

The labyrinth of nutrient cycles and buffers in wetlands: results based on research in the Camargue (southern France)*

In grateful memory of Prof. Dr A. W. H. van Herk and Prof. Dr R. Brouwer, who each in his own way contributed to my work

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Abstract

Wetlands, especially in the Mediterranean area, are subject to severe eutrophication. This may upset the equilibrium between phytoplankton production in undesirable quantities and a quantitatively desirable macrophyte production. In order to manage this equilibrium, a quantitative knowledge of nutrient input and fluxes is essential and the role of sediments in these processes must be understood. This knowledge can be useful even for agriculture, e.g. rice cultivation, where optimal utilization of fertilizers can lead to an economic benefit.

In this article different aspects of nutrient cycles are discussed in view of approaching a sufficiently precise quantification. The nutrient input balance of the Camargue was therefore measured which showed that the input of nutrients with the irrigation water, taken from the river Rhone, roughly equals the quantity of fertilizers added.

Phytoplankton growth can be approached reasonably with the Monod model, although there are still many practical problems, such as the influence of the pH on P uptake and the problem of measuring P uptake in the field. The situation is worse for macrophyte growth; quantitative data are scarce and studies have often been carried out with unrealistic nutrient concentrations or without addressing the influence of the sediment. This influence can also include negative factors, such as high concentrations of Fe^{2+} , H_2S or FeS, but cannot yet be quantified.

The nitrogen cycle in wetlands is dominated by denitrification. Most wetlands have sediments with high concentrations of organic matter, therefore with a large reducing capacity. Besides this process, we have shown that denitrification can also be controlled by FeS. In the Camargue sediments this denitrification is mediated by bacteria from the sulfur cycle; this appeared to be the major pathway. It was shown that a stoichiometric relation exists between nitrate reduced and sulphate produced. The influence of the temperature was quantified and appeared to be stronger at high organic matter concentrations than at lower ones. Denitrification with FeS means that the bacteria use nitrate also for their N demands, while this is not necessarily the case during denitrification with organic matter.

Mineralization of macrophytes is a much slower process than that of phytoplankton, probably because of their high C/N ratio. We could, however, not confirm the general assumption that the addition of nitrogen stimulates this mineralization. On the contrary, we found that two amino acids both with a C/N ratio of 6 had different mineralization rates. The amino acid composition of dead macrophytes and the C/N ratio may be of equal importance.

Unlike nitrogen, phosphate is always strongly adsorbed onto sediments. The two mechanisms of the adsorption of inorganic phosphate onto sediments, i.e. the adsorption onto $\text{Fe}(\text{OOH})$ and the precipitation of apatite, have been quantified. The adsorption of phosphate onto $\text{Fe}(\text{OOH})$ can be satisfactorily described with the Freundlich adsorption isotherm: $P_{\text{ads}} = A \cdot (\text{o-P})^B$. The adsorption coefficient A depends on the pH of the system and the

* This paper, giving an overview of the research in the sediments of the Camargue, was read during the symposium Nutrient Cycles –

A Joy Forever, on the occasion of my retirement, 19th of May 1993 at the I.H.E. in Delft (Netherlands).

Ca^{2+} concentration of the overlying water and can be quantified preliminarily by $A = a \cdot 10^{(-0.416 \cdot \text{pH})} \cdot (2.86 - (1.86 \cdot e^{-\text{Ca}^{2+}}))$. B can be approached by 0.333, which means the cube root of the phosphate concentration. The second mechanism is the solubility of apatite. We found a solubility product of 10^{-50} for hard waters. The two mechanisms are combined in solubility diagrams which describe equilibrium situations for specific lakes.

The conversion of $\text{Fe}(\text{OOH})$ to FeS has a strong influence on phosphate adsorption, although the partial reduction of $\text{Fe}(\text{OOH}) \approx \text{P}$ by H_2S does not release significant quantities of phosphate. Even after complete conversion to FeS only a small part of the bound phosphate was released.

Besides the two inorganic phosphate compounds, we established the existence of two organic pools, one soluble after extraction with strong acid (ASOP), the other one with strong alkali. The first pool is probably humic bound phosphate, while the larger part of the second pool was phytate. The ASOP was remineralized during the desiccation of a Camargue marsh; this drying up oxidized FeS , thus improving the phosphate adsorption and decreasing the denitrification capacity. It can, therefore, be an important tool for management. The phytate was strongly adsorbed onto $\text{Fe}(\text{OOH})$, which explains the non-bioavailability towards bacteria.

The fact that the sediment phosphate concentration can be approached by multiplying the relevant sediment adsorption constant with $\sqrt[3]{\text{o-P}}$ concentration has the consequence that much larger quantities of phosphate accumulate in the sediments than in the overlying water. This means that even if the phosphate input is stopped, the eutrophication will only be reversed very slowly, and not at all, if the shallow waters in wetlands have no through flow – as is often the case in many marshes in Mediterranean wetlands.

Abbreviations used:

o-P = dissolved ortho phosphate (or its concentration);

N_{part} , P_{part} = particulate N or P;

Tot- N_{inorg} = Total inorganic nitrogen (= $\text{NH}_3 + \text{NO}_2^- + \text{NO}_3^-$).

Ex Pondere et Numero Veritas (Kolthoff & Sandell, 1946)

Introduction

Nutrients are needed to sustain primary production in ecosystems. Either nitrogen or phosphate is generally the nutrient limiting this production. In a normal, i.e. non-polluted, situation the concentrations of N and P are low compared to the demand and, as a result, by far the larger part of the nutrients is in the vegetation. Recycling after the death of the plants and phytoplankton is needed to maintain primary production.

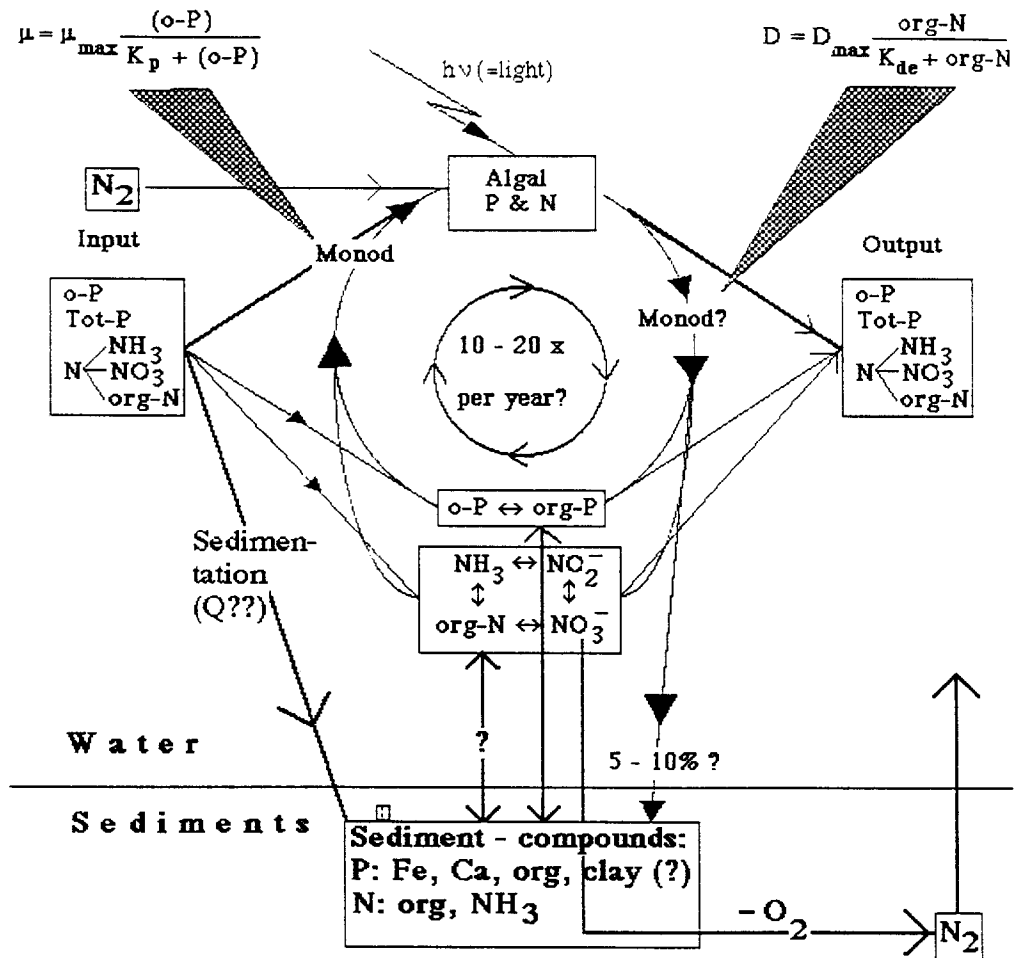
The recycling processes can be distinguished in two major processes. Nutrients from phytoplankton will be remineralized in a few days, while the phytoplankton is still suspended in the water. This process occurs on a nearly daily basis. On the contrary, most macrophytes die late in autumn and sink to the sediments with which they are then mixed. Recycling processes pass through the sediments and may take months, and for some fractions of organic nutrients years or decades.

In open water lakes 95–99% of primary production is phytoplankton production. Only in shallow lakes

or marshes macrophyte production becomes quantitatively significant, but it will never exceed 50% even in extreme cases.

The main processes of the N and P cycles are reproduced in Fig. 1a. On the first view macrophytes are not included – the main nutrient flux is through the phytoplankton. For the protection of shallow lakes such as occur frequently in wetlands and are important for wildlife, the balance between primary production and macrophyte growth is precarious and must be maintained in favour of the macrophytes. Therefore some remarks on primary production nutrient cycling are included in this article. For a successful protection of wetlands concrete measures, based on quantitative knowledge are needed.

There are several possible definitions of ‘a wetland’; some of those include all freshwater bodies. In this lecture I restrict ‘wetland’ to shallow (0.1–2 m) water bodies with submerged or emergent vegetations. They form an interface between the coastal seas and the continental freshwater habitats. They contain salt, brackish or freshwater, often more or less stagnant during certain periods. They are often situated in deltas or estuaries of (large) rivers; because of this geological situation they contain much clay and silt. Many of



The nutrient labyrinth -- a joy forever!

Fig. 1a. Main pathways of N and P in freshwater ecosystems.

these rivers are heavily polluted, and the pollutants will accumulate on these clays and silts, which have a large adsorption capacity. As a result the question is often raised: 'Are these water bodies, with their important sediment layers, a 'sink' or a 'source' for nutrients?'. The question is wrongly put: in the sediments these nutrients are in equilibrium between input and binding capacity of the system and since mixing is intensive, due to the limited depth and macrophytes root deeply, they will not be buried definitively. The position of the equilibrium controls whether input or output dominates; this may vary over the seasons depending on events in the system and may change over the years.

The interest in nutrient cycles in wetlands is rather recent. In Chapman's (1977) review of Wet Coastal

Ecosystems they are only mentioned twice, qualitatively. An exception is the work of Gaudet (1976) who studied nutrients in a papyrus swamp in Uganda. Gaudet demonstrated the strong accumulation of most nutrients except K and Na, in the detrital sediments, i.e. the peat and sludge layers. These swamps act as important buffers especially for N and P.

