Freshwater Biology (2009) **54,** 1819–1831 doi:10.1111/j.1365-2427.2009.02229.x

Could rising aquatic carbon dioxide concentrations favour the invasion of elodeids in isoetid-dominated softwater lakes?

P. SPIERENBURG*,‡, E.C.H.E.T. LUCASSEN†, A.F. LOTTER‡ AND J.G.M. ROELOFS*
Department of Aquatic Ecology and Environmental Biology, Radboud University Nijmegen, Nijmegen, The Netherlands
†B-WARE Research Centre, Radboud University Nijmegen, Nijmegen, The Netherlands
‡Institute of Environmental Biology, Palaeoecology, Utrecht University, Utrecht, The Netherlands

SUMMARY

1. During the past century, isoetid vegetation types in softwater lakes have often been invaded by faster-growing elodeids. In these C-limited systems, this may be related to rising aquatic CO_2 levels.
2. In a laboratory experiment we tested the growth response of two elodeid species, *Myriophyllum alterniflorum* and *Callitriche hamulata*, at four different CO_2 levels, ranging from 20 to 230 μmol L^{-1}. In addition, we tested the effect of the nutrient status of the sediment on the growth of *C. hamulata* at the different CO_2 levels.
3. Shoot and root growth increased with rising CO_2 availability. Irrespective of sediment type, growth was minimal to negative at the lowest CO_2 treatment level, while becoming positive at CO_2 levels around 40–50 μmol L^{-1}. Substantial growth was only obtained when the macrophytes were growing on mesotrophic sediments. The plants reached close to maximal growth at CO_2 levels of *c.* 100 μmol L^{-1}.
4. Within this experiment, the growth of *C. hamulata* at CO_2 levels above 90 μmol L^{-1} may have been limited by N and P availability in both sediment types. The growth rate of *M. alterniflorum* did not seem to be limited by N and P availability, most likely due to its much higher relative root production.
5. The experimental results show that neither *M. alterniflorum* nor *C. hamulata* is able to invade isoetid-dominated softwater lakes at very low aquatic CO_2 concentrations. However, if the sediments contain enough nutrients, a rise in aquatic CO_2 could allow the invasion of elodeid species leading to the subsequent disappearance of slow-growing isoetids.

Keywords: CO_2 increase, *Callitriche hamulata*, macrophyte growth, *Myriophyllum alterniflorum*, softwater lakes

Introduction

Softwater lakes frequently occur in boreal and temperate regions and at higher elevations in the subtropics, mostly on poorly buffered siliceous bedrock or on non-calcareous sandy soils (Gacia *et al*., 1994; Murphy, 2002). They are mainly fed by rainwater and shallow local groundwater flows, are generally nutrient-poor and contain very low amounts of dissolved inorganic carbon (DIC) in the water column. Macrophyte production in such lakes is often C-limited (Murphy, 2002). The HCO_3^- concentration in softwater lakes is generally below 200 μmol L^{-1}. Such low HCO_3^- concentrations would probably not contribute substantially to the C pool needed for photosynthesis by macrophytes, able of using HCO_3^- additionally to

---

Correspondence: Peter Spierenburg, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ, Nijmegen, The Netherlands. E-mail: p.spierenburg@science.ru.nl

CO_2 as a C source (Madsen, Maberly & Bowes, 1996). Consequently, to meet their C requirements, macrophytes in softwater lakes have to rely on the uptake of CO_2, which is only available in low concentrations down to 6 μmol L^{-1} (Robe & Griffiths, 1992; Murphy, 2002). The submerged vegetation in pristine softwater lakes is therefore dominated by macrophytes that are adapted to low C availability, including isoetid species, such as *Isoëtes* spp., *Lobelia dortmanna* L., *Littorella uniflora* (L.) Asch. and *Eriocaulon aquaticum* (Hill) Druce. To overcome the nutrient and C stress, isoetids possess several morphological and physiological adaptations, such as the ability to utilise pore water CO_2, and a crassulacean acid metabolism in several *Isoëtes* species and *L. uniflora* (Wium-Andersen, 1971; Søndergaard & Sand-Jensen, 1979; Richardson *et al.*, 1984; Keeley, 1998). However, as isoetids have low production rates, they might easily be outcompeted by faster-growing elodeid species, such as *Myriophyllum alterniflorum* D.C. and *Callitriche hamulata* Kütz. ex W.D.J. Koch, which are quite common species in unproductive lakes with a low alkalinity, provided nutrient and C availability is sufficient (Sand-Jensen & Søndergaard, 1979; Roelofs, Schuurkes & Smits, 1984; Nielsen & Sand-Jensen, 1997).

During the past century, isoetid-dominated vegetation types have seriously declined and have been replaced by faster-growing elodeid species. This change has previously been attributed to increased nutrient availability (increased anthropogenic eutrophication) and/or acidification as an effect of increased sulphur and nitrogen-depositions (Roelofs, 1983; Arts & Leuven, 1988; Roelofs *et al.*, 1995; Brouwer, Bobbink & Roelofs, 2002; Smolders, Lucassen & Roelofs, 2002; Pedersen *et al.*, 2006). However, eutrophication in itself might not fully explain the decline of isoetid vegetation and the appearance of elodeids. An extensive survey focussing on the distribution of macrophytes in relation to water chemistry in the Netherlands showed that *M. alterniflorum* and *L. uniflora* appeared at similar aquatic phosphate levels, with an average of 1.1 μmol L^{-1} (de Lyon & Roelofs, 1986). *Callitriche hamulata* even preferred waters with lower phosphate levels, with an average of 0.5 μmol L^{-1}. Dense stands of *M. alterniflorum* and *C. hamulata* are found in SW Norwegian softwater lakes with orthophosphate (PO_4^{3-}) levels between 0.1 and 0.3 μmol L^{-1} (P. Spierenburg & E.C.H.E.T. Lucassen, unpubl. data). Moreover, Roelofs *et al.* (1984) observed in an experimental setup that at higher aquatic phosphate levels (>0.5 μmol L^{-1}), *M. alterniflorum* was extensively covered by epiphytes, and eventually died off.

Because macrophyte production in many softwater lakes is C-limited (Murphy, 2002), an increase in aquatic CO_2 levels might also be responsible for the replacement of isoetids by faster-growing macrophytes, such as *Juncus bulbosus* L., *M. alterniflorum* and *C. hamulata*.

Aquatic CO_2 concentrations in softwater lakes might rise as a result of liming of lakes or their catchment area, followed by reacidification of the lake water. When softwater lakes in SW Norway reacidified after liming, the elevated CO_2 and NH_4^+ availability resulted in luxurious growth of *J. bulbosus* (Schuurkes *et al.*, 1987; Roelofs, Brandrud & Smolders, 1994; Lucassen *et al.*, 1999), while in Sweden, liming of softwater lakes has led to massive expansions of *M. alterniflorum* (Roelofs, 1983; Dickson *et al.*, 1995; Brandrud, 2002).

Besides the rise in atmospheric CO_2, various other factors, such as rising temperatures, liming, decreasing soil water pH and changes in hydrology can all result in higher dissolved organic carbon (DOC) inputs, from terrestrial sources, like wetlands and forests in lakes (Freeman *et al.*, 2001, 2004; Hudson, Dillon & Somers, 2003; Evans *et al.*, 2006; Fenner *et al.*, 2007a,b). Indeed, DOC concentrations in lakes and streams in the U.K., the U.S.A. and many north-west European countries have increased considerably over the past 15 years, and are expected to increase even more in the near future (Evans, Monteith & Cooper, 2005; Skjelkvale *et al.*, 2005) and a strong positive correlation has been found between lake DOC and CO_2 concentrations (e.g. Hope, Kratz & Riera, 1996; Sobek, Tranvik & Cole, 2005).

