Freshwater Biology (1996) **36,** 661–671

Effects of CO_2 enrichment on mineral accumulation and nitrogen relations in a submersed macrophyte

JOHN E. TITUS* AND JOHN H. ANDORFER†
Department of Biological Sciences, Binghamton University, Binghamton, NY 13902, U.S.A.
**Author to whom correspondence should be sent*
†Present address: Department of Biology, University of South Florida, Tampa, FL 33620, U.S.A.

SUMMARY

1. Six- to eight-week greenhouse experiments with independent control of pH and dissolved CO_2 evaluated the potential for CO_2 enrichment to stimulate the accumulation of Al, Fe, P and N in shoots of *Vallisneria americana*, particularly at pH 5. These minerals were provided only as they occurred in natural lake sediments.
2. The effect of CO_2 enrichment at pH 5 *v* pH 7.3 on growth and tissue N concentration was also determined.
3. CO_2 enrichment at pH 5 effected 5.5- and 7-fold increases in total shoot accumulation of Al and Fe, respectively. In a two-way factorial experiment, CO_2 enrichment yielded 6- to 11-fold greater total shoot P accumulation in plants grown on less and more fertile sediments, respectively.
4. In a three-way factorial experiment, CO_2 enrichment stimulated *Vallisneria* growth, especially at pH 5, and resulted in a 31–58% reduction in tissue [N] for different pH × sediment combinations. These are greater reductions than previously reported. It also increased total shoot N accumulation up to 6-fold, and there were significant interactions with pH and sediment source: the CO_2 enrichment effect on shoot N accumulation was greater at pH 5 than at pH 7.3, and it was greater with the more fertile sediment at pH 5.
5. Water chemistry (pH and/or [CO_2]) and sediment fertility thus both indirectly influenced the accumulation of sediment-derived minerals in macrophyte shoots within the water column.

Introduction

Sculthorpe (1967) cited the availability of photosynthetic carbon sources as a principal environmental factor influencing productivity in submersed macrophytes. Dissolved inorganic carbon (DIC) enrichment has repeatedly been demonstrated to stimulate carbon uptake rates in submersed macrophytes (e.g. Steeman Nielsen, 1947; Titus & Stone, 1982; Wetzel *et al.*, 1985). In longer term greenhouse experiments, CO_2 enrichment has overcome growth limitations occurring at low pH (Titus, Feldman & Grisé, 1990) and on infertile sediments (Barko, Smart & McFarland, 1991b; Titus, 1992). Further, DIC concentration has been positively correlated with submersed macrophyte growth in experimental field studies (Overath *et al.*, 1991; Rattray, Howard-Williams & Brown, 1991). These findings are important because DIC concentration varies widely among lakes and streams, even at the same pH (Titus *et al.*, 1990; Rebsdorf, Thyssen & Erlandsen, 1991). In this paper, we examine two potential correlates of growth stimulation by CO_2 enrichment: more rapidly growing plants may accumulate greater quantities of minerals from the sediment in their shoots, and shoot tissue quality may be altered.

Sediment is the principal source of phosphorus (P) and several other minerals for submersed macrophytes (Barko, Gunnison & Carpenter, 1991a), and thus these plants may act to mobilize minerals from the sediment into the water column (Barko & Smart, 1980; Carignan

© 1996 Blackwell Science Ltd

& Kalff, 1980). Similarly, potentially toxic metals may be transported from the sediment into macrophyte shoots (Welsh & Denny, 1979). In some cases, this mechanism may account for substantial internal loading of minerals into water bodies (Landers, 1982; Smith & Adams, 1986). Simply by promoting macrophyte growth, high CO_2 concentrations may increase internal mineral loading into freshwater ecosystems.

The first objective of this paper is to evaluate the potential for DIC enrichment, primarily as CO_2 enrichment at pH 5, to stimulate the accumulation of sediment-derived minerals in shoots of the submersed macrophyte *Vallisneria americana* Michx. (hereafter *Vallisneria*). This potential was tested in greenhouse experiments in which the selected minerals (Al, Fe, P and N) were provided only in the sediment, and water column DIC concentrations were elevated either via CO_2 and HCO_3^- enrichment at constant pH, or as HCO_3^- enrichment concomitant with a pH increase at a constant free CO_2 concentration ($[CO_2]$). Experiments were carried out with different natural lake sediments to assess the role of sediment fertility in P and N accumulation.

A second outcome of CO_2 enrichment is that macrophyte tissue quality may change. Recent literature on terrestrial plants has repeatedly documented a decline in tissue N concentration with CO_2 enrichment (e.g. Coleman *et al.*, 1991). Focus on N is warranted because of the importance of nitrogen to photosynthetic capacity (Field & Mooney, 1986; Madsen & Baattrup-Pedersen, 1995), herbivory (Lincoln, Couvet & Sionit, 1986) and decomposition (Godshalk & Wetzel, 1978; Enriquez, Duarte & Sand-Jensen, 1993). In uncontrolled field environments, *Vallisneria americana* grew much more rapidly in a high DIC site, but showed a sharp decline in tissue [N] relative to lower DIC sites (Overath *et al.*, 1991). In contrast, in a controlled greenhouse environment the same species showed an increase in tissue [N] with CO_2 enrichment at pH 5 on two of the three sediments tested (Titus, 1992). In the latter experiment, however, HNO_3 was used to control pH in the growth medium, and may have confounded the issue.

Our second objective was to determine the effect of CO_2 enrichment on tissue quality without added HNO_3. Effects on tissue [N] were tested at two different pH levels and for plants grown on two different natural lake sediments. The factorial design used also allowed us to determine the relative impact on growth of CO_2 enrichment at low and high pH.

Materials and methods

Study species

Vallisneria americana (Hydrocharitaceae) develops in late spring (in east-central North America) from a buried tuber into a basal rosette of ribbon-like leaves. Rosettes give rise to additional rosettes on elongated axillary stolons throughout the growing season. A 'shoot' in this paper refers to all the rosettes and interconnecting stolons derived from a single tuber at the beginning of the growing season, and associated sexual reproductive structures when present. The relatively shallow root system (Titus & Stephens, 1983) develops adventitiously below the rosette base. Toward the end of the growing season, some stolons grow down into the sediment and form tubers prior to the senescence and decay of the parent plant in autumn.

