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 CO₂ levels.

2. In a laboratory experiment we tested the growth response of two elodeid species, Myriophyllum alterniflorum and Callitriche hamulata, at four different CO₂ 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 CO₂ levels.

3. Shoot and root growth increased with rising CO₂ availability. Irrespective of sediment type, growth was minimal to negative at the lowest CO₂ treatment level, while becoming positive at CO₂ 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 CO₂ levels of c. 100 μmol L⁻¹.

4. Within this experiment, the growth of C. hamulata at CO₂ 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 CO₂ concentrations. However, if the sediments contain enough nutrients, a rise in aquatic CO₂ could allow the invasion of elodeid species leading to the subsequent disappearance of slow-growing isoetids.

Keywords: CO₂ increase, Callitriche hamulata, macrophyte growth, Myriophyllum alterniflorum, soft-water 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 HCO₃⁻ concentration in softwater lakes is generally below 200 μmol L⁻¹. Such low HCO₃⁻ concentrations would probably not contribute substantially to the C pool needed for photosynthesis by macrophytes, able of using HCO₃⁻ additionally to
CO₂ 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 CO₂, 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 Ericaëulon 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 CO₂ 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, if 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 focusing 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 (PO₄³⁻) 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 CO₂ 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 CO₂ 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 CO₂ and NH₄⁺ 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 CO₂, 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 CO₂ concentrations (e.g. Hope, Kratz & Riera, 1996; Sobek, Tranvik & Cole, 2005).

The aquatic CO₂ 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, CO₂ levels can drop far below the saturation point as a result of CO₂ uptake by primary producers (J.G.M. Roelofs, unpubl. data). Hence, it is expected that the predicted rise in atmospheric CO₂ concentrations to levels of 720–1020 ppm in the year 2100 (Meehl et al., 2007) will result in increased aquatic CO₂ levels.
Henry’s law with temperature correction ($K_H = 0.034$ $\text{M atm}^{-1}$, $K_S = 0.041$ $\text{M atm}^{-1}$), a maximum aquatic CO$_2$ increase to levels of around 40–50 $\mu$mol L$^{-1}$ can be calculated at temperatures of 20–10 $^\circ\text{C}$.

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 $\mu$mol 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 $^\circ\text{C} \pm 1$ $^\circ\text{C}$), 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 $\mu$mol 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) 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.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mesotrophic</th>
<th>Oligotrophic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content (%FM)</td>
<td>21 (0.6)</td>
<td>16 (1.3)</td>
</tr>
<tr>
<td>Total organic matter (%DM)</td>
<td>1.1 (0.2)</td>
<td>0.5 (0.2)</td>
</tr>
<tr>
<td>PO$_4^{3-}$ (µmol kg DM$^{-1}$)</td>
<td>514 (23)</td>
<td>70 (5)</td>
</tr>
<tr>
<td>NO$_3$ (µmol kg DM$^{-1}$)</td>
<td>88 (11)</td>
<td>63 (13)</td>
</tr>
<tr>
<td>NH$_4$ (µmol kg DM$^{-1}$)</td>
<td>43 (6)</td>
<td>63 (20)</td>
</tr>
</tbody>
</table>

The values within parentheses are expressed as SE.

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

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>224</td>
</tr>
<tr>
<td>K</td>
<td>11</td>
</tr>
<tr>
<td>Mg</td>
<td>24</td>
</tr>
<tr>
<td>Ca</td>
<td>29</td>
</tr>
<tr>
<td>Cl</td>
<td>175</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>20</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>49</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>2</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>23</td>
</tr>
<tr>
<td>PO$_4^{3-}$</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>
Compressed air was mixed with pure CO$_2$ at Veenendaal, the Netherlands. Before mixing, the controllers (EL-FLOW select F201CV; Bronkhorst, Netherlands) 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 ± 2.3 $10^{-3}$ and 0.02 ± 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 aed at 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 DIC concentrations and the pH were determined by taking samples after 5 days in 20 mL acid-rinsed, iodised 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). 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$(W_{rf}$ + $W_{rd}$)–ln$W_{ri}$](t)$^{-1}$, where $W_{ri}$ 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 SPSS 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 CO2 levels in the water column was tested with an independent factorial ANOVA. Growth responses in relation to CO2 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 CO2 level, were tested using ANCOVA.

Results

Aquatic CO2

The CO2 levels differed significantly between all treatments (one-way ANOVA, P < 0.05; Games–Howell P < 0.05, Table 3). The variation in CO2 concentrations within each container was low (mean SE < 5%). Containers holding mesotrophic sediment showed slightly but significantly higher mean CO2 levels (independent factorial ANOVA, P = 0.01). There was no effect of macrophyte species on mean CO2 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 PO43−, TP and Fe, whereas NO3−, NH4+ and Mg2+ 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 CO2 levels (Table 5, Fig. 1). The RGR of the shoots best fitted an inverse model (R2 = 0.87–0.96; ANOVA; P < 0.05, Fig. 1a), with the curve levelling off at around 100 μmol CO2 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 CO2 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, Pcorrected model < 0.001, Ptreatment < 0.001, Psediment < 0.001). The absolute biomass gain during the experiment was quite similar for M. alterniflorum and C. hamulata on mesotrophic sediment (Table 5, ANCOVA, Pcorrected model < 0.001, Ptreatment < 0.001, Pspecies ≤ 0.001).

