and DENIKE, 1974). In aerobic nitrogen fixers ammonium both represses the synthesis of nitrogenase and inhibits the nitrogenase activity (BROTONEGORO, 1974; MULDER and BROTONEGORO, 1974).

In this part of the study the influence of inorganic combined nitrogen and the influence of the organic substrate on the acetylene-reducing activity of the Pluss-See sediments is discussed simultaneously because of the interrelationship between these two factors.

4.2. Experimental results.

Figure 9 shows the results of an experiment in which the effect of added mannitol on the acetylene-reducing activity of a sediment sample was investigated. Two phases can be distinguished. In the first phase the acetylene-reducing activity in the amended sediment is higher than in the control sediment. The ethylene production is linear with time as in the control sediment. In the second phase an exponential increase of the ethylene production can be observed.

In a further experiment the influence of mannitol on the acetylene reduction of sediment samples of different origin was investigated. Figure 10 shows the results of an experiment in which samples from the surficial sediments of the littoral, from the surficial sediments

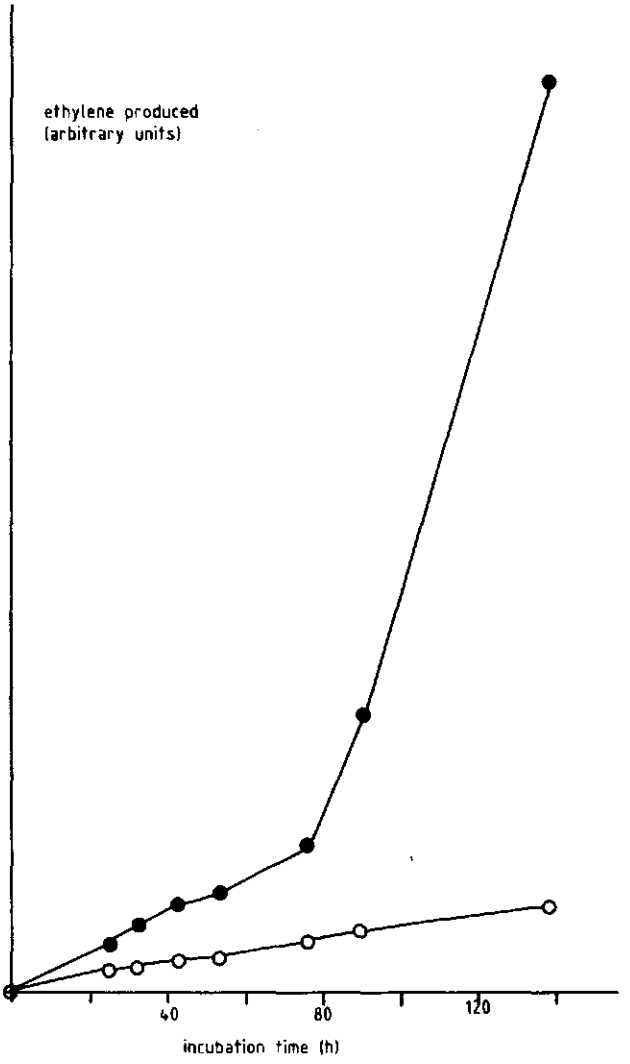

Figure 9. Ethylene production in acetylene reduction assays of a Pluss-See sediment sample from 29 m water depth (upper 5 cm).
Incubation temperature 27°C. pC_2H_2 0.2 atm.
●: 1% mannitol;
○: control.

at 29 m water depth and from the sediments at 9-18 cm below the sediment surface at 29 m water depth were amended with 1% mannitol and incubated anaerobically at 27°C. After an initial period of 20 hours

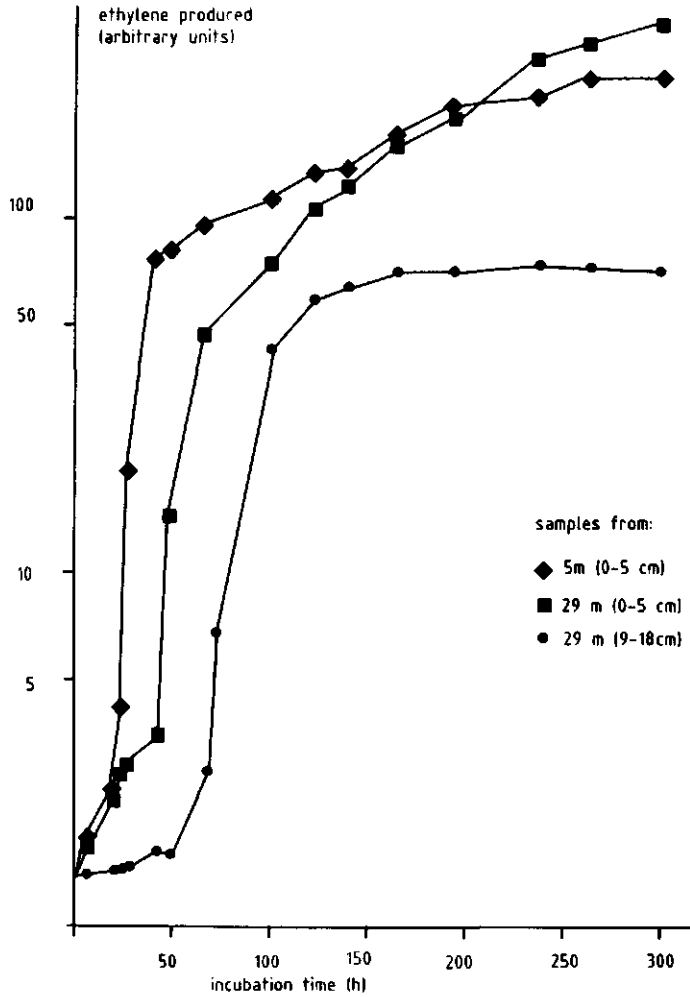

Figure 10. Ethylene production in acetylene reduction assays of samples of Pluss-See sediments enriched with 1% mannitol. Incubation temperature 27°C. pC_2H_2 0.2 atm.

the ethylene production increased exponentially in the littoral sediment sample. The same increase occurred in the surficial sediments from 29 m water depth 29 hours later. Still later, after 70 hours of incubation a similar increase was observed in the sediment from 9-18 cm below the sediment surface at 29 m water depth. The course of

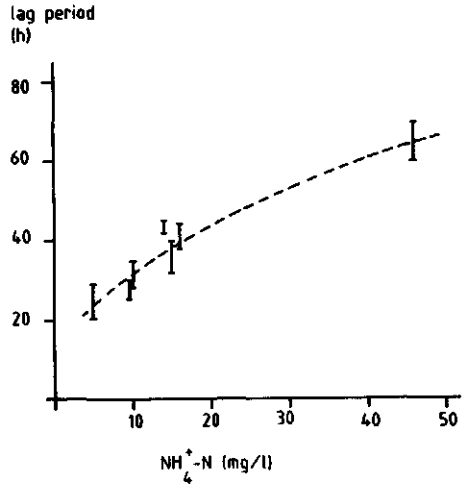

Figure 11. The relation between the NH_4^+-N concentration in the interstitial water of the sediment and the time between the addition of glucose and the onset of the exponential synthesis of nitrogenase in the sediment.

the ethylene production showed an exponential increase over a short period of 20 hours in all sediment samples (see figure 10) after which the production flattened off. The production in the sediment from 9-18 cm below the sediment-water interface at 29 m water depth even came to a complete stand-still. The amount of ethylene produced in this sediment was remarkably less than in the other samples.

The differences in the onset of the exponential increase of the acetylene reduction were thought to be caused by differences in the interstitial ammonium concentration that was different for the three sediment samples: 4.6 mg NH_4^+-N.l^{-1} in the littoral sediment sample, 13.3 mg NH_4^+-N.l^{-1} in the surficial sediment sample from 29 m water depth and 46.1 mg NH_4^+-N.l^{-1} in the sample from 9-18 cm below the sediment surface at 29 m water depth. This possibility was affirmed by a comparable experiment in which the effect of added glucose on more samples of different origin was investigated. Figure 11 shows the relationship between the initial interstitial ammonium concentration and the length of the period between the addition of the mono-

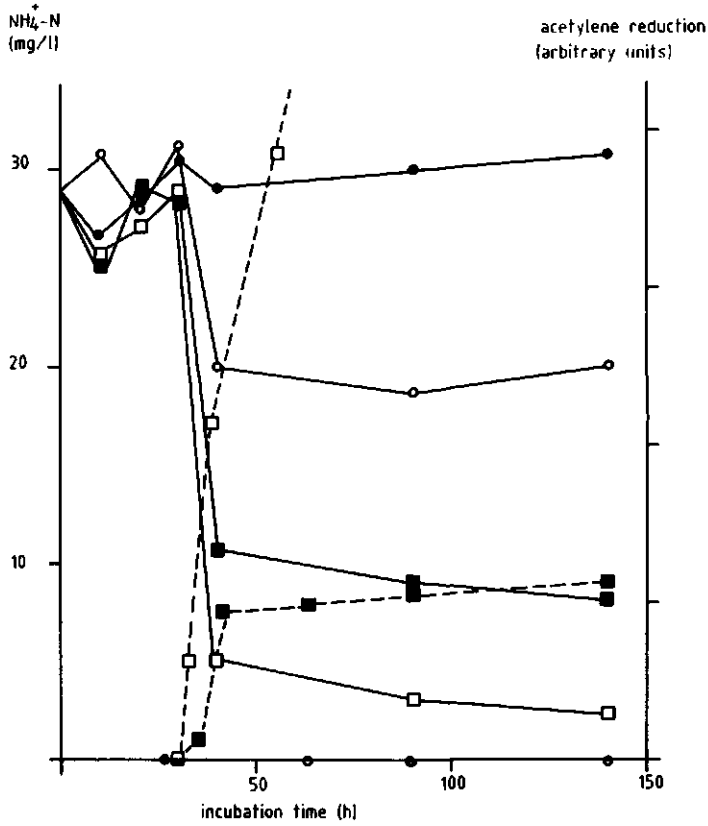

Figure 12. The NH_4^+-N concentration in the interstitial water and the acetylene-reducing activity of the sediment after addition of glucose to the sediment.
_____ : NH_4^+-N concentration __ __ __ : acetylene reduction.
glucose concentration: ● 0.1%; ○ 0.25%; ■ 0.5%; □ 1.0% surficial sediment from 29 m water depth; incubation temperature: 27°C;
pC_2H_2: 0.2 atm.

saccharide and the onset of the exponential increase. This period increased with increasing interstitial ammonium concentration.

Figure 12 shows the results of an experiment that was performed to investigate the influence of the concentration of the added monosaccharide (in this case glucose) on the acetylene-reducing activity and

the interstitial ammonium concentration of a sediment sample. The interstitial ammonium concentration of a sample amended with 1% glucose sharply decreased after 30 hours of anaerobic incubation at 27°C to values between 2 and 5 mg NH_4^+-N.l^{-1}. At the same time the acetylene reduction started. In the sediment with 0.5% glucose the interstitial ammonium concentration also decreased after 30 hours of incubation, but the final concentration was higher (about 10 mg NH_4^+-N.l^{-1}) than in the sample with 1% glucose. Acetylene reduction started simultaneously with the decrease of the ammonium concentration. Contrary to the sample with 1% glucose the ethylene production was low and came to a standstill shortly after the onset of the production. No significant acetylene reduction at all occurred in the samples with added glucose concentration lower than 0.5%. Sediment amended with 0.25% glucose showed a decrease of the interstitial ammonium concentration after 30 hours of incubation from 30 to 20 mg NH_4^+-N.l^{-1}. Sediment with 0.1% glucose did not show any significant decrease of the interstitial ammonium concentration.

This experiment shows that the synthesis of nitrogenase or at least the activity of nitrogenase is repressed at interstitial ammonium concentrations of at least 20 mg N.l^{-1}. If the added glucose is not enough to lower the concentration below this threshold no significant acetylene reduction occurs. If, however, the glucose concentration is sufficient, the remaining glucose is (at least partly) used for the reduction of acetylene. In the experiment described here almost all of the 0.5% glucose is used for the decrease of the ammonium concentration. Within 10 hours of the onset of the acetylene reduction the glucose is exhausted and the process stops. This is in contrast to the sediment amended with 1% glucose.

From the above experiment no conclusion can be drawn about the inactivating effect of ammonium on nitrogenase: both inhibition of the nitrogenase activity and repression of the synthesis of nitrogenase are possible. If ammonium would inhibit the nitrogenase activity, addition of ammonium would negatively affect the acetylene-reducing activity. This is not the case as was shown in an experiment in which ammonium upto a concentration of 50 mg NH_4^+-N.l^{-1} was added to profundal sediment samples. No significant effect of the added amounts could be observed, as shown in figure 13. From this results

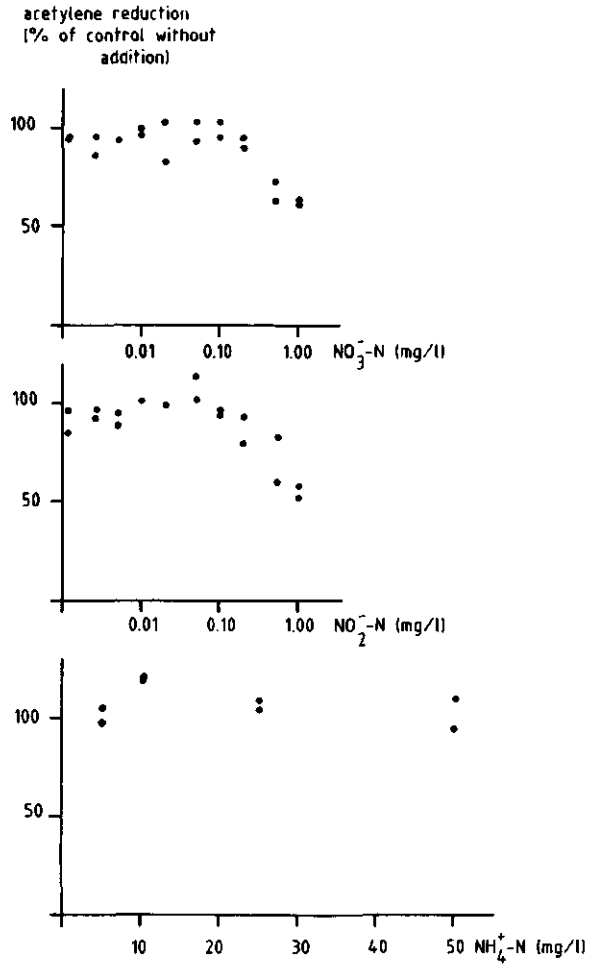

Figure 13. Influence of added inorganic nitrogen on the acetylene reduction rate of the sediments of the Pluss-See.

the conclusion has to be drawn that ammonium only represses the synthesis of nitrogenase in the sediments. The onset of the exponential increase of the acetylene reduction (see figure 9) apparently marks the derepression of the synthesis of nitrogenase. The acetylene reduction in the first phase, before the derepression, represents the activity of the nitrogenase already present in the sediment, whose activity is not affected by ammonium (DAESCH and MORTESON, 1972) and

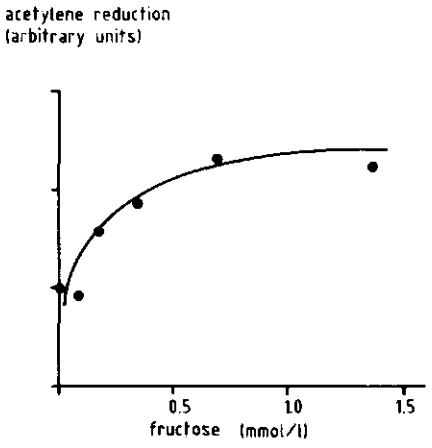

Figure 14. The effect of the addition of different fructose concentrations on the acetylene-reducing activity of profundal (29 m) surficial (0-5 cm) sediments from the Pluss-See.
Anearobic incubation for 43 h. at 27°C.

whose synthesis (originally in the sediment) apparently was not repressed.The increased acetylene reduction in this phase reflects the increased substrate availability by the addition of the monosaccharide. The linearity of the acetylene reduction rate with time in the first phase supports the above conclusion that no significant nitrogenase synthesis occurs above the threshold. The influence of the substrate concentration on the acetylene reduction in the first phase has been investigated in more detail. The resulting curve (see figure 14) showed resemblance with a saturation curve, again supporting the conclusion, that no significant nitrogenase synthesis occurs and that the first phase after monosaccharide addition reflects the increased substrate availability for the already present nitrogenase. The measured *in situ* ammonium concentration in the interstitial water is often higher than the concentration above which nitrogenase synthesis is repressed. However, also in such periods, which under natural conditions can last several months (see 6.3.1) acetylene-reducing activity can be detected in the sediments. As shown in 3.2.2 acetylene reduction is a process that can be related to actively protein-synthesizing cells. Inhibition of the nitrogenase synthesis by ammo-

nium might be expected in the long run to result in a decline of the acetylene-reducing activity in the sediments, because of death and lysis of microorganisms normally occurring under natural conditions. Such a decline apparently did not occur during these periods. Two possible explanations can be given:

(1) Nitrogenase is synthesized in spite of the high ammonium concentrations; (2) There is an input of nitrogenase from elsewhere into the sediment system. In order to investigate this an experiment was set up in which transport of nitrogenase from elsewhere was excluded by separating the sediment from its environment. In a series of 30 Hungate tubes, sediment samples were stored anaerobically in the dark

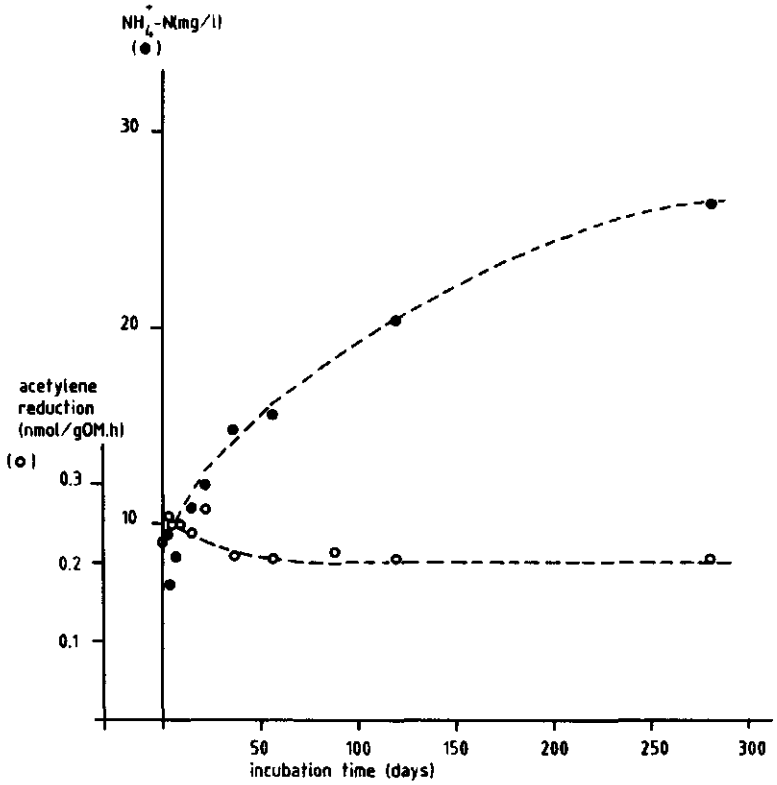

Figure 15. Acetylene-reducing activity and interstitial ammonium concentration of a sediment sample from 29 m water depth during a long-term experiment. Incubation temperature: 4°C.

at *in situ* temperature (4°C) during a long period. At several intervals the acetylene-reducing activity, the ammonium concentration of the interstitial water and the dry matter content of the sediment were measured. During the first 50 days of incubation the acetylene reduction of the sediments showed a light decrease after which it remained constant for a period of at least 230 days (see figure 15). At the same time the ammonium concentration of the interstitial water had increased from 10 to 25 mg NH_4^+-N.l^{-1} after 280 days of incubation. The last 160 days of this period the ammonium concentration was above the threshold concentration. Nevertheless no significant decrease of the acetylene reduction could be detected. It has to be concluded that most probably nitrogenase synthesis takes place also at interstitial ammonium concentrations above the threshold, in spite of the inhibition of this synthesis, as shown above.

Nitrogen fixation is a process that consumes relatively much energy. In order to get information about the relation between the amount of organic matter (= energy) consumed in the sediment and the amount of nitrogen fixed, a series of experiments was carried out to evaluate the influence of the concentration and the type of organic substrate. The efficiency of the acetylene reduction was estimated by calculating the extra amount of ethylene produced in the sediments per unit of added carbohydrate. The results (see figure 16) showed comparable results for the different monosaccharides. The efficiency of the acetylene reduction increased with decreasing substrate concentration. Maximum efficiency for the observed concentration range was about 200 nmol acetylene per mg monosaccharide for all applied monosaccharides except for galactose. The maximum efficiency for this substrate was 300 nmol C_2H_2.mg^{-1}.

In the experiments shown so far only the effect of monosaccharides has been investigated. The addition of long-chained carbohydrates like cellulose did not result in any increase of the acetylene-reducing activity during an incubation period of 170 hours. However, that cellulose might be a potential energy source for nitrogen fixation in the sediments can be concluded from the measured increase of the acetylene-reducing activity after addition of cellulase and hemicellulase to the sediments (see table I).

As shown above ammonium did not influence the activity of the exis-

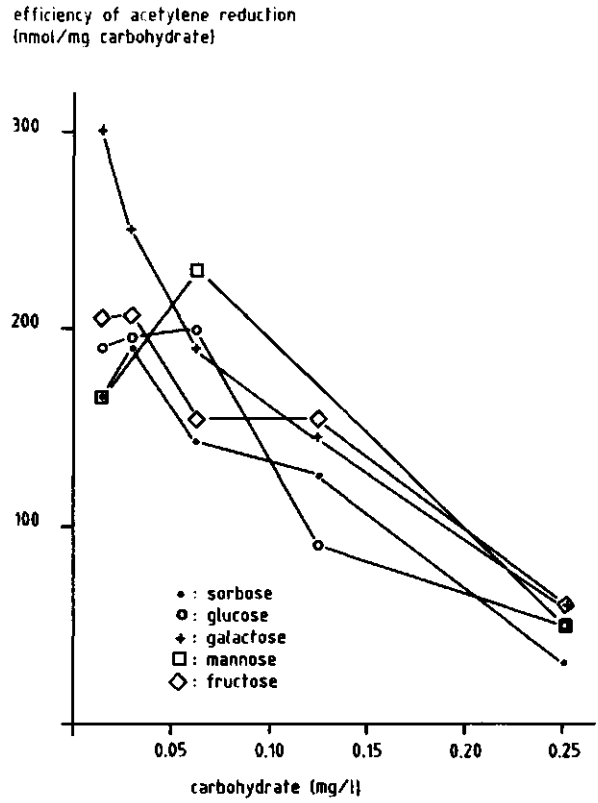

Figure 16. The efficiency of the acetylene reduction in assays of surficial sediments (0-5 cm) from 29 m water depth enriched with different concentrations of 5 carbohydrates. Incubation temperature: 27°C; pC_2H_2: 0.2 atm.

ting nitrogenase in the sediment. The influence of nitrate and nitrite was different. Both forms of combined nitrogen caused a strong inhibition of the acetylene-reducing activity of the sediments above concentrations of 0.1 mg N/l (see figure 13).

4.3. Discussion.

From the above described experiments two, at first sight, contradictory conclusions can be drawn: the synthesis of nitrogenase is repressed above a so-called repression-derepression threshold; at the

same time synthesis of nitrogenase seems to take place *in situ* at in-

Table I. The influence of cellulase and hemicellulase on the acetylene-reducing activity of surficial, profundal (29 m) sediments of the Pluss-See.
Anaerobic incubation (0.8 atm. Ar; 0.2 atm c_2H_2) of 17 hours at 27°C.

	Acetylene-reducing activity (arbitrary units and standard deviation)	(% of control)
control	322 ± 4	100
+ cellulase (1 $mg.l^{-1}$)	677 ± 30	210
+ hemicellulase (1 $mg.l^{-1}$)	517 ± 26	161

terstitial ammonium concentrations above this threshold.
In pure cultures of nitrogen-fixing micro-organisms synthesis of nitrogenase does not start until the ammonium concentration approaches zero (DROZD *et al.*, 1972; DAESCH and MORTESON, 1972; SHAH *et al.*, 1972). KNOWLES and DENIKE (1974) found that the repression-derepression threshold is higher according as the organic matter content of the soil is higher. The values they found for this threshold can not be compared with the above found threshold concentration, because information on the water content of their soil system fails. The authors explained the relation with the organic matter content by pointing at the role that organic matter plays in the adsorption of ammonium. The role of the ammonium adsorption is discussed elsewhere in this paper (see chapter 6 and 7). KNOWLES and DENIKE (1974) do not mention acetylene-reducing activity in unamended soil systems and hence do not discuss the question of why acetylene reduction (nitrogen fixation) occurs and why therefore nitrogenase has to be produced at ammonium concentrations above the threshold.
The intracellular ammonium concentration and not the extracellular

concentration regulates the nitrogenase synthesis. If nitrogenase synthesis takes place at a certain concentration of ammonium, this means that at this concentration the intracellular ammonium concentration is below a certain intracellular threshold. Assuming that this intracellular threshold is the same *in vitro* as *in situ*, the conclusion can be drawn that nitrogenase is protected from the interstitial ammonium concentration. The contradictory conclusions can be explained by assuming that nitrogenase or the nitrogen-fixing microorganisms *in situ* are not in direct contact with the interstitial water. Nitrogenase synthesis at sites more directly in contact with the interstitial water will not take place untill the interstitial ammonium concentration is lowered drastically by adding monosaccharides that enable the development of an aerobic bacteria which utilize ammonium nitrogen for growth. These conclusions are in agreement with the conclusions about the causes of the non-saturation of nitrogenase with acetylene (see 3.2).

JAEGER and WERNER (1977) found a positive relationship between the concentration of added ammonium and the acetylene-reducing activity in the sediments of Harkortsee (FRG). No such relationship has been found in the present study (figure 13).

A positive relationship has been found between the added monosaccharide concentration and the activity of the nitrogenase originally present in the sediments (see figure 14). This means that the activity of nitrogenase is substrate-limited. This conclusion is in agreement with the hypothesis of STEWART (1969) that nitrogen fixation of free-living heterotrophic nitrogen fixers in nature is limited by substrate availability.

The nitrogenase activity of sediments can be described in terms of potential and actual activity, as done for nitrogen fixation in pea and field bean by van MIL (1981). In natural, unamended sediments the actual nitrogenase activity is lower than the potential activity, because of shortage of organic substrate (= energy). By addition of monosaccharides the actual activity increases. At the same time the interstitial ammonium concentration decreases, resulting in the onset of nitrogenase synthesis, *i.e.* the increase of the potential nitrogenase activity, after the interstitial ammonium concentration is decreased below the repression-derepression threshold. Apparently

below this threshold nitrogenase synthesis is allowed to take place also at sites which are in closer contact with the interstitial water than the sites, in which nitrogen-fixation is taking place in the natural, unamended sediments.

It has been shown that cellulose is a potential substrate for nitrogen fixation. HUNGATE (1950) described anaerobic, cellulolytic bacteria. LESCHINE and CANALE-PAROLA (1983) described mesophilic cellulolytic *Clostridia* from freshwater sediments. An interesting question is the relation between the latter anaerobes and the free-living heterotrophic anaerobic nitrogen fixers.

The theoretical efficiency of nitrogen fixation in aerobic bacteria is nearly 280 mg N_2 per gram of sugar consumed, provided that all of the consumed carbohydrate is available for N_2-fixation (MULDER, 1975). In anaerobic bacteria this value is only 20-25 mg per g of sugar. Free-living nitrogen-fixing bacteria fix relatively small amounts of nitrogen. The maximum efficiency for anaerobes measured in the present study (see figure 16) is 200-300 nmol C_2H_2/mg monosaccharide or 4-6 mg N_2/gC, assuming a 3:1 molar ratio of acetylene reduced to N_2 fixed, i.e. in the same range as the values reported for *Clostridia* (5-10 mg N_2/g of carbon compound; MULDER, 1975).

The efficiency of the nitrogen fixation in the sediments is lower than the efficiency calculated by PATRIQUIN and KNOWLES (1975) in very nutrient-poor, marine skeletal carbonate sand (23.3 mg N_2/gC) and by O'TOOLE and KNOWLES (1973) in sandy loam soil (upto 75 mg N_2/gC). This means that in the sediments of the Pluss-See relatively more substrate is used for other processes. The sediments of the Pluss-See therefore seem to be a less favourable place for bacterial nitrogen-fixing populations than the systems studied by the above authors. O'TOOLE and KNOWLES (1973) claimed that the inverse relationship between the efficiency and the substrate concentration suggests, that the efficiency under natural conditions with low substrate concentration will be very high. In the present study also the *in situ* efficiency has been calculated from the sedimentation of organic carbon and the observed nitrogen fixation in the sediments. This will be discussed later in this paper (see 8.10).

In the present study it has been shown that conditions at the microlevel can differ considerably from conditions measured by macrome-

thods which neglect concentration gradients at the microlevel. Processes are taking place which do not occur under average conditions, but they do take place at microsites, where conditions are favourable for nitrogen fixation. This aspect has to be considered especially if predictions are made about natural processes and if an attempt is made to transfer laboratory data to *in situ* conditions.

5. The influence of temperature on the acetylene-reducing activity in the sediments of the Pluss-See.

The effect of temperature on the acetylene-reducing activity of sediments was studied by performing the assay under anaerobic conditions at 8 different temperatures. In figure 17a the produced ethylene after 24 hours of incubation is shown in relation to the incubation temperature. Maximum acetylene reduction was measured at 31°C. At higher temperature acetylene-reducing activity sharply declined. Figure 17a was transformed into an Arrhenius plot (see figure 17b). The slope of this plot (b) can be used to calculate the activation energy E according to:

$$E = -19.159\ b\ (J.mol^{-1}).$$

It resulted into a value of 47.6 $kJ.mol^{-1}$ (= 11.4 $kcal.mol^{-1}$) for the data given in figure 17b. The sediments showed an exponential response between 4 and 31°C (i.e. the linear part of the Arrhenius plot). No discontinuity could be observed as reported by several authors to be characteristic for the nitrogenase complex (WAUGHMAN, 1977; BURNS and BULEN, 1965; HARDY *et al.*, 1968). The non-biphasic pattern of the Arrhenius plot is in agreement with the results of PANKHURST and SPRENT (1975) and LINDE *et al.* (1969). According to HADFIELD and BULEN (1969) the activity of the enzyme is not adversely affected up to at least 40°C. The sharp decline of the acetylene-reducing activity above 31°C is in close agreement with the results of WAUGHMAN (1977). He hypothesized that not the nitrogenase but other essential enzymes become inactivated at these high temperatures.

The activation energy measured in the sediments of the Pluss-See falls in the lower range of data collected by WAUGHMAN (1977). He states that there is some evidence that bacteria in the soil are less sensitive to temperature changes than under the conditions of pure culture. This appears to be valid for bacteria in lake sediments too. The activation energy measured in the Pluss-See sediments is in the range of the values reported by WAUGHMAN (1977) for the activation energy above the discontinuity. This is in agreement with the values reported for soil systems (KNOWLES *et al.*, 1973; WAUGHMAN, 1976). If this discontinuity is caused by the existence of two discrete states of the enzyme (BURNS and HARDY, 1975) the state of the enzyme at

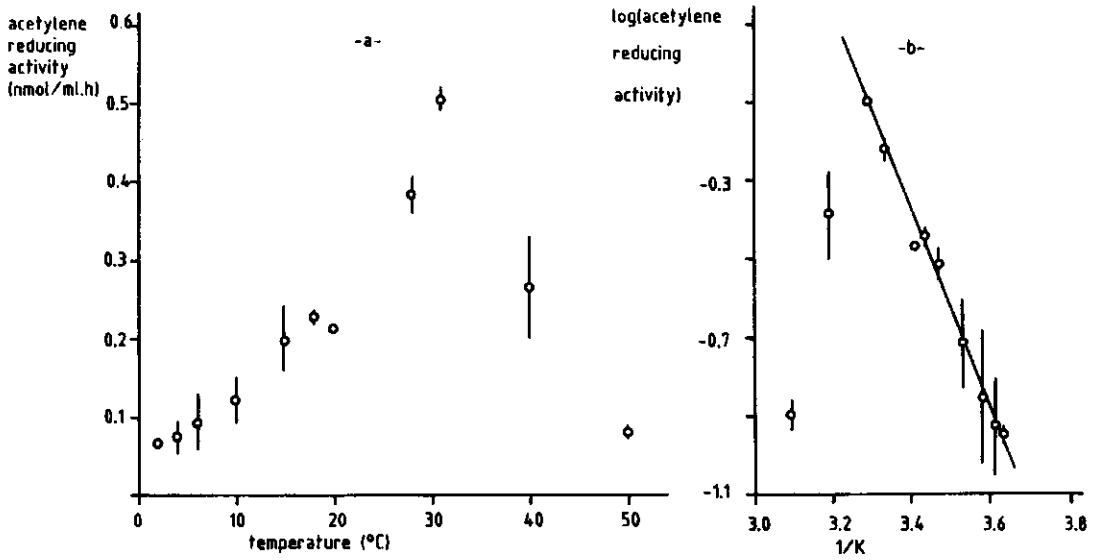

Figure 17. Influence of temperature on the acetylene-reducing activity in surficial (0-5 cm) sediments from 29 m water depth.
a: conventional presentation
b: Arrhenius plot

higher temperature in pure cultures or extracts might be present in the field also at lower temperatures. A different but, in the light of the above mentioned hypothesis of Waughman, more plausible explanation for the low activation energy and for the absence of the discontinuity in the Arrhenius plot is that acetylene-reducing activity is controlled by other rate-limiting steps. The temperature dependency of acetylene reduction in that case actually represents the temperature dependency of the rate-limiting process, which has a lower activation energy and no discontinuity.

If the nitrogen fixation by heterotrophic asymbiotic bacteria is limited by the availability of organic substrate, as hypothesized by STEWART (1969), the measured activation energy of the acetylene-reducing activity is actually the activation energy of the energy delivering process, i.e. the uptake and mineralization of organic substrate. This is supported by the fact that the measured activation energy of the acetylene-reducing activity of Pluss-See sediments is comparable with the activation energy measured by TOERIEN and CAVARI (1982) for the heterotrophic glucose uptake and the turn-over-rates of glucose in lake sediments. The availability of substrate is not limiting in pure and enrichment cultures. In such systems therefore the activation energy of the nitrogenase system itself is measured, resulting in a biphasic Arrhenius plot. The biphasic plots described by KNOWLES *et al.* (1973) for natural samples are in agreement with this conclusion because the natural samples were amended with organic substrate. These natural samples can therefore be characterized as enrichment cultures.