The aquatic CO_2 concentrations in many pristine softwater lakes are in equilibrium with the atmosphere or just slightly oversaturated (Dillon & Molot, 1997; Riera, Schindler & Kratz, 1999; Jonsson, Karlsson & Jansson, 2003). Especially during summer days, CO_2 levels can drop far below the saturation point as a result of CO_2 uptake by primary producers (J.G.M. Roelofs, unpubl. data). Hence, it is expected that the predicted rise in atmospheric CO_2 concentrations to levels of 720–1020 ppm in the year 2100 (Meehl *et al.*, 2007) will result in increased aquatic CO_2 levels. From

Henry's law with temperature correction ($K_H^{\ominus}$ = 0.034 M atm^{-1}, K_H = 0.041 M atm^{-1}), a maximum aquatic CO_2 increase to levels of around 40–50 μmol L^{-1} can be calculated at temperatures of 20–10 °C.

Softwater lakes usually contain nutrient-poor water, with the sediment as the most important nutrient source for macrophytes (Carignan & Kalff, 1980; Brouwer *et al.*, 2002). Because the nutrient requirements of elodeids are generally higher than those of isoetids, it is expected that rising CO_2 availability will mainly result in the appearance of elodeid species in those softwater lakes with sediments containing higher (mesotrophic) nutrient concentrations.

In the present study we undertook a laboratory experiment to test the effect of elevated aquatic CO_2 on the growth of two elodeid softwater species on sediments of different nutrient status. We grew plants at four different aquatic CO_2 concentrations, ranging from close to ambient atmospheric equilibrium conditions (20 μmol L^{-1} CO_2 in the water column) to a 10-fold increase. We hypothesised that elodeid production rate would increase under elevated CO_2 levels, especially if the sediment is sufficiently rich in nutrients to support extensive growth. Extensive growth of the elodeids may imply that, under similar CO_2 and sediment conditions, isoetid vegetation can be invaded or even replaced by faster-growing elodeid macrophytes, with possible consequences for the whole softwater ecosystem.

Methods

Experimental design

The growth rates of two elodeid species, *M. alterniflorum* and *C. hamulata*, were tested at four aquatic CO_2 levels and on two sediment types. To this end, 48 glass containers (length: 26 cm, width: 13 cm, height: 32 cm) were placed in a water bath at a constant temperature (19 °C ± 1 °C), regulated by means of a recirculation chiller (NESLAB Merlin M-75; Thermo Scientific, Newington, NH, U.S.A.). Irradiance was provided by six 400 W HPS lamps (Hortilux-Schréder, Monster, The Netherlands) for a photoperiod of 16 h at PAR (400–700 nm) of 235 μmol m^{-2} s^{-1}.

Thirty-two glass containers were filled with 8 cm fresh sediment (2.7 L). A nutrient-enriched sediment was obtained by using a mixture of sediments from two softwater lakes in the 'Valkenberg' nature reserve (the Netherlands, 51°27′N, 6°12′E). The remaining 16 glass containers were filled with fresh sediment from the 'Broekse Wielen' softwater lake (the Netherlands, 51°43′N, 5°46′E). Based on the organic matter and nutrient contents, the sediment mixture from 'Valkenberg' was defined as mesotrophic, whereas the sediment from 'Broekse Wielen' was defined as oligotrophic (Table 1). Both sediments are representative of natural situations in softwater lakes. The overlying water column was created using an oligotrophic culture medium (Table 2). The water column (6.8 L per glass container) in the glass containers was continuously refreshed (3.6 L day^{-1}) with culture medium, pumped into the aquaria by peristaltic pumps (Masterflex, 7015–20; Cole-Parmer, Vernon Hills, IL, U.S.A.) from black covered 110 L storage containers. Black tubing was used to prevent algal growth within the tubes. The water level in the glass containers was kept constant at 20 cm above the sediment, using an overflow system.

Healthy specimens of *M. alterniflorum* were collected from Lake 'De Nieuwe Wiel' (the Netherlands, 51°41′N, 5°11′E, CO_2 concentration in the water column on 2 October 2007: 75 μmol L^{-1}), whereas

Table 1 Properties (mean) of the two sediment types at the start of the experiment

Parameter	Mesotrophic	Oligotrophic
Water content (%FM)	21 (0.6)	16 (1.3)
Total organic matter (%DM)	1.1 (0.2)	0.5 (0.2)
PO_4^{3-} (μmol kg DM^{-1})	514 (23)	70 (5)
NO_3 (μmol kg DM^{-1})	88 (11)	63 (13)
NH_4 (μmol kg DM^{-1})	43 (6)	63 (20)

The values within parentheses are expressed as SE.

Table 2 Chemical composition (μmol L^{-1}) of the culture medium used in the experiment

Constituent	Concentration
Na	224
K	11
Mg	24
Ca	29
Cl	175
HCO_3^-	20
SO_4^{2-}	49
NH_4^+	2
NO_3^-	23
PO_4^{3-}	<0.1

C. hamulata was collected from Lake Valkenberg (CO_2 in the water column on 4 October 2007: 50 μmol L^{-1}). All plants were washed and epiphytic algae were removed.

Half of the glass containers with mesotrophic sediments were each planted with 10 apical, rootless shoots of *M. alterniflorum* (length 8 cm), while the other half were each planted with ten apical rootless shoots of *C. hamulata* (length 8 cm). *C. hamulata* was only planted in the glass containers with oligotrophic sediments (10 apical shoots, 8 cm). The mean (±SE) fresh masses (FM, g) per glass container were 2.10 ± 0.04 and 0.46 ± 0.01 for *M. alterniflorum* and *C. hamulata*, respectively, corresponding to mean (±SE) initial dry masses (DM, g) of $0.13 \pm 2.3\ 10^{-3}$ and $0.02 \pm 0.35\ 10^{-3}$ for *M. alterniflorum* and *C. hamulata*, respectively.

To create four different CO_2 levels in the water column, compressed air was mixed with pure CO_2 at four constant proportions by means of mass flow controllers (EL-FLOW select F201CV; Bronkhorst, Veenendaal, the Netherlands). Before mixing, the compressed air was scrubbed with sodalime to remove CO_2 and subsequently filtered over a 15 μm inline filter.

Each glass container was randomly assigned to one of the four treatments and aerated with a fixed flow of 0.15 L min^{-1} with the appropriate compressed air/CO_2 mixture. Each plant species, sediment type and treatment combination was replicated four times.

Water measurements

During the experiment, water pH was measured using a Cyberscan pH 300 series pH meter (Eutech, Singapore) with a double Ag/AgCl reference pH electrode (HI 1043; Hanna Instruments, IJsselstein, The Netherlands). Every 4 days, 1 mL water samples were taken from all containers and immediately analysed for DIC using an infrared carbon analyser (Advance Optima, ABB, Cary, NC, U.S.A.). CO_2 levels were calculated from the DIC concentrations and the pH according to Stumm & Morgan (1996).

The contents of Ca, Fe, K, Na, Mg and total-P (TP) in water were determined by taking samples after 5 days in 20 mL acid-rinsed, iodised polyethylene terephthalate (PET) bottles and preserving them by adding 10 μL HNO_3 (65%) per mL. The measurements were made using an inductively-coupled plasma emission spectrophotometer (ICP-OES model IRIS Intrepid II XDL; Thermo Fisher Scientific, Waltham, MA, U.S.A.). NH_4^+, NO_3^-, PO_4^{3-} and Cl^- in the water were determined by taking samples in 20 mL acid-rinsed, iodised PET bottles and preserving them with 4 μL citric acid (650 μM) per mL. NO_3^-, NH_4^+ and PO_4^{3-} were colorimetrically determined using hydrazine sulphate, salicylate and ammonium molybdate on an Auto Analyser (model III, Bran & Luebbe, Nordstedt, Germany). Chloride was determined by flame photometry (Auto Analyser, model III, Bran & Luebbe).

Plant measurements

The plants were harvested after 40 days, separating roots and shoots. Shoot length, the number of branches, FM (g) and DM (g, 24 h, 70 °C) were determined. Root FM was determined, and root plaques were removed using the cold-DCB method (18 °C) developed by Taylor & Crowder (1983), before the root DM (24 h, 70 °C) was determined.

