Hydrobiologia **275/276**: 349–358, 1994.
E. Mortensen et al. (eds), Nutrient Dynamics and Biological Structure in Shallow Freshwater and Brackish Lakes.

Kinetics of luxury uptake of phosphate by algae-dominated benthic communities

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Key words: benthic algae, phosphate, luxury uptake, eutrophication, intracellular P

Abstract

The uptake of phosphate by benthic communities, dominated by living algae, previously exposed to different levels of external nutrient loading, exhibited first-order kinetics with respect to the intracellular P-deficit. This deficit is the difference between the maximum and the actual intracellular P-concentration.

The maximum storage capacity of P per unit of dry weight was positively correlated to the level of external nutrient loading, whereas the phosphate uptake rate constant was negatively correlated.

The observed internal P concentrations in the benthic layer of test ditches over a period of two and a half years, indicated a slight decrease towards a minimum value in a ditch with a low external P-input. In a medium loaded ditch the internal P-concentration did not change significantly. In a high loaded ditch increasing internal P-concentrations over time were observed, towards P-saturation of the benthic community.

Introduction

The control of growth of photo-autotrophic organisms by rate-limiting environmental factors has since long been a central issue in water research. Numerous publications have appeared on photosynthesis-light relationships (see e.g. Kirk (1983) for a review) and on the effects of nutrient limitation (e.g. Droop, 1974; Kunikane & Kaneko, 1984).

In natural waters often periodic changes in the phosphorus concentration occur. The efficiency at which a species is capable of uptake and storage of phosphorus during periods of enhanced availibility, for use during periods of limitation, determines its competitive ability.

After addition of dissolved phosphorus to a P-limited community of algae a considerable uptake can follow (Bierman, 1976; Riegman, 1985) far in excess of the immediate needs for growth and maintenance. An internal feedback mechanism, that restricts the uptake rate progressively when the intracellular P concentration increases is responsible for an asymptotic approach towards internal P-saturation of the cells.

In contrast to the fairly large number of experiments performed with pure cultures of one single species (e.g. Okada *et al.*, 1982; Kunikane *et al.*, 1984; Riegman, 1985), information on phosphate uptake kinetics in natural communities is rather scarce. Extrapolation of parameter values obtained in the laboratory on single species to natural systems with a variety of species under fluctuating environmental conditions is often questionable.

Different species may perform different uptake kinetics and therefore contribute to a different extent to the phosphate uptake kinetics of the whole community. Furthermore, the composition of the natural community itself may change following the level of phosphate supply.

It has since long been recognised that growth rates are controlled by internal rather than extracellular nutrient concentrations (Droop, 1974; Nyholm, 1978). Depending on the biomass present in a system and its nutritional status, luxury uptake, which is nutrient uptake in excess of the amount immediately needed for growth, may cause the extracellular dissolved phosphorus concentration to return fast to a low level after a step-wise phosphorus addition. The effects of the nutrient dosage remain visible in an increased primary productivity over a much longer period (Portielje & Kersting, 1992), caused by the availibility of the internally stored phosphorus for growth.

This paper presents the results of laboratory experiments on the kinetics of phosphorus-uptake by algae dominated natural benthic communities. Parameters derived from these experiments, combined with field data on the accumulation of biomass and internal phosphorus in artificial ditches subject to different rates of external nutrient loading, are subsequently used to estimate the phosphate uptake capacity of the benthic communities of these ditches after step-wise nutrient dosages. The aim of the study is to gain insight into the role of the benthic community in the nutrient household of the whole system. The phosphate uptake capacity of the benthic community and the kinetics of phosphate uptake in relation to the level of external phosphorus input are studied. The immediate phosphorus uptake by the benthic community after a dosage acts as a buffer of the system to step-wise phosphorus loadings. Due to this buffering capacity the dissolved phosphate concentration decreases rapidly to low values. This may to a substantial extent determine the resistance of the ecosystem towards transition to a higher trophic state. After saturation of this buffer capacity the increasing phosphorus concentrations in the water phase will enable phytoplankton to become the dominant primary producers.

