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Sediment-related growth limitation of *Elodea nuttallii* as indicated by a fertilization experiment

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SUMMARY

1. A fertilization experiment was performed to identify the limiting nutrient for the growth of submerged vegetation in ditches of a peat-grassland system in the Netherlands, in which restoration measures involved ceasing fertilization, exporting nutrients by removal of above-ground plant mass and large-scale introduction of calcium-rich, nutrient-poor artesian water.
2. Growth of *Elodea* was significantly enhanced by enrichment with nitrogen alone, and by fertilization with nitrogen in combination with phosphorus, and by nitrogen in combination with phosphorus and potassium.
3. Plant tissue nutrient concentrations increased significantly, for nitrogen by enrichment with nitrogen alone, and with nitrogen in combination with phosphorus and potassium; for phosphorus by enrichment with phosphorus alone and with phosphorus in combination with nitrogen and potassium; tissue concentrations of potassium were not enhanced by any treatment.
4. The elemental ratios of treated plants indicated that nitrogen, rather than phosphorus, was limiting in all treatments, except in those involving nitrogen and NK enrichment (when phosphorus was limiting).
5. The efficiency with which plants used nutrients declined with increased supply of nitrogen and phosphorus, but was unchanged when potassium was increased. Efficiencies were similar to those of other aquatic macrophytes.

Introduction

It has long been recognized that the nature of bottom sediments influences the growth of rooted macrophytes (reviews by Sculthorpe, 1967 and Hutchinson, 1975). It remains difficult to predict or manage the growth of submerged plants, however, because the sediment and plant characteristics governing the growth of particular species are imperfectly understood.

Many submerged macrophytes take up nutrients via roots and shoots (Sytsma & Anderson, 1993b). The degree to which either source is used depends on the nature of the element (Barko, 1982) and on nutrient concentrations in the interstitial and ambient water (Carignan & Kalff, 1980). Most evidence indicates, however, that the sediment is the major nutrient source (Denny, 1972, 1980; Barko & Smart, 1981; Smart & Barko, 1985).

In the centre of the Netherlands a project is in progress to restore former species diversity in the 'De Veenkampen' peat-grassland by reduction of the availability of nutrients in the soil. These measures consist of ceasing fertilization, exporting nutrients by removing above-ground plant mass twice a year, and by hydrological manipulation. The latter involves raising the water table in the ditches which crisscross the area and by subsurface irrigation (via pipes at a depth of 0.7 m below the soil surface). The irrigation water was from two artesian wells and had a low concentration of solutes but was rich in Ca^{2+} ions. This hydrological manipulation of the system is intended to

Table 1 Chemical characteristics of sediment and surface water in ditches within a peat–grassland system, after seven years of treatment with nutrient-poor, Ca^{2+}-rich artesian water. Samples were collected at sites from which *E. nuttallii* had disappeared

	Sediment		Water	
Characteristic	Mean	SD	Mean	SD
Nutrients				
Total organic carbon	285 g kg^{-1}*	149	6.21***	3.72
Bicarbonate	—		96.33***	3.08
Total nitrogen	16.46 g kg^{-1}*	8.42	—	
NO_3–N	0.31 mg kg^{-1}*	0.46	0.003*****	0.003
NH_4–N	22.70 mg kg^{-1}*	14.55	0.06****	0.04
Phosphorus	0.45 g kg^{-1}*	0.14		
Water extr. phosphorus	4.32 mg kg^{-1}*	4.28		
Desorbable phosphorus	11.81 mg kg^{-1}*	6.54		
o-Phosphorus			0.13****	0.12
Sodium	0.26 g kg^{-1}**	0.05	11.9*****	
Potassium	0.49 g kg^{-1}*	0.36	0.18****	0.15
NH_4OAc extr. potassium	0.06 mg kg^{-1}*	0.03		
Calcium	26.17 g kg^{-1}*	13.86	32.36****	10.20
NH_4OAc extr. calcium	16.62 mg kg^{-1}*	6.79		
Magnesium	4.05 g kg^{-1}**	0.85	0.48****	0.15
Iron	1.93 g kg^{-1}**	0.36	0.21*****	
Aluminium	2.72 g kg^{-1}**	0.37		
pH	6.54*	0.08	8.01****	0.13
Density (g DW ml^{-1})	0.32*	0.15		
Moisture	773 g kg^{-1}*	132		
Organic matter	521 g kg^{-1}*	245		

Water parameters in mg l^{-1}. Mean values and standard deviations. Extr., extractable.
* Annual average field cores (seven dates, $n = 5$); ** Incidental cores (one date, $n = 5$).
*** Annual average field samples (10 dates, $n = 2$). **** Water used for the experiment (three dates, $n = 2$). ***** One sample (July, 1992; D. van der Hoek, unpublished).

reduce the availability of nitrogen (by reducing the nitrogen content and the decomposition rate) and phosphate (via the presence of calcium) in the artesian water. These changes were expected to help reduce the primary production of terrestrial and aquatic vegetation and enhance the colonization of new, less competitive, species (Wisheu & Keddy, 1989; Gough & Marrs, 1990).

