Chapter 13
Rhizome, Root/Sediment Interactions, Aerenchyma and Internal Pressure Changes in Seagrasses

Kasper Elgetti Brodersen, Michael Kühl, Daniel A. Nielsen, Ole Pedersen and Anthony W. D. Larkum

Abstract Life in seawater presents several challenges for seagrasses owing to low O_2 and CO_2 solubility and slow gas diffusion rates. Seagrasses have evolved numerous adaptations to these environmental conditions including porous tissue providing low-resistance internal gas channels (aerenchyma) and carbon concentration mechanisms involving the enzyme carbonic anhydrase. Moreover, seagrasses grow in reduced, anoxic sediments, and aerobic metabolism in roots and rhizomes therefore has to be sustained via rapid O_2 transport through the aerenchyma. Tissue aeration is driven by internal concentration gradients between leaves and belowground tissues, where the leaves are the source of O_2 and the rhizomes and roots function as O_2 sinks. Inadequate internal aeration e.g., due to low O_2 availability in the surrounding water during night time, can lead to sulphide intrusion into roots and rhizomes, which has been linked to enhanced seagrass mortality. Under favourable conditions, however, seagrasses leak O_2 and dissolved organic carbon into the rhizosphere, where it maintains oxic microzones protecting

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the plant against reduced phytotoxic compounds and generates dynamic chemical microgradients that modulate the rhizosphere microenvironment. Local radial O_2 loss from belowground tissues of seagrasses leads to sulphide oxidation in the rhizosphere, which generates protons and results in local acidification. Such low-pH microniches can lead to dissolution of carbonates and protolytic phosphorus solubilisation in carbonate-rich sediments. The seagrass rhizosphere is also characterised by numerous high-pH microniches indicative of local stimulation of proton consuming microbial processes such as sulphate reduction via root/rhizome exudates and/or release of alkaline substances. High sediment pH shifts the sulphide speciation away from H_2S towards non-tissue-penetrating HS^- ions, which can alleviate the belowground tissue exposure to phytotoxic H_2S. High sulphide production can also lead to iron and phosphorus mobilization through sulphide-induced reduction of insoluble Fe(III)oxyhydroxides to dissolved Fe(II) with concomitant phosphorus release to the porewater. Adequate internal tissue aeration is thus of vital importance for seagrasses as it ensures aerobic metabolism in distal parts of the roots and provides protection against intrusion of phytotoxins from the surrounding sediment.

13.1 Introduction

When higher (seed) plants evolved about 400 million years ago, the challenge was to maintain an adequate water balance through the development of a leaf cuticle, stomata, intercellular spaces and xylem, i.e., to become homiohydric (Raven 1977). However, life in air also enables much faster rates of gaseous exchange, since diffusion coefficients for CO_2 and O_2 are approximately 10,000 times higher in air than in water (Armstrong 1979). When some angiosperms returned to an aqueous environment about 100 million years ago, freshwater hydrophytes and marine seagrasses thus had to overcome constraints on gas exchange imposed by the slower gas diffusion as well as a much lower solubility of O_2 and CO_2 in water (Table 13.1). Additionally, seagrasses had to contend with mechanical stress such as wave action and other difficulties of living in seawater, for example, ion regulation and sediment-related potential phytotoxins, especially sulphide that occurs in large quantities in marine sediments due to high sulphate levels ($\sim$25 mM) in seawater and the prevalence of sulphate reducing bacteria as a major component in anoxic mineralization of organic material (Jørgensen 1982). Seagrasses show many adaptations found in hydrophytes such as aerenchyma, i.e., airspaces in their tissues providing low-resistance internal gas pathways in both roots and shoots, as well as (i) a photosynthetic leaf epidermis, (ii) loss of stomata, and (iii) reinforced structures to withstand wave-action, such as thick shoot bases and tough strap-shaped leaves (with the major exception of the genus *Halophila* and to some extent *Amphibolis*) (e.g. Armstrong 1979; Larkum et al. 2006a, b).

13.1.1 Gas Exchange in Seagrasses

Molecular oxygen (O_2) and carbon dioxide (CO_2) are substrates and products in respiration and photosynthesis; thus transport processes affecting these gases are of vital importance for seagrasses. The dissolution of O_2 in seawater is straightforward, obeying Henry's law, but the dissolution of CO_2 is more complex as it is part of the pH-dependent speciation of dissolved inorganic carbon (DIC) in seawater:

$$\begin{array}{ccccccc} & & K_{+1} & & K_{+2} & & K_{+3} \\ CO_2 + H_20 & \leftrightarrow & H_2CO_3 & \leftrightarrow & HCO_3^- + H^+ & \leftrightarrow & CO_3^{2-} + 2H^+ \\ & & K_{-1} & & K_{-2} & & K_{-3} \end{array} \quad (1.1)$$

According to Eq. 1.1, CO_2 dissolves in water to form carbonic acid, which is a relatively slow reaction that can be increased by the enzyme carbonic anhydrase (CA) in many biological systems. H_2CO_3 at normal pH of seawater (7.5–8.4) disproportionates rapidly into bicarbonate and a proton. At more alkaline pH, bicarbonate disproportionates into carbonate and another proton. The action site of CA is indicated in Eq 1.1 by a grey box. CA can be located both intra- and extra-cellularly (e.g. Badger and Price 1994) and there is good evidence in many photosynthetic systems for the secretion of extracellular CA into the cell wall facilitating an enhanced uptake of CO_2 via HCO_3^- conversion (e.g. Badger and Price 1994). The presence of CAs in seagrasses has been much debated but their presence had until recently only been inferred by inhibitor studies (e.g. Larkum and James 1996; Beer et al. 2002). However, CA coding genes have now been found to be expressed in the transcriptome of *Zostera muelleri* spp. *capricorni* (Golicz et al. 2015). Although the precise location of CA in the seagrass tissue has not been resolved, previous evidence indicated their presence in the outer cell wall of the leaf epidermal layers of many seagrasses (Larkum and James 1996; Beer et al. 2002; Borum et al. 2015).

It appears that seagrasses have developed mechanisms to enhance the uptake of DIC (Beer et al. 2002; Larkum et al. 2006a, b), and such mechanisms are discussed in detail in Chap. 16. For the present purpose, it is enough to know that photosynthetic carbon fixation is facilitated by several mechanisms that either passively or actively transports DIC from the surrounding seawater into the cytoplasm and chloroplasts of the epidermal cells, where CO_2 fixation takes place and O_2 is

Table 13.1 Concentration and diffusion coefficients of O_2, CO_2 and bicarbonate ion in air and air-saturated seawater (salinity of 35) at a temperature of 25 °C (Larkum et al. 1989)

	Concentration (mmol m^{-3})			Diffusion coefficients (m^2 s^{-1})		
	CO_2	HCO_3^-	O_2	CO_2	HCO_3^-	O_2
Air	17	0	9375	1.56×10^{-5}	NR	1.97×10^{-5}
Seawater	11.5	2000	206	1.55×10^{-9}	$1.00 \cdot 10^{-9}$	2.26×10^{-9}

Data is calculated based upon an atmospheric CO_2 concentration of 401 ppm

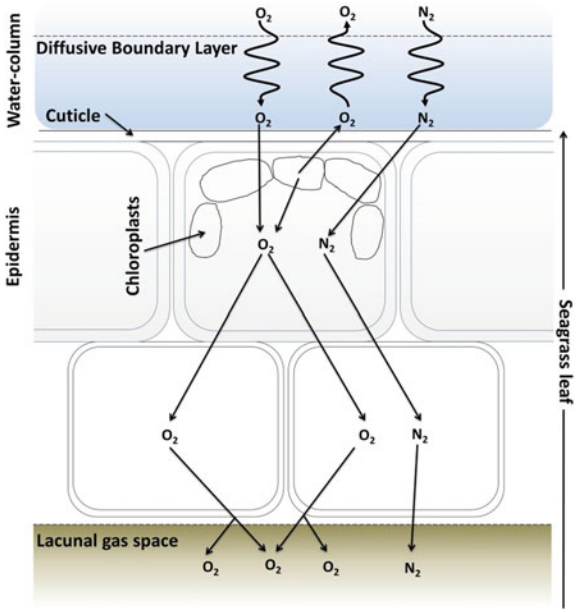

Fig. 13.1 Conceptual diagram showing the major diffusional transport routes for O_2 and N_2 from the ambient medium to the lacunal space (under non-pressurised conditions) in a seagrass leaf. Data modified from Larkum et al. (1989)

produced. Photosynthesis-generated O_2 moves via diffusion to either the surrounding seawater or inwards from the epidermal cells into the surrounding tissue and the aerenchymal spaces depending on the actual concentration gradient and the resistance to diffusion (Fig. 13.1; e.g. Colmer 2003).

