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

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Abstract. Seagrass wasting disease (SWD), an infection believed to be caused by *Labyrinthula zosterae*, has been linked to seagrass declines in several places around the world. However, there is uncertainty about the mechanisms of disease and the potential involvement of opportunistic colonising microorganisms. Using 16S rRNA gene amplicon sequencing, we compared the microbiome of SWD lesions in leaves of *Zostera muelleri* with communities in adjacent asymptomatic tissues and healthy leaves. The microbiome of healthy leaf tissues was dominated by *Pseudomonas* and *Burkholderia*, while the most predominant taxa within adjacent tissues were *Pseudomonas* and *Rubidimonas*. Members of the *Saprospiraceae*, potential macroalgal pathogens, were over-represented within SWD lesions. These pronounced changes in microbiome structure were also apparent when we examined the core microbiome of different tissue types. While the core microbiome associated with healthy leaves included three OTUs classified as *Burkholderia*, *Cryomorphaceae*, and the SAR11 clade, a single core OTU from the *Arenicella* was found within adjacent tissues. *Burkholderia* are diazotrophic microorganisms and may play an important role in

seagrass nitrogen acquisition. Contrastingly, some members of the *Arenicella* have been implicated in necrotic disease in other benthic animals. Moreover, microbiome structure was maintained across sites within healthy tissues, but not within SWD lesions or the tissues immediately adjacent to lesions. Predicted functional profiles revealed increased photoautotrophic functions in SWD tissues relative to healthy leaves, but no increase in pathogenicity/virulence. Notably, we demonstrated the presence of *L. zosterae* in SWD lesions by PCR, but only in one of the two sampled locations, which indicates that other microbiological factors may be involved in the initiation/development of SWD-like symptoms. Our study suggests that the dynamics of the seagrass microbiome should be considered within the diagnosis and management of SWD.

Additional keywords: bacteria, benthos, biodiversity, ecology, environmental monitoring, population dynamics.

Running page head: Microbial shifts in seagrass wasting disease.

Introduction

Many benthic marine plants and animals establish tight ecological relationships with their consortia of associated microorganisms (i.e. their microbiome) (Hernandez-Agreda, Gates *et al.* 2017), which can in turn have important impacts on ecosystem function and health (Hollants, Leliaert *et al.* 2013). For instance, the seagrass microbiome can play essential roles in key chemical cycling processes, including nitrogen fixation (Bagwell, La Rocque *et al.* 2002; Garcias-Bonet, Arrieta *et al.* 2016), sulphate reduction and oxidation (García-Martínez, López-López *et al.* 2009; Jensen, Kuhl *et al.* 2007; Küsel, Trinkwalter *et al.* 2006), iron reduction (Küsel, Trinkwalter *et al.* 2006), phosphate solubilisation (Ghosh, Subhashini *et al.* 2012) and carbon remineralization (Trevathan-Tackett, Seymour *et al.* 2017). Conversely, in many benthic organisms like corals, sponges, macrophytes (i.e. higher plants and macroalgae), members of the microbiome can also have detrimental impacts on the host, including causing disease (Amend, Burgaud *et al.* 2019; Pollock, Morris *et al.* 2011; Sutherland, Porter *et al.* 2004).

Within seagrasses, there are at least three known genera of pathogenic or parasitic protists and oomycetes that can have substantial detrimental effects on seagrass health (Sullivan, Trevathan-Tackett *et al.* 2018), including *Labyrinthula* (Martin, Chiari *et al.* 2016), *Phytomyxea* (Walker and Campbell 2009) and *Phytophthora* (Govers, Man-in-'t-Veld *et al.* 2016). Several mass losses of seagrass habitats have been documented in multiple species since the 1930's (Orth, Carruthers *et al.* 2006; Short and Wyllie-Echeverria 1996; Waycott, Duarte *et al.* 2009) when the first SWD pandemic outbreak decimated populations of *Zostera marina* (*Z. marina*) along the Atlantic coast of North America and Europe (Muehlstein 1989; Petersen 1933). Subsequent studies of seagrass disease have since been mainly focused on *L. zosterae* (Short, Muehlstein *et al.* 1987; Sullivan, Sherman *et al.* 2013; Young 1943), which based on Koch's postulates was proposed as the primary aetiological agent of SWD in *Z. marina* (Short, Muehlstein *et al.* 1987). Further investigations demonstrated that *L. zosterae* is a host-specific pathogen of *Z. marina*, transferred by direct contact of plants (Muehlstein 1989; Muehlstein, Porter *et al.* 1991).

SWD is an infection typically evidenced as dark, necrotic lesions on the blades of the seagrass leaf shoot (Short, Muehlstein *et al.* 1987), which can result in wide-spread seagrass die-offs often related with increased susceptibility (Blakesley, Berns *et al.* 2002; Muehlstein 1989) and/or environmentally-driven immunosuppression of the host (Giesen, van-Katwijk *et al.* 1990; Groner, Burge *et al.* 2016; Vergeer, Aarts *et al.* 1995). The particular factors that contribute to an increased prevalence of SWD are poorly understood, although it seems to be affected by the combined effects of multiple environmental parameters (Burge, Kim *et al.* 2013; Jakobsson-Thor, Toth *et al.* 2018; Sullivan, Trevathan-Tackett *et al.* 2018; Trevathan-Tackett, Lauer *et al.* 2013) and intrinsic features of the plant (Groner, Burge *et al.* 2014; Groner, Burge *et al.* 2016). There are multiple measurements of SWD prevalence, including the proportion of each leaf affected by the disease as measured by leaf lesions (Bull, Kenyon *et al.* 2012; Jakobsson-Thor, Toth *et al.* 2018), the proportion of diseased leaves relative to the total number of leaves per shoot (Groner, Burge *et al.* 2014), or the number of *L. zosterae* cells per mg of seagrass dry weight (Jakobsson-Thor, Toth *et al.* 2018). However, most of the studies agree in that SWD is most prevalent in summer (Bull, Kenyon *et al.*

2012), at higher salinities (Jakobsson-Thor, Toth *et al.* 2018) and shallower depths (Groner, Burge *et al.* 2014; Jakobsson-Thor, Toth *et al.* 2018). Even though disease prevalence is very variable at the biogeographical scale (Groner, Burge *et al.* 2016; Sullivan, Sherman *et al.* 2013), it has been suggested that *L. zosterae* is present in a chronic reservoir (i.e. where an infectious agent can persistently or recurringly cause disease).

Multiple haplotypes of *Labyrinthula* species have been characterised microscopically and morphologically (Sullivan, Robinson *et al.* 2017). In addition, the phylogeny (Martin, Chiari *et al.* 2016), spatial and temporal distribution (Bockelmann, Beining *et al.* 2012), and virulence (Brakel, Werner *et al.* 2014) of this microorganism have been described elsewhere, with a primary focus on the pathogen-host relationship with *Zostera* seagrass species. Multiple studies have isolated *Labyrinthula* sp. from other seagrass species, such as *Halodule uninervis*, *Halophila ovalis, Thalassodendron ciliatum*, *Phyllospadix scouleri*, and *Phyllospadix torrey*, but its pathogenicity was not tested or could not be confirmed (Sullivan, Robinson *et al.* 2017; Sullivan, Trevathan-Tackett *et al.* 2018; Vergeer and den-Hartog 1994). Given that *L. zosterae* is ubiquitous in the marine environment, with global distribution in multiple seagrass species in both healthy and infected but still asymptomatic specimens (Bockelmann, Beining *et al.* 2012; Chitrampalam, Goldberg *et al.* 2015; Vergeer and den-Hartog 1994), *Labyrinthula* has been considered primarily an opportunistic pathogen (Sullivan, Robinson *et al.* 2017; Sullivan, Trevathan-Tackett *et al.* 2018; Vergeer and den-Hartog 1994).

Disease-related changes of the microbiome have been demonstrated in other benthic organisms. For instance, in corals, significant shifts in bacterial community composition and functional profiles have been observed between healthy-colonies and Necrotic Patch Disease-affected tissues (Quintanilla, Ramirez-Portilla *et al.* 2018). This pattern is often associated with microbial dysbiosis, which is broadly defined as a change in a host organism's microbiome relative to that found in individuals deemed healthy (Petersen and Round 2014).

Similarly, relative to healthy individuals, the microbiome of diseased macroalgae showing symptoms of a bleaching type disease is characterised by taxonomic and functional shifts (Fernandes, Steinberg *et al.* 2012; Kumar, Zozaya-Valdes *et al.* 2016; Zozaya-Valdes, Egan *et al.* 2015). These investigations highlight the likely involvement of the microbiome in disease onset, but as yet the potential role of different components of the microbiome, and particularly that of the seagrass bacterial microbiome in SWD has not been considered. Within this context, there is emerging evidence that diseases of many benthic marine organisms can often be classified as 'polymicrobial' (Kumar, Zozaya-Valdes *et al.* 2016; Miller and Richardson 2011), whereby multiple causative agents are often difficult to tease apart from broad-scale shifts in the host organism's microbiome. Prominent examples of polymicrobial diseases in benthic marine organisms include Black Band Disease in corals, whereby a complex cascade of shifts in microbial community structure and function occur during disease progression (Sato, Civiello *et al.* 2016), and bleaching events in macroalgae, for which multiple opportunistic microbes are believed to be responsible (Kumar, Zozaya-Valdes *et al.* 2016). Shifts in microbiome structure are sometimes considered within the context of dysbiosis (Meres, Ajuzie *et al.* 2012; Zozaya-Valdés, Roth-Schulze *et al.* 2017) and can either precede the onset of a disease or syndrome (Schaubeck, Clavel *et al.* 2016) or occur following an initial infection that compromises host health (Oh, Freeman *et al.* 2013).

The remaining uncertainty about the role of potentially opportunistic pathogens in SWD results from i) the paucity of paired studies with pathogenicity testing for *L. zosterae* isolates (Martin, Chiari *et al.* 2016), and ii) the myriad of interacting factors (not only microbial) likely involved in disease initiation and development of SWD symptoms (Sullivan, Trevathan-Tackett *et al.* 2018). Within this context, and in light of the invaluable insight that 16S rRNA gene sequence analysis has provided into our understanding of microbial genetic diversity and community structure of environmental samples (Sanschagrin and Yergeau 2014; Shokralla, Spall *et al.* 2012), we did not attempt to isolate putative bacterial pathogens or address Koch's postulates. Instead, we used a purely genomic approach to account for as yet uncultivable microorganisms. Given our limited understanding of the ecology of SWD and the dynamic nature of the

seagrass microbiome (Hurtado-McCormick, Kahlke *et al.* 2019), we examined patterns in the *Z. muelleri* bacterial microbiome associated with SWD, with the goal of determining the potential involvement of opportunistic colonising microorganisms other than *L. zosterae* in SWD. We hypothesise that bacterial community composition and predicted function will differ between plants deemed healthy to those exhibiting symptoms of SWD. Changes in the microbiome may either follow *L. zosterae* infection or be linked to the development of SWD-like symptoms without the involvement of *L. zosterae.*

Materials and methods

Study sites

Leaf samples from healthy and SWD affected specimens of *Z. muelleri* were collected from Rose Bay (33°52'20.1'S 151°15'43.7'E) and Lake Macquarie (33°09'29.4'S 151°31'54.9'E), which are representative habitats colonized by seagrass meadows in New South Wales (NSW), Australia (Green and Short 2003). Rose Bay is a coastal site situated within a large urban estuary, while Lake Macquarie is an enclosed coastal estuarine lagoon (Office of Environment & Heritage - NSW Government 2015). These two sampling locations are separated by 120 km of coastline and were chosen to capture different environmental conditions and levels of anthropogenic impact representative of seagrass beds in NSW. The two sites were previously characterised in Hurtado-McCormick, Kahlke *et al.* (2019), and habitat feature data relevant for this study is provided in **Supplementary Table 1**. Sampling was conducted between October and November 2015 (Austral spring), with both sites surveyed during low-tide conditions (< 2 m depth). Healthy plants and specimens exhibiting SWD-like symptoms were spatially interspersed within mixed meadows containing the seagrass species *Zostera muelleri* and *Halophila ovalis*, with no obvious structure in the location of diseased plants. All sampled meadows were located near to the shoreline in lower intertidal zones. At the time of sampling, water physicochemical properties (i.e. temperature and conductivity as a salinity indicator, see **Supplementary Table 2**) were measured *in situ* using a multi-probe meter (WTW Multi 3430, Germany).