Vallisneria occurs on various sediment types in a range of oligotrophic to eutrophic sites, but is most abundant in hardwater systems (J. Titus, unpublished observations). It is hardy and easily transplanted when collected as a tuber or as a rosette prior to the elongation of its first roots in spring. Growth by *Vallisneria* is responsive to CO_2 enrichment at pH 5 on a variety of sediments (Titus, 1992).

Experimental design and statistical analysis

We report data from three greenhouse experiments, each completed in a different year. In experiment A, *Vallisneria* was grown at ambient CO_2 concentration (i.e. with aqueous free CO_2 in equilibrium with atmospheric CO_2) and at 3.2 and 10 times ambient CO_2, all at pH 5. These relatively high CO_2 enrichment concentrations are in accord with the degree of CO_2 oversaturation in many acidic freshwaters (Titus *et al.*, 1990). The same relatively fertile sediment was used in all treatments. Tissue metal concentration data from experiment A, previously reported in a paper in the context of growth responses and metal toxicity at low pH (Titus *et al.*, 1990), are here reanalysed and multiplied by shoot biomass to yield total shoot content of Al and Fe, i.e. data relevant to internal metal loading. All data were log-transformed to reduce

13652427, 1996, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2427.1996.00135.x by Mississippi State University, Wiley Online Library on [13/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

heteroscedasticity or non-normality, and analysed with one-way ANOVA using initial weight class (see below) as a blocking variable. Significant outcomes were tested further with Tukey's Studentized range test (SAS Institute, 1985).

In experiment B, *Vallisneria* was grown at ambient and 10 times ambient CO_2 levels, again at pH 5, but on two contrasting naturally occurring sediment types (described below) as part of a two-way factorial design. Tissue [P] data from experiment B, also previously reported (Titus, 1992), are here reanalysed and multiplied by shoot biomass to yield total shoot P content. Dry mass data were log-transformed so that a significant interaction would indicate a disproportionate effect of CO_2 enrichment on one of the two sediment types. All data were analysed with both one-way and two-way ANOVA with initial weight class as a blocking variable. No Bonferroni correction was necessary when means were used in more than one ANOVA because of the very low probability values involved.

Experiment C plants were grown at ambient and eight times ambient CO_2 levels, on two sediments differing in fertility, and at pH 5 and 7.3 for a three-way factorial design. A key difference between pH 5 and 7.3 is the composition of DIC: at pH 5 (and 23 °C), 96% of DIC is free CO_2 and 4% is HCO_3^-, whereas at pH 7.3, 10% of DIC is free CO_2 and 90% is HCO_3^-. Experiment C yielded data on dry mass, tissue [N], and total shoot N content. Dry mass and tissue [N] data were log-transformed to alleviate heteroscedasticity and non-normality, respectively, and all data were analysed with three-way ANOVA incorporating a split-plot nested design: in this experiment, both sediment types were tested within each tank ($n = 15$ plants per sediment type in each tank), and replicate tanks were nested within each CO_2 level at pH 5. Insufficient tanks were available for tank-level replication in other treatments reported here, but when tested, 'tank effects' have consistently been small in comparison with main treatment effects (including this experiment: see Table 3 below). Methods described below refer to all three experiments unless a particular experiment is cited.

Sediment

A well-buffered silty sediment from alkaline Otsego Lake (Otsego County, NY; latitude 42°41′40″, longitude 74°55′18″) was used in all experiments. This sediment has supported robust growth of *Vallisneria* in the field (Overath *et al.*, 1991). In experiments B and C, sediment from anthropogenically acidified Big Moose Lake (Herkimer County, NY; latitude 43°49′02″, longitude 74°51′23″) was also used. Sediments were collected with an Ekman dredge or with plastic buckets by hand from Hyde Bay in Otsego Lake and at the Inlet site in Big Moose Lake. Sediments were returned to the Binghamton University greenhouse and mixed thoroughly before being allocated to pots.

Sediments were characterized by determining organic content as percentage of dry weight loss on ignition at 600 °C (Allen, Grimshaw & Rowland, 1986), bulk density as weight after drying at 105 °C per unit volume of fresh sediment, and exchangeable NH_4^+ by extraction of 5 g fresh sediment shaken in 50 ml of 2 N KCl (Bremner, 1965), followed by NH_4^+ analysis with a salicylate method (Kempers & Zweers, 1986). The total extractable NH_4^+ per pot was estimated by multiplying the quantity of extractable NH_4^+ per g fresh sediment by the total sediment weight in each pot. Porewater pH, soluble reactive P (SRP), and dissolved NH_4^+ are also reported. Compared with Big Moose sediment, Otsego Lake sediment had about 65% lower organic content, 2-fold higher bulk density, higher porewater pH, about 3- to 5-fold higher values for porewater SRP, porewater dissolved NH_4^+, and exchangeable NH_4^+ concentrations, and about 7-fold higher total exchangeable NH_4^+ (Table 1). Collectively, these parameter values indicate that Otsego Lake sediment is more fertile than Big Moose Lake sediment, so Otsego Lake sediment will be referred to as 'fertile' and Big Moose Lake sediment as 'infertile'.

