

The Seagrass Rhizosphere

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Thesis submitted for the degree of Doctor of Philosophy at the University of Technology Sydney I June 10, 2016

CERTIFICATE OF ORIGINAL AUTHORSHIP

This thesis is the result of a research candidature conducted jointly with another University

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I also certify that the thesis has been written by me. Any help that I have received in my

research work and the preparation of the thesis itself has been acknowledged. In addition, I

certify that all information sources and literature used are indicated in the thesis.

Signature of Student:

Date: June 10, 2016

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ACKNOWLEDGEMENTS

I would like to thank my supervisors: Michael Kühl, Daniel A. Nielsen, Ole Pedersen and Peter J. Ralph, without whom I would never have been able to complete this PhD thesis and who all provided editorial assistance to this work. Michael you have been the greatest support of all and the best ever imagined mentor during my PhD project. Thank you very much. Daniel you were my solid support at UTS, and patiently helped me sorting my mind especially during the first year of my PhD, which made me stay on track and keep focussed. Thanks. Ole you appeared a bit later in my PhD project, but I will always remember our voyage back to shore at Narrabeen Lagoon (both mentally and physically) with all our sensitive equipment and data in the rubber dinghy; and how you guided me through that challenging phase of my PhD. Peter you have provided the perfect research facility to overcome the challenge of submitting this PhD thesis, and I could not have wished for a better place to undertake my PhD candidature. Thanks.

Friends and colleges: Mads Lichtenberg, Klaus Koren, Jakob Santner, Mathieu Pernice, Nahshon Siboni, Justin Seymour, Paul York, Michael Rasheed, Maria Mosshammer, Kathrine Jul Hammer, Stacey M. Trevathan-Tackett, Laura-Carlota Paz, Sofie L. Jakobsen, Tony Larkum, Anja Frøytrup, Milan Szabo, Katherina Petrou, Dale Radford, Michele Fabris, Joey Crosswell, Jean-Baptiste Raina, Katie Chartrand, Bojana Manojlovic, Peter Davey, Alex Thomson, Jeff Kelleway, Jessica Tout, Daniel Wangpraseurt, Verena Schrameyer, and all the people I have forgotten; thanks for all the fun experiences in the laboratory, good companionships, long scientific and less-scientific talks and nice research. I am looking very much forward to continue our collaborations in the future.

Thanks to my overseas family for supporting me on this personal dream of mine; of both undertaking a PhD, but as much experiencing the adventures and challenges of moving to and living in Australia.

Lastly, in my mind a PhD is like a journey from a to b, where you in the beginning of your PhD stand at the bottom of a mountain, a big impressive mountain with a threatening glacier close to the summit, and you know that somehow in the end of this journey you will have to find yourself on the top of that mountain planting your personal mark, for forever

sight, into the solid rocky ground. But as in all other aspects of life, it is important to

remember to focus on the journey itself and not on reaching the final goal, as else you will

often find yourself forgetting the purpose of your challenge and thus lose yourself on your

way ("journey") to the summit. I therefore dedicate my PhD thesis to my little, much

beloved, family. Karla, Rufus and Verena, without you guys I could never have done this, and

for that I am you forever grateful. Thanks, with all the love of my heart.

Kasper Elgetti Brodersen

June 10, 2016

IV

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Figure II. Conceptual diagram, illustrating internal aeration, the below-ground oxic microshield and potential hydrogen sulphide (H₂S) intrusion in seagrasses. (a) Rhizospheric oxic microshield present as a result of a sufficient O₂ supply from the leaves. Radial O₂ loss (ROL) from below-ground tissue leads to spontaneous chemical re-oxidation of phytotoxic H₂S to non-toxic sulphate (SO₄²⁻). (b) Inadequate internal aeration may result in H₂S intrusion, enhancing the risk of seagrass mortality, owing to chemical suffocation. Transversal sections (blue) visualize the extensive internal lacunar system (i.e. aerenchyma) of seagrasses. Black shadow indicates that O₂ is present. Redrawn from Pedersen et al. (1998) with permission from Ole Pedersen (University of Copenhagen, Denmark).

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Figure 1.1. Schematic drawing of the applied split flow-chamber (top view) visualising the position of the examined seagrass specimen, with leaves in the "water" compartment and roots/rhizome in the "sediment" compartment (Detailed drawings are available in the supplementary information).

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- **Figure S1.3.** Schematic drawing of the split flow-chamber (side view). Illustrating the inlet wall at the side of the chamber and the division of the chambers.
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- **Figure 2.2.** The dynamics of the below-ground chemical microenvironment of *Zostera muelleri* under experimentally changed environmental conditions as mapped with microelectrodes, illustrating the rhizome region including basal meristems with leaf sheath: (a) shoot 1; (b) shoot 2. The x- and y-axis are organized spatially, thus reflecting the actual orientation of the below-ground microsensor measurements. Y=0 indicate the below-ground tissue surface. Error bars are ±SD. n=2-4. The illustration of *Zostera muelleri*

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/)).

