Aquatic adventitious roots of the wetland plant *Meionectes brownii* can photosynthesize: implications for root function during flooding

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Summary

- Many wetland plants produce aquatic adventitious roots from submerged stems. Aquatic roots can form chloroplasts, potentially producing endogenous carbon and oxygen. Here, aquatic root photosynthesis was evaluated in the wetland plant *Meionectes brownii*, which grows extensive stem-borne aquatic roots during submergence.
- Underwater photosynthetic light and CO₂ response curves were determined for aquatic-adapted leaves, stems and aquatic roots of *M. brownii*. Oxygen micro-electrode and ¹⁴CO₂-uptake experiments determined shoot inputs of O₂ and photosynthate into aquatic roots.
- Aquatic adventitious roots contain a complete photosynthetic pathway. Underwater photosynthetic rates are similar to those of stems, with a maximum net photosynthetic rate (P_max) of 0.38 µmol O₂ m⁻² s⁻¹; however, this is c. 30-fold lower than that of aquatic-adapted leaves. Under saturating light with 300 mmol m⁻³ dissolved CO₂, aquatic roots fix carbon at 0.016 µmol CO₂ g⁻¹ DM s⁻¹. Illuminated aquatic roots do not rely on exogenous inputs of O₂.
- The photosynthetic ability of aquatic roots presumably offers an advantage to submerged *M. brownii* as aquatic roots, unlike sediment roots, need little O₂ and carbohydrate inputs from the shoot when illuminated.

Introduction

Root function often becomes impeded in flooded soils (Armstrong & Drew, 2002). Dissolved O₂ within sediments is depleted through root and microbial respiration and slow gas diffusion rates impede O₂ entry, leading to soil anoxia (Drew & Lynch, 1980). Roots in anoxic sediments rely on shoots not only for carbohydrates via the phloem, but also for O₂, which is supplied internally via the aerenchyma (Armstrong, 1979; Colmer, 2003).

In many flood-tolerant terrestrial species, growth of adventitious roots into the surface sediments is induced by flooding (Jackson & Drew, 1984). These new roots are beneficial for flooding tolerance, as a result of morphological adaptations such as aerenchyma and barriers to radial O₂ loss, which contribute to their superior internal transport of O₂ (Armstrong, 1979; Justin & Armstrong, 1987; Colmer, 2003), enabling growth into anoxic sediments. Roots growing in these anoxic environments receive O₂ from shoots via the aerenchyma. This O₂ can be atmospheric when shoots are above water, or when shoots are completely submerged, O₂ can be sourced via inward diffusion from the water column or from shoot photosynthesis when light is sufficient (Colmer, 2003; Voesenek *et al.*, 2006). Photosynthesis by submerged shoots can, however, be restricted by low light and/or decreased availability of CO₂ as a result of low dissolved concentrations, slow diffusion in water, boundary layers impeding entry, and stomatal closure (Smith & Walker, 1980; Mommer *et al.*, 2005). Thus, sediment roots of submerged plants can experience large diurnal fluctuations in O₂.
availability, dependent on shoot photosynthetic rate as CO₂ supply changes (e.g. rice (Oryza sativa); Colmer & Pedersen, 2008) and periods of anoxia can occur during the night (Waters et al., 1989; Pedersen et al., 2006; Holmer et al., 2009).

Growth of aquatic adventitious roots (hereafter referred to as aquatic roots) from stems into the floodwaters can also be initiated as a response to flooding in many wetland species (Khan et al., 1981; Etherington, 1984; Naidoo & Naidoo, 1992; Rich et al., 2008). Aquatic roots share many morphological features with sediment adventitious roots, and in addition, as aquatic roots can be exposed to light, these can form photosynthetically active chloroplasts, which we hypothesize could contribute to their O₂ and carbohydrate status (Rich et al., 2008).

This study evaluated photosynthesis in aquatic roots and the capacity of these roots to produce some of their requirements (i.e. O₂ and CO₂ fixation) independently from the shoot. A herbaceous wetland species Meionectes brownii (syn. Haloragis brownii; Moody & Les, 2007) was chosen as it quickly grows an extensive pigmented aquatic root system when submerged and is therefore a good model system for examining the physiology of aquatic roots. Field-collected material was used to determine photosynthetic O₂ production rates in aquatic roots, and the movement of endogenously produced O₂ from shoots to aquatic roots. Propagated material was used in a series of ¹⁴CO₂-uptake experiments confirming carbon fixation in these roots and investigating carbon input from the shoots. This study demonstrates for the first time the presence of a complete photosynthetic pathway, capable of both O₂ evolution and carbon fixation, in the aquatic roots of a terrestrial wetland plant.

