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THE EFFECTS OF VARYING NITROGEN ON BRACKISH SUBMERSED AQUATICS UNDER VARYING LEVELS OF PHOSPHORUS IN SEDIMENTS

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FINAL REPORT

COASTAL RESOURCES DIVISION
TIDEWATER ADMINISTRATION
MARYLAND DEPARTMENT OF NATURAL RESOURCES

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PROJECT OFFICER

DECEMBER 1989

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BACKGROUND

Since the restoration of submersed aquatic vegetation (SAV) is one of the objectives in the current efforts to clean up Chesapeake Bay, it is important to target the water quality parameters necessary for its reestablishment. Previous studies during the EPA Bay Program established that the most probable causes of the Baywide decline during the 1970s included excess nutrient loadings and reduced light levels in the shallows (with herbicide damage sporadically exacerbating the problem in areas adjacent to agricultural fields). However, due to the ending of the phase I (i.e. research) of the EPA program, (in order to begin the implementation phase), little was accomplished in terms of establishing the actual water quality necessary to support SAV. This data gap was especially problematic in regard to the issue of whether nitrogen or phosphorus was more a problem in encouraging epiphytic overgrowth on SAV leaves, one of the main hypothesis' for the decline in the upper Bay.

In 1985 we began an intensive survey of sites in the Choptank River, where revegetation was re-occurring because of the low runoff patterns associated with reduced precipitation. (Low runoff produces decreased levels of nutrients, sediment and herbicides in the eastern shore tributaries of the bay because they are dominated by non-point source pollution, see Lomax and Stevenson 1982). Several conclusions emerged from an intensive field study of the Choptank River with supporting data from the Wye River and the Severn River. Perhaps most significant was the finding that areas supporting the regrowth of SAV during the growing seasons in 1985, 1986 and 1987 could be defined with a small number of key water quality parameters. Generally,

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at protected sites in the mesohaline lower reaches of the estuary (10-20 ppt salinity), regrowth of SAV was observed when mean ambient growing season (April - September) concentrations were less than values in Table 1:

Table 1. Water quality values associated with growth of submersed aquatic vegetation in the Choptank River between 1985 and 1989.

Chl *a*	15 $\mu g\ l^{-1}$
SESTON	20 $mg\ l^{-1}$
Light (Kd)	2.0 m^{-1}
DIN	0.14 $mg\ l^{-1}$
DIP	0.01 $mg\ l^{-1}$

Among the management values of these concentrations is that they might be used as water quality criteria which could be used to set standards for effluents of sewage treatment plants (STP) discharging into tidal waters or upstream tributaries as well as for targeting where non-point source cleanup is necessary. The Bay cleanup targets might be set (at least in part) by the water quality requirements of SAV. However, there is one limitation in a strictly field approach which needs to be resolved before these values gain broad acceptance by management agencies. That is the fact that these concentrations usually co-vary and effects of nitrogen (N) for example can not be readily distinguished from phosphorus (P). Thus we can not directly answer the question: what might happen if we just removed P from the STP and left the N at existing levels?

One approach which can be used to circumvent the covariance of field variables

is through the use of microcosm experiments.

Previous studies (Twilley et al. 1985; Staver 1984) used microcosm and mesocosm experiments to establish the link between N and P enrichment in the water column and reductions in SAV growth. However, they did not distinguish between the effects of nitrogen versus phosphorus enrichment. Since SAV are capable of obtaining nutrients from the sediment pool via their root system (Chen and Barko 1988), these effects could be partitioned out by making one available in the sediment while increasing the other in the water column.

Phosphorus is rapidly adsorbed onto sediment particles under field conditions, becoming entrained in the sediment. Therefore if P inputs to the estuary were reduced, water column concentrations could become very low, creating P limitation in species which cannot access the sediment pool. Thus, algal growth may be suppressed, even though N concentrations could remain very high. This is scenario which seems to exist in the Potomac River since the Blue Plains STP was upgraded to remove P in 1982, and at Havre de Grace, MD, where the water flowing in from the Susquehanna is low in P due to high sedimentation rates upstream behind Conawingo Dam. Whether or not similar conditions in brackish water would promote SAV growth is an open question, and the primary one which we have attempted to address here.

In the current study, mesocosms (large fiberglass tanks) were used to examine the effects on SAV growth of increasing nitrogen in the water column when phosphorus is available only in the sediment. The experiment was conducted at ambient salinity in the Choptank estuary at Horn Point, which was 7 ppt at the beginning of the study.

METHODS

The recirculating fiberglass tank system located in the Horn Point Environmental Laboratory greenhouse (described in detail in Stevenson and Staver 1989) was used to conduct this study. Each pair of tanks was filled with filtered (5 μm) Choptank River water and was maintained at 27 ± 2°C throughout the study. Salinity was maintained at 7 ppt by the addition of deionized water (to compensate for evaporation) at approximately two week intervals.

Three native SAV species, *Ruppia maritima*, *Potamogeton perfoliatus* and *P. pectinatus* were started four weeks before the experiment was begun in fiberglass raceways in which filtered (Vortex XL diatom filter) river water was recirculated. *Ruppia* was grown from seeds, while the two *Potamogetons* were grown from dormant tubers.

Phosphorus, in the form of sodium phosphate, was incorporated into Difco Bitek complete nutrient agar, 100 ml of which was poured into individual 0.9 liter plastic containers to yield a final P fertilization rate of 15 g P m^{-2}. A second set of containers received unamended agar. On top of the agar each container received approximately 0.5 liter of a sediment mixture consisting of one part silty sediment (from the Choptank Estuary) and 2 parts sand (for a final organic content of 1.5%). The roots of immature (nonflowering) plants of each species were rinsed free of sediment and the entire plants were transplanted into these pots. Two cm of clean, commercially available "play-sand" was added to the top to slow the diffusion of nutrients from the sediment into the water column. One plant was placed in each pot, with triplicate pots of each species being planted for plain agar and P amended agar at each of the six levels of nitrogen

enrichment (for a total of 108 pots).

Potomogeton perfoliatus and *Ruppia* plants were grown as part of an SAV propagation project (Stevenson and Staver 1989), while *P. pectinatus* plants were obtained from the experimental ponds at Horn Point. Plants of comparable size were chosen, with each of the propagation plants having been grown from a single seed or tuber. The *P. pectinatus* plants were harvested as plugs, placed in plastic pots and left in the pond for approximately three weeks before being transplanted into the experimental pots. All of the experimental plants were allowed to acclimate for one week before the addition of nitrogen to the aqueous medium began. Five levels of nitrogen fertilization were maintained by weekly additions of ammonium nitrate dissolved in water (taken from the tanks) to final concentrations of 10, 20, 40, 80, 160 μM. A sixth pair of unfertilized tanks was the control. Beginning on day 28 additions were made twice weekly after nutrient analysis of the tanks showed that water column concentrations were declining to low levels very rapidly, presumably due to plant uptake.

