Oxygen loss from seagrass roots coincides with colonization of sulphide oxidizing cable bacteria and reduces sulphide stress

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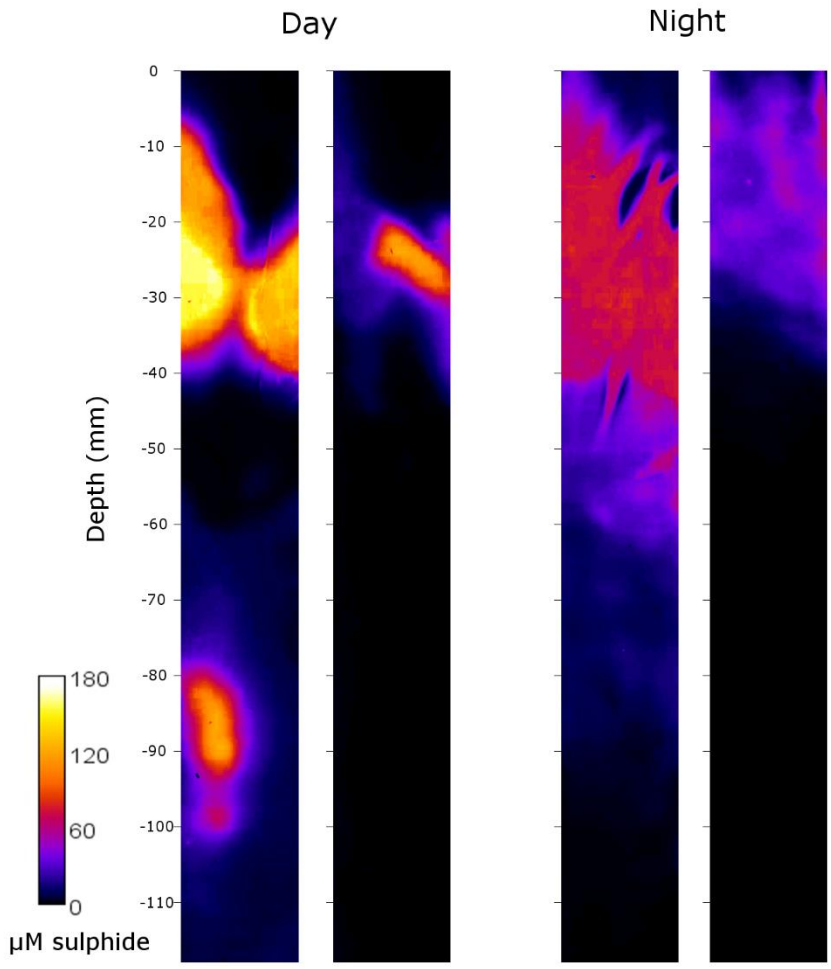

Fig. S1 | *In situ* distribution of sulphide in sediments colonised by both *Halophila ovalis* and *Zostera muelleri*. Time-integrated sulphide concentrations were measured using Diffusive Gradients in Thin Films (DGTs; DGT Research) that were deployed for 12 hours during the day and 12 hours during the night in the Swan River. The width of sulphide gels was 18 mm and total path of diffusion was 780 µm. Samplers were carefully pushed into the sediment while keeping the upper 30 mm of the probe window in the water column. 0 mm marks the sediment-water interface.