**Materials and Methods**

**Plant material**

*Meionectes brownii* Hook. f. is a herbaceous perennial commonly found in wetland fringes across southern Australia and is highly tolerant of prolonged inundation to 1 m (Romanowski, 1998). When these plants are partially or fully submerged they grow an extensive aquatic root system (Fig. 1), and after several weeks of submergence form thin, highly dissected, aquatic-adapted leaves. *Meionectes brownii* was collected from an unnamed ephemeral wetland c. 8 km southeast of Albany, Western Australia (35.0779°S, 117.9267°E), over two collection trips in successive years during October (i.e. during spring). In both years, at the time of collection, the wetland was flooded to a depth of c. 0.3 m. Floodwaters were clear with photosynthetically active radiation (PAR) at 0.25 m of c. 100 l mol m⁻² s⁻¹, when incident light at the surface was 520 l mol m⁻² s⁻¹. Plants were collected at dawn and wrapped individually in paper towel dampened with wetland water, placed in plastic sample bags within an insulated cool-box and transported on the same day to Perth, Western Australia. Plants not used within 3 d for internal O₂ measurements were transported to The Freshwater Biological Laboratory, Hillerød, Denmark. For transport to Denmark, apical shoots 100 mm in length were wrapped individually in paper towel dampened with tap water. Following arrival in Denmark c. 24 h later, these shoots were used to establish plants in waterlogged, washed quartz sand containing NPK slow-release fertilizer (Osmocote, Scotts Professional, Camarillo, CA, USA; 3 g kg⁻¹). Plants collected on the second trip were maintained in aquaria (as above) for 7 d while photosynthetic measurements were conducted.

For ¹⁴CO₂-incorporation experiments, axial shoots from the established plants were propagated as described above. These new plants were grown in a controlled environment for 4–6 wk to generate a root system (12 h : 12 h dark : light; mean PAR 130 μmol m⁻² s⁻¹; ambient temperature min : max 15°C : 24°C). Before submergence, plants had all sand washed from the sediment roots and were placed onto a hypoxic nutrient solution containing (in mol m⁻³):
mounted in the incubator, and bottles without tissues served beads to facilitate mixing. The bottles were immediately segments) were placed into each filled bottle, with two glass bottles were immediately adjusted to 6.5 with KOH. After 24 h, plants were moved onto deoxygenated nutrient solution (composition as above) also containing 0.1% (w/v) agar for 48 h.

Individual plants were then transferred into 500-ml brown glass bottles with freshly deoxygenated nutrient–0.1% agar solution. Plants were sealed into the bottles with a foam plug 10 mm above the root: shoot junction and plastic film (Parafilm; Pechiney Plastic Packaging Company, Chicago, IL, USA) to prevent nutrient leakage. Trials had shown that partial submergence promoted the most aquatic root growth (data not shown), so the basal third of the shoot of these plants was submerged in a solution containing (in mol m\(^{-3}\)): Ca\(^{2+}\), 0.50; Mg\(^{2+}\), 0.25; Cl\(^{-}\), 1.00; SO\(_4^{2-}\), 0.25; K\(^+\), 1.00; HCO\(_3^{-}\), 1.00. The submergence solution was maintained at 20°C and at pH 6.0, giving a free CO\(_2\) concentration of 200 mmol m\(^{-3}\). Dissolved CO\(_2\) was held constant by adding pressurized CO\(_2\), regulated by a pH controller (CO\(_2\)/pH control 12V; JBL GmbH & Co. Neuhofen, Germany). The submergence solution was refreshed weekly. Plants received 12 h light (underwater PAR at shoot level 100–150 μmol m\(^{-2}\) s\(^{-1}\)) and 12 h darkness. Plants were submerged for 21–28 d before being used and, as axial shoots were not submerged, aquatic-adapted leaves did not form on these plants.

**Underwater net photosynthesis (P\(_{\text{n}}\)) and respiration**

Underwater photosynthesis by tissues of field-collected plants was measured as net O\(_2\) evolution in closed, clear-glass, 50-ml bottles mounted on a rotating wheel incubator (Colmer & Pedersen, 2008). The bottles contained submergence solution (described in the previous section) with dissolved CO\(_2\) treatments imposed by adding varying amounts of KHCO\(_3\) with pH adjusted to pH 6.0 using KOH, to provide a range of CO\(_2\) concentrations from 50 to 1000 mmol m\(^{-3}\) (Stumm & Morgan, 1996). K\(_2\)SO\(_4\) was added as required in the various treatments so that K\(^+\) concentration was equal across treatments. Measurements were started with dissolved O\(_2\) at c. 50% of the air equilibrium concentration (bubbling solution with N\(_2\) and air in 1 : 1 volumes), flushed solutions were gently mixed and KHCO\(_3\) was injected just before filling the bottles. Tissues (two to three leaves, or eight 50-mm root segments, or three 30-mm stem segments) were placed into each filled bottle, with two glass beads to facilitate mixing. The bottles were immediately mounted in the incubator, and bottles without tissues served as blanks. Following incubations of c. 1.5 h (exact times recorded) at 20°C, dissolved O\(_2\) concentrations in the incubation solutions were measured using an O\(_2\) microelectrode with a protection cap (Revsbech, 1989; OX-25; Unisense A/S, Aarhus, Denmark) connected to a pico-ammeter (PA2000; Unisense A/S). Light response curves were obtained with 500 mmol m\(^{-3}\) dissolved CO\(_2\), and CO\(_2\) response measurements were at PAR of 430 ± 7 μmol m\(^{-2}\) s\(^{-1}\).