Theory

After addition of phosphorus to a P-limited culture of algae, the algae can readily take up significant amounts of the added phosphorus. The uptake rate V(g P [g dry matter hour]$^{-1}$) is usually described using Michaelis-Menten kinetics:

$$V = V_{\max} [\mathrm{P}] / (K_p + [\mathrm{P}]) \qquad (1)$$

in which $V_{\max}$ is the maximum uptake rate, [P] the external dissolved phosphorus concentration and K_{p} the concentration at which the uptake rate is at half-maximum. $V_{\max}$ depends on the P-deficiency of the cells, expressed as the difference between the maximum intracellular P-content $\mathrm{P}_{\mathrm{int,max}}$ and the actual intracellular P-content $\mathrm{P}_{\mathrm{int}}$, and a first-order rate constant k, also defined as an adaptation rate constant (Riegman, 1985). This thus defines the rate at which a P-deficiency can be eliminated as:

$$V_{\max} = k\,(\mathrm{P}_{\mathrm{int,max}} - \mathrm{P}_{\mathrm{int}})^{\mathrm{n}} \qquad (2)$$

n is the order of the process.

Both n and k are parameters that reflect the enzymatically controlled uptake system, which may comprise more than one step. Bierman (1976) used a two-step mechanism to describe the uptake of phosphate by cyanobacteria. The first step represented the transport across the cell membrane by means of an assumed membrane carrier. The driving force for this transport is the gradient from external dissolved phosphate to internal dissolved phosphate. The second step is the intracellular storage of excessive P as a solid compound, usually polyphosphates (Nyholm, 1978). The level of the internal dissolved phosphate concentration is determined by the internal stored phosphate.

$P_{int,max}$ may be subject to long-term adaptation and selection mechanisms of the community to environmental conditions. For convenience the difference between $P_{int,max}$ and P_{int} is expressed as a deficit D, and the decrease of the deficit during a period of enhanced uptake, is:

$$dD/dt = -V \qquad (3)$$

At high external concentrations the Michaelis-Menten term approaches unity, so $V \approx V_{max}$. The solution of equation (3), after substitution of (1) and (2), with initial conditions $t = 0$ $D = D_{t=0}$, is:

$$D_t = [D_{t=0}^{1-n} + (n-1)kt]^{1/(1-n)} \qquad (4)$$

In case of a first-order uptake proces, with $n = 1$, the solution is simply:

$$D_t = D_{t=0} \exp(-kt) \qquad (5)$$

The values of the parameters k, n and $P_{int,max}$ can be estimated through least-squares optimization from the changes in the intracellular P-concentration as calculated from measured extra-cellular P concentrations. For each parameter the 90% confidence contours are calculated using (Draper & Smith, 1966):

$$S = S_{min}\left\{1 + \frac{p}{(n-p)}\, F(p, n-p, 90\%)\right\} \qquad (6)$$

with S the sum of squares at the 90% confidence contour and S_{min} the minimum sum of squares. n is the number of samples and p the number of parameters to be estimated. $F(p, n-p, 90\%)$ is the F-distribution according to Fisher.

Materials and methods

For the uptake experiments benthic communities from three test ditches receiving different levels of external P- and N-loading, in this paper respectively referred to as reference, medium and high loaded ditch, were used.

The ditches are located near Renkum in the pleistocene part of the Netherlands. The length of the ditches is 40 m and the width is 1.6 m at the bottom and 3.3 m at the water surface. A 25 cm layer of sand was brought in as bottom material. The banks have a slope of 30° and consist of gravel. The water depth is maintained at 0.5 m. The average retention time of the water is about 0.25 years. A detailed technical description of the ditches is given by Drent & Kersting (1993). Initially the conditions in all three ditches were identical. The sediments had a low phosphorus content of about 0.10 mg P [g dry matter]$^{-1}$. This was measured as the sum of the individual steps of the extraction scheme according to Hieltjes & Lijklema (1980). Since May 1989 the ditches received different levels of external phosphorus loading. The phosphorus is added manually as K_2HPO_4, dissolved in tap water, and homogeneously distributed over the water surface every six months, in early May and late October. The annual loading rates are:

	External loading rate (g P m^{-2} yr^{-1})
Reference ditch	0.0
medum loaded ditch	0.4
High loaded ditch	1.2

A background loading originates from atmospheric deposition and the occasional inlet of groundwater with low nutrient concentrations which serves to maintain a constant water level during dry periods. This loading is estimated at about 0.11 g P m^{-2} yr^{-1}, and equal for all three ditches.