All plant material was ground in liquid N. Ground-up shoot (1 mg) and root (0.3 mg) material was digested in 4 mL HNO_3 (65%) and 1 mL H_2O_2 (35%), using an Ethos D microwave (Milestone, Sorisole Lombardy, Italy) (Kingston & Haswell, 1997). The digestives were analysed for elemental composition with an ICP-OES (model IRIS Intrepid II XDL, Thermo Fisher Scientific). C and N contents of ground-up dry plant material, weighed in pressed, ultralight-weight tin capsules, were analysed by an elemental analyser (EA 1110, Carlo Erba; Thermo Fisher Scientific).

The relative growth rate (RGR, day^{-1}) of the shoots was calculated as $RGR = (\ln W_{sf} – \ln W_{si})(t)^{-1}$, where W_{si} is the initial and W_{sf} the final DM (g) of the shoots. To be able to calculate the RGR of roots a modified equation was used: $RGR = [\ln(W_{si} + W_{rf}) – \ln W_{si}](t)^{-1}$, where W_{rf} is the final DM (g) of the roots and, since all plant material started without roots, equals the gain in biomass during the experiment.

Statistical analysis

All statistics were performed using SPPS 14.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.). Care was taken that the test assumptions were met. Differences in CO_2 levels in the water column were tested using a one-way ANOVA followed by a Games–Howell *post hoc* procedure (as population variance was not equal between groups). The influence of species or sediment type on the CO_2 levels in the water column was tested with an independent factorial ANOVA. Growth responses in relation to CO_2 levels were fitted using curve estimation regression with an inverse or logarithmic model, and subsequently tested with an ANOVA or *t*-test. Relations between three variables e.g. RGR, sediment type and CO_2 level, were tested using ANCOVA.

Results

Aquatic CO_2

The CO_2 levels differed significantly between all treatments (one-way ANOVA, $P < 0.05$; Games–Howell $P < 0.05$, Table 3). The variation in CO_2 concentrations within each container was low (mean SE < 5%). Containers holding mesotrophic sediment showed slightly but significantly higher mean CO_2 levels (independent factorial ANOVA, $P = 0.01$). There was no effect of macrophyte species on mean CO_2 levels (independent factorial ANOVA, $P = 0.71$).

Surface and pore water

The mean chemical composition of the water after 5 days is given in Table 4. The water above the mesotrophic sediment showed elevated concentrations of PO_4^{3-}, TP and Fe, whereas NO_3^-, NH_4^+ and Mg^{2+} were slightly higher in the water above oligotrophic sediments (independent *t*-test, $P < 0.05$).

Biomass production

Growth of shoots and roots increased at higher CO_2 levels (Table 5, Fig. 1). The RGR of the shoots best fitted an inverse model ($R^2 = 0.87$–0.96; ANOVA; $P < 0.05$, Fig. 1a), with the curve levelling off at around 100 μmol CO_2 L^{-1} for plants growing on mesotrophic sediments and around 50 μmol L^{-1} for *C. hamulata* plants on the oligotrophic sediment. The increase in the RGR of *C. hamulata* at higher CO_2 levels was especially marked on the mesotrophic sediment (Fig. 1a). Shoot production of *C. hamulata*, expressed as RGR, was significantly higher on mesotrophic sediment (ANCOVA, $P_{\text{corrected model}} < 0.001$, $P_{\text{treatment}} < 0.001$, $P_{\text{sediment}} < 0.001$). The absolute biomass gain during the experiment was quite similar for *M. alterniflorum* and *C. hamulata* on mesotrophic sediment (Table 5, ANCOVA, $P_{\text{corrected model}} < 0.001$, $P_{\text{treatment}} < 0.001$, $P_{\text{species}} \leq 0.001$).

With higher CO_2 availability, the plants generally produced more branches. Apart from the lowest CO_2 treatment, where there was hardly any biomass production, the number of branches per cm of the plant was higher at elevated CO_2 concentrations (Table 5, ANOVA, *C. hamulata* on oligotrophic sediment, $P = 0.02$, *C. hamulata* on mesotrophic sediment $P = 0.04$, *M. alterniflorum* on mesotrophic sediment $P = 0.01$).

The RGR of roots increased logarithmically with CO_2 level (Fig. 1b). The RGR of *M. alterniflorum* was lower than that of *C. hamulata* on mesotrophic sediment (ANCOVA, $P_{\text{corrected model}} < 0.001$, $P_{\text{treatment}} < 0.001$, $P_{\text{species}} \leq 0.003$). The absolute gain in root biomass, however, was higher for *M. alterniflorum* (Table 5, ANCOVA, $P_{\text{corrected model}} < 0.001$, $P_{\text{treatment}} < 0.001$, $P_{\text{species}} \leq 0.001$).

The trend for the RGR of *C. hamulata* roots growing on mesotrophic sediment just failed to be significant ($P_{\text{corrected model}} < 0.001$, $P_{\text{treatment}} < 0.001$, $P_{\text{sediment}} \leq 0.061$), but the RGR of the roots of *C. hamulata* was significantly higher at the highest CO_2 treatment (*t*-test, $P \leq 0.001$).

Table 3 Mean pH, DIC, CO_2 and HCO_3^- concentrations (μmol L^{-1}) during the experiment in the water layer

CO_2 treatment	20 μmol L^{-1}		45 μmol L^{-1}		90 μmol L^{-1}		220 μmol L^{-1}	
Sediment type	Oligo	Meso	Oligo	Meso	Oligo	Meso	Oligo	Meso
pH	6.2 (0.04)	6.4 (0.02)	6.0 (0.04)	6.2 (0.02)	5.8 (0.03)	6.0 (0.01)	5.5 (0.03)	5.7 (0.01)
DIC	34 (1.7)	55 (1.9)	60 (2.6)	88 (2.3)	111 (4.2)	132 (3.2)	238 (7.6)	278 (5.3)
CO_2	19 (0.5)	25 (0.6)	41 (1.2)	52 (0.8)	85 (2.5)	94 (1.8)	206 (6.5)	231 (4.1)
HCO_3^-	15 (1.4)	30 (1.4)	19 (2.0)	36 (1.7)	26 (2.2)	38 (1.7)	32 (2.4)	47 (1.5)

The values within parentheses are expressed as SE. The pH values are geometric means.
DIC, dissolved inorganic carbon; Oligo, oligotrophic sediment; Meso, mesotrophic sediment.

Table 4 Mean chemical composition (μmol L^{-1}) of the water layer above the oligotrophic and mesotrophic sediments

Element	Oligotrophic	Mesotrophic
K^+	11.1 (1.0)	9.71 (0.8)
Ca^{2+}	34.4 (0.9)	34.6 (0.5)
Mg^{2+}*	26.1 (0.3)	22.9 (0.2)
Cl^-	253 (9.4)	259 (4.9)
NH_4^+*	3.95 (0.5)	1.01 (0.1)
NO_3^-*	19.7 (1.3)	5.29 (0.7)
PO_4^{3-}*	0.05 (0.01)	0.26 (0.01)
Total-P*	0.15 (0.03)	0.53 (0.03)
Total Fe*	0.23 (0.06)	0.53 (0.04)

The values within parentheses are expressed as SE. Elements marked with an asterisk (*) differed significantly between the sediment types (independent *t*-test, $P < 0.05$).

The root : shoot ratio (R : S) was higher at elevated CO_2 levels. The observed relation between R : S and CO_2 levels best fitted a logarithmic function and was highly significant ($R^2 = 0.77–0.92$; ANOVA, $P < 0.001$, Fig. 2). This relationship was strong for macrophytes growing on mesotrophic sediment but only moderate for *C. hamulata* growing on oligotrophic sediment. The R : S of *C. hamulata* on oligotrophic sediment was 1.5–2 units higher than that of *C. hamulata* growing on mesotrophic sediment (ANCOVA, $P_{\text{corrected model}} < 0.001$, $P_{\text{treatment}} = 0.001$, $P_{\text{sediment}} < 0.001$). *M. alterniflorum* showed the largest increase in R : S with elevated CO_2 concentration, resulting in the highest relative root production at CO_2 levels above 100 μmol L^{-1}.

