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Rhizosphere O_2 dynamics in young *Zostera marina* and *Ruppia maritima*

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ABSTRACT: *Zostera marina* and *Ruppia maritima* often share the same habitat, but *R. maritima* appears more resistant to environmental stress. We investigated the impact of light intensity and water column O_2 concentrations on radial oxygen loss (ROL), in young specimens of *Z. marina* and *R. maritima*. Planar optode imaging revealed that ROL of *Z. marina* was localized to the root tip, while *R. maritima* showed ROL along extensive root sections. The total root biomass of the 2 species was similar, but, while *R. maritima* had only 1 root, of which 33% of its length showed ROL, *Z. marina* had 2 to 5 individual roots, where only 2 to 3 exhibited O_2 leakage, but then only at root tips. ROL resulted in an oxic volume of 4.26 ± 0.51 mm^3 $plant^{-1}$ for *Z. marina* and 5.39 ± 0.47 mm^3 $plant^{-1}$ for *R. maritima* (n = 3). ROL per plant at light saturation was 2.32 ± 0.30 and 2.89 ± 0.38 nmol h^{-1} for *Z. marina* and *R. maritima*, respectively. These values declined by 71 and 60% in darkness. However, both species were able to maintain ROL as long as ambient O_2 levels remained >50% air saturation. The calculated ROL integrated over a 24 h cycle was 48.8 ± 10.6 nmol O_2 $plant^{-1}$ d^{-1} (n = 3) for *R. maritima* and 30% less for *Z. marina*. The ability of *R. maritima* to maintain higher ROL than *Z. marina* could be an important feature defining its potential for colonizing and maintaining growth in eutrophic sediments.

KEY WORDS: Radial oxygen loss · Seagrass · Benthic oxygen dynamics · Rhizosphere · Planar optodes

INTRODUCTION

Seagrass beds are important ecosystems in coastal environments; they protect against coastal erosion and act as nurseries for many organisms (Duarte 2000, Marbá et al. 2006). Furthermore, seagrass beds are often characterized by intense mineralization activity and thereby play an important role in coastal carbon and nutrient cycling (Smith et al. 1984, Blaabjerg et al. 1998, Lee et al. 2007). Seagrasses have been widely distributed in northern Europe, but disease and eutrophication have for decades led to massive decline in plant coverage of many coastal regions (Borum et al. 2006, Waycott et al. 2009).

Recent reductions in eutrophication and improved O_2 and light availability in many coastal waters has, however, still not resulted in a full recovery of the original *Zostera marina* (eelgrass) coverage (Boström et al. 2003). It has been suggested that lack of recovery of *Z. marina* in formerly suitable habitats is linked to sensitivity towards sediment resuspension (Valdemarsen et al. 2010, Delefosse & Kristensen 2012), low light availability (Holmer & Bondgaard 2001), short-term periods of extreme salinity and temperature conditions (Evans et al. 1986, Höffle et al. 2011), as well as low ambient O_2 concentrations and elevated benthic H_2S levels (Mascaró et al. 2009, Pulido & Borum 2010).

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Z. marina often shares habitat with another seagrass, *Ruppia maritima* (Larkum et al. 2006), that appears more resistant to anthropogenic forcing. *R. maritima* has apparently replaced *Z. marina* in many areas, including the Odense Fjord, Denmark (Petersen et al. 2009). The growth of *Z. marina* is limited to subtidal areas (Evans et al. 1986); it produces shoots with 3 to 7 elongated green leaves that measure from 2 to 10 mm in width and may reach over a meter in length (Borum et al. 2004). *R. maritima*, on the other hand, is a smaller seagrass typically having leaves <20 cm long and <1 mm wide (Verhoeven 1979), living both in the intertidal and the subtidal zones.

The aerobic roots of *Z. marina* and *R. maritima* are surrounded by anoxic, organically enriched and occasionally sulfidic sediment (Borum et al. 2006). Seagrasses withstand these conditions by ensuring transport of O_2 to the rhizosphere (Pedersen et al. 1998, Sand-Jensen et al. 2005) via well-developed aerenchyma tissue (Larkum et al. 1989). Some parts of the roots remain impermeable to O_2, and presumably also other gasses such as H_2S, while other parts allow significant radial oxygen loss (ROL) (Barnabas 1996, Connell et al. 1999, Armstrong et al. 2000). Since these permeable and metabolically active root sections are involved in nutrient assimilation, the surrounding oxidized microenvironment protects the plant from phytotoxins such as H_2S, reduced metal ions and certain organic acids (Thursby 1984, Goodman et al. 1995). H_2S derived from microbial SO_4^{2-} reduction is typically the most potent toxin present in coastal sediments. H_2S inactivates metallo-enzymes through the formation of metal sulfides inside the plant tissue (Fürtig et al. 1996). Differences in the ability to maintain an effective ROL and the efficiency to assimilate sediment-derived nutrients could therefore be important for regulating the distribution of these seagrass species in eutrophic coastal environments.

The establishment of *Z. marina* and *R. maritima* is dependent on seed dispersal and the subsequent growth of seedlings and later formation of vegetative shoots (Cho & Poirrier 2005, Greve et al. 2005). Most research on seagrass performance has focused on mature plants and relatively few efforts have been dedicated to seedlings and juvenile plants. Yet, this early life stage may be the most sensitive to H_2S intrusion and regulate successful establishment of the seagrass meadows. The purpose of this study was therefore to investigate O_2 dynamics in rhizospheres of young *Z. marina* and *R. maritima* under different environmental conditions using planar O_2 imaging (Glud et al. 1996, Frederiksen & Glud 2006). The data were used to evaluate and discuss the ability of the 2 plant species to withstand stressful periods of reduced O_2 and light availability.

