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Oxygen dynamics in the rhizosphere of *Cymodocea rotundata*

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ABSTRACT: The spatial distribution of oxygen and the dynamics of the oxic microzone around roots of *Cymodocea rotundata* were studied using oxygen microelectrodes under constant light conditions and during light-dark transitions. Under daylight steady state conditions, oxygen was present at concentrations up to 75% of air saturation at the root surface, and the oxic microzone around the roots was 80 µm thick. Steady state oxygen concentrations were reached within 1.5 h after light-dark shifts. Under darkness, free oxygen, at about 20% of air saturation, was still present on the root surface at steady state, but the thickness of the oxic microzone shrank to 50 µm. The oxygen present in the rhizosphere during darkness was supplied from the water column to roots via, primarily, gas-phase diffusion in leaves and rhizomes. The oxic microzone around roots comprised about 0.5% of the total volume of the seagrass rhizosphere, and the root-mediated oxygen supply was estimated to be insignificant for the whole sediment oxygen budget contributing about 1% of total oxygen consumption only. However, the continuous oxygen supply may ensure a persistent oxic environment for below-ground tissues of *C. rotundata* and, hence, protect the plant from reduced phytotoxins.

KEY WORDS: Seagrasses · Roots · Sediments · Oxygen · Respiration

The rhizosphere of submerged rooted plants is usually deprived of oxygen due to high respiration within the sediment and slow diffusion of oxygen from the water column to the sediment. Below-ground plant tissues may function temporarily on anaerobic metabolism, but the energy yield is 10-fold lower than by aerobic respiration, and oxic conditions must be restored within hours for root and rhizome survival (Vartapetian & Jackson 1997). All higher plants living in reduced soils and sediment, therefore, depend on efficient oxygen transport from above-ground tissues to rhizomes and roots.

Part of the oxygen transported to roots and rhizomes escapes from the plant and diffuses into the rhizosphere (Armstrong et al. 1994), and this oxygen loss varies substantially among aquatic macrophytes (Sand-Jensen et al. 1982, Caffrey & Kemp 1991). Freshwater species of isoetids in particular lose a large fraction of the photosynthetically generated oxygen to the sediment, where it may create oxic horizons with distinct diurnal oxygen fluctuations within the sediment (Christensen et al. 1994, Pedersen et al. 1995). The root oxygen loss in seagrasses has only been examined for eelgrass and it appears to be much lower than that in isoetids (Sand-Jensen et al. 1982, Caffrey & Kemp 1991). The low oxygen release will most likely generate oxic microzones around roots and rhizomes, as Caffrey & Kemp (1991) reported for *Potamogeton perfoliatus*. Still this modest oxygen release may suffice to protect the plant from anaerobic phytotoxins in the sediment and to promote the aerobic degradation of organic matter within the rhizosphere.

In the present study we used microelectrode techniques to describe steady state oxygen profiles around roots of the tropical seagrass *Cymodocea rotundata* both in the light and in the darkness. The rates of oxygen loss were computed from the profiles and we provide an approximate estimate of diurnal oxygen flux from the roots to the sediment of a Philippine seagrass bed. We also describe the time course of oxygen release switching between light and dark conditions in order to assess the dynamic nature of rhizosphere oxygen conditions and to evaluate whether oxygen diffusion within the seagrass is based upon gas- or liquid-phase diffusion in the lacunal tissues.

Material and methods. Shoots of *Cymodocea rotundata* were collected from a silty location at Santa Barbara, Bolinao in the Philippines (Terrados et al. 1998). The shoots were gently washed to remove the sediment and subsequently divided into 10 to 20 cm long

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rhizome fragments containing 2 to 5 ramets. The shoots were planted into 1 l transparent glass beakers containing homogenised silty sediment from Santa Barbara, and some of the roots were carefully arranged horizontally towards the glass wall. The beakers were thereafter wrapped with aluminium foil and kept for 48 h in a large outdoor concrete pool, which was continuously flushed with fresh seawater, to allow reestablishment of the chemical profiles around the roots. All measurements were carried out under natural light (200 to 400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and constant temperature (30°C).

Mapping of free oxygen in the rhizosphere was carried out using commercially available Clark-type microelectrodes (Unisense, Denmark). The microelectrode is small (tip diameter of <10 μm), fast responding (90% response <1 s), with a stirring sensitivity of <1%, and the tip of the electrode was extraordinarily thin to provide optimal flexibility. This allowed a set-up, where the flexible oxygen microelectrode was moved vertically with a micromanipulator (Märzhäuser, Germany) in steps of 10 μm towards the root surface. Determination of the position of the root surface was made partly by visual inspection through the glass beaker by means of a dissection microscope (Olympus, Japan) and partly from the electrode signal (Revsbech et al. 1998a).