Nutrient concentrations in the tanks were monitored weekly, and additionally just prior to and shortly after fertilization in the beginning of the study. Samples were analyzed for NO_2, NO_3, NH_4 and PO_4 on a Technicon Auto Analyzer II using standard techniques. Chl *a* was measured on a weekly basis from 60 ml samples filtered through Whatman GFC glass fiber filter. Determination of chl *a* was made fluorometrically (Standard Methods). The tanks were not filtered, cleaned or disturbed in any way during the experiment, so estimates of chl *a* and epiphytes reflected actual algal populations.

The experimental plants were harvested after eight weeks of growth. Epiphytes growing on the plants were estimated by the method of Staver (1984). Three undisturbed segments of plant stems were collected in a 500 ml bottle underwater and shaken vigorously to remove attached epiphytes. The plants were removed and dried at 60 °C, then weighed on a Mettler AT 250 balance. The epiphyte/water mixture was homogenized in a Waring blender, and two 10 ml aliquots were filtered onto a pre-weighed, pre-ashed Whatman GFC glass fiber filter (nominal retention 1.2 μm). Estimates for all plant species at each treatment level were made.

The plants were subsequently harvested and divided into aboveground (AG) and belowground (BG) material and dried to constant weight in a large forced draft oven (PGC Aminco) at 60 °C. They were weighed on a top-loading Ohaus balance and aboveground material was ground in a Wiley Mill (Arthur H. Thomas Co.) with a 1 mm mesh sieve. The three aboveground replicates were combined for subsequent analysis after it was determined that there was not sufficient plant material in some samples to do all of the desired tests. The following analysis were conducted on the combined replicates. Ash-free dry weight was determined by combustion at 500 °C for four hours. Carbon and nitrogen content were determined by combustion in a Control Equipment modified Perkin-Elmer 240B C-H-N Analyzer. Phosphorus content was measured by acid digestion followed by colorimetric determination using the molybdate-vanadate method.

RESULTS AND DISCUSSION

Actual water column N concentrations slowly built up over the course of the experiment (Figure 1). DIN concentrations remained close to zero in the control and 10 μM tanks but ranged up to over 700 μM in the highest enrichment tank. A buildup of N occurred in the higher enrichment treatments apparently because nitrogen additions exceeded plant and sediment uptake.

Growth of *Potomogeton perfoliatus* and *Ruppia maritima* responded positively to increasing nitrogen concentrations in the water column. Increases over controls in both total aboveground (AG) biomass (Figures 3a and 4a) and ash free dry weight (AFDW) (Table 2) were observed from the 20 μM N treatment to the 40-80 μM N treatments and then levelled off at the highest levels of enrichment. These data contrast sharply with the results obtained in brackish ponds by Twilley et al. (1985) in which increasing N and P was added in the water column resulting in declines in SAV biomass.

In addition to Twilley et al. (1985), Staver (1984) showed that when the water column is enriched with both N and P, epiphyte communities develop which reduce the amount of light reaching SAV leaves, ultimately resulting in reductions in SAV biomass and reproductive potential. In our experiment, P was introduced to the root system of the plants via agar in the bottom of the culture vessels, and was not added directly to the water column. A small amount of P diffused through the sediment into the water, but generally concentrations were very low, thus limiting the growth of algae in the systems.

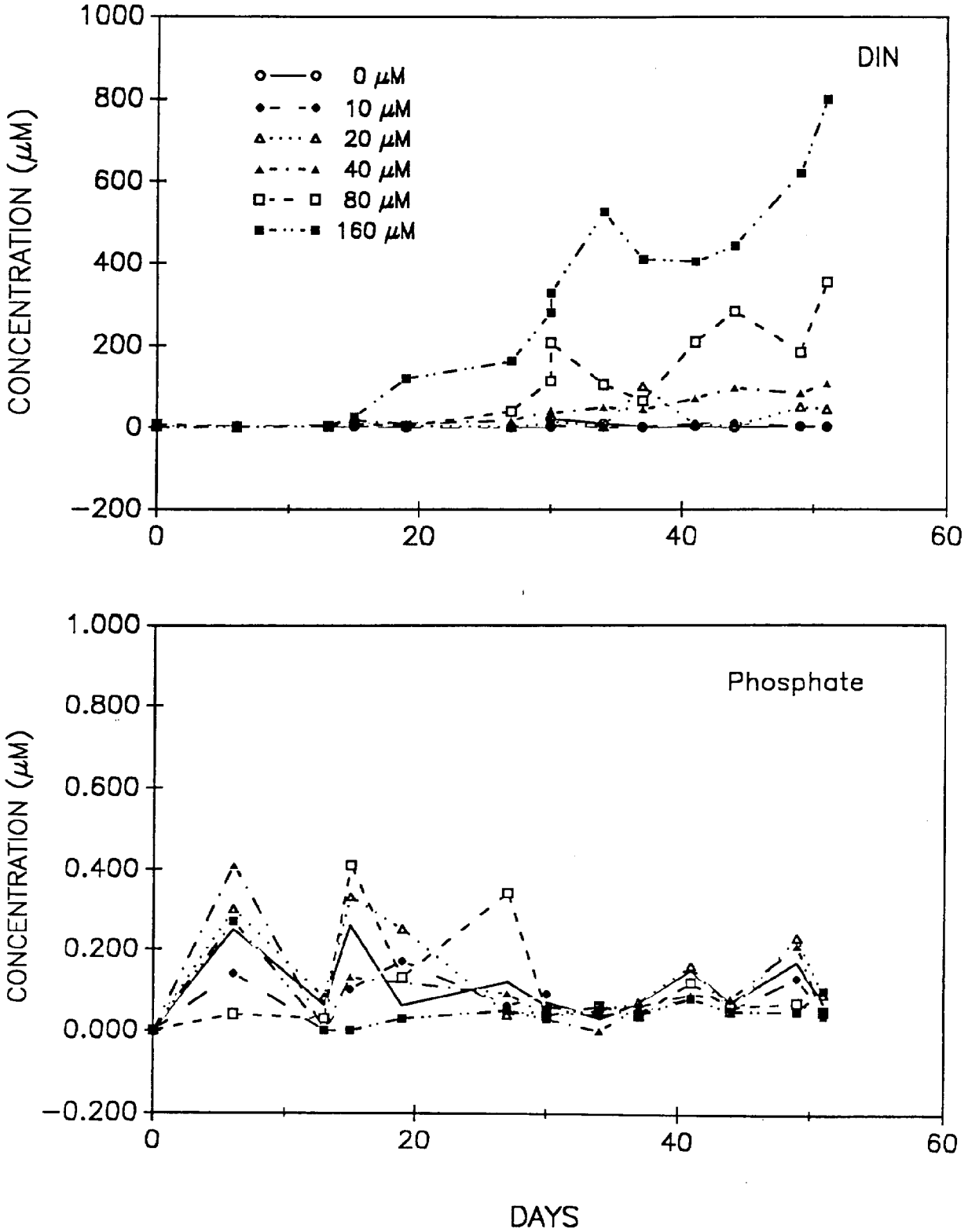


Figure 1. Dissolved inorganic nitrogen and phosphorus concentrations (μM) in the experimental tanks during the growing period.