An important consideration for the movement of gases in seagrasses is the diffusional constraints on the movement of CO_2 and O_2 both in solution and in the gas phase (Larkum et al. 1989, 2006a, b). The diffusive gas transport is described by Fick's first law:

$$J_j = D_j(C_a - C_s)/l \tag{1.2}$$

where J_j is the flux of gas j (mol m^{-2} s^{-1}), D_j is the diffusion coefficient of the gas j (m^2 s^{-1}) (in water or in air; at a given temperature and salinity), l is the distance over which diffusion occurs (m), C_a and C_s are the concentrations of the gas j (mol m^{-3}) at the source and sink, respectively. In this formulation it is assumed that there is (i) a net flux of gas from source to sink, (ii) no net consumption or production of the diffusing species underway, and (iii) that C_a and C_s are constant—see Nobel (1990). In the following sections, O_2 will mainly be expressed as a concentration (in µmol L^{-1}) when in solution and as a partial pressure (in kPa) when in the gas phase; where ~240 µmol L^{-1} (depending on salinity and temperature) and ~20.6 kPa represents 100% air saturation in a marine environment.

The diffusive transport of a gas across a given plant tissue compartment can be conceptualized as a set of electrical resistances in series (and parallel) (Van den Honert 1948; Raven 1977; Armstrong 1979; Nobel 1990):

$$J_j = D_j(C_a - C_s)/l = DF/R_{jT} \tag{1.3}$$

where $D_j/l = 1/R_{jT}$ and R_{jT} is the total resistance of the gas transport pathway to species j, having the units, s m^{-1}. DF is the driving force or the concentration gradient (C_a–C_s). And each component of the pathway (catenary) can be assigned as previously described in Larkum et al. (1989):

$$J_j = DF/R_{jT} = DF_a/R_{ja} = DF_b/R_{jb} = DF_c/R_{jc}, \text{ etc.} \tag{1.4}$$

and therefore

$$R_{jT} = R_{ja} = R_{jb} = R_{jc}, \text{ etc.} \tag{1.5}$$

where the subscripts T, a, b, c, etc. refer to the total sequence of diffusional steps (T = total) and to the individual steps (a, b, c, etc.); for example, a represents the diffusive boundary layer, b the cuticle, c the epidermal cell wall, etc. (Fig. 13.1).

Using this formulation, it is possible to set out a resistance circuit for the movement of CO_2 and O_2 across the epidermal cell and hypodermal cell of a seagrass, respectively. Such a formulation can then be used to calculate the flux of O_2 either outwards from the epidermal cell or inwards into the aerenchymal spaces (Fig. 13.1; see Larkum et al. 2006a, b) and as we show in the next section, this has important implications for our understanding of the aeration of seagrasses.

13.1.2 Diffusive Boundary Layers and Water Motion Around Seagrass Leaves

Gas exchange between aquatic macrophytes and the surrounding water is impeded by the presence of a diffusive boundary layer (DBL) (e.g. Jørgensen and Revsbech 1985; Hurd 2000; Brodersen et al. 2015a). As flow declines towards the plant surface, the viscosity of water dampens out turbulences, forming the DBL as a thin layer of water just above the tissue surface where molecular diffusion governs solute exchange between tissue and water. The thickness of the DBL is affected by water flow and surface rugosity (e.g. Jørgensen and Des Marais 1990; Larkum et al. 2003), where low flow and/or more coarse topography lead to a thicker DBL than fast flow and/or a more smooth surface. The DBL thickness is an important factor controlling solute exchange as diffusion time increases with the square of the DBL thickness. Therefore, the DBL can present a major barrier to plant solute exchange, especially under low flow conditions or e.g. in the presence of epiphytes on seagrass leaves that increase rugosity (Brodersen et al. 2015a). Larkum et al. (1989)

Table 13.2 Gaseous composition of the lacunal system of several seagrasses (Larkum et al. 1989)

Species	O_2 (%)	N_2 (%)	CO_2 (%)	% of O_2 flux to lacunae	Max. lacunal pressure (kPa)
Zostera muelleri subsp. *capricorni*	32.3	67.6	–	13	–
Cymodocea serrulata	34.3	65.4	0.0095	16	22.0
Syringodium isoetifolium	32.2	64.4	0.140	17	8.18
Halophila ovalis	34.1	66.1	–	–	10.0
Enhalus acoroides	33.5	67.0	0.1017	12.6	–
Amphibolis antarctica	31.7	67.8	–	8	–
Halodule uninervis	–	–	0.0037	–	–

reported a DBL thickness on seagrass leaves ranging from ~50 μm under maximal flow to 200–1000 μm under medium to low flow conditions, and this range was later confirmed by microsensor measurements (e.g. Binzer et al. 2005; Borum et al. 2006; Brodersen et al. 2015a). For seagrasses, this means that under natural conditions there will be a strong diffusion resistance to movement of solutes into the leaves from the surrounding seawater (Fig. 13.1).

With respect to O_2, the DBL at the outer surface of the leaves means that, despite the proximity of the epidermal cell layer to the surrounding seawater, the diffusion resistance for molecules moving out of the epidermis or into the airspace system of the aerenchyma can be similar. Thus, during medium to high light exposure, when photosynthesis is active and O_2 is being produced at high rates, accumulating O_2 pressurizes the aerenchyma (Table 13.2; Larkum et al. 1989; Bodensteiner 2006). This can be seen in many seagrass species where, around midday, the leaves become more erect, and gas bubbles can often be seen escaping from wounds in the leaf surface. The pressurisation of the leaf during active photosynthesis leads to increased O_2 partial pressure (pO_2), which may increase O_2 supply to the roots and rhizome. However, the longitudinal transport to the below-ground tissues is greatly restricted by the diminished aerenchymal spaces in the shoot base manifold (further described in Sect. 13.2).

13.2 Internal Aeration

The leaves of seagrasses are generated at the base of the shoot, i.e., the basal leaf meristem that in mono-meristematic leaf-replacing species such as *Zostera* and *Posidonia* is a combined rhizome/basal leaf meristem area at the root-shoot junction (Fig. 13.2). Three other forms exists, i.e., di-meristematic leaf-replacing species (such as *Thalassia* and *Cymodocea*), mono-meristematic non-leaf-replacing species (mostly *Halophila*), and di-meristematic non-leaf-replacing species (few species of *Halophila*) (e.g. Short and Duarte 2001), but in the following we will mainly focus

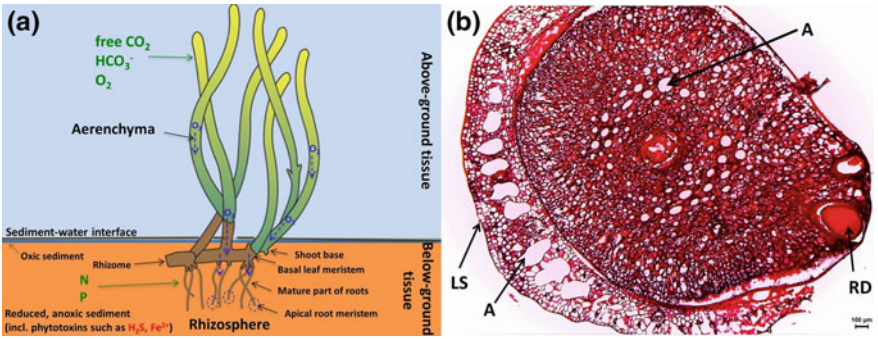

Fig. 13.2 **a** Conceptual diagram of the aerenchymal system in seagrass. **b** Cross-sectional image of a shoot base with leaf sheath of *Zostera muelleri* spp. *capricorni* showing the extended air lacunal system at the meristematic region of the rhizome. Scale bar = 100 µm. LS = indicate the leaf sheath; A = aerenchyma; RD = initial root development. Data modified from Brodersen et al. (2015b). Copyright 2015 John Wiley & Sons Ltd.

