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Use of sediment CO_2 by submersed rooted plants

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• *Background and Aims* Submersed plants have different strategies to overcome inorganic carbon limitation. It is generally assumed that only small rosette species (isoetids) are able to utilize the high sediment CO_2 availability. The present study examined to what extent five species of submersed freshwater plants with different morphology and growth characteristics (*Lobelia dortmanna*, *Lilaeopsis macloviana*, *Ludwigia repens*, *Vallisneria americana* and *Hydrocotyle verticillata*) are able to support photosynthesis supplied by uptake of CO_2 from the sediment.
• *Methods* Gross photosynthesis was measured in two-compartment split chambers with low inorganic carbon availability in leaf compartments and variable CO_2 availability (0 to >8 mmol L^{-1}) in root compartments. Photosynthetic rates based on root-supplied CO_2 were compared with maximum rates obtained at saturating leaf CO_2 availability, and ^{14}C experiments were conducted for two species to localize bottlenecks for utilization of sediment CO_2.
• *Key Results* All species except *Hydrocotyle* were able to use sediment CO_2, however, with variable efficiency, and with the isoetid, *Lobelia*, as clearly the most effective and the elodeid, *Ludwigia*, as the least efficient. At a water column CO_2 concentration in equilibrium with air, *Lobelia*, *Lilaeopsis* and *Vallisneria* covered >75% of their CO_2 requirements by sediment uptake, and sediment CO_2 contributed substantially to photosynthesis at water CO_2 concentrations up to 1000 μmol L^{-1}. For all species except *Ludwigia*, the shoot to root ratio on an areal basis was the single factor best explaining variability in the importance of sediment CO_2. For *Ludwigia*, diffusion barriers limited uptake or transport from roots to stems and transport from stems to leaves.
• *Conclusions* Submersed plants other than isoetids can utilize sediment CO_2, and small and medium sized elodeids with high root to shoot area in particular may benefit substantially from uptake of sediment CO_2 in low alkaline lakes.

Key words: Submersed rooted plants, CO_2 uptake, sediment CO_2, *Lobelia dortmanna*, *Lilaeopsis macloviana*, *Ludwigia repens*, *Vallisneria americana*, *Hydrocotyle verticillata*.

INTRODUCTION

Photosynthesis of submersed aquatic plants is often limited by low availability of dissolved inorganic carbon (DIC; Madsen and Maberly, 1991). Although the concentration of CO_2 in water can be orders of magnitude higher than in air due to CO_2 accumulation from respiration or groundwater input, the availability in water is in general lower because molecular diffusion of dissolved gases is about 10^4 times slower in water than in air. Hence, the transport of CO_2 through the diffusive boundary layer represents a bottleneck to the DIC supply of submersed plants (Maberly and Spence, 1989). The ability to utilize bicarbonate is probably the most important adaptation to overcome DIC limitation of underwater photosynthesis since bicarbonate is most often present in much higher concentration than free CO_2 (Prins and Elzenga, 1989; Madsen and Sand-Jensen, 1991; Maberly and Madsen, 2002). In addition to bicarbonate utilization, crassulacean acid metabolism (CAM) and C_4-like metabolism function as carbon-concentrating mechanisms facilitating inorganic carbon acquisition in some submersed plants (Maberly and Madsen, 2002). However, free CO_2 in either the water column or the interstitial water of the sediment is a more favourable source if available

and if it can be exploited by the plants (Maberly and Madsen, 2002; Jones, 2005).

Due to mineralization of organic matter within the sediment, the CO_2 concentration in interstitial water is often 10–250 times higher than atmospheric equilibrium (approx. 16 μmol CO_2 L^{-1}; Wium-Andersen and Andersen, 1972; Pedersen *et al.*, 1995). The use of sediment CO_2 is well described for isoetids (Wium-Andersen, 1971; Boston *et al.*, 1987; Madsen *et al.*, 2002). Isoetids are small aquatic rosette plants with extensive root systems and well-developed air lacunae ensuring efficient gas transport between roots and shoot. Sand-Jensen and Søndergaard (1978) found that 95% of the CO_2 fixed by the isoetid *Lobelia dortmanna* was obtained directly from the sediment, but the role of sediment CO_2 has only been evaluated for a few non-isoetid species.

As sediment CO_2 can exceed 5 mmol L^{-1}, its use has a substantial potential to supplement water column-derived DIC and alleviate restrictions on CO_2 supply to submersed plants. The role of sediment CO_2 should, however, depend on large root surface areas relative to photosynthetic leaf area, gas-permeable root walls, proportionally high and well-connected root, stem and leaf aerenchyma, and moderate transport distances. *Lobelia dortmanna*, which relies almost exclusively on sediment CO_2, has a very large biomass of highly gas-permeable roots, large lacunae connecting roots directly with

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leaves, and short leaves in a rosette ensuring short transport distance (Madsen *et al.*, 2002). Hence, species with similar morphology and tissue characteristics should potentially be able to take substantial advantage of the high sediment CO_2 concentrations. In some aquatic plant species, root walls have low gas permeability which minimizes radial O_2 loss to the sediment and ensures effective O_2 supply to the root tip (Colmer, 2003), and this feature is common among wetland species, floating-leaved plants and seagrasses growing in reduced sediments (Armstrong, 1979; Smits *et al.*, 1990; Colmer, 2003; Jensen *et al.*, 2005). If submersed freshwater species also produce almost impermeable roots, it could have adverse effects on root uptake of sediment CO_2, although concentration gradients for CO_2 can be much steeper than O_2 gradients between roots and sediment (Greenway *et al.*, 2006).

Evidence of sediment CO_2 uptake already exists for several plant species, including but not limited to, the isoetids. The emergent *Phragmites australis* has the ability to use sediment CO_2, although only 1% of the fixed carbon was derived from this source (Brix, 1990). The submersed *Vallisneria americana* is also able to mediate a substantial transport of CO_2 from roots to leaves, with up to 85% of CO_2 assimilation being supported by sediment CO_2 (Kimber *et al.*, 1999). The only published attempt to establish whether this phenomenon is important *in situ* was conducted by Loczy *et al.* (1983). They used sediments with $[^{14}C]DIC$ to test if *Myriophyllum spicatum*, *Vallisneria americana* and *Heteranthera dubia* were able to use sediment CO_2, and concluded that none of these species used significant amounts of sediment CO_2 in shoot photosynthesis. Accordingly, apart from the single record on *Vallisneria* (Kimber *et al.*, 1999), documentation of significant supply of sediment CO_2 has not been published for non-isoetid species.