Summarizing it can be concluded that the above results support the hypothesis of STEWART (1969), that nitrogen fixation by free-living heterotrophic microorganisms *in situ* is limited by the availability of organic substrate.

6. The relation between nitrogen fixation (acetylene reduction) in the sediments and some properties of the sediments.

6.1. Introduction.

In the previous chapters attention has been paid to the application of the acetylene reduction assay to the sediments and to the effect of amending the sediments with several substances. In this chapter the relation between the acetylene-reducing activity of the (unamended) sediments and some properties of the sediments and the overlying water will be discussed as well as an estimation of the yearly fixed nitrogen in the sediments of the Pluss-See. In fact part of the field observations will be discussed here in addition to the laboratory studies in the first two chapters.

6.2. Methods.

From August 1977 to August 1978 the acetylene-reducing activity of the sediments at the three sampling stations was measured at regular intervals (ca. every three weeks). Vertical distribution of acetylene-reducing activity was measured under anaerobic conditions at a standard temperature of 27°C. Core samples were split up into fractions of 3 cm thickness (0-15 cm) and 5 cm thickness (15-30 cm). In these fractions the dry weight, organic matter content, carbon and nitrogen contents, interstitial ammonium concentration and pH were measured. In some core samples also adsorbed ammonium was measured. Specific weight was determined in samples of the littoral sediment.
In the surficial 5 cm of the sediments the anaerobic acetylene-reducing activity was measured at 27°C as well as at the *in situ* temperature, while acetylene-reducing activity at 27° C was measured also under aerobic conditions. The aerobic acetylene-reducing activity of the littoral samples was measured during the whole observation period, that of the profundal sediments only in 1978. In the surficial sediments the same parameters were measured as in the core samples. In addition the glucose uptake kinetic parameters of the heterotrophic populations were measured. The results of these measurements will be discussed in the next chapter. For a description of the applied measurement techniques see chapter 2.

6.3. Results.

6.3.1. Composition of the sediments, the interstitial water and the contact water.

Dry weight.

The dry weight of the profundal sediments of the Pluss-See, expressed as the percentage of the fresh weight, was low. At the sediment-water interface the mean dry matter content was 2% at 29 m water depth and 2.5% at 15 cm water depth (see figure 18). Dry matter content increa-

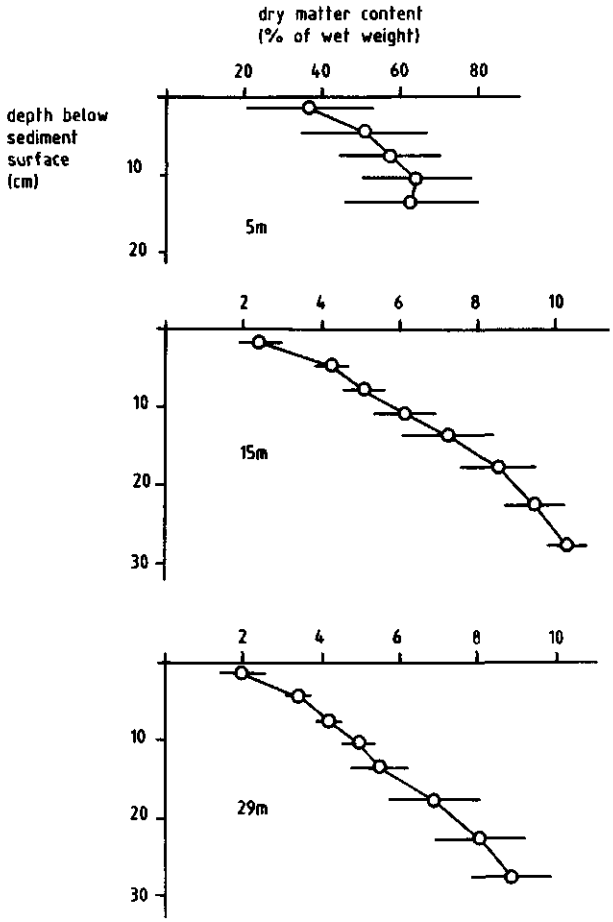

Figure 18. Profiles of dry matter content of the sediments at 29,15 and 5 m water depth. Mean and s.d. of 19 core samples.

sed with depth into the sediments up to 8.9% and 10.3% at 25-30 cm below the sediment-water interface at 29 and 15 m water depth, respectively. The dry matter content of the littoral sediments was considerably higher than of the profundal sediments. The mean dry matter content varied from 37% at the sediment surface to 63% at 12-15 cm below the surface. At the same time the standard deviation of the values in the littoral was much higher than in the profundal sediments, pointing at the more homogeneous character of the latter. The standard deviation of the mean dry matter content decreased with depth below the sediment surface at all measuring stations.

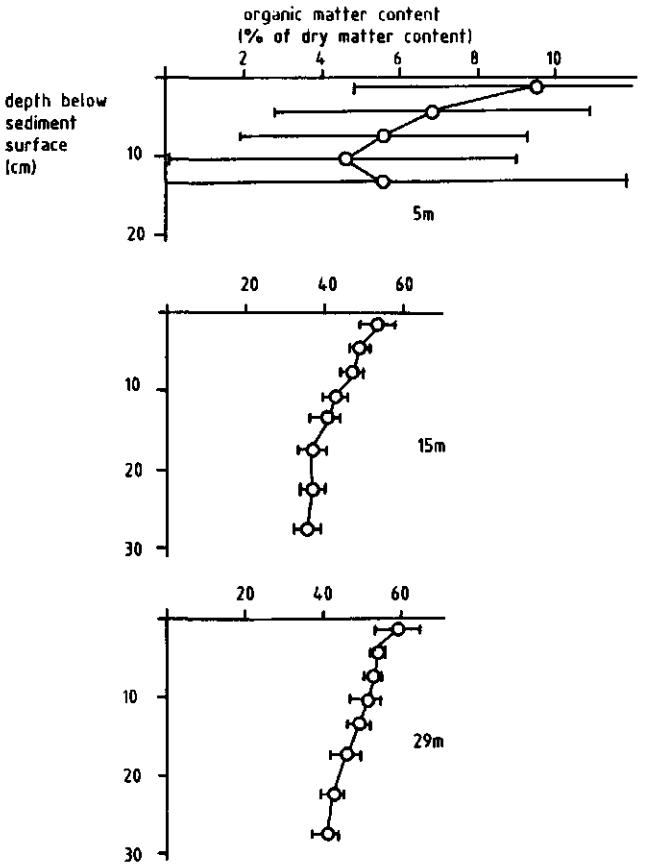

Figure 19. Profiles of organic matter content of the sediments at 29,15 and 5 m water depth. Mean and s.d. of 19 core samples.

Organic matter content.

The organic matter content of the sediments was expressed as percent ash free dry weight of the dry matter content. The profundal sediments showed a high organic matter content (see figure 19) with mean values at the sediment surface of 59% at 29 m water depth and 53.6% at 15 m water depth. The organic matter content decreased with increasing depth below the sediment surface as a result of the decomposition of the organic matter. The organic matter content decreased till 40% at 25–30 cm below the sediment surface at 29 m water depth. At 15 m water depth the decrease of the organic matter contents stopped at 15–20 cm below the sediment surface. Below this depth the organic matter content stabilized.

The organic matter content of the littoral sediments was considerably lower. At the sediment surface the mean content amounted to only 9.6% of the dry matter content. This value decreased with increasing depth into the sediments down to about 5.5% at 12–15 cm depth.

The standard deviation of the mean values in the littoral was considerably higher than in the profundal sediment.

Carbon.

The carbon content of the sediments consisted mainly of organic carbon. In preliminary investigations it was found that the calcium carbonate content of the sediments was negligible. The carbon content showed the same pattern as the organic matter content (see figure 20). In the profundal sediments the carbon content was high and decreased with depth into the sediments: at 29 m water depth the mean carbon content decreased from 30.3% at the sediment surface down to 21% at 25–30 cm depth; at 15 m water depth the mean carbon content decreased from 26.8% at the sediment surface to 18.6% at 25–30 cm depth. The mean carbon content in the littoral sediments was low. At the sediment surface it amounted to 4.5% of the dry matter content.

The course of the carbon content through the measuring period did not show any significant tendencies. Comparison between the carbon and the organic matter content showed that 50–55% of the organic matter consists of carbon.

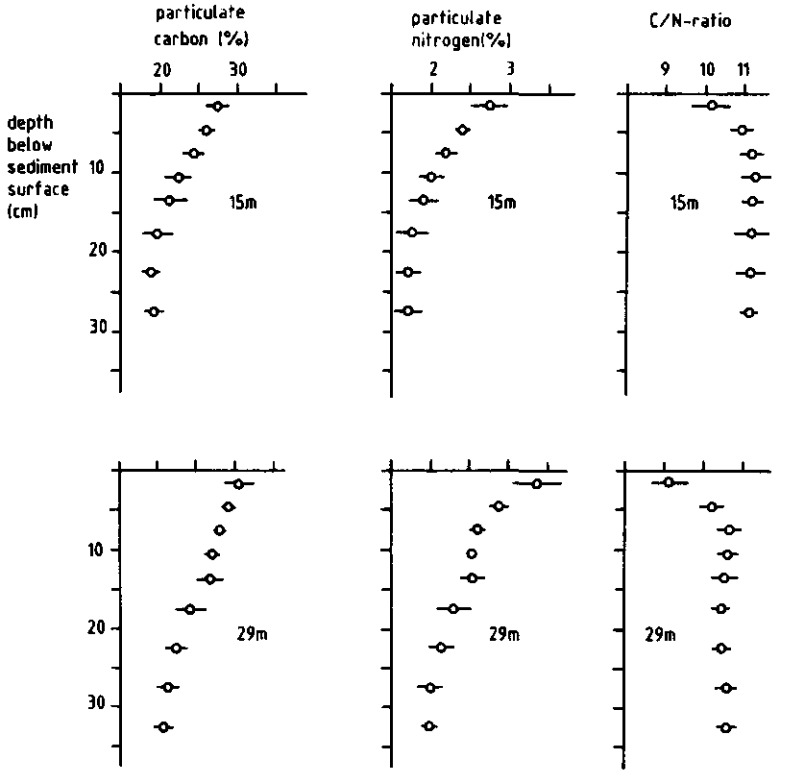

Figure 20. Distribution of particulate carbon, particulate nitrogen and their ratio in the sediments. Mean and s.d. of 19 cores.

Nitrogen.

The nitrogen content of the sediment at 29 m water depth was on the average 3.3% of the dry matter content at the sediment-water interface (see figure 20), below which it decreased down to around 2% at 25-30 cm depth. The nitrogen content decreased thus 40% in the first 30 cm of the sediment, i.e. 10% more than the carbon content decreased in the same sediment layers. The nitrogen decrease at 15 m water depth was also higher than the carbon decrease: at the surface the mean nitrogen content was 2.7%, at 25-30 cm below the surface 1.7%. The nitrogen content of the littoral sediment was relatively low. At the sediment surface it amounted to 0.31%.

The course of the nitrogen content in the first 5 cm of the sediments

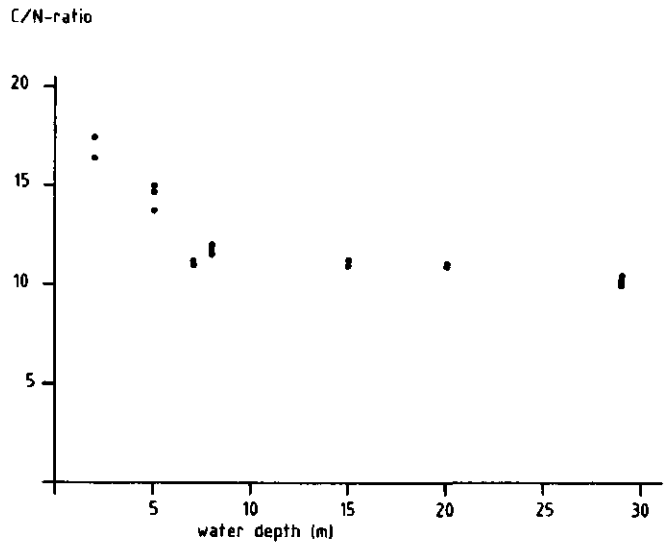

Figure 21. The C/N ration of the sediment at different water depth.

at 29 m water depth did show maximum values during the winterperiod and in May 1978 (up to 3.5%). No clear pattern could be observed at 15 and 5 m water depth.

C/N ratio.

Lowest values of the C/N ratio were observed at the sediment surface at 29 m water depth. The mean value of these sediments was 9.1 (see figure 20). The C/N ratio increased with depth into the sediments down to 6-9 cm below the sediment surface at 29 as well as 15 m water depth. Below this depth it remained rather constant with increasing depth. At 29 m water depth this constant ratio is about 10.5, at 15 m 11. The C/N ratio at 15 m water depth was higher than that at 29 m for every comparable depth below the sediment surface. At the surface it was 10.2 and it increased with depth up to 11.2 at 12-15 cm. The C/N ratio of the littoral sediments was relatively high. The high littoral C/N values are evident from a transect sampling of the sediments (see figure 21). The values in the profundal were low and relatively constant (about 10). In the littoral a sharp increase with decreasing water depth or decreasing distance from the shore line

could be observed.

The C/N ratio of the surficial sediments at 29 m water depth showed minimal values in February, whereas in the summer period of 1978 also low values were measured. No clear pattern could be observed at 15 and 5 m water depth.

pH.

The pH of the interstitial water decreased with increasing depth of

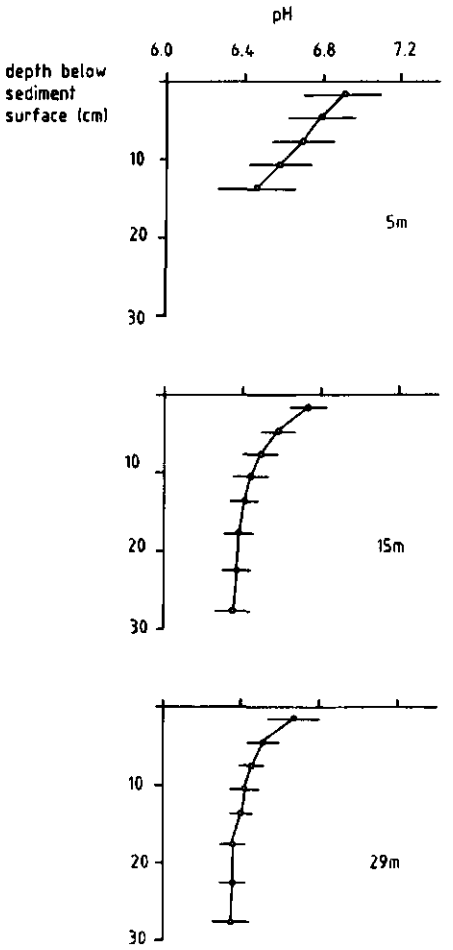

Figure 22. Profiles of pH of the sediments at 29,15 and 5 m water depth. Mean and s.d. of 19 core samples.

the sediments and with increasing depth below the sediment surface (see figure 22). The surficial sediments in the littoral showed a mean pH of 6.92. At 15 and 29 m water depth these figures were 6.74 and 6.67, respectively. In the profundal sediments pH values stabilized below 15-20 cm at 6.36 - 6.38. In the littoral no such stabilization could be observed, because these sediments were only sampled to 15 cm below the sediment surface.

Ammonium.

Ammonium, produced by the decomposition of organic nitrogen compounds, exists in three forms (ROSENFELD, 1979):

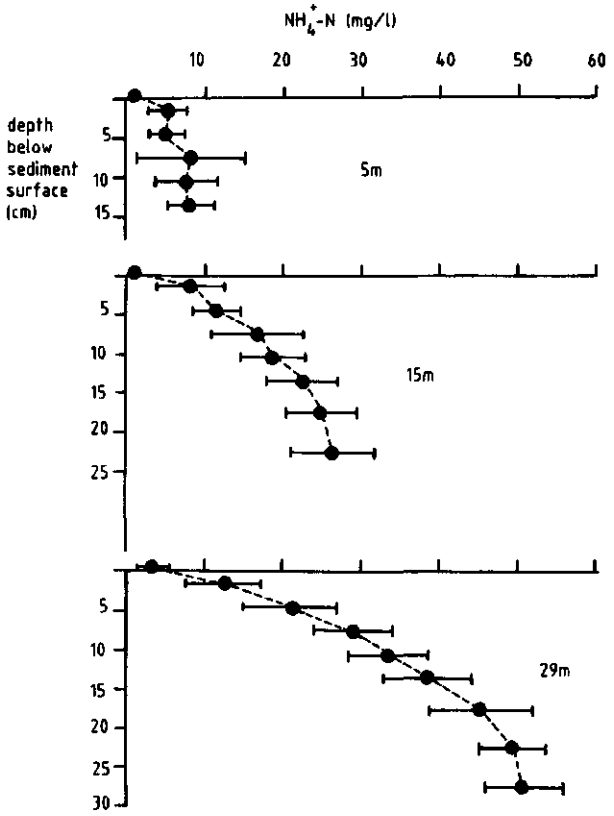

Figure 23. Profiles of ammonium-N in the interstitial water of core samples from 5,15 and 29 m water depth. Mean and s.d. of 19 cores.

- dissolved ammonium in the interstitial water;
- exchangeable ammonium:adsorbed by means of an ion exchange reaction at the surface of clay particles and organic matter;
- fixed ammonium: adsorption in the interlayers of clay structures and not easily replaced by other cations.

In the present study no attention is paid to the latter form of ammonium.

Dissolved ammonium.

Figure 23 shows the mean ammonium concentration in the interstitial

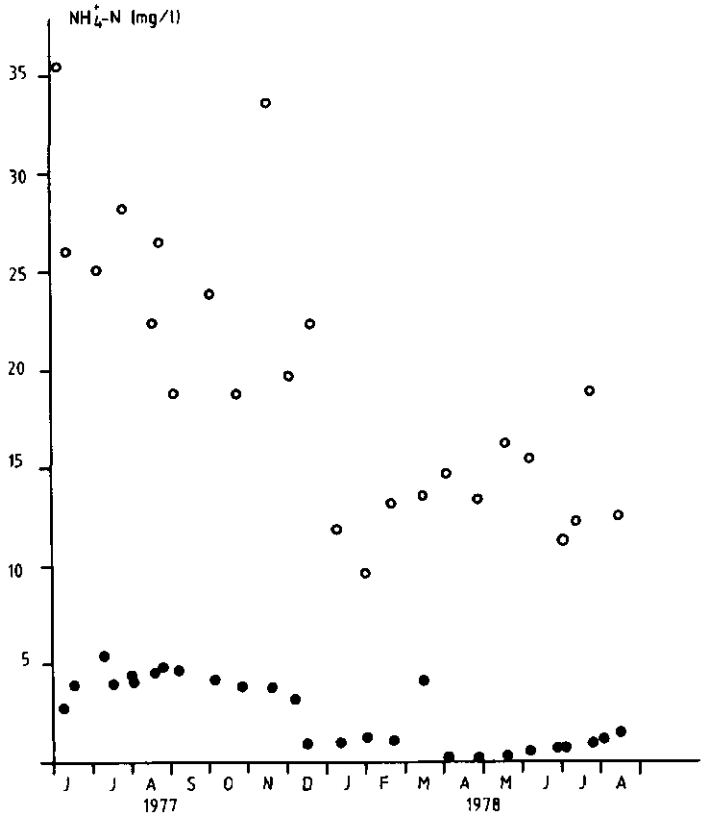

Figure 24. Ammonium concentration in the interstitial water of the surficial sediments (0-5 cm) at 29 m water depth (O) and in the water at the sediment-water interface (●).

water of the sediments and the mean concentration in the water just above the sediment surface (= contact water) at the three measuring stations. The ammonium concentration increased with increasing water depth. In a sediment core the ammonium concentration increased with depth below the sediment-water interface. The concentration gradient within a core increased with water depth.

At 29 m water depth the interstitial ammonium concentration increased from 12 mg/l at the sediment-water interface up to 50 mg/l NH_4^+-N at 25-30 cm below the sediment surface. At 15 m water depth the interstitial concentrations were considerably lower, from 8 mg/l NH_4^+-N at the sediment surface to 27 mg/l NH_4^+-N at 25-30 cm below the sediment surface. The concentrations in the littoral sediments were still lower: on the average lower than 10 mg/l.

The ammonium concentrations in the water just above the sediment surface (see figure 24) were high in 1977 at 29 m water depth until the destratification in the beginning of December. During wintertime and spring the concentrations stayed low, after which a steady increase in the summertime could be observed. The interstitial concentrations showed a much more irregular pattern. Nevertheless a similarity with the course in the contact water can be recognized: high concentrations in 1977 until destratification, after which concentrations are considerably lower. Concentrations before and after the destratification were significantly ($p<0.0005$) different.

At 15 m water depth the same pattern could be observed (see figure 25). In the contact water the concentrations were relatively high before destratification and low hereafter. During wintertime concentrations showed a slight increase, after which concentrations stayed low during the observation period. In the interstitial water the difference between the period before and after turn-over was less clear. Nevertheless the difference was significant ($p<0.05$). In contrast to the parallelism of the concentrations in the contact water and the interstitial water in the profundal, the concentrations in the littoral showed an inverse relationship (see figure 25). In the contact water concentrations were relatively high in the winter period and low during the other seasons, whereas interstitial concentrations were low in the winter and high during the summer period.

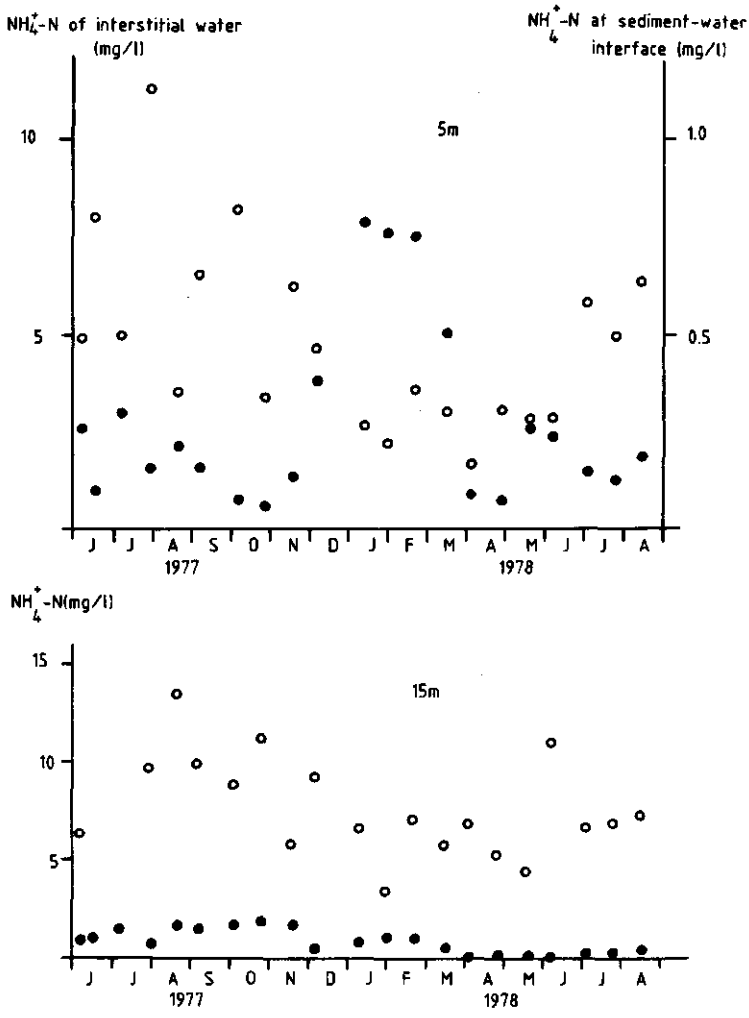

Figure 25. Ammonium concentration in the interstitial water of the surficial sediments (0-5 cm) (O) and in the water at the sediment-water interface (●) at 5 m water depth (upper figure) and 15 m water depth (lower figure).

Exchangeable ammonium.

Exchangeable ammonium, expressed as mg nitrogen per gram of dry sediment, was highest at the sediment surface. Deeper in the sediments lower and more or less constant values were observed (see figure 26).

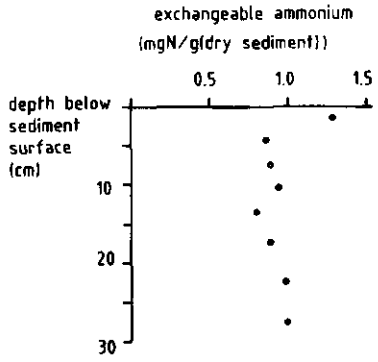

Figure 26. Profile of the exchangeable ammonium content in a sediment core from 29 m water depth (22.02.78).

The exchangeable ammonium content of the surficial sediments at 29 m water depth ranged from 0.2 to 1.2 mgN/g of dry sediment. Mean content during the observation period was 0.80 mgN/g of dry sediment. Lower values were observed in the surficial sediments at 15 m water depth: mean content was 0.43 mgN/g dry sediment. No consistent pattern could be observed at this depth. Nor was this the case in the littoral sediments. Mean content in these sediments was 0.04 mgN/g of dry sediment. ROSENFELD (1979) showed that the concentration of exchangeable ammonium is predominantly associated with the organic matter in the sediment. Therefore the relatively low values of the littoral sediments were thought to be caused by the low organic matter content of these sediments. No significant correlation between the carbon content and the exchangeable ammonium content could be observed. If, however, the C/N ratio of the sediments was held constant, the partial correlation coefficient (see 6.3.3) between the exchangeable ammonium content and the carbon content was significant ($r = +0.556$; $p<0.01$). Reversely, the partial correlation coefficient between the exchangeable ammonium content and the C/N ratio was significant ($r = -0.530$; $p<0.02$), if the carbon content was held constant.

The ratio between exchangeable and dissolved ammonium.

Between exchangeable and dissolved (interstitial) ammonium a rapid equilibrium exists (ROSENFELD, 1979). He found a linear relationship between these two forms. If the adsorption sites in the sediments are not saturated with ammonium, the ratio between these two forms gives information on the adsorption coefficient of the sediments. The ratio can be expressed in different ways:

1. The ratio between the quantity of exchangeable and dissolved ammonium per gram of wet sediment according to:

$$K = \frac{\text{exchangeable ammonium (mgN/g of dry sediment) x dry matter content (\%)}}{\text{dissolved ammonium (mgN/l) x water content (\%)}}$$

   In the surficial sediments at 29 m water depth K ranged from 0.48 to 4.10 l/g with a mean value of 1.47. At 15 m water depth mean K was slightly higher: 2.04 l/g, which means that at the average two times more ammonium is adsorbed than dissolved. K ranged at this depth from 0.56 to 4.14 l/g. The proportion of exchangeable ammonium was much higher in the littoral sediments: K ranged from 2.5 to 13.1 l/g (mean 8.0).

2. K depends on the water content of the sediments. Its value gives information on the adsorption properties of the sediment system as a whole, not of the adsorbing medium itself, i.e. the particulate fraction of the sediments. Therefore the ratio between both forms of ammonium has also been calculated according to:

$$K_e = \frac{\text{exchangeable ammonium (mgN/g dry sediment)}}{\text{dissolved ammonium (mgN/l)}}$$

   K_e gives information on the adsorption properties of the particulate fraction of the sediments. In the surficial sediments at 29 m water depth maximum values were observed in February and during the summerperiod of 1978. K_e values ranged from 0.007 to 0.115 l/g (mean 0.045). Comparable values were observed in the surficial sediments at 15 m water depth, ranging from 0.020 to 0.130 l/g (mean 0.060). Relatively low values were measured in the littoral sediments: from 0.002 to 0.021 l/g (mean 0.014).

3. As shown above, the exchangeable ammonium was associated mainly with organic matter. Because the organic matter content of the

littoral sediments showed large variation, K_e for these sediments has also been calculated with reference to the organic carbon content:

$$K_e(orgC) = \frac{\text{exchangeable ammonium (mgN/g of organic carbon)}}{\text{dissolved ammonium (mgN/l)}}$$

Mean K_e (orgC) for the littoral sediments was 0.237 l/g, slightly higher than the mean K_e(orgC) of the surficial sediments at 29 m water depth: 0.185 l/g. K_e(orgC) values of the littoral sediments were significantly correlated with C/N ratios of the organic matter ($n=20$; $r=-0.538$; $p<0.02$).

No significant correlation existed between K_e and C/N for the profundal station at 15 m water depth. A weak correlation was found at 29 m water depth ($n=25$; $r=-0.370$; $p<0.10$). At both stations K_e was significantly correlated with the pH ($n=22$; $r=0.558$; $p<0.01$ and $n=22$; $r=0.462$; $p<0.05$ respectively).

6.3.2. Acetylene-reducing activity in the sediments.

Rates of acetylene reduction were expressed per gram of organic carbon in the sediments. The acetylene-reducing activity in the vertical fractions of the core samples from 29 m water depth showed relatively high rates at the sediment surface and a strong decrease with depth below the sediment surface. Below 20 cm no significant acetylene reduction occurred (see figure 27).

The same pattern was observed at 15 m water depth (see figure 27). The mean acetylene-reducing activity at the sediment-water interface, however, was substantially lower than at 29 m water depth. The vertical distribution of the acetylene-reducing activity in the sediment cores from the littoral showed an irregular, but rather comparable pattern with high rates at the sediment surface and low rates in the deeper sediment layers (see figure 27).

Figure 28 shows the acetylene-reducing activity in the surficial sediments at the three sampling stations during the observation period. The *in situ* temperature at 15 and 29 m water depth was 4°C throughout this period. The acetylene-reducing activity at 29 m water depth was relatively low in 1977 followed by some high rates in February 1978. During the spring and the summer of 1978 the rates

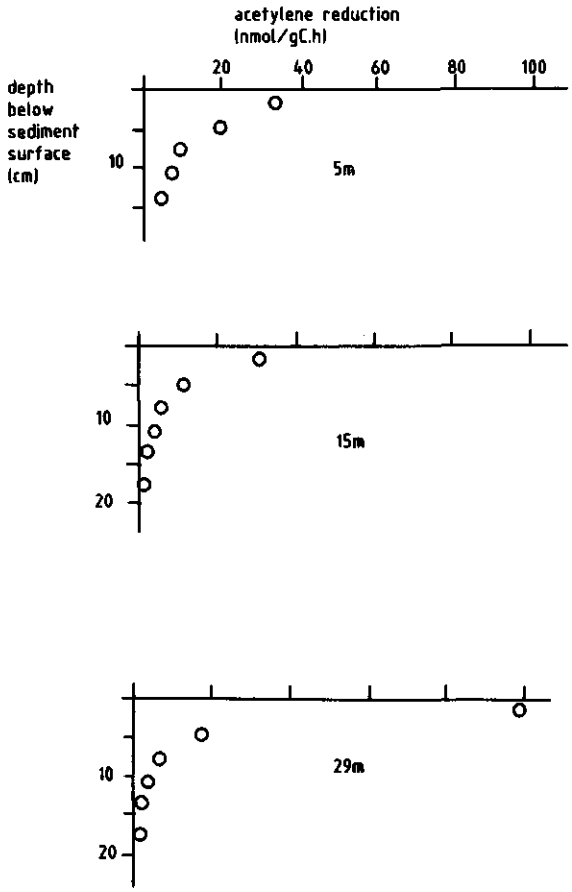

Figure 27. Profiles of acetylene-reducing activity of the sediments at 29,15 and 5 m water depth. Mean and s.d. of 19 core samples.

were low again, however, higher than those in the previous year. Mean value over the whole period was 9.7 nmol. $gC^{-1}.h^{-1}$. The anaerobic acetylene-reducing activity at 27°C showed the same pattern. Correlation of acetylene reduction at *in situ* temperature with acetylene reduction at 27°C was highly significant ($n=24$; $r=0.951$; $p<0.001$). Mean value of acetylene-reducing activity at standard temperature was 36.1 nmol. $gC^{-1}.\ h^{-1}$, i.e. 3.7 times higher than the mean rate at *in situ* temperature. Aerobic acetylene-reducing activity at 27°C was measured only during half the observation period. Rates were rela-

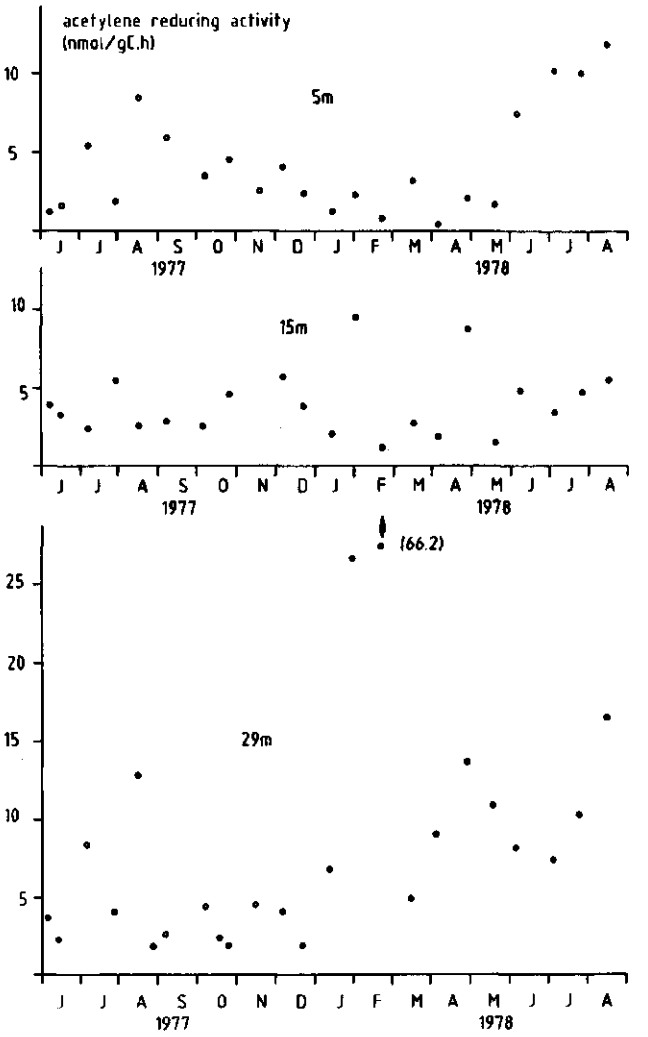

Figure 28. Acetylene-reducing activity at *in situ* temperature in the surficial (0-5 cm) sediments at 5,15 and 29 m water depth.

tively low. Mean activity was 7.0 nmol, $gC^{-1}.h^{-1}$, i.e. 5 times lower than the rates under anaerobic conditions. Aerobic rates were significantly correlated with anaerobic rates at the same temperature ($n=11$; $r=0.890$; $p<0.001$).