MATERIALS AND METHODS

Sediment, water and plant collection

Sediment, water and *Ruppia maritima* plants were collected on several occasions from June 2011 to October 2011 at Bregnør Bay in the outer part of Odense Fjord, Denmark (55.48118°N, 10.61002°E). Salinity varied from 15 to 25, and the water depth ranged between 0.3 and 0.7 m, depending on tide and wind direction. Sampled surface sediment was sieved through a 1 mm mesh, homogenized and transported to the laboratory in 10 l containers. Here it was transferred to measuring aquaria (see 'Experimental setup'), or larger containers for plant nursing.

Sediment porosity was calculated using wet density (weight of a known volume of sediment) and water content (weight loss after drying wet sediment for 24 h at 105°C). Organic content was determined as weight loss of dry sediment after ignition (LOI) for 5 h at 520°C. The volume specific sediment O_2 consumption rate (R_{sed}) was measured using slurried sediment samples as described in Frederiksen & Glud (2006).

R. maritima were collected by careful sieving, and recovered plants were replanted in the nursery. Plants were kept in aerated water with a constant salinity of 20 and a temperature of 17°C on a 12 h light:12 h dark cycle with 200 µmol photons m^{-2} s^{-1}. Young apical shoots were selected and used for the experiments.

Zostera marina seeds used for germinating seedlings were collected in the first 2 wk of July around the island of Fyn, Denmark, using the protocol of Wyllie-Echeverria et al. (2003). Seeds were planted in the nursery and kept under the same conditions as described above for *R. maritima*. After germination, the *Z. marina* seedlings were kept in the nursery until they reached approximately the same size as *R. maritima* apical shoots (plants with 2 to 4 leaves of about 3 to 5 cm length). Aboveground biomass was 6.4 ± 2.2 mg dry weight (DW) for *R. maritima* and 3.7 ± 1.6 mg DW for *Z. marina*, while root biomass was 4.2 ± 3.4 mg DW for *R. maritima* and 4.0 ± 2.5 mg DW for *Z. marina* (n = 4) (Table 1).

Similar-sized plants of both species were randomly selected and carefully taken from the nursery and planted next to an O_2 sensitive planar optode

Table 1. Leaf and root characteristics (basic dimensions and growth rates) of the young *Zostera marina* and *Ruppia maritima* plants used in experiments. Data are means ± SD

Characteristic	*Z. marina*	*R. maritima*	n
No. of leaves	3	4	5
Leaf length (mm)	34 ± 9	52 ± 25	5
Leaf width (mm)	1.2 ± 0.2	0.9 ± 0.1	5
Leaf area (mm^2)	81 ± 33	94 ± 48	5
Leaf biomass (mg)	3.7 ± 1.6	6.4 ± 2.2	4
Leaf growth rate (mm d^{-1})	6.0 ± 1.8	9.7 ± 5.0	5
No. of roots	2–5	1	5
Root length (mm)	44 ± 31	29 ± 4	5
Root diameter (mm)	0.2 ± 0.1	0.3 ± 0.1	5
Rhizome diameter (mm)	–	3.2 ± 0.2	3
Root area (mm^2)	52 ± 36	59 ± 20	5
Root biomass (mg)	4.0 ± 2.5	4.2 ± 3.4	4
Root growth rate (mm d^{-1})	4.3 ± 0.9	3.9 ± 0.8	3

mounted on the front plate of measuring aquaria (see next subsection). Basic plant characteristics of *Z. marina* and *R. maritima* plants were measured before and after the experiments (Table 1), and the health of all plants was evaluated under a stereomicroscope. For a plant to be healthy it should have intact vascular tissue in the root tips, roots with a green to white/beige color, a hard and white meristem and green leaves. Leaf and root growth rates were calculated from time-lapse black-and-white images obtained from planar optode imaging. Root inspections before and after the experiments documented that the roots appeared very similar and in good conditions. Furthermore, roots that had been growing along the sensor foil and in the interior of the chamber appeared very similar.

Experimental setup

The setup with measuring aquaria used to monitor O_2 distribution in the rhizosphere of the 2 seagrasses allowed the control of light intensities and O_2 concentrations in the overlying water. The dimensions of the applied aquaria were 16 × 14 × 1 cm in the lower part, containing 0.22 l of sediment, while the upper, wider part allowed space for 1.22 l of seawater (Fig. 1). Water in the upper part was constantly recirculated with a larger separate reservoir. The front plate of each aquarium was equipped with a highly transparent, O_2-sensitive planar optode (PO). *Z. marina* seedlings and *R. maritima* apical shoots were positioned simultaneously at the front plate, with their roots fixed along the PO by pieces of string (Jensen et al. 2005). Subsequently, sediment was filled into the lower part of the aquarium, after the front plate with plants had been mounted, and left for 48 h, for the plants to acclimatize and sediment to settle before any measurements were performed. The aquaria were tilted at a 45° inclination, with the front plate facing down to assure root growth next to the optode. To prevent microalgal growth on the front plate and potential interference from ambient light, the front plate and the camera system were covered with black cloth during the acclimatization period and all subsequent measurements.