on the mono-meristematic leaf-replacing species. The meristematic region of the rhizome has poorly developed aerenchyma owing to the compact anatomy of the tissue, and thereby O_2 diffusion to this area is impeded. To alleviate this structural limitation to O_2 movement, the surrounding leaf sheath has an extensive distribution of large, internal gas channels (Fig. 13.2). The shoot base also produces fiber-rich tissue that provides biomechanical strengthening of the root/shoot base against wave action, and many seagrasses also have adventitious roots that anchor the shoot base into the sediment. As a consequence, the aerenchyma system, which consists of long gas channels or lacunae that stretch through the leaves and roots, peter out in the shoot base, where it is replaced by a much more tenuous intercellular pathway for gas transport (Fig. 13.2). The net result of this extended and reinforced gas pathway in the shoot base manifold causes O_2 to diffuse laterally into the surrounding sediment and tissues, especially to the young developing leaves in addition to the downwards diffusion to the rhizome and roots (e.g. Pedersen et al. 1998, 1999; Jensen et al. 2005; Frederiksen and Glud 2006; Brodersen et al. 2014; Koren et al. 2015; Brodersen et al. 2015a, b). Hence, while the root/shoot manifold forms a hindrance to the passage of O_2 from shoot to root, it alleviates mechanical stress from wave action and secures the O_2 supply to the young meristematic tissues, thereby enabling a protection against intrusion of sediment-produced H_2S.

13.2.1 Internal O_2 Concentration Gradients

The sediment surrounding the rhizosphere is largely anoxic and thus roots and rhizomes are unable to take up O_2 from the sediment environment. Instead, O_2 moves along a concentration gradient from the above-ground shoot to the rhizome and root-tips by means of molecular gaseous diffusion. As described above, diffusion in

the liquid phase is slow and not effective over distances larger than a few mm. As a consequence, seagrasses have evolved a network of porous gas-filled spaces (aerenchyma) in all tissues where gas phase diffusion enables sufficient O_2 transport to the below-ground tissues.

The driving force of O_2 transport is the strong internal gradient in O_2 partial pressure (pO_2) from shoot to root tip. The gradient develops as a result of (i) O_2 consumption of the tissues, and (ii) radial O_2 loss (ROL) from the aerenchyma to the environment. In the mature zones of rhizomes and roots, tissue respiration is moderate since the metabolic processes primarily serve to support maintenance respiration, and barriers to ROL exists that reduce the loss of O_2 along the diffusion pathway (Colmer 2003). In the apical zones of rhizomes and in the root-tips, on the other hand, cell division requires additional energy and thus increased O_2 consumption, resulting in a steep decline in tissue pO_2. The root-tips are highly permeable to O_2 and ROL is extensive (Jensen et al. 2005; Pedersen et al. 1998), resulting in a steep gradient in pO_2 inside the aerenchyma from shoot to root-tip. This gradient drives a steady flux of O_2 to the O_2 demanding tissues. Figure 13.3 demonstrates how tissue pO_2 systematically declines with distance to the shoot, which acts as entry point of O_2 in the dark and a site of O_2 production in the light.

In the dark, the pO_2 of roots and rhizomes is strongly correlated to water-column pO_2 (e.g. Greve et al. 2003), which is reflected by decreasing tissue pO_2 following a decline in water-column pO_2 (Fig. 13.3). A strong dependence of water-column O_2 on the night-time tissue respiration has also been demonstrated in situ (Sand-Jensen et al. 2005; Borum et al. 2005). Sand-Jensen et al. (2005) reported that at dusk, when photosynthesis ceased ($\sim$8 p.m., Fig. 13.4), tissue pO_2 declined rapidly to a point where the decline followed water-column pO_2 (Fig. 13.4), and the shoot base

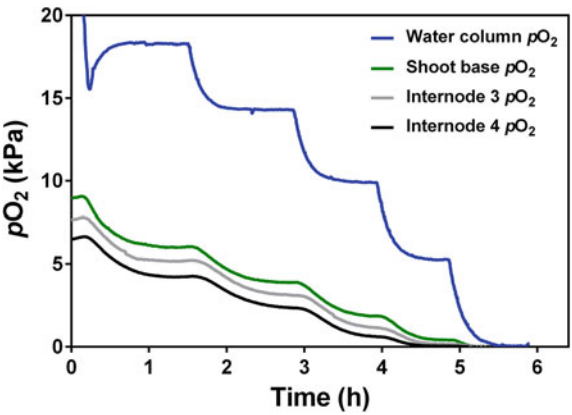

Fig. 13.3 Below-ground tissue pO_2 as a function of water-column pO_2 in darkness measured in *Zostera marina*. The O_2 microelectrodes were inserted into the shoot base close to the leaf meristem, which was buried approximately 5 mm into the sediment, and in the 3rd and the 4th internode of the rhizome. The pO_2 of the water-column was successively reduced in steps of 4–5 kPa over a timeframe of 6 h and kept at 20 °C. Data modified from Pedersen et al. (2004)

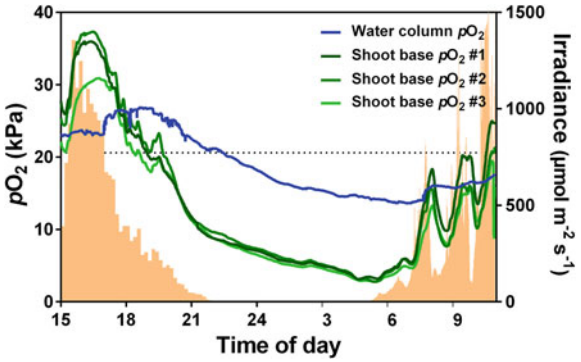

Fig. 13.4 In situ pO_2 of the shoot base of 3 replicate plants of *Zostera marina* and the water-column over a diurnal cycle measured in Roskilde Fjord, Denmark. The O_2 microelectrodes were inserted into the shoot base close to the leaf meristem, which was buried approximately 5 mm into the sediment. The dotted line indicates air equilibrium of dissolved O_2. Irradiance of the PAR spectrum (400–700 nm) measured at the canopy surface is shown in orange colour. Data modified from Sand-Jensen et al. (2005)

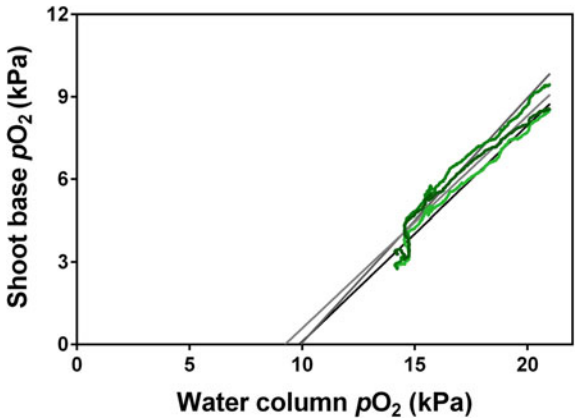

Fig. 13.5 Water-column pO_2 versus shoot base pO_2 during night-time of 3 replicate plants of *Zostera marina*. The data are extracted from Fig. 13.4 in the time period of 10 p.m. to 5 a.m. The grey lines represent linear regression of each replicate plant and are extrapolated to interception with the horizontal axis (as this gives an estimate of at which water-column pO_2 the vulnerable shoot base tissue becomes anoxic). Data modified from Borum et al. (2006)

became anoxic at a water-column pO_2 of approximately 9–10 kPa (~50% air saturation; Fig. 13.5). The water-column pO_2 required to prevent shoot base anoxia depends on the above-ground:below-ground tissue ratio since the shoot acts as site of O_2 uptake, whereas roots and rhizomes are sinks due to respiration and ROL. At a relatively low ratio, the critical water-column pO_2 for shoot base anoxia would be higher compared to a situation, where the ratio is higher. Implications of tissue

anoxia encompass (i) low energy yield when anaerobic fermentation takes over from respiration, (ii) reduced nutrient uptake by the roots, (iii) impeded translocation of carbohydrates and nutrients between leaves and roots, and (iv) anoxic rhizosphere conditions near the root-tips potentially leading to sulphide intrusion (see below; e.g. Zimmerman and Alberte 1996; Pedersen et al. 2004; Borum et al. 2006; Brodersen et al. 2015b).