Sample collection

Necrotic lesions, characteristic of SWD have been previously described as black-brown dots or streaks on the leaves, which develop into patches, larger blackened spots and longer streaks (Muehlstein 1989; Muehlstein, Porter *et al.* 1991; Short, Muehlstein *et al.* 1987). Using these previous descriptions as symptomatic criteria, we defined 'healthy individuals' as plants that were completely asymptomatic (i.e. did not show any necrotic lesions on a green leaf surface), and 'diseased individuals' as plants that presented single or multiple necrotic lesions (i.e. black-brown dots or streaks on the leaf surface). It should be noted that this classification was based on macroscopic observations of SWD-like symptoms, and therefore putatively 'diseased individuals' could have been impacted by other processes including senescence or grazing scarring. Leaf samples of *Z. muelleri* were collected with sterile-gloved hands, rinsed with non-filtered seawater collected on-site, and placed into plastic bags (Ziploc®) partially filled with the same water. All samples were transported to the laboratory on ice and processed within 3 h of collection. A total of 10 diseased plants were collected (3rd rank leaf, 5 biological replicates per site). From these, 10 tissue samples were individually excised from SWD lesions (2.5 - 3 cm^2 of surface area per sample), and 10 samples were taken from asymptomatic tissues located immediately adjacent to the lesions collected (2.5 - 3 cm^2 of surface area per sample, at least 2 cm away from an active lesion). These samples are here denoted as 'SWD lesion' and 'adjacent' tissues, respectively. Samples were also collected from 12 entirely healthy specimens (2.5 - 3 cm^2 of surface area per sample, 6 biological replicates per site), which were different from the diseased individuals. Upper and lower sections of the 3rd leaf of plants located within the same meadows were excised from each plant and are here denoted as 'healthy' tissues (**Fig. 1**). The two sections were sampled in order to account for the heterogeneous distribution of SWD lesions on the leaf surface, and subsequently pooled based on our observations in a previous study of the statistical similarities between microbiomes associated with different fractions of the seagrass leaf (Hurtado-McCormick, Kahlke *et al.* 2019). All samples (i.e. healthy, adjacent and SWD lesion tissues, $N = 32$) were collected from the 3rd oldest leaf, which has previously been shown to be where there is the highest *L. zosterae* prevalence/abundance (Bockelmann, Beining *et al.* 2012). Samples were rinsed

with Milli-Q water (Millipore Corporation, Billerica, MS, USA), snap-frozen with liquid nitrogen and stored at -80 °C prior to analysis.

Microbial community DNA isolation

Community DNA was extracted from all washed-leaf tissue samples using a bead-beating and chemical lysis-based DNA extraction kit (PowerSoil® DNA Isolation Kit, MoBio Laboratories, Carlsbad, CA, USA), following the manufacturers standard protocol, with the following minor modification: rather than 0.25 g of material, all of the collected plant tissue was used. DNA concentration was determined in a Nanodrop (Nanodrop - 1000, ThermoScientific, NanoDrop Products, Wilmington, DE, USA). Samples were diluted (1:50 - 1:100) for PCR when concentrations exceeded 50 ng/μL (range of DNA content of the extractions = 3 - 10 ng/μL), and 2 μL of template DNA were used for each PCR reaction.

PCR detection of L. zosterae

To determine whether the symptomatology observed in SWD tissues was associated with infection by *L. zosterae*, an 80 bp region located between the internal transcribed spacers (ITS) 1 and 2 was amplified using the forward primer Laby_ITS_Taq_f: 5'-TTGAACGTAACATTCGACTTTCGT-3' and the reverse primer Laby_ITS_Taq_r: 5'-ACGCATGAAGCGGTCTTCTT-3', which were specifically designed to detect *L. zosterae* in seagrass leaves (Bockelmann, Beining *et al.* 2012). PCR reactions were carried out using standard conditions recommended by the manufacturer using the 12.5 μL 2X ImmoMix Red™ Master Mix (Bioline, Memphis, TN, USA), in a 25 μL reaction volume: 2 μL template DNA, 1 μL of each primer (10 μM), and 8.5 μL Mili-Q water (Millipore Corporation, Billerica, MS, USA). PCR cycling conditions involved an initial activation step at 95°C for 10 min, followed by a step down protocol including: i) 10 cycles of: denaturation at 95°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 30 s, and ii) 10 cycles of: denaturation at 95°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 30 s. This was finally followed by a holding stage at 72°C for 10 min. The resultant amplicons were visualized on 2% agarose gel with GelRed (1:10000), and light intensities were compared against a synthetized 140 bp positive control designed with the consensus sequence of *L. zosterae* (GenBank

accession number JN121409.1). Positive amplifications of the expected 80 bp band were confirmed by running the same PCR twice on all SWD samples.

16S rRNA gene amplicon sequencing

Bacterial communities associated with the three different seagrass tissue types were characterised using 16S rRNA gene amplicon sequencing. The 16S rRNA gene was amplified separately from DNA extracted from 3-5 replicates of each sample type per site using the universal bacterial primer set 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 519R (5'-CGGTTACCTTGTTACGACTT-3') (Weisburg, Barns *et al.* 1991), specifically targeting the hypervariable regions V1-V3 of the bacterial 16S rRNA gene (Mao, Zhou *et al.* 2012). PCR reactions were performed in 25 μL volumes containing 12.5 μL GoTaq Green Master Mix, 0.4 μL of each primer (10 μM), and 2 μL of template DNA. PCR cycling conditions involved an initial activation step at 95 °C for 120 s, followed by 30 cycles of: denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 90 s, followed by a final extension stage at 72 °C for 10 min and a holding stage at 4 °C. DNA sequencing libraries were constructed with the Illumina TruSeq DNA library preparation protocol and sequenced on the Illumina MiSeq platform (bTEFAP® diversity assay, Molecular Research LP, Shallowater, TX, USA) following the manufacturer's guidelines. Paired sequencing reads of 300 bp were subsequently processed.

Bacterial 16S rRNA gene sequences for each sample were analysed separately, and paired sequencing files were processed independently using a customised pipeline (Kahlke 2018). Briefly, paired-end DNA sequences were de-multiplexed using MOTHUR, v1.41.1 (Schloss, Westcott *et al.* 2009), then joined using FLASH, v1.2.11 (Magoc and Salzberg 2011), quality-filtered using MOTHUR, and finally de-replicated using VSEARCH, v2.3.2 (Rognes, Flouri *et al.* 2016). Quality filtering involved both, trimming of ambiguous bases in each of the sequences and removal of short fragments with low-quality scores from the data set. Operational Taxonomic Units (OTUs) were defined at 97% sequence identity and subsequently clustered using VSEARCH. The same tool was also used to detect and remove chimeric sequences. Taxonomy assignment of OTUs was performed in QIIME, v1.9.1 (Caporaso, Kuczynski *et al.*

2010), using BLAST, legacy BLAST from NCBI (BLAST, v2.2.22) (Altschul, Gish *et al.* 1990), and the SILVA database, v132 (Glöckner, Yilmaz *et al.* 2017). Processed sequences were rarefied to the same depth (1012 sequences per sample) to remove the effect of sampling effort upon analysis, after removing a high proportion of chloroplast and mitochondria sequences (50%) likely from the seagrass host. Chloroplast and mitochondria sequences were removed from the OTU table using the filter_taxa_from_otu_table.py script in QIIME, v1.9.1 (Caporaso, Kuczynski *et al.* 2010), with the terms 'D_3__Chloroplast', and 'D_4__Mitochondria' as negative taxa.

Bacterial community analyses

In order to estimate alpha diversity, the Chao1 and Shannon's diversity indices were calculated in QIIME, v1.9.1 (Caporaso, Kuczynski *et al.* 2010). The true Shannon's diversity (i.e. effective number of species) was calculated by applying the exponential function to the Shannon's diversity index, an approach previously used to estimate alpha diversity of the *Arabidopsis thaliana* root microbiome (Lundberg, Lebeis *et al.* 2012). Mixed modelling was employed to test for differences in alpha diversity between seagrass tissues and between sampling sites, while controlling for the non-independence of samples for the paired diseased and adjacent replicates. For Chao1, a linear mixed model was used, and a generalised linear mixed model (Gamma distribution with identity link) was applied for the exponentiated Shannon's diversity. Both models included terms for site (two level fixed factor) and tissue (three level fixed factor), a site x tissue interaction term, and a random effect term for plant replicate. Where indicated by significant terms in the ANOVA output, post-hoc pair-wise tests were used to determine which levels of factors generated the significant results. All p-values were corrected for multiple testing using the Benjamini-Hochberg's FDR approach.

Differences in bacterial community composition between samples (Bray-Curtis dissimilarity) were analysed using permutational ANOVA (Monte-Carlo, 9999 draws). Differences between seagrass tissues and between sampling sites were tested in an interaction model with the same model structure (fixed effect terms for site, tissue and the site x tissue interaction) used for alpha diversity, here using stratified permutations (i.e. drawing single replicates from a given plant replicate in each permutation) to

account for the non-independence of data. Where applicable, the permutational ANOVA was re-run using data subsets including only the levels of the factors of interest, with p-value correction for multiple testing (Benjamini-Hochberg's FDR). The statistical analyses for alpha and beta diversity were conducted in R v4.0.2 (R Core Team 2020) using the packages lme4 (Bates, Mächler *et al.* 2015), lmerTest (Kuznetsova, Brockhoff *et al.* 2017), emmeans (Lenth, Singmann *et al.* 2020), and vegan (Oksanen, Blanchet *et al.* 2019).

Similarity percentages (SIMPER) analyses (two-way crossed) were also performed on Bray-Curtis similarity matrices to identify the OTUs most responsible for the discrimination of microbial assemblages in the different tissue types, here denoted as 'discriminatory' OTUs. The nMDS and SIMPER analyses were all performed in PRIMER-E, v7.0.13 (Clarke 1993; Clarke and Gorley 2015; Clarke, Gorley *et al.* 2014). To examine patterns in the occurrence of OTUs within the three tissue types and identify the extent of 'unique' and 'shared' associations, an interaction network was generated using the QIIME, v1.9.1 pipeline for bipartite networks (Caporaso, Kuczynski *et al.* 2010), employing the force-directed layout edge-weighted spring embedded according to e-weights. Networks were subsequently visualised in Cytoscape, v3.7.1 (Smoot, Ono *et al.* 2011).

Core microbiomes for each sampling site and seagrass tissue were separately defined using a PERL custom script that estimates core species (OTUs) based on occurrence and abundance thresholds by identifying the microbial species that are shared among all samples or groups of interest (Kahlke 2017). Consistent with the approaches used by Lundberg, Lebeis *et al.* (2012), we accounted for any possible outliers in the data by including a replicate threshold. Hence, any OTU present (relative abundance > 0%) in all biological replicates minus one (occurrence = n - 1), across both sites or all three tissues, was classified as a core OTU. We determined the existence of core microbiome members that were persistently present in the healthy, adjacent and SWD lesion tissues, respectively.

Potential functional roles of OTUs were estimated using the FAPROTAX, v1.1 pipeline (Louca, Parfrey *et al.* 2016). Briefly, predicted functional profiles were generated by associating individual OTUs with metabolic functions of cultured prokaryotes. For this, an annotation database created on the basis of genomic complement of sequenced genomes was used, and the functional assignment was based on annotated functional categories from previously identified bacteria at the genus or species level. Therefore, the functional categories estimated for each sample should only be assumed as putatively present within the studied bacterial microbiomes, and further formal functional methods should be used to corroborate these predictions.

Redundancy analysis (RDA) was employed to determine which predicted functional categories differed most strongly among the three tissue types. To remove confounding effects of location and control for the repeated measurements of adjacent tissues (i.e. non-independence of adjacent and SWD samples), location and plant replicate were included as conditioning terms in the RDA model, so that variation attributable to these factors could be removed from the analysis. Prior to analysis, predicted functional categories that were perfectly correlated were removed from the dataset so that a single, representative function per group of correlated functions was kept for further analysis. The Hellinger transformation (Legendre and Gallagher 2001) was performed on the remaining data. Loadings for predicted functional categories from the RDA and the relative position of sample scores within tissue type were then used to determine which functions most contributed to the differences between tissue types. Values of the samples for the first and second RDA axes were subsequently tested for statistical significance, using a linear model and ANOVA. Where the tissue term was significant, pair-wise tests were performed to assess which tissues differed significantly, and p-values were corrected for multiple testing using the Benjamini-Hochberg's FDR approach. The nested conditioning factor (i.e. plant replicates nested within sites) made using RDA with site as a constraining factor infeasible. Therefore, we used a similar approach to test for differences in predicted functions between the two sampling locations: principal components analysis (PCA). Here, PCA was conditioned on plant replicate, using the same dataset and transformation as those used for the RDA. The first and second PCA axes values were subsequently tested for statistical significance,

using linear model and ANOVA, to determine if significant patterns in the functional profiles existed between locations. These statistical analyses were conducted in R v4.0.2 (R Core Team 2020), using the packages vegan (Oksanen, Blanchet *et al.* 2019) and emmeans (Lenth, Singmann *et al.* 2020). RDA/PCA scores and, when applicable, relative abundances of predicted functions discriminating between sample types were visualized in biplots and beeswarm plots, respectively. These plots were generated in R v4.0.2 (R Core Team 2020) using the package beeswarm (Eklund 2016).