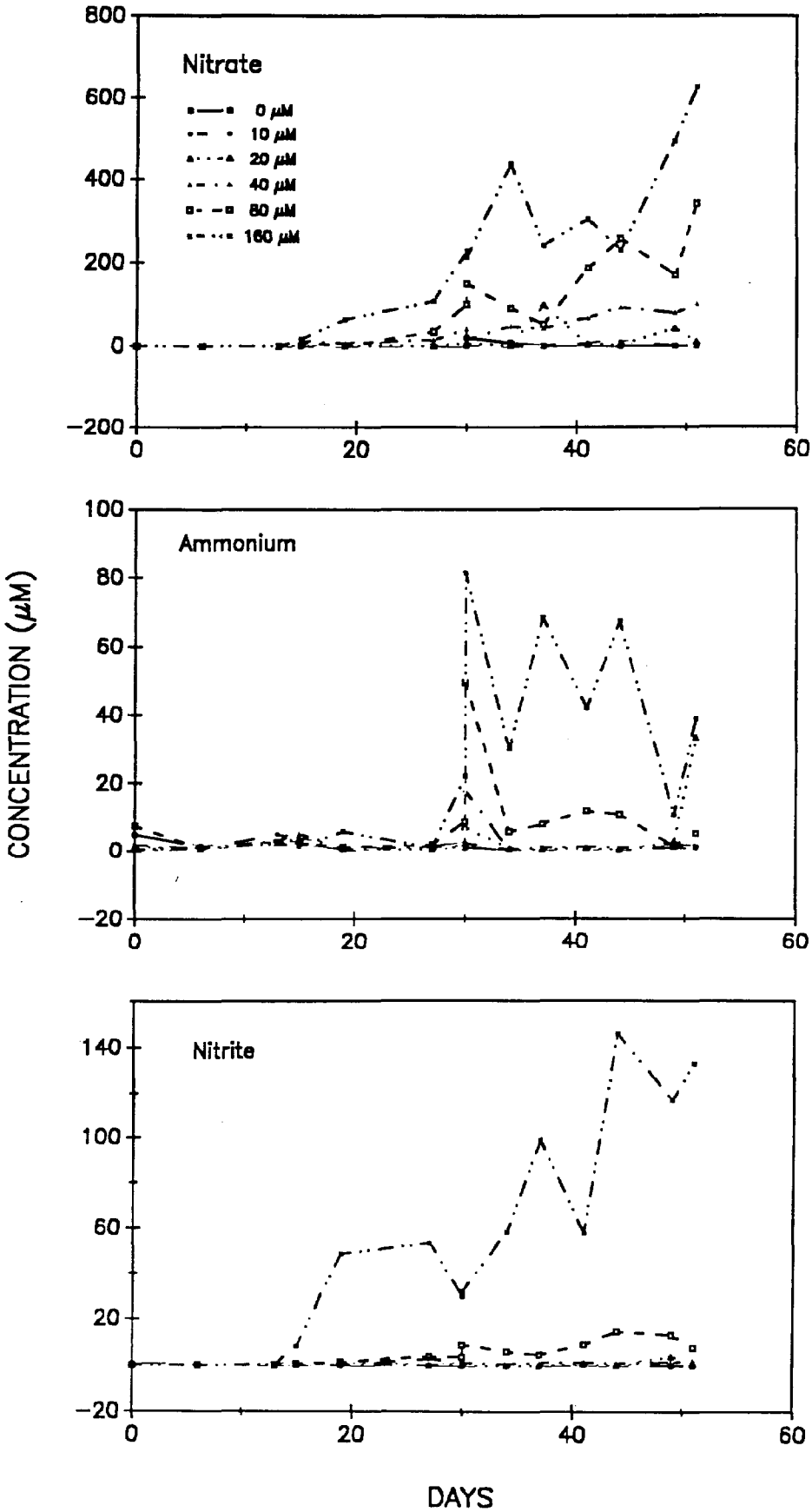


Figure 2. Nitrate, ammonium and nitrite concentrations (μM) in the experimental tanks during the growing period.

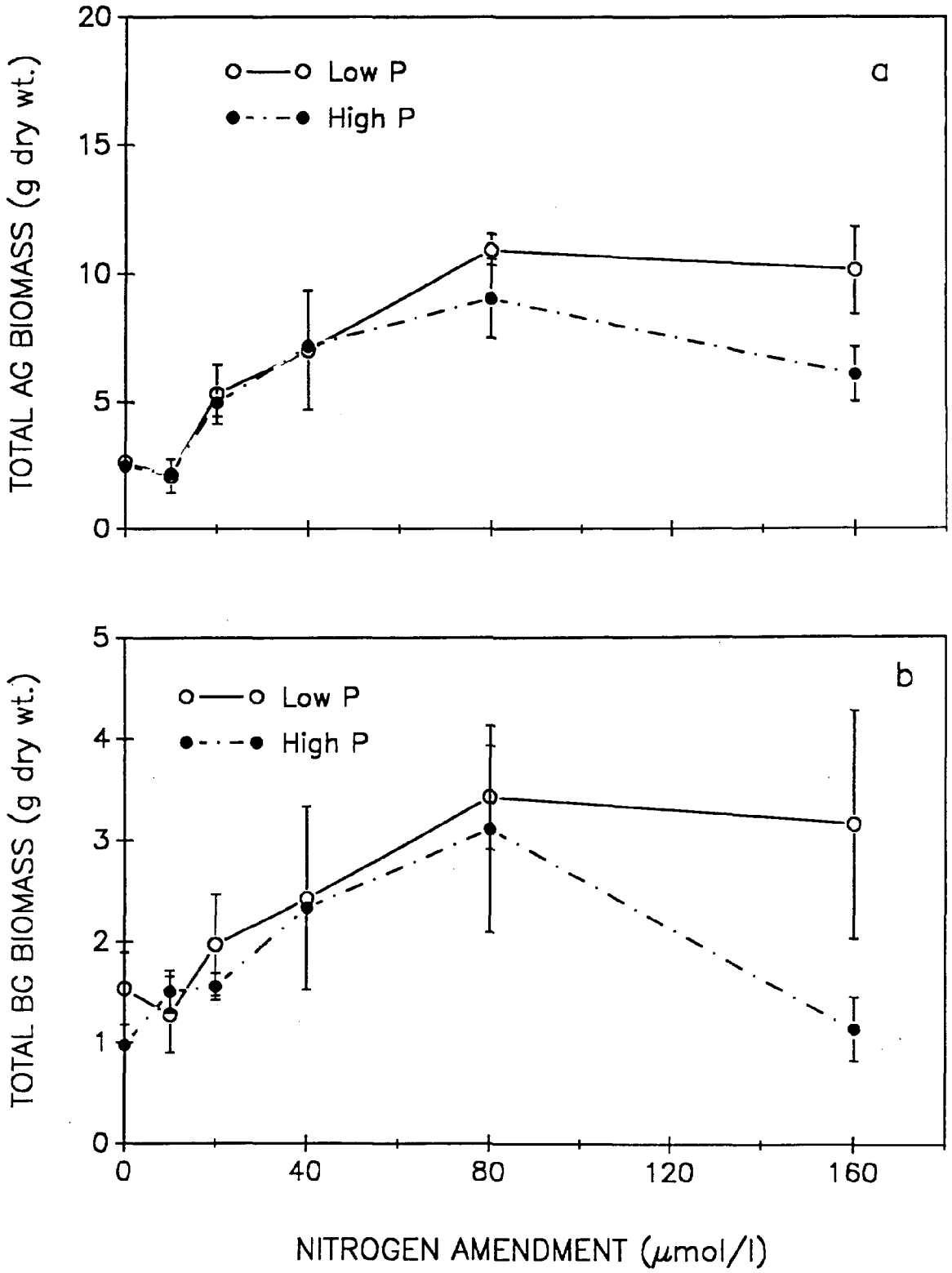


Figure 3. Total aboveground (a) and belowground (b) biomass (g dry weight) of *Potamogeton pectinatus* over the range of water column nitrogen concentrations.