The aerenchyma has been suggested to function as an important reservoir of O_2 for respiration in the dark. However, in the case of *Z. marina*, the pool of O_2 initially captured in the aerenchyma would only be able to support respiratory demands for 8–13 min, assuming an initial pO_2 near atmospheric equilibrium (i.e. 20.6 kPa) (Sand-Jensen et al. 2005). Moreover, shoot and apical root tissues are highly gas permeable and the pool of O_2 quickly equilibrates with the environment (e.g. Fig. 13.3) further shortening the time that stored O_2 can meet respiratory demands. Thus, seagrasses primarily rely on a reservoir of O_2 in the water-column surrounding the leaves to support night-time respiration in their tissues, and this makes them vulnerable to O_2 depletion in the water-column during night-time or periods of low irradiance, e.g. due to low water transparency.

Photosynthetically produced O_2 supports daytime respiration in both above- and below-ground seagrass tissues. In the light, shoot base tissue pO_2 can reach 40 kPa or more (Fig. 13.4) and thereby significantly exceed water-column pO_2. As previously mentioned, the high tissue pO_2 in the leaves in the light results in a steep O_2 gradient to the surrounding water-column and also internally from shoot to root-tips. This facilitates that even the most distant root-tips can experience daytime pO_2 of close to air equilibrium as shown for e.g., *Cymodocea rotundata* and *Zostera marina* (Jensen et al. 2005; Pedersen et al. 1998).

The strong relationship between below-ground tissue pO_2 and photosynthesis during the day is illustrated in Fig. 13.6 showing a saturation of shoot base pO_2 with increasing irradiance (measured at leaf canopy height) with a shape resembling typical photosynthesis *versus* irradiance curves. The data for Fig. 13.6 are extracted from the light period between 6 and 11 a.m. in Fig. 13.4, and show that with a

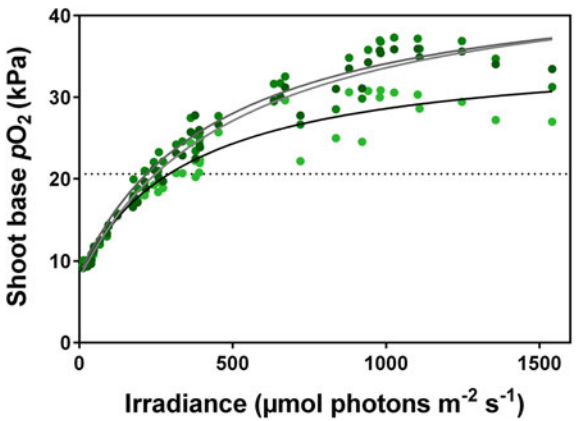

Fig. 13.6 Irradiance versus shoot base pO_2 during day-time of 3 replicate plants of *Zostera marina*. The data are extracted from Fig. 13.4 in the time period of 6 p.m. to 11 a.m. on day 2. The grey lines represent non-linear regression of each replicate plant applying a Jassby and Platt (1976) model. The dotted line represents air saturation of dissolved O_2. Data modified from Borum et al. (2006)

photon irradiance of approximately 250–300 µmol photons m^{-2} s^{-1} and above, shoot base pO_2 exceeds the atmospheric equilibrium of 20.6 kPa. Thus, in shallow transparent waters governing good light conditions, the below-ground tissues of seagrasses exhibit a beneficial intra-plant O_2 status due to photosynthetic O_2 production during the day.

13.3 Seagrass-Sediment Interactions

Seagrasses are generally found in highly reduced sediments enriched with organic matter (Borum et al. 2006) including remnants of seagrass plants. The high productivity of seagrass meadows and the resulting continuous contribution of organic matter to the sediment, both from seagrass debris and exudates from roots and rhizomes (Moriarty et al. 1986; Pollard and Moriarty 1991), as well as from enhanced sedimentation due to diminished flow in dense seagrass beds (Ward et al. 1984; Madsen et al. 2001), supports high rates of microbial carbon mineralization in the sediment. The O_2 solubility in seawater is limited (typically 284–196 µM in air-saturated seawater at 10–30 °C and a salinity of 34) as compared to terrestrial systems, and the O_2 supply to the sediment from the seawater can be impeded by the diffusive boundary layer (DBL) (e.g. Jørgensen and Revsbech 1985; Jørgensen and Des Marais 1990; Kühl and Revsbech 2001) and slow internal sediment diffusion rates (Glud et al. 2007). Aerobic respiration and re-oxidation of reduced chemical species diffusing towards the sediment-water interface rapidly deplete O_2 in the upper mm's of the sediment. In anoxic marine sediments, microbial sulphate reduction is the dominant anaerobic respiratory process (Jørgensen 1982), whereby sulphate is reduced to sulphides that exhibit a pH dependent speciation (with a pK_a value of ~pH 7) into dissolved hydrogen sulphide gas (H_2S) and hydrogen sulphide ions (HS^-) at typical sediment pH values. Sulphide species react efficiently with oxidized molecules such as Fe(III), causing a further reduction of the sediment. Due to its high toxicity to aerobic organisms, high H_2S concentrations are generally detrimental to plants and animals living in sulphidic sediments (Lamers et al. 2013). It has therefore long been speculated that seagrasses must have a capacity to alleviate H_2S exposure in order to sustain their own growth.

13.3.1 H_2S Intrusion at Low Water-Column O_2 Concentrations

Water-column hypoxia during night-time can lead to H_2S intrusion into below-ground tissues if the O_2 flux across the DBL is insufficient to maintain ROL at the basal leaf meristem and root-tips. The fast growing root-tips are highly gas permeable because they lack a structural barrier to ROL (Connell et al. 1999),

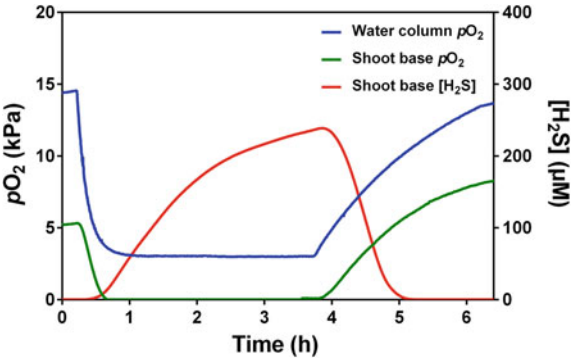

Fig. 13.7 Shoot base pO_2 and shoot base H_2S as a function of water-column pO_2 in *Zostera marina*. The O_2 and H_2S microelectrodes were inserted into the shoot base close to the leaf meristem, which was buried approximately 5 mm into the sediment. Water-column pO_2 was manipulated in steps of about 10 kPa and kept at 20 °C. Data modified from Pedersen et al. (2004)

but during conditions with normal water-column pO_2, the ROL results in the formation of a so called "oxic microshield" in the rhizosphere around the basal leaf meristem and root-tips (Jensen et al. 2005; Frederiksen and Glud 2006; Brodersen et al. 2015b). The released O_2 can oxidize sulphide and thereby prevent H_2S from diffusing into the young, structurally unprotected tissue (further described in Sect. 13.3.2 below; Brodersen et al. 2015b). During water-column hypoxia, however, the ROL may become insufficient to maintain these oxic shields in the rhizosphere, increasing the risk of H_2S exposure and intrusion (Fig. 13.7).