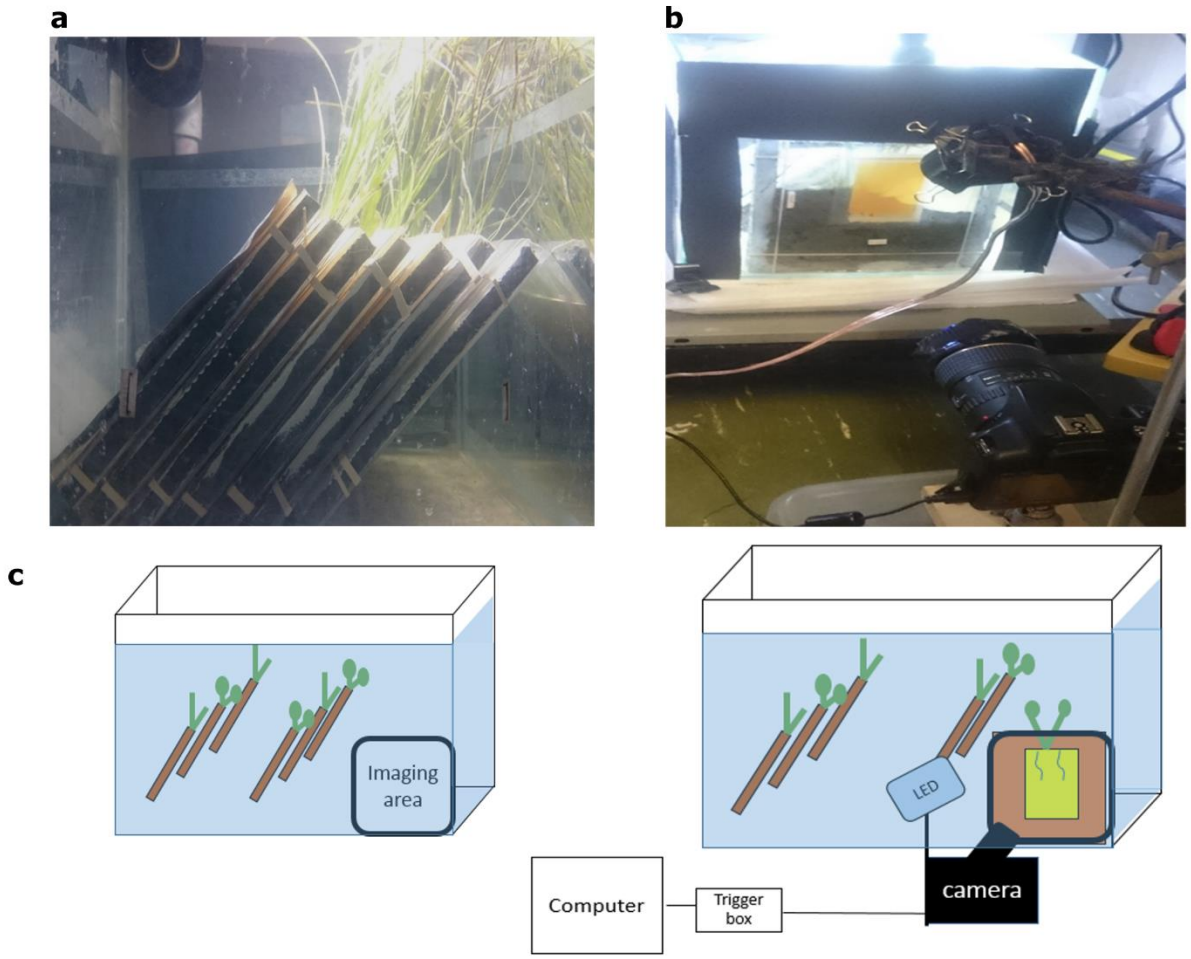

Fig. S2 | Rhizoboxes and optode imaging experimental set-up. a, photograph of rhizoboxes (20 cm x 20 cm x 2 cm) containing either *Zostera muelleri* or *Halophila ovalis* sieved sediment and fitted with an oxygen planar optode. **b**, photograph of imaging set-up for obtaining oxygen distribution in the rhizosphere. **c**, schematic drawing of oxygen optode imaging set-up, where 7 blue LEDs (λ-peak = 447.5 nm) equipped with a dichroic blue filter excited both dyes to create duel emission; red luminescence from the O_2 sensitive dye and green luminescence from the reference dye. Emission was captured using a modified digital SLR (Canon EOS 1000D) equipped with a macro lens (Sigma 50 mm F2.8) and a long-pass emission filter that were controlled via USB using the software 'Look@RGB'. Four rhizoboxes were also fitted with sulphide diffusive gradients in thin films (DGTs). These sulphide gels were mounted behind the optode sheet, covered with a 10 µm thick nucleopore membrane and deployed for one hour.