The aim of this study was to examine whether the sediment can be a quantitatively important CO_2 source for submersed aquatic plants other than isoetids. The hypothesis was that the importance of sediment CO_2 uptake is affected by: (*a*) life form; (*b*) tissue porosity; and (*c*) area-based root to shoot ratio. The sediment CO_2 uptake for whole shoot photosynthesis was assessed at varying CO_2 concentrations around the roots in split chambers. Four macrophyte species of different morphology and life form, *Ludwigia repens*, *Lilaeopsis macloviana*, *Hydrocotyle verticillata* and *Vallisneria americana*, were used in the experiments and compared with the isoetid, *Lobelia dortmanna* (Fig. 1). In addition, root $^{14}CO_2$ uptake experiments were conducted with *V. americana* and *L. repens* in order to illustrate spatial differences in carbon fixation and thereby identify bottlenecks to utilization of sediment CO_2.

MATERIALS AND METHODS

Plant material

Five species with different life form and morphology were selected to represent potentially different abilities to take up sediment CO_2 and transport CO_2 by means of molecular gas-phase diffusion from roots to leaves. *Lobelia dortmanna* L. (water lobelia) is a small submersed isoetid with short, stiff leaves in rosettes. It inhabits shallow waters of

FIG. 1. The five plant species studied. From left to right: *Lobelia dortmanna*, *Lilaeopsis macloviana*, *Vallisneria americana*, *Ludwigia repens* and *Hydrocotyle verticillata*.

low-alkaline, oligotrophic lakes across the Northern hemisphere. *Ludwigia repens* Forst., is a fast-growing perennial stem plant native to North America. It is found in both slow-flowing streams and stagnant waters. *Lilaeopsis macloviana* A.W. Hill has hollow, cylindrical leaves emerging directly from a horizontal rhizome. It originates from South America, from the highlands to brackish marshes. *Hydrocotyle verticillata* Thunb. (umbrella plant) is a small plant with single leaves on long petioles emerging directly from a horizontal rhizome. It is a cosmopolitan species usually growing in stagnant waters. *Vallisneria americana* Michx. is a perennial plant with long strap-shaped leaves in a rosette. It is an almost cosmopolitan species found in flowing waters and shallow lakes in America, Asia and Australia. *Ludwigia*, *Lilaeopsis*, *Hydrocotyle* and *Vallisneria* were all one genotype obtained as tissue culture-grown plants from Tropica Aquarium Plants, Denmark, whereas *Lobelia* was collected in Lake Skånes Värsjö, Southern Sweden.

All species, except *Lobelia* and *Vallisneria*, were grown under water from apical shoots of emergent plants. The plants were submersed in high-alkaline tap water (alkalinity = approx. 5 meq. L^{-1}) at 23 °C with 250 µmol photons $m^{-2} s^{-1}$ (PAR) in a 12:12 h light:dark cycle. The water was fertilized with liquid fertilizer (Tropica AquaCare *liquid +*) containing N and P plus micronutrients. The potting soil (washed river sand) was fertilized with 4–5 pellets per plant of a slow release fertilizer (Osmocote Extract-Scotts, standard). The pH was maintained at 6·8 in the water column using a pH stat (Dupla Aquaristik, Grafschaft-Gelsdorf, Germany) through periodically bubbling with CO_2 providing approx. 1·2 mmol L^{-1} of free CO_2 in the water. Water was changed twice a week to prevent excess accumulation of planktonic algae.

Porosity and leaf area measurements

To estimate the potential for gas-phase diffusion of CO_2 from roots to shoot, porosity (% gas space per unit tissue

volume) was measured on samples of roots, leaves and stems by determining tissue buoyancy before and after vacuum infiltration of the gas spaces with water (Raskin, 1983), using the equations for gas volume as modified by Thomson *et al.* (1990).

Root area may serve as a bottleneck for root-mediated CO_2 uptake and, thus, root area was calculated from measurements of the length and diameter of the main and lateral roots using a dissecting microscope. Leaf area was measured using a Li-Cor portable area meter model Li-3000 (Li-Cor, Lincoln, NE, USA). Tissue dry mass was determined after drying for 24 h at $105°C$.

Split-chamber experiments

The aim of the experiment was to describe whole-plant photosynthesis as a function of varying CO_2 concentration around the roots. Whole-plant photosynthesis was measured in two-compartment split chambers, where roots and shoot were hydraulically isolated into two separate water volumes using a Perspex cradle around the base of the plants tightened with hydrophobic lanolin and hardened coconut oil. In this way, CO_2 availability could be manipulated around the roots without affecting the CO_2 concentration in the shoot compartment.

Photosynthetic O_2 production was measured using oxygen electrodes (Unisense OX-500 with a PA2000 pA meter connected to a laptop computer running Picolog for data-logging), which were inserted into both root and shoot compartments. The pH was continuously measured in the root chamber (Radiometer pH electrode with Radiometer pH meter PHM 92) and in the shoot compartment at the beginning and end of each experiment. The chamber was positioned horizontally at $20°C$ in a temperature-controlled water jacket of demineralized water. Two halogen light sources were used for *Lobelia* and *Lilaeopsis* providing 640 µmol photons $m^{-2} s^{-1}$ (PAR). For *Hydrocotyle*, *Vallisneria* and *Ludwigia*, additional irradiance was needed to light saturate photosynthesis, and two extra fluorescent light sources increased the photon flux to 920 µmol $m^{-2} s^{-1}$.

