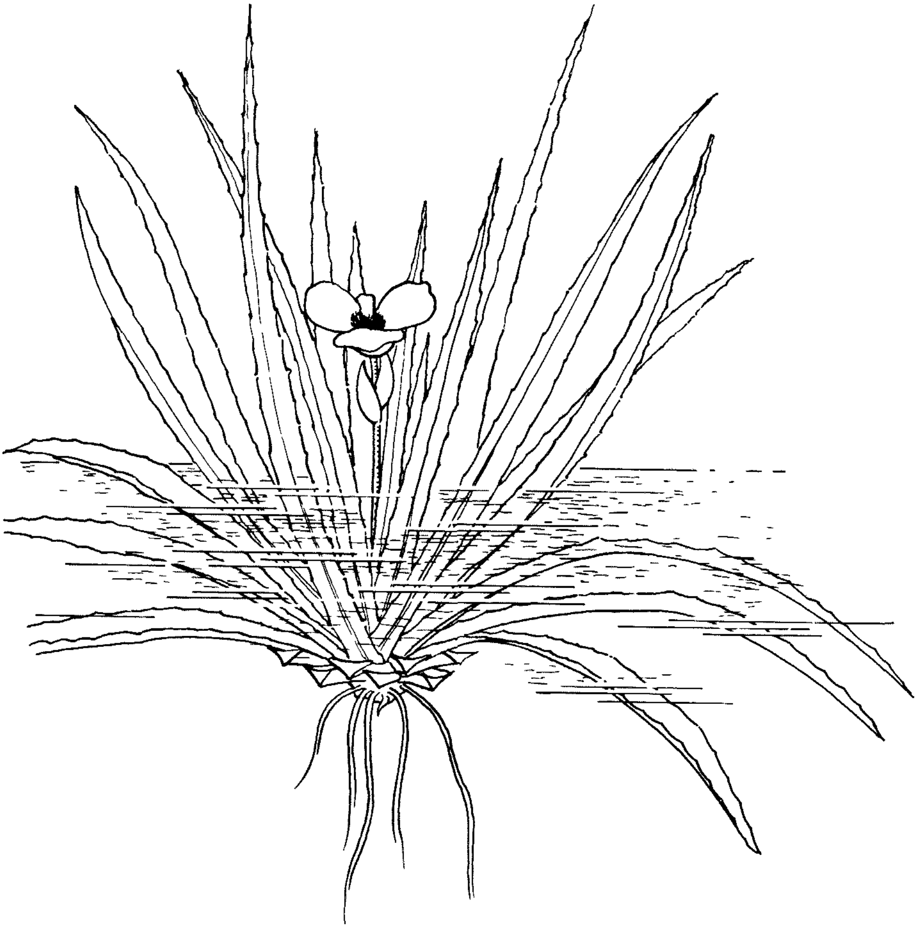
VEGETATION UNDER CHEMICAL STRESS: EFFECTS OF ACIDIFICATION, EUTROPHICATION AND ALKALINISATION



JAN ROELOFS

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VEGETATION UNDER CHEMICAL STRESS: EFFECTS OF ACIDIFICATION, EUTROPHICATION AND ALKALINISATION

een wetenschappelijke proeve op het gebied van de natuurwetenschappen,
in het bijzonder de biologie

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JOHANNES GISBERT MARIA ROELOFS

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Aan mijn ouders,
Voor José, Gijs, Anke en Janneke.

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VOORWOORD:

Het voor u liggende proefschrift wijkt af van een gangbaar proefschrift omdat niet één afgerond onderzoek wordt gepresenteerd. Dit hangt samen met het verloop van mijn onderzoekscarrière in de afgelopen 13 jaar. Telkens als een onderzoek goed op gang was gekomen toonden derden (Ministeries van VROM en Landbouw en Visserij) hier belangstelling voor. Door toegekende onderzoekssubsidies kon het onderzoek vervolgens uitgebreid worden en konden extra medewerkers worden aangetrokken. Om ook deze medewerkers de kans te geven een proefschrift te schrijven moest ik bepaalde onderzoekslijnen aan deze onderzoekers overlaten en derhalve zelf nieuw onderzoek starten.

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CHAPTER 1.

General introduction.

GENERAL INTRODUCTION.

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A short view on the titles in this thesis might lead to the conclusion that some of the described environmental changes and their biological implications have nothing in common. One chapter deals with the problem of shortage of "natural water" in peaty regions and the consequences of inlet of alkaline river water into surface waters in these regions. Two other chapters deal with the problem of water acidification as a result of atmospheric deposition of air pollutants and chapters 5-8 concern the effects of deposition of air pollutants on forest ecosystems.

However, a closer look at the involved mechanisms in biogeochemistry and plant nutrition shows that there are a lot of similarities. A lowering of the ground water level and drought stimulate acidification in both aquatic and terrestrial ecosystems. This is caused by a change in the balance between alkalinity producing and consuming biogeochemical conversions or nutrient uptake by plants.

Tabel 1.

Important acid consuming biogeochemical processes :

Sulphate reduction : $SO_4^{2-} + 2CH_2O + 2H^+ \text{---->} H_2S + 2H_2O + 2CO_2$
Denitrification : $4NO_3^- + 3CH_2O + 4H^+ \text{---->} 2N_2 + 7H_2O + 5CO_2$
Nitrate uptake : $NO_3^- + R.OH + H^+ \text{---->} R.NH_2 + 2O_2$

Important acid producing biogeochemical processes :

Sulphide oxidation : $H_2S + 2O_2 \text{---->} SO_4^{2-} + 2H^+$
Nitrification : $NH_4^+ + 2O_2 \text{---->} NO_3^- + 2H^+ + H_2O$
Ammonium uptake : $NH_4^+ + R.OH \text{---->} R.NH_2 + H^+ + H_2O$

In more or less organic wet soils reducing, acid consuming sulphur and nitrogen conversions take place, while in dry, aerated soils acid producing sulphur and nitrogen oxidation processes can be important (Stumm and Morgan, 1981). Denitrification as an alkalinity generating process does generally not lead to accumulation of potentially acidifying nitrogen compounds because of the fact that N_2 or N_2O diffuses from the soil into the atmosphere.

On the contrary, sulphur reduction leads to accumulation of potentially acidifying reduced sulphur components (Kelly and Rudd, 1982, 1984; Rudd et al., 1986; Schuurkes et al., 1988). This means that often a long term loading of those ecosystems with airborne nitrogen and sulphuric acid does not lead to severe acidification as long as the ecosystems remain wet. However, as a result of an increased water extraction for industrial and human purposes and a changed land use, there is a trend of decreasing water levels, and thus an increase in periods in which the upper soil layers of many naturally wet heathlands, peatlands and wetlands become aerated. Under these condition large quantities of sulphides, stored as e.g. FeS, are oxidized to sulphates, causing strong soil and water acidification (van Breemen, 1975; van Dam, 1987; Schuurkes et al., 1988).

In moorland pools this phenomenon could be observed in the years after the extremely dry year of 1976. The pH of the water dropped from circumneutral to values of on an average 3.8 (Roelofs and Schuurkes, 1983). The lowest pH recorded was as low as 2.9.

Between 1980 and 1988, a period with a number of wet years, the pH increased slightly. Investigations of van Dam (1987) made clear that the changes in diatom assemblages in Dutch moorland pools are strongly correlated with changes in pH. Changes in fish populations, macrofauna and aquatic macrophytes also appeared to be strongly correlated to water acidification (Roelofs and Schuurkes, 1983; de Lyon and Roelofs, 1986; Schuurkes, 1987; Leuven, 1988; Arts, 1990). Also the disappearance of a number of plant species from originally wet heathlands was clearly related to soil acidification. Species like *Lycopodium inundatum* L. and *Pedicularis sylvatica* L. appeared to be restricted to soils with a pH > 4.2, while places where these plant species disappeared had a pH < 4.0 (Roelofs et al., 1989; Houdijk, 1990).

In a part of the nature reserve "Staverden", a former wet heathland, the pH of the soil has dropped to values between pH 3.6 and 4.2 after drainage. Only acid resistant species like *Molinia caerulea* (L.) Moench. developed well under these conditions. After the building of a few dams in the drainage systems the upper soil layer became wet again and the soil water pH increased to values up to pH 5.8 at places with the highest ground water level (Cals and Roelofs, 1990; Cals et al., 1991).

In many peatlands in the Netherlands nowadays a landscape exists consisting of canals, dikes, ponds and shallow lakes. These waters are remnants of peat digging in former centuries. As a result of a changed land use and increased water extraction there is a shortage of water in most of these peatlands in summer. In order to prevent the peat from shrinking and acidification of the top layer, and also to meet the demand for agricultural purposes, an increasing amount of alkaline river water, rich in bicarbonate and sulphate, is led into these ecosystems (van Wirdum, 1979; 1989; Bloemendaal and Roelofs, 1988).

It has already been known for a long time that there is a correlation between water inlet and changes in water quality and macrophyte development (van Wirdum, 1979; 1989). The response of the dense stands of the water soldier (*Stratiotes aloides* L.) is most striking. In all systems (except for some small ditches) this vegetation disappeared after inlet of alkaline river water. The water became turbid and only a few plant species survived. It was not clear, however, why the *Stratiotes* plants disappeared. It is often stated that the direct eutrophication as a result of the inlet of river water rich in nutrients is at least partly responsible for the decreased water quality (Koerselman and Verhoeven, 1989). Alkalinisation, however, can also stimulate mineralisation processes by neutralizing organic acids (Kok et al., 1990). This not only leads to internal eutrophication but also to changes in the carbon sources. Prins and De Guia (1986) mention that *Stratiotes* needs relatively high amounts of carbon dioxide, while alkalinisation leads to a decrease in the CO_2 levels in the water layer.

The strongly increased sulphate concentrations can also contribute to the observed eutrophication processes (Curtis, 1989). Possibly not only the increased mobility of phosphorus, but also the increased levels of reduced components such as sulphides and ammonium/ammonia play a role (Bloemendaal and Roelofs, 1988).

It was also observed that there were changes in the balance between oxidized and reduced nitrogen compounds in acidifying moorland pools and heathlands. As a result of decreased nitrification rate and high atmospheric inputs of ammonium the nitrate levels decreased and the ammonium levels increased (Roelofs and Schuurkes, 1983; Schuurkes, 1987; Bloemendaal and Roelofs, 1988; Boxman et al., 1988).

In forest ecosystems, however, the situation is more complicated. There are forest ecosystems in which airborne ammonium sulphate, even in very acid soils, is quickly nitrified (van Breemen et al., 1982; 1983). This nitrification causes severe soil acidification. In other acid forest soils, however, the nitrification is inhibited, which leads to increasing levels of ammonium in the top soil layer (Kriebitzsch, 1978; Boxman et al., 1988).

It is clear from a number of studies that the changes in aquatic and terrestrial vegetation during alkalinisation and acidification are correlated with the pH of water and soil. It is not clear, however, whether these changes are caused directly by changes in the proton load or by the concomittant changes in mineral balances or toxic components in aquatic and terrestrial ecosystems.

The work, presented in this thesis, deals with three topics:

1. Alkalinisation in peatlands (chapter 2).
2. Acidification and eutrophication in soft water ecosystems (chapter 3 and 4).
3. Soil acidification and nitrogen enrichment in heathlands and forest ecosystems (chapter 5, 6 , 7 and 8).

In all studies the same research strategy was followed. Each investigation started with a correlative field study on a number of sites in which plant distribution and/or plant vitality were correlated with a number of environmental factors in order to formulate a working hypothesis.

Causalities between plant development and/or vitality and a selected number of abiotic parameters were studied in the laboratory, using various ecophysiological techniques.

Finally, in order to translate laboratory studies more or less to the field situation, culture experiments were carried out in small-scale ecosystems under fully controlled conditions in the laboratory and under less controlled conditions in e.g. concrete tanks in the open air.

In chapter 2 the results are presented of an investigation on the effects of inlet of alkaline river-type water, rich in sulphate, into surface waters in peaty lowlands. During field studies a comparison was made between two identical shallow surface waters. One of the investigated waters was isolated from incoming river-type water and characterized by dense stands of the water soldier (*Stratiotes aloides*). In the second water the *Stratiotes* plants had disappeared after inlet of alkaline, river- type water from the IJsselmeer.

In the laboratory a number of eco-physiological and culture experiments were carried out with *Stratiotes aloides* plants and, finally, inlet of alkaline water rich in sulphate was simulated in artificial, small scale ecosystems.

In the chapters 3 and 4 the results are presented of an investigation on the effects of acidification and eutrophication on macrophyte communities in soft water ecosystems. The investigations started with a correlative field study on the distribution of soft water species in relation to a number of environmental factors. The results of these investigations are presented in chapter 3. Finally a number of eco-physiological and culture experiments were carried out with plants from poorly buffered sites, eutrophicated sites and acidified sites. These results are presented in chapter 4.

A short review on the effects of airborne sulphur and nitrogen deposition on aquatic and terrestrial heathland vegetation is presented in chapter 5.

Chapter 6 deals with the effects of airborne ammonium sulphate deposition on forest stands. Precipitation at various distances from chicken farms and fields dressed with animal slurry has been analyzed. Soil and needle analyses were carried out in a number of stands of *Pinus nigra* var. *maritima* (Ait.) Melville (corsican pine). Ecophysiological experiments were carried out in order to estimate whether ammonium deposition stimulates nutrient leaching in the crown.

In chapter 7 the results are presented of an investigation on the effect of changed nitrogen nutrition on nutrient fluxes in *Pinus sylvestris* L. (scots pine) seedlings.

Chapter 8 is a review paper concerning changes in nutrient fluxes in canopies and roots of coniferous trees as affected by nitrogen enriched air pollution, and finally, chapter 9 gives a synthesis of all the results presented in this thesis.

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CHAPTER 2.

Inlet of alkaline river water into peaty lowlands: Effects on water quality and *Stratiotes aloides* stands.

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Inlet of alkaline river water into peaty lowlands: effects on water quality and *Stratiotes aloides* L. stands

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ABSTRACT

Roelofs, J.G.M., 1991. Inlet of alkaline river water into peaty lowlands: effects on water quality and *Stratiotes aloides* L. stands. *Aquat. Bot*, 39: 267–293.

As a result of changed land use and concomitantly increased drainage, in combination with increased use of water for industrial, agricultural and other human purposes, there is an increasing shortage of "natural" water in many areas of The Netherlands in summer. To compensate for this shortage, increasing amounts of hard water from the Rivers Rhine and Meuse are diverted to these lowlands.

It is commonly assumed that the disappearance of *Stratiotes aloides* L. in peaty lowlands is caused by a decline of the water quality, owing to high concentrations of nutrients in the incoming water. However, field observations in "de Weerribben", a large mire complex in The Netherlands, indicated that the decline of the water quality in this area is caused by an altered ion composition of the water. The incoming Rhine-type water from Lake IJsselmeer is very alkaline, rich in HCO_3^- and SO_4^{2-}. These anions appear to be responsible for a change in the decomposition processes. Consequently, the peat becomes very fine and soft, and because of increased bioturbation the water becomes turbid. As a result of increased reduction processes in the soil there is an increased mobility of phosphorus, leading to internal eutrophication and greatly increased levels of reduced compounds such as S^{2-}, CH_4 and NH_4^+ in the soft, upper layer of the bottom. Transplantation experiments with *Stratiotes aloides* plants showed that the plants started to decay in winter, after migration to the soft, upper sapropel layer. Culture experiments proved that the S^{2-} and NH_4^+ levels in this layer are lethal to *Stratiotes* plants.

INTRODUCTION

As a result of changed land use and waterwinning there is often shortage of water in many lowlands in The Netherlands in summer. Lowering of the water level can cause serious damage, particularly in peatlands, because of the fact that the peat shrinks, and this is an irreversible process. To prevent damage and also to meet the demands of agriculture, an increasing amount of water,

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mostly river water, is brought into these lowlands. Already more than 60% of the surface waters in The Netherlands are partly fed by the River Rhine (Bloemendaal and Roelofs, 1988). In the same period the water quality in these surface waters decreased rapidly.

The surface waters in peatlands, which originate from peat digging in earlier centuries, were characterised by dense stands of the water soldier (*Stratiotes aloides* L.) until quite recently. It had already been observed (1950–1970) that an increased input of river water leads to changes in water quality and the composition of the macrophyte flora (van Wirdum, 1979, 1989). Most of the aquatic macrophytes, such as *Potamogeton compressus* L., *Potamogeton alpinus* Balb., *Potamogeton obtusifolius* Mert. & Koch, *Potamogeton acutifolius* Link and also several Characeae disappeared or decreased in numbers. The most striking change, however, was the disappearance of the dense *Stratiotes* stands. The water became very turbid, often without any submerged macrophyte species.

Up to now it has been supposed that eutrophication as a result of supply with heavily (P and N) polluted river water was the direct cause for the changes in the composition of the flora. However, in waters supplied with river water poor in nutrients, the same changes in water quality and macrophyte development occurred. Therefore, it is considered possible that the alkalinisation of the surface waters in the peatlands, as a result of the supply of alkaline sulphate- and bicarbonate-rich water, leads to internal eutrophication in these shallow, peaty, aquatic ecosystems (Bloemendaal and Roelofs, 1988; Roelofs and Cals, 1989). Several authors state that alkalinisation leads to high sulphide and ammonium concentrations and to P-mobilisation from the sediment (Traaen, 1980; Canfield, 1983; Schindler, 1985; Brock et al., 1985; Schindler et al., 1986; van Katwijk and Roelofs, 1988; Curtis, 1989).

In this study, the role of alkalinisation and sulphate enrichment in the eutrophication processes has been examined. Investigations have been carried out during one year in two surface waters in a peatland. One of the waters was connected to a canal system and in dry summer periods it received Rhine-type water from the IJsselmeer. The second water was isolated from the first by a 20-m wide peat strip.

The isolated system was characterised by a dense stand of *Stratiotes aloides*, whereas in the open system this plant species was absent. Apart from physical and chemical analyses of plants, water and soil, transplantation experiments with *Stratiotes* plants from the isolated to the open system have been carried out. The effects of increasing bicarbonate, sulphate, chloride or combinations of these ions on water quality development have been studied in a series of artificial ponds in the grounds of the Catholic University of Nijmegen. Further, culture experiments with *Stratiotes aloides* plants have been carried out in the laboratory under fully controlled conditions in staged gradients of sulphide and ammonium. Finally, the effect of increased ammonium concentrations on nutrient fluxes in *Stratiotes* plants has been studied.

STUDY SITES

Two small, shallow waters were selected in the mire complex "De Weerribben" in the northwestern part of the province of Overijssel in The Netherlands (NE). In this large peatland area, hundreds of waters exist as a result of the removal of peat in former centuries. Most of them are small and shallow, 100–200 m long, 30–50 m wide and 0.5–1.5 m deep. The waters are separated from each other by peat strips 5–20 m wide. Most of these waters, called "petgaten", are connected to each other by a system of canals and have received river- (Rhine) type water from the IJsselmeer for a few decades. Prior to the introduction of IJsselmeer water into the system, most of these waters were characterised by dense stands of *Stratiotes aloides*. Nowadays, dense stands of *Stratiotes* are restricted to waters that are isolated from the canal system.

One of the two investigated waters is isolated, thus receiving no river-type water. It is characterised by a dense stand of *Stratiotes* and is referred to as "Weerribben isolated" (W.I.). The second petgat is separated from the first by a 20-m-wide peat strip. This water is connected to the canal system and receives river-type water from the IJsselmeer in dry periods. It is referred to as "Weerribben open" (W.O.). In this water the *Stratiotes* vegetation has disappeared.

MATERIALS AND METHODS

Field studies

From January to December 1981, water samples were collected fortnightly at three sampling sites in De Weerribben. One of the sites was situated in the open system (W.O.) and two were situated in the isolated system (W.I.), one inside and one outside the dense *Stratiotes aloides* vegetation.

Each sample consisted of 21 subsamples of 400 ml, collected at seven locations at depths of 10, 30 and 60 cm. Immediately after sampling the pH was measured using a Metrohm Model E488 pH meter and a Model EA152 combined electrode. Oxygen was measured using a Yellow Springs Model 54 oxygen meter and turbidity with a Denton FN5 turbidity meter. Alkalinity was estimated by titrating 100 ml of water with 0.01 M HCl down to pH 4.2. From the remaining water, 200 ml were passed through a Whatman GF/C filter and stored at −28 °C until analysis.

Soil samples were taken every month with an Ekman soil sampler. On each location three subsamples were collected in a polyethylene bag. After 24 h the redox potential was determined using a Metrohm type E488 pH/mV meter and a combined platinum electrode. The water content was estimated by weighing, drying (24 h, 105°C) and reweighing of 500-ml soil samples. The soil water was collected by centrifugation of a 400-ml soil sample (10 min, 5000 rpm).

For chemical analysis of the soil, 100 mg of a well-mixed, dry soil sample were combusted (in triplicate) under pressure in a mixture of 0.5 ml perchloric acid and 0.2 ml sulphuric acid, according to Kotz et al. (1972). For all analyses calcium, magnesium, iron and manganese were estimated with a Beckman Model 1272 Atomic Absorption Spectrophotometer, and sodium and potassium were estimated flame-photometrically, using a Technicon I Auto Analyzer. The following were measured colorimetrically with a Technicon II Auto Analyzer: orthophosphate according to Henriksen (1965), nitrate according to Kamphake et al. (1967), ammonia according to Grasshoff and Johannsen (1977), chloride according to O'Brien (1962) and sulphate according to Technicon Auto Analyzer Methodology (1981). Total phosphorus and total nitrogen were determined after UV destruction with a Technicon II Auto Analyzer and sulphide by means of a type Orion 94-16A sulphide ion-specific silver electrode. A double junction calomel electrode served as a reference. Both electrodes were connected to a Metrohm Model E488 pH/mV meter. Samples of 10 ml were taken and immediately fixed with a sulphide anti-oxidant buffer (SAOB), containing sodium hydroxide, sodium EDTA and ascorbic acid (van Gemerden, 1984). Dissolved organic carbon (DOC) and carbon dioxide were estimated with a modified Horiba PIR 2000 carbon analyser according to Roelofs (1983). Methane (CH_4) production was measured after incubation of 50 ml soil in 200-ml bottles under anaerobic conditions in the dark (20°C), using a gas chromatograph.

On 13 May 1981, 60 well-developed *Stratiotes aloides* plants from the isolated pond (W.I.) were transferred to the non-isolated pond (W.O.). To keep the plants together, the plot of ~ 16 m^2 was surrounded by wire netting. Every 4 weeks five plants were collected for analysis from both the natural stand and the artificial plot. Vitality was expressed as the percentage of the plant parts that were green (excluding the roots).

Experiments in artificial ponds

Experiments were conducted in 12 coated 720-l concrete tanks (150 cm long, 80 cm wide, 60 cm deep). After adding 60 l of freshly dug peat from an undisturbed peatland in the neighbourhood of the investigated ponds W.I. and W.O., the tanks were filled with 600 l of tap water. By adding different amounts of HCl, Na_2SO_4, NaCl and $NaHCO_3$ to the tap water in the different tanks, four staged gradients of bicarbonate, sulphate, chloride and a combination of these anions were created. The concentrations of these elements at the beginning of the experiment are shown in Table 1. In every tank seven

TABLE 1

Effects of the initial macro-ion composition on water quality and the vitality of *Stratiotes aloides* plants in artificial aquatic ecosystems (coated concrete tanks) with an undisturbed peat layer. Values of five measurements from June to November 1982. DOC, S^{2-} and vitality of the *Stratiotes aloides* plants were estimated in August 1982. The vitality is expressed as a percentage of the leaf area that is green

	Anion composition (μmol l^{-1})			pH			O_2 (mg l^{-1})			DOC (mg C l^{-1})	Turbidity (ppm Pt l^{-1})			Total P (μmol l^{-1})			Total N (μmol l^{-1})			S^{2-} (μmol l^{-1})	Vitality (% green)
	HCO_3^-	SO_4^{2-}	Cl^-	Mean	Min.	Max.	Mean	Min.	Max.		Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.		
a	500	250	1000	5.9	5.4	6.6	5.6	3.2	9.2	1.8	8	7	10	0.95	0.75	1.25	86	65	100	0.00	90
	1000	250	1000	6.4	5.5	7.0	3.5	0.5	8.1	4.3	40	16	46	2.10	1.50	2.25	220	130	275	2.20	80
	3000	250	1000	7.7	7.3	8.6	2.4	1.4	4.1	6.5	70	28	88	3.25	2.25	3.50	257	170	350	2.50	50
b	1000	250	1000	6.4	5.5	7.0	3.5	0.5	8.1	4.3	40	16	46	2.10	1.50	2.25	220	130	275	2.20	80
	1000	500	1000	8.1	6.8	9.3	6.0	4.1	7.9	2.3	5	4	5	0.75	0.50	1.00	81	65	95	0.00	80
	1000	1000	1000	7.9	7.0	8.5	6.2	5.1	7.2	1.7	5	4	8	1.45	1.25	1.75	99	80	115	0.00	70
	1000	2000	1000	7.8	6.7	8.5	5.7	4.2	8.1	1.8	4	3	7	1.30	1.00	1.75	86	70	105	0.00	80
c	1000	250	1000	6.4	5.5	7.0	3.5	0.5	8.1	4.3	40	16	46	2.10	1.50	2.25	220	130	275	2.20	80
	1000	250	3000	7.5	6.6	8.0	5.3	4.2	7.8	2,2	3	2	5	0.90	0.50	1.25	93	80	105	0.90	80
	1000	250	5000	7.6	6.4	8.2	4.4	2.8	7.3	1.6	4	3	8	2.00	0.75	3.75	91	75	100	1.20	55
	1000	250	7000	8.0	6.6	8.9	5.9	3.5	8.0	1.1	5	3	13	1.35	0.75	2.25	87	70	95	1.50	50
d	500	250	1000	5.9	5.4	6.6	5.6	3.2	9.2	1.8	8	7	10	0.95	0.75	1.25	86	65	100	0.00	90
	2000	600	1700	7.3	6.5	7.7	4.5	1.5	9.3	4.7	25	20	36	2.05	1.30	2.25	215	105	280	3.00	55
	3300	950	2400	7.8	7.2	8.4	3.1	0.5	6.9	5.7	30	14	36	1.80	1.25	2.25	227	125	300	9.40	45

healthy *Stratiotes aloides* plants, collected in De Weerribben, were introduced on 12 February 1982. To estimate the effect of bicarbonate on sulphate and sulphate reduction, two tanks were arranged as described above. Both tanks had an initial sulphate concentration of 500 μmol l^{-1} and bicarbonate concentrations of 380 and 1000 μmol l^{-1}.

Each month, from June to November, oxygen, pH, alkalinity, turbidity and sulphide were measured directly and water samples were taken for chemical analysis. On 3 August, the vitality of the *Stratiotes* plants was estimated by determining the percentage of the leaf surface that was green.

Culture experiments with Stratiotes aloides

Technical equipment

The experiments were conducted in glass containers (25 cm long, 25 cm wide, 31 cm deep), which were placed in a stainless steel water bath. The temperature was maintained at 17 °C by means of a Neslab type coolflow 75 cooling/heating aggregate. The culture medium was refreshed continuously (1 l h^{-1}) from black polyethylene 120-l stock containers by means of multichannel peristaltic pumps. Black silicone tubing was used to prevent nutrient consumption by algal growth. Irradiance in this facility was provided by 400-W high pressure metal halide lamps at a photo-period of 14 h at 250 μE m^{-2} s^{-1}.

Culture medium

The basic culture medium (Table 2) was composed synthetically by adding chemicals to twice demineralized water. The concentration of each element was within the range of the observed concentrations in waters with natural stands of *Stratiotes aloides* (de Lyon and Roelofs, 1986). A staged ammo-

TABLE 2

Chemical composition of the basic culture medium

Major components	(μmol l^{-1})	Trace elements	(μmol l^{-1})
Na^+	620 or 1120	Fe	2 00
K^+	150 or 100	Cu	0 40
Mg^{2+}	60	Mn	2 70
Ca^{2+}	125	Zn	0 30
Cl^-	610	Mo	0 50
SO_4^{2-}	250	Co	0 01
NH_4^+	10 or 0	Sr	0 02
NO_3^-	20 or 0	Bo	0 04
PO_4^{3-}	0.5	Br	0 06
HCO_3^-	0 or 500		

nium gradient was created by adding different amounts of NH_4Cl to the stock solution and a sulphide gradient was created by continuous inflow of equivalent amounts of Na_2S and HCl from 1 M stock solutions by means of a multichannel peristaltic pump. Hydrochloric acid was added to keep the pH constant, as Na_2S reacts as a base. In both the ammonium and sulphide gradients two plants were used for each step. However, the ammonium gradient was set up in duplicate, the sulphide gradient was not. After a cultivation period of 10 weeks, the vitality of the plants was estimated as the percentage of the plant parts that were green (excluding the roots). The used plant material was collected from the *Stratiotes* stands in De Weerribben. Before starting the experiments with ammonium and sulphide, the plants were growing in the experimental setup in basic culture medium for 2 weeks.

Nutrient uptake

For these experiments *Stratiotes* plants from the investigated stand in De Weerribben were pre-incubated in the basic culture medium in the experimental setup as described above. After an acclimatisation period of 7 weeks in media containing nitrate or nitrate plus ammonium, two plants (replicated five times) were brought into glass incubation chambers consisting of two compartments: a plant compartment of 20 l, and a root compartment of 2 l. The plant and root compartments were separated by a silicone septum. The experimental media in both compartments were continuously refreshed ($1\ l\ h^{-1}$ and $0.1\ l\ h^{-1}$ for the plant and root compartments, respectively) by means of multichannel peristaltic pumps. Experiments were carried out in culture media containing 0 or 100 $\mu mol\ NH_4^+\ l^{-1}$ and 0 or 500 $\mu mol\ HCO_3^-\ l^{-1}$ under both light ($250\ \mu E\ m^{-2}\ s^{-1}$) and dark conditions.

The nutrient fluxes were calculated from the nutrient concentrations measured in inflow and outflow of the plant and root compartments. Also the oxygen levels in both the plant and root compartments were measured during a light and dark period of 8 h each.

RESULTS

Field investigations

Table 3 shows clearly the influence of the hard IJsselmeer water on the chemical composition of the non-isolated shallow, peaty, softwater ecosystem W.O. (Weerribben open). In contrast to what was expected, it appeared that the nutrient levels of Lake IJsselmeer were not higher than those in the isolated peaty softwater system W.I. The concentrations of the major ions sodium, magnesium, calcium, chloride and bicarbonate are low in the isolated system and much higher in the non-isolated system. Only the sulphate level had hardly increased as a result of water supply from the IJsselmeer. Although

TABLE 3

Physico-chemical properties of the investigated aquatic ecosystems during 1982. Weerribben Open (W.O.) is receiving water from Lake IJsselmeer. Water analyses were carried out twice a month (except for Lake IJsselmeer), soil water and soil analyses once a month

(A) Surface water

Water	n	PO_4^{3-} (µmol l^{-1})			Total P (µmol l^{-1})			NH_4^+ (µmol l^{-1})		
		Mean	Min.	Max.	Mean	Min.	Max	Mean	Min	Max
Weerribben isolated (W.I.)	24	0.69	0.30	1.15	2.4	0.2	6.8	6.4	2.5	10.0
Weerribben open (W.O.)	24	0.90	0.32	2.34	2.4	0.5	3.9	6.7	4.0	35.4
Lake IJsselmeer	2	0.90	-	-	-	-	-	5.7	-	-

Water	NO_3^- (µmol l^{-1})			HCO_3^- (µmol l^{-1})			SO_4^{2-} (µmol l^{-1})			Cl^- (µmol l^{-1})		
	Mean	Min	Max.	Mean	Min	Max.	Mean	Min	Max.	Mean	Min	Max
Weerribben isolated (W.I.)	2.8	1.0	6.0	420	150	470	220	85	260	520	440	540
Weerribben open (W.O.)	2.4	0.0	34.6	1840	1340	2013	250	160	440	1150	910	1240
Lake IJsselmeer	1.4	-	-	2900	-	-	1535	-	-	2095	-	-

Water	Ca^{2+} (µmol l^{-1})			Mg^{2+} (µmol l^{-1})			K^+ (µmol l^{-1})			Na^+ (µmol l^{-1})		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min	Max.
Weerribben isolated (W.I.)	90	63	140	150	119	165	40	20	70	420	380	505
Weerribben open (W.O.)	435	275	510	400	306	450	90	65	120	1150	955	1240
Lake IJsselmeer	730	-	-	520	-	-	235	-	-	2600	-	-

(B) Soil-water

	n	Eh (mV)	pH			S^{2-} (µmol l^{-1})		
			Mean	Min.	Max.	Mean	Min	Max.
Weerribben isolated (W.I.)	12	−105	5.7	5.2	6.1	2.3	0.3	3.6
Weerribben open (W.O.)	12	−172	6.7	6.1	6.9	12.9	6.7	19.1

	PO_4^{3-} (µmol l^{-1})			NH_4^+ (µmol l^{-1})			NO_3^- (µmol l^{-1})		
	Mean	Min	Max	Mean	Min.	Max.	Mean	Min	Max.
Weerribben isolated (W.I.)	2.3	0.4	4.8	199	81	301	3.4	0.5	10.0
Weerribben open (W.O.)	24.1	4.4	45.8	638	150	1020	5.4	0.5	15.0

	HCO_3^- (µmol l^{-1})			CO_2 (µmol l^{-1})			CH_4 (µmol l^{-1})		
	Mean	Min.	Max.	Mean	Min.	Max	Mean	Min	Max
Weerribben isolated (W.I.)	1121	650	1670	3772	880	6150	60	-	-
Weerribben open (W.O.)	3892	2080	5720	4733	950	10010	568	-	-

(C) Soil (mmol kg^{-1} dry weight)

	P			N			C		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min	Max.
Weerribben isolated (W.I.)	26	19	34	1397	1077	1717	27.8×10^3	19.8×10^3	35.1×10^3
Weerribben open (W.O.)	33	24	45	1465	1192	1737	28.8×10^3	21.1×10^3	35.4×10^3

–, Data not available.

the actual nutrient levels in the open system were not higher than those in the isolated system in the summer, there were clear signs of eutrophication. The water was more turbid and there was no well-developed vegetation of aquatic plants. Only in very shallow sites was there some development of the non-rooted species *Utricularia vulgaris* L. and *Ceratophyllum demersum* L. In less shallow sites there were only some small stands of the nymphaeid *Nuphar lutea* (L.)Sm. The water in the isolated system, however, was clear and showed a well-developed aquatic vegetation dominated by *Stratiotes aloides*. Abundant accompanying species were *Utricularia minor* L., *Hydrocharis morsus-ranae* L., *Lemna trisulca* L. and *Nymphaea alba* L. and less abundant *Potamogeton obtusifolius* Mert. & Koch and *Equisetum fluviatile* L. (Table 4). The water was less turbid and had consistently low inorganic nitrogen levels.

In the open system, however, both the inorganic and organic nitrogen levels increased markedly in the winter (Fig. 1). The total phosphorus concentration also was much higher during the winter and spring. In the summer, however, the free inorganic phosphorus concentration decreased very rapidly to very low levels, while in the same period the opposite took place in the isolated system in the water layer below the emerged *Stratiotes* vegetation (Fig.

TABLE 4

Species composition of the aquatic vegetation in the investigated petgaten

Species	Open petgat W.O.	Isolated petgat W.I.
Stratiotes aloides L.		+100
Hydrocharis morsus-ranae L.		+15
Lemna trisulca L.		+10
Nymphaea alba L.		+5
Nuphar lutea (L.) Sm.	+40	+
Potamogeton compressus L.		+
Potamogeton obtusifolius Mert. & Koch		+
Riccia fluitans L.		+
Utricularia minor L.		+
Utricularia vulgaris L.	+	+
Ceratophyllum demersum L.	+40	
Lemna minor L.	+	+
Spirodela polyrhiza (L.) Schleiden	+	+
Berula erecta (Hudson) Coville	+5	+5
Thelypteris palustris Schott		+5
Equisetum fluviatile L.		+
Phragmites australis (Cav.) Trin. ex Steud.	+	+
Total number of species	7	16
Total coverage of water surface (%)	10	60

+100, Maximum covering 100% in a square of 100 m^2.
+, Maximum covering less than 5% in a square of 100 m^2.

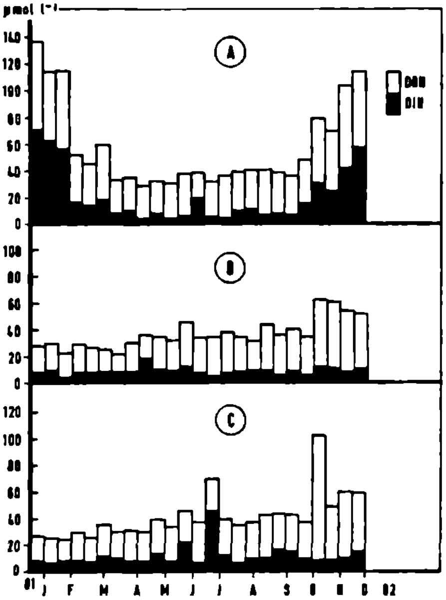

Fig 1. The seasonal change in dissolved organic (DON) and inorganic (DIN) nitrogen concentrations in the water of: (A) the open system (W O.), (B) the isolated system (W I.) outside the *Stratiotes* vegetation; (C) the isolated system in the *Stratiotes* vegetation.