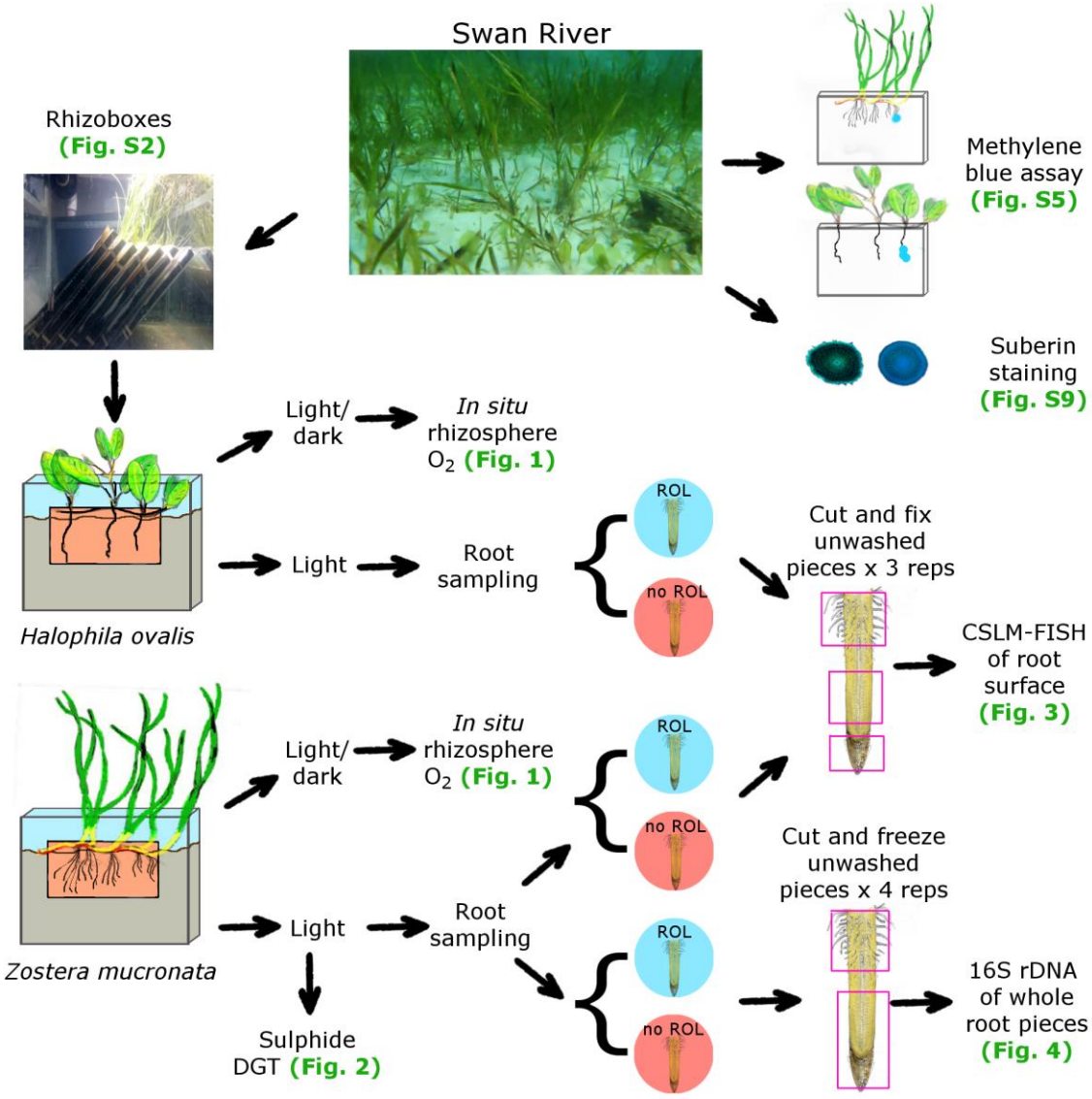

Fig. S3 | Methods summary. *Halophila ovalis*, *Zostera muelleri* and sediment were collected from the Swan River estuary. Plants were either grown in rhizoboxes, placed in methylene blue assay chambers or stained to visualised suberin content. Rhizoboxes were fitted with oxygen planar optodes to investigate *in situ* rhizosphere oxygen in both light and dark transitions. Four additional rhizoboxes were also fitted with sulphide diffusive gradients in thin films (DGTs). Both roots leaking oxygen and roots not leaking oxygen were sampled to investigate the influence of radial oxygen loss on the root microbial community using either confocal laser scanning microscopy – fluorescent in situ hybridisation (FISH-CLSM) on root surfaces, or 16S rRNA sequencing (for *Zostera muelleri* only).

