

PHYSIOLOGY

Oxygen Exchange by Entire Root Systems of *Cyperus involucratus* and *Eleocharis sphacelata*.

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ABSTRACT

Net oxygen exchange between entire root systems of the sedges *Cyperus involucratus* Rottb. and *Eleocharis sphacelata* R. Br. was measured in a bi-compartment apparatus, fitted with a polarographic oxygen electrode and a platinum wire electrode in the root chamber. The roots of both species consumed oxygen from water in the root chamber, with no net exchange when the oxygen partial pressure (pO_2) in the chamber was zero. Rates of oxygen uptake by roots of intact plants were always lower than those of excised roots, suggesting a contribution by oxygen transport from the shoots to the root respiratory demand. The contribution of oxygen transported from the shoots increased with diminishing pO_2 in the root medium, approaching the total oxygen demand as pO_2 fell to zero. The roots released oxygen when titanium (III) citrate redox buffer ($E_H = -350$ mV) was used in the root chamber to mimic the redox potential of natural sediments. Rates of oxygen release into the reduced solutions were 21 ± 5 and $55 \pm 7 \mu\text{mol O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ root dry weight from *C. involucratus* and *E. sphacelata*, respectively, in the light, and 16 ± 3 and $9 \pm 3 \mu\text{mol O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ root dry weight in the dark (mean values ± 1 standard deviation). These results suggest that an agitated body of water alone is not a suitable medium for measuring root oxygen release by entire root systems. A solution with a high oxygen demand is more appropriate.

Key words: rhizosphere oxidation, waterlogging, redox, respiration, lacunar transport.

INTRODUCTION

Oxygen release from roots, as a consequence of internal gas transport in the lacunar system, has been documented for

many aquatic plant species (Armstrong 1982). Root oxygen release establishes an oxidized rhizosphere, which may reduce the assimilation of reduced phytotoxins by roots (Reddy *et al.* 1989). Information on oxygen transport by aquatic plants is of interest for their management in natural wetlands where their ability to aerate underground organs and oxidize the rhizosphere is essential for growth in deep water and reducing sediments (Armstrong 1982, Chen and Barko 1988). Root oxygen release is also an important process in constructed wetlands used for wastewater treatment, as it apparently enhances nitrification and reduces the biological and chemical oxygen demand in effluents (Reddy *et al.* 1989).

Plants differ considerably in the ability to oxidize their rhizospheres. Only some aquatic species are capable of significantly increasing the sediment redox potential above that of adjacent unvegetated sites (Chen and Barko 1988, Boor and Sorrell 1991). These differences between species must be largely due to the density and biomass of roots produced since rates of oxygen release by individual roots are remarkably similar across a wide range of submerged and emergent taxa (Sorrell and Dromgoole 1987). Oxygen release from single roots can be estimated *in vitro* (Armstrong 1982, Caffrey and Kemp 1991). However, it is difficult to measure the total oxygen exchange by root systems of large plants which may be more relevant to rhizosphere oxidation *in situ*. The large volumes of solution needed to immerse whole root systems in oxygen exchange chambers can dilute gas exchange processes. Furthermore, the redox potential (E_H) of wetland sediments is much lower than that of anoxic water (DeLaune *et al.* 1990).

In this study, we measured oxygen exchange by entire root systems, using an *in vitro* method that mimics reducing sediments. Our objectives were (i) to measure the net oxygen exchange by entire root systems, by using traditional polarographic methods in oxygen-depleted water; (ii) to measure the net oxygen exchange in a reduced redox buffer (DeLaune *et al.* 1990), and compare this with results obtained in water; and (iii) to use these methods to compare root oxygen exchange between two emergent plants, *Cyperus involucratus* Rottb. is

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a marginal species, while *Eleocharis sphacelata* R. Br. grows in water up to 2 m deep.