The length (m) : mass (DM, g) ratios (L : M) showed a distinct pattern for both species growing on mesotrophic sediment (Fig. 3). The L : M was relatively low at the lowest CO_2 concentrations (<30 μmol L^{-1}), but it increased strongly at CO_2 levels around 40–50 μM and then decreased logarithmically with further increase in CO_2 concentration ($R^2 = 0.87–0.95$; ANOVA, $P < 0.001$ for both plants species on mesotrophic sediment). The L : M ratio of *C. hamulata* growing on oligotrophic sediments is decreasing logarithmically with higher CO_2 availability (from $CO_2 \geq 40$ μmol L^{-1}, $R^2 = 0.77$; ANOVA, $P < 0.001$).

Stoichiometry

Mass ratios of C : N and C : P in shoots generally rose with increasing CO_2 availability (Fig. 4). Initial C : N and C : P ratios of planted shoots of *M. alterniflorum* were 12 ± 0.2 and 74 ± 0.7, respectively. Initial *C. hamulata* C : N was 12 ± 0.3 and C : P was 97 ± 8.4.

At all treatment levels, the C : N ratios of the shoots at the end of the experiment differed from the initial values, with the exception of the 45 μmol L^{-1} treatment (independent *t*-test, $P < 0.05$). At the lowest CO_2 level, the C : N ratio of the shoots sank below that of the starting conditions, whereas the ratio

Table 5 Absolute gain in shoot and root biomass and the final total shoot length, number of branches and branch density at the end of the experiment

Species	Sediment type	CO_2 treatment (μmol L^{-1})	Shoot growth (DM, mg)	Root growth (DM, mg)	Length (cm)	Number of branches	Branch density number per cm
Callitriche hamulata	Oligo	20	24 (12.3)	5 (0.5)	88.9 (3.23)	21 (1.1)	0.24 (0.020)
		45	78 (13.0)	18 (2.0)	213.5 (19.82)	30 (2.1)	0.14 (0.007)
		90	102 (23.0)	27 (3.1)	137.9 (10.27)	25 (0.9)	0.18 (0.008)
		220	163 (32.7)	37 (16.3)	137.9 (12.43)	31 (1.8)	0.23 (0.015)
C. hamulata	Meso	20	18 (2.1)	1 (0.3)	83.6 (5.42)	26 (1.8)	0.31 (0.033)
		45	125 (22.5)	7 (2.3)	468.1 (43.99)	62 (7.6)	0.13 (0.006)
		90	450 (33.8)	45 (1.9)	842.3 (128.00)	86 (6.8)	0.11 (0.012)
		220	665 (84.2)	91 (14.5)	551.0 (65.89)	91 (5.2)	0.17 (0.013)
Myriophyllum alterniflorum	Meso	20	7 (22.2)	3 (0.4)	100.7 (6.14)	28 (2.9)	0.28 (0.019)
		45	182 (24.5)	21 (7.9)	394.5 (7.80)	76 (4.4)	0.19 (0.008)
		90	334 (78.5)	113 (38.1)	396.3 (42.33)	76 (5.1)	0.20 (0.011)
		220	589 (90.6)	327 (66.5)	352.3 (27.20)	91 (6.5)	0.26 (0.009)

The values within parentheses are expressed as SE. The length and number of branches were calculated per glass container as the sum of the shoot length (main shoot and branches) and the sum of the number of branches of all plants present. The branch density is calculated as number of branches per cm of total plant length at the end of the experiment.
Oligo, oligotrophic; Meso, mesotrophic.

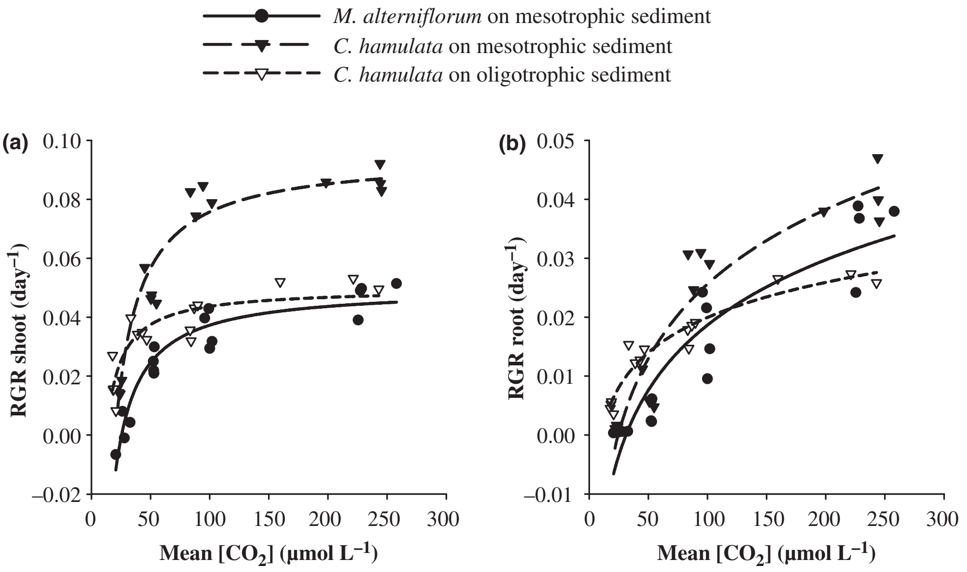

Fig. 1 (a) Relative growth rate (RGR, day^{-1}) of shoot in relation to mean CO_2 levels. Regression lines: *Myriophyllum alterniflorum* on mesotrophic sediment, RGR = $(-1.28\cdot[CO_2]^{-1}) + 0.05$, $R^2 = 0.91$; *Callitriche hamulata* on mesotrophic sediment, RGR = $(-1.91\cdot[CO_2]^{-1}) + 0.09$, $R^2 = 0.94$; *C. hamulata* on oligotrophic sediment, RGR = $(-0.62\cdot[CO_2]^{-1}) + 0.05$, $R^2 = 0.76$. (b) RGR of root in relation to mean CO_2 levels. Regression lines: *M. alterniflorum* on mesotrophic sediment, RGR = $0.016\cdot\ln[CO_2] - 0.055$, $R^2 = 0.86$; *C. hamulata* on mesotrophic sediment, RGR = $0.018\cdot\ln[CO_2] - 0.059$, $R^2 = 0.90$; *C. hamulata* on oligotrophic sediment, RGR = $0.009\cdot\ln[CO_2] - 0.020$, $R^2 = 0.93$.

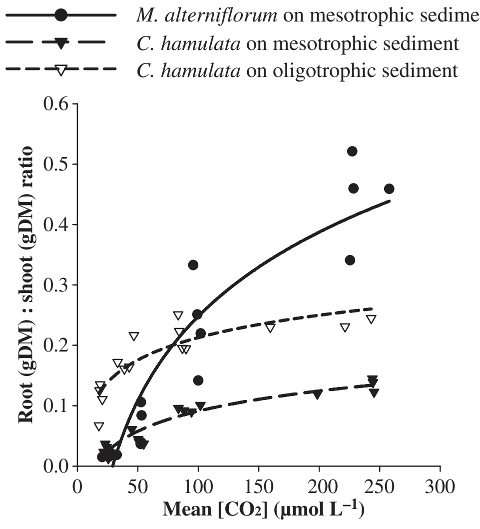

Fig. 2 Root (g DM) : shoot (g DM) ratio (R : S) at the end of the experiment. Regression lines: *Myriophyllum alterniflorum* on mesotrophic sediment, R : S = $0.20\cdot\ln[CO_2] - 0.68$, $R^2 = 0.87$; *Callitriche hamulata* on mesotrophic sediment, R : S = $0.05\cdot\ln[CO_2] - 0.0.13$, $R^2 = 0.92$; *C. hamulata* on oligotrophic sediment, R : S = $0.05\cdot\ln[CO_2] - 0.03$, $R^2 = 0.77$.