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Figure S2.1. The vertical distribution of $[O_2]$, $[H_2S]$ and pH values in the immediate rhizosphere of *Zostera muelleri* (Plant 1) as compared to in the reduced artificial sediment

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Figure S2.2. The vertical distribution of $[O_2]$, $[H_2S]$ and pH values in the immediate rhizosphere of *Zostera muelleri* (Plant 2) as compared to in the reduced artificial sediment elucidating the effect of the experimentally changed environmental conditions as well as differences between plant-vegetated and non-vegetated areas. Grey lines represent profiles in the bulk artificial sediment; Black lines represent profiles in the immediate rhizosphere. Vertical microprofiles in the immediate rhizosphere were performed at the basal meristem. Y=0 indicate the surface of the nitrogen bubbled seawater (oxygen sink). The artificial sediment surface is at ~10 mm depth; the below-ground tissue at ~25 mm depth. Error bars are \pm SD. n=2. Notice as fewer measurements were performed on the roots-system of plant 2 the total culture time in the artificial sediment of this plant decreased. Hence, plant 2 had less time to modify the biogeochemical condition in the immediate rhizosphere.

Figure S2.3. Conceptual diagram roughly illustrating the approximate position of the microprofile measurements (black dots), as well as the in this study defined zones of interest within the artificial sediment (area enclosed by dotted lines). Note that the chemical microprofiles of the below-ground tissue, describing the dynamics of the chemical microenvironment of *Z. muelleri* (abbreviated microprofiles in the figure), covers both the oxic microzone and the immediate rhizosphere (solid black line). IR represents the profiles measured within the immediate rhizosphere of *Z. muelleri* (i.e. the plant-vegetated area) as compared to the bulk artificial sediment (Bulk). Reference is the approximate position of the basal meristem H₂S concentration reference. Arrows indicate the respective distances from the below-ground tissue surface.

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Figure 3.1. A: Experimental setup. The below-ground tissue of the seagrass is embedded in the artificial sediment containing the O_2 sensitive nanoparticles. A SLR camera and LED are mounted perpendicular to the transparent chamber wall. Gas supply and reference optode are immersed in the overlaying water. B: Calibration curve of the sensor nanoparticles in the artificial sediment. Symbols and error bars represent means \pm SD (n=3). The red curve shows a fit of an exponential decay function to the calibration data ($R^2 > 0.999$).

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Figure 3.4. A: False color image of the O_2 concentration around the seagrass roots taken after 90 min illumination of the leaves at 500 μ mol photons m⁻² s⁻¹. Oxygen dynamics pictures visualizing the change in oxygenation between the time points 0 min (light) and 135 min (anoxic water) (B) and between the time points 135 min (anoxic water) and 405 min (airsaturated water) (C). D-E: time profile of the 6 ROIs. F: line profile across some small roots at the time points 0, 120 and 240 min.

Figure S3.1. Visualization of the calibration process. Acquired images were split into red, green, and blue channels and analyzed using the freely available software ImageJ (http://rsbweb.nih.gov/ij/). In order to obtain O₂ concentration images the following steps were performed: First the red channel (oxygen sensitive emission of PtTFPP) and green channel (emission of the reference dye MY) images were divided using the ImageJ plugin Ratio Plus (http://rsb.info.nih.gov/ij/plugins/ratio-plus.html). This gave pictures as shown in the top left. For the calibration the obtained pictures were correlated to the measured O₂ levels in the water column. There different regions were measured and used to generate the calibration plot. This calibration cure could then be used to transfer a ratio image to an O₂ image (top right).

Figure S3.2. Oxygen pictures (scale in % air saturation) in focus and out of focus. It can be seen that in the focal plane of the rhizome the greatest level of detail can be obtained. Out of focus the picture gets blurry and only parts of the structures can be visualized. For planar optrodes this can be a resolution limiting factor as close contact of the rhizome to the optrode is needed.

Figure 4.1. Schematic diagram of the experimental setup, showing the custom-made aquarium equipped with the narrow split flow-chamber and the ratiometric bio-imaging camera system (a). Image of the below-ground plant tissue structure during O₂ measurements (b). Image visualising the below-ground plant tissue structure during pH measurements (c). Note that the difference in brightness seen on the structural images (b, c) is due to the specific long pass filters used for luminescence imaging.

Figure 4.2. Vertical O_2 concentration microprofiles measured towards the leaf tissue surface of *Z. marina* during light-dark transitions (incident irradiance (PAR) of 500 μ mol photons m⁻² s⁻¹) at the two experimental temperatures (~16 and 24 °C). Y = 0 indicate the leaf tissue surface. Symbols with error bars represent the mean ±SD. n = 3; leaf level replicates. **147**

Figure 4.3. O_2 distribution and microdynamics within the rhizosphere of *Zostera marina* L. determined via optical nanoparticle-based O_2 sensors (O_2 colour coded image). The steady-state O_2 images were obtained at two different temperatures (16 and 24 °C) during light-dark transitions (photon irradiance (PAR) of 500 µmol photons m⁻² s⁻¹). Legends depict the

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Figure 4.6. Selected regions of interest (ROI) within the immediate rhizosphere of *Zostera marina* L. used to determine the pH heterogeneity and dynamics during light-dark transitions (incident irradiance (PAR) of 500 μ mol photons m⁻² s⁻¹) at the two experimental temperatures (~16 and 24 °C). Boxes and numbers indicate the measured ROI. Mean pH values representing the entire ROI are presented in Table 4.2. Note that the white areas on leaves/prophyllums (marked with black arrows on the figure) should be interpreted with caution as some of these high pH microniches (pH of \geq 9) seemed to be caused by epiphyte-derived red background luminescence (Notes S4.1; Figure S4.6).