The shoot compartment was filled with artificial lake water without addition of HCO_3^- to keep DIC at a minimum (Smart and Barko, 1985). A phosphate buffer (1 mmol L^{-1}) was used to stabilize the pH at 8 to make sure that DIC in the form of free CO_2 entering the shoot compartment would mainly be converted to HCO_3^-. At pH 8 and concentrations of DIC <0.1 mmol L^{-1}, 98% of the DIC occurs in the form of HCO_3^- and the concentration of free CO_2 is ≤ 2 µmol L^{-1}. The root compartment was filled with DIC-rich water (>10 mmol DIC L^{-1}) made from tap water (approx. 5 mmol DIC L^{-1}; pH 8·5) enriched with equal molar amounts of $KHCO_3$ and $NaHCO_3$.

The experiments were initiated at a pH of approx. 9 (adjusted by addition of 0·1 mol L^{-1} NaOH) in the root chamber to ensure low CO_2 availability (<20 µmol L^{-1}) for root uptake since most DIC occurs as HCO_3^- at this pH. The plants were pre-incubated in light until the oxygen concentration in both the root and shoot chamber decreased at a steady rate, reflecting that pools of DIC within the plant tissue were depleted and concentrations of free CO_2 in the

water were below CO_2 compensation points. After the pre-incubation period, the pH was lowered stepwise in the root chamber by injecting small amounts of HCl (0·1 mol L^{-1}), converting HCO_3^- to free CO_2, and O_2 evolution in both the root and shoot chamber was recorded at each CO_2 concentration. When rates of O_2 evolution in the chambers reached maximum, reflecting that photosynthesis was CO_2 saturated, the experiment was stopped.

Control experiments were conducted to ensure that the plant was only taking up CO_2 from the root chamber and not CO_2 or HCO_3^- leaking from the root to the shoot chamber during the experiment. At the end of each experiment, the pH was raised to 9 in the root chamber by injecting NaOH to remove virtually all free CO_2 from the root medium. If the oxygen concentration then declined, it was assumed that there had been no leakage of free CO_2 into the shoot compartment. As an additional control, water samples were taken from the shoot compartment at the beginning and end of each experiment and analysed for DIC on an infrared gas analyser (ADC, 225-MK3) to verify that the DIC concentration in the shoot compartment remained low and did not increase during the experiment. After each experiment, leaf and root area and dry mass were quantified.

Maximum leaf photosynthesis

For all species, leaf photosynthesis based on water column CO_2 was measured as a function of free CO_2 to allow comparison with photosynthesis based on sediment CO_2 and estimates of the relative importance of sediment and water column as sources of CO_2 under different DIC availabilities. Leaf sections of approx. 10–20 mg dry mass were cleaned of epiphytes and mounted in a temperature-controlled Perspex chamber. Whole leaves of *Lobelia* and *Lilaeopsis* exhibited low photosynthetic rates, possibly due to the presence of cuticle or wax, and were therefore cut longitudinally to offset leaf surface resistance towards gas diffusion and enhance DIC transport to photosynthetic tissues. The medium was made from one part tap water and one part demineralized water, with a resulting alkalinity of 2·83 meq. L^{-1}. A halogen light source provided 270 µmol photons $m^{-2} s^{-1}$ of PAR, which should be sufficient to light saturate photosynthesis of individual leaves (Binzer *et al.*, 2006). The availability of free CO_2 was changed stepwise by changing the pH from a maximum of 9·2 through addition of 0·1 mol L^{-1} HCl. This procedure was repeated until the oxygen evolution reached a maximum, reflecting CO_2-saturated photosynthesis. The oxygen concentration was measured and logged together with pH as described for whole-plant photosynthesis. After each experiment, leaf dry mass and area were measured as described earlier.

^{14}C incorporation in Vallisneria americana and Ludwigia repens

Root $^{14}CO_2$ uptake experiments were conducted with *V. americana* and the elodeid species *L. repens* to describe spatial patterns in carbon fixation and identify potential bottlenecks in utilization of sediment CO_2. The plants were mounted with their roots in closed PVC chambers and the shoots exposed to a common bulk chamber holding 13·5 L. A

water-tight seal made of a Perspex cradle and lanolin was placed around the base of each plant. The root medium was made from tap water (alkalinity 5·7 meq. L^{-1}) enriched with $NaHCO_3$ and $KHCO_3$ to raise the alkalinity to 7·8 meq. L^{-1}. The pH was lowered to 6·1 using HCl to achieve a free CO_2 concentration of 5 mmol L^{-1}.^{14}C was added as dissolved inorganic carbon to a specific activity of 1290 Bq mL^{-1}. The shoot bulk chamber was filled with a DIC-poor medium ($<$0·1 mmol DIC L^{-1}; Smart and Barko, 1985) at a temperature of 21°C.

The plants were incubated for 4 h, with one set of plants kept at 1490 μmol photons m^{-2} s^{-1} and another set in darkness. A sample of the shoot medium was analysed every hour to detect if ^{14}C had leaked from the root chamber to the bulk water. To check further for any ^{14}C leakage into the bulk water, shoots of *Ludwigia* with no access to the root medium were placed freely floating in the shoot medium and later analysed for ^{14}C incorporation in the light. Both controls showed that no leakage of ^{14}C had occurred. After incubation, the plants were harvested and cut into segments of stems or leaves, dried at 70°C for 24 h and homogenized with a pestle in a mortar. Approximately 1–8 mg of tissue was added to 20 mL plastic vials and 500 μL of ethylene glycol was injected to dissolve the plant tissue. After 24 h, 10 mL of Ultima Gold scintillation liquid were added (Perkin Elmer and Analytical Sciences). The vials were placed on a rotating wheel for 3 d to dissolve and homogenize the samples further, and then counted on a tri-carb 2800 TR scintillation counter. To compensate for differences in quenching with different amounts of ^{14}C-labelled plant material, counting efficiency was estimated by adding inorganic ^{14}C to variable amounts of non-labelled plant material dissolved in ethylene glycol and scintillation liquid.

Statistics

Data were analysed statistically using Graph Pad Prism 5 and a 0·05 significance level. Some data were log transformed to ensure variance homogeneity (Bartlett's test). One- or two-way analysis of variance (ANOVA) with a Tukey *post hoc* test or Student's *t*-test were used to test for significant differences.