Planting, harvesting and analysis of plant tissue

Young *Vallisneria* rosettes were collected from Chenango Lake (Broome County, NY; latitude 42°12′18″, longitude 75°50′13″), weighed and sorted into four (experiment C) or five (experiments A and B) weight classes, transplanted in mid-June into plastic pots containing a total sediment volume of 2.2 l (experiments A and B) or 3 l (experiment C), and randomly positioned within 1200-l fibreglass tanks. Initial weight class was used as a blocking variable: equal numbers from each class were used in each of the treatments, which were randomly assigned to tanks. Tank water initially was a solution of 0.63 mM $CaCl_2$, 0.15 mM $KHCO_3$ and 0.28 mM $MgSO_4$ in deionized water, modi-

13652427, 1996, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2427.1996.00135.x by Mississippi State University, Wiley Online Library on [13/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 1 Characteristics of the lake sediments used. Porewater chemistry (pH, soluble reactive phosphate and [NH_4^+]) from Titus (1992) with permission. Means shown for n = 5–18. One-way analyses of variance were significant ($P < 0.05$) for all parameters

			Porewater				
Source lake	Loss on ignition (%)	Bulk density (g ml^{-1})	pH	[SRP] (μg P l^{-1})	[NH_4^+] (mg N l^{-1})	Exchangeable NH_4^+ (μg g^{-1} fresh wt)	Total NH_4^+ (mg pot^{-1})
Big Moose	9.7	0.43	6.3	0.36	1.99	2.95	8.5
Otsego	3.4	1.08	7.0	1.29	10.42	14.7	57.7

fied from Smart & Barko (1985) by the removal of $NaHCO_3$. We increased water depth above the sediment surface stepwise to 70 cm as the plants grew. Remcor refrigerated circulators maintained tank temperatures at 23 ± 2 °C. Plants were exposed to natural light regimes in the Binghamton University greenhouse.

Horizon 5997 pH controllers maintained water pH within 0.2 units of desired levels by automatic dropwise addition of acid or base (1 N acid comprised of 70% H_2SO_4 and 30% HNO_3, and 1 N NaOH in experiments A and B; 0.4 N HCl and 0.4 N NaOH in experiment C). Tylan FC-280 mass flow controllers regulated the enrichment of compressed air lines with pure CO_2. The compressed air, with and without CO_2 enrichment, was then continuously bubbled through tank water to attain different DIC levels. The refrigerated circulators and bubbling air streams provided continuous mixing. In this paper, 'CO_2 enrichment' is used for simplicity, but it should be understood that, even at pH 5, CO_2 enrichment is accompanied by some degree of HCO_3^- enrichment. At pH 7.3, CO_2 enrichment leads to a much greater degree of HCO_3^- enrichment.

Monitoring in each tank included water temperature as a check on the circulators, total DIC with infrared gas analysis (Analytical Development Co., Model 225 Mk II gas analyser and Hewlett–Packard 3390 A Integrator) of CO_2 sparged from acidified tank water samples, and specific conductance with a Radiometer CDM 80 conductivity meter. One-third of the tank volume was replaced with fresh solution (as above) if conductance rose above 600 μS cm^{-1}. Tank waters were filtered with diatomaceous earth filters as needed to maintain water clarity.

Plants were harvested after 6, 7 and 8 weeks for experiments A, B, and C, respectively. Shoots (leaves, stolons, and flowers if present) were rinsed thoroughly, dried at 85 °C (70 °C in experiment C for tissues to be analysed for N), and in most cases ground with a stainless steel Wiley mill to pass a no. 40 screen (0.43 mm square meshes). Small plants were ground in porcelain mortars with liquid nitrogen. Ground samples were digested in a mixture of hydrogen peroxide and sulphuric acid (Allen *et al.*, 1986) in a Technicon block digester and analysed for phosphate content with a molybdenum blue procedure (Murphy & Riley, 1962), for aluminium with a hydroxyquinoline method (James, Clark & Riha, 1983), for iron with atomic absorption spectrophotometry using a Perkin–Elmer 2380 spectrophotometer, and for nitrogen with a salicylate method (Kempers & Zweers, 1986). Nitrogen data are not reported for experiments A and B because HNO_3 was used for pH control, and may have altered tissue N content due to foliar uptake. Analyses of selected tissue samples for NO_3^- (Cataldo *et al.*, 1975) revealed negligible levels, so that N concentrations reflect reduced nitrogen forms. Analytical methods were verified by analysing digested certified National Bureau of Standards orchard leaves (no. 1571) or citrus leaves (no. 1572). We assume below that the Al, Fe, P and N analysed for *Vallisneria* shoots were derived from the sediment because we did not add these minerals to experimental tank water, and because previous work has strongly implicated root uptake from sediments (Barko *et al.*, 1991a; Jackson *et al.*, 1991), although we have not ruled out foliar uptake of minerals released directly from sediment into the water column.

Results

CO_2 enrichment: effects on Al, Fe and P accumulation at pH 5

CO_2 enrichment increased dry mass accumulation in experiment A 10-fold and decreased tissue [Al] by 47%, but did not significantly change tissue [Fe] (Fig. 1a–c).

13652427, 1996, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2427.1996.00135.x by Mississippi State University, Wiley Online Library on [13/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