The production of the terrestrial vegetation in the hydrologically manipulated system has slowly declined. Potassium probably limited production 2–4 years after hydrological manipulation had started (Best, Oomes & Berendse, 1992). Biomass of aquatic vegetation in the hydrologically manipulated system was far lower than in the immediate surroundings 3–7 years after manipulation began. The submerged vegetation in the ditches was dominated by *Chara globularis* Thuill. close to the artesian wells. It was composed largely of narrow-leaved Potamogetonaceae (*Potamogeton acutifolius* Link, *P. crispus* L., *P. obtusifolius* Mert. et Koch, *P. pectinatus* L. *P. pusillus* L., *P. trichoides* Cham. et Schld.) and some *Elodea nuttallii* (Planch.) St John at sites further away from the wells. At the margins of the restored area, in ditches still more remote from the wells, submerged macrophytes disappeared and only some filamentous algae remained. In ditches surrounding the manipulated system, Lemnaceae (mainly *Lemna gibba* L., *L. minor* L. and *Spirodela polyrhiza* (L.) Schleiden) grew abundantly often alternating with Callitrichaceae (largely *Callitriche platycarpa* Kutz.) and *Potamogeton crispus*, *P. pusillus* and *P. trichoides*. As the objective of the project was to restore species diversity to that observed before severe impact by agriculture (Pas, 1975), the occurrence of 'bare areas' (i.e. those with only filamentous algae) was of concern. Therefore, a fertilization experiment was performed in the laboratory to investigate whether the growth of the submerged vegetation was being limited by one or more nutrients on these bare areas. The experiment was carried out under circumstances

mimicking field conditions. *Elodea nuttallii* (hereafter called *Elodea* in the text) seemed a suitable test plant, as this species had previously been common in sites now devoid of vegetation.

Materials and methods

Sediment collection

Cores of sediment were obtained from Veenkampen ditch sites from which *Elodea* had disappeared, using a manual core sampler containing a Perspex tube 40 cm long and of 4.5 cm inner diameter. After coring, the contents of the tube were slowly expelled; the portion below 10 cm soil depth was discarded and the portion above 10 cm soil depth was transferred with as little disturbance as possible into 15 cm high, 4.5 cm inner diameter Perspex tubes. Depth was measured from the soil surface. The bottom ends of the cores were capped tightly. The upper surfaces of the cores were covered by a 1-cm layer of nutrient-free sand. This prevented the direct exchange of the injected fertilizer solution with the surface water, which was checked by similarly injecting a Sudan Blue solution into the sediments and subsequently measuring the colour of the surface water over a period of 12 h. Sediment cores were equilibrated for 1 week submerged in fresh Veenkampen water at 15 °C, prior to fertilization. The soil contents of the cores were determined at the beginning and at the end of the incubation period. Each core contained a mean dry weight of 45.71 g (SD 8.01) of soil.

Experimental environment and procedures

The fertilization experiment was conducted in a greenhouse. Ambient irradiance (March–April, 1993) was slightly reduced by the glass roof the greenhouse and the glass lids of the aquaria in which the sediment cores were incubated. Water temperature was maintained at 15 ± 2 °C. Solution composition is given in Table 1. Bicarbonate was added as $(NaH)CO_3$ to achieve a final concentration of 200 mg HCO_3^- l^{-1} to prevent growth being limited by inorganic carbon due to the relatively small volume of water. The HCO_3^- concentration was never lower than 152.8 ± 9.5 mg l^{-1}. The water in the aquarium was aerated to ensure mixing and sufficient carbon supply.

The fertilization treatments followed a factorial design in blocks, in which all combinations of nitrogen, phosphorus and potassium at natural and enriched level were included. The experiment involved seven treatments and a control, all replicated twelve times. Each block consisted of a 34.5 l aquarium (effective volume 30.22 l), with one replicate of each treatment and one control. The plant growth response (increase in ash-free dry weight, AFDW) and the tissue concentrations of nitrogen, phosphorus and potassium were measured at the beginning and at the end of a 56-day cultivation period.

The concentrations of each fertilizer solution were chosen such that injecting 0.3 ml per core would enhance each fraction of the elemental concentration thought to be most important for plants in the interstitial water by 0.380 mg ml^{-1}. The fractions thought to be most important to plants of the elements added were: for nitrogen, NO_3^- and NH_4^+; for phosphorus, the $H_2PO_4^-$ fraction extractable by deionized water; for potassium, the potassium fraction extractable by 1 N NH_4OAc solution. The sediments of each treated core were fertilized by injecting fertilizer in solution into the core using a syringe. The syringe was fitted with a 10-cm needle, which allowed the solution to be injected below the oxidized zone of the sediment. The fertilizer solution was bubbled with N_2 gas for 15 min prior to injection to extrude oxygen and thus prevent as much as possible disturbance of the redox potential in the cores. The oxygen concentration in the fertilizer solution was checked before adding the nutrient solutions: it varied between 0.6 and 1.0 p.p.m. oxygen. The nutrient solutions were added as follows: nitrogen (180 g NH_4Cl l^{-1}), phosphorus (210 g $NaH_2PO_4.2H_2O$ l^{-1}) and potassium (90 g KCl l^{-1}).

The cores were fertilized in mid-February and they were equilibrated for 1 week (submerged in fresh, aerated, Veenkampen water). Six sediment cores were then harvested to allow the initial soil nutrient concentrations in sediment water to be determined.