Results

Detection of L. zosterae in diseased plants

Using a PCR assay specific for the presence of *L. zosterae*, we detected *L. zosterae* in only four of 32 seagrass leaf tissue samples. Positive detections all occurred in putative SWD lesion samples from Rose Bay (four out of five technical replicates, see **Supplementary Fig. 1**). However, *L. zosterae* was not detected in any putative SWD tissues from Lake Macquarie.

Increased bacterial richness and diversity in SWD

A significant difference in both Chao1 ($\chi^2 = 23.08$, DF = 2, $p < 0.0001$) and Shannon's diversity ($\chi^2 = 7.98$, DF = 2, $p = 0.02$) among tissues was detected (**Fig. 2a** and **Supplementary Table 3**). Microbiomes associated with SWD lesions displayed significantly higher levels of richness compared to both healthy ($p < 0.0004$) and adjacent tissues ($p = 0.02$), and greater diversity relative to healthy tissues ($p < 0.03$). Both Chao1 ($\chi^2 = 12.69$, DF = 1, $p = 0.0004$) and Shannon's indices ($\chi^2 = 9.51$, DF = 1, $p = 0.002$) were significantly lower at Rose Bay relative to Lake Macquarie (**Fig. 2b** and **Supplementary Table 3**). No significant tissue x site interaction was found for Chao1 ($\chi^2 = 2.01$, DF = 2, $p = 0.4$) or Shannon's index ($\chi^2 = 2.06$, DF = 2, $p = 0.4$). Taken together, these results indicate that microbiomes associated with SWD lesions are more diverse, and that this pattern is not necessarily linked to the detection of *L. zosterae* in seagrass leaves.

The leaf microbiome displays variable community composition

A significant tissue x site interaction was found in community composition (permutational ANOVA: $F_{2,31} = 1.73$, $p = 0.02$), as well as significant differences between both tissue types (permutational ANOVA: $F_{2,31} = 4.92$, $p = 0.0001$) and sites (permutational ANOVA: $F_{1,31} = 5.30$, $p = 0.0001$). All tissue types were significantly different at Rose Bay (p values < 0.03), while at Lake Macquarie only samples from healthy and adjacent tissues ($p = 0.03$), and from healthy and SWD tissues ($p = 0.02$) differed significantly between each other (**Fig. 3** and **Supplementary Table 4**). The extent of these differences between healthy and SWD tissues (i.e. effect) was stronger at Rose Bay ($p = 0.002$), where *L. zosterae* was detected, and weaker at Lake Macquarie ($p = 0.02$). These statistical patterns were apparent in the nMDS plot (**Fig. 4**), whereby three discrete clusters of samples, associated with the different seagrass leaf tissue types, were clearly differentiated. Within this plot, samples associated with healthy leaf tissues at Rose Bay exhibited tighter clustering than the other two tissue types, which is consistent with the higher statistical similarities (SIMPER analyses) that we observed within healthy leaves (average similarity = 36%) when compared to adjacent (average similarity = 22%) and SWD tissues (average similarity = 17%) (see **Supplementary Table 4**). The opposite trend was observed in samples from Lake Macquarie, where SWD tissues were more similar to each other (average similarity = 41%) and clustered tighter in the MDS plot than either adjacent (average similarity = 27%) or SWD samples (average similarity = 25%). Notably, the absence of *L. zosterae* in one of the samples at Rose Bay (point distant from the SWD cluster in **Fig. 4**) resulted in the higher inter-replicate variability within SWD tissues that we observed at Rose Bay. Our comparisons across the two sampling sites showed that bacterial communities were similar on healthy samples at both sites (permutational ANOVA: $F_{1,11} = 1.35$, $p = 0.3$), but SWD-associated communities (permutational ANOVA: $F_{1,9} = 3.75$, $p = 0.007$) and assemblages from adjacent tissues (permutational ANOVA: $F_{1,9} = 3.12$, $p = 0.007$) differed significantly between sites. Altogether, these results indicate that microbiomes associated with healthy tissues are more similar between sites than the communities associated with adjacent tissues or SWD lesions, and that the discrimination of tissues is clearer when *L. zosterae* is detected.

We used a co-occurrence network approach to characterise the level of specificity of OTUs associated with healthy, adjacent and SWD lesion microbiomes by identifying 'unique' and 'shared' associates across the three different seagrass tissue types (**Fig. 5**). Unique OTUs were defined here as OTUs exclusively associated with only a single seagrass tissue type, whereas those that were associated with two or all three tissue types were classified as shared OTUs. To remove the potential spurious impact of rare sequences, we only considered as 'unique' those microorganisms present in more than two samples. Following removal of seagrass sequences and rarefication, a total of 299 OTUs were identified across all samples, and 6% of these were unique to an individual seagrass tissue. Of these 19 unique OTUs, 2 (10%), 2 (10%) and 15 (80%) OTUs were associated with healthy, adjacent and SWD lesion samples, respectively. The unique OTUs cumulatively spanned more than 13 bacterial orders and 8 classes across 6 phyla. On the other hand, 22% of the OTUs were shared across all three seagrass tissues (nodes in the centre of the network in **Fig. 5**), of which a single OTU affiliated with the genus *Algitalea* was the most abundant bacteria. Moreover, 27% of all OTUs were associated with only two tissue types. Of these, 2% occurred in both healthy and adjacent leaves, 7% in both healthy and SWD lesions, and 18% in both adjacent and SWD lesions.

The variability within bacterial assemblages across the different seagrass tissues was driven by several 'discriminatory' bacteria, here defined as OTUs that contributed > 1% to the differences between tissue types using SIMPER analyses (see **Supplementary Table 5**). Two OTUs classified as members of the *Pseudomonas* genus were the principal drivers of the differences between all three tissues due to their relative over-representation within both the healthy and adjacent microbiomes, relative to the SWD lesion tissues. A differential over-representation of these OTUs within the healthy and adjacent microbiomes substantiated their contribution to the differences between the two tissues. Additionally, a single OTU classified as *Burkholderia* was over-represented in the healthy tissues, relative to the adjacent and SWD lesion samples. On the other hand, two OTUs classified as members of the *Saprospiraceae* family were over-represented within the SWD lesion microbiomes, relative to those associated with healthy tissues. Two OTUs belonging to the *Rubidimonas* genus were most responsible

for the differences in community composition between the adjacent tissue and the other two sample types, given that these microorganisms were more abundant within the adjacent tissue-associated microbiomes. Notably, when only the microbiomes associated with SWD lesions were compared between sites, two OTUs affiliated with the bacterial genera *Schizothrix* and *Hellea* dominated the communities of samples collected at Lake Macquarie (representing 12% and 11% of these microbiomes, respectively, **Fig. 3**), and contributed the most (3.2% and 3.0%, respectively) to the significant ($p = 0.00044$) differences observed between SWD samples from Lake Macquarie and Rose Bay.

Disease-associated core microbiomes are not maintained at large spatial scales

To further characterise the differences between the microbiomes of the three tissue types, we identified persistent members of microbiomes associated with healthy, adjacent and SWD lesion tissues (**Fig. 6**). These core microorganisms are considered potentially likely to impart critical ecological functions to the host due to their preservation within each community (i.e. core microbiome)(Shade and Handelsman 2012). We identified three OTUs that were maintained across all healthy leaves collected at both sampling locations. These OTUs were affiliated with the genus *Burkholderia*, the family *Cryomorphaceae*, and the SAR11 clade within the *Alphaproteobacteria*. Within the SWD lesion samples, only a single OTU from the *Arenicella* genus could be characterised as a core microbiome member, whereas no core OTUs were observed across the adjacent tissue samples. No single OTU was maintained across all sample types (see **Supplementary Fig. 2a**), meaning that no overall 'seagrass leaf core microbiome' was found for these three different stages of the development of SWD. Similarly, no core microbiomes were observed within each sampling location (see **Supplementary Fig. 2b**). However, it should be noted that low replication limits the power of our approach to identify core microbiomes and may consequently reduce the number of OTUs classified as core members within a given environment. Therefore, we recommend bigger sample sizes for future studies that aim to characterise the core microbiome.

Predicted functional potential differs amongst tissue types and sites

We calculated putative functional group abundance profiles from prokaryotic taxon abundance profiles, using the method developed by Louca, Parfrey *et al.* (2016), whereby individual microorganisms were associated with previously annotated metabolic functions of particular ecological relevance for marine and lake biogeochemistry. Predicted functional profiles were subsequently compared across sample types to identify 'discriminatory' metabolic or ecologically relevant functions between the three seagrass tissues and the two sampling locations, separately. Out of the 299 OTUs, 31% were assigned to 29 functional groups.

Putative functional profiles differed significantly between seagrass tissues on both the RDA1 ($F_{2,29} = 21.96$, $p < 0.0001$) and RDA2 ($F_{2,29} = 13.30$, $p < 0.0001$) axes. These tissue-to-tissue differences were clearly evidenced in RDA clustering patterns mimicking those we observed for community composition (**Fig. 7**). Predicted functional profiles of healthy leaf tissues and SWD lesion samples were significantly different on the RDA1 axis ($p < 0.0001$), with 9 putative functional categories most contributing to the differences between these two tissue types (see **Supplementary Table 6**). More specifically, five functional categories were more abundant in the healthy samples, relative to SWD tissues. These included 'nitrate respiration' (2 OTUs) and 'nitrate reduction' (3 OTUs). On the other hand, 'oxygenic phototrophy' and associated functions (15 OTUs), including 'cyanobacteria', 'photoautotrophy', and 'phototrophy' were more abundant in SWD lesion samples than in healthy tissues. Adjacent tissues differed significantly from healthy ($p = 0.039$) and SWD tissues ($p = 0.0003$) and showed intermediate abundance levels of these functional categories. The significant separation among tissue types on the RDA2 axis was driven by higher abundance of the functional category 'intracellular parasites' in adjacent tissues, relative to healthy leaves ($p = 0.0002$) and tissues exhibiting SWD-like lesions ($p = 0.0002$). In this axis, no significant differences were found between healthy and SWD tissues ($p = 0.9$). Predicted functional profiles were also compared between the two sampling locations, but no differences were found when testing axes of conditioned PCA for statistical significance (**Supplementary Fig. 3** and **Supplementary Table 7**).

Discussion

Seagrass cover is declining across the globe at an accelerated rate (Waycott, Duarte *et al.* 2009), with seagrass losses in several regions attributed to SWD (Sullivan, Trevathan-Tackett *et al.* 2018). However, our understanding of the specific mechanisms underlying disease progression and the potential interplay between *L. zosterae* and other members of the seagrass microbiome is very limited. Here, we characterised the bacterial communities associated with healthy leaf tissues and SWD lesions in *Z. muelleri*, an approach extensively used to ascertain the role of microbes in disease initiation and the subsequent progression of the infection process (Gilbert, Quinn *et al.* 2016; Hamady and Knight 2009). We hypothesized significant differences between healthy microbiomes and those associated with SWD lesions, and the predominance of opportunistic bacteria within seagrass tissues showing SWD symptoms. In other benthic marine organisms, changes in the microbiome of healthy individuals that take place gradually through disease progression have been observed irrespective of the intrusion of an aetiological agent (Lloyd and Pespeni 2018; Longford, Campbell *et al.* 2019), and potential pathogens often occur exclusively in diseased phenotypes (Lee, Davy *et al.* 2015; Roder, Arif *et al.* 2014; Rosenberg, Kushmaro *et al.* 2009; Webster 2007; Webster, Xavier *et al.* 2008).

SWD is not always caused by L. zosterae

L. zosterae was only detected in samples putatively classified as SWD that were collected at Rose Bay, while putative SWD-like symptoms at Lake Macquarie were not explained by the presence of *L. zosterae*. Notably, this pattern was accompanied by significant differences between the microbiomes of SWD samples collected at Rose Bay and Lake Macquarie. We propose that at Rose Bay, the SWD tissues experienced an infection by *L. zosterae*, followed by a shift in the bacterial community as a (secondary) response to *L. zosterae* infection. On the other hand, the significant bacterial shifts that we observed between healthy and SWD samples from Lake Macquarie occurred in the absence of *L. zosterae* and might therefore reflect either a role of the microbiome in the initiation of this SWD syndrome or a secondary microbial response to an as yet undefined cause of SWD. The possibility that pathogenic eukaryotes other than *L. zosterae* may cause the SWD-like symptomatology that we observed remains to be investigated. Therefore, our results indicate both that infection by *L. zosterae* can lead

to measurable shifts in the seagrass microbiome, and that SWD-type symptoms can occur in the absence of *L. zosterae*.