Gaseous H_2S spreads by molecular diffusion inside the aerenchyma from areas of high concentration near the root-tips towards the shoot. In the gas phase, oxidation of H_2S with O_2 is a relatively slow spontaneous chemical reaction, and both O_2 and H_2S can thus coexist for some time in the same tissues (Borum et al. 2005; Pedersen et al. 2004). Figure 13.7 shows an example, where water-column pO_2 was experimentally manipulated, and where a decline from 15 to 3 kPa O_2 resulted in H_2S intrusion. At the shoot base, H_2S was detected in the tissue before complete O_2 depletion, where after H_2S continued to rise up to $\sim$250 µM. When the surrounding water-column was brought back to atmospheric equilibrium, pO_2 in the shoot base increased, while H_2S was depleted. However, O_2 and H_2S co-existed in the same tissue for >1 h until O_2 reached the root-tips, and H_2S intrusion was once again restricted by the oxic sediment microshield. Recent studies also suggest an internal H_2S detoxification mechanism, whereby H_2S is oxidized to elemental sulphur (an intermediate in sulphide oxidation) precipitating on the inner walls of the aerenchyma (e.g. Holmer and Hasler-Sheetal 2014; Hasler-Sheetal and Holmer 2015).

Intrusion of H_2S into seagrass tissue has also been demonstrated in situ, where sulphide poisoning has been suggested to result in localised die-off events (e.g. Borum et al. 2005; Carlson Jr et al. 1994). Florida Bay in the U.S. has been severely affected by such die-off events, and Borum et al. (2005) showed that gaseous H_2S

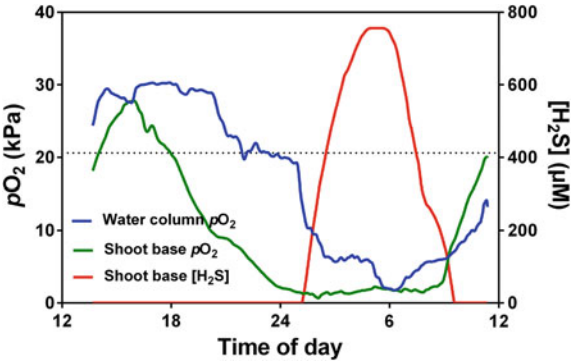

Fig. 13.8 In situ pO_2 and H_2S of the shoot base of *Thalassia testudinum* and the water-column pO_2 over a diurnal cycle measured in a die-off patch at Barnes Key, Florida Bay, USA. The O_2 and H_2S microelectrodes were inserted into the shoot base close to the leaf meristem, which was buried approximately 20 mm into the sediment. The dotted line indicates air equilibrium of dissolved O_2. Data modified from Borum et al. (2005)

started penetrating the below-ground tissues and spread to the shoot base of seagrasses at a water-column pO_2 of approximately 50% air equilibrium (10 kPa) (Fig. 13.8). As the water-column experienced further hypoxia during the night, H_2S reached a tissue concentration of more than 750 µM in the shoot base. In line with the observations from laboratory experiments (Fig. 13.7), the shoot base never became anoxic and H_2S and molecular O_2 coexisted throughout the night (Fig. 13.8). Tissue H_2S then started declining following sunrise, as photosynthetically produced O_2 resulted in higher pO_2 in the below-ground tissues, but H_2S persisted in the shoot base tissue until 10 a.m., i.e., >4 h after sunrise. Sulphide intrusion into the below-ground tissue of seagrasses is thus strongly linked to the O_2 status of the plants.

Koren et al. (2015) found that the oxygenated region around the seagrass rhizome of *Z. muelleri* was diminished during night-time (Fig. 13.9), likely in response to lowering of the internal pO_2 and thereby a reduction in the O_2 gradient from the rhizome to the anoxic sediment. A combination of darkness and low water-column pO_2 ($\sim$50% air equilibrium) has previously been shown to enable H_2S to reach the root and rhizome of the plant, thus exposing the plant to potential poisoning (Fig. 13.10). Seagrasses may thus be sensitive to diminished water flow, light and/or pollution that can affect the O_2 transport to the lower tissue regions of the plant. Pollution effects include sediment re-suspension from dredging, which lowers the photosynthetically active radiation (PAR) reaching the leaves (Erftemeijer and Lewis 2006), and eutrophication-induced algal blooms lowering light availability and water-column O_2 concentrations through increased night-time respiration and degradation of settled algal biomass in the sediment. Growth of epiphytic algae on the seagrass leaf can also reduce PAR and increase the DBL thickness and thereby impede O_2 transport into the leaf (Drake et al. 2003; Brodersen et al. 2015a).

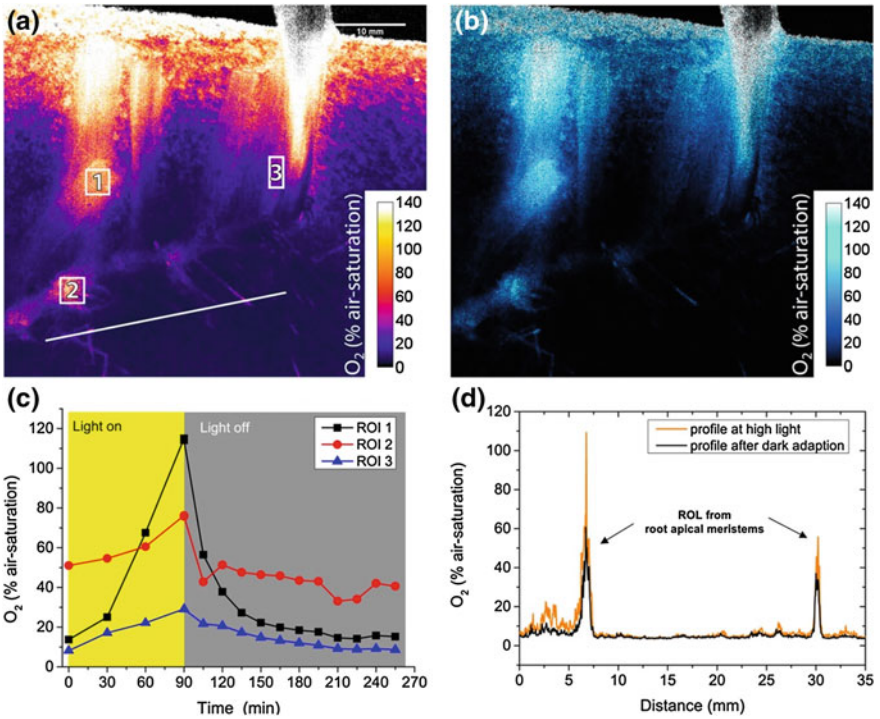

Fig. 13.9 **a** Colour coded O_2 image acquired via novel optical nanoparticle-based O_2 sensors, visualising the O_2 distribution in the seagrass rhizosphere under an incident photon irradiance of 500 μmol photons m^{-2} s^{-1}. **b** The relative difference in the below-ground tissue oxidation capacity between measurements in light and darkness. **c** Real-time O_2 concentrations within selected regions of interest (ROIs, as shown in panel A) during a light/dark transition. Black symbols and profile represents measurements at the prophyllum (ROI 1), red symbols and profile represent measurements at the root-shoot junction (ROI 2), blue symbols and profile represent measurements at the basal leaf meristem (ROI 3). **d** The extracted line profile from the O_2 image (shown in panel A) across 2 roots, visualising radial O_2 loss (ROL) from the root apical meristems during a light/dark transition. Partly redrawn with permission from Koren et al. (2015). Copyright 2015 American Chemical Society

13.3.2 Oxic Microshields and Below-Ground Tissue Oxidation Capacity

The passive, internal aeration system of the seagrass plant not only serves to aerate various tissue parts including the below-ground portions (see Sects. 13.1 and 13.2). Aerenchymatic gas transport to the below-ground tissue and ROL to the surrounding sediment enables oxidation of the immediate rhizosphere microenvironment and alleviates exposure to phytotoxins such as H_2S (Brodersen et al. 2015b). The release of O_2 from roots and rhizomes has been demonstrated on multiple occasions