A series of 3 experiments was conducted. Expt 1 followed the plants' O_2 dynamics in a 24 h cycle of 12 h light:12 h darkness (200 µmol photons m^{-2} s^{-1}) and 100% air-saturated water. In Expt 2, light intensity was increased in steps of 50 µmol photons m^{-2} s^{-1} from 0 to 200 µmol photons m^{-2} s^{-1}, at an ambient O_2 level of 100% air-saturated water. Different irradiance levels were obtained by changing the distance of a growth lamp (PAR 38 17 W 12, light-emitting diode [LED] spotlights with an integrated lens; http://aliteled.com) relative to the plants. Absolute irradiance was measured using a LI-Cor 250A light meter equipped with a LI-190 quantum sensor (http://licor.com). In Expt 3, O_2 levels in the overlying water

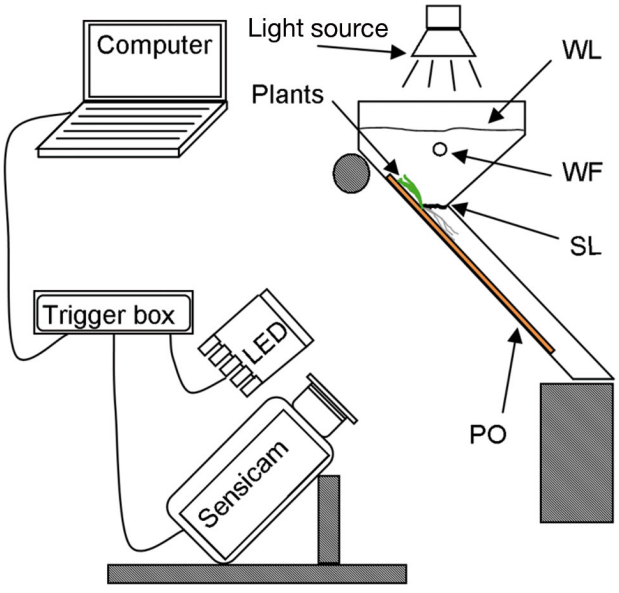

Fig. 1. Experimental setup. Position of the planar optode (PO) is shown as a yellow line on the front plate of the aquarium. Water (WL) and sediment (SL) levels are indicated in the upper part of the aquarium, and the plant position is shown near the sediment surface. Water flow is enabled by pumping recirculating water through the inlet (WF). O_2 level in the water was manipulated with a gas mixer (not shown). The Sensicam camera for recording O_2 images and LED excitation light was connected to a computer via a trigger box

were changed in steps of 25% of air-saturated water from 0 to 100%. This was done in darkness. The O_2 levels were manipulated by flushing the overlying water with a mixture of nitrogen and atmospheric air regulated by two 5850S mass flow controllers connected to a Type 0154 digital control/readout unit (http://brooksinstrument.com). The O_2 concentration in the overlying water was continuously monitored with a separately calibrated point optode connected to a micro-fiber optic O_2 meter (Microx TX3; www.presense.de). Porewater samples (0.9 ml) for H_2S concentration measurements were taken using a syringe and long needle, directly after each experiment, from the bulk sediment (10 cm deep) not affected by roots. Samples were fixed in 1 M ZnAc (volume ratio 1:9) and analyzed according to Cline (1969). Each of the 3 experiments was repeated 3 times, each time using 2 new *Z. marina* and 2 *R. maritima* plants in parallel. Subsequently, data were chosen from the plants which had succeeded growing roots along the optode, ending with 3 replicates of each species. Measurements for each plant were repeated at a frequency of 5 to 10 times h^{-1} over periods of 2 to 12 h depending on the experiment. Data are presented as means for the 3 plants, integrating the respective experimental periods.

Planar optode system

To record planar O_2 dynamics, we used a 12-bit peltier cooled camera (SensiCam; www.pco.de) equipped with an f1.4/25 mm lens (Tevidon Docter Optics). The camera was positioned perpendicularly to the aquarium and next to the excitation light, consisting of 4 blue high-power LEDs (λ-peak 445 nm; LXHL-LR3C, Luxoen) (Fig. 1). A 590 nm longpass emission filter (Schott OG590) was mounted on the camera lens, while a 470 nm shortpass filter (blue dichroic color filter; www.uqgoptics.com) was mounted on the LED housing to eliminate stray light. Synchronized operation of camera and LEDs was ensured by a computer-controlled trigger box, with the custom-developed software Look@Molli (Holst et al. 1998, Holst & Grunwald 2001). Images were recorded every 15 min, and calculations were done from images taken at steady state. Processing of the acquired images and subsequent extraction of concentration profiles were done using the custom-made software CalMolli and the freeware ImageJ (http://rsbweb.nih.gov/ij/).

The O_2-sensitive PO was prepared by knife-coating a pre-made sensing cocktail onto 125 µm thick transparent polyester foil (http://goodfellow.com). The cocktail consisted of 1% wt wt^{-1} O_2 indicator dye, platinum (II) meso-tetra (pentafluorophenyl)-porfyrin (PtTFPP) (www.frontiersci.com) and 2% wt wt^{-1} antenna dye Coumarin dye 545T (www.sigma aldrich.com) dissolved in a 4% wt wt^{-1} polystyrene matrix (www.goodfellow.com) using toluene as solvent (Mayr et al. 2009, Larsen et al. 2011). PtTFPP has excellent photostability and sensitivity in the lower concentration range (Borisov & Klimant 2007), and has a relatively high dynamic range and long-lived luminescence decay (Khalil et al. 2005).