At 15 m water depth (figure 28) acetylene-reducing activity at *in*

situ temperature (4°C) showed an irregular pattern. Mean activity at *in situ* temperature was 3.9 nmol.$gC^{-1}.h^{-1}$, substantially lower than the mean rate at 29 m water depth. Activity at 27°C showed the same irregular pattern. Mean rate was 15.6 nmol.$gC^{-1}.h^{-1}$, 4 times higher than the mean rate at the *in situ* temperature. Aerobic acetylene-reducing activity at 27°C was much lower than anaerobic activity (1.4 nmol.$gC^{-1}.h^{-1}$), in fact 11.5 times lower. In contrast with the results at 29 m water depth aerobic rates were not significantly correlated with anaerobic activity.

In the littoral at 5 m water depth acetylene-reducing activity at *in situ* temperature showed high values during the summer season and low values in the cold season (figure 28). Mean activity at this station was 4.2 nmol C_2H_2.$gC^{-1}.h^{-1}$. Anaerobic activity at 27°C showed an irregular pattern without striking trends. The same holds for aerobic acetylene-reducing activity at this temperature, except for a relatively high value in December 1977, the only time that aerobic incubation yielded higher values than anaerobic incubation. As was found at 15 m water depth, no significant correlation existed between anaerobic and aerobic activity. Mean acetylene-reducing activity under aerobic conditions (3.0 nmoles.$gC^{-1}.h^{-1}$) was 3.6 times lower than under anaerobic conditions (11.0 nmoles.$gC^{-1}.h^{-1}$).

An estimation of the yearly fixed amount of nitrogen at the three sampling stations was based on the following assumptions:

- Referring to the discussion in 3.2.3 the yearly fixed nitrogen is estimated with various conversion factors:
  - 3, the theoretical conversion factor;
  - 1.5, assuming non-saturation of the nitrogenase complex with acetylene under the assay conditions, but saturation with N_2 under natural conditions;
  - 6.9, assuming saturation with acetylene, but non-saturation with N_2. Besides an exceptional high factor of 25, this is the highest average conversion factor reported by HARDY *et al.* (1973);
- nitrogen fixation in the surficial 5 cm of the sediments for the different stations was assumed to be 57%, 41% and 66% of total sedimental nitrogen fixation for 5,15 and 29 m water depth respectively. These values were calculated from the mean vertical distribu-

tion of acetylene reduction in the sediments (see figure 27);

- the period between two sampling dates was split up into two equal parts. Acetylene-reducing activity in the first period was assumed to be equal to the activity measured at the first sampling date, the activity in the second period to the activity measured at the next sampling date;
- specific weight of the sediments at 15 and 29 m water depth was assumed to be unity. For littoral sediments the measured specific weight was used for the transformation of the activity/g fresh sediment to the activity/m^2.

Based on these assumptions the total fixed nitrogen between 17.8.77 and 16.8.78 was calculated to be:

depth (m)	fixed N ($g.m^{-2}$) ratio 1:6.9	ratio 1:3	ratio 1:1.5
5	0.17	0.38	0.77
15	0.15	0.35	0.70
29	0.24	0.55	1.10

6.3.3. The relationship between the acetylene-reducing activity and the composition of the sediments.

Figure 29 shows the relationship between the acetylene-reducing activity of the sediments and the ammonium concentration in the interstitial water, as measured in the annual cycle (see figures 28, 24 and 25). In the profundal sediments the interstitial ammonium concentration seems to set the upper limit for acetylene-reducing activity: the lower the ammonium concentration, the higher the acetylene-reducing activity *can* be. Realization of this potential acetylene-reducing activity seems to depend on other environmental conditions. Acetylene-reducing activity at 29 m water depth is weakly correlated with the interstitial ammonium concentration ($n=25$; $r=-0.361$; $p<0.10$). A slightly stronger correlation exists at 15 m water depth: $n=19$; $r=-0.518$; $p<0.05$. A positive relationship seems to exist in the littoral sediments: in these sediments high acetylene-reducing activity coincidenced with high ammonium concentrations. Some data deviate from this relationship. These values are associated with low K_e(orgC). If only sediment with K_e(orgC) >0.20 is taken into consideration acetylene reduction is significantly correlated with the interstitial ammonium concentration ($n=14$; $r=0.704$; $p<0.01$).

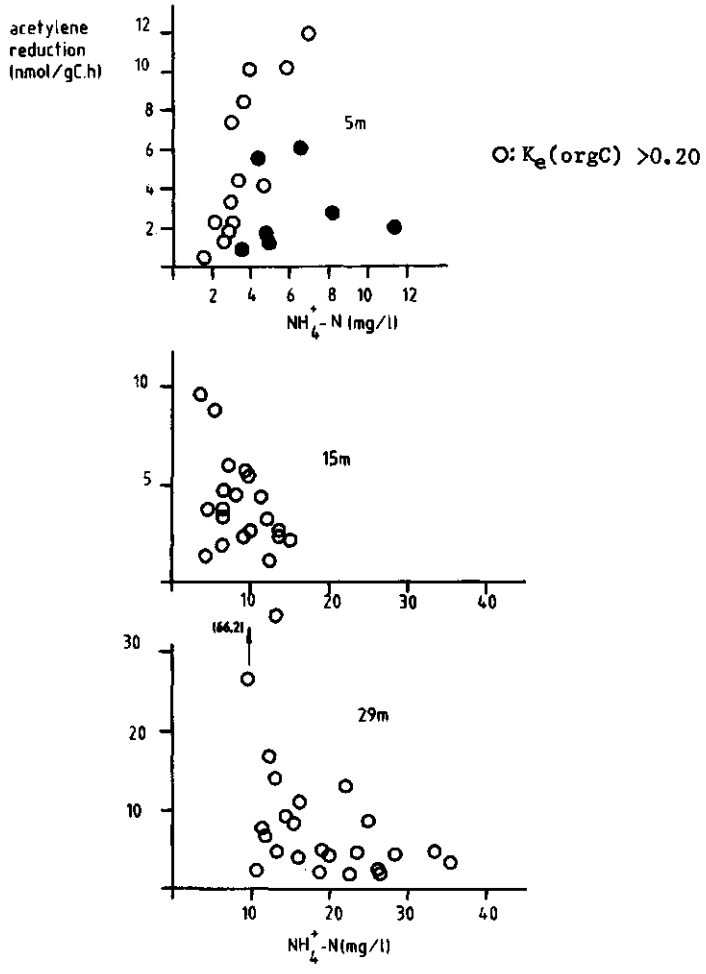

Figure 29. Relation between the ammonium concentration in the interstitial water and the acetylene-reducing activity of the surficial sediments (0-5 cm).

As shown in chapter 3 nitrogenase or the nitrogen-fixing microorganisms are not in direct contact with the ammonium in the interstitial water. One factor that may explain this screening mechanism is the capacity of the sediments to adsorb ammonium. Acetylene-reducing activity in the sediments at 29 m water depth was positively correlated with the adsorbed ammonium content ($n=25$; $r=+0.498$; $p<0.02$). No cor-

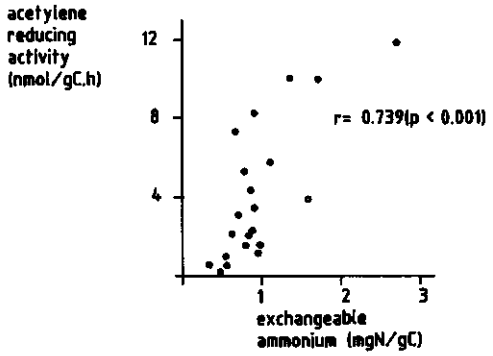

Figure 30. The relation between the exchangeable ammonium content and the acetylene-reducing activity in the littoral sediments.

with temperature ($r=0.760$; $n=21$; $p<0.001$). No significant temperature relation was found at 15 m and 5 m water depth. In 6.3.1 it is shown that the organic matter content of the sediments is one of the factors governing the exchangeable ammonium adsorption. In the littoral sediment samples the organic matter content varied considerably. To correct for this variation, adsorbed ammonium of the littoral sediment samples was expressed per gram of organic carbon. Regression analysis of the acetylene-reducing activity on these values revealed a strong correlation ($r=0.739$; $n=21$; $p<0.001$) (see figure 30).

As described in 6.3.1 the ratio between adsorbed and dissolved (interstitial) ammonium (K_e) gives information on the adsorption properties of the sediments, if at least the adsorption sites are not saturated with ammonium. Linear regression analysis of the acetylene-reducing activity values on the K_e-values of the sediment samples from the deepest part of the lake showed a significant correlation ($n=25$; $r=0.573$; $p<0.01$) between these two parameters (see figure 31). This correlation is still more significant, if one extreme value is left out of the analysis: $n=24$; $r=0.725$; $p<0.001$. At 15 m water depth a slighty less significant correlation was found: $n=19$; $r=0.539$; $p<0.02$. K_e(orgC) and the acetylene-reducing activity in the littoral sediments were also slightly correlated ($n=21$; $r=0.458$; $p<0.05$).

The important role of temperature in the explanation of the variation of the acetylene-reducing activity in the littoral sediments is shown in figure 32. Acetylene-reducing activity is significantly correlated

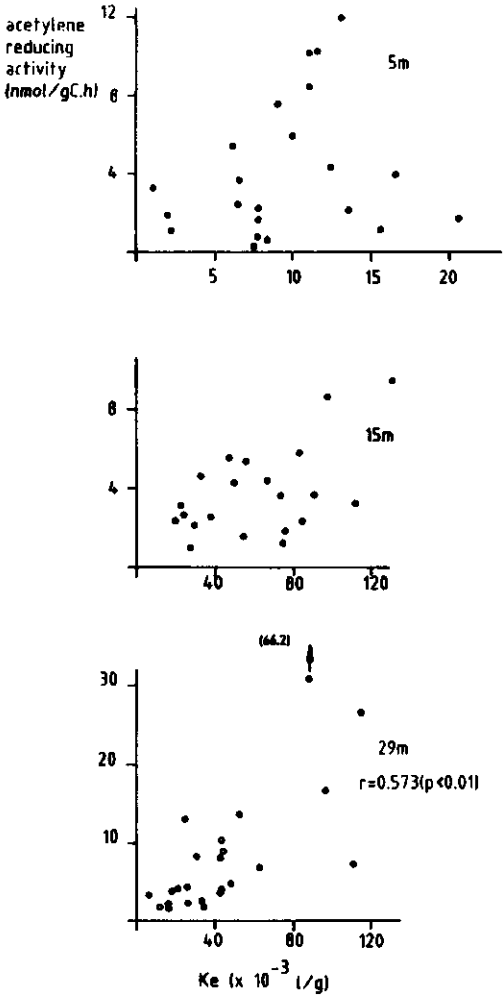

Figure 31. Relation between K_e and the acetylene-reducing activity of the surficial sediments (0-5 cm) at 5,15 and 29 m water depth.

fluctuations have been observed in the profundal sediments.

An inverse relationship was found to exist between acetylene-reducing activity and C/N ratio of the sediments at 29 m water depth (see figure 33). At C/N ratios below 10 acetylene-reducing activity apparently increased in an exponential way. The magnitude of the increase

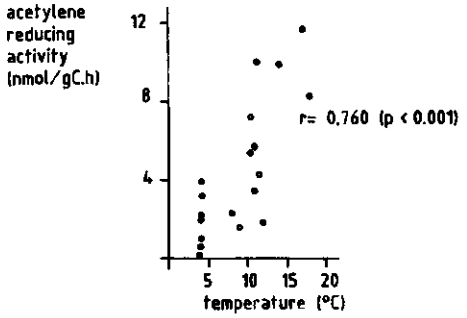

Figure 32. The relation between temperature of the incubated samples and the acetylene-reducing activity in the surficial (0-5 cm) littoral sediments.

seems to depend on the interstitial ammonium concentration in the surficial sediments. In fact two curves can be drawn through the data: one for concentrations higher than 17 mg NH_4^+-N/l and one for lower concentrations. Linear regression analysis of acetylene-reducing activity on the C/N ratio of the sediments at 29 m depth gives a correlation that is significant ($n=25$; $r=-0.476$; $p<0.02$). If the same analysis is run for sediments with interstitial ammonium concentrations below 17 mg NH_4^+-N/l the correlation is more significant ($n=11$; $r=-0.762$; $p<0.01$).

At 15 m water depth the acetylene-reducing activity of the sediments was also correlated significantly with the C/N ratio of the sediments ($n=19$; $r=-0.655$; $p<0.01$). A weak correlation was observed in the littoral sediments ($n=21$; $r=-0.444$; $p<0.05$).

In table II the correlations between the acetylene-reducing activity (expressed as nmol $C_2H_2.gC^{-1}.h^{-1}$.) and the different parameters for the three sampling stations are summarized.

In order to reveal interdependence of the different parameters, partial correlation coefficients have been calculated. The partial correlation coefficient between two parameters (A and B) is the correlation coefficient in a cross section of individuals all having the same value of the parameter C. This parameter C is held constant, so that only A and B are involved in the correlation. Partial correlation coefficients have also been calculated with two parameters

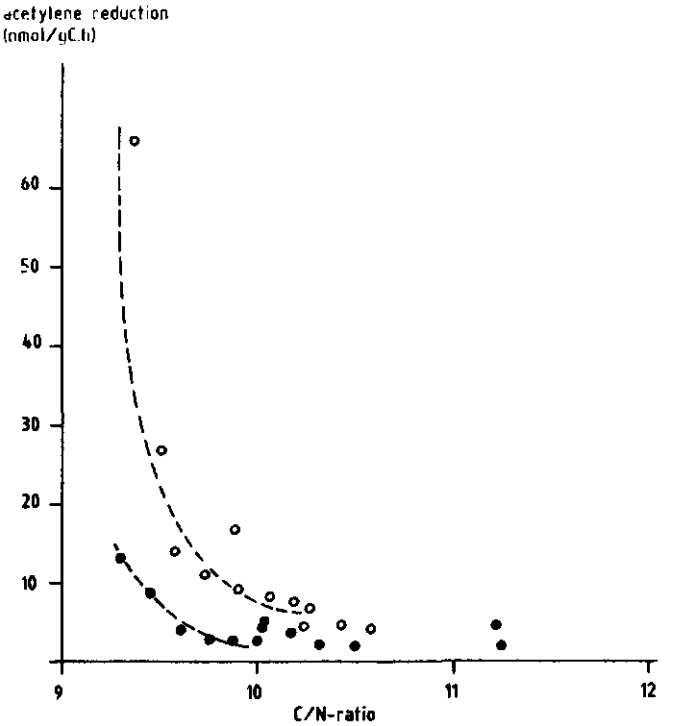

Figure 33. Relation between the C/N ratio and the acetylene-reducing activity of the surficial sediment from 29 m water depth.
: relatively high NH_4^+ -concentrations (>17 mg NH_4^+-N.l^{-1}) in the interstitial water at the sediment-water interface.
: relatively low NH_4^+-concentrations (<17 mg NH_4^+-N.l^{-1}) in the interstitial water at the sediment-water interface.

(C and D) held constant.

In the littoral sediments the partial correlation coefficient of the acetylene-reducing activity with the interstitial ammonium concentration, keeping temperature and exchangeable ammonium constant, was insignificant ($r=-0.423$; $n=17$). The partial correlation coefficients of the acetylene-reducing activity with temperature and with exchangeable ammonium, keeping the other two parameters constant, were both significant ($n=17$; $r=-0.660$; $p<0.01$ and $n=17$; $r=0.648$; $p<0.01$ respectively). Temperature and exchangeable ammonium therefore seem to be

Table II: Correlation coefficients of the acetylene-reducing activity (nmol $C_2 \cdot gC^{-1} \cdot h^{-1}$) with the interstitial ammonium concentration, the exchangeable ammonium content, K_e, the C/N ratio and the temperature of the surficial sediments at the three sampling stations in the Pluss-See.

Water depth (m)	n	Interstitial ammonium (mgN/l)	Exchangeble ammonium (mgN/g of dry sediment)	K_e (1/g)	C/N ratio	Temperature (°C)
5	21	+0.174	+0.739***1)	+0.458*2)	-0.444*	+0.760****
	17[4]	+0.239	+0.774****1)	+0.419*2)	-0.399	+0.735****
15	19	-0.518*	+0.130	+0.539**	-0.655***	- 3)
29	25	-0.361	+0.498**	+0.573***	-0.476**	- 3)
	16[4]	-0.589**	+0.527*	+0.760****	-0.555*	- 3)

* : $p<0.05$
** : $p<0.02$
*** : $p<0.01$
****: $p<0.001$

1): exchangeable ammonium as mgN/g of carbon;
2): correlation coefficient with K_e(orgC);
3): no significant temperature variation observed at this depth;
4): correlation coefficients for the same sample set as for table V.

the most important of the studied parameters for the explanation of the variation of the acetylene-reducing activity in the littoral sediments. Multiple regression of temperature, exchangeable ammonium and the C/N ratio revealed that these parameters explained 75.8% of the variance of the acetylene-reducing activity.

The observed correlation of the acetylene-reducing activity with K_e in the sediments at 15 m water depth is caused by intercorrelation of K_e with the C/N ratio and the interstitial ammonium concentration: with these two parameters held constant r=0.099. The C/N ratio and the interstitial ammonium concentration were not correlated (n=19; r=-0.361).

The partial correlation coefficient of the acetylene-reducing activity and K_e in the sediments at 29 m water depth, with the C/N ratio held constant, is significant (n=24; r=0.694; $p<0.001$. The partial correlation coefficient with the C/N ratio, keeping K_e constant, is significant too (n=24; r=-0.451; $p<0.05$). Multiple regression showed that K_e and the C/N ratio explained 55.9% of the variance of the acetylene-reducing activity in the sediments at this depth.

Summarizing the following conclusions can be drawn from the field measurements:

1. in the littoral sediments acetylene-reducing activity is mainly correlated with temperature and adsorbed ammonium;
2. interstitial ammonium concentration sets the upper limit for the acetylene-reducing activity in the profundal sediments;
3. acetylene-reducing activity in the profundal sediments at 15 m water depth is correlated with the C/N ratio and the interstitial ammonium concentration of the sediments;
4. acetylene-reducing activity in the profundal sediments at 29 m water depth is correlated with K_e and the C/N ratio. At interstitial ammonium concentrations lower than 17 mg NH_4^+-N/l the correlation with the C/N ratio was more significant.

6.4. Discussion.

6.4.1. Composition of sediments, interstitial water and contact water.

Sediments.

Lake bottoms can be subdivided into areas of accumulation, with se-

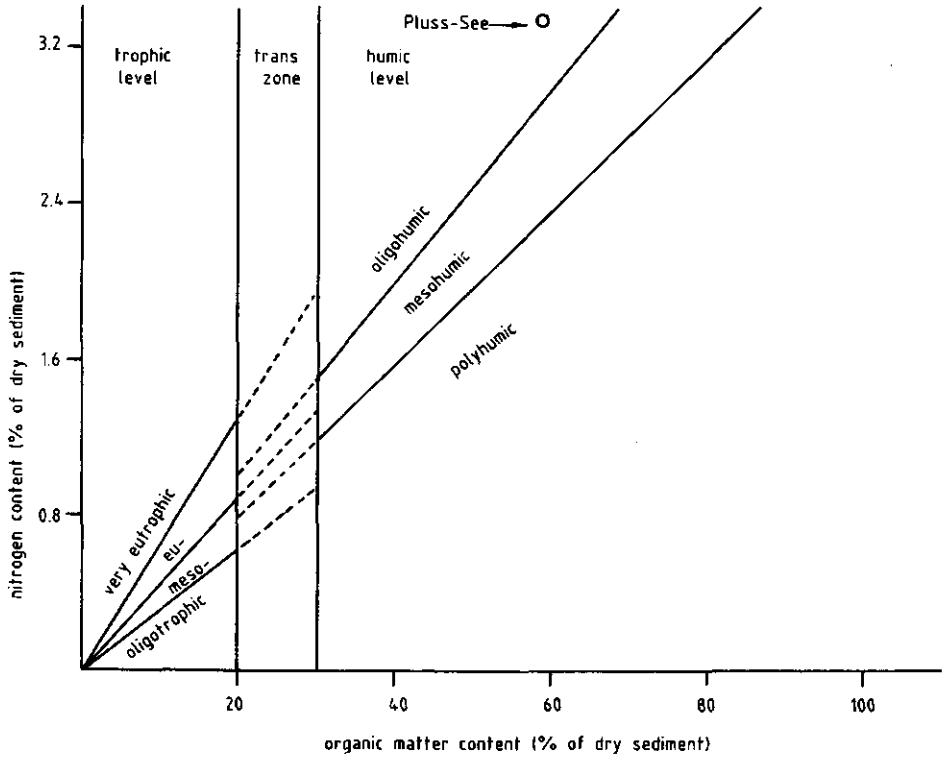

Figure 34. Diagram illustrating the relationship between nitrogen concentration and loss on ignition of surficial sediments (0-1 cm) relative to lake trophic level and lake humic level. (adapted from: HÅKANSON, 1984).

diments having a water content >75%, areas of erosion with a water content of the sediments <50% and an area of transport between them. (HÅKANSON, 1981). From the dry matter content of the sediments it can be seen that both profundal sediment sampling stations in the Pluss-see are situated in the accumulation area and that this is not the case for the littoral station. Sediment sampling at different depths in the Pluss-see showed that accumulation of soft sediments occurred up to at least 7 m water depth. At 6 m water depth no permanent, soft sediments have been observed. Hence the borderline of the accumulation area seems to be situated somewhere between 6 and 7 m water depth.

The differences in composition between sediments at 15 and 29 m water depth (lower C, N, OM and higher DM at 15 m) point at the more decomposed state of the sediments at 15 m water depth. Similar differences in Lake Esrom have been observed by HARGRAVE and KAMP-NIELSEN (1977). They explained these differences as being caused by the declination of the bottom slope. This might be the case in the Pluss-See too: the 15 m station is situated at a steep slope of the lake bottom. The influence of lake morphometry in general on the distribution of the sediments is further discussed in chapter 8.

The organic matter contents of the sediments, as represented by C, N and OM, is high and comparable with the data presented by UNGEMACH (1960). The organic matter content of the profundal sediments is at the higher range of the data presented bij HÅKANSON (1984) for 71 lakes. HARGRAVE (1975) showed for a series of lakes a positive correlation between sedimentation of suspended matter and the percentage organic matter of the surface sediment. From this relationship a very high sedimentation might be expected in the Pluss-See.

HÅKANSON (1984) used the ratio between OM and N of sediments for the classification of lakes. Given the high organic content of the Pluss-See the ratio does not characterize the trophic level of the lake, but the humic level. The position of the Pluss-See in the diagram, presented by HÅKANSON (1984) is shown in figure 34. The Pluss-See can be classified as a lake on the borderline between oligo- and mesohumic conditions. This is confirmed by the C/N ratio of the sediments, increasing from 9 at the sediment surface to 11 in deeper layers. HANSEN (1961) defines oligohumic lakes as lakes having

C/N ratios <10 and polyhumic lakes as lakes having C/N ratios >10-15. The same limit (C/N ratio=10) can be used for the discrimination between dy and gyttja sediments (HANSEN, 1959).

The sediments of the Pluss-See can be characterized as dy-gyttja (LUNDBECK, 1926; STABEL, 1984; UNGEMACH, 1960). KOPPE (1924) observed significant allochtonous influence in the littoral zone of the lake, while in deeper parts the sediments may be classified as gyttja. This difference between littoral and profundal sediments is clearly shown by the C/N ratio of the sediments, having high values in the littoral and relatively low values in the profundal (see figure 21). However, the C/N ratio of the sediments is not only an index for humosity. The average C/N-ratio of natural planktonic material is 5.6 (HÅKANSON, 1984), although this value may vary strongly owing to the physiological state of the phytoplankton community (BANSE, 1974; GOLDMAN, 1980). During the decomposition of detritus relatively rich in nitrogen (e.g. dead fytoplankton) the C/N ratio tends to increase (KOYAMA and TOMINO, 1968; OTSUKI and HANYA, 1972; BLACKBURN and HENRIKSEN, 1983; GRAF *et al.*, 1982). Organic matter in aquatic ecosystem can be thought to consist of a number of fractions with differing susceptibilities to bacterial attack (LANCELOT and BILLEN, 1985). If it is assumed that the more labile fraction has a lower C/N ratio than the more resistant one, an increase of the C/N ratio would be observed during decomposition (LANCELOT and BILLEN, 1985). In this way the C/N ratio can be used as an index for the degradability of the organic matter, i.e. the availability of organic substrate for the microbial heterotrophic population. This can be demonstrated by the vertical distribution of the C/N ratio in the sediments (see figure 20). At the sediment-water interface the C/N ratio is relatively low. This material has recently been deposed and has not yet been subjected to such an intensive decomposition as the deeper and older sediment layers, having higher C/N ratio. Similar increases have been observed in marine sediments (ROSENFELD, 1981). Differences in humosity between lakes and sites within lakes (caused by differences in allochtonous influences) make it, however, impossible to use the C/N ratio as an absolute index for substrate availability. Its use is limited to C/N-differences at one site, e.g. the vertical C/N distribution in the sediment.

Interstitial water

The observed ammonium concentrations in the interstitial water are similar to those reported by OHLE (1964), who showed the relation between the interstitial ammonium concentration and the productivity of the lake. The Pluss-See showed both relatively high interstitial ammonium concentrations and high primary productivity (OHLE, 1964). The seasonal fluctuations in the littoral were similar to those reported by KAMIYAMA *et al.* (1979) in shallow water regions of Lake Biwa. As in the Pluss-See these fluctuations were accompanied by seasonal changes in the sediment temperature. The high temperatures in the summer time combined with the higher input of organic matter by sedimentation of primary products results in higher mineralization and hence higher ammonium concentrations. The fluctuations of the interstitial ammonium concentration in the profundal sediments were accompanied by fluctuations of the ammonium concentration in the water just above the sediment surface. These fluctuations in the water were caused by the circulation and mixing of the water during homothermy and the build up of ammonium nitrogen in the hypolimnion during the stratification of the lake.

The interstitial ammonium concentration increased with depth into the sediment. Several workers have shown that vertical distributions can be explained by mathematical models that include the mineralization of organic nitrogen in the sediments, the diffusional flux of ammonium from the sediment into the overlaying waters and the compaction of the sediments (BERNER, 1977; KAMIYAMA, 1978 and 1979; ANIKOUCHINE, 1967; VAN DER BORGHT *et al.*, 1977). Differences in ammonium concentration between the two profundal stations may be explained in terms of these models: in the deep parts of the lake the mixing and turbulences of the water will be reduced compared to shallower regions. As a result there is a build up of ammonium at this depth, causing a decrease of the concentration gradient responsible for the diffusional transport of ammonium out of the sediments. As a consequence the ammonium concentration in the interstitial water increases until the (increasing) concentration gradient creates a diffusional flux that is in equilibrium with the mineralization rate in the sediments. Besides this effect of the reduced turbulences at this depth, the input of organic nitrogen and thus the mineralization

rate at the deepest point of the lake is higher as a consequence of sediment focusing (see chapter 8).

The adsorption of ammonium has been shown to be predominantly associated with the organic matter content of sediments (ROSENFELD, 1979). This could also be shown in the present study for the littoral sediments. The variation of the organic matter content in the profundal sediments was too small to produce a significant correlation with the exchangeable ammonium. The observed range of exchangeable ammonium in the surficial sediments (2-4 mgN/g organic carbon) is slightly higher than the values reported by KEENEY *et al.* (1970) and KONRAD *et al.* (1970) for some Wisconsin lakes and by KEMP and MUDROCHOVA (1972) for a Lake Ontario sediment core.

ROSENFELD (1979) observed a rapid equilibrium between exchangeable and dissolved ammonium. He found a linear relationship between these two forms of ammonium in anoxic, marine sediments. From the slope of the adsorption isotherm he calculated the adsorption coefficient K. K can also be calculated from field data by taking the ratio of exchangeable to dissolved ammonium for each individual sample, as was done in the present study. In this way, K-values are overestimated at lower dissolved ammonium concentrations. (ROSENFELD, 1979). However, in the present study not the absolute values for K are of interest, but variation of K in relation to the variation of other parameters.

The ammonium adsorption coefficient K, as used by ROSENFELD (1979), is a characteristic of the sediment system as a whole. The K_e value calculated in the present study describes the adsorption properties of the particulate fraction of the sediments, if at least the particulate fraction is not saturated with ammonium. If adsorption is predominantly associated with organic matter (as shown by ROSENFELD, 1979), K_e is a characteristic of the organic matter in the sediments, describing the adsorption properties of the organic matter. K_e and especially K_e(orgC) is then an index for the number of adsorption sites (in fact ion exchange sites) per unit surface of organic matter.

In the littoral sediments it could be shown that the adsorbed ammonium content was correlated both with the organic matter content and the quality of the organic matter (see 6.3.1). K_e(orgC) was significantly correlated with the C/N ratio of the sediments.

Also from the vertical distribution of the exchangeable ammonium in

the profundal sediments it could be shown that ammonium adsorption is not only associated with organic matter, but moreover with the quality of this organic matter (see figure 26). The exchangeable ammonium content of the recently deposed, degradable sediments at the sediment surface is higher than of the refractory permanent sediments at 30 cm below the sediment surface, inspite of the much higher dissolved ammonium concentration at this depth. This means that the relative number of ion exchange sites on the organic matter decreases during decomposition. This might be partly caused by the decrease of pH with depth in the sediments (figure 22). The positive correlation between K_e and pH might be explained by the lower number of hydrogen ions at higher pH competing for adsorption sites. Increasing cation exchange with increasing pH has been reported for amorphous minerals by KEENEY *et al.* (1970).

6.4.2. Acetylene reduction in the sediments and its relation with the composition of the sediment.

Annual rate of nitrogen fixation

The annual rate of nitrogen fixation in the sediments of the Pluss-See has been estimated to be 0.15-1.1 $gN.m^{-2}.year^{-1}$ (see 6.3.2). From table III it can be concluded that these rates fall within the lower range of the values reported in the literature. This is in agreement with the conclusion of OLÁH *et al.* (1983) that nitrogen fixation in lake sediments is inversely correlated with lake depth. From this relationship higher rates should be expected at the 15 and 5 m sampling station. No attempts have been made to measure redox-potential and oxygen at the sediment-water interface *in situ*. From data on the oxygen distribution given by ALBRECHT *et al.* (1978) and STABEL (unpublished results), it can be concluded that conditions for nitrogen fixation at 15 m water depth and in the littoral were less favourable with respect to the redox-potential. This might be an explanation for the relatively low nitrogen fixation rates at 5 and 15 m water depth. Moreover these low rates may be explained by the low organic carbon input because of the focusing of sediments (see chapter 8). The ambient temperature at 29 m water depth is low during the whole year, but even if the annual average was between 10 and 20°C, fixation rates would be still within the lower range of the

Table III. Estimation of amounts of nitrogen yearly fixed in the sediments of some freshwater and estuarine ecosystems.

	Nitrogen fixation ($g.N.m^{-2}.year^{-1}$)	Reference	
freshwater ecosystems:			
Keszthely Basin, Lake Balaton	4.7	OLAH *et al*	(1983)
Körös backwater reservoir	6.5	OLAH *et al*	(1983)
polycultural fishpound	0.49	OLAH *et al*	(1983)
liquid manure oxidation fishpound	0.61	OLAH *et al*	(1983)
Harkortsee	8.4	JÄGER and WERNER	(1977)
Lake Erie [1)]	20.4	HOWARD *et al*	(1970)
Lake Mendota	0.23 - 0.27	MACGREGOR *et al*	(1973)
Lake Wingra	0.25 - 0.29	MACGREGOR *et al*	(1973)
Trout Lake	0.05	MACGREGOR and KEENEY	(1973)
Pluss-See 5 m	0.4 - 0.8	this study	
Pluss-See 15 m	0.4 - 0.7	this study	
Pluss-See 29 m	0.6 - 1.1	this study	
estuaries:			
Waccasassa	0.37	BROOKS *et al*	(1971)
Rhode River	0.24	MARSHO *et al*	(1975)
Duplin River	8 - 14	HANSON	(1983)

1): based on relatively constant rates, measured over a three month period by the authors, assuming a molar ratio of 1:3, a specific weight of the sediments = 1 and that acetylene reduction took place at the given rates in the top 20 cm layer of the sediments.

values given in table III.

In general, nitrogen fixation rates in plain lake sediments are low compared with sediments with rhizomes of macrophytes. The conditions in the rhizosphere are favourable for nitrogen fixation, because ammonium is scavenged and useful substrates are released by the rhizomes. For instance, PATRIQUIN and KNOWLES (1972) observed in rhizosphere sediments from *Thalassia testudinum* stands 10-100 times higher rates than in sediments without rhizomes. They suggested that the productive seagrass beds and marsh grasses are primarly supported by nitrogen fixation. In contrast to this, McROY *et al.* (1973) concluded that nitrogen fixation was no important source of nitrogen in seagrass beds. Results of TEAL *et al.* (1979) are in support of this conclusion.

Relation with the composition of the sediments.

STEWART (1969) expected that nitrogen fixation by free-living heterotrophic microorganisms would be limited by the shortage of organic substrate (= energy). The present study has shown that substrate availability, as expressed by the C/N ratio of the sediments, plays indeed an important role in the nitrogen fixation of the sediments.

At the same time the important role of ammonium has been demonstrated. Ammonium represses the synthesis of nitrogenase (DAESCH and MORTESON, 1972; BROTONEGORO, 1974; KNOWLES and DENIKE, 1974). In chapter 4 it has been shown that the interstitial ammonium concentration has to decrease below a repression-derepression threshold, before nitrogenase synthesis can occur throughout the whole sediment. Above this threshold, nitrogenase (synthesis) appears to be limited to microsites with relatively low ammonium concentrations. This threshold could be demonstrated to exist in the field too: at ammonium concentration >17 mg N . l^{-1} the relation between the C/N ratio and the acetylene-reducing activity was different from that at ammonium concentrations <17 mg N . l^{-1} (see figure 33).