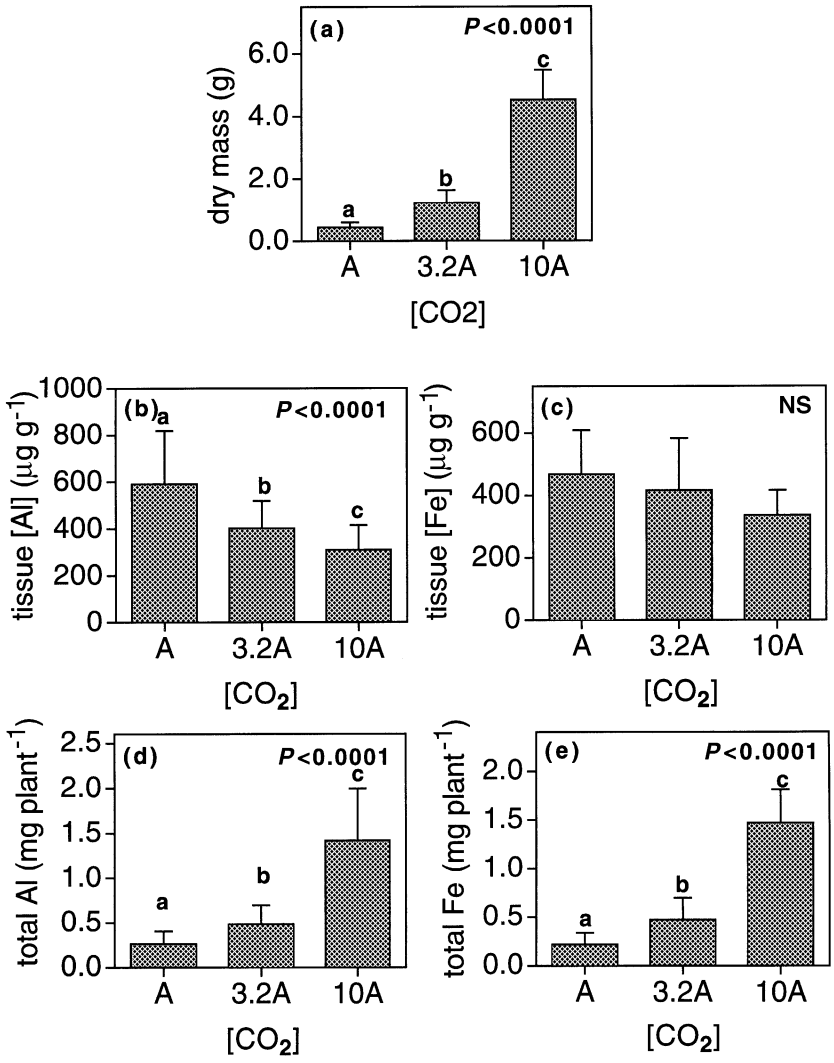

Fig. 1 Effects of CO_2 enrichment on: (a) dry mass; (b) tissue [Al]; (c) tissue [Fe]; (d) total Al; and (e) total Fe for *Vallisneria* shoots grown at pH 5. 'A' ('ambient') represents the free CO_2 concentration for water in equilibrium with the atmosphere. Means (+ 1 SD) for $n = 20$ (dry mass, Al) or 10 (Fe). P values from one-way ANOVAs on log-transformed data; means with differing letters differ significantly ($P < 0.05$) according to Tukey's Studentized range test. [Al] and [Fe] data of Titus *et al.* (1990) reanalysed with permission.

The biomass increase was proportionately so much greater than the tissue [Al] decrease that the total Al contained in *Vallisneria* shoots increased 5.5-fold ($P < 0.0001$) from 0.26 mg Al per plant at ambient CO_2 to 1.42 mg Al per plant at ten times ambient CO_2 (Fig. 1d). Similarly, total shoot Fe increased 7-fold ($P < 0.0001$) from 0.21 to 1.47 mg Fe per plant (Fig. 1e), largely because of the growth response to CO_2 enrichment.

In experiment B, dry mass increased about 15-fold with CO_2 enrichment on both low and high fertility sediments (Fig. 2a, b), whereas tissue [P] decreased 56% with low fertility sediment and 26% with relatively high fertility sediment (Fig. 2c, d). Again, the proportionate growth increase was so much greater than the decline in tissue [P] that total shoot P increased sharply ($P < 0.0001$ from one-way ANOVAs) on both sediments with CO_2 enrichment (Fig. 2e, f), approximately 6-fold with relatively infertile Big Moose sediment and 11-fold with more fertile Otsego sediment. These very highly significant CO_2 effects are supported by two-way ANOVA (Table 2).

Sediment type also significantly influenced dry mass (Table 2), which was about 30% higher with fertile sediment at both CO_2 levels (Fig. 2a, b). The consistent proportionate increase resulted in an insignificant $CO_2 \times$ sediment interaction for the log-transformed data. On the other hand, the much greater decline with CO_2 enrichment in tissue [P] with low fertility sediment than with high fertility sediment (Fig. 2c, d) resulted in a significant $CO_2 \times$ sediment interaction (Table 2). Because total shoot P increased much more sharply with CO_2 enrichment on high fertility sediment than on low fertility sediment (Fig. 2e, f), there

13652427, 1996, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2427.1996.00135.x by Mississippi State University, Wiley Online Library on [13/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

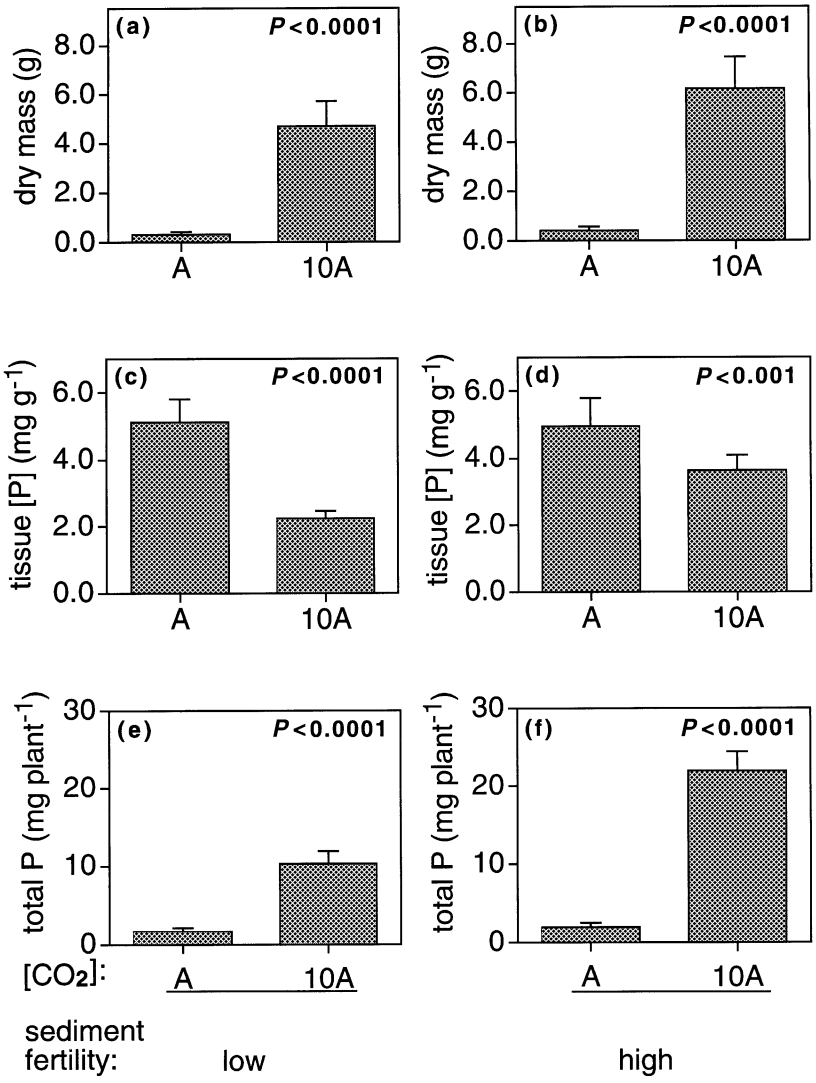

Fig. 2 Effects of CO_2 enrichment on: (a,b) dry mass; (c,d) tissue [P]; and (e,f) total P in *Vallisneria* shoots grown at pH 5 on low fertility (a,c,e: Big Moose Lake) or high fertility (b,d,f: Otsego Lake) sediments. 'A' ('ambient') represents the free CO_2 concentration for water in equilibrium with the atmosphere. Means (+ 1 SD) for n = 10. P values from one-way ANOVAs. [P] data of Titus (1992) reanalysed with permission.

was also a significant $CO_2 \times$ sediment interaction for total P (Table 2).