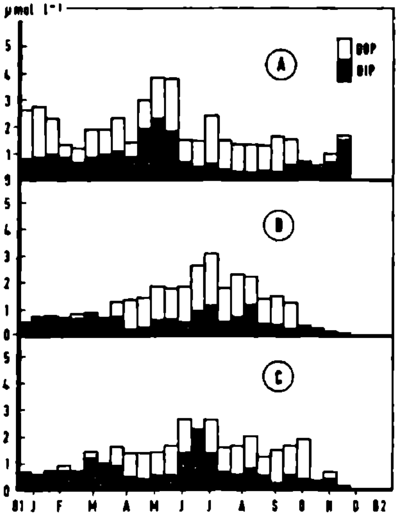

Fig. 2. The seasonal change in the dissolved organic (DOP) and inorganic (DIP) phosphorus concentrations in the water of. (A) the open system (W.O.), (B) the isolated system (W.I) outside the *Stratiotes* vegetation; (C) the isolated system in the *Stratiotes* vegetation.

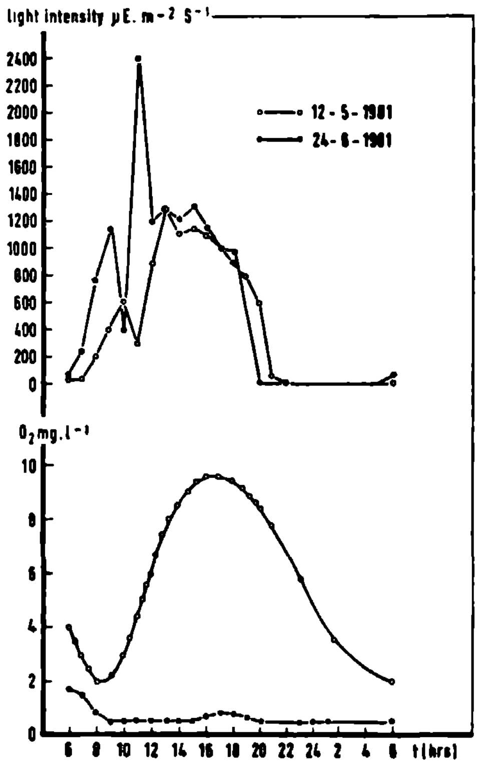

Fig. 3. The diurnal pattern in light intensity (above) and oxygen (below) concentration in the water between *Stratiotes* plants in May, when the plants are submerged and in June when the plants have emerged.

2). The latter phenomenon is correlated strongly with the oxygen level in the water layer (Fig. 3). In May, when all *Stratiotes* plants are still submerged, there is a clear daily rhythm in the oxygen levels of the water correlated with the light intensity. During daytime the oxygen level increases up to 9.5 ppm, while in the night it decreases to 2.0 ppm O_2. In June, however, when all plants have emerged, the oxygen level of the water is constantly very low (~0.5 ppm O_2), indicating that the net amount of oxygen produced during assimilation probably is released to the atmosphere by the emerged leaves.

Assimilation–respiration experiments with whole *Stratiotes* plants during a light and dark period showed that there is only a net efflux of oxygen in the leaf compartment and a net influx of oxygen in the root compartment (Fig. 4). This means that in dense stands of *Stratiotes* in the summer the plants also contribute to the observed low oxygen levels in the water layer. Phosphorus is more mobile in the water layer under these conditions.

A comparison between the total phosphorus level in the soil and the mobile orthophosphate level in the soil solution, however, demonstrated that the *o*-PO_4 concentration is more than ten times higher in the soil solution of the

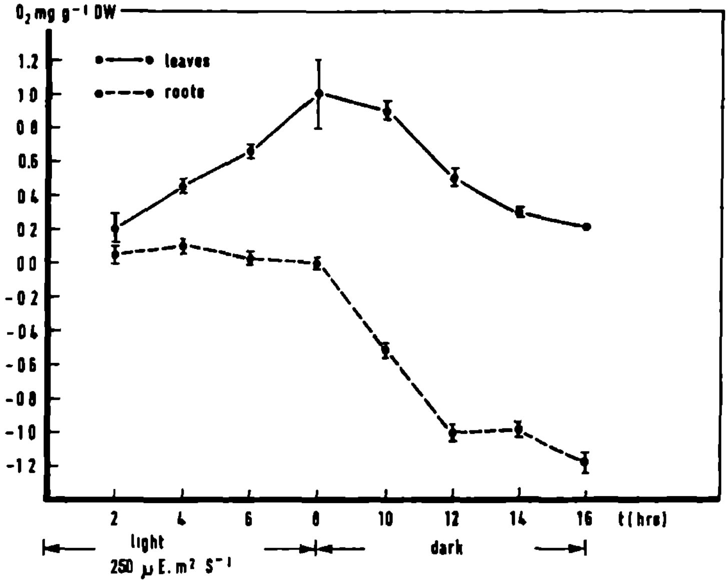

Fig. 4. The net oxygen flux from leaves and roots of whole *Stratiotes* plants during a light and dark period.

open system, than in the soil solution in the isolated system at a site without *Stratiotes* plants, whereas the total phosphorus level in the soil is in the same order of magnitude in both systems (Figs. 5 and 6).

It is well known that under circumneutral conditions, the availability of phosphorus strongly depends on the redox potential in the soil (Patrick et al., 1973). At low redox potentials, iron phosphate complexes such as strengite ($FePO_4 \cdot 2H_2O$) undergo partial or complete dissolution. From the results shown in Fig. 7 it is clear that during the whole year the redox potential is much lower in the soil of the open system. A comparison between the phosphorus levels in the soil solution and the redox potential shows that there is a clear correlation.

In January and February, when the redox potential in the soil of the isolated system is above the level at which Fe^{3+} is reduced (+77 mV; Stumm and Morgan, 1981) the orthophosphate level in the soil solution of the isolated system is extremely low (Fig. 5B). During the rest of the year the bulk potential fluctuates around +50 mV, which is only slightly below the reduction potential for Fe^{3+} while the phosphorus level is only slightly increased compared with January and February. In the open system, however, only in February is the bulk potential higher than the reduction potential for Fe^{3+} and indeed in this month only the phosphorus level in the soil solution is very low (Fig. 5A). In spring and summer the potential is very low and in the range of sulphate and carbon dioxide reduction, while the phosphorus level in the soil solution is high. The lowest bulk potential is reached in June, while

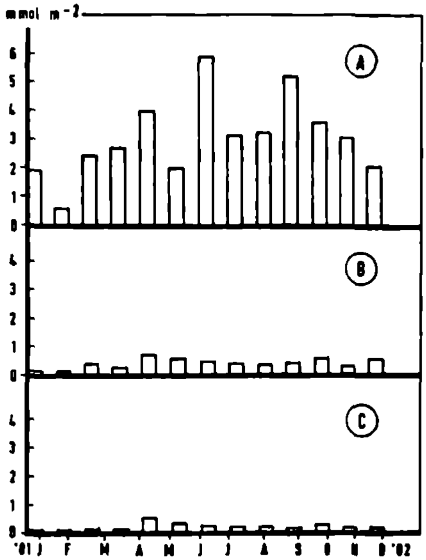

Fig. 5. The seasonal change in the orthophosphate concentration in the soil-water solution of: (A) the open system (W.O.); (B) the isolated system (W.I.) outside the *Stratiotes* vegetation; (C) the isolated system in the *Stratiotes* vegetation.

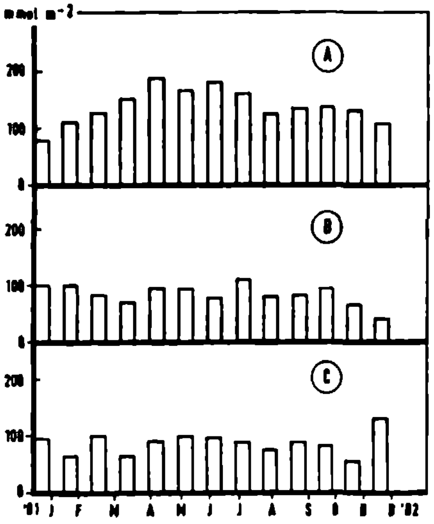

Fig. 6. The seasonal change in the total phosphorus concentration in the upper soil layer (10 cm) of: (A) the open system (W.O.); (B) the isolated system (W.I.) outside the *Stratiotes* vegetation; (C) the isolated system in the *Stratiotes* vegetation.

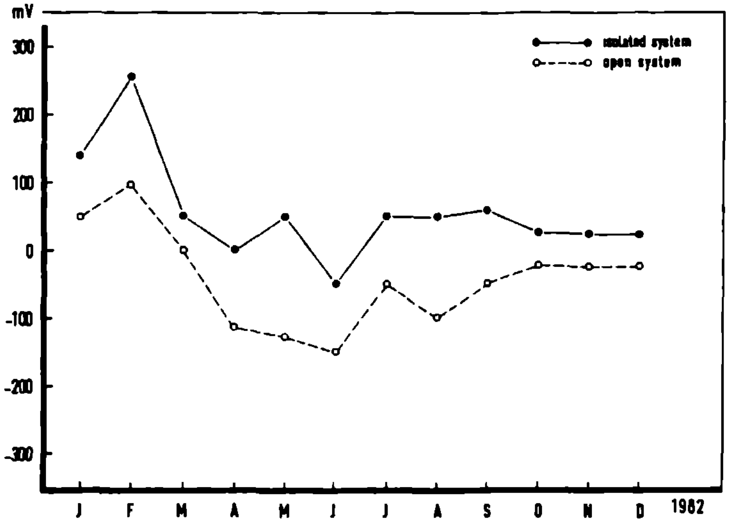

Fig. 7. The seasonal change in redox potential of the soil solution in the open and isolated systems.

in the same month the phosphorus level is the highest. As a result of these reduction processes, the levels of reduced compounds such as NH_4^+, S^{2-} and CH_4 are high in the soft upper mud layer of the open system and much lower in the isolated system (Table 3).

Transplantation experiments

The *Stratiotes* plants that were transplanted in May from the isolated system to a confined part of the open system grew very well during the summer. The nitrogen content of the plants increased very rapidly, probably as a result of the higher nitrogen availability. At the end of the growth season the nitrogen content of the transplanted plants was twice as high as that of the non-transplanted plants (Fig. 8). The first signs of necrosis were observed in February, 3 months after the migration of the *Stratiotes* plants from the water surface to the soft mud layer. Two months later, in April, the plants had decomposed almost completely, whereas the plants in the isolated system were still green and very vital (Fig. 9), which is normal for these evergreen plants. After April no living parts of the transplanted plants were found and in the following growth season no new *Stratiotes* plants developed in the open system.

Culture experiments

Culture experiments were carried out with submerged *Stratiotes* plants in early spring (between 25 February and 7 April 1982). It is clear from the results shown in Fig. 10 that a reduced oxygen content of the water in com-

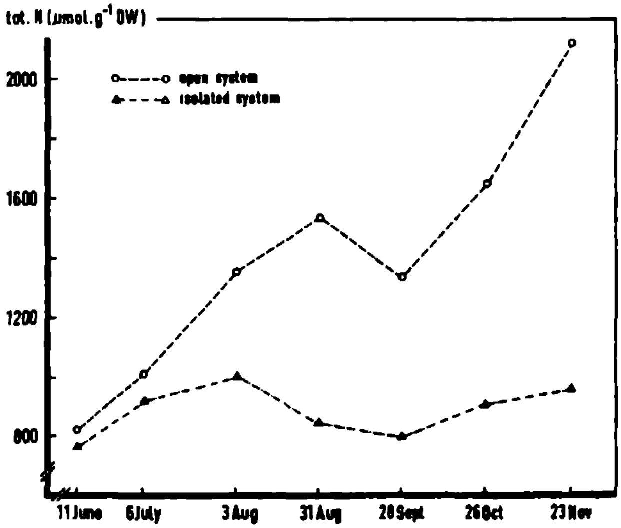

Fig. 8. The nitrogen content of *Stratiotes aloides* plants in the open and isolated systems in De Weerribben.

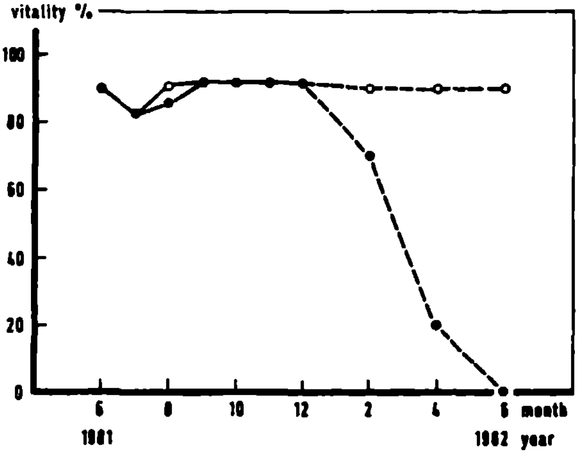

Fig. 9. The vitality of transplanted (●) and non-transplanted (○) *Stratiotes aloides* plants, expressed as the percentage of the plant parts that are green: ——, emerged plants; – – –, submerged plants.

bination with a high concentration of methane has hardly any effect on the vitality of the *Stratiotes* plants after a cultivation period of 10 weeks. Even at oxygen concentrations ranging from 0.0 ppm at night up to only 0.2 ppm during the day, more than 80% of the plant material was still green and photosynthetically active after 10 weeks. Increasing the ammonium and sulphide levels

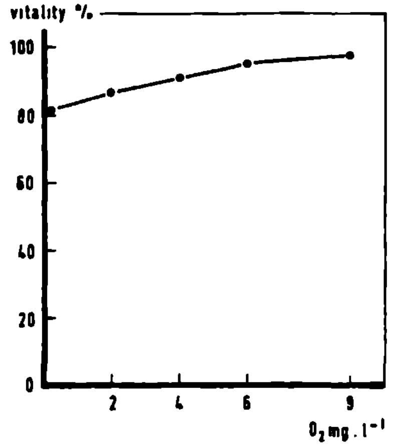

Fig. 10. The vitality of *Stratiotes aloides* plants, expressed as the percentage of the plant parts that are green, after a cultivation period of 10 weeks in a staged gradient of O_2, created by bubbling different amounts of CH_4 through the cultivation medium.

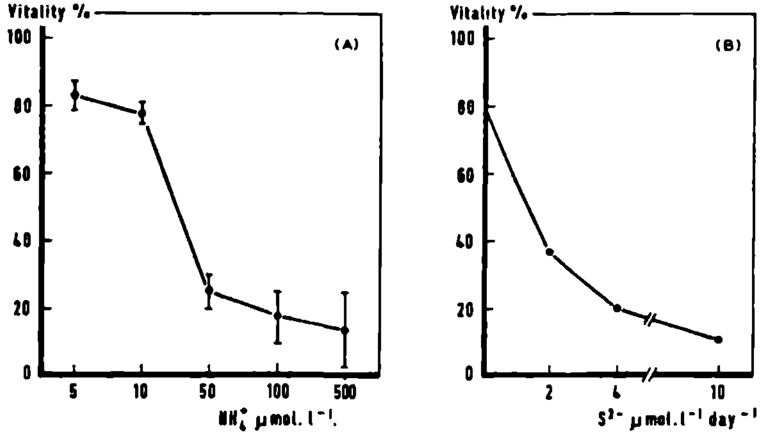

Fig. 11. The vitality of *Stratiotes aloides* plants after a cultivation period of 10 weeks in a staged gradient of (A) ammonium and (B) sulphide.

in the water had much more effect (Fig. 11). After 7 weeks at ammonium levels of 50 µmol l^{-1} or more the plants showed the same signs of necrosis as the transplanted plants in the open system in the Weerribben and after 10 weeks more than 80% of the originally green parts were decaying. The same results were obtained in an environment containing low levels of sulphide. At sulphide input levels between 4 and 10 µmol l^{-1} day^{-1} more than 80% of the plant material was decaying after 10 weeks.

Ammonium and nutrient fluxes in Stratiotes aloides *plants*

Plants that had already been growing for 7 weeks in a medium containing only nitrate as nitrogen source and then were introduced into a medium containing equivalent amounts of nitrate, ammonium and potassium, showed an equivalent influx of NO_3^- and K^+ during the first hours, but after 2 or 3 h the net influx of NO_3^- decreased strongly, while K^+ changed from a net influx to a net efflux and NH_4^+ from a zero to a net influx equivalent to the efflux of K^+ (Fig. 12).

Plants that had already been growing for 7 weeks in a medium containing 10 μmol l^{-1} ammonium nitrate before they were introduced into a medium containing 50 μmol l^{-1} NH_4NO_3, 50 μmol l^{-1} KCl and 500 μmol l^{-1} $NaHCO_3$ showed a net influx of NH_4^+ by both leaves and roots and an equivalent efflux of K^+ in the dark (Fig. 13). There was no net influx or efflux of NO_3^- and HCO_3^-.

At a light intensity of 250 μE m^{-2} s^{-1}, however, the influx of NH_4^+ was as high as under dark conditions, but the net efflux of K^+ had been reduced by ~50% as compared with that under dark conditions (Fig. 14).

The reduction in K^+ efflux was as high as the net influx of HCO_3^- under light conditions. When no HCO_3^- was available in the medium (Fig. 15), the net NH_4^+ influx was compensated for by a net efflux of K^+ and H^+. The net efflux of H^+ was as high as the net influx of HCO_3^- in a medium containing bicarbonate. So it is likely that in the light in a medium containing HCO_3^- and NH_4^+ the plants take up NH_4^+ and excrete K^+ and H^+. The H^+ reacts with HCO_3^- to give CO_2, which will be taken up by the plants. So it seems that there is no direct influx of bicarbonate into the plants.

From the ion exchange experiments it can be concluded that when the me-

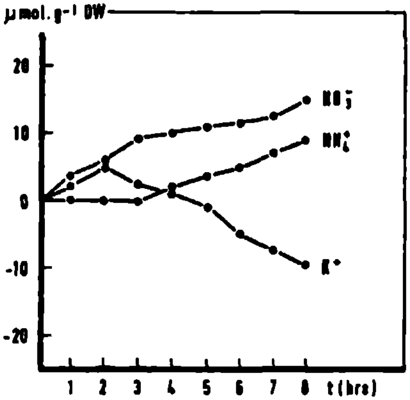

Fig. 12. The nitrate, ammonium and potassium fluxes in *Stratiotes aloides* plants in a medium containing 50 μmol ammonium nitrate (after a cultivation period of 7 weeks in an ammonium-free, nitrate-containing medium).

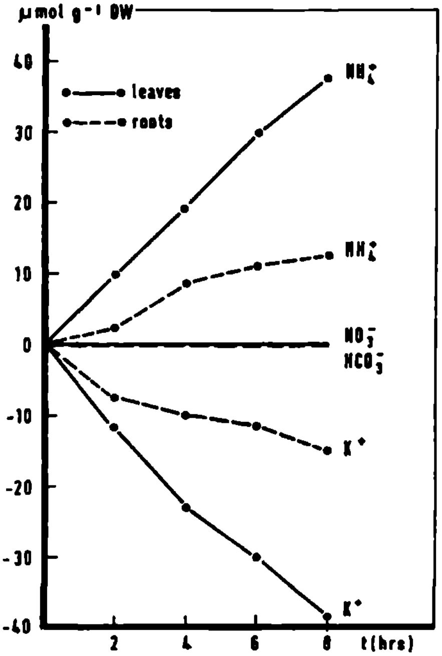

Fig. 13. Nutrient fluxes in leaves and roots of *Stratiotes aloides* plants in the dark in basic culture medium containing 50 μmol l^{-1} ammonium nitrate, 50 μmol l^{-1} potassium chloride and 500 μmol l^{-1} sodium bicarbonate.

dium in the root and leaf area of *Stratiotes* plants is never depleted of ammonium, there will be a net efflux of base cations which can cause nutrient deficiencies. Comparing the nitrogen, potassium and magnesium levels in the tissue of *Stratiotes* plants it is clear that after a cultivation period of 7 weeks in a staged gradient of ammonium in the culture medium, there is indeed an increase of the nitrogen level and a decrease of both the potassium and the magnesium levels at ammonium levels of ≥ 50 μmol l^{-1} (Fig. 16).

Experiments with artificial ponds

In Table 1 the results are summarised of the effects of changes in the macroionic composition on several water quality parameters in artificial ponds containing a non-disturbed, freshly dug peaty soil layer from the nature reserve De Weerribben. Increasing the bicarbonate level at low sulphate and chloride concentrations has a very strong effect on the water quality. The average oxygen concentration decreases, while the dissolved organic carbon concentration strongly increases from 1.8 mg l^{-1} at a bicarbonate level of 500 μmol l^{-1}

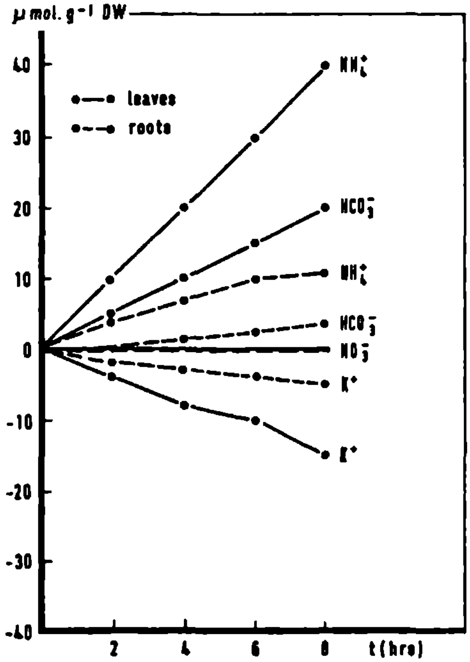

Fig. 14. Nutrient fluxes in leaves and roots of *Stratiotes aloides* plants in the light in basic culture medium containing 50 μmol l^{-1} ammonium nitrate, 50 μmol l^{-1} potassium chloride and 500 μmol l^{-1} sodium bicarbonate.

up to 6.5 mg l^{-1} at a bicarbonate level of 3000 μmol l^{-1}. The turbidity of the water also strongly increases from 8 ppm Pt l^{-1} at 500 μmol l^{-1} HCO_3^- up to 70 ppm Pt l^{-1} at 3000 μmol l^{-1} HCO_3^-. There is also a strong increase of the total phosphorus, nitrogen and sulphide concentrations by increasing the bicarbonate level and the water is much more coloured.

Increasing the sulphate or chloride levels at a moderate bicarbonate concentration (1000 μmol l^{-1}) has a positive effect on the measured water quality data. The average oxygen concentration is much higher and the turbidity, colour and total nitrogen concentration are much lower than under low sulphate and chloride levels.

From experiments with combined staged gradients of bicarbonate, sulphate and chloride, it is clear that in general the results are comparable with those obtained by changing only the bicarbonate level. Only the decrease of the oxygen concentration and the increase of the DOC and turbidity are not as high, while the increase in the sulphide (S^{2-}) concentration is higher.

From the field situation (Table 3) it was clear that an inlet of hard, bicarbonate- and sulphate-rich water led to a large increase in the bicarbonate level

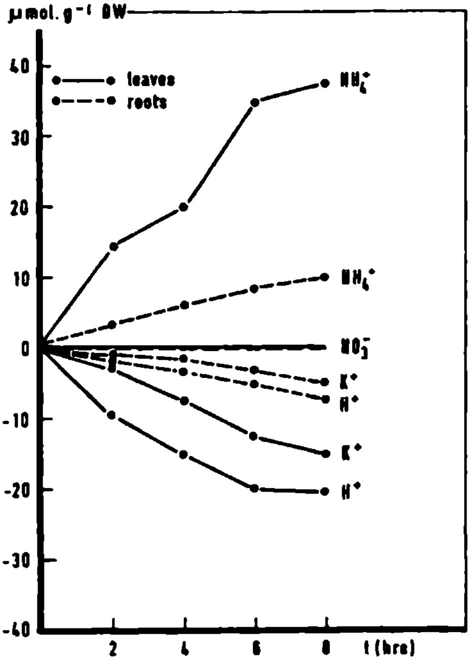

Fig. 15. Nutrient fluxes in leaves and roots of *Stratiotes aloides* plants in the light in basic culture medium containing 50 μmol l^{-1} ammonium nitrate and 50 μmol l^{-1} potassium chloride.

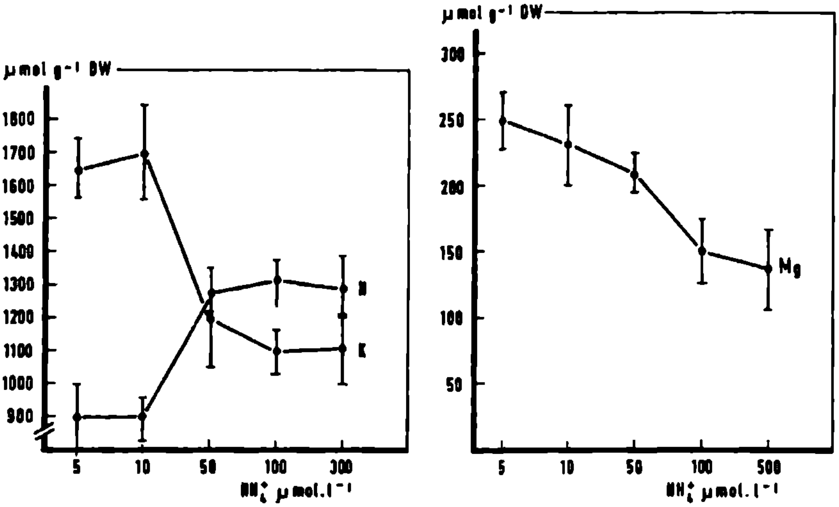

Fig. 16. The nitrogen, potassium and magnesium content of *Stratiotes* after a cultivation period of 7 weeks in a staged gradient of ammonium.

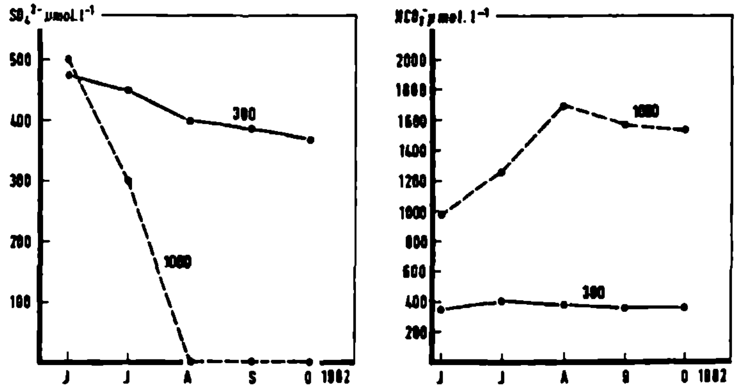

Fig. 17. The changes in sulphate and bicarbonate concentrations in artificial ponds with differences in initial bicarbonate concentrations (380 and 1000 µmol l^{-1} HCO_3^- respectively).

but not in the sulphate level in the water layer. This could also be observed in two artificial ponds which initially contained 500 µmol l^{-1} SO_4^{2-} and 380 and 1000 µmol l^{-1} HCO_3^- (Fig. 17). In the pond with the low bicarbonate level, the sulphate level decreased only very slowly during the summer, while the bicarbonate level did not change. In the pond with the moderate bicarbonate level, however, the sulphate concentration decreased from 500 to 0 µmol l^{-1} within 2 months, while the bicarbonate level increased from 1000 µmol l^{-1} in June to 1500 µmol l^{-1} in October.

DISCUSSION

The major changes in the originally peaty soft water ecosystems which caused the disappearance of *Stratiotes aloides* are the result of an increased water hardness. Inlet of water rich in bicarbonate and sulphate leads to internal eutrophication of these ecosystems, probably as a result of increased mineralisation of organic matter (peat) and a concomitant decrease of the redox potential. The somewhat higher pH of the soil water of the open system (pH 6.5) compared with the isolated system (pH 6.0) cannot be responsible for the strongly increased concentration of orthophosphate in the soil water of the open system. The solubility of strengite ($FePO_4 \cdot 2H_2O$) decreases with increasing pH in the given range (Patrick et al., 1973), but increases very strongly with a decreasing redox potential of the soil solution. This is fully in agreement with the results in this study, in which the orthophosphate level in the soil solution is clearly correlated with the redox potential (Figs. 5 and 7). In summer the redox potential of the soil solution of the open system is on average 150 mV below that of the isolated system, while the *o*-PO_4 concentration is ten times higher than that of the isolated system. It is clear from Figs. 5 and 7 that the concentration of o-PO_4 in the soil solution is a function of the redox potential. At times when the potential is above the reduction potential of Fe^{3+} (+70 mV; Stumm and Morgan, 1981), the o-PO_4 concentration in the soil solution is extremely low (below 0.7 μmol l^{-1}). The highest o-PO_4 concentration (41 μmol l^{-1}) and the lowest redox potential (−150 mV) were recorded in June.

In both ecosystems the redox potential is below the reduction potential of nitrate, and indeed the nitrate concentration in the soil solution is very low. On the other hand, the ammonium levels are high, but in the soil solution of the open system they are much higher than in the isolated system.

Alkalinisation and internal eutrophication

It is clear that the increased trophic state of the ecosystem, caused by input of alkaline water rich in bicarbonate and sulphate, can be a result of both biological and chemical processes. Changes in the binding capacity of soil complexes, often regulated by the redox potential and microbial processes, play an important role. It seems that the combination of a high level of bicarbonate and sulphate (which will be reduced to sulphide) leads to eutrophication. On the basis of experimental research it appears that bicarbonate initiates the process of eutrophication, while sulphate intensifies it. It seems that reduction of sulphate in an acid environment has no influence on the phosphate release from the sediment, while in an alkaline water the same process leads to an increased release of phosphate from the sediment (Curtis, 1989).

Several authors mention that alkalinisation, alone or in combination with high sulphide concentrations as a result of sulphate reduction, increases P mobilisation from the sediment (Canfield, 1983; Curtis, 1989). Support for this hypothesis is found in an investigation of 600 freshwater ecosystems in The Netherlands by van Katwijk and Roelofs (1988). It was found that the orthophosphate concentrations and the bicarbonate alkalinity in water and soil-water solutions in ordination diagrams changed in the same direction, which means that a high alkalinity usually corresponds with a high concentration of orthophosphate.

It is well known that decomposition of organic material is inhibited in acid waters compared with alkaline waters (Traaen, 1980; McKinley and Vestal, 1982; Hoeniger, 1985). The hypothesis of Brock et al. (1985) that bicarbonate neutralises decay-inhibiting acids has been confirmed by Kok and van de Laar (1990). They found that the decomposition rate of organic material is strongly correlated with the internal pH of the detritus. The internal pH and the decomposition rate of the detritus appeared to be much more a function of the buffer capacity of the surrounding water than of the pH.

The increased decomposition and mineralisation rate can lead to a higher trophic state of the water and a higher oxygen demand in the soil. The redox potential is, apart from pH and temperature, an important regulating factor in the mobilisation of P and N from the soil to the water layer (Mortimer, 1941–1942; Fillos and Swanson, 1975; Bostrom et al., 1982; Hosomi et al., 1982). Under anaerobic conditions ferric iron (Fe^{3+}) will be reduced to the soluble Fe^{2+} ion and as a result phosphorus will also be released.

When the water is both rich in bicarbonate and sulphate this process will be accelerated. As a result of the sulphate reduction there will be a high production of sulphides, which in their turn will react with reduced iron to FeS. As a result of this iron consumption in the soil solution there will be a shift in the equilibrium of the redox reaction from Fe^{3+} to Fe^{2+}, which will lead to phosphorus mobilisation. Formation of FeS coupled with P mobilisation occurs mainly in alkaline, well-buffered waters with a high sulphate flux (Stauffer, 1981). Furthermore, sulphate reduction leads to bicarbonate production (Baker et al., 1986; Cook et al., 1986; Schindler et al., 1986; Psenner, 1988; Bloemendaal and Roelofs, 1988):

$$2CH_2O+SO_4^{2-}\rightarrow 2H_2O+S^{2-}+2CO_2$$

$$CO_2+2H_2O\rightarrow HCO_3^-+H_3O^+$$

$$S^{2-}+H_3O^+\rightarrow HS^-+H_2O$$

$$2CH_2O+SO_4^{2-}\rightarrow HCO_3^-+HS^-+H_2O+CO_2$$

The bicarbonate alkalinity produced gives a positive feedback to the eutrophication process. For this reason, internal alkalinisation and eutrophication can continue, when external eutrophication already has stopped (Stauffer, 1985; Schindler et al., 1986; Bloemendaal and Roelofs, 1988; Curtis, 1989) or does not occur.

According to Schindler (1988), alkalinity production will be determined by the biological reduction of sulphate and nitrate (Schindler, 1986; Baker et al., 1986; Cook et al., 1986; Rudd et al., 1986; Schindler et al., 1986), while the cation exchange is of uncertain importance. Then not only sulphate but also nitrate affects internal alkalinisation and eutrophication. Andersen (1982) showed that nitrate is even more efficient than oxygen in acting as a redox buffer. In keeping the redox potential high it inhibits the P release from the sediment. Also, van Katwijk and Roelofs (1988) found a clear correlation between nitrate and the ammonium to nitrate ratio and the phosphorus level in the water layer.

Toxicity of NH_3/NH_4^+ and H_2S/HS^- to Stratiotes aloides

A further consequence of increased reductive microbial activity is the formation of potentially toxic components such as ammonia and sulphides. In soils rich in iron the sulphide concentrations remain low, because iron reacts immediately with S^{2-} to give FeS. In peaty ecosystems, however, or in ecosystems which are not influenced by groundwater rich in iron, the iron pool can be exhausted. Under these conditions sulphide accumulates in the soil-water solution. This is the case in the soil solution of the non-isolated peaty ecosystem (Table 3) and also in the experimental setup with artificial ponds (Table 1).

Ammonium/ammonia also accumulates in the soil solution after the alkalinisation processes described above (Table 3). From culture experiments (Kohler et al., 1973; Glänzer et al., 1977) it was already clear that ammonia can be toxic to many aquatic plant species. From the results of the culture experiments in this study, it was clear that both the ammonium and the sulphide levels in the soft upper sapropel layer are sufficiently high to kill the *Stratiotes* plants in winter and early spring, when they are overwintering in this soft organic layer. From the transplantation experiments it was already clear that the *Stratiotes* plants grew very well during the summer in both the isolated and the open alkalinised peaty ecosystems. In the aerobic, upper water layer, the levels of both sulphides and ammonium are always very low, probably as a result of sulphide and ammonium oxidation.

Alkalinisation, carbon budgets and macrophyte development

Although alkalinisation in general leads to an increased inorganic carbon content in the water layer, the CO_2 concentration decreases as a result of an increasing pH. This means that in general there is a trend of increasing carbonate/bicarbonate levels and decreasing carbon dioxide concentrations. Plant species from peaty environments, however, are merely adapted to CO_2 uptake. Prins and De Guia (1986) found that *Stratiotes aloides* plants take up only very small quantities of bicarbonate: for a good rate of growth they require high CO_2 levels in the water layer. Prins and coworkers concluded that, as a result of alkalinisation, the CO_2 levels in the water layer become too low for good development of the plants. The nutrient uptake experiments carried out during this study also support this hypothesis (Fig. 14) as a relatively low bicarbonate uptake rate for *Stratiotes aloides* has been measured. It is likely that other aquatic macrophyte species that disappear during alkalinisation also suffer from lack of CO_2 supply. It is striking that after alkalinisation, only plant species which are known to use bicarbonate or organic carbon occur, such as *Ceratophyllum demersum* and *Utricularia vulgaris*.

One of the most striking phenomena after alkalinisation of surface waters on peaty soils is the greatly increased turbidity of the water. This turbidity is not only caused by algal bloom as a result of the internal eutrophication, but more particularly by changes in the peaty soil. The peat becomes very fine and weak and as a result of gas production, bubbling and turbation by the wind, fish or recreation activity, the water becomes turbid. This process of turbation has been observed in soil-water columns in the laboratory (Bloemendaal and Roelofs, 1988). It appeared that the increase in turbidity of the water after alkalinisation was almost as high in the dark as it was in the light (van Loon, 1981).

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CHAPTER 3.

Impact of acidification and eutrophication on macrophyte communities in soft waters in The Netherlands.
I. Field observations.

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IMPACT OF ACIDIFICATION AND EUTROPHICATION ON MACROPHYTE COMMUNITIES IN SOFT WATERS IN THE NETHERLANDS
I. FIELD OBSERVATIONS

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(Accepted for publication 15 July 1983)

ABSTRACT

Roelofs, J.G.M., 1983. Impact of acidification and eutrophication on macrophyte communities in soft waters in The Netherlands. I. Field observations. *Aquat. Bot.*, 17: 139—155.

During the last decades a strong decline has been noticed in the number of waters dominated by "*Littorellion*" species, mostly isoetids such as *Lobelia dortmanna* L., *Isoetes lacustris* L. and *Littorella uniflora* (L.) Aschers. Sixty-eight waters, which were known to be dominated by *L. uniflora* after 1950 were investigated. In 1980, *L. uniflora* appeared to be absent or to have strongly decreased in 53 (78%) of the waters. In 41 of them, *Littorella* had been replaced by submerged *Juncus bulbosus* L. and/or *Sphagnum* spp. These changes seem to have been caused by changed inorganic carbon budgets as a consequence of acidification.

In the remaining 12 waters, eutrophication of the water and/or sediment seems to be responsible for the changes in the plant communities. Enrichment with phosphate of the mineral sediment alone, leads to luxurious growth of submerged, rooted macrophyte species such as *Myriophyllum alterniflorum* DC and *Ranunculus peltatus* Schrank, whereas phosphate-enrichment of both sediment and water leads to luxurious growth of pleustophytes such as *Riccia fluitans* L. and *Lemna minor* L. in small, shallow waters, and to plankton bloom and luxurious growth of epiphytes in larger, deeper waters.

In these cases light limitation seems to be responsible for the disappearance or decline of the "*Littorellion*" species.