Figure 4.7. Cross tissue line sections (CTS) determining the pH microdynamics at the plant/rhizosphere interface and on the plant tissue surface. The steady-state cross tissue line sections were determined at the two experimental temperatures (i.e. $^{\sim}16$ and 24 $^{\circ}$ C) during light-dark transitions (under an incident photon irradiance (PAR) of 500 μ mol photons m⁻² s⁻¹). (a) Structural image of the seagrass *Z. marina* L. embedded in the artificial,

transparent sediment with pH sensitive nanoparticles (pH colour coded image), illustrating the positions of the respective cross tissue line sections (CTS1-5). (b) Line microprofile across internode 3 with attached prophyllum (CTS1). (c) Line microprofile across internode 4 with prophyllum close to nodium 4 (CTS2). (d) Line microprofile across root from root-bundle 6 (CTS3). (e) Line microprofile across internode 7 with propyllum at the base of the prophyllum (CTS4). (f) Line microprofile across nodium 9 at the end of the rhizome with degraded prophyllum (CTS5). n = 3. Note that the white areas on leaves/prophyllums (marked with black arrows on the figure) should be interpreted with caution, as some of these high pH microniches (pH of \geq 9) seemed to be caused by epiphyte-derived red background luminescence (Notes S4.1; Figure S4.6).

Figure 4.8. Vertical pH microprofiles (VM) illustrating the pH heterogeneity and microdynamics in the rhizosphere of Z. marina L. The vertical pH microprofiles were determined at steady-state conditions during light-dark transitions (photon irradiance (PAR) of 500 μmol photons m⁻² s⁻¹) at ~16 and 24 °C. (a) Structural image of the *Z. marina* L. plant illustrating the spatial positions of the vertical pH microprofiles (colour coded image). (b) Vertical pH microprofile from the water/sediment interface across the first prophyllum and the basal meristem with leaf sheath to the bottom of the artificial sediment (VM1). (c) Vertical pH microprofile from the water/sediment interface across the base of the fifth prophyllum and the rhizome (internode 7) to the bottom of the artificial sediment (VM2). (d) Vertical pH microprofile from the water/sediment interface across the root-shoot junction at nodium 8 to the bottom of the artificial sediment (VM3). Y-axis = 0 indicate the artificial sediment surface. The approximate position of the below-ground tissue is indicated on the graphs by means of colour coded boxes (i.e. P = Prophyllum (blue), BM = Basal meristem with leaf sheath (green), R = Roots (brown); IN7P = Internode 7 at the base of the prophyllum (green); N = Nodium 8 (green)). n = 3. Note that the white areas on leaves/prophyllums (marked with black arrows on the figure) should be interpreted with caution, as some of these high pH microniches (pH of ≥9) seemed to be caused by epiphytederived red background luminescence (Notes S4.1; Figure S4.6). *156*

Figure S4.1. Luminescence spectra of the optical pH nanosensors in alkaline (pH 10; green) and acidic (pH 3; orange) solutions, showing a marked drop in luminescence in the yellow-orange-red wavelength interval (~550-675 nm) combined with an increase in the violet-

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Figure S4.2. Calibration of pH nanosensor luminescence. Ratio images, i.e., the ratio of red and blue channels extracted from the recorded RGB image, were quantified in small transparent glass vials with pH nanoparticle-containing agar buffered to defined pH levels spanning pH 4-10.

Figure S4.3. Calibration curves for optical pH nanoparticle-based sensors at the two experimental temperatures 16 and 24 °C. Mean ratio values were fitted with a sigmoidal function ($r^2 = 0.99$ and 0.97, respectively). Error bars are \pm SD (n=3).

Figure S4.4. pH microprofiles measured in the bulk, artificial sediment containing pH sensitive nanoparticles with a pH microelectrode (red symbols; mean \pm SD; n=3) and with the optical nanoparticle-based sensors (black line). Y = 0 indicates the artificial sediment surface.

Figure S4.5. Calibration curves of optical O_2 nanoparticle-based sensors measured at the two experimental temperatures (16°C and 24°C). Mean ratio values were fitted with an exponential decay function ($r^2 = 0.99$ for both curves). Legend depicts the different temperatures. Error bars are \pm SD. n=3.

Figure S4.6. Visualization of potential artefacts in the obtained pH images (images are from the 16°C treatment). The blue and red channel images are obtained by splitting the original RGB picture into its respective colour channels. The blue channel image (A) appears quite homogeneous in terms of intensity, while the red channel image (B) shows several high intensity regions. When merging the two channels (C) it can be seen that most of the picture appears in a homogeneous pink colour, while the hotspots in the red picture remain. This subsequently leads to very high apparent pH values at those spots as the ratio of red and blue channel leads to the final pH image (D). In contrast to other regions (e.g. low pH hotspot at the rhizome; A) those spots do not change over time and in response to the altered light levels and/or temperature. An additional artefact is presented by the region on top of the artificial sediment (e.g. square in the pH image; D). In this region the measured

intensities are not due to the optical nanoparticle based sensors and only represent noise such as scattered light, wherefore this region has been excluded.