CO_2 interactions with pH and sediment: effects on growth, tissue [N] and N accumulation

Carbon dioxide enrichment produced 3.4- and 11-fold increases in dry mass at pH 5 with low and high fertility sediments, respectively, but only 1.6- and 2.0-fold increases at pH 7.3 (Fig. 3a, b). The significant effect of CO_2 enrichment, when expressed in proportional terms, was clearly much greater at low pH, as reflected in the significant $CO_2 \times$ pH interaction (Table 3). Sediment type also interacted with CO_2 enrichment (Table 3): plants grown on fertile sediment showed a much greater increase in dry mass with CO_2 enrichment than those grown on infertile sediment (compare Fig. 3b with 3a). The far greater growth response to CO_2 enrichment at pH 5 with fertile sediment (Fig. 3b) than at any other pH $\times$ sediment combination exemplifies the significant $CO_2 \times$ pH $\times$ sediment interaction (Table 3).

Tissue [N] consistently decreased with CO_2 enrichment at all pH $\times$ sediment combinations: by 58% and 47% for plants grown at pH 5 on low and high fertility sediments, respectively, and by 31% and 46% at pH 7.3 (Fig. 3c, d; Table 3). The CO_2 enrichment effect is reduced at high pH with infertile sediment relative to the other pH $\times$ sediment combinations (Table 3: significant $CO_2 \times$ pH $\times$ sediment interaction).

Carbon dioxide enrichment, pH and sediment type all had significant influences on total shoot N accumu-

13652427, 1996, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2427.1996.00135.x by Mississippi State University, Wiley Online Library on [13/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

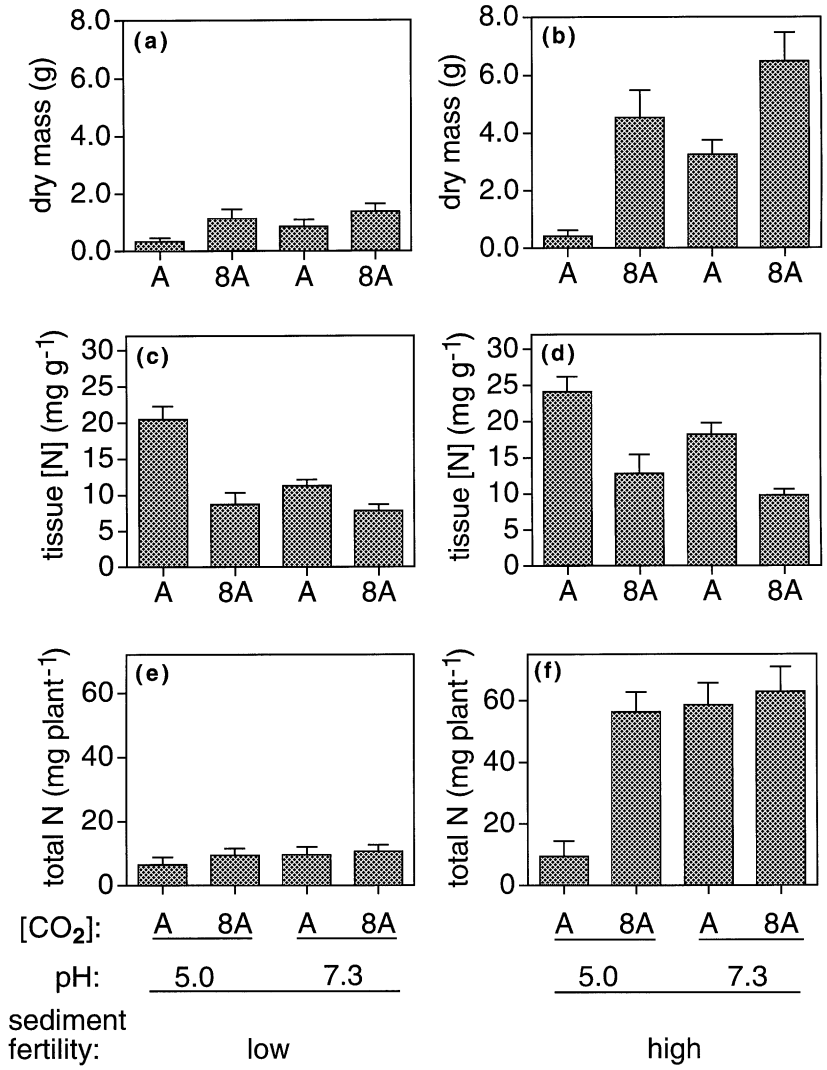

Fig. 3 Effects of CO_2 enrichment at pH 5 or pH 7.3 on: (a,b) dry mass; (c,d) tissue [N]; and (e,f) total N for *Vallisneria* shoots grown on low fertility (a,c,e: Big Moose Lake) or high fertility (b,d,f: Otsego Lake) sediments. 'A' ('ambient') represents the free CO_2 concentration for water in equilibrium with the atmosphere. Means (+ 1 SD) for n = 26–30 (pH 5) or 14–15 (pH 7.3).

lation by *Vallisneria* (Fig. 3e, f; Table 3), but the significant interactions are vital for interpreting CO_2 effects because both pH and sediment type modify the effect of CO_2 enrichment on N accumulation. The tissue [N] declines observed at pH 7.3 essentially compensated for the dry mass increases with CO_2 enrichment so that there was no effect of $[CO_2]$ on total shoot N content at that pH. In contrast, CO_2 enrichment greatly stimulated N accumulation at pH 5, particularly in *Vallisneria* grown on fertile sediment (Fig. 3f; $CO_2 \times$ pH $\times$ sediment interaction in Table 3).