Dynamic time traces of the oxygen concentration at the root surface were obtained during steady state in light, during switches from light to darkness and during steady state in darkness. The microelectrode was positioned at the root surface and the signal from the electrode was logged on a datalogger (PicoLog, England) connected to the picoammeter (Unisense, Denmark). Steady state conditions were allowed to develop in light, after which the aquarium was covered with aluminium foil and steady state oxygen concentrations developed in darkness. Finally, the aluminium foil was removed to reestablish steady state light conditions.

The oxygen flux under steady state conditions in light and darkness was calculated from the cylindrical version of Fick's first law:

$$J = D \frac{dC}{dr} 2\pi r$$

where J is the radial flux, D is the effective diffusion coefficient, dC/dr is the radial concentration gradient and r is the radius of the root.

Results. The steady state oxygen profiles around the roots of *Cymodocea rotundata* showed the presence of a thin but nevertheless permanent oxic microzone (Fig. 1). In light, the root surface oxygen concentration was 75% of air saturation but it decreased dramatically to reach anoxic conditions only 80 μm away from the

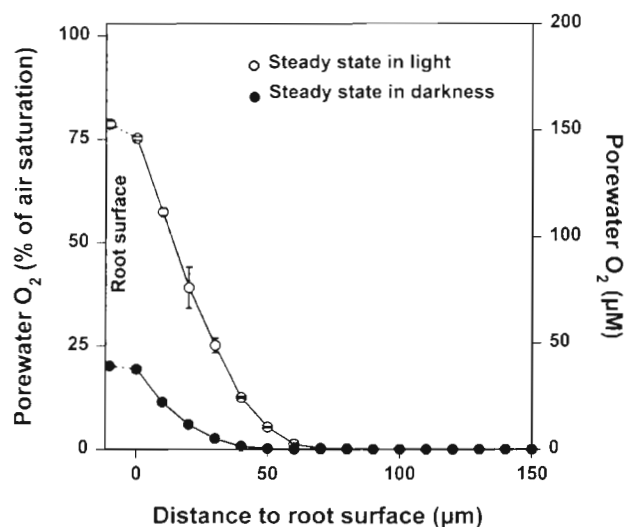


Fig. 1. Steady state microdistribution of porewater O_2 versus distance to the root surface in *Cymodocea rotundata*. Steady state conditions were defined from the time trace of Fig. 3. (O) Light values, (●) dark values. The measurements were repeated 3 times by withdrawing the microelectrode, moving it 200 μm along the root axis and repeating the profiling. Mean values \pm SD

root surface. In darkness, the oxygen concentration was 20% of air saturation at the root surface and the thickness of the oxic zone declined to 50 μm . Hence, the oxic microzone persisted although the oxygen supply from shoot photosynthesis ceased. The dotted line in Fig. 1 indicates the relative uncertainty associated with the determination of the actual root surface, i.e. the data point measured at the root surface may be displaced $\pm 5 \mu\text{m}$ at the horizontal axis. Repeated measurements in more sandy sediments showed no difference from the measurements in silty sediments with respect to the thickness of the oxic zone (data not shown).

The oxygen flux from the root into the sediment was calculated from Eq. (1). Microscopic examination of the root (Fig. 2) showed an almost circular cross-section with a radius of 0.05 cm. Hence, we treated the root as a hollow cylinder when calculating oxygen fluxes. The effective diffusion coefficient of silty sediments has previously been determined to be $1.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ by Revsbech et al. (1998a, b). The concentration gradient (dC/dr) was calculated from the slope of the linear parts of Fig. 1 to be 33.1 in light and 13.2 $\text{nmol O}_2 \text{ cm}^{-4}$ in darkness. Accordingly, the oxygen flux was calculated to be $5.25 \mu\text{mol O}_2 \text{ m}^{-2} \text{ root h}^{-1}$ in light and to be $2.28 \mu\text{mol O}_2 \text{ m}^{-2} \text{ root h}^{-1}$ in darkness. Assuming 12 h light and 12 h darkness, the 24 h estimate amounts to $90.28 \mu\text{mol O}_2 \text{ m}^{-2} \text{ root d}^{-1}$. If we correct for the actual root surface in a typical mixed seagrass bed of the Philippines ($0.95 \text{ m}^2 \text{ root m}^{-2} \text{ sediment}$; Duarte et al.