A leaf-area meter (Li-3000; Li-Cor, Lincoln, NE, USA) was used to measure leaf surface area (rates of net photosynthesis were calculated for the area of both sides of leaves, that is, total surface area, so that comparisons could be made with stems and roots). Stems and aquatic root diameters were measured using digital callipers and the surface area was calculated using the formula for a cylinder. Dry mass of each sample was determined after freeze drying.

**Intra-tissue O\(_2\) dynamics**

Microelectrode studies were carried out to determine the movement of O\(_2\) from photosynthetically active shoots into chlorophyll-containing aquatic roots that were illuminated at different light intensities. Plants were transferred into a shallow tank (length 0.8 m) made from 150-mm-diameter PVC pipe sliced lengthways in half, with caps at each end. The tank was filled with submergence solution (described in the section ’Plant material’) also containing 5.0 mol m\(^{-3}\) MES, with pH adjusted to 6.00 using KOH (Pedersen et al., 2009). The plant was fully submerged and held in place (for electrode insertion) on Perspex stands using putty (BlueTac; Bostick, Wauwatsa, WI, USA). The aquatic roots to be measured were submerged in two smaller Perspex subtanks within the main tank (length 200 mm). To prevent photosynthesis, one subtank was opaque and, during measurements, the surface of this subtank was covered with black polyethylene sheeting to prevent light penetration (PAR < 3 μmol m\(^{-2}\) s\(^{-1}\)). The root subtanks contained deoxygenated 0.1% (w/v) agar solution of the same nutrient composition as that used in the submergence solution; therefore, any O\(_2\) measured within the roots was either endogenously produced by the root or derived from the shoot, via movement through the aerenchyma. The O\(_2\) concentration in the bulk medium of the subtanks was checked at the end of experiments using an O\(_2\) microelectrode (OX-25; Unisense A/S), and had not increased above the very low levels present initially.

Clark-type O\(_2\) microelectrodes with a guard cathode and tip diameter of 25 μm (OX-25; Unisense A/S) were inserted into aquatic roots in both subtanks (50 mm from the root–shoot junction) and into the stem (within 50 mm of both aquatic root bases) using micromanipulators (MM5; Märzhäuser, Wetzlar, Germany). The microelectrodes were connected to a pico-ammeter (PA2000; Unisense A/S) and the outputs were logged every 10 s on a computer using an analogue-to-digital converter (ADC-20; Pico Technology, St Neots, UK). Once steady-state readings were achieved, the aquatic roots were severed from the stem and the
responses of internal $pO_2$ continued to be recorded. Four replicate plants were evaluated.

Experiments were conducted in a 20°C room. The temperature of the submergence solution was logged using a pendant logger (Hobo Pendant Temp/Light Data Logger UA-001-08; Onset Computer Corporation, Pocasset, MA, USA) and varied from 19 to 23°C; microelectrode drift attributable to temperature was corrected. During periods with light, PAR (350–400 $\mu$mol m$^{-2}$ s$^{-1}$) was provided by halogen spotlights.