Discussion

CO_2 enrichment: effects on Al, Fe and P accumulation

Others have shown that macrophytes accumulate sediment-derived metals in their shoots (Jackson *et al.*, 1994b), and that metal concentrations in macrophyte tissues may be greater at low pH (Grisé, Titus & Wagner, 1986). Our data suggest a far greater potential for macrophyte transport of metals from the sediment into the water column at pH 5 in higher CO_2 environments than at lower CO_2 concentrations. For this potential to be realized in nature: (i) high CO_2 environments must exist at low pH; (ii) there must be considerable rooted macrophyte production in such systems; and (iii) macrophyte tissues must decay to release constituent metals into the water column. Low pH lakes with CO_2 concentrations well in excess of atmospheric equilibrium values do exist (Titus *et al.*, 1990), and Wile *et al.* (1985) have reported high submersed macrophyte standing crop in a low pH lake, although standing crop surely varies among acidic lakes. Given eventual decay releasing minerals from macrophyte

13652427, 1996, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2427.1996.00135.x by Mississippi State University, Wiley Online Library on [13/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 2 Two-way ANOVA table summarizing effects of CO_2 (C) and sediment (S) on dry mass, tissue [P] and total P accumulation by *Vallisneria* shoots in experiment B. Initial weight class was used as a blocking variable. NS, $P > 0.05$

		Dry mass			Tissue [P]			Total P		
Source	df	Mean square	*F*	*P*	Mean square	*F*	*P*	Mean square	*F*	*P*
CO_2	1	74.9	1634	< 0.0001	0.439	156	< 0.0001	20.6	1069	< 0.0001
Sediment	1	0.62	13.6	< 0.001	0.038	13.5	< 0.001	3.56	185	< 0.0001
C × S	1	0.006	0.1	NS	0.062	22	< 0.0001	3.18	165	< 0.0001
Weight class	4	0.49	10.7	< 0.0001	0.009	3.4	< 0.05	0.058	3	< 0.05
Error	32	0.046			0.003			0.019		

shoots (Jackson, Rasmussen & Kalff, 1994a), the potential exists for high CO_2 concentrations to stimulate macrophyte transport of metals from sediments into the water, provided that metals are available in the sediment (Jackson *et al.*, 1991).

The potential for CO_2 enrichment to enhance rates of P 'pumping' (Carignan & Kalff, 1980) by macrophytes from the sediment also exists at low pH. The release of P to the water column upon subsequent macrophyte senescence and decay may contribute to water column enrichment (Carpenter, 1980; Landers, 1982). It is also clear that the quantitative significance of P pumping depends on sediment type. We observed greater P accumulation in plants grown on Otsego sediment, which had significantly higher porewater P concentrations (Table 1). Lower P availability in Big Moose sediments may have restricted P pumping by plants grown on those sediments, especially at high CO_2.

CO_2 interactions with pH and sediment

Effects on growth. The greater impact of CO_2 enrichment on dry mass accumulation at pH 5 than at pH 7.3 probably results from the relative abundance of HCO_3^- at pH 7.3: without CO_2 enrichment, much more DIC is available for photosynthesis at pH 7.3 than at pH 5, so that DIC (including CO_2) is apparently less limiting to growth. The greater dry mass at ambient CO_2 and pH 7.3 relative to pH 5 on both sediments supports this interpretation. The limited growth response to CO_2 enrichment at pH 7.3 appears to be in line with Barko *et al.* (1991b), who reported greater sensitivity of this species to light intensity and sediment fertility than to DIC enrichment at relatively high pH.

Mineral nutrient availability may limit growth responses to CO_2 enrichment, as suggested for other systems (e.g. Oechel *et al.*, 1994). Our finding of a disproportionate effect of CO_2 enrichment with the fertile sediment *v* the infertile sediment, however, is puzzling because it opposes an earlier report of proportionate CO_2 enrichment effects on growth of the same species with sediment from the same two sites (Titus, 1992). In particular, *Vallisneria* grown on infertile Big Moose sediment at high CO_2 attained much greater biomass in the earlier experiment than in this experiment. Sediment heterogeneity within the collection site may account for this puzzle. A second possibility is that the HNO_3 used for pH control in the earlier experiment (but not in our experiment C) countered mineral nutrient limitation.

Plants at pH 5 on fertile sediment may have been particularly responsive to CO_2 enrichment because of acute DIC limitations accompanying low pH, and the availability of mineral nutrients to support greatly increased growth.

Effects on tissue [N]. We report sharp declines in tissue [N] with CO_2 enrichment, as opposed to the increases previously reported (Titus, 1992), and greater than any decreases we have noted in the literature on terrestrial plants. We now suspect that plants in the earlier experiment were able to absorb considerable NO_3^- because of our use of HNO_3 to control pH, although we have no explanation for why tissue [N] should be greater in the more rapidly growing, high CO_2 treatment plants. The sharp declines we report here may be due to 'growth dilution' of N as carbohydrates accumulate in high DIC environments, and may arise because of the wide range of DIC concentrations we used. Use of a wide range, however, is supported by existing variations in DIC regimes among freshwater systems (Titus *et al.*, 1990; Rebsdorf *et al.*, 1991). The consequences of a sharp decline in tissue [N] for

13652427, 1996, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2427.1996.00135.x by Mississippi State University, Wiley Online Library on [13/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 3 Three-way ANOVA table summarizing effects of CO_2 (C), pH (P), sediment (S) and interactions on dry mass, tissue [N] and total N accumulation by *Vallisneria* shoots in experiment C. The *F* value for the main effect of CO_2 is based on the mean square for tanks nested within CO_2 levels. All other effects were tested with the error mean square as the denominator. NS, $P > 0.05$