Given their rapid response to environmental change, microbes have been proposed as early warning indicators for environmental stress and coral reef health (Glasl, Webster *et al.* 2017). Similarly, it has been proposed that microbial communities associated with seagrasses could be used as descriptors of ecological status in seagrass meadows, based on variations in the relative abundance of some bacterial phylotypes in response to local environmental conditions (Mejia, Rotini *et al.* 2016). Our observations of both SWD symptoms and conspicuous changes in the microbiome associated with SWD lesions in the absence of *L. zostearae* are consistent with previously suggested approaches to monitor marine ecosystems that incorporate microbial communities as a putative bioindicator. The microbial shifts observed here are indicative of the potential to use seagrass epiphytic bacterial communities as a diagnostic tool for assessing seagrass health, which needs further investigation.

Schizothrix and *Hellea* dominated the communities associated with putative SWD lesions collected at Lake Macquarie, where *L. zosterae* was absent. It is notable that the only species within the *Hellea* genus, *H. balneolensis*, has previously been observed within epiphytic bacterial communities associated with macroalgae (Tujula, Crocetti *et al.* 2010), and has been shown to be particularly enriched in samples from *Delisea pulchra* (*D. pulchra*) suffering from a seaweed bleaching disease (Zozaya-Valdés, Roth-Schulze *et al.* 2017). Therefore, we suggest that *H. balneolensis* might be an alternative cause of SWD lesions in *Z. muelleri*. Nevertheless, the possibility that negative PCR amplifications resulted from other potentially pathogenic eukaryotes at Lake Macquarie should be further investigated. Given that our classification of putative SWD samples was based on previous descriptions of macroscopic SWD-like symptomatology, it is also possible that the lesions observed in putative SWD samples from Lake Macquarie could be physiological responses not necessarily associated with SWD.

SWD lesions harbour more diverse bacterial communities

SWD-associated microbiomes showed significantly higher levels of microbial richness (Chao1 index) and diversity (Shannon's index) compared to the communities associated with healthy samples. This is in contrast with the decreased microbial diversity typically associated with disease or preceding dysbiosis events (Abrahamsson, Jakobsson *et al.* 2014; Candela, Rampelli *et al.* 2012; Dobbler, Procianoy *et al.* 2017; Frank, Amand *et al.* 2007; Kusstatscher, Cernava *et al.* 2019; Lloyd and Pespeni 2018). However, there are also examples of increased microbial diversity in diseased tissues of marine benthic organisms such as corals (Closek, Sunagawa *et al.* 2014; Roder, Arif *et al.* 2014; Sunagawa, DeSantis *et al.* 2009) and macroalgae (Fernandes, Steinberg *et al.* 2012). In these systems, the lower microbial diversity in healthy microenvironments possibly reflects the response of opportunistic microbes to changes in the local environment. Similar associations between higher microbial diversity and disease have also been observed in terrestrial plants (Luo, Ran *et al.* 2010; Shen, Penton *et al.* 2018).

We suggest that the increase in microbiome diversity observed in SWD lesion tissues is perhaps indicative of colonisation by multiple opportunistic microorganisms after the initial infection event, rather than by a single aetiological agent, which might be expected to eventually dominate the microbiome of infected tissue. A proposed potential mechanism for this response involves, as previously well-documented, an initial infection by *L. zosterae*, which penetrates the plant tissue while triggering metabolic changes induced in the seagrass host (e.g. release of secondary metabolites with antimicrobial properties (Arnold and Targett 2002), near SWD lesions (Steele, Caldwell *et al.* 2005; Vergeer, Aarts *et al.* 1995; Vergeer and Develi 1997)). Other chemical changes associated with SWD lesions could also influence bacterial community diversity within these tissues. For instance, decomposition of infected tissues could mobilise organic matter (Opsahl and Benner 1993), resulting in increased bacterial activity (Blum and Mills 1991) and potential shifts in community composition. Several nutrients are known to be released from seagrass necromass, including organic and inorganic forms of carbon, nitrogen and phosphorus, phenolics and lipids (Prasad, Ganguly *et al.* 2019; Walker, Pergent *et al.* 2001); all of which could be mineralised by different bacterial and archaeal communities, leading to greater bacterial diversity (Trevathan-Tackett, Jeffries *et al.* 2020).

The preservation of the Z. muelleri microbiome across sites is lost under SWD conditions

Our two sampling locations were separated by 120 km and characterised by dissimilar environmental conditions (see **Supplementary Tables 1** and **2**). When microbiomes associated with each tissue type were compared across sites, no significant differences between healthy samples were found. In contrast, SWD lesions and samples taken from tissues immediately adjacent to lesions differed significantly in their composition across locations. Consistent with this, healthy samples displayed bacterial community structures that were more similar to each other (SIMPER analyses, average similarity = 29%) than either the microbiomes of SWD (average similarity = 16%) or adjacent (average similarity = 13%) samples (see **Supplementary Table 4**). This is consistent with previous observations in seaweed-associated bacterial and archaeal communities, whereby disease shaped microbiome composition to an extent that was comparable to geographic shifts in assemblage structure (Marzinelli, Campbell *et al.* 2015). However, the authors of this previous work observed opposite patterns in the kelp species *Ecklonia radiata*, whereby microbial communities on stressed individuals were more similar to each other among locations than those on healthy hosts.

The similarities between healthy samples observed here are also in line with our previous observations of the regional conservation of leaf-associated bacterial assemblages within the *Z. muelleri* microbiome (Hurtado-McCormick, Kahlke *et al.* 2019). Indeed, the composition of bacterial communities associated with healthy leaves resembles that seen in our previous study, as members of the *Burkholderiaceae* and *Pseudomonadaceae* (known to discriminate the seagrass leaf from other seagrass and surrounding microenvironments) also dominated the healthy phyllosphere studied here and were over-represented in these tissues. In other 16S rRNA gene studies on seagrasses, leaf-enriched taxa included the *Betaproteobacteria* and *Planctomycetia* classes (Fahimipour, Kardish *et al.* 2017), which we also observed largely represented in healthy leaf tissue samples (i.e. *Delfia* and *Blastopirellula*). Less abundant bacteria in healthy leaves collected at Lake Macquarie included *Winogradskyella, Taeseokella* and *Portibacter*. These families belong to the Bacteroidetes, a phylum that has been

669 previously reported on leaves of many *Zostera* species (Crump, Wojahn *et al.* 2018;
670 Kurilenko, Ivanova *et al.* 2007).
671
672 Our results indicate that the stability across sites of the healthy leaf microbiome was not
673 maintained in the SWD tissues, where we observed significant differences in bacterial
674 community composition between the two sites. Moreover, we observed higher levels of
675 inter-replicate variability within SWD samples (SIMPER analyses, average similarity =
676 16%), relative to healthy leaves (average similarity = 29%). These patterns are
677 consistent with those observed in the bleaching disease of the red macroalgae *D.*
678 *pulchra*, where microbiomes of bleached individuals are significantly more divergent
679 from one another than those of healthy algae (Kumar, Zozaya-Valdes *et al.* 2016). This
680 increase in community divergence has been previously correlated with disturbance to
681 natural community structure (O'Connor 2013; Rubal, Veiga *et al.* 2014; Séguin, Gravel
682 *et al.* 2014).
683
684 The increased heterogeneity in microbiome structure observed in SWD lesions was also
685 apparent in the adjacent tissues (SIMPER analyses, average similarity = 13%),
686 indicating that SWD influences the microbiome in the tissues adjacent to visible lesions.
687 Indeed, the significant differences in microbiome structure between the adjacent tissue
688 samples and both the healthy and SWD lesion samples at Rose Bay is perhaps
689 indicative of the existence of 'transitional communities' in these samples. This is
690 supported by our observation that the bacterial communities associated with
691 asymptomatic seagrass tissue adjacent to SWD lesions share proportions of members
692 with either healthy- (42%), SWD-associated assemblages (71%), or both (39%) larger
693 than the amount of OTUs exclusively associated with adjacent tissues (26%). In fact,
694 20% of the 299 OTUs were common (i.e. present in both) to adjacent and both healthy
695 and diseased tissues, whereas only 7% were common to healthy and diseased samples.
696 The potential transitional state of the adjacent tissues from Rose Bay was further
697 supported by the nMDS plot, showing an intermediate positioning of the adjacent tissue
698 samples between the healthy and SWD lesion samples from this specific sampling
699 location. A similar pattern has been reported in the macroalgal species *D. pulchra*,
700 whereby microbiomes associated with adjacent tissues lacking visible signs of

bleaching exhibited similarities to both healthy and bleached samples (Campbell, Harder *et al.* 2011). Adjacent and SWD samples from Lake Macquarie, however, did not display such a pattern and instead clustered together in the nMDS plot. This is consistent with the statistical similarities observed for the two tissues at this particular location and suggests that the proposed transitional state of the adjacent tissues is influenced by the presence of *L. zosterae,* which was only detected at Rose Bay.

A single OTU identified as *Algitalea* was the most abundant bacteria amongst shared OTUs. Despite not having been reported as seagrass associates, members of this genus are common and predominant within epiphyte bacterial communities associated with different species of macroalgae (Comba-González, Niño-Corredor *et al.* 2021; Paix, Carriot *et al.* 2020; Yoon, Adachi *et al.* 2015), and have also been isolated from coral back-reef-associated environments (Kegler, Hassenruck *et al.* 2018). Nevertheless, the proportion of shared OTUs (22%) in the co-occurrence network was markedly lower than that of the OTUs exclusively associated with a single tissue type (51%), indicating that microbiomes are mostly tissue specific. When excluding OTUs present in two or less samples, 6% of all OTUs were exclusively associated with a single tissue type, suggesting a low level of partitioning of microbes between the three tissues. Notably, proportions of these unique OTUs were highest (15 out of the 19 unique OTUs) within the SWD lesion samples relative to the other two tissue types, which is in line with our observations of higher levels of richness and diversity within these samples. We suggest that these patterns are indicative of plant colonisation of SWD lesions by diverse groups of opportunistic bacteria, rather than by a single aetiological agent. More specifically, we identified four key taxa that were responsible for the differences observed between the three tissue types, including *Pseudomonas*, *Burkholderia* and *Saprospiraceae*.

Marine *Pseudomonas* are well-recognised seagrass leaf-associated microbes (Lujan, Eisen *et al.* 2017; Mishra and Mohanraju 2018), and some species are well-characterised carboxydotrophic organisms with the ability to oxidize carbon monoxide in coastal marine environments (Tolli, Sievert *et al.* 2006). In addition, there is evidence of the antifouling potential of other species within the genus, such as *P. aeruginosa*, which inhibits the growth of other microorganisms in experimental coatings exposed to

seawater of different climatic zones (Kharchenko, Beleneva *et al.* 2012). Therefore, we speculate that the two *Pseudomonas* OTUs that we predominantly observed in healthy and adjacent tissues likely exploit sugars excreted from the leaf surface (Hirano and Upper 2000).

Many *Burkholderia* species are effective nodulating rhizobia with the ability to fix nitrogen (Elliott, Chen *et al.* 2007), and some of them are well recognised endophytic diazotrophic members within rhizosphere microbiomes in terrestrial plants (Govindarajan, Balandreau *et al.* 2008). Given this, and the extensive evidence for the presence (Crump, Wojahn *et al.* 2018; Garcias-Bonet, Arrieta *et al.* 2016; Weidner, Arnold *et al.* 2000) and potential importance of a wide diversity of diazotrophs in seagrass tissues (Patriquin 1972; Sun, Zhang *et al.* 2015; Welsh 2000), the *Burkholderia* OTU that we predominantly observed in healthy samples may play an important role in seagrass nitrogen acquisition. Moreover, our functional profiling results suggest that additional bacterial genera might be associated with nitrogen cycling processes within healthy seagrass leaves. Specifically, *Kordia* might undertake functions related with nitrate reduction, whereas *Pseudomonas* and *Stenotrophomonas* might be involved in both nitrate reduction and nitrogen respiration.