The loading rates were based on empirical relationships between the external phosphorus loading and the observed concentrations of total phosphorus as a function of mean water depth and mean hydraulic retention time, developed by a Dutch advisory committee (Lijklema *et al.*, 1989). The medium loading rate corresponds through these relationships to an expected total phosphorus concentration of 0.15 mg P l^{-1}, a general Dutch standard to which all surface waters should comply as a minimum requirement. The high loading rate is three times this standard.

On top of the sand a benthic layer consisting mainly of living algae has developed in all three ditches, and, because macrophytes and phytoplankton were virtually absent, this layer is the main source of primary production in the reference ditch and the ditch with the medium loading rate (Portielje & Kersting, 1993). In the ditch with the high loading rate also a bloom of filamentous algae, mainly *Cladophora*, occurred, but this vanished by a sudden transition to a phytoplankton dominated system in the spring of 1991.

The species composition of the benthic community was determined microscopically as the visually estimated fraction of total biomass for individual species.

Phosphorus uptake experiments

Samples of the benthic communities were collected from three intact sediment cores per ditch, each with a diameter of 5.3 cm. The cores were pushed into the sand to a depth of at least 10 cm. A benthic layer of consisting of mainly living algae was present as a clearly distinct layer on top of the sand. It was removed by means of resuspension and decantation of the algal suspension after settling of the sand. This was repeated several times until all the algal material was removed and only the clear sand remained in the core. The algal material of the three cores was mixed for each ditch.

In the mixtures the dry weight (105 °C) concentrations were determined as the dry rest after evaporation of a subsample of the mixture.

The initial intracellular P-content was determined by drying a subsample of the mixture at 40 °C, complete destruction of the dried material with a H_2SO_4-Se mixture, using H_2O_2 as an oxidator, and subsequent P analysis (Novozamsky *et al.*, 1983).

Phosphorus uptake experiments were conducted in moderately stirred 250 ml suspensions (dilutions of the original suspension) with a known dry weight concentration at room temperature, allowing exposure to the daily light variation as experienced in the laboratory. After addition of phosphate to a concentration of 6 mg P l^{-1}, added as dissolved K_2HPO_4 and pH adjustment to 7, samples of 10 ml were taken at regular time intervals. These were immediately filtered through a 0.45 μm membrane filter, and dissolved phosphorus was determined in the filtrate on a Skalar SA-40 Autoanalyser, using the modified molybdate-blue method according to Murphy & Riley (1962). The choice of the initial ratio of the dry weight and P concentration was based on the results of preliminary experiments (not included here), and chosen in such a way that an accurate measurement of the time course of the phosphate concentration was possible. Furthermore, the P-concentrations were chosen high enough to allow omission of the Michaelis-Menten term in equation (1) for the whole duration of the experiments. This enhances the quality of the parameter estimation for k and P_{max}. However, K_p is not estimated. The tailing-off of the uptake at low phosphate concentrations as expressed by the Michaelis-Menten term therefore is not considered, but it is felt (and can be shown by simulation) that for long term changes this term hardly affects the results.

The results of the experiments are used to estimate the contribution of the benthic layer to the removal of dissolved phosphorus from the water phase after a dosage.

Results

Algal species composition

Visual observation revealed that the benthic material consisted for the major part of living algae, and that detritus and bacteria only contributed a small fraction.

In the reference ditch the dominant species in the algal community was *Gloeocystis*, which was estimated to cover about 80% of the total algal biomass. Other species present in appreciable amounts (estimated at >1% of the total algal biomass) were *Oscillatoria limnetica* (≈10–15%), *Coelosphaerium* (5%) and *Closterium* (2%).

In the medium loaded ditch, *Gloeocystis* was also the dominant species and estimated at about

60–70%. *Aphanocapsa delicatissima* contributed an estimated 20% of the total algal biomass, and *Oscillatoria limnetica* about 10%. Further on, there were a few percents of diatoms (pennales).