In each of the remaining sediment cores three 10 cm long, preweighed, *Elodea* shoots were planted 4 cm deep. Plant material composed by an equal number of shoots of approximately the same weight as those planted were harvested and their initial fresh and dry mass and nutrient contents were determined. Initial plant mass per core was 0.093 g ash-free dry weight (SD 0.015). *Elodea* was obtained from an outdoor pond of the institute, and had been cultivated previously

for 5 weeks in nutrient-rich sediment and bicarbonate-enriched Veenkampen water, changed every 2 weeks.

The planted sediment cores were incubated for 56 days, during which the Veenkampen water was changed and enriched with HCO_3^- three times. At the end of the incubation period all sediment cores and plants were harvested. Water chemistry was determined at the beginning and at the end of each time the water was changed.

Sediment, plant tissue and water analysis

The contents of each core were mixed. The sediments were analysed for NO_3-N and NH_4^+-N, water extractable-phosphorus, and for NH_4OAc extractable potassium as measures of plant-available macronutrients. The total contents of nitrogen, phosphorus, potassium, organic matter, water and pH were measured. Each analysis was duplicated.

The NH_4^+ and NO_3^- concentrations of the soil were determined by extracting 40 g fresh soil with 100 ml 1 N KCl within 24 h of being collected. The concentrations were then measured colorimetrically (respectively, Nanocolor Test 05 and Nanocolor Test 63). Water extractable-phosphorus was determined by successively suspending a volume of 1.2 ml of air-dried soil in 2 ml deionized water at 20 °C for 22 h and then extracting in 70 ml Veenkampen water by shaking for 1 h (Houba *et al*., 1989). The phosphate concentration was measured colorimetrically as phospho-molybdate complex using a Technicon autoanalyser. The NH_4OAc extractable-potassium concentrations of the soil were determined by extracting 0.5 g dry soil with 10 ml 1 N NH_4OAc pH 7.0 for 15 min under rotation, followed by centrifugation (four repetitions), pooling of the supernatants and dilution with the extractant to a final volume of 50 ml (Liu & Bates, 1990). The potassium concentrations in the extracts were measured by atomic absorption spectrometry. Total nitrogen concentration of the soil was measured using a Carlo Erba Elemental Analyser. Total phosphorus concentration was determined by digesting 0.5 g dry soil with 10 ml of a 1 : 1 mixture of concentrated H_2SO_4 and HNO_3. The phosphate concentration was measured as described above. Total potassium concentration of the soil was determined by digesting 2 g dry soil with 15 ml concentrated HNO_3 at 130 °C for 1 h. The solution was evaporated by heating and the residue was extracted twice with 2 ml concentrated HCl and then evaporated. The residue was dissolved in 25 ml 1 N HCl overnight. The solution was amended with 15 ml of a 0.7% (v/v) trichloro-acetic acid in 3% (v/v) HCl mixture and 0.5 ml 1.5 N $BaCl_2$ and final extraction lasted 3 h. Potassium was measured using atomic absorption spectrometry (method Chemical Department AB-DLO). Water content of the soil was determined gravimetrically (drying to constant weight at 105 °C). Ash was determined as in the plant material. The pH was measured in 1 M KCl solution in a soil–liquid ratio of 1 : 2.5 (w/v). The pH_{KCl} was converted to pH_{water} using a regression formula of $pH_{water} = (0.677 \times pH_{KCl}) + 2.35$ (R.H.Kemmers, Winand Staring Centre, Wageningen, the Netherlands, personal communication).

The fresh and dry weight of the plant material, roots included, and its ash and macronutrient contents were measured. The harvested material was rinsed with tap water to remove soil particles and most of the periphyton. The material was weighed after drying for 24 h at 105 °C. It was subsequently ground and the ash and nutrient concentrations were measured. Ash was determined by loss on ignition at 550 °C for 1.5 h. Nitrogen concentrations were determined using a Carlo Erba Elemental Analyser. Phosphorus was determined colorimetrically in H_2SO_4/HNO_3 digests using a modified molybdate method. Potassium was determined in 16 N HNO_3 digests using atomic absorption spectrometry (method Chemical Department AB-DLO).

The chemical composition of the surface water was determined as follows. Organic carbon was measured in 50 ml freeze-dried samples using a mass spectrometer. pH and HCO_3^- were measured by titration with 0.1 N HCl (Metrohm 672 titroprocessor with dosimat). Water samples were subsequently filtered using 0.45 µm Millipore filters. NO_3^- and NH_4^+ were measured using a TRAACS autoanalyser and *o*-phosphate using a Technicon autoanalyser. Na^+, K^+, Ca^{2+} and Mg^{2+} were measured using high performance liquid chromatography (IC-Pak C M/D column; Waters 431 conductivity detector).

Data analysis

Analysis of variance (ANOVA; Statgraphic Plus Package, Version 6.0) was used to assess the sensitivity of the growth response and final nutrient concentrations of *Elodea* to fertilization treatment.