In trying to explain why the threshold-concentration depends on the organic matter content of the sediments, KNOWLES and DENIKE (1974) pointed to the role of ammonium adsorption by the organic matter as affecting nitrogen fixation. Because of this adsorption at least part of the ammonium will be in a non-active state. OLÄH *et al.* (1983),

using methods that exactly determine the dissolved ammonium content in the interstitial water, concluded that high rates of nitrogen fixation in sediments rich in ammonium cannot be explained by the unavailability of inorganic nitrogen. In the present study the dissolved ammonium content is measured too. Relatively high concentrations, expecially in the sediments at 29 m water depth, were found to be associated with high acetylene-reducing activity. However, at the same time the important role of the ammonium adsorption characteristics of the sediments, as expressed by K_e, have been demonstrated. Ammonium adsorption is mainly governed by the organic matter (ROSENFELD, 1979). The present study has shown that preferentially organic matter with relatively high degradability (low C/N ratio) adsorbs ammonium. As shown in chapter 3 sediments ought not to be considered as a homogeneous substance. It can be expected that also the distribution of ammonium throughout the sediments is not homogeneous. At sites relatively isolated from the bulk of the interstitial water and with relatively high K_e low dissolved ammonium concentrations are probable. If these sites are also characterized by the high degradability of the organic matter, conditions are favourable for nitrogen fixation.

OLÄH *et al.* (1983) did not find any correlation between acetylene reduction and the organic matter content of the sediments. From the data of the present study the conclusion can be drawn that the quality of the organic matter is very important for the acetylene reduction. Therefore not the bulk of the organic matter should be considered with respect to nitrogen fixation, but that part of the organic matter, that can be characterized as readily decomposable.

The C/N ratio has often been used in studies concerning nitrogen fixation to indicate the relative poorness or richness in nitrogen of an environment. High C/N ratios have often been shown to favour nitrogen fixation. For instance BOHLOOL (1978) found high acetylene reduction rates in intertidal sediments influenced by effluent of an apple cannery, having high C/N values. However, in lake sediments high C/N ratio ought not to be associated with the relative nitrogen poorness of the environment in the first place, but with the refractory character of the organic matter.

JAEGER and WERNER (1977) found a positive correlation between the am-

monium concentration and the acetylene reduction of the sediments of the Harkortsee. Ammonium should not only be considered as an inhibitor of the nitrogenase synthesis, but also as a product of the mineralization of organic matter. If other conditions are the same, high decomposition rates will be associated both with high ammonium concentrations and with high substrate availability for the nitrogen-fixing population. A positive correlation between ammonium concentration and acetylene-reducing activity may actually be caused by a positive correlation between acetylene reduction and substrate availability. This might be the explanation for the positive correlation between the acetylene-reducing activity in the littoral and both the interstitial ammonium concentration and the adsorbed ammonium content. The temperature dependence of the ammonium concentration in the interstitial water of the littoral sediments are in support of this.

Summarizing the conclusions, the field observations showed the important role of both ammonium adsorption and the substrate availability for the acetylene-reducing activity of the sediments. Counteracting the repressing influence of ammonium, adsorption of ammonium produces locally favourable conditions for nitrogen fixation in sediments with high ammonium concentrations in the interstitial water. These results are in support of the conclusions drawn from the experiments involving amendment of sediment samples in the laboratory (chapter 4).

7. The relation between the acetylene-reducing activity and the uptake of ^{14}C-glucose by heterotrophic microorganisms in the sediments of the Pluss-See.

7.1. Introduction.

Because of the absence of light energy, nitrogen fixation in profundal sediments will be mainly a heterotrophic process. In studying the factors controlling nitrogen fixation in lake sediments it is necessary to investigate the relation between the energy delivering heterotrophic processes and nitrogen fixation. As an aspect of heterotrophic activity the uptake of ^{14}C-D-glucose and the mineralization of this substrate have been measured. This technique, originally developed for use in lake water (WRICHT and HOBBIE, 1966), has been applied for the measurement of organic solutes by heterotrophic populations in the sediment too (HALL *et al.*, 1972; HANSON and GARDNER, 1978; NOVITSKY, 1983). It is based on the assumption that the uptake of organic solutes by natural heterogeneous populations obeys Michaelis-Menten kinetics.

Maximum uptake velocity (V_m), turn-over-time (Tt) as well as the sum of the substrate affinity (K_t) and the natural substrate concentration (S_n) can then be calculated (see 2.6). If the natural substrate concentration is known, K_t and the *in situ* turn-over-time of the naturale substrate concentration can be calculated. Glucose has been chosen as a substrate because it is one of the common substrates for nitrogen-fixing anaerobic heterotrophic bacteria as *Clostridium pasteurianum*.

Uptake kinetics of ^{14}C-glucose have been measured in the surficial 5 cm sediments at the three stations in the Pluss-See parallel to measurements of acetylene reduction.

7.2. Methodological aspects.

7.2.1. The use of disturbed, diluted sediments.

The measurement of the kinetic parameters of the glucose uptake has been performed by diluting the sediment samples with anoxic, filtrated water from the hypolimnion of the Pluss-See (see chapter 2). This procedure was necessary to allow homogeneous distribution of the radio-active glucose in the sample and to slow down the uptake of

glucose in order to avoid a significant decrease of the substrate concentration within the incubation period. However, by diluting and stirring the sediments, the environmental conditions of the microorganisms in the sediments will change more or less. Several authors have discussed this problem. HALL *et al.* (1972) compared the uptake of diluted sediments with the uptake in undisturbed sediment cores. They found that uptake activity was one order of magnitude less in the undisturbed cores. The same comparison and the same conclusions were made by NOVITSKY (1983). In both studies uptake in undisturbed sediment cores was measured by injecting radio-active glucose into the overlying water. The latter author concluded that the importance of substrate diffusion into the sediments as a controlling factor of microbial activity was shown. However, it seems to be very improbable that the main glucose source for the microbial populations in the sediments is a glucose flux from the overlying water. It will be more realistic to assume that sedimented particulate carbohydrates release glucose by enzymatic hydrolysis and that this glucose is taken up immediately by the natural microbial population. The relation between the glucose source and the glucose-consuming microorganisms will be very intimate and diffusion will not be a limiting factor. The diffusion as described by NOVITSKY (1983) is an artefact introduced by a method in which an artificial substrate is introduced from outside into the system.

MEYER-REILL (1978) using sandy sediments performed the uptake measurement by replacing the interstitial water by artificial interstitial water with the radio-active glucose. Although the distance between the microorganisms and the radio-active glucose is smaller than with the method described by HALL *et al.* (1972) and NOVITSKY (1983), glucose has to diffuse from the interstitial water to the microorganisms, if they are not in direct contact with the bulk of the interstitial water. As shown in chapter 4 this is the case for acetylene reducing bacteria in the sediments of the Pluss-See.

The method of MEYER-REILL (1978) cannot be applied to the dy-gyttja sediments of the Pluss-See. The method described by HALL *et al.* (1972) and NOVITSKY (1983) will seriously underestimate heterotrophic activity in introducing an artificial limiting factor that can be avoided by diluting and mixing the sediments. As CHRISTIAN and WIEBE

(1978) put it, microhabitats are certainly disrupted and this most likely results in the stimulation of some populations and inhibition of others. The measured activity has to be considered as a reflection of the potential activity (CHRISTIAN and WIEBE, 1978) or realized rates in the reaction system (HANSON and GARDNER, 1978).
It is evident that it is unrealistic to distribute the radio-active glucose homogeneously through the natural glucose pool, maintaining its inhomogeneous distribution within the sediments. Hence it is impossible to measure natural glucose uptake rates by introducing an artificial substrate into the sediments. In a general way this conclusion holds for every method for the measurement of "natural" rates that involves the introduction of an artificial substrate into the sediments and the disruption of microhabitats. FLEISCHER (1975) stated that if one is interested in *in situ* rates, turn-over-times of sediment sugars studied with incubated labelled substrate in diluted or stirred samples should be taken with reservation. From the above discussion the conclusion can be drawn that his statement also holds for studies using undisturbed sediment cores.

7.2.2. The assumption of Michaelis-Menten kinetics. (Explanation of kinetic symbols in 2.6).

The assumption that the uptake of organic solutes by natural microbial populations obeys Michaelis-Menten kinetics is a simplification. It assumes that the whole natural population has a similar affinity for the substrate. Certainly this is not the case. WILLIAMS (1973) showed mathematically that uptake will deviate from Michaelis-Menten kinetics if the transport constant (K_t) varies over a wide range within the natural population. Such deviations have been observed by AZAM and HODSON (1981) and could also be observed in the sediments of the Pluss-See. Figure 35 shows the results of an uptake experiment applying a wide concentration range of the labelled glucose. The Lineweaver-Burk plot shows a similar deviation as the one described by WILLIAMS (1973). The consequences of this deviation for the calculated kinetic parameters is shown in figure 35. The value of the kinetic parameters varies considerably with the concentration range that is used for the calculation of the parameters. Especially V_m and K_t+S_n appear to be very sensitive to a change in the concentration

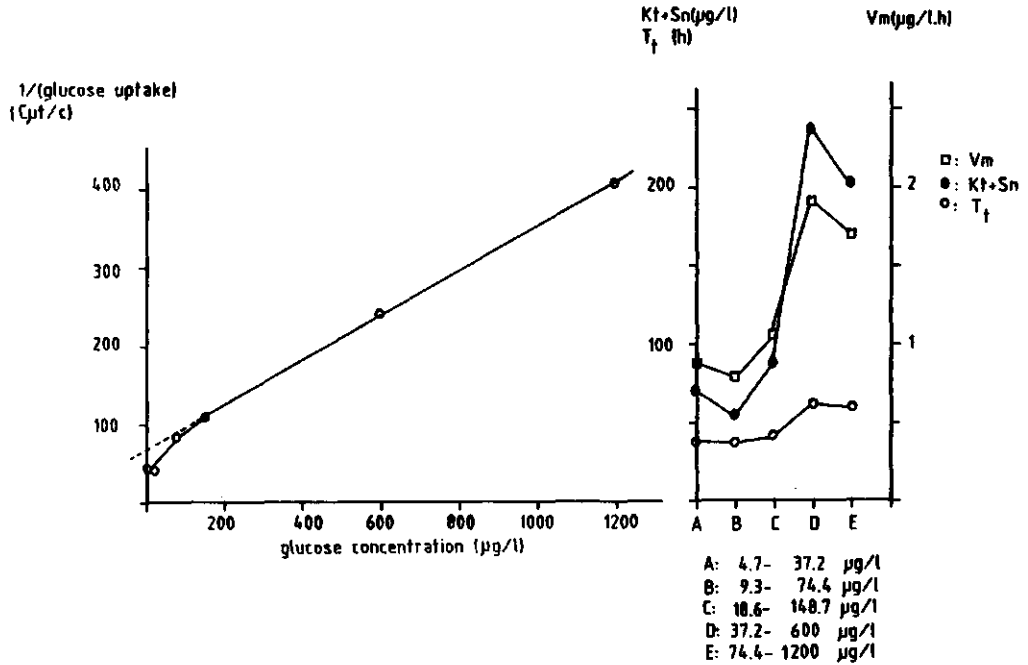

Figure 35. Influence of applied concentration range on the calculated kinetic parameters for the uptake of ^{14}C -glucose (for explanation of symbols see 2.6.).

range. Turn-over-time values are rather stable. These results show that the values of the kinetic parameters cannot be interpreted in an absolute way, but can only be compared with parameters calculated from the same concentration range. The kinetic parameters therefore give only relative information on the heterotrophic activity of the microbial population.

Recently SMITH *et al.* (1984) discussed similar deviations from linearity of the Lineweaver-Burk plot, caused by errors in the background estimation. The method they used involves a constant concentration of the labelled substrate and varying concentrations of the added unlabelled substrate. As a consequence they used only one blank, in contrast to this study, where blanks were used for every added concentration of the labelled substrate. Therefore no such artefactual deviations may be expected in this study.

7.3. Results.

V_m-values of the littoral sediments (expressed as $\mu g\ ^{14}C$-glucose.g (sediment carbon)$^{-1}$.h^{-1}) showed an irregular pattern, with maximum values in the summer of 1978 (see figure 36). V_m was significantly correlated with temperature ($n=16$; $r=0.692$; $p<0.01$). At the two profundal sampling stations high V_m-values were observed during the winter period, low values in the summer of 1977 and elevated values in the spring of 1978. Except for the relatively high value in February V_m-values at 29 m water depth are significantly correlated with the V_m-values at 15 m water depth ($n=17$; $r=0.728$; $p<0.001$). K_t+S_n both at 28 m and at 15 m water depth showed a steady increase during the observation period (see figure 37) and were significantly correlated with each other ($n=18$; $r=0.670$; $p<0.01$). Values ranged from 10 up to 100 $\mu g\ .l^{-1}\ ^{14}C$-glucose $.\ l^{-1}$. The same range was found in the littoral sediments, but no consistent pattern could be observed. This is in contrast to the turn-over-time (T_t)-values in these sediments. During the winter time the observed T_t-values were relatively high, whereas low values were measured in the rest of the observation period (see figure 38). A negative correlation could be observed between T_t and temperature of the littoral sediments ($n=19$; $r=-0.55$; $p<0.02$). T_t ranged from about 0.05 up to 1 hour. The same range was observed at the two profundal sampling stations. An irre-

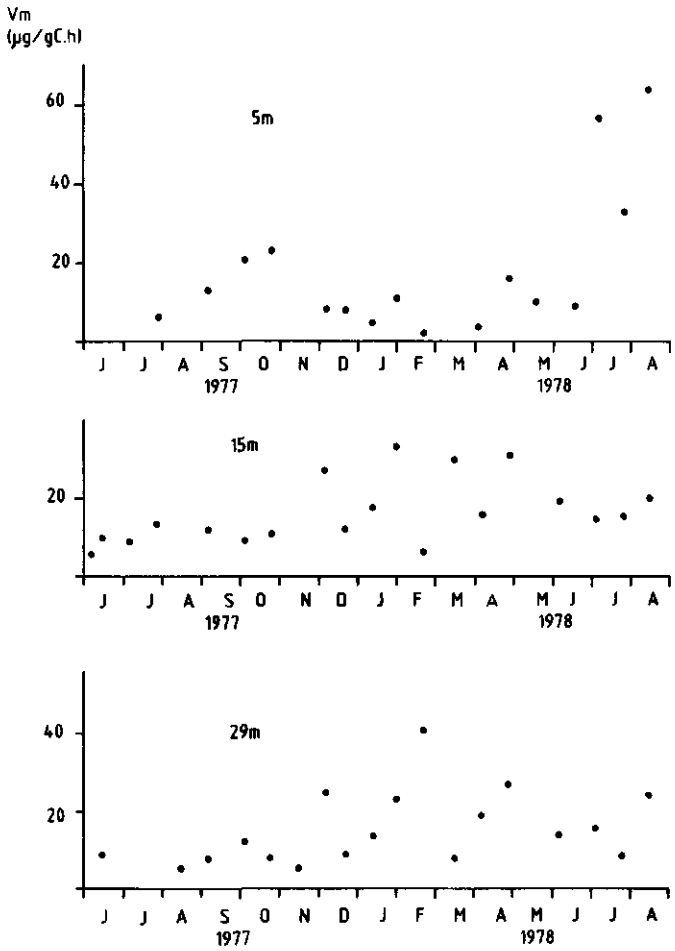

Figure 36. Maximum uptake velocity (V_m) of glucose by the microbial populations in the surficial (0-5 cm) sediments from 5,15 and 29 m water depth.

gular pattern was observed at 15 m water depth with higher variations in 1978 than in 1977. At 29 m water depth low values were observed in the spring of 1977, after which T_t increased during summer and autumn. In the winter period a sharp drop of T_t occurred, after which again relatively high values during the spring and summer of 1978 were observed. Mean Tt was lowest in the littoral sediments (0.23 h). Slightly higher values were found in the profundal: 0.35 and 0.45 h

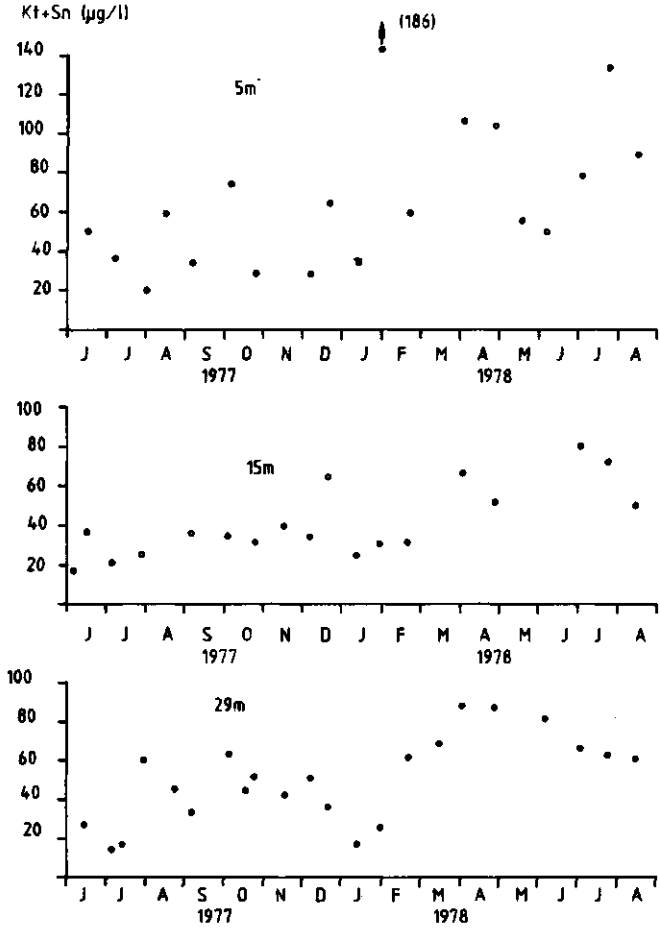

Figure 37. K_t+S_n in the surficial (0-5 cm) sediments at 5,15 and 29 m water depth.

at 15 and 29 m water depth, respectively.

The mean mineralization rate was highest in the profundal sediments. At 29 m water depth mean mineralization was 16.5% of the total uptake and at 15 m 13.9% (see figure 39). In the littoral sediments the mean mineralization rate amounted to only 8.8% of the total uptake. The course of the mineralization did not show any regular pattern in the produndal sediments. In the littoral high mineralization rates were measured in the summer period and low rates in the winter period. In

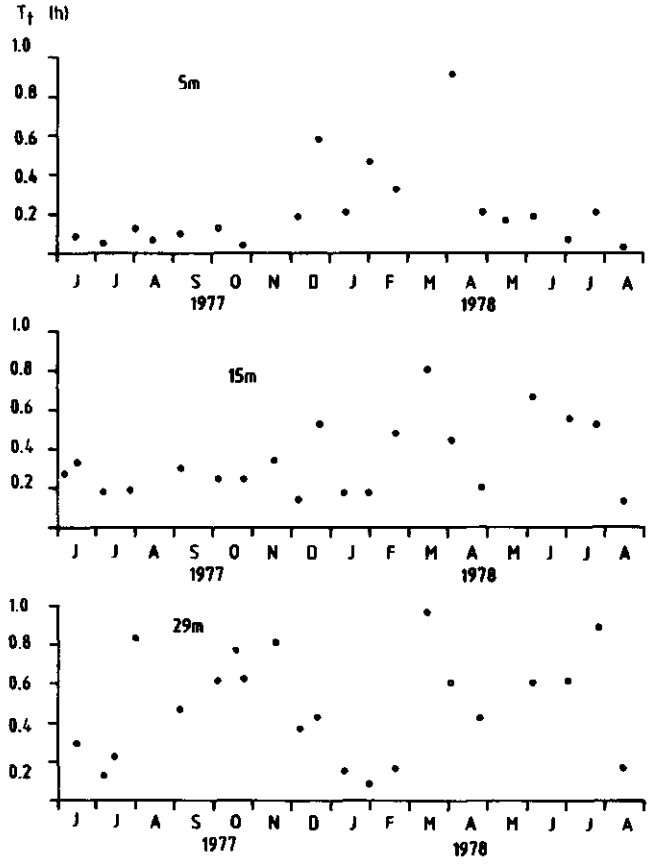

Figure 38. Turn-over time (T_t) of ^{14}C-glucose in the surficial (0-5 cm) sediments at 5,15 and 29 m water depth.

Table IV. Mean maximum glucose uptake velocities (V_m) of the surficial (0-5 cm) sediments at 5,15 and 29 m waterdepth in the Pluss-See.

Water depth (m)	Mean V_m ($mgC.m^{-2}.h^{-1}$)
5	11.4
15	6.6
29	6.0

fact, the mineralization at this depth was significantly correlated with temperature (n=16; r=0.692; p<0.01; see figure 40).
In table IV V_m-values were recalculated to $gC^{14}C$-glucose.$m^{-2}.h^{-1}$. From this table it can be seen that the heterotrophic glucose-uptake activity at an areal base is relatively high in the littoral and

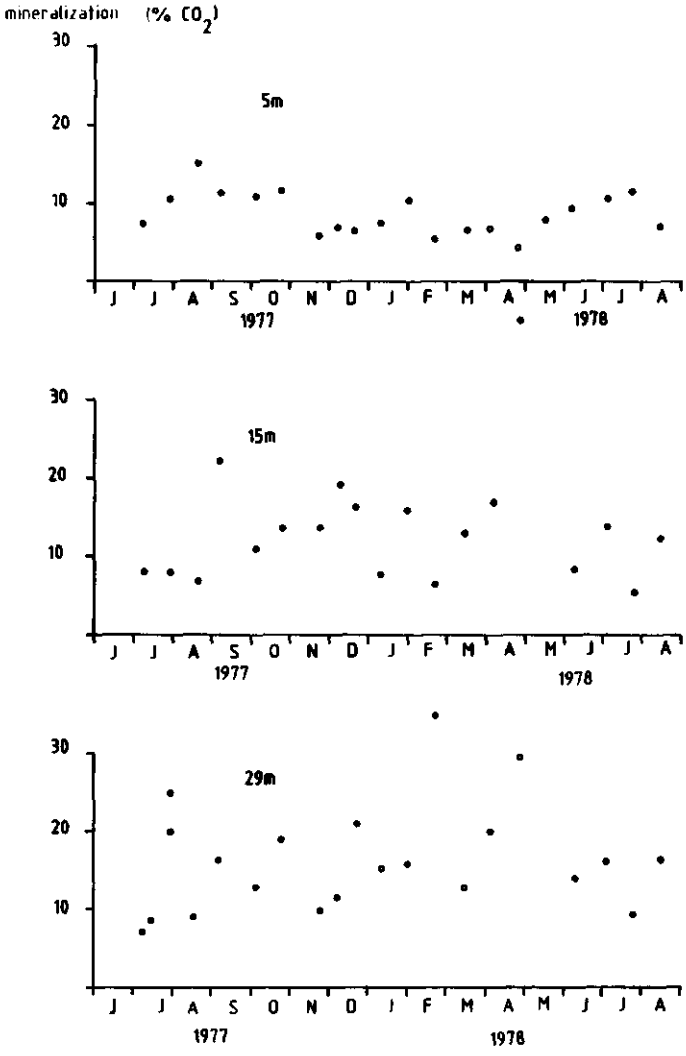

Figure 39. Percentage of ^{14}C-glucose gross uptake that is mineralized in the surficial (0-5 cm) sediments at 5,15 and 29 m water depth.

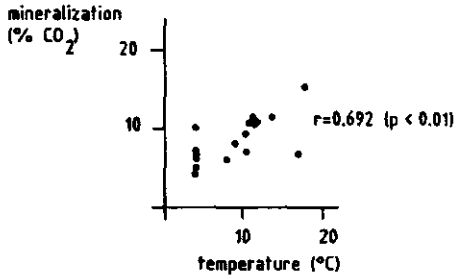

Figure 40. The relation between temperature and the mineralization of glucose in the surficial (0-5 cm) littoral sediments.

equally low at the two profundal sampling stations. Taking into account the higher mean temperature in the littoral zone it can be concluded that heterotrophic uptake activity in the sediments is more or less the same for the three sampling stations.

Relation of V_m with acetylene reducing activity and composition of the sediments.

At all depths V_m showed a very significant correlation ($p<0.001$) with the acetylene-reducing activity of the sediments (see figure 41). Correlation coefficients of Vm with some measured properties of the sediments are summarized in table V. At 29 m water depth V_m was positively correlated with K_e ($p<0.02$) and negatively correlated with the interstitial ammonium concentration ($p<0.02$) and the C/N ratio of the sediments ($p<0.02$). At 15 m water depth V_m was negatively correlated with the C/N ratio ($p<0.05$). No significant correlation was found between V_m and both the interstitial ammonium concentration and K_e, nor was this the case for the littoral sediments. However, if K_e(orgC)-values smaller than 0.2 were not taken into consideration the correlation between V_m and the interstitial ammonium concentration was found to be highly significant ($p<0.001$) in the littoral. This correlation was positive in contrast to the negative correlation found at 29 m water depth. V_m of the littoral sediments was also found to be positively correlated with the exchangeable ammonium content (expressed as mg NH_4-N. gC^{-1}) and temperature. No correlation was found between the C/N ratio and V_m in the littoral sediments.

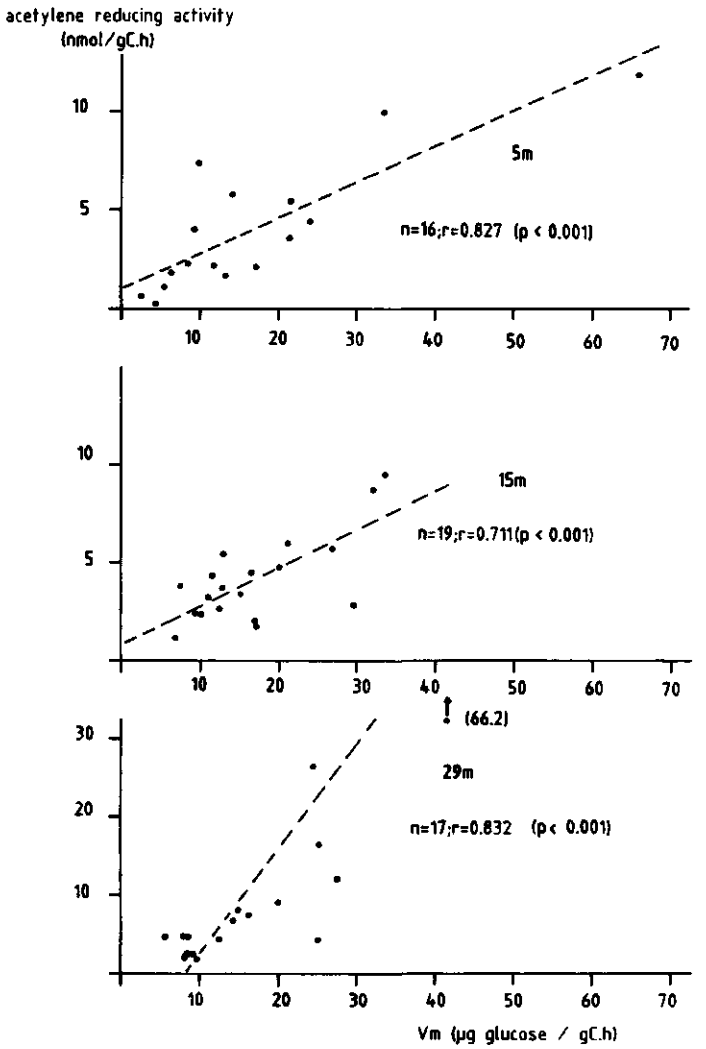

Figure 41. Relation between the ^{14}C-glucose maximum uptake velocity and the acetylene-reducing activity of the surficial sediments at 5,15 and 29 m water depth.

Comparison with the correlation coefficients in table II is not possible for the sediments at 5 and 29 m water depth, because these are based on more sediment samples. Therefore in table II also the correlation coefficients are presented which are based on the same set of

Table V: Correlation coefficients of V_m (μg glucose.$gC^{-1}.h^{-1}$) with the interstitial ammonium concentration, the exchangeable ammonium content, K_e the C/N ratio and the temperature of the surficial sediments at the three sampling stations in the Pluss-See.

Water depth (m)	n	Interstitial ammonium (mgN/l)	Exchangeble ammonium (mgN/g of dry sediment)	K_e (1/g)	C/N ratio	Temperature (°C)
5	17	+0.272	+0.774****1)	+0.343[2)]	-0.299	+0.774****
15	19	-0.291	-0.136	+0.255	-0.511*	- [3)]
29	16	-0.579**	+0.470	+0.584**	-0.600**	- [3)]

* : $p<0.05$
** : $p<0.02$
*** : $p<0.01$
****: $p<0.001$

1): exchangeable ammonium as mgN/g of carbon;
2): correlation coefficient with K_e(orgC);
3): no significant temperature variation observed at this depth.

sediment samples as in table V. From these tables it can be seen that in general K_e is less correlated with V_m than with the acetylene-reducing activity. At 15 m water depth also the correlation of the interstitial ammonium concentration with V_m is different from the correlation with the acetylene-reducing activity.

K_e does not seem to be important for explaining the variation of V_m in the sediments, but it does apparently for the variation of the acetylene-reducing activity. The difference in behaviour between these two parameters becomes more striking in the sediments at 29 m water depth, if some partial correlation coefficients are calculated. The partial correlation coefficient between the acetylene-reducing activity and K_e, with the C/N ratio held constant, is significant ($n=16$; $r=0.658$; $p<0.01$), the same correlation between V_m and K_e is not ($n=16$; $r=0.388$). If only sediment samples with interstitial ammonium concentration $<17 mgN.l^{-1}$ are taken into consideration, this difference is even more striking: $r=0.952$ ($n=8$; $p<0.001$) and $r=0.313$ ($n=8$; n.s.) respectively.

7.4. Discussion.

Maximum uptake velocity (V_m).

V_m and K_t+S_n have been shown to be affected by the applied glucose concentration range. Hence care should be taken in comparing the observed values with literature data. V_m for glucose in the overlying water varies between 0.1 and 1 $\mu g.l^{-1}.h^{-1}$ (OVERBECK, 1974). If expressed per liter V_m-values observed in this study ranged up to 350 $\mu g.\ l^{-1}.h^{-1}$. Although the applied concentration range is considerable lower in uptake assays of lake water, the high values found here demonstrate the well known high bacterial activity of surficial sediments (WETZEL, 1975). Comparable differences between water and sediments were found by WOOD and CHUA (1973), WOOD (1973), MEYER-REIL *et al.* (1978) and NOVITSKY (1983).

HALL *et al.* (1972), WOOD and CHUA (1973) and TOERIEN and CAVARI (1982) applied comparable concentration ranges of glucose. V_m of polluted Toronto Harbour sediments (WOOD and CHUA, 1973) was about one order of magnitude higher than the values observed in the Pluss-See sediments. The latter V_m-values were slightly lower than those

reported by TOERIEN and CAVARI (1982) for Lake Kinneret sediments and were comparable with the values observed by HALL *et al.* (1972) in the shallow oligotrophic Marion Lake. Although higher values might be expected in the eutrophic Pluss-See, the observed agreement can be explained by the very efficient decomposition of organic matter in the water column (OHLE, 1962) resulting in a relatively low input of organic matter into the sediments.

V_m in the sediments at 29 and that at 15 m water depth were found to be significantly correlated. From this it can be concluded that V_m is governed by the same process at both sites, e.g. by the sedimentation of organic matter or by the conditions of the overlying water. V_m in the littoral sediments was found to be correlated with temperature. Temperature dependency of V_m was observed by TOERIEN and CAVARI (1982) in Lake Kinneret sediment by assaying the same sediment sample at different temperatures. In the present study, however, different samples were assayed at different temperatures during the observation period. Because higher sediment temperature coincidens with higher primary production and higher sedimentation of organic matter, higher uptake rates might be the combined effect of temperature and input of organic matter into the sediments. HALL *et al.* (1972) did not find a linear increase of V_m with temperature. They speculated that this is caused by the presence of microorganisms with low temperature optima, as were found by ALLEN (1971) in epiphytic *Caulobacter* strains. No evidence was found for psychrophylic bacteria in either littoral or profundal sediments of the Pluss-See. As shown in chapter 5, nitrogen-fixing microorganisms in the profundal sediments with permanently low temperatures showed maximum acetylene-reducing activity at 31°C.

K_t + S_n.

WITZEL (1980) found low K_t-values at low temperatures. He suggests that the microbial population compensates for the lower uptake activities at low temperature with higher affinities for the substrate. In this study no relation was found between temperature and K_t+S_n. The observed values for K_t+S_n fall in the same range as reported by HALL *et al.* (1972) and TOERIEN and CAVARI (1982). Considerably higher values are reported by WOOD and CHUA (1973) in polluted Toronto Harbour sediments. Because of the sediment dilution changes of K_t+S_n

will be mainly caused by changes of K_t (HALL *et al.*, 1972; BLAUW, 1978). The increase of K_t+S_n, in fact the decrease of the substrate affinity of the microbial population in the profundal sediments during the observation period, might be caused by the adaptation of the population to higher substrate concentrations.

Turn over time.

As shown in 7.2.2 the turn-over-time (T_t) is less clearly affected by the applied concentration range and can therefore be compared with literature data. Observed T_t values fall in the lower range of the values reported in the literature. They can be compared with T_t measured by HALL *et al.* (1972) in Marion Lake sediments (0.061-0.4 h), WOOD and CHUA (1973) in Toronto Harbour sediments (0.03-0.17 h), and KING and KLUG (1982) in eutrophic Wintergreen Lake sediments (0.017 h). In contrast TOERIEN and CAVARI (1982) reported turn-over-times ranging from 1.4 to 9.7 h in Lake Kinneret sediments. CHRISTIAN and WIEBE (1978) reported T_t-values upto 5 hours in *Spartina alterniflora* marsh soils and ANDERSON *et al.* (1978) up to 2.6 h in Lake Tjärnesjön sediments. From the relatively low T_t values found in the Pluss-See sediments the conclusion can be drawn that the heterotrophic population is taking up glucose very efficiently and that glucose is probably a commom substrate in these sediments. KING and KLUG (1982) did not observe seasonal or site differences in T_t values. In the Pluss-See the T_t increased with water depth, demonstrating the more intensive glucose metabolism in the littoral. Comparable seasonal fluctuation of T_t values as found in the littoral has been reported by HALL *et al.* (1972).