In the highest loaded ditch *Dimorphococcus* made up about 65% of the biomass. *Gomphosphaerium* was estimated at 25% and *Monoraphidium* at 5%. Present at low abundancies (< 1%) were *Astasia fasus*, *Phacus helicoides*, several species of *Euglena*, *Pediastrum duplex* and *Scenedesmus spp.*

Most species are of planktonic origin but in the ditches they are present in the benthic layer, which is most probably due to the stagnant conditions in the water and an enhanced nutrient availibility in the near bottom region.

Gloeocystis, *Aphanocapsa delicatissima*, *Dimorphococcus* and *Gomphosphaerium* are present in the benthic layers as colonies of cells surrounded by mucus.

Phosphate uptake experiments

The amounts of dry weight in the benthic layer in the three ditches and the initial intracellular P-concentrations within this layer are given in Table 1.

Figure 1 shows the results of the uptake experiments. The experiments were performed in duplo and the averages of P_{int} are plotted versus time. Fitting of equation (4) versus the measured data revealed that the parameters k and n are strongly correlated, which is reflected in wide confidence contours (data are not included). Therefore the simplified equation (5) was used. The simulated course of P_{int} with the optimum parameter set is represented in Fig. 1 by the lines. The optimum parameter set obtained by fitting of (5) is given in Table 2. Figure 2 presents the optimum values for k and $P_{int,max}$ and the 90% confidence contours. The quality of the best fit, in terms of the least-squares sum, using two parameters (k and $P_{int,max}$, with n arbitrarily set at 1), did not deteriorate much as compared to the three parameter model (equation 4), in which n is also estimated.

Table 1. Dry weight and initial intracellular P-concentrations in the benthic layer of the three ditches.

Ditch	g DW m^{-2}	mg P [g DW]$^{-1}$
Reference	352	0.57
Medium loaded	460	1.70
Highest loaded	563	4.09

Table 2. Optimum parameter set obtained from least-squares optimization of the measurfed data.

	k	$P_{int,max}$
	min^{-1} 10^4	mg P [g dm]$^{-1}$
Reference	30	3.60
Medium loaded	24	4.29
High loaded	22	5.14

Figure 3 displays the measured time-course of the dissolved phosphorus concentration in the highest loaded ditch after a dosage in April 1992. It is compared with the simulated uptake by the benthic community, using the estimated optimum values of the parameters ($k = 22\ 10^{-4}\ min^{-1}$, $P_{int,max} = 5.14$ mg P [g dry matter]$^{-1}$). The initial external dissolved phosphorus concentration at $t = 0$ was, as calculated from the supplied dosage, 1.80 mg P l^{-1}. The figure shows that the simulated uptake by the benthic community is responsible for a major part of the fast decrease in dissolved phosphorus concentrations during the first day after the dosage. After that no significant additional uptake by the benthic layer is likely to take place. Sedimentary uptake, by the underlying sand, is responsible for the remaining decrease in dissolved phosphorus which continues after the first day, as shown by the lower line in Fig. 3. The model used for the simulation of sedimentary uptake is described by Portielje & Lijklema (1993), and describes vertical transport in the sediment and the dynamics of adsorption onto the sand particles. In Fig. 3a K_p value of 100 μg P l^{-1} has been used, but the sensitivity for K_p and k is small, and affects the calculated concentration only during the first day. Neglection of diffusion limitation in the benthic layer may have overestimated the uptake rate during the first day,