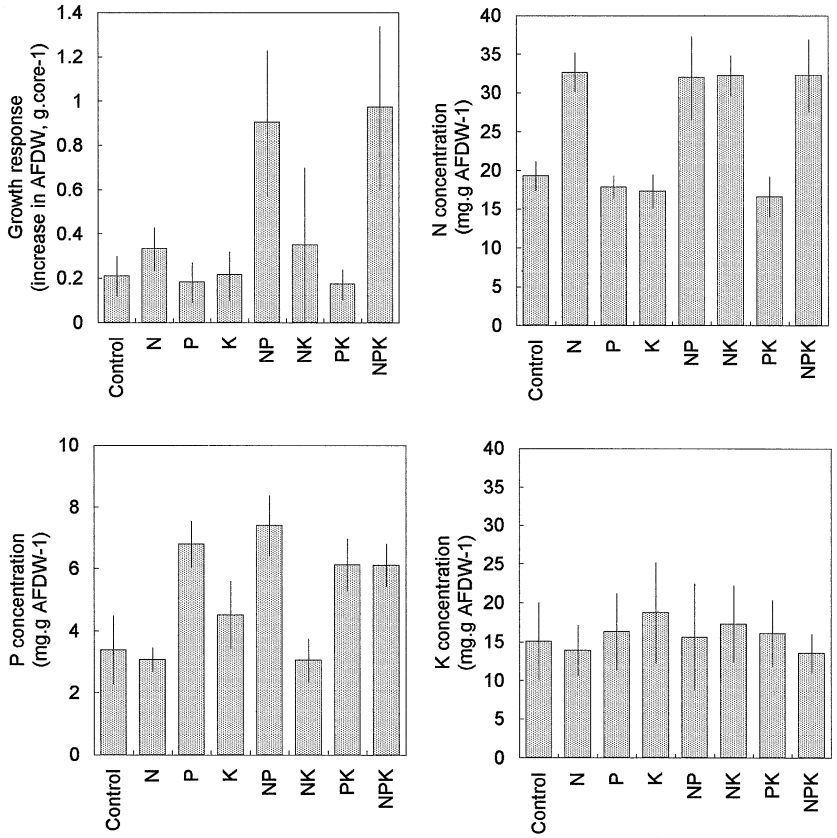

Fig. 1 Growth response and final tissue nutrient concentrations of *E. nuttallii* at the end of a 56-day cultivation period on sediment cores previously fertilized following a factorial design in blocks. Mean values and standard deviations.

The nutrient use efficiency of *Elodea* was estimated at low (tentatively nutrient-limiting) and at high nutrient supply for nitrogen, phosphorus and potassium. Nutrient use efficiency at low elemental supply was estimated from the slope of a regression of total plant biomass on the total elemental mass in the plants at the end of the cultivation period (Shaver & Melillo, 1984), that had not been fertilized with that element. For example, for nitrogen at low nitrogen supply, the biomass data of all treatments in which nitrogen had not been added were regressed on the total plant nitrogen mass. Nutrient use efficiency at high elemental supply, conversely, was estimated from the slope of a regression of total plant biomass on the total mass of that element in the plants, which had been fertilized with that element.

Results

Characterization of sediment and water of De Veenkampen

The Veenkampen sediment, collected in ditches from which *Elodea* had disappeared, consisted largely of peat (high organic matter content) and had a low density, but it also contained some clay (relatively low C concentration; Table 1). As to the macronutrients, the NH_4^+–N concentration was usually high and the NO_3^-–N, water extractable-phosphorus and NH_4OAc extractable-potassium concentrations low.

The basic water was relatively rich in *o*-phosphorus and K^+, but poor in HCO_3^- (*c.* 1.5 mM) and NO_3^+–N and NH_4^-–N (Table 1).

Growth response and nutrients in Elodea

The growth response of *Elodea* was stimulated most by fertilization with nitrogen, applied alone, in combination with phosphorus and in combination with phosphorus and potassium (Fig. 1; Table 2). Growth was not stimulated by phosphorus and/or potassium. The effects of single fertilization with nitrogen, combined fertilization with nitrogen and phosphorus, and combined fertilization with nitrogen, phosphorus and potassium on the growth response were significant (Table 2). The effect of nitrogen was larger than that of phosphorus and that of both elements combined

Table 2 Fertilization effects on growth response and final tissue nutrient concentrations of *E. nuttallii* after a 56-day cultivation period; multifactorial ANOVA with block as cofactor

Effect	d.f.	MS	*F*-ratio	*P*-value
Growth response				
N	1	0.085	7.804	0.010
P	1	0.006	0.723	0.413
K	1	< 0.001	0.002	0.964
NP	1	2.864	45.764	< 0.001
NK	1	0.116	7.140	0.014
PK	1	0.009	1.218	0.282
NPK	1	3.469	40.845	< 0.001
Tissue N concentration				
N	1	1064.663	182.827	< 0.001
P	1	13.290	3.893	0.061
K	1	24.265	5.044	0.035
NP	1	958.473	52.421	< 0.001
NK	1	995.906	164.449	< 0.001
PK	1	43.520	7.558	0.012
NPK	1	1002.952	68.392	< 0.001
Tissue P concentration				
N	1	2.275	11.365	0.002
P	1	57.279	131.112	< 0.001
K	1	4.034	6.777	0.016
NP	1	82.146	122.776	< 0.001
NK	1	2.414	6.215	0.021
PK	1	35.697	79.349	< 0.001
NPK	1	35.086	88.837	< 0.001
Tissue K concentration				
N	1	8.186	0.400	0.540
P	1	8.909	0.315	0.586
K	1	80.659	2.146	0.157
NP	1	1.830	0.050	0.827
NK	1	29.633	1.315	0.264
PK	1	6.342	0.284	0.605
NPK	1	14.450	0.810	0.387

d.f., degree of freedom; MS, mean square.