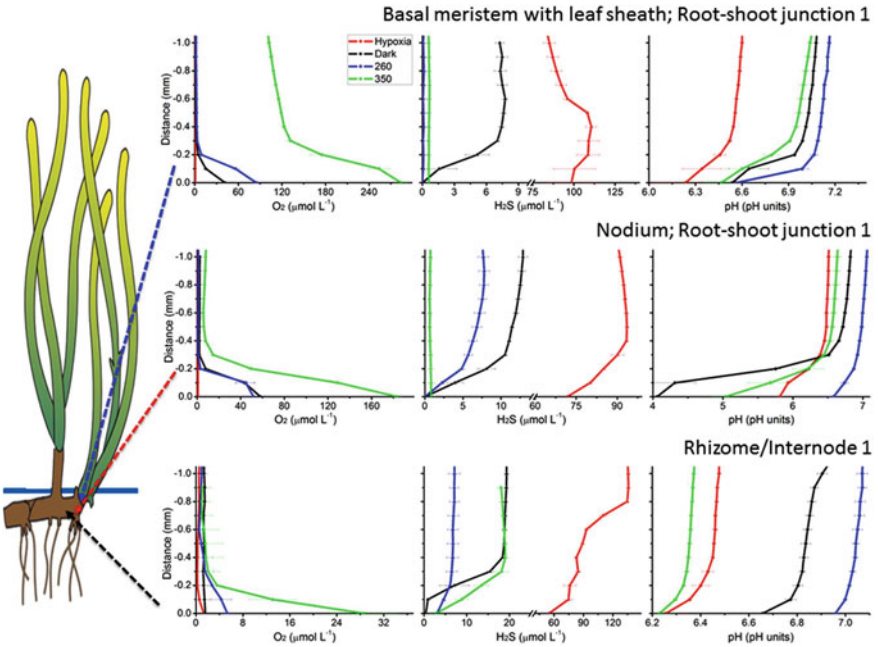

Fig. 13.10 Seagrass-derived sediment detoxification as a result of below-ground tissue radial O_2 loss into the immediate rhizosphere. Concentration profiles of O_2, H_2S and pH were measured with microelectrodes in darkness (black profiles), at an incident photon irradiance of 260 (blue profiles) and 350 (green profiles) µmol photons m^{-2} s^{-1}, and in darkness with hypoxic conditions in the water-column (red profiles). *Upper panels* represents measurements at the basal leaf meristem with leaf sheath, *intermediate panels* (horizontally) at the root-shoot junction and *lower panels* at the rhizome. *Left panels* represent the immediate rhizosphere O_2 concentration, *intermediate panels* (vertically) represents the immediate rhizosphere H_2S concentration and *right panels* represents the immediate rhizosphere pH. Y = 0 indicate the below-ground tissue surface. Error bars are ±SD. n = 2–4. Note the break on the x-axis of panels illustrating the immediate rhizosphere H_2S concentration. The illustration of *Z. muelleri spp. capricorni* originates from the IAN/UMCES symbol and image libraries (Diana Kleine, Integration and Application Network (IAN), University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/)). Data modified from Brodersen et al. (2015b). Copyright 2015 John Wiley & Sons Ltd.

(Pedersen et al. 1998, 1999; Jensen et al. 2005; Frederiksen and Glud 2006; Brodersen et al. 2015a, b; Jovanovic et al. 2015; Koren et al. 2015) (Fig. 13.9), but only recently has the direct connection between O_2 release and removal of H_2S around the below-ground tissue been confirmed (Brodersen et al. 2014, 2015b). By applying a split flow chamber with artificial, transparent sediment, Brodersen and co-workers used microsensors to measure the O_2 release from the below-ground tissue from *Z. muelleri* spp. *capricorni* and could align such oxic microzones with the concomitant detection of H_2S depletion towards the roots and rhizomes resulting from chemical oxidation (Fig. 13.10). While the leakage of O_2 varied across the rhizome, a several hundred µm thick oxic microshield was detected at the point of

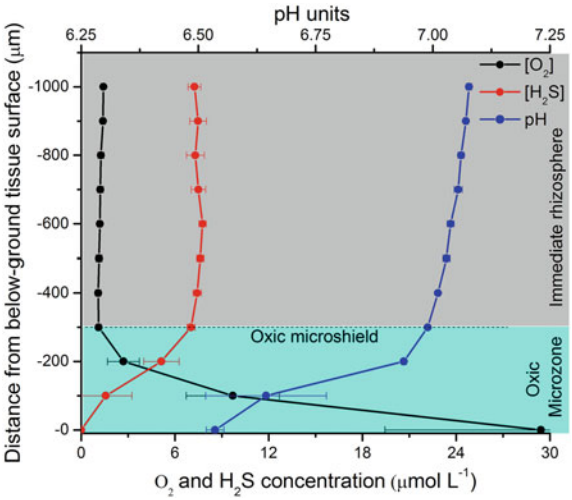

Fig. 13.11 Oxic microshields surrounding the root/shoot junctions (including the basal leaf meristem with leaf sheath), the rhizome and the apical root meristems of seagrasses. Black symbols and profile represents [O_2]; red symbols and profile represents [H_2S]; and blue symbols and profile represents pH. The shown microelectrode microprofiles are from the meristematic region of the rhizome. Y = 0 indicate the below-ground tissue surface. Error bars are ±SD. n = 3. Data modified from Brodersen et al. (2015b). Copyright 2015 John Wiley & Sons Ltd.

radial O_2 loss (Fig. 13.11), which was sufficient to oxidize most of the H_2S before it reached the tissue surface (Figs. 13.10 and 13.11).

In contrast to previous observations in temperate *Z. marina* plants (Jensen et al. 2005), *Z. muelleri*, which extends into subtropical regions, showed no or very little ROL from the root-tips, and the highest ROL was found around the basal leaf meristem with leaf sheaths, where new leaves are generated (Brodersen et al. 2014, 2015b; Koren et al. 2015). While mature seagrass tissue has a high resistance to radial O_2 transport (Jensen et al. 2005; Frederiksen and Glud 2006) partly due to the presence of Casparian-band like structures (Barnabas 1996), the new tissue being formed in the meristem has a poorly developed lacunal system and little resistance to radial gas transport. As such, the concomitant higher lateral movement of O_2 to the meristematic tissue of *Z. muelleri* is likely an adaptation to protect this crucial but vulnerable tissue of the plant against exposure to H_2S, both internally and externally. However, to what extent this is a general feature of seagrasses needs further evaluation.

Anoxic, reduced sediment conditions have also been shown to induce development of below-ground tissue gas barriers owing to accumulation of suberin lamellae in the hypodermal tissue of seagrasses (Enstone et al. 2003; Armstrong and Armstrong 2001, 2005). Adequate internal aeration is thus a key prerequisite for healthy seagrass communities, as the intra-plant O_2 status and thereby the

below-ground tissue oxidation capacity to a large extent determines the resilience of the plants towards sediment-produced H_2S and environmental disturbances, such as nutrient loadings and dredging operations leading to markedly reduced light availability and O_2 conditions in the water-column (Brodersen et al. 2015a, b).

Apart from its protective function, ROL into the rhizosphere may also stimulate aerobic heterotrophic bacteria leading to increased local remineralisation and mobilization of nutrients of potential benefit to the seagrasses (Blaabjerg et al. 1998; Brodersen et al. 2017a; Hansen et al. 2000; Nielsen et al. 2001) (see also Chap. 17). Nutrient mobilization can also happen through a change in the rhizosphere pH as a result of ROL (Brodersen et al. 2017a). Brodersen et al. (2015b) showed that the pH microenvironment around the below-ground tissue was affected by ROL with pH decreasing by 1–2 pH units inside the oxic microshield relative to the surrounding buffered, artificial sediment (Fig. 13.10). This drop in pH is likely a result of the release of protons (H^+) from re-oxidation of H_2S, and this mechanism has been proposed to be of significance for the mobilization of phosphate in carbonate-rich sediments (Fourqurean and Zieman 2002; Holmer et al. 2006; Brodersen et al. 2015b; Brodersen et al. 2017a).