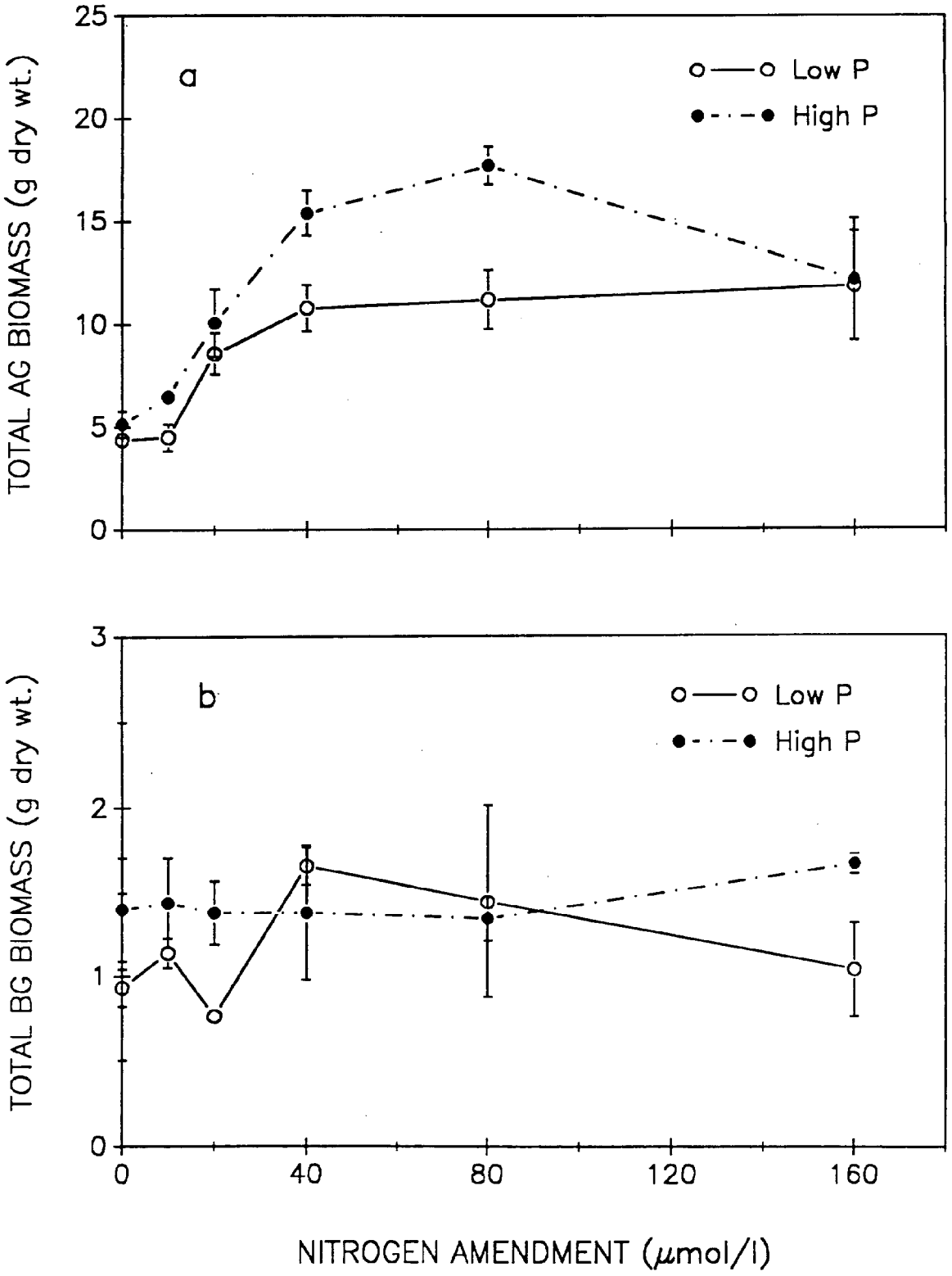


Figure 4. Total aboveground (a) and belowground (b) biomass (g dry weight) of *Ruppia maritima* over the range of water column nitrogen concentrations.

