Supplementary material

Shifts in the seagrass leaf microbiome associated with wasting disease in *Zostera muelleri*

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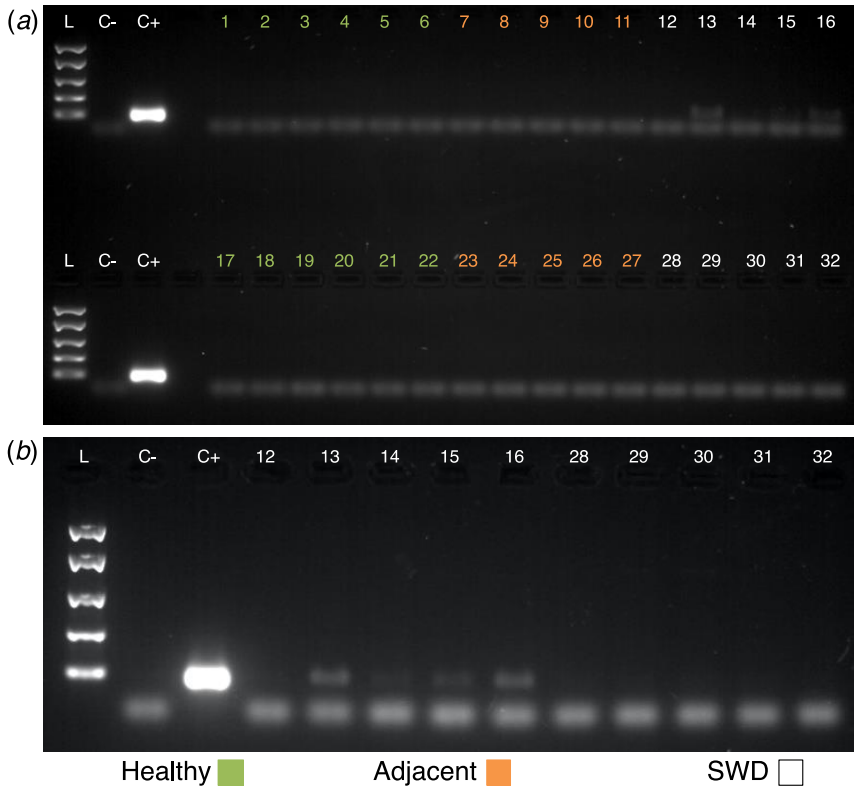

Fig. S1. PCR detection of genomic DNA from *L. zosterae*. Presence of the putative pathogen involved in seagrass wasting disease-the protozoan *Labryinthula zosterae*, was detected by polymerase chain reaction (PCR) with primers specifically designed for *L. zosterae* detection on seagrass leaves. All samples were initially tested (*a*) and positive amplifications of the 80-bp region between the internal transcribed (ITS) spacers 1 and 2 were subsequently confirmed by running the same detection assay only in SWD samples (*b*). L: molecular weight ladder (EasyLadder I, Bioline); C-, negative control (Mili-Q water instead of DNA template); C+, synthetised positive control designed with the consensus sequence of *L. zosterae* (GenBank accession number JN121409.1); lines 1–32: *Z. muelleri* leaves gDNA (Rose Bay: 1–16, Lake Macquarie: 17–32). Samples are coloured by tissue type.