Mineralization.

Mineralization percentages are low compared with values reported for lake water (up to one third of total glucose uptake (HOBBIE and CRAWFORD, 1969)). The measured values can be compared with mineralization percentages reported by TOERIEN and CAVARI (1982). WOOD (1970) supposed that under the anaerobic conditions fermentation products are formed resulting in a lower CO_2-production. TOERIEN and CAVARI (1982), however, did not find any difference between anaerobic and aerobic assay conditions. KING and KLUG (1982) supposed that this

might be caused by the maintenance of anaerobic conditions in microenvironments within the aerobic sediments. Aerobic incubation of profundal Pluss-See sediments significantly decreased the uptake of glucose, probably caused by the killing or inhibition of the strictly anaerobic population. The mineralization percentage, however, was the same, supporting the existence of anaerobic microenvironments in the aerobic sediments.

KING and KLUG (1982) reported higher mineralization percentages with increasing incubation time from 18% after 10 minutes to 67% after 34 h of incubation caused by the progressing isotopic dilution. If formation of fermentation products, as found by KING and KLUG (1982) and CHRISTIAN and WIEBE (1978), is also important in the Pluss-See sediments, the measured uptake rates underestimate the actually realized uptake rates.

TOERIEN and CAVARI (1982) suggest that the mineralization percentage is constant under different environmental conditions. As shown above this might be true with respect to the oxygen concentration. They found also constant percentages at different temperatures. Their results are in agreement with those of GRIFFITHS *et al.* (1984), who found that temperature has little effect on percent mineralization over the temperature ranges normally encountered *in situ*. In the littoral Pluss-See sediments, however, a significant correlation between mineralization percentage and temperature was observed, suggesting higher metabolism with increasing temperature. A same relation was found by TISON and POPE (1980).

Relation with acetylene reduction and sediment composition.

At all stations V_m was highly significantly correlated with the acetylene-reducing activity of the sediments. In fact V_m showed similar relationships with sediment properties as acetylene-reducing activity. The importance of the C/N ratio for the heterotrophic activity is not surprising. As described in chapter 5 the C/N ratio can be considered as an index for substrate availability. Low C/N values are associated with relatively high degradability of the organic matter and therefore with high heterotrophic activity. WOOD and CHUA (1973) could not find any relation between glucose uptake velocity and the organic matter content of the sediments. In the Pluss-See it could be

shown that not in the first place the organic matter content, but especially the quality of the organic matter is important for the heterotrophic activity. The acetylene-reducing activity was shown (see chapter 4) to be limited by the substrate availability. A similar relationship with the C/N ratio therefore can be expected.
The significant correlation between V_m and the acetylene-reducing activity affirms the substrate limitation of the nitrogen fixation in the sediments.
The significant correlation of V_m with both the interstitial and the adsorbed ammonium content in the littoral sediments supports the explanation that is given in 6.4.2 for the correlation of the acetylene-reducing activity with both forms of ammonium: higher heterotrophic activity, i.e. higher decomposition of organic matter will result in higher concentrations of mineralization products, e.g. ammonium.
The correlation of the acetylene-reducing activity with K_e was significant, but not the correlation of V_m with K_e (see tables II and V). From this difference it can be concluded, that the adsorption capacity of the sediments, and especially of the organic matter in the sediments plays an important role in the nitrogen fixation in making ammonium inactive by adsorption. The interstitial (dissolved) ammonium concentration itself seems to be less important. This might be explained by the earlier results (see chapter 4) that the nitrogen-fixing sites are not in direct contact with the interstitial water. The higher the adsorption capacity of the organic matrix, the more the ammonium transport from the interstitial water to the nitrogen fixing sites is hindered.
As described in chapter 6 K_e gives information on the adsorption properties of the sediments, if these sediments are not already saturated with ammonium. In fact, K_e represents the adsorption coefficient, if the adsorbed/dissolved ratio is within the linear part of the adsorption isotherm and if ammonium concentrations are not too low (ROSENFELD, 1979). The correlation of the acetylene-reducing activity and K_e of the sediments at 29 m water depth is more significant, if only sediment samples with interstitial ammonium concentrations <17 $mgN.l^{-1}$ are taken into consideration. This might be explained by the possible saturation of the sediments with ammonium at concentrations

>17 $mgN.l^{-1}$. This concentration has been shown to be about equal to the repression-derepression threshold (see chapter 6). It certainly would be an interesting point to ask if this threshold actually represents the concentration at which sediments and soils are saturated with ammonium.

8. Sedimentation of suspended matter in the Pluss-See and its relation with sediment composition and nitrogen fixation (acetylene reduction) in the sediments.

8.1. Introduction.

In the previous section acetylene-reducing activity in the sediments of the Pluss-See was discussed in relation to some properties of the sediments and the interstitial water. It has been shown that acetylene-reducing activity in the sediments is governed both by the organic substrate (= energy) availability and by the ammonium concentration. Ammonium is generated by the breakdown of organic matter, i.e. the energy source of the nitrogen fixation process, and by this process itself. The organic matter is not produced in the sediments but in the photogenic zone of the lake (autochthonous organic matter) or outside the lake (allochthonous organic matter) and subsequently transported to the sediment system by sedimentation and other physical processes. In this section these processes and their relation with the sediment composition and the acetylene reduction in the sediments are discussed. The discussion is based on the measurement of the sedimentation of suspended matter with sedimentation traps.

8.2. Methodological aspects of the sedimentation measurement.

The sedimentation traps used were similar to those described by ZEITSCHEL (1978), apart from the automatically changing mechanism that not was applied. For description of the traps, see chapter 2.
The opening to volume ratio of these traps is equivalent to a cylinder with a height to width ratio of 4:1. SMETACEK *et al.* (1978) discussing the functioning of these traps concluded that they cannot be regarded *per se* as having either underestimated or exaggerated actual sedimentation rates. As shown by GARDNER (1980) the use of baffles at the top of funnels improves the catch efficiency considerably. Taking into account the relatively low flow velocities in a protected lake as the Pluss-See the conclusion can be drawn that there will be no serious undertrapping by resuspension of already trapped material out of the funnel. The advantage of the applied traps is that within short periods of exposure enough material for analysis can be collected. This is particularely important in lakes with intensive decompo-

sition in the watercolumn and therefore low sedimentation rates as in the Pluss-See (OHLE, 1962).

In order to prevent decomposition of organic matter in the traps chloroform was added to the collection tubes at the beginning of the observation period. This addition was stopped after it was found that occasionally massive amounts of vertically migrating zooplankton were caught in the traps during periods of homothermy. The problem of artificial decomposition in the traps is discussed thoroughly by BLOESCH and BURNS (1980). The literature they reviewed revealed contradictory results. As a conclusion they advise making exposure time as short as possible. The exposure time should not exceed three weeks if questions of balances, settling fluxes of particulate organic matter and sediment accumulation rates are considered. In this study exposure time was 1 week, except for the winter period during ice cover. Maximum exposure time during this period was four weeks. A possible effect of artificial decomposition will therefore be maximal in the winter period. This will, however, be counteracted by the low temperatures throughout the lake and the fact that the trapped material in this period consists mainly of resuspended bottom material (see 8.4) that has already been exposed to intensive decomposition at the sediment surface. The degradability of this material will be relatively low. It can therefore be assumed that no serious artificial decomposition has taken place in the collecting tubes of the sedimentation traps. No attempts have been made to measure dissolved decomposition products in the traps, as done by VERDOUW and DEKKERS (1982) for dissolved nitrogen in order to prevent underestimations caused by decomposition.

No parallel traps could be used. To test the reliability of the data the traps were exposed near to each other (mutual distance 10 m) just below the thermocline at 9 m water depth in the pelagial. Coefficient of variation of the measured rates was smaller than 5%. This was regarded to satisfy the purpose of the measurement in regard of the literature data on accuracy of parallel traps and parallel moorings reviewed by BLOESCH and BURNS (1980).

8.3. Field observations.

As described in chapter 2 the sedimentation of particulate matter was measured at 4 stations in the lake at approximately 1 m above the sediment surface. One trap was positioned in the littoral zone, where water depth was 5 m. The other traps were exposed in the profundal: one at a place, where the water depth was 15 m and the steepness of the slope of the sediment surface was approximately maximum, one at the deepest part of the lake just at the base of this slope and one about 10 m remote from the base of this slope (see figure 1).

The measured sedimentation rates of carbon and nitrogen, expressed as $g.m^2.day^{-1}$, are presented in figure 42. In the littoral maximum values were measured in the summer period (up to 0.95 $gC.m^{-2}.day^{-1}$). The rates in the winter period were low in contrast with the sedimentation in the profundal. Especially in the deepest part of the lake, sedimentation was very intensive during the winter period. Maximum values were measured in February 1978. The differences in the measurements between the two traps at the deepest part (29-0 and 29-N) are striking. Peaks of sedimentation could also be observed in the summer period, but these peaks were considerably lower than those in the littoral during the same period. The data of 15 m water depth showed an intermediate pattern. Winter maxima were lower than those at the deepest part of the lake, summer maxima were lower than the littoral maxima and higher than the summer maxima at 29 m water depth.

One of the peaks could be followed clearly on its way down to the deepest part of the lake. The peak caused by the collapse of a phytoplankton bloom reached the littoral trap in the week between 28.6.78 and 5.7.78 (mean rate for this period: 0.95 $gC.m^{-2}.day^{-1}$). Two weeks later (in the period from 12.7.78 to 19.7.78) a maximum could be observed at 15 m water depth (0.58 $gC.m^{-2}.day^{-1}$). In the same period a maximum could be observed in the traps at 29 m water depth (0.36 $gC.m^{-2}.day^{-1}$ both in 29-0 and 29-N). Obviously the sedimentation from 5 m to 15 m took longer than the sedimentation from 15 m to 29 m water depth. From the height of the observed peaks no conclusions can be drawn about the breakdown of organic matter in the water column. Besides the breakdown of organic matter a dispersional effect of differences in sedimentation velocity of the particles will

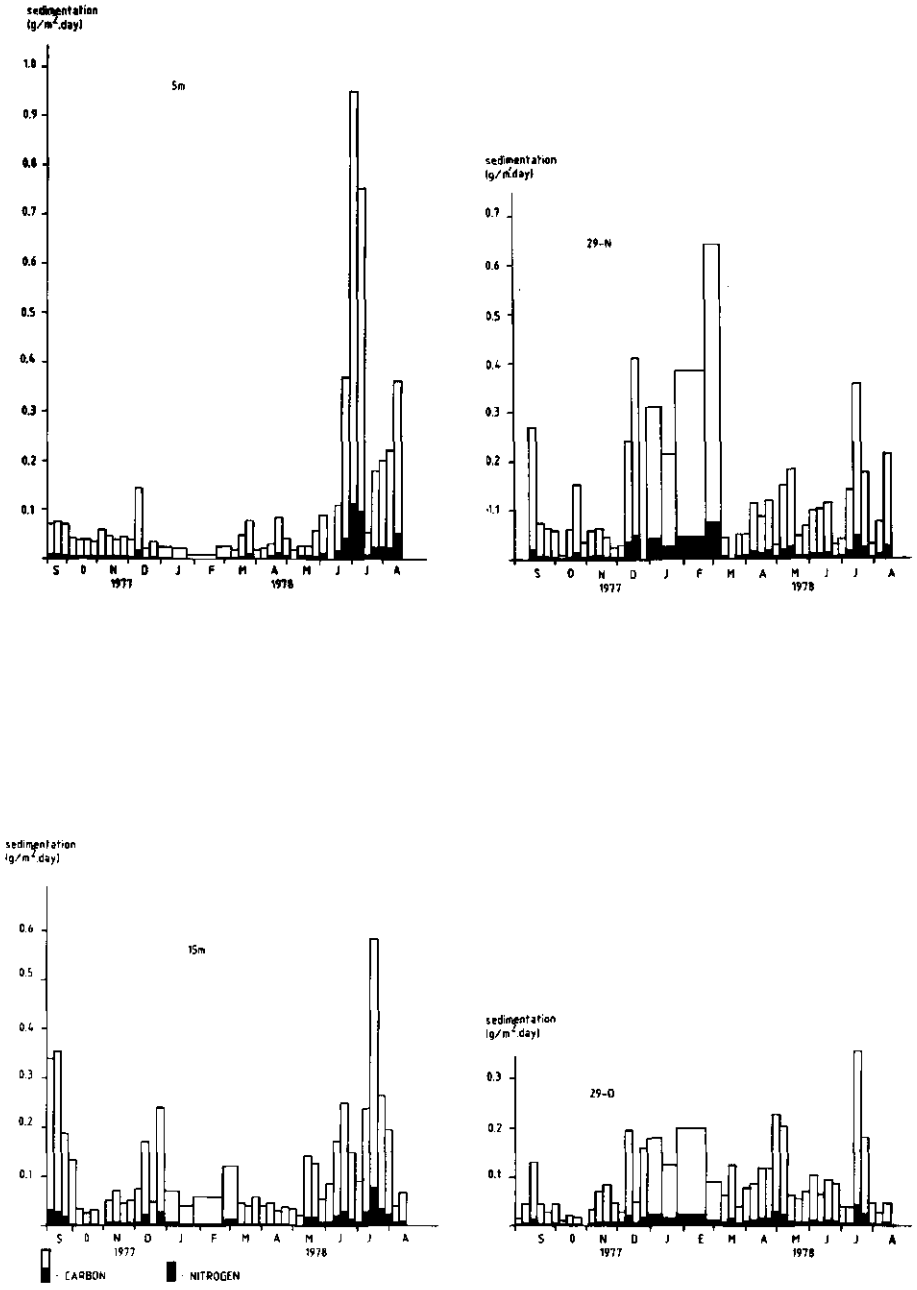

Figure 42. Sedimentation of carbon and nitrogen at 5, 15 and 29 m water depth.

Table VI. Total sedimentation, resuspension and primary sedimentation of carbon and nitrogen at the sampling stations in the Pluss-See.

Sampling station	Total sedimentation $g.m^{-2}.year^{-1}$		Resuspension $g.m^{-2}.year^{-1}$		Primary sedimentation $g.m^{-2}.year^{-1}$		% of primary production (=200gC.m^{-2}.$year^{-1}$; STABEL, unpublished)
	N	C	N	C	N	C	
5 m	4.6	37.7	1.4	11.8	3.2	25.9	13.0
15 m	5.3	43.6	1.3	10.8	4.0	32.8	16.4
29 m O	4.5	36.7	2.6	21.5	1.9	15.2	7.6
29 m N	7.3	60.5	5.3	43.7	2.0	16.8	8.4

Table VII. Carbon content of the surficial (0-5 cm) sediments and the settling material in the Pluss-See (mean and standard deviation expressed as % of dry matter).

Water depth (m)	Sediment	Settling material	
		12.09.77-16.08.78	28.12.77-08.03.78
5	4.5 ± 1.7	31.8 ± 5.5	28.0 ± 2.1
15	25.9 ± 1.9	35.4 ± 7.5	24.7 ± 2.6
29-O	28.5 ± 2.1	32.1 ± 5.5	25.9 ± 3.0
29-N		32.6 ± 7.0	26.4 ± 4.2

lower the maxima at greater water depth.

The total amounts of settled organic carbon and organic nitrogen during the observation period are presented in table VI. Although large differences in spread of sedimentation over the observation period could be observed between the different depths, the total amounts of settled organic carbon and nitrogen are almost the same, except for trap 29-N. The total amounts measured with this trap were considerably higher than those measured by the other traps. The higher catch of this trap is mainly caused by the extremely high sedimentation rates in the winter period (see figure 42).

In figure 43 the carbon and nitrogen contents of the settling material at the different stations are presented. Especially at 15 m water depth a seasonal trend could be observed. Carbon and nitrogen content of the settling material during the winter period were considerably lower than in the other periods. Similar but less distinctive trends could be observed at the other stations. Table VII shows that the differences between the carbon content of the settling material and the surficial sediments are smallest during the winter period. At 15 m water depth the carbon content of the settling material equals the carbon content of the surficial sediments at this water depth. The carbon content of the settling material at 29 m water depth was even lower than the carbon content of the surficial sediment at the deepest part of the lake. The differences at 5 m water depth in the littoral are large during the whole measuring period, because the carbon content of the surficial sediments in the littoral is relatively low.

No trend at any of these stations could be observed in the C/N ratio of the settling material. The mean C/N ratio was lowest at 15 m water depth (7.68). At the other stations these mean values were approximately equal (8.36 at 5 m; 8.33 at 29-O; 8.62 at 29-N).

8.4. Correction for resuspension.

In the profundal zone maximum sedimentation rates were observed during the winter period, although no substantial primary production has been measured during this period (STABEL, unpublished results). Therefore it can be assumed that the measured rates are caused by the resuspension of surficial sediments into the water column by tur-

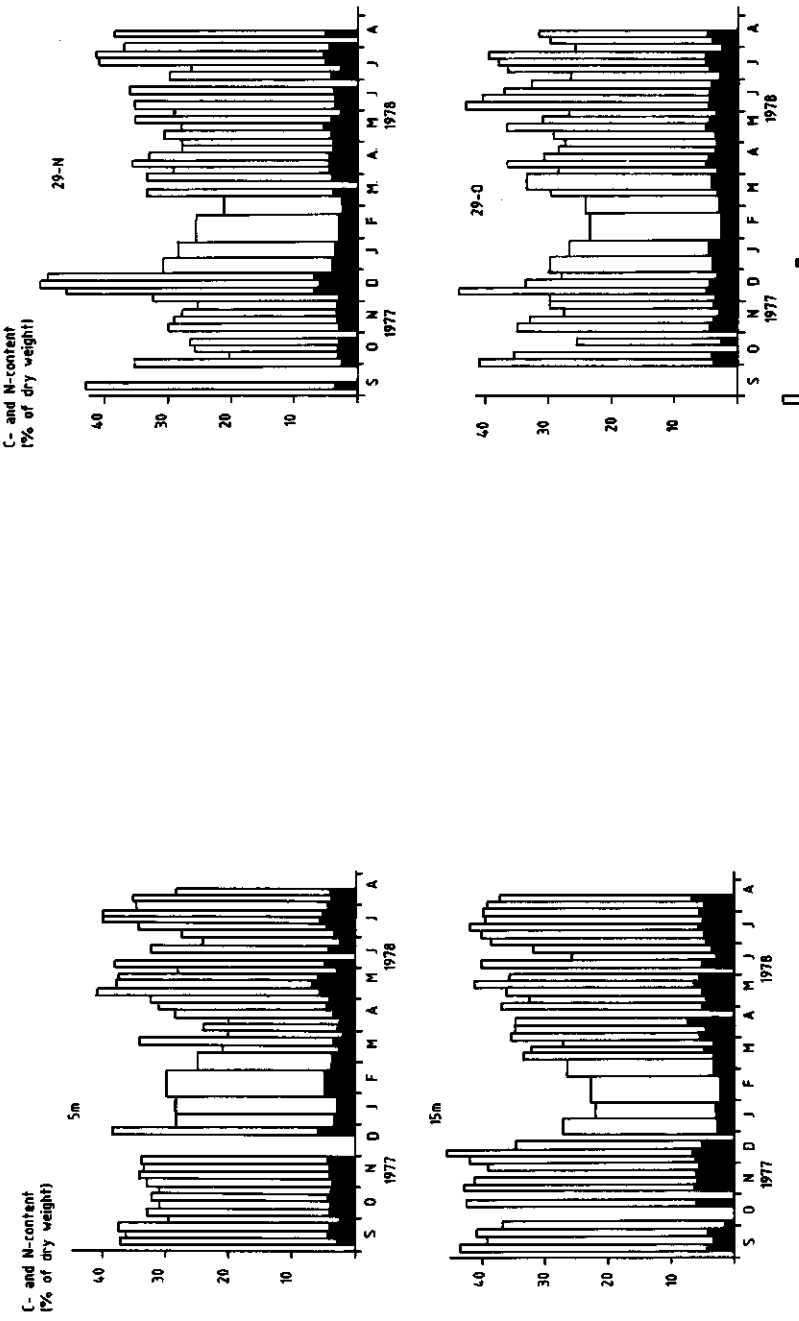

Figure 43. Carbon and nitrogen contents of settling particulate material at 5, 15 and 29 m water depth in the Pluss-See.

bulent water movements that can freely reach the bottom of the lake during periods of homothermy. This conclusion is confirmed by the composition of the trapped particulate material during this period. In contrast to other periods the carbon content of the trapped material equals the carbon content of the profundal sediments (see table VII).

The process of resuspension has been recognized in many studies (e.g. DAVIS, 1968; DAVIS, 1973; GASITH, 1976; LASTEIN, 1976). It disturbs the measurement of sedimentating particulate material originating from the photogenic zone or from outside the lake (in this study both are designated as primary sedimentation).

This process plays an important role in the transport and sorting out of material from shallower regions to deeper parts of the lake (DAVIS, 1973; DAVIS *et al.*, 1971). This transport of sediment resulting in a faster accumulation of sediments in the deeper parts of the lake (e.g. DEEVEY, 1955; RIGG, 1958; PEWE *et al.*, 1965; WIECKOWSKI, 1969) is called sediment focusing (LIKENS and DAVIS, 1975). HILTON (1985) distinguishes five processes which redistribute settled material or cause sediment focusing. Three of them are relevant for the Pluss-See:

- Sliding and slumping on slopes: movement of sedimentary material parallel to a sloping bed (sliding) or movement initiated by a rotational failure of the sediment (slumping) moves material from shallow region to deeper water. In a conical basin this will cause obvious sediment focusing, if slopes are >4%;
- Intermittent complete mixing: homogeneous resuspension of sediments during the autumn overturn throughout the water column, followed by sedimentation. The mass of particulate material is directly proportional to the depth of the overlying water;
- Peripheral wave action: turbulence in the littoral region of the lake creating resuspension of material in this zone and subsequent redeposition in deeper water.

To separate primary sedimentation from resuspension, STEELE and BAIRD (1972) tried to use the difference in C/N ratio between tripton and surficial sediments, but they found no significant differences. In the present study significant differences have been found. However, the C/N ratio of the settling organic matter did not show the expec-

ted pattern, i.e. higher values during periods with resuspension dominating the gross sedimentation. Therefore the C/N ratio could not be used to correct sedimentation data for resuspension. GASITH (1975) used the differences in organic matter content between the tripton and the surficial sediments. In this study a similar method has been applied, using the differences in carbon content between the primary settling material and the resuspended surficial sediments.

Measured rates were corrected according to:

$$S_p = S_t \cdot \left(\frac{C_x - C_s}{C_p - C_s}\right)$$

where:

S_p = primary sedimentation (g dry weight . m^{-2}.day^{-1});
S_t = gross sedimentation (g dry weight . m^{-2}.day^{-1});
C_x = carbon content of trapped material (= gross sedimentation) (%);
C_s = carbon content of the resuspended surficial sediment (%);
C_p = carbon content of primary sedimentation material (%).

The problem of applying Gasith's method to the data of the Pluss-See is to find values for C_s and C_p. As can be seen clearly from the data on carbon content of the trapped material in 29-O and 29-N during the winter period with only resuspension (see figure 43) C_s has no constant value. At the beginning of the resuspension period carbon content is relatively high (30%) and decreases to 21-24% at the end of this period. Apparently this decrease reflects the vertical distribution of the carbon content in the sediments: the top-layer with relatively high carbon content will be resuspended first, after which other layers with lower carbon content will follow. If C_s has to be known exactly, the very origin of the resuspended material and thickness of the layer that has been resuspended has to be known. Within the scope of this study this was not possible. For correction of the Pluss-See data therefore following assumptions have been made:

- the data measured from 4.1.78 till 8.3.78 do represent resuspension only. No correction is necessary: primary sedimentation = 0;

- for the rest of the observation period C_s = 25.0%, i.e. the mean carbon content of the caught material during the period from 4.1.78 till 8.3.78 at 15 and 29 m water depth which was assumed to consist entirely of resuspended sediments.
- C_p can be measured in periods, without significant resuspension, i.e. in periods with relatively high primary sedimentation rates. From figure 43 it can be seen that this is approximately 40%. For

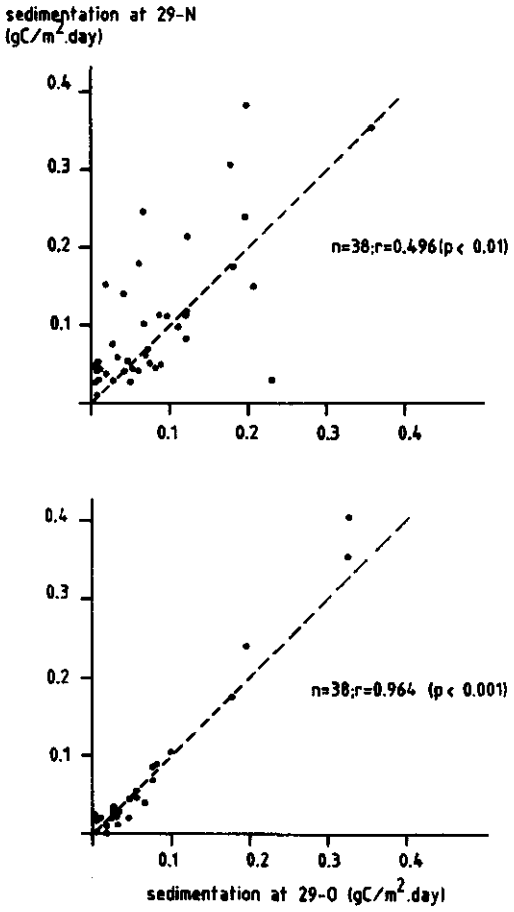

Figure 44. Relation between the sedimentation rates measured at station 29-0 and station 29-N. Upper figure: raw data; lower figure: data after correction for resuspension.

the correction of the Pluss-See data this value is used for C_p.
With the above assumptions the correction method has been applied to the data measured at 29 m water depth.
As shown in figure 44 the gross sedimentation rates measured by the two traps at 29 m water depth showed great differences, although the rates were significantly correlated. After correction of the data in the described way the thus generated primary sedimentation rates showed much better agreement: the correlation coefficient increased from 0.68 to 0.98 for the data corrected with the above formula. The differences in measurements between the two traps coincided with differences in carbon content. In the average, resuspension rates at 29-N were higher than at 29-O. This points to strong local differences in resuspension. These local differences may be caused by the position of the 29-N trap at the base of the steep slope of the lake bottom which may propagate a slumping or sliding sediment movement downward. From the strong local differences the conclusion can be drawn that surficial sediments are not resuspended in a homogeneous way throughout the lake and then resettled. The density of resuspended sediments will be higher near the sediment surface. Sediment transport resulting in sediment focusing will therefore occur mainly near the sediment surface.
In figures 45 the results of the correction are shown for the different stations. It should be kept in mind that this figure is influenced by the assumptions that have been made. It will not, however, change drastically if these assumptions are changed. From the figures it can be seen that resuspension is not confined to the winter period. Throughout the whole year resuspension can take place.
In table VI the total primarily settling carbon and nitrogen during the observation period is summarized. In contrast to total sedimentation rates remarkable differences existed between the different depths. Primary sedimentation rates at both 29 m traps are almost equal.

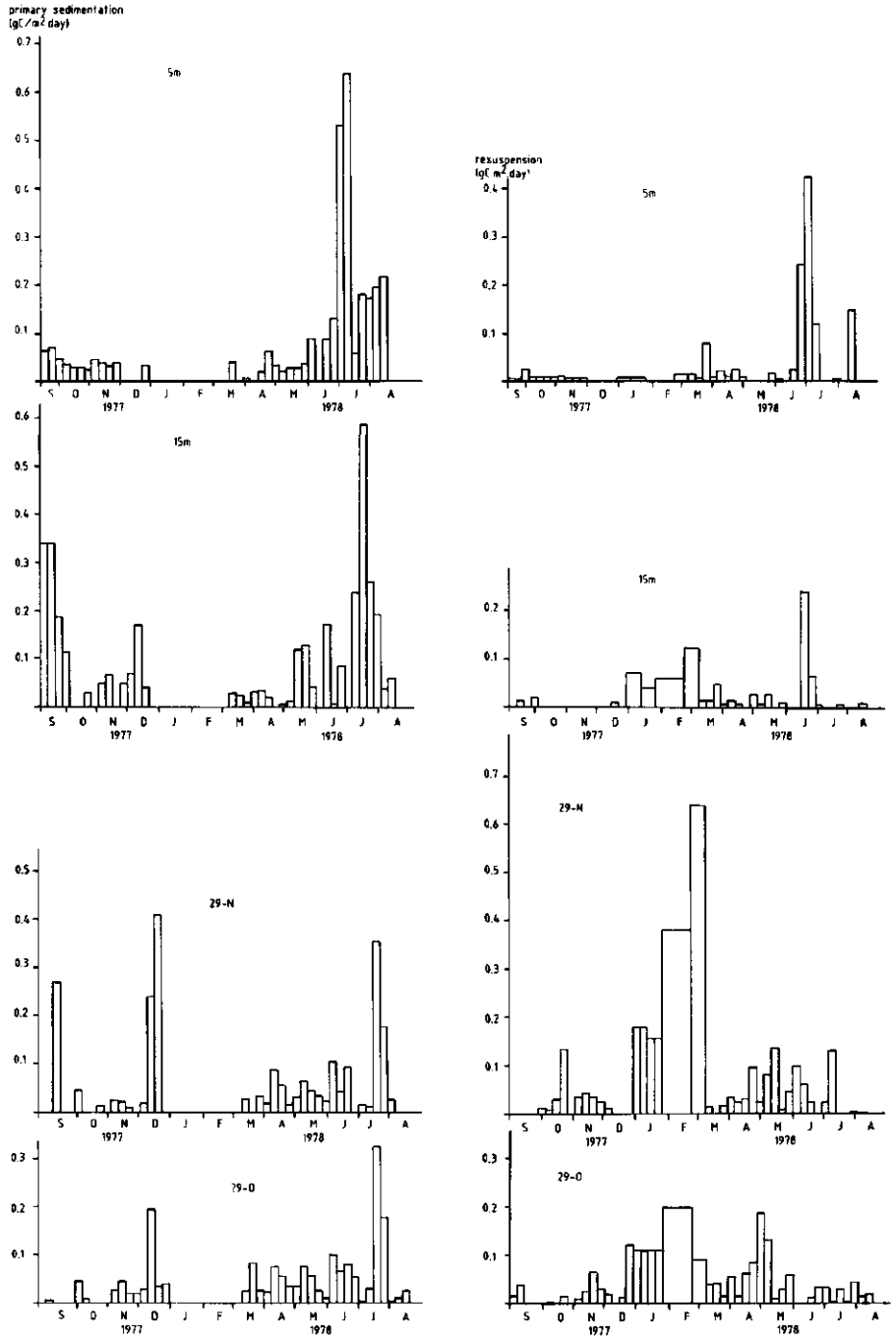

Figure 45. Primary sedimentation and resuspension of organic carbon at 5, 15 and 29 water depth.

8.5. The relation between sedimentation and primary production.

In table VI the data on annual primary sedimentation are compared with the primary production during this period measured by STABEL (unpublished results). It can be seen that at 15 m water depth a higher percentage of the primary production is settling than at 5 m water depth. This is partly caused by the fact that below 5 m water depth primary production took place (STABEL, unpublished results). In addition it might be caused by the patchiness of the primary production in the lake. The distance between the littoral station and the three other, profundal, stations is relatively large. No data, however, are available on horizontal differences in primary production in the Pluss-See.

Eight percent of the primary production reaches the deepest point of the lake. The calculation of this value differs from the calculation given by OHLE (1962). This difference originates from a different conception of the focusing effect in the lake. Ohle's "Trichtereffekt" results in a lower percentage: 0.6%. Although these differences have significant implications for the sediment accumulation, both percentages point to the very efficient decomposition of organic matter in the water column of the Pluss-See, described by OHLE (1962, 1984) as the "kurzgeschlossene Stoffkreislauf" (short circuit metabolism).

The sedimentation in the Pluss-See is low compared with other lakes. LASTEIN (1976) concluded from the literature he reviewed that in dimictic eutrophic lakes the sedimentation is about 500-1000 g (dry matter) $.m^{-2}.year^{-1}$. The sedimentation in the Pluss-See is about 50 g (dry matter) $.m^{-2}.year^{-1}$. Comparison with the literature data listed by CANFIELD *et al.* (1982) shows that the sedimentation in the Pluss-See is in the lower range of the given values. HARGRAVE (1975) compared sedimentation, organic carbon supply, mixing depth and the surface sediment organic content. From the correlations he described, it can be calculated that in the Pluss-See with a mixing depth of 5 m, 50% of the primary production should reach the deepest point of the lake. It is clear from the previous data that the Pluss-See does not fit into these correlations.

In figure 46 the relation between the maximum depth and the percentage of the primary production that is settling at the deepest part

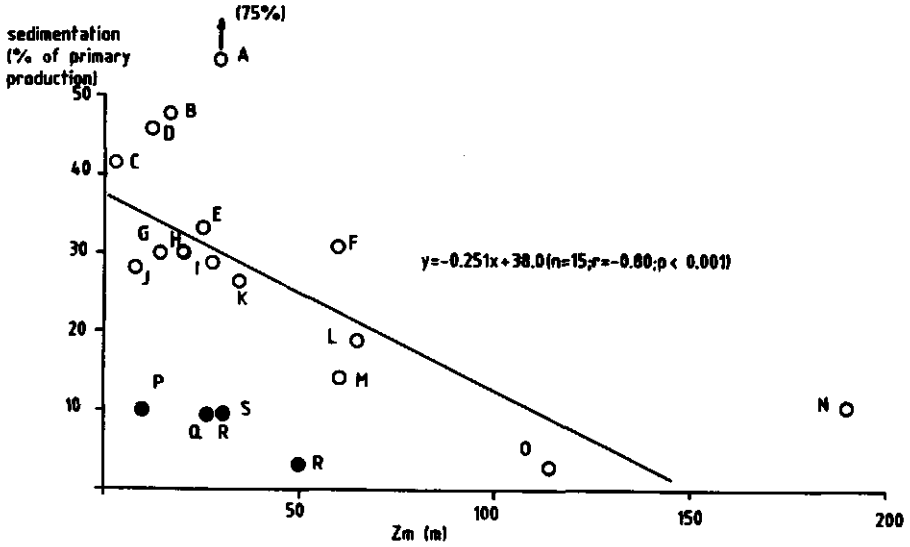

Figure 46. The relation between maximum lake depth (z_m) and percentage of primary product settling at z_m. For explanation characters see next page.
● : not involved in linear regression analysis.

of the lake is presented for some lakes described in the literature and for the Pluss-See. Apparently a trend can be observed of lower percentage at greater maximum depth ($n=12$; $r=-0.77$; $p<0.01$). However, some lakes seem to deviate from this trend, having lower sedimentation in relation to the maximum depth: Müggelsee, Char Lake, Lake Biwa and Pluss-See. These lakes are characterized by a very efficient decomposition of organic matter in the water column.
Apparently the Pluss-See differs in a rather essential way from most other lakes. This might be explained by the rather stable nature of the thermocline that retards the sedimentation of particles. As will be described in 8.6 the morphology of the lake results in an extremely low wind influence which allows the development of a relatively stable thermocline. OVERBECK (1973) mentioned increased heterotrophic activity in the metalimnion of the Pluss-See. This observation is in support of the above explanation.