MATERIALS AND METHODS

Plant material. Seeds of *C. involucratus* and *E. sphacelata* were germinated in sand trays in a mist propagator. Seedlings were grown in waterlogged sand in individual polyethylene bags, and sprayed weekly with a commercial nutrient solution (Aquasol, Hortico, Sydney) until they were 4 to 6 months old. Plants were removed from the bags and used in experiments when the shoots were about 300 mm long, with mature root systems but only short young rhizomes. There were 50 to 70 adventitious roots on each plant, up to 120 mm in length, with frequent development of lateral roots < 25 mm long. Any broken, bent or dead roots were removed. The volume of roots was always < 15% of the root chamber described below.

Experimental chamber. We measured root oxygen exchange by enclosing intact plants in an acrylic bi-compartment apparatus, with the shoots and roots isolated in separate chambers. Air was pumped continuously (1 L min^{-1}) through the shoot chamber (length 0.8 m, diameter 0.1 m) and mixed with a small electric fan. The root chamber (length 0.17 m, diameter 0.07 m) was covered with foil to prevent light penetration. The total volume of distilled water in this chamber, including a side-arm for the electrodes (see below), was 744 ml. It was cooled by a thermostatically controlled 20C water jacket. The two chambers were separated by an opaque PVC plate which prevented light penetrating into the root chamber. The plants were held in position by sealing the root-shoot junction in the plate with a flexible sealing compound ("Blu-Tak"). The seal was made gas-tight with a thin film of petroleum jelly, and a 5-mm layer of water. In preliminary tests we found no oxygen leakage through this seal into de-oxygenated water in the root chamber.

The oxygen partial pressure ($p\text{O}_2$) in the root chamber was measured with a Clark-type oxygen electrode (Orion 97-08) and recorded on a strip-chart recorder. The electrode was calibrated in the chamber in air-saturated and de-oxygenated water. A magnetically coupled impeller provided rapid mixing in the chamber and a flow-independent electrode response. A mesh barrier above the impeller prevented it from damaging the roots. The temperature in the root chamber was monitored with a temperature probe. A circular bank of twelve 36-W fluorescent tubes and two 60-W incandescent bulbs gave a photon irradiance within the shoot chamber of $220 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (PAR). The root chamber was shielded from the lights with an aluminum foil cover, painted black on the inside. With the lights on, the temperature in the root chamber remained stable at $23 \pm 0.5\text{C}$. For dark treatments,

the shoot chamber was covered with a black cloth and foil, leaving the lights on to minimize temperature changes.

Oxygen exchange experiments. Experiments were begun with either $p\text{O}_2 = 20 \text{ kPa}$ or 1 kPa in the root chamber. In some experiments, the air in the darkened shoot chamber was replaced with oxygen-free nitrogen, to demonstrate the role of oxygen transport in root oxygen exchange. At the end of experiments, the roots were excised and their oxygen uptake rates measured in the closed root chamber. Dry weights of roots were measured after drying for 24 hr at 70C . Rates of oxygen exchange were calculated by differentiation of the slope of the recorder trace (Sorrell and Dromgoole 1987), after data were transferred to a spreadsheet file, to enable smooth curves of the oxygen responses to be produced.

In a preliminary series of experiments, the significance of epiphytic microbial respiration on the measured oxygen exchange was tested. There was no measurable effect on the rates of root oxygen exchange when antibiotics (20 mg L^{-1} nalidixic acid and streptomycin) were added to the chamber. Hence, microbial growth on the roots of these plants is an insignificant component of their oxygen exchange.

Effects on redox conditions. We measured oxygen transport in reducing conditions by adjusting the redox potential (E_{H}) in the root chamber with titanium (III) citrate buffer. Ti^{3+} is a nonphytotoxic reductant, and the buffer was prepared as described by DeLaune *et al.* (1990), except that we used 1.0 M Tris buffer to maintain pH 6.5 in the chamber, rather than saturated sodium carbonate. Titanium (III) citrate was injected into the chamber through a self-sealing septum. E_{H} was measured with a platinum wire electrode, which was cleaned and calibrated as described elsewhere (Boon and Sorrell 1991). The reference electrode was a saturated calomel electrode (Radiometer K4040), and 244 mV was added to readings to give E_{H} values. Readings were not corrected for pH and temperature differences between runs, as these were not significant (< 0.1 pH units and < 0.5C). E_{H} was +420 mV in air-saturated water, and +360 mV in de-oxygenated water. The Ti^{3+} buffer reduced E_{H} to < -300 mV at the beginning of each experiment.