O_2-sensitive quenching was quantified from the luminescent lifetime using 2 well-defined periods on the luminescent decay curve (Holst & Grunwald 2001, Frederiksen & Glud 2006). A modified Stern-Volmer equation (Klimant et al. 1995) solved for O_2 concentration was used to convert luminescent lifetime images into O_2 concentration images according to:

$$[O_2] = \frac{\tau_0 - \tau}{K_{SV} \times (\tau - \alpha \times \tau_0)} \quad (1)$$

where τ is the luminescence lifetime at O_2 concentration $[O_2]$, τ_0 is the corresponding value at anoxic condition, α is the non-quenchable fraction of the luminescent signal (α = 0.2), while K_{SV} represents the Stern-Volmer quenching constant. A 2-point calibration was performed from the average luminescent lifetime using areas with known concentration 0% (deep anoxic sediment) and 100% air saturation (aerated water phase). A 16-image average was applied to improve the signal to noise ratio.

Planar distortion and calculations

The O_2 images only captured ROL of the roots that grew directly along the wall. However, after each experiment all plants were recovered, and, for plant-specific calculations, roots that grew within the interior of the chamber were included in the calculations assuming that they exhibited the same average performance as the roots observed at the wall. However, the fact that measurements are performed along an O_2-impermeable wall results in a distortion of the otherwise radial diffusion geometry (Wenzhöfer & Glud 2004, Meysman et al. 2010). To account for this, the calculated volume of oxidized sediment around the root tips at the wall was assumed to be hemispheric, and it was assumed that the total oxic volume remains unaffected by the presence of the wall (Frederiksen & Glud 2006).

RESULTS

Sediment

The sediment was composed of organic-poor sand (LOI: 0.39 ± 0.03%, n = 5), with an average particle size of 300 µm and a porosity of 0.41 ± 0.03 (n = 3) (Table 2). Average O_2 penetration depth at the sediment surface was 3.8 ± 0.8 mm, as calculated from vertical O_2 profiles extracted from the PO images. Volume-specific O_2 consumption rates (R_{SWI}) calculated from the profiles using the approach of Berg et al. (1998) were 0.15 ± 0.08 nmol cm^{-3} s^{-1} (n = 5), which is typical for coastal sediments (Glud 2008). This theoretically derived value was similar to the measured rate of 0.21 ± 0.02 nmol cm^{-3} s^{-1} (n = 4) obtained from slurried sediment samples (R_{sed}) (Table 2). For further calculations we decided to use the R_{SWI} as slurry incubations are known to overestimate O_2 reaction rates (Hansen et al. 2000). H_2S concentrations were low with concentrations of 6.1 ± 5.0 µM (Table 1).

Table 2. Basic sediment characteristics and volume-specific sediment respiration calculated from slurry experiments (R_{sed}) and from O_2 profiles at the sediment–water interface (R_{SWI}). Data are means ± SD. LOI: weight loss of dry sediment after ignition

Characteristic	Value	n
Porosity (vol. $vol.^{-1}$)	0.41 ± 0.03	3
LOI (%)	0.39 ± 0.03	3
O_2 penetration depth (mm)[a]	3.8 ± 0.8	5
R_{sed} (nmol cm^{-3} s^{-1}) (slurry expt)	0.21 ± 0.02	4
R_{SWI} (nmol cm^{-3} s^{-1})[a]	0.15 ± 0.08	5
Porewater H_2S (µM)	6.1 ± 5.0	15

[a]Profiles extracted from planar optode image

Plants

The selected plants had 3 to 4 green leaves and mostly white or white-beige roots. *Ruppia maritima* plants had 1 root each, while *Zostera marina* plants had between 2 and 5 roots, of which 2 to 3 leaked O_2 from the tips. Leaf growth rate during the experiments was 6.0 ± 1.8 and 9.7 ± 5.0 mm d^{-1} (n = 3) and root growth rate was 4.3 ± 0.9 and 3.9 ± 0.8 mm d^{-1} (n = 3) for *Z. marina* and *R. maritima*, respectively (Table 1). All plants appeared healthy throughout the 20 to 30 h long experiments.

24 h light/dark cycle

O_2 images documented that ROL was observed only at the very root tips (2 to 3 mm) of *Z. marina*, whereas *R. maritima* exhibited ROL along longer sections of the roots (Fig. 2). At a light intensity of 200 µmol photons m^{-2} s^{-1} and 100% air saturation in the