Previous 16S rRNA gene investigations have shown that members of the *Saprospiracea* family are common in marine environments (McIlroy and Nielsen 2014) and important members of the seagrass phyllosphere (Ugarelli, Laas *et al.* 2019). Notably, the *Saprospiraceae* discriminate between microbial communities of bleached and healthy *D. pulchra*, with OTUs assigned to the family more abundant in bleached samples (Zozaya-Valdes, Egan *et al.* 2015). Along with other algicidal bacteria within the *Cytophaga*/*Flavobacterium*/*Bacteroidetes* (CFB) group, these bacteria have been suggested as potential macroalgal pathogens (Zozaya-Valdes, Egan *et al.* 2015). This implies that the two *Saprospiracea* OTUs that we predominantly observed within SWD tissues may cause harm to the seagrass host.

Taken together, these results show that the preservation of the seagrass microbiome across different sites, associated with healthy leaves ($p = 0.3$, see **Supplementary**

765 **Table 4**), is lost under the conditions of SWD. This is due to clear compositional shifts
766 of bacterial assemblages from healthy to diseased plants at both sampling locations.
767 According to our statistical analyses, these between-tissues shifts are larger (average
768 similarities <15%) than those observed within healthy tissues across sites (average
769 similarity = 28%). This is in line with the evident discrimination of microbiomes
770 associated with the three different tissue types studied here, and it is driven by four
771 predominant bacterial taxa that have been previously reported in similar studies as
772 predominant and even discriminatory microorganisms within the seagrass phyllosphere.
773 We therefore investigated the possible existence of 'core' members within these very
774 distinct communities to point out potential functionally relevant members.
775
776 *Core members change transiently from healthy to diseased plants*
777 Across a broad range of host-microbe systems, the 'core microbiome' has been defined
778 as the assemblage of microbes that displays spatio-temporal persistence, implying that it
779 is likely to impart key functions for the development, health and physiology of its host
780 (Björk, O'Hara *et al.* 2018). Changes in the core microbiome have been related to
781 diseases in a range of benthic marine hosts. For instance, the depletion of healthy-state
782 core associated taxa in conjunction with a substantial increase of bacterial diversity in
783 diseased tissues is common in gorgonian corals (i.e. sea fans of the *Pacifigorgia* spp.)
784 affected by a necrotic-patch disease (Quintanilla, Ramirez-Portilla *et al.* 2018).
785 Comparable drastic changes in the entire microbiome (e.g. differential abundance of
786 discriminatory OTUs) of corals affected by Yellow Band Disease (Closek, Sunagawa *et*
787 *al.* 2014), White Plague Disease (Roder, Arif *et al.* 2014; Sunagawa, DeSantis *et al.*
788 2009), Black Band Disease (Meyer, Gunasekera *et al.* 2016), and in necrotic and
789 unusual coral lesions (Meyer, Paul *et al.* 2014; Ransome, Rowley *et al.* 2014) have also
790 been reported elsewhere. Moreover, there is evidence of a specific signature of
791 microbial community shifts that correlates with the different stages of the progression of
792 Sea Star Wasting Disease (Lloyd and Pespeni 2018).
793
794 Here, we defined core microbiomes associated with each tissue type across both
795 sampling locations, and core communities associated with each site across all three
796 tissue types. We did not observe a unified core microbiome across all three tissues or

across the two sites. The absence of a unified core microbiome across all sampled tissue types indicates that the seagrass tissues examined here represent markedly different microbial niches and, therefore, that SWD causes pronounced changes in the inherent characteristics of the *Z. muelleri* leaf microbiome. We identified three core OTUs that were maintained within healthy tissues across the two sites, including members of the *Burkholderia*, *Cryomorphaceae*, and SAR11 clade. As *Burkholderia* is a predominant family within healthy-associated assemblages, its presence in the core microbiome reinforces its potential importance in the seagrass leaf. This is in line with our previous identification of an OTU from the *Burkholderiales* order as a core member of the seagrass lower leaf microbiome (Hurtado-McCormick, Kahlke *et al.* 2019). The other two core members, *Cryomorphaceae* and the SAR11 clade, are dominant bacteria within pelagic microbial assemblages (Bowman 2014; Giovannoni 2017).

The lack of a core microbiome in the adjacent tissue samples provides further support to our proposition that these samples might represent a transitory state between the healthy and diseased status of the seagrass host, whereby higher levels of spatio-temporal heterogeneity and stochasticity might be expected. Indeed, adjacent samples were the most dissimilar between each other (only 14% of average similarity) of the three tissue types. Within SWD lesions, a single core OTU was observed and identified as a member of the *Arenicella* genus. Described species within this genus include aerobic, non-motile bacteria that have been isolated from marine sediments (Romanenko, Tanaka *et al.* 2010) and a range of benthic macroorganisms, including sea urchins (Nedashkovskaya, Cleenwerck *et al.* 2013), lobsters (Feinman, Martínez *et al.* 2017), oysters (Garcia Bernal, Trabal Fernandez *et al.* 2016) and brown algae (Balakirev, Krupnova *et al.* 2012). In macroalgae, the *Arenicella* genus is associated with the meristem of distinct algal morphological forms adapted to different depths and is not always form-specific (Balakirev, Krupnova *et al.* 2012). Notably, members of *Arenicella* have been implicated in necrotic disease in some benthic animals (Feinman, Martínez *et al.* 2017; Whitten, Davies *et al.* 2014).

These patterns are indicative of conspicuous shifts in core members of the microbiome between healthy and SWD-affected individuals, which are concomitant with changes in

predominant microbes within the seagrass phyllosphere. To provide further insight into the processes associated with these shifts and their potential implications, we used taxonomy-based functional profiling.

Functional shift from heterotrophy to autotrophy

Microbial community shifts between healthy and SWD seagrass leaf tissues were also apparent when we compared predicted functional profiles. Relative to healthy tissues, autotrophic functions affiliated with phototropism and photosynthesis were over-represented within SWD predicted profiles. Contrastingly, heterotrophic functions associated with nitrate respiration/reduction were under-represented within SWD tissues relative to healthy leaves. These results indicate that reduction of nitrogen cycling functions may be one of the consequences of SWD progression in *Z. muelleri*, which is notable considering that seagrass primary production is largely regulated by nitrogen availability (Capone and Taylor 1980; Herbert 1999). This putative functional shift is consistent with our observations of an increase in *Cyanobacteria*, from 1.3% (relative abundance, 2 OTUs) within healthy samples to 14.5% (14 OTUs) within SWD lesion-associated microbiomes. Similar microbial shifts to assemblages dominated by *Cyanobacteria* have been observed in other diseases in marine benthic organisms, such as corals, where *Cyanobacteria* proliferate within individuals infected with Black Band Disease (Meyer, Gunasekera *et al.* 2016).

Our predicted functional analysis also revealed evidence for an increase in functions associated with intracellular parasites within the adjacent tissues, with OTUs affiliated with this functional category belonging to the order Rickettsiales. Notably, several plant diseases, including the papaya bunchy top disease (Davis, Ying *et al.* 1998) and yellows-type diseases in strawberries (Streten, Waite *et al.* 2005) have been associated with Rickettsia-like organisms.

Final remarks

Our results provide evidence for a conspicuous shift of the seagrass leaf microbiome, probably in response to the changing conditions caused by SWD. We demonstrated that such changes occur at both the taxonomic and putative functional levels, leading to

substantially distinct bacterial communities of diseased vs. healthy individuals of *Z. muelleri*. We also observed measurable shifts in the specificity and stability of different bacterial taxa associated with healthy, adjacent and SWD lesion tissues, and these patterns were also apparent when we examined the core microbiome of different tissue types. The lack of evidence for an increase in bacterial pathogenic and virulent functions within tissues affected by SWD, as well as the increased bacterial richness and diversity that we observed within SWD tissues, are not supportive of a bacterial-mediated infection. Nevertheless, the predominance of *Hellea* within the microbiomes of SWD samples collected at Lake Macquarie, where *L. zosterae* was not detected, might suggest the influence of a prokaryote in the initiation and/or development of SWD. On the other hand, other eukaryotic species elsewhere implicated as potential, albeit rarer, causes of SWD (e.g. *Phytomyxea* (Walker and Campbell 2009) and *Phytophthora* (Govers, Man-in-'t-Veld *et al.* 2016)) may also have contributed to SWD symptoms in the samples where *Labyrinthula* was not present. Our observations that drastic changes in the microbiome and SWD-type symptoms still occur in the absence of *L. zosterae* suggest that microbial shifts could potentially be used as a diagnostic tool to assess seagrass health. However, there is also the possibility that, in samples where *L. zosterae* was not detected, the lesions observed are not necessarily related with SWD and could have been caused by other processes including grazing.

While we did not observe clear evidence for the involvement of a seagrass bacterial pathogen in the development of SWD, our results point to a marked shift in the microbiome of putatively infected leaves, which is indicative of a dysbiosis event. We propose that this shift might result from the opportunistic colonisation by bacteria, presumably after infection by *L. zosterae* and the subsequent appearance of the SWD-type symptomatology observed. Nevertheless, our observations of shifts in bacterial assemblages within putative SWD tissues where *L. zosterae* was not detected suggest that changes in the microbiome may not necessarily depend on infection by *L. zosterae*. Additional studies of SWD events across geographically disparate sampling sites, with increased intra-site replication, would provide insights into how local environmental conditions influence the disease process and its associated changes in the microbiome.

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Conflict of interest

The authors declare no conflicts of interest.

Other information

Data availability

Raw sequencing data (i.e. dataset of files in FASTQ format) generated during and analysed during the current study are available in the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA542187 (Hurtado-McCormick 2019).

Author contributions

VH-M conceived the study, designed the sampling strategy, conducted the fieldwork and lab work, developed the methodological approaches, analysed and interpreted the data, drafted the manuscript, prepared the figures and tables and obtained the approval of the final submission. DK performed the statistical analyses on diversity and functional data and provided essential support on their interpretation. BT supported the initial curation and quality-check of the data and made important contributions to the editing process of the final manuscript. PR supervised the study. JS conceived the study, designed the sampling strategy, provided the regular supervision of VH-M throughout the data analysis and interpretation, drafted the manuscript and substantially contributed to its intellectual content. All authors edited the final manuscript and agreed to approve the submitted version and be accountable for the content of the work.

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References

Abrahamsson, T.R., Jakobsson, H.E., Andersson, A.F., Björkstén, B., Engstrand, L., and Jenmalm, M.C. (2014) Low gut microbiota diversity in early infancy precedes asthma at school age. *Clinical & Experimental Allergy* **44**(6), 842-850.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic Local Alignment Search Tool. *Journal of Molecular Biology* **215**(3), 403-410.

Amend, A., Burgaud, G., Cunliffe, M., Edgcomb, V.P., Ettinger, C.L., Gutiérrez, M.H., Heitman, J., Hom, E.F.Y., Ianiri, G., Jones, A.C., Kagami, M., Picard, K.T., Quandt, C.A., Raghukumar, S., Riquelme, M., Stajich, J., Vargas-Muniz, J., Walker, A.K., Yarden, O., and Gladfelter, A.S. (2019) Fungi in the marine environment: open questions and unsolved problems. *mBio* **10**(2), e01189-18.

Arnold, T.M., and Targett, N.M. (2002) Marine tannins: the importance of a mechanistic framework for predicting ecological roles. *Journal of Chemical Ecology* **28**(10), 1919-1934.

Bagwell, C.E., La Rocque, J.R., Smith, G.W., Polson, S.W., Friez, M.J., Longshore, J.W., and Lovell, C.R. (2002) Molecular diversity of diazotrophs in oligotrophic tropical seagrass bed communities. *FEMS microbiology ecology* **39**(2), 113-119.

Balakirev, E.S., Krupnova, T.N., and Ayala, F.J. (2012) Symbiotic associations in the phenotypically-diverse brown alga *Saccharina japonica*. *PLoS ONE* **7**(6), e39587.

Bates, D., Mächler, M., Bolker, B.M., and Walker, S.C. (2015) Fitting linear mixed-effects models using lme4. *Journal of statistical software* **67**(1), 1-48.

Björk, J.R., O'Hara, R.B., Ribes, M., Coma, R., and Montoya, J.M. (2018) The Dynamic Core Microbiome: Structure, Stability And Resistance. *bioRxiv*, 137885.