For each experiment, data on leaf and whole-plant photosynthesis were fitted to a modified Jassby and Platt (1976) model:

$$Y = P_{\max}\tanh(\alpha x/P_{\max}) \qquad (1)$$

where $P_{\max}$ is the maximum photosynthetic rate (μmol O_2 g^{-1} d. wt leaf h^{-1}), α is the initial slope of the curve [μmol O_2 g^{-1} d. wt leaf h^{-1} (mmol CO_2 L^{-1})$^{-1}$] and x is the CO_2 concentration (μmol CO_2 L^{-1}). Standard linear regression was used to estimate α because the model (eqn. 1) did not fit some of the replicates of each species properly. The α values were analysed by two-way ANOVA and a Bonferroni *post hoc* test which requires values for each replicate.

TABLE 1. *Tissue porosity (% gas volume) in the five species of aquatic plants*

	Leaf	Root	Stem/petiole
Lobelia dortmanna	33·6 ± 6·5[c]	63·3 ± 10·3[a]	–
Lilaeopsis macloviana	62·2 ± 5·5[a]	27·7 ± 9·6[b]	–
Vallisneria americana	43 ± 8·6[b]	34·4 ± 8·1[b]	–
Ludwigia repens	13·5 ± 2·8[d]	20·3 ± 3·3[c]	19 ± 10·5[a]
Hydrocotyle verticillata	4·2 ± 0·6[e]	12·8 ± 5·7[c]	17·5 ± 11·8[a]

For *Hydrocotyle*, porosity was measured in petioles and for *Ludwigia* in stems. Values are mean ± s.d., $n = 3$ except for *Lilaeopsis* where $n = 5$, and for the stem of *Hydrocotyle* where $n = 2$. One-way ANOVA and Tukey *post hoc* tests were used to test differences among species within each group of tissues, and significant differences are indicated by different letters ($P \leq$ 0·05). –, data not available.

RESULTS

Plant morphology and tissue porosity

The shoot to root area ratio was highly variable among species, i.e. 0·76 for *Lilaeopsis* to 56·4 for *Hydrocotyle*. The shoot to root area ratio was 0·98 for *Lobelia* and 0·99 for *Ludwigia*, and only *Vallisneria* (3·1) and *Hydrocotyle* had ratios $>$1·0.

Porosity varied significantly among species, being higher in *Lobelia*, *Vallisneria* and *Lilaeopsis* than in *Hydrocotyle* and *Ludwigia* (Table 1). Porosity ranged from 4·2 to 62·2% in leaves and from 12·8 to 63·3% for roots. The highest leaf porosity was found in *Lilaeopsis* and the lowest in *Hydrocotyle*. Root porosity was significantly higher in *Lobelia* than in the other species. Root porosity exceeded leaf porosity in *Lobelia*, while the opposite was found for *Lilaeopsis*. No significant differences between leaf, root and stem porosity were found for the remaining species.

Split-chamber experiments and maximum leaf photosynthesis

The split-chamber experiments showed that four of the five species were able to sustain photosynthesis based on sediment CO_2 supply (Table 2). The response to increasing CO_2 concentrations around the roots followed saturation curves (Fig. 2) very similar to that for leaf photosynthesis based on water CO_2 supply (data not shown). Only *Hydrocotyle* did not follow this pattern as increasing CO_2 around the roots did not stimulate photosynthesis at all. Maximum gross photosynthesis ($P_{\max\ (\text{root } CO_2)}$) based on sediment CO_2 varied from negative rates for *Hydrocotyle* to 432 μmol O_2 g^{-1} d. wt h^{-1} in *Lobelia* (Table 2). Maximum gross photosynthesis based on water column CO_2 supply ($P_{\max\ (\text{leaf } CO_2)}$) varied only 2-fold from 303 to 630 μmol O_2 g^{-1} d. wt h^{-1}.

The initial slope of the relationship between photosynthesis and CO_2 concentration reflects the efficiency with which the plant is able to utilize CO_2 at low CO_2 availability in the sediment or water column, with steep slopes reflecting low resistance to CO_2 uptake (Table 2). The initial slopes were consistently steeper for water column CO_2 supply, with no significant differences among species. When CO_2 was taken up through the roots, the initial slopes varied significantly among species but with some overlap. *Lobelia* was the most efficient species to utilize sediment CO_2 (i.e. exhibited the

TABLE 2. *Maximum gross photosynthetic rates (P_{max}), initial slope of photosynthesis vs. CO_2 (α) and CO_2 saturating concentrations for the five plant species with leaf and root supply of CO_2*

	P_{max} (μmol O_2 g^{-1} d. wt h^{-1})		α [μmol O_2 g^{-1} d. wt h^{-1} (mmol CO_2 L^{-1})$^{-1}$]		Saturating concentration (mmol CO_2 L^{-1})	
	Leaf-supplied CO_2	Root-supplied CO_2	Leaf-supplied CO_2	Root-supplied CO_2	Leaf-supplied CO_2	Root-supplied CO_2
Lobelia dortmanna	630 ± 106[a]	432 ± 83[a]	687 ± 319[ab]	132 ± 43[a]	1·6	2·7
Lilaeopsis macloviana	535 ± 104[a]	134 ± 36·1[a]	1388 ± 216[ab]	82 ± 27[ab]	0·25	1·3
Vallisneria americana	597 ± 76[a]	101 ± 101[ab]	2052 ± 1270[a]	50 ± 73[b]	0·34	8
Ludwigia repens	303 ± 63[a]	10·1 ± 8·8[b]	1602 ± 1073[ab]	21 ± 20[b]	0·29	9·5
Hydrocotyle verticillata	576 ± 257[a]	−8·6 ± 7[b]	225 ± 166[b]	–	2·25	–

Student's *t*-test was used to test for differences in P_{max} values (mean ± s.d., $n = 4$–6, $P = 0.05$). Two-way ANOVA was used to analyse for differences in initial slope between the two CO_2 sources (mean ± s.d., $n = 4$–6, $P = 0.05$), and one-way ANOVA and Tukey *post hoc* to analyse differences among species for each CO_2 source (mean ± s.d., $n = 4$–6, $P = 0.05$). Within each column, significant differences are marked by different letters. –, data not available.

steepest slope), followed by *Lilaeopsis* and *Vallisneria*, while *Ludwigia* was rather inefficient. The initial slope could not be estimated for *Hydrocotyle*. For *Lobelia*, the initial slope was not significantly lower when CO_2 was supplied by the roots even though leaves had been sliced open to facilitate uptake in the experiment with water column CO_2 supply (Student's *t*-test).