It is therefore clear that much more quantitative research still has to be done before management based on scientific understanding becomes possible. My interest is in the nutrient cycles (mainly N and P) in these ecosystems and their influence on the aquatic vegetation, and in the way in which these nutrients accumulate here. This article addresses mainly Mediterranean permanent freshwater marshes with

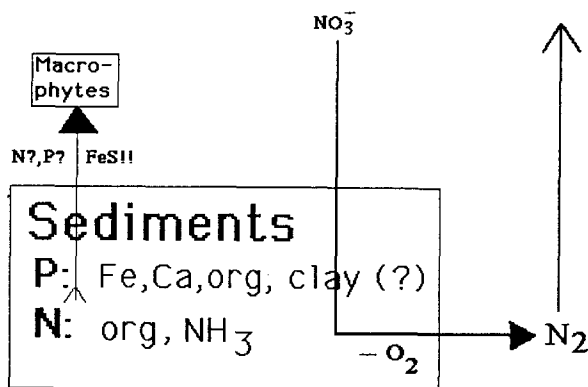


Fig. 1b. Enlargement of sediment compartment of Figure 1a.

Table 1. Input of nutrients into the aquatic systems of the Camargue. Irrigation water taken from the river Rhone. Fertilizers for dry cultures not included. (After El Habr, 1987)

Loading at the entrance of the irrigation channel		Loading at the end of the irrigation channel	
o-P	= 56 t	o-P	= 36.4 t
Tot-N _{inorg}	= 576 t	Tot-N _{inorg}	= 576 t
P _{part}	= 24 t	P _{part}	= 34 t
N _{part}	= 36 t	N _{part}	= 51.5 t
Fertilizer used		Fertilizer used minus losses (denitrification)	
o-P	= 250 t	o-P	= 250 t
Tot-N _{inorg}	= 1500 t	Tot-N _{inorg}	= 300 t

important emergent vegetation, but also with a large nutrient input. As an example of how large the loading of the Mediterranean deltas can be, Table 1 gives the quantities we have estimated to enter the Camargue.

These data will be combined with the hydrological data of Godin (1990) in order to arrive at a quantitative nutrient flux model of the Camargue.¹ Flux models are essential for a scientific management of wetlands.

Quantitative knowledge of the nutrient status in aquatic ecosystems begins with simple statements on relative quantities. For phytoplankton biomass production about 7–10% N and 0.5–1% P (of dry weight)

¹ After this paper was read Arnold (1994) prepared an hydrological spreadsheet model of the hydrology of the Camargue. Hydrology and nutrient fluxes of the three deltas of the Ebro, Po and Rhone were being synthesized by C. J. De Groot. His sudden death left this work unfinished; his contribution to the knowledge of nutrient processes will remain an impressive monument of his scientific capacity; we will remember him as excellent scientist and good colleague.

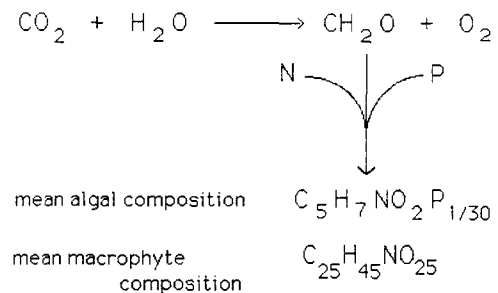


Fig. 2. Schematic presentation of primary production in relation to nutrient supply.

are needed (see Fig. 2), for macrophytes roughly 5 times less. Therefore, for a given quantity of nutrients, macrophytes may build up as much as 5 times more organic matter (mostly as cellulose etc.) than phytoplankton. This is important for the understanding of recycling after dying off and of the influence of decaying macrophytes on the oxygen regime; because of the relatively large input of dead organic matter during a short period in the autumn, the sediments will be depleted of O₂. Usually this happens only the next spring, as soon as temperature rises. O₂ is then depleted rapidly, but org-N is mineralized slowly, because of the high C/N ratio in the dead macrophyte material. This contrasts sharply with the decomposition of dead phytoplankton, which is a much more continuous and rapid process and which rapidly releases much NH₃ because of the low C/N ratio of phytoplankton (see 3.2).

While low concentrations of N and P (as used to exist in pristine systems) may lead to ecosystems with a low primary production, excess of nutrients will lead to serious problems of eutrophication. This implies a too large phytoplankton production and automatically, because of decreasing light penetration, a shift away from macrophytes towards phytoplankton, which in this kind of wetlands is highly undesirable. For the optimization of primary production it is, therefore, necessary to control the nutrient levels; it is essential to know all fluxes and processes and to quantify the influence of the local factors controlling them in order to calculate nutrient budgets.

A different approach was made by Howard-Williams (1985) who examined the cycling and retention of N and P in wetlands. In a theoretical overview

the author gave a careful analysis of the different processes. He further examined the influence of the rate of water flow on processes in the N and P cycle and next treated these systems as black box models, discussing and applying the different constants that are needed which were, however, often not measured but taken from other systems or reactions: for instance, the constants for decomposition processes that were used were the same for P, N and C. The paper is important for everybody beginning a research programme on wetland nutrient fluxes.

In this article I will discuss some examples of nutrient studies from the literature, with some emphasis on the more generalized articles, and summarize some of our own results² obtained in the wetlands of the Camargue (Delta of the Rhone, Southern France). The processes that will be considered are

- (a) for the N cycle, denitrification and mineralization;
- (b) for the P cycle, adsorption onto sediments;
- (c) for the N and P cycles, uptake the phytoplankton and macrophytes.

The result will demonstrate the long way we still have to go before a mathematical description of the cycles of N and P can be developed.

The work in the Camargue is part of the C. E. C. sponsored programme on the deltas of the Ebro, Po and Rhone. A report on this work (Goltermann, Comin & Ferrari, 1992) is available on request.

Growth and nutrient uptake

Phytoplankton growth

If culture characteristics such as pH, light, temperature and, therefore, the cell carbon concentration remains constant during growth, phytoplankton growth can be described (for summaries see Goltermann, 1975, Van Liere & Walsby, 1982) with the equation of Monod (1949):

$$\mu = \mu_{\max} \frac{\text{Nu}}{\text{K}_s + \text{Nu}},$$

where μ = growth rate (d^{-1}); μ_{\max} = maximal growth rate (d^{-1}); Nu = concentration of growth limiting nutrient; K_s = constant (= concentration of Nu where growth rate = $0.5 * \mu_{\max}$).

There is no conceptual model behind this equation. Monod (1949) wrote: 'But it is both convenient and logical to adopt a hyperbolic function'. Testing this

equation is usually done by curve fitting. There is, however, a practical problem: formerly curve fitting was done by plotting μ^{-1} against Nu^{-1} ; this still happens today with the smaller personal computers, with which curve fitting became too easy. The disadvantage is, that the point at the lowest concentration become the highest values for the curve fit, having thus an overrated influence. (Goltermann, 1991a). Non-linear curve fitting is nowadays possible, but as yet rarely applied.

The experiments are always done in the laboratory at a constant pH, usually pH = 7. As the pH may have a strong influence on the P-uptake, one may expect the pH to have a strong influence on the growth rate. One example has been published by Goltermann (1977), who found that *Scenedesmus* had a growth rate of 2 d^{-1} at pH = 8.5 and 0.9 d^{-1} at pH > 9.5. As growth will affect the pH a buffer must be added, which is not usually done. There are as yet very few observations in lakes in the literature. Bentzen & Taylor (1991) studied the Michaelis-Menten kinetics of P-uptake of natural plankton. They discussed the problem that this technique yields means for the different populations and that, as o-P in the summer epilimnion of temperate lakes cannot be measured precisely, the sum of K_s and Nu is measured. They discussed statistical means to circumvent this problem, which often introduce new uncertainties. They did not discuss the problem inherent to $^{32}\text{PO}_4$ addition, that physical exchange and biological uptake cannot really be distinguished.

The problem is especially important in shallow lakes, as during growth the pH will increase considerably, while the sediments will buffer the o-P concentration as a large amount is fixed in the sediments (see below, 3). This adsorption depends on pH as well, but the quantification is still difficult. Altogether, there is as yet no satisfactory way of describing phytoplankton growth quantitatively in marshes and shallow lakes.

The situation is even less satisfactory for the mineralization process. Mineralization rates of phytoplankton P and N are fast, in the order of hours and days respectively, but cannot yet be described as a simple function of e.g. time and temperature. There is evidence that mineralization in lakes increases with increasing concentrations of the dead organic matter (Saunders *et al.*, 1980) in agreement with Sakamoto's (1966) finding that the chlorophyll concentration in lakes increases exponentially with the P-concentration. (For discussion, see Golterman & Kouwe, 1980). After death phytoplankton cells will be remineralized rapidly. Golterman (1988a) has suggested that for this rem-

² The work in the Camargue was carried out by 'L'Equipe de Limnologie'; the names are given in the acknowledgement.

eralization a Monod equation may also be used, with org-N as the limiting factor. This concept attributes to N concentrations a possible influence on the variability always found in models of the relation between P loading and primary production, (e.g. the O.E.C.D. model, O.E.C.D., 1982) but maintains the P loading as the overall controlling factor.

Aspects of nutrient uptake by macrophytes

Not only are the nutrient fluxes through the macrophyte compartment small compared with those through the phytoplankton (they become visible in Fig. 1a only after enlargement, see Fig. 1b), but if we compare our knowledge of nutrient uptake by phytoplankton or by rooted vegetation, there is a large difference as well. As to emergent macrophytes, we know far less about the influence of nutrients on their growth rate. I have chosen a few examples from the literature to demonstrate this point.

Cary and Weerts (1984) studied growth of *Salvinia* and *Typha* in water cultures in a waterbath in a greenhouse. They showed optimal growth to occur at 25 °C with 70 mg l⁻¹ of N and 20 mg l⁻¹ of P. Poor growth occurred at respectively 2 mg l⁻¹ of N and 1 mg l⁻¹ of o-P. At 16 and 20 °C growth decreased till respectively 1/3 and 2/3 of the maximal growth, which reduced the need for N and P. Ulrich and Burton (1985) cultured *Phragmites* in sand cultures and applied 64 different treatments with nutrients. NO₃⁻ was dosed at 0–46 g m⁻² and o-P at 0–12 g m⁻² (These quantities are far above the quantities of fertilizers used in rice-fields). They stated that K additions did not show any effect at all, while optimal growth was obtained at the highest quantities of N and P added. They demonstrated that after a higher N dose, the plants accumulated more N. Bornkamm & Raghi-Atri (1986) studied the growth of *Phragmites* in sand cultures at low P and N concentrations (both 0.14 mM) and at high concentrations of P (16.6 mM) and N (14.8 mM). When *Phragmites* was grown over a period of 4 years at high concentrations of P and N, during the first 2 years effects of P were observed, while later the high N concentration caused a higher biomass production, taller and thicker shoots and increased concentrations of N in the plant. Hill (1979) studied uptake (which was measured as percentage of final dry weight) and release of nutrients by several aquatic macrophytes and arrived at decomposition rate constants of 0.03–0.04 d⁻¹; no differences were given for dry weight, C, N and P, although the curves look rather different.

These studies, important as they may be as a start, are rather far removed from the natural conditions under which these plants grow. Carignan & Kalff (1980), e.g., showed that rooted plants use phosphate from the sediments and not from the water. Barko and Smart (1980) demonstrated that 4 different species of submerged macrophytes could use N and P from the sediments (but not K). Best & Mantai (1978) showed that *Myriophyllum spicatum* can meet its N requirements by uptake via the roots and by adsorption from the water while P was adsorbed from the sediments and transported to the shoots. A synergistic relationship between N and P was found: o-P in the water affected NO₃⁻ uptake from the water. Another problem in establishing the relationship between nutrients and growth rate is the fact that the most rapid uptake of nutrients may occur earlier than the maximal growth rate (Boyd, 1970). Experiments should therefore cover the full vegetative period and studies on growth rate should permit a full factorial analysis.