8.6. The relation between observed sedimentation rates and the input of suspended matter into the sediment system.

As shown above, surficial sediments are not homogeneously resuspended throughout the lake. Locally, strong differences in sedimentation of resuspended matter could be observed. In this light one may question the implicit assumption that the measured sedimentation rate does represent the input of resuspended matter into the sediments just below the trap. The traps might for instance underestimate this input if there is a non-vertical transport of resuspended sediment more or less along the sediment surface, as it was put in 8.4 as a possible explanation for the strong differences in sedimentation of resuspended matter. At the same time it is possible that the suspended matter near the sedimentation trap is not reaching the sediment surface just below the trap, but is transported by water movements to some other place.
In order to relate sedimentation data to data on sediment composition and bacterial activity in the sediments it is therefore necessary to describe the process of sediment transport within lakes. There are two main factors governing this process: wind-induced water currents and gravity. The way in which wind affects the hydrodynamics of the lake and consequently the bottom dynamics highly depends on the

morphology and the topography of the lake. One aspect of morphology, the bottom slope, determines the way in which gravity acts on the sediment transport. The steeper this slope the less turbulence is needed to induce a sediment transport along the lake bottom. The extreme case will be sediment sliding by gravity alone over the lake bottom towards the deeper parts of the lake.

A further aspect of importance for the sediment transport is the nature of the surficial sediments. Coarse deposits with relatively high specific weight are not as easily transported by water currents as fine flocculent material. Water currents in this way do effect a sorting-out of bottom deposits, resulting generally in coarse deposits in the littoral zone and fine flocculent material in the profundal. With respect to this fine flocculent material the lake bottom can be subdivided into areas of erosion, areas of transport and areas of accumulation (HÅKANSON, 1981). In areas of accumulation there is a continuous deposition of fine materials. These areas are characterized by soft deposits with a water content of the surficial (0-1 cm) sediments >75%. In areas of transport there is a discontinuous deposition of fine materials. The physical, chemical and biological characteristics of such areas are therefore very variable. In erosion areas rock surfaces, coarse deposits and relict or consolidated materials prevail. The water content of the surficial sediments is usually <50%.

HÅKANSON (1981) describes how the size of the accumulation area is related to morphological and topographical features of the lake. He distinguishes three factors governing the size of this area: the energy factor which describes the relation between wind energy and bottom dynamics; the slope factor, describing the relation between bottom slope and sediment tranport, and the form factor which characterizes how lakes with a convex hypsographic curve have larger bottom areas subject to erosion and transportation than lakes with a concave hypsographic curve.

The energy factor (E) is expressed as:

$$E = [\sqrt{a}/z_m - 0.2]$$

where:

a = lake area (km^2);

z_m = maximum depth (m).

The value of E for the Pluss-See is 0.187, i.e. less than the smallest value reported by HÅKANSON (1981). This means that the influence of wind-induced water currents on the tranport of sediments in the Pluss-See is very small compared with other lakes.

The slope factor is expressed as:

$S = \log (60.6\ \bar{z}\ /\ \sqrt{a})$

where $\bar{z}$ = mean depth (m). The value of S for the Pluss-See is 3.179, i.e. higher than the maximum value given by HÅKANSON (1981). This high value, originating from the very high mean slope of the Pluss-See, points at the important role that the bottom slope plays in the sediment transport processes of the Pluss-See.

The form factor is expressed as $z_{max}/3\bar{z}$, i.e. the inverted volume development (V_d^{-1}). For the Pluss-See this value is 1.026, i.e. approximately the ideal cone form $(V_d^{-1}=1)$.

Comparison of the values of these factors for the Pluss-See with those of other lakes given by HÅKANSON (1981) reveals the extreme position of the Pluss-See.

HÅKANSON (1981) combines the three factors in the following way to calculate the percentage of a given lake area dominated by accumulation processes (a_A):

$a_A = 80.7 - 52.0 \times E \times V_d^{-1} \times S.$

For the Pluss-See a_A = 49.0%. From field observations it could be concluded that permanent accumulation takes place at depths greater than 6 m, i.e. 64.3% of the lake surface. The accumulation area is therefore larger than predicted by the model of HÅKANSON (1981). This might be caused by the fact that the Pluss-See has not only an extremely low energy factor (low wind influence because of lake morphology), but is also protected from wind influence by the forested hills surrounding the lake (low wind influence because of landscape morphology). Besides this, HÅKANSON (1981) states that his model especially applies to lakes with areas from 1 to 5000 km^2. The surface of the Pluss-See is only 0.14 km^2. The underestimation of the accumulation area by the model is in agreement with EVANS and RIGLER (1983) who stated that the model tends to overestimate the areas

which they define as shallow, because the model does not discriminate between transportation and erosional areas.

HÅKANSON (1977) states that fine deposits rarely stay permanently on slopes declining more than 4-5%. At every depth of the Pluss-See except at the deepest point of the lake the bottom slope is far more. If no permanent accumulation would occur at these slopes, a frustum-like accumulation pattern would have been the result. The observation of permanent accumulation at every depth below 6 m and the bathymetric map of the Pluss-See contradict this conclusion. This might be explained by the combination of the extreme low energy factor and the position of the lake surrounded with forested hills that moreover attenuates the wind influence.

Summarizing, the Pluss-See can be described as an almost conical lake with an extremely low wind influence, in which sediment transport will be mainly governed by the bottom slope. Because of the sharp inclination of this slope, sediment will tend to slide over the lake bottom by gravity alone. In this way the observed strong local differences in sedimentation of resuspended sediment can be explained: weak turbulences are enough to initiate sediment transport along the lake bottom. These turbulences are not strong enough to resuspend sediment in a homogeneous way throughout the lake water. Therefore a concentration gradient of suspended matter is established in vertical direction with high concentrations near the sediment surface. As a result the sedimentation trap near the lower end of the bottom slope (29-N) will catch more of the sediment transported along the sediment surface than the more remote trap 29-O.

Main resuspension of sediments occurred during the period of homothermy following the autumn overturn. From this it can be concluded that intermittent complete mixing is an important process for sediment focusing in the Pluss-See. As shown before this process does not result in a homogeneous distribution of resuspended meterial throughout the lake. Intermittent complete mixing seems to initiate sediment sliding or slumping. This underlines the important role of the bottom slope in the focusing process within the Pluss-See, as predicted by the model of HÅKANSON (1981) and is in agreement with the model constructed by HILTON (1985) to predict the importance of the different processes for sediment redistribution and focusing.

This model predicts that slope will dominate almost completely the redistribution of sediments in the Pluss-See, but that also intermittent complete mixing will play a role.
Sediment transport along the lake bottom has been observed by other authors. WIECKOWSKI (1969) observed in lakes of north-east Poland that sediment is sliding as a semifluid layer into bottom depressions. LUDLAM (1974) describes sediment slumping on the sides of the Fayetteville Green Lake basin in New York State, resulting in turbidity currents. Because of the varved structure of the sediments this slumping could be studied in an excellent way in this lake, contrary to the Pluss-See, where sediments are homogeneous without varves. VERDOUW and DEKKERS (1982) described sediment transport along the edge of Lake Vechten (The Netherlands).
From the importance of sediment transport along the lake bottom the conclusion can be drawn that the sedimentation of suspended matter as measured with sedimentation traps does not represent the total input of suspended matter into the sediments just below the trap. Sediment transport along the lake bottom is not or only partly measured with the traps. This sediment transport results in the focusing of the sediments within lakes, i.e. the phenomenon that sediments accumulate faster in the deeper parts of the lake.
The equation given by HÅKANSON (1981) estimates the size of the accumulation area. However, if the relation between input of organic matter and bacterial activity in the sediments has to be known, the sediment transport and the spatial variation of the accumulation within the accumulation area have to be described. Because no methods were available to measure this transport directly, this problem is approached in a theoretical way as described below.

8.7. Focusing models and their implications.

Dating sediment cores by several methods is one way to get an impression of the history of a lake, especially of the productivity and the sediment accumulation in former years. Most of these studies are based on a single sediment core, mostly taken at the deepest part of the lake. The effect of sediment focusing was recognized, but it was assumed that this effect was constant during lake filling. Changes in accumulation rates were believed to be attributed to changes in

sedimentation only. In order to explain accumulation rates measured by LIKENS and DAVIS (1975) in Mirror Lake, New Hampshire, LEHMAN (1975) constructed models that describe the focusing process as a function of lake morphology. The output of these models is the ratio between sedimentation and accumulation of sediments at the deepest point of the lake (z_m). In fact these models showed that for some morphometric types the ratio between the (primary) sedimentation and the accumulation at the deepest point of the lake changes during the filling of the lake.

The focusing models described by LEHMAN (1975) are based on the volume depth distributions given by JUNGE (1966). JUNGE (1966) described the volume depth distribution of several lake types: sinusoids, hyperboloids, ellipsoids. Hyperboloid and ellipsoid distributions can both be described by:

$$V_{(u)} = 1 - \frac{6u - 3(1-p)u^2 - 2pu^3}{3+p} \qquad \text{(JUNGE, 1966)} \qquad (1)$$

where:

$V_{(u)}$ = the fraction of the total volume which lies below the contour line corresponding to some fraction (u) of the maximum depth ($u = z/z_m$);

$$p = 6(\bar{z} / z_m) - 3 \qquad (1/3 < \bar{z} / z_m < 2/3) \qquad (2)$$

$\bar{z} / z_m$ is the ratio of mean depth ($\bar{z}$) to maximum depth (z_m) or the "mean depth ratio".

Sinusoid distributions can be described by:

$$v_{(u)} = g(1-r(1-u))/g(1-r) \qquad \text{(JUNGE, 1966)} \qquad (3)$$

$$g(x) = x(\cos^{-1}x)^2 + 2(1-x) - 2(1-x^2)^{\frac{1}{2}} \cdot (\cos^{-1}x) \qquad (4)$$

where g is a function used to define V, x is a dummy variable and r is a parameter related to the mean depth ratio (JUNGE, 1966):

$$\bar{z} / z_m = \frac{2}{r}\left(\frac{(2r-r^2)^{\frac{1}{2}}}{\cos^{-1}(1-r)} - \frac{r}{(\cos^{-1}(1-r))^2}\right) - \frac{1-r}{r} \qquad (5)$$

r represents the position of the lake surface in relation to the axis of the sinusoid: r=1, if the axis of the sinusoid is located at the lake surface: r=2, if the axis of the sinusoid is located at u=0.5. The mean depth ratios of hyperboloids are limited to values between 0.333 and 0.667, of ellipsoids between 0.5 and 0.667. The mean depth ratio of sinusoids is limited to values between 0.297 and 0.50. The limits of the range of mean depth ratios of hyperboloids are the ratios of a cone (0.333) and a paraboloid (0.50).

The mean depth ratio of the Pluss-See is 0.325 (calculated from data, given by KRAMBECK (1974). Theoretically this means that only a sinusoid distribution is possible. In figure 47 the volume depth distribution of the Pluss-See (KRAMBECK, unpublished) is compared with a conic distribution and with a sinusoid distribution with r=1.9879 which corresponds with $\bar{z} / z_m = 0.325$ (see 5). The volume depth distribution lies in between. In fact the distribution is conic in the shallow region (low u) and sinusoid in the deep region. In figure 48 the mean radius of the lake area at different depth is given (based on data of KRAMBECK, unpublished). In the profundal

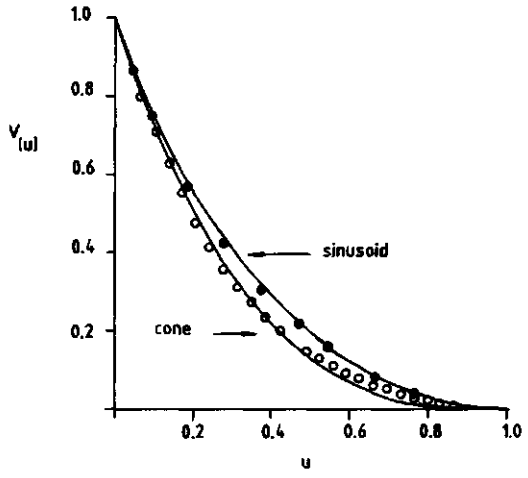

Figure 47. Volume-depth distributions of a conic and a sinusoid lake basin, of the total Pluss-See basin (O) and of the Pluss-See basin deeper than 8 m (●). For explanation of symbols, see text 8.7.

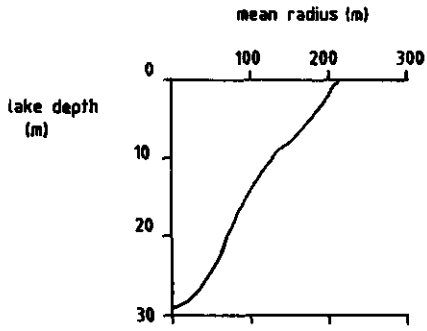

Figure 48. Mean radius of horizontal section of the Pluss-See basin in relation to lake depth.

region the mean radius shows a sinusoid development, followed by a discontinuity at 8 m water depth. This discontinuity is caused by a relatively large littoral plain in the north-west of the lake (see figure 1). The volume depth distribution of the part of the lake deeper than 8 m fits the sinusoid distribution perfectly well with r=1.9895, corresponding to $\bar{z} / z_m = 0.323$, the mean depth distribution of the part of the lake deeper than 8 m (see figure 47).

Based on the volume depth distributions of JUNGE (1966) and some assumptions about the way the lake basin is getting filled with (permanent) sediments, LEHMAN (1975) gives for some distributions the ratio of the sedimentation to the accumulation of the sediments in the deepest point of the lake, namely for a frustrum, a hyperboloid, a sinusoid and an ellipsoid distribution. As the volume depth distribution of the Pluss-See is situated between the sinusoid and conic distribution, only these distributions are taken into further consideration. For the sinusoid and the conic distribution, if they maintain this conformation during lake filling, the sedimentation accumulation ratio is constant. For both distributions the following relationship holds:

$$S_{(t)} = - \bar{z} \; / \; z_m \cdot \frac{dz}{dt} \qquad (6)$$

where:

$S_{(t)}$ = influx of sediment per unit lake surface (= accumulation of sediments assuming homogeneous sedimentation);

$\frac{dz}{dt}$ = accumulation rate at the deepest point of the lake.

In studying the relationship between input of organic matter and the heterotrophic activity in the sediments, not only the deepest point of the lake is of importance, but any part of the lake bottom. Based on the focusing models of LEHMAN (1975) it is possible to calculate the sedimentation-accumulation ratio in shallower parts of the lake. For the conic and the sinusoid distribution this ratio is linear with depth:

- cone:

$$\left(\frac{S_{(t)}}{dz/dt}\right)_u = - \bar{z} \; / \; z_m \; .u = - 0.333.u \qquad (7)$$

- sinusoid:

$$\left(\frac{S_t}{dz/dt}\right)_u = \frac{u}{\bar{z} \; / \; z_m} = \frac{z/z_m}{\bar{z} \; / \; z_m} = \frac{z}{\bar{z}} \qquad (8)$$

Equations (7) and (8) assume accumulation throughout the lake basin. Reference has to be made to the erosion and transportation area. This can be done, if all parameters in (7) and (8) are considered to be parameters of that part of the basin that lies below the contour line corresponding to the border line of the accumulation area. To account for the sediment transport out of the erosion and transportation area (7) and (8) has to be divided by a_A:

- cone:

$$\left(\frac{St}{d_{z_A/dt}}\right)u_A = 0.333 \; \frac{z_A}{z_{m_A}} \cdot \frac{1}{a_A} \qquad (9)$$

- sinusoid:

$$\left(\frac{St}{d_{z_A/dt}}\right)u_A = \frac{z_A}{\bar{z}_A} \cdot \frac{1}{a_A} \qquad (10)$$

The models describe the result of the focusing process, not the process itself, i.e. the transport of sediment into deeper parts of the lake. For a paleolimnologist only the result of this transport matters not the process itself. On the contrary it does matter in studies concerning the relationship between the input of organic matter into the sediments and the heterotrophic activities in these sediments. It makes a difference, if the transport is a continuous or a discontinuous process, only taking place during homothermy. If the process of focusing is discontinuous, the settled organic matter will be broken down (at least partly) at the place, where it reaches the lake bottom. There will be a direct relation between the rate of sedimentation and the heterotrophic activity in the sediments (if this activity is not limited by other factors than the input of organic matter). If sediment focusing is a continuous process, the settling particle will be transported into deeper regions of the lake as soon as it reaches the lake bottom and will be broken down in these deeper regions. The relationship between sedimentation and heterotrophic activity in the sediments is then not straightforward. The focusing models can predict the nature of this relationship.
In figure 49 a conic lake basin is shown. Two different situations

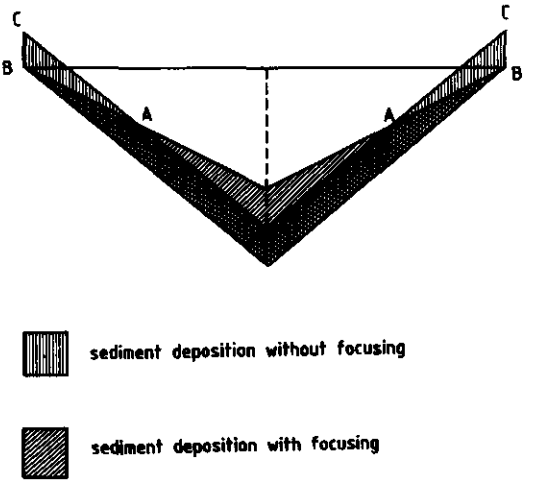

Figure 49. Conic lake basin, showing sediment deposition with and without focusing of sediments. For explanation of symbols see text 8.7.

are compared: a theoretical situation without focusing, resulting in a homogeneous sediment layer and a situation with sediment focusing. The area ABC represents the amount of the homogeneous sediment that has been transported through point A since the lake was formed in order to get the situation with focusing. In fact A is not a point, but the isobath, where the sedimentation-accumulation ratio equals unity. It is possible to calculate the transport of sediment through this and every other isobath. Dividing this transport by the length of the isobath a sediment flux per meter isobath and per "lake age" results.

For the simple conic distribution this flux T is:

$$T = 1/3 \cdot b \cdot d_s \cdot \left(\frac{x}{z_m} - \frac{x^2}{z_m^2}\right) \left(m^3 \cdot m^{-1} \cdot (\text{lake age})^{-1}\right) \qquad (11)$$

where:

b = means radius of the lake surface (m);

d_s = thickness of accumulated sediments at z_m (m).

T is maximal for $dT/dx = 0$ that means for $x = \frac{1}{2}z_m$. Figure 50 shows the relationship between the focusing transport and the water depth. A more complicated, but essentially comparable relation holds for the sinusoid distribution. Formula (11) does not account for the fact that no sediments accumulate in the littoral zone. Absence of permanent accumulation in the littoral zone will result in enhanced sediment transport. This is, however, counteracted by the fact that the homogeneous sediment distribution in figure 49 is irrealistic in this zone. A sediment layer thicker than the water depth of the original lake basin is not possible. Therefore formula (11) overestimates sediment transport in this zone. No account has been made for these boundary deviations, because they will not change the general pattern of sediment transport as shown in figure 50.

If the focusing transport is a rather continuous process, the depth-dependence of the flux will not only be manifest in the long run, but also in the present and will therefore influence the heterotrophic processes in the sediments directly. If this transport is mainly along the lake bottom, as it is in the Pluss-See (see 8.5), this

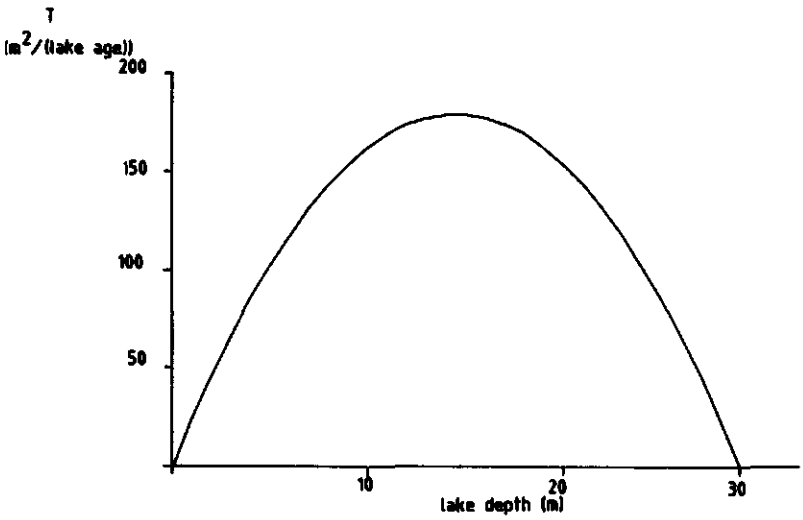

Figure 50. Relation between focusing transport (T) and lake depth for a conic lake basin with b = 213; d_s = 10 and z_m = 29 (see 8.7.; p.88).

transport represents the movement of sedimented particles over the sediment surface into the deeper parts of the lake, i.e. the fluid or semi-fluid suspension WIECKOWSKI (1969) described. The more intensive this transport is, the less influence there will be of the primary sedimentation on the heterotrophic activity in the sediments.

8.8. Verification of the model.

Below 6 m water depth permanent accumulation of sediments has been observed in the Pluss-See. The accumulation area is therefore assumed to be the area with depth >6 m. The mean depth of the waterbody below 6 m is 7.05 m; the maximum depth is 23 m. The percentage of the lake area deeper than 6 m is 64.5. Hence a_A = 0.645. The accumulation-sedimentation ratio is therefore $\frac{1}{0.645} \times \frac{23}{7.05} = 5.06$.

At 15 m water depth this ratio is $\frac{9}{23} \times 5.06 = 1.98$.

If the accumulation area is considered to be conical, maximum transport intensity will be at $\frac{1}{2} z_{m_A} = 16.5$ m.

Two aspects have to be verified: the accumulation-sedimentation ra-

tio at z_m and the linearity between depth and accumulation rate within the accumulation area.

- accumulation of sediments at z_m.

By means of pollen analysis AVERDIECK (1983) estimated the accumulation rate from the year 1500 till now at 26 m water depth to be 2.34 mm.year^{-1}. There are no indications for a severe, cultural eutrophication in the recent history of the lake. Quantitative data are only available for het last two decades. Comparison of data on primary production in 1960 (OHLE, 1962), 1977-1978 (STABEL, unpublished results) and 1981 (MEFFERT and OVERBECK, 1985b) (see 2.1) points to a constant productivity in the last twenty years. It can be assumed, therefore, that no dramatical increase of sedimentation has occurred in the recent history of the lake.

From figures 18, 19 and 20 it can be seen that the dry matter, the organic matter and the organic carbon content of the sediments stabilize at about 10%, 40% and 20% respectively at 30 cm below the sediment surface. Assuming the specific weight of the wet sediments to be unity, the annual accumulation of permanent sediments at the deepest part of the lake can be calculated to be 0.0023 x 10^2 x 0.1 x 0.4 = 92 g organic matter .m^{-2} or 46 gC.m^{-2}. From the vertical distribution of organic carbon in the sediments (see figure 20) and the carbon content of the settling suspended matter (32.3%; table VII) it can be calculated, that 38% of the settled particulate carbon remains as permanent sediment. The annual input of organic carbon at the sediment surface, therefore, amounts to $\frac{46}{0.38}$ = 121 gC.m^{-2}. Compared to the measured primary sedimentation rate of 15-17 gC.m^{-2}.year^{-1} (see 8.5), this figure points to a considerable sediment focusing. From the theoretical accumulation-sedimentation ratio of 5.06 at 29 m an annual primary sedimentation rate of $\frac{121}{5.06}$ = 24 gC.m^{-2} can be calculated, which is slightly higher than the measured rate. The difference might be partly caused by the fact that allochthonous input of organic matter was not taken into account. As described in 2.1 the Pluss-See is surrounded by forested hills. A considerable input of leaf litter might be expected. The C/N ratio of the sediments points also to a considerable allochthonous input of organic matter (see 6.4.1).

GASITH and HASLER (1976) estimated the annual transport of airborne litterfall in lake Wingra to be 320 $gC.m^{-1}$ of wooded shoreline. Similar values were estimated by JORDAN and LIKENS (1975) in Mirror Lake. For the Pluss-See with a shore line of about 1400 m this would mean an annual tansport of airborne litterfall of 480 kg $C.year^{-1}$ or 3.4 $gC.m^{-2}.year$.

An other source of error might be the estimation of the accumulation area a_A. From field observation it was concluded that a_A is 64,5% of the lake area. The model of HÅKANSON (1981) predicts for the Pluss-See a_A = 49% (see 8.6). From this value an accumulation-sedimentation ratio of 6.2 can be calculated. With this ratio and the accumulation rate of AVERDIECK (1983) a primary sedimentation of 19.5 $gC.m^{-2}.year^{-1}$ can be calculated, which is in close agreement with the measured primary sedimentation rate of 15-17 $gC.m^{-2}.year^{-1}$.

It can be concluded that the model explains to a considerable degree the ratio between measured primary sedimentation and measured sediment accumulation at z_m.

- linearity between water depth and sediment accumulation.

The linearity between water depth and sediment accumulation has been demonstrated to exist in Bob Lake by EVANS and RIGLER (1980) and in two small Shield lakes by EVANS and RIGLER (1983). They used the ^{210}Pb-dating technique. No such technique was available in the present study. Some evidence for existence of a linear relationship can be drawn from the vertical decrease of the carbon content in the profundal sediments representing the breakdown of organic matter. The breakdown of organic matter in the sediments can be described as a first order reaction (OGURA, 1972; BERNER, 1974).

$$\ln \frac{c'_t}{c'_o} = - k \cdot t \qquad (2)$$

where:

c'_t = concentration of degradable organic carbon in the sediments at time t;

c'_o = concentration of degradable organic carbon at time o, c.i. at the sediment surface;

k = reaction constant.

Organic carbon content values were expressed as percentage of dry sediments (see 2.3). These values cannot be compared with each other without taking into account the loss of dry matter during the breakdown of organic matter. To correct for this loss the organic carbon content values were converted to concentrations. These concentrations were corrected for sediment compaction. For this correction it was assumed that the ash concentration only changed because of sediment compaction.

The organic carbon content was expressed as concentration in compacted sediments according to:

$$c_{d_p} = \frac{c_d}{100} \times \frac{DM_d}{100} \times 1000 \times \frac{(100-OM_p)}{(100-OM_d)} \times \frac{DM_p}{DM_d} =$$

$$= c_d \times 10^{-1} \times DM_p \times \frac{(100-OM_p)}{(100-OM_d)} \qquad (1)$$

where:

- c_{d_p} = organic carbon concentration (g/l) at depth d below the sediment surface, corrected for compaction;
- c_d = organic carbon content (% of dry sediment) at depth d below the sediment surface;
- DM_d = dry matter content (% of wet sediment) at depth d below the sediment surface;
- DM_p = dry matter content (% of wet sediment) of compacted permanent sediments;
- OM_d = organic matter content (% of dry sediment) at depth d below the sediment surface;
- OM_p = organic matter content (% of dry sediment) of compacted, permanent sediments.

DM_p and OM_p can be estimated from the vertical distribution of DM and OM in the sediments (see figures 18 and 19).

The degradable organic carbon concentration at depth d below the sediment surface can be approximated by:

$$c'_{d_p} = c_{d_p} - c_p, \text{ where:} \qquad (3)$$

c_p = total organic carbon concentration of the permanent sediment.

c_p can be estimated from the vertical distribution of total organic carbon in the sediments.

If the accumulation rate (A) is constant in terms of consolidated, permanent sediment, then:

$$t = \frac{1}{A} \cdot d_p \qquad (4)$$

where d_p = depth below the sediment surface assuming complete consolidation and breakdown of the sediments to permanent sediments.
If it is assumed that the ash-concentration in a vertical profile only changes by compaction, d_p can be calculated from the real depth (d) below the sediment surface following:

$$d_p = d \cdot \frac{DM_{(o-d)}}{DM_p} \cdot \frac{(100-OM_{(o-d)})}{(100-OM_p)} \qquad (5)$$

where:

$DM_{(o-d)}$ = mean dry matter content (% of wet sediment) of the surficial sediment layer with thickness d;

$OM_{(o-d)}$ = mean organic matter content (% of dry sediment) of the surficial sediment layer with thickness d.

Substitution of (3) and (4) into (2) gives:

$$\ln \frac{c_{d_p} - c_p}{c_{o_p} - c_p} = - k \cdot \frac{1}{A} \cdot d_p \qquad (6)$$

For a single core $\frac{k}{A} = k'$ is a constant. k' can be determined graphically or by linear regression analysis, because of the linear relationship between $\ln \frac{c_{d_p} - c_p}{c_{o_p} - c_p}$ and d_p. If the reaction constant k

is considered to be constant throughout the accumulation area, the ratio between k' at depth z and k' at z_m is equal to the ratio between the accumulation rates at these depths:

$$\frac{k'_z}{k'_{z_m}} = \frac{k}{A_z} \cdot \frac{A_{z_m}}{k} = \frac{A_{z_m}}{A_z} = F \qquad (7)$$

where F is the "focusing ratio". Applying (7) the spatial variation of the accumulation rate within the accumulation area can be determined by means of three simple parameters (vertical distribution of dry matter, total organic matter and organic carbon content within the sediments), if the breakdown of organic matter in the sediments can be described as a first-order reaction and the accumulation rate has not been subjected to severe changes in the recent history of the lake.

The k' values have been calculated from the mean vertical distribution of the dry matter, organic matter and organic carbon content in the sediments at 15 and 29 m water depth, based on 20 cores each (see figures 18, 19 and 20).

Figure 51a shows that a linear relationship between $\ln \frac{c_{d_p} - c_p}{c_{o_p} - c_p}$ and d_p exists at both water depths. From the k'-values F for 15 m water depth can be calculated to be 0.465 which is slightly higher than the predicted value (see figure 51b) (For 29 m (= z_m) F = 1 by definition).

F can be estimated in a more simple, but less accurate way by measuring the depth (d_q) below the sediment surface at which the sediments are broken down to a permanent state, i.e. the depth below which the organic carbon content is more or less constant. This is shown for three sediment cores from 10, 15 and 29 m water depth respectively in figure 52a.

The vertical distribution of the dry matter, organic matter and organic carbon content was measured at 1 cm intervals. Depth below the sediment surface was corrected for compaction according to (5). The organic carbon content was converted to concentration in compacted sediments according to (1).

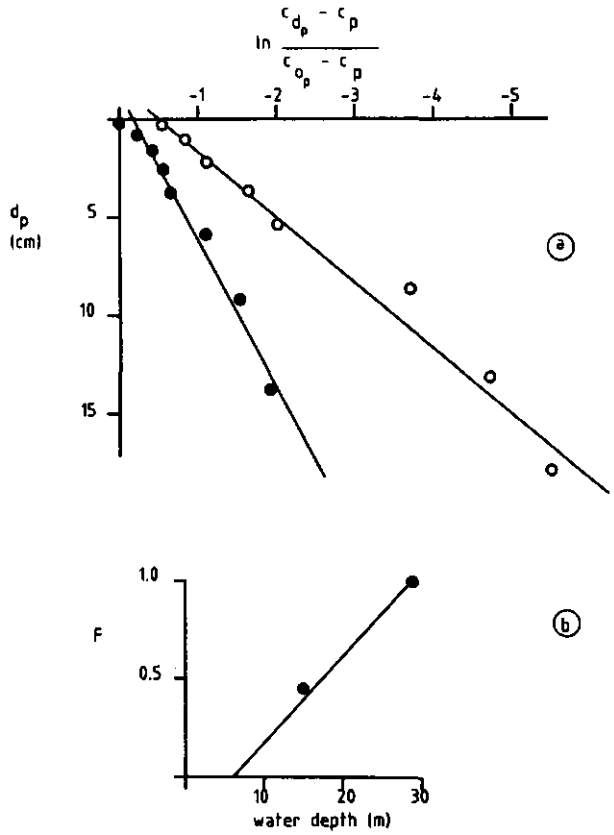

Figure 51 a. Relation between $\ln \frac{c_{d_p} - c_p}{c_{o_p} - c_p}$ and d_p at 15 m (O) and 29 m (●) water depth, calculated from figures 18, 19 and 20. For explanation of symbols, see text 8.8.
b. Relation between water depth and focusing ratio (F). Drawn line: predicted value.
●: calculated from figure 51a.

From figure 52a it can be estimated that at d_q = 1, 2.5 and 6 cm for the cores from 10, 15 and 29 m water depth respectively no further breakdown of organic matter occurs. F has been estimated as the fraction of d_q at 29 (= z_m). From figure 52b it can be seen that

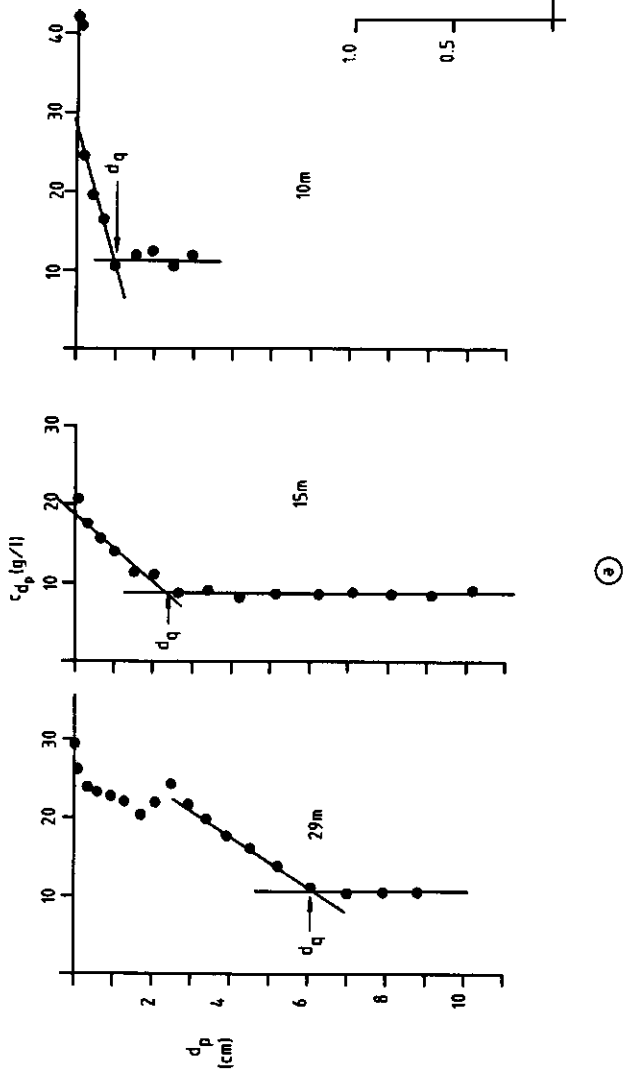

Figure 52 a. Vertical distribution of organic carbon in sediment cores from 10, 15 and 29 m water depth. For explanation of symbols, see text 8.8.
b. Relation between water depth and focusing ratio (F). Drawn line: predicted value. (●): calculated from figure 52 a.