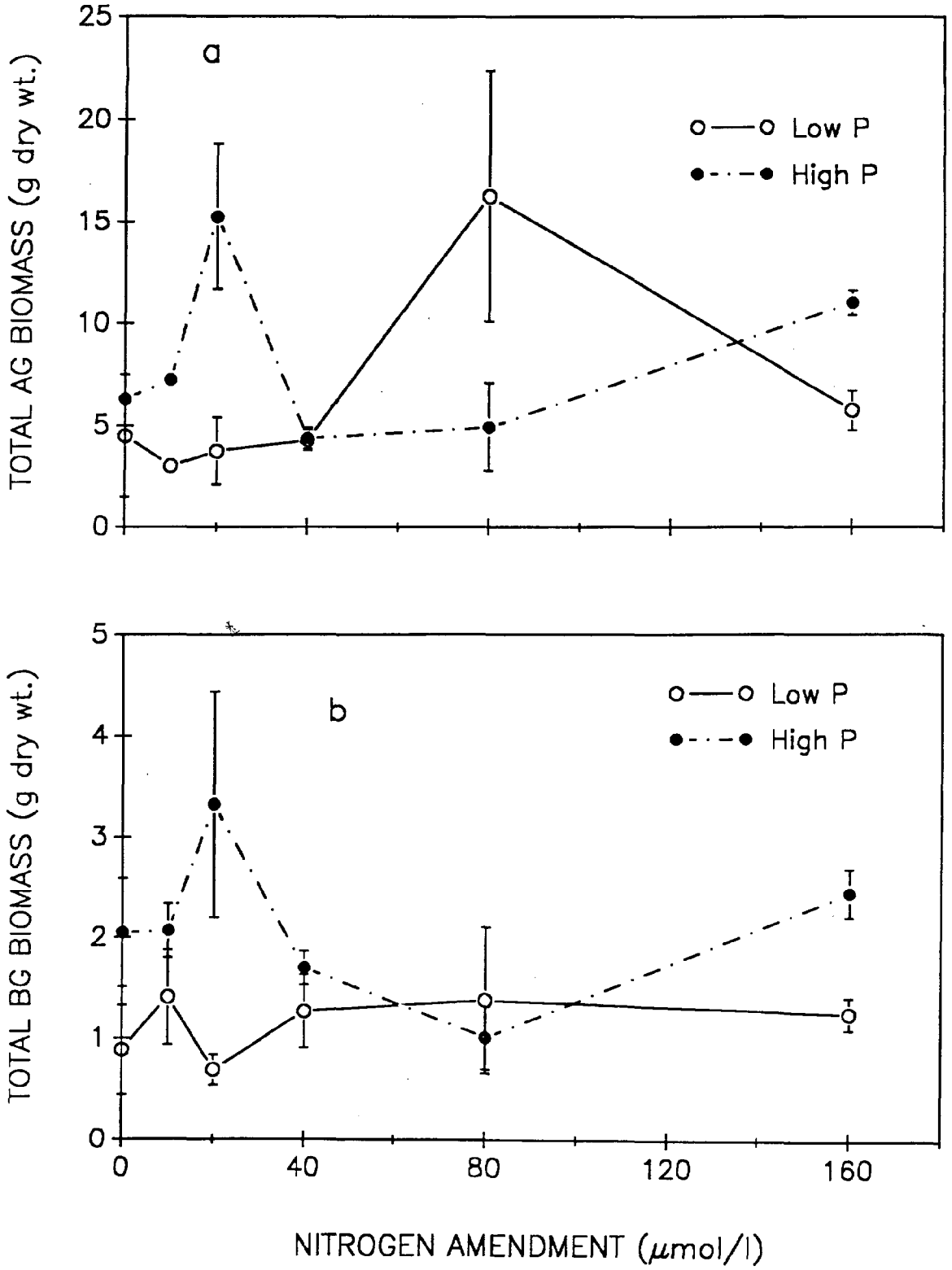


Figure 5. Total aboveground (a) and belowground (b) biomass (g dry weight) of *Potamogeton perfoliatus* over the range of water column nitrogen concentrations.

Table 2. Ash free dry weight (g) of experimental plants grown with high or low sediment phosphorus over a range of water column N concentrations.

N addition ($\mu mol\ l^{-1}$)	Species					
	P. perfoliatus		*P. pectinatus*		*Ruppia maritima*	
	High P	Low P	High P	Low P	High P	Low P
0	3.32	2.42	2.19	2.27	4.55	3.56
10	3.04	1.98	1.76	1.72	5.10	3.58
20	3.97	1.50	4.16	4.43	8.13	6.97
40	2.94	2.70	6.07	5.77	13.08	9.25
80	2.37	7.11	7.34	8.77	14.60	9.01
160	5.96	3.22	4.85	8.28	9.81	9.74

Ref. ashfree.tab

Chl *a* concentrations (Figure 6) remained below 5 $\mu g\ l^{-1}$ throughout the fertilization period. They rose to 15 and 20 $\mu g\ l^{-1}$ in the 40 and 160 tanks, respectively, late in the study, but fell off again shortly thereafter. Nutrient samples from this period which are still being analyzed should explain this brief excursion. Epiphyte loadings on the plants ranged from a low of 0.002 gdw/gdw plant on *Potomogeton pectinatus* to 0.836 gdw/gdw plant on *P. perfoliatus*. These values are in the lowest range of those reported by Staver (1984), who found epiphytes ranging from 0.26-3.10 gdw/gdw of plant material. Furthermore the highest values in our study, which occurred on *P. perfoliatus*, were inflated by the presence of $CaCO_3$ on the leaves.

Thus increases in N alone appear to cause increases in SAV biomass, provided P is available in the sediment and is low enough in the water column to preclude algal growth. The question remains, then, what caused the levelling off of biomass in our experiment at high nitrogen concentrations? Actual water column nitrogen concentrations suggested that some factor other than nitrogen limitation was responsible for preventing further increases in growth. Nitrogen accumulated to concentrations of several hundred μM toward the end of the experiment under the highest N treatments (Figure 1a), showing that N uptake was not keeping up with inputs. Tissue N concentrations of all three species increased with water column N enrichment over the range tested, but increases slowed considerably above the 40 μM N treatment (Figure 7). This corresponds well with actual water column N values, which increased markedly above the 40 μM N treatment (Figure 1). It can be inferred from this data that N was limiting in the control through 40 μM N treatments, but not at higher levels of enrichment (which correspond to actual water column N concentrations exceeding 100

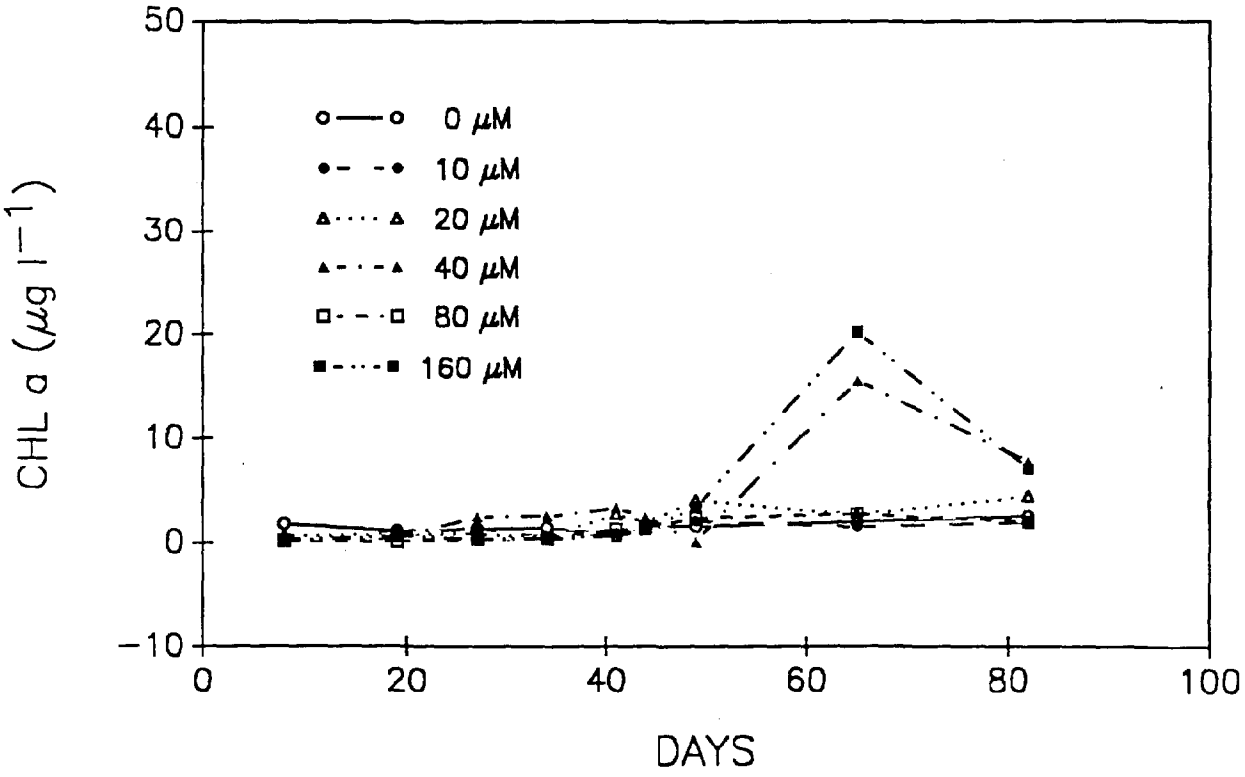
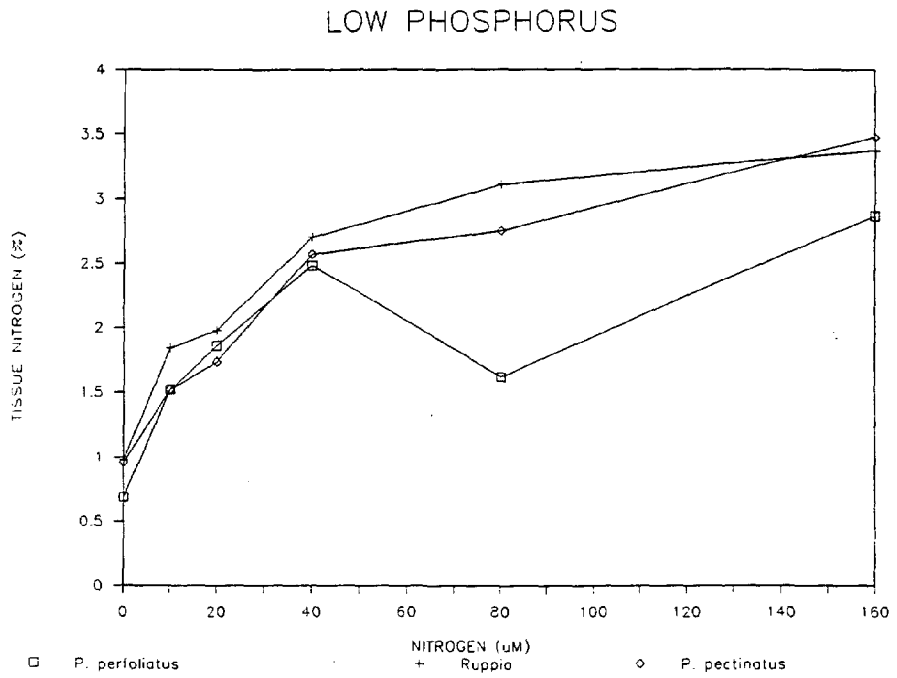