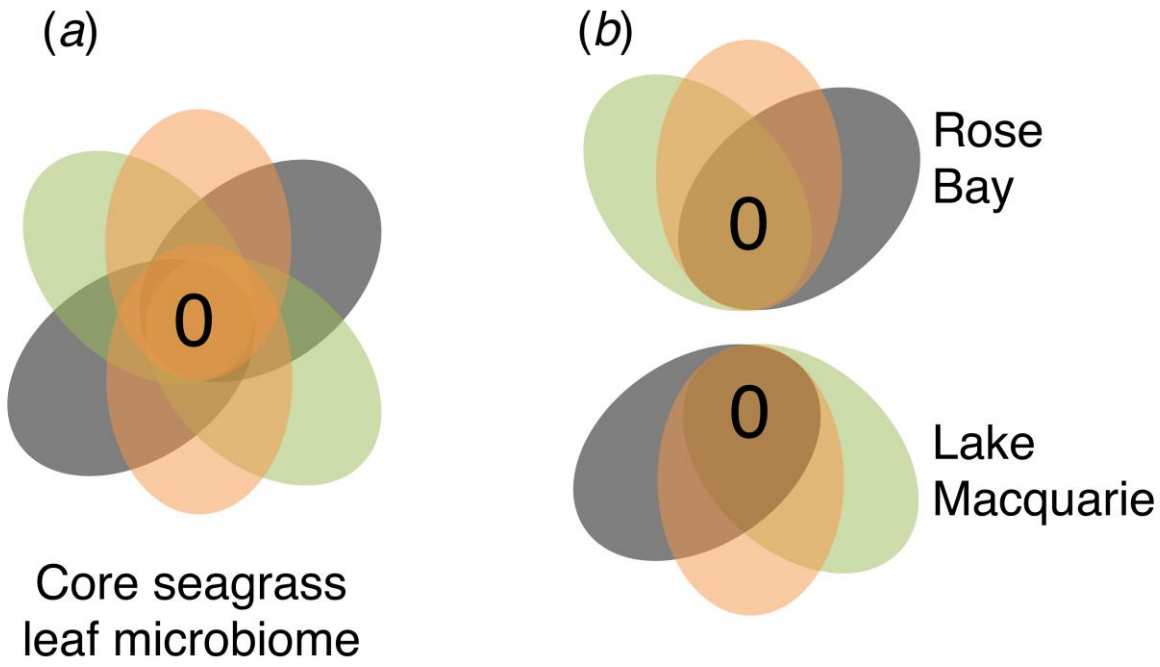

Fig. S2. Unified core microbiome. Bacterial core OTUs were identified as those microorganisms consistently present (relative abundance > 0) in most of the samples ($n - 1$), across all samples (*a*) and within each sample location (*b*). Numbers in the middle represent the amount of core OTUs identified (i.e. core size). The absence of a unified core microbiome indicates that the microbiomes investigated here are mostly tissue specific.