		Dry mass			Tissue [N]			Total N		
Source	df	Mean square	*F*	*P*	Mean square	*F*	*P*	Mean square	*F*	*P*
CO_2	1	17.7	31	< 0.05	3.48	46	< 0.05	13600	100	< 0.01
Tank	2	0.565			0.076			136		
pH	1	6.36	331	< 0.0001	0.673	289	< 0.0001	6950	313	< 0.0001
Sediment	1	8.84	460	< 0.0001	0.743	319	< 0.0001	50900	2295	< 0.0001
C × P	1	3.24	168	< 0.0001	0.122	53	< 0.0001	4690	212	< 0.0001
C × S	1	1.63	85	< 0.0001	0.007	3	NS	9340	422	< 0.0001
P × S	1	0.804	42	< 0.0001	0.010	4	< 0.05	5760	260	< 0.0001
C × P × S	1	0.532	28	< 0.0001	0.101	43	< 0.0001	3970	179	< 0.0001
Error	159	0.019			0.002			22		

photosynthetic rates (Madsen & Baattrup-Pedersen, 1995), herbivory (Fajer, Bowers & Bazzaz, 1989) and decomposition rates (Enriquez *et al.*, 1993) may be substantial.

The greater impact of CO_2 enrichment on tissue [N] at pH 5 than at pH 7.3 can be accounted for by growth dilution, in that a much greater growth response to CO_2 enrichment was observed at pH 5 than at pH 7.3.

A final observation on tissue [N] data is that the concentrations we found at high CO_2 (and at ambient CO_2 on low fertility sediment at pH 7.3) are at or below the value reported to limit *Vallisneria* growth (13 mg g^{-1}; Gerloff & Krombholz, 1966), yet these values are from slowly and rapidly growing plants alike. Even the large plants grown on fertile sediment at pH 7.3 and high CO_2, however, may not have been growing rapidly at the experiment's end. Overath *et al.* (1991) make the related point that low tissue nutrient concentrations may arise both in low nutrient, low DIC environments associated with slow macrophyte growth and in high nutrient, high DIC environments associated with rapid growth.

Effects on N accumulation. The 6-fold increase in shoot N accumulation with CO_2 enrichment at pH 5 on fertile sediment was clearly driven by the 11-fold increase in dry mass for these plants, only partially countered by their 47% decline in tissue [N]. The limited increase in N accumulation by *Vallisneria* shoots in response to CO_2 enrichment at other combinations of pH and sediment type may be due to depletion of sediment N by the plants: quantities of N in shoots at the experiment's end were similar to total exchangeable NH_4^+ in pots without plants. This seems particularly likely for plants grown on low fertility sediment, in which exchangeable NH_4^+ was depleted *c*. 70% from already low values (data not shown). Despite the relatively large sediment volume (3 l) for the relatively small plants grown on the low fertility sediment, a greater sediment volume might have enabled greater N transport from the sediment into macrophyte shoots.

In any case, it is clear that the degree of sediment N depletion depends on water chemistry and that CO_2 enrichment has great potential to enhance N accumulation by macrophyte shoots, but only under selected conditions: the greatest CO_2 effect by far occurred at low pH with the more fertile Otsego sediment (Fig. 3f). Conversely, we can expect the least effect of high CO_2 concentration on macrophyte N accumulation at relatively high pH with relatively infertile sediments.

Conclusion

We conclude that CO_2 enrichment stimulates *Vallisneria* growth to a much greater degree at pH 5 than at pH 7.3, and results in a sharp reduction in tissue [N] under all conditions tested. We also conclude that CO_2 enrichment stimulates greater metal and mineral nutrient accumulation in *Vallisneria* shoots at pH 5 on different sediments, although not at pH 7.3. The response of mineral accumulation to CO_2 enrichment roughly parallels the response of biomass accumula-

13652427, 1996, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2427.1996.00135.x by Mississippi State University, Wiley Online Library on [13/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

tion: conditions more favourable to growth such as higher CO_2 concentration, higher HCO_3^- concentration, or more fertile sediment often (but not always) effected greater mineral accumulation in *Vallisneria* shoots. Because DIC regimes vary widely among freshwater ecosystems (e.g. Titus *et al.*, 1991; Rebsdorf *et al.*, 1991), macrophyte shoot accumulation of sediment-derived minerals should also vary widely due to water chemistry alone.

Acknowledgments

We thank David Grisé and Drs Richard Feldman and Gary Sullivan for assistance, and Dr Leland Jackson for constructive comments on an earlier draft of the manuscript. This material is based in part upon work supported by the National Science Foundation under Grant No. BSR 8506730.

References

Allen S.E., Grimshaw H.M. & Rowland H.P. (1986) Chemical analysis. *Methods in Plant Ecology*, 2nd edn (Eds P.D. Moore and S.B. Chapman), pp. 285–344. Blackwell Scientific, Oxford.

Barko J.W. & Smart R.M. (1980) Mobilization of sediment phosphorus by submersed freshwater macrophytes. *Freshwater Biology*, **10**, 229–238.

Barko J.W., Gunnison D. & Carpenter S.R. (1991a) Sediment interactions with submersed macrophyte growth and community dynamics. *Aquatic Botany*, **41**, 41–65.

Barko J.W., Smart R.M. & McFarland D.G. (1991b) Interactive effects of environmental conditions on the growth of submersed aquatic macrophytes. *Journal of Freshwater Ecology*, **6**, 199–207.

Bremner J.M. (1965) Inorganic forms of nitrogen. *Methods of Soil Analysis*, Part 2 (Ed. C.A. Black), pp. 1179–1237. American Society of Agronomy, Madison, WI.

Carignan R. & Kalff J. (1980) Phosphorus sources for aquatic weeds: water or sediment. *Science*, **207**, 987–989.

Carpenter S.R. (1980) Enrichment of Lake Wingra, Wisconsin, by submersed macrophyte decay. *Ecology*, **61**, 1145–1155.

Cataldo D.A., Haroon M., Schrader L.E. & Youngs V.L. (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis*, **6**, 71–80.

Coleman J.S., Rochefort L., Bazzaz F.A. & Woodward F.I. (1991) Atmospheric CO_2, plant nitrogen status and the susceptibility of plants to an acute increase in temperature. *Plant Cell Environment*, **14**, 667–674.