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Figure 5.1. Microbial diversity in the rhizosphere of the seagrass *Zostera muelleri* determined via 16S rRNA amplicon sequencing. The phylogenetic tree denotes the spatial separation of the microbial consortia as determined via beta diversity analysis by Jackknife comparison of the weighted sequences data. The heat-map shows the abundance of the respective bacterial class/genus within the selected regions of interest, where (o) and (f) denote order and family classification, respectively. The heat-map includes taxonomic groups within each sample that represent >1% of the total sequences, which cumulatively represents >85% of the total sequenced data. Diagrams (in %) show the mean relative abundance of designated bacterial classes present within the selected regions of interest of the artificial sediment matrix. All data originate from reduced, artificial sediment with added native pore water microbes (described in the Supplementary Materials and Methods; Notes S5.1). n = 2-3.

Figure 5.2. The below-ground chemical microenvironment at the basal leaf meristem, i.e., the meristematic region of the rhizome of the seagrass *Zostera muelleri*. (a) and (b) represent microsensor measurements in an artificial sediment matrix with added pore water microbes. (c) and (d) represent microsensor measurements in a sterilized environment, i.e., sterilized artificial sediment matrix and below-ground tissue surface. (a) and (c) show measurements in darkness. (b) and (d) show measurements in light (photon irradiance of ~150 μ mol photons m⁻² s⁻¹). Black line and symbols show the O₂ concentration; Red line and symbols show the H₂S concentration; Blue line and symbols show pH. The dotted lines indicate the thickness of the plant-derived oxic microzone, and X = 0 indicates the surface of the basal leaf meristem. Symbols with error bars represent means ± S.D (n = 3-4 technical replicates; biological replication of the below-ground chemical microenvironment dynamics is shown in the Supplementary Results; Fig. S5.1 and S5.2). 189

Figure S5.1. Chemical microenvironment at the interface between the surface of the meristematic region of the rhizome and the immediate rhizosphere. Biological replication #2.

- **Figure S5.2.** Chemical microenvironment at the interface between the surface of the meristematic region of the rhizome and the immediate rhizosphere. Biological replication #3.
- **Figure S5.3.** Principal component analysis (PCA) of the bacterial community composition within the seagrass rhizosphere and the bulk sediment. RAM = root apical meristem area; BLM = basal leaf meristem area; BS = bulk sediment. This PCA explained more than 75% of the variances of our samples.
- **Figure S5.4.** Spatial distribution of rhizosphere microbes around the root apical meristem (RAM) of the seagrass *Zostera muelleri* as determined via epifluorescence microscopy of DAPI-stained bacteria.
- **Figure S5.5.** Conceptual diagram visualizing sampling areas within the reduced, artificial sediment.
- Figure 6.1. Schematic diagram of the experimental setups. (a) Above-ground light and O_2 microsensor measurements. (b) Measurements on the below-ground chemical microenvironment with Clark-type O_2 microsensors. (c) Measuring light transmission spectra at the seagrass leaf surface.
- **Figure 6.2.** Profiles of photon scalar irradiance measured at two different downwelling photon irradiances (50- and 200 μ mol photons m⁻² s⁻¹) on *Z. marina* leaves with- and without epiphyte cover. Left panels show the scalar irradiance 0-10 mm from the leaf surface measured in 1 mm steps. Right panels show the scalar irradiance 0-1 mm from the leaf surface measured in 0.1 mm steps (enlarged plots of the scalar irradiance showed in the left panels). Data points represents means \pm S.D. n=3; leaf level replicates.
- **Figure 6.3.** Spectral scalar irradiance measured over *Z. marina* leaves under an incident irradiance of 50 and 200 μ mol photons m⁻² s⁻¹ with- (right panels) and without epiphytes (left panels). Coloured lines represents spectra collected at the given depths in mm above the leaf surface expressed as % of incident irradiance on a log-scale. n=3; leaf level replicates.

Figure 6.4. Spectra of photon scalar irradiance transmitted through *Z. marina* leaves with-(red line) and without (black line) epiphyte cover and at two different downwelling irradiances (50- and 200 μ mol photons m⁻² s⁻¹). Dashed lines represents \pm S.D. n=4; leaf level replicates.

Figure 6.5. Vertical microprofiles of the O_2 concentration measured towards the leaf surface under 4 different incident irradiances (0, 50, 100 and 200 μ mol photons m⁻² s⁻¹). Red symbols and lines represent leaves with 21% epiphyte-cover, Black symbols and lines represent leaves without epiphyte-cover. y = 0 indicates the leaf surface. Symbols and errors bars represent means \pm SD. n = 3-4; leaf level replicates.

Figure 6.6. Net photosynthesis rates as a function of downwelling photon irradiance. Rates were calculated for the 4 different incident irradiances (0, 50, 100 and 200 μ mol photons m² s⁻¹) and were fitted with a hyperbolic tangent function (Webb *et al.*, 1974) with an added term to account for respiration (Spilling *et al.*, 2010) (R² = 0.99). Red symbols and line represent leaves with ~21% epiphyte-cover. Black symbols and line represent leaves without epiphyte-cover. Error bars are \pm SD. n = 3-4; leaf level replicates.