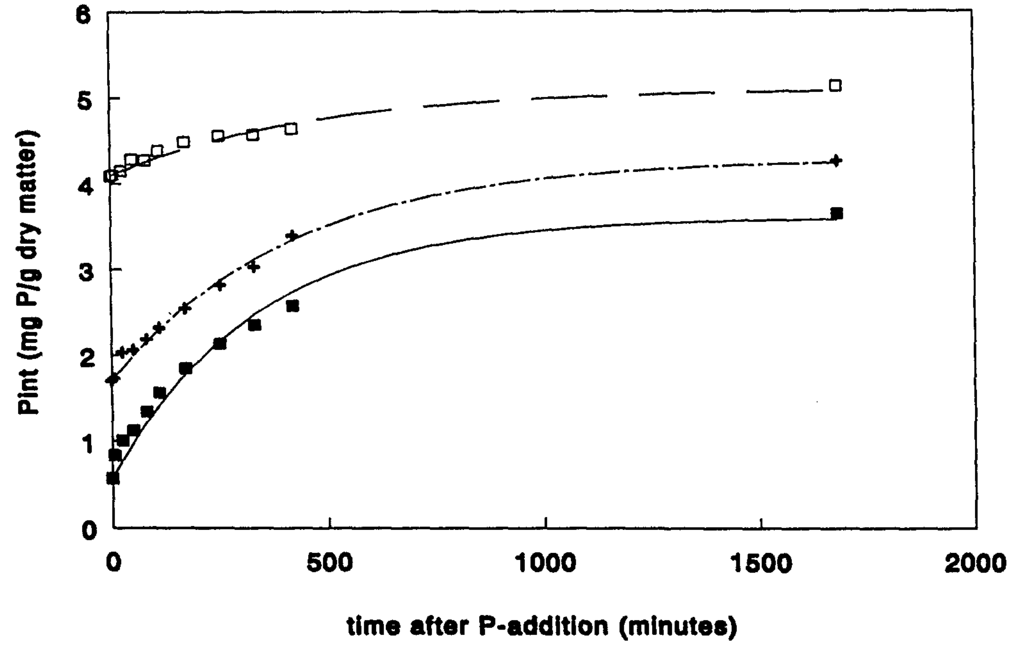

Fig. 1. Changes in internal P-concentrations after a pulse addition of 6 mg P l^{-1} to suspensions of benthic algae. originating from the three ditches. (■ = reference, + = medium loaded, □ = highest loaded)

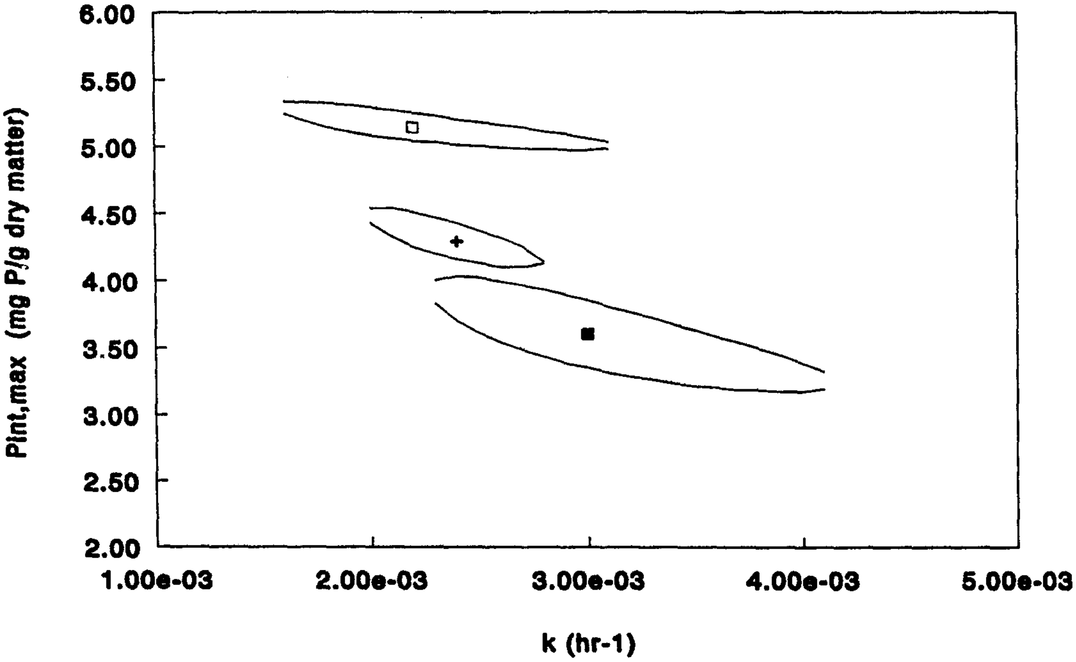

Fig. 2. Optimum values and 90% confidence contours for *k* and $P_{int,max}$ as estimated from least-squares optimization. *(■ = reference, + = medium loaded, □ = highest loaded)*