Figure 6. Chlorophyll *a* concentrations ($\mu g\ l^{-1}$) in the experimental tanks during the growing period.



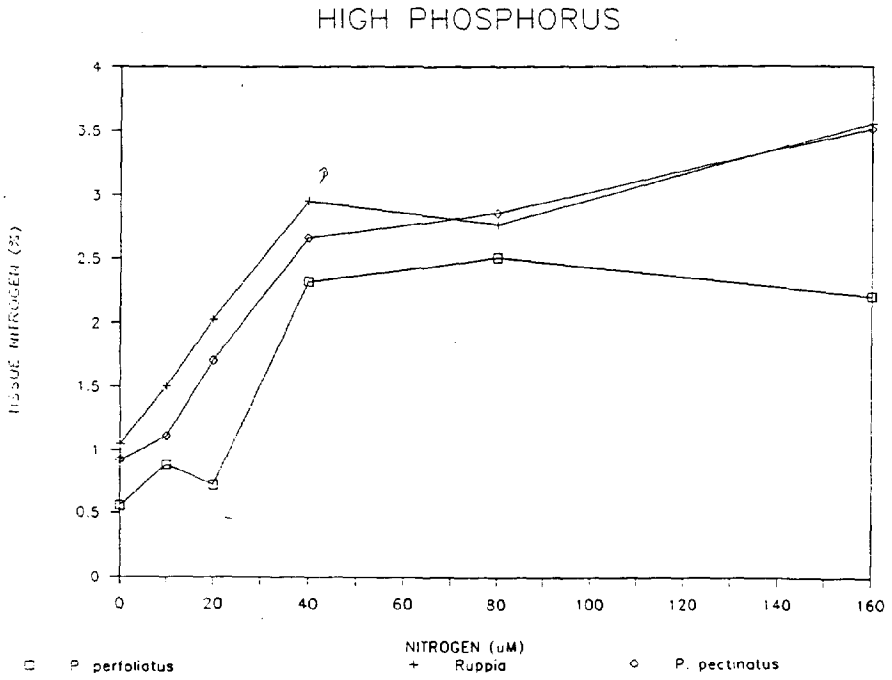


Figure 7. Tissue nitrogen concentrations (%) in the species tested under low (a) and high (b) sediment phosphorus conditions over the range of water column nitrogen concentrations.

μM from day 19 and 30 for the 80 μM and 160 μM treatments, respectively). These data agree with the results of Van Wijk (1989) which indicate that a N concentration of 57 μM NO_3 (the maximum tested) was limiting to *P. pectinatus* growth under lab conditions. However, Gerloff and Krombholz' (1966) critical value for tissue N, 1.3%, was exceeded in all but the control plants (low P) and control and 10 μM plants (high P), suggesting that this value is not valid for the plants studied here.

Several factors could have contributed to limit growth at the highest N levels, including self shading, which occurs when stem density becomes very high; carbon limitation, which occurs when high densities of plants are grown in confined systems; or a shortage of micronutrients resulting from plant uptake. Self shading undoubtedly occurred toward the end of the study when plant densities were at their maximum. In addition, pH shifts above 9 were recorded in the latter half of the study during mid-day despite our attempts to minimize carbon limitation by bubbling the tanks continuously with ambient air; therefore carbon limitation may have come into play. Micronutrient limitation was probably not an issue due to the small demand by aquatic plants compared to the large volume of water in which they were grown.

Belowground (BG) biomass trends reflect the different reproductive strategies of *Potomogeton pectinatus* and *Ruppia maritima* (Figure 3b and 4b). While *Ruppia* belowground biomass shows little apparent influence of N concentration, *P. pectinatus* BG biomass increases up to 80 μM N at both P concentrations. Presumably this is due to a buildup of tubers in the sediment (although tubers were not counted), which results from enhanced nutritive status at higher N concentrations.

Ruppia overwinters as both seed and rhizomes. While overwintering rhizomes probably have a head start over seeds in the spring, they are more susceptible to the ravages of the weather, which can be quite severe in the shallow embayments where *Ruppia* typically occurs. Winter winds create wave action which can dislodge the rhizomes, which are generally located within the top few centimeters of sediment, and low winter tides can cause exposure of sediments resulting in freezing or dessication. Ice scouring can also occur, also causing disruption of surface sediments containing rhizomes. Seeds of this species are particularly resilient to harsh conditions (compared to soft seeds like *Zostera marina* which can be easily damaged -R. Orth pers. com. 1989), making reproduction by seed essential to help prevent possible winterkill.