INTRODUCTION

During the past 20 years the deleterious effects of acid precipitation on aquatic and terrestrial ecosystems have become a growing problem. Reports from The First International Symposium on Acid Precipitation and Forest Ecosystem (Dochinger and Seliga, 1975), the United Nations Conference of the Human Environment (Engstrom, 1971) and of the International Conference on the Impact of Acid Precipitation (Drabløs and Tollan, 1980) emphasize the gravity of the phenomenon.

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Aquatic habitats appear to be very susceptible to the effects of acidification. The severe depletion of fish stocks and the decreased diversity of algae and invertebrates in many Swedish and Norwegian waters can be ascribed to the effects of acidification. It is only fairly recently that a few reports have been published which express alarm about acidifying waters on poorly buffered sediments in The Netherlands (van Dam and Kooyman-van Blokland, 1978; van Dam et al., 1981; van Zellem, 1981) and Belgium (Vangenechten et al., 1980).

The mechanisms of acidification, however, appear to be very complex and are not yet clearly understood. In many cases the impact of acidification cannot be ascribed to pH decrease alone. Brooktrout, *Salvelinus fontinalis* (Mitchell), for instance, seem to suffer mainly from toxic aluminium concentrations mobilized by increasing acidification, as they are able to tolerate substantial additions of sulphuric acid (Daye and Garside, 1975). It is also known that heavy metals are more toxic to algae at low pH (Voigt, 1979). Inhibition of nitrogen-turnover processes (Tamm et al., 1977; Alexander, 1980) and leaching (Stuanes, 1980) are likely to cause future nutrient deficiencies in forest ecosystems.

However, little is known about the impact of acidification on freshwater macrophytes. Grahn et al. (1974) and Grahn (1977) mention a suppression of isoetid species in Scandinavian waters by luxurious *Sphagnum* growth as a result of acidification of the water and Nilssen (1980) mentions a luxurious growth of *Juncus bulbosus* L. in acidified Scandinavian waters.

In The Netherlands there has been a dramatic decline in the number of stands belonging to the phytosociological alliance *Littorellion* during the last 30 years. These stands are characterized by isoetids, such as *Littorella uniflora* (L.) Aschers., *Lobelia dortmanna* L., *Isoetes lacustris* L., etc. (Schoof-van Pelt, 1973). These "*Littorellion* communities" mainly occurred in moorland pools, in small lakes and dune lakes on mineral sandy soils. It is precisely these poorly buffered waters that have become strongly acidified during the last decades (van Dam and Kooyman-van Blokland, 1978; van Dam et al., 1981). The coastal dune lakes appeared to be less susceptible to acidification, due to the higher content of calcium carbonate of the bottom.

Eutrophication has been generally considered to be the main cause of the decline of the *Littorellion* communities (Schoof-van Pelt, 1973; Westhoff et al., 1973; Westhoff, 1979; Pott, 1982; Wittig, 1982a,b; Wittig and Pott, 1982); Westhoff (1979) also mentioned inadequate maintenance in nature reserves as a secondary cause. Acidification was not mentioned by these authors as being a possible cause.

The present study was carried out in order to establish which part of the decline of the "*Littorellion*" communities can be ascribed to acidification, which part is due to eutrophication and possible other causes, and which changes in the composition of the macrophyte communities occur as a result of these processes.

STUDY SITES

Sixty-eight low alkaline waters were selected, where, according to Schoof-van Pelt (1973), Westhoff et al. (1971) and H.M. van der Steeg, C. den Hartog, D.T.A. van der Ploeg, E. Weeda and J. Hofstra (personal communication), after 1950 a plant community occurred, in which *Littorella uniflora* was one of the dominant species.

Low alkaline waters in The Netherlands are restricted to the higher situated, poorly buffered, sandy soils in the southern and eastern part of the country and to the coastal region (Fig. 1). In the provinces of Noord Brabant, Limburg, Gelderland, Overijssel and Drenthe mainly moorland pools and some small lakes were studied and only dune pools in the province of Zuid-Holland and on the isles of Texel, Terschelling and Schiermonnikoog.

Fig. 1. Study areas. One dot may represent more than one investigated water.

MATERIALS AND METHODS

During 1979 and 1980 each water was visited four times. On location, the floral composition was investigated and pH-measurements were carried out with a Metrohm model E 488 pH-meter and a model EA 152 combined electrode. Alkalinity was estimated by titration of 100 ml of water with 0.01 M HCl down to pH 4.2. Water samples were taken in 200-ml iodated polyethylene bottles, immediately passed through a Whatman GF/C filter and fixed with 1 ml of a 200 mg l^{-1} $HgCl_2$ solution. In the micro-habitats of each investigated plant species (Tables III and IV) sediment samples were taken with a brass tube (length 10 cm, diameter 5.6 cm). On each location 6 subsamples were taken and decanted into a 2-l iodated polyethylene bottle. All samples were transported to the laboratory in a refrigerated container.

After arrival at the laboratory, the water samples were immediately frozen and stored at −20°C until use. Redox potential measurements in the sediment samples were carried out within 24 h with a Metrohm model E 488 pH/mV meter and a model EA 217 platinum electrode. For the analysis of the interstitial water 20 g of wet well-mixed sediment were weighed into a 400-ml conical flask and shaken on a Gerhardt model LS 20 shaker during 1 h, after addition of 200 ml bidistilled water. After centrifugation in a Heraeus Crist model 111 labofuge (10 min, 5000 r.p.m.) the acidity was estimated by titration of 50 ml of the supernatant with 0.01 M NaOH up to pH 8.2. The alkalinity was estimated by titration of 50 ml of the supernatant with 0.01 M HCl down to pH 4.2. One-hundred ml of the supernatant was fixed by adding 0.5 ml of a 200 mg l^{-1} $HgCl_2$ solution and stored at −20°C until use. The water content of the sediment samples was estimated by weighing, drying (24 h, 105°C) and reweighing. One-hundred mg of dried sediment were digested in a mixture of 0.5 ml concentrated perchloric acid and 0.2 ml sulphuric acid under pressure (4 h, 170°C), according to Kotz et al. (1972), in order to obtain the elemental composition. For all samples, calcium was estimated with a Beckman model 1272 Atomic Absorption Spectrophotometer, sodium and potassium were estimated flame-photometrically using a Technicon I Auto Analyzer and colorimetrically with a Technicon II Auto Analyzer: orthophosphate according to Hendriksen (1965), nitrate according to Kamphake et al. (1967), ammonia according to Grasshoff and Johannsen (1977), chloride according to O'Brien (1962) and sulphate according to Technicon methodology (1981). The carbon dioxide content was calculated from acidity and that of hydrogen carbonate was calculated from alkalinity, both with a correction for pH.

RESULTS

Macrophyte composition

In 1979, only 15 (~ 22%) of the 68 waters investigated, in which *Littorella uniflora* was one of the dominant species after 1950, were still dominated by this species; most of these stands occurred in the coastal region (Table Ia).

The 53 waters in which *L. uniflora* had disappeared or had strongly decreased can be divided into two groups. The major group (group 1: 41 waters) is characterized by the presence of submerged *Juncus bulbosus* and/or *Sphagnum* spp. and a very low turbidity of the water (Table Ib). Twenty-five of these waters were dominated by *J. bulbosus*, 12 of them by *Sphagnum* spp. and in four of them no submerged plants were present except *J. bulbosus* and *Sphagnum* spp. in very small quantities. The waters of the minor group (group 2: 12 waters) are characterized by the absence of submerged *J. bulbosus* and/or *Sphagnum* spp. (Table Ic). Five of these waters were dominated by *Myriophyllum alterniflorum* DC, three of them by *Riccia fluitans* L. and *Lemna minor* L. and four of them were free of submerged macrophytes. In these last four waters the turbidity was very high as a result of luxurious plankton growth. Considerable differences were also noticed in the composition and coverage of the accompanying species (Tables Ia—c).

Eleocharis acicularis (L.) R. et S. is common and generally covered more than 5% of the bottom in the waters dominated by *Littorella uniflora*, it occurred only sporadically with a low coverage in waters dominated by *J. bulbosus*, and was absent in waters dominated by *Sphagnum* spp. In waters dominated by *M. alterniflorum*, *E. acicularis* showed a mean coverage and a frequency equal to those in waters dominated by *L. uniflora*, whereas *E. acicularis* was absent in waters dominated by *Riccia fluitans* and *Lemna minor*.

Luronium natans (L.) Raf. showed a similar pattern. It is common in waters dominated by *Littorella uniflora*, is less numerous in waters dominated by *J. bulbosus* and is scarce in waters dominated by *Sphagnum* spp. In the waters dominated by *M. alterniflorum*, *Luronium natans* is frequent, whereas in waters dominated by *Riccia fluitans* and *Lemna minor* it is absent.

Potamogeton gramineus L. is restricted to waters dominated by *Littorella uniflora*. *Polygonum amphibium* L. and *Eleocharis palustris* (L.) R. et S. were both frequent in waters dominated by *Littorella uniflora*, *M. alterniflorum* and *Riccia fluitans*/*Lemna minor*; and were scarce in waters dominated by *J. bulbosus* and *Sphagnum* spp.

Carex rostrata Stokes and *Eriophorum angustifolium* Honck. occurred more frequently in the waters dominated by *J. bulbosus*. *Eleocharis multicaulis* (Sm.) Sm. was absent in waters dominated by *Littorella uniflora* (without *J. bulbosus*), *M. alterniflorum* and *Riccia fluitans*. *Ranunculus peltatus* Schrank and *Elodea nuttallii* (Planch.) St. John both occurred only in waters dominated by *Myriophyllum alterniflorum*.

TABLE Ia

The floral composition of the waters in which *Littorella uniflora* was one of the dominant plant species in 1979—1980

Number of waters	Dominant species	Accompanying species	Frequency*
11	*Littorella uniflora* (L.) Aschers.	*Eleocharis acicularis* (L.) R. et S.	7 +
		Eleocharis palustris (L.) R. et S.	6
		Hydrocotyle vulgaris L.	6
		Echinodorus ranunculoides (L.) Engelman	5
		Luronium natans (L.) Raf.	5 +
		Potamogeton gramineus L.	5 +
		Ranunculus flammula L.	5
		Carex rostrata Stokes	4
		Elatine hexandra (Lapierre) DC	4 +
		Myosotis scorpioides L.	4
		Peplis portula L.	3
		Polygonum amphibium L.	3 +
		Chara globularis Thuill.	2
		Carex lasiocarpa Ehrh.	2
		Echinodorus repens (Lamk.) Kern et Reichg.	2
		Myriophyllum alterniflorum DC	2
		Eriophorum angustifolium Honck.	1
		Hypericum elodes L.	1
		Menyvanthes trifoliata L.	1
		Nuphar lutea (L.) Sm.	1
		Ranunculus ololeucos Lloyd	1
4	*Littorella uniflora* (L.) Aschers. and *Juncus bulbosus* L.	*Hydrocotyle vulgaris* L.	3
		Sphagnum spp.	3 +
		Carex rostrata Stokes	2
		Eleocharis multicaulis (Sm.) Sm.	2 +
		Eleocharis palustris (L.) R. et S.	2
		Lobelia dortmanna L.	2
		Ranunculus flammula L.	2
		Echinodorus repens (Lamk.) Kern et Reichg.	1
		Hypericum elodes L.	1
		Luronium natans (L.) Raf.	1 +
		Isoëtes lacustris L.	1 +

*Frequency: the number of waters in which the species was present. Species marked with + often had a coverage of >5%.

TABLE Ib

The floral composition of the waters in which *Littorella uniflora* was not one of the dominant species and in which submerged *Juncus bulbosus* and/or *Sphagnum* occurred in 1979—1980

Number of waters	Dominant species	Accompanying species	Frequency
25	*Juncus bulbosus* L.	*Sphagnum* spp.	12 +
		Carex rostrata Stokes	11 +
		Hypericum elodes L.	8
		Eleocharis palustris (L.) R. et S.	8
		Hydrocotyle vulgaris L.	7
		Luronium natans (L.) Raf.	7
		Eriophorum angustifolium Honck.	7 +
		Utricularia minor L.	7 +
		Nymphaea alba L.	6
		Ranunculus flammula L.	6
		Ranunculus ololeucos Lloyd	6
		Peplis portula L.	6
		Potamogeton natans L.	6
		Carex lasiocarpa Ehrh.	2 +
		Echinodorus ranunculoides (L.) Engelman	2
		Eleocharis acicularis (L.) R. et S.	2
		Menyanthes trifoliata L.	1
		Nuphar lutea (L.) Sm.	1
		Polygonum amphibium L.	1
12	*Sphagnum* spp. (most *S. cuspidatum* Hoffm.)	*Carex rostrata* Stokes	10 +
		Eriophorum angustifolium Honck.	9 +
		Hydrocotyle vulgaris L.	8
		Eleocharis multicaulis (Sm.) Sm.	6
		Utricularia minor L.	5 +
		Eleocharis palustris (L.) R. et S.	4
		Menyanthes trifoliata L.	4
		Hypericum elodes L.	4
		Ranunculus flammula L.	4
		Carex lasiocarpa Ehrh.	3
		Juncus articulatus L.	3
		Luronium natans (L.) Raf.	2
		Nymphaea alba L.	2
		Polygonum amphibium L.	2
4	Clear water no submerged macrophytes except *Juncus bulbosus* and *Sphagnum* spp. in very small quantities		

TABLE Ic

The floral composition of the waters in which *Littorella uniflora* was not one of the dominant species and in which no submerged *Juncus bulbosus* and/or *Sphagnum* spp. occurred in 1979—1980

Number of waters	Dominant species	Accompanying species	Frequency
5	*Myriophyllum alterniflorum* DC	*Eleocharis palustris* (L.) R. et S.	5 +
		Hydrocotyle vulgaris L.	4
		Littorella uniflora (L.) Aschers.	4 +
		Luronium natans (L.) Raf.	4 +
		Myosotis scorpioides L.	4
		Ranunculus peltatus Schrank	4 +
		Ranunculus flammula L.	4
		Apium inundatum (L.) Rchb.f.	2
		Chara globularis Thuill.	2 +
		Eleocharis acicularis (L.) R. et S.	2 +
		Echinodorus ranunculoides (L.) Engelman	2
		Elatine hexandra (Lapierre) DC	2 +
		Nuphar lutea (L.) Sm.	2
		Polygonum amphibium L.	2 +
		Nymphaea alba L.	1
		Peplis portula L.	1
		Elodea nuttallii (Planch.) St. John	1
3	*Riccia fluitans* L. *Lemna minor* L.	*Lemna trisulca* L.	3 +
		Eleocharis palustris (L.) R. et S.	2
		Hydrocotyle vulgaris L.	2
		Myosotis scorpioides L.	2
		Nuphar lutea (L.) Sm.	2 +
		Ranunculus flammula L.	2
		Rorippa amphibia (L.) Besser	2
		Peplis portula L.	1
4	Turbid water (algal bloom) no submerged macrophytes		

Frequency: the number of waters in which the species was present. Species marked with + often had a coverage >5%.

Chemistry of water and sediments

Water chemistry

The results are shown in Table II. Waters dominated by *Littorella uniflora* had a mean alkalinity of 0.50 meq. l^{-1} and those dominated by *J. bulbosus* of 0.02 meq. l^{-1}; however, it has to be noted that most waters with *J. bulbosus* as a dominant species had no buffer capacity at all. The waters dominated by *Sphagnum* spp. had a mean alkalinity of 0.01 meq. l^{-1}, those dominat-

Water chemistry

	n*	pH "average"	Alkalinity (HCO_3^- mmol l^{-1})		SO_4^{2-} (μmol l^{-1})		Cl^- (μmol l^{-1})		Ca^{2+} (μmol l^{-1})	
			Mean	90%	Mean	90%	Mean	90%	Mean	90%
Littorella uniflora	33	6.5	0.50	0.1—1.0	336	100—1000	638	40—4000	262	100— 300
Juncus bulbosus	107	3.9	0.02	0.0—0.2	780	300—2000	666	80—1500	310	100— 500
Sphagnum spp.	59	3.8	0.01	0.0—0.2	530	100—1500	448	80— 500	160	100— 300
Myriophyllum alterniflorum	25	6.9	0.80	0.2—2.0	660	500—1500	795	100—1500	620	400—1500
Ranunculus peltatus	62	6.8	1.10	0.4—2.0	822	200—2000	979	200—2000	710	300—1500
Riccia fluitans	31	6.4	0.80	0.2—2.0	607	200—1000	687	250—1000	564	300—1500
Lemna minor	91	6.5	0.90	0.2—4.0	836	200—2000	1001	80—4000	679	200—1500

*Number of samples investigated.

TABLE IIb

Water chemistry

	n*	NH_4^+ (μmol l^{-1})		NO_3^- (μmol l^{-1})		PO_4^{3-} (μmol l^{-1})		K^+ (μmol l^{-1})	
		Mean	90%	Mean	90%	Mean	90%	Mean	90%
Littorella uniflora	33	5	0— 10	10	0— 50	0.16	0.0— 0.5	134	50—300
Juncus bulbosus	107	40	2— 200	7	0— 50	0.21	0.0— 0.5	163	10—400
Sphagnum spp.	59	46	2— 400	7	0— 50	0.19	0.0— 0.5	79	10—200
Myriophyllum alterniflorum	25	23	0— 50	44	0— 100	0.22	0.0— 0.5	139	50—300
Ranunculus peltatus	62	44	0— 100	162	0— 600	0.44	0.0— 5.0	176	20—400
Riccia fluitans	31	27	2— 50	85	0— 10	2.20	0.1—20.0	190	50—400
Lemna minor	91	68	2—1000	224	0—2000	1.70	0.1—20.0	210	50—750

*Number of samples investigated.

ed by *M. alterniflorum* and *Riccia fluitans* and/or *Lemna minor* had a mean alkalinity of 0.80 meq. l^{-1}, thus higher than that of the *Littorella*-dominated waters. From the investigated plant species, *J. bulbosus* and *Sphagnum* spp. occurred mainly in acid waters (pH on an "average" 3.8) while the other species mostly occur in circumneutral waters.

The mean sulphate concentration was the lowest in waters dominated by *Littorella uniflora* and clearly higher in the waters dominated by *J. bulbosus, M. alterniflorum* and *R. fluitans.* The mean ammonium concentration was low in the waters dominated by *L. uniflora* and clearly higher in the waters dominated by *J. bulbosus* and *Sphagnum* spp. However, the mean nitrate concentration was the lowest in waters dominated by *J. bulbosus* and *Sphagnum* spp., somewhat higher in waters dominated by *L. uniflora,* and much higher in the waters dominated by *M. alterniflorum* and *R. fluitans.*

The mean concentrations of ortho-phosphate were practically identical in the waters dominated by *L. uniflora, J. bulbosus, Sphagnum* spp. and *M. alterniflorum* and much higher in waters dominated by *R. fluitans* and *Lemna minor.*

The mean concentrations of potassium, calcium and chloride did not differ much in the investigated waters, except for waters dominated by *Sphagnum* spp., in which they were lower, and for waters dominated by *M. alterniflorum*, in which they were higher.

Sediment analysis (Tables III and IV)

The mean HCO_3^- concentration of the interstitial water was the lowest for *J. bulbosus,* somewhat higher for *Sphagnum* spp. and *Littorella uniflora* and much higher for *M. alterniflorum* and *R. fluitans.*

The mean CO_2 concentration, however, was the highest for *J. bulbosus* and *Sphagnum* spp., clearly lower for *M. alterniflorum* and the lowest for *L. uniflora.*

The mean concentrations of ortho-phosphate in the interstitial waters were almost equal for *L. uniflora, J. bulbosus* and *Sphagnum* spp. and clearly higher for *M. alterniflorum* and *R. fluitans.* The mean nitrate concentration was the highest for *L. uniflora,* lower for *J. bulbosus* and *Sphagnum* spp. and much lower for *Lemna minor* and *R. fluitans.*

The mean ammonium concentrations, however, were the lowest for *Littorella uniflora,* higher for *J. bulbosus* and *Sphagnum* spp. and the highest for *Lemna minor* and *R. fluitans.*

The mean calcium concentration was almost equal for *Littorella uniflora, J. bulbosus* and *Sphagnum* spp. and much higher for *M. alterniflorum, Ranunculus peltatus, Lemna minor* and *Riccia fluitans.*

The mean total N concentration in the sediment was the lowest for *Littorella uniflora,* somewhat higher for *J. bulbosus* and *M. alterniflorum* and much higher for *Sphagnum* spp., *Lemna minor* and *R. fluitans.*

The mean total P concentration also was the lowest for *Littorella uniflora,* higher for *J. bulbosus, Sphagnum* spp., *M. alterniflorum, R. fluitans* and the highest for *Lemna minor.*

TABLE III

Chemical composition of the interstitial water

	n*	HCO_3^- (mmol l^{-1})		CO_2 (mmol l^{-1})		PO_4^{3-} (µmol l^{-1})		NO_3^- (µmol l^{-1})		NH_4^+ (µmol l^{-1})		Ca^{2+} (µmol l^{-1})	
		Mean	90%	Mean	90%	Mean	90%	Mean	90%	Mean	90%	Mean	90%
Littorella uniflora	9	1.02	0.06— 3.00	0.90	0.09—1.50	5.1	0.0— 7.5	90	15—180	267	150— 390	708	200—1500
Juncus bulbosus	19	0.42	0.00— 2.10	3.33	0.90—9.60	5.4	0.0— 15.0	60	15—180	510	150—1200	627	200—1500
Sphagnum spp.	9	0.78	0.00— 3.00	5.01	1.20—9.90	5.4	0.0— 15.0	51	15— 75	564	150—1500	675	200—1500
Myriophyllum alterniflorum	9	4.80	1.20— 9.00	1.65	0.00—3.90	11.4	1.5— 30.4	73	15—180	381	150— 600	1650	750—3000
Ranunculus peltatus	15	3.60	0.90— 9.90	1.86	0.00—7.50	24.0	3.6— 75.0	81	15—180	333	150— 900	1650	750—3000
Riccia fluitans	10	7.06	1.10—36.20	0.50	0.00—1.30	29.9	3.1—154.0	16	0— 35	1270	182—3790	1700	600—3000
Lemna minor	11	7.20	2.20—18.00	1.05	0.24—2.20	33.0	26.3— 68.8	29	9—106	1250	900—2400	1890	600—4000

*Number of samples investigated.

TABLE IV

Physico-chemical properties of the sediment

	n*	Redoxpotential (mV)	Total N (μmol g^{-1})		Total P (μmol g^{-1})		Total Ca (μmol g^{-1})		Organic weight (% dry weight)	
		Mean	Mean	90%	Mean	90%	Mean	90%	Mean	90%
Littorella uniflora	9	+70	35	14— 90	1.8	0.7— 5.0	7	0— 17	2.9	0.7— 5.0
Juncus bulbosus	19	+102	67	14— 90	4.8	1.0— 10.0	14	1— 80	8.1	0.9—20.0
Sphagnum spp.	9	+194	235	50— 300	7.2	2.0— 25.0	16	0— 82	20.0	5.3—60.0
Myriophyllum alterniflorum	9	−40	65	10— 300	10.5	2.0— 40.0	56	10—170	6.5	0.8—25.0
Ranunculus peltatus	15	+47	109	14— 400	20.0	5.0—100.0	40	5—180	5.6	0.8—20.0
Riccia fluitans	10	−150	544	70—1300	14.2	7.0— 34.0	75	8—180	23.0	4.3—78.0
Lemna minor	11	−152	247	47— 820	22.0	11.0— 33.0	64	8—103	11.4	3.4—41.6

*Number of samples investigated.

The mean total Ca concentrations show similar results: the lowest for *Littorella uniflora* and the highest for *R. fluitans*.

The mean organic weight of the sediment was the lowest for *L. uniflora*, followed by *Ranunculus peltatus*, *M. alterniflorum* and *J. bulbosus* and much higher for *Sphagnum* spp. and *Riccia fluitans*.

DISCUSSION

All observations indicate that acidification of the water caused by acid precipitation leads to a decrease and finally to a disappearance of *Littorella uniflora* and other "*Littorellion*"-species, coincident with the appearance and often luxurious growth of *J. bulbosus*, *Sphagnum* spp., *Eleocharis multicaulis*, *Eriophorum angustifolium*, etc. (Table Ib). The pH of the water declines strongly, on an average from pH 6.5 for *L. uniflora* down to pH 3.8 for *J. bulbosus* and *Sphagnum* spp. Alkalinity decreases on an average from 0.50 meq. l^{-1} for *L. uniflora* down to 0.02 and 0.01 meq. l^{-1} for *J. bulbosus* and *Sphagnum* spp., respectively Wiegleb (1978) mentions similar data for waters in the Federal German Republic: a mean alkalinity of 0.50 meq. l^{-1} for *L. uniflora* and 0.05 meq. l^{-1} for *J. bulbosus*. Sand-Jensen and Rasmussen (1978) also found *J. bulbosus* to be the only vascular plant species in strongly acidified Scandinavian waters and Nilssen (1980) mentions a luxurious growth of *J. bulbosus* in acidified waters in Norway. Grahn et al. (1974) and Grahn (1977) mention a suppression of isoetid species in Scandinavian waters by luxurious *Sphagnum* growth as a result of acidification of the water. Investigations by Spence (1964, 1967) clearly show that "*Littorellion*" species such as *L. uniflora* and *Lobelia dortmanna* mainly occur in non-acid, poorly buffered waters.

Acidification of the water brings about not one, but a number of changes in the physical and chemical properties of water and sediment, and it is difficult to establish which of these is responsible for the changes in the macrophyte composition. For instance, the sulphate concentration in *J. bulbosus*-dominated, acidified waters on an average is more than twice as high as in non-acidified *Littorella uniflora*-dominated waters, probably due to precipitation containing sulphuric acid and a possibly reduced sulphate reduction, though it is doubtful whether these sulphate concentrations have any influence on the observed changes in the macrophyte composition, as the maximum SO_4^{2-} concentration at which *L. uniflora* occurs is considerably higher than the mean SO_4^{2-} concentration for *J. bulbosus*.

There are also changes in the nitrogen and phosphorus concentrations. The total amount of mineral nitrogen in waters dominated by *J. bulbosus* and *Sphagnum* spp. (including the interstitial water) is on an average much higher than in waters dominated by *L. uniflora*, but whether this is of any importance is doubtful, as phosphorus is normally the limiting factor in moorland pools (Table IIb).

In particular, the mean ammonium concentrations of water and interstitial water in acidified *J. bulbosus* and *Sphagnum* spp. dominated water bodies are much higher, undoubtedly due to the strongly decreased nitrification as a result of the low pH.

Whether these high ammonium concentrations are toxic to *L. uniflora* under these circumstances has to be doubted. It is true that ammonium toxicity to various species of aquatic macrophytes has been reported (Glänzer et al., 1977), but these experiments were carried out at a relatively high pH, at which part of the ammonium occurs as toxic NH_3 (Warren, 1962). However, the pH in the acidified waters is low enough to keep all ammonium as non-toxic NH_4^+. Possibly, however, differences among the various plant species may occur in the ability to use ammonium instead of nitrate as a nitrogen source.

The mean concentration of ortho-phosphate in the water as well as in the interstitial water is almost equal in *L. uniflora*-, *J. bulbosus*- and *Sphagnum* spp.-dominated waters. Therefore, it is not to be expected that the changes in the macrophyte composition are due to changes in the phosphate budget, although the mean total P concentration in the upper sediment layer of the acidified waters is clearly higher than in the non-acidified waters. The accumulation of organic material as a result of strongly decreased microbial activity could partly be responsible, just as the strongly increased redox potential in the upper sediment layer, which leads to accumulation of phosphate coming from the deeper, anaerobic sediment layers. The latter process has been described by Damman (1978) for ombrotrophic peat bogs.

It is likely that the tremendous changes in the inorganic carbon content which occur as a result of acidification, play a very important role. In acidifying waters the inorganic carbon content of the water decreases to practically zero and the submerged macrophytes fully depend on the sediment, as the diffusion of CO_2 from the air into stagnant acid water is insufficient. Sand-Jensen and Søndergaard (1979) discovered that the size of *L. uniflora* plants in water poor in CO_2 depends on the CO_2 level in the sediment.

The data presented here clearly show that in acidified waters dominated by *J. bulbosus* the mean CO_2 concentration of the interstitial water is much higher than in non-acidified, *L. uniflora*-dominated waters. The CO_2 concentration of the interstitial water is highest in the *Sphagnum* spp.-dominated waters. This increase of CO_2 is no doubt due to the acid water layer acting upon the HCO_3^- and CO_3^{2-} in the sediment. This leads to the hypothesis that changes in the macrophyte composition are mainly dependent on the changes in the CO_2 concentration in the sediment, which depends on the HCO_3^- content of the interstitial water and the CO_3^{2-} content of the sediment. Field observations support this hypothesis. In acidifying dune pools above relatively CO_3^{2-}-rich sediments (e.g. van Hünenplak, Terschelling) the very tall *Littorella* plants became fully overgrown by *Sphagnum* spp., whereas in acidifying moorland pools above relatively CO_3^{2-} -poor sediments (e.g., Groothuisven near Tilburg) *L. uniflora* and *Lobelia dortmanna* became overgrown by *J. bulbosus*.

In other acidifying moorland pools, above mainly CO_3^{2-}-free sediments (e.g., Staalbergven near Oisterwijk; Galgenven near Tilburg) *Littorella uniflora* and *Luronium natans* gradually became smaller and almost disappeared, without any other macrophyte species appearing in their place.

Carbon dioxide measurements in the interstitial water of these locations also confirmed the above hypothesis. There is also an explanation for the fact that in some places "*Littorellion*" communities can persist for a very long period after acidification, as was observed in a few cases during this study and has also been mentioned by Pietsch (1972) for some waters in Central Europe. Luxurious growth of *J. bulbosus* and *Sphagnum* spp. can only occur after an increased CO_2 level in the sediment when phosphate or nitrogen are not limiting.

Eutrophication, in its classical meaning (increased input of nutrients such as phosphate and nitrogen), often mentioned as the main cause of the disappearance or decline of "*Littorellion*" species (Schoof-van Pelt, 1973; Pietsch, 1977; Wittig, 1980), has been a less important factor in the investigated waters during the last 30 years than acidification.

A change in the phosphate content seems to be responsible for the changes in all 12 waters where "*Littorellion*" species had strongly declined or had disappeared and no submerged *Sphagnum* spp. or *J. bulbosus* occurred. A phosphate enrichment of the mineral soil, with a strongly increased amount of ortho-phosphate in the interstitial water, but hardly any increased amount of phosphate in the water (e.g., environments rich in iron) leads to luxurious growth of submerged macrophytes, mainly *M. alterniflorum* and/or *Ranunculus peltatus* and a suppression of "*Littorellion*" species. However, after a number of years of luxurious plant growth the submerged plant species disappear again because the sediment becomes more or less organic. It is not clearly understood which processes underlie this phenomenon. Possibly the strongly lowered redox potential and the concomitantly strongly decreased CO_2 and strongly increased NH_4^+ concentration of the sediment which are found under these circumstances are responsible (Tables III and IV). A phosphate enrichment of the system with a clearly increased ortho-phosphate concentration not only in the sediment, but also in the water leads to luxurious growth of non-rooted macrophytes such as *Riccia fluitans* and *Lemna minor* in small, shallow waters. In larger and deeper water bodies the water becomes turbid as a result of algal bloom and/or strong development of epiphytes on the macrophytes. In this case, light reduction seems to be responsible for the decrease or disappearance of the submerged macrophytes (Sand-Jensen and Søndergaard, 1981).

In order to support the preceding hypotheses, and to obtain causal relations between the changes in the physico—chemical environment and plant development, "in vitro" eco-physiological and culture experiments have been carried out with the plant species involved. The results of these experiments will be discussed in part II of this study.

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CHAPTER 4.

Impact of acidification and eutrophication on macrophyte communities in soft waters.

II. Experimental studies.

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IMPACT OF ACIDIFICATION AND EUTROPHICATION ON MACROPHYTE COMMUNITES IN SOFT WATERS. II. EXPERIMENTAL STUDIES

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ABSTRACT

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In The Netherlands, there has been a dramatic decline during the last 30 years in the number of stands belonging to the phytosociological alliance *Littorellion*. Generally, the communities classified within this alliance occur in poorly buffered, oligotrophic waters, with very low phosphate, nitrogen and carbon dioxide levels in the water layer and considerably higher nutrient levels in the sediment. The plant species dominating these communities are isoetids such as *Littorella uniflora* (L.) Aschers., *Lobelia dortmanna* L. and *Isoetes lacustris* L., which show various adaptations to make successful growth possible under these conditions.

Field observations showed that the waters where *Littorella uniflora* had disappeared or strongly decreased could be divided into two groups. A major group (77%) was characterized by the presence of submerged *Juncus bulbosus* L. and/or *Sphagnum* species. These waters appeared to be strongly acidified ($pH < 4.5$) and had increased nitrogen levels with ammonium as the dominant N-source. Within this group, the waters with luxuriant growth of *Juncus bulbosus* and/or *Sphagnum* spp. had strongly increased carbon dioxide levels in both sediment and water.

Different types of experiments proved causal relationships between the observed changes in macrophytes and the changed physico-chemical parameters. Ecophysiological experiments showed that *Juncus bulbosus* lacks the typical adaptations of the isoetid plant species, i.e. it uses very low amounts of sediment-CO_2 and releases only a little oxygen from the roots. However, *Juncus bulbosus* is more able than *Littorella uniflora* to use CO_2 from the water layer. From the nutrient-uptake experiments, the decreased nitrate and increased ammonium levels seem to be favourable to *Juncus bulbosus*. The culture experiments clearly demonstrated that the biomass of *Juncus bulbosus* only increased strongly when the sediment was poorly buffered and the pH of the water was low. When combining factors like CO_2 enrichment of the sediment, with and without phosphate, and/or ammonium enrichment of the water in the culture experiments, it is clearly shown that phosphate and/or ammonium enrichment without CO_2 enrichment do not lead to an increase in biomass of *Juncus bulbosus*. Therefore, it is obvious that the changes in the macrophyte community can be ascribed primarily to changes in the carbon budget as a result of acidification.

A minor group of waters (23%) was characterized by the absence of submerged *Juncus bulbosus* and/or *Sphagnum* spp. In most of these waters, submerged plant species occurred, such as *Myriophyllum alterniflorum* DC or non-rooted species such as *Riccia fluitans* L. These waters were not acidified, and generally had an increased alkalinity and higher nitrogen and phosphate levels of sediment and/or water. Culture experiments showed that phosphate enrichment of the sediment alone leads to luxuriant growth of submerged macrophyte species such as *Myriophyllum alterniflorum*, whereas phosphate enrichment of both sediment and water leads to mass development of non-rooted plant species such as *Riccia fluitans* .

INTRODUCTION

In The Netherlands, there has been a dramatic decline during the last 30 years in the number of stands belonging to the phytosociological alliance *Littorellion*. These stands are characterized by isoetids, such as *Littorella uniflora* (L.) Aschers., *Lobelia dortmanna* L., *Isoetes lacustris* L., etc. (Schoof-van Pelt, 1973).

Field observations showed that the waters in which *Littorella uniflora* had disappeared or strongly decreased could be divided into two groups (Roelofs, 1983a). The major group (77%) was characterized by the presence of submerged *Juncus bulbosus* L. and/or *Sphagnum* species, an acid water layer (pH on average 3.9) and increased ammonium levels. On locations with luxuriant growth of *Juncus bulbosus* and/or *Sphagnum* spp., the CO_2 levels of the sediment were particularly high. It is possible that the changed inorganic carbon and/or nitrogen budgets, as a result of acidification, are responsible for the observed changes in the macrophyte communities.

In the minor group of waters (23%), in which no submerged *Juncus bulbosus* and/or *Sphagnum* spp. occurred, the water layer was not acidified. In waters with a luxuriant growth of *Myriophyllum alterniflorum* DC, the nutrient levels of the sediment, particularly the phosphate level, were clearly higher than those in *Littorella uniflora*-dominated waters, whereas the phosphate levels in the water layer were very low. In waters with a luxuriant growth of pleustophytes, such as *Riccia fluitans* L. and *Lemna minor* L., the nutrient levels of both water and sediments were clearly higher.

In order to establish causal relationships between the observed changes in the macrophyte communities and the physical—chemical environment, a number of ecophysiological and culture experiments have been conducted with the plant species most involved.

Nitrogen availability and uptake

The utilization of nitrogen by aquatic macrophytes has not yet been investigated thoroughly. Only a few workers have considered the selective utilization, when ammonium and nitrate are supplied together. Investigations by Ferguson and Bollard (1969), Kopp et al. (1974) and Schwoerbel and Tilmans (1974) show that ammonium is used first. This might be due to inhibition of nitrate uptake by ammonium. Aquatic macrophytes which can use ammonium as well as nitrate as their sole N-source show different morphological characteristics when growing in a NH_4^+- or NO_3^--medium.

Melzer (1979) studied nitrate reductase activities of submerged macrophytes using ecophysiological experiments. In waters containing high levels of ammonium, most plant species had low nitrate reductase activity; only a few showed high activity. In nitrate-rich waters, both species with high and species with low activity were found. When plants were transplanted from NH_4^+-rich to NO_3^--rich waters, there was usually little or no rise of nitrate reductase activity, and Melzer emphasizes that these plants might not survive, due to N-starvation. Considering these regulation mechanisms, it could be expected that in field situations the used nitrogen source depends on the ammonium and nitrate levels in the water. It is obvious that the experiments of Melzer (1979) give little information on ammonium or nitrate utilization by submerged macrophytes. Nitrogen uptake can more easily be determined by "in vitro" experiments, in which the decrease in N-concentration of the nutrient medium can be followed.