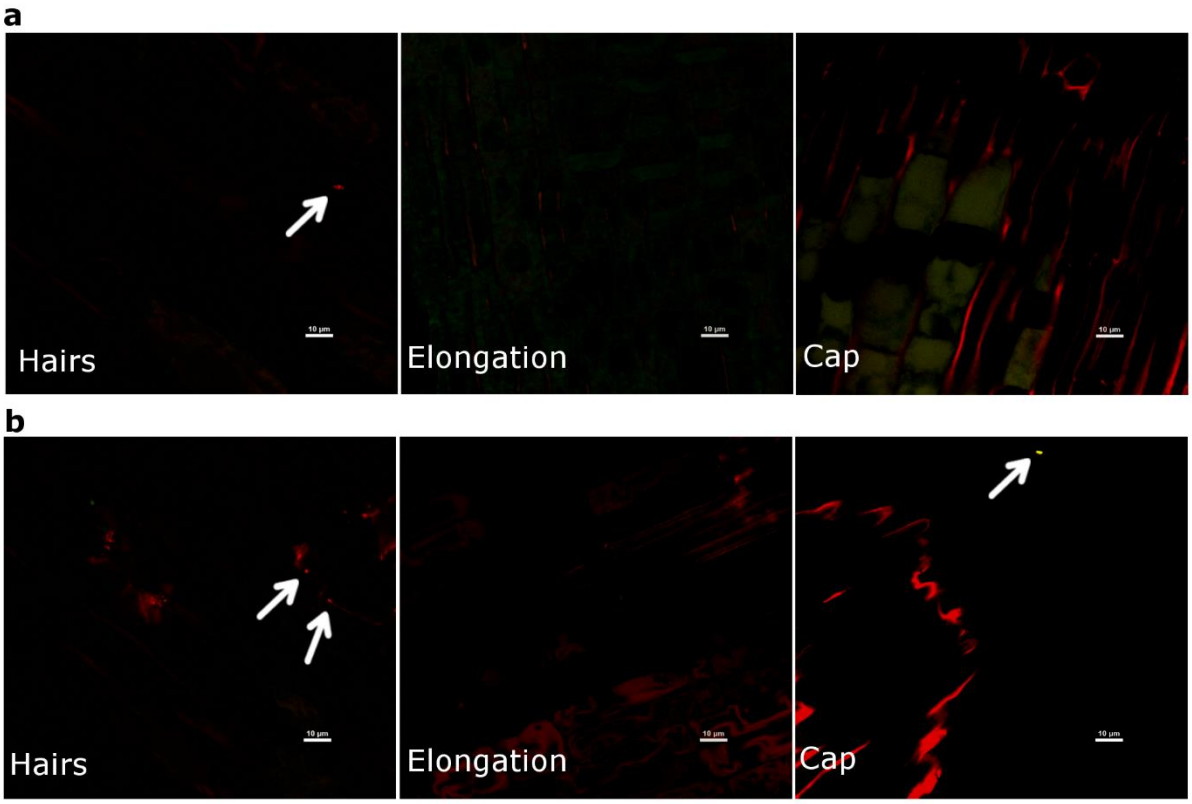

Fig. S4 | Confocal laser scanning microscopy (CLSM) micrographs of seagrass roots stained with negative control probes (NONEUB) for each dye combination (Cy5-Cy5, 6-Fam-6-Fam, Atto 565). a, *H. ovalis* root regions. **b**, *Z. muelleri* root regions. Auto-fluorescence in the red laser channel (640 nm) can be seen from the root cap of both species. Small non-specific binding the size of bacterial cells (indicated by arrows) were counted as 'cells' and deducted from sample counts. Scale bar is 10 µm.

Table S1. General 16S rRNA sequencing statistics of *Z. muelleri*. Sequence target was 341F (CCTAYGGGRBGCASCAG) - 806R (GGACTACNNGGGTATCTAAT) and read length was 300bpPE. A 'blank' sample of the reaction buffers was also sequenced and any sequences aligning to this sample were subtracted from all other samples.

Root region	**Root type**	**Rep**	**Reads pre-filtering**	**Reads post-filtering**	**# Family's**	**Sequences aligning to *Desulfobulbceae***	**Sequences aligning to *Desulfobulbus***
Elong.	Leaking	1	58966	358	33	10	1
		2	76574	598	38	10	1
		3	80370	9416	99	120	5
		4	109403	1095	40	16	12
	Non-Leaking	1	95060	12887	98	106	22
		2	113390	6400	62	42	10
		3	86390	26949	95	82	4
		4	93151	12090	99	123	15
Root hairs	Leaking	1	108905	38803	132	829	61
		2	101187	32265	133	1124	172
		3	105746	42290	129	954	34
		4	96781	35438	132	933	110
	Non-leaking	1	161152	68004	138	3875	613
		2	120440	51853	139	1961	535
		3	86177	35480	129	826	112
		4	112092	38034	136	1857	131
Sediment	Sediment	1	94724	36383	131	1551	101
		2	74032	26862	125	979	73
		3	106619	37969	124	1492	86
		4	88214	33715	118	1388	69
Blank		1	1167	88	4	0	0