Saturating concentrations for CO_2 supplied by the roots varied from 1·3 mmol CO_2 L^{-1} for *Lilaeopsis* to 9·5 mmol CO_2 L^{-1} for *Ludwigia*. Since *Hydrocotyle* was unable to use root-supplied CO_2, no saturating concentration could be estimated for root uptake (Table 2). The saturating CO_2 concentrations estimated for water column uptake were much lower than for root-mediated uptake, except for *Lobelia*.

The role of sediment CO_2 supply for whole-plant gross photosynthesis at different water column CO_2 concentrations was estimated by combining data from the fits of photosynthesis of leaf segments supplied by CO_2 from the water column with estimated maximum rates for whole shoots in split-chamber experiments (Table 3). For *Lobelia*, sediment CO_2 supplied >85% of photosynthesis up to water column CO_2 concentrations of 100 μmol L^{-1} even when water column uptake was facilitated by slicing leaves open, resulting in very conservative estimates of the role of sediment uptake.
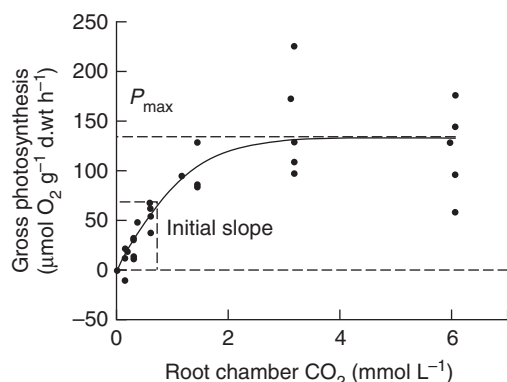
For *Lilaeopsis*, leaves were similarly sliced to facilitate water column CO_2 supply, and roots supplied >50% of all CO_2 as long as water column concentrations were <100 μmol CO_2 L^{-1}. For *Vallisneria*, sediment uptake supplied >34% of total DIC uptake at water column CO_2 concentrations up to 100 μmol CO_2 L^{-1}, while sediment contributed <30% of total CO_2 uptake in *Ludwigia* even at water column CO_2 concentrations in air equilibrium (16 μmol L^{-1}).

Using data for root area and $P_{max\ (root\ CO_2)}$ for each of the five plant species, a maximum root CO_2 uptake was calculated (Table 4). The highest root uptake was found in *Lobelia* of 123 pmol CO_2 cm^{-2} root s^{-1}, which was significantly higher than for the other species (Tukey *post hoc*). *Lilaeopsis* and *Vallisneria* had significantly higher CO_2 uptake per root surface area than *Ludwigia* (Table 4).

^{14}C root uptake, transport and assimilation in shoots

The experiment with ^{14}C supplied to the root medium showed that both *Vallisneria* and *Ludwigia* were able to take up inorganic ^{14}C via the roots and incorporate it in the shoots (Fig. 3). Water samples from the shoot chamber and suspended shoots of *Ludwigia* with no access to labelled ^{14}C in the root chamber showed no incorporation of ^{14}C above background levels (data not shown), confirming that seals

FIG. 2. Gross photosynthesis of *Lilaeopsis macloviana* vs. CO_2 concentration around the roots in split chambers with CO_2 concentrations close to zero around the leaves. Five replicates were fitted to a photosynthesis model (Jassby and Platt, 1976) in order to estimate P_{max}, and the initial slope was estimated by linear regression.

TABLE 3. *Contribution to total plant photosynthesis (%) of root CO_2 uptake ($P_{max\ (root\ CO_2)}$) at variable water column CO_2 concentrations and sediment CO_2 concentrations supporting maximum rates of photosynthesis (>10 mmol L^{-1})*

	Water CO_2 concentration (μmol L^{-1})			
Species	16	100	500	1000
Lobelia dortmanna	98	86	58	46
Lilaeopsis macloviana	86	50	23	20
Vallisneria americana	75	34	15	14
Ludwigia repens	28	6	3	3
Hydrocotyle verticillata	–	–	–	–

Estimates of root contributions were conservative for *Lobelia* and *Lilaeopsis* since leaves were cut open to facilitate photosynthesis based on supply of CO_2 from the water column. –, data not available.

TABLE 4. *Maximum root CO$_2$ uptake per root area for the five plant species*

	Maximum root CO$_2$ uptake (pmol CO$_2$ cm^{-2} root s^{-1})
Lobelia dortmanna	123 ± 20·3[a]
Lilaeopsis macloviana	55·3 ± 10·6[b]
Vallisneria americana	56·7 ± 6·3[b]
Ludwigia repens	5·5 ± 22·6[c]
Hydrocotyle verticillata	–

A Tukey *post hoc* test was used to test for differences among species (mean ± s.d., $n = 4–6$, $P = 0·05$). Significant differences are marked with different letters. –, data not available.

between root and shoot chambers were tight and that transport occurred only inside the plants.

For both species, ^{14}C fixation decreased systematically with increasing distance to the roots (one-way ANOVA, $P < 0·05$; Fig. 3). In *Vallisneria*, the bulk of the ^{14}C was assimilated in the lower 0–2 cm sections of the leaves, while transport to leaf tips (8–11 cm) was negligible. In *Ludwigia*, transport of ^{14}C to stems and leaves followed a similar decline with distance to the roots (Fig. 3). ^{14}C assimilation was much higher in stems of *Ludwigia* than in leaves, except in the shoot apex, where ^{14}C fixation peaked immediately below the apex.