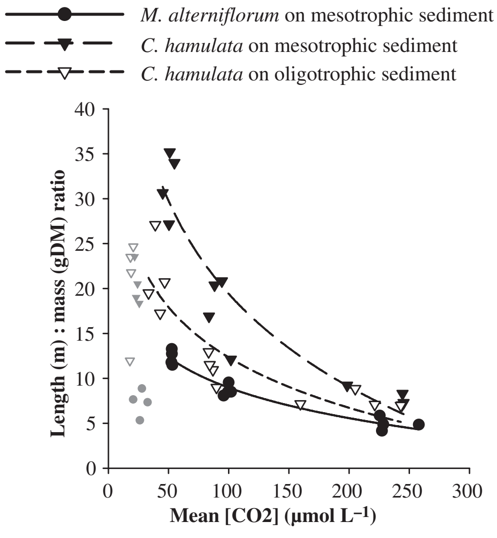

Fig. 3 Shoot length (m) : mass (DM, g) ratios (L : M) at the end of the experiment. Regression lines: *Myriophyllum alterniflorum* on mesotrophic sediment, L : M=$-4.90\cdot\ln[CO_2] + 31.53$, $R^2 = 0.95$; *Callitriche hamulata* on mesotrophic sediment, L : M=$-14.96\cdot\ln[CO_2] + 88.30$, $R^2 = 0.87$; *C. hamulata* on oligotrophic sediment, L : M=$-8.06\cdot\ln[CO_2] + 49.60$, $R^2 = 0.77$. Samples at the lowest CO_2 level (smaller, grey symbols) were not included in the regressions.

strongly increased at CO_2 availabilities of 90 and 220 µmol L^{-1}.

Initial C : P values in shoots were at all times different from the ratio in plants cultured at CO_2 levels of 90 and 220 µmol L^{-1}. At CO_2 levels of 45 µmol L^{-1}, only the C : P ratio of *M. alterniflorum* shoots was significantly raised compared to initial

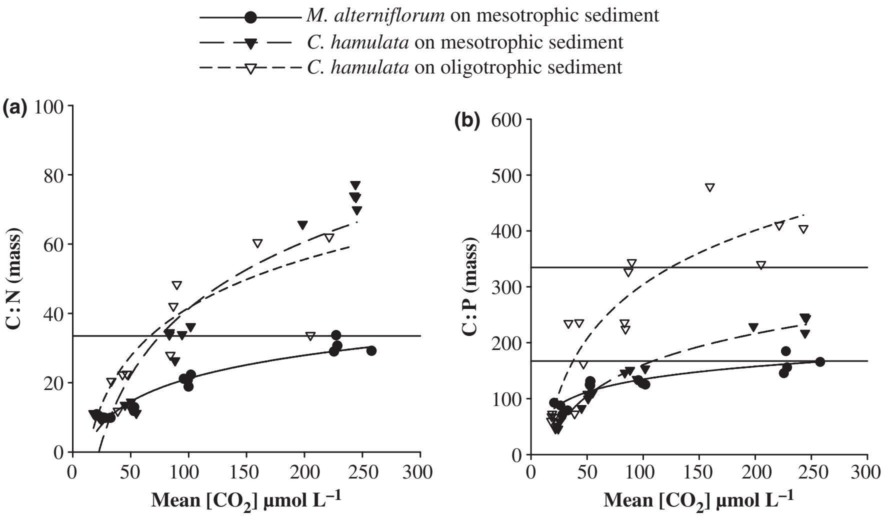

Fig. 4 Shoot C : N (a) and C : P (b) ratios in relation to mean CO_2 levels, at the end of the experiment. Regression lines for C : N ratios: *Myriophyllum alterniflorum* on mesotrophic sediment, C : N = 0.97·ln[CO_2] + 8.43, R^2 = 0.92; *Callitriche hamulata* on mesotrophic sediment, C : N = 27.81·ln[CO_2]-86.72, R^2 = 0.89; *C. hamulata* on oligotrophic sediment, C : N = 20.15·ln[CO_2] − 51.20, R^2 = 0.81. Regression lines for C : P ratios: *M. alterniflorum* on mesotrophic sediment, C : P = 33.07·ln[CO_2] − 17.73, R^2 = 0.80; *C. hamulata* on mesotrophic sediment, growth = 80.32·ln[CO_2] − 209.36, R^2 = 0.98; *C. hamulata* on oligotrophic sediment, growth = 139.76·ln[CO_2] − 339.59, R^2 = 0.84. Critical C : N level for maximal growth at 33.5, calculated from (Gerloff & Krombholz, 1966). Critical C : P: level for maximal growth at 334 and for maximal yield at 167 calculated from (Gerloff & Krombholz, 1966), low at 169, calculated from (Colman *et al*., 1987).

C : P ratios. At a CO_2 availability of 20 µmol L^{-1}, only the shoots of *C. hamulata* growing on mesotrophic sediments showed a C : P ratio that was significantly lower than initial values (independent *t*-test, $P < 0.05$).

The response of tissue C : N to the CO_2 treatment was similar for *C. hamulata* on both oligotrophic and mesotrophic sediments, while *M. alterniflorum* C : N ratios increased less with higher CO_2 availability (ANCOVA, $P_{\text{corrected model}} < 0.001$, $P_{\text{treatment}} < 0.001$, $P_{\text{species}} = 0.001$). Changes in C : P ratio with CO_2 level were comparable for *C. hamulata* and *M. alterniflorum* on mesotrophic sediment. The C : P of *C. hamulata* appeared to increase slightly faster than that of *M. alterniflorum*, but this difference was not statistically significant (ANCOVA, $P_{\text{corrected model}} < 0.001$, $P_{\text{treatment}} < 0.001$, $P_{\text{species}} = 0.915$). The increase in the C : P of *C. hamulata* on oligotrophic sediment with higher CO_2 availability was larger than that in the C : P of plants on mesotrophic sediment (ANCOVA, $P_{\text{corrected model}} < 0.001$, $P_{\text{treatment}} < 0.001$, $P_{\text{sediment}} < 0.001$).

The N : P ratios showed a weak but significant negative linear relationship (data not shown) with CO_2 level, for both *C. hamulata* ($R^2 = 0.38$) and *M. alterniflorum* ($R^2 = 0.56$) on mesotrophic sediment.

Discussion

Growth and CO_2 availability

In our experiment, higher CO_2 availability clearly increased the total biomass production of both *C. hamulata* and *M. alterniflorum* growing on mesotrophic and oligotrophic sediments. Growth at the lowest CO_2 level of 19–25 µmol L^{-1} was close to zero, or even negative, for both species, but especially for *M. alterniflorum*. In contrast, growth was positive and increasing at CO_2 levels ≥41–52 µmol L^{-1}. This indicates that CO_2 levels in the lowest CO_2 treatment were hardly sufficient or even insufficient to support the energy and C demands for respiration over the 40-day experimental period. Consequently, hardly any roots were formed at the lowest CO_2 levels. These plants had to rely for their nutrient demand on internal reservoirs and the small amounts of nutrients dissolved in the water.

The growth response to CO_2 level was highest for *C. hamulata* growing on mesotrophic sediment, whereas *C. hamulata* growing on oligotrophic sediment showed a markedly smaller growth response to CO_2 level. The RGR of *M. alterniflorum* (on mesotrophic sediment) showed the smallest increase in response to elevated CO_2, although the absolute biomass gain was comparable to that of *C. hamulata* growing on mesotrophic sediment. This might be related to the higher initial biomass of *M. alterniflorum.* Compared to *C. hamulata*, the lower RGR of *M. alterniflorum* in relation to CO_2 concentrations might also be connected to the lower CO_2 affinity of the latter species. As pointed out by Maberly & Madsen (1998, 2002), aquatic plants that are capable of taking up HCO_3^-, as is the case with *M. alterniflorum* (Allen & Spence, 1981), generally have a lower affinity for CO_2 than species restricted to CO_2 as a C source, such as *C. hamulata* (Adamec, 1997). The lower CO_2 affinity of *M. alterniflorum* might, especially at low CO_2 levels, have resulted in lower CO_2 uptake rates, resulting in a lower photosynthetic rate and possibly to a lower RGR.