Figure 6.7. Radial O_2 loss from the root-cap of *Z. marina* (~1 mm from the root-apex) to the immediate rhizosphere measured at two different irradiances (0 and 200 μ mol photons m⁻² s⁻¹). Left panel show radial O_2 loss from seagrass with leaf epiphyte-cover, right panel show radial O_2 loss from seagrass without leaf epiphyte-cover. X = 0 indicates the root surface. Error bars are ±SD. n = 3-5; root level replicates.

Figure 7.1. *In situ* distribution of phytotoxic sulfide during light (photon irradiance of 500 μmol photons m⁻² s⁻¹) and dark conditions in a sediment colonised by the tropical seagrass species *Cymodocea rotundata*, *Cymodocea serrulata*, *Halophila ovalis*, *Halodule uninervis*, *Syringodium isoetifolium* and *Thalassia hemprichii* as determined with sulfide sensitive Agl DGT probes (a). The width of all deployed DGT gels was 18 mm (b). Distribution of sulfide concentrations in the rhizosphere of *Cymodocea serrulata* during light and dark conditions (c). All images are color coded, where the color scale depicts the sediment sulfide concentration.

Figure 7.2. (a) Rhizospheric pH heterogeneity and phosphorus distributions in carbonaterich sediment with the tropical seagrass *Cymodocea serrulata* during light (photon irradiance of 500 μmol photons m⁻² s⁻¹) and dark conditions. The enlarged plot focusses on the basal leaf meristem area, i.e., the meristematic region of the rhizome. (b) Rhizospheric pH and phosphate concentrations during light and dark conditions as obtained from the extracted cross tissue line profiles shown in (a). All images are color coded, where the color scales depict the sediment pH and phosphate concentrations.

Figure 7.3. Co-distributions of seagrass-mediated rhizospheric phosphorus and Fe(II) solubilisation coupled to the plant-generated pH microheterogeneity at the root/sediment interface during light (photon irradiance of 500 μmol photons m⁻² s⁻¹) and dark conditions in carbonate-rich marine sediment inhabited by the tropical seagrass Cymodocea serrulata. Panel (a) show the rhizospheric pH, Fe(II) and phosphorus concentrations within the selected region of interest, as shown on the provided illustration of the below-ground plant tissue structure (a; Extended Data Fig. 7.3). Panel (b) represent the line profiles (P1-4) as indicated on the two-dimensional chemical images (a), showing the cross tissue Fe(II) and phosphorus concentrations during light and dark conditions. All images are color coded, where the color scales depict the sediment pH, Fe(II) and phosphorus concentrations, respectively (a). The red arrow on the phosphorus scale bar indicates the detection limit for the applied phosphorus sensitive multi-ion gel (Zr-oxide - SPR-IDA) probe (a). Note the different scales on the y-axes in panel (b). Panel (c) shows a conceptual diagram of the seagrass-derived rhizospheric phosphorus and iron mobilization mechanisms in carbonaterich sediments. *256*

Figure ED7.1. Distribution and dynamics of O_2 concentration within the rhizosphere of the tropical seagrass *Cymodocea serrulata*. Seagrasses were exposed to dark and light conditions (incident photon irradiance of ~500 μ mol photons m⁻² s⁻¹). Arrows indicate seagrass-derived oxic microzones. The color bar depicts the O_2 concentration in % air saturation. The seagrasses were transplanted into sieved (<1mm sediment fraction) natural sediment from the sampling site to exclude any larger animals and bivalves, as well as to ensure natural ratios of essential nutrients and rhizosphere microbes.

Figure ED7.2. pH heterogeneity and dynamics within the seagrass rhizosphere of two specimens of the tropical seagrass *Cymodocea serrulata* during dark and light conditions (incident photon irradiance of \sim 500 μ mol photons m⁻² s⁻¹). The color coding depicts the pH value. The seagrasses were transplanted into sieved (<1mm sediment fraction) natural sediment from the sampling site to exclude larger animals and bivalves, as well as to ensure natural ratios of essential nutrients, buffering salts and microbes, respectively.

Figure ED7.3. Rhizospheric Fe(II), phosphorus and pH conditions during dark and light conditions (incident photon irradiance of ~500 μmol photons m⁻² s⁻¹) (a,c). Data is shown from the tropical seagrass species *Cymodocea serrulata*. Images are colour coded. Legends depict the analyte concentration (a). The red arrow on the phosphate calibration bar denotes the method detection limit (MDL) of the LA-ICPMS measurement (a). No such arrow is shown for Fe as the MDL was negligibly small in this case. Marked areas depict the selected regions of interest (b), as shown on the chemical images (panel a; and on figure 7.3 in the main text). Note the different scale on the y-axis.

Figure ED7.4. Distribution and dynamics of Ca concentration within the rhizosphere of the tropical seagrass *Cymodocea serrulata*. Seagrasses were exposed to dark and light conditions (incident photon irradiance of \sim 500 µmol photons m⁻² s⁻¹). The color bar depicts the relative Ca concentration. The seagrasses were transplanted into sieved (<1mm sediment fraction) natural sediment from the sampling site to exclude any larger animals and bivalves, as well as to ensure natural ratios of essential nutrients and rhizosphere microbes.