DISCUSSION

Widespread use of sediment CO$_2$

Four out of the five studied plant species were able to use CO$_2$ taken up by the roots, although with highly variable efficiency. *Lobelia* was the most efficient to use CO$_2$ supplied via the roots. It had the highest $P_{max \; (root \; CO_2)}$ and the highest CO$_2$ uptake per root area, rendering it extremely efficient at supplying CO$_2$ from the root to the shoot. This agrees with the conclusion of Sand-Jensen and Søndergaard (1978) that 95% of the assimilated CO$_2$ in *Lobelia* is usually derived from the sediment in accordance with its natural distribution in DIC-poor lakes. The results also showed that *Lilaeopsis* and *Vallisneria* are able to use sediment CO$_2$, and *Lilaeopsis* in particular used sediment CO$_2$ very effectively. *Ludwigia* was capable of sustaining low rates of photosynthesis with sediment CO$_2$, but its $P_{max \; (root \; CO_2)}$ was >40 times lower than that of *Lobelia* and ten times lower than in *Vallisneria*. *Hydrocotyle* was, however, unable to sustain photosynthesis with root-supplied CO$_2$ even at very high CO$_2$ concentrations around the roots.

The fact that both *Vallisneria* and *Lilaeopsis* could use CO$_2$ supplied from the sediment challenges the general perception that only isoetids possess this capability (Loczy *et al.*, 1983; Madsen and Sand-Jensen, 1991). Previously, it has been shown that species of *Lilaeopsis* and *Vallisneria* also have CAM metabolism similar to some isoetids (Webb *et al.*, 1988), suggesting that these two non-isoetid plants have several mechanisms which can at least partly ameliorate inorganic carbon limitation in waters with low availability of free CO$_2$. While this study concludes that some life forms such as *Ludwigia* and *Hydrocotyle* are able to use little, or no,
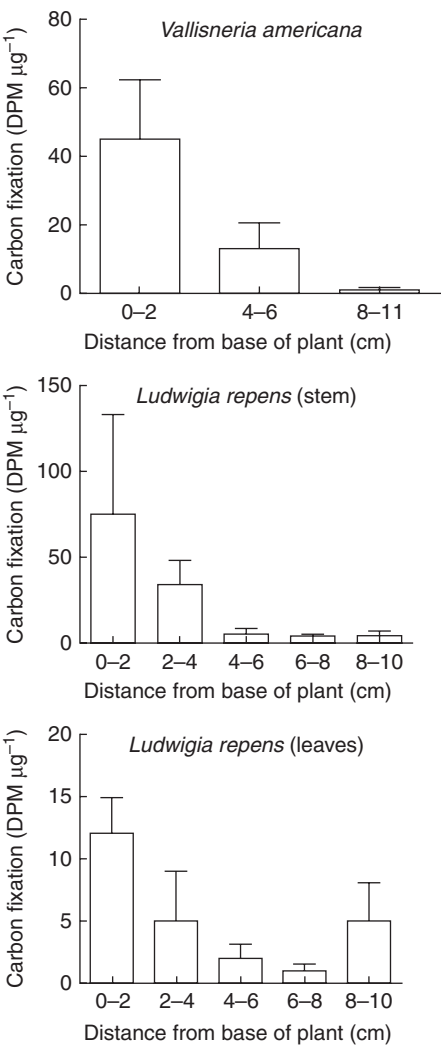

FIG. 3. Carbon fixation (mean ± s.d., $n = 5$) presented as ^{14}C activity (disintegrations per minute, DPM, µg^{-1} d. wt) in different shoot sections of *Vallisneria americana* and *Ludwigia repens* after incubation in the light with ^{14}C-labelled DIC added to the root chamber at a CO$_2$ concentration of 5 mmol L^{-1}. Values were corrected for uptake and transport in darkness.

sediment CO$_2$, submersed plants other than isoetids are certainly able to cover a substantial part of their CO$_2$ requirements by root-mediated CO$_2$ uptake when the DIC availability in the water column is low. The variable ability among species to utilize sediment CO$_2$ may depend on several factors: (*a*) the concentration of CO$_2$ in the interstitial pore water relative to that of the water column; (*b*) root area relative to shoot area; (*c*) CO$_2$ permeability of root walls; (*d*) root, stem and leaf porosity to support internal transport via gas phase diffusion; (*e*) internal diffusion barriers and morphological bottlenecks; and (*g*) distance from the roots to the leaves.

CO$_2$ availability

The specific CO$_2$ concentrations in the root chamber at which leaf photosynthesis saturated varied greatly among the four species that were able to use sediment CO$_2$. Photosynthesis of *Lobelia* and *Lilaeopsis* based on sediment

CO$_2$ saturated well below the sediment concentrations of 5 mmol CO$_2$ L^{-1} reported for different types of sediment (Wium-Andersen and Andersen, 1972). For both species, even with the conservative estimates due to the sliced leaves during the measurements, sediment CO$_2$ uptake played a major role at water column CO$_2$ concentrations up to 100 μmol L^{-1}, which is well above the atmospheric equilibrium of 16 μmol L^{-1}. The photosynthesis of *Vallisneria* and *Ludwigia* saturated at somewhat higher root concentrations of 8–10 mmol L^{-1} and none of these species was able to achieve as high photosynthetic rates as when saturated with CO$_2$ from the water column, indicating constraints on CO$_2$ supply from the sediment other than the relative availability of CO$_2$ from the two sources. For *Vallisneria*, the importance of water column DIC is further strengthened by its ability to utilize bicarbonate (Titus and Stone, 1982).

The effect of shoot to root area ratio on uptake of root-mediated CO$_2$

There was a negative correlation between $P_{\text{max (root CO}_2)}$ and shoot to root area ratio for *Lobelia*, *Lilaeopsis* and *Vallisneria*, indicating that for these species a high root area relative to leaf area enhances the use of sediment CO$_2$ (Fig. 4). In general, an area-based shoot to root ratio around or below 1·0 for these species seemed to be a suitable ratio for root uptake of CO$_2$, as also suggested by Madsen and Sand-Jensen (1991). *Ludwigia* had a low shoot to root area ratio but low sediment-based photosynthesis, suggesting that the root supply of CO$_2$ was limited by factors other than the area available for root CO$_2$ uptake. In contrast, the fact that *Hydrocotyle* was unable to use CO$_2$ taken up through the roots agrees well with its extremely high shoot to root area ratio.