Furthermore, in the shallow marshes other environmental factors may have a strong influence on the vegetation. For example, Van Wijck *et al.* (1992) have demonstrated that the presence of reduced compounds such as Fe²⁺, S²⁻ and FeS may have a negative effect on the development of several aquatic emergent macrophytes – regardless of the amount of nutrients present.

Aspects of the nitrogen cycle

Probably one of the most complete N-flux models of a wetland is that of Valiela & Teal (1979), who measured all important N processes in the Great Sippewissett Marsh, the results of which were published in a number of papers, summarized in 1979. They showed that quantitatively the most important processes were N-input, with both ground and surface water, output towards the sea, N₂ fixation and denitrification (see Table 2). Most processes were measured 10–12 times a year and a mean value was taken. The variability and its controlling factors, were however, not taken into account. For example, Kaplan and Valiela (1979) measured denitrification, which depended largely on the temperature, but it is clear from their results that other factors – certainly FeS – may be equally important. As the results of their experiments vary irregularly over time, one has to know the factors which control the variability in order to take a weighted mean. We also have to know these controlling factors in order to use

Table 2. Nitrogen budget for Great Sippewissett Marsh (USA). From Valiela and Teal, 1979. (rounded off values, in kg ha^{-1} ; minor processes omitted)

Processes	Input	Output	Net exchange
<i>Precipitation</i>			
NO ₃ -N	110		
NH ₃ -N	70		
DON	190		
Part-N	15		
Total	380		380
<i>Groundwater flow</i>			
NO ₃ -N	2920		
NH ₃ -N	460		
DON	2710		
Total	6100		6100
N ₂ fixation		3280	3280
<i>Tidal water exchange</i>			
NO ₃ -N	390	1210	
NH ₄ -N	2620	3540	
DON	16300	18500	
Part-N	6740	8200	
Total	26200	31600	5350
Denitrification		4120	-6940 (+2820)
Sedimentation		1300	-1300
Overall total	35990	39860	-3870

their effects when applying them to other wetlands. As an example I will demonstrate the difficulty of quantifying the denitrification process.

Denitrification

Denitrification is the process by which NO₃⁻ is reduced to N₂; heterotrophic bacteria are generally supposed to be responsible for this process. The process needs energy, which is derived from the oxidation of organic matter or other reducing compounds. Minzoni *et al.* (1988) showed that nitrogen added to rice fields in the Camargue, both as NH₄⁺ or NO₃⁻, was rapidly lost in 4–6 days (see Fig. 3a,b) – well before the rice could use it. In order to prove that the responsible process was indeed denitrification we tried to measure the quantity

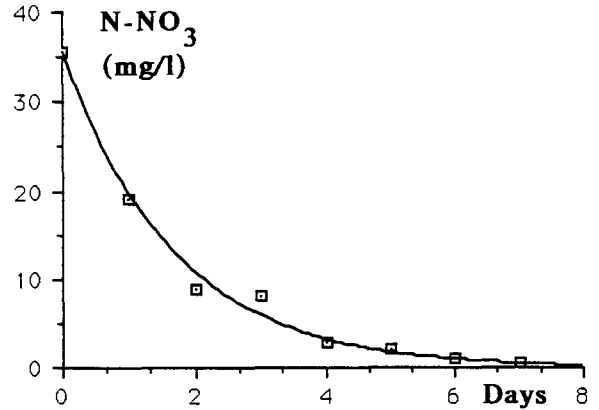


Fig. 3a. Disappearance of nitrate in a rice field in the Camargue. Fitted curve: $\text{N-NO}_3 = 35.7 * 10^{-0.259d}$, $R^2 = 0.99$.

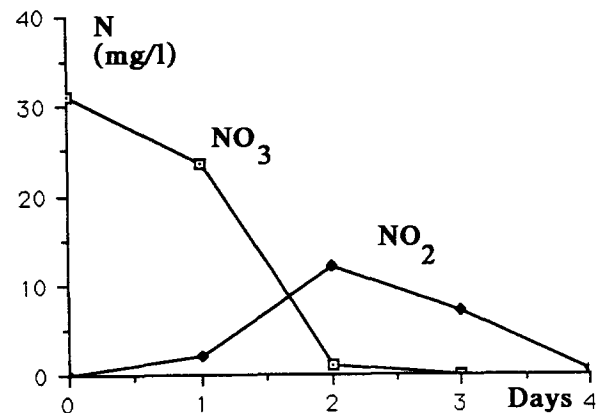


Fig. 3b. Disappearance of nitrate and production of nitrite in a rice field in the Camargue.

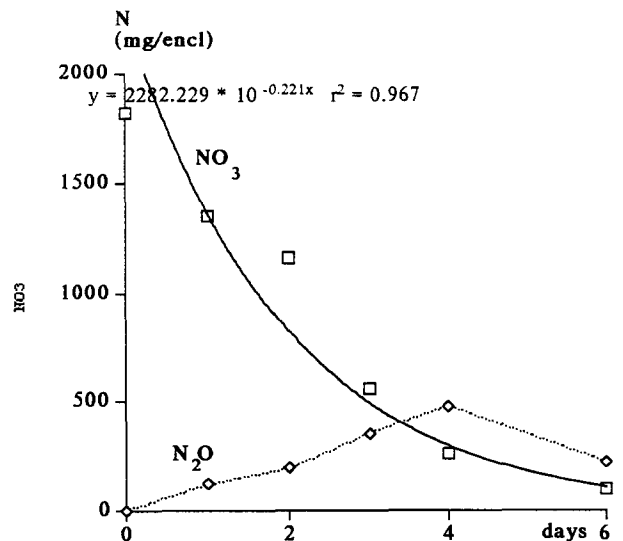


Fig. 3c. NO₃ disappearance and N₂O production in an enclosure in a rice field in the Camargue. Fitted curve: $\text{N-NO}_3 = 22280 * 10^{-0.22}$; $R^2 = 0.97$.

Table 3. NO_3^- disappearance rate (in $\text{mg l}^{-1} \text{d}^{-1}$ of N) in two Camargue sediments with high or low organic matter concentration

	Temperature				
	25 °C		15 °C		0 °C
NO_3^- addition:	First	Second	First	Second	First
Organic matter					
High	5.28	6.49	3.77	3.68	1.45
Low	1.91	3.59	1.58	2.59	1.07
High/Low	2.76	1.81	2.39	1.42	1.35

of N_2O which is formed when the final reduction to N_2 is inhibited by acetylene. We could, however, only recover about 50% of the NO_3^- lost (Fig. 3c). This discrepancy was caused by the set-up in the field, were we applied enclosures to which NO_3^- and acetylene were added. It was more or less automatically assumed that the N_2O formed would – like the N_2 – escape upwards as gas. We realized however that N_2O is much more soluble than N_2 . Downward diffusion with the irrigation water which penetrates into the ground water might be as important as upward diffusion (see Fig. 4). We therefore added N_2O to an identical enclosure and found that the N_2O decreased at a constant rate of 20% per day. The N_2O production rate could then be calculated from the quantity recovered in the overlying water plus the air space after correction for the loss of N_2O . The result is given in Fig. 5; it can be concluded that about 70–80% of the NO_3^- added to shallow waters is lost as N_2 . Roughly 10% appeared as NO_2^- , the turnover rate of which is discussed below. Large quantities of NO_2^- are found in all our experiments in situ and in vitro and will be discussed further below. This N balance leaves the possibility that about 10% of the fertilizers added to rice fields will enter the underground water table.

The next step was to quantify the rate controlling factors in order to be able to extrapolate from one situation to another. Organic matter and NO_3^- concentrations, pH and temperature come to the mind automatically. By adding NO_3^- to alkaline ($\text{pH} \pm 8$) mud/water systems in vitro, El Habr and Golterman (1990) have demonstrated that, if the sediments were relatively rich in organic matter (about 5%), the temperature had a strong influence on the denitrification rate, while if the organic matter was about 2%, this influence was feebler (see Fig. 6). It is unexpected that the NO_3^-

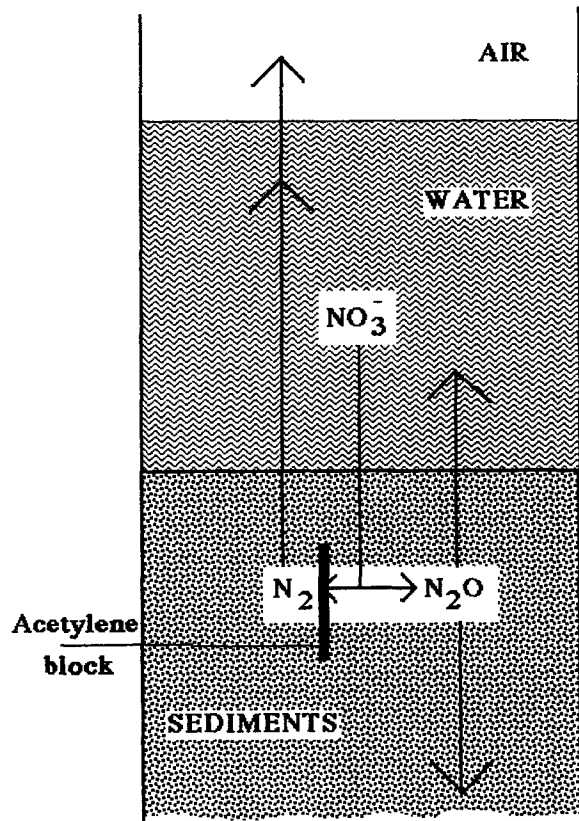


Fig. 4. Upward N_2 diffusion and upward and downward N_2O diffusion in an enclosure in shallow waters.

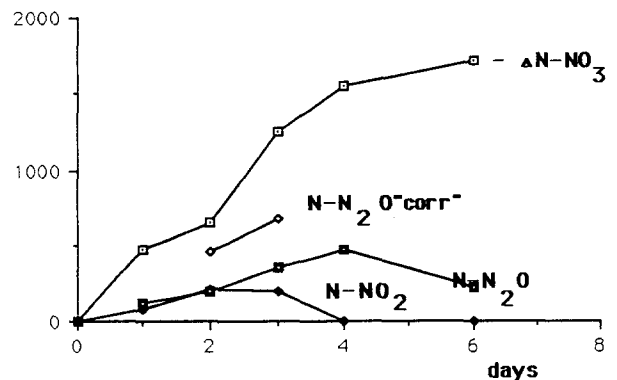


Fig. 5. NO_3^- disappearance with NO_2^- and N_2O production in a rice field in the Camargue. N_2O production: drawn line = measured values; after correction for the N_2O disappearance rate: $\text{N-N}_2\text{O}$ 'correct'.

concentration decreased linearly with time: a bacterial process should cause an exponential decrease. I think that this means that there is a physical limiting factor,

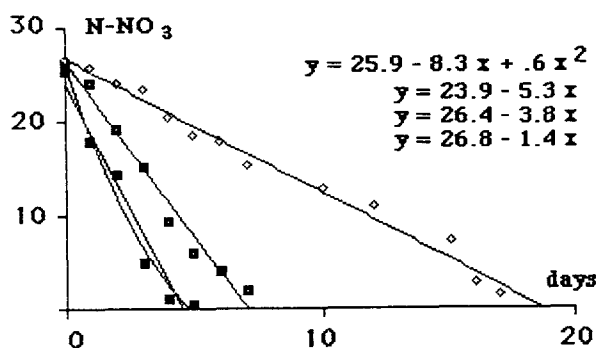


Fig. 6. Influence of the temperature on denitrification rate in a mud/water system with sediment from a Camargue marsh. (From El Habr and Golterman, 1990). Shown are linear rates at 0, 15 and 25 °C and the quadratic rate at 25 °C.