Ecophysiological studies on nutrient uptake by plant species of soft waters have not been carried out up to now. This part of the study was set up in order to determine whether the observed replacement of *Littorella uniflora* by *Juncus bulbosus*, caused by acidification, could be related to changed N-availability and pH. Waters dominated by *Littorella uniflora* generally have low nitrogen levels, nitrate often being the dominant N-source, whereas waters dominated by *Juncus bulbosus* generally have higher nitrogen levels, ammonium being the dominant N-source. This study examined in what way the different N-circumstances might be favourable for *Littorella uniflora* or *Juncus bulbosus.*

Carbon dioxide availability and photosynthesis

Field investigations show that the CO_2 levels in poorly buffered non-acidified waters are often very low (6—40 μmol l^{-1}). However, the CO_2 levels of the interstitial water can reach concentrations up to 2—4 mmol l^{-1} (Nygaard, 1965; Wium-Andersen and Andersen, 1972; Roelofs, 1983a).

Isoetid plant species, such as *Littorella uniflora, Isoetes lacustris* and *Lobelia dortmanna*, have several morphological and biochemical adaptations to enable successful growth under these conditions.

- *Lobelia dortmanna* (Wium-Andersen, 1971) and *Littorella uniflora* (Søndergaard and Sand-Jensen, 1979) are able to absorb free CO_2 by the roots.
- Søndergaard and Sand-Jensen (1979) estimated that *Littorella uniflora* and *Lobelia dortmanna*, plants with extensive lacunal systems, can recapture a large part of their photo-respired CO_2; in this way contributing to a more efficient assimilation.
- The underground biomass of these plant species depend on the nutrient levels of the sediment (Sand-Jensen and Søndergaard, 1979).

- *Littorella uniflora* (Keeley, 1983) and many species of the genus *Isoetes* (Keeley, 1981a, b, 1982a, b) have developed a diurnal acidification/deacidification cycle very similar to Crassulacean acid metabolism (C.A.M.).
- Sand-Jensen et al. (1982) recorded a high oxygen release by the roots of isoetid plant species.

The macrophyte succession, in particular the replacement of *Littorella uniflora* by *Juncus bulbosus* and *Sphagnum* spp., is one of the most typical characteristics accompanying acidification. As already discussed (Roelofs, 1983a), this change in the submerged vegetation is probably due to a changed carbon budget in the ecosystem. In order to acquire a deeper understanding of this phenomenon, the photosynthetic response of *Littorella uniflora* and *Juncus bulbosus* to several inorganic carbon conditions was studied.

Culture experiments

Transplantation experiments are often used in order to study the effect of environmental factors on aquatic macrophyte growth (Agami et al., 1975; Giesy and Tessier, 1979; Carignan and Kalff, 1980). One of the disadvantages of this method, however, is the fact that a number of factors will always change, which makes it difficult to estimate which environmental factor is responsible for the observed changes in macrophyte development.

Fully controlled culture experiments in the laboratory, in which only one factor or a certain combination of factors is varied, are more suitable. Glänzer et al. (1977) carried out such experiments with river plants.

In this study, similar laboratory experiments have been conducted with soft-water macrophytes. Culture experiments have been conducted in staged pH/alkalinity gradients of the water on both carbonate-free and low-carbonate-containing sediments, and in staged ammonium gradients of the water at both low and relatively high pH. Effects of phosphate and/or ammonium enrichment of the culture medium were studied at a low pH on carbonate-free and low-carbonate-containing sediments. Finally, the effect of phosphate enrichment of the sediment in combination with a phosphate-free culture medium was studied.

MATERIALS AND METHODS

Chemical and physical analyses of the media, sediments and plant materials were carried out according to Roelofs (1983a). Carbon dioxide measurements were carried out using an Oceanography International model 0525 HR infrared carbon analyzer; the CO_2 was stripped from the acid media using N_2-gas.

Nutrient uptake

In Experiment 1, N- and P-uptake were determined under different pH conditions. In Experiments 2 and 3, the ammonium and/or nitrate uptake was studied after different incubation periods in a N-free medium. The nutrient uptake was followed during 24 h.

The experiments were conducted with *Littorella uniflora* and *Juncus bulbosus*. For Experiments 1 and 2, both *Littorella* and *Juncus* were obtained from the "Staalbergven" near Oisterwijk. For Experiment 3, *Littorella* was obtained from the Nieuwkuykse Wiel" and *Juncus* from the "Ven bij Schaijk". The physical and chemical characteristics of those sampling sites are given in Table I; they are comparable in the concentrations of most nutrients. All waters have relatively high levels of ammonium and nitrate. However, the "Staalbergven" and the "Ven bij Schaijk" have recently been acidified, whereas the "Nieuwkuykse Wiel" is not acidified and still poorly buffered.

In the laboratory, the plants were washed free of sediment and epiphytes and incubated in a N-free basic culture medium. 30 g (wet weight) of plant material was added to the nutrient media in 2-l perspex tanks. The chemical composition of the media is given in Table II; all solutions were made with de-ionized bi-distilled water. In Experiment 1, pH 3.5 was obtained by

TABLE I

Some chemical and physical characteristics of the water and sediment of the sampling-sites

	Water						
	pH	Alk.	NH_4^+	NO_3^-	PO_4^{3-}	K^+	
Nieuwkuykse Wiel	7.3	0.5	23	24	0.4	181	
Staalbergven	4.4	0.0	35	41	0.4	80	
Ven near Schayk	4.1	0.0	14	35	0.3	72	
	Soil extract (1:10)						
	pH	Alk.	Acid.	NH_4^+	NO_3^-	PO_3^{3-}	K^+
Nieuwkuykse Wiel	6.1	0.05	0.02	1.3	0.0	0.0	15
Staalbergven	5.3	0.0	0.06	13	0.6	0.2	—
Ven near Schayk	6.6	0.04	0.02	12.5	0.6	0.2	—
	Total sediment						
	Loss on ignition	Anorg. C	Soil-type	N	P		
Nieuwkuykse Wiel	1.0	0.1	Sand	21	4		
Staalbergven	4.1	0.1	Sand	45	5		
Ven near Schayk	1.0	0.1	Sand	45	14		

All values are expressed as μmol l^{-1} except: pH; alkalinity and acidity (meq l^{-1}); loss on ignition (%); and total sediment (μmol g^{-1} dry sediment).

TABLE II

Chemical composition of the incubation medium and the media of the nutrient-uptake experiments

	Incubation-medium	Nutrient-medium
Experiment 1 pH = 3.5	N + P-free basic culture medium	Basic culture medium +50 μmol l^{-1} NH_4NO_3 + 5 μmol l^{-1} K_2HPO_4 $3H_2O$
Experiment 2 pH = 3.5	N + P-free basic culture medium	Basic culture medium +50 μmol l^{-1} NH_4NO_3 +40 μmol l^{-1} K_2HPO_4 $3H_2O$
Experiment 3 pH = 4.0	N-free basic culture medium	Basic culture medium +50 μmol l^{-1} KNO_3 +50 μmol l^{-1} NH_4Cl

adding 1 N HCl, and pH 6.5 by adding 0.5 mmol l^{-1} $NaHCO_3$ (alkalinity 0.5 meq l^{-1}). All experiments were carried out at a light intensity of 250 μE m^{-2} s^{-1} at 20°C using the cultivation set-up as described later.

The nutrient uptake was followed during 24 h by collecting water samples from the 2-l perspex tanks at 0, 2, 4, 6, 12 and 24 h after the start of the experiment. For each experiment, a tank without plants served as a control. The samples were fixed with 0.1 ml of a 200 mg l^{-1} $HgCl_2$-solution and stored at 4°C until analysis. The chemical analyses were carried out immediately after completion of the experiments. Only when there was a linear relation between uptake and time was the nutrient uptake rate per gram dry weight calculated. Corrections were made for volume loss as a consequence of sampling and for the control.

Plant adaptations, carbon dioxide availability and photosynthesis

At several field locations and from two culture experiments, the root to leaf ratio of *Littorella uniflora* and *Juncus bulbosus* plants was estimated (on dry weight basis) and correlated to the carbon dioxide concentrations of the interstitial water. Carbon dioxide measurements of the water layer were also carried out in a number of recently acidified waters with great differences in macrophyte development.

The photosynthetic responses of *Littorella uniflora* and *Juncus bulbosus* at different carbon dioxide levels were studied in a perspex incubation chamber (Fig. 1). The leaves could be separated from the roots using a perspex diaphragm. The opening was sealed with chewing gum and agar agar to ensure a waterproof separation. Both the leaf and the root compartment had in- and out-flow openings. The in-flow opening was connected to two bottles containing, respectively, a bicarbonate and an acid solution. Samples were

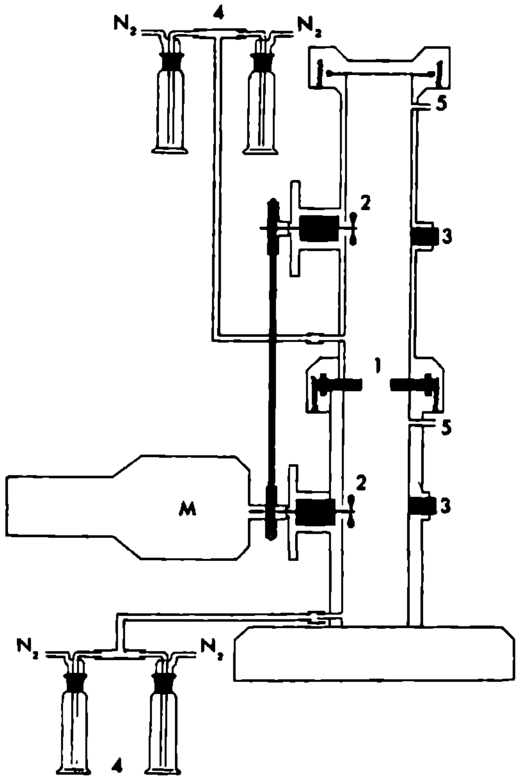


Fig. 1. Perspex incubation chamber for measuring the photosynthetic response, with: (1) diaphragm; (2) winged stirrers with M = motor; (3) septa for sampling; (4) bottles with experimental solutions; (5) out-flow opening.

taken with a glass syringe through an opening sealed with a septum. The four bottles were connected to plastic bags filled with N_2 in order to prevent oxygen in-flow. Two winged stirrers continuously mixed the incubation media in both compartments in order to stimulate gas exchange and to obtain a homogeneous solution.

Light was provided by two cold-light sources (Schott KL 150 B) with an intensity of 350 μE m^{-2} s^{-1} which saturated photosynthesis of both species.

Prior to the experiments, the media were bubbled with N_2 to lower the O_2-content to approximately 30 μmol l^{-1} and the plants were placed in the light until O_2-evolution had ceased. This was necessary to exhaust an eventual internal C-source of the plants.

The experiments lasted 2 h. 1.5-ml samples were taken every 30 min from both compartments with a glass syringe. Corrections were made for the dilution due to the sampling. Oxygen measurements were carried out with a Gilson oxygraph model K-IC equipped with a fast-response Clark electrode.

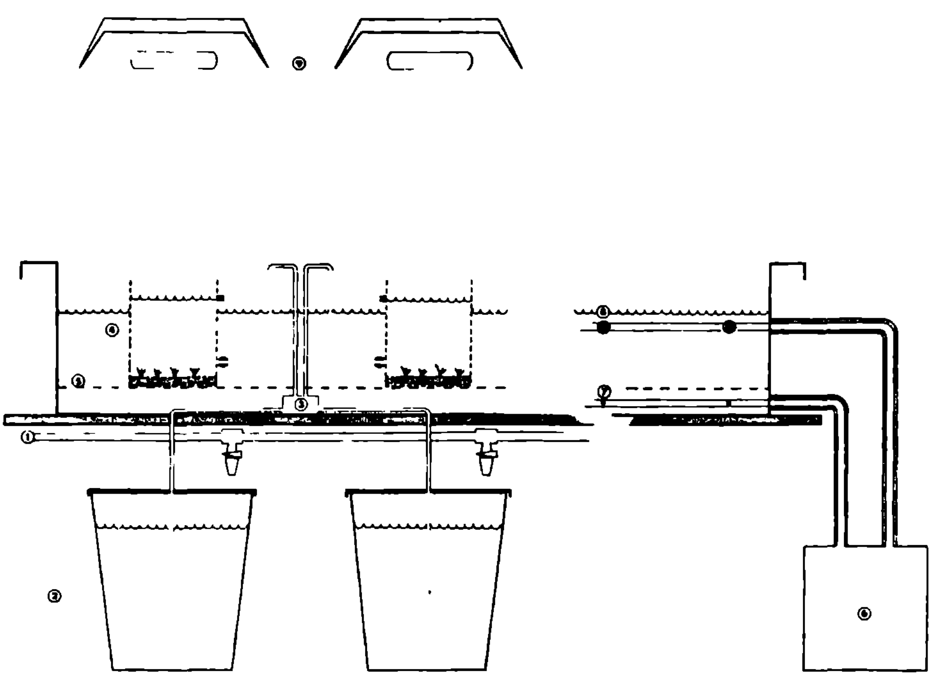


Fig. 2. Experimental set-up for culture experiments, with: (1) demi-water supply; (2) polyethylene stock containers; (3) peristaltic pumps; (4) glass aquaria; (5) waterbath; (6) cooling and heating aggregate; (7) in-flow and (8) out-flow openings, (9) metal halide lamps.

Culture experiments

Technical equipment

The experiments were conducted in glass containers (l.b.h. 25 × 25 × 31 cm) which were placed in a stainless steel waterbath (Fig. 2). The temperature was maintained at 17°C by means of a Neslab-type coolflow 75 cooling aggregate. The culture medium was continuously refreshed (1 l h^{-1}) from black polyethylene 120-l stock containers by means of Colora-type 3610 multi-channel peristaltic pumps. Black silicone tubing was used in order to prevent nutrient consumption by algal growth. Irradiance in this facility was provided by 6 Philips-type HP (1) 400 W high-pressure metal halide lamps at a photoperiod of 14 h at 250 $\mu E\ m^{-2}\ s^{-1}$.

Sediments

The mineral sandy soils used in Experiments 4—7 were obtained from the "Staalbergven" near Oisterwijk. They were washed in acidified water from the sampling site in order to remove carbonates. Samples were taken to check whether residual carbonates were still present. The sediment used in Experiment 8 was obtained from "De Rouwkuilen" near Venray. The sediments, used in Experiments 5—7, and part of those used in Experiment 8, were enriched with 50 mmol $CaCO_3$ kg^{-1} soil (wet weight) and thoroughly mixed.

The mineral sandy soils used in Experiments 9 and 10 were obtained from the littoral border of "De Broekse Wielen" near Grave. Phosphate was removed from the sediment used in Experiment 10 by washing with 0.1 M HCl and 1.0 M NaOH. After neutralization, a 6-step phosphate range was created by adding $Ca_3(PO_4)_2$.

The different additions to the sediments used in the culture experiments are summarized in Table III.

TABLE III

Additions to sediments and media of the different culture experiments

Experiment	Washed sediment	Basic culture medium
4	—	+ pH/HCO_3^- gradient
5	+ $CaCO_3$	+ pH/HCO_3^- gradient
6	+ $CaCO_3$	+ NH_4Cl gradient (pH = 4.0)
7	+ $CaCO_3$	+ NH_4Cl gradient (pH = 7.0)
8	+/– $CaCO_3$	+ Na_2HPO_4 and/or $(NH_4)_2SO_4$
9	—	+ Na_2HPO_4 gradient
10	+ $Ca_3(PO_4)_2$ gradient	– phosphate

TABLE IV

Chemical composition of the basic culture medium

Major components (μmol l^{-1})		Trace-elements (μmol l^{-1})	
Na^+	620 or 1120	Fe	2.00
K^+	150	Cu	0.40
Mg^{2+}	60	Mn	2.70
Ca^{2+}	125	Zn	0.30
Cl^-	610	Mo	0.50
SO_4^{2-}	250	Co	0.01
NH_4^+	10	Sr	0.02
NO_3^-	20	Bo	0.04
PO_4^{3-}	0.5	Br	0.06
HCO_3^-	0 or 500		

Culture medium

The basic culture medium (Table IV) was composed synthetically by adding certain chemicals to twice-demineralized water, the concentration of each element being within the range of the observed concentrations in waters in which *Littorella uniflora* occurs (Roelofs, 1983a).

In Experiments 4 and 5, a 6-step pH/alkalinity gradient was created by adding sulphuric acid (H_2SO_4) and $NaHCO_3$ to the basic culture medium without HCO_3^-.

In Experiment 6, a 6-step ammonium range was created by adding ammonium chloride (NH_4Cl) to the basic culture medium without NH_4^+ and HCO_3^- and then acidifying with sulphuric acid down to pH 4.0.

In Experiment 7, the same NH_4^+ range was created by adding NH_4Cl to the basic culture medium containing 0.5 mmol HCO_3^- l^{-1}.

In Experiment 8, part of the culture medium was enriched with Na_2HPO_4 (5 μmol l^{-1}) or $(NH_4)_2SO_4$ (2000 μmol l^{-1}) or both. In this experiment, all media were acidified with HCl down to pH 5.0.

A 6-step phosphate range was created in Experiment 9 by adding Na_2HPO_4 to the basic culture medium without phosphate.

In Experiment 10, only basic culture medium without phosphate was used.

The additions to, and gradients of, the media are given in Table III. As a control, water and interstitial water samples were taken and analyzed regularly; pH measurements were also carried out. When necessary, the pH was corrected.

Plant material

Plants of *Juncus bulbosus, Littorella uniflora, Sphagnum recurvum* P. Beauv. and *Sphagnum cuspidatum* were collected from the "Staalbergren" near Oisterwijk, whereas *Myriophyllum alterniflorum* and *Riccia fluitans* were collected from "De Broekse Wielen" near Grave.

After thoroughly washing and removing epiphytes, ca. 10 g (wet weight) of each plant species were planted in a container. In order to estimate the initial biomass, 3 × 10 g (wet weight) of each plant species were dried (24 h; 105°C) and weighed.

At the end of the 10-week experiments, the plants were collected and carefully cleaned, after which the dry weight was estimated.

RESULTS

Nutrient uptake

The results of Experiment 1 (Table V) show that the ammonium-, nitrate- and orthophosphate-uptake rates of *Littorella uniflora* and *Juncus bulbosus* were hardly affected by pH or alkalinity. Experiments 2 and 3 (Table V), as well as Experiment 1, demonstrate that the two plant species (obtained from waters containing both nitrate and ammonium) only used ammonium as a N-source when NO_3^- and NH_4^+ were supplied together. However, after 7—14 days of N-starvation, they also used some nitrate.

In most cases the N- and P-uptake rates of both plant species increased when the incubation period in the N- and P-free medium was longer. How-

TABLE V

Nutrient-uptake rate (μmol g^{-1} DW h^{-1}) of *Littorella uniflora* and *Juncus bulbosus*

(a) At different pH of the medium (Experiment 1)

	NH_4^+		NO_3^-		PO_4^{3-}	
	pH = 3.5	pH = 6.5	pH = 3.5	pH = 6.5	pH = 3.5	pH = 6.5
L. uniflora	0.42	0.46	0.0	0.0	0.08	0.11
J. bulbosus	0.11	0.18	0.0	0.0	0.26	0.21

(b) After an incubation period of (A) 2 days, (B) 7 days, and (C) 14 days in a N-free medium (Experiments 2 and 3)

		Experiment 2				Experiment 3			
		NH_4^+	NO_3^-	PO_4^{3-}	K^+	NH_4^+	NO_3^-	PO_4^{3-}	K^+
L. uniflora	A	0.3	0.0	0.3	—	1.7	0.0	—	-1.9
	B	—	—	—	—	4.2	1.0	—	-1.2
	C	2.8	0.4	0.6	-2.0	5.5	1.3	—	-1.2
J. bulbosus	A	0.5	0.0	0.4	—	1.2	0.0	—	0.2
	B	—	—	—	—	2.1	0.4	—	0.4
	C	0.3	0.0	0.8	-0.8	3.4	0.5	—	0.5

ever, this increase was much stronger for *Littorella uniflora*, especially for nitrate uptake. Potassium was excreted by *Littorella uniflora*, whereas *Juncus bulbosus* showed a positive K-uptake rate in Experiment 3.

Plant adaptations, carbon dioxide availability and photosynthesis

The results as expressed in Table VI clearly show that the below-ground biomass is related to the carbon dioxide level of the interstitial water. However, under all conditions, the root biomass of *Littorella uniflora* is much higher than that of *Juncus bulbosus*.

Oxygen release by the roots (Table VII) is related to the root biomass, and was relatively high for *Littorella uniflora* and very low for *Juncus bulbosus*, even when it had a high root biomass.

The carbon dioxide measurements of the water in recently acidified waters (Table VIII) clearly show that the carbon dioxide level is very low at localities without submerged macrophyte growth (50—60 μmol l^{-1}), and much higher at localities with luxuriant growth of *Juncus bulbosus* and/or *Sphagnum cuspidatum* (150—870 μmol l^{-1}).

When adding carbon dioxide to the whole plants, *Juncus bulbosus* showed saturated photosynthesis at a concentration of only 0.5 mmol l^{-1} (Fig. 3),

TABLE VI

The root/leaf ratio of ***Littorella uniflora*** and ***Juncus bulbosus*** at different CO_2-levels of the interstitial water

	CO_2 (mmol l^{-1})	Root/leaf ratio (dry wt.)	
		L. uniflora	*J. bulbosus*
(Culture) Exp. 4	0.3	2.3	0.56
(Culture) Exp. 5	3.5	1.2	0.16
Ven near Bakkeveen	0.2	7.5	0.76
Beuven	1.3	1.8	–
Lobelia-baai	1.2	1.3	–
Ven near Schayk	2.5	–	0.15

TABLE VII

Oxygen release by roots* of *Littorella uniflora* and *Juncus bulbosus*

	% root biomass	% oxygen
L. uniflora	26	23.0
	40	38.5
J. bulbosus	45	2.5
	55	3.0

*Expressed as % of the total O_2-release.

TABLE VIII

The CO_2-level of the water at some acidified locations with differences in macrophyte development

Location	Macrophyte vegetation	pH (water)	CO_2(μmol l^{-1})
Staalbergven	No submerged macrophytes	4.0	66
Ven near Schayk	No submerged macrophytes	3.7	55
Vogelvijvers	No submerged macrophytes	4.7	60
Vogelvijvers	*Juncus bulbosus*	4.0	150
Rouwkuilen	*Juncus bulbosus*	4.1	170
Ven near Schayk	*J. bulbosus + Sphagnum cuspidatum*	3.9	840
Mariaveen	*Sphagnum cuspidatum*	3.6	870

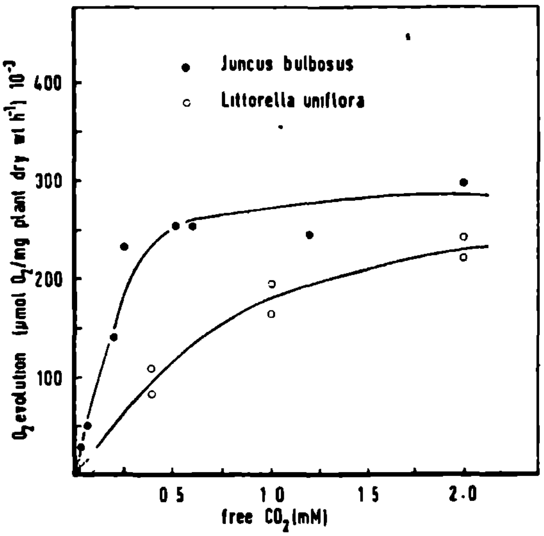


Fig. 3. Photosynthetic response of *Littorella uniflora* and *Juncus bulbosus* at different carbon dioxide concentrations.

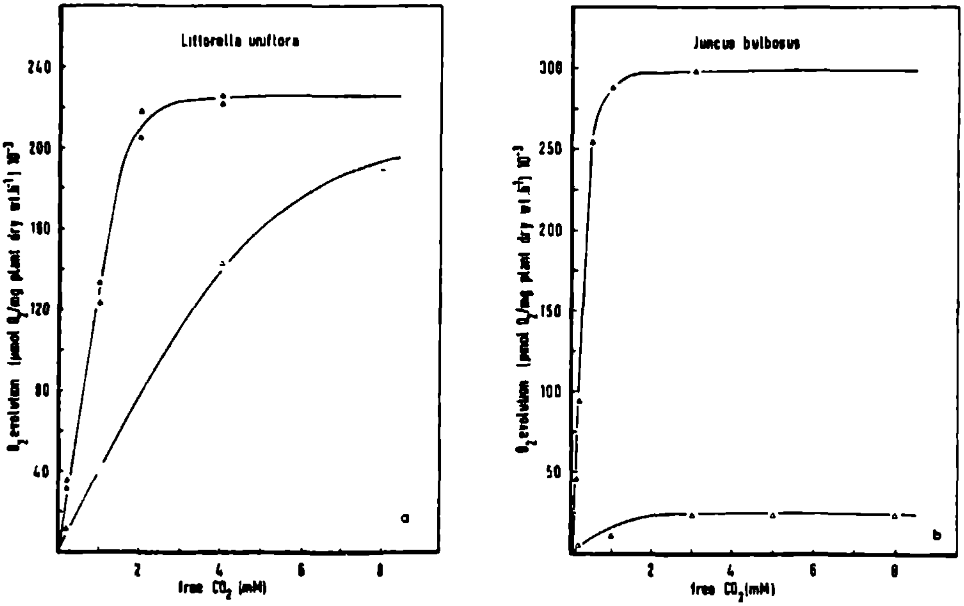


Fig. 4. Photosynthetic response of (a) *Littorella uniflora* and (b) *Juncus bulbosus* when carbon dioxide is offered to the roots (▵) or to the leaves (▴).

whereas *Littorella uniflora* showed no saturated photosynthesis until a concentration of 2 mmol l^{-1} (Fig. 3).

However, when carbon dioxide was offered to the roots only, *Littorella uniflora* showed a relatively high photosynthetic response (>80% of the maximum photosynthesis at 8 mmol $l^{-1}CO_2$; Fig. 4a), whereas *Juncus bulbosus* showed a very low photosynthetic response (< 10% of the maximum photosynthesis at 8 mmol $l^{-1}CO_2$; Fig. 4b).

Culture experiments

The results of Experiment 4 clearly show that increase of biomass of *Littorella uniflora* was only observed in a carbonate-free sediment when the water was poorly buffered (alkalinity 0.5 mmol l^{-1}; Fig. 5a); at a low pH there was a strong decrease in biomass. *Juncus bulbosus* showed a decrease in biomass in a carbonate-free sediment at each pH and alkalinity of the water. However, this decrease was the lowest at a high alkalinity (Fig. 5b).

In sediments containing little carbonate (Experiment 5), *Littorella* grew at each pH and alkalinity of the water, although the increase in biomass was the highest at a low pH and a high alkalinity (Fig. 5a). *Juncus bulbosus* showed a decrease in biomass at a high pH and low-to-medium alkalinities of the water. However, a strong increase in biomass occurred at a low pH and

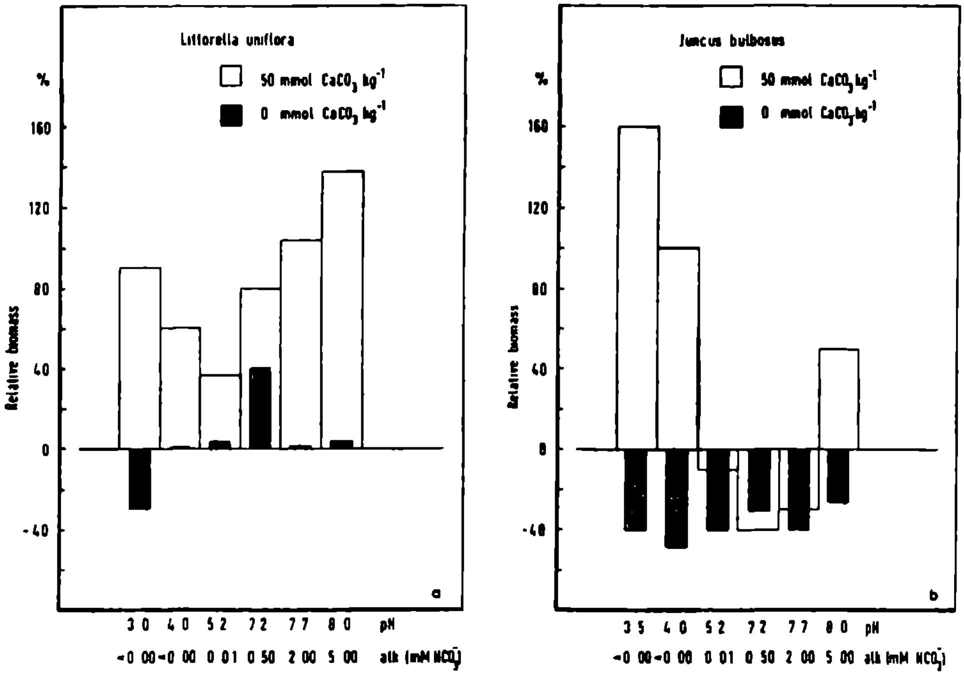


Fig. 5. Relative change in biomass of (a) *Littorella uniflora* and (b) *Juncus bulbosus* in a staged pH/alkalinity gradient of the medium on carbonate-free and low-carbonate sediment (Experiments 4 and 5).

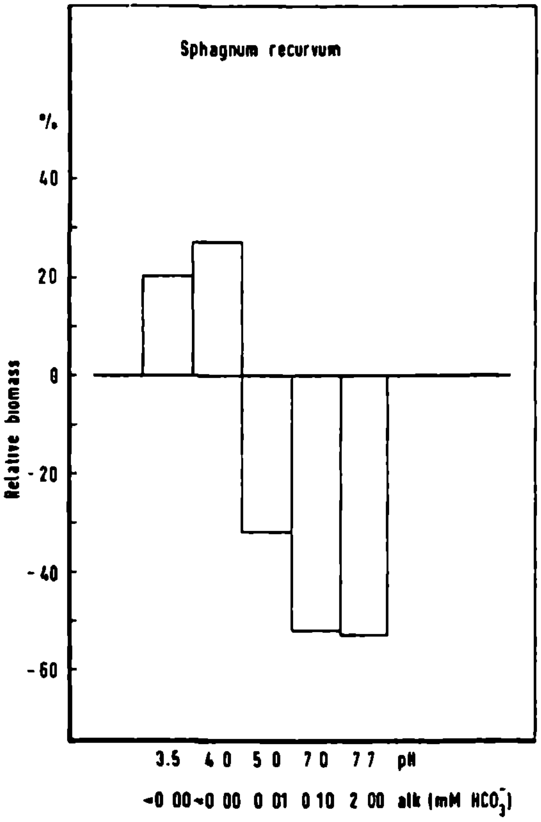


Fig. 6. Relative change in biomass of *Sphagnum recurvum* in a staged pH/alkalinity gradient of the medium (Experiment 4).

high alkalinities (Fig. 5b). *Sphagnum recurvum* only showed an increase in biomass at a low pH (Fig. 6). However, it showed a strong decrease in biomass at both low and medium alkalinities of the water.

Experiments 6 and 7 clearly show that at a high pH, the maximum growth of *Littorella uniflora* occurred at low NH_4^+ levels of the medium. At a low pH, there is a strong increase in biomass at each ammonium concentration. The strongest growth, however, occurred in both NH_4^+-free medium and in medium with a very high NH_4^+ level (Fig. 7a). At a high pH, *Juncus bulbosus* only showed some increase in biomass at very high ammonium levels; at a low NH_4^+ level it showed a strong decrease. At a low pH, a strong increase in biomass occurred at each ammonium level, but at high levels the increase is about twice as high (Fig. 7b). Maximum growth of *Sphagnum cuspidatum* was also observed at a low pH and high ammonium levels of the medium (Fig. 8).

Experiment 8 (Table IX) shows that phosphate enrichment of the acid culture medium, both NH_4^+-free and NH_4^+-rich, combined with a carbonate-

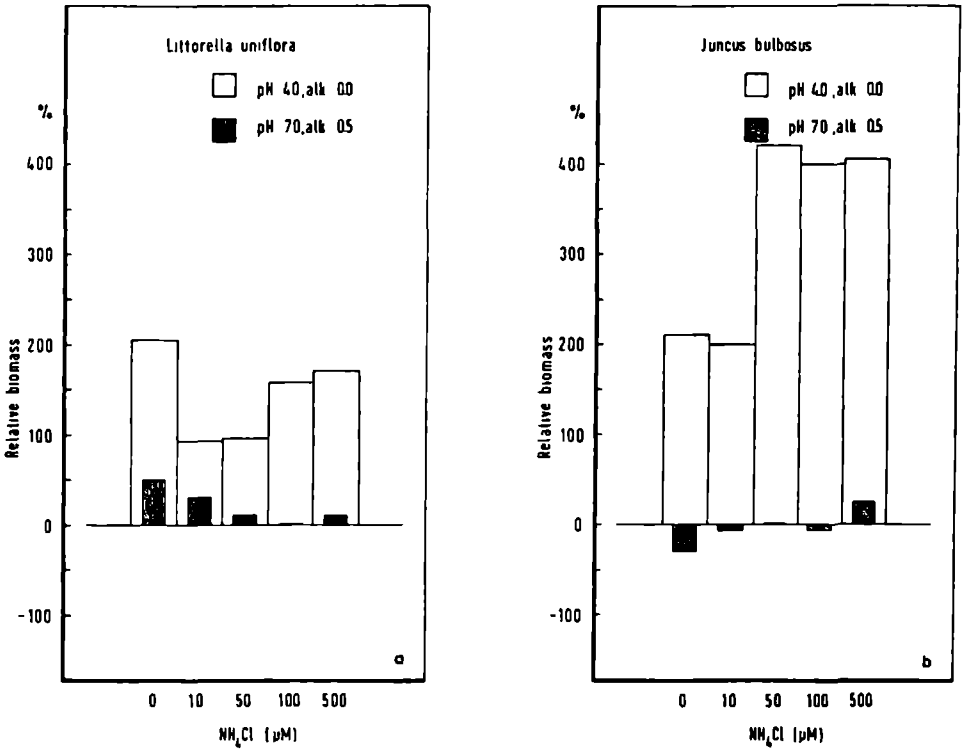


Fig. 7. Relative change in biomass of (a) *Littorella uniflora* and (b) *Juncus bulbosus* in a staged NH$_4$Cl-gradient at different pH/alkalinity (Experiments 6 and 7).

free sediment, did not result in an increase in biomass of the two investigated species. In a phosphate-rich medium, *Juncus bulbosus* did not show a decrease in biomass either, but in a phosphate-free culture medium, the biomass decreased strongly. *Littorella uniflora* showed a clear decrease in biomass in both phosphate-free and phosphate-rich culture media. Both plant species only showed a strong increase in biomass in sediments containing little carbonate, although this increase is much stronger for *Juncus bulbosus* than for *Littorella uniflora*. The latter did not show any increase in biomass at high ammonium levels.

Experiment 9 (Fig. 9) shows that *Littorella uniflora* and *Myriophyllum alterniflorum* only grew with very low phosphate levels in the medium. At increased phosphate levels ($\geqslant$0.5 μmol l^{-1}), a strong epiphyte development occurred and *Myriophyllum alterniflorum* died off completely within the 10-week experiment. *Riccia fluitans*, a non-rooted plant species, showed luxuriant growth at somewhat higher phosphate levels of the medium, whereas at high phosphate levels, the growth was less and a strong development of algae occurred.

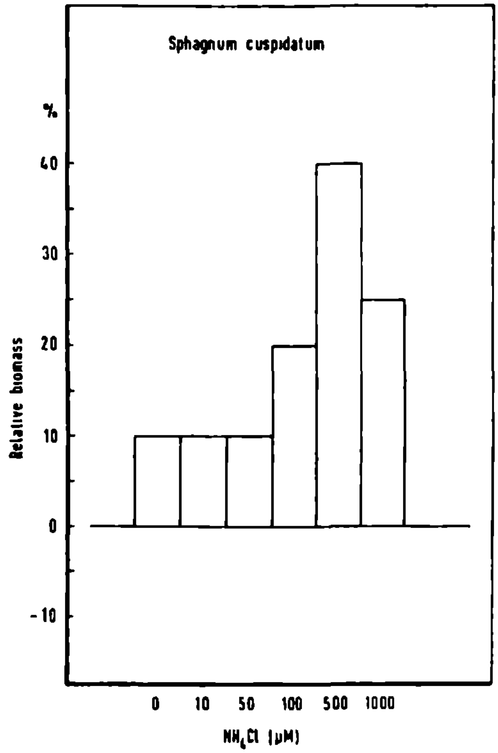


Fig. 8. Relative change in biomass of *Sphagnum cuspidatum* in a staged NH_4Cl-gradient at pH = 4.0 (Experiment 6).