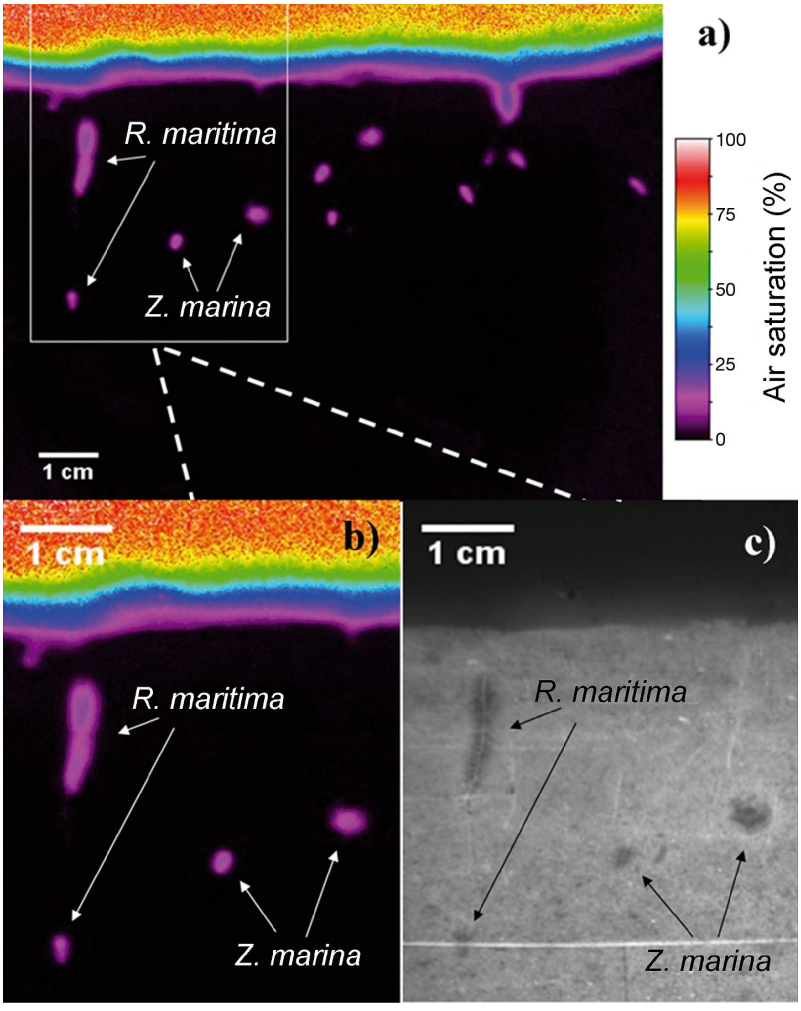

Fig. 2. (a) Planar optode image of radial oxygen loss (ROL) at a light intensity of 200 µmol photons m^{-2} s^{-1} and 100% air saturation in the overlying water, (b) magnified part of the planar optode image and (c) magnified black-and-white image. All images show oxidized zones around *Ruppia maritima* and *Zostera marina* root tips

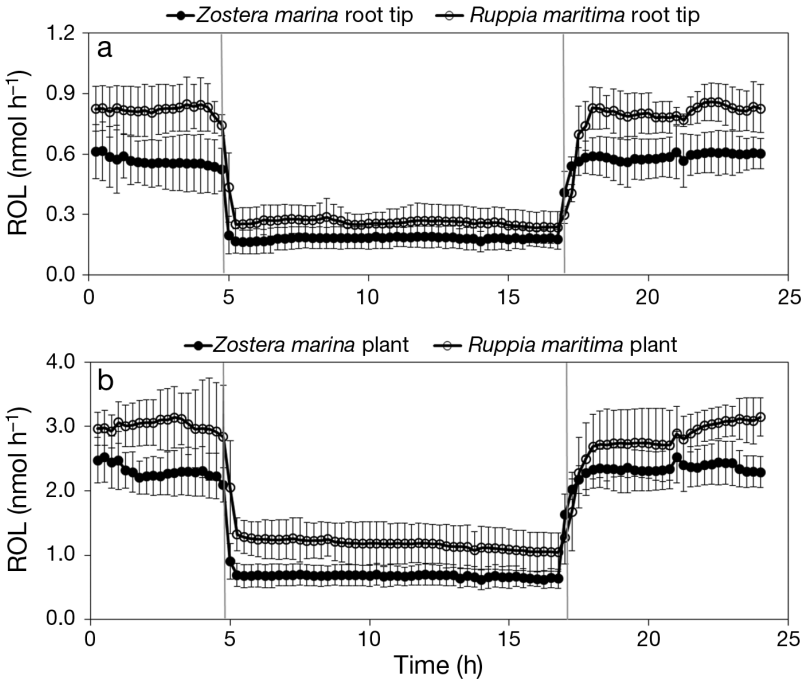

Fig. 3. Temporal changes in radial oxygen loss (ROL; nmol h^{-1}) from (a) the root tips and (b) per plant for *Ruppia maritima* (open circles) and *Zostera marina* (filled circles) during the light/dark cycle with 100% air saturation in the overlying water and a light intensity of 200 µmol photons m^{-2} s^{-1} during the light part of the cycle. Periods of light and darkness are divided by vertical lines. Error bars indicate standard deviation (n = 3)