$^{14}C$ incorporation from dissolved $^{14}CO_2$

Carbon fixation in $M.\ brownii$ stems, leaves and aquatic roots was determined by measuring $^{14}C$ incorporation when underwater. Experiments were conducted in shallow rectangular Perspex tanks with lids. Tanks were divided into two main subtanks, connected via two slits (3 mm) and a 0.35-l buffer zone between the two main subtanks (to ensure no leakage of $^{14}C$ between tanks). During $^{14}CO_2$-uptake experiments, entire plants were submerged; plants were placed in the larger 2.5-l subtank and the aquatic roots from several stem nodes were threaded through the buffer zone into the second subtank of 1.0 l. A water-tight seal between the compartments was made using putty (BlueTac). Two experiments were conducted: in the first, the entire plant was provided with dissolved $^{14}CO_2$ in the form of KH$^{14}CO_3$ (0.6 $\mu$Ci l$^{-1}$), and incubated for 3 h in the light; in the second, the entire plant, except for aquatic roots in the 1.0-l subtank, was provided with dissolved $^{14}CO_2$ as above and incubated for 10 h in the light. Control plants were incubated in the dark so diffuse entry and movement of $^{14}C$ could be determined. Tanks were filled to 5 mm below the lid with submergence solution (described in the section ‘Plant material’) also containing 5.0 mol m$^{-3}$ MES, and the pH adjusted to 6.00 using KOH to achieve a dissolved CO$2$ concentration of 300 mmol m$^{-3}$. The submergence solution in each subtank was mixed gently using a 25-mm magnetic stir bar on the tank wall against a small magnetic stir plate (M-motor; Lego, Bilund, Denmark). In experiments using light, intensities were 457 ± 50 $\mu$mol m$^{-2}$ s$^{-1}$ PAR. Five replicates of plants in individual tanks were used in each experiment.

Aliquots were taken from all tanks before and at the conclusion of the experiments and CO$2$ liberated with 3% (v/v) nitric acid so that the total inorganic carbon (TIC) concentration could be determined using an infrared gas analyser (ADC-225-MK3; Analytical Development Co. Ltd, Hoddesdon, UK). Dissolved CO$2$ was calculated from solution TIC, temperature and pH. The $^{14}C$ content of these aliquots was also measured (as described in the following paragraph), and showed that no leakage of isotope occurred between the subtanks.

Following incubations, plants were rinsed three times to remove any loosely bound $^{14}C$ (30 s for each rinse, in submergence solution adjusted to pH 3–4 with HCl). Plants were then separated into tissue types (stems, leaves, sediment and aquatic roots), oven-dried at 105°C for 24 h, and finely ground using a mortar and pestle. Ground tissue (1–5 mg) was dissolved in 500 $\mu$L of ethylene glycol for 24 h at room temperature. Scintillation liquid (10 ml; Ultima Gold; PerkinElmer, Waltham, MA, USA) was added to each sample, and mixed on a rotating wheel for 48 h. $^{14}C$ in the samples was determined by scintillation counting (tri-carb 2800 TR; PerkinElmer). To compensate for differences in quenching from counting different masses of labelled plant material and variation in pigmentation in the different tissue types, counting efficiency was ascertained by adding inorganic $^{14}C$ to 1–5 mg of nonlabelled plant material before counting. These controls were used to create quenching curves for each tissue type.

Anatomy, porosity and pigment analyses

Fresh 10-mm segments (within 50–100 mm of the root–shoot junction) of field-collected aquatic roots were embedded in 1% (w/v) agar and transverse sections were cut by hand. Sections were viewed with a light microscope (Zeiss Axioskop2; Carl Zeiss Pty. Ltd, Hamburg, Germany), with chloroplast visualization augmented by ultraviolet (UV) light, which causes red chlorophyll autofluorescence. Images were captured with a digital camera (Zeiss AxiosCam MRC Rev.3; Carl Zeiss Pty. Ltd) and manipulated for light intensities, contrast and brightness using the Zeiss software package AxiosVision Rel. 4.6 (Carl Zeiss Pty. Ltd).

For field-collected plants, tissue porosity and biomass partitioning were determined. Porosity (% gas volume per unit tissue volume) of aerial and submerged stems, leaf tissue, and aquatic and sediment roots was measured by determining tissue buoyancy before and after vacuum infiltration of the gas spaces with water (Raskin, 1983), using the equations as modified by Thomson et al. (1990). Plant biomass was determined by separating whole plants into tissue fractions, oven-drying at 60°C for 72 h and then weighing.

Chlorophyll concentration in tissues was determined in both field-collected and propagated plants. Tissue was frozen on dry ice, freeze-dried and ground in a ball mill. Cold 100% methanol was added to 10–20 mg dry mass and the samples were incubated in darkness, on ice. After 30 min, samples were centrifuged at 9300 g for 10 min at 4°C, the supernatants were removed and their absorbance was determined at 470, 665.2 and 652.4 nm, using a glass cuvette in a UV-visible spectrophotometer (model 1601; Shimadzu, Chiba, Japan). The concentrations of chlorophyll $a$ (Chl$a$) and $b$ (Chl$b$) were calculated using the equations from Wellburn (1994).

Data analyses

Photosynthetic models were fitted with GraphPad Prisma 5.0 (GraphPad Software Inc., La Jolla, CA, USA).
Hill–Whittingham equation (Hill & Whittingham, 1955) was used to model CO₂ response curves, and was chosen as it accounts for diffusion resistance to CO₂ across the liquid boundary layer. Light response data were fitted to the hyperbolic tangent equation (Jassby & Platt, 1976).