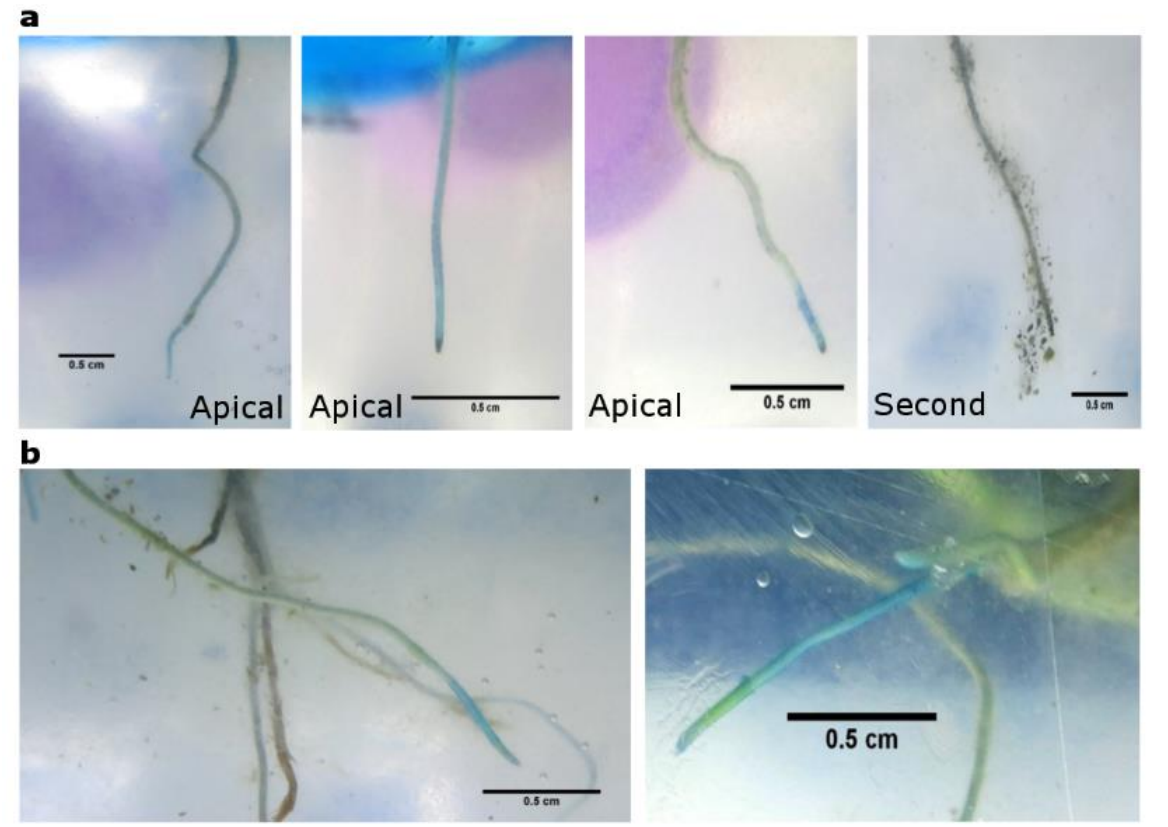

Fig. S5 | Roots of *Halophila ovalis* and *Zostera muelleri* transferred into an anoxic agar solution containing methylene blue. Methylene blue is colourless when reduced and blue when oxidised. **a**, oxygen loss in *H. ovalis* was limited to the apical root, and extended back to 0.25 – 1 cm from the tip. No ROL was observed from older roots of *H. ovalis*. **b**, oxygen loss in *Z. muelleri* was limited to 0.25 – 0.5 cm from the root tip. No ROL was observed from older roots of *Z. muelleri*.

For methylene blue staining, 0.1 % (w/v) agar was dissolved in seawater (30 psu). Once the solution had cooled, 13 mg L^{-1} methylene blue was added followed by 130 mg L^{-1} of the reducing agent $Na_2S_2O_4$ and the solution then gently shaken until colourless. This solution was then poured into clear acrylic containers that were sealed at the top by waterproof foam. *Zostera muelleri* and *Halophila ovalis* plants with intact roots (including apical roots) were selected from recently collected cores and the roots were then threaded through a small slit in the foam and sealed in at the junction with the rhizome using non-toxic silicone grease to prevent oxygenated water entering the container. These containers were then transferred into a larger aerated aquarium so that the shoots were submerged in the aquaria while roots remained in the anoxic methylene-blue solution. Plants were left for 12 hours under constant light (250 µmol photons m^{-2} s^{-1}) and constant temperature (24 °C) and roots monitored

continuously for any colour development. Any observed changes in colour were recorded using a Canon S110 camera.

Table S2. Oxygen loss from *Halophila ovalis* and *Zostera muelleri* roots during light and dark (30 minute darkness). Measurements are averages ± 1 SD taken from 9 replicate plants of each species for the light measurements (250 µmol photons m^{-2} s^{-1}) and four replicate plants of each species for the dark measurements. Total width of ROL was taken from the widest point and includes the diffusion of O_2 from both sides of the root.

Oxygen loss parameter	***Halophila ovalis* (light)**	***Halophila ovalis* (dark)**	***Zostera muelleri* (light)**	***Zostera muelleri*(dark)**
Maximum O_2 (µmol L^{-1})	59.0 ± 19.0	26.4 ± 1.0	59.0 ± 21.7	14.1 ± 20.1
Maximum O_2 (% air equilibrium)	22.6 ± 7.3	10.1 ± 0.4	22.5 ± 8.3	5.4 ± 7.7
Mean O_2 (µmol L^{-1})	27.7 ± 9.7	7.8 ± 2.1	30.5 ± 11.7	5.7 ± 7.8
Mean O_2 (% air equilibrium)	10.6 ± 3.7	3.0 ± 0.8	11.7 ± 4.5	2.2 ± 3.0
Total width of ROL (mm)	1.5 ± 0.7	1.0 ± 0.2	1.3 ± 0.8	0.4 ± 0.5
Length along root of ROL(mm)	10.6 ± 4.8	10.2 ± 5.3	4.2 ± 2.7	1.25 ± 1.8