Figure S7.1. Schematic drawing of the custom-made, narrow experimental chambers positioned within the 20 L seawater reservoirs. Note the position of the optode and DGT gels on opposite sides of the investigated roots. During measurements we carefully ensured good contact between the below-ground biomass and the optode or the DGT gels, respectively.

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Figure S7.3. (A) Chemical structures of the indicators and references dyes used in the O_2 and pH optodes, respectively. (B) Images of an O_2 and pH optode positioned next to each other and exposed to different analyte concentrations (i.e. O_2 and pH levels). The images were obtained with a SLR camera (EOS 1000D, Canon, Japan) and the optodes were excited using a hand-held UV lamp. In this setup, the O_2 sensor had no additional optical isolation layer.

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Figure S7.4. Calibration plots of the O_2 and pH optodes used in the study. All data points with erro bars represent mean values with the corresponding standard deviation (n=3-6). For the O_2 optode a single exponential decay function was fitted (dashed line; $R^2 > 0.98$) and this fit was used for calibrating the experimental O_2 images. The pH optode response was fitted using a sigmoidal function (dashed line; $R^2 > 0.98$). For practical reasons (i.e. the applied software ImageJ does not support this type of fit) a linear fit in the range pK_a±1 was used. The used linear fit is depicted as the black line in the calibration plot above (pH range 7-9). Within the chosen pH range this type of linear fit describes the sensor response to changing pH values very well ($R^2 > 0.98$), without notable experimental errors.

Figure S7.5. Calibration plot of the sulfide binding AgI gel used in this study. All data points represent mean values \pm S.D. (n=3-6) and were fitted using the following function: y=b*ln(x-a); (R² > 0.99).

Figure S7.6. Calibration plot of the PO_4^{3-} binding precipitated Zr-oxide gel used in this study. The curve shows a calibration of gels made in Denmark and shipped to Australia (Calibration 1) and one of gels made at the actual remote study site (Green Island, Cairns, Australia; Calibration 2). Data points with error bars represent mean values \pm S.D. (n=3-6) and were fitted using the following function: $y=y_0 + A*e^{R0*x}$; ($R^2 > 0.98$).

Figure 8.1. Vertical O_2 concentration profiles measured towards the leaf surface under incident photon irradiances of 0, 75, 200 and 500 μ mol photons m⁻² s⁻¹. Red symbols and

lines represent leaves with silt/clay-cover; black symbols and lines represent control plants, i.e., leaves without silt/clay-cover. Upper panels are measurements in water with a reduced O_2 level of ~40% of air equilibrium (mimicking night-time water-column O_2 conditions, approximately 8.2 kPa); Lower panels are measurements in water at 100% air equilibrium (mimicking day-time water-column O_2 conditions, 20.6 kPa). y = 0 indicates the leaf surface. Symbols and error bars represent means \pm SE; n = 3-4.

Figure 8.2. Vertical depth profiles of the O_2 concentration measured towards the leaf surface of plants with a microbially active silt/clay-cover (red symbols and lines), with an inactivated silt/clay-cover (obtained by pre-heating the added silt/clay to 120°C in an oven for 2 h; blue symbols and lines), and without silt/clay-cover (control plants; black symbols and lines). All measurements were performed in darkness. y = 0 indicates the leaf surface. Symbols and error bars represent means \pm SE; n = 4.

Figure 8.3. Apparent net photosynthesis rates as a function of downwelling photon irradiance (PAR, 400-700 nm) of plants with leaf silt/clay-cover (red symbols and lines) and without leaf silt/clay-cover (control plants; black symbols and lines). Rates were calculated for incident photon irradiances of 0, 75, 200 and 500 μ mol photons m⁻² s⁻¹ and were fitted with an exponential function (Webb *et al.*, 1974) with an added term to account for respiration (Spilling et al. 2010) ($R^2_{40\%AS,control}$ =0.93; $R^2_{40\%AS,silt-cover}$ =0.98; $R^2_{100\%AS,control}$ =0.99; $R^2_{100\%AS,silt-cover}$ =0.99). The upper panel represents measurements in water kept at 40% air equilibrium, while the lower panel represents measurements in water kept at 100% air equilibrium. Error bars are ± SE; n = 3-4.

Figure 8.4. In situ measurements of diel changes in the O_2 concentration and temperature of the water-column (A, B), the light availability at leaf canopy height (A, B), and of the O_2 partial pressure and H_2S concentration in the meristematic tissue of Zostera muelleri plants with and without leaf silt/clay-cover, respectively (C, D) from Narrabeen Lagoon, NSW, Australia. The O_2 and H_2S microsensors were inserted into the shoot base close to the basal leaf meristem, which was buried \sim 2 cm into the sediment. The horizontal, dashed line in panels A and B corresponds to 100% atmospheric O_2 partial pressure. Legends depict the physical/chemical water-column parameters (A, B) and the chemical species (C, D). Panels A and C are from measuring day #1 (representing a sunny day), while panels B and D are from

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Figure 8.5. In situ intra-plant O_2 status as a function of the O_2 partial pressure in the surrounding water-column during night-time. The data were extracted from Figure 4 approximately 2h after sunset. The grey lines represent a linear regression and are extrapolated to interception with the horizontal x-axis, to provide an estimate of the water-column O_2 level where the meristematic tissue at the shoot base becomes anoxic $(R^2_{control,day\#1} = 0.97; R^2_{control,day\#2} = 0.70; R^2_{silt-cover,day\#1} = 0.97; R^2_{silt-cover,day\#2} = 0.94)$. Upper panels (A, B) are measurements from control plants (black symbols), while lower panels (C, D) are measurements from plants with a silt/clay-cover on the leaves (red symbols).