TABLE IX

Growth of *Littorella uniflora* and *Juncus bulbosus* in Experiment 8 with different combinations of N-, P- and C-addition to water and sediment

Basic culture medium (pH = 5.0) plus (μmol l^{-1}):		Sediment (5% org. wt.) (carb.-free sed.) plus (mmol kg^{-1})	Relative change in biomass (%)	
$(NH_4)_2SO_4$	NaH_2PO_4	$CaCO_3$	*Littorella*	*Juncus*
—	5	—	−12	+ 4
—	—	50	+35	+73
2000	—	—	−32	−58
2000	—	50	0	+65
2000	5	—	−32	− 4

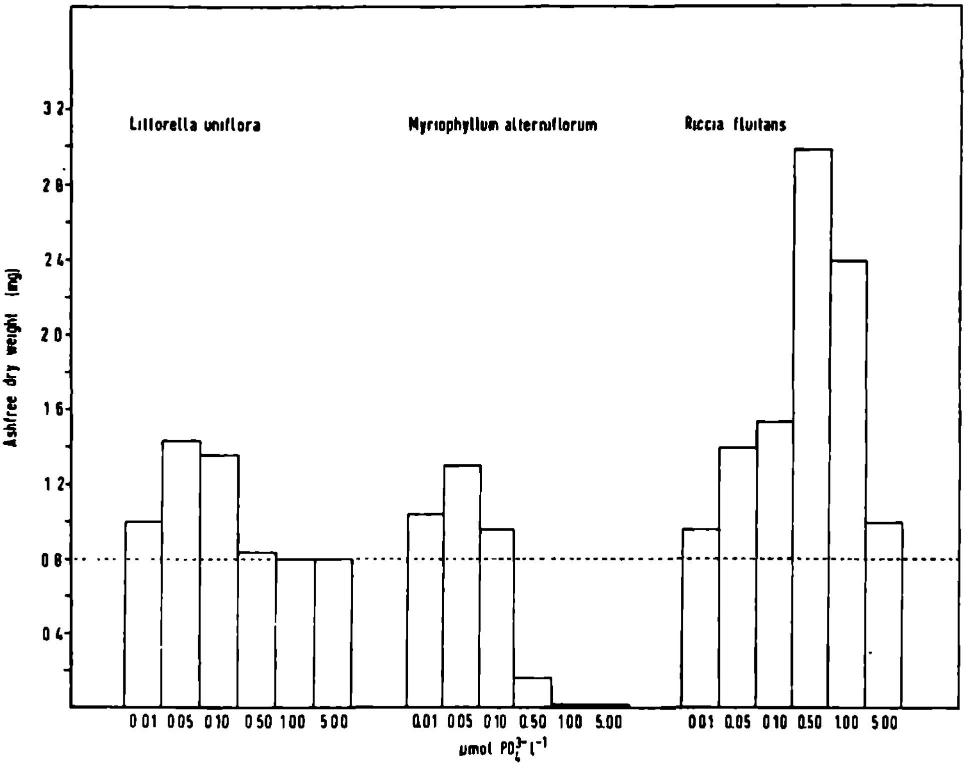
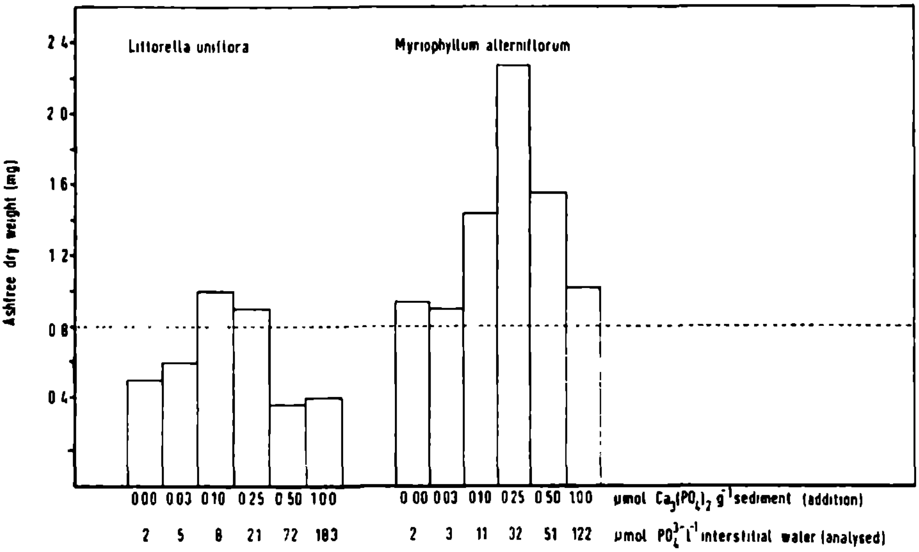


Fig. 9. Biomass (ashfree dry weight) of *Littorella uniflora*, *Myriophyllum alterniflorum* and *Riccia fluitans* after a cultivation period of 10 weeks in a staged phosphate gradient of the medium (Experiment 9) (dotted line = initial biomass).



Experiment 10 (Fig. 10) clearly shows *Myriophyllum alterniflorum* growing very well at higher phosphate levels in the sediment. The strongest increase in biomass was found at 32 μmol PO_4^{3-} l^{-1} interstitial water. The strongest growth of *Littorella uniflora* was found at 8 μmol PO_4^{3-} l^{-1} interstitial water. Both plant species showed a strong epiphyte development at very high phospate levels in the sediment.

DISCUSSION

All observations support the hypothesis that changes in the macrophyte communities are mainly due to changed carbon and/or nitrogen budgets, as a result of acidification. In non-acidified, poorly buffered oligotrophic waters, the CO_2-level of the water is very low (6—40 μmol l^{-1}), whereas the CO_2-level of the sediment is 10—100 times higher. Plants growing under these conditions, e.g. *Littorella uniflora*, *Isoetes lacustris* and *Lobelia dortmanna* show certain adaptations to make successful growth possible (CO_2-uptake by the roots, high root biomass at low CO_2-levels of the sediment, high oxygen release by the roots, etc.). The ecophysiological experiments prove that *Juncus bulbosus* lacks the adaptation to use sediment-CO_2, and that it shows only a very low oxygen release by the roots. Also, the root biomass is very low, compared to *Littorella uniflora*. However, the experiments also show that *Juncus bulbosus* is more capable than *Littorella uniflora* of using CO_2 from the water layer.

When the above-mentioned, poorly buffered waters are acidified and the sediment contains little carbonate, a temporary increase of the CO_2-concentration in the sediment will occur (up to 10 mmol l^{-1}; Roelofs, 1983a). The observed increased CO_2-levels in the water layer are probably due to diffusion of CO_2 from the sediment to the water layer. Under these conditions, there is often a luxuriant growth of *Juncus bulbosus* and/or *Sphagnum* spp., which suppresses the isoetid plant species. The culture experiments clearly show that the biomass of *Juncus bulbosus* only increased strongly when the sediment contained little carbonate and the pH of the water was low; when the water was poorly buffered and not acidified, there was a strong decrease in biomass. *Littorella uniflora*, however, showed an increase in biomass at each pH and alkalinity of the water on sediment containing a little carbonate. On carbonate-free sediments, *Juncus bulbosus* showed a strong decrease in biomass at each pH of the water, whereas the biomass of *Littorella uniflora* strongly decreased at a low pH and increased when the water was poorly buffered and not acidified. So it is obvious that the observed changes in the inorganic carbon budgets are completely dependent on the carbonate content of the sediment.

Fig. 10. Biomass (ashfree dry weight) of *Littorella uniflora* and *Myriophyllum alterniflorum* after a cultivation period of 10 weeks in a staged phosphate gradient of the sediment (Experiment 10) (dotted line = initial biomass).

Although the changes in the macrophyte communities can primarily be ascribed to changed carbon budgets, the changed nitrogen budgets also play an important role. Generally, the non-acidified, poorly buffered waters are poor in nitrogen with nitrate being the dominant N-source. The nutrient-uptake experiments clearly demonstrate that *Littorella* is more adapted to these conditions than *Juncus*. The culture experiments also show that *Littorella* has the strongest increase in biomass in a culture medium without ammonium. The acidified waters generally have strongly increased nitrogen levels, with ammonium as the dominant N-source. Field investigations showed that acidified waters, particularly in areas with intensive stock breeding, had very high ammonium levels, which were clearly correlated to the ammonium levels of the precipitation (Roelofs, 1983b). The nutrient uptake experiments show that *Littorella* had a net potassium release in culture media with relatively high ammonium levels, whereas *Juncus bulbosus* showed a net potassium uptake under these conditions. A net potassium release by macrophytes might lead to starvation, due to lack of K^+. The culture experiments demonstrate that both *Juncus bulbosus* and *Sphagnum cuspidatum* had the strongest increase in biomass at high ammonium levels in the medium. Whether this stronger increase in biomass is only the result of a better nitrogen supply is doubtful, as *Littorella* also showed a stronger increase in biomass at very high ammonium levels in the medium and a low carbonate level in the sediment, while it showed hardly any increase at high ammonium levels in the medium when the sediment was free of carbonate. Analyses of the interstitial water (Table X) show that a strong nitrification of ammonium occurred when the medium was rich in ammonium and the sediment contained little carbonate. This nitrification process causes acidification of the soil (van Breemen et al., 1982), which leads to a supplementary transformation of HCO_3^- and CO_3^{2-} into CO_2. So it seems plausible that these higher carbon dioxide levels are responsible for the stronger increase in biomass. When combining factors like CO_2 enrichment of the sediment with

TABLE X

Soil acidification as a result of nitrification processes
(Experiment 8, after 8 weeks)

Medium	Interstitial water	
NH_4^+ (μmol l^{-1})	pH	NO_3^- (μmol l^{-1})
0	5.7	<10
50	5.7	<10
100	5.7	<10
500	5.3	150
1000	5.4	120

and without phosphate and/or ammonium enrichment of the water in the culture experiments, it is clearly shown that phosphate and/or ammonium enrichment without CO_2 enrichment do not lead to an increase in biomass of *Juncus bulbosus* (Table IX).

The waters in which *Littorella* had disappeared or strongly decreased and in which no submerged *Juncus bulbosus* or *Sphagnum* spp. occurred appeared not to be acidified. The alkalinity of the waters with a luxuriant growth of *Myriophyllum alterniflorum* was on an average 60% higher than those dominated by *Littorella uniflora*. The mean phosphate level of the water was hardly any higher, whereas the phosphate level of the interstitial water was more than twice as high. Investigations by Best and Mantai (1978) and Barko and Smart (1980) show that another species of the genus *Myriophyllum*, namely *M. spicatum* L. takes up phosphate mainly by roots, and Carignan and Kalff (1980) found the same for both *Myriophyllum spicatum* and *Myriophyllum alterniflorum*, even at higher phosphate levels of the water layer. The culture experiments show that *Myriophyllum alterniflorum* only increased in biomass at very low phosphate levels of the medium ($\leqslant 0.10$ μmol PO_4^{3-} l^{-1}). At higher phosphate levels, the plants became fully overgrown with epiphytes and light limitation was probably responsible for the decrease in biomass (Sand-Jensen and Søndergaard, 1981).

Culture experiments in a phosphate-free medium at different phosphate levels of the sediment show that *Myriophyllum alterniflorum* had a much stronger increase in biomass at higher phosphate levels of the interstitial water than *Littorella uniflora* (32 and 8 μmol l^{-1}, respectively). Under optimal PO_4^{3-}; conditions for both plant species, the increase in biomass was much stronger for *Myriophyllum alterniflorum*. At very high phosphate levels of the sediment, a strong development of epiphytes occurred, which resulted in a lower increase in the biomass. Field observations had shown that in small and shallow waters, a luxiriant growth of pleustophytes, such as *Riccia fluitans*, occurred when the water was not acidified and had an increased phosphate level. Culture experiments show that *Riccia fluitans* increased very strongly in biomass at phosphate levels in the water of 0.50 and 1.00 μmol l^{-1}. At high phosphate levels (5 μmol l^{-1}), a much lower increase in biomass was observed, probably due to the strong development of filamentous algae.

FINAL CONCLUSIONS

(1) Acidification of the water is the main cause for the decline of *Littorellion* species in The Netherlands.

(2) Acidification of a water body above a non-buffered, carbonate-free sediment leads to disappearance of all submerged aquatic macrophytes due to lack of carbon dioxide.

(3) When nitrogen and phosphate are not limited, acidification of a water body above a poorly buffered sediment containing little carbonate leads to

a suppression of the isoetid plant species by luxurious growth of *Juncus bulbosus* and/or *Sphagnum* spp. as a result of raised carbon dioxide levels in the water layer.

(4) The increased ammonium and decreased nitrate levels in acidified waters, as a result of reduced nitrification processes, are favourable to *Juncus bulbosus.*

(5) In acidified waters above poorly buffered sediments, very high ammonium levels in the water layer, as a result of high ammonium sulphate deposition from the atmosphere in areas with intensive stock breeding, lead to an even stronger development of *Juncus bulbosus* and *Sphagnum* spp. This can probably be ascribed to soil acidification as a result of nitrification processes, which causes temporarily increased carbon dioxide levels.

(6) In non-acidified waters with increased alkalinity, phosphate enrichment of the sediment alone leads to luxuriant growth of rooted macrophytes such as *Myriophyllum alterniflorum.* Phosphate enrichment of both sediment and water leads to a mass development of non-rooted macrophyte species such as *Riccia fluitans* in small and shallow waters, and to a plankton bloom and a strong benthic algae development in larger and deeper waters.

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CHAPTER 5.

The effect of airborne sulphur and nitrogen deposition on aquatic and terrestrial heathland vegetation.

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The effect of airborne sulphur and nitrogen deposition on aquatic and terrestrial heathland vegetation

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Summary. As research on the effects of acidification on heathlands is scarce, the data discussed mainly concern western Europe and particularly the Netherlands. The change from heather into grass dominated heathlands appeared to be caused by the high atmospheric nitrogen enrichment. Acidification of oligotrophic, poorly buffered pools in heathland areas finally results in the disappearance of all submerged plant species, caused by the too low CO_2 levels in the water layer. The vitality of the pine-forests decreases; apart from the premature shedding of needles as a result of nutrient deficiences, they become more susceptible to secondary stress factors such as NH_3, O_3, drought and fungal diseases.

Key words. Acidification; nitrogen enrichment; nutrient leaching; heathland vegetation; afforestation.

Introduction

Heathlands and peatlands are often neglected areas in acid rain research[9], probably due to the low economic value of these areas. In western Europe, heathlands occur mainly on oligotrophic, weakly buffered sandy soils. Oligotrophic, poorly buffered ecosystems are characterized by plant communities consisting of persistent, slowly growing species[25]; in water these are mainly the tough, isoetid *Littorellion* species such as *Littorella uniflora* (L.) Aschers. and *Lobelia dortmanna* L., on the poor, oligotrophic sandy soils typical heathland species such as *Calluna vulgaris* (L.) Hull and *Erica tetralix* L. In former days part of the heathlands were planted with coniferous trees, particularly *Pinus sylvestris* L. (Scotch Pine), and regionally also *Pinus nigra* var. *maritima* (Ait.) Melville (Corsican Pine), *Picea abies* (L.) Karst (Norway spruce), *Picea sitchensis* (Bong.) Karr. (Sitka spruce) and *Pseudotsuga menziesii* (Mirb.) Franco (Douglas Fir).

Mainly during the last decades dramatic changes have taken place in the plant communities of these areas. The number of moorland pools and small lakes in which *Littorellion* species occur has declined drastically[24], a lot of heathlands have turned into grasslands and particularly in areas with intensive stockbreeding the condition of the pine forests is bad[13,15].

The change of heathlands into grasslands

The most obvious phenomenon in many heathlands during the last decades is the changing from heathland into grassland[11,12,25]. Particularly *Molinia caerulea* (L.) Moench and *Deschampsia flexuosa* (L.) Trin. expand strongly, at the expense of *Calluna vulgaris* and other heathland species. In order to estimate whether this phenomenon is related to changes in the physical-chemical environment, 70 grass-dominated and heather-dominated heathlands have been investigated[25]. Many parameters, such as the pH, showed hardly any differences. However, the nitrogen levels in grass-dominated heathlands appeared to be much higher (table 1). Both in grass-dominated and heather-dominated heathlands the ammonium levels were 10 to 20 times higher than the nitrate levels.

Investigations clearly show that a major part of the nitrogen originates from atmospheric deposition. In clean air this atmospheric nitrogen deposition is only a few kg ha^{-1} yr^{-1}. At the present time in the Netherlands the wet deposition (rain) alone is already on average 15 kg N ha^{-1} yr^{-1} and in areas with intensive stockbreeding 20–60 kg N ha^{-1} yr^{-1}; 75–90% of the nitrogen is deposited as ammonium sulphate. In raindrops and on wet surfaces ammonia reacts with sulphur dioxide, forming $(NH_4)_2SO_4$. As a result of the alkalinization by NH_3 the solubility of SO_2 increases considerably. The total nitrogen deposition, however, is considerably higher than the above values, because there is also dry deposition on soil and plants[1,2,7].

Impact of ammonium on heathland vegetation

1. Soil acidification. Although heathland soils are mostly acidic by nature, there are often certain spots where, due to natural causes (loamy places or upwelling deeper ground water) or to human activities (digging, paths, cattle drinking-places) the soil has become slightly buffered and thus less acidic[25]. Here plant species occur which are restricted to these slightly buffered, less acidic sediments. In the course of time a heterogeneous environment with a rich plant community has developed. The deposited ammonium at these slightly buffered locations is transformed into nitrate very quickly by nitrification, which causes acidification of the soil[6,7,25].

Laboratory experiments[25] with artificially buffered heathland soils show that nitrification stops or is strongly inhibited in this type of soil at pH 4.1. This appeared also to be the case for the average pH-value in both grass-dominated and heather-dominated heathlands, which indicates that the pH in heathlands is probably determined by the nitrification limit. The final result of NH_4-deposition is that the differences in pH disappear and thus also the plant species of the slightly buffered locations. A poor plant community remains, consisting of only a few acid-resistant species.

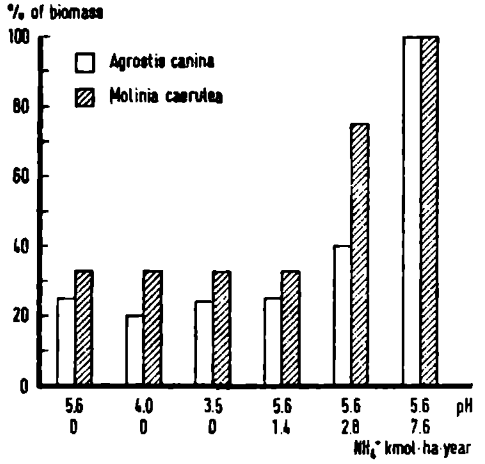
Table 1. The pH (H_2O) and average nutrient concentrations in the soil-solution of 70 investigated heathlands

Species	Coverage	pH (H_2O)	NH_4^+ μmoles kg^{-1}	NO_3^-	PO_4^{3-}	K^+
Erica tetralix	> 60%	4.1	55	0.0	4.0	37
Calluna vulgaris	> 60%	4.1	84	1.4	4.4	46
Molinia caerulea	> 60%	4.2	248	17.2	4.7	88
Deschampsia flexuosa	> 60%	4.1	429	29.0	6.0	182

2. Nitrogen enrichment. If the soil on which ammonium is deposited is acidic, a strong accumulation of nitrogen occurs in the upper soil layer, because ammonium is bound much more strongly to the soil absorption complex than nitrate. When there is competition between heather species such as *Erica tetralix* and *Calluna vulgaris* and grasses such as *Molinia caerulea* the grasses profit from these higher nitrogen levels[4,11,12,25,32]. Field fertilization experiments have shown that nitrogen enrichment indeed stimulates the development of grasses in heathlands[11,12]. However, the problem with these field fertilization experiments is that the high atmospheric nitrogen deposition was not taken into account. For this reason, experiments were carried out in a greenhouse. A number of small heathlands were created, using undisturbed, natural heathland soils. Precipitation experiments during one year showed that the biomass development of the grasses *Agrostis canina* L. and *Molinia caerulea* is not influenced by the acidity of the precipitation (fig.). If the precipitation contained ammonium sulphate, a strong increase in biomass with increasing NH_4^+ deposition was observed. The chosen annual ammonium deposition was comparable with the real field deposition. The highest deposition was comparable with the field deposition in areas with very intensive stockbreeding. The results of these experiments show that the NH_4^+ deposition level in cleaner areas of the Netherlands can already cause a marked increase in biomass of the two grass species. For this reason it can be concluded that the high atmospheric nitrogen enrichment is a main cause for changes from heather dominated into grass dominated heathlands.

Aquatic vegetation

In oligotrophic, poorly buffered pools and lakes in heathland areas of western Europe a plant community commonly occurs which belongs to the phytosociological alliance *Littorellion*[31,39]. The stands of this community are often characterized by the presence of isoetids such as *Littorella uniflora*, *Lobelia dortmanna* and *Isoetes* sp.[24]. Field investigations in the Netherlands[24] showed, that the waters in which *Littorella uniflora* was one of the dominant species appeared to be poorly buffered (alkalinity on an average 0.5 meq l^{-1}) and had a circumneutral pH (around 6.5), and the nutrient levels in the water layer were low (table 2), as were the CO_2 levels in the water layer (6–40 μmoles l^{-1}). The nutrient levels in the soil solution were 10–100 times higher.



The relative biomass development of *Agrostis canina* and *Molinia caerulea* on natural heathland soil during a one year treatment with precipitation with different pH and ammonium concentrations in a greenhouse.

Isoetid plant species, such as *Littorella uniflora*, *Isoetes* sp. and *Lobelia dortmanna*, have several morphological and biochemical adaptations to make successful growth possible under these conditions.

- *Lobelia* and *Littorella* are able to absorb free CO_2 by the roots[34,40].
- *Littorella* and *Lobelia*, plants with extensive lacunal systems, can recapture a considerable part of the photorespired CO_2, in this way contributing to a more efficient assimilation[33].
- The underground biomasses of these plant species depend on the nutrient levels of the sediment[30].
- *Littorella* and many species of the genus *Isoetes* have developed a diurnal acidification – deacidification cycle very similar to the Crassulacean Acid Metabolism (C.A.M.)[16,17].
- Isoetid plant species have a high oxygen release from the roots[27,29].

Acidification of the water

Field investigations in the Netherlands showed that over 80% of all naturally poorly-buffered pools and lakes in heathland areas have been strongly acidified during the last decades (pH at present on average 3.8). In most cases the original vegetation has been replaced by submerged *Juncus bulbosus* L. and/or *Sphagnum species*[6,24]. In these locations the CO_2 levels in the soil solution and the water layer were strongly increased (tables 2 and 3)[24,25,27]. Several authors also mention a strong development of *Juncus bulbosus* and/or *Sphagnum* sp. in acidifying Scandinavian waters[10,23,28].

Ecophysiological experiments have proved that *Juncus bulbosus* lacks the typical adaptations which enable suc-

Table 2. The average chemical composition of water and soil-solution on locations with *Littorella uniflora*, *Juncus bulbosus* and *Sphagnum* sp. (μM)

	L. uniflora	*J. bulbosus*	*Sphagnum*
Water			
n	33	107	59
pH	6.5	3.9	3.8
HCO_3^-	500	20	10
NH_4^+	5	40	46
NO_3^-	10	7	7
PO_4^{3-}	0.16	0.21	0.19
Soil solution			
n	9	19	9
HCO_3^-	1020	420	780
CO_2	900	3330	5010
NH_4^+	267	510	564
NO_3^-	90	60	51
PO_4^{3-}	5.1	5.4	5.4

Table 3 The CO_2-level of the water 10 cm above the soil at some acidified locations with differences in macrophyte development

Location	Macrophyte vegetation	pH (water)	CO_2 (μmoles l^{-1})
Staalbergven	No submerged macrophytes	4 0	66
Ven near Schayk	No submerged macrophytes	3 7	55
Vogelvijvers	No submerged macrophytes	4 7	60
Vogelvijvers	*Juncus bulbosus*	4 0	150
Rouwkuilen	*Juncus bulbosus*	4 1	170
Ven near Schayk	*J bulbosus + Sphagnum cuspidatum*	3 9	840
Mariaveen	*Sphagnum cuspidatum*	3 6	870

cessful growth in poorly buffered, oligotrophic waters[27] Under all conditions the root biomass of *Juncus* was much lower than that of *Littorella uniflora*, and *Juncus bulbosus* had a very low oxygen release by the roots, even when it had a relatively high root biomass When only the roots of the plants were provided with carbon dioxide, *Juncus bulbosus* showed a very low photosynthetic response, even at very high CO_2 levels, whereas *Littorella uniflora* showed a relatively high photosynthetic response When the leaves of the plants were provided with CO_2, *Juncus bulbosus* showed saturated photosynthesis at a concentration of 200 μmoles CO_2 l^{-1}, whereas *Littorella uniflora* showed no saturation until a concentration of 2000 μmoles CO_2 l^{-1} was reached

Nitrogen uptake experiments demonstrated that the two plant species, when obtained from waters containing both nitrate and ammonium, used ammonium as their sole nitrogen source Only after a few days of NH_4^+ starvation did they also use some nitrate The nitrogen uptake rate increased when the incubation period in the ammonium-free culture medium was longer, however, this increase was much stronger for *Littorella uniflora* particularly with respect to nitrate uptake When ammonium was used, *Littorella* showed a net potassium release, whereas *Juncus bulbosus* showed a positive K uptake

Culture experiments during ten weeks under fully controlled conditions showed that *Juncus bulbosus* only had a strong increase in biomass on sediments containing little carbonate when the pH of the water was low When the pH of the water was higher, a marked decrease in biomass occurred *Littorella uniflora*, however, showed an increase in biomass at each pH of the water In carbonate-free sediments, *Juncus bulbosus* showed a strong decrease in biomass at each pH of the water, whereas *Littorella* showed a decrease in biomass at a low pH and an increase when the water was poorly buffered *Juncus bulbosus* and *Sphagnum cuspidatum* Hoffm had the strongest increase in biomass at high ammonium levels in the water, whereas *Littorella* had the highest biomass in ammonium free water containing nitrate

It can be concluded that acidification of a water above a sediment which is not yet acidified leads to luxuriant growth of *Juncus bulbosus* and *Sphagnum* spec as a result of increased carbon dioxide and ammonium levels in the water layer (tables 2 and 3) These plant species can utilize these nutrients much more efficiently[27,30,37,33] The increased carbon dioxide level is a result of the strongly increased CO_2 level in the sediment[4], caused by the acid water layer acting upon the HCO_3^- buffering system and the microbial activities still present The diffusion of CO_2 from the sediment causes a strong over-saturation with CO_2 in the deeper water layers (tables 2 and 3) The increased NH_4^+ and decreased NO_3^- levels can be ascribed to airborne ammonium sulphate deposition and reduced nitrification After acidification of the sediment the CO_2 level in the sediment decreases strongly as a result of the reduced microbial activity and the absence of carbonates The diffusion of CO_2 to the water layer also decreases strongly, resulting in the disappearance of all submerged plant species, caused by the too low CO_2 levels in the water layer

Afforestation

In the past, mainly at the beginning of this century, large parts of the heathlands in western Europe were planted with coniferous trees Species often used are Scotch Pine (*Pinus sylvestris*) and Norway Spruce (*Picea abies*) and regionally also Corsican Pine (*Pinus nigra* var *maritima*), Douglas Fir (*Pseudotsuga menziesii*), Sitka Spruce (*Picea sitchensis*), Giant Fir (*Abies grandis* Lindl) and others

In the Netherlands more than 60% of the present forests are situated on former heathland soils[3] The condition particularly of coniferous forests is often poor[3] A nationwide investigation, carried out by the Dutch State Forest Service[3] in 1983, showed that 7–38% of all trees in Scotch Pine forests had less than one complete year-class of needles In Douglas Fir forests 29–70% of the trees had less than 50% of the leaves considered normal for this climatic zone 64% of the unhealthy Scotch Pine forests and 41% of the unhealthy Douglas Fir forests showed grey-green or yellow-green discolored needles A comparable investigation in 1984 showed a further decline with respect to 1983[3]

In Germany the vitality of the forests has also decreased strongly Air pollution is generally believed to be the main cause Ulrich[36] ascribes the phenomena mainly to soil acidification as a result of acid or acidifying substances from the atmosphere The high Al/Ca ratio, resulting from the dissolving of aluminium by mineral acids and from leaching of calcium is harmful to the root system

Other authors[19], however, believe that the observed nutrient deficiencies and concomitant damage to the trees can be ascribed mainly to leaching as a result of ozone damage to the leaves In the forests on former heathlands in the Netherlands, however there is no correlation between the mean ozone concentration in the atmosphere and the condition of the forest[2,3] but there is a clear correlation between the ammonia emission and the condition of the forest[1,3]

Some authors mention a relationship between intensive stockbreeding and the condition of pine trees[13,15] Van Breemen et al[6,7] state that ammonia from manure reacts with sulphur dioxide from the atmosphere, causing wet and dry deposition of large amounts of ammonium sulphate on trees and soil[15] Nitrification of the ammonium[7,18] caused a marked pH-decrease of the soil (temporarily down to pH 2 8)

Whether deposition of ammonium sulphate on acidic forest soils will result in strong acidification depends on the type of forest and soil Kriebitzsch[20], who conducted

Table 4 pH and chemical composition of soil-distilled water extracts (1:3) of A) healthy, B) moderately damaged and C) severely damaged *Pinus nigra* and *Pseudotsuga menziesii* forests (µmoles kg^{-1} dry soil)

	n	pH (H_2O) mean	min.	max.	NH_4^+ mean	NH_4^+(KCl)* mean	NO_3^- mean	K^+ mean	Mg^{2+} mean	Ca^{2+} mean	Al^{3+} mean
Pinus nigra											
A)	20	4.1	3.5	4.6	334	687	271	137	77	153	191
B)	16	4.0	3.4	4.9	384	751	130	47	45	128	158
C)	20	4.1	3.7	4.4	509	1346	117	60	26	43	183
Pseudotsuga menziesii											
A)	10	4.1	3.9	4.4	245	499	164	89	60	106	214
B)	10	4.1	3.8	4.3	562	733	153	67	48	69	211
C)	11	4.3	4.0	4.6	692	1240	157	67	22	36	211

*0.5 M KCl extract

nitrification experiments in many types of acidic forest soils, divided them into four groups: A, B, C and D. In the soils belonging to group A there was no nitrification and ammonium was the only nitrogen source. In the soils belonging to groups B and C there was partial nitrification and in those belonging to group D there was total nitrification. The investigations in this study showed that heathland and *Pinus* soils mainly belong to group A. Field studies in the Netherlands in *Pinus nigra*[26] and *Pseudotsuga menziesii* forests on former heathland soils showed indeed only partial or no nitrification. The nitrate levels were low, whereas the ammonium levels were high (table 4). The soils of healthy, moderately damaged and severely damaged forests had on an average a pH (H_2O) of 4.1, which indicates that also in this type of forest soil the pH is determined by the nitrification limit. Analysis of precipitation in open plots and canopy throughfall in a *Pinus nigra* var. *maritima* forest in an area with intensive stockbreeding showed that NH_4^+ and SO_4^{2-} are quantitatively by far the most important ions[26]. In open plots SO_4^{2-} is fully compensated by NH_4^+; however, in canopy throughfall SO_4^{2-} is only partially compensated by NH_4^+ (table 5). Some authors mention foliar uptake of NH_4^+[21,26].

Ecophysiological experiments with needles of *Pinus nigra* var *maritima* indeed showed uptake of ammonia, which was compensated by excretion of potassium, magnesium and calcium. Needle analyses in healthy and damaged *Pinus nigra* forests showed that premature shedding of the needles in damaged forests was related to K and/or Mg deficiencies in the needles[26]. As a result of the NH^+ uptake the nitrogen levels were significantly higher in the needles of damaged forests. Whether the observed cation excretion will result in disturbed nutrient budgets depends mainly on the soil condition. A high ammonium sulphate deposition leads to accumulation of NH_4^+ and leaching of K, Mg and Ca from the soil. Field investigations in *Pinus nigra* var. *maritima* and *Pseudotsuga menziesii* forests demonstrated that the NH_4^+ levels were significantly higher and the Mg and Ca levels significantly lower in soils of severely damaged forests (table 6). As a result the NH_4/K, NH_4/Mg and the Al/Ca ratios increased. It is well-known that increased NH_4/K and NH_4/Mg ratios inhibit K^+ and Mg^{2+} uptake[14,22], while an increased Al/Ca ratio can damage the root system. Ulrich[36] mentions an Al/Ca ratio $\geq$ 5 in the soil solution as very critical for root damage. In both *Pseudotsuga menziesii* and *Pinus nigra* forests the NH_4/K and NH_4/Mg ratios are relatively low in healthy forests and significantly higher in damaged forests, while the average Al/Ca ratio is significantly higher in severely damaged forests and, particularly in *Pseudotsuga* forests, far above the critical value (table 6).

Apart from the premature shedding of needles as a result of nutrient deficiencies, the forests become more susceptible to secondary stress factors such as NH_3, O_3, drought and fungal diseases. In some regions of the Netherlands the condition of *Pinus nigra* and *Pinus sylvestris* rapidly decreases as a result of a certain fungal disease (*Diplodia pinea* (Desm.) Kickx). Needle analyses in infected and non-infected forests demonstrated that the nitrogen levels were significantly higher in *Diplodia*-infected forests[26].

Table 5 The average chemical composition of precipitation in a) open plots and b) throughfall in a *Pinus nigra* forest in an area with intensive stockbreeding (µmoles l^{-1})

	NH_4^+	NO_3^-	K^+	Mg^{2+}	Ca^{2+}	SO_4^{2-}	Na^+	Cl^-	H^+
a)	597	71	53	35	71	318	170	197	1.7
b)	2283	147	196	94	150	1379	346	462	0.6

Table 6 The ratios of some nutrients in soil extracts of A) healthy, B) moderately damaged and C) severely damaged *Pinus nigra* and *Pseudotsuga menziesii* forests (mol/mol)

	n	NH_4^+/K^+ mean	min	max	NH_4^+/Mg^{2+} mean	min	max	Al^{3+}/Ca^{2+} mean	min	max
Pinus nigra										
A)	21	4.7	0.5	14.0	6.4	1.1	24.3	2.0	0.4	5.6
B)	17	9.2	0.8	36.8	10.0	1.8	26.3	1.3	0.2	2.8
C)	21	11.3	1.9	51.8	22.1	1.6	57.2	5.5	1.7	16.7
Pseudotsuga menziesii										
A)	10	3.8	0.5	11.8	4.5	0.6	10.0	6.6	0.8	40.9
B)	10	8.7	1.5	31.2	19.3	2.0	51.1	8.9	0.7	46.7
C)	11	18.2	3.8	64.5	47.6	7.9	118.0	15.6	1.4	54.0

Final conclusions

- Acidification of poorly buffered heathland soils as a result of acid or acidifying precipitation leads to a strong decline in the number of plant species
- Acidification of a water body above a carbonate-free sediment leads to disappearance of all submerged macrophytes due to lack of carbon dioxide
- Acidification of a water body above a sediment containing little carbonate, as a result of ammonium-containing acidifying precipitation, leads to suppression of the isoetid plant community by luxuriant growth of *Juncus bulbosus* and/or *Sphagnum* species as a result of increased carbon dioxide and ammonium levels in the water layer
- Airborne ammonium deposition on acidic heathland soils leads to a succession from heather-dominated into grass-dominated heathlands
- Ammonium sulphate deposition on pine forests leads to ammonium uptake, and excretion of essential nutrients such as potassium and magnesium by the needles. As a result of ammonium accumulation and cation leaching in the soil the uptake of K^+ and Mg^{2+} is also inhibited This combination of effects leads to a disturbed nutrient budget in the trees, resulting in premature shedding of needles and increased susceptibility to secondary stress factors, which may finally lead to severely damaged forests.

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CHAPTER 6.

The effect of airborne ammonium sulphate on *Pinus nigra* var. *maritima* in The Netherlands.

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The effect of air-borne ammonium sulphate on *Pinus nigra* var. *maritima* in the Netherlands

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Summary As a result of air pollution, considerable deposition of ammonium sulphate occurs on vegetation and soil in the vicinity of chicken farms and fields dressed with animal slurry. A clear relation exists between this ammonium sulphate deposition and the distance to certain agricultural activities. Field investigations and ecophysiological experiments both show that the needles of *Pinus nigra* var. *maritima* (Ait.) Melville take up ammonium and excrete potassium, magnesium and calcium. This often results in potassium and/or magnesium deficiencies and may lead to premature shedding of needles. The high levels of nitrogen in the needles are strongly correlated to fungal diseases.

Whether the osberved cation leaching will result in disturbed nutrient budgets depends mainly on soil conditions. Leaching of K, Mg and Ca from the soil, caused by ammonium sulphate, may further inhibit nutrient uptake.

Field investigations show a clear correlation between increased ratios of NH_4 to K, Mg and Ca in the soil solution and the damage to pine forests.

Introduction

Recent publications concerning forest damage in Western-Europe have expressed considerable alarm, particularly in Germany where the vitality of the forests has decreased rapidly. Air pollution is generally believed to be the main cause. Ulrich[20] ascribes the phenomena mainly to soil acidification as a result of acid or acidifying substances from the atmosphere. The high Al/Ca ratio resulting from dissolution of aluminium by mineral acids and from leaching of calcium is harmful to the root system. Krause *et al.*[11], however, believe that the observed nutrient deficiencies and concomitant damage to the trees can be ascribed mainly to leaching as a result of ozone damage to the leaves. In the Netherlands the condition of pine forests is often poor. In 1983 a nation-wide investigation, carried out by the Dutch State Forest Service (den Boer and Bastiaens[2]), showed that 7 to 38% of all trees in *Pinus sylvestris* L. forests had less than one complete year-class of needles. In *Pseudotsuga menziesii* (Mirb.) Franco forests, 29 to 70% of the trees had less than 50% of the leaves considered normal for this climatic zone. 64% of the unhealthy *Pinus sylvestris* forests and

41% of the unhealthy *Pseudotsuga menziesii* forests showed grey-green or yellow-green discoloured needles.

Janssen[8] and Hunger[6] noticed a relation between intensive stock-breeding and the condition of pine trees.

On an average the total deposition of acid and potential acidifying substances in The Netherlands is ± 6 kmol equivalent $H^+ \cdot ha^{-1} \cdot yr \cdot^{-1}$ of which ± 80% as $(NH_4)_2SO_4$[1].