Since the oxidation of Ti^{3+} is stoichiometric (DeLaune *et al.* 1990), rates of oxygen release by the roots can be calculated from the rate of change of E_{H} . We established the relationship between E_{H} and oxygen release by titrating Ti^{3+} solutions in the chamber with oxygen-saturated water.

RESULTS AND DISCUSSION

Oxygen exchange. Time courses of $p\text{O}_2$ changes in the chamber show that the root systems of intact plants consumed oxygen from the root chamber (Figure 1) until the water was totally oxygen-depleted. Net oxygen uptake by the roots was

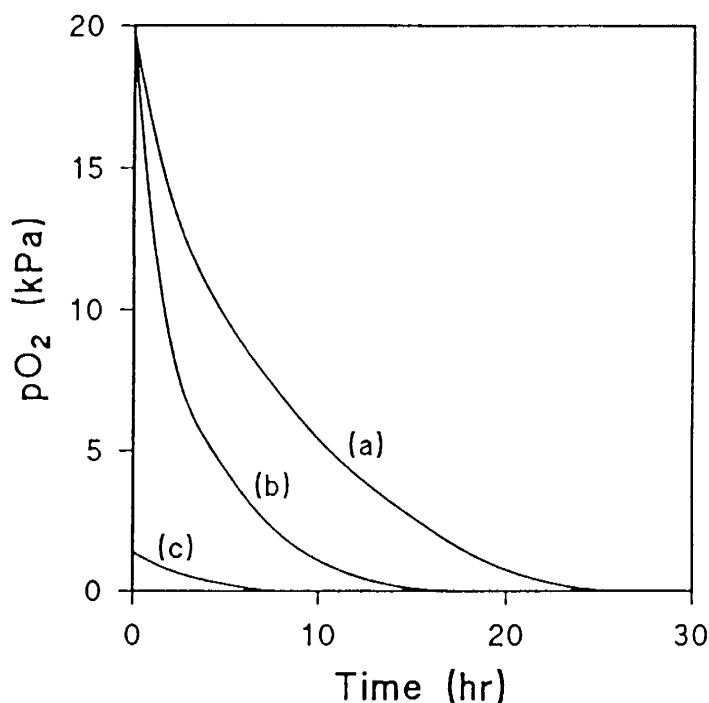


Figure 1. Chart traces of oxygen partial pressure (pO_2) in the root solution showing oxygen uptake by the roots of a *Cyperus involucratus* plant. (a) Intact plant, shoots in the dark with air in the shoot chamber; (b) Excised roots of the same plant sealed in closed root chamber; (c) As for (a), but initial $pO_2 = 1$ kPa in the root chamber. Root dry weight: 0.866 g.

evident for both species, irrespective of whether the shoot chamber was illuminated or darkened, or contained air or nitrogen. The rate of oxygen depletion was always lower for roots of intact plants than for the same root system when excised (Figure 1). In addition, root oxygen uptake by intact plants from the root chamber was lower when the shoot chamber contained air than when it contained nitrogen (Figure 2A, B), and was higher when the shoots were darkened than when they were illuminated (Figure 2C, D). It is clear that some of the oxygen consumed by the roots comes from the shoots. These data are all consistent with a light-enhanced oxygen transport from shoots to roots contributing oxygen to the respiratory demand of the roots.