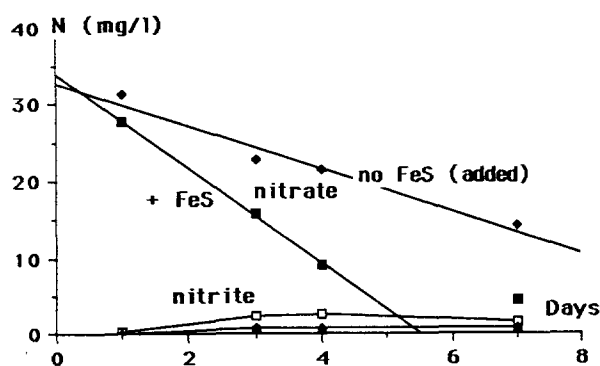
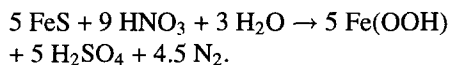


Fig. 7. Denitrification rate with and without extra FeS added at ambient temperature. (From Golterman, 1991).

diffusion. Only when the NO_3^- concentration became low, a non-linear denitrification rate was found.

When in these experiments NO_3^- became the limiting factor for bacterial metabolism or growth, NO_3^- was added a second time to the same systems. Especially in the sediments poor in organic matter the results were different: the disappearance rates after the second addition were always higher than in the first experiment, but in the sediments poor in organic matter more than in the rich ones (see Table 3). In the first instance we thought that adaptation might cause this difference, but it seemed unlikely that, if denitrification was indeed caused by a mixed flora of heterotrophs, such a sharp contrast would occur. We therefore looked for other possibilities and realized that, besides organic matter, FeS might be a reductant for denitrification. In fact, if FeS was added to sediments, denitrification rate increased considerably (Golterman, 1991b; see Fig. 7). Furthermore we could show that the amount of NO_3^- reduced roughly equalled the amount of SO_4^{2-} pro-

duced (see Table 4). I think that the following reaction may be proposed:

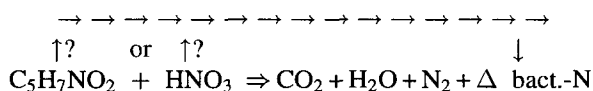


It seems likely, that the reducing power of the Fe^{2+} will also be used, although it is not certain.

In denitrification experiments in mud/water systems *in vitro*, large quantities of NO_2^- were produced and released into the medium (see Fig. 8a,b). In this experiment the disappearance rate of NO_2^- was also measured. (Fig. 8c,d) It appeared that a rapid oxidation to NO_3^- occurred in the O_2 containing overlying water. When FeS was present, much less NO_3^- was found, most likely because of the denitrification with FeS as reductant. The NO_2^- production depended on the presence of FeS and amounted in some experiments to concentrations of 30% of the NO_3^- added. García-Gil & Golterman (1993) showed that the reaction rate of this kind of denitrification followed Monod kinetics with the concentration of FeS as the controlling factor. It is certain that bacteria of the sulfur cycle are involved, but more research is needed.

The production of NO_2^- did not only happen in *in vitro* experiments. In the field, too, much NO_2^- was produced, which in marshes or wetlands is of course highly undesirable. The question must be raised why other bacteria than those of the S-cycle do not immediately use this energy source. An important variable that may influence denitrification rate in the field is the diffusion rate into the sediments; if the reduction of NO_2^- takes place in deeper layers than the reduction of NO_3^- , further downward diffusion will take time, while part of the formed NO_2^- will automatically 'escape' upward. Theoretical work on this aspect is in progress and has shown the great importance of the geometry of the experimental design, including the depth of the sediment layers.

For the N-balance of the ecosystem it makes much difference whether organic matter containing N is the substrate for the denitrification or FeS. In the first case the N for the growth of the bacteria may come either from the NO_3^- , or, more likely, from the organic matter:



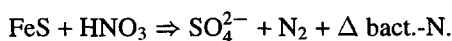
In this case there is therefore no increase in the N content of the soil (sediments). But if FeS is the substrate, the ΔN can only be taken from the NO_3^- :

Table 4. Denitrification experiments with FeS as reducing agent in sediments of the Camargue. The sediments were enriched with FeS and nitrate; nitrate, sulphate and FeS were measured after 5, 9 or 14 days and are expressed in mmol l^{-1}

First experiment								
FeS	∂NO_3		∂SO_4		$\partial\text{S}/\partial\text{N}^{1)}$	$\partial\text{S}/\partial\text{N}$ after correction for 'org-Cdenitrification'		
	5 days	14 days	5 days	14 days				
--	2.36	4.43	0	0.37				
--	1.71	4.71	0	0.38				
++	5.24	5.93	3.17	3.85	0.55;	0.64		
++	4.86	5.86	3.11	3.81	0.54;	0.64		
Second experiment								
	5 days		9 days		5 days		9 days	
--	2.5	2.5	0	0				
++	6.14	7	2.4	2.8	0.46;	0.4	0.66;	0.63
++	7.57	7.86	2.4	2.8	0.32;	0.4	0.58;	0.53

¹⁾ The theoretical value of $\partial\text{S}/\partial\text{N}$ should be 0.56.

N.B. The $\Sigma (\text{H}_2\text{S} + \text{H}_2\text{SO}_4)$ was always recovered as $100 \pm 10\%$.



In the first place we need now to find out whether these mechanisms exist in other marshes than those of the Camargue and to measure the influence of the FeS concentration and temperature on the denitrification rate before extrapolations from one wetland to another can be made.

Mineralization

Dead aquatic plant material will be remineralized by bacteria – several studies have addressed the problem. Phytoplankton, when dying off, releases P and N, the first by autolytic action, the second by bacterial action (Golterman, 1975). The mineralization of N is due to the fact that phytoplankton contains a high concentration of proteins, which, when used as energy supply, will be remineralized and for only a small part used for the new bacterial biomass (see Fig. 9). For macrophytes the situation is different: because of the high C/N ratio in the plant material N seems to be needed in order to make bacterial growth possible. The usual approach to the study of mineralization is to put dead material in so-called litter bags and to follow the decrease of dry weight in time, as is often done in terrestrial ecology. In this way Carpenter & Adams (1979)

followed the influence of temperature and N-content on the decay rates of Coarse Particulate Organic Matter, CPOM. They found decay rates of CPOM in the range of $0.01\text{--}0.141 \text{ d}^{-1}$. The N content of both water and tissue and the temperature had a strong influence ($Q_{10} = 3$), while extra added P did not. Furthermore they showed large differences between experiments in the field and in the laboratory, supposedly due to the presence of animals in the field but not in the laboratory. Remineralization of the different elements, however, was not studied.

Hill (1979) followed uptake and release of nutrients by aquatic macrophytes and published decay rate constants (the same for dry weight, N, P and C) of $0.03\text{--}0.04 \text{ d}^{-1}$.

Forés *et al.* (1988) showed that the decomposition rate of rice-straw is much lower ($k \approx 0.008 \text{ d}^{-1}$) because of the high mineral content of the rice-straw. After 139 days of decomposition 75% of the biomass, 70% of the C, 50% of the N and 30% of the P remained in the rice detritus. They showed furthermore that the loss rates were for $\text{N} < \text{C} < \text{P}$.

Menéndez *et al.* (1989) studied the decomposition *Ruppia cirrhosa*. They showed that the relative exponential nutrient loss rates were high during the first three days, but not different for C, N and P, and were

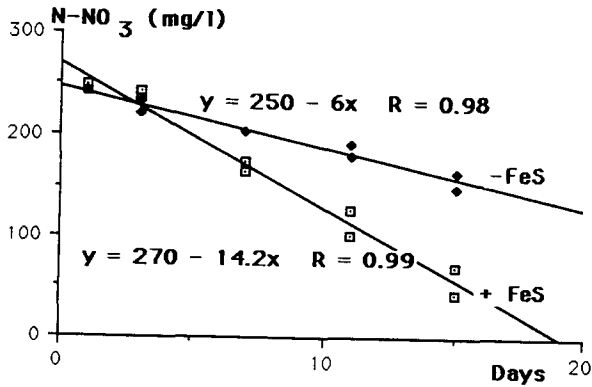


Fig. 8a. Disappearance rate of nitrate added to a 2 layer mud/water system with and without FeS added.

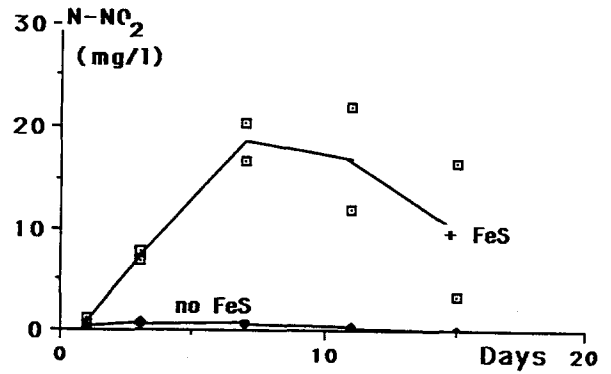


Fig. 8b. Production of nitrite in the same experiment as Figure 8a.

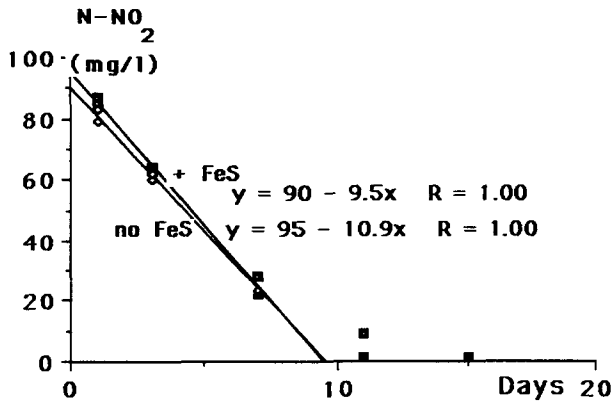


Fig. 8c. Disappearance rate of nitrite added to a 2 layer mud/water system with and without FeS added.

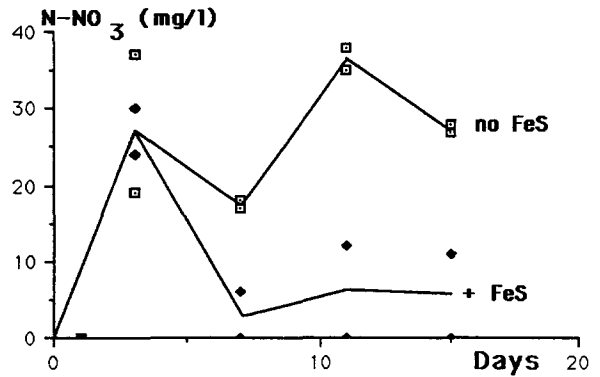


Fig. 8d. Production of nitrate in the same experiment as Figure 8c.

strongly influenced by the macroinvertebrate populations. In a critical study Wieder and Lang (1982) pointed out that studies to measure decay rates in the field should be set up differently from studies trying to analyse the factors controlling these decay rates. In the latter case the set-up should allow a test of variance, which asks for a different approach.