Results

Meionectes brownii forms chlorophyll-containing aquatic roots

In flooded conditions, aquatic roots can be a significant component of the biomass of M. brownii. In field-collected plants, which had been partially submerged for several months, aquatic root dry mass was 26% of the total (Table 1), accounting for 36% of the submerged dry mass. In propagated plants, 3 wk of partial submergence resulted in 15% of the total plant dry mass being aquatic roots (Table 2). When transverse sections of aquatic roots were excited with UV light, red autofluorescence was detected in discrete spherical and elongate structures in the cortical cells (Fig. 2), indicating the presence of chlorophyll within these plastids. This was confirmed by chlorophyll analysis (Table 1). Aquatic root Chlₐ concentration did not differ significantly from that of either submerged or aerial stems; however, aquatic roots had c. 5-fold less Chlₐ than aquatic and aerial leaves. Sediment roots had no detectable chlorophyll. Ratios of Chlₐ : Chl₇ did not differ significantly between plant organs, having a grand mean (± SE) of 2.5 ± 0.8.

Aquatic roots contain a complete photosynthetic pathway

Production of O₂ by underwater net photosynthesis was measured for submerged stems, aquatic roots and aquatic-adapted leaves (Fig. 3). There was no significant difference between calculated maximum net photosynthetic rates (Pₘₐₓ) values) for stems and aquatic roots (Table 1), but aquatic leaf Pₘₐₓ was significantly higher at 11.2 μmol O₂ m⁻² s⁻¹. Photosynthesis of aquatic roots and stems was light-saturated (Iₘₐₓ) at < 200 μmol quanta m⁻² s⁻¹ (Fig. 3; Table 1), whereas Iₘₐₓ for aquatic leaves was 665 μmol quanta m⁻² s⁻¹. Dark respiration rates (surface area basis) of the aquatic roots were similar to those of aquatic leaves and c. 3-fold those of submerged stems and sediment roots (Table 1).

The presence of a complete photosynthetic pathway within aquatic roots of M. brownii was confirmed through the fixation of dissolved ¹⁴CO₂ (Table 2). When the entire plant was submerged in a solution enriched with dissolved ¹⁴CO₂ and provided with light, all organs, apart from sediment roots, fixed carbon (Table 2). Plants used in these experiments had not developed aquatic-adapted leaves and therefore the fixation rates reported in Table 2 for leaves are for submerged aerial leaves which fix carbon 3.4-fold faster than aquatic roots (as compared with aquatic-adapted leaves which have a Pₘₐₓ c. 30-fold higher than that of aquatic roots; Table 1). Radioactivity in aquatic roots provided with ¹⁴C over a 3-h incubation period was 41.7 Bq mg⁻¹ DM, 4-fold higher than the activity of the nonphotosynthetic sediment roots over the same period (Table 2). To confirm that the ¹⁴C in aquatic roots was fixed endogenously, ¹⁴C was provided to the entire plant except the aquatic roots and the partitioning of fixed ¹⁴C was determined after 10 h of illuminated incubation. When all organs were in well-lit conditions, the photosynthetic aquatic roots did not appear to be a sink for shoot-fixed ¹⁴C as they had a low radioactivity (0.54 Bq mg⁻¹ DM) whereas activity was substantial in sediment roots (Table 2).

Photosynthesis in aquatic roots enhances tissue O₂ status

The availability of O₂ is often limited for sediment roots and submerged plant tissues. To ascertain whether aquatic roots

Table 1 Dry mass (DM) partitioning and associated characteristics of field-collected Meionectes brownii organs

<table>
<thead>
<tr>
<th>Sediment roots</th>
<th>Aquatic roots</th>
<th>Submerged stems</th>
<th>Aerial stems</th>
<th>Aquatic leaves</th>
<th>Aerial leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass (% total DM)</td>
<td>4.5 ± 0.97</td>
<td>26.1 ± 2.7</td>
<td>43.7 ± 3.4</td>
<td>6.9 ± 0.85</td>
<td>6.7 ± 1.2</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>nd</td>
<td>27.9 ± 0.24</td>
<td>26.3 ± 0.71</td>
<td>21.1 ± 0.37</td>
<td>3.9 ± 0.42</td>
</tr>
<tr>
<td>Chlₐ (mg g⁻¹ DM)</td>
<td>bd</td>
<td>0.87 ± 0.24</td>
<td>1.34 ± 0.22</td>
<td>0.77 ± 0.22</td>
<td>4.05 ± 0.8</td>
</tr>
<tr>
<td>Rₙ (μmol O₂ m⁻² s⁻¹)</td>
<td>0.09 ± 0.01</td>
<td>0.32 ± 0.02</td>
<td>0.11 ± 0.00</td>
<td>nd</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>Pₘₐₓ (μmol O₂ m⁻² s⁻¹)¹</td>
<td>0.38 ± 0.14</td>
<td>0.55 ± 0.08</td>
<td>nd</td>
<td>11.2 ± 2.1</td>
<td>nd</td>
</tr>
<tr>
<td>Pₘₐₓ (μmol O₂ g⁻¹ DM s⁻¹)²</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.00</td>
<td>nd</td>
<td>0.35 ± 0.19</td>
<td>nd</td>
</tr>
<tr>
<td>Iₘₐₓ (μmol quanta m⁻² s⁻¹)²</td>
<td>183 ± 5.6</td>
<td>190 ± 1.8</td>
<td>nd</td>
<td>665 ± 15.8</td>
<td>nd</td>
</tr>
</tbody>
</table>