The saturating pO_2 values for oxygen uptake were highly variable; many responses were not fully saturated over the range of pO_2 values examined (Figure 2). The contribution of lacunar oxygen transport from the shoot to the root oxygen demand varied with the pO_2 in the root chamber. Plots of the fraction of the root oxygen demand satisfied by oxygen transport from the shoot against pO_2 (Figure 2) demonstrate that the contribution of oxygen transport to the root oxygen demand increases with decreasing pO_2 in the root chamber. At $pO_2 = 10$ kPa, the fractions were often < 0.5 , but they increased steadily to 1.0 as pO_2 approached zero. The root

systems never had a net oxygen release, and hence oxygen transport from shoots to roots can only be inferred indirectly from oxygen exchange measurements in the root chamber especially at $pO_2 = 0$. Similar results have been obtained for other emergent macrophytes when oxygen transport is measured in oxygen-depleted water (Bedford *et al.* 1991). Both plant species responded similarly to light, dark, root excision and shoot nitrogen treatments.

When attempting to measure root oxygen release, one must recognize that the immersion of entire root systems in large volumes of water does not closely mimic their behavior in nature. The roots will be supported by axial oxygen transport during growth in waterlogged substrates, but may be subject to quite different radial gradients once plants are transferred to experimental chambers. The consequence of this initial growth of roots beyond the length that internal transport can support in the chamber will be an excess of root oxygen demand over supply, and hence failure to detect any root oxygen release. Such problems are evident in other studies where entire root systems are bathed in large volumes of water or nutrient solution (*e.g.* Bedford *et al.* 1991). Even if some roots are adequately aerated and release oxygen into

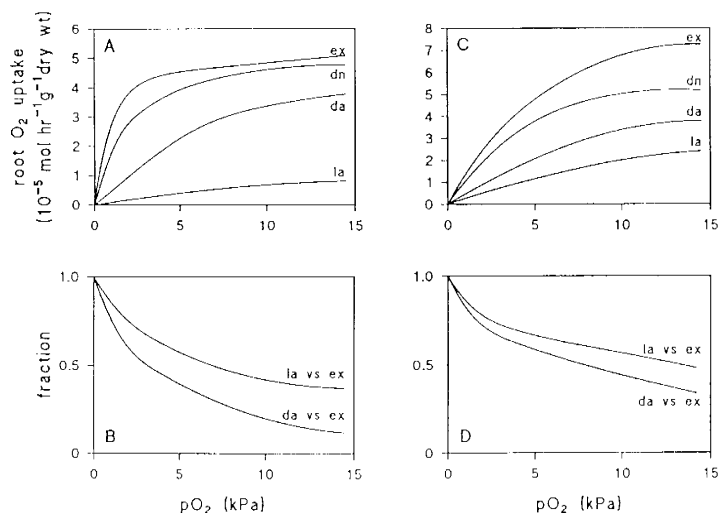


Figure 2. Examples of the response of root oxygen uptake rate to oxygen partial pressure (pO_2) in the root solution. Each graph presents curves derived from continuous oxygen electrode traces obtained from a single plant: A. *Cyperus involucratus*, root oxygen uptake response to pO_2 ; B. Fractions of root oxygen demand in A satisfied by oxygen transport from the aerial tissues; C. *Eleocharis sphacelata*, root oxygen uptake response to pO_2 ; D. Fractions of root oxygen demand in C satisfied by oxygen transport from the aerial tissues. Key: ex = oxygen uptake by excised roots; dn = root oxygen uptake with shoots in N_2 in the dark; da = root oxygen uptake with shoots in air in the dark; la = root oxygen uptake with shoots in air in the light; la vs ex = root oxygen uptake with shoots in air in the light (la) as a fraction of oxygen uptake by excised roots (ex); da vs ex = root oxygen uptake with shoots in air in the dark (da) as a fraction of oxygen uptake by excised roots (ex).

the water, dissolved oxygen in the root chamber is more readily available to respiring root tissue than the gaseous oxygen in the shoot chamber, and any oxygen released from the permeable root surfaces would be quickly resorbed.