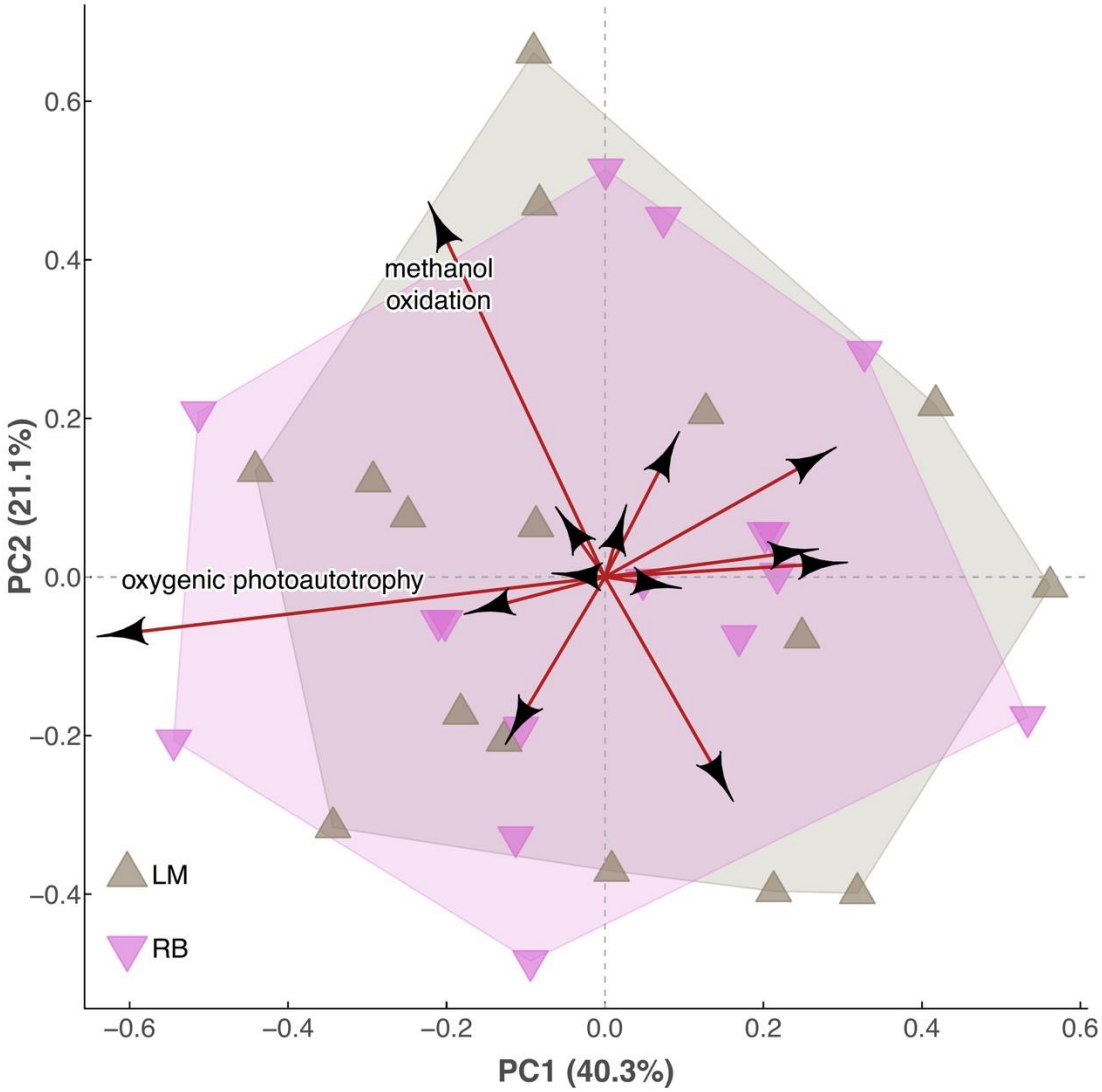

Fig. S3. Site-associated predicted functional profiles. Conditioned principal components analysis (CPCA) was used to identify predicted functional categories that best discriminated between the two sampling locations. The PCA biplot is shown, with scores of site samples represented as points (key at bottom left; LM, Lake Macquarie; RB, Rose Bay) and putative functions loadings displayed as vectors (rescaled for clarity; see Table S7 for unscaled loadings). The relative percentage of the variance explained in the two PCA axes is shown next to each axis label. Only vectors that most contributed to the separation among sites were labelled. Predicted functional profiles were generated from *16S* rRNA gene sequencing data using an annotation database created on the basis of genomic complement of sequenced genomes and the Functional Annotation of Prokaryotic Taxa (FAPROTAX) pipeline. Each taxonomically annotated OTU was compared against each FAPROTAX annotation rule.

Table S1. Study sites selection criteria and categorisation based on anthropogenic impact ranking

T, temperature; S, salinity (conductivity). Reference site, Reference site type, Reference sanitary inspection category, Reference microbial assessment category and Reference suitability grade are according to State of the Beaches 2014–2015 Report (BeachWatch, Office of Environment and Heritage). Distance to closest contamination source is from environmental survey observations (distances measured in Google Earth). Relative abundance was quantified by qPCR (average for triplicates per site). Overall categorisation is from the final assessment of anthropogenic impact level for a total of four sampling locations (1 = very low risk, 4 = very high risk)

Study site name	Region	Coordinates (latitude, longitude)	*T* (°C)	*S* (mS cm^{-1})	Reference site	Reference site type	Reference sanitary inspection category	Reference microbial assessment category	Reference suitability grade	Distance to closest contamination source (m)	Relative abundance of the clinical class 1 integron-integrase gene *intI1* (gene copy number L^{-1})	Overall categorisation
Rose Bay	Central Sydney	33°52′20.1″S 151°15′43.7″E	24.0	52.9	Rose Bay Beach	Estuarine	Moderate	Category B	Good	927 to Point Piper Marina	1203.36	3
Lake Macquarie	Hunter Region	33°09′29.4″S 151°31′54.9″E	30.3	50.4	Sunshine	Lagoon or lake	Moderate	Category B	Good	1323 to the Vales Point Power Station	285.78	2

Table S2. Water physicochemical properties

Temperature and Conductivity were measured in-situ using a multi-probe meter (WTW Multi 3430, Germany). Conductivity is a salinity indicator

Site	Temperature (°C)	Conductivity (mS cm^{-1})
Rose Bay	24.0	52.9
Lake Macquarie	30.3	50.4

Table S3. Statistical analyses for α diversity

Differences in alpha diversity between sample types were tested for statistical significance using mixed modelling. Significant values at the 0.05 level are shown in bold. h, healthy; a, adjacent; swd, diseased, na, not applicable. The exponential function was applied to the Shannon's diversity index to estimate the effective number of species. d.f. were calculated from a Satterthwaite approximation for LMM