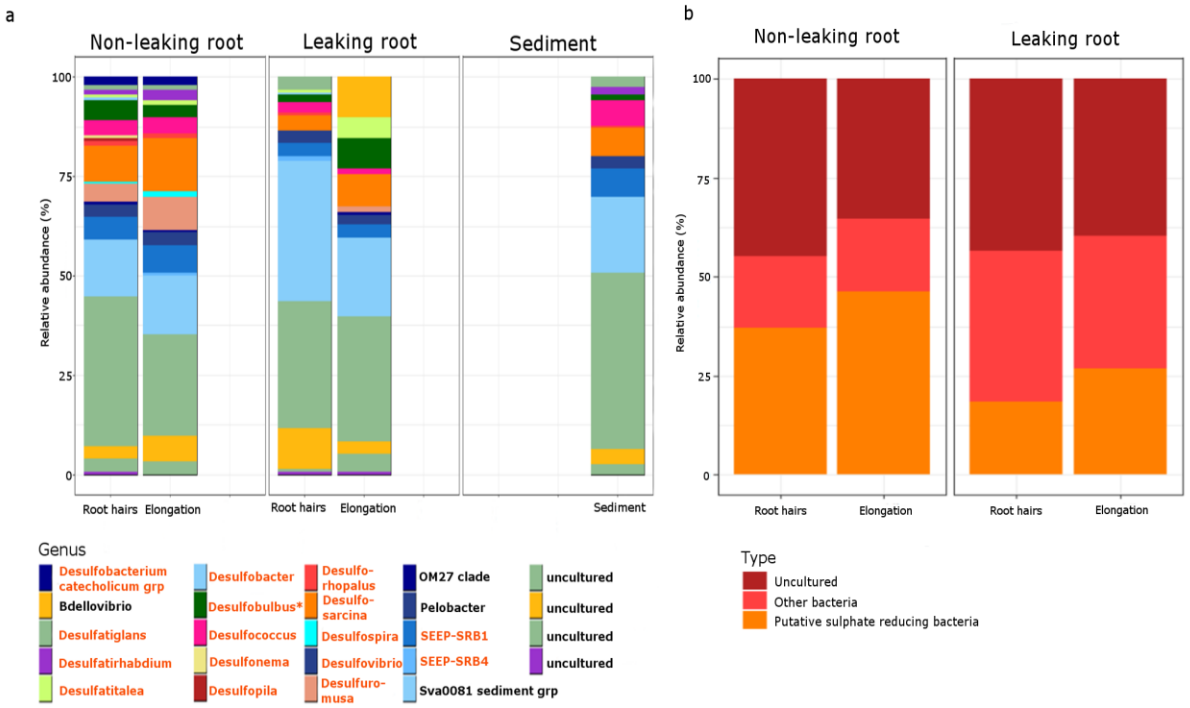

Fig. S6 | Relative abundance of δ-proteobacteria across sample types. a, Putative sulphate reducing genera are highlighted in red text. **b,** relative abundance of δ-proteobacteria genera in leaking and non-leaking roots of the seagrass *Zostera muelleri* grouped as either uncultured δ-proteobacteria, non-sulphate reducing bacteria (other bacteria) and putative sulphate reducers.