Gas permeability

The gas permeability of the root surface is essential for the ability of CO$_2$ to diffuse into the roots, and therefore highly important for uptake of sediment CO$_2$. Some plant groups (wetland species, seagrasses and aquatic plants with floating leaves) growing in anoxic soils or sediments exhibit low gas permeability in the roots to minimize radial O$_2$ loss to the
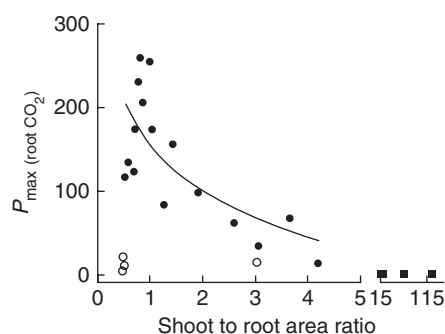
sediment and ensure oxygen supply to the root tips (Armstrong, 1979; Smits *et al.*, 1990; Colmer, 2003). The same trait may be present in submersed freshwater plants and may also hold for exchange of CO$_2$, although gradients in CO$_2$ are much steeper than O$_2$ gradients. Since *Lobelia* relies almost entirely on sediment CO$_2$ and releases most of its photosynthetically produced oxygen to the sediment, it must have a high gas permeability (Sand-Jensen *et al.*, 1982; Pedersen *et al.*, 1995; Møller and Sand-Jensen, 2008). Since both *Lilaeopsis* and *Vallisneria* can effectively use root-mediated CO$_2$, it is likely that their roots are also highly gas permeable although the estimated maximum CO$_2$ uptake per root area was about half of that for *Lobelia*. However, as for *Ludwigia*, which exhibited very low CO$_2$ uptake rates per root area, it is not possible to separate constraints on uptake from those of transport processes.

Tissue porosity

Root, stem and leaf porosity is obviously essential for efficient transport and use of sediment CO$_2$. Once CO$_2$ has penetrated into the roots, it needs to be transported by molecular diffusion in the air-filled lacunae to the photosynthetically active tissue (Brix, 1990; Madsen and Sand-Jensen, 1991). According to Fick's first law of diffusion, the rate depends on the cross-sectional area of the lacunae and the steepness of the concentration gradient determined by the difference in CO$_2$ concentrations divided by the length of the lacunae. Hence, an estimate of the potential role of CO$_2$ transport from roots to shoots of different lengths can be made by assuming no CO$_2$ consumption along the diffusion path, as presented in a review by Madsen and Sand-Jensen (1991). Based on their calculations, Madsen and Sand-Jensen (1991) concluded that CO$_2$ transport inside the lacunae of submersed plants would only be significant over short distances. However, applying the cross-sectional area of the lacunae in *Ludwigia* (0·93 mm^2), which had a moderate stem porosity of 19%, revealed that diffusion could supply 40 pmol CO$_2$ s^{-1} at a distance of 200 cm and 130 pmol CO$_2$ s^{-1} at 60 cm if the root CO$_2$ concentration was 5 mmol L^{-1} (Fig. 5). These supply

FIG. 4. Relationship between maximum photosynthesis based on root CO$_2$ uptake ($P_{\text{max (root CO}_2)}$) and plant shoot/root area ratio for *Lobelia*, *Vallisneria*, *Lilaeopsis*, *Ludwigia* and *Hydrocotyle*. *Ludwigia* and *Hydrocotyle* were not used to fit the model. Filled circles, *Lobelia*, *Vallisneria* and *Lilaeopsis*; open circles, *Ludwigia*; filled squares, *Hydrocotyle*.
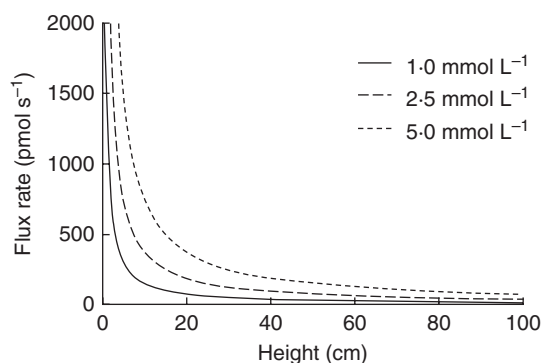
FIG. 5. Calculated fluxes of CO$_2$ in the internal lacunae from the root medium to the apex of *Ludwigia* as a function of shoot length. The rates are calculated from Fick's first law of diffusion assuming root CO$_2$ concentrations of 1, 2·5 and 5 mmol L^{-1}, and apical concentrations of 0 mmol L^{-1}. Exchange of CO$_2$ along the length of the shoot is not taken into account. The cross-sectional area of the lacunae was estimated to be 0·93 mm^2. Modified from Madsen and Sand-Jensen (1991).

rates can be compared with average photosynthetic CO$_2$ requirements of between 40 and 125 pmol cm^{-2} leaf s^{-1} for elodeids as done by Madsen and Sand-Jensen (1991). Although the estimates and comparison are based on rather simple assumptions, the calculations show that root supply of CO$_2$ by diffusion is certainly not trivial even over distances of $\geq$20 cm and especially not for species with high tissue porosity and large cross-sectional area of lacunae in stem and leaves as in *Lobelia*, *Lilaeopsis* and *Vallisneria* (Table 1).

Although tissue porosity was relatively high in *Vallisneria*, the ^{14}C experiment and the role of sediment CO$_2$ estimated from the split-chamber experiment showed a moderate CO$_2$ contribution from the sediment, when water column CO$_2$ was above air saturation. This could probably be due to the less favourable shoot to root area ratio, since *Vallisneria* had 3·1 cm^2 of leaf to be supplied with CO$_2$ per cm^2 of root surface. *Ludwigia* had a low shoot to root area ratio and reasonable tissue porosity, but was nevertheless not very well supplied by sediment CO$_2$, since <30% of maximum gross photosynthesis could be supported by root uptake even at very low water column CO$_2$ availability. Either low gas permeability of roots or diffusion barriers between tissues could limit CO$_2$ uptake or transport. For example, ^{14}C fixation was much higher in the green *Ludwigia* stems than in leaves, suggesting transport constraints in the short petioles.

Hydrocotyle was virtually unable to utilize sediment CO$_2$, and this agrees well with its morphology and life form. Although the plant is small with short transport distances, the use of sediment CO$_2$ would be restricted by the very high shoot to root area ratio and the low tissue porosity. In addition, diffusion barriers may be present between the long petiole and the leaf.