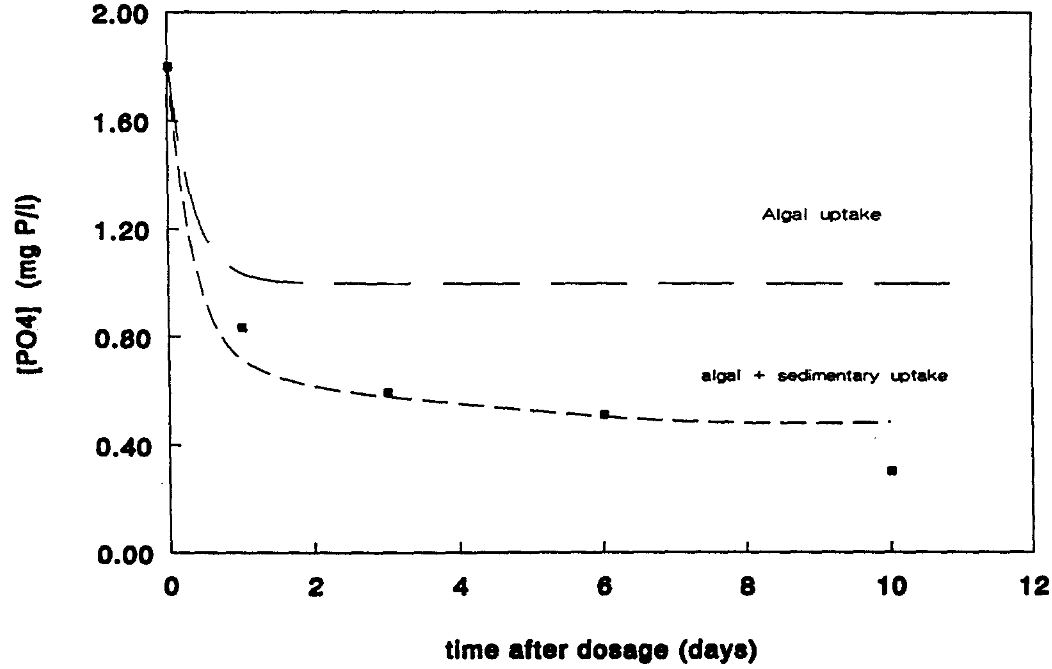

Fig. 3. Simulated algal uptake and sedimentary uptake after a pulse loading of 1.80 mg P l^{-1} to the highest loaded ditch in May 1992 and observed P concentrations.

as can be seen from Fig. 3. Inclusion would have shifted the curve slightly to the right. The thickness of the diffusive boundary layer strongly depends on the turbulent conditions in the water column and therefore varies in time. The fraction of phosphate taken up by the benthic community as compared to sedimentary uptake is however not affected to any appreciable extent by this imperfection.

Figure 4 presents the global change of the internal P-content of the benthic material in the three ditches since the start of the loading program in May 1989. In the highest loaded ditch P_{int} is continuously increasing, despite the net production of biomass. The continuous increase of P_{int} and the small additional uptake capacity measured in the uptake experiments suggest that the benthic community will approach saturation with P upon continuation of the present level of external phosphorus input. In the medium loaded ditch P_{int} remains more or less unchanged. In the reference ditch a slight but irregular decrease is observed, which may be due to a still on-going net production of cell material (Portielje & Kersting, 1993), resulting in dilution of the internal P-pool. It should be noted however that this interpretation is slightly biassed by the restricted number of observations and the fact that the last data were taken in May, whereas the others originate from October. All observations however were made just before a new semi-annual phosphorus dosage, so a steady state of the nutritional status of the algae with respect to the external loading at the time of sampling can be assumed.

Conclusions and discussion

Measured kinetics of phosphorus uptake by algae dominated benthic communities can be described well as a function of the intracellular P deficit, defined as the difference between maximum storage capacity of phosphorus and the actual intracellular P-content.