Main test						
LMM (all tissue types)						
Index	Factor	χ^2	d.f.	*P*		
Chao1	Tissue	23.08	2	< **0.0001**		
	Site	12.69	1	**0.0004**		
	Tissue × Site	2.01	2	0.4		
Index	Factor	χ^2	d.f.	P		
Shannon's	Tissue	7.98	2	**0.02**		
	Site	9.51	1	**0.002**		
	Tissue × Site	2.06	2	0.4		
Pair-wise tests						
LMM (all tissue types)						
Index	Contrast	Estimate	s.e.	d.f.	*t*-ratio	*P*
Chao1	a - swd	-19.33	6.19	8.36	-3.12	**0.02**
	a - h	18.96	8.43	22.75	2.25	**0.03**
	swd - h	38.29	8.43	22.75	4.54	**0.0004**
Index	Contrast	Estimate	s.e.	d.f.	z ratio	P
Shannon's	a - swd	-39.51	33.16	na	-1.19	0.2
	a - h	33.90	21.46	na	1.58	0.2
	swd - h	73.41	28.00	na	2.62	**0.03**
ANOVA (healthy v. *diseased)*						
Index	Contrast	Estimate	s.e.	d.f.	*t*|*z* ratio	*P*
Chao1	swd - h	38.29	7.78	18	4.92	**0.0001**
Shannon's	swd - h	73.41	22.64	18	3.24	**0.005**

Table S4. Statistical analyses for β diversity

Differences in bacterial community composition between tissue types and sites were tested for statistical significance using permutational ANOVA. Significant values at the 0.05 level are shown in bold. rb, Rose Bay; lm, Lake Macquarie; h, healthy; a, adjacent; swd, diseased

Main test							
Factor	d.f.	SS	R^2	*F*	*P*		
Site	1	1.52	0.12	5.30	**0.0001**		
Tissue	2	2.83	0.22	4.92	**0.0001**		
Site × Tissue	2	1.00	0.08	1.73	**0.02**		
Residual	26	7.47	0.58				
Total	31	12.82	1.00				
Pair-wise tests							
Tissue type across sites							
Tissue	Terms	d.f.	SS	R^2	*F*	*P*	
h	Site	1	0.31	0.12	1.35	0.3	
	Residual	10	2.31	0.88			
	Total	11	2.63	1.00			
a	Site	1	1.05	0.28	3.12	**0.007**	
	Residual	8	2.70	0.72			
	Total	9	3.75	1.00			
swd	Site	1	1.15	0.32	3.75	**0.007**	
	Residual	8	2.46	0.68			
	Total	9	3.61	1.00			
Tissue types within sites							
Site	Factor levels	Terms	d.f.	SS	R^2	*F*	*P*
rb	h - swd	Tissue	1	1.40	0.37	5.24	**0.002**
		Residual	9	2.40	0.63		
		Total	10	3.79	1.00		
	h - a	Tissue	1	1.29	0.36	5.16	**0.002**
		Residual	9	2.25	0.64		
		Total	10	3.54	1.00		
	swd - a	Tissue	1	0.73	0.20	2.01	**0.03**
		Residual	8	2.89	0.80		
		Total	9	3.62	1.00		
lm	h - swd	Tissue	1	1.01	0.30	3.84	**0.02**
		Residual	9	2.37	0.70		
		Total	10	3.38	1.00		
	h - a	Tissue	1	0.86	0.24	2.79	**0.03**
		Residual	9	2.77	0.76		
		Total	10	3.62	1.00		
	swd - a	Tissue	1	0.38	0.15	1.36	0.1
		Residual	8	2.26	0.86		
		Total	9	2.64	1.00		

Average similarities (%)

Between or within sites

Site	Rose Bay	Lake Macquarie
rb	13.22	
lm	7.70	22.76

Between or within tissues

Tissue	h	a	swd
h	29.44		
a	9.13	13.80	
swd	5.88	12.01	16.08

Between or within sites × tissues

Site × Tissue	rb × h	rb × a	rb × swd	lm × h	lm × a	lm × swd
rb × h	35.67					
rb × a	10.10	22.39				
rb × swd	3.99	9.25	17.32			
lm × h	28.52	9.59	2.91	25.42		
lm × a	2.35	5.21	4.35	14.47	26.68	
lm × swd	1.35	3.28	5.52	15.27	31.17	41.23