With higher CO2 availability, the plants generally produced more branches. Apart from the lowest CO2 treatment, where there was hardly any biomass production, the number of branches per cm of the plant was higher at elevated CO2 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 CO2 level (Fig. 1b). The RGR of M. alterniflorum was lower than that of C. hamulata on mesotrophic sediment (ANCOVA, Pcorrected model < 0.001, Ptreatment < 0.001, Pspecies ≤ 0.003). The absolute gain in root biomass, however, was higher for M. alterniflorum (Table 5, ANCOVA, Pcorrected model < 0.001, Ptreatment < 0.001, Pspecies ≤ 0.001).

The trend for the RGR of C. hamulata roots growing on mesotrophic sediment just failed to be significant (Pcorrected model < 0.001, Ptreatment < 0.001, Psediment ≤ 0.061), but the RGR of the roots of C. hamulata was significantly higher at the highest CO2 treatment (t-test, P ≤ 0.001).

<table>
<thead>
<tr>
<th>CO2 treatment</th>
<th>20 μmol L−1</th>
<th>45 μmol L−1</th>
<th>90 μmol L−1</th>
<th>220 μmol L−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment type</td>
<td>Oligo</td>
<td>Meso</td>
<td>Oligo</td>
<td>Meso</td>
</tr>
<tr>
<td>pH</td>
<td>6.2 (0.04)</td>
<td>6.4 (0.02)</td>
<td>6.0 (0.04)</td>
<td>6.2 (0.02)</td>
</tr>
<tr>
<td>DIC</td>
<td>34 (1.7)</td>
<td>55 (1.9)</td>
<td>60 (2.6)</td>
<td>88 (2.3)</td>
</tr>
<tr>
<td>CO2</td>
<td>19 (0.5)</td>
<td>25 (0.6)</td>
<td>41 (1.2)</td>
<td>52 (0.8)</td>
</tr>
<tr>
<td>HCO3−</td>
<td>15 (1.4)</td>
<td>30 (1.4)</td>
<td>19 (2.0)</td>
<td>36 (1.7)</td>
</tr>
</tbody>
</table>

The values within parentheses are expressed as SE. The pH values are geometric means.

DIC, dissolved inorganic carbon; Oligo, oligotrophic sediment; Meso, mesotrophic sediment.

© 2009 Blackwell Publishing Ltd, *Freshwater Biology*, 54, 1819–1831
The root : shoot ratio (R : S) was higher at elevated CO\textsubscript{2} levels. The observed relation between R : S and CO\textsubscript{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\textsubscript{2} concentration, resulting in the highest relative root production at CO\textsubscript{2} levels above 100 $\mu\text{mol L}^{-1}$.

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\textsubscript{2} concentrations (<30 $\mu\text{mol L}^{-1}$), but it increased strongly at CO\textsubscript{2} levels around 40–50 $\mu\text{mol}$ and then decreased logarithmically with further increase in CO\textsubscript{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\textsubscript{2} availability (from CO\textsubscript{2}‡ 40 $\mu\text{mol L}^{-1}$, $R^2 = 0.77$; ANOVA, $P < 0.001$).

Stoichiometry

Mass ratios of C : N and C : P in shoots generally rose with increasing CO\textsubscript{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 : 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 $\mu\text{mol L}^{-1}$ treatment (independent $t$-test, $P < 0.05$). At the lowest CO\textsubscript{2} level, the C : N ratio of the shoots sank below that of the starting conditions, whereas the ratio

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Mean chemical composition ($\mu\text{mol L}^{-1}$) of the water layer above the oligotrophic and mesotrophic sediments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>Oligotrophic</td>
</tr>
<tr>
<td>K$^+$</td>
<td>11.1 (1.0)</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>34.4 (0.9)</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>26.1 (0.3)</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>253 (9.4)</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>3.95 (0.5)</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>19.7 (1.3)</td>
</tr>
<tr>
<td>PO$_4^{3-}$</td>
<td>0.05 (0.01)</td>
</tr>
<tr>
<td>Total-P</td>
<td>0.15 (0.03)</td>
</tr>
<tr>
<td>Total Fe$^+$</td>
<td>0.23 (0.06)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Table 5</th>
<th>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</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Sediment type</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>$Callitriche\ hamulata$</td>
<td>Oligo</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meso</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>$C.\ hamulata$</td>
<td>Meso</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

© 2009 Blackwell Publishing Ltd, *Freshwater Biology*, 54, 1819–1831
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 45 µmol L$^{-1}$, only the C : P ratio of *M. alterniflorum* shoots was significantly raised compared to initial
C: P ratios. At a CO₂ 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 CO₂ treatment was similar for *C. hamulata* on both oligotrophic and mesotrophic sediments, while *M. alterniflorum* C:N ratios increased less with higher CO₂ availability (ANCOVA, *P* corrected model < 0.001, *P* treatment < 0.001, *P* species = 0.901). Changes in C : P ratio with CO₂ 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* corrected model < 0.001, *P* treatment < 0.001, *P* species = 0.915). The increase in the C : P of *C. hamulata* on oligotrophic sediment with higher CO₂ availability was larger than that in the C : P of plants on mesotrophic sediment (ANCOVA, *P* corrected model < 0.001, *P* treatment < 0.001, *P* species = 0.915).