One may wonder, however, whether C and N have the same disappearance rates, and whether the N will be remineralized to NH_3 , or will partially enter one of the organic-N pools in the sediments. The bioavailability of org-N in sediments is not equal. Bonetto *et al.* (1988) showed that, if a fractionation of the soil org-N was carried out by a sequential extraction with NaOH and H_2SO_4 , three fractions with different bio-activity could be obtained. We have, however, so far been unable (because of technical difficulties) to find out into which pool the mineralizing N enters before it becomes NH_3 , which may take a consider-

able time. Another unexpected finding was that the addition of inorganic N did not stimulate mineralization of rice straw, contrary to a generally accepted idea in soil science (Russel, 1973). We could furthermore not demonstrate the importance of the C/N ratio for the mineralization process. Leucine and lysine, each with C/N = 6, showed a quite different mineralization rate. I think that amino acid composition may be a factor which could have an influence on the bacterial growth on material with high C/N.

The mineralization process in nature takes a long time, especially as after macrophyte death temperatures are often low. During this time burial of sediments may withdraw the org-N from the available layer, making quantification at this moment impossible.

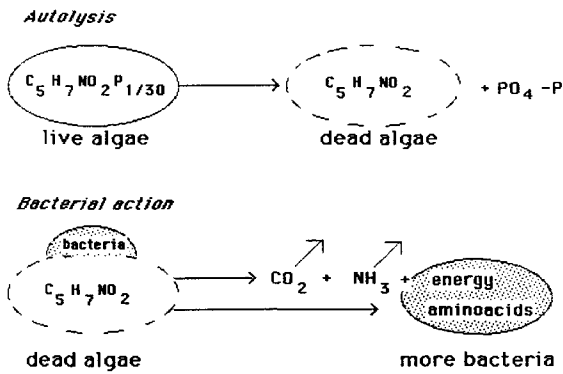


Fig. 9. Schematic presentation of autolytic and bacterial decomposition processes on dead algal material.

Aspects of phosphate binding onto sediments

Inorganic phosphates

When phosphate enters a waterbody it will be distributed over the different compartments, water and sediments, as follows:

$$P_{in} = P_{sed} + P_{out} + \Delta(o-P)_w \quad (I)$$

where P_{in} = phosphate entering the lake ($g\ m^{-2}\ y^{-1}$); P_{out} = phosphate leaving the lake ($g\ m^{-2}\ y^{-1}$); P_{sed} = phosphate entering the sediments ($g\ m^{-2}\ y^{-1}$); $\Delta(o-P)_w$ = increase in phosphate concentration in water* depth ($g\ m^{-2}\ y^{-1}$).

The P entering the sediment compartment, P_{sed} , does not only come from decaying plant material; there is active, chemical adsorption as well. Because of their high content in clay, silt or Fe(OOH), wetlands have a large phosphate binding capacity. As general processes plant and microbial uptake may have some influence, but after the death of the vegetation most of the phosphate is rapidly remineralized; the microbial pool is only small, but it may have a rapid turnover. Adsorption of o-phosphate by mineral compounds is several times more important. A first quantification of the binding capacity was made by Olsen (1964), who showed that the adsorption could be described by the Freundlich adsorption isotherm:

$$P_{sed} = A * (o-P_w)^B \quad (II)$$

where P_{sed} = P concentration on sediments ($mg\ g^{-1}$); $o-P_w$ = P concentration in water ($mg\ l^{-1}$); A and B = constants. B is often about 0.3–0.4.

'A' reflects the adsorption capacity of sediments and is related to the Ca^{2+} and Fe(OOH) concentra-

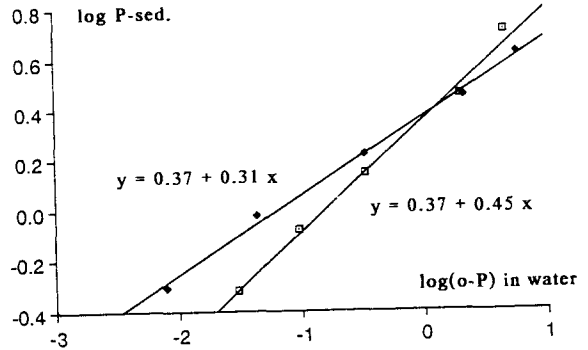


Fig. 10. o-P adsorption on sediments from Lake Puyvalador (Pyrenees, France) and from a rice-field in the river Po (Italy) watershed. Log transformation from data from Golterman, 1984. Both $R^2 > .99$.

tions. Two examples of such isotherms are given in Fig. 10.

Golterman (1984), in a review of this process based on more experimental work, strongly supported earlier proposals to describe the adsorption equilibrium with the Freundlich adsorption isotherm. He suggested that Fe(OOH) is one of the important mechanisms adsorbing o-P to sediments, even in hard water systems.

Richardson (1985) discussed some of the mechanisms of phosphate retention capacity in different freshwater wetland types and demonstrated the large quantities that can be adsorbed. He showed large differences for the different soil types in which he believed extractable Al to be the most important adsorbent, as it showed a higher correlation with a proposed 'phosphate adsorption index' than e.g. Fe(OOH) did. This is probably caused rather by the fact that there are several forms of iron in soils which are not equally active in phosphate adsorption. His equilibrium concentrations of o-P in the water ranging from 16–210 $mg\ l^{-1}$, are, however, unrealistically high, and do not suggest a real adsorption mechanism. In an experimental study with a marine clay Golterman (1994a) showed that $Al(OH)_3$ does not play a role in the adsorption process and argued that, theoretically, there are strong arguments against Al as adsorbent for phosphate in aquatic sediments.

The adsorption process onto Fe(OOH)

The importance of Fe(OOH) as an adsorbent in sediments was understood long ago (Mortimer, 1941, 1942; Olsen, 1964; Golterman, Viner and Lee, 1977; Lijklema, 1977, 1980). The equilibrium can be described by the same kind of equation as equation

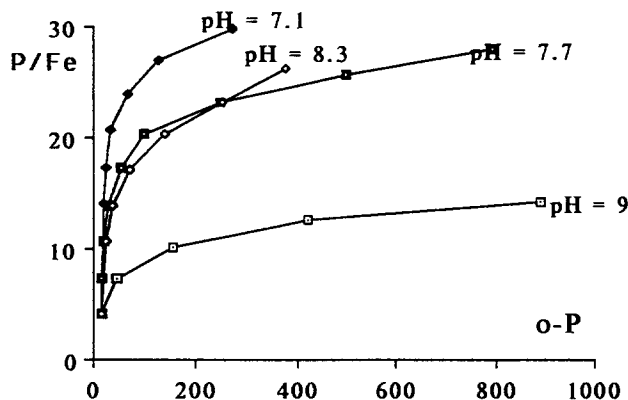


Fig. 11. Adsorption of o-P onto Fe(OOH). Y axis: $\mu\text{g P mg}^{-1}\text{ Fe}$; X axis o-P concentration in $\mu\text{g l}^{-1}$.

(II) and some examples are given in Fig. 11. Lijklema (1977) used a logarithmic description taking into account the influence of the pH on the position of the equilibrium:

$$P_{\text{ads}}/\text{Fe} = 0.298 - 0.031 \text{ pH} + 0.201 * (\text{o-P})^{0.5}. \quad (\text{III})$$

His data were later recalculated by Golterman (1982) as a Freundlich adsorption isotherm:

$$P_{\text{ads}}/\text{Fe} = A * (\text{o-P})^B. \quad (\text{IV})$$

A and B are constants under certain boundary conditions, while the constant A depends on the pH. Golterman (1991c) proposed originally $A = (\alpha + \beta * \text{pH})$ and $B = 0.2$, which values were only valid for $\text{pH} < 10.5$. From later and more experiments in which I measured the adsorption of o-P onto Fe(OOH) at different pH values, I found that the exponential curve

$$A = 23626.10^{-0.416 * \text{pH}} \quad (R^2 = 0.95; N = 13) \quad (\text{V})$$

fitted the data more satisfactorily (Golterman, 1995a). B fell in the range 0.25–0.6, but with B set at 0.333 the regression coefficient is not significantly smaller than with the values obtained by curve fitting. This means that the concentration in the sediments can be approached by the cube root of the o-P concentration, a fact we will use below (see page 54).

The constant A was found to depend not only on the pH, but on the Ca^{2+} concentration as well (4.2).

The question has been raised why the empirical Freundlich isotherm gives such a good fit of the observed data, as there is no chemical or physical model behind it. Theoretically I have now shown (Golterman, 1995b) that equation (II) is numerically correct, if we suppose that, for 1 molecule of P adsorbed, two Fe(OOH) molecules become inactive. A complex mathematical

formula is then obtained which under certain conditions is simplified to (IV). This argues strongly against the Langmuir adsorption isotherm, which supposes that there is a maximum amount of adsorption sites. In the sediments the amount of free Fe(OOH) and the pH control the adsorption capacity, through the parameters of the Freundlich adsorption constant A.

The amount of Fe(OOH) \approx P fixed in the sediments can be estimated by means of extraction with Ca-NTA under reducing conditions (Golterman & Booman, 1988, De Groot & Golterman, 1990). It is not yet known to what extent Fe-P-humic complexes are involved; the Ca-NTA extracts are always dark brown and interactions seem possible. But the main characteristic of this compound is Fe(OOH) \approx P, while humic acid is likely to be (loosely?) attached to it. The Ca-NTA extracts usually contain very little org-P, except with organic rich sediments as found e.g. in the Doñana.

Fe(OOH) constitutes a limited percentage of the Tot-Fe in shallow lakes and marshes, where a substantial part of Tot-Fe may be in the form of FeS during certain periods. Shifts between Fe(OOH) and FeS are likely to occur, depending on changes in redox and pH. Therefore, Fe(OOH) in Camargue and Garonne sediment suspensions was reduced to FeS by adding different quantities of Na_2S (De Groot, 1991). The changes in o-P and Fe(OOH) \approx P induced were followed in time. The increase in the o-P in the water was found to be smaller than predicted and an iron-bound phosphate fraction was shown to remain present, even when Na_2S was added in excess. This is supposedly caused by the formation of an iron-phosphate salt: FePO_4 or $\text{Fe}_3(\text{PO}_4)_2$ (vivianite) during the reduction of the Fe(OOH).

Next, the changes in the o-P were studied during the reduction of part of a Fe(OOH) suspension onto which o-P was adsorbed and during the oxidation of part of the FeS in a FeS suspension. These experiments confirmed the results of the experiments with mud. Furthermore, when Fe(OOH) \approx P suspension were treated with H_2S gas, only very small amounts of o-P were released even when the amount of H_2S converted about 90% of the Fe(OOH) into FeS (Golterman, 1995). Phosphate release under anoxic conditions normally does not come from this inorganic P-pool.

I think that changes in the Fe(OOH)/FeS system are more likely to have a significant impact on the o-P adsorption in shallow lakes and marshes than in deeper lakes, where only a small part of the Fe will be in the form of FeS because of the limited amount of reduc-

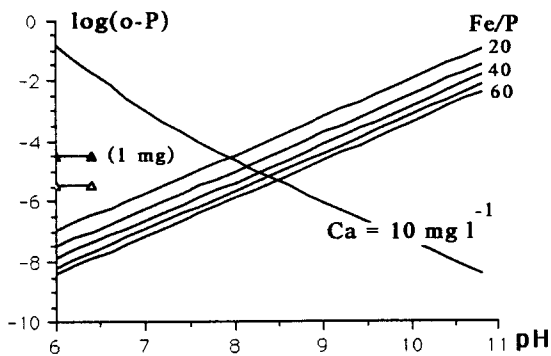


Fig. 12a. Solubility diagram for o-P as function of Ca^{2+} concentration in the water, $\text{Fe}(\text{OOH})$ concentration in the sediment and pH. The influence of the Ca^{2+} concentration on the adsorption of o-P onto $\text{Fe}(\text{OOH})$ is taken into account. (From Golterman, 1995) ($\text{Ca} = 10 \text{ mg l}^{-1}$; $\text{Fe}(\text{OOH}) = 5.6 \text{ mmol l}^{-1}$).