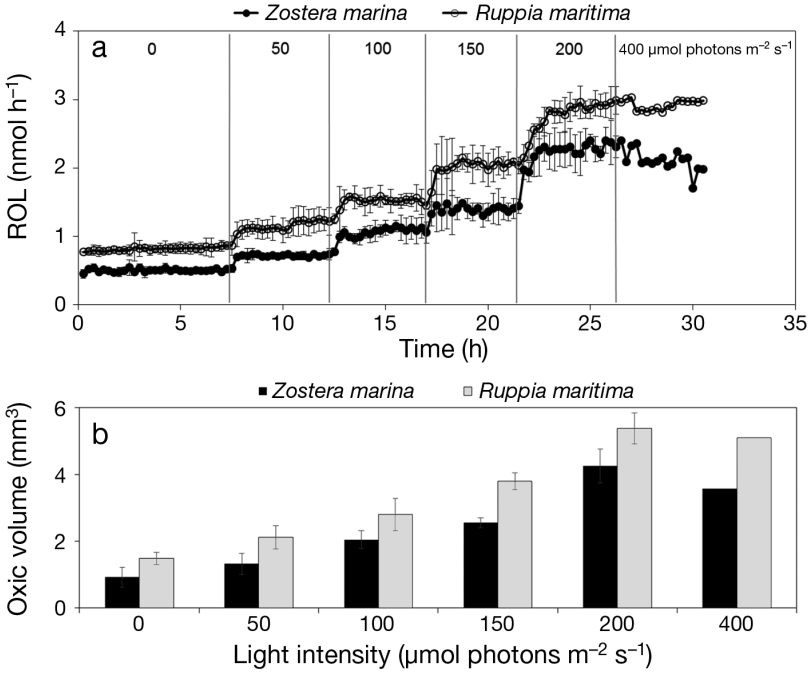

Fig. 4. Temporal changes at different light intensities of (a) radial oxygen loss (ROL; nmol h^{-1}) in rhizospheres of *Ruppia maritima* (open circles) and *Zostera marina* (filled circles) and (b) the sediment oxic volume (mm^3) in rhizospheres of *R. maritima* and *Z. marina*. The O_2 level in the overlying water was kept at 100% air saturation. Error bars indicate standard deviation (n = 3)

overlying water, 8 and 33% of the root length were found to have measurable ROL for *Z. marina* and *R. maritima*, respectively.

Volume-integrated sediment O_2 consumption was calculated from the oxic volume as assessed from the PO images and the volume-specific sediment O_2 consumption rate, R_{SWI}. For whole plants under normal conditions (100% air-saturated water and full light, i.e. 200 µmol photons m^{-2} s^{-1}), oxic sediment volumes around roots were 4.26 ± 0.51 mm^3 $plant^{-1}$ for *Z. marina* and 5.39 ± 0.47 mm^3 $plant^{-1}$ for *R. maritima* (n = 3).

ROL for the single root tip of *Z. marina* was 0.67 ± 0.02 nmol h^{-1} in full light and 73% lower in darkness (0.18 ± 0.007 nmol h^{-1}). The corresponding values for *R. maritima* were 0.81 ± 0.03 nmol h^{-1} and 0.26 ± 0.01 nmol h^{-1}, respectively, i.e. a reduction of 68% between light and darkness. Accordingly, *R. maritima* had 17 and 31% higher ROL than *Z. marina* in light and darkness, respectively (Fig. 3a). When entire plants are considered, the reduction was 71% from light to darkness in *Z. marina* (from 2.32 ± 0.30 to 0.67 ± 0.02 nmol h^{-1}) and 60% in *R. maritima* (2.89 ± 0.38 to 1.17 ± 0.07 nmol h^{-1}) (Fig. 3b). Despite the pronounced reduction of ROL in darkness, the measurements indicated that both plants were able to sustain ROL without photosynthesis as long as the overlying water remained fully oxic (Fig. 3).

Light-intensity change

ROL of both seagrasses appeared to be directly proportional to light intensity. A stepwise increase in light intensity from darkness to full light (200 µmol photons m^{-2} s^{-1}) increased ROL for whole plants from 0.50 ± 0.05 to 2.30 ± 0.22 nmol h^{-1} for *Z. marina* and 0.80 ± 0.07 to 2.90 ± 0.18 nmol h^{-1} for *R. maritima* (Fig. 4a). The corresponding oxic rhizos-

phere for *Z. marina* increased from 0.92 ± 0.3 to 4.26 ± 0.5 mm^3 and for *R. maritima* from 1.48 ± 0.18 to 5.39 ± 0.47 mm^3, respectively (Fig. 4b). Plants were apparently light saturated at 200 µmol photons m^{-2} s^{-1} as further increase in light had no apparent effect on ROL.

O_2 concentration change

ROL of *Z. marina* and *R. maritima* kept in darkness responded proportionally to the O_2 level in the overlying water (Fig. 5). Neither *Z. marina* nor *R. maritima* showed any ROL at ambient O_2 levels of 0 and 25% air saturation (Fig. 5), making both species vulnerable to H_2S intrusion when these conditions prevail during night. ROL for the entire rhizosphere reemerged when ambient O_2 levels reached 50% air saturation and higher, more rapidly for *R. maritima* than for *Z. marina*. Thus, the oxic volumes for *Z. marina* and *R. maritima* rhizospheres were 2.17 and 3.27 mm^3, respectively, at 75% air saturation (Fig. 5b), which corresponds to a ROL of 1.17 ± 0.15 and 1.77 ± 0.25 nmol h^{-1}, respectively (Fig. 5a). The oxic volume of *R. maritima* rhizospheres was, on average, 39% larger than that of *Z. marina*.