Table S5. SIMPER analysis (taxonomy)

Discriminatory OTUs with the highest contribution (Contrib% ≧ 1%) to the differences between seagrass tissue types. Two way-crossed similarity percentages analysis (SIMPER, site × tissue). Top-3 contributor OTUs are shown in bold. OTUs pooled at the lowest taxonomic level (cumulative contributions); h, healthy; a, adjacent; swd, diseased

OTUs	Contrib%
a - swd	
Rubidimonas	**5.15**
Pseudomonas	**3.49**
Saprospiraceae	**3.02**
Hellea	2.09
Lacinutrix mariniflava	1.57
Phormidesmis	1.55
Schizothrix	1.46
Cellvibrionaceae	1.35
Delftia	1.32
Cloacibacterium	1.31
Algitalea	1.25
Micrococcus luteus	1.19
Pirellula	1.17
Candidatus *Megaira*	1.11
Stenotrophomonas rhizophila	1.10
Microtrichaceae	1.08
a - h	
Burkholderia	**9.82**
Pseudomonas	**5.90**
Rubidimonas	**5.74**
Stenotrophomonas	3.66
Cloacibacterium	2.72
Delftia	2.30
Lacinutrix mariniflava	1.82
Phormidesmis	1.54
Cryomorphaceae	1.49
Candidatus *Megaira*	1.36
Winogradskyella	1.26
Moraxella osloensis	1.20
Alphaproteobacteria (SAR11)	1.12
Micrococcus luteus	1.12
Sphaerotilus	1.01
swd - h	
Burkholderia	**8.85**
Pseudomonas	**3.96**
Saprospiraceae	**2.85**
Stenotrophomonas	1.94
Rubidimonas	1.90
Schizothrix	1.74
Delftia	1.66
Phormidesmis	1.62
Pirellula	1.32
Hellea	1.30
Cryomorphaceae	1.24
Cellvibrionaceae	1.22
Algitalea	1.15
Pleurocapsa	1.00

Table S6. Predicted functions loadings from RDA analysis (functional predictions compared between tissue types)

Total OTUs assigned to each putative function. Predicted functional categories were generated from *16S* rRNA gene sequencing data using FAPROTAX. Functions that most contributed to the separation among tissue types are shown in bold

Associated OTUs	Putative function	RDA1	RDA2
6	Aerobic chemoheterotrophy	0.0844	–0.0205
2	Cellulolysis	–0.0456	0.0213
13	Chemoheterotrophy	0.0596	–0.0806
3	Dark oxidation of sulfur compounds	–0.0263	–0.0236
5	Fermentation	0.0863	–0.0312
7	Human pathogens	0.2258	0.0142
10	**Intracellular parasites**	**0.0225**	**–0.1211**
4	Ligninolysis	–0.05	–0.0748
1	Methanol oxidation	–0.0661	–0.0748
9	**Nitrate reduction**	**0.1986**	**0.1774**
8	**Nitrate respiration**	**0.2318**	**0.1708**
12	**Oxygenic photoautotrophy**	**–0.5226**	**0.1337**
11	Predatory or exoparasitic	–0.1394	0.033

Table S7. Predicted functions loadings from conditioned PCA analysis (functional predictions compared between sites)

Total OTUs assigned to each putative function. Predicted functional categories were generated from *16S* rRNA gene sequencing data using FAPROTAX. Functions that most contributed to the separation among sites are shown in bold

Associated OTUs	Putative function	PC1	PC2
6	Aerobic chemoheterotrophy	0.1463	–0.2556
2	Cellulolysis	–0.1097	–0.1842
13	Chemoheterotrophy	0.0655	–0.0096
3	Dark oxidation of sulfur compounds	–0.0356	0.0025
5	Fermentation	0.0795	0.1554
7	Human pathogens all	0.2644	0.1483
10	Intracellular parasites	0.0182	0.0611
4	Ligninolysis	–0.0442	0.0629
1	**Methanol oxidation**	**–0.2096**	**0.445**
9	Nitrate reduction	0.2383	0.0313
8	Nitrate respiration	0.2747	0.0165
12	**Oxygenic photoautotrophy**	**–0.6099**	**–0.0714**
11	Predatory or exoparasitic	–0.1474	–0.0388