In contrast, *P. pectinatus* is extremely plastic in its reproductive strategies and the allocation of resources into tubers or seeds is heavily influenced by environmental conditions (Kautsky 1987). It often relies on tubers located up to 10 or more cm deep in the sediment, making them less susceptible to the problems associated with winter weather than the shallow *Ruppia* rhizomes. In our study seed production generally appeared to be much lower in *P. pectinatus* than in *Ruppia*, although we did not quantify either seed or tuber production.

There was no apparent trend in the response of *P. perfoliatus* growth to increasing N concentrations at either P concentrations (Figure 5a). There was a large build-up of calcium carbonate ($CaCO_3$) on the leaves of *P. perfoliatus* in all tanks, and a comparison of dry weight to ash free dry weight shows the exaggerating effect that this material can have on biomass estimates (Table 2).

Calcium carbonate deposition occurs when the leaves of *P. perfoliatus* become polarized during photosynthesis, creating micro-zones of very high pH close to the upper surfaces of the leaves (Prins and Elzenga 1989). Increased temperature at the upper leaf surfaces, which occurs when sunlight irradiates them, also helps create conditions favorable for $CaCO_3$ precipitation.

Patterns of belowground biomass were similar to AG biomass in *P. perfoliatus* (Figure 5b). As with *P. pectinatus*, tubers are an important method of reproduction (Stevenson and Confer 1978) and their production is directly related to AG biomass (Goldsborough and Kemp 1988). Therefore the similarity is expected.

The response of AG plant growth to the two sediment P concentrations varied with species (Figures 3a, 4a and 5a). *Ruppia* growth was consistently higher at high P concentrations, while *P. pectinatus* growth was similar up to 40 μM N, but was greater under low P concentrations at higher N concentrations. This suggests that *Ruppia* was P limited at high N concentrations, while *P. pectinatus* was not. *P. perfoliatus* growth showed no clear trend with regard to sediment P.

Tissue P concentrations (Figure 8) were highest in *P. pectinatus*, lowest in *P. perfoliatus*, and were not distinctly different between the two sediment P concentrations except in *P. pectinatus* at the highest N concentrations. Mean tissue P concentrations in *Ruppia*, *P. pectinatus*, and *P. perfoliatus* were 0.17 %, 0.22 % and 0.15 %, respectively, which indicates no P limitation based on Gerloff and Krombholz' (1966) critical tissue concentration of 0.13 % and Van Wijk's (1989) 0.15 %.

Tissue carbon concentrations were consistent within each species over all N and P treatments (Figure 9). A rather large difference existed, however, between species, with

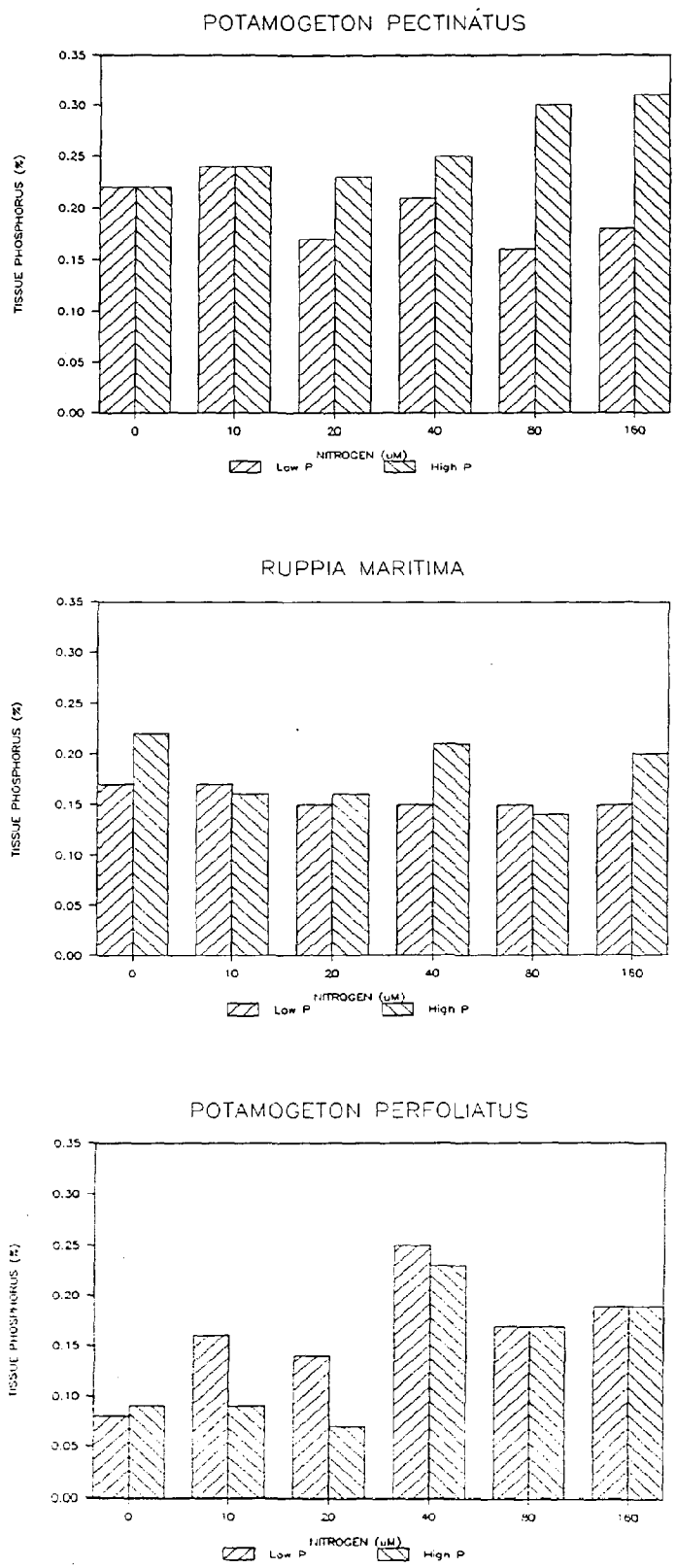


Figure 8. Tissue phosphorus concentrations (%) in *Potamogeton pectinatus* (a), *Ruppia maritima* (b) and *Potamogeton perfoliatus* (c) grown under low and high sediment phosphorus concentrations over the range of water column nitrogen concentrations.