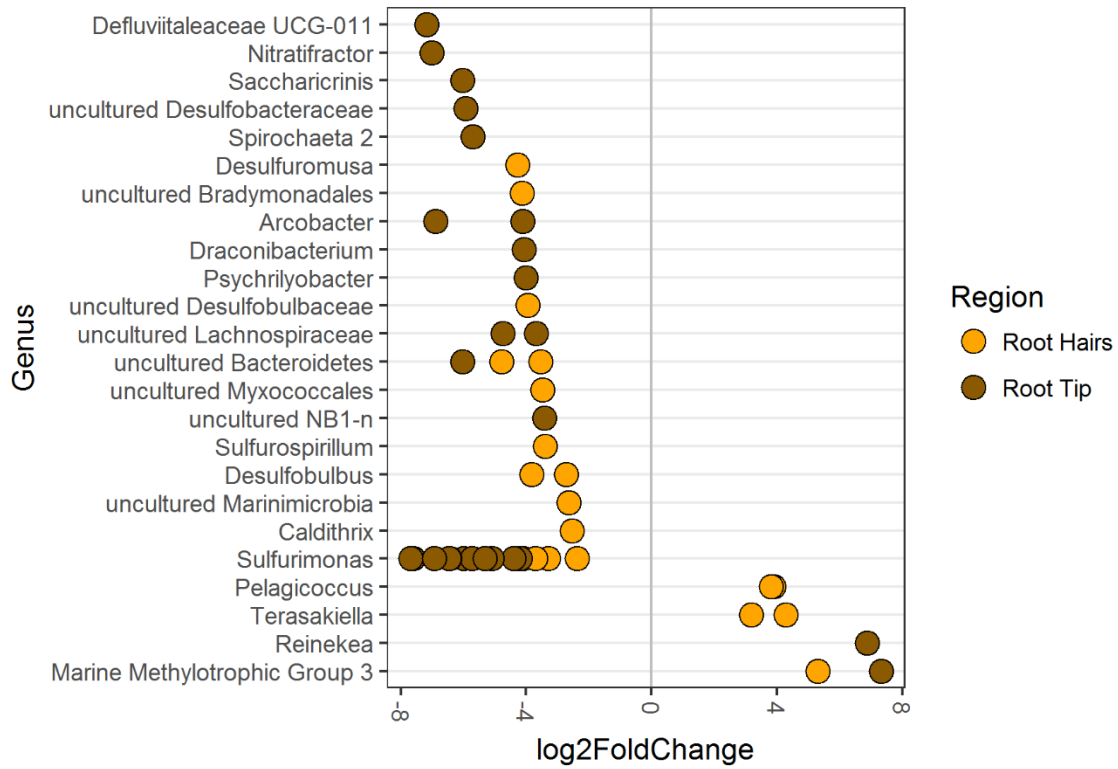

Fig. S7 | Differentially abundant OTUs (significance of $p < 0.05$) between oxygen leaking and non-leaking root regions of *Z. muelleri*. OTUs are arranged by increasing significance of their adjusted p-values and the direction of the $\log_2$ fold change. Greater than 0 $\log_2$ fold change indicates OTUs more differentially abundant in leaking roots.

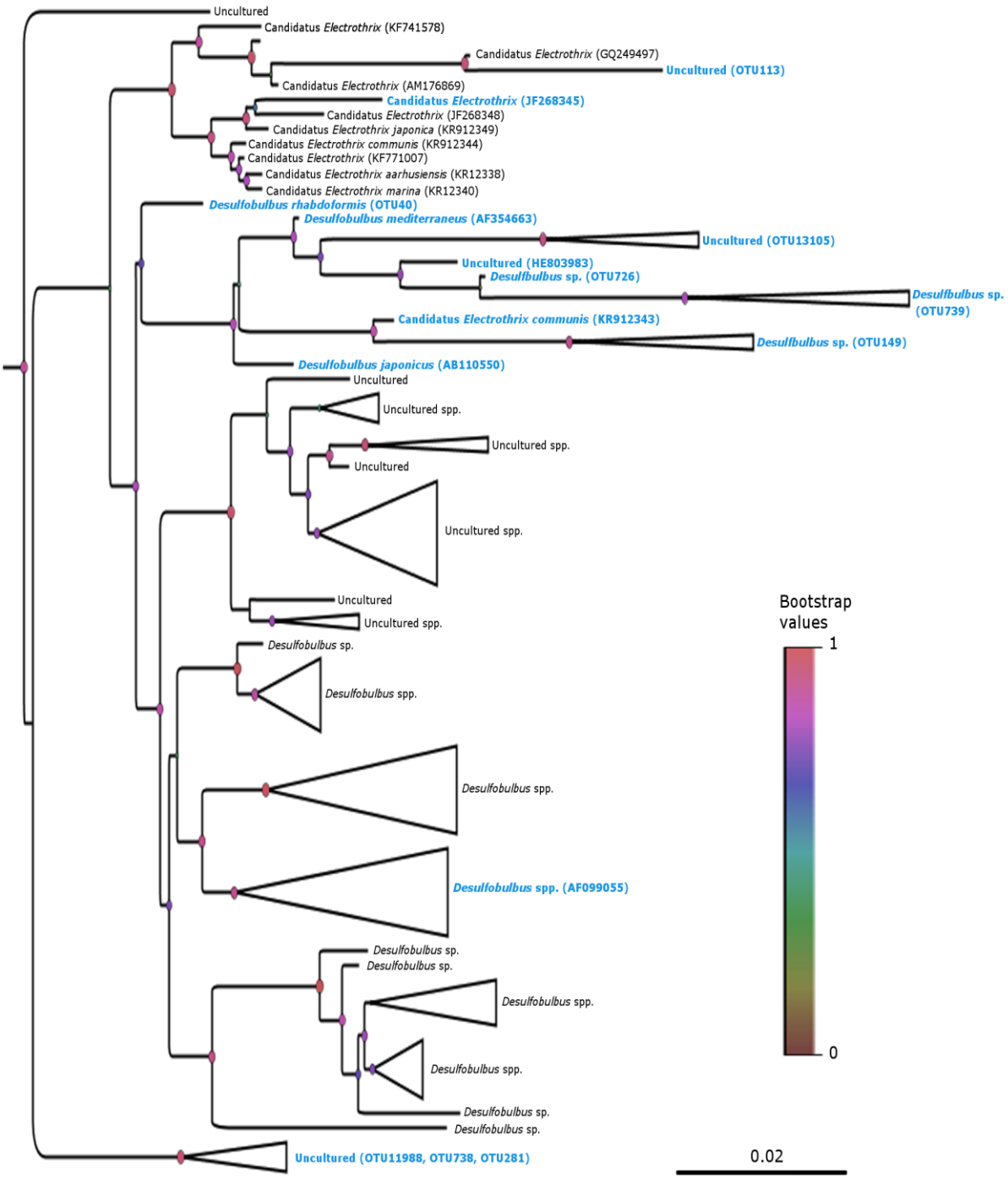

Fig. S8 | Maximum-likelihood tree of 16S rRNA sequences of cable bacteria (Candidatus *Electrothrix*) and closely related *Desulfobulbaceae*. Phylogenetic tree was constructed using FastTree (General Time Reversible model and gamma rate) from *Desulfobulbaceae* sequences that were re-aligned to the most recent SILVA database (release 132) using SINA aligner (v1.2.11) with a minimum identity of 0.9 and a sequence cut-off of 0.7. Sequences from this study are highlighted in blue text with corresponding accession numbers in parentheses. Bootstrap values are indicated by coloured circles as each node.