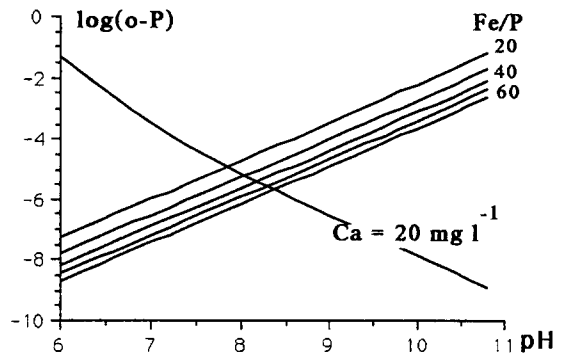


Fig. 12b. As Fig. 12a, but for $\text{Ca} = 20 \text{ mg l}^{-1}$.

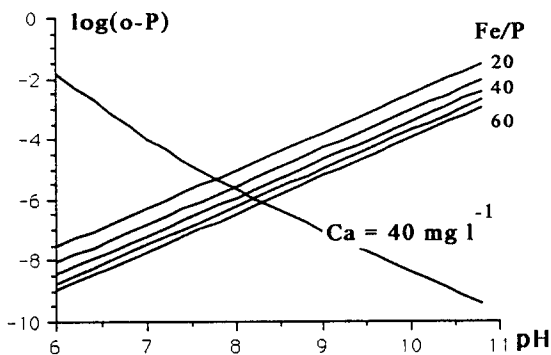


Fig. 12c. As Fig. 12a, but for $\text{Ca} = 40 \text{ mg l}^{-1}$.

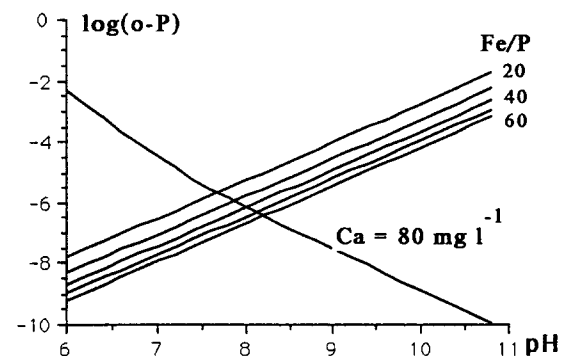


Fig. 12d. As Fig. 12a, but for $\text{Ca} = 80 \text{ mg l}^{-1}$.

ing power available (i.e. sinking detritus produced by phytoplankton (Golterman, 1984).

The precipitation process

A second factor controlling the equilibrium of o-P between water and sediments is the Ca^{2+} concentration in the water. The low solubility of apatite, $\text{Ca}_5(\text{PO}_4)_3\text{OH}$, is known to control the solubility of o-P in water (see review by Golterman, 1984). The precise value of the solubility product has remained a problem for a long time. Values between 10^{-50} and 10^{-60} have been proposed. A practical solution was given by Golterman and Meyer (1985), who determined the ionic product $(\text{Ca})^{5*}(\text{PO}_4)^{3*}(\text{OH})$ from 924 samples from the two hard water rivers, Rhine and Rhone. They found a mean value of 10^{-50} with a standard deviation of $10^{-0.3}$. It think it is now reasonable to use this solubility product for calculations of the solubility of o-P in hard water in equilibrium with the air. A discussion on the precision of this calculation

is given by Clymo and Golterman, 1985. Work is still needed on two uncertainties: the influence of ion activity coefficients and the great uncertainty of the K_3 of H_3PO_4 . It is amazing how poorly this constant has been measured.

When lake sediments acidify (e.g. by CO_2 production by mineralization), part of the $\text{CaCO}_3 \approx \text{P}$ will be solubilized, but if $\text{Fe}(\text{OOH})$ is present, it will be readsorbed onto this compound which has a stronger adsorption at lower pH values (see solubility diagram, Fig. 12).

The adsorption and precipitation processes taken together

Golterman (1988b) calculated the $\text{Fe}(\text{OOH}) \approx \text{P}$ and the $\text{CaCO}_3 \approx \text{P}$ equilibria together in a solubility diagram (see Fig. 12). This diagram describes the solubility of o-P as a function of the pH, the $\text{Fe}(\text{OOH})$ concentration in the sediment and the Ca^{2+} concentration in the water. The CaCO_3 concentration in the sediment is not important.

The diagram was intended as a first approach to modelling the equilibrium of phosphate between sediments and overlying hard water. The diagram, however, did not yet take into account the presence of humic-Fe(OOH)-phosphate complexes, nor the influence of Ca^{2+} on the adsorption of phosphate onto Fe(OOH), a rather recent finding. This development makes correction of the solubility diagram necessary. Our adsorption studies have always been carried out with Fe(OOH) in H_2O . We have now shown that NaCl, MgCl_2 and CaCl_2 , normally occurring compounds in freshwater, largely increase the phosphate adsorption onto Fe(OOH); when normal concentrations of these cations were added the amount of phosphate remaining in solution decreased by $> 50\%$. With NaCl and MgCl_2 this does not cause further problems as their influence can be quantified; with CaCl_2 we have the problem that the influence of Ca^{2+} on the adsorption process cannot yet be distinguished from the $\text{CaCO}_3 \approx \text{P}$ precipitation itself: Ca^{2+} added to the adsorption mixture disappeared from the solution. Work in this direction is in progress but difficult. At low o-P and pH values the o-P is so strongly adsorbed that little remains in solution; at higher concentrations and pH values apatite precipitation becomes important. I have shown experimentally (Golterman, 1995a) that the Freundlich adsorption constant can be approached by

$$A = 23626.10^{-0.416 \cdot \text{pH}} \cdot (2.86 - (1.86 \cdot e^{-\text{Ca}^{2+}})) \quad (\text{VI})$$

($N = 32$; $R^2 = 0.9$); ($10^{-0.416 \cdot \text{pH}}$ can be written as $(\text{H}^+)^{0.416}$).

With this equation the solubility diagram of o-P has been recalculated (see Figs 12a–12d). For each Ca^{2+} concentration a different solubility diagram must be calculated with Equation VI.

In shallow lake systems this leads to an equilibrium between o-P in water and sediments, which causes the sediments to accumulate much P. Furthermore, the presence of Ca^{2+} in the overlying water strongly limits the solubility of o-P, causing a precipitation reaction of phosphate above concentrations controlled by Ca^{2+} , pH and temperature.

A large part of phosphate in the sediments is available for phytoplankton. Space does not permit to treat bioavailability and fractionation of P-compounds in sediments, but as bioavailability is strongly related to the chemical nature of the phosphate compounds, we developed a fractionation scheme for sediment phosphate (Golterman & Booman, 1988; De Groot & Golterman, 1990). The finally developed scheme is

given in annex I. Bioavailability has been discussed by Golterman (1988c), while De Graaf Bierbrauwer-Würtz and Golterman (1989) showed that the sum of Ca and Fe phosphate in sediments is bioavailable for phytoplankton growth. This was an unexpected finding as apatite was supposed not to be available. This is probably true for the larger bluff erosion material, but not for apatite coprecipitated with CaCO_3 . Secondly, the equilibrium between $\text{Fe(OOH)} \approx \text{P}$ and $\text{CaCO}_3 \approx \text{P}$ will shift towards $\text{Fe(OOH)} \approx \text{P}$ when the $\text{CaCO}_3 \approx \text{P}$ is depleted by algae. Little is known about the availability of sediment nutrients for rooted macrophytes.

In several rivers Grobler & Davies (1979) and reservoirs in South Africa Grobler & Davies (1981) have shown that phosphate extractable with NTA (i.e. $\text{Fe(OOH)} \approx \text{P}$ plus $\text{CaCO}_3 \approx \text{P}$) was correlated ($R = 0.97$) with phosphate available for *Selenastrum capricornutum* in culture. Grobbelaar (1983) has shown that in the Amazon, the water of which contains low Ca^{2+} concentrations, most of the particulate phosphate (i.e. adsorbed onto the sediments as $\text{Fe(OOH)} \approx \text{P}$) was available for phytoplankton.

For shallow waters, where the o-P concentration is in equilibrium with the o-P concentrations, Equation (I) can be written as

$$P_{\text{ads}} + \text{o-P} = n \cdot L \quad \text{or} \quad A \cdot (\text{o-P})^B + \text{o-P} = n \cdot L, \quad (\text{VI})$$

where L = the net annual loading ($\text{g m}^{-2} \text{y}^{-1}$) and n = number of years.

As for natural sediments B is very often near 0.33 as well, we can rewrite (Eq. VI) as

$$\sqrt[3]{\text{o-P}} + \text{o-P} - n \cdot L = 0. \quad (\text{VII})$$

This equation has only one real root, which can be calculated (see Fig. 13). In this Figure it is shown how at first phosphate mainly enters the sediments while the concentration in the water increases only very slowly; later this concentration increases rapidly. Furthermore I have indicated in this Figure how the phosphate concentration will decrease, but only very slowly, when the input is eventually stopped: because of their buffering capacity the sediments will for a long time continue to release o-P into the water, which process may be slower than the building up stage. (For further details see Golterman, 1991c). In this way, we can visualize the principle of the influence of the phosphate binding capacity on the phosphate transport through, and its accumulation in, wetlands, but a precise quantification is unfortunately not yet possible. The situation is not different for other values of B , (e.g. between 0.3 and 0.45), but the calculations become difficult.

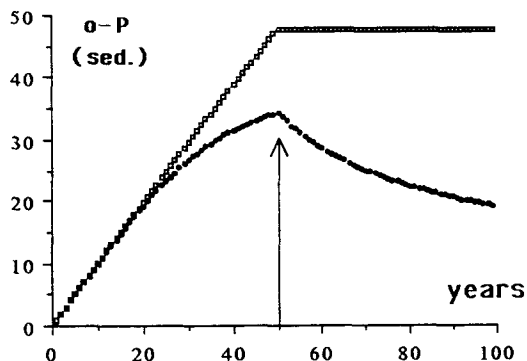


Fig. 13a. Changes over time of o-P_{sed} (mg m^{-2}) in a shallow mud/water system with a flushing rate of 1 y^{-1} (lower curve) and without flushing (upper curve).

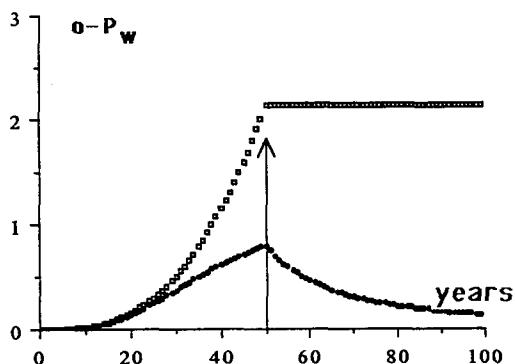


Fig. 13b. Changes over time of o-P_w (mg m^{-2}) in a shallow mud/water system with a flushing rate of 1 y^{-1} (lower curve) and without flushing (upper curve).