Figure 8.6. *In situ* intra-plant O_2 status as a function of incident photon irradiance (PAR) during daytime. The data were extracted from Figure 8.4 at sunrise (measuring day #1). The intra-plant O_2 evolution during the light-limiting phase of PAR were fitted with an exponential model (Grey lines; Webb *et al.*, 1974) ($R^2_{control} = 0.95$, $\alpha_{control} = 0.149$; $R^2_{silt-cover} = 0.95$, $\alpha_{silt-cover} = 0.098$). Upper panel (A) shows measurements from control plants (Black symbols), while the lower panel (B) shows measurements from plants with a silt/clay-cover on the leaves (red symbols).

Figure S8.1. Depth microprofiles of O_2 concentration across the water/sediment interface. Y = 0 indicate the sediment surface. All microsensor measurements were performed in darkness. The investigated marine sediment originated from Narrabeen Lagoon, NSW, Australia. Symbols and error bars are mean \pm SEM. n = 4.

Figure S8.2. Net photosynthesis rates of the three investigated *Zostera muelleri* spp. *capricorni* plants as a function of incident photon irradiance. Black symbols and lines represent measurements on control plants; red symbols and lines represent measurements on plants with fine sediment particles (i.e. leaf silt/clay-cover). Left panels are measurements at 40% air saturation in the water-column (mimicking water-column O₂ conditions during darkness and at sunrise). Right panels are measurements in a 100% air saturated water-column (mimicking water-column O₂ conditions at mid-day). The O₂ fluxes are fitted with a saturated exponential function (Webb *et al.*, 1974) amended with a term, R, to account for the respiration (Spilling *et al.*, 2010).

- **Figure 9.1.** 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).
- **Figure 9.2.** (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 = $100 \, \mu m$. LS = indicate the leaf sheath; A = aerenchyma; RD = initial root development. Data modified from Brodersen et al. (2015b). Copyright 2015 John Wiley & Sons Ltd.
- **Figure 9.3.** 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 3^{rd} and the 4^{th} 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).
- **Figure 9.4.** 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 measured at the canopy surface is shown in orange colour. Data modified from Sand-Jensen et al. (2005).
- **Figure 9.5.** Water-column pO_2 versus shoot base pO_2 during night-time of 3 replicate plants of *Zostera marina*. The data are extracted from Figure 9.4 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).
- **Figure 9.6.** Irradiance versus shoot base pO_2 during day-time of 3 replicate plants of *Zostera marina*. The data are extracted from Figure 9.4 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).

Figure 9.7. 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).

Figure 9.8. 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).

Figure 9.9. (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.

Figure 9.10. 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) μ mol 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 O₂ concentration, intermediate panels (vertically) represents the immediate rhizosphere H₂S 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 H₂S 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 for Environmental of Maryland Center Science (ian.umces.edu/imagelibrary/)). Data modified from Brodersen et al. (2015b). Copyright 2015 John Wiley & Sons Ltd. 347

Figure 9.11. 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 \pm SD. n = 3. Data modified from Brodersen et al. (2015b). Copyright 2015 John Wiley & Sons Ltd.

Figure 9.12. 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.

Figure 9.13. 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°C 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.

Figure 9.14. Conceptual diagram visualising seagrass-derived sediment detoxification. (a) O₂ 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 O₂ 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 H₂S and shifting the geochemical sulphide speciation towards non-tissue-permeable HS⁻ ions, respectively. (b) Oxic microshield protecting the vulnerable basal leaf meristem. O₂ released from the below-ground tissue drives chemical re-oxidation of sediment-produced H₂S within the oxic microniches. (c) Inadequate internal aeration may lead to H₂S 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. 355

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Table 2.1. Radial oxygen release (O_2 flux), oxygen concentration at the below-ground tissue surface, H_2S consumption within the oxic microshield, total sulphide concentration at the tissue surface (S) and at the oxic microzone-reduced sediment interface (I), and ΔpH through the oxic microshield. Calculated for each of the four different treatments: dark, 260 μmol photons m^{-2} s⁻¹, 350 μmol photons m^{-2} s⁻¹ and hypoxia, at the: Basal meristem with leaf sheath (BM); Node (N); Internode (IN); mature zone of roots (RM); and apical meristem region of roots (RA). Mean values ± SE; except internode and root values which are given as mean ±SD; and the total sulphide concentration which is given as a mean (n = 3-15). As flux rates are calculated from mean values only SE are provided. (-) Indicates zero flux rate. Additional statistical information confirming the resemblance between the examined plants is provided in the supporting information (Figure S2.5).