Table 3 N : P : K ratio in *E. nuttallii* mass at the beginning and end of a 56-day cultivation period. Mean values ($n = 6$ at the beginning, $n = 12$ at the end of the experiment)

Treatment	N:	P:	K
Initial	4.0	1	3.2
Control	5.7	1	4.5
N	10.6	1	4.5
P	2.6	1	2.4
K	3.8	1	4.2
N + P	4.3	1	2.1
N + K	10.5	1	5.6
P + K	2.7	1	2.6
N + P + K	5.3	1	2.2

showed a strong interaction. The effect of fertilization with potassium was not statistically significant.

Tissue nitrogen concentration was greatly enhanced by fertilization with nitrogen alone, and with nitrogen in any combination with phosphorus and potassium (Fig. 1; Table 2). The tissue phosphorus concentration increased after fertilization with phosphorus alone, and with phosphorus in any combination with nitrogen and potassium (Fig. 1; Table 2). The potassium concentration was less affected by fertilization than either the nitrogen or the phosphorus concentrations (Fig. 1). None of the latter effects was significant (Table 2).

The nutrient use efficiencies of *Elodea* were calculated using the regression coefficients of the lines fitted to the plant biomass and tissue nutrient concentration data (Fig. 2). They were, at low nutrient supply, 0.05 g AFDW mg N^{-1}, 0.20 g AFDW mg P^{-1} and 0.04 g AFDW mg K^{-1}, and, at high nutrient supply, 0.03 g AFDW mg N^{-1}, 0.15 g AFDW mg P^{-1} and 0.06 g AFDW mg K^{-1}.

The elemental ratios in *Elodea* at the beginning and end of the experiment are presented in Table 3. The nitrogen–phosphorus ratios range from 2.6 to 10.6 and the potassium–phosphorus ratios from 2.1 to 5.6.

Nutrient concentrations in the sediment

Nutrient concentrations were determined in the sediment cores at the beginning and end of the experiment, and in the water at each change of solution.

Fertilization with NH_4Cl resulted in a mean NH_4^+–N concentration of 171 μg g^{-1} DW (Table 4), about 45% of the target value of 329 μg N g^{-1} DW (recalculated from elemental concentration on volume basis; see Materials and methods). Fertilization with NaH_2PO_4 resulted in a mean water extractable-phosphorus concentration of 435 μg g^{-1} DW, about 132% of the target value of 329 μg water extractable-phosphorus g^{-1} DW. Fertilization with KCl resulted in a mean NH_4OAc extractable-potassium concentration of 433 μg g^{-1} DW, about 96% of the target value of 469 μg NH_4OAc extractable-potassium g^{-1} DW. In the case of fertilization with nitrogen, over 50% of the administered amount was not in the form of NO_3^- or NH_4^+. This may have been because of denitrification, or biological/chemical fixation to the soil. The latter phenomenon is most likely in view of