Blakesley, B.A., Berns, D.M., Merello, M.F., Hall, M.O., and Hyniova, J. (2002) The dynamics and distribution of the slime mold *Labyrinthula* sp. and its potential impacts on *Thalassia testudinum* populations in Florida. In Seagrass Managenement: It's not just nutrients! Symposium. (Ed. HS Greening) pp. 199-207. (Tampa Bay Estuary Program: St. Petersburg, Florida, USA)

969 Blum, L.K., and Mills, A.L. (1991) Microbial growth and activity during the initial
970 stages of seagrass decomposition. *Marine Ecology Progress Series* **70**(1), 73-82.
971
972 Bockelmann, A., Beining, K., and Reusch, T.B.H. (2012) Widespread occurrence of
973 endophytic *Labyrinthula* spp. in northern European eelgrass *Zostera marina* beds.
974 *Marine Ecology Progress Series* **445**, 109-116.
975
976 Bowman, J.P. (2014) The Family *Cryomorphaceae*. In The Prokaryotes. (Eds. E
977 Rosenberg, EF DeLong, S Lory, E Stackebrandt and F Thompson). (Springer: Berlin,
978 Heidelberg)
979
980 Brakel, J., Werner, F.J., Tams, V., Reusch, T.B.H., and Bockelmann, A. (2014) Current
981 European *Labyrinthula zosterae* Are Not Virulent and Modulate Seagrass (*Zostera*
982 *marina*) Defense Gene Expression. *PLoS ONE* **9**(4), e92448.
983
984 Bull, J.C., Kenyon, E.J., and Cook, K.J. (2012) Wasting disease regulates long-term
985 population dynamics in a threatened seagrass. *Oecologia* **169**(1), 135-142.
986
987 Burge, C.A., Kim, C.J.S., Lyles, J.M., and Harvell, C.D. (2013) Special issue oceans
988 and humans health: the ecology of marine opportunists. *Microbial ecology* **65**(4), 869-
989 879.
990
991 Campbell, A.H., Harder, T., Nielsen, S., Kjelleberg, S., and Steinberg, P.D. (2011)
992 Climate change and disease: bleaching of a chemically defended seaweed. *Global*
993 *Change Biology* **17**(9), 2958-2970.
994
995 Candela, M., Rampelli, S., Turroni, S., Severgnini, M., Consolandi, C., De Bellis, G.,
996 Masetti, R., Ricci, G., Pession, A., and Brigidi, P. (2012) Unbalance of intestinal
997 microbiota in atopic children. *BMC Microbiology* **12**(1), 95.
998
999 Capone, D.G., and Taylor, B.F. (1980) Microbial nitrogen cycling in a seagrass
1000 community. *Estuarine Perspectives*, 153-161.
1001
1002 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello,
1003 E.K., Fierer, N., Gonzalez Peña, A., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley,
1004 S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge,
1005 B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A.,
1006 Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. (2010) QIIME allows
1007 analysis of high-throughput community sequencing data. *Nature Methods* **7**(5), 335-
1008 336.
1009
1010 Chitrampalam, P., Goldberg, N., and Olsen, M.W. (2015) *Labyrinthula* species
1011 associated with turfgrasses in Arizona and New Mexico. *European Journal of Plant*
1012 *Pathology* **143**(3), 485-493.
1013
1014 Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community
1015 structure. *Australian Journal of Ecology* **18**, 117-143.
1016

Clarke, K.R., and Gorley, R.N. (2015) 'PRIMER v7: User Manual/Tutorial.' (PRIMER-E Ltd: Plymouth, UK)

Clarke, K.R., Gorley, R.N., Somerfield, P.J., and Warwick, R.M. (2014) 'Change in marine communities: an approach to statistical analysis and interpretation.' 3rd edn. (Primer-E Ltd: Plymouth, UK)

Closek, C.J., Sunagawa, S., DeSalvo, M.K., Piceno, Y.M., DeSantis, T.Z., Brodie, E.L., Weber, M.X., Voolstra, C.R., Andersen, G.L., and Medina, M. (2014) Coral transcriptome and bacterial community profiles reveal distinct Yellow Band Disease states in *Orbicella faveolata*. *The ISME Journal* **8**(12), 2411-2422.

Comba-González, N.B., Niño-Corredor, A.N., López-Kleine, L., and Montoya-Castaño, D. (2021) Temporal changes of the epiphytic bacteria community from the marine macroalga *Ulva lactuca* (Santa Marta, Colombian-Caribbean). *Current Microbiology* **78**, 534-543.

Crump, B.C., Wojahn, J.M., Tomas, F., and Mueller, R.S. (2018) Metatranscriptomics and Amplicon Sequencing Reveal Mutualisms in Seagrass Microbiomes. *Frontiers in microbiology* **9**, 388.

Davis, M.J., Ying, Z., Brunner, B.R., Pantoja, A., and Ferwerda, F.H. (1998) Rickettsial relative associated with papaya bunchy top disease. *Current Microbiology* **36**(2), 80-84.

Dobbler, P.T., Procianoy, R.S., Mai, V., Silveira, R.C., Corso, A.L., Rojas, B.S., and Roesch, L.F.W. (2017) Low microbial diversity and abnormal microbial succession is associated with necrotizing enterocolitis in preterm infants. *Frontiers in Microbiology* **8**, 2243.

Eklund, A. (2016) beeswarm: the bee swarm plot, an alternative to stripchart. R package. 0.2.3 edn.

Elliott, G.N., Chen, W., Chou, J., Wang, H., Sheu, S., Perin, L., Reis, V.M., Moulin, L., Simon, M.F., Bontemps, C., Sutherland, J.M.B., R., de Faria, S.M., Trinick, M.J., Prescott, A.R., Sprent, J.I., and James, E.K. (2007) *Burkholderia phymatum* is a highly effective nitrogen-fixing symbiont of *Mimosa* spp. and fixes nitrogen *ex planta*. *New Phytologist* **173**(1), 168-180.

Fahimipour, A.K., Kardish, M.R., Lang, J.M., Green, J.L., Eisen, J., and Stachowicz, J. (2017) Global-Scale Structure of the Eelgrass Microbiome. *Applied and Environmental Microbiology* **83**(12), e03391-16.

Feinman, S.G., Martínez, A.U., Bowen, J.L., and Tlusty, M.F. (2017) Fine-scale transition to lower bacterial diversity and altered community composition precedes shell disease in laboratory-reared juvenile American lobster. *Diseases of Aquatic Organisms* **124**(1), 41-54.

Fernandes, N., Steinberg, P., Rusch, D., Kjelleberg, S., and Thomas, T. (2012) Community structure and functional gene profile of bacteria on healthy and diseased thalli of the red seaweed *Delisea pulchra*. *PLoS ONE* **7**(12), e50854.

Frank, D.N., Amand, A.L.S., Feldman, R.A., Boedeker, E.C., Harpaz, N., and Pace, N.R. (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences* **104**(34), 13780-13785.

Garcia Bernal, M., Trabal Fernandez, N., Saucedo Lastra, P.E., Medina Marrero, R., and Mazón-Suástegui, J.M. (2016) *Streptomyces* effect on the bacterial microbiota associated to *Crassostrea sikamea* oyster. *Journal of Applied Microbiology* **122**(3), 601-614.

García-Martínez, M., López-López, A., Calleja, M.L., Marbà, N., and Duarte, C.M. (2009) Bacterial Community Dynamics in a Seagrass (*Posidonia oceanica*) Meadow Sediment. *Estuaries and Coasts* **32**(2), 276-286.

Garcias-Bonet, N., Arrieta, J.M., Duarte, C.M., and Marbà, N. (2016) Nitrogen-fixing bacteria in Mediterranean seagrass (Posidonia oceanica) roots. *Aquatic Botany* **131**, 57-60.

Ghosh, U., Subhashini, P., Dilipan, E., Raja, S., Thangaradjou, T., and Kannan, L. (2012) Isolation and characterization of phosphate-solubilizing bacteria from seagrass rhizosphere soil. *Journal of Ocean University of China* **11**(1), 86-92.

Giesen, W.B.J.T., van-Katwijk, M.M., and den-Hartog, C. (1990) Temperature, salinity, insolation and wasting disease of eelgrass (*Zostera marina* L.) in the Dutch Wadden Sea in the 1930's. *Netherlands Journal of Sea Research* **25**(3), 395-404.

Gilbert, J.A., Quinn, R.A., Debelius, J., Xu, Z.Z., Morton, J., Garg, N., Jansson, J.K., Dorrestein, P.C., and Knight, R. (2016) Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature* **535**(7610), 94-103.

Giovannoni, S.J. (2017) SAR11 bacteria: the most abundant plankton in the oceans. *Annual Review of Marine Science* **9**, 231-255.

Glasl, B., Webster, N.S., and Bourne, D.G. (2017) Microbial indicators as a diagnostic tool for assessing water quality and climate stress in coral reef ecosystems. *Marine Biology* **164**(4), 91.

Glöckner, F.O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., Bruns, G., Yarza, P., Peplies, J., Westram, R., and Ludwig, W. (2017) 25 years of serving the community with ribosomal RNA gene reference databases and tools. *Journal of Biotechnology* **261**, 169-176.

Govers, L.L., Man-in-'t-Veld, W.A., Meffert, J.P., Bouma, T.J., van-Rijswick, P.C.J., Heusinkveld, J.H.T., Orth, R.J., van-Katwijk, M.M., and van-der-Heide, T. (2016)

Marine *Phytophthora* species can hamper conservation and restoration of vegetated coastal ecosystems. *Proceedings of the Royal Society B: Biological Sciences* **283**(1837), 20160812.

Govindarajan, M., Balandreau, J., Kwon, S., Weon, H., and Lakshminarasimhan, C. (2008) Effects of the inoculation of *Burkholderia vietnamensis* and related endophytic diazotrophic bacteria on grain yield of rice. *Microbial Ecology* **55**(1), 21-37.

Green, E.P., and Short, F.T. (2003) 'World atlas of seagrasses.' (University of California Press: Berkeley, California)

Groner, M.L., Burge, C.A., Couch, C.S., Kim, C.J.S., Siegmund, G., Singhal, S., Smoot, S.C., Jarrell, A., Gaydos, J.K., Harvell, C.D., and Wyllie-Echeverria, S. (2014) Host demography influences the prevalence and severity of eelgrass wasting disease. *Diseases of Aquatic Organisms* **108**(2), 165-175.

Groner, M.L., Burge, C.A., Kim, C.J.S., Rees, E., Van Alstyne, K.L., Yang, S., Wyllie-Echeverria, S., and Harvell, C.D. (2016) Plant characteristics associated with widespread variation in eelgrass wasting disease. *Diseases of Aquatic Organisms* **118**(2), 159-168.

Hamady, M., and Knight, R. (2009) Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. *Genome Research* **19**(7), 1141–1152.

Herbert, R.A. (1999) Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiology Reviews* **23**(5), 563-590.

Hernandez-Agreda, A., Gates, R.D., and Ainsworth, T.D. (2017) Defining the Core Microbiome in Corals' Microbial Soup. *Trends in Microbiology* **25**(2), 125-140.

Hirano, S.S., and Upper, C.D. (2000) Bacteria in the Leaf Ecosystem with Emphasis on *Pseudomonas syringae* - a Pathogen, Ice Nucleus, and Epiphyte. *Microbiology and Molecular Biology Reviews* **64**(3), 624-653.

Hollants, J., Leliaert, F., De-Clerck, O., and Willems, A. (2013) What we can learn from sushi: a review on seaweed–bacterial associations. *FEMS microbiology ecology* **83**(1), 1-16.

Hurtado-McCormick, V. (2019) PRJNA542187: Seagrass leaf microbiome associated with seagrass wasting disease (SWD, 16s amplicon sequencing). (National Center for Biotechnology Information (NCBI): Sequence Read Archive (SRA))

Hurtado-McCormick, V., Kahlke, T., Petrou, K., Jeffries, T., Ralph, P.J., and Seymour, J.R. (2019) Regional and Microenvironmental Scale Characterization of the *Zostera muelleri* Seagrass Microbiome. *Frontiers in Microbiology* **10**, 1011. [In English]

Jakobsson-Thor, S., Toth, G.B., Brakel, J., Bockelmann, A., and Pavia, H. (2018) Seagrass wasting disease varies with salinity and depth in natural *Zostera marina* populations. *Marine Ecology Progress Series* **587**, 105-115.

Jensen, S.I., Kuhl, M., and Prieme, A. (2007) Different bacterial communities associated with the roots and bulk sediment of the seagrass *Zostera marina*. *FEMS Microbiology Ecology* **62**(1), 108-117.

Kahlke, T. (2017) panbiom. Version 1.0 edn. (Gitbub. https://github.com/timkahlke/panbiom)

Kahlke, T. (2018) Ampli-Tools. Version 1.0 edn. (Zenodo. https://doi.org/10.5281/zenodo.1137872)

Kegler, H.F., Hassenruck, C., Kegler, P., Jennerjahn, T.C., Lukman, M., Jompa, J., and Gardes, A. (2018) Small tropical islands with dense human population: differences in water quality of near-shore waters are associated with distinct bacterial communities. *PeerJ* **6**, e4555.