Table 2.2. Maximum and effective quantum yields of PSII measured at the center of the leaf in the middle of the leaf canopy of both vertical shoots. The after hypoxia measurements were conducted after a 24h (12:12h) light-dark cycle. Leafs were exposed to a 100% air saturated water column during the 24h recovery time. Means \pm SD (n = 2-4).

Table 3.1. Maximum (F_v/F_m) and effective (Y(II)) quantum yields of PSII-related photosynthestic electron transport in seagrass leaves of plants mounted in the experimental setup with artificial sediment + O_2 nanoparticles (mean \pm SD; n = 4-6). (-) indicates no photosynthetic activity.

Table 4.1. O_2 concentrations at selected regions of interest (ROI) within the immediate rhizosphere of *Zostera marina* L. Boxes and numbers indicate the measured ROI. O_2 concentrations are given in both % air saturation and μ mol L^{-1} at ~16 and 24 °C during light-dark transitions.

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Table S5.1. Photosynthetic capability and below-ground tissue oxidation capacity based on PAM measurements and above- to below-ground biomass ratio, respectively, of all investigated *Zostera muelleri* specimens.

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Table 6.1. O_2 fluxes across the leaf surface and radial O_2 loss from the root-cap (~1 mm from the root-apex). (-) indicate no data points. Negative values denote net O_2 uptake. Rates are mean±S.D. n=3-5; leaf/root level replicates. ^{a,b}indicates significant difference between seagrasses with leaf epiphyte cover as compared to seagrasses without leaf epiphyte cover (control plants) (^a Two-way ANOVA, $F_{3,3}$ (PAR) = 2931.2, $F_{1,3}$ (epiphytes) = 3555.1, p < 0.01; ^b Mann-Whitney test, p < 0.05).

Table S6.1. Two-way ANOVA for O_2 evolution (nmol O_2 cm⁻² h⁻¹). TMT = Experimental treatments, i.e., with or without leaf epiphytes; PAR = incident irradiance (0, 50, 100 and 200 μ mol photons m⁻² s⁻¹).

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Table 8.3. Photosynthetic parameters derived from the light response curves in Fig. 8.3. Including photosynthetic activity, compensation irradiance, onset of photosynthesis saturation and respiration rates of investigated *Zostera muelleri* spp. *capricorni* plants withand without (i.e. control plants) fine sediment particles on leaves. All photosynthetic related parameters were determined at both 40 % of air equilibrium and in air equilibrium. n = 3.

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Table 9.1. Concentration and diffusion coefficients of O_2 , CO_2 and bicarbonate ion in air and air-saturated seawater (35 % salinity) at a temperature of 25°C (Larkum et al. 1989). **331**

Table 9.2. Gaseous composition of the lacunal system of several seagrasses (Larkum et al. 1989).

Summary

Seagrass meadows are important marine ecosystems providing an array of ecosystem services to aquatic and terrestrial environments including sediment stabilisation, acting as shelter, feeding and nursery grounds for numerous marine species and even mitigating climate change through their ability to capture and store carbon in the sediment for millennia. However, owing to anthropogenic interference, seagrass meadows worldwide are shrinking, putting essential ecosystem functions at risk. Understanding the basic mechanisms that control the fitness of seagrasses is necessary in order to elucidate how human activities and changing environmental conditions is affecting the seagrass ecosystems and what can be done to better manage them. Through a series of experiments employing high-resolution measuring techniques including luminescence imaging, microsensors and novel optical sensor nanoparticles, this thesis explores the mechanisms of seagrass sediment detoxification and nutrient mobilisation, and the effect of environmental stressors on these essential processes.

We show that radial O₂ loss from the below-ground tissue leads to formation of oxic microshields that re-oxidates phytotoxic H₂S in the rhizosphere and thus results in sediment detoxification; a vital seagrass-derived chemical defence mechanism that is adversely affected by water-column hypoxia. These seagrass-driven alterations of the rhizosphere biogeochemistry modulate the microbial community composition at the plant/sediment interface, potentially increasing the rhizospheric nitrogen availability owing to microbial-mediated nitrogen fixation. We also found that the leaf microenvironment largely controls the intra-plant O₂ conditions and thus the below-ground tissue oxidation capacity, where sediment deposition and epiphyte overgrowth on leaves negatively affects the internal plant aeration through multiple pathways, such as (i) enhancing the thickness of the mass transfer impeding diffusive boundary layer around the leaves, (ii) reducing the light availability/quality for photosynthesis, and (iii) enhancing the over-night respiration rates in the phyllosphere. Finally, we show that seagrass-driven alterations of the rhizosphere pH microenvironment leads to development of low-pH microniches around the below-ground tissue, corresponding to the seagrass-derived oxic microzones, that results in pronounced

rhizospheric phosphorus and iron mobilization for seagrasses colonizing phosphorouslimited carbonate-rich sediments.

The results of this thesis brings to light the overarching importance of internal tissue aeration in seagrasses through its effect on rhizospheric biogeochemical processes and conditions, and thus underlines the need for minimizing environmental stressors leading to inadequate internal aeration, such as water-column hypoxia and sediment re-suspension, for seagrass health in changing oceans.