In benthic communities previously exposed to different levels of external nutrient supply, and as

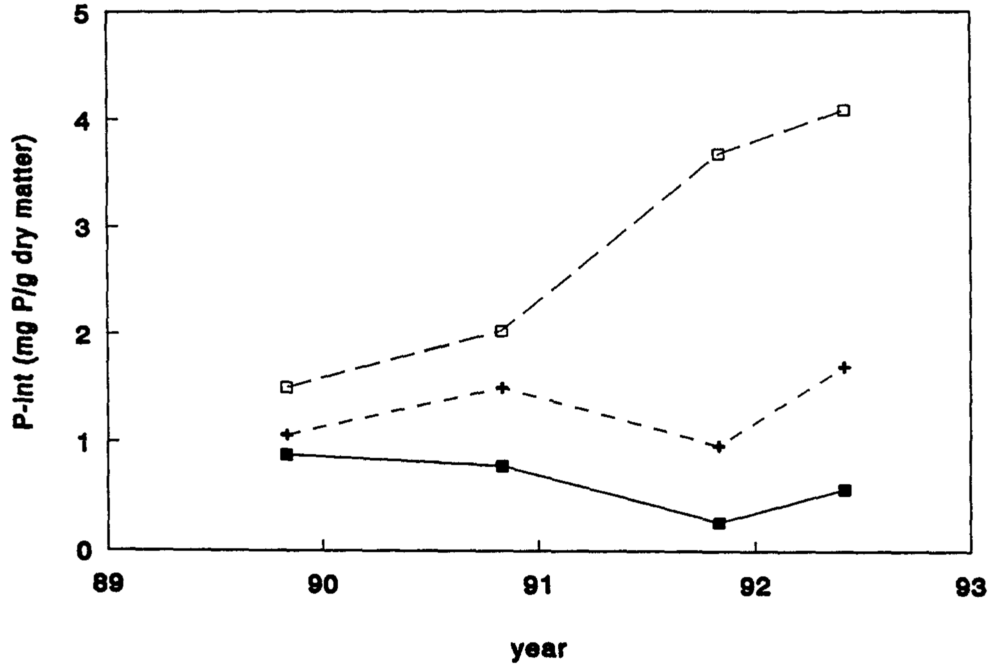

Fig. 4. Change in measured internal P-concentrations in benthic algal communities at three different trophic levels. *(■ = reference, + = medium loaded, □ = highest loaded)*

a result with different composition and intracellular P-contents, the maximum storage capacity for phosphorus was found to be correlated to the initial intracellular P-content, indicating a shift in the maximum storage capacity of the community in relation to the ambient trophic level. This may also be partly due to species selection related to the availibility of phosphate.

The uptake rate constant did not differ significantly for the three communities, although there is a slight tendency for higher k-values at lower internal P-concentrations.

The species composition of the medium loaded ditch is to a large extent (≈75%) identical to that of the reference ditch. The main difference is an estimated 20% contribution to total algal biomass of *Aphanocapsa delicatissima* in the medium loaded ditch, which was absent in the reference ditch. Adaptation in maximum storage capacity caused by adaptive mechanisms within a species is a possible explanation for the differences in the maximum P storage capacity between these two ditches. The species composition of the highest loaded ditch is different from that of the other two ditches, and this may also cause the higher maximum storage capacity of phosphorus in the highest loaded ditch.

Although algae contribute the largest part of the biomass in the benthic communities, bacteria may also to a certain extent determine the kinetics of phosphate uptake and the phosphate storage. Some bacterial species can reach very high internal P-concentrations (Marais *et al.*, 1983). Redox conditions determine storage or release of P by bacteria (Fuhs and Chen, 1975). Anaerobic conditions and the resulting microbial mediated P-release may cause a decrease in the P-pool of the sedimentary top layer of well over 30% during a few weeks (Boström *et al.*, 1985). Calculations on the oxygen budget of the ditches revealed that anaerobic conditions due to oxygen consuming processes within the benthic layers only occur at very low vertical dispersion coefficients (results will be published elsewhere), but that during most of the year the O_2-depletion within the benthic layer during night is too small to cause anoxia.

The benthic layers have a very loose structure (porosities are well over 90%).

The mucilage surrounding the algal colonies may be a growing site for bacteria (Brunberg & Boström, 1992), and interactions between algae and attached bacteria may determine the survival of the algal colonies, but possibly also the kinetics of P-uptake.