13.3.3 Rhizosphere pH Heterogeneity and pH-Mediated Sulphide Detoxification

While seagrass O_2 dynamics has been investigated in several studies, much less is known about spatio-temporal pH dynamics in the seagrass rhizosphere. By means of novel nanoparticle-based optical pH imaging, Brodersen et al. (2016) recently documented pronounced spatio-temporal pH heterogeneity in the immediate rhizosphere of the seagrass *Z. marina* L. Imaging of the sediment pH distributions in 2D revealed several distinct micro-niches of low and high pH within the seagrass rhizosphere as compared to the bulk sediment pH (Fig. 13.12). Light exposure of the canopy and an experimental temperature increase from 16 to 24 °C, i.e., to the temperature optimum for oxygenic photosynthesis in summer acclimated *Z. marina* L. plants (Staehr and Borum 2011), lead to elevated pH levels in the seagrass rhizosphere with rhizome/root surface pH increasing by up to 0.9 pH units relative to the sediment pH. This photosynthesis/temperature-dependent pH effect may be due to: (i) secretion of allelochemicals like amines by the plant, (ii) CO_2 uptake by the below-ground tissue changing the carbonate equilibrium in the rhizosphere (Colmer 2003; Larkum unpublished data), and/or (iii) enhanced root/rhizome exudates stimulating sulphate reducing bacteria in the rhizoplane consuming protons through their microbial metabolism (Pollard and Moriarty 1991). Previous studies have shown an increase in sulphate reduction rates (SRR) within seagrass-vegetated sediment and on the below-ground tissue surface of seagrass during photosynthesis, and such stimulation of SRR was attributed to increased

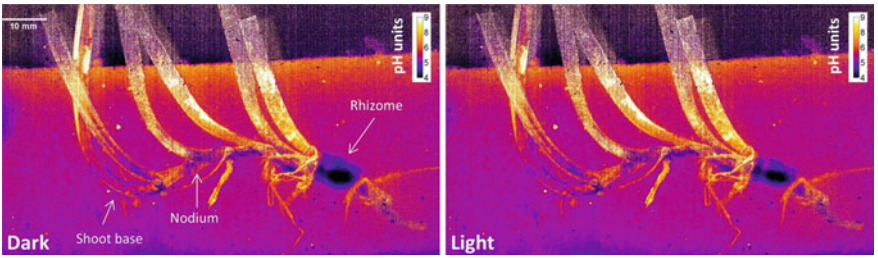

Fig. 13.12 pH heterogeneity and dynamics in the seagrass rhizosphere determined via novel optical nanoparticle-based pH sensors during a light/dark transition (incident irradiance of 500 μmol photons m^{-2} s^{-1}). Colour coded pH image; Legend depicts the pH units. *Left panel* represents measurements in darkness; *right panel* represents measurements in light. The colour coded pH images are the average of three measurements. Data modified from Brodersen et al. (2016). Copyright 2015 John Wiley & Sons Ltd.

exudation of carbohydrates and amino acids (Isaksen and Finster 1996; Moriarty et al. 1986; Blaabjerg et al. 1998; Hansen et al. 2000; Nielsen et al. 2001). The dissolved organic carbon (DOC) exudation from the seagrass rhizome and roots has been estimated to account for 0.7–18% of the total carbon fixed via photosynthesis (e.g. Wetzel and Penhale 1979; Moriarty et al. 1986; Blaabjerg et al. 1998; Hansen et al. 2000).

The chemical speciation of sulphide is pH-dependent, where H_2S predominates at pore-water pH < 7 and HS^- ions at pH > 7. A plant-induced increase in rhizosphere pH can thus shift the chemical speciation towards the non-tissue-permeable HS^- ion, thereby reducing H_2S exposure of the below-ground tissues (Brodersen et al. 2015b, 2016). Brodersen and co-workers (2015b, 2016) showed that regions of the rhizosphere with low pH (down to pH 4) correlated with the presence of plant-mediated oxic microniches (Figs. 13.11 and 13.12), while the tissue surface pH generally was higher than in the bulk sediment. A pH drop within the oxic microshield of the rhizosphere, as a result of the formation of sulphuric acid (i.e. $2O_2 + H_2S \rightarrow 2H^+ + SO_4^{2-}$), can lead to dissolution of carbonates and a concomitant release of sediment-bound phosphorus (Brodersen et al. 2017a; Fourqurean and Zieman 2002; Holmer et al. 2006; Lambers et al. 2009), which then become available for plant assimilation.

An overview of the effect of plant activity on the rhizosphere pH microenvironment at plant/sediment- and oxic/anoxic interfaces is given in Fig. 13.13. Close to selected root/shoot junctions (Fig. 13.13c, e) either a pronounced decrease in pH towards the tissue surface was observed (Fig. 13.13e) indicating chemical re-oxidation of H_2S via ROL and thereby sediment detoxification in these regions, or an increase in pH towards the approximate oxic/anoxic interface was observed, followed by a rapid decrease in pH towards and on the below-ground tissue surface (Fig. 13.13c). The latter is indicative of proton consuming processes, such as

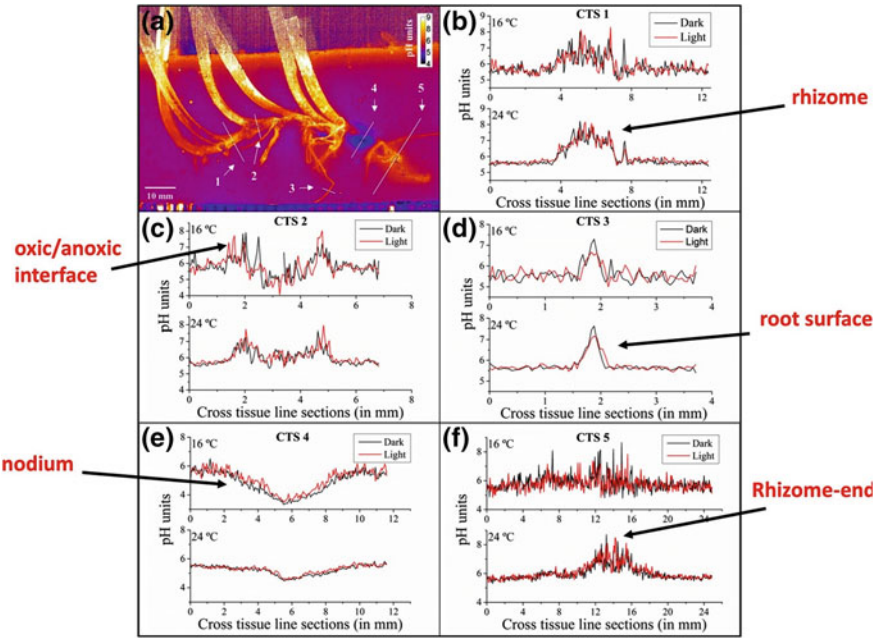

Fig. 13.13 pH microdynamics in the seagrass rhizosphere at plant/sediment- and oxic/anoxic interfaces measured via novel optical nanoparticle-based pH sensors during light/dark transitions and at temperatures of 16 and 24 °C (where 24 °C represents the temperature optimum for oxygenic photosynthesis in *Zostera marina* L.). **a** Colour coded pH image visualising the extracted cross tissue line profiles in the seagrass rhizosphere. **b–f** Cross tissue line section 1–5 as shown in panel a, determining pH microdynamics at plant/sediment- and oxic/anoxic interfaces. Data modified from Brodersen et al. (2016). Copyright 2015 John Wiley & Sons Ltd.

sulphate reduction, at the oxic/anoxic interface followed by chemical re-oxidation of H_2S at the plant/sediment interface (Brodersen et al. 2016). High sediment SRR may lead to a sulphide-induced release of sediment-bound phosphor, from the reduction of Fe(III)oxyhydroxides to Fe(II), as this results in the release of previously sequestered phosphate into the surrounding pore-water (Brodersen et al. 2017a; Pollard and Moriarty 1991; Pagès et al. 2011, 2012). It is therefore intriguing to speculate that a mutual beneficial relationship may exist between the plant hosts and sulphate reducing bacteria based on a reciprocal exchange of nutrients.

Thus there is first experimental evidence that seagrasses can modulate their rhizosphere pH microenvironment. Such changes in pH potentially present an important additional chemical defence mechanism, whereby seagrass plants can further alleviate H_2S toxicity by shifting the sulphide speciation towards non-tissue-permeable HS^- ions.