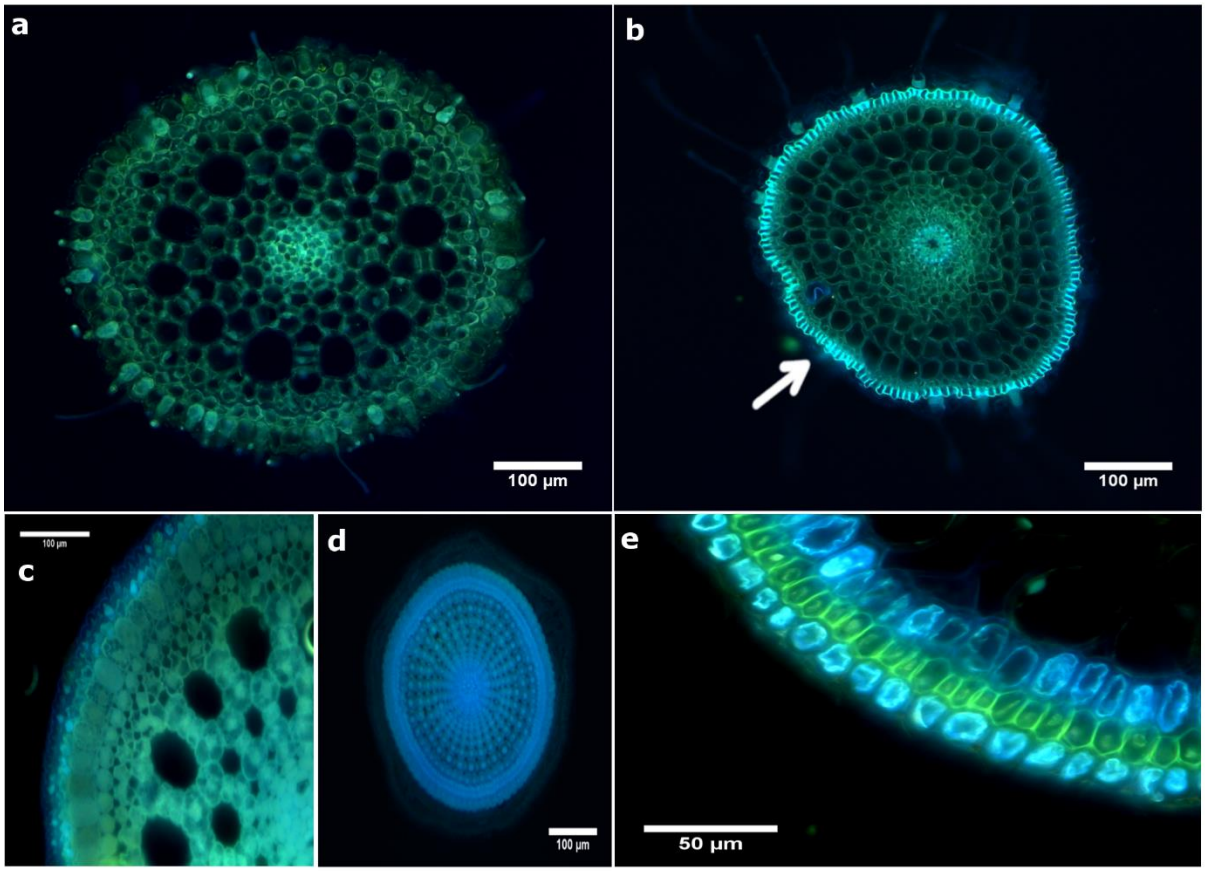

Fig. S9 | Root anatomy and suberin deposition of *H. ovalis* and *Z. muelleri*. To visualize suberin, roots were stained for 1 h with 0.1% Fluorol Yellow 088 in polyethylene glycol-glycerol and viewed under UV light (Zeiss Axioscope). **a**, *H. ovalis* mature root hair zone with no obvious suberin deposition. **b,** suberized cell wall of *Z. muelleri* root hair zone. Arrow indicates fluorescent suberized outer wall. **c**, *H. ovalis* root tip with no suberin. **d**, *Z. muelleri* root tip with no suberin. **e**, suberized cell wall of *Z. muelleri* root hair zone.