Therefore, it is doubtful that low oxygen release rates are characteristic of roots in the field. Rates of oxygen transport and root oxygen release are stimulated by increasing external oxygen demand (Caffrey and Kemp 1991). Hence, we believe that realistic estimates of root oxygen release, and by implication internal oxygen transport, are unlikely to be obtained by experimental designs in which large amounts of root tissue are enclosed in oxygen-depleted water. Such designs differ greatly from studies where oxygen transport is measured on single roots with sleeving microelectrodes or in small-volume chambers (Armstrong 1982, Sorrell and Dromgoole 1987, Caffrey and Kemp 1991). In such cases the re-absorption problem is avoided, and the oxygen exchange rates are quite similar to those that occur *in situ* (Caffrey and Kemp 1991).

Ti³⁺ oxidation. In these experiments, the root medium was titanium (III) citrate buffer, which has a high oxygen demand and provided E_H values as low as those that occur naturally in sediments. These E_H values remained stable during control experiments without plants in the chamber. Excised root systems of both species lowered E_H to a minimum of approximately -400 mV (Figure 3). This can only be explained by the generation of a reductant, presumably ethanol, by the excised roots. In contrast, the roots of intact plants raised E_H . For *C. involucratus*, the rate of increase was only marginally greater in the light than in the dark, whereas rates of increase were much more light-dependent in *E. sphacelata*. With nitrogen in the shoot chamber, the roots of both species failed to raise E_H . Data on rates of oxygen release into these solutions (Table 1) emphasize (i) that roots of intact plants raise E_H , whereas excised roots or roots of plants with shoots in nitrogen reduce E_H , (ii) that the rates of root oxygen release by *C. involucratus* are scarcely affected by light/dark treatments on the shoots, (iii) that root oxygen release by *E. sphacelata* is much higher in the light than in the dark. We conclude from (iii) that there is a mechanism accelerating root oxygen release by *E. sphacelata* in the light. Possible explanations include differences in stomatal behavior between the species, or that internal pressurization and mass flow of gases in the shoots, which is significant in *E. sphacelata* but not in *C. involucratus* (Brix *et al.* 1992), increases the pO_2 at the rhizome-root junctions of *E. sphacelata*.

While oxygen transport in the shoots and rhizomes of emergent aquatic plants can occur by pressurized flow (Brix *et al.* 1992), diffusion is the only significant mechanism