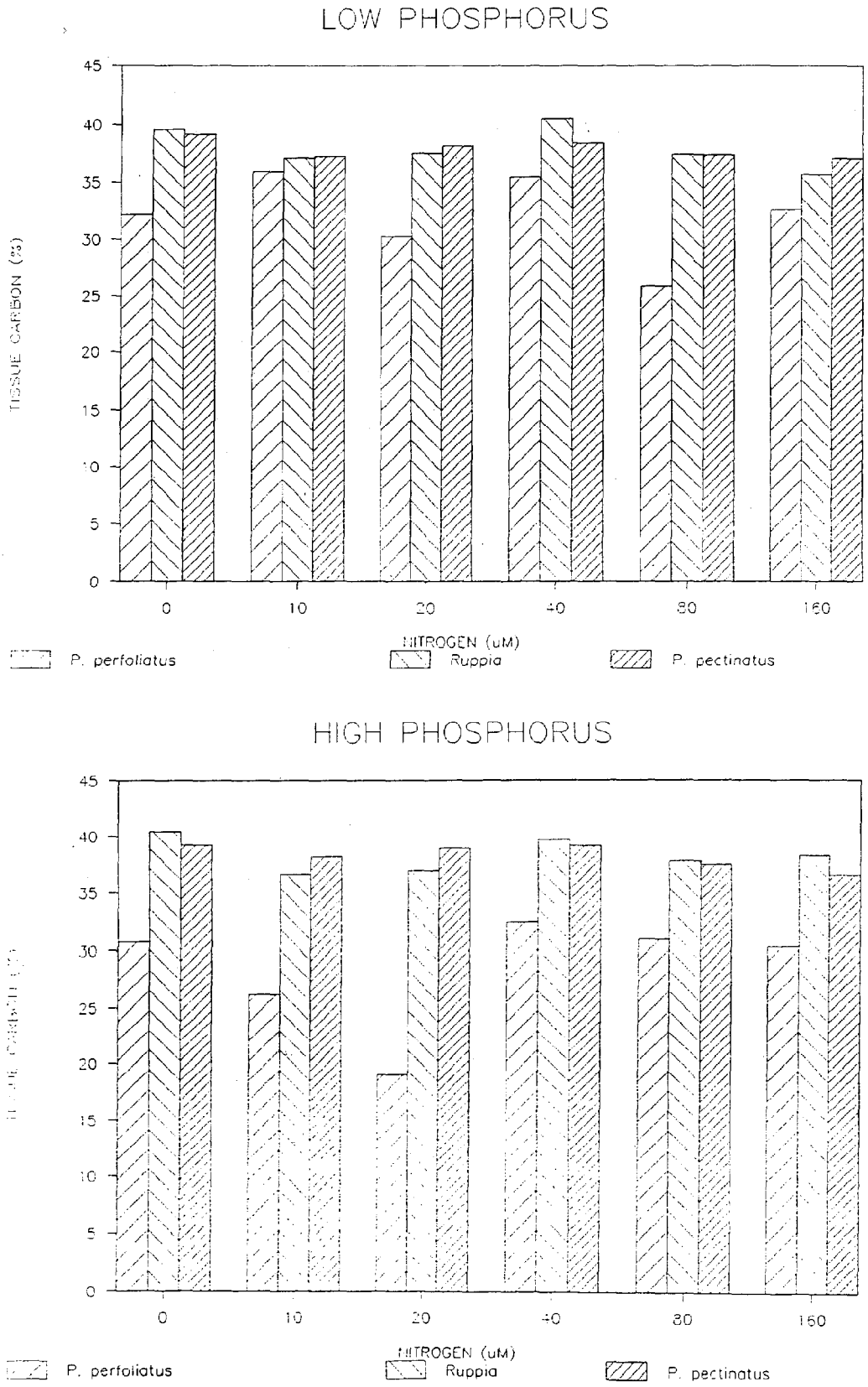


Figure 9. Tissue carbon concentrations (%) of the species tested under low (a) and high (b) sediment phosphorus concetrations over the range of water column nitrogen concentrations.

P. perfoliatus C concentrations being lower than the other two species under all treatments. *P. pectinatus* and *Ruppia* were similar under all treatments, ranging between 35 and 40 % C.

The generally lower tissue C levels in *P. perfoliatus* may be attributable to less efficient bicarbonate uptake by this species. As plant biomass increased in the tanks, pH was driven up during daylight hours due to photosynthesis. At pH 9, which was recorded on sunny days, most dissolved inorganic carbon (DIC) is in the form of HCO_3. The inability to use HCO_3 efficiently could lead to depressed tissue C concentrations.

An interesting aside in this experiment was buildup of nitrite (NO_2) in the water column (Figure 2). Ammonium nitrate was added, but immediately following the onset of fertilization nitrite appeared in the tank receiving 160 μM N and to a much lesser extent in the 80 μM tank. Ammonium was apparently being oxidized to nitrite more quickly than it could be assimilated by the plants in those tanks, while at lesser dosing rates ammonium uptake equalled inputs.

MANAGEMENT IMPLICATIONS

The debate over whether N or P is limiting in coastal waters has been going on since Redfield's (1958) work. As Ryther pointed out (1959) this is not only an academic question, and it has recently become important in efforts to manage nutrient inputs for the benefit of the Bay's living resources. Data from the Choptank River (Stevenson et al. 1989) has suggested that for the mesohaline to oligohaline portions of Chesapeake Bay tributaries, both nutrients can be limiting to algal growth at different times of the year and in different parts of the river. The variability in which nutrient is limiting is due in part to the source of inputs, with N entering the estuary primarily by diffuse source loading from agricultural land and residential sewage while P is entering primarily from sewage treatment plants (STP) (Fisher 1988).

In contrast to the Choptank Estuary, the tidal fresh section of the Potomac River and the mouth of the Susquehanna near Havre de Grace, MD are characterized by high N and low P inputs, leading to P limitation in the water column most of the year. Both of these areas support large and healthy populations of submersed aquatics.

Low water column P concentrations were achieved in the Potomac through reductions in effluent P concentrations at the Blue Plains STP (Carter and Rybicki 1986). The resurgence in SAV following those reductions imply that they were crucial in reversing the competitive balance so that SAV were favored over algal species. Although management of tributaries like the Choptank may be based on similar objectives, there has been debate over the possibility that comparable conditions in brackish water would not lead to the same results.

Our study was designed to assess the impact on SAV of reduced water column P concentrations over a range of N concentrations. We have demonstrated here the effectiveness of keeping P inputs to the water column low (basically only diffusion in this experiment) in limiting algal growth in brackish water. When N inputs exceeded SAV requirements, the excess N was not taken up to any large extent by algae because their production was limited by P. In fact, without the presence of a large pool of phosphorus in the water column, SAV growth is enhanced by nitrogen enrichment, up to a point.

The nitrogen "saturation" point, the concentration above which further increases in SAV biomass are small or zero, can also be determined from the data collected here. We found that above actual water column N concentrations of about 100 μM there was little increase in AG biomass. Thursby (1984) and Dennison et al. (1987) found similar saturation points for *Ruppia maritima* and *Zostera marina*, respectively. Thus N loading rates which produce concentrations exceeding 100 μM are not beneficial to SAV.

Based on the data produced here, it appears that reductions in phosphorus inputs would be an efficient way to restore water quality to a level which will support healthy SAV populations. Since P inputs in the Choptank are dominated by point sources which can be cost effectively removed by precipitation, P reductions could be achieved more readily than N reductions, which will involve a more complex array of strategies to deal with the various sources, and may not ultimately be as important to the submersed aquatics. In the particular cases of estuaries on the eastern shore of Chesapeake Bay which have extremely high N concentrations, special attention should be paid to P removal. This underscores the necessity for advanced P treatment at STP receiving wastewater from rapidly growing towns such as Easton which has already impacted SAV growth in the Choptank Estuary.

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