Organic phosphates

Besides the inorganic phosphates occurring in the sediments, organic phosphates are also found. Their extraction has so far been dubious. With the NTA and EDTA extractions usually only small amounts of org-P are extracted (Only in sediments rich in organic matter, such as are found in the Doñana National Park (Spain), somewhat larger amounts of org-P were found in these extracts). As it is unlikely that any inorganic P compounds remain in the pellet after extractions of iron and calcium bound phosphate, it is usually understood that the remaining P is organic. In the past attempts to extract these compounds failed, as, after the necessary hydrolysis, the o-P formed was rapidly adsorbed onto the $\text{Fe}(\text{OOH})$. Since in the present extraction scheme $\text{Fe}(\text{OOH})$ and CaCO_3 are extracted as well, this danger is no longer present. Therefore De Groot (1990) and De Groot & Golterman (1991) were able to analyse the org-P compounds further. It was found that a

considerable quantity of the org-P could be extracted with strong acids at low concentrations (e.g. 0.5 M H_2SO_4), but in a relatively short period, 30 min. This pool, called Acid Soluble Organic Phosphate (ASOP) appeared to be biologically active. When a Camargue marsh was left to dry, it was converted into o-P. (De Groot & Fabre, 1993). Work in progress has suggested that this pool is a humic bound phosphate – with the phosphate some Fe^{3+} always goes into solution as well, normally at a mol/mol ratio of 2 – 3. As the phosphate released at the same time becomes o-P, we have no evidence that the P was indeed bound to humic compounds, but it seems very likely.

The remaining P in the pellet can be extracted with hot 2 M NaOH. The P goes into solution as org-P; with 'phytase 2' it can be hydrolysed to o-P. (We notice that alkaline phosphatase did not release o-P.) This strongly suggests that the org-P was inositolhexaphosphate, phytate (De Groot & Golterman, 1993). Experiments with test compounds showed that it is probably $\text{Fe}_4\text{phytate}$. This compound could be prepared by adsorbing phytate onto $\text{Fe}(\text{OOH})$ and was shown not to be soluble in HCl, while Ca and Mg salts were soluble in HCl. Furthermore, $\text{Fe}_4\text{phytate}$ prepared in the laboratory was not extracted by Ca-NTA nor by 0.5 M HCl, and only partly by EDTA, but it was extracted by hot 2 M NaOH. $\text{Fe}(\text{OOH})$ with adsorbed phytate appeared to be protected against an attack with acid or chelating compounds, while free $\text{Fe}(\text{OOH})$ went into solution with H^+ . A white precipitate then formed with a ratio of 4.3 Fe per 6 o-P. Therefore, we think that we have obtained strong, but not yet conclusive evidence of the presence of humic bound phosphate and Fe-phytate in the org-P fraction of sediments. Further work on the identification of the organic part of these molecules seems to be important, together with analyses of other sediments.

Phosphate release during anoxic conditions in sediments will most likely come from these organic pools. After mineralization by bacteria, the part laying on top of the sediments can diffuse into the overlying water without interaction with $\text{Fe}(\text{OOH})$.

The question has been raised in the past, how organic phosphate in sediments could occur in such large amounts, most of these compounds being biodegradable. The presence of humic bound and inositol phosphate explains this, as both compounds form complexes which are highly resistant to bacterial attacks.

Conclusions

Although most processes concerning nutrients in wetlands are known, precise quantifications are still impossible, because the effects of the rate-controlling factors on these processes are still unknown. Nutrient budgets of wetlands, therefore, suffer from a great uncertainty and cannot as yet be used for management. In view of the large economic and other important aspects of the wetlands, studies should be directed towards a precise, quantitative understanding of denitrification, N fixation, mineralization, P binding onto sediments and the relationship between nutrient input and plant growth. This relationship is important for the quantification of the eutrophication problem, which in shallow lakes and marshes starts slowly, but once under way is difficult to reverse.

Denitrification is usually considered by environmentalists to be a Good Thing as it will partially protect against eutrophication in those systems where N is the plant growth limiting factor, but N-accumulation due to bacterial growth may occur. For the farmers it means an important economic loss and they will tend to use more and more nitrogen fertilizer. Its positive effect is, therefore, for wetland systems rather limited especially as some of the nitrate will be used for the increase of the bacterial populations(s) and thus will accumulate in the sediments. Denitrification in rice fields may be avoided by using urea as N-fertilizer. Urea rapidly diffuses into the anoxic layer of the sediments, where it is hydrolysed by urease to NH_3 . As in these deeper layers there is no O_2 and the NH_3 will be adsorbed onto the 'Cation Exchange Capacity', it is longer protected against losses than NH_3 used as fertilizer. In this way the NH_3 remains available a few weeks longer for the rice than added NH_3 (Golterman, Schouffoer & Bruijn, 1994).

Denitrification furthermore creates, in combination with phosphate binding, a system favourable for N-binding by cyanophytes or blue-green algae, as the two processes together yield a phosphate rich, but nitrogen poor system. The development of these organisms must be considered highly undesirable because of their nuisance properties. And when the blue-green algae have established themselves in the natural shallow waters of wetlands, it will be very difficult to eradicate them again; this is partly due to the accumulation of phosphate in the sediments. It is nearly impossible to remove the accumulated phosphate.

Golterman (1991c) found an unexpected link between S, N and P cycles: the denitrification by

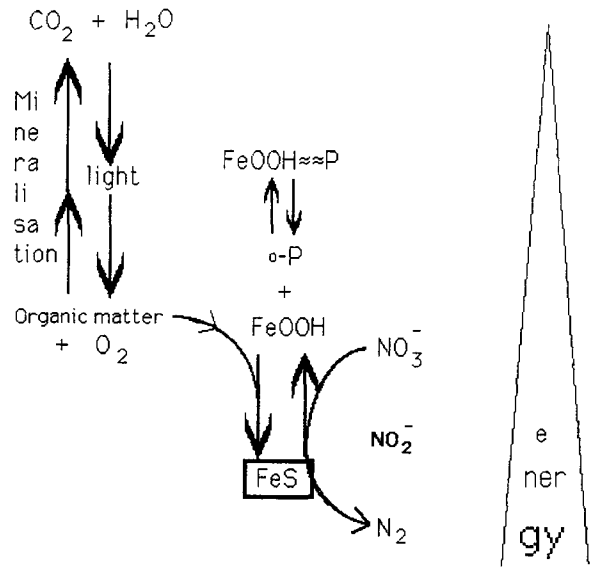


Fig. 14. Schematic relations between primary production, denitrification and phosphate binding onto sediments.

FeS produces $\text{Fe}(\text{OOH})$ which will strongly enhance P binding onto sediments, which in turn will become more oxidized and lose their toxic character for some parts of the vegetation. (Golterman, 1991b; De Groot, 1991). Figure 14 represents a schematic relation between these processes, but it remains difficult to develop a mathematical model combining them. The overall result is a loss of nitrogen compounds and a stronger fixation of phosphate, which together will produce a strong pressure towards blue green algal development. This will especially be the case in productive wetlands, where it is not desirable.

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References

- Arnold, E., 1994. Integrated water management in an irrigation-basin comprising wetlands and rice crops in the Camargue (South France). M. Sc. thesis, Faculty of Civil Engineering, University of Delft.
- Barko, J. W. & R. M. Smart, 1981. Sediment based nutrition of submersed macrophytes. *Aquat. Bot.* 10: 339–352.
- Bentzen, E. & W. D. Taylor, 1991. Estimating Michaelis-Menten parameters and lake water phosphate by the Rigler bioassays. *Can. J. Fish. Aquat. Sci.* 48: 73–83.
- Best, M. D. & K. E. Mantai, 1978. Growth of *Myriophyllum*: sediment or lake water as the source of nitrogen and phosphorus. *Ecology* 59: 1075–1080.
- Bonetto, C., F. Minzoni & H. L. Golterman, 1988. The Nitrogen cycle in shallow water sediment systems of rice fields, Part II. Fractionation and bioavailability of organic nitrogen compounds. *Hydrobiologia* 159: 203–210.
- Bornkamm, R. & F. Raggi-Atri, 1986. On the effects of different nitrogen and phosphate concentrations on the development of *Phragmites australis*. *Arch. Hydrobiol.* 105: 423–441.
- Boyd, C. E., 1970. Production, mineral accumulation and pigment concentrations in *Typha latifolia* and *Scirpus americanus*. *Ecology* 51: 285–290.
- Carignan, R. & J. Kalf, 1980. Phosphorus source for aquatic weeds: water or sediments? *Science* 207: 987–989.
- Carpenter, S. R. & M. S. Adams, 1979. Effects of nutrients and temperature on decomposition of *Myriophyllum spicatum* in a hard-water eutrophic lake. *Limnol. Ocean.* 24: 520–529.
- Cary, P. R. & P. G. J. Weerts, 1984. Growth and nutrient comp. of *Typha* as affected by temperature, N. and P. *Aquat. Bot.* 19: 105–118.
- Chapman, V. J., 1977. *Wet Coastal Ecosystems*. Elsevier Scientific Publishing Cy, Amsterdam, Oxford, New York, 428 pp.
- Clymo, R. S. & H. L. Golterman, 1985. Precision and accuracy of the determination of the ionic product of hydroxy-apatite. *Hydrobiologia* 126: 31–34.
- De Graaf Bierbrauwer-Würtz, I. M. & H. L. Golterman, 1989. Fosfaatfracties in de bodem van een aantal Nederlandse meren. (in Dutch, with English summary). *H₂O* 22: 411–414.
- De Groot, C. J., 1990. Some remarks on the presence of organic phosphates in sediments. *Hydrobiologia* 207: 303–309.
- De Groot, C. J., 1991. The influence of FeS on the inorganic phosphate system in sediments. *Verh. int. Ver. Limnol.* 23: 3029–3035.
- De Groot, C. J., 1992. Flux et transformations du phosphate et de l'azote dans les zones humides: La Camargue (Delta du Rhône). Thèse Université Claude Bernard, Lyon I.
- De Groot, C. J. & H. L. Golterman, 1990. Sequential fractionation of sediment phosphate. *Hydrobiologia* 192: 143–148.
- De Groot, C. J. & A. C. Fabre, 1993. The impact of desiccation of a freshwater marsh (Garcines Nord, Camargue, France) on the sediment–water–vegetation interactions. Part 3: The fractional composition and the phosphate adsorption characteristics of the sediment. *Hydrobiologia* 252: 105–116.
- De Groot, C. J. & H. L. Golterman, 1989. Gefractioneerde extracties van bodemfosfaten. *H₂O* 22: 66–67 (in dutch with English summary).
- De Groot, C. J. & H. L. Golterman, 1990. Sequential fractionation of sediment phosphate. *Hydrobiologia* 192: 143–148.
- De Groot, C. J. & H. L. Golterman, 1993. On the presence of organic phosphate in some Camargue sediments: evidence for the importance of phytate. *Hydrobiologia* 252: 117–126.
- De Groot, C. J. & C. Van Wijck, 1993. The impact of desiccation of a freshwater marsh (Garcines Nord, Camargue, France) on the sediment–water–vegetation interactions. Part 1: The sediment chemistry. *Hydrobiologia* 252: 83–94.
- El Habr, H., 1987. Les Elements nutritifs du Rhône: Leur Devenir dans les Canaux d'Irrigation et les Marais de Camargue. Thèse (N° d'ordre: 104–87), Université Claude Bernard, Lyon I.
- El Habr, H. & H. L. Golterman, 1990. In vitro and in situ studies on nitrate disappearance in water-sediment systems of the Camargue (southern France). *Hydrobiologia* 192: 223–232.
- Forés, E., M. Menéndez & F. A. Comin, 1988. Rice straw decomposition in rice-field soil. *Plant and Soil* 109: 145–146.
- Garcia-Gil, J. & H. L. Golterman, 1993. Influence of FeS on the denitrification rate in sediments from Camargue (France). *FEMS Microbiol. Ecol.* 13: 85–92.
- Gaudet, J. J., 1976. Nutrient relationships in the detritus of a tropical swamp. *Arch. Hydrobiol.* 78: 213–239.
- Godin, L., 1990. Impact de la Riziculture sur l'Hydrologie et la Qualité des Eaux en Camargue. Thèse, Université d'Avignon.
- Golterman, H. L., 1975. *Physiological Limnology. An approach to the Physiology of Lake Ecosystems*. Elsevier, Amsterdam, Oxford, New York, 489 pp.
- Golterman, H. L., 1977. Sediments as a source of phosphate for algal growth. In Golterman, H. L. (ed.), *Interactions between Sediments and Freshwater*. Dr W. Junk Publishers, The Hague: 313–317.
- Golterman, H. L., 1982. Loading concentration models in shallow lakes. *Hydrobiologia* 91: 169–174.
- Golterman, H. L., 1984. Sediments, modifying and equilibrating factors in the chemistry of freshwaters. *Verh. int. Ver. Limnol.* Vol. 22: 23–59.
- Golterman, H. L., 1988a. Chlorophyll – phosphate relationships as tool for water management. In Round, F. E. (ed.), *Algae and the aquatic Environment. Contributions in honour of J. W. G. Lund*. Biopress: 205–225.
- Golterman, H. L., 1988b. The calcium- and iron bound phosphate phase diagram. *Hydrobiologia* 159: 149–151.
- Golterman, H.L., 1988c. Reflections on fractionation and bioavailability of sediment bound phosphate. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 30: 1–4.
- Golterman, H. L., 1991a. Reflections on post O.E.C.D. eutrophication models. *Hydrobiologia* 218: 167–176.
- Golterman, H. L., 1991b. The influence of FeS on the denitrification rate. *Verh. int. Ver. Limnol.* Vol. 24: 3025–3028.
- Golterman, H. L., 1991c. Nutrient cycle interactions in the sediments of the Camargue wetlands. 4th Symposium Italian Ecologists, S.I.T.E., Atti 12: 801–806.
- Golterman, H. L., 1995a. The role of the ferric hydroxide-phosphate-sulphide system in sediment water exchange. *Hydrobiologia* 297: 43–54.
- Golterman, H. L., 1995b. Theoretical aspects of the adsorption of ortho-phosphate onto ironhydroxide. *Hydrobiologia* 315: 59–68.