Conclusion and implications

All examined species except *H. verticillata* were able to utilize sediment CO$_2$, although with variable efficiency. As expected, and as well described in the literature, the isoetid *L. dortmanna* was the most effective sediment CO$_2$ user. *Lilaeopsis macloviana* was almost as efficient as *Lobelia* and it can be expected that quite a number of species with a life form and morphology involving low shoot to root area, high tissue porosity and close connection between leaves and roots can take considerable advantage of the high concentrations of free CO$_2$ in the sediment. For such species, the diffusion model shows a potential for substantial CO$_2$ supply over decimetres to even metres if the cross-sectional area of lacunae is large. If uptake or diffusion constraints occur, as in *V. americana* and *L. repens*, the role of sediment CO$_2$ is smaller but still not trivial. Only species with poor root development and/or low tissue porosity may not benefit from sediment CO$_2$ at all. Accordingly, sediment CO$_2$ supply may play an important role for many small and medium-sized submersed plant species especially in low alkaline lakes with low CO$_2$ concentrations in the water column.

ACKNOWLEDGEMENTS

LITERATURE CITED

Armstrong W. 1979. Aeration in higher plants. *Advances in Botanical Research* **7**: 225–332.

Binzer T, Sand-Jensen K, Middelboe AL. 2006. Community photosynthesis of aquatic macrophytes. *Limnology and Oceanography* **51**: 2722–2733.

Boston HL, Adams MS, Pienkowski TP. 1987. Utilization of sediment CO$_2$ by selected North-American isoetids. *Annals of Botany* **60**: 485–494.

Brix H. 1990. Uptake and photosynthetic utilization of sediment-derived carbon by *Phragmites australis* (Cav) Trin Ex Steudel. *Aquatic Botany* **38**: 377–389.

Colmer TD. 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell and Environment* **26**: 17–36.

Greenway H, Armstrong W, Colmer TD. 2006. Conditions leading to high CO$_2$ (>5 kPa) in waterlogged–flooded soils and possible effects on root growth and metabolism. *Annals of Botany* **98**: 9–32.

Jassby AD, Platt T. 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography* **21**: 540–547.

Jensen SI, Kuhl M, Glud RN, Jorgensen LB, Prieme A. 2005. Oxic microzones and radial oxygen loss from roots of *Zostera marina*. *Marine Ecology-Progress Series* **293**: 49–58.

Jones JI. 2005. The metabolic cost of bicarbonate use in the submerged plant *Elodea nuttallii*. *Aquatic Botany* **83**: 71–81.

Kimber A, Crumpton WG, Parkin TB, Spalding MH. 1999. Sediment as a carbon source for the submerged macrophyte *Vallisneria*. *Plant, Cell and Environment* **22**: 1595–1600.

Loczy S, Carignan R, Planas D. 1983. The role of roots in carbon uptake by the submersed macrophytes *Myriophyllum spicatum*, *Vallisneria americana*, and *Heteranthera dubia*. *Hydrobiologia* **98**: 3–7.

Maberly SC, Madsen T. 2002. Freshwater angiosperm carbon concentrating mechanisms: processes and patterns. *Functional Plant Biology* **29**: 292–405.

Maberly SC, Spence DHN. 1989. Photosynthesis and photorespiration in freshwater organisms: amphibious plants. *Aquatic Botany* **34**: 267–286.

Madsen TV, Maberly SC. 1991. Diurnal variation in light and carbon limitation of photosynthesis by two species of submerged freshwater macrophyte with a differential ability to use bicarbonate. *Freshwater Biology* **26**: 175–187.

Madsen TV, Sand-Jensen K. 1991. Photosynthetic carbon assimilation in aquatic macrophytes. *Aquatic Botany* **41**: 5–40.

Madsen TV, Olesen B, Bagger J. 2002. Carbon acquisition and carbon dynamics by aquatic isoetids. *Aquatic Botany* **73**: 351–371.

Møller CL, Sand-Jensen K. 2008. Iron plaques improve the oxygen supply to root meristems of the freshwater plant, *Lobelia dortmanna*. *New Phytologist* **179**: 848–856.

Pedersen O, Sand-Jensen K, Revsbech NP. 1995. Diel pulses of O$_2$ and CO$_2$ in sandy lake sediments inhabited by *Lobelia dortmanna*. *Ecology* **76**: 1536–1545.

Prins HBA, Elzenga JTM. 1989. Bicarbonate utilization – function and mechanism. *Aquatic Botany* **34**: 59–83.

Raskin I. 1983. A method for measuring leaf volume, density, thickness, and internal gas space. *Hortscience* **18**: 698–699.

Sand-Jensen K, Søndergaard M. 1978. Growth and production of isoetids in oligotrophic Lake Kalgaard, Denmark. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **20**: 659–666.

Sand-Jensen K, Prahl C, Stokholm H. 1982. Oxygen release from roots of submerged aquatic macrophytes. *Oikos* **38**: 349–354.

Smart RM, Barko JW. 1985. Laboratory culture of submersed freshwater macrophytes on natural sediments. *Aquatic Botany* **21**: 251–263.

Smits AJM, Laan P, Thier RH, Vandervelde G. 1990. Root aerenchyma, oxygen leakage patterns and alcoholic fermentation ability of the roots

of some nymphaeid and isoetid macrophytes in relation to the sediment type of their habitat. *Aquatic Botany* **38**: 3–17.

Thomson CJ, Armstrong W, Waters I, Greenway H. 1990. Aerenchyma formation and associated oxygen movement in seminal and nodal roots of wheat. *Plant, Cell and Environment* **13**: 395–403.

Titus JE, Stone WH. 1982. Photosynthetic response of 2 submersed macrophytes to dissolved inorganic carbon concentration and pH. *Limnology and Oceanography* **27**: 151–160.

Webb DR, Rattray MR, Brown JMA. 1988. A preliminary survey for crassulacean acid metabolism (CAM) in submerged aquatic macrophytes in New Zealand. *New Zealand Journal of Marine and Freshwater Research* **22**: 231–235.

Wium-Andersen S. 1971. Photosynthetic uptake of free CO$_2$ by roots of *Lobelia dortmanna*. *Physiologia Plantarum* **25**: 245–248.

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