Kharchenko, U., Beleneva, I., and Dmitrieva, E. (2012) Antifouling potential of a marine strain, *Pseudomonas aeruginosa* 1242, isolated from brass microfouling in Vietnam. *International Biodeterioration & Biodegradation* **75**, 68-74.

Kumar, V., Zozaya-Valdes, E., Kjelleberg, S., Thomas, T., and Egan, S. (2016) Multiple opportunistic pathogens can cause a bleaching disease in the red seaweed *Delisea pulchra*. *Environmental Microbiology* **18**(11), 3962-3975.

Kurilenko, V.V., Ivanova, E.P., and Mikhailov, V.V. (2007) Peculiarities of adhesion of epiphytic bacteria on leaves of the seagrass *Zostera marina* and on abiotic surfaces. *Microbiology* **76**(4), 502-506.

Küsel, K., Trinkwalter, T., Drake, H.L., and Devereux, R. (2006) Comparative evaluation of anaerobic bacterial communities associated with roots of submerged macrophytes growing in marine or brackish water sediments. *Journal of experimental marine biology and ecology* **337**(1), 49-58.

Kusstatscher, P., Cernava, T., Harms, K., Maier, J., Eigner, H., Berg, G., and Zachow, C. (2019) Disease incidence in sugar beet fields is correlated with microbial diversity and distinct biological markers. *Phytobiomes Journal* **3**, 22-30.

Kuznetsova, A., Brockhoff, P.B., and Christensen, R.H.B. (2017) lmerTest package: tests in linear mixed effects models. *Journal of statistical software* **82**(13), 1-26.

Lee, S.T.M., Davy, S.K., Tang, S., Fan, T., and Kench, P.S. (2015) Successive shifts in the microbial community of the surface mucus layer and tissues of the coral *Acropora muricata* under thermal stress. *FEMS Microbiology Ecology* **91**(12), fiv142.

Legendre, P., and Gallagher, E.D. (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia* **129**(2), 271-280.

Lenth, R., Singmann, H., Love, J., Buerkner, P., and Herve, M. (2020) Package 'emmeans': Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.7. *CRAN R Development Core Team*, 1-79.

Lloyd, M.M., and Pespeni, M.H. (2018) Microbiome shifts with onset and progression of Sea Star Wasting Disease revealed through time course sampling. *Scientific Reports* **8**(1), 16476.

Longford, S.R., Campbell, A.H., Nielsen, S., Case, R.J., Kjelleberg, S., and Steinberg, P.D. (2019) Interactions within the microbiome alter microbial interactions with host chemical defences and affect disease in a marine holobiont. *Scientific Reports* **9**(1), 1363.

Louca, S., Parfrey, L.W., and Doebeli, M. (2016) Decoupling function and taxonomy in the global ocean microbiome. *Science* **353**(6305), 1272-1277.

Lujan, K.M., Eisen, J.A., and Coil, D.A. (2017) Draft Genome Sequences of *Pseudomonas moraviensis* UCD-KL30, *Vibrio ostreicida* UCD-KL16, *Colwellia* sp. Strain UCD-KL20, *Shewanella* sp. Strain UCD-KL12, and *Shewanella* sp. Strain UCD-KL21, Isolated from Seagrass. *Genome Announcements* **5**(13), e00023-17.

Lundberg, D.S., Lebeis, S.L., Herrera Paredes, S., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Glavina del Rio, T., Edgar, R.C., Eickhorst, T., Ley, R.E., Hugenholtz, P., Green Tringe, S., and Dangl, J.L. (2012) Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488**(7409), 86-90.

Luo, J., Ran, W., Hu, J., Yang, X., Xu, Y., and Shen, Q. (2010) Application of bio-organic fertilizer significantly affected fungal diversity of soils. *Soil Science Society of America Journal* **74**(6), 2039-2048.

Magoc, T., and Salzberg, S.L. (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**(21), 2957-2963.

Mao, D., Zhou, Q., Chen, C., and Quan, Z. (2012) Coverage evaluation of universal bacterial primers using the metagenomic datasets. *BMC Microbiology* **12**(1), 66.

Martin, D.L., Chiari, Y., Boone, E., Sherman, T.D., Ross, C., Wyllie-Echeverria, S., Gaydos, J.K., and Boettcher, A.A. (2016) Functional, phylogenetic and host-geographic signatures of *Labyrinthula* spp. provide for putative species delimitation and a global-scale view of seagrass wasting disease. *Estuaries and Coasts* **39**(5), 1403-1421.

Marzinelli, E.M., Campbell, A.H., Zozaya Valdes, E., Vergés, A., Nielsen, S., Wernberg, T., de Bettignies, T., Bennett, S., Caporaso, J.G., Thomas, T., and Steinberg, P.D. (2015) Continental-scale variation in seaweed host-associated bacterial

communities is a function of host condition, not geography. *Environmental Microbiology* **17**(10), 4078-4088.

McIlroy, S.J., and Nielsen, P.H. (2014) The Family *Saprospiraceae*. In The Prokaryotes. (Eds. E Rosenberg, EF DeLong, S Lory, E Stackebrandt and F Thompson) pp. 863-889. (Springer: Berlin, Heidelberg)

Mejia, A.Y., Rotini, A., Lacasella, F., Bookman, R., Thaller, M.C., Shem-Tov, R., Winters, G., and Migliore, L. (2016) Assessing the ecological status of seagrasses using morphology, biochemical descriptors and microbial community analyses. A study in *Halophila stipulacea* (Forsk.) Aschers meadows in the northern Red Sea. *Ecological Indicators* **60**, 1150-1163.

Meres, N.J., Ajuzie, C.C., Sikaroodi, M., Vemulapalli, M., Shields, J.D., and Gillevet, P.M. (2012) Dysbiosis in epizootic shell disease of the American lobster (*Homarus americanus*). *Journal of Shellfish Research* **31**(2), 463-472.

Meyer, J.L., Gunasekera, S.P., Scott, R.M., Paul, V.J., and Teplitski, M. (2016) Microbiome shifts and the inhibition of quorum sensing by Black Band Disease cyanobacteria. *The ISME Journal* **10**(5), 1204-1216.

Meyer, J.L., Paul, V.J., and Teplitski, M. (2014) Community shifts in the surface microbiomes of the coral *Porites astreoides* with unusual lesions. *PLoS ONE* **9**(6), e100316.

Miller, A.W., and Richardson, L.L. (2011) A meta-analysis of 16S rRNA gene clone libraries from the polymicrobial black band disease of corals. *FEMS Microbiology Ecology* **75**(2), 231-241.

Mishra, A.K., and Mohanraju, R. (2018) Epiphytic Bacterial Communities in Seagrass Meadows of Oligotrophic Waters of Andaman Sea. *Open Access Library Journal* **5**(3), e4388.

Muehlstein, L. (1989) Perspectives on the wasting disease of eelgrass *Zostera marina*. *Diseases of Aquatic Organisms* **7**, 211-221.

Muehlstein, L., Porter, D., and Short, F.T. (1991) *Labyrinthula Zosterae* Sp. Nov., The Causative Agent of Wasting Disease of Eelgrass, *Zostera Marina*. *Mycologia* **83**(2), 180-191.

Nedashkovskaya, O.I., Cleenwerck, I., Zhukova, N.V., Kim, S.B., and De Vos, P. (2013) *Arenicella chitinivorans* sp. nov., a gammaproteobacterium isolated from the sea urchin *Strongylocentrotus intermedius*. *International Journal of Systematic and Evolutionary Microbiology* **63**(11), 4124-4129.

O'Connor, N.E. (2013) Impacts of sewage outfalls on rocky shores: Incorporating scale, biotic assemblage structure and variability into monitoring tools. *Ecological Indicators* **29**, 501-509.

Office of Environment & Heritage - NSW Government (2015) State of the Beaches 2014–2015.

Oh, J., Freeman, A.F., Park, M., Sokolic, R., Candotti, F., Holland, S.M., Segre, J.A., Kong, H.H., and NISC Comparative Sequencing Program (2013) The altered landscape of the human skin microbiome in patients with primary immunodeficiencies. *Genome Research* **23**(12), 2103-2114.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., and Wagner, H. (2019) vegan: Community Ecology Package. R package version 2.5-6. *CRAN R Development Core Team*, 1-296.

Opsahl, S., and Benner, R. (1993) Decomposition of senescent blades of the seagrass *Halodule wrightii* in a subtropical lagoon. *Marine Ecology Progress Series* **94**, 191-205.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck-Jr., K.L., Hughes, R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., and Williams, S.L. (2006) A Global Crisis for Seagrass Ecosystems. *BioScience* **56**(12), 987-996.

Paix, B., Carriot, N., Barry-Martinet, R., Greff, S., Misson, B., Briand, J., and Culioli, G. (2020) A multi-omics analysis suggests links between the differentiated surface metabolome and epiphytic microbiota along the thallus of a Mediterranean seaweed holobiont. *Frontiers in Microbiology* **11**, 494.

Patriquin, D. (1972) The origin of nitrogen and phosphorus for growth of the marine angiosperm *Thalassia testudinum*. *Marine Biology* **15**(1), 35-46.

Petersen, C., and Round, J.L. (2014) Defining dysbiosis and its influence on host immunity and disease. *Cellular Microbiology* **16**(7), 1024-1033.

Petersen, H.E. (1933) Wasting disease of eelgrass (*Zostera marina*). *Nature* **132**(3348), 1004.

Pollock, F.J., Morris, P.J., Willis, B.L., and Bourne, D.G. (2011) The urgent need for robust coral disease diagnostics. *PLOS pathogens* **7**(10), e1002183.

Prasad, M.H.K., Ganguly, D., Paneerselvam, A., Ramesh, R., and Purvaja, R. (2019) Seagrass litter decomposition: an additional nutrient source to shallow coastal waters. *Environmental Monitoring and Assessment* **191**(1), 5.

Quintanilla, E., Ramirez-Portilla, C., Adu-Oppong, B., Walljasper, G., Glaeser, S.P., Wilke, T., Reyes-Munoz, A., and Sanchez, J.A. (2018) Local confinement of disease-related microbiome facilitates recovery of gorgonian sea fans from necrotic-patch disease. *Scientific Reports* **8**(1), 14636.

R Core Team (2020) R: A language and environment for statistical computing. (Foundation for Statistical Computing: Vienna, Austria)

Ransome, E., Rowley, S.J., Thomas, S., Tait, K., and Munn, C.B. (2014) Disturbance to conserved bacterial communities in the cold-water gorgonian coral *Eunicella verrucosa*. *FEMS Microbiology Ecology* **90**(2), 404-416.

Roder, C., Arif, C., Bayer, T., Aranda, M., Daniels, C., Shibl, A., Chavanich, S., and Voolstra, C.R. (2014) Bacterial profiling of White Plague Disease in a comparative coral species framework. *The ISME Journal* **8**(1), 31-39.

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016) VSEARCH: a versatile open source tool for metagenomics. In PeerJ. Vol. eCollection 2016.

Romanenko, L.A., Tanaka, N., Frolova, G.M., and Mikhailov, V.V. (2010) *Arenicella xantha* gen. nov., sp. nov., a gammaproteobacterium isolated from a marine sandy sediment. *International Hournal of Systematic and Evolutionary Microbiology* **60**(8), 1832-1836.

Rosenberg, E., Kushmaro, A., Kramarsky-Winter, E., Banin, E., and Yossi, L. (2009) The role of microorganisms in coral bleaching. *The ISME Journal* **3**(2), 139-146.

Rubal, M., Veiga, P., Reis, P.A., Bertocci, I., and Sousa-Pinto, I. (2014) Effects of subtle pollution at different levels of biological organisation on species-rich assemblages. *Environmental Pollution* **191**, 101-110.

Sanschagrin, S., and Yergeau, E. (2014) Next-generation sequencing of 16S ribosomal RNA gene amplicons. *Journal of Visualized Experiments*(90), e51709.

Sato, Y., Civiello, M., Bell, S.C., Willis, B.L., and Bourne, D.G. (2016) Integrated approach to understanding the onset and pathogenesis of black band disease in corals. *Environmental Microbiology* **18**(3), 752-765.

Schaubeck, M., Clavel, T., Calasan, J., Lagkouvardos, I., Haange, S.B., Jehmlich, N., Basic, M., Dupont, A., Hornef, M., von-Bergen, M., Bleich, A., and Haller, D. (2016) Dysbiotic gut microbiota causes transmissible Crohn's disease-like ileitis independent of failure in antimicrobial defence. *Gut* **65**(2), 225-237.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., B.B., O., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., and Weber, C.F. (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology* **75**(23), 7537-7541.