¹Values predicted from the Hill–Wittington equation (r² = 0.91–0.96).
²Values predicted from the hyperbolic tangent equation (r² = 0.97–0.99).
bd, below the detection limit; nd, not determined.

Mean values are shown for the percentage of total DM, organ porosity (percentage gas volume per unit tissue volume), chlorophyll a (Chlₐ) concentration, dark respiration (Rₙ), predicted maximum underwater net photosynthetic rate (Pₘₐₓ) and predicted light saturation levels for underwater net photosynthesis (Iₘₐₓ). Rates are expressed on a total surface area basis (i.e. both sides of leaves) so direct comparisons can be made between the various organs. Values are mean ± SE. n = 10 for DM; n = 5 for all other parameters.
were producing enough O₂ via photosynthesis to support their respiratory needs, or whether they were reliant on O₂ from the submerged stem, plants were completely submerged and the partial pressure of oxygen (pO₂) was monitored in an illuminated aquatic root and in a shaded (nonphotosynthesizing) aquatic root, as well as within the stem. For these experiments, the shoot was submerged in a solution bubbled with air, but the roots were in subtanks containing deoxygenated stagnant agar, so that any O₂ in the roots was either transported from the shoots or produced endogenously. Once steady-state readings were reached, the aquatic roots were severed from the stem while tissue pO₂ continued to be measured. An example of the pO₂ responses of the different organs to the treatments is shown in Fig. 4, and mean values for replicated data at each new steady state are summarized in Table 3. When illuminated, the pO₂ values of both the stem and the nonshaded aquatic root increased by 71%; however, the shaded aquatic root, which was unable to photosynthesize, showed only a 27% increase in pO₂ upon illumination of the shoot. Once the aquatic roots were severed from the stem, the illuminated aquatic root showed no change in O₂ status; by contrast, the pO₂ of the shaded aquatic root rapidly declined to very low values following excision. Movement of O₂ into the nonphotosynthesizing root when intact and in an O₂-free medium was presumably via diffusion through the highly developed aerenchyma system, as both stems and roots show high porosity (Table 1). There was no significant difference between the porosity of aerial or submerged stems and that of aquatic roots (Table 1), with a grand mean (± SE) of 25.1 ± 0.9%.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Leaves</th>
<th>Stems</th>
<th>Aquatic roots</th>
<th>Sediment roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (% total)</td>
<td>40.8 ± 3.0</td>
<td>37.9 ± 4.4</td>
<td>15.5 ± 4.0</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>Chla (mg g⁻¹ DM)</td>
<td>6.75 ± 0.68</td>
<td>0.77 ± 0.12</td>
<td>0.92 ± 0.09</td>
<td>bd</td>
</tr>
<tr>
<td>PN (μmol CO₂ g⁻¹ DM s⁻¹)</td>
<td>0.05 ± 0.007</td>
<td>0.03 ± 0.003</td>
<td>0.02 ± 0.003</td>
<td>bd</td>
</tr>
<tr>
<td>Incorporation of radioactivity (8q mg⁻¹ DM) for dissolved ¹⁴CO₂ provided to the entire plant, illuminated for 3 h</td>
<td>63.7 ± 15.7</td>
<td>45.9 ± 7.5</td>
<td>41.7 ± 5.2</td>
<td>9.8 ± 0.2¹</td>
</tr>
<tr>
<td>Incorporation of radioactivity (8q mg⁻¹ DM) for dissolved ¹⁴CO₂ provided to the entire plant EXCEPT for aquatic roots, illuminated for 10 h</td>
<td>139.7 ± 23</td>
<td>77.3 ± 7.3</td>
<td>0.54 ± 0.05²</td>
<td>41.9 ± 1.7¹</td>
</tr>
</tbody>
</table>

¹These organs cannot photosynthesize, and therefore measured ¹⁴C must have been translocated.
²These organs were not provided with ¹⁴CO₂, and therefore measured ¹⁴C must have been translocated.

bd, below the detection limit.