The degree of shoot extension was related to the CO_2 availability. The L : M ratio decreased logarithmically from 45 to 220 μmol L^{-1} CO_2. This was caused by a greater increase in DM compared to the gain in length at higher CO_2 levels. This shows that shoot extension is relatively more important for shoot expansion at lower CO_2 levels. Shoot extension can have favourable effects on plant survival, since it increases the surface area and enhances the potential for diffusive CO_2 and nutrient uptake from the water, without using many resources. Moreover, it can also give an advantage in the competition for light with algae and other macrophytes.

Strikingly, the L : M ratio was relatively low at the lowest CO_2 treatment level of 20 μmol L^{-1}, being the same as at the start of the experiment. At very low CO_2 levels, the plants on mesotrophic sediment hardly showed any shoot extension, possibly due to the C-limitation and consequently energy limitation as a result of the very low CO_2 availability.

Root production also increased with rising aquatic CO_2 availability. It seems that maximum root production was not yet attained during this experiment, possibly because root production was still limited by C availability even at the highest aquatic CO_2 levels of around 220 μmol L^{-1}.

At increased CO_2 levels, *M. alterniflorum* and *C. hamulata* first mainly increased shoot production, thereby increasing their photosynthetic capacity. Subsequently, as the increase in shoot growth levelled off at around 100 μmol L^{-1} CO_2, root production became more important. This is also reflected in the increasing R : S ratio with rising CO_2 levels, a pattern also found for other aquatic macrophytes (e.g. Svedäng, 1992; Pagano & Titus, 2007). The higher relative investment in root biomass is probably the result of an increasing nutrient demand (mainly P and N) with increasing growth rates (Chapin, 1980).

The relative root production of *C. hamulata*, expressed as the R : S ratio, was higher for the plants on oligotrophic sediments (Fig. 2). The increased biomass investment allows the plant to access more nutrients from the sediment.

Nutrient status of the sediment

The nutrient status of the sediment proved to be important for plant growth in relation to CO_2 levels. The production increase by *C. hamulata* with elevated CO_2 was greater on mesotrophic sediment than on oligotrophic sediment. The mesotrophic sediment had a seven-fold higher PO_4^{3-} content, while TN values were comparable to those in the oligotrophic sediment. This suggests that the additional P availability in the mesotrophic sediment was responsible for the difference in the macrophyte growth response to CO_2 availability on the two sediment types. The mesotrophic sediments allowed high growth rates of *C. hamulata*, while the oligotrophic sediments did not support high biomass production. At CO_2 levels $\geq$100 μmol L^{-1}, the RGR of *C. hamulata* on mesotrophic sediments was twice as high as that of *C. hamulata* on oligotrophic sediments. Shoot RGR values were $\geq$0.08 and 0.04 day^{-1} for *C. hamulata* on mesotrophic and oligotrophic sediments, respectively. This difference was even more pronounced in terms of absolute biomass gain. At CO_2 levels $\geq$90 μmol L^{-1}, *C. hamulata* showed a three- to four-fold higher absolute shoot growth on mesotrophic compared to oligotrophic sediment.

Various studies measuring the RGR of *Callitriche cophocarpa* Sendtn at high CO_2 and nutrient availabilities found RGR values around 0.09 day^{-1}, close to the values we found for *C. hamulata* on mesotrophic sediment (Nielsen & Sand-Jensen, 1991; Vadstrup & Madsen, 1995; Olesen & Madsen, 2000). Although the RGR of *M. alterniflorum* on mesotrophic sediment in our experiment was not as high as that of *C. hamulata*, its growth rate was comparable to previously reported growth rates for other *Myriophyllum* species. Nielsen & Sand-Jensen (1991) measured an RGR of 0.046 day^{-1} for *Myriophyllum spicatum* at high DIC concentrations, while Pagano & Titus (2007) reported an RGR of 0.025 day^{-1} for *Myriophyllum sibericum* at 175 μmol L^{-1} CO_2.

Stoichiometry and growth limitation

The mass C : N and C : P ratios of plant shoots always increased with CO_2 availability, which means that relatively more C was incorporated into the plant tissue at higher aquatic CO_2 levels. Plants are known to be able to change their elemental ratio in response to relative elemental availability in their environment, which can have major consequences for plant–herbivore food web structures (Sterner & Elser, 2002).

Sediment type also had an effect on plant C : P ratios. *Callitriche hamulata* shoots growing on the mesotrophic sediments, which were richer in P, showed C : P ratios which were generally 1.7–1.9 times lower than those in shoots of the same species growing on the oligotrophic sediments. The shoot C : N ratios, however, did not differ between *C. hamulata* growing on mesotrophic and on oligotrophic sediments, with similar initial TN contents.

The increase in C : P and C : N ratios at elevated CO_2 was smaller for *M. alterniflorum* than for *C. hamulata*. At CO_2 levels ≥100 μmol L^{-1}, these ratios were lower than those in *C. hamulata* growing on mesotrophic sediment. Since the absolute biomass gain in this situation was similar for the two species, this implies that *M. alterniflorum* was better able to increase N and P uptake rates with rising growth rates. The concentrations of P and N in the water were extremely low, making the sediment the most important source for uptake by *M. alterniflorum*. The lower C : P and C : N ratios in *M. alterniflorum* shoots were correlated to the high production of roots in this species, as shown by the absolute gain in root biomass, as well as the high R : S ratio at CO_2 levels >50 μmol L^{-1}. The higher root biomass will have made it possible for *M. alterniflorum* to access more nutrients in the sediment pore water.

Discrepancies between C, N and P uptake at varying elemental supply rates can be used to estimate nutrient limitation of plant growth and yield. A C : N mass ratio above 33.5 is indicative of N-limitation for maximal macrophyte growth (calculated from % N data, adopted from Gerloff & Krombholz, 1966). For shoot C : P levels, this critical level lies at 334 while the critical C : P level for maximal yield is 167 (calculated from % P data, adopted from Colman *et al*., 1987 and Gerloff & Krombholz, 1966). Applying these critical levels to the results of *M. alterniflorum* on mesotrophic sediment suggests that N was not limiting the growth of *M. alterniflorum* at the CO_2 range we used. It was only at the highest CO_2 concentrations that there might have been some P-limitation on growth. On the other hand, C : N ratios in *C. hamulata* increased more with CO_2 level than those in *M. alterniflorum*, and reached the critical level at around 75 μmol L^{-1}, irrespective of the sediment type. A major C : P ratio increase with CO_2 availability was observed when *C. hamulata* was growing on oligotrophic sediment. The C : P ratio indicated a P-limited growth at CO_2 levels >75–110 μmol L^{-1}. The specimens growing on mesotrophic sediment showed a moderate increase in C : P and possible P-limitation at CO_2 levels >90–225 μmol L^{-1}. These results indicate that the growth of *C. hamulata* was primarily C-limited up to CO_2 concentrations of approximately 75 μmol L^{-1}, after which N availability started to have an additional effect on growth rate. For the specimens growing on oligotrophic sediment, P may also have reduced production rates at CO_2 levels >50 μmol L^{-1}.

Implications for softwater lakes

Since the plants showed no growth, or even negative growth, at the lowest CO_2 level, and were not even able to extend their shoots, it is conceivable that both *M. alterniflorum* and *C. hamulata* are unable to form sustainable populations at the very low CO_2 availability of 19–25 μmol L^{-1}. Under natural field conditions, plant survival will be further hampered by competition with other primary producers, such as isoetids and algae. The inability of *M. alterniflorum* and *C. hamulata* to grow at very low CO_2 levels is supported by field observations of the closely related species *C. cophocarpa*. This species was unable to grow at ambient CO_2 in two Danish softwater lakes (Vadstrup & Madsen, 1995). Recent measurements in Norwegian and Dutch softwater lakes dominated by isoetid vegetation and not featuring *M. alterniflorum* or *C. hamulata* also found mean CO_2 levels <40 μmol L^{-1} (J.G.M. Roelofs, unpubl. data). Isoetid species are very well capable of surviving and reproducing at very low aquatic C levels, even at levels <15 μmol L^{-1}, because of their adaptations to low aquatic C availability, such as the ability to utilise CO_2 from the sediment pore water. CO_2 concentrations in the pore water are generally up to a 100 times higher than those in the water column (Wium-Andersen & Andersen, 1972).