- Golterman, H. L. & A. Booman, 1988. Sequential extraction of iron-phosphate and calcium-phosphate from sediments by chelating agents. *Verh. int. Ver. Limnol.* Vol. 23: 904–909.
- Golterman, H. L., F. A. Comin & I. Ferrari, 1992. Synthesis report to the E. C. Studies of the Nitrogen and Phosphate cycles in the Deltas of the Mediterranean rivers Ebro, Po and Rhone.
- Golterman, H. L. & F. A. Kouwe, 1980. Chemical budgets and nutrient pathways. Chapter 4. In *Functioning of freshwater ecosystems*. Ed. by E. D. Le Cren & R. H. Lowe-McConnell, IBP 22. Cambridge University Press, London, 588 pp.
- Golterman, H. L. & M. L. Meyer, 1985. The geochemistry of two hard water rivers, the Rhine and the Rhone. Part 4. The apparent solubility product of hydroxy-apatite. *Hydrobiologia* 126: 25–30.
- Golterman, H. L., A. B. Viner & G. F. Lee, 1977. Preface in Golterman, H. L. (ed.), *Interactions between Sediments and Freshwater*. Dr W. Junk Publishers, The Hague: 1–9.
- Grobbelaar, J. U., 1983. Availability to algae of N and P adsorbed on suspended solids in turbid waters of the Amazon River. *Arch. Hydrobiol.* 96: 302–316.
- Grobler, D. C. & E. Davies, 1979. The availability of sediment phosphate to algae. *Water SA* 5: 114–123.
- Grobler, D. C. & E. Davies, 1981. Sediments as a source of phosphate; A study of 38 impoundments. *Water SA* 7: 54–60.
- Hill, B. H., 1979. Uptake and release of nutrients by aquatic macrophytes. *Aquat. Bot.* 7: 87–93.
- Forés, E., M. Menéndez & F. A. Comin, 1988. Rice straw decomposition in rice-field soil. *Plant and Soil* 109: 145–146.
- Howard-Williams, C., 1985. Cycling and retention of N + P in wetlands: a theoretical approach. *Freshwat. Biol.* 15: 391–431.
- Kaplan, W. & I. Valiela, 1979. Denitrification in a salt marsh ecosystem. *Limnol. Oceanogr.* 24: 726–734.
- Kolthoff, I. M. & E. B. Sandell, 1946. *Textbook of Quantitative Inorganic Analysis*. Macmillan Company, New York, 794 pp.
- Lijklema, L., 1977. The role of iron in the exchange of phosphate between water and sediment. In Golterman, H. L. (ed.), *Interactions between Sediments and Freshwater*. Dr W. Junk Publishers, The Hague: 313–317.
- Lijklema, L., 1980. Interaction of ortho-phosphate with iron (III) and Aluminium hydroxides. *Envir. Sci. Technol.* 14: 537–541.
- Menéndez, M., E. Forés & F. A. Comin, 1989. *Ruppia cirrhosa* – Decomposition in a coastal temperate lagoon as affected by macroinvertebrates. *Arch. Hydrobiol.* 117: 39–48.
- Minzoni, F., 1987. Studio di processi legati ai cicli dell'azoto e del fosforo nell'ecosistema risaia. Thèse, Université de Parma (It.).
- Minzoni, F., C. Bonetto & H. L. Golterman, 1988. The nitrogen cycle in shallow water sediment systems of rice fields. Part I: The denitrification process. *Hydrobiologia* 159: 189–202.
- Monod, J., 1949. The growth of bacterial cultures. *Ann. Rev. Microbiol.* 3: 371–394.
- Mortimer, C. H., 1941. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.* 29: 280–329.
- Mortimer, C. H., 1942. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.* 30: 147–201.
- Murphy, J. & J. P. Riley, 1962. A modified single-solution method for the determination of soluble phosphate in sea water. *J. Mar. Biol. Ass. U. K.* 37: 9.
- O.E.C.D., 1982. *Eutrophication of waters: Monitoring, Assessment and Control*. Report of the O.E.C.D. Cooperative programme on Eutrophication. Prepared by Vollenweider, R. A. & J. Kerekes, O.E.C.D., Paris, 164 pp.
- Olsen, S., 1964. Phosphate equilibrium between reduced sediments and water, laboratory experiments with radioactive phosphorus. *Verh. int. Ver. Limnol.* Vol. 13: 915–922.
- Richardson, C. J., 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Science* 228: 1424–1427.
- Russell, E. W., 1973. *Soil conditions and plant growth*. 10th edn. Longman, London & New York, 840 pp.
- Sakamoto, M., 1966. Primary production by phytoplankton community in some Japanese lakes and its dependence on depth. *Arch. Hydrobiol.* 62: 1–28.
- Saunders, G. W., K. C. Cummins, D. Z. Gak, E. Pieczynska, V. Straskrabova & R. G. Wetzel, 1980. Organic matter and Decomposers. Chapter 7. In *Functioning of freshwater ecosystems*, E. D. Le Cren & R. H. Lowe-McConnell (eds), IBP 22. Cambridge University Press, London, 588 pp.
- Ulrich & Burton, 1985. Effects of nitrate, phosphate and K fertilization on growth and nutrient uptake patterns by *Phragmites*. *Aquat. Bot.* 21: 53–62.
- Valiela, I. & J. M. Teal, 1979. The N-budget of a salt marsh ecosystem. *Nature* 280: 652–657.
- Van Liere, L. & A. E. Walsby, 1982. Interactions of Cyanobacteria with light. In Carr, N. G. & B. A. Whitton (eds), *The Biology of Cyanobacteria*. Botanical Monographs Vol. 19. Blackwell Scientific Publications, Oxford: 9–47.
- Van Wijck, C., C. J. de Groot & P. Grillas, 1992. The effect of anaerobic sediment on the growth of *Potamogeton pectinatus* L.: the role of organic matter, sulphide and ferrous iron. *Aquat. Bot.* 44: 31–49.
- Wieder, R. K. & G. E. Lang, 1982. A critique of the analytical methods used in examining decomposition data obtained from litter bags. *Ecology* 63: 1636–1642.

Annex I

Extraction scheme

It may be useful for the understanding of the present work to describe the extraction scheme used to estimate the amounts of $\text{Fe(OOH)} \approx \text{P}$ or $\text{CaCO}_3 \approx \text{P}$. Golterman & Booman (1988) and De Groot & Golterman (1989, 1990) developed a sequential extraction scheme using Ca-NTA (with dithionite) to extract $\text{Fe(OOH)} \approx \text{P}$, followed by extraction of the $\text{CaCO}_3 \approx \text{P}$ by Na-EDTA. The results are reproducible and all test compounds were extracted as expected. After the removal of inorg-P by these extractions, a considerable amount of P remained fixed on the sediments. As the chelating extractants are milder than the classical extractants (NaOH and H_2SO_4), we believe that the remaining pool is organic – and is larger than has often been supposed (De Groot, 1990). A considerable part of the remaining pool is extractable with H^+ (Acid soluble org-P, ASOP); in the classical extractions this pool was extracted as $\text{CaCO}_3 \approx \text{P}$. After the ASOP extraction some phosphate still remains in the pellet. Nearly all of this can be extracted with hot NaOH; this phosphate is for the larger part a phytate/ Fe(OOH) complex. The complete extraction scheme is:

sediment		
↓ extraction with Ca-NTA, 0.02 M	→	$\text{Fe(OOH)} \approx \text{P}$
pellet		
↓ extraction with EDTA, 0.05 M	→	$\text{CaCO}_3 \approx \text{P}$
pellet		
↓ extraction with 0.5 M HCl (20 °C, 30 min)	→	ASOP (humic-P? see page 55)
ROP (Residual organic P)		
↓ extraction with 2 M NaOH (90 °C, 30 min)	→	probably phytate, see page 55
pellet nearly		
phosphate free		

NTA and EDTA solutions have the disadvantage that they interfere with the P determination: not more than 5 ml of the Ca-NTA solution can be taken and not more than 4 ml of the EDTA solution. However, the interference can also be prevented by taking a molybdate solution with 25 g l^{-1} of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in stead of the usual 9.6 g l^{-1} (Murphy & Riley, 1962). The molecular extinction coefficient does not change when 25 ml of Ca-NTA or 20 ml of EDTA are added in stead of 5 ml, but for the later extractant 10 ml acetone must be added (final volume 50 ml) in order to prevent precipitation of the blue colour; the absorbance maximum shifts to 815 nm, but does not decrease. If the molybdate with the higher concentration is used, it permits either larger volumes of the extractants for the colorimetric analysis (sediments with low $\text{Fe(OOH)} \approx \text{P}$ concentrations), or higher concentrations (sediments with high $\text{Fe(OOH)} \approx \text{P}$ concentrations). I found that with 0.04 M of Ca-NTA the extraction of Camargue sediments became more efficient, so that all $\text{Fe(OOH)} \approx \text{P}$ could be extracted in less extractions. The EDTA extractions are more efficient with EDTA, 0.1 M at $\text{pH} = 4.5$.