Reported values for the uptake rate constant k are an order of magnitude lower than those published by Riegman (1985) for cyanobacteria. He found for seven phosphorus starved species k values ranging from 120 to 370 10^4 min^{-1}. This may be due to differences in algal species composition. Cyanobacteria are known to exhibit the largest uptake rates under P-limited conditions. As in the present experiments the major part of the algae (*Gloeocystis*, *Aphanocapsa delicatissima*, *Dimorphococcus*, *Gomphosphaerium*) was present as colonies surrounded with mucus, this may have reduced diffusion in the boundary layer around the cells.

Although there is no proven law that first order kinetics apply to phosphorus uptake mechanisms, it is generally used and shown to describe observed uptake satisfactorily. The simplification of the model to first-order kinetics ($n = 1$ in equation (4)) did not significantly deteriorate the accuracy of the description with respect to the optimal parameter set with $n \neq 1$. This is in agreement with Riegman (1985) who also found first-order kinetics to work satisfactory.

Luxury uptake by the algae-dominated benthic community is responsible for most of the fast decrease in the dissolved phosphorus concentration in the water phase, observed during the initial phase after a dosage in the highest loaded ditch. After the first day no significant additional uptake by the benthic community will take place, and from that time on the observed decrease in the dissolved phosphorus concentration is mainly due to uptake by the sandy sediment, where adsorption processes determine the phosphate storage.

The value of K_p affects the tailing off of phosphate uptake after a step-wise addition but is not important for long term simulations. Bierman (1976) suggests a value for K_p of about 30 μg P l^{-1} for cyanobacteria and 60 μg P l^{-1} for diatoms and green algae. Riegman (1985) reported values of approximately 7 μg P l^{-1} for the cyanobacteria *Oscillatoria limnetica* and *Anabaena flos-aquae*.

Benthic microbial layers often perform a high activity per unit of volume. In natural systems of benthic microbial layers often distinct horizontal layers have been distinguished (Revsbech *et al.*, 1983). Differences between different species in uptake kinetics combined with their spatial separation makes application of measured mixed culture kinetics to field situations more difficult, as physical transport starts to play a role.

On a longer term the increased intracellular P-concentrations cause a higher net production of cell material if no other factors are limiting. This then results again in 'dilution' of the internal P-concentrations.

From extrapolation in time of the internal P-concentrations in the benthic communities of the three ditches, it may be inferred that at the level of phosphorus loading in the reference ditch, net production of biomass will continue until the internal P-concentration reaches a minimum value. Our measured internal P-concentrations in the reference ditch are lower than those calculated from data from Bierman (1976): 2.3 mg P [g dry matter]$^{-1}$ for green algae and 0.7 mg P [g dry matter]$^{-1}$ for non N-fixing blue-green algae. The mucus surrounding the colonies may have added to the total amount of dry matter in the ditches, thereby lowering the P-fraction, and a small contribution of dead organic matter with lower P-content can also not be precluded. At the level of the medium loaded ditch the internal P-concentrations are rather constant, while in the highest loaded ditch they are increasing and likely to reach saturation upon continued external loading, given the reduced uptake capacity after a phosphate addition as shown by phosphate uptake experiments. The benthic and phytoplankton community will then lose its function as a buffer site for pulse loadings of phosphate. In this ditch a transition from a system dominated by benthic algae to a system dominated by phytoplankton has been observed. The reason for this transition may be due to the competitive advantage of phytoplankton species over benthic algal species in light limited systems. Another explanation may be that the replacement of P-limitation by light and/or inorganic carbon limitation can induce mechanisms by which algae can perform active vertical movement by means of buoyancy regulation. In the literature (e.g. Booker & Walsby, 1981) evidence has been provided for a positive effect of a nutrient dosage on the buoyancy of P-limited cyanobacteria. Klemer *et al.* (1982) showed that C-limitation and N-limitation have opposite effects on the buoyancy of *Oscillatoria*, with respectively an increase and a decrease. It can not be proven that these mechanisms have caused the transition towards a phytoplankton dominated system in the highest loaded ditch, but it may be an explanation. The ability of alternating presence in the euphotic zone and in nutrient-richer deeper layers is of ecological advantage.

Acknowledgements

The authors wish to thank Rudi Roijackers and Rob Suijkerbuijk for the identification of the algal species, and two anonymous reviewers for their valuable comments.

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