Rising aquatic CO_2 concentrations can change a softwater lake into a more suitable habitat for elodeid species such as *M. alterniflorum* and *C. hamulata*. In our experiment, the growth of both species was positively influenced by CO_2 concentrations of at least 45 μmol L^{-1}, with a strong increase in RGR up to approximately 100 μmol L^{-1}, after which their RGR on mesotrophic sediment levelled off (Fig. 1). The RGR reached values close to the maximum in this experiment, with a CO_2 increase to only about 75 μmol L^{-1}, a small increase which has previously been observed in SW Norwegian softwater lakes with dense stands of either *M. alterniflorum* or *C. hamulata*. Measurements of aquatic CO_2 levels in these lakes have found values between 43 and 156 μmol L^{-1}, depending on season, time of the day and amount of solar irradiance (E.C.H.E.T. Lucassen, unpubl. data). A rise in temperature might further amplify the growth response to rising CO_2 levels. Olesen & Madsen (2000) found a more pronounced positive RGR response of *C. cophocarpa* to high CO_2 levels with increased temperatures. Isoetids, by contrast, show low growth rates, which hardly change with rising CO_2 levels. Pagano & Titus (2007) found a small, non-significant increase in RGR as CO_2 levels were increased from 31 to 175 μmol L^{-1} CO_2. *Lobelia dortmanna* showed mean RGR values of around 0.001 and 0.002 day^{-1} at low and high CO_2 levels, respectively. Corresponding values for *Isoëtes lacustris* L. were around 0.010 and 0.015 day^{-1} and those for *I. echinospora* Durieu around 0.020 and 0.025 day^{-1}. Within the isoetid group, *L. uniflora* shows the greatest growth response to elevated CO_2. Andersen, Andersen & Pedersen (2006) reported RGR values for *L. uniflora* of 0.01 and 0.02 day^{-1} in a field experiment with low (17 μmol L^{-1}) and high (175 μmol L^{-1}) CO_2 levels, respectively. A laboratory study found an RGR for *L. uniflora* of around 0.004 day^{-1} at CO_2 levels of 15 μmol L^{-1}, and of 0.021 day^{-1} at 150 μmol L^{-1} (Andersen & Andersen, 2006).

Elevated CO_2 levels not only favour elodeid species, they may even be disadvantageous to some isoetids. The leaves of isoetids contain many gas-filled lacunae, providing the plant with substantial buoyancy. Normally, this buoyancy is counterbalanced by the roots, which provide anchoring in the sediment. When CO_2 levels increase, the R : S ratio of isoetids becomes drastically reduced, decreasing the plant's grip on the sediment (Smolders *et al.*, 2002; Bagger & Madsen, 2004). When the R : S ratio becomes small enough, there is a drastically increased risk of the plants being uprooted. When this happens, the isoetids float to the surface and eventually die off. The risk of uprooting is especially high for isoetids growing on more organic sediment types, as is the case in many SW Norwegian isoetid-dominated softwater lakes, as well as in softwater lakes in agricultural areas. But even on more sandy sediments, isoetids can be massively uprooted during storms, as has been observed for *L. uniflora* in a Dutch softwater lake (Spierenburg *et al.*, in prep).

The elodeids we tested in our experiments needed to grow on mesotrophic sediment to enable substantial growth at elevated CO_2 levels. Many softwater lakes, especially in the Atlantic region, do have such mesotrophic sediments, but are still dominated by isoetid vegetation because of the very low C availability (Brouwer *et al.*, 2002). With rising CO_2 availability, it is especially these softwater lakes which will become vulnerable to invasion by elodeids, since P and N are already sufficiently available in the sediments. Furthermore, isoetid vegetation in softwater lakes with oligotrophic sediments will be threatened by a combination of eutrophication and rising CO_2 levels, as has been shown by our experiment.

Acknowledgments

This research was funded by the Centre for Wetland Ecology (CWE). We wish to thank Martin Versteeg, Jelle Eygensteijn, Germa Verheggen, Kimberly Koens and Jeroen Graafland for their valuable help NSG publication number 20090401.

References

Adamec L. (1997) Relations between K^+ uptake and photosynthetic uptake of inorganic carbon by aquatic plants. *Biologia Plantarum*, **39**, 599–606.

Allen E.D. & Spence D.H.N. (1981) The differential ability of aquatic plants to utilize the inorganic carbon supply in freshwaters. *New Phytologist*, **87**, 269–283.

Andersen F.Ø. & Andersen T. (2006) Effects of arbuscular mycorrhizae on biomass and nutrients in the aquatic plant *Littorella uniflora*. *Freshwater Biology*, **51**, 1623–1633.

Andersen T., Andersen F.Ø. & Pedersen O. (2006) Increased CO_2 in the water around *Littorella uniflora* raises the sediment O_2 concentration. *Aquatic Botany*, **84**, 294–300.

Arts G.H.P. & Leuven R.S.E.W. (1988) Floristic changes in shallow soft waters in relation to underlying environmental factors. *Freshwater Biology*, **20**, 97–111.

Bagger J. & Madsen T.V. (2004) Morphological acclimation of aquatic *Littorella uniflora* to sediment CO_2 concentration and wave exposure. *Functional Ecology*, **18**, 946–951.

Brandrud T.E. (2002) Effects of liming on aquatic macrophytes, with emphasis on Scandinavia. *Aquatic Botany*, **73**, 395–404.

Brouwer E., Bobbink R. & Roelofs J.G.M. (2002) Restoration of aquatic macrophyte vegetation in acidified and eutrophied softwater lakes: an overview. *Aquatic Botany*, **73**, 405–431.

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

Chapin F.S. III (1980) The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*, **11**, 233–260.

Colman J.A., Sorsa K., Hoffmann J.P., Smith C.S. & Andrews J.H. (1987) Yield- and photosynthesis-derived critical concentrations of tissue phosphorus and their significance for growth of Eurasian water milfoil, *Myriophyllum spicatum* L. *Aquatic Botany*, **29**, 111–122.

Dickson W., Borg H., Ekstrom C., Hornstrom E. & Gronlund T. (1995) Reliming and reacidification effects on lakewater. Chemistry, plankton and macrophytes. *Water, Air, and Soil Pollution*, **85**, 919–924.

Dillon P.J. & Molot L.A. (1997) Dissolved organic and inorganic carbon mass balances in central Ontario lakes. *Biogeochemistry*, **36**, 29–42.

Evans C.D., Monteith D.T. & Cooper D.M. (2005) Long-term increases in surface water dissolved organic carbon: observations, possible causes and environmental impacts. *Environmental Pollution*, **137**, 55–71.

Evans C.D., Chapman P.J., Clark J.M., Monteith D.T. & Cresser M.S. (2006) Alternative explanations for rising dissolved organic carbon export from organic soils. *Global Change Biology*, **12**, 2044–2053.

Fenner N., Freeman C., Lock M.A., Harmens H., Reynolds B. & Sparks T. (2007a) Interactions between elevated CO_2 and warming could amplify DOC exports from peatland catchments. *Environmental Science and Technology*, **41**, 3146–3152.

Fenner N., Ostle N.J., McNamara N., Sparks T., Harmens H., Reynolds B. & Freeman C. (2007b) Elevated CO_2 effects on peatland plant community carbon dynamics and DOC production. *Ecosystems*, **10**, 635–647.

Freeman C., Evans C.D., Monteith D.T., Reynolds B. & Fenner N. (2001) Export of organic carbon from peat soils. *Nature*, **412**, 785.

Freeman C., Fenner N., Ostle N.J., Kang H., Dowrick D.J., Reynolds B., Lock M.A., Sleep D., Hughes S. & Hudson J. (2004) Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. *Nature*, **430**, 195–198.