The N : P ratios showed a weak but significant negative linear relationship (data not shown) with CO₂ level, for both *C. hamulata* (*R²* = 0.38) and *M. alterniflorum* (*R²* = 0.56) on mesotrophic sediment.

**Discussion**

**Growth and CO₂ availability**

In our experiment, higher CO₂ availability clearly increased the total biomass production of both *C. hamulata* and *M. alterniflorum* growing on mesotrophic and oligotrophic sediments. Growth at the lowest CO₂ 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 CO₂ levels ≥41–52 μmol L⁻¹. This indicates that CO₂ levels in the lowest CO₂ 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 CO₂ 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 CO₂ level was highest for *C. hamulata* growing on mesotrophic sediment, whereas *C. hamulata* growing on oligotrophic sediment showed a markedly smaller growth response to CO₂ level. The RGR of *M. alterniflorum* (on mesotrophic sediment) showed the smallest increase in response to elevated CO₂, 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 CO₂ concentrations might also be connected to the lower CO₂ affinity of the latter species. As pointed out by Maberly & Madsen (1998, 2002), aquatic plants that are capable of taking up HCO₃⁻, as is the case with *M. alterniflorum* (Allen & Spence, 1981), generally have a lower affinity for CO₂ than species restricted to CO₂ as a C source, such as *C. hamulata* (Adamec, 1997). The lower CO₂ affinity of *M. alterniflorum* might, especially at low CO₂ levels, have resulted in lower CO₂ uptake rates, resulting in a lower photosynthetic rate and possibly to a lower RGR.

The degree of shoot extension was related to the CO₂ availability. The L : M ratio decreased logarithmically from 45 to 220 μmol L⁻¹ CO₂. This was caused by a greater increase in DM compared to the gain in length at higher CO₂ levels. This shows that shoot extension is relatively more important for shoot expansion at lower CO₂ levels. Shoot extension can have favourable effects on plant survival, since it increases the surface area and enhances the potential for diffusive CO₂ 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 CO₂ treatment level of 20 μmol L⁻¹, being the same as at the start of the experiment. At very low CO₂ 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 CO₂ availability.

Root production also increased with rising aquatic CO₂ 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 CO₂ levels of around 220 μmol L⁻¹.

At increased CO₂ 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 CO₂, root production became more important. This is also reflected in the increasing R : S ratio with rising CO₂ levels, a pattern also found for other aquatic macrophytes (e.g. Svedång, 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 CO₂ levels. The production increase by *C. hamulata* with elevated CO₂ was greater on mesotrophic sediment than on oligotrophic sediment. The mesotrophic sediment had a seven-fold higher PO₄³⁻ 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 CO₂ 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 CO₂ levels ≥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 ≥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 CO₂ levels ≥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 CO₂ 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 $\mu$mol 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 $\geq$100 $\mu$mol 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 $>50$ $\mu$mol 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 $\mu$mol 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 $\mu$mol 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 $\mu$mol L$^{-1}$. These results indicate that the growth of C. hamulata was primarily C-limited up to CO$_2$ concentrations of approximately 75 $\mu$mol 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 $\mu$mol 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 $\mu$mol 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 $\mu$mol 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 $\mu$mol 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...
Rising aquatic CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ levels. Olesen & Madsen (2000) found a more pronounced positive RGR response of *C. cophocarpa* to high CO₂ levels with increased temperatures. Isoetids, by contrast, show low growth rates, which hardly change with rising CO₂ levels. Pagano & Titus (2007) found a small, non-significant increase in RGR as CO₂ levels were increased from 31 to 175 μmol L⁻¹ CO₂. *Lobelia dortmanna* showed mean RGR values of around 0.001 and 0.002 day⁻¹ at low and high CO₂ levels, respectively. Corresponding values for *Isoetes lacustris* L. were around 0.010 and 0.015 day⁻¹ and those for *L. echinospora* Durieu around 0.020 and 0.025 day⁻¹. Within the isoetid group, *L. uniflora* shows the greatest growth response to elevated CO₂. 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) CO₂ levels, respectively. A laboratory study found an RGR for *L. uniflora* of around 0.004 day⁻¹ at CO₂ levels of 15 μmol L⁻¹, and of 0.021 day⁻¹ at 150 μmol L⁻¹ (Andersen & Andersen, 2006).

Elevated CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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


Rising CO₂ and macrophyte invasions


(Manuscript accepted 11 March 2009)