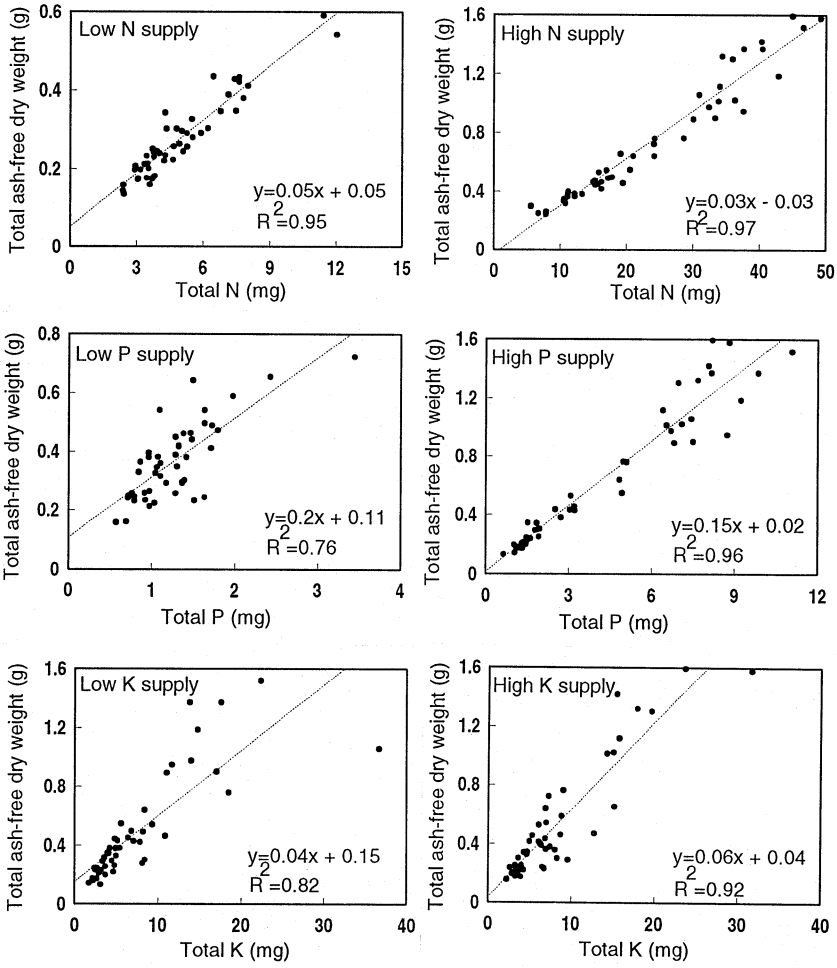

Fig. 2 Estimation of nutrient use efficiencies of *E. nuttallii* for N, P and K at low (left side) and at high (right side) nutrient supply by regression of total plant biomass (three plants) on N, P and K mass (three plants). Note different axes for high and low nutrient supplies in each case.

Table 4 Sediment nutrient concentrations on dry mass basis 1 week after fertilization, before planting *E. nuttallii*. Mean values and standard deviations (n = 6)

	Elemental concentration													
	t-N mg g^{-1}		NO_3–N µg g^{-1}		NH_4–N µg g^{-1}		t-P µg g^{-1}		Water extr. P µg g^{-1}		t-K µg g^{-1}		NH_4OAc extr. K µg g^{-1}	
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	15.0	1.8	3.0	0.8	17.3	9.0	0.665	0.087	20.0	8.1	1.403	0.311	168.3	15.7
N	21.1	5.2	3.9	1.4	171.2	14.1	0.537	0.158	11.7	3.7	1.078	0.327	195.0	59.4
P	18.0	2.1	4.1	0.9	18.3	6.4	1.598	0.322	435.0	190.9	1.207	0.223	118.3	16.7
K	16.6	2.4	4.2	0.8	17.5	5.6	0.742	0.091	20.0	5.8	1.597	0.358	433.3	241.0
NP	18.5	2.8	4.9	1.5	152.2	29.5	1.668	0.198	418.3	134.6	1.252	0.217	135.0	24.3
NK	18.7	2.6	3.8	1.0	99.5	29.1	0.527	0.084	10.0	5.8	1.535	0.275	925.0	288.0
PK	16.1	1.5	3.8	1.4	12.6	2.2	1.755	0.387	316.7	157.6	1.846	0.248	510.0	212.2
NPK	17.2	1.7	3.7	0.5	168.1	33.5	1.960	0.385	528.3	287.0	2.026	0.237	825.0	167.6

the greatly increased t-nitrogen concentration in the sediment. After fertilization with potassium alone or with KP, the NH_4OAc extractable-potassium concentrations in the sediment were usually similar to the target concentrations. They were, however, greatly enhanced by simultaneous fertilization with potassium

Table 5 Summary of the tentative growth limitation in *E. nuttallii* as indicated by various parameters measured in a fertilization experiment. ***, effect significant at $P < 0.01$

	Parameter									
		Plant-tissue nutrient concentration			Plant elemental ratio		Calculated elemental shortage			
Treatment	Plant growth response	N	P	K	N : $P < 7.8$	N : $P > 8.4$	N	P	K	Tentative limitation
Control					+		+			N
N	+***	+***				+				P
P			+***		+					N
K					+					N
NP	+***	+***	+***		+		+		+	N
NK		+***				+	+			P
PK			+***		+					N
NPK	+***	+***	+***		+		+			N

and nitrogen, possibly by the expulsion of K^+ ions attached to the clay particles and their replacement by the NH_4^+ ions.

Discussion

Concomitant assessment of sediment nutrient availability and the nutrient status of submerged macrophytes can elucidate the dynamics of macrophyte communities and nutrient cycling in aquatic ecosystems, thus providing information useful for devising strategies for managing aquatic plants.

Evidence that nitrogen can be a major sediment-related factor limiting growth

The present experiment yielded information on various aspects of nutrient limitation to the growth of submerged plants. The growth response of the test plant *Elodea* was stimulated appreciably by nitrogen, NP and NPK fertilization (Table 5). In several cases luxury consumption may have occurred, as indicated by the greatly enhanced tissue nutrient concentrations, notably nitrogen concentration after fertilization with nitrogen, NP, NK and NPK, and phosphorus concentration after fertilization with phosphorus, NP, PK and NPK. The reason that the nitrogen concentration was not statistically significant enhanced when nitrogen fertilization was combined with the addition of potassium may be that the K^+ ions interfered with the uptake of NH_4^+ ions; both ions having the same charge and similar mass and therefore having similar binding capacity to and mobility in the sediment.

Chambers & Fourqurean (1991) pointed out the danger of reliance upon a single criterion for the assessment of nutrient status. Elemental ratios may provide additional criteria to evaluate nutrient limitation of growth. Vascular plants require a balanced ratio of elements for optimal growth. Plants should show this same ratio of elements in their tissues, unless an element is limiting growth or an element is stored in excess of demand (luxury consumption; Chapin & Van Cleve, 1989). Therefore, elemental ratios can be useful indicators of relative deficiency when there are multiple deficiencies.