Van Breemen *et al.*[3,4] state that ammonia from manure reacts with sulphur dioxide from the atmosphere, causing wet and dry deposition of large amounts of ammonium sulphate on trees and soil[19]. In a woodland near Warnsveld nitrification of the ammonium caused a marked pH decrease of the soil (temporarily down to pH 2.8).

In the present study precipitation analyses have been carried out at various distances from chicken farms and fields dressed with animal slurry. The chemical compositions of open field precipitation and forest canopy throughfall have been compared.

In the South-east of the Netherlands soil and needle analyses have been carried out on *Pinus nigra* var. *maritima* (Ait.) Melville at 58 locations and the results were correlated with the condition of the trees. Acid and artificially buffered soils were incubated after adding various amounts of ammonium sulphate to investigate nitrification processes. Eco-physiological experiments were carried out in order to estimate the leaching of nutrients from needles caused by ammonium sulphate.

Materials and methods

Field investigations and experimental studies

During 1983, fortnightly precipitation was collected and analysed, using black polyethylene bottles (1 l) and funnels (ϕ 10 cm), containing 1 ml of a 200 mg l^{-1} $HgCl_2$ fixing solution. In the spring and autumn 58 locations with *Pinus nigra* var. *maritima* were visited At each location soil samples were taken, consisting of six subsamples of the mineral sandy soil, taken just below the litter layer to a depth of 15 cm, using a brass tube (length 15 cm, ϕ 18 cm). The samples were transported to the laboratory in a refrigerated container.

Needles were collected from each year class from trees between 30 and 50 years old. The vitality was determined from the number of year classes of needles. Trees with 2–3 year classes were estimated as healthy, those with 1–2 year classes as moderately damaged and those with 0–1 year class as severely damaged. In order to analyse an aqueous soil extract 70 g of fresh, well mixed soil were weighed into a 500 ml polyethylene bottle together with 200 ml of twice-distilled water and shaken on a Gerhardt model LS 20 shaker for 1 hour. The pH was then measured with a Metrohm model E 488 pH/mV meter and a model EA 152 combined electrode. After centrifugation in a Heraeus Christ model 3 labofuge (10 min, 5000 rpm) 100 ml of the supernatant were fixed with 0.5 ml of 200 mg.l^{-1} $HgCl_2$ solution and stored at – 28°C until analysis. For the tissue analyses 50 mg of dried (72 h 55°C) and ground needles were digested according to Nkonge[13].

Nitrification and soil acidification experiments were conducted with a mixture of ten acidic forest soils (pH H_2O 4.1). A portion of the mixture was artificially buffered with

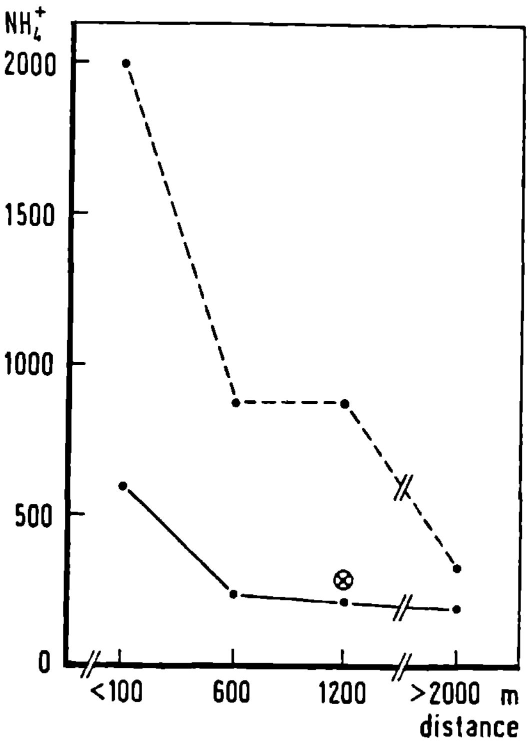

Fig. 1. The ammonium concentration in —— open field precipitation (μM) and ----- forest soil (μmol kg^{-1} dry wt) as a function of the distance to chicken farms.

Table 1. pH and chemical composition (averages) of soil distilled water extract (1:3) in A: healthy, B: moderately and C: severely damaged *Pinus nigra* var. *maritima* forests (μmol.kg^{-1} dry soil)

		pH (H_2O)									
	n	Mean	Min	Max	NH_4	NH_4(KCl)	NO_3	K	Mg	Ca	Al
A	20	4.1	3.5	4.6	334	687	271	137	77	153	191
B	16	4.0	3.4	4.9	384	751	130	47	45	128	158
C	20	4.1	3.7	4.4	509	1346	117	60	26	43	183

50 mmol $CaCO_3$. kg^{-1} (pH H_2O 6). To 600 g portions of the artificially buffered fresh soil (moisture content 21%), 0, 5, 10, 15, 20, 25 and 38 mmol of $(NH_4)_2SO_4$ were added per kg of fresh soil; the untreated mixture received 25 mmol . kg^{-1}. All soils were incubated at 20°C for 114 days, covered with glasswool to prevent evaporation. The pH was measured fortnightly and the chemical composition was estimated at the beginning and end of the 114 days period.

Nutrient uptake/release experiments were carried out with $\frac{1}{2}$ year old needles of a healthy *Pinus nigra* var. *maritima* tree from a forest near Nijmegen. After collection, the needles were washed thoroughly with twice distilled water. Five 50 g (wet weight) portions of needles were incubated (24 h, 20°C) in duplo in 2 l perspex containers, containing 0.5 l bidistilled water and 1. 0 μM $(NH_4)_2SO_4$, 2. 250 μM $(NH_4)_2SO_4$, 3. 250 μM Na_2SO_4, 4. 2500 μM $(NH_4)_2SO_4$ and 5. 2500 μM Na_2SO_4. The containers were placed on a Gerhardt model 20 shaker and irradiated with a Philips type HP (I) 400 W high pressure metal halide lamp at a light intensity of 500 μF $m^{-2} \cdot s^{-1}$. During the experiment the pH in all media was 4.35 ± 0.15. 10 ml water samples were taken at 0, 1, 4, 8 and 24 h after the start of the experiment and analysed immediately after completion of the experiment.

Calcium and magnesium contents were estimated with a Beckman model 1272 A.A.S., and aluminium with a LI type V11 flameless A.A.S. Potassium was estimated flame-photometrically using a Technicon I Auto-Analyzer. A Technicon II Auto-Analyzer was used for the colorimetric analyses of nitrate according to Kamphake *et al*[9], ammonia by the method of Grasshof and Johannsen[5], sulphate by the Technicon methodology[18] and chloride according to O'Brien[15]. Statistical analyses of the results were carried out according to Kruskal-Wallis.

Results

Effects on soil

The ammonium levels of the forest soils and the ammonium sulphate levels in the precipitation increase strongly with decreasing distance from chicken farms (Fig. 1). In all forest soils investigated, however, nitrate levels were low and ammonium appeared to be the preponderant nitrogen source.

The average pH (H_2O) was the same for healthy, moderately damaged and severely damaged forests (pH 4.1, Table 1). The results from the incubation experiments demonstrated that nitrification proceeded rapidly in artificially buffered soils leading to a rapid decline in pH (Fig. 2). With sufficient ammonium sulphate present the pH decreased to 4.1. The pH did not decrease further in the presence of ammonium sulphate even after 114 days (Fig. 3).

The NH_4 levels (Table 1) in soil solutions of severely damaged forests are significantly higher than in healthy forests ($P = 0.045$), whereas the Mg and Ca concentrations were significantly lower ($P = 0.008$ and 0.004). Also the K concentrations seem to be lower in the soils of severely damaged forests but these differences are not significant ($P = 0.08$). The aluminium concentrations were almost equal for healthy, moderately damaged and severely damaged forests. As a result the NH_4/K, NH_4/Mg and Al/Ca ratio are relatively low in healthy forests and significantly higher in severely damaged forests ($P = 0.010$, $P = 0.003$ and $P = 0.017$). (Table 2).

Effects on leaves

Typically characteristic of damaged trees are premature shedding of needles and the frequent occurrence of fungal diseases. Tissue analyses show that there are only slight differences between the potassium and magnesium levels in one, two and three year old needles of non-damaged

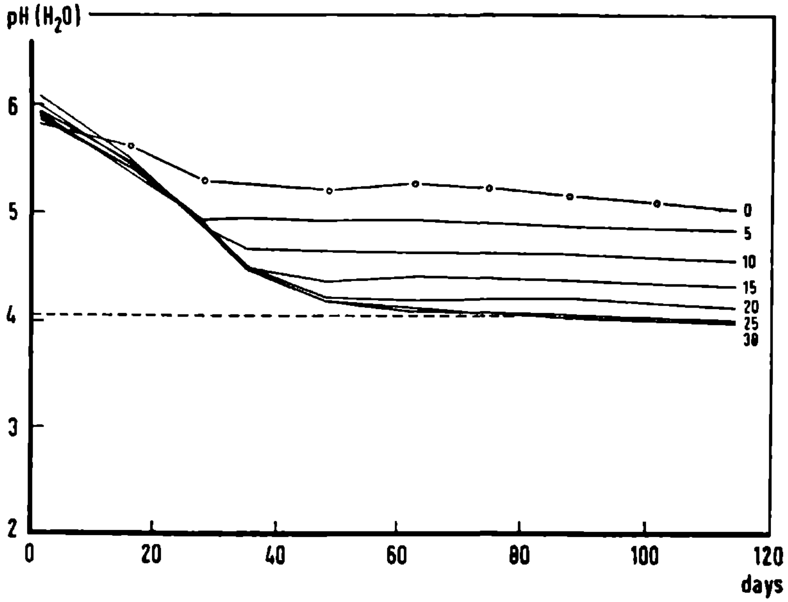

Fig. 2. The change in pH of ----- acidic and —— artificially buffered heathland soil as a function of the amount of ammonium sulphate added (mmol . kg^{-1} dry wt).

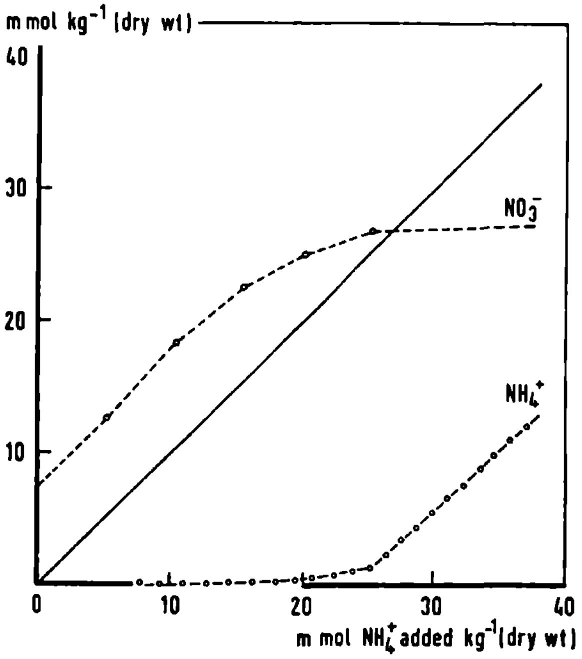

Fig. 3. Ammonium and nitrate concentrations in a moderately buffered heathland soil after incubation for 114 days (20°C) with addition of different amounts of ammonium sulphate.

Table 2 Some nutrient ratios in soil extracts of A healthy, B moderately damaged and C severely damaged *Pinus nigra* var *maritima* forests (mol/mol)

		NH_4/K			NH_4/Mg			NH_4/Ca			Al/Ca		
	n	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
A	21	4.7	0.5	14.0	6.4	1.1	24.3	4.0	0.3	9.8	2.0	0.4	5.6
B	17	9.2	0.8	36.8	10.0	1.8	26.3	2.9	0.5	9.2	1.3	0.2	2.8
C	21	11.3	1.9	51.8	22.1	1.6	57.2	10.1	0.7	19.8	5.5	1.7	16.7

Table 3 Potassium, magnesium and nitrogen levels in 1, 2 and 3 years old needles of A healthy, B moderately damaged and C severely damaged *Pinus nigra* var *maritima* forests (μmol gr^{-1} dry weight)

Year class	A			B			C		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Potassium									
1	123.8	81.3	150.0	103.2	50.0	128.2	137.0	65.7	325.0
2	112.6	68.8	187.5	43.8	0.0	65.7	Not present		
3	119.4	71.9	215.5	Not present			Not present		
Magnesium									
1	44.1	17.6	80.0	30.1	12.5	51.3	34.1	10.6	55.0
2	33.5	20.0	45.0	22.7	13.1	42.5	Not present		
3	31.8	21.3	56.3	Not present			Not present		
Nitrogen									
1	944	800	1117	1006	844	1478	1269	883	1517
2	878	656	1094	969	544	1561	Not present		
3	828	628	1044	Not present			Not present		

trees (Table 3), but the levels in two year old needles of damaged trees appear to be significantly lower (K $P = 0.0002$, Mg $P = 0.036$). The potassium levels and generally the magnesium levels also, are lower than the observed minimum levels in the needles of non-damaged trees. The nitrogen levels in one year old needles of severely damaged trees are significantly higher ($P = 0.0058$).

All trees infected by the fungi *Brunchorstia pinea* (Karst) Hohnel and/or *Diplodia pinea* (Desm.) Kickx had nitrogen levels in the needles exceeding 1150 μmol.g^{-1} (dry wt), whereas for all non-infected trees they were below 1150 μmol.g^{-1} (dry wt) (Table 4).

Analyses of canopy throughfall and open field precipitation show clear differences in chemical composition (Table 5). In open field precipitation sulphate is fully compensated by ammonium, but only ± 80% in canopy throughfall, the remainder by potassium, magnesium and calcium. Ammonium sulphate levels in canopy throughfall can increase to *ca.* 4000 μmol.l^{-1} in areas with high ammonium emission.

The ecophysiological experiments show extensive ammonium uptake by needles and K, Mg and Ca release, when they are incubated

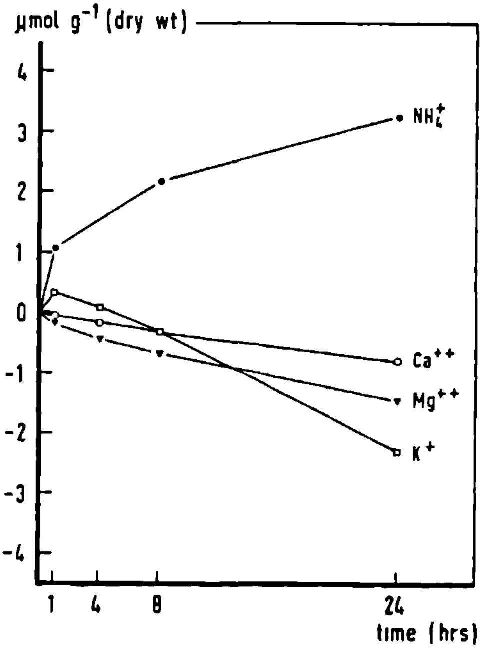

Fig 4 Ammonium uptake and potassium, magnesium and calcium release from pine needles in a 250 μM ammonium solution

Table 4 Nitrogen levels in one year old needles of trees infected by ***Brunchorstia pinea*** (Karst) Hohnel and/or *Diplodia pinea* (Desm) Kickx and in non-infected trees (μmol gr $^{-1}$ dry weight)

Infected (n = 11)			Non infected (n = 17)		
Mean	Min	Max	Mean	Min	Max
1355	1189	1517	956	800	1117

Table 5 The average chemical composition of precipitation in a open plots (2) and b through-fall (4) in a *Pinus nigra* forest in an area with intensive stockbreeding (μmol l^{-1})

	NH_4^+	NO_3^-	K^+	Mg^{2+}	Ca^{2+}	SO_4^{2-}	Na^+	Cl^-	H^+
a	597	71	53	35	71	318	170	197	1 7
b	2283	147	196	94	150	1379	346	462	0.6

in an ammonium containing medium (Fig. 4). This process proceeds continuously. When the needles are added to a medium with a certain salt concentration, rapid excretion of K, Mg and Ca takes place initially, irrespective of whether $(NH_4)_2SO_4$ or Na_2SO_4 has been used. In a medium containing Na_2SO_4 however, the excretion of cations decreases rapidly after a few hours and gradually becomes comparable with that of needles in twice distilled water, whereas in a medium containing $(NH_4)_2SO_4$ the needles continue to excrete relatively

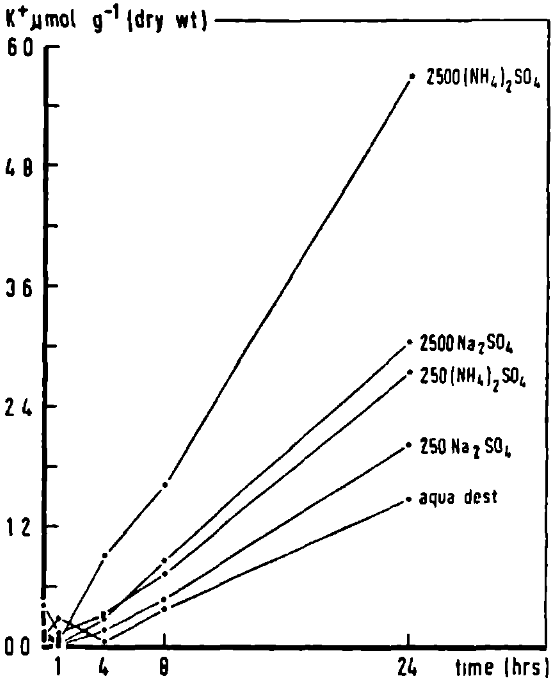

Fig 5 Potassium release from pine needles in distilled water and in solutions containing ammonium and sodium sulphate (μM)

large amounts of K, Mg and Ca (Figs 5, 6, 7) The duplo experiments varied within a few percent. In media with relatively low $(NH_4)_2SO_4$ levels, the excretion of Ca and Mg is more than ten times higher than in distilled water.

Discussion

A clear relation exists between the distance from stock farms and the ammonium sulphate deposition[16]. The deposited ammonium sulphate affects the forest ecosystem. The nutrient release/uptake experiments show that needles of *Pinus nigra* var. *maritima* take up ammonium from $(NH_4)_2SO_4$ solutions and compensate by excreting potassium, magnesium and calcium, which cannot be ascribed exclusively to an osmoregulatory adaptation. This phenomenon has also been described for aquatic macrophytes in acidified $(NH_4)_2SO_4$ containing waters[17]. Forest canopy throughfall analyses suggest a further confirmation of this exchange mechanism Tissue analyses demonstrate that the observed premature shedding of the needles of damaged trees is related to postassium and/or magnesium deficiencies in older needles Krause *et al* also indicate nutrient deficiencies as a result of

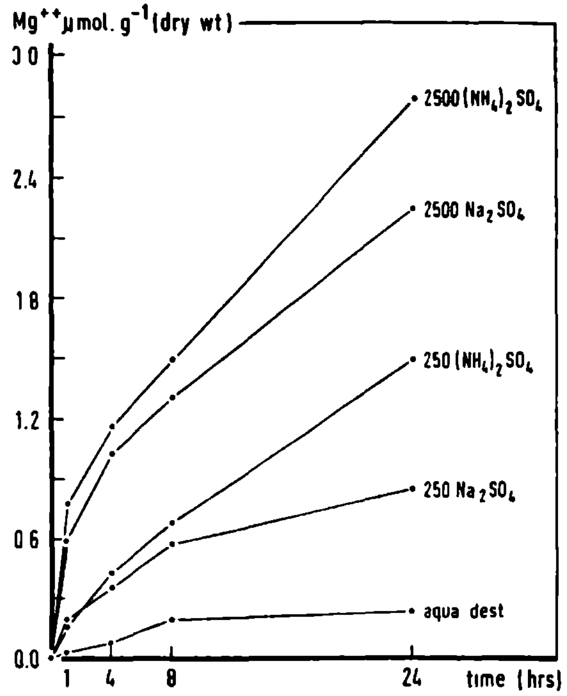

Fig. 6. Magnesium release from pine needles in distilled water and in solutions containing ammonium and sodium sulphate (*µM*).

cation leaching in German forests, but they ascribe this mainly to needle damage caused by ozone. However, this cation leaching is rather low compared with that caused by ammonium sulphate deposition.

The above mentioned reactions help to explain the high nitrogen levels in pine needles.

All trees investigated and infected by *Brunchorstia pinea* (Karst) Höhnel and *Diplodia pinea* (Desm.) Kickx showed higher total N in the needles. A possible causal relation between high N levels and susceptibility to fungal diseases has to be further investigated.

Several authors[3,4,10,12] mention nitrification of ammonium and the concomitant soil acidification in acidic forest soils. In this study the acidic forest soils investigated seem to show little or no nitrification; the nitrate levels were relatively low and ammonium levels were high. The incubation experiments with artificially buffered soils demonstrated that in these soils nitrification stops or is strongly inhibited at pH (H_2O) < 4.1. Soils of healthy, moderately damaged and severely damaged forests had an average pH (H_2O) of 4.1, indicating that the soil pH probably is determined by the nitrification limit. It should be noted that all the *Pinus nigra* forests investigated had been planted on former sandy acidic heathland soils, so the nitrification results are

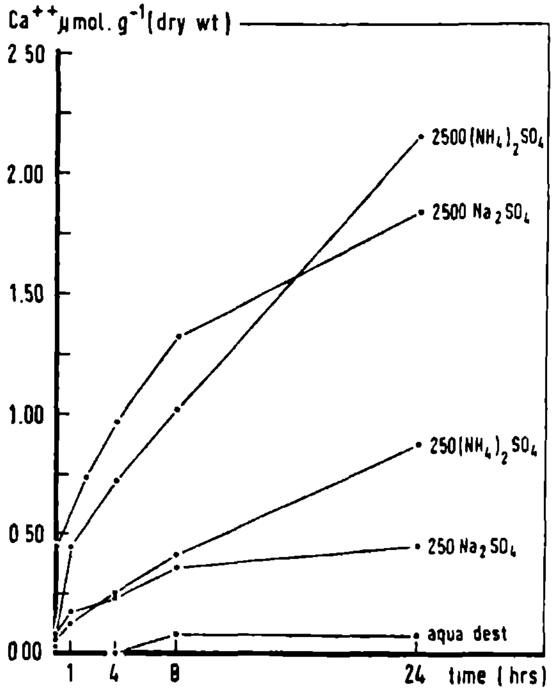

Fig. 7. Calcium release from pine needles in distilled water and in solutions containing ammonium and sodium sulphate (µM).

consistent with those of Kriebitzsch[12], who conducted nitrification experiments in many types of acidic forest soil.

The observed ammonium sulphate deposition also results in cation exchange and consequent cation leaching (K, Mg and Ca) from the soil. The NH_4/K, NH_4/Mg and NH_4/Ca ratios are much higher in soils of severely damaged trees. It has already been stated by other authors[7,14] that these increased ratios, especially in soils with a low nutrient level, inhibit nutrient uptake.

The aluminium levels in soils of non-damaged forests are almost equal to those in moderately and severely damaged forests. However, as a result of calcium leaching, the Al/Ca ratio is much higher in soils of severely damaged forests.

Ulrich[20] mentions an Al/Ca ratio > 5 as very critical for root damage. In the soils of severely damaged forests this ratio was indeed above 5, but some of the healthy forests also showed Al/Ca ratios far above this limit. However, according to Ulrich[20] a high Al/Ca ratio does not necessarily result in damage to trees. Organic acids in the soil can detoxify aluminium by forming organic aluminium complexes.

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CHAPTER 7.

Some effects of nitrate versus ammonium nutrition on the nutrient fluxes in *Pinus sylvestris* seedlings. Effects of mycorrhizal infection.

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Some effects of nitrate versus ammonium nutrition on the nutrient fluxes in *Pinus sylvestris* seedlings. Effects of mycorrhizal infection

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As a result of the high deposition of ammonium compounds in The Netherlands the forest trees are under a severe nitrogen stress. Effects of increasing amounts of ammonium on the major nutrient fluxes by Scots pine seedlings have been studied. In addition, the effect of mycorrhization of the roots has been taken into account. With nitrate N nutrition only, the seedlings accumulate calcium, magnesium, potassium, and protons. When a low ammonium concentration is present, the seedlings lose their cations. When the medium is depleted of ammonium, a reuptake of the cations occurs. In the presence of a high ammonium concentration (based on field studies) a continuous loss of cations from the roots takes place. The reverse has been found for phosphate uptake. Ammonium stimulates phosphate accumulation. It is shown that ammonium inhibits the unidirectional influx rate of ^{86}Rb, while concurrently stimulating the unidirectional efflux rate. Mycorrhization of the roots has a beneficial effect on the nutrient fluxes. With nitrate N nutrition mycorrhizal seedlings accumulate more nitrate, calcium, magnesium, and protons than nonmycorrhizal seedlings. With high ammonium N nutrition mycorrhizal seedlings lose fewer cations than nonmycorrhizal seedlings. In conclusion, the ecological significance of ammonium deposition on forest stands is discussed.

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Les arbres forestiers, suite aux retombées élevées de composés ammoniacaux, en Hollande, sont soumis à un stress sévère d'azote. Les effets des quantités croissantes d'ammonium sur les flux principaux de nutriments dans de jeunes plants de pin écossais font l'objet de cette étude. L'effet de la mycorrhization des racines a aussi été considéré. Avec la nutrition azotée nitrique seule, les plants accumulent le calcium, le magnésium, le potassium et les protons. Lorsque la concentration en sels ammoniacaux est basse, les plantes perdent des cations. Lorsque le milieu ne contient pas de sels ammoniacaux, il y a reprise de l'absorption des cations. Une perte continue de cations des racines a lieu quand la concentration en sels ammoniacaux est élevée (ce résultat est basé sur des études au champ). L'inverse a été constaté pour l'absorption du phosphate. En effet, les sels ammoniacaux stimulent l'accumulation du phosphate et ils inhibent la vitesse d'influx unidirectionnel de ^{86}Rb alors qu'en même temps, se produit une stimulation de la vitesse d'efflex unidirectionnel. La mycorrhization des racines a un effet bénéfique sur les flux de nutriments. Avec la nutrition azotée nitrique, les plants mycorrhizés accumulent plus de nitrate, de calcium, de magnésium et de protons que les plants non mycorrhizés. En milieu riche en ammonium, les plants mycorrhizés perdent moins de cations que les plants non mycorrhizés. La signification écologique des retombées d'ammonium sur les forêts est discutée.

[Traduit par la revue]

Introduction

The Netherlands and large parts of western Europe deal with an excess nitrogen deposition. While the background deposition is estimated to be 5 to 10 kg N ha^{-1} year^{-1} at present 10 to 20 times those values have been reported. Locally even values exceeding 500 kg N ha^{-1} year^{-1} have been measured (Anonymous 1986*a*). According to Ågren (1983) pine and spruce forests will be saturated with nitrogen after an input of 25 kg N ha^{-1} year^{-1} for 25 to 50 years. The deposition of nitrogen compounds occurs mainly as ammonium sulphate and this deposition has strong effects on vegetation and soil (Breemen *et al.* 1982; Roelofs *et al.* 1985; Nihlgård 1985). According to the Dutch National Forest Service, the condition of the forests is alarming. The 1986 survey revealed that more than 50% of the forest is less vigorous, based on vitality criteria as developed by the Dutch National Forest Service (Anonymous 1986*b*). The geographical damage pattern does not fit very well with the occurrence of sulphur dioxide, nitrogen oxides, or ozone in the air, but a good correlation has been observed with the occurrence of ammonium (Boer 1986).

Most of the Dutch forests have been planted on former heathlands, which are acidic and poor in nutrients. In this type of soil the nitrification rate is very low or even absent (Kriebitzsch 1978). This means that ammonium is accumulated in the soil and this phenomenon can actually be observed in the forest stands (Roelofs *et al.* 1985). Whereas in the soil water extracts of healthy stands of *Pinus nigra* var. *maritima* (Ait.) Melville and *Pseudotsuga menziesii* (Mirb.) Franco the ammonium to nitrate ratio is 1:2 and 1:5, respectively, this ratio has increased to 4:4 for both types of trees in the soil water extracts of severely damaged stands. This implies that not only the ratio between both N forms has been changed but also the total N content of the soil (Roelofs 1986; Roelofs and Boxman 1986). Nitrogen is no longer growth limiting (Cole and Rapp 1981), which means that another nutrient becomes growth limiting. Excess ammonium may have severe biochemical and biophysical effects on the plant. Ammonium inhibits photosynthetic phosphorylation (Eerden 1982), starch synthesis (Matsumoto *et al.* 1969; Waikiuchi *et al.* 1971), protein synthesis (Patnaik *et al.* 1972; Zedler *et al.* 1986), and chlorophyll synthesis (Puritch and Barker 1967; Patnaik *et al.* 1972) and saturates membrane lipids, whereby the membrane becomes more permeable (Eerden 1982). Because of the inhibition of protein synthesis a sharp rise in amino acid content of the plant tissue has been found (Patnaik *et al.* 1972; Aronsson 1985; Zedler *et al.* 1986; Dijk and Roelofs 1987).

On increasing the ammonium to nitrate ratio the major nutrient fluxes may also change. In general, with ammonium-N nutrition anion accumulation is increased, while cation

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accumulation is decreased. With nitrate-N nutrition exactly the reverse has been found (Nelson and Selby 1974; Kurvits and Kirkby 1980; Driessche 1978; Runge 1981). Rygiewicz *et al.* (1984*a*, 1984*b*) found for three North American coniferous species a stimulation of the net potassium and calcium efflux on applying ammonium nutrition at physiological pH values with respect to nitrate nutrition. In studies with *Pinus sylvestris* L. seedlings it was found that nitrate alone was an inferior source of N, resulting in lower dry weights and chlorosis of the needles (Nelson and Selby 1974).

In this study the effects of the nitrogen source on the potassium, calcium, magnesium, phosphate, ammonium, nitrate, and proton fluxes of Scots pine seedlings were determined. One situation was also created whereby excess ammonium was added. In addition, the effect of mycorrhization of the roots on these fluxes was studied. In The Netherlands the number of symbiotic fructifications has been found to be strongly decreased (Arnolds 1985). A possible correlation with enhanced ammonium deposition was demonstrated by Boxman *et al.* (1986), who found a strong decrease of the lateral symbiotic fungus growth on increasing the ammonium concentration of the growth medium. Meyer (1986) found a strong decrease in the number of symbiotic infections of spruce roots with increasing ammonium treatment.

Materials and methods

Surface sterilized seeds of *P. sylvestris* were germinated on a sterilized Podzolic soil. One week the seedlings received distilled water and the other week, medium. The composition of the medium was as follows (micromolar): NH_4Cl, 100; KNO_3, 90; KH_2PO_4, 10; $CaCl_2 \cdot 2H_2O$, 100; $MgCl_2 \cdot 6H_2O$, 50; $Na_2SO_4 \cdot 10H_2O$, 10; $FeCl_3 \cdot 6H_2O$, 1; $ZnSO_4 \cdot 7H_2O$, 0.7; $MnCL_2 \cdot 4H_2O$, 0.8; $CuSO_4 \cdot 5H_2O$, 0.2; thiamine hydrochloride, 0.3; H_3BO_3, 0.8; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.008, adjusted to pH 4.0 with HCl. When the seedlings were 2 months old, they were inoculated with the ectomycorrhizal fungus *Rhizopogon luteolus* Fr. Inoculation occured with three mycelial discs of 6 mm in diameter, cut from a freshly grown mycelial mat, per seedling. About 5 months later the seedlings had formed mycorrhizas (25 to 35% of the root tips were infected, whereas the noninoculated seedlings had less than 1% infection). The seedlings were grown in a climate room at 20°C at a relative humidity of 60 to 70%. The light regime was 16 h of light (150 $\mu E \cdot m^{-2} \cdot s^{-1}$) and 8 h of darkness. Before use the seedlings were carefully removed from the soil and washed with double-distilled water. Then, the seedlings were preincubated for 48 h in medium diluted $\times 10^{-2}$ pH 4.0, under aerobic conditions. Next, the seedlings were preincubated for 10 min in full-strength medium to equilibrate the cell wall and free space. After the preincubation 10 seedlings were incubated in 100 mL fresh medium at 20°C. At regular time intervals 5 mL medium samples were taken and analyzed for nutrients (Dijk and Roelofs 1987). Changes in medium concentrations were calculated as changes per gram dry roots. In radiotracer uptake studies the label, $H_2{}^{32}PO_4$ or ^{86}Rb, was added after the equilibration period. At regular time intervals the seedlings were harvested and washed with ice-cold double-distilled water (for ^{32}P uptake) or with 50 mM ice-cold $MgCl_2$ solution (for ^{86}Rb uptake) to remove extracellularly bound label (Borst Pauwels *et al.* 1973). The roots of the seedlings were dried (24 h, 105°C) and analyzed for radioactivity. Changes in label content were calculated as changes per gram dry roots. In radioactive efflux studies carrier-free ^{86}Rb was added to the medium during the preincubation period. Next, the seedlings were preincubated for 10 min in a rubidium free medium to desorb cell wall and free space (Rygiewicz and Beldsoe 1984). After the 10-min equilibration period the seedlings were transferred into a fresh rubidium free medium. At regular time intervals 0.5-mL medium samples were taken and analyzed for radioactivity. Changes in label concentrations were calculated as changes per gram of dry roots. Statistical analyses were carried out according to Kruskal–Wallis

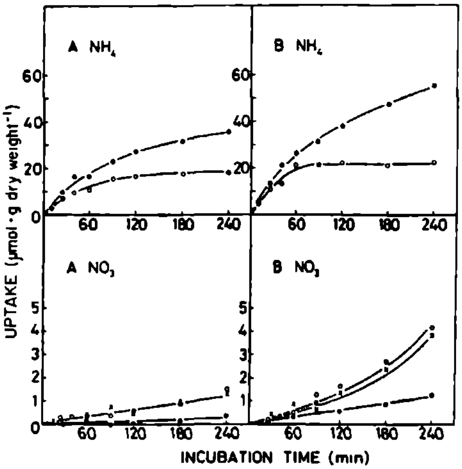

FIG. 1. The time course of ammonium and nitrate uptake by (A) noninoculated and (B) inoculated Scots pine seedlings at pH 4.0. ×, 90 μM nitrate; ○, 90 μM nitrate + 100 μM ammonium; ●, 90 μM nitrate + 1000 μM ammonium.

Results

It is shown in Fig. 1 that mycorrhizal seedlings accumulated ammonium at a faster rate than nonmycorrhizal seedlings did. Mycorrhizal seedlings exhausted the medium, initially containing 100 μM ammonium, in approximately 90 to 120 min, whereas nonmycorrhizal seedlings accumulated the ammonium in approximately 180 to 240 min. At the ammonium concentrations applied the ammonium uptake is concentration dependent. Increasing the ammonium concentration led to an increased initial rate of ammonium influx. The nitrate uptake rate under all conditions applied was higher in mycorrhizal seedlings than in nonmycorrhizal seedlings. With mycorrhizal seedlings it was found that in the absence of added ammonium or at a low ammonium concentration the uptake of nitrate was not linear but increased more than proportional with time. Addition of 100 μM ammonium had no effect on the nitrate uptake by both mycorrhizal seedlings and nonmycorrhizal seedlings. Addition of 1000 μM ammonium, however, inhibited the nitrate uptake by both nonmycorrhizal seedlings and mycorrhizal seedlings.