Enriquez S., Duarte C.M. & Sand-Jensen K. (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C : N : P content. *Oecologia*, **94**, 457–471.

Fajer E.D., Bowers M.D. & Bazzaz F.A. (1989) The effects of enriched carbon dioxide atmospheres on plant–insect herbivore interactions. *Science*, **243**, 1198–2000.

Field C. & Mooney H.A. (1986) The photosynthesis–nitrogen relation in wild plants. *On the Economy of Plant Form and Function* (Ed. T.J. Givnish), pp. 25–55. Cambridge University Press, Cambridge.

Gerloff G.C. & Krombholz P.H. (1966) Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. *Limnology and Oceanography*, **11**, 529–537.

Godshalk G.L. & Wetzel R.G. (1978) Decomposition of aquatic angiosperms. II. Particulate components. *Aquatic Botany*, **5**, 301–327.

Grisé D., Titus J.E. & Wagner D.J. (1986) Environmental pH influences growth and tissue chemistry of the submersed macrophyte *Vallisneria americana*. *Canadian Journal of Botany*, **64**, 306–310.

Jackson L.J., Rasmussen J.B., Peters R.H. & Kalff J. (1991) Empirical relationships between the element composition of aquatic macrophytes and their underlying sediments. *Biogeochemistry*, **12**, 71–86.

Jackson L.J., Rasmussen J.B. & Kalff J. (1994a) A mass-balance analysis of trace metals in two weedbeds. *Water Air and Soil Pollution*, **75**, 107–119.

Jackson L.J., Rowan D.J., Cornett R.J. & Kalff J. (1994b) *Myriophyllum spicatum* pumps essential and non-essential trace elements from sediments to epiphytes. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 1769–1773.

James B.R., Clark C.J. & Riha S.J. (1983) An 8-hydroxyquinoline method for labile and total aluminum in soil extracts. *Soil Science Society of America Journal*, **47**, 893–897.

Kempers A.J. & Zweers A. (1986) Ammonium determination in soil extracts by the salicylate method. *Communications in Soil Science and Plant Analysis*, **17**, 715–723.

Landers D.H. (1982) Effects of naturally senescing aquatic macrophytes on nutrient chemistry and chlorophyll *a* of surrounding waters. *Limnology and Oceanography*, **27**, 428–439.

Lincoln D.E., Couvet D. & Sionit N. (1986) Response of an insect herbivore to host plants grown in carbon dioxide enriched atmospheres. *Oecologia*, **69**, 556–560.

Madsen T.V. & Baattrup-Pedersen A. (1995) Regulation of growth and photosynthetic performance in *Elodea*

13652427, 1996, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2427.1996.00135.x by Mississippi State University, Wiley Online Library on [13/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

canadensis in response to inorganic nitrogen. *Functional Ecology*, **9**, 239–247.

Murphy J. & Riley J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31–36.

Oechel W.C., Cowles S., Grulke N., Hastings S.J., Lawrence B., Prudhomme T., Riechers G., Strain B., Tissue D. & Vourlitis G. (1994) Transient nature of CO_2 fertilization in arctic tundra. *Nature*, **371**, 500–503.

Overath R.D., Titus J.E., Hoover D.T. & Grisé D. (1991) The influence of field site and natural sediments on the growth and tissue chemistry of *Vallisneria americana* Michx. *Journal of Freshwater Ecology*, **6**, 135–145.

Rattray M.R., Howard-Williams C. & Brown J.M.A. (1991) The photosynthetic and growth rate responses of two freshwater angiosperms in lakes of different trophic status: responses to light and dissolved inorganic carbon. *Freshwater Biology*, **25**, 399–407.

Rebsdorf A., Thyssen N. & Erlandsen M. (1991) Regional and temporal variation in pH, alkalinity, and carbon dioxide in Danish streams, related to soil type and land use. *Freshwater Biology*, **25**, 419–435.

SAS Institute, Inc. (1985) *SAS User's Guide: Statistics*, version 5 edn. SAS Institute, Cary, NC.

Sculthorpe C.D. (1967) *The Biology of Aquatic Vascular Plants*. Edward Arnold, London.

Smart R.M. & Barko J.W. (1985) Laboratory culture of submersed freshwater macrophytes on natural sediments. *Aquatic Botany*, **21**, 251–263.

Smith C.S. & Adams M.S. (1986) Phosphorus transfer from sediments by *Myriophyllum spicatum*. *Limnology and Oceanography*, **31**, 1312–1321.

Steeman Nielsen E. (1947) Photosynthesis of aquatic plants with special reference to the carbon-sources. *Dansk Botanisk Arkiv*, **12**, 1–71.

Titus J.E. (1992) Submersed macrophyte growth at low pH. II. CO_2–sediment interactions. *Oecologia*, **92**, 391–398.

Titus J.E. & Stephens M.D. (1983) Neighbor relations and seasonal growth patterns for *Vallisneria americana* in a mesotrophic lake. *Oecologia*, **56**, 23–29.

Titus J.E. & Stone W.H. (1982) Photosynthetic response of two submersed macrophytes to dissolved inorganic carbon concentration and pH. *Limnology and Oceanography*, **27**, 151–160.

Titus J.E., Feldman R.S. & Grisé D. (1990) Submersed macrophyte growth at low pH. I. CO_2 enrichment effects with fertile sediment. *Oecologia*, **84**, 307–313.

Welsh R.P.H. & Denny P. (1979) The translocation of lead and copper in two submerged aquatic angiosperm species. *Journal of Experimental Botany*, **30**, 339–345.

Wetzel R.G., Brammer E.S., Lindström K. & Forsberg C. (1985) Photosynthesis of submersed macrophytes in acidified lakes II. Carbon limitation and utilization of benthic CO_2 sources. *Aquatic Botany*, **22**, 107–120.

Wile I., Miller G.E., Hitchen G.G. & Yan N.D. (1985) Species composition and biomass of the macrophyte vegetation of one acidified and two acid-sensitive lakes in Ontario. *Canadian Field Naturalist*, **99**, 308–312.

(*Manuscript accepted 7 August 1996*)

13652427, 1996, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2427.1996.00135.x by Mississippi State University, Wiley Online Library on [13/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License