If critical elemental ratios published for other submerged macrophytes are universal, it can be expected that nitrogen–phosphorus ratios < 7.8 indicate that nitrogen was limiting growth in that plant material whereas ratios > 8.4 indicate that phosphorus was limiting (Sytsma & Anderson, 1993a). Following this line of reasoning, nitrogen should have been more limiting than phosphorus in all treatments except at nitrogen and NK fertilization, and phosphorus should have been limiting at nitrogen and NK fertilization. We know of no other published elemental ratios for potassium–phosphorus.

Nutrient-use efficiency is a function of nutrient availability, biomass allocation and growth rate (Chapin, 1980, 1988). A large supply of nutrients may boost growth and therefore relatively less biomass is produced per milligram of element absorbed (dilution effect). When only one element is abundant, however,

Table 6 Nutrient use efficiencies (g dry weight per mg nutrient accumulated) of emergent and submerged aquatic macrophytes. The data of the referenced studies are derived from Sytsma & Anderson, 1993. The data of this study have been recalculated from ash-free dry weight basis to dry weight basis, using the appropriate ash contents measured

	Low nutrient supply			High nutrient supply			
Species	N	P	K	N	P	K	Reference
Calamagrostis canadensis (Michaux) Beauvois	0.14–0.18	1.16–1.50		0.070–0.072	0.48–0.55		Shaver & Melillo, 1984
Typha latifolia L.	0.10–0.13	0.71–0.87		0.05–0.07	0.36–0.39		Shaver & Melillo, 1984
Hydrilla verticillata (L.f.)Caspary	0.05	0.65					Barko & Smart, 1986
Myriophyllum spicatum	0.04	0.20					Barko & Smart, 1986
Myriophyllum aquaticum (Vell.)Verdc.	0.13	0.59		0.09	0.36		Sytsma & Anderson, 1993
Elodea nuttallii	0.05	0.20	0.04	0.03	0.15	0.06	This study

others may limit growth and then the excess element may accumulate (luxury consumption). The nutrient-use efficiencies of *Elodea* for nitrogen and phosphorus reported in this study were in the same range as those published for other aquatic macrophytes (Table 6; Shaver & Melillo, 1984; Barko & Smart, 1986; Sytsma & Anderson, 1993a). They were for nitrogen and phosphorus 1.5 times higher at low than at high elemental supply. The response of *Elodea* to changes in nutrient supply was quantitatively similar to that of *Myriophyllum aquaticum* but was less than in *Calamagrostis canadensis* and *Typha latifolia*. The efficiency of use of potassium by *Elodea* was, contrary to expectations, lower at low than at high potassium supply (Fig. 2; Table 6). This discrepancy may stem from the rather poor fit of the line to the data, caused largely by two datapoints in the high range of the low potassium supply. Lines fitted to plant biomass data and plant-potassium concentrations, but excluding the latter datapoints, resulted in equal regression coefficients. The latter may indicate either that the K^+ supply was never below that required to sustain plant growth or that K^+ was available to a limited extent to the plant. Limited K^+ availability may occur in submerged macrophytes that are unable to absorb K^+ from the sediment and that therefore rely for their potassium uptake on the ambient water (where concentrations are usually low; Barko, 1982). If *Elodea* took up K^+ from the water column only, the growth response at any treatment, including potassium fertilization, should have been very small because it was permanently limited by potassium. The greatest growth response (at NPK fertilization), however, was 0.027 g g^{-1} AFDW day^{-1}, i.e. normal for summer conditions in the Netherlands (Best & Dassen, 1987; Best, unpublished). Thus it can be concluded that *Elodea* can take up K^+ from the sediment and that K^+ was not limiting growth.

Initial nutrient concentration in sediment and water and nutrient accumulation in *Elodea* during the 56-day cultivation period were calculated and compared (Table 7). This revealed that more nitrogen accumulated in the plant material in all treatments (except fertilization with PK) than expected if the initial NO_3^- and NH_4^+ concentrations in sediment and water indeed indicated the plant-available nitrogen pool. During the 56-day cultivation period, however, the sediment rich in organic matter probably mineralized and therefore more nitrogen should have been available. From Veenkampen field data a mineralization rate of at least 0.436 mg N $core^{-1}$ $period^{-1}$ at 15 °C was derived (Best, unpublished). Nevertheless, even if the nitrogen freed by mineralization supplemented the plant-available nitrogen pool, a shortage of nitrogen had to be expected in the plant material of the control treatment and after fertilization with NP, NK and NPK (Table 5). Accumulation of phosphorus in the plant material never exceeded the pool composed by water extractable-phosphorus of the sediment and *o*-phosphorus of the water. Potassium accumulated in the plant material to a greater extent at fertilization with NP than expected if NH_4OAc extractable-potassium of the sediment and K^+ of the water composed the plant-available potassium pool.