It is shown in Fig. 2 that with nitrate-N nutrition a net efflux of calcium occurred in nonmycorrhizal seedlings. In mycorrhizal seedlings a transient net efflux of calcium was found. After approximately 60 min a reuptake of calcium was found. Ammonium at 100 μm stimulated net calcium efflux, which was more pronounced in mycorrhizal seedlings. Addition of 1000 μM ammonium led to a strong increase in net calcium efflux. Under these conditions it was found that the net calcium efflux was significantly lower ($p < 0.05$) in mycorrhizal seedlings than in nonmycorrhizal seedlings. For magnesium similar fluxes were found. With nonmycorrhizal seedlings and nitrate-N nutrition a transient net influx was found, which was followed by a release of magnesium after approximately 60 min. With mycorrhizal seedlings a continuous net magnesium uptake was found. Addition of 100 μM ammonium stimulated net magnesium efflux, which was not

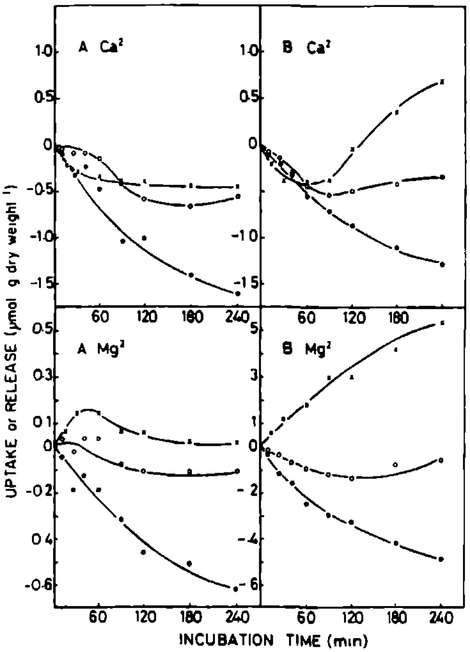

FIG 2 The time course of calcium and magnesium fluxes by (A) noninoculated and (B) inoculated Scots pine seedlings at pH 4 0 ×, 90 μM nitrate, ○, 90 μM nitrate + 100 μM ammonium ● 90 μM nitrate + 1000 μM ammonium

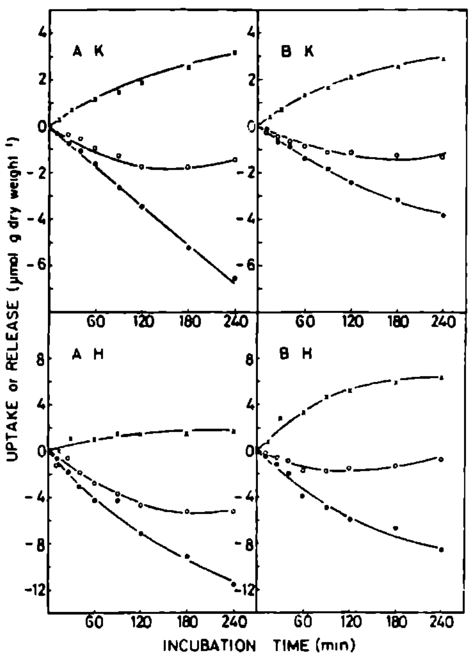

FIG 3 The time course of potassium and proton fluxes by (A) noninoculated and (B) inoculated Scots pine seedlings at pH 4 0 ×, 90 μM nitrate, ○, 90 μM nitrate + 100 μM ammonium ●, 90 μM nitrate + 1000 μM ammonium

significantly different for both types of seedlings Again, a reuptake of magnesium was found after approximately 120 min with mycorrhizal seedlings and after 180 min with nonmycorrhizal seedlings, respectively Addition of 1000 μM ammonium led to a continuous net efflux of magnesium, which was significantly higher ($p < 0\ 05$) with nonmycorrhizal seedlings as compared with mycorrhizal seedlings

Figure 3 also illustrates that a net potassium uptake occurred with nitrate-N nutrition, although no differences between nonmycorrhizal and mycorrhizal seedlings could be demonstrated With 100 μM ammonium both types of seedlings released potassium, again followed by a reuptake, after approximately 120 min Under these conditions no significant differences between both types of seedlings were found In the presence of high ammonium concentrations a continuous release of potassium was found, but in this case the nonmycorrhizal seedlings released significantly ($p < 0\ 05$) more potassium than mycorrhizal seedlings did

Because preliminary experiments only showed a slight variation in pH under these conditions (maximal 0 25 pH units), an unbuffered nutrient solution was used The buffer capacity of the medium was neglected since it was very low and did not differ for the media containing both types of seedlings (data not shown) Therefore, changes in medium pH were calculated as changes in proton content of the roots With nitrate-N nutrition there was a consumption of protons as seen by an increase in medium pH With nitrate-N nutrition the medium pH increased 0 13 and 0 05 pH units with mycorrhizal seedlings and nonmycorrhizal seedlings, respectively, after 4 h incubation With ammonium-N nutrition a decrease in medium pH was found In the presence of 100 and 1000 μM ammonium the pH dropped 0 10 and 0 19 pH units, respectively, after 4 h of incubation with nonmycorrhizal seedlings With mycorrhizal seedlings the pH decreased 0 04 and 0 16 pH units, respectively in the presence of 100 and 1000 μM ammonium The time course of the net proton fluxes is shown in Fig 3 The net proton uptake was significantly higher ($p < 0\ 01$) in mycorrhizal seedlings than in nonmycorrhizal seedlings with nitrate-N nutrition In the presence of 100 μM ammonium, again, a transient net release of protons was found This release was significantly higher from nonmycorrhizal seedlings ($p < 0\ 05$) than from mycorrhizal seedlings After 90 min incubation a reuptake of protons was found in mycorrhizal seedlings, after 180 min in nonmycorrhizal seedlings In the presence of 1000 μM ammonium, again, a continuous release of protons was found, which was significantly higher ($p < 0\ 05$) from nonmycorrhizal seedlings than from mycorrhizal seedlings

In Fig 4 the effect of the N form applied on the orthophosphate uptake is shown In this situation the reverse was found with respect to the cation fluxes Ammonium-N nutrition stimulated phosphate uptake with respect to nitrate-N nutrition ($p < 0\ 01$) However, no differences were found between the two types of seedlings or between the presence of 100 and 1000 μM ammonium

In the Figs 1 to 3 only net cation fluxes were shown It was also examined whether the constituents of net fluxes, i e , the influx and (or) efflux were influenced by the form of N nutrition It is shown in Fig 5A that seedlings accumulated more

TABLE 1. The effect of the N form applied on the initial uptake and efflux rate of ^{86}Rb by mycorrhizal Scots pine seedlings at pH 4.0

	Initial uptake rate		Initial efflux rate,
	µmol g dry weight^{-1} min^{-1}	%	%
90 µM nitrate	0.032*a*	100	100*a*
90 µM nitrate + 100 µM ammonium	0.013*b*	41	146*b*
90 µM nitrate + 1000 µM ammonium	0.006*c*	18	524*c*

NOTE: Values are the mean of duplicate measurements. Values followed by a different letter are significantly different at $p < 0.01$.

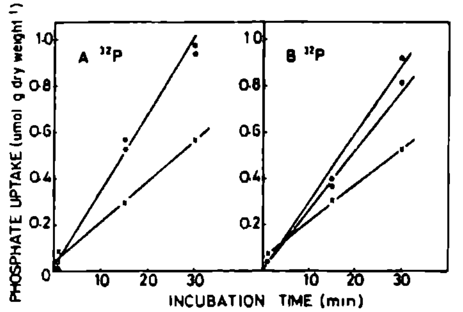

FIG. 4. The time course of ^{32}P uptake by (A) noninoculated and (B) inoculated Scots pine seedlings at pH 4.0. ×, 90 µM nitrate; ○, 90 µM nitrate + 100 µM ammonium; ●, 90 µM nitrate + 1000 µM ammonium.

rubidium under conditions of nitrate-N nutrition than with ammonium-N nutrition. On increasing the ammonium concentration to 1000 µM a further decrease in rubidium accumulation was found. The initial uptake rates under the various conditions applied were calculated using a nonlinear curve fitting programme and are shown in Table 1. Again the highest uptake rate was observed with nitrate-N nutrition and uptake decreased when the ammonium concentration was raised. Figure 5B shows the effect of the N form applied on the rubidium efflux from prelabelled seedlings into a rubidium-free medium. With nitrate-N nutrition the lowest efflux was measured. Addition of ammonium led to a stimulation of the efflux. The initial rates of rubidium efflux were calculated using nonlinear curve fitting and are shown in Table 1. Because the rubidium concentration in the roots is not known, the efflux rates are given in arbitrary units (radioactivity released per gram dry roots). With nitrate-N nutrition the lowest initial rate of rubidium efflux was found. When the ammonium concentration was raised, a strong increase in the initial rate of rubidium efflux was found.

Discussion

Inoculation of *P. sylvestris* seedlings with the ectomycorrhizal fungus *R. luteolus* had marked effects on the net fluxes of the various nutrients. Mycorrhizal seedlings accumulated ammonium at a faster rate than nonmycorrhizal seedlings did and thus facilitated medium exhaustion. The nonlinear uptake of nitrate is possibly due to the induction of the nitrate reductase enzyme (Kessler 1964). Presence of 100 µM ammonium had no effect on the nitrate uptake, although ammonium is known to inhibit nitrate uptake (Runge 1981). The ammonium to nitrate ratio at which inhibition occurs may vary among different species. It was found in Douglas fir and lodgepole pine (*Pinus contorta* Dougl.) seedlings that a 1 3 4 molar mixture of nitrate to ammonium inhibited the nitrate reductase activity per gram dry roots by 30 to 35% as compared with nitrate-N nutrition only (Bigg and Daniel 1978). No deviations from a linear nitrate uptake were found in nonmycorrhizal seedlings and a low ammonium concentration also had no effect on the nitrate accumulation. This suggests a possible important role of the symbiotic partner when nitrate uptake is concerned. The nitrate reductase activity may occur to a greater extent in the fungus. It has been found earlier that symbiotic infections increase the accumulation of ammonium and nitrate by different kinds of conifer seedlings (Littke 1982; Bledsoe and Zazoski 1983; and Rygiewicz *et al.* 1984*a*, 1984*b*). It can be concluded from Fig. 1, however, that Scots

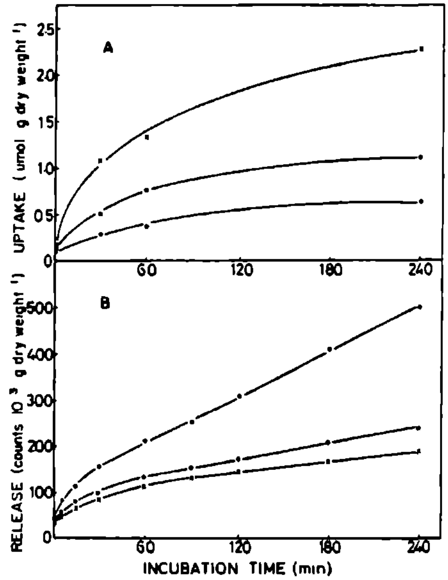

FIG. 5. The time course of ^{86}Rb uptake (A) and release (B) by mycorrhizal Scots pine seedlings at pH 4.0. ×, 90 µM nitrate; ○, 90 µM nitrate + 100 µM ammonium; ●, 90 µM nitrate + 1000 µM ammonium.

pine seedlings preferred ammonium as the main source of nitrogen, since ammonium accumulation was much higher than nitrate accumulation This is not surprising since these pines are commonly found on acidic soils, where ammonium is the dominant N form, although nitrates also are found in these soils (Roelofs and Boxman 1986) In general, conifers grow better in the presence of ammonium or in mixtures of nitrate and ammonium (Driessche 1971, Nelson and Selby 1974, Ingestad 1979) That the seedlings lose their cations when ammonium is present in the medium is in accordance with the generally observed phenomenon that ammonium N nutrition stimulates anion accumulation, while nitrate-N nutrition stimulates cation accumulation (Nelson and Selby 1974, Ingestad 1979, Kurvits and Kirkby 1980)

Rygiewicz *et al* (1984*a*, 1984*b*) and Bledsoe and Rygiewicz (1986) also found a net loss of potassium and calcium for different conifer seedlings at physiological pH values, although the loss is dependent on the type of conifer With nitrate N nutrition they found a strongly reduced loss This may of course depend on the experimental conditions applied and the combination of conifer and symbiont In forest stands several symbiotic partners can be found with one tree Bledsoe and Rygiewicz (1986) found no clear differences between cation fluxes from inoculated and noninoculated Douglas-fir seedlings but ascribe this to the long preincubation with ammonium Littke (1982) also found a release of potassium during ammonium accumulation with Douglas-fir seedlings Just as shown in Fig 3, a reuptake was found as soon as the medium was depleted of ammonium For tobacco seedlings (*Nicotiana tabacum*) a similar flux relationship has also been found and this relationship seems to be dependent on changes in the membrane potential during ammonium uptake (Scherer *et al* 1984) Depolarization of the cell membrane may lead to an efflux of potassium (Smith and Walker 1978) Possibly the same relationship is true for the calcium and magnesium fluxes since the net efflux of these cations is only transient and reverses when the medium is depleted of ammonium (Fig 2) Not only changes in membrane potential but also changes in intracellular pH will be important Ammonium uptake is coupled to proton efflux, while nitrate uptake is coupled to proton influx (Fig 3) Theuvenet and Borst-Pauwels (1977) have shown that the V_{max} of the ^{86}Rb uptake in yeast (*Saccharomyces cerevisiae*) is dependent on the intracellular pH Inoculation of the seedlings with a mycorrhizal fungus facilitates cation accumulation in the case of nitrate-N nutrition, while in the case of ammonium-N nutrition the net cation efflux from mycorrhizal seedlings is less or at most equal to that from nonmycorrhizal seedlings

With the uptake of phosphate exactly the reverse has been found as compared with cation fluxes The phosphate uptake was stimulated by ammonium-N and no differences were found between the two types of seedlings or between the ammonium concentrations applied Why excess ammonium had no effect on the phosphate uptake is not known at this moment A stimulation of the phosphate uptake by ammonium has been found earlier with Scots pines (Ingestad 1979) In a pot experiment with Douglas-fir seedlings Bledsoe and Zazoski (1983) also found a stimulation of the phosphate uptake by ammonium and attributed this to mobilization of phosphate due to a decreased rhizosphere pH during ammonium uptake This may be an important phenomenon in the soil but not in short-term hydroculture experiments, where the pH of the medium hardly changed In addition to changes in medium pH, changes in intracellular pH may be important Borst-Pauwels and Peters (1977) have shown a relationship between the cellular pH and the rate of phosphate uptake in yeast

When the influx and efflux of ^{86}Rb were studied separately, it appeared that ammonium exerted a dual effect on the net flux Not only the initial rate of rubidium influx was decreased but also a strong stimulation of the initial rate of rubidium efflux was found In studies with the isolated symbiont *R luteolus* it has been found that ammonium inhibited the rubidium uptake in a competitive way (Boxman *et al* 1986) Similar results have been found with roots of barley (*Hordeum vulgare* L 'Fergus' and 'Herta') Siddiqui and Glass (1984) showed that ammonium decreased the unidirectional influx of ^{45}Ca, whereas the unidirectional efflux of ^{45}Ca was stimulated by ammonium

In the soil water extracts of most pine forests in The Netherlands ammonium is the dominant N form The trees will accumulate fewer other cations than anions as long as ammonium is present Because ammonium is bound to a great extent to the soil adsorption complex, its mobility is low and the rhizosphere may soon be depleted of ammonium The mobility of nitrate is much greater, because it is predominantly dissolved in the soil water The nitrate supply is determined by diffusion and by mass flow, along with the water uptake (Runge 1981) When ammonium is exhausted from the rhizosphere, a reuptake of cations may occur In this way a balanced nutrient uptake is possible When ammonium is present in excess, a critical situation may arise for the trees A continuous ammonium load may lead to a great loss of other cations and, in fact, nutrient deficiencies in conifer needles have been established in areas with a high ammonium input In those forest stands showing potassium and (or) magnesium deficiencies in the needles, ammonium levels in the soil solution are always high (>500 μM ammonium) (Roelofs 1986) These findings are in good agreement with the results of this study, in which pronounced effects on nutrient fluxes have been found with ammonium levels of the same order of magnitude

Parallel with the nitrogen enrichment of the soil, the number of symbiotic macrofungi has decreased (Arnolds 1985), the fungal lateral growth has been found to be inhibited (Boxman *et al* 1986), and the number of symbiotic infections has decreased (Meyer 1986) The beneficial aspect of the fungi will be decreased, not only with respect to the nutrient fluxes but also to pathogenic fungal attack (Marx 1969)

In conclusion, the form of nitrogen in the soil solution is of great importance to the tree with respect to tree nutrition. Excess nitrate-N stimulated cation accumulation, while excess ammonium-N stimulated cation loss With low ammonium concentrations a reuptake of cations was found as soon as the medium was depleted of ammonium Therefore a well-balanced nitrate to ammonium ratio seems the most favourable condition Mycorrhization of the roots, generally, stimulated nutrient accumulation in the case of nitrate-N nutrition or protected the roots from a too great a loss of nutrients, in the case of ammonium-N nutrition

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CHAPTER 8.

Nutrient fluxes in canopies and roots of coniferous trees as affected by nitrogen-enriched air-pollution.

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Nutrient fluxes in canopies and roots of coniferous trees as affected by nitrogen-enriched air-pollution.

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Summary

Enhanced deposition of nitrogen compounds -particularly ammonium- plays an important role in coniferous forest deterioration in The Netherlands. As a result of canopy interception the input of nitrogen to the forest floor increases considerably. Whether ammonium accumulates in the soil (70% of the investigated sandy soils) or is being nitrified (30% of the soils) is dependent on the soil type. In nitrifying soils the aluminium concentration in the soil solution increases. Excess of ammonium and aluminium has strong negative effects on mycorrhizas and plants. Mycorrhizal growth and uptake of, particularly, calcium and magnesium become inhibited. A strong reduction in contents of both ions in the plant tissue is observed under these conditions. This correlates very well with the deficiency symptoms observed in many forest stands. Ammonium stress leads to a high nitrogen-content of the plant tissue, which is negatively correlated with chlorophyll and positively correlated with the arginine content of the needles.

1. Introduction

The contribution of nitrogen compounds to the acid rain problem has been underestimated for a long time. In the 1980-ies the first reports on the role of nitrogen in forest decline appeared (1-3). At present, there is a general agreement that changes in the condition of forests are due to a number of stress factors, of which air-pollution plays a major role. In the Dutch situation enhanced nitrogen deposition is of extreme importance, although enhanced deposition of nitrogen compounds also takes place in parts of Belgium, Denmark, France, Germany, Italy, Poland and Scandinavia (4,5).

Growing forests need 5 to 8 kg (±350 to 570 mol) $N.ha^{-1}.yr^{-1}$ for the production of new biomass (6). In natural ecosystems, such as forests and heathlands, nitrogen is often the growth limiting factor (7,8). This means that biomass production is limited by the amount of available nitrogen. An increase in nitrogen supply stimulates the biomass production, causing a relative shortage of other nutrients like magnesium, potassium, phosphorous, molybdenum and boron (3). In large parts of Europe the nitrogen content of the precipitation exceeds the natural background of 5 to 10 kg (±350 to 700 mol) $N.ha^{-1}.yr^{-1}$ (5). According to Asman (5) the total NH_x deposition in Central Europe amounts to 11 kg (±800 mol) $N.ha^{-1}.yr^{-1}$ from (wet + dry) deposition. In the Netherlands the total deposition of NH_x from (wet + dry) deposition has increased from ±3 kg (±200 mol) $N.ha^{-1}.yr^{-1}$ in 1870 to 8.4 kg (±600) $N.ha^{-1}.yr^{-1}$ in the 1940's. During the past decades a sharp rise in NH_x deposition to 21 kg (±1500 mol) $N.ha^{-1}.yr^{-1}$ has been observed (4,5).

The scope of this study is to give a view on the effects of nitrogen- with emphasis on ammonium- deposition, on conifer trees in the Netherlands. Figure 1 provides schematically the processes involved; these will be dealt with in the next sections.

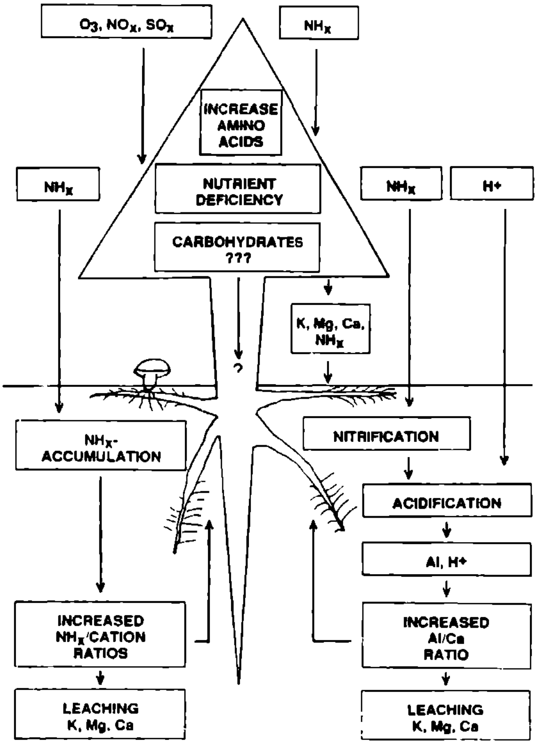

Figure 1. Schematic presentation of processes involved in forest decline

2. Ammonium-canopy interactions

Due to the filtering action of tree canopies, gaseous ammonia, sulphur dioxide and ammonium sulphate are deposited on the foliage. Therefore, the deposition in forests is considerably higher than in the surrounding meadows (1-3, 9).

From studies in areas with a relatively low deposition of ammonium it appears that the ammonium concentration in the throughfall is lower than in the open-air bulk precipitation (9-12). These observations suggest an uptake of ammonium by the foliage. This has been confirmed by Verry and Timmons (13), who found that fertilization of the foliage with ammonium resulted in a rapid uptake and metabolism of ammonium. Roelofs *et al.*, (2) and Roelofs and van Dijk, (12), have shown that needles of *Pinus sylvestris*, *Pinus nigra* and *Pseudotsuga menziesii* accumulate ammonium from artificial (500 µM) ammonium sulphate containing rain, in exchange for potassium, magnesium and calcium.

Table 1

The average chemical composition of precipitation (µM) in open plots and throughfall in *Pinus nigra* forests in three different regions of the Netherlands during 1984.

	H^+	NH_4^+	K^+	Na^+	Ca^{2+}	Mg^{2+}	NO_3^-	Cl^-	SO_4^{2-}
North (Terschelling)									
open	100	65	25	308	47	62	48	370	72
troughfall	400	59	345	6700	460	1120	110	8000	860
South (Heeze)									
open	45	130	19	60	43	17	54	73	70
throughfall	6	1060	170	310	200	113	216	350	760
South-east (Venray)									
open	2	200	20	40	30	15	49	50	90
throughfall	1	2421	216	175	278	100	147	462	1400

Table 1 shows precipitation data from different locations in the Netherlands. On the location with a low ammonium deposition (Terschelling) the sodium, magnesium and cloride levels are high, clearly as a result of seaspray. Also the proton load is relatively high. In the troughfall the proton concentration is four times higher and most of the other ion concentrations are 10 to 20 times higher as compared to open-air bulk precipitation. Only the ammonium concentration is lower in the troughfall.

In the Southern and South-eastern locality the sodium, magnesium and cloride levels are much lower as a result of a greater distance to the sea. The proton load is much lower here, due to the neutralization of (sulphuric) acid by ammonia. In the throughfall, and on the South-eastern locality even in the bulk precipitation, the acid is completely neutralized. In these areas with high ammonium deposition the ammonium concentration in the throughfall is higher than in the precipitation, which means that the deposition on the canopies is much larger than the absorbing capacity of the foliage. In the bulk precipitation sulphate is fully compensated by ammonium, whereas in the throughfall only 70 to 86% is compensated.

In moderately polluted areas (South of Sweden) ammonium deposition from throughfall analyses indicate a deposition of 20 to 40 kg (±1430 to 2860 mol) $N.ha^{-1}.yr^{-1}$ to the forest floor. The absorbing capacity of the canopy is estimated to be 10 to 15 kg (±715 to 1070 mol) $N.ha^{-1}.yr^{-1}$ (9). In The Netherlands an average nitrogen deposition of 80 kg (±5720 mol) $N.ha^{-1}.yr^{-1}$ on the forest floor was observed in pine forests. In heavely polluted areas (South and South-east of the Netherlands) an extremely high deposition of ammonium on the forest floor was observed. Ammonium inputs ranging from 119 to 270 kg (±8500 to 19300 mol) $N.ha^{-1}.yr^{-1}$ were measured.

Not only foliar ammonium uptake but also ammonium uptake via the soil will provide the tree with nitrogen. As the latter quantity is high enough, effects on the foliar ammonium uptake will be found (14). The time of the year also seems to be an important factor. In the winter, when biological activity is low, there seems to be only a slight foliar uptake, whereas considerable uptake in the summer is observed, due to biomass production of the vegetation (9).

3. Ammonium-soil interactions

Ammonium deposition on the forest floor may lead to acidification or ammonium accumulation. Whether the one or the other process takes place is strongly dependent on deposition levels and soil conditions.

On non-calcareous soil types, where most of the pines can be found, clear effects of ammonium deposition were found. Whether ammonium acts as an acidifying or eutrophicating agent depends on the soil type. According to Kriebitzsch (15), acidic forest soils may be divided into four classes, ranging from soils with a complete nitrification to soils with no or a very low nitrification.

In the Netherlands a nationwide investigation was carried out to establish in which soils and to what extent deposition of ammonium leads to a disturbed mineral balance in the soil (16). During nine months canopy throughfall was collected monthly in 36 randomly chosen *Pinus nigra, Pinus sylvestris* and *Pseudotsuga menziesii* stands. At each location soil samples were taken every two months and the nutritional state was analysed.

By comparing the ratios of ammonium to nitrate in throughfall deposition and soil solution, nitrification rates have been estimated. A small deviation between these ratios (less than 20%) indicates a soil with a very low nitrification rate. In soils with low or moderate nitrification rates deviations of 20 to 40% or 40 to 80% were found. When the difference between the NH_4/NO_3 ratio in throughfall and in soil is very large, nitrification rates possibly are very high.

Table 2 shows that in 64% of the investigated soils the deviation between the ratios is less than 40%, indicating a low nitrification rate. Comparisons are also made between the NH_4/K and NH_4/Mg ratios in throughfall deposition and soil solution. In 89% of the forests where the NH_4/K and/or the NH_4/Mg ratios in the soil reflect the ratios in throughfall deposition (deviation < 20%), nitrification rates were low (deviation < 40%) (Table 2). In these soils ammonium concentrations in throughfall are directly responsible for the value of the NH_4/K and NH_4/Mg ratios in the soil solution. Ammonium exchange processes take place at the soil absorption complex, resulting in leaching of potassium, magnesium and sometimes

Table 2

Correlations between NH_4/NO_3, NH_4/Mg and NH_4/K ratios in the soil and canopy throughfall

	NH_4/NO_3 < 40% (1)	NH_4/NO_3 > 40% (1)
number of locations	23 (64%)	13 (36%)
NH_4/Mg and/or NH_4/K <20% (2)	89%	11%
NH_4/Mg and/or NH_4/K >20% (2)	56%	44%

(1) NH_4/NO_3 < | > 40%: the NH_4/NO_3 ratio in the soil water deviates less/more than 40% from this ratio in canopy throughfall

(2) NH_4/Mg and/or NH_4/K < | > 20%: the NH_4/Mg and/or NH_4/K ratio in the soil water deviates less/more than 20% from this ratio in the canopy throughfall

calcium. Nationwide investigations revealed a strong correlation between the vitality of *Pinus nigra, Pseudotsuga menziesii* and *Quercus robur* stands and the NH_4/K, NH_4/Mg and sometimes the Al/Ca ratio in the soil solution (17, 18).

In soils with a high nitrification rate proton exchange processes take place, resulting in leaching of magnesium, potassium and calcium ions. Apart from nutrient leaching, soil acidification leads to aluminium dissolution. High aluminium to calcium ratios may lead to damage of the root system of trees.

4. Ammonium-plant interactions

Direct effects on above-ground parts

Direct effects of NH_x depositions are the result of sufficiently high concentrations in or on the plant tissue. In the immediate surroundings of emission sources direct effects of NH_3, and at greater distances direct effects of NH_4^+ may be expected. In general, ammonia is more toxic to plants than ammonium, since ammonium bears no net charge and diffuses easily through membranes. Under normal conditions NH_3 is an intermediate in the nitrogen metabolism of the cell, but the conversion to amino acids is very fast and occurs in the chloroplasts and not in the cytoplasm (19, 20). Therefore concentrations in the cytoplasm are very low. At high external ammonia/ammonium concentrations, however, the concentrations in the cytoplasm may become toxic. Deposition on the outside of the needless affect the surface wax layer (21). Net uptake of ammonium by the foliage has also been demonstrated (2, 12).

It can be concluded from ecotoxicological studies that:

- ammonium is an uncoupler of the photosynthetic phosphorylation. The ATP content decreases and, as a result, also the carbohydrate production (22, 23).
- ammonium inhibits starch synthesis, resulting in accumulation of glucose (24, 25).
- ammonium saturates membrane lipids, as a results of which the membrane becomes more permeable and less flexible (22).
- ammonium inhibits protein synthesis, as a result of which the aminoacid content strongly increases, in particular arginine (26-29).
- ammonium inhibits chlorophyl synthesis, leading to chlorosis of the green tissue (26, 29, 30).

Table 3

N-content (% dry weight), chlorophyll content (mg.g^{-1} fresh weight), arginine content (µg.g^{-1} fresh weight) and percentage of free-arginine-N of total-N (% of total-N) of *Pinus sylvestris* needles, collected in April 1986.

	vital (n=8)	non-vital (n=10)
1985 Needles		
N	1.95	2.79
chlorophyll a	1.04	0.32
chlorophyll b	0.25	0.06
arginine	8.6	95.7
% free-arg N	4.6	37.8

Field observations with *Pinus sylvestris* show indeed a strong negative correlation between the N-content of the needles and the chlorophyll and arginine content of the needles (29) (see Table 3). The same phenomena were observed in a greenhouse experiment, where pine and spruce were treated with artificial N-enriched rain (van Dijk, pers. comm.).

Toxic effects of ammonia are commonly observed in winter season, in combination with low temperatures, and mostly in the direction of the source and within a radius of approximately 300 m from the source (31). When the biological activity is low, the trees are not able to detoxify the ammonia. When the temperature rises, damage patterns can be observed, resulting in pale-brown and, later on violet-red needles. This effect is mostly only transient and disappears later on in the growing season. Direct ammonia effects are only observed with evergreen species.

Direct effects on underground parts

Direct effects of NH_x depositions are the result of sufficiently high concentrations in or near the absorbing organs of the plant, which are the roots and the fungi living symbiotically with the fine roots.

During the past decades the number of symbiotic and saprophytic fungi in the Dutch forests has changed. Actually the number of fruitbodies of mycorrhizal fungi has decreased and many common species became rare or even disappeared from the forest stands (32). The strongest reduction was observed for the fungi growing on nutrient poor, acidic soils. A decrease in the number of saprophytic fungi was less clear, although a shift towards the more nitrophilous species was observed.

A recent survey on the mycoflora in Douglas stands revealed a negative correlation between the number of mycorrhizal fruitbodies and the degree of air pollution (33). Especially in the southeastern part of the Netherlands, where the deposition of nitrogen in the form of ammonium-N to the forest floor amounts to more than 100 kg $N.ha^{-1}.yr^{-1}$, it was estimated that the number of mycorrhizal species has been reduced with 60%. In Sweden at the Lisselbo experiment, observations on the fungal flora have also shown that an increased nitrogen supply decreased the number of mycorrhizal fungi (34).

Table 4

The effect of increased NH_4/K ratios on the lateral growth and biomass of mycorrhizal fungi (n=14) in pure culture at pH=5.5. The effect of increased Al/Ca ratios on the lateral growth of mycorrhizal fungi (n=11) in pure cultures at pH=4.0. Mean of triplicate measurements.

NH_4/K	lateral growth (%)	biomass after growth period (%)	Al/Ca	lateral growth (%)
1	100	100	0.02	100
5	57	210	0.5	93
10	44	221	1.8	72
20	35	209	4.1	68
40	31	173	11.6	63
			19.4	50

Table 4 shows that either enhanced ammonium or enhanced aluminium concentrations decrease the fungal lateral growth (35). In the presense of ammonium the fungal biomass initially increases, which can be explained by incorporation of ammonium into fungal chitin (36). The results indicate a protection of the fungi to ammonium by forming more biomass and remaining rather small. In this way the absorption capacity will be decreased, which enables the cells to survive. At very high ammonium concentrations the biomass production decreases again, which could mean that the fungi are not capable to detoxify all the ammonium.

Apart from a reduction in lateral growth, ammonium excerts a direct influence on the nutrient uptake. It has been shown that the uptake of potassium, calcium and orthophosphate by the isolated mycorrhizal fungus *Rhizopogon luteolus* became inhibited on raising the ammonium concentration (16, 37).

It has frequently been found that an increase in soil nitrogen, particularly ammonium-N, leads to a decreased rate of mycorrhizal infection. It must be emphasized, however, that most experiments were conducted from a fertilization point of view. According to Björkman (38) a strong decrease in mycorrhizal infection can be found after nitrogen fertilization. Björkman pointed out that after a nitrogen gift the protein synthesis was stimulated, leading to a decrease in soluble carbohydrates. Richards and Wilson (39) found, however, no correlation between the soluble carbohydrates and the mycorrhizal frequency, but they did find a correlation between the the mycorrhizal frequency and the ratio between the soluble carbohydrates and total nitrogen in the tissue. According to Slankis (40) high ammonium concentrations inhibit fungal auxin synthesis, resulting in too low concentrations of growth substances.

Alexander and Fairly (41) showed that nitrogen fertilization in a 35 year old *Picea sitchensis* stand (300 kg N.ha-1 as ammonium sulphate granules) resulted in a decrease of 15% of the mycorrhizal formation in the second year after the fertilization. Meyer (42) showed a strong negative correlation between the mycorrhizal frequency and the soil nitrogen content. The mycorrhizal frequency he found with spruce (*Picea abies*) seedlings was optimal (± 70%) when the soil N content was approximately 40 mg mineral N.kg^{-1} dry soil. Increasing the soil N content to aproximately 350 mg mineral N.kg^{-1} dry soil reduced the mycorrhizal frequency to less than 5%. In these experiments no correlation with the soil pH (water) was found. The pH varied between 3.35 and 3.57. In a greenhouse experiment, with *Pinus sylvestris* and *Pinus nigra* on a podzolic soil (16) and in an experiment with *Pinus nigra* on hydro cultures also a decrease in mycorrhizal frequency was found after increasing the ammonium gift (see Figure 2). This Figure also shows that the mycorrhizal frequency of the fine roots is negatively correlated with the aluminium content of the fine roots at aluminium >0.5 % dry matter.

Figure 3 shows that with increasing ammonium-N nutrition the shoot/root ratio increases. This means that a relatively decreasing root biomass must provide a relatively increasing shoot biomass with water and nutrients. With increasing Al/Ca ratios in the culture solution virtually no effect on the shoot/root ratio was found. This has also been found with *Betula pendula* seedlings (43).

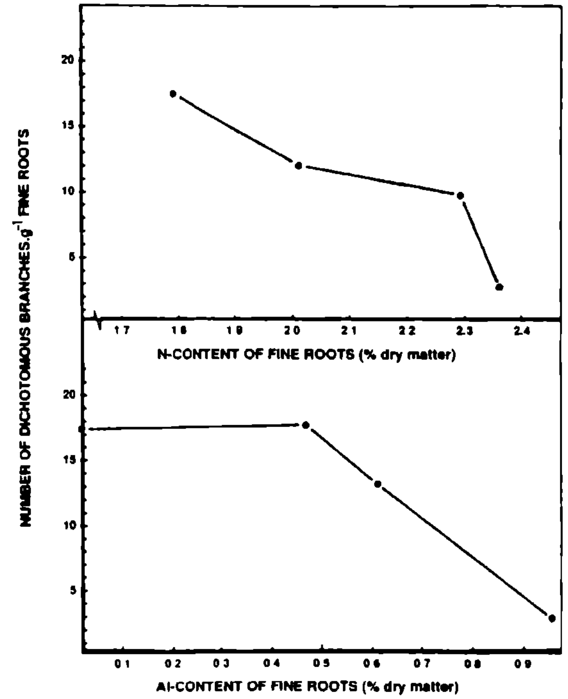

Figure 2. Relations between the N- and Al-content of the fine roots and the number of dichotomous branched fine roots. *Pinus nigra* in solution cultures at pH=4.0, after two years of incubation. Mean of duplicate measurements.

5. Ammonium-plant nutrition interactions

Soils with ammonium accumulation

Total N removal from a forest ecosystem is estimated to be 14 to 22 kg (±1000 to 1600 mol) $N.ha^{-1}.yr^{-1}$ for conifer and 31 to 48 kg (±2200 to 3400 mol) $N.ha^{-1}.yr^{-1}$ for deciduous forest stands, based on N leaching from the soil and N uptake by the vegetation, for an average soil type (44). Based on throughfall analysis this amount may be somewhat higher (see section 2). Given deposition data, ranging from 70 to 170 kg (±5300 to 12000 mol) $N.ha^{-1}.yr^{-1}$, this will mean an accumulation of ammonium in the soil, resulting in a mineral imbalance of the soil. A negative correlation has been found between the vitality of the tree and the disturbed ammonium/basic cation and the ammonium/nitrate ratio (17, 18, 45). In general, the vegetation of acidic soils prefers ammonium-N as the main source of nitrogen, although nitrate-N is also found. Conifers grow better in the presence of ammonium or in

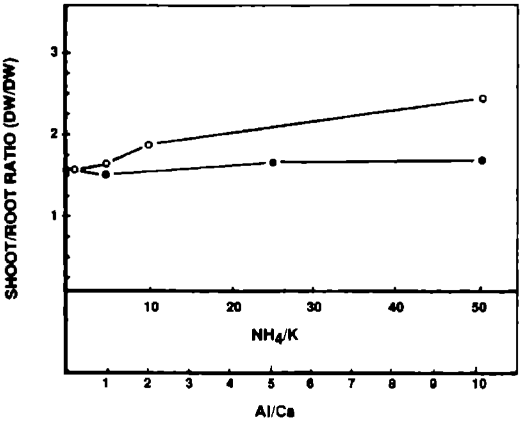

Figure 3. Impact of increasing NH_4/K (o) and Al/Ca (•) ratios on the shoot/root ratio (dry weight basis) of *Pinus nigra* in solution cultures at pH=4.0, after two years of incubation. Mean of duplicate measurements.

mixtures of ammonium and nitrate. In studies with *Pinus sylvestris* seedlings it was found that nitrate alone was an inferior source of nitrogen, resulting in lower dry weights and chlorosis of the needles (46). It has been generally observed that ammonium-N nutrition decreases cation accumulation, while nitrate-N nutrition decreases anion accumulation. Ingestad (47) has shown that *Pinus sylvestris* and *Picea abies* seedlings, on varying the ammonium to nitrate ratio from 0:100 to 100:0, lose a great deal of their cations, while the phosphorous content showed only a slight increase. Others have demonstrated the same phenomenon (46, 48, 49, 51). On increasing the ammonium to nitrate ratio, the enzyme nitrate reductase becomes inhibited, although the ratio at which inhibition occurs may vary among the species. With *Pseudotsuga menziesii* and *Pinus contorta* seedlings a 1:3.4 molar mixture of nitrate to ammonium decreases the nitrate reductase activity per gram dry roots by 30 and 35%, respectively as compared to nitrate nutrition only (52).