Plants in these experiments were partially submerged and had not formed aquatic-adapted leaves and therefore leaf values are for submerged aerial leaves. Dry mass (DM; % total) and chlorophyll a (Chl a) concentration are means for plants grown alongside those used in ¹⁴CO₂-uptake experiments. During ¹⁴CO₂-uptake experiments, entire plants were submerged in a split tank system, with aquatic roots from several nodes in a separate subtank from the rest of the plant. Two experiments were conducted: in the first, the entire plant was provided with dissolved ¹⁴CO₂ from KH¹⁴CO₃ (0.6 μCi l⁻¹) and incubated for 3 h in light; in the second, the entire plant, except for aquatic roots in a separate subtank, was provided with dissolved ¹⁴CO₂ and incubated for 10 h in light. Net photosynthesis (PN) was determined from the first experiment. In the light, photosynthetically active radiation (PAR) was 457 ± 50 μmol m⁻² s⁻¹. Values are mean ± SE (n = 10 for DM; n = 5 for all other parameters).

![Fig. 2](image-url)

**Fig. 2** Transverse hand section taken from within 50–100 mm of the root–shoot junction of an aquatic root of *Meionectes brownii*. (a) Under bright field illumination cortical cells (A), part of the stele (B) and aerenchyma (C) are seen. Cortical cells contain elliptical green pigmented plastids (arrowheads), which autofluoresce red with ultraviolet light excitation (b). Bar, 50 μm.
Discussion

When partially or fully submerged, *M. brownii* forms extensive aquatic adventitious roots, contributing over 85% of the total root biomass (Table 1). This response indicates poor survival of *M. brownii* roots in anoxic sediments and the likely superior functioning of new aquatic roots within the water column. Aquatic roots would presumably have better access to O2 compared with sediment roots in anoxic soil, as the water column contains O2, aquatic roots are close to the source of shoot-produced O2, and aquatic roots also typically grow into an illuminated environment, allowing chloroplast biosynthesis (Pyke, 2007) and the endogenous production of O2 (Figs 3 and 4).

As a mechanism for flooding tolerance, aquatic root photosynthesis has received limited attention. Dromgoole (1988) quantified CO2 fixation by pneumatophores of the mangrove *Avicennia marina*, which showed fixation rates (on a surface area basis) that were 66% of those published for mangrove leaves (Attwill & Clough, 1980). However, these measurements were conducted in air and it is unlikely the pneumatophores would achieve these rates when submerged by tides, as a result of diffusion constraints underwater restricting CO2 entry to the roots. Rich *et al.* (2008) measured photosynthetic O2 production by aquatic roots of the succulent, flood-tolerant, terrestrial species *Tecticornia pergranulata*, which has an apparent maximum capacity for underwater net photosynthesis of 0.45 μmol O2 m⁻² s⁻¹, 2-fold higher than that of the 4-fold thicker and less porous succulent stems when underwater (Pedersen *et al.*, 2006). In *M. brownii*, aquatic-adapted leaf photosynthesis is significantly higher than that of aquatic roots and stems (Fig. 3), presumably because of the 5-fold higher...
Chl$\text{a}$ concentration and higher surface area to volume ratio in the aquatic-adapted leaves, as compared with the stems and roots. The large biomass of submerged stems and aquatic roots in the field (submerged stems and aquatic roots account for 70% of the total plant biomass, and aquatic leaves only 7%; Table 1) potentially result in the products of root and stem photosynthesis being more significant than their relatively low photosynthetic rates suggest. With saturating light and $\text{CO}_2$, aquatic roots could be contributing 17% and stems 26% of the total for submerged plant organs (calculated from biomass data and photosynthetic rates from Table 1).

The carbohydrate status of roots can determine tolerance of anoxic conditions and recovery post-stress (Webb & Armstrong, 1983; Gibbs & Greenway, 2003). Although $M.\text{ brownii}$ aquatic root photosynthetic rates are substantially lower than those of leaves (Table 1), carbon fixation in aquatic roots appears to be high enough that significantly less input of shoot fixed carbon is required by the aquatic roots, compared with the nonphotosynthetic sediment roots (Table 2). Moreover, regardless of the source of photosynthates (endogenous or shoot), field-collected aquatic roots have 2-fold higher concentrations of total soluble sugars than sediment roots at dusk (SM Rich, unpublished); this availability of substrate presumably puts aquatic roots in a favourable position to tolerate the hypoxic or even anoxic conditions that might occur overnight (Pedersen et al., 2006; Holmer et al., 2009).