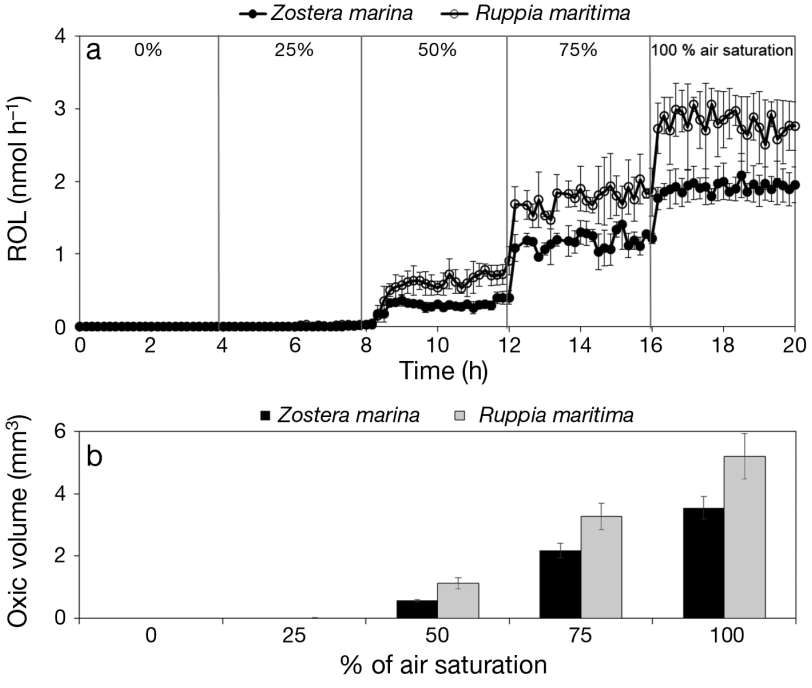

Fig. 5. Temporal changes at different O_2 levels in overlying water (in darkness) of (a) radial oxygen loss (ROL; nmol h^{-1}) in rhizospheres of *Ruppia maritima* (open circles) and *Zostera marina* (filled circles) and (b) sediment oxic volume (mm^3) in rhizospheres of *R. maritima* and *Z. marina*. Error bars indicate standard deviation (n = 3)

DISCUSSION

High concentrations of Fe^{3+}, Mn^{4+} and H_2S commonly occur in reduced sediments of seagrass habitats (Pedersen et al. 2004). These substances can be toxic at high concentrations, and aquatic plants have consequently developed 2 main strategies to deal with this challenge: (1) leakage of O_2 to the surrounding sediment forming a protective oxidative shield against the toxins (Sand-Jensen et al. 1982, Pedersen et al. 1998) and (2) formation of roots impermeable to these toxins (Barnabas 1996).

The first strategy creates oxic microzones in otherwise anoxic sediment. Previous studies have documented the development of oxic microzones and physical barriers, such as Casparian band-like structures, against chemical exchange in roots of both *Zostera marina* (Barnabas 1996, Jensen et al. 2005, Frederiksen & Glud 2006) and *Ruppia maritima* (Barnabas 1996). However, the present study documents in detail that young *Z. marina* and *R. maritima* apparently have somewhat different strategies to counteract the damaging effects of toxins. *Z. marina* has a very strong barrier towards ROL, and O_2 leakage is confined to the root tip, extending only a few millimeters up the root. *R. maritima*, on the other hand, leaks greater amounts of O_2 at the root tip, but it also has a weaker barrier against ROL along the upper root sections, and thereby maintains extensive sections of the rhizosphere oxic. Accordingly, *R. maritima* leaks more O_2 per plant than *Z. marina* and maintains a larger oxic volume in the sediment even though *Z. marina* has a greater number of O_2-leaking root tips (2–3×). *R. maritima* has shorter and fewer roots than *Z. marina* (Table 2), accommodating faster O_2 transport from the leaves to the roots (Sand-Jensen et al. 2005) (Fig. 2). O_2 released through ROL is consumed by biogeochemical processes in the rhizosphere. Here especially H_2S and NH_4^+ oxidation could be beneficial for the plant (Holmer & Laursen 2002). Both processes occur in the rhizospheres of seagrasses (Iizumi et al. 1980, Caffrey & Kemp 1990, Azzoni et al. 2001, Hebert & Morse 2003) and can help detoxify these compounds at high concentrations. H_2S is a known phytotoxin with sensitivity levels <0.5 mM H_2S for *Z.*

marina and between 3.2 and 5.9 mM H_2S for *R. maritima* (M. Ø. Pedersen & E. Kristensen unpubl. data). In our experiment the sediment H_2S concentrations were too low to be a threat to the plants (6.1 ± 5.0 µM). Moreover, NH_4^+, the preferred N source for seagrasses (Thursby & Harlin 1982, 1984), can also be toxic at high concentrations, but this effect is far less studied than the impacts of H_2S (van Katwijk et al. 1997, Britto & Kronzucker 2002, van der Heide et al. 2008).

The difference in O_2 permeability in the roots of the 2 seagrass species may also affect the root-mediated nutrient uptake. *R. maritima* can efficiently assimilate and use NO_3^- as a nitrogen source (Pulich 1989), but NH_4^+ remains the most available nutrient in most coastal sediments. Thursby & Harlin (1982, 1984) examined the uptake kinetics of NH_4^+ in the roots of both plants, and found that *Z. marina* has a lower affinity (K_s of 104 µM) and higher capacity (V_{max} of 211 µmol g^{-1} DW h^{-1}) for NH_4^+ uptake in the roots than does *R. maritima* (K_s of 2.8 to 12.7 µM and V_{max} of 48 to 56 µmol g^{-1} DW h^{-1}). However, there have to our knowledge been no studies that directly link ROL to nutrient uptake in seagrasses, but low K_s for NH_4^+ in *R. maritima* could be a result of high permeability and thereby easier access of porewater solutes to the plant tissues where nutrients are assimilated. If this hypothesis holds, *R. maritima* is better suited for NH_4^+ uptake at low porewater concentrations, while *Z. marina* is most efficient at higher porewater concentrations. *In situ* NH_4^+ concentrations typically range from 30 to 160 µM inside non-eutrophic seagrass beds (Kenworthy et al. 1982, Williams & Ruckelshaus 1993, Hemminga et al. 1994), while unvegetated sandy areas may have concentrations in the range of 5 to 60 µM (Kristensen 1993). The higher root permeability and lower K_s for NH_4^+ will therefore potentially give *R. maritima* an advantage in colonizing new sandy sediments which concurrently can be H_2S rich due to low iron content.