Table 5 shows that net cation uptake is stimulated by nitrate-N nutrition and mycorrhization of the roots is more benificial to uptake than non-mycorrhization of the roots. Increasing the ammonium to nitrate ratio to 1.1 results initially in a net efflux of the cations, but as soon as the medium is depleted of ammonium, a reentry of the cations occurs (45). Under this condition the phosphate uptake is stimulated and , again, mycorrhization is benificial. When the ammonium to nitrate ratio is increased to 11.1, a continuous net efflux of calcium, magnesium, potassium, and protons was found. Mycorrhizal seedlings lost significantly less other cations than non-mycorrhizal seedlings did. Under high ammonium conditions no effect on the phosphate uptake was found.

Table 5

The effect of the N-form applied on some net nutrient fluxes by ***Pinus sylvestris*** seedlings at pH=4.0, after an incubation of 4 hrs. Effect of inoculation with the mycorrhizal fungus ***Rhizopogon luteolus***. I: 90 µ.M nitrate, II: 90 µ.M nitrate + 100 µ.M ammonium, III: 90 µ.M nitrate + 1000 µ.M ammonium. Fluxes in μ.mol.g^{-1}. dry roots. (+): net uptake, (-): net efflux. NM: non-mycorrhizal seedlings, MYC: mycorrhizal seedlings.

	Ca^{2+}		Mg^{2+}		K^+		H^+		PO_4^{3-}	
	NM	MYC	NM	MYC	NM	MYC	NM	MYC	NM	MYC
I	-0.5	+0.7	+0.1	+0.5	+3.2	+2.9	+ 1.8	+6.4	+0.6	+0.5
II	-0.6	-0.3	-1.2	-0.6	-1.5	-1.4	- 3.3	-0.7	+0.9	+0.9
III	-1.6	-1.3	-6.3	-4.9	-6.6	-3.9	-11.5	-8.4	+1.0	+0.8

Rygiewicz *et al.* (48, 49) also found, with ammonium-N nutrition only and a NH_4/K ratio of 3.5, a net loss of potassium and calcium for different conifer seedlings at physiological pH values, although the loss was dependent on the kind of conifer. With nitrate-N nutrition they found a strongly reduced loss. This may of course depend on the experimental conditions applied and the combination of conifer and symbiont. In forest stands several symbiotic partners can be found with one tree. Bledsoe and Rygiewicz (53) found no clear differences between cation fluxes from inoculated and non-inoculated Douglas seedlings, but ascribe this to the long preincubation with ammonium. Littke (50) also found a release of potassium during ammonium accumulation with Douglas seedlings. Also for tobacco seedlings (*Nicotiana tabacum*) a similar flux relation has been found and this relation seems to be dependent on changes in the membrane potential during ammonium uptake (54). Depolarization of the cell membrane may lead to an efflux of potassium (55). Possibly the same relation is true for the calcium and magnesium fluxes since the net efflux of these cations is only transient and reverses when the medium is depleted of ammonium. Not only changes in membrane potential but also changes in intracellular pH will be important. Ammonium uptake is coupled to proton efflux, while nitrate uptake is coupled to proton influx.

Table 6 also shows that particularly the content of calcium and magnesium in ***Pinus nigra*** is negetively correlated with the N-content of the plant tissue. The potassium and phosphorus content of the above ground parts showed minor variations, although in the fine roots a significant decrease in potassium content and, at high ammonium concentrations, also in phosphorus content was found.

In the water extracts of the upper mineral soil layer of pine forests in the Netherlands ammonium is mostly the dominant N-form. The trees will accumulate less other cations than anions as long as ammonium is present. Due to the fact that ammonium is bound to a great extent to the soil adsorbtion complex its mobility is low and the rhizosphere may soon be depleted of ammonium. The mobility of nitrate is much greater, as it is predominantly dissolved in the soil water. The nitrate supply is determined by diffusion and by mass flow, along with the water uptake (51). When the ammonium is exhausted from the rhizosphere a

Table 6

Effects of ammonium nutrition on the nutrient content of *Pinus nigra* in hydro cultures at pH=4.0, after two years of incubation. Contents: µmoles.g^{-1} DW. Mean of duplicate measurements.

Nutrients in the needles

NH_4^+ (µM)	N	Ca^{2+}	Mg^{2+}	K^+	P
100	909	148	75	160	35
500	979	172	51	200	42
1000	1149	61	46	170	39
5000	1650	35	26	152	43

Nutrients in the stem

NH_4^+ (µM)	N	Ca^{2+}	Mg^{2+}	K^+	P
100	488	190	79	70	23
500	694	149	72	64	26
1000	764	150	76	100	37
5000	1302	66	38	72	39

Nutrients in the roots(coarse, ø > 2 mm)

NH_4^+ (µM)	N	Ca^{2+}	Mg^{2+}	K^+	P
100	928	70	54	82	57
500	1012	56	36	68	61
1000	1090	50	42	50	46
5000	1316	48	41	52	40

Nutrients in the roots (fine, ø ≤ 2 mm)

NH_4^+ (µM)	N	Ca^{2+}	Mg^{2+}	K^+	P
100	1268	69	51	162	75
500	1482	66	37	104	71
1000	1636	51	36	74	72
5000	1684	33	14	36	49

re-uptake of cations may occur. In this way a balanced nutrient uptake is possible. When ammonium is present in excess a critical situation may arise for the trees. A continuous ammonium load may lead to a great loss of cations and, in fact, nutrient deficiences in conifer needles have been established in areas with a high ammonium input. In those forest stands showing potassium and/or magnesium deficiencies in the needles the ammonium levels in the soil solution are always high ($NH_4/K \geq 5$ and $NH_4/Mg \geq 10$) (17).

Acidifying soils and aluminium

In this type of soil ammonium will be oxidized to nitrate with concomitant release of two mol H^+ per mol ammonium. Together with the direct input of H^+, nitrification will contribute to the proton load of the soil. When the pH of the soil solution decreases, proton exchange processes at the soil-absorption complex take place and aluminium becomes available, resulting in increased aluminium to calcium and magnesium ratios.

Physiological effects of aluminium are (56):

- inhibition of cell division of the roots
- inhibition of root respiration
- inhibition of polysaccharide synthesis in the cell wall
- effects on water and nutrient uptake
- formation of aluminium-nucleic acid complexes

As shown in Figure 4 aluminium stress leads, in particular, to a severe reduction of the divalent cation uptake. Even at low aluminium concentrations the net uptake of calcium and magnesium is reversed, leading to a net efflux of both cations. The net uptake of ammonium and phosphorus is hardly affected. 100 µM aluminium stimulates net potassium uptake and 100 and 500 µM aluminium stimulate nitrate uptake.

The observations are in accordance with the observations as shown in Table 7. A strong reduction of magnesium and calcium was found in all plant parts. This is a common effect of aluminium (56-58). According to Hütermann (59) aluminium drives calcium out of the root cell wall and the magnesium content was also greatly reduced. These effects were already found at not-visibly damaged roots. The potassium content of the needles increased strongly, although calculations did not explain a reallocation of potassium from the fine roots to the needles. This means a stimulation of the potassium uptake and also a stimulation of the translocation to the needles. According to Cumming *et al.* (60) aluminium concentrations upto 150 µM stimulate potassium uptake by *Picea rubens*. Thornton *et al.* (58) found a decrease in root potassium content of *Picea rubens*, while the potassium content of the needles remained approximately equal on the average. It can be concluded from the work of Hutchinson *et al.* (61) that spruce is more sensitive to aluminium than pine.

In the long term, aluminium excess may have severe consequences for pine growth. At Al/Ca ratios greater than 1 a strong reduction of particularly the calcium and magnesium content takes place. Ultimately the trees may develop severe deficiency symptoms as already are frequently found some pine forest stands.

6. Conclusions

- Due to canopy interception, the input of nitrogen compounds to the forest floor is enhanced as compared to the bulk precipitation.
- In the Netherlands approximately 70% of the forest soils has a low nitrification rate, leading to ammonium accumulation and mineral imbalances. 30% of the forest soils has a moderate or high nitrification rate, leading to aluminium excess and mineral imbalances.

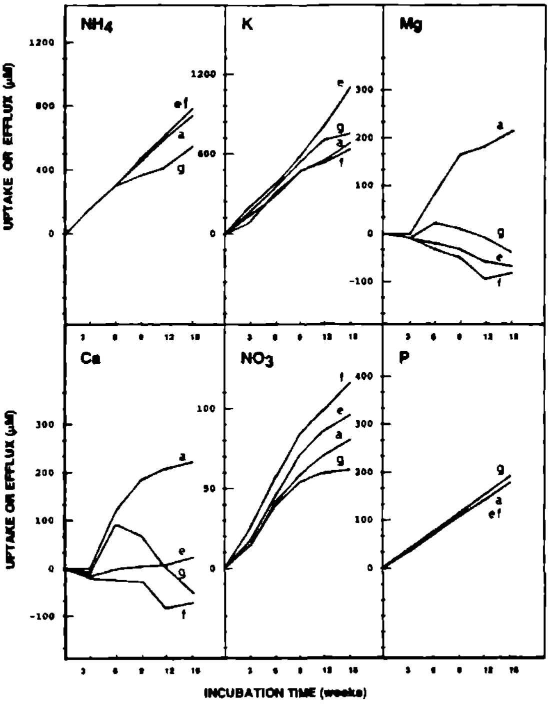

Figure 4. Effects of incresed aluminium concentrations on the nutrient uptake by ***Pinus nigra*** during 15 weeks at pH=4.0 in hydro cultures. a: control, 0 µM Al; e: 100 µM Al; f: 500 µM Al; g: 1000 µM Al. Mean of duplicate measurements.

- Ammonium and aluminium have negative effects on mycorrhizal growth.
- Ammonium stress leads to enhanced shoot/root ratios.
- Due to ammonium stress nutrient uptake is inhibited and this results in deficiencies of particularly calcium and magnesium in the plant. A high N-content of the tissue is negatively correlated with chlorophyll and positively correlated with arginine content of the needles.
- Aluminium stress leads to deficiencies of particularly calcium and magnesium in all plant parts.

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Table 7

Effects of increasing aluminium concentrations on the nutrient content of *Pinus nigra* in hydro cultures at pH=4.0, after two years of incubation. Contents: µmoles.g^{-1} DW. Mean of duplicate measurements. (*: single measurement).

Nutrients in the needles

Al^{3+} (µM)	N	Ca^{2+}	Mg^{2+}	K^+	P
0	909	148	75	160	35
100	835	89	36	270	37
500 (*)	920	101	29	288	53
1000 (*)	906	63	20	300	39

Nutrients in the stem

Al^{3+} (µM)	N	Ca^{2+}	Mg^{2+}	K^+	P
0	488	190	79	70	23
100	643	170	88	124	50
500 (*)	481	112	29	84	26
1000 (*)	453	64	15	92	21

Nutrients in the roots (coarse, ø > 2 mm)

Al^{3+} (µM)	N	Ca^{2+}	Mg^{2+}	K^+	P
0	928	70	54	82	57
100	821	60	44	56	55
500 (*)	679	64	46	52	39
1000 (*)	863	54	38	60	57

Nutrients in the roots (fine, ø ≤ 2 mm)

Al^{3+} (µM)	N	Ca^{2+}	Mg^{2+}	K^+	P
0	1268	69	51	162	75
100	1140	41	32	82	100
500 (*)	1047	34	20	76	70
1000 (*)	906	35	28	48	82

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CHAPTER 9.

Synthesis.

VEGETATION UNDER CHEMICAL STRESS: ACIDIFICATION, EUTROPHICATION AND ALKALINISATION.
A SYNTHESIS.

The same procedure has been followed in the study of the various environmental changes, viz. alkalinisation of aquatic ecosystems in peaty lowlands, water acidification and forest decline:

1. A widely spread or intensive field investigation on the distribution and/or development of involved plant species in relation to a large number of environmental factors.
2. After postulation of a working hypothesis derived from the field investigations ecophysiological experiments with a selected number of plants and abiotic parameters were carried out in order to establish causal relations between observed biological and chemical parameters during field investigations.
3. Culture experiments and/or experiments in more or less controlled small-scale ecosystems were carried out in order to get a better translation from laboratory investigations to the field situation and vice versa.

After field studies it was possible to postulate a working hypothesis for all three investigated topics.

The ecophysiological experiments provided information on the causality of the observed correlations between plant development and environmental factors.

During cultivation experiments in an experimental set up or in artificial ponds it could be observed that the results, obtained from field investigations and ecophysiological experiments, could be reproduced by changing one or more important environmental factors.

The field investigations in the nature reserve "Weerribben" demonstrated that problably not the inlet of water from the IJsselmeer led to a direct eutrophication of the surface waters, since water from the northern part of the IJsselmeer is poor in nutrients. The Rhine type water from the IJsselmeer, however, was very alkaline and rich in bicarbonate and sulphate (chapter 2). A comparison between a water body receiving water from the IJsselmeer (in dry periods) and an identical but isolated water body made clear that, especially in the soft upper soil layer, the mobility of phosphorus was much higher in the not isolated system compared to the isolated system and also the concentrations of reduced components like H_2S, CH_4 and NH_4^+ were much higher. From transplantation experiments with *Stratiotes aloides* L. plants from the isolated to the open system it became clear that the plants started to decay not in summer, but in the winter when the plants had migrated to the weak upper sapropel layer. Ecophysiological and culture experiments proved that the ammonium and sulphide levels in this layer were lethal to *Stratiotes* plants.

It has been discussed in chapter 2 that the inlet of alkaline, Rhine type water rich in bicarbonate and sulphate leads to internal alkalinisation, eutrophication and a high production of methane, sulphides and ammonium. The upper peaty soil layer becomes soft and as a result of gas bubbling and bioturbation the water becomes more or less turbid. Probably not only the toxic components are of importance, but also the fact that in winter and spring the *Stratiotes* plants become covered by fine detritus, which , in combination with the higher turbidity of the water layer, has a negative effect on photosynthesis and so on the plant development.

This process of internal alkalinisation, eutrophication and production of toxic components could also be reproduced in artificial small-scale aquatic ecosystems (700 l. concrete tanks). Indeed bicarbonate and sulphate appeared to be responsible for the observed changes in water quality.

Also the changes in inorganic carbon sources as a result of the alkalinisation processes have a negative effect on the growth of *Stratiotes* plants (Prins and De Guia, 1986). It is striking that also in acidifying moorland pools the change in nitrogen and carbon budgets appeared to be the main cause for the disappearance of many plant species from naturally weakly buffered oligotrophic waters (chapter 3 and 4). The high atmospheric input of NH_x in combination with the reduced nitrification rate in acidifying waters leads to an increased NH_4^+/NO_3^- ratio in the water layer. It appeared that plants from weakly buffered, nitrate dominated environments excrete nutrients like potassium when they predominantly take up ammonium in acidified, ammonium enriched environments.

The same phenomenon has been observed in heathlands. Many endangered plant species from heathlands, such as *Thymus serpyllum* L., *Polygala serpyllifolia* Hose, *Pedicularis sylvatica* L. and *Lycopodium inundatum* L. normally occur at places with low NH_4^+ concentrations in the soil water. After acidification also in these environments the NH_4^+/NO_3^- ratio increases as a result of a decreased nitrification rate and a high input of atmospheric ammonium (Houdijk, 1990), leading to the disappearance of the above mentioned plant species.

It appeared from laboratory experiments that not the decrease or increase in pH value of the water or soil water is directly responsible for the changes in vegetation and the disappearance of certain plant species, but much more the concomitant changes in inorganic carbon and nitrogen or accumulation of toxic compounds.

Acidification of lakes leads to at least a temporarily increase of the CO_2 level in the water layer. Plant species like *Juncus bulbosus* L. and *Sphagnum cuspidatum* Hoffm. benefit from this. When also the ammonium concentration is relatively high, this results in a high production of these plant species and a suppression of many plant species from oligotrophic soft water ecosystems (chapter 3 and 4).

During alkalinisation in lowland peatlands the CO_2 level in the water layer decreases as a result of an increasing pH. Productive plant species such as *Stratiotes aloides* are negatively affected by this because they mainly use CO_2 for photosynthesis (Prins and De Guia, 1986). Plants which can use HCO_3^- more efficiently, such as *Ceratophyllum demersum* L. or planktonic algae, profit from this situation.

Schuurkes et al. (1986) carried out some experiments on the N nutrition of plants from both weakly buffered and from acid or acidified environments. They found that the first group of plants predominantly took up nitrate from a medium containing ammonium nitrate, while the second (acid) group of plants predominantly took up ammonium. It was clear from treatments with artificial rain water in small-scale ecosystems in a greenhouse that water acidification as a result of sulphuric acid containing rain did not lead to a luxurious growth of *Juncus bulbosus* . However, water acidification caused by ammonium sulphate containing precipitation did lead to a mass development of *Juncus bulbosus* and a complete suppression of isoetid plant species from oligotrophic, poorly buffered environments (Schuurkes et al., 1987).

This was in good agreement with the observations during field investigations (chapter 3). In acidified waters with a luxurious growth of *Juncus bulbosus* ammonium was the main nitrogen source, while in waters dominated by the soft water macrophyte *Littorella uniflora* (L.) Aschers. the dominant nitrogen source was nitrate.

Ecophysiological experiments with *Stratiotes aloides, Littorella uniflora, Juncus bulbosus* and *Thymus serpyllum* showed that these plants, which normally occur in environments in which nitrate is the main nitrogen source, have problems in taking up base cations like potassium and magnesium in situations with decreasing nitrate and increasing ammonium levels. *Juncus bulbosus,* the successful plant species in ammonium dominated, acidified environments, has no problems taking up potassium in solutions rich in ammonium (chapter 2, 3 and 4; Houdijk, 1990).

The same phenomenon seems to be responsible for at least a part of the forest decline in areas with high ammonium deposition levels. In the Netherlands this is the case in the whole country, with the exception of the coastal region, where the ammonium deposition level is relatively low (Houdijk, 1990). Particularly in acid forest soils with a low nitrification rate input of ammonium leads to a disturbed mineral balance. The increased NH_4^+/K^+ and NH_4^+/Mg^{2+} ratios lead also in coniferous trees to inhibition of the uptake of base cations (chapter 6, 7, 8; Boxman et al., 1987; 1988a; 1988b; Houdijk, 1990). This often results in a disturbance of the mineral balance in the trees (chapter 6, 7, 8; van Dijk and Roelofs, 1988; van Dijk et al., 1990; Boxman et al., in press.). In those situations the trees can suffer from severe nutrient deficiencies or become more susceptible to drought, frost, insects or fungal diseases like *Sphaeropsis sapinea* (Fr.) Dyko & Sutton and Roelofs and van Dijk, 1987; De Kam et al., 1989).

Sphaeropsis sapinea causes much damage in Corsican Pine (*Pinus nigra* var. *maritima* (Ait.) Melville) and Scots Pine (*Pinus sylvestris* L.) stands in heavily polluted areas, e.g. the Dutch Peel region. It appeared that this fungal attack is very significantly correlated with an increased nitrogen content in the plant tissue (chapter 6) and also with the nitrogen concentration in the soil (Boxman and van Dijk, 1988).

The knowledge about the effect of high ammonium concentrations on the potassium and magnesium nutrition is not new. Mulder (1956) and Jacob (1958) already found that high ammonium concentrations in agricultural soils inhibited the magnesium uptake in agricultural crops, leading to severe Mg^{2+} deficiencies in both crops and animals.

In acid forest soils the inhibition of magnesium uptake may also be caused by aluminium (chapter 6, 8; (Boxman et al., in press; Evers, 1983; Thornton et al., 1987; Keltjens and van Loenen, 1989). In the Dutch situation, with a high input of ammonium sulphate and a relatively low input of protons, severe soil acidification and the concomitant leaching of Ca^{2+} and dissolution of aluminium in the rooted upper soil layer mainly occur in soils with a moderate to high nitrification rate (van Breemen et al., 1982; 1983; chapter 6; Boxman et al., 1988).

So, in soils with a low nitrification rate as well as in soils with high nitrification rates high ammonium input can cause magnesium deficiencies in the trees.

Main conclusions.

1. Lowering of the (ground)water level stimulates the process of acidification. A compensation of water loss in surface waters in peatlands by inlet of alkaline water rich in bicarbonate and sulphate leads to internal alkalinisation, eutrophication and increased levels of reduced components like CH_4, H_2S/HS^- and NH_4^+/NH_3. The high concentrations of sulphides and ammonium in the weak upper soil layer can be lethal to *Stratiotes aloides* plants.

2. Acidification of soft water ecosystems as a result of nitrogen containing, acidifying deposition leads to at least a temporarily strong development of *Juncus bulbosus* and submerged *Sphagnum* species which suppresses isoetid plant species such as *Littorella uniflora* and *Lobelia dortmanna* L.

3. Both acidification of soft water ecosystems and alkalinisation of surface waters in peaty lowlands lead to changes in the vegetation which are, at least partly, due to changes in the carbon budgets and nitrogen compounds.

4. The change in nitrogen compounds, i.e. the increase in the NH_4^+/NO_3^- ratio during alkalinisation in peatlands, and NH_4^+ input and a reduced nitrification rate after acidification in aquatic and terrestrial heathland vegetation and some type of forests, leads to inhibition of K^+ and Mg^{2+} uptake. This can cause severe nutrient deficiencies and plant damage. On the contrary, the successful plants in nitrogen enriched, acidified environments like *Juncus bulbosus* are able to take up base cations such as K^+ from solutions containing high concentrations of ammonium.

5. Mycorrhizal infection of roots (pine seedlings) can reduce the efflux of base cations in soil solutions rich in ammonium. However, the growth of the mycorrhiza forming fungi is strongly inhibited in soil solutions rich in ammonium.

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SAMENVATTING.

In dit proefschrift worden drie onderzoeksitems besproken, welke alle te maken hebben met milieuveranderingen onder invloed van menselijk handelen. Het eerste onderzoek heeft betrekking op de gevolgen van de aanvoer van gebiedsvreemd, alkalisch IJsselmeerwater op de waterkwaliteit en vegetatieontwikkeling in laagveenplassen in Noord-west Overijssel. Het tweede onderzoek handelt over de effecten van waterverzuring op waterplantengemeenschappen in zwak-gebufferde oppervlaktewateren, terwijl het derde onderzoek de effecten van verzurende en stikstofhoudende neerslag op bosoecosystemen beschrijft.

In alle drie de onderzoeken is dezelfde onderzoeksstrategie gevolgd, te weten:

1. Een uitgebreid of intensief veldonderzoek naar de verspreiding en/of ontwikkeling van de betreffende plantesoorten is uitgevoerd in relatie tot een groot aantal milieufactoren, teneinde een werkhypothese te formuleren.
2. Na formuleren van een werkhypothese werden oecofysiologische experimenten met een geselecteerd aantal plantesoorten en abiotische parameters uitgevoerd om causale verbanden te kunnen onderscheiden van indirecte relaties.
3. Vervolgens werden cultuurexperimenten en/of experimenten in kleinschalige oecosystemen uitgevoerd om een betere vertaling te krijgen van laboratoriumonderzoek naar de veldsituatie en omgekeerd.

Na het veldonderzoek kon voor alle drie de onderzoeksitems een werkhypothese opgesteld worden. De oecofysiologische experimenten leverden informatie op met betrekking tot causale verbanden tussen plantenontwikkeling en milieufactoren. Uit de cultuurexperimenten in het laboratorium of in vijvers bleek, dat de resultaten uit het veldonderzoek en de oecofysiologische experimenten gereproduceerd konden worden door een of meer belangrijke milieufactoren te veranderen.

Het veldonderzoek in natuurreservaat "De Weerribben" toonde aan dat waterinlaat uit het IJsselmeer waarschijnlijk niet tot directe eutrofiëring van de oppervlaktewateren leidde, daar IJsselmeerwater weinig nutriënten bevat. Het Rijntype water uit het IJsselmeer is echter zeer alkalisch en bevat veel bicarbonaat en sulfaat (hoofdstuk 2). Een vergelijkend onderzoek tussen een water waarin in droge perioden water uit het IJsselmeer ingelaten wordt en een identiek, geisoleerd water, toonde aan dat met name in de weke bovenste bodemlaag de mobiliteit van fosfor in het niet geisoleerde water veel hoger was dan in het geisoleerde water, evenals de concentraties aan gereduceerde verbindingen als H_2S, CH_4 en NH_4^+.
Uit transplantatie-experimenten met *Stratiotes aloides* L. (Krabbescheer) planten vanuit het geisoleerde naar het open systeem werd duidelijk dat de planten niet in de zomer, maar in de winter begonnen af te sterven, toen ze gemigreerd waren naar de weke, bovenste sapropeliumlaag.

Oecofysiologische en cultuurexperimenten toonden aan dat de ammonium- en sulfideconcentraties in deze laag lethaal waren voor *Stratiotes* planten.

In hoofdstuk 2 is besproken, dat de inlaat van alkalisch Rijntype water dat veel bicarbonaat en sulfaat bevat leidt tot interne alkalinisering, eutrofiëring en een hoge productie van methaan, sulfide en ammonium. De bovenste, venige bodemlaag wordt week en als gevolg van gasborreling en bioturbatie wordt het water min of meer troebel. Waarschijnlijk zijn niet alleen de toxische verbindingen van belang, maar ook het feit dat in de winter en in de lente de *Stratiotes* planten bedekt worden met fijn detritus, dat, in combinatie met de grotere troebelheid van het water, een negatief effect heeft op de fotosynthese en dus op de plantenontwikkeling.
Dit proces van interne alkalinisering, eutrofiëring en productie van toxische stoffen kon ook gereproduceerd worden in kunstmatige, kleinschalige aquatische oecosystemen (betonnen bakken van 700 l.). Bicarbonaat en sulfaat bleken inderdaad verantwoordelijk te zijn voor de geobserveerde veranderingen in de waterkwaliteit.

Ook de veranderingen in de anorganische koolstofhuishouding van het water als gevolg van de alkaliniseringsprocessen hebben een negatief effect op de groei van *Stratiotes* planten. Opvallend is, dat ook in verzurende heidevennen de veranderingen in stikstof- en koolstofhuishouding van het water de hoofdoorzaken bleken te zijn voor het verdwijnen van vele plantesoorten van normaliter zwak gebufferde oligotrofe wateren (hoofdstuk 3 en 4). De hoge atmosferische depositie van NH_x, in combinatie met de verminderde nitrificatie in verzurende wateren, leidt tot een verhoogde NH_4^+/NO_3^- ratio in de waterlaag. Hetzelfde verschijnsel is geobserveerd in heidemilieus. Veel bedreigde plantesoorten van heidemilieus, zoals *Thymus serpyllum* L. (wilde tijm), *Polygala serpyllifolia* Hose (liggende vleugeltjesbloem), *Pedicularis sylvatica* L. (heidekartelblad) en *Lycopodium inundatum* L. (moeraswolfsklauw) komen normaal voor op plaatsen met lage ammoniumconcentraties in het bodemwater. Na verzuring neemt ook in deze milieus de NH_4^+/NO_3^- ratio toe als gevolg van verminderde nitrificatie en een hoge depositie van ammonium uit de atmosfeer, met als gevolg verdwijnen van deze plantesoorten.

Uit laboratoriumexperimenten bleek, dat niet de toename of afname van de pH van water of bodemwater verantwoordelijk is voor de veranderingen in vegetatie en het verdwijnen van bepaalde plantesoorten, maar veel meer de daarmee gepaard gaande veranderingen in anorganische koolstof- en stikstofhuishouding of de accumulatie van toxische verbindingen. Verzuring van meren leidt, op zijn minst tijdelijk, tot een toename van de CO_2-concentratie in de waterlaag. Plantesoorten als *Juncus bulbosus* L. (knolrus) en het veenmos *Sphagnum cuspidatum* Hoffm. profiteren hiervan. Wanneer ook de ammoniumconcentratie relatief hoog is resulteert dit in een hoge productie van deze plantesoorten en een verdringing van vele plantesoorten van oligotrofe, zacht water oecosystemen (hoofdstuk 3 en 4).

Gedurende alkalinisering in veengebieden daalt het CO_2 niveau in de waterlaag als gevolg van de pH verhoging. Op productieve plantesoorten als *Stratiotes aloides* heeft dit een negatief effect omdat zij voornamelijk CO_2 gebruiken voor de fotosynthese. Plantesoorten welke efficiënter HCO_3^- kunnen benutten , zoals *Ceratophyllum demersum* L. (gedoornd hoornblad), of planktonische algen, profiteren van deze situatie.

Schuurkes verrichtte in 1986 onderzoek naar de stikstofvoeding van planten zowel uit zwakgebufferde als uit zure of verzuurde milieus. Hij vond dat de eerste groep planten bij voorkeur nitraat opnam uit een voedingsmedium dat ammoniumnitraat bevatte, terwijl de tweede groep planten bij voorkeur ammonium opnam.
Uit resultaten van behandelingen met kunstmatig regenwater in kleinschalige oecosystemen in een kas bleek duidelijk dat waterverzuring als gevolg van zwavelzuur bevattende regen niet tot een weelderige groei van *Juncus bulbosus* leidde. Waterverzuring veroorzaakt door ammoniumsulfaat bevattende regen leidde echter wel tot massale ontwikkeling van *Juncus bulbosus* en een complete verdringing van isoetide plantesoorten van oligotrofe, zwakgebufferde milieu's.

Dit stemde goed overeen met de resultaten van het veldonderzoek (hoofdstuk 3). In verzuurde wateren met een weelderige groei van *Juncus bulbosus* was ammonium de belangrijkste stikstofbron, terwijl in wateren die gedomineerd werden door de zachtwater plant *Littorella uniflora* (L.) Aschers. (oeverkruid) nitraat de belangrijkste stikstofbron was. Oecofysiologische experimenten met *Stratiotes aloides, Littorella uniflora, Juncus bulbosus* en *Thymus serpyllum* toonden aan dat planten, welke normaal voorkomen in milieu's waarin nitraat de belangrijkste stikstofbron is, problemen hebben met de opname van basische kationen als kalium en magnesium in situaties met afnemende nitraat- en toenemende ammoniumconcentraties. *Juncus bulbosus*, de succesvolle plantesoort in ammonium gedomineerde verzuurde milieu's, heeft geen problemen met de opname van kalium uit voedingsmedia met veel ammonium (hoofdstuk 2, 3 en 4).

Hetzelfde verschijnsel lijkt verantwoordelijk te zijn voor tenminste een deel van de bossterfte in gebieden met hoge ammoniumdepositieniveau's. In Nederland is dit het geval in het gehele land, met uitzondering van de kuststreek, waar het ammonium depositieniveau relatief laag is. Met name in zure bosbodems met een lage nitrificatiesnelheid leidt de depositie van ammonium tot een verstoorde mineralenbalans. De verhoogde NH_4^+/K^+ en NH_4^+/Mg^{2+} ratio's leiden ook in naaldbomen tot een geremde opname van basische kationen (hoofdstuk 6, 7, 8). Dit resulteert vaak in een verstoring van de mineralenbalans in de bomen (hoofdstuk 6, 7, 8).
In deze situaties kunnen de bomen lijden aan ernstig nutriëntengebrek of een verhoogde gevoeligheid vertonen voor droogte, vorst, insecten of schimmelziekten als *Sphaeropsis sapinea* (Fr.) Dyko et Sutton en *Brunchorstia pinea* (Karst) Honel (hoofdstuk 6).
Sphaeropsis sapinea veroorzaakt veel schade in *Pinus nigra* var. *maritima* (Ait.) Melville (corsicaanse den) en *Pinus sylvestris* L. (grove den) opstanden in zwaar

verontreinigde gebieden als de Peel. De aantasting door deze schimmel bleek zeer significant gecorreleerd te zijn met het stikstofniveau in het planteweefsel (hoofdstuk 6) en tevens met de stikstofconcentratie in de bodem.

De kennis met betrekking tot het effect van hoge ammoniumconcentraties op de kalium- en magnesiumvoeding is niet nieuw. Mulder (1956) en Jacob (1958) vonden reeds dat hoge ammoniumconcentraties in landbouwgronden de magnesiumopname in landbouwgewassen afremden, wat leidde tot emstig magnesiumgebrek in zowel gewas als dier.

In verzuurde bosbodems kan de remming van de magnesiumopname ook veroorzaakt worden door aluminium (hoofdstuk 6, 8). In de Nederlandse situatie, met een hoge depositie van ammoniumsulfaat en een relatief lage input van protonen, komen emstige bodemverzuring en het daarmee gepaard gaande lekken van calcium en het oplossen van aluminium in de bovenste, bewortelde bodemlaag voornamelijk voor in bodems met een matig tot hoge nitrificatiesnelheid (hoofdstuk 6).

Dus, zowel in bodems met een lage als met een hoge nitrificatie kan een hoge ammoniumdepositie magnesiumgebrek in de bomen veroorzaken.

Hoofdconclusies.

1. Verlaging van het (grond)waterniveau stimuleert het verzuringsproces. Compensatie voor het waterverlies in oppervlaktewateren in veengebieden door inlaat van alkalisch water met veel bicarbonaat en sulfaat leidt tot interne alkalinisering, eutrofiëring en verhoogde concentraties van gereduceerde verbindingen als CH_4, H_2S/HS^- en NH_4^+/NH_3. De hoge concentraties aan sulfiden en ammonium in de weke bovenste bodemlaag kan lethaal zijn voor *Stratiotes aloides* L. planten.

2. Verzuring van zachtwateroecosystemen als gevolg van stikstofbevattende, verzurende depositie leidt tot een, op zijn minst tijdelijke, sterke ontwikkeling van *Juncus bulbosus* L. en ondergedoken *Sphagnum* soorten welke isoetide plantesoorten als *Littorella uniflora* (L.) Aschers. en *Lobelia dortmanna* L.(waterlobelia) verdringen.

3. Zowel verzuring van zachtwateroecosystemen als alkalinisering van oppervlaktewateren in laagveengebieden leidt tot veranderingen in de vegetatie welke, op zijn minst gedeeltelijk, te wijten zijn aan veranderingen in de koolstof- en stikstofhuishouding van deze wateren.

4. De verandering in stikstofvorm, d.w.z. de toename van de NH_4^+/NO_3^- ratio gedurende alkalinisering in veengebieden en ammoniumdepositie en een verminderde nitrificatiesnelheid na verzuring in aquatische en terrestrische vegetaties en sommige bostypen, leidt tot remming van de kalium- en magnesiumopname. Dit kan emstig

nutriëntengebrek en beschadiging van planten veroorzaken. Aan de andere kant zijn de succesvolle plantesoorten in stikstofverrijkte, verzuurde milieus, zoals *Juncus bulbosus* L., in staat basische kationen zoals kalium op te nemen uit oplossingen die hoge concentraties ammonium bevatten.

5. Vorming van mycorrhiza's in wortels (denne-zaailingen) kan de efflux van basische kationen in bodemwater met veel ammonium verminderen. De groei van mycorrhiza-vormende schimmels wordt echter sterk afgeremd in bodemwater met veel ammonium.

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CURRICULUM VITAE.

J.G.M. Roelofs werd geboren op 17 juni 1950 te Venlo. Van 1962 tot 1966 doorliep hij de Lagere en van 1966 tot 1969 de Middelbare Tuinbouwschool te Venlo. Na een jaar werkzaam te zijn geweest op een bloemenkwekerij volgde hij van 1970 tot en met 1973 een chemische opleiding op HBO-niveau aan de Hogeschool Venlo.

Begin 1974 trad hij in dienst bij het Laboratorium voor Aquatische Oecologie van de Katholieke Universiteit te Nijmegen. Hij werkte hier eerst als analist; vanaf 1978 voerde hij zelfstandig onderzoek uit naar relaties tussen waterplanten en waterkwaliteit.

Dit onderzoek, met name de aspecten waterverzuring en effecten van de landbouw op waterkwaliteit, trok de aandacht van de ministeries van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer en Landbouw, Natuurbeheer en Visserij. Vanaf eind 1982 is hij projectleider van het verzuringsonderzoek en het onderzoek naar de relaties tussen waterplanten en waterkwaliteit. Aan deze, door externe opdrachtgevers gefinancierde, onderzoeken werken gemiddeld ca. 8 wetenschappelijke onderzoekers.

Vanaf 1989 is hij voorzitter van twee deskundigenteams: oppervlaktewateren en heide- en schraallanden. Deze adviseren het departement Natuurbeheer van het ministerie van Landbouw, Natuurbeheer en Visserij omtrent effectgerichte maatregelen tegen verzuring en eutrofiëring. Tevens is hij leider van een projectgroep, bestaande uit 5 wetenschappelijk onderzoekers, welke in opdracht van de directie Natuur-, Milieu- en Faunabeheer van het ministerie van Landbouw, Natuurbeheer en Visserij onderzoek verricht naar effectgerichte maatregelen tegen verzuring en eutrofiëring.

Hij is coördinator van het onderdeel vegetatie van een internationaal onderzoek naar effecten van stikstofverzadiging (Nitrex). Aan dit onderzoek, gefinancierd door nationale overheden en de E.G., nemen 11 instituten uit 7 landen deel.

Verder maakt hij deel uit van een internationale commissie, welke zich bezighoudt met het vaststellen van kritische depositieniveaus van verzurende en eutrofiërende stoffen.

Naast het onderzoek verzorgt hij al vele jaren onderwijs. In Nijmegen doceert hij Aquatische Oecologie en Oecofysiologie. Aan het International Institute of Hydraulic Engineering te Delft doceert hij het onderwerp Acidification of the Environment in de internationale Post Doc. cursus Applied Ecology. Aan diverse binnenlandse en buitenlandse universiteiten geeft hij regelmatig gastcolleges, o.a.: T.U. Delft, L.U. Wageningen, Universität Oldenburg, Justus Liebig Universität Giessen en de Universität Basel.

In april 1989 is hij aangesteld als Universitair Docent aan de Katholieke Universiteit te Nijmegen.