Séguin, A., Gravel, D., and Archambault, P. (2014) Effect of Disturbance Regime on Alpha and Beta Diversity of Rock Pools. *Diversity* **6**(1), 1-17.

Shade, A., and Handelsman, J. (2012) Beyond the Venn diagram: the hunt for a core microbiome. *Environmental Microbiology* **14**(1), 4-12.

Shen, Z., Penton, C.R., Lv, N., Xue, C., Yuan, X., Ruan, Y., Li, R., and Shen, Q. (2018) Banana *Fusarium* wilt disease incidence is influenced by shifts of soil microbial communities under different monoculture spans. *Microbial Ecology* **75**(3), 739-750.

Shokralla, S., Spall, J.L., Gibson, J.F., and Hajibabaei, M. (2012) Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology* **21**(8), 1794-1805.

Short, F.T., Muehlstein, L.K., and Porter, D. (1987) Eelgrass wasting disease: cause and recurrence of a marine epidemic. *The Biological Bulletin* **173**(3), 557-562.

Short, F.T., and Wyllie-Echeverria, S. (1996) Natural and human-induced disturbance of seagrasses. *Environmental Conservation* **23**(1), 17-27.

Smoot, M.E., Ono, K., Ruscheinski, J., Wang, P., and Ideker, T. (2011) Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics* **27**(3), 431-432.

Steele, L., Caldwell, M., Boettcher, A., and Arnold, T. (2005) Seagrass–pathogen interactions:'pseudo-induction'of turtlegrass phenolics near wasting disease lesions. *Marine Ecology Progress Series* **303**, 123-131.

Streten, C., Waite, G.K., Herrington, M.E., Hutton, D.G., Persley, D.M., and Gibb, K.S. (2005) Rickettsia-like-organisms and phytoplasmas associated with diseases in Australian strawberries. *Australasian Plant Pathology* **34**(2), 157-164.

Sullivan, B.K., Robinson, K.L., Trevathan-Tackett, S.M., Lilje, E.S., Gleason, F.H., and Lilje, O. (2017) The first isolation and characterisation of the protist Labyrinthula sp. in southeastern Australia. *Journal of Eukaryotic Microbiology* **64**(4), 504-513.

Sullivan, B.K., Sherman, T.D., Damare, V.S., Lilje, O., and Gleason, F.H. (2013) Potential roles of *Labyrinthula* spp. in global seagrass population declines. *Fungal Ecology* **6**(5), 328-338.

Sullivan, B.K., Trevathan-Tackett, S.M., Neuhauser, S., and Govers, L.L. (2018) Host-pathogen dynamics of seagrass diseases under future global change. *Marine Pollution Bulletin* **134**, 75-88.

Sun, F., Zhang, X., Zhang, Q., Liu, F., Zhang, J., and Gong, J. (2015) Seagrass (*Zostera marina*) Colonization Promotes the Accumulation of Diazotrophic Bacteria and Alters the Relative Abundances of Specific Bacterial Lineages Involved in Benthic Carbon and Sulfur Cycling. *Applied and Environmental Microbiology* **81**(19), 6901-6914.

Sunagawa, S., DeSantis, T.Z., Piceno, Y.M., Brodie, E.L., DeSalvo, M.K., Voolstra, C.R., Weil, E., Andersen, G.L., and Medina, M. (2009) Bacterial diversity and White

Plague Disease-associated community changes in the Caribbean coral *Montastraea faveolata*. *The ISME Journal* **3**(5), 512-521.

Sutherland, K.P., Porter, J.W., and Torres, C. (2004) Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. *Marine ecology progress series* **266**, 273-302.

Tolli, J.D., Sievert, S.M., and Taylor, C.D. (2006) Unexpected diversity of bacteria capable of carbon monoxide oxidation in a coastal marine environment, and contribution of the *Roseobacter*-associated clade to total CO oxidation. *Applied Environmental Microbiology* **72**(3), 1966-1973.

Trevathan-Tackett, S.M., Jeffries, T.C., Macreadie, P.I., Manojlovic, B., and Ralph, P. (2020) Long-term decomposition captures key steps in microbial breakdown of seagrass litter. *Science of The Total Environment* **705**, 135806.

Trevathan-Tackett, S.M., Lauer, N., Loucks, K., Rossi, A.M., and Ross, C. (2013) Assessing the relationship between seagrass health and habitat quality with wasting disease prevalence in the Florida Keys. *Journal of Experimental Marine Biology and Ecology* **449**, 221-229.

Trevathan-Tackett, S.M., Seymour, J.R., Nielsen, D.A., Macreadie, P.I., Jeffries, T.C., Sanderman, J., Baldock, J., Howes, J.M., Steven, A.D., and Ralph, P.J. (2017) Sediment anoxia limits microbial-driven seagrass carbon remineralization under warming conditions. *FEMS microbiology ecology* **93**(6), fix033.

Tujula, N.A., Crocetti, G.R., Burke, C., Thomas, T., Holmström, C., and Kjelleberg, S. (2010) Variability and abundance of the epiphytic bacterial community associated with a green marine *Ulvacean* alga. *The ISME journal* **4**(2), 301-311.

Ugarelli, K., Laas, P., and Stingl, U. (2019) The Microbial Communities of Leaves and Roots Associated with Turtle Grass (*Thalassia testudinum*) and Manatee Grass (*Syringodium filliforme*) are Distinct from Seawater and Sediment Communities, but Are Similar between Species and Sampling Sites. *Microorganisms* **7**, 4.

Vergeer, L.H.T., Aarts, T.L., and de-Groot, J.D. (1995) The 'wasting disease'and the effect of abiotic factors (light intensity, temperature, salinity) and infection with *Labyrinthula zosterae* on the phenolic content of *Zostera marina* shoots. *Aquatic Botany* **52**(1-2), 35-44.

Vergeer, L.H.T., and den-Hartog, C. (1994) Omnipresence of Labyrinthulaceae in seagrasses. *Aquatic Botany* **48**(1), 1-20.

Vergeer, L.H.T., and Develi, A. (1997) Phenolic acids in healthy and infected leaves of *Zostera marina* and their growth-limiting properties towards *Labyrinthula zosterae*. *Aquatic Botany* **58**(1), 65-72.

Walker, A.K., and Campbell, J. (2009) First records of the seagrass parasite *Plasmodiophora diplantherae* from the northcentral Gulf of Mexico. *Gulf and Caribbean Research* **21**(1), 63-65.

Walker, D.I., Pergent, G., and Fazi, S. (2001) Seagrass decomposition. In Global Segrass Research Methods. (Eds. FT Short and RG Coles). (Elsevier)

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., and Williams, S.L. (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences* **106**(30), 12377-12381.

Webster, N.S. (2007) Sponge disease: a global threat? *Environmental Microbiology* **9**(6), 1363-1375.

Webster, N.S., Xavier, J.R., Freckelton, M., Motti, C.A., and Cobb, R. (2008) Shifts in microbial and chemical patterns within the marine sponge *Aplysina aerophoba* during a disease outbreak. *Environmental Microbiology* **10**(12), 3366-3376.

Weidner, S., Arnold, W., Stackebrandt, E., and Pühler, A. (2000) Phylogenetic analysis of bacterial communities associated with leaves of the seagrass Halophila stipulacea by a culture-independent small-subunit rRNA gene approach. *Microbial ecology* **39**(1), 22-31.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. (1991) 16S Ribosomal DNA Amplification for Phylogenetic Study. *Journal of Bacteriology* **173**(2), 697-703.

Welsh, D.T. (2000) Nitrogen fixation in seagrass meadows: Regulation, plant-bacteria interactions and significance to primary productivity. *Ecology Letters* **3**(1), 58-71. [In English]

Whitten, M.M.A., Davies, C.E., Kim, A., Tlusty, M., Wootton, E.C., Chistoserdov, A., and Rowley, A.F. (2014) Cuticles of European and American lobsters harbor diverse bacterial species and differ in disease susceptibility. *Microbiology Open* **3**(3), 395-409.

Yoon, J., Adachi, K., and Kasai, H. (2015) Isolation and characterization of a novel marine *Bacteroidetes* as *Algitalea ulvae* gen. nov., sp. nov., isolated from the green alga *Ulva pertusa*. *Antonie van Leeuwenhoek* **108**(2), 505-513.

Young, E.L. (1943) Studies on *Labyrinthula*. The etiologic agent of the wasting disease of eel-grass. *American Journal of Botany* **30**(8), 586-593.

Zozaya-Valdes, E., Egan, S., and Thomas, T. (2015) A comprehensive analysis of the microbial communities of healthy and diseased marine macroalgae and the detection of known and potential bacterial pathogens. *Frontiers in Microbiology* **6**, 146.

1539 Zozaya-Valdés, E., Roth-Schulze, A.J., Egan, S., and Thomas, T. (2017) Microbial
1540 community function in the bleaching disease of the marine macroalgae *Delisea pulchra*.
1541 *Environmental Microbiology* **19**(8), 3012-3024.
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Figures

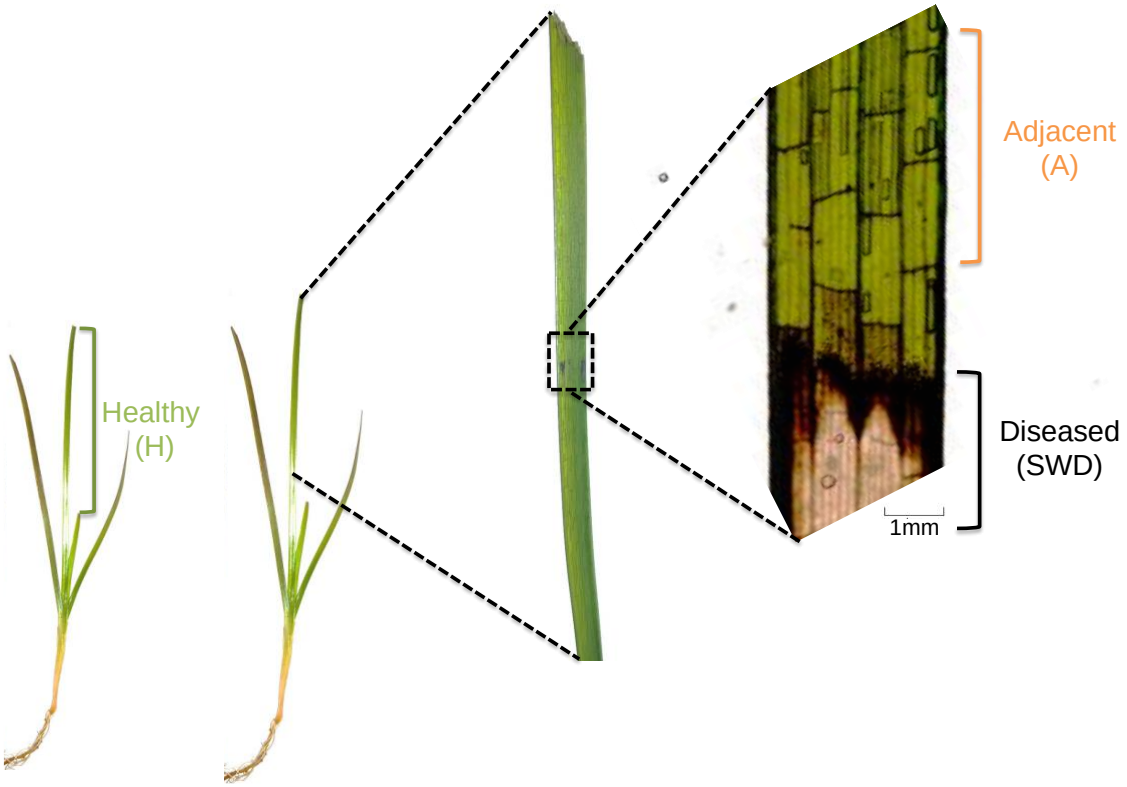

Fig. 1. Sampling strategy. Leaf samples from healthy (i.e. completely asymptomatic, *n* = 12) specimens of *Z. muelleri* were collected and compared with diseased (i.e. presenting single or multiple necrotic lesions typical of SWD, *n* = 10) and asymptomatic leaf tissues immediately adjacent to active lesions (*n* = 10). Healthy tissues were collected from a different plant. Sample collection took place between October and November 2015 at Rose Bay and Lake Macquarie (New South Wales, Australia), which are two locations separated by 120 km that offer different environmental conditions (see **Supplementary Tables 1** and **2**). Seagrass leaf images were generated using the Precipoint's M8 dual microscope and scanner.