Those cases where the elemental ratio indicated a deficiency did not always coincide with those in which 'elemental shortage' was calculated. Reasons for this may be due to the following. First, the case

Table 7 Sediment nutrients (NO_3^-–N + NH_4^+–N; water extractable-P; NH_4OAc extractable-K), water nutrients (NO_3^-–N + NH_4^+–N; *o*-P; K^+) and nutrient accumulation in *E. nuttallii* at the end of a 56-day cultivation period. Values are means, calculated as products of, respectively, sediment mass and associated nutrient concentration ($n = 6$), water mass and associated nutrient concentration ($n = 3$), and as difference between the products of plant mass and associated nutrient concentrations at the beginning and end of the growth period. It was assumed that on average 12.5% of the volume of aquarium water was available per planted core

	Elemental mass (mg planted core^{-1})								
	N			P			K		
Treatment	Sediment initial	Water initial	Plants final–initial	Sediment initial	Water initial	Plants final–initial	Sediment initial	Water initial	Plants final–initial
Control	0.93	0.24	1.83	0.91	0.49	0.15	7.68	0.68	1.42
N	8.00	0.24	8.55	0.53	0.49	0.46	8.91	0.68	2.85
P	1.02	0.24	0.98	19.88	0.49	0.66	5.41	0.68	1.19
K	0.99	0.24	1.58	0.91	0.49	0.43	19.80	0.68	2.38
NP	7.18	0.24	26.88	19.12	0.49	6.17	6.17	0.68	12.49
NK	4.72	0.24	9.05	0.46	0.49	0.51	42.27	0.68	4.31
PK	0.75	0.24	0.60	14.47	0.49	0.48	23.30	0.68	0.99
NPK	7.85	0.24	29.40	24.14	0.49	5.44	37.70	0.68	11.52

where phosphorus limitation is indicated by the elemental ratio, but is not calculated as a shortage from the nutrient budgets, after nitrogen fertilization (Table 5). The mobility of the relatively large $H_2PO_4^-$ ion in the sediments' interstitial water may have been impeded by the large amount of small NH_4^+ ions, introduced by fertilization, leading to a lower uptake than potential. Secondly, the case where nitrogen limitation is indicated by the elemental ratio but is not calculated as a shortage from the nutrient budgets, after phosphorus, potassium and PK fertilization. Here, the possibility cannot be excluded that the nitrogen mineralization rates were lower than estimated. This is not likely, however, as the field data (E.P.H. Best, unpublished) originated from unplanted submerged cores of which the mineralization rate is expected to be somewhat lower than that of planted cores where the plant roots aerate part of the sediment. Another explanation could be that the plants could not absorb nitrogen below certain low critical concentrations. Thirdly, in the case where nitrogen limitation is indicated by the elemental ratio but is not calculated as a shortage from the nutrient budgets, after potassium fertilization. Here less nitrogen may have been taken up by the plant material than is potentially possible, because of the introduction of K^+ ions into the interstitial water and its inherent inhibiting effect on NH_4^+ mass flow in the interstitial water. High K^+ uptake due to the relatively high permeability of plant membranes for this ion may also have interfered with the NH_4^+ uptake (Pitman & Luttge, 1983). Fourthly, in the case where nitrogen limitation is indicated by the elemental ratio but is not calculated as a shortage from the nutrient budgets, after NK fertilization. Here more nitrogen may have been absorbed from the sediment by K^+ translocation from shoots to roots and the subsequent exchange of K^+ to NH_4^+ (the hypothesis of Barko *et al*., 1988). Another explanation could be, however, that more nitrogen was available for plant growth as a result of bacterial lysis in the sediment during the cultivation period and more scavenging from the water column occurred (> 12% of water volume). Finally, it cannot be excluded that the elemental ratio alone may be a poor indicator of growth limitation.

The data from this study on the nitrogen–phosphorus ratio indicate that nitrogen limits the growth of *Elodea* under unfertilized conditions and after fertilization with phosphorus, potassium, NP, PK and NPK, but that phosphorus limitation occurs after fertilization with nitrogen and NK. In this study, interactive effects between sediment density and nutrient uptake (Barko & Smart, 1986) do not play a part, as the experiment was performed on one type of sediment. Thus, at the 'De Veenkampen' field site the growth of submerged macrophytes

with a nutritional strategy similar to that of *Elodea* is limited by nitrogen, provided that no upward seepage and concomitant nutrient import occurs. The latter condition is fulfilled, as the *in situ* upward seepage is suppressed by the hydrological manipulation (Best, Van der Schaaf & Oomes, 1995).

Growth limitation in submerged aquatic macrophytes

Submerged macrophyte production in aquatic ecosystems largely depends on irradiance and carbon availability (Barko *et al*., 1988). As to growth-limiting elements for primary producers in general, so far the emphasis has been largely on phosphorus. In most of these studies other elements have not been considered. However, recently, the nutritional ecology of submerged macrophytes as a whole has been highlighted and nitrogen has been singled out as the prime candidate as a growth-limiting nutrient (Barko, Gunnison & Carpenter, 1991). If nitrogen availability is indeed a major growth-limiting factor, it may heavily influence production, species composition and succession in aquatic ecosystems because of the resource-based competition by the macrophytic species, just as in terrestrial systems (Tilman, 1982). So far, however, nitrogen limitation of growth of submerged macrophytes has been found in only a few cases (Anderson & Kalff, 1986; Anderson & Kalff, 1988; Barko *et al*., 1988; Sytsma & Anderson, 1993; this study).

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