Gacia E., Ballesteros E., Camarero L., Delgado O., Palau A., Riera J.L. & Catalan J. (1994) Macrophytes from lakes in the eastern Pyrenees – community composition and ordination in relation to environmental-factors. *Freshwater Biology*, **32**, 73–81.

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.

Hope D., Kratz T.K. & Riera J.L. (1996) Relationship between P-CO_2 and dissolved organic carbon in northern Wisconsin lakes. *Journal of Environmental Quality*, **25**, 1442–1445.

Hudson J.J., Dillon P.J. & Somers K.M. (2003) Long-term patterns in dissolved organic carbon in boreal lakes: the role of incident radiation, precipitation, air temperature, southern oscillation and acid deposition. *Hydrology and Earth System Sciences*, **7**, 390–398.

Jonsson A., Karlsson J. & Jansson M. (2003) Sources of carbon dioxide supersaturation in clearwater and humic lakes in northern Sweden. *Ecosystems*, **6**, 224–235.

Keeley J.E. (1998) CAM photosynthesis in submerged aquatic plants. *Botanical Review*, **64**, 121–175.

Kingston H.M. & Haswell S.J. (1997) *Microwave Enhanced Chemistry: Fundamentals, Sample Preparation and Applications*. American Chemical Society, Washington, DC.

Lucassen E.C.H.E.T., Bobbink R., Oonk M.M.A., Brandrud T.E. & Roelofs J.G.M. (1999) The effects of liming and reacidification on the growth of *Juncus bulbosus*: a mesocosm experiment. *Aquatic Botany*, **64**, 95–103.

de Lyon M.J.H. & Roelofs J.G.M. (1986) *Aquatic Plants in Relation to Water Quality and Sediment Characteristics (in Dutch)*. Laboratorium voor Aquatische Oecologie, Katholieke Universiteit Nijmegen, Nijmegen.

Maberly S.C. & Madsen T.V. (1998) Affinity for CO_2 in relation to the ability of freshwater macrophytes to use $HCO^-{}_3$. *Functional Ecology*, **12**, 99–106.

Maberly S.C. & Madsen T.V. (2002) Freshwater angiosperm carbon concentrating mechanisms: processes and patterns. *Functional Plant Biology*, **29**, 393–405.

Madsen T.V., Maberly S.C. & Bowes G. (1996) Photosynthetic acclimation of submersed angiosperms to CO_2 and HCO_3^-. *Aquatic Botany*, **53**, 15–30.

Meehl G.A., Stocker T.F., Collins W.D. *et al.* (2007) Global climate projections. In: *The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (S. Solomon, D. Qin, M. Manning. *et al.*), pp 747–845, Cambridge University Press, NY.

Murphy K.J. (2002) Plant communities and plant diversity in softwater lakes of northern Europe. *Aquatic Botany*, **73**, 287–324.

Nielsen S.L. & Sand-Jensen K. (1991) Variation in growth-rates of submerged rooted macrophytes. *Aquatic Botany*, **39**, 109–120.

Nielsen S.L. & Sand-Jensen K. (1997) Growth rates and morphological adaptations of aquatic and terrestrial forms of amphibious *Littorella uniflora* (L.) Aschers. *Plant Ecology*, **129**, 135–140.

Olesen B. & Madsen T.V. (2000) Growth and physiological acclimation to temperature and inorganic carbon availability by two submerged aquatic macrophyte species, *Callitriche cophocarpa* and *Elodea canadensis*. *Functional Ecology*, **14**, 252–260.

Pagano A.M. & Titus J.E. (2007) Submersed macrophyte growth at low pH: carbon source influences response to dissolved inorganic carbon enrichment. *Freshwater Biology*, **52**, 2412–2420.

Pedersen O., Andersen T., Ikejima K., Hossain M.Z. & Andersen F.Ø. (2006) A multidisciplinary approach to understanding the recent and historical occurrence of the freshwater plant, *Littorella uniflora*. *Freshwater Biology*, **51**, 865–877.

Richardson K., Griffiths H., Reed M.L., Raven J.A. & Griffiths N.M. (1984) Inorganic carbon assimilation in the Isoetids, *Isoetes lacustris* L. and *Lobelia dortmanna* L. *Oecologia*, **61**, 115–121.

Riera J.L., Schindler J.E. & Kratz T.K. (1999) Seasonal dynamics of carbon dioxide and methane in two clear-water lakes and two bog lakes in northern Wisconsin, USA. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 265–274.

Robe W.E. & Griffiths H. (1992) Seasonal-variation in the ecophysiology of *Littorella-uniflora* (L) Ascherson in acidic and eutrophic habitats. *New Phytologist*, **120**, 289–304.

Roelofs J.G.M. (1983) Impact of acidification and eutrophication on macrophyte communities in soft waters in the Netherlands I. field observations. *Aquatic Botany*, **17**, 139–155.

Roelofs J.G.M., Schuurkes J.A.A.R. & Smits A.J.M. (1984) Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. *Aquatic Botany*, **18**, 389–411.

Roelofs J.G.M., Brandrud T.E. & Smolders A.J.P. (1994) Massive expansion of *Juncus bulbosus* L after liming of acidified SW Norwegian lakes. *Aquatic Botany*, **48**, 187–202.

Roelofs J.G.M., Smolders A.J.P., Brandrud T.E. & Bobbink R. (1995) The effect of acidification, liming and reacidification on macrophyte development, water quality and sediment characteristics of soft-water lakes. *Water Air and Soil Pollution*, **85**, 967–972.

Sand-Jensen K. & Søndergaard M. (1979) Distribution and quantitative development of aquatic macrophytes in relation to sediment characteristics in oligotrophic Lake Kalgaard, Denmark. *Freshwater Biology*, **9**, 1–11.

Schuurkes J.A.A.R., Elbers M.A., Gudden J.J.F. & Roelofs J.G.M. (1987) Effects of simulated ammonium sulphate and sulphuric acid rain on acidification, water quality and flora of small-scale soft water systems. *Aquatic Botany*, **28**, 199–226.

Skjelkvale B.L., Stoddard J.L., Jeffries D.S. *et al.* (2005) Regional scale evidence for improvements in surface water chemistry 1990–2001. *Environmental Pollution*, **137**, 165–176.

Smolders A.J.P., Lucassen E.C.H.E.T. & Roelofs J.G.M. (2002) The isoetid environment: biogeochemistry and threats. *Aquatic Botany*, **73**, 325–350.

Sobek S., Tranvik L.J. & Cole J.J. (2005) Temperature independence of carbon dioxide supersaturation in global lakes. *Global Biogeochemical Cycles*, **19**, 1–10.

Søndergaard M. & Sand-Jensen K. (1979) Carbon uptake by leaves and roots of *Littorella uniflora* (L.) Aschers. *Aquatic Botany*, **6**, 1–12.

Sterner R.W. & Elser J.J. (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, NJ.

Stumm W. & Morgan J.J. (1996) *Aquatic Chemistry*. John Wiley & Sons, Inc, New York.

Svedäng M.U. (1992) Carbon-dioxide as a factor regulating the growth dynamics of *Juncus bulbosus*. *Aquatic Botany*, **42**, 231–240.

Taylor G.J. & Crowder A.A. (1983) Use of DCB technique for extraction of hydrous iron oxides from roots of wetland plants. *American Journal of Botany*, **70**, 1254–1257.

Vadstrup M. & Madsen T.V. (1995) Growth limitation of submerged aquatic macrophytes by inorganic carbon. *Freshwater Biology*, **34**, 411–419.

Wium-Andersen S. (1971) Photosynthetic uptake of free CO_2, by the roots of *Lobelia dortmanna*. *Physiologia Plantarum*, **25**, 245–248.

Wium-Andersen S. & Andersen J.M. (1972) Carbon dioxide content of the interstitial water in the sediment of Grane Langsø, a Danish Lobelia lake. *Limnology and Oceanography*, **17**, 943–947.

(Manuscript accepted 11 March 2009)