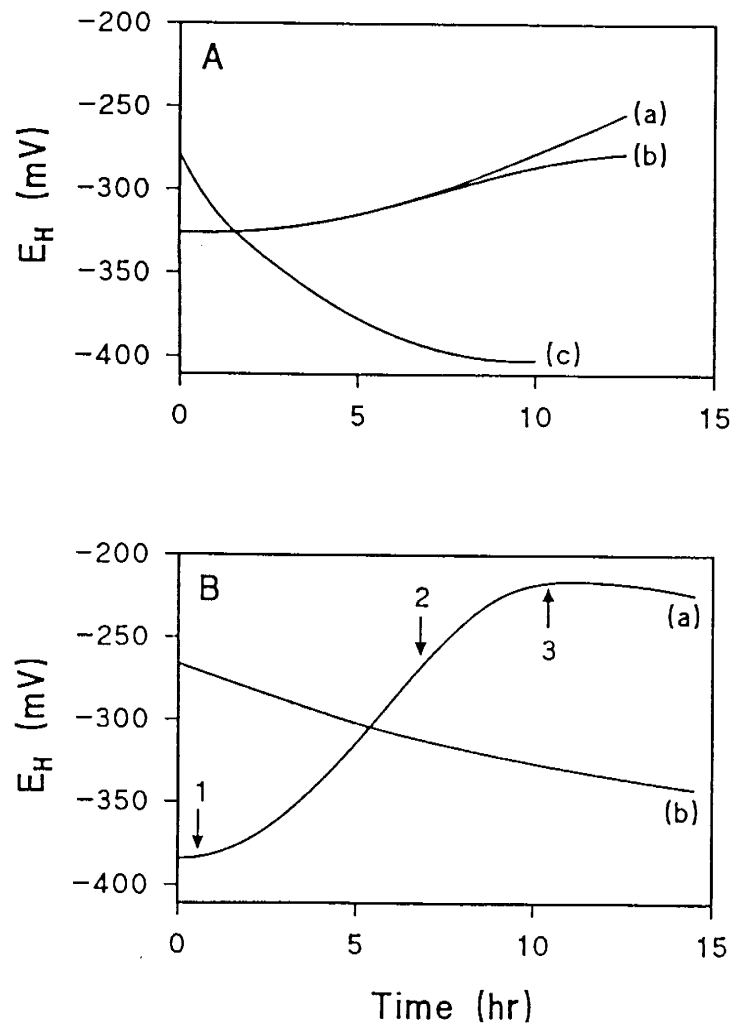


Figure 3. Examples of chart traces showing effect of root oxygen exchange on redox potential (E_H) in titanium citrate buffer. A. *Cyperus involucratus*: (a) shoots in the light; (b) shoots in the dark; (c) excised root system. B. *Eleocharis sphacelata*: (a) intact plant; (b) excised root system. Treatments: (1) Shoots in the light; (2) Shoots in the dark, and (3) nitrogen in the shoot chamber.

TABLE 1. RATES OF OXYGEN RELEASE BY ROOTS OF *CYPERUS INVOLUCRATUS* AND *ELEOCHARIS SPHACELATA* INTO TITANIUM CITRATE BUFFER AT $E_H = -350$ mV. RATES ARE GIVEN FOR EXCISED ROOTS AND ROOTS WITH THE AERIAL TISSUES IN THE DARK AND LIGHT, RESPECTIVELY. DATA ARE MEANS \pm 1 STANDARD DEVIATION (n=3).

Sedge	Excised	Dark	Light
<i>Cyperus involucratus</i>	-12 \pm 2	16 \pm 3	21 \pm 5
<i>Eleocharis sphacelata</i>	-37 \pm 5	9 \pm 3	55 \pm 7

Rates are $\mu\text{mol O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ root dry weight.

within roots and into the rhizosphere (Beckett *et al.* 1988). The rate of transport within the roots and across the root wall depends upon the relative resistances of radial and axial pathways, as well as the pO_2 gradients, which can be modified by the external oxygen demand (Armstrong *et al.* 1990). When titanium citrate is used the oxygen released by aerobic parts of the root system can be detected, because Ti^{3+} oxidation is not easily reversible. This explains the release of oxygen from entire root systems of plants into titanium (III) citrate buffer, but not into oxygen-depleted water. Rates of oxygen transport and release by plants are therefore not fixed, but vary with external pO_2 and oxygen demand (Sorrell and Dromgoole 1987, Caffrey and Kemp 1991). Low rates of release into water are evident in recent data from Bedford *et al.* (1991), who found $< 2.5 \mu\text{mol O}_2 \text{ hr}^{-1} \text{ plant}^{-1}$ released by the roots of several wetland species. In contrast, *E. sphacelata* and *C. involucratu*s released no oxygen into water, but their rates of release into titanium citrate (Table 1) were as high as $50 \mu\text{mol O}_2 \text{ hr}^{-1} \text{ plant}^{-1}$ (*E. sphacelata* in the light).

Internal oxygen transport in aquatic plants allows aerobic metabolism and growth of roots in reducing sediments. Most species also release a substantial amount of oxygen from the roots, and significant sediment oxidation has been unequivocally demonstrated in several studies for species with high root biomass (Tessenow and Baynes 1975, Chen and Barko 1988, Boon and Sorrell 1991). The actual rates of oxygen release by roots depend on factors such as plant size and growth stage, season, root length, and temperature (Reddy *et al.* 1989, Caffrey and Kemp 1991). In this study we have demonstrated that root oxygen release by entire root systems is minimized when they are enclosed in solutions with no external oxygen demand. With this experimental design the roots are the only sink, so any oxygen that is released will most likely be resorbed by the respiring root tissue. Oxygen-depleted water is therefore not a suitable medium for estimating root oxygen release by entire root systems; a solution such as the titanium citrate buffer, which to some extent mimics the sediment oxygen demand, is more appropriate.

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