The sediment root system of $M.\text{ brownii}$ breaks down during prolonged flooding (SM Rich, unpublished), suggesting that aquatic roots take over all the functions of sediment roots, excluding anchorage. The roles that aquatic roots of flooded terrestrial plants play in nutrient and water uptake, or in hormone production, are largely unknown. Significantly higher nutrient uptake per unit root mass has been shown in aquatic roots compared with sediment roots of deepwater rice ($^{15}\text{N}$; Khan et al., 1981) and watercress ($Rorippa\text{nasturtium aquaticum}$) ($^{59}\text{Fe}$ and $^{86}\text{Rb}$; Cumbus & Robinson, 1977). To our knowledge, no studies on water uptake or hormone production within aquatic roots have been undertaken. Because of the ease of foliar uptake of water and nutrients when plants are submerged (Sculthorpe, 1967), there is disagreement in the literature over the role sediment roots of submerged plants play in nutrient uptake (Carignan & Kalff, 1980; Waisel et al., 1982; Rattray et al., 1991), and aquatic roots have hardly been considered. More research is needed on the functioning of aquatic roots, with comparisons to sediment roots, of plants in flooded environments.

The present study has demonstrated for the first time the presence of a complete photosynthetic pathway, capable of both $\text{O}_2$ evolution and carbon fixation, within flood-induced aquatic roots of a terrestrial wetland species. In $M.\text{ brownii}$, which responds to flooding with the growth of an extensive aquatic root system, this photosynthetic ability presumably offers an advantage to submerged plants as aquatic roots need little $\text{O}_2$ and carbohydrate inputs from the shoot during the day, unlike sediment roots which are completely reliant on the shoot.

Acknowledgements

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References


Table 3 Steady-state internal $\text{O}_2$ partial pressure ($p\text{O}_2$) in submerged $Meionectes\text{ brownii}$ organs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dark</th>
<th>Light</th>
<th>Severe internal $p\text{O}_2$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ</td>
<td>Intact internal $p\text{O}_2$ (kPa)</td>
<td>Intact internal $p\text{O}_2$ (kPa)</td>
<td>Intact internal $p\text{O}_2$ (kPa)</td>
</tr>
<tr>
<td>Illuminated aquatic stems</td>
<td>$10.8 \pm 0.02^a$</td>
<td>$22.6 \pm 0.01^b$</td>
<td>$22.9 \pm 0.01^b$</td>
</tr>
<tr>
<td>Illuminated aquatic roots</td>
<td>$12.7 \pm 0.03^a$</td>
<td>$21.3 \pm 0.01^b$</td>
<td>$21.5 \pm 0.01^b$</td>
</tr>
<tr>
<td>Shaded aquatic roots</td>
<td>$12.3 \pm 0.01^a$</td>
<td>$15.6 \pm 0.01^c$</td>
<td>$0.22 \pm 0.02^d$</td>
</tr>
</tbody>
</table>

$Meionectes\text{ brownii}$ shoots with attached aquatic roots were submerged and $p\text{O}_2$ monitored in an illuminated and a shaded (nonphotosynthesizing) aquatic root, and in the stem. The shoot was submerged in a solution bubbled with air, but the aquatic roots were submerged in subchambers containing deoxygenated stagnant agar. Once steady-state readings were achieved, the aquatic roots were severed from the stem and the response in $p\text{O}_2$ recorded. Values are mean ± SE ($n = 4$) for plants submerged at 19–23°C; microelectrode drift attributable to temperature was corrected. In the light, photosynthetically active radiation (PAR) was 350–400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Different superscript letters indicate significant differences at $P < 0.05$ (comparisons across the full matrix).

Webb & Armstrong (1983) suggest that different plant species have different strategies for dealing with flooding. Some plants, such as $M.\text{ brownii}$, maintain photosynthesis in their submerged organs during prolonged flooding (SM Rich, unpublished), indicating that aquatic roots take over all the functions of sediment roots, excluding anchorage. The roles that aquatic roots of flooded terrestrial plants play in nutrient and water uptake, or in hormone production, are largely unknown. Significantly higher nutrient uptake per unit root mass has been shown in aquatic roots compared with sediment roots of deepwater rice ($^{15}\text{N}$; Khan et al., 1981) and watercress ($Rorippa\text{nasturtium aquaticum}$) ($^{59}\text{Fe}$ and $^{86}\text{Rb}$; Cumbus & Robinson, 1977). To our knowledge, no studies on water uptake or hormone production within aquatic roots have been undertaken. Because of the ease of foliar uptake of water and nutrients when plants are submerged (Sculthorpe, 1967), there is disagreement in the literature over the role sediment roots of submerged plants play in nutrient uptake (Carignan & Kalff, 1980; Waisel et al., 1982; Rattray et al., 1991), and aquatic roots have hardly been considered. More research is needed on the functioning of aquatic roots, with comparisons to sediment roots, of plants in flooded environments.

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