1998), the root-mediated oxygen loss amounted to about $85.8 \mu\text{mol O}_2 \text{ m}^{-2}$ seagrass bed d^{-1} or down to $10.8 \mu\text{mol O}_2 \text{ m}^{-2}$ seagrass bed d^{-1} if the root contribution of only *Cymodocea rotundata* is taken into account ($0.12 \text{ m}^2 \text{ root m}^{-2}$ sediment; Duarte et al. 1998).

The oxygen concentration at the root surface responded rapidly to changes in light conditions (Fig. 3). It took less than 1.5 h to reach a new steady state after switching from darkness to light and the changes from light to darkness were equally fast. These measurements also showed that oxic conditions persist at the root surface under darkness.

We can obtain a rough idea of the permeability of the transport pathway from leaves to roots by modelling the time trace obtained from Fig. 3 (Revsbech & Jørgensen 1986). We applied the 1-dimensional version of Fick's second law of diffusion, assuming an indefinitely long root and an instantaneous change from 75 to 25% of air saturation at the source (the lacunae within the leaves) upon switching from light to darkness. In addition, we assumed that the whole pathway from leaves to roots may be gas-phase diffusion and only the last part from the root lacunae and out through the root cortex to the tip of the electrode would be liquid-phase ($225 \mu\text{m}$; Fig. 2). The time trace in Fig. 3 was obtained 5.5 cm from the leaf base and it showed a 50% decrease of the total decline in oxygen after 28 min. The model predicted a 50% decrease after 28 min to occur 7.8 cm from the source, or close to the actual 5.5 cm.

Discussion. These results provide the first experimental description of the microdistribution of free oxygen around roots of a seagrass. The oxygen concentration at the root surface of *Cymodocea rotundata* was relatively high (about 75% of air saturation in light) but rapid oxygen consumption of the silty sediment resulted in steep gradients and confined the thickness of the oxic microzones to $80 \mu\text{m}$. This is considerably less than the $350 \mu\text{m}$ microzone found for roots of rice in waterlogged soil (Revsbech et al. 1998a) and the several mm thick oxic zone measured around rhizomes of *Potamogeton pectinatus* (Caffrey & Kemp 1991). The oxygen loss from *C. rotundata* was, furthermore, much