13.4 Effects of Anthropogenic Impacts on Seagrass Habitats and the Rhizosphere Microenvironment

Human activity in coastal marine areas such as boating activities, coastal and harbour development, dredging-induced sediment re-suspension and nutrient loadings, can have profound and adverse effects on the health of adjacent seagrass meadows (Brodersen et al. 2017b; Erftemeijer and Lewis 2006; Orth et al. 2006; Waycott et al. 2009). In fact, seagrass meadows are declining worldwide at an alarming rate (Waycott et al. 2009). This is often a result of synergetic negative impacts on the surrounding environment and thereby seagrass fitness, such as (i) lower light availabilities in the water-column caused by, for example, nutrient-driven algal blooms and/or increased water turbidity from anthropogenic-induced land run-off, adversely regulating rates of leaf photosynthesis during day-time (e.g. Dennison 1987; Short and Burdick 1995; Erftemeijer and Lewis 2006), (ii) enhanced water-column respiration rates during night-time, reducing the water-column O_2 conditions and thus the passive O_2 influx into the aerenchyma (e.g. Borum et al. 2006), (iii) impeded gas exchange with the surrounding water owing to, for example, nutrient-driven enhanced leaf epiphyte growth further reducing the passive, diffusive O_2 exchange and the CO_2 uptake of the leaves, thereby potentially leading to inadequate internal aeration and photorespiration (e.g. Maberly 2014; Brodersen et al. 2015a), as well as (iv) high sediment H_2S concentrations, as a response to high sediment SRR fuelled by nutrient inputs, leading to enhanced rhizosphere O_2 demands and sediment toxicity (Borum et al. 2005). Enhanced seagrass mortality has thus often been linked to low light availability (e.g. Kim et al. 2015; York et al. 2015) and low night-time water-column O_2 conditions (Greve et al. 2003; Pedersen et al. 2004; Borum et al. 2005; Brodersen et al. 2015b) coupled with high sediment O_2 demands and H_2S production/concentrations (e.g. Carlson et al. 1994; Borum et al. 2005). These factors can strongly reduce the intra-plant O_2 status owing to a reduction in the O_2 source and/or an increase in the O_2 sink, as the plant-derived rhizosphere oxic microshields, described above, generally ensures protection against phytotoxic H_2S intrusion (Brodersen et al. 2015b). These effects highlight the importance of minimizing environmental disturbance activities in close proximity to seagrass meadows, and pose a challenge for making the increasing exploration of natural resources, e.g. causing increased harbour developments in Australia, environmentally sustainable.

13.5 Conclusions

The aerenchyma system of seagrasses ensures aeration of the shoots, rhizomes, roots and, in many cases, the rhizosphere. The shoot and shoot-base manifold are important components of this aeration system and have to be understood to fully

understand the aeration of the roots and rhizomes. In particular, the shoot-base manifold seems to ensure that O_2 is supplied to the young leaf meristem, and this may be particularly important when H_2S penetrates the roots and rhizomes. However, perhaps the major conclusion in this chapter is that seagrasses can actively alter their rhizosphere microenvironment via release of O_2 and allelochemicals into the sediment surrounding their below-ground tissue. This exudation provides a chemical defence mechanism, whereby seagrasses can detoxify their immediate rhizosphere through (i) chemical oxidation of sediment-derived H_2S via plant-released O_2, or (ii) shifting the chemical sulphide speciation towards non-tissue-permeable and thus non-phytotoxic HS^- ions by local increase of the rhizosphere pH (Fig. 13.14). Radial O_2 loss mainly occurs at the meristematic regions of the rhizome and roots forming oxygenated microzones around the most essential and vulnerable parts of the plants in the otherwise reduced, anoxic sediment environment. The capacity of seagrass below-ground tissue to oxidize the rhizosphere is predominantly regulated by light availability during day-time and by water-column O_2 levels during night-time. Overnight water-column hypoxia may lead to inadequate internal aeration of the seagrass, which in turn may result in

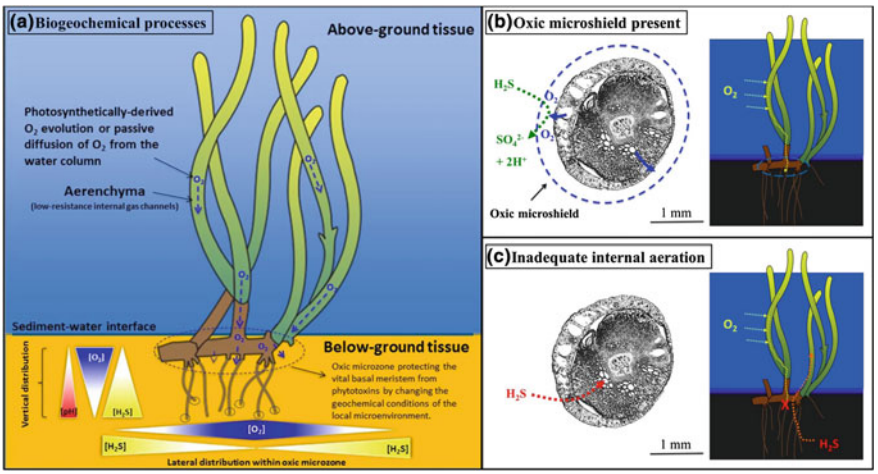

Fig. 13.14 Conceptual diagram visualising seagrass-derived sediment detoxification. **a** O_2 transported down to the below-ground tissue via the aerenchyma is released from the meristematic region of the rhizome (basal leaf meristem), the rhizome and from root apical meristems into the immediate rhizosphere. Radial O_2 loss from the below-ground tissue maintaining protective oxic microniches in the immediate rhizosphere, and plant-derived sediment pH changes, chemically detoxifies the surrounding sediment by re-oxidizing sediment-produced H_2S and shifting the geochemical sulphide speciation towards non-tissue-permeable HS^- ions, respectively. **b** Oxic microshield protecting the vulnerable basal leaf meristem. O_2 released from the below-ground tissue drives chemical re-oxidation of sediment-produced H_2S within the oxic microniches. **c** Inadequate internal aeration may lead to H_2S intrusion which in turn may kill the plants as a result of chemical asphyxiation. Data modified from Brodersen et al. (2015b). Copyright 2015 John Wiley & Sons Ltd.

sulphide intrusion and thereby increased seagrass mortality owing to chemical asphyxiation. Seagrass plants are thus most vulnerable to phytotoxin intrusion at night-time, where O_2 supply to the below-ground tissue to sustain aerobic metabolism and maintain protective oxic microniches in the immediate rhizosphere is completely dependent on passive diffusion of O_2 from the surrounding water-column into the aerenchymal tissue of the leaves. The most important structural adaptation of seagrasses to life in sulphidic sediment habitats is therefore, most likely, the formation of suberin tissue barriers to ROL, where the low cross tissue gas permeability ensures efficient transport of O_2 to distal parts of the plants and at the same time impedes H_2S intrusion.

Plant-mediated low pH hotspots in the rhizosphere may lead to a concomitant release of sediment-bound phosphorus, which is often the limiting nutrient in carbonate-rich sediments. Modification of rhizosphere pH may thus be important for nutrient mobilization allowing seagrasses to grow in nutrient-limited marine environments. The relatively higher pH levels on below-ground tissue surfaces also indicate secretion of allelochemicals and/or plant-derived stimulation of proton consuming microbial metabolisms, such as sulphate reduction. However, such mechanisms remain speculative and call for direct experimental confirmation.

The chemical defence systems of seagrasses, described in this chapter, are of great importance for the plants. They ensure protection against sediment-produced phytotoxins and provide oxygenated microniches for the growing seagrass roots. Overnight water-column hypoxia and DBL-impeded O_2 transport/evolution in the leaves may result in the degradation of the below-ground oxic microshields and H_2S exposure of the below-ground tissue surface. Anthropogenic-induced environmental disturbances causing O_2 depletion in coastal marine environments thus represents a major threat to seagrass meadows, as low intra-plant O_2 conditions during night-time is a key factor causing events of seagrass die-backs.

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