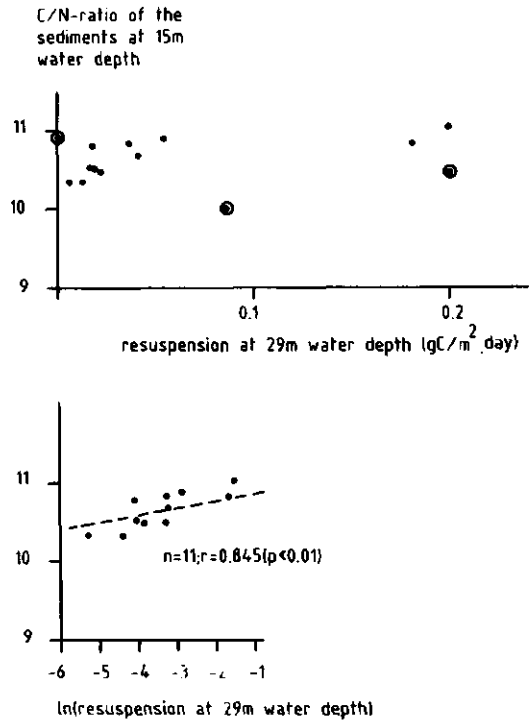

Figure 53. Relation between the resuspension rate at 29 m water depth and the C/N ratio of the sediments at 15 m water depth.
◎: not involved in linear regression analysis.

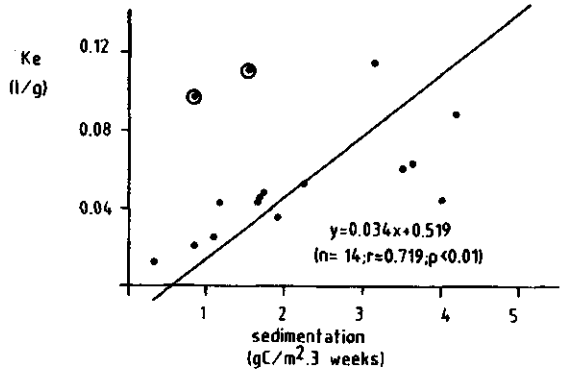

Figure 55. Relation between total sedimentation of particulate carbon and K_e of the surficial (0-5 cm) sediments at 29 m water depth.
◎ : not involved in linear regression analysis.

these values are in close agreement with the values predicted by the model.

The results support the hypothesis of EVANS and RIGLER (1980) that in morphometrically typical temperate lakes depth would be the main factor causing variation in the rate of sediment accumulation. They found that water depth explained 72 and 57% of sediment accumulation in Costello and Red Chalk Lake respectively.

Summarizing it can be concluded that the focusing model explains the difference between the observed primary sedimentation rate and the reported accumulation rate, and the observed variation of the mean vertical distribution of carbon in the sediments within the accumulation area. In addition to the model of HÅKANSON (1981) that predicts the size of the accumulation area, the presented focusing model describes bottom dynamics within the accumulation area. The implications of this model should be kept in mind when discussing the relation between sedimentation and sediment properties.

8.9. Relation between sedimentation, sediment composition and acetylene-reducing activity in the sediments.

In chapter 6 the relation between sediment composition and acetylene-reducing activity has been discussed. Especially the C/N ratio and the ammonium adsorption capacity of the sediments appeared to be important parameters for explaining the observed variation of the acetylene-reducing activity. In this section attention will be paid to the relation of both sediment composition and acetylene-reducing activity with the sedimentation of particulate organic matter.

- sediment composition (C, N content etc.).

Settling material, reaching the sediment surface, will change the composition of the surficial sediments, if there is a substantial difference in composition between the surficial sediments and the settling material and if the sedimentation rate is high enough.

In table VII the mean carbon content of the settling material and the surficial sediments are compared for the different depths. Especially at 15 m water depth these differences are considerable, except for the winter period with high rates of resuspension. No relation, however, could be observed between the total sedimentation of carbon

and C/N or K_e of the surficial sediments. At this depth surficial sediments are resuspended and (at least partly) transported to the deeper parts of the lake. The material deposited at 29 m water depth partly comes from the surficial sediments at 15 m water depth. An indication for this is the weak tendency of the C/N ratio of the surficial sediments at 15 m water depth to increase with increasing sedimentation rates of resuspended material at 29 m water depth, as shown in figure 53.

The C/N ratio of the surficial sediments at 29 m water depth is

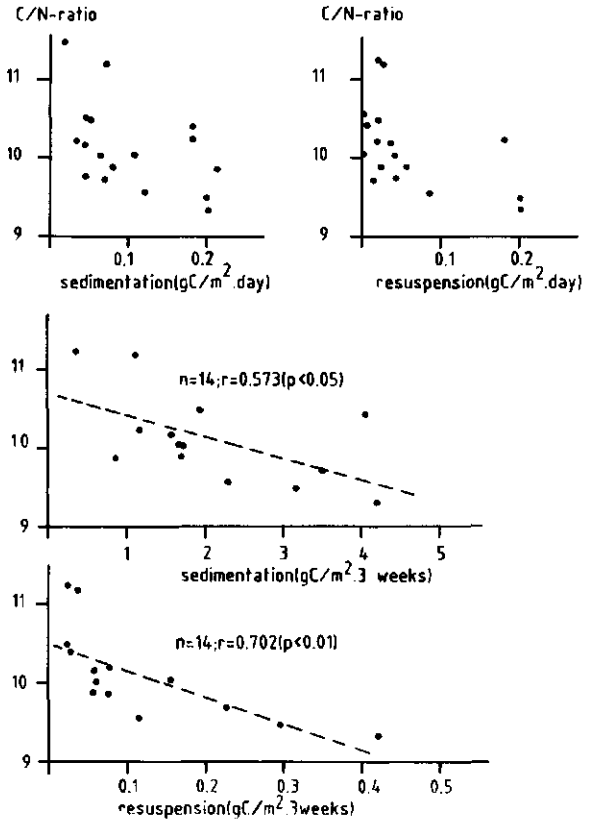

Figure 54. The relation between sedimentation and resuspension at 29 m water depth and the C/N ratio of the surficial sediment at this depth.

significantly negatively correlated with the sedimentation rate, especially if sedimentation of resuspended matter over longer periods (3 weeks) is taken into consideration (see figure 54).
A significant positive correlation was observed between K_e of the surficial sediments at 29 m water depth and both the total sedimentation rate and the sedimentation rate of resuspended material, if two extreme values are not involved in the linear regression analysis (see figure 55; see page 138).
From this the conclusion can be drawn that the composition of the sediments at 29 m water depth is governed mainly by the transport of sediments from other parts of the lake by resuspension and transport along the sediment surface.
In the littoral sediments no correlation could be observed between the sedimentation rate and the C/N ratio, nor between the sedimentation rate and K_e either.

- *acetylene reduction in the sediments.*

Figure 56 shows that both in the littoral sediments and in the profundal sediments at 29 m water depth the acetylene-reducing activity was positively correlated with the total sedimentation of organic carbon. No correlation could be observed at 15 m water depth. At 29 m water depth the acetylene reduction was positively correlated with the resuspension rate and not with the primary sedimentation rate. The opposite holds for the littoral sediment.

The results can be summarized as follows:

1. in the littoral acetylene-reducing activity is significantly governed by primary sedimentation of organic carbon, the sediment composition is not;
2. no correlation exists between the measured properties of the sediments and the acetylene-reducing activity at 15 m water depth. At this depth bottom material is resuspended and transported to deeper regions of the lake;
3. the sediment composition and the acetylene reduction of the sediments at the deepest point of the lake is mainly governed by sedimentation of resuspended sediments from shallower regions.

The previous results can be explained by the sediment transport

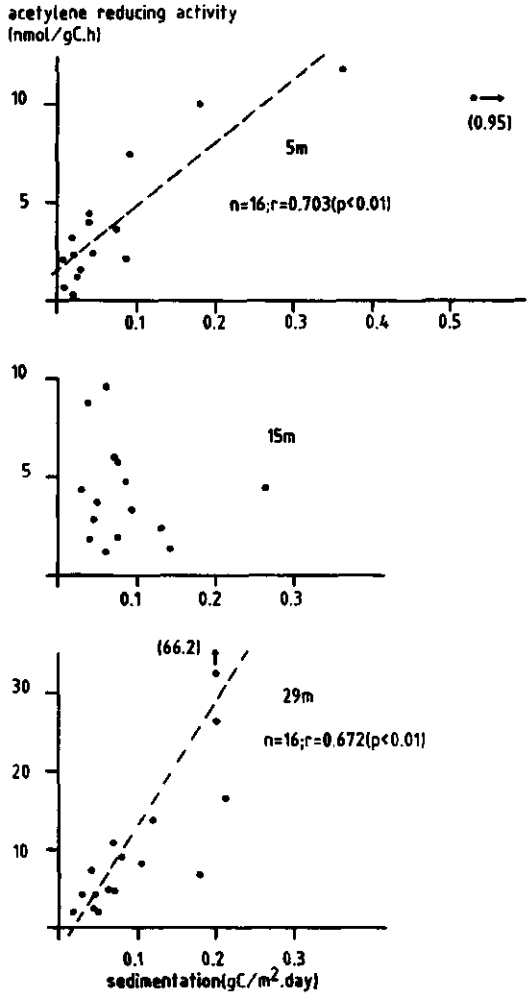

Figure 56. Relation between the sedimentation rate and the acetylene-reducing activity of the surficial sediments, measured at the end of exposure time of sedimentation traps.

process, as described by the focusing model. The situation half-way down the bottom slope, i.e. approximately near the 15 m sampling station, can be described as follows: the particulate material at the sediment-water interface does not represent material deposited at this place, but material deposited in shallower regions and

thereafter transported to deeper parts of the lake. Consequently there will be no direct relation between this material and the material settling directly at this place, because there will be a considerable retardation during the transport along the sediment surface. Sediment composition and acetylene reduction at this depth will be mainly governed by the sediment transport along the sediment surface. At this depth this transport will be nearly maximum (see figure 50). As a consequence no significant correlation between sedimentation and both sediment composition and acetylene reduction can be expected.

In the littoral, transport intensity is very low (see figure 50). Direct sedimentation will be of importance in relation to the transport along the sediments surface. Moreover the retardation at this depth is considerably less than at 15 m water depth. The material settling in the littoral and the material transported along the sediments in this region will be closely correlated. Changes in the measured sedimentation rates will be accompanied by changes in the transport along the lake sediment surface. As a consequence a positive correlation between sedimentation and sediment activity might be expected. Because deposited material is transported to deeper regions, sediment composition will not be changed. The absence of a significant correlation between sedimentation and sediment composition might be explained by this.

At 29 m water depth the transport intensity is as low as in the littoral (see figure 50). The transport along the sediment surface is partly measured by the traps. The measured rates will therefore be correlated with the total input of organic matter at this depth. No transport of sediments from this depth will take place. Changes in total input of organic matter will therefore be reflected in the composition of the sediments and consequently in the heterotrophic activity and the acetylene-reducing activity of the sediments.

Summarizing the correlations between sedimentation and sediment composition on one hand and acetylene-reducing activity on the other can be well explained by the focusing model and its consequence for the bottom dynamics. It demonstrates the usefullness of whole lake models in explaining the spatial variation of composition and heterotrophic activity in lake sediments.

8.10. Efficiency of nitrogen fixation.

In chapter 4 the efficiency of nitrogen fixation (acetylene reduction) is described in sediment samples amended with several monosaccharides. From the measured acetylene reduction rates *in situ* and the estimated carbon flux into the sediments the efficiency of nitrogen fixation under natural conditions can be calculated. This has been done only for the deepest point of the lake. At the other sampling stations the determination of the carbon input is complicated by the sediment transport along the lake bottom.

Nitrogen fixation at 29 m water depth has been estimated to be 0.24-1.10 $gN.m^{-2}.year^{-1}$ (see 6.3.2). The total carbon input (including the sediment transport along the bottom) has been estimated to be about 81 $gC.m^{-2}.\ year^{-1}$ (see 8.8). Hence the efficiency of nitrogen fixation under natural conditions is 3-14 $mgN.gC^{-1}$. Approximately 40% of the total input consists of refractory organic matter that finally forms the permanent sediment. Solely referring to the decomposable organic matter, the efficiency is 5-23 $mgN.gC^{-1}$.

Efficiencies in amended sediments amounted to approximately 200 nmoles $C_2H_2.g(carbohydrate)^{-1}$ (see 4.2). With the various conversion factors used in 6.3.2 this can be calculated to be 0.8-3.7 $mgN.gC^{-1}$. So the efficiency under natural conditions is higher than in amended sediments. This will be still higher if the number of nitrogen-fixing microorganisms is taken into account. No attempts have been made in this study to count these numbers, but as the amended sediments may be considered to be enrichment cultures for nitrogen-fixing microorganisms, numbers in the amended sediments will be higher than in natural sediment. Thus having lower numbers of nitrogen-fixing microorganisms, sediments under natural conditions showed considerably higher efficiency of nitrogen fixations than sediments amended with carbohydrates. The inverse relationship between substrate concentration and efficiency of nitrogen fixation led O'TOOLE and KNOWLES (1973) to the suggestion that efficiency may be very high in the natural environment. The results show that this is indeed the case in the profundal sediments of the Pluss-See.

The estimated carbon flux into the sediments represents the carbon supply for the whole heterotrophic population in the sediments, not

only the nitrogen-fixing microorganisms. Comparing the estimated efficiency under natural conditions with the efficiencies for free-living nitrogen-fixing bacteria and the theoretical efficiency of the nitrogen-fixing process, given by MULDER (1975), leads to the conclusion, that a significant part of the carbon (= energy) supply is used by nitrogen-fixing microorganisms. Nitrogen-fixing microorganisms therefore seem to make up an important part of the heterotrophic population in the sediments. The correlation between the acetylene-reducing activity and the glucose uptake velocity in the sediments, as described in chapter 7, can therefore be partly attributed to the relatively large part of the glucose-consuming population that consists of nitrogen-fixing microorganisms. No evidence can be given for this conclusion, because no counts of nitrogen-fixers in the sediments are available.

NOVITSKY (1983) found by autoradiography that only a small part of the total population in the sediments took up glucose, in contrast to other substrates. The enumeration of bacteria in the sediments of the Pluss-See by the epifluorescence technique showed that total numbers of bacteria amounted to approximately 10^9 cells per gram of wet sediment (BLAUW, unpublished results). If the proportion in the Pluss-See is the same as found by NOVITSKY (1983) this would mean that appoximately 10^7 microorganisms per gram of sediment take up glucose. OLAH *et al.* (1983) showed *Clostridium* counts of up to 10^6 per gram in sediments with low carbon content 2-9% compared to the Pluss-See sediments (30%). Hence some indication is available for the possibility that a considerable proportion of the glucose-uptaking population consists of nitrogen-fixing microorganisms.

9. Transport of ammonium through the sediment-water interface.

To value the estimated nitrogen fixation rates in the sediments, it is useful to compare this nitrogen input with other relevant nitrogen fluxes in the sediment-water system. In this section the transport of ammonium through the sediment-water interface into the overlying water is discussed.

The ammonium transport can be calculated in two ways:

1. from the vertical distribution of the organic nitrogen in the sediments;
2. from the vertical distribution of ammonium in the interstitial water.

The vertical distribution of organic nitrogen in the sediments.

The nitrogen content in the sediments at 29 m water depth (see figure 20) decreases from 3.34% at the sediment-water interface to about 2% at 30 cm below the sediment surface. This means that 60% of the nitrogen compounds have been broken down after they have been deposited at the bottom. At 30 cm below the sediment surface the water content of the sediment is approximately 90%. If all the mineralized nitrogen stayed in the interstitial water, the ammonium concentration at 30 cm below the sediment surface would be about 3000 mg $N.l^{-1}$. The measured ammonium concentration at this depth is ca. 50 mg $N.l^{-1}$. Hence almost all of the mineralized nitrogen is transported back into the overlying water. Assuming steady-state conditions over the years, 60% of the settled nitrogen compounds is mineralized and transported back to the overlying water. From the input of organic nitrogen into the sediments a mean transport of ammonium into the overlying water can be calculated. Taking into account an accumulation: sedimentation ratio of 5.06 (see 8.8), the input of nitrogen at 29 m water depth can be estimated from table VI to be about 10 g $N.m^{-2}.year^{-1}$. Therefore the mean ammonium transport from the sediments into the overlying water can be estimated to be 10 x 0.6 = 6 g $N.m^{-2}.year^{-1}$.

The vertical distribution of ammonium in the interstitial water.

If the transport of ammonium proceeded only by diffusion, it would obey Fick's first law. Allowing for sediment porosity (Φ), this law

states that:

$J = - \Phi . D . \frac{\delta c}{\delta x}$ (BERNER, 1971), where:

J = diffusion flux in mass per unit area of sediment per unit time;

D = diffusion coefficient for the sediment, including effects of tortuosity;

$\frac{\delta c}{\delta x}$ = concentration gradient of the ammonium concentration at depth x below the sediment surface.

The concentration gradient at the sediment-water interface can be estimated from the vertical distribution of ammonium in the inter-

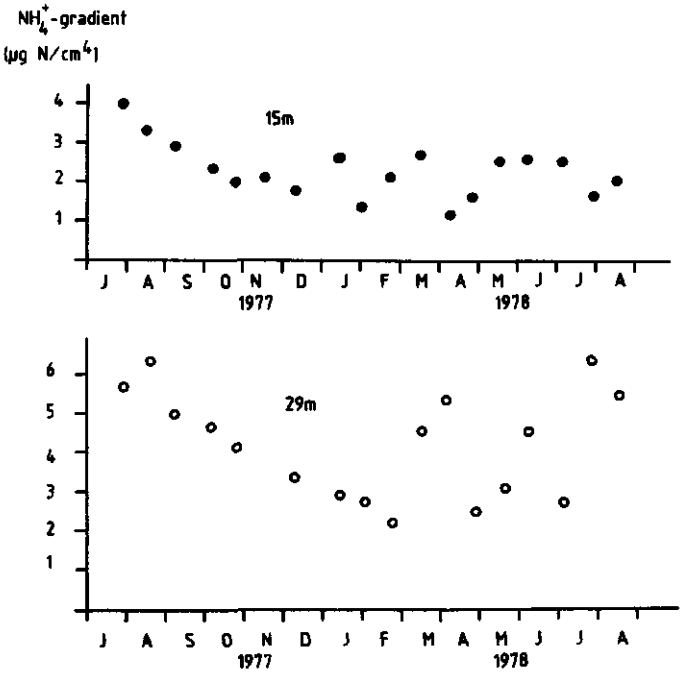

Figure 57. Gradient of ammonium-N in the surficial sediments at 15 and 29 m water depth.

stitial water and the water at the sediment-water interface. As shown in figure 57 these concentration gradients exhibited seasonal fluctuations, with low values in the wintertime and relatively high values in the summertime. Similar trends have been observed by KAMIYAMA *et al.* (1979) in Lake Biwa. Mean gradients at the profundal

stations were 2.27 and 3.92 mg NH_4-N.L^{-1}.cm^{-1} at 15 and 29 m water depth respectively.

DUURSMA (1966) reported D for monovalent ions to be 0.59 x $10^{-5}cm^2.s^{-1}$ at 20°C. At 4°C (the ambient temperature in the profundal of the Pluss-See) this would be about 3.8 x $10^{-6}cm^2.s^{-1}$. BERNER (1974) calculated for D a value of 3.5 x $10^{-6}cm^2.s^{-1}$ for ammonium in Somes Sound sediments with a porosity of 80%. From the diagram presented by MANHEIM (1970) D for sediments with porosity of 98% (the porosity of the Pluss-See sediments in the profundal at the sediment-water interface) can be estimated to be about 2 x $10^{-5}cm^2.s^{-1}$. Although it is not specified in this paper, these values refer to a temperature of 20°C. At 4°C the value of D will be about 1.3 x $10^{-5}cm^2.s^{-1}$. HESSLEIN (1980) measured in soft sediments of Lake 227 a diffusion coefficient for tritiated water of 0.3-0.8 x $10^{-5}cm^2.s^{-1}$, at 4°C. From these data it might be expected that the diffusion coefficient of ammonium in the Pluss-See sediments will be between 0.3 and 1.3 x $10^{-5}m^2.s^{-1}$. Therefore the flux of ammonium from the sediments at 29 m water depth into the overlying water can be estimated to be between 5.5 and 24.0 $gN.m^{-2}.year^{-1}$. At 15 m water depth this flux is between 3.2 and 13.9 $gN.m^{-2}.year^{-1}$.

Comparison with the flux calculated from the vertical distribution of organic nitrogen shows that the lower estimate agrees well with the flux calculated from the vertical distribution of organic nitrogen in the sediments. The latter estimate depends largely on the estimation of the input of organic nitrogen into the sediments which has been shown previously to correspond with the data on sediment growth given by AVERDIECK (1983). Therefore it may be concluded that the ammonium flux from the sediments into the overlying water can be estimated to be 5.5-6 $gN.m^{-2}.year^{-1}$ and that the diffusion coefficient of ammonium in the sediments of the Pluss-See will be about 0.3 x $10^{-5}m^2.s^{-1}$, i.e. at the lower range of the values given in the literature. This diffusion coefficient represents in fact not only diffusional transport, but the total ammoniumtransport from the sediments into the overlying waters. A more appropiate name for this coefficient is "transport coefficient". From the relatively low value of this transport coefficient compared with the reported values of the

diffusion coefficient it can be concluded that molecular diffusion plays a major role in the transport of ammonium. Other transport forms seem to play a minor role which can be explained by the extremely low energy factor of the Pluss-See (see 8.6).

10. Contribution of sedimental nitrogen fixation to the nitrogen economy of the lake.

In the previous chapters the annual nitrogen fixation in the sediments (chapter 6) and the annual deposition of nitrogen into the sediments (chapter 8) have been estimated. If it is assumed that these are the only inputs of nitrogen into the sediment system, the importance of nitrogen fixation to the nitrogen economy of the sediments can be evaluated. In table VIII the data on deposition and nitrogen fixation are summarized. The comparison of nitrogen fixation with nitrogen deposition is complicated by the focusing transport. However, these complications can be overcome by comparing nitrogen fixation and nitrogen deposition on a whole-lake scale, because focusing transport is only important to the spatial variation of nitrogen input into the sediments within the lake. For the comparison on a whole-lake scale it has been assumed that the littoral sediments (from 0 to 6 m water depth) are represented by the sediments at the littoral sampling station, the profundal sediments between 6 and 25 m water depth by the sediments at the sampling station at 15 m water depth and the profundal sediments deeper than 25 m water depth by the

Table VIII. Deposition of organic nitrogen and nitrogen fixation at the three sampling stations in the Pluss-See ($gN.m^{-2}.year^{-1}$).

	Sampling station at water depth		
	5 m	15 m	29 m
sedimentation (measured)	4.6	5.3	4.5 - 7.3
primary sedimentation	3.2	4.0	2.0
total deposition (sedimentation + focusing)	0.0 *)	8.0	10.0
nitrogen fixation	0.2 - 0.8	0.2 - 0.7	0.2 - 1.1

*) The sampling station at 5 m water depth is located in the erosion area, where no permanent accumulation of sediments occurs.

sediments at the sampling station at 29 m water depth. At a whole-lake scale nitrogen fixation in the sediments has to be compared with the primary sedimentation of nitrogen. From table IX it can be seen that 5-17% of the total nitrogen input into the sediments originates from sediment nitrogen fixation. Because these calculations are based on only three sampling stations in the lake the results have to be considered with due reserve. Nevertheless the conclusion can be drawn that nitrogen fixation plays a small but significant role in the nitrogen economy of the sediments.

The organic matter deposited by sedimentation and focusing transport has already been subjected to intensive microbial decomposition. A large proportion of this organic matter is not or slowly decomposed, resulting in the build up of permanent sediments. As shown in chapter 9 40% of the deposited organic nitrogen is finally buried as permanent sediments and 60% is decomposed and transported back into the lake water. In contrast to this the combined nitrogen produced by the nitrogen-fixing process is built in into freshly synthesized cellular constituents of the nitrogen-fixing microorganisms. A relatively large proportion of this organic matter will be finally decomposed in the sediments and the fixed nitrogen will be released as ammonium and transported back into the lake water. Only 8% of the primary production is deposited at 29 m water depth (table V). From this settled matter 38%, i.e. 3% of the primary production, remain as

Table IX. Nitrogen fixation and deposition of organic nitrogen and their contribution to the transport of ammonium from the sediments into the water of the Pluss-See ($kgN.year^{-1}$).

	N-input into sediments ($kg.year^{-1}$)	(%)	Ammonium transport from ($kg.year^{-1}$)	(%)
deposition	522	83-95	313	67-89
nitrogen fixation	27-107	5-17	26-104	8-25
total	549-629	100	339- 417	100

permanent sediment (see 8.8). If it is assumed, that also 3% of the biomass of the nitrogen fixers in the sediments remains as permanent sediments, it can be calculated that 8-25% of the ammonium transported from the sediments into the overlying water originates from nitrogen fixation in the sediments (see table IX).

In order to value the importance for the nitrogen economy of the whole lake, sedimental nitrogen fixation has to be compared with other nitrogen in- and outputs of the system. No quantitative information about these was available. From data on the nitrogen content of the lake in 1974 it can be seen (figure 58) that the total nitrogen mass in the lake water fluctuated between 1000 and 2000 kg. This fluctuation of 1000 kg represents the net result of gains and losses throughout the year. Part of this fluctuation can be explained by the sedimentation of particulate nitrogen and transport of ammonium back into the water. From an ecosystem point of view only the permanent burial of nitrogen in the sediments can be considered as a nitrogen-output, not the organic nitrogen that after settling on the lake bottom is broken down to ammonium and transported back into the water. Exchange between water and sediments can be considered as

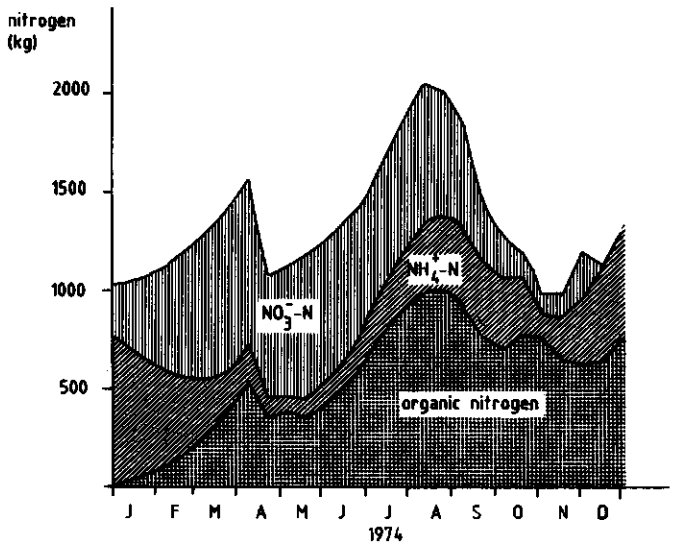

Figure 58. Variation of total mass of nitrate-, ammonium- and organic nitrogen of the Pluss-See in 1974. Drawn from data compiled by KRAMBECK (unpublished).

a process within the ecosystem. This exchange amounts to circa 300 kg/year (ammonium transport in table IX). If the fluctuations of the nitrogen content in the water in 1977-1978 are the same as in 1974 at least 700 kg/year (1000-300) can be attributed to in- and outputs. In fact in- and outputs of nitrogen are higher, because processes which lead to nitrogen loss (denitrification, formation of permanent sediments, outflow) occurre simultaneously with processes which lead to nitrogen input (transport from the drainage area, nitrogen fixation, atmospheric deposition). Therefore it can be concluded that the contribution of sedimental nitrogen fixation to the nitrogen economy (27-107 kgN/year, table IX) is small. From table IX it can be calculated that sedimental nitrogen for 13-50% compensated the loss of nitrogen to the permanent sediments (about 200 kg/year). Assuming that organic matter is broken down to the same degree as in the sediments of the Pluss-See, it can be expected that in lakes where a larger proportion of the primary production reaches the lake bottom sedimental nitrogen fixation completely makes up for the nitrogen loss to the permanent sediments.

There are only a few studies that evaluate the role of nitrogen fixation in the sediments for the nitrogen economy of the lake. JÄGER and WERNER (1977) calculated that sedimental nitrogen fixation contributed for 0.44% to the total nitrogen input of Lake Harkort (FRG). MACGREGOR *et al.* (1973) concluded that 5-8% of the total nitrogen input of Lake Mendota originated from nitrogen fixation in the top 10 cm of the sediments. In contrast TORREY and LEE (1976) estimated that only 0.3% of the total nitrogen input of the same lake is derived from nitrogen fixation in the sediments. No explanation is given for this large difference. HOWARD *et al.* (1970) concluded that nitrogen fixation in the sediments may play a significant role in the overall nitrogen economy of Lake Erie. However, they did not present data that confirm this supposition. The same holds for KEIRN and BREZONIK (1971) who stated that nitrogen fixation in the sediments may contribute in substantial quantities to the nitrogen supply of the lake basin as a whole and may in this sense be geochemically significant.

In estuarine and marine environments the contribution of sedimental nitrogen fixation to the overall nitrogen economy seems to be small.

BROOKS *et al.* (1971) concluded that the phenomenon is probably not important for the overlying waters in the Waccasassa estuary because of the low rates found and the location of activity in compact sediments. MARSHO *et al.* (1975) found that nitrogen fixation in the sediments contributed in amounts of about 4% to the total nitrogen input of the Rhode River estuary. They concluded that the significance of nitrogen fixation to the total budget of the estuary appears to be minor. HERBERT (1975) supposes that heterotrophic nitrogen fixation in the sediments probably plays a role in the nitrogen budget of the Kingoodie Bay at certain times of the year. To confirm these results more data would be required. HERBERT (1975) concludes that the data obtained sofar confirm the statements by STEWART (1969) that lack of oxidizable substrates may restrict heterotrophic nitrogen fixation in natural waters. Therefore it is not surprising that high rates of nitrogen fixation are found in environments with relatively high inputs of such substrates, e.g. in the rhizosphere of aquatic macrophytes (HANSON, 1977; 1983; PATRIQUIN, 1973; SYLVESTER-BRADLEY, 1976) and near effluent discharges (BOHLOOL, 1978; KNOWLES *et al.*, 1974).

11. General discussion.

In this study the problem of nitrogen fixation in lake sediments has been approached in several ways:

1. In the first place nitrogen fixation in sediments was studied under controlled conditions in the laboratory (chapters 3,4 and 5). From these experiments the conclusion has been drawn that the activity of the existing nitrogenase (i.e. the actual activity) depends on the availability of organic matter as an energy source and that the propagation of nitrogenase throughout the sediments (i.e. the increase of the potential activity) is controlled by the dissolved ammonium concentration. A crucial element in the arguments leading to these conclusions is the existence of concentration gradients at the micro-level, resulting in micro-sites with favourable conditions for nitrogen fixation.
2. In the second place the relation between nitrogen fixation and some properties of the sediments was studied under natural conditions (chapter 6 and 7). These field observations confirmed the conclusions from the laboratory experiments. Furthermore the field observations pointed to the important role of the degradability of the organic matter (indexed by its C/N ratio) and the ammonium adsorption for the nitrogen fixation.
3. Finally nitrogen fixation in the sediments was studied on a whole-lake scale. This part of the study describes the transport of sediment and (re-)suspended matter within the lake and its implications for the sediment composition and the processes within the sediments. This approach allows to indicate the role of sedimental nitrogen fixation for the nitrogen economy of the lake. It further allows the determination of nitrogen fixation efficiency under natural conditions. The conclusion was drawn that nitrogen fixation in the sediments is of importance for the nitrogen economy of the sediment but unimportant for the nitrogen economy of the whole lake. Efficiency of nitrogen fixation is relatively high under natural conditions.

In this chapter three aspects will be discussed:

1. microsites;
2. organic matter as a master controlling factor;
3. relation between sediment transport and sediment characteristics.

11.1. Microsites.

Two different phenomena led to the postulation of the existence of concentration gradients at the micro-level in the sediments:

1. the barrier for the transport of acetylene on its way from the interstitial water to the nitrogen-fixing sites;
2. the observation that nitrogenase synthesis may occur at observed measured ammonium concentrations above the repression-derepression threshold. From this observation the conclusion has been drawn that the activity of the existing nitrogenase is controlled by the availability of organic matter and that the synthesis is limited by the dissolved ammonium concentration.

The existence of microsites within the sediments implicates the maintenance or continuous renewal of microgradients. This can be effected both by low diffusion coefficients resulting in low transport rates and by processes that consume or produce substances at specific sites in the sediments resulting in the counterbalancing of the levelling effect of diffusional transport on the concentration gradients.

Low diffusion coefficients might be effected by the presence of organic structures leading to a compartmentation within the sediments. RAMEY (1972) observed elevated numbers of bacterial cells within discrete fecal pellets in the sediments of Marion Lake. GOWING and SILVER (1983) suggest that the metabolic activities of bacteria inside these pellets may produce microhabitats that could differ considerably from that of the pellet exteriors. Also the quaternary structure of macromolecules might lead to a compartmentization of the sediments. The observed thixotropic behaviour of the sediments sustains this possibility.