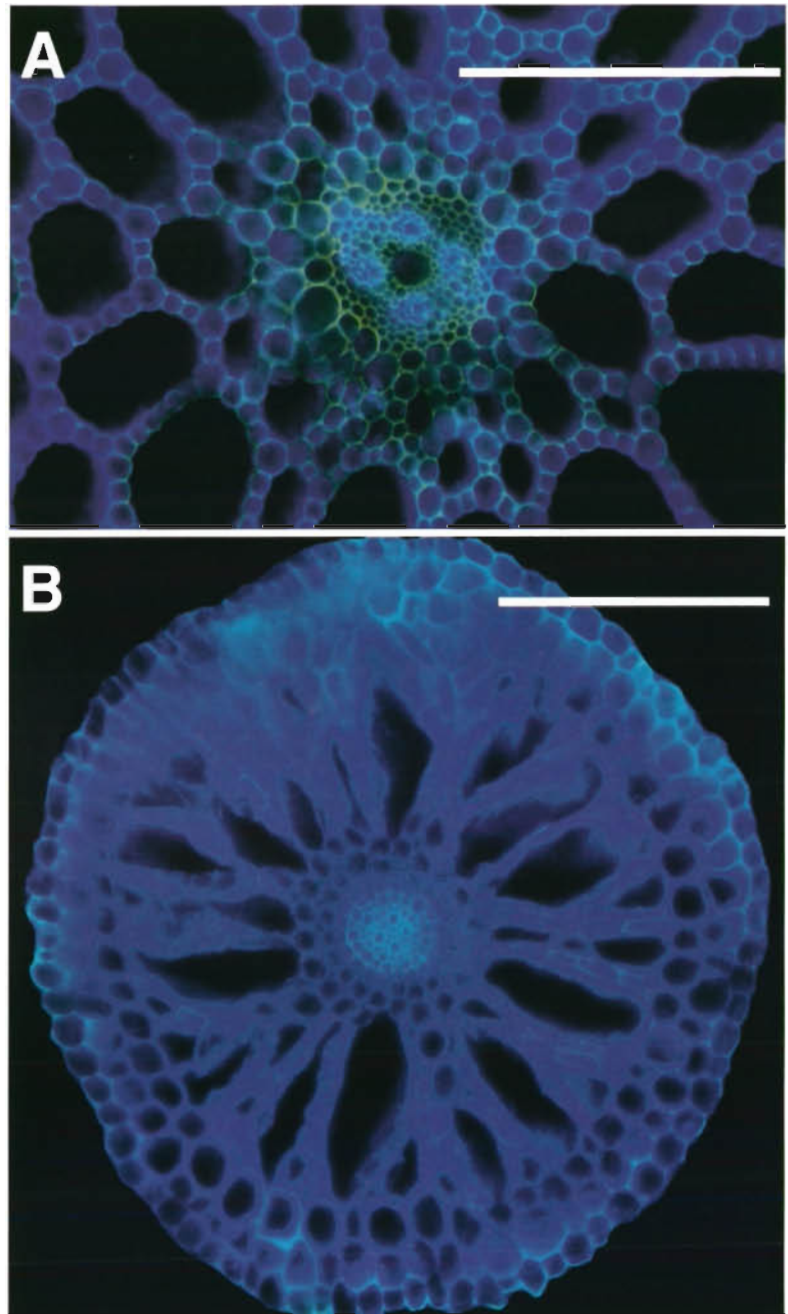


Fig. 2. Transversal sections of (A) the rhizome and (B) the root of *Cymodocea rotundata*. The tissue was stained in Calcofluor White and examined by epifluorescence microscopy. Bar = $500 \mu\text{m}$

lower than the oxygen loss from roots of the freshwater isoetids, *Littorella uniflora* (Christensen et al. 1994) and *Lobelia dortmanna* (Pedersen et al. 1995), which create extensive oxic horizons within the sediment. However, these latter species are especially adapted to facilitate gas exchange with the sediment since pore-water CO_2 is their main inorganic carbon source for photosynthesis (Pedersen & Sand-Jensen 1992). The

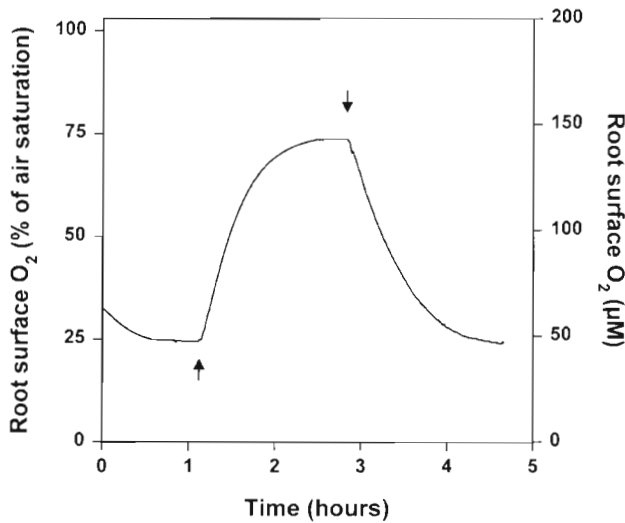


Fig. 3. Time trace of the oxygen concentration on the root surface of *Cymodocea rotundata* in a dark-light-dark cycle. The arrows denote the time at which the steady state micro-distribution profiles for Fig. 1 were obtained

modest rates of root oxygen loss found for *C. rotundata* (this study) and for *Zostera marina* (Sand-Jensen et al. 1982) suggest that seagrasses may, in general, leak less oxygen to the sediment than rooted freshwater macrophytes.

The finding of free oxygen in the seagrass rhizosphere agrees with the observations by Oremland & Taylor (1977) who detected diel changes of oxygen concentrations in gas bubbles from the sediment of a *Thalassia testudinum* bed. Although some studies have failed to detect free oxygen in the rhizosphere of *Zostera noltii* and *Z. marina* (citations in Risgaard-Petersen & Jensen 1997), we suggest that oxic micro-zones around roots of seagrasses must be present, in general, at least when the plants are illuminated. If aerobic respiration is to be maintained in the outer root epidermis cells, then some leakage of oxygen to the surrounding medium must occur to give rise to an oxygen containing rhizosphere. The well-developed aerenchyma of *Cymodocea rotundata* rhizomes and roots (Fig. 2) allows the plant to efficiently support the root cells with oxygen for aerobic respiration and similar aerenchyma have been found in other seagrasses (Barnabas 1987, 1991).