Z. marina has a similar root biomass to that of *R. maritima* (4.0 and 4.2 mg, respectively), but a lower ROL when normalized to root biomass under optimal conditions (100% air saturation and light). ROL for the entire root biomass at these conditions was 0.58 ± 0.08 and 0.69 ± 0.09 nmol mg^{-1} DW h^{-1} (n = 3), for *Z. marina* and *R. maritima*, respectively. These values were reduced by 60 and 75% (0.15 ± 0.01 and 0.28 ± 0.02 nmol mg^{-1} DW h^{-1}; n = 3) in darkness. ROL normalized to root biomass over an entire day (12 h light:12 h dark) was 9.0 ± 1.0 nmol mg^{-1} DW d^{-1} (n = 3) for *Z. marina* and 11.6 ± 1.3 nmol mg^{-1} DW d^{-1} (n = 3) for *R. maritima*, corresponding to 23% lower daily root O_2 leakage for *Z. marina*.

When related to whole plants during a 12 h light: 12 h dark cycle, ROL for *R. maritima* with 1 root was 48.8 ± 10.6 nmol O_2 $plant^{-1}$ d^{-1} (n = 3), while the concurrent ROL of *Z. marina* having 2 to 3 O_2-leaking root tips was 35.9 ± 10.0 nmol O_2 $plant^{-1}$ d^{-1} (n = 3). These values can be extrapolated to seagrass bed area applying typical values for shoot density of 1200 shoots m^{-2} for *R. maritima* (Burkholder et al. 1994) and 905 shoots m^{-2} for *Z. marina* (Olesen & Sand-Jensen 1994). Thus, O_2 translocation into seagrass bed rhizospheres should be 0.059 mmol m^{-2} d^{-1} for *R. maritima* and 0.032 mmol m^{-2} d^{-1} for *Z. marina*. Since diffusive O_2 uptake (DOU) over the sediment–water interface, calculated from O_2 profiles, was 28.4 mmol m^{-2} d^{-1}, ROL corresponds to only 0.2 and 0.1% of DOU for *R. maritima* and *Z. marina*, respectively. The above calculations for *Z. marina* beds only account for young plants that are leaking much less O_2 into the rhizosphere as compared to adult plants. Previous reports using a similar approach showed that total plant-mediated O_2 translocation into the rhizosphere of adult *Z. marina* seagrass beds (using the same plant density) found rates of 2.16 to 2.28 mmol m^{-2} d^{-1} (Jensen et al. 2005, Frederiksen & Glud 2006). The difference between adults and seedlings is simply attributed to size, the ~20× larger leaf area, ~21× higher root biomass and 2 to 3× more root tips of adult plants; these attributes determine the adult individuals' orders of magnitude higher plant-specific ROL.

Our results show that ROL in young *Z. marina* and *R. maritima* during darkness is highly dependent on the O_2 concentration in the overlying water. Young plants of both species were unable to maintain oxic zones around root tips at 0 and 25% air saturation in the overlying water. For adult *Z. marina*, the threshold has been reported to 25% air saturation (Frederiksen & Glud 2006). Conditions with O_2 concentration in the water dropping to or below 25% air saturation can occur during calm summer nights and make both species vulnerable to H_2S intrusion. However, as the O_2 concentration in the water column increased, *R. maritima* was more successful per rhizosphere in reestablishing ROL, compared to *Z. marina*.

R. maritima has more advantages for quickly colonizing new sediment areas, including high growth rates, its ability to quickly develop from seeds to mature plants (Wetzel et al. 1981) and an apparent ability to exploit water column nutrients better than other seagrasses (Thursby & Harlin 1984). However, the present study also documents that young *Z. marina* always leak less O_2 into the rhizosphere than

R. maritima — whether per biomass, per root tip, or per plant. This was caused by a difference in permeable root surface area between the 2 plants and partly by a difference in aboveground biomass. Both total leaf area and leaf biomass are smaller in *Z. marina* (81 ± 8 mm^2 and 3.7 ± 1.6 mg) than in *R. maritima* (94 ± 16 mm^2 and 6.4 ± 2.2 mg) giving *R. maritima* a larger surface for O_2 production via photosynthesis in light and O_2 uptake over the leaf surface in darkness. The overall larger ROL in *R. maritima* than in *Z. marina* protects the former species better from H_2S stress through oxidation of H_2S. In coastal eutrophic sediments, such as in Odense Fjord, >50% of the benthic O_2 consumption is related to direct or indirect H_2S oxidation (Kristensen 2000). However, rhizosphere-mediated H_2S oxidation (i.e. 8 to 16 µmol m^{-2} d^{-1}, assuming a 1:1 stoichiometry) clearly plays a minor role in the total benthic H_2S-oxidation rate. However, locally, ROL induces a refuge, and this could partly explain why *R. maritima* is more successful in recolonizing shallow coastal sediments (Petersen et al. 2009). Such areas are often exposed to high benthic metabolism, resuspension events, or shading from floating macroalgae (i.e *Ulva lactuca* and *Chaetomorpha* sp.) that cause local O_2 depletion, reduce light availability and increase H_2S production (Krause-Jensen et al. 1996, Canal-Vergés et al. 2010, Valdemarsen et al. 2010) providing *R. maritima* with a competitive advantage, as long as the O_2 concentration in the water does not drop to critical values. Overall, young *Z. marina* plants appear more vulnerable towards anthropogenic forcing, leading to high porewater H_2S concentrations because they have low aboveground biomass and less ROL compared to *R. maritima*.

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