Ammonium-consuming processes within the sediments are not very probable, because decomposition processes result in the production of ammonium. Ammonium adsorption has been shown to play an important role as already suggested by BROOKS *et al.*, (1971). Adsorption of am-

monium is mainly governed by organic matter (ROSENFELD, 1979; chapter 6). Recently deposed detritus showed a high capacity to adsorb ammonium (chapter 6) which may lead to the maintenance of concentration gradients. The high adsorption capacity of the surficial sediments might be partly caused by the relatively high pH of the recent sediments (chapter 6). It also might be caused by the production of organic substances that specifically adsorb ammonium. Extracellular products of microorganisms might play a role. The functions of extracellular products as a protective mechanism in general is widely accepted (HARRIS and MITCHELL, 1973). Production of slimes under natural conditions has been proposed as a mechanism to protect nitrogenase against ammonium and oxygen by JÆGER and WERNER (1977). The existence of microenvironments might be the consequence of the bacterial attachment to the particulate fraction of the sediments. FLOODGATE (1972) described three stages in the attachment-process. During the first, reversible stage physical and physico-chemical forces predominate. In the second irreversible phase the predominant factor is the adhesive material holding the bacterial cell in place. In the third or biological phase the cell may grow and divide so that eventually a microenvironment is developed on the surface of the particulate fraction.

11.2. Organic matter as a master controlling factor.

Organic matter appears to be the main factor controlling the nitrogen-fixing process in the sediments. It forms the energy source for this heterotrophic process. At the same time organic matter (and especially the degradable part) (see chapter 6) plays an important role in the adsorption of ammonium, the inhibitor of nitrogenase synthesis that is produced in the decomposition process. In this way the organic matter produces favourable conditions for the nitrogen fixation, a process in which molecular nitrogen functions as an acceptor of the reduction equivalents produced by the decomposition of organic matter. As the decomposition process proceeds, the adsorption capacity of the organic matter is reduced (see chapter 6), resulting in an increasing inhibition of the nitrogenase synthesis by NH_4^+. Simultaneously, however, the availability of organic substrate is reduced, resulting in a decreasing production of

reduction equivalents. The role of organic matter as a main controlling factor of heterotrophic nitrogen fixation in the sediments determines for a great deal the significance of this process for the nitrogen economy of the sediments. A considerable part of the particulate organic matter, produced in the photogenic zone of the lake and finally settling on the lake bottom, consists of nitrogen. The efficiency of this process, expressed as mgN per gram of organic carbon deposed (see 8.10) can therefore also be expressed as mgN per gram of organic nitrogen deposed. The importance of nitrogen fixation for the nitrogen economy of the sediments can be evaluated by comparing its contribution with the total nitrogen input into the sediments, i.e. nitrogen fixation plus nitrogen deposition (see chapter 10). This means that the importance of nitrogen fixation for the nitrogen economy of the sediment system is directly related to the efficiency of this process. Because this efficiency has its limits, the significance of heterotrophic nitrogen fixation for the nitrogen economy of the sediments will be limited too.

Efficiency of nitrogen fixation has been related to the amount of carbon consumed by microorganisms (chapter 4), i.c. efficiency of microorganisms and to the amount of carbon deposed at the sediments (chapter 8), i.c. efficiency of the sediments. Efficiency of nitrogen fixation can also be related to the amount of particulate carbon produced in the photogenic zone. Expressing efficiency in this way, the suitability of a lake for nitrogen fixation in the sediments can be characterized. In fact, an indication is given of the proportion of the energy, fixed by photosynthesis, that is finally used for nitrogen fixation in the sediments. This "lake-efficiency" largely depends on the proportion of the primary production that reaches the lake bottom. This proportion has been shown (see figure 46) to be correlated mainly with depth. Therefore not only productivity, but also depth will influence nitrogen fixation in the sediments. Indeed, OLÁH *et al.* (1983) showed that both parameters were correlated with nitrogen fixation in the sediments. Because of the high mineralization rates in the water column the efficiency of the Pluss-See for nitrogen fixation in the sediments can be described as low. Higher efficiencies can be expected in lakes in which a higher percentage of the primary production reaches the lake bottom. In this

kind of lakes the contribution of sedimental nitrogen fixation to the nitrogen economy will be higher. With the same efficiency of the sediments more nitrogen will be fixed and transported into the water column. Especially in lakes where internal loading is important, i.e. where the nitrogen input is relatively unimportant compared with exchange between sediment and water, sedimental nitrogen fixation can be expected to be a significant factor in the nitrogen economy of a lake.

The results of this study show that the question of why nitrogen fixation is taking place in environments rich in combined nitrogen is not appropriate: the conditions at the nitrogen-fixing sites are apparently favourable for this process. It has been shown that it is not the nitrogen richness, but the richness in degradable organic matter (= energy; = ammonium-adsorption) of the sediments that governs the nitrogen-fixing process. The answer given by OLAH *et al.* (1983) on the above question is trivial. They speculated that nitrogen fixation might be a way to remove reduction equivalents from reduced sediments. However, this answer is just one of the ways to describe the nitrogen fixation process. Molecular nitrogen acts as an acceptor of reduction equivalents. If nitrogen fixation takes place in a reduced environment, it is apparently one of the ways of the system to remove reduction equivalents. The environmental conditions favouring the development of nitrogen-fixing microorganisms concern in the first place the absence of available nitrogen compounds. If this were not the case this group of microorganisms could probably not compete with microorganisms being able to live under the same environmental conditions but, instead of spending considerable amounts of energy for a highly endergonic process, using them for growth. That nitrogen-fixing microorganisms (mainly clostridia) would be able to use molecular nitrogen as an electron acceptor because of shortage of other electron-acceptors has to be excluded.

11.3. Relation between sediment transport and sediment characteristics.

Knowledge of sediment transport within a lake is necessary to explain the spatial variation of sediment composition and processes within the sediment. Moreover it allows conclusions about the role of

sedimental nitrogen fixation in the nitrogen economy of the lake. In many studies sediment focusing has been recognized as an important process for the redistribution of sediments within lakes. A first quantitative approach of this process has been made by LEHMAN (1975) in order to explain the vertical variation of accumulation rate within a single sediment core. The model presented by HÅKANSON (1981) quantifies the area of the lake bottom where accumulation of soft sediments is taking place. It does not give the variation of accumulation rate within the accumulation area. However, this very rate is important to explain the spatial variation of process rates in the sediment system. The focusing model presented in this study is an elaboration of Lehman's model in order to explain the spatial variation within the accumulation area.

All studies concerning sediment focusing were directed to the result of the focusing process, i.e. the accumulation of sediment. However, in studies concerning processes within the sediment, not only the accumulation of sediments is important, but also the dynamics of the focusing process itself. Therefore it has been tried in this study to deduce implications for the spatial variation of transport intensity from the result of the focusing process. The presented model is a rude simplification of the sediment transport finding place in reality. Nevertheless it could explain to a considerable extent the ratio between sedimentation and accumulation rate at the deepest part of the lake. Moreover it could explain the relation between the measured sedimentation rates and the measured sediment characteristics (composition and activity). This might be partly due to the extreme morphology of the Pluss-See. It can be assumed that a more sophisticated model will be necessary for lakes with a more complex morphology. Moreover in lakes with a significant energy factor, morphology will not be the only factor controlling the distribution of organic matter (HÅKANSON, 1981).

Because the model is deduced from the result of the focusing process, it contains the implicit assumption of a process with a constant rate. Therefore it cannot explain the temporal variation of sediment composition and sediment activities. It does, however, explain why at certain places a correlation is found between sedimentation and sediment characteristics (5 and 29 m water depth) and not at other

places (15 m water depth). To explain the temporal variation of sediment characteristics from the temporal variation of sediment transport, more information about the dynamics of this transport is necessary.

12. Summary.

Sediments of productive lakes are usually rich in organic matter and, except for a thin surficial layer, anaerobic. These conditions seem to be favourable for heterotrophic nitrogen fixation. However, these sediments also contain relatively high ammonium concentrations. Ammonium represses the synthesis of the enzyme nitrogenase. Moreover, ammonium inhibits the activity of the enzyme in aerobic nitrogen fixers. These effects of ammonium seem to be functional. Nitrogen fixation is a highly endergonic process. Therefore it is more economic to use combined nitrogen (e.g. ammonium) than atmospheric nitrogen as a nitrogen source. Nevertheless, a number of workers have detected nitrogen fixing activity in ammonium rich sediments.

In the present investigation the significance of heterotrophic nitrogen fixation in the sediments for the nitrogen economy of the Pluss-See has been studied. Special attention has been paid to the role of organic matter supply and ammonium.

The surface area of the lake is 14 ha, the maximum depth is 29 m. Every year a stable thermal stratification develops in the lake, usually with an anaerobic hypolimnion.

The problem of nitrogen fixation in lake sediments has been approached in three ways:

1. Nitrogen fixation in the sediments was studied under controlled conditions in the laboratory;
2. The relation between nitrogen fixation and some properties of the sediments was studied under natural conditions;
3. The relation between nitrogen fixation in the sediments and processes within the lake was studied under natural conditions.

Laboratory studies (chapters 3, 4 and 5).

Nitrogen fixating activity was measured with the acetylene reduction assay. One of the requirements for this assay, the saturation of nitrogenase with acetylene, was not met (figure 5), because nitrogen fixation apparently occured in protected microsites with poor accessibility for acetylene (3.2.2). This protection may have also consequences for the measurement of nitrogen fixation with $^{15}N_2$

(3.2.3).

Nitrogenase activity of the sediments was stimulated by the addition of organic substrates, as mannitol, glucose, fructose etc., suggesting that the activity of nitrogenase *in situ* in these sediments was limited by the availability of organic substrate (4.2). This suggestion is affirmed by the absence of a discontinuity in the Arrhenius plot (figure 17b).

Upon addition of organic substrate to the sediments two phases could be observed (figure 9). In the first phase acetylene reducing activity is constant, but higher than in control sediments. In this phase the activity of the already present nitrogenase is stimulated (increase of the actual activity). In the second phase, after the interstitial ammonium concentration has dropped below a certain threshold-value, the synthesis of nitrogenase is derepressed and an exponential increase of nitrogenase activity can be observed (increase of the potential activity). Because nitrogenase synthesis *in situ* had to be assumed above the derepression threshold, the conclusion was drawn that the dissolved ammonium concentration within the protected microsites was lower than in the bulk of the sediments (4.3). Apparently nitrogen fixation occures in ammonium rich sediments, because nitrogen fixers are not in contact with these high concentrations.

Field obeervations; relation between the nitrogenase activity and some other properties of the sediments (chapters 6 and 7).

During a year nitrogenase activity and some other characteristics of the sediments were measured at three stations in the lake: in the littoral sediments at 5 m water depth and in the profundal sediments at 15 and 29 m water depth. Highest nitrogenase activity was measured at the sediment surface at the deepest part of the lake (figure 27). Especially in the winter period very high rates were observed (figure 28). In the sediments at the deepest part of the lake the yearly fixed amount of nitrogen was estimated to be 0.24-1.10 $g.m^{-2}$, depending on the conversion factor used. In the shallower regions this amount was estimated to be 0.15-0.77 $g\ N.m^{-2}$.

Acetylene reduction in the littoral sediments was correlated with temperature (table II). In the profundal sediments no significant temperature variation could be observed. Acetylene reducing activity in the profundal sediments was correlated with the C/N ratio (figure 33), which could be shown to be an index for substrate availability in these sediments. In both littoral and profundal sediments acetylene reducing activity was highly significantly correlated with the maximum glucose uptake velocity (V_m) of the heterotrophic population in the sediments (figure 41). The repression-derepression threshold of the interstitial ammonium concentration could be observed under natural conditions (figure 33). Acetylene reducing rates were higher at ammonium concentrations below this threshold. The ammonium adsorption coefficient (Ke) of the sediments seemed to be more important for the acetylene reducing activity than the ammonium concentration it self (table II). This finding suggests that the dissolved ammonium concentration in the protected microsites is lowered by adsorption.

Field obeervations; the relation between nitrogen fixation in the sediments and the sedimentation of suspended matter (chapters 8, 9 and 10).

Sedimentation of particulate organic matter was measured at the three stations. The measured rates were corrected for resuspension using the differences in carbon content between the settling particulate material and the carbon content of the surficial sediments (8.4; figure 47). Eight percent of the primary production reached the bottom at the deepest part of the lake (table VI). Redistribution of sediments resulting in sediment focusing is important in the lake. Both intermittent complete mixing and sliding of sediments on slopes are important for the focusing process. A correlation between sedimentation and acetylene reducing activity could be observed in the littoral sediments and at the deepest part of the lake (figure 63). No correlation was found at 15 m water depth. Only at the deepest part of the lake a correlation was found between the sedimentation and both the C/N ratio and Ke of the sediments (figure 61 and 62). These correlations and non-correlations could be explained by the transport of sediments within the lake, described by a

simple focusing model (8.7). Using this model the efficiency of nitrogen fixation under natural conditions could be estimated to be high compared to the efficiency measured in pure and enrichment cultures (8.10).

Also using this model it could be shown (10) that nitrogen fixation may be important to the nitrogen economy of the sediments but not for the nitrogen economy of the whole lake. Nitrogen fixation is expected to be more important in lakes with a larger proportion of the primary production reaching the bottom.

13. Kurzfassung.

Die Sedimente euproduktiver Seen haben im Durchschnitt einen hohen Gehalt an organischer Substanz und sind normalerweise anaerob, abgesehen von einer dünnen Schicht an der Sedimentoberfläche. Diese Bedingungen sind günstig für heterotrophe Stickstoffixierung. Jedoch, diese Sedimente enthalten dazu häufig relativ hohe Ammoniumkonzentrationen. Das Ammonium unterdrückt die Synthese des Enzyms Nitrogenase. Darüberhinaus hemmt Ammonium die Enzymaktivität von aeroben Stickstoffixierern. Diese Effekte des Ammoniums können als zweckmässig betrachtet werden. Die Reduktion des molekularen Stickstoffs ist ein energieaufwendiger Prozess. Es ist wirtschaftlicher die schon vorhandene Stickstoffverbindungen (wie Ammonium) für den Stickstoffbedarf zu verwenden. Trotzdem wurde von verschiedenen Untersuchern Stickstoffixierung in Sedimenten mit hohen Ammoniumkonzentrationen festgestellt.

In dieser Studie wurden die Bedeutung der heterotrophen Stickstoffixierung im Sediment für den Stickstoffhaushalt des Plussees (BRD) sowie die Faktoren, die diesen Prozess *in situ* kontrolieren, untersucht. Besonders die Rolle der organischen Substanz und des Ammoniums wurde berücksichtigt.

Die Wasserfläche des Plussees beträgt 14 ha, die Maximaltiefe 29 m. Jährlich entwickelt sich eine stabile Sprungschicht, meistens mit einem anaeroben Hypolimnion.

Das Problem der Stickstoffixierung im Sediment wurde auf verschiedenen Weisen untersucht:

1. Im Labor wurde die Stickstoffixierung unter kontrolierten und manipulierten Bedingungen untersucht;
2. Im Freiland wurden die Beziehungen zwischen der Stickstoffixierung und der Beschaffenheit des Sediments untersucht;
3. Im Freiland wurden die Beziehungen zwischen der Stickstoffixierung im Sediment und die Sedimentation des suspendierten organischen Materials im Wasser untersucht.

Laborexperimente (Abschnitte 3, 4 und 5).

Die Stickstoffixierung wurde mit Hilfe des Azetylenreduktionsverfahren gemessen. Eine der Voraussetzungen dieser Methode, die

Sättigung des Enzyms Nitrogenase, wurde nicht erfüllt (Figur 5), da die Stickstoffixierung an abgeschirmten Stellen erfolgt, die schwer zugänglich sind für Azetylen (3.2.2). Die Abschirmung wird wahrscheinlich auch für die Messung der Stickstoffixierung mit $^{15}N_2$ Konsequenzen haben (3.2.3).

Die Nitrogenase-Aktivität im Sediment wurde durch Zugabe organischer Substanzen, wie Mannit, Glukose, Fruktose usw. erhöht (4.2). Die *in situ* Aktivität des Enzyms ist offenbar substratlimitiert. Auch die Abwesenheit einer Diskontinuität in der Arrheni'schen Darstellung der Temperaturabhängigkeit weist auf eine Substratlimitation hin (Figur 17b). Nach Zugabe organischer Substanz zu Sedimentproben konnten zwei Phasen nachgewiesen werden (Figur 9). In der ersten Phase konnte eine konstante Äthylenproduktion festgestellt werden, die höher war als in der Blindprobe (Zunahme der aktuellen Aktivität). In der zweiten Phase, nachdem die Konzentration des interstitialen Ammoniums unterhalb einer bestimmten Schwelle gesunken war, konnte eine exponentielle Zunahme der Nitrogenase-Aktivität festgestellt werden. Diese Zunahme wurde der Aufhebung der repressiven Wirkung des Ammoniums auf die Nitrogenase-Synthese zugeschrieben (Zunahme der potentiellen Aktivität). Trotzdem wird Nitrogenase *in situ* offenbar auch bei höheren Konzentrationen synthetisiert. Deshalb wurde die Schlussfolgerung gezogen, dass die Ammoniumkonzentration an den abgeschirmten Stellen niedriger ist als im restlichen Sediment (4.3). Stickstoff wird offenbar in Sedimenten mit hohen Ammoniumkonzentrationen fixiert, weil die Stickstoffixierer nicht in direktem Kontakt met diesen hohen Konzentrationen stehen.

Freilanduntersuchungen; die Beziehungen zwischen Stickstoffixierung und der Beschaffenheit des Sediments (Abschnitte 6 und 7).

Für die Dauer eines Jahres wurden die Nitrogenase-Aktivität und einige andere Sedimentparameter an drei Stationen im See gemessen: im Littoralsediment bei 5 m Wassertiefe und im Profundalsediment bei 15 und 29 m Wassertiefe. Die höchste Nitrogenase-Aktivität wurde an der Sedimentoberfläche bei 29 m Wassertiefe gemessen (Figur 27). Besonders im Winter konnten sehr hohe Aktivitäten festgestellt werden (Figur 28). Die jährlich fixierte Menge Stickstoff wurde an der tiefsten Stelle des Sees, abhängig von dem Umrechnungsfaktor, auf

0.24-1.10 $g.m^{-2}$ geschätzt. Im flacheren Bereich auf 0.15-0.77 $g.m^{-2}$. Die Azetylenreduktion im Littoralsediment war mit der Temperatur korreliert (Tabelle II). Im Profundalsediment konnten keine signifikanten Temperaturschwankungen festgestellt werden. Die Azetylenreduktion war hier mit dem Kohlenstoff/Stickstoff Verhältnis korreliert (Figur 33). Es konnte gezeigt werden, dass dieses Verhältnis im Profundalsediment einen Mass für die Verfügbarkeit der organischen Substanz darstellt. Sowohl im Littoral- als im Profundalsediment war die Azetylenreduktion sehr signifikant mit der maximalen Glukoseaufnahmegeschwindigkeit (V_m) der heterotrophen Population im Sediment korreliert (Figur 41).

Die Derepressionsschwelle der Ammoniumkonzentration konnte auch im Freiland festgestellt werden (Figur 33). Bei Konzentrationen unterhalb dieser Schwelle war die Nitrogenase-Aktivität höher. Der Ammonmiumadsorptionskoeffizient (K_e) des Sediments ist offenbar wichtiger für die Nitrogenase-Aktivität als die Ammoniumkonzentration an sich (Tabelle II).

Die Ammonium/adsorption könnte die niedrigen Ammoniumkonzentrationen an den abgeschirmten Stellen im Sediment erklären.

Freilanduntersuchungen; die Beziehungen zwischen Stickstoffixierung im Sediment und die Sedimentation des suspendierten organischen Materials im Wasser (Abschnitte 8, 9 und 10).

Gleichzeitig mit der Azetylenreduktion im Sediment wurde die Sedimentation des suspendierten Materials im Wasser an den drei Stationen gemessen. Die Daten wurden für Resuspension korrigiert. Dafür wurde der Unterscheid zwischen dem Kohlenstoffgehalt des Sinkstoffes und dem Kohlenstoffgehalt an der Oberfläche des Sediments benutzt (8.4; Figur 47). Acht Prozent des Primärprodukts wurde an der tiefsten Stelle des Sees abgesetzt (Tabelle VI).

Ein wichtiger Prozess im Plussee ist die Fokussierung des Sediments. Der Sedimenttransport erfolgt hauptsächlich kurz über die Sedimentoberfläche, grossenteils während der Vollzirkulation.

Im Littoral bereich und an der tiefsten Stelle des Sees war die Sedimentation signifikant mit der Azetylenreduktion korreliert (Figur 63). Keine Korrelation konnte bei 15 m Wassertiefe nachgewiesen

werden. Nur an der tiefsten Stelle war die Sedimentation mit dem Kohlenstoff/Stickstoff Verhältnis und K_e des Sediments korreliert (Figur 61 und 62). Ein einfaches Modell für die Fokussierung des Sediments konnte die Beziehungen zwischen der gemessenen Sedimentationsraten einerseits und der Azetylenreduktion und der Zusammensetzung des Sediments andererseits erklären (8.7). Mit diesem Model konnte die Effizienz der Stickstoffixierung unter natürlichen Bedingungen eingeschätzt werden. Die Effizienz zeigte sich viel höher als in Rein- und Anreicherungskulturen (8.10). Mit Hilfe dieses Modells konnte auch gezeigt werden (10), dass Stickstoffixierung für den Stickstoffhaushalt des Sees zwar ohne Bedeutung ist, aber dass dieser Prozess für den Stickstoffhaushalt des Sediments durchaus wichtig sein kann: etwa 8-25% des Ammoniums, das vom Sediment in die Wassersäule transportiert wurde, stammte von Stickstoffixierung im Sediment. Dieser Prozess wird für den Stickstoffhaushalt eines Sees von mehr Bedeutung sein, wenn ein grosser Teil des Primärproduktes den Seeboden erreicht.

14. Samenvatting.

Biologische stikstofbinding is het proces, waarbij moleculaire stikstof onder invloed van het enzym nitrogenase wordt gereduceerd tot ammonium-stikstof. Dit proces wordt heterotroof genoemd, als de geassimileerde koolstof afkomstig is van organische verbindingen en ook de benodigde energie als regel geleverd wordt door de afbraak van organische stof. Het vermogen tot stikstofbinding is een eigenschap van een beperkte groep microorganismen. Het proces vindt plaats bij een zeer lage redoxpotentiaal en kost relatief veel energie.

In het sediment van meren zijn in het algemeen anaërobe (zuurstofloze) omstandigheden aanwezig en is de redoxpotentiaal dientengevolge laag. Tevens is de bodem van een meer een verzamelplaats van organisch materiaal afkomstig uit het bovenstaande water. Beide omstandigheden zijn gunstig voor heterotrofe stikstofbinding door anaërobe bacteriën. In het sediment kunnen echter ook hoge ammoniumconcentraties voorkomen. Ammonium onderdrukt de synthese van het enzym nitrogenase; bij aërobe stikstofbinders remt ammonium ook nog de werking van het enzym. Vanuit een oogpunt van optimale energiehuishouding is dit effect van ammonium begrijpelijk. Waarom zouden microörganismen op een zo kostbare wijze in hun stikstofbehoefte voorzien, als ammonium-stikstof in ruime mate aanwezig is. In een veelal zo ammoniumrijk milieu als het sediment lijkt stikstofbinding een niet voor de hand liggend proces. Niettemin is dit proces veelvuldig in dergelijke milieus aangetoond.

Het doel van dit onderzoek was de betekenis van heterotrofe stikstofbinding in sedimenten voor de stikstofhuishouding van een meer duidelijk te maken en aan te geven welke factoren dit proces onder natuurlijke omstandigheden controleren. Daarbij is vooral aandacht besteed aan de rol van organische stof en ammonium.

Het onderzoek is uitgevoerd in de Plussee, een meertje ten noorden van Plön in Holstein (BRD). Dit meer, waarin veel onderzoek verricht wordt door het Max Planck Instituut voor Limnologie te Plön, heeft een zeer extreme morfologie. Het is in verhouding tot zijn oppervlakte (14 ha; gemiddelde doorsnede 422 m) zeer diep (de maximale diepte is bijna 30 m). Het heeft bijna de vorm van een ideale, omgekeerde kegel. Door zijn vorm en zijn beschutte ligging kan zich

elk jaar een zeer stabiele temperatuursgelaagdheid in het water ontwikkelen. De matig voedselrijke (mesotrofe) toestand van het meer en de daarmee samenhangende afbraakprocessen zorgen ervoor, dat het diepe en koude deel van het water (het hypolimnion) voor een groot deel zuurstofloos is.

De problematiek is op drie manieren benaderd:

1. in laboratorium-experimenten met sediment-monsters uit het meer is onder gecontroleerde omstandigheden het effect van kunstmatig aangebrachte veranderingen op het stikstofbindend vermogen van het sediment onderzocht;
2. door middel van waarnemingen in het veld is getracht relaties te leggen tussen het stikstofbindend vermogen van het sediment en andere eigenschappen van dat sediment;
3. door middel van waarnemingen in het veld is getracht relaties te leggen tussen enkele eigenschappen van het sediment (waaronder het stikstofbindend vermogen) en de sedimentatie van gesuspendeerde organische stof in het meer.

Laboratoriumexperimenten (hoofdstukken 3, 4 en 5).

De stikstofbindende activiteit van het sediment werd gemeten met behulp van de acetyleen-reductie-methode. Echter, aan een van de voorwaarden voor deze methode, namelijk de verzadiging van het enzym nitrogenase met acetyleen, werd niet voldaan (figuur 5). Het bleek, dat de oorzaak hiervan gelegen was in het feit, dat de nitrogenase zich op plaatsen in het sediment bevindt, die niet in direct contact staan met het poriewater (3.2.2.). Hierdoor is de acetyleen-concentratie ter plekke van de nitrogenase lager dan theoretisch te verwachten zou zijn op grond van de oplosbaarheid van acetyleen in water. Het is te verwachten dat een dergelijke afscherming ook problemen oplevert voor een andere methode om stikstofbinding te meten, namelijk de $^{15}N_2$-methode (3.2.3.).

De acetyleen-reductie in het sediment wordt gestimuleerd door de toediening van organische stoffen als mannitol, glucose en fructose (4.2.). Dit wijst erop, dat de nitrogenase-activiteit onder natuurlijke omstandigheden gelimiteerd wordt door de voorziening van de stikstofbindende microorganismen met organische stof. Dit wordt ondersteund door de aard van de temperatuursafhankelijkheid van de

nitrogenase-activiteit (5).
In de reductie van acetyleen na toediening van organische stoffen zijn twee fasen te herkennen (figuur 9). In de eerste fase is een versnelde, maar constante reductie te zien. In de tweede fase neemt deze reductie exponentieel toe. Deze toename begint nadat de ammoniumconcentratie beneden een bepaalde drempelwaarde is gedaald. In deze fase vindt er productie plaats van nitrogenase (verhoging van de potentiële activiteit). In de eerste fase is het effect te zien van een verhoogde substraatvoorziening op de al aanwezige nitrogenase (verhoging van de actuele activiteit). Blijkbaar kan nitrogenase pas geproduceerd worden beneden een bepaalde ammoniumconcentratie. Toch is onder natuurlijke omstandigheden boven deze concentratie nitrogenase aanwezig en dus geproduceerd. Een mogelijke verklaring hiervoor is, dat nitrogenase geproduceerd wordt in de hierboven al genoemde, afgeschermde plaatsen in het sediment, omdat daar de ammoniumconcentratie lager is dan in de rest van het sediment (4.3.). De vraag, waarom stikstofbinding voorkomt in sediment met relatief hoge ammoniumconcentraties, zou dan beantwoord kunnen worden: de (opgeloste) ammoniumconcentratie ter plaatse van de stikstofbindende microorganismen is blijkbaar lager dan in de rest van het sediment.

Veldwaarnemingen; relatie tussen de stikstofbindende activiteit en andere eigenschappen van het sediment (hoofdstukken 6 en 7).
Gedurende een jaar werd de stikstofbindende activiteit op drie verschillende plaatsen in het meer vervolgd, tesamen met een aantal andere eigenschappen van het sediment. De hoogste nitrogenase-activiteit werd waargenomen in het diepste deel van het meer juist aan de sedimentoppervlakte (figuur 27). Vooral in het winterseizoen werden hier zeer hoge waarden gemeten (figuur 28). Geschat wordt, dat in het sediment in het diepste deel van het meer 0,24-1,10 g stikstof per vierkante meter per jaar wordt gebonden, in ondieper gelegen sediment 0,15-0,77 g per vierkante meter per jaar.
In de bodem van de oeverzône (de litorale zône) kon een duidelijke correlatie met de temperatuur waargenomen worden (tabel II). In het onder dieper water gelegen sediment (de profundale zône) heerst een nagenoeg constante temperatuur van ongeveer 4°C.
De nitrogenase-activiteit was duidelijk gecorreleerd met parameters,

die iets zeggen over de beschikbaarheid van organisch materiaal, zoals de verhouding tussen het koolstof- en het stikstofgehalte (de C/N verhouding) van het sediment en de maximale glucose-opname-snelheid (V_m) van de heterotrofe populatie in het sediment. Tevens kon een invloed van ammonium waargenomen worden. Ook onder natuurlijke omstandigheden kon een drempelwaarde aangetoond worden (figuur 33). Bij ammoniumconcentraties lager dan deze waarde werd een hogere nitrogenase-activiteit vastgesteld. Niettemin bleek, vooral in het sediment op de diepste plaats, de (positieve) correlatie met de capaciteit van het sediment om ammonium te adsorberen sterker (tabel II). Tussen de adsorptie-coëfficiënt van V_m, een wat bredere parameter voor heterotrofe activiteit, bestond nauwelijks een correlatie (tabel V). Blijkbaar gaat het hier om een effect van ammonium-adsorptie specifiek op het functioneren danwel de synthese van nitrogenase. De eerder genoemde lage ammoniumconcentraties op de afgeschermde plaatsen in het sediment zouden verklaard kunnen worden door de sterke adsorptie van ammonium ter plaatse.

Veldwaarnemingen; relatie tussen enkele eigenschappen van het sediment (waaronder de nitrogenase activiteit) en de sedimentatie van gesuspendeerde organische stof in het meer (hoofdstukken 8, 9 en 10). Gelijktijdig met de meting van de nitrogenase-activiteit is de sedimentatie van gesuspendeerd materiaal gemeten, met name van gesuspendeerde organische stof afkomstig van de primaire productie in de bovenste lagen van het meer. Enerzijds wordt door de sedimentatie organisch materiaal (= energie) aangevoerd voor de heterotrofe micro-organismen in het sediment (waaronder de stikstofbinders). Anderzijds kan door de meting van de aanvoer van stikstof ten gevolge van sedimentatie een schatting gemaakt worden van de belangrijkheid van de stikstofbinding voor de stikstofhuishouding van het sediment en het totale meer.

De meting van sedimentatie werd bemoeilijkt, omdat in het meer resuspensie plaats vond van reeds gesedimenteerd materiaal. Gecorrigeerde meetgegevens toonden aan, dat ongeveer 8% van het organisch materiaal, dat in de bovenste lagen van het water geproduceerd wordt, uiteindelijk het sediment op het diepste punt bereikt (tabel VI). In de litorale zône en op het diepste punt van het meer was de se-

dimentatie positief gecorreleerd met de nitrogenase-activiteit (figuur 63). Op het andere meetstation bij 15 m waterdiepte werd geen correlatie vastgesteld. Een correlatie met de C/N verhouding en V_m werd slechts op het diepste punt waargenomen. Als gesuspendeerd materiaal ergens in het meer op de bodem terecht komt, blijft het daar meestal niet liggen. Zowel vanwege de relatief steile hellingen als tengevolge van turbulenties in het water, vooral tijdens perioden zonder temperatuursgelaagdheid, vindt er een transport van sediment plaats naar diepere delen van het meer. Dientengevolge komt uiteindelijk op het diepste punt meer sediment terecht dan door rechtstreekse sedimentatie, terwijl in de litorale zône juist minder sediment terecht komt. In de Plussee vindt dit sedimenttransport vooral plaats vlak boven de sedimentoppervlakte. Een eenvoudig model van dit transport kon de relaties tussen de sedimentatie enerzijds en anderzijds enkele eigenschappen van het sediment, waaronder de nitrogenase-activiteit, verklaren (8.7.). Met het model kon ook het totale transport van organisch materiaal naar het sediment berekend worden. Dit maakte het mogelijk een schatting te maken van de efficiency van de stikstofbinding onder natuurlijke omstandigheden. Zoals reeds in de literatuur voorspeld was, bleek deze aanmerkelijk hoger te zijn dan in rein- en ophopingsculturen (8.10). Tevens kon een stikstofbalans van het sediment opgesteld worden. Hieruit bleek, dat de rol van stikstofbinding voor de stikstofhuishouding van het sediment niet is te verwaarlozen: ongeveer 8-25% van het ammonium, dat naar het bovenstaande water getransporteerd wordt, is afkomstig van stikstofbinding (10). Niettemin is de rol voor het hele meer onbeduidend, juist omdat het sediment van minder belang is voor de stikstofhuishouding van het meer. Het is daarom te verwachten, dat in meren, waarin het sediment in dit opzicht wel een rol van betekenis speelt, de stikstofbinding in het sediment ook belangrijker zal zijn. Met name geldt dit voor meren, waarin een groter deel van de primaire productie het sediment bereikt.

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Tjeerd Sytze Blauw werd op 27 augustus 1949 geboren te Terwispel (gemeente Opsterland). Van 1961 tot 1968 bezocht hij het Openbaar Lyceum "Schoonoord" te Zeist, waar hij het gymnasium β behaalde. In 1968 liet hij zich inschrijven als student aan de Landbouwhogeschool te Wageningen. In 1976 studeerd hij af in de richting Milieuhygiëne (N-42). De doctoraalvakken waren: waterzuivering, toxicologie, microbiologie, natuurbeheer en -behoud.
Van 1976 tot 1980 was hij werkzaam bij het Max Planck Institut für Limnologie te Plön (BRD), met als onderzoeksgebied de stikstofbinding in sedimenten van meren. Vanaf september 1978 was hij tevens belast met de uitvoering van een project van de Deutsche Forschungsgemeinschaft over extracellulaire algenprodukten.
In september 1980 trad hij in dienst van de provincie Zeeland. Hier is hij sindsdien werkzaam als wetenschappelijk medewerker waterkwaliteitsbeheer bij de Provinciale Waterstaat.