We did not expect, however, that the oxygen release from the roots of *Cymodocea rotundata* would be maintained during darkness. The oxic microzone clearly shrank and the oxygen concentration at the root surface declined to about 20% of air saturation at steady state, but the persistent oxygen profiles document that *C. rotundata* was able to sustain an efficient oxygen supply to the roots also in darkness. This oxygen cannot have been supplied by leaf photosynthesis.

The steep decline in Fig. 3 indicates that the photosynthetic oxygen pool present within the aerenchyma may supply respiration and oxygen leakage to the sediment for less than 2 h before a new steady state is reached. Under the steady state condition, respiration and the root-mediated oxygen loss to the sediment must be supplied by constant rate diffusion, where oxygen diffuses from the water surrounding the leaves, into the leaf lacunae and down to the roots via the aerenchyma. As verified by the modelling of the diffusive pathway, the transport must primarily occur as gas-phase diffusion with only a few barriers of liquid-phase diffusion between the leaf and the root surface. This pathway allows for the rapidly attained steady states (less than 1.5 h) upon switching between dark and light and for the continuous oxygen supply of roots in the dark. According to Smith et al. (1984) the oxygen supply for aerobic respiration in roots of *Zostera marina* derives from leaf photosynthesis, and root metabolism switches to anaerobic respiration after a few hours in darkness. This was obviously not the case for *C. rotundata*, and we suggest that other seagrass species with similar morphology and moderate distances between above- and below-ground tissues may also supply aerobic root respiration by passive diffusion of oxygen from leaves to roots during the night.

The importance of root-mediated oxygen leakage by seagrasses for total aerobic decomposition in the sediment is likely small. The sediment consumption of oxygen may amount to 20–131 mmol O₂ m⁻² d⁻¹ (Blackburn et al. 1994), which is more than 200-fold higher than our rough estimate of total oxygen supply via roots (0.085 mmol O₂ m⁻² d⁻¹) in the Philippine seagrass bed. Although the total seagrass root surface may be large, the sediment volume kept oxic by root oxygen leakage is probably modest. If we assume a mean root diameter of 1 mm and a constant oxic microzone around all roots of 80 µm, less than 100 cm³ (equivalent to 0.5%) of the sediment would be kept oxic per m² of our mixed seagrass bed containing 0.95 m² root surface. Some aerobic microbial processes may, however, rely primarily on the root-mediated oxygen leakage because the roots are excreting organic carbon, which may stimulate specific bacteria (Hough & Wetzel 1975). Sediment processes confined to the oxic-anoxic interphase (e.g. coupled nitrification-denitrification) may also be stimulated by oxygen leakage from the roots of submerged macrophytes (Christensen & Sørensen 1986, Caffrey & Kemp 1992, Risgaard-Petersen & Jensen 1997). Moreover, the permanent oxidized rhizosphere is probably of vital importance for the plant in detoxifying reduced phyto-toxins (e.g. sulphide) in the near-root sediment (Penhale & Wetzel 1983, van Wijck et al. 1992, Carlson et al. 1994, Goodman et al. 1995).

Our findings point towards several important aspects that remain subjects for critical examination. For example, we expect that there are considerable oxygen gradients along the axis of the seagrass roots as found for both maize (Armstrong et al. 1994) and rice (Revsbech et al. 1998a). Also, it has been shown that the longitudinal oxygen permeability varies from the base of the root towards the tip with large permeability near the root tip (Armstrong 1971, Smirnoff & Crawford 1983, Armstrong & Armstrong 1988, Koncalova 1990, Smits et al. 1990). This phenomenon is probably of high adaptive value since it enhances the diffusion to the root tip and thus allows greater penetration of roots into anaerobic sediments. We cannot exclude, however, that distal parts of the *Cymodocea rotundata* roots may be forced into anaerobic respiration during unfavourable conditions. Also, we do not know whether the measured oxygen leakage applies to all age stages of the roots. Christensen et al. (1994) present data suggesting those young roots of *Littorella uniflora* leak more oxygen than old roots. Finally, the oxygen dynamics of the rhizosphere of *C. rotundata* may greatly depend on rates of plant respiration and sediment oxygen consumption.

In summary, we have documented the permanent presence of microzones with free oxygen around roots of *Cymodocea rotundata*. During darkness the oxygen is not derived from leaf photosynthesis but rather from the large oxygen pool of the water column. The root-mediated oxygen leakage seems unimportant for the total aerobic metabolism in the sediment but it may be vital for root-associated microbial processes and for detoxifying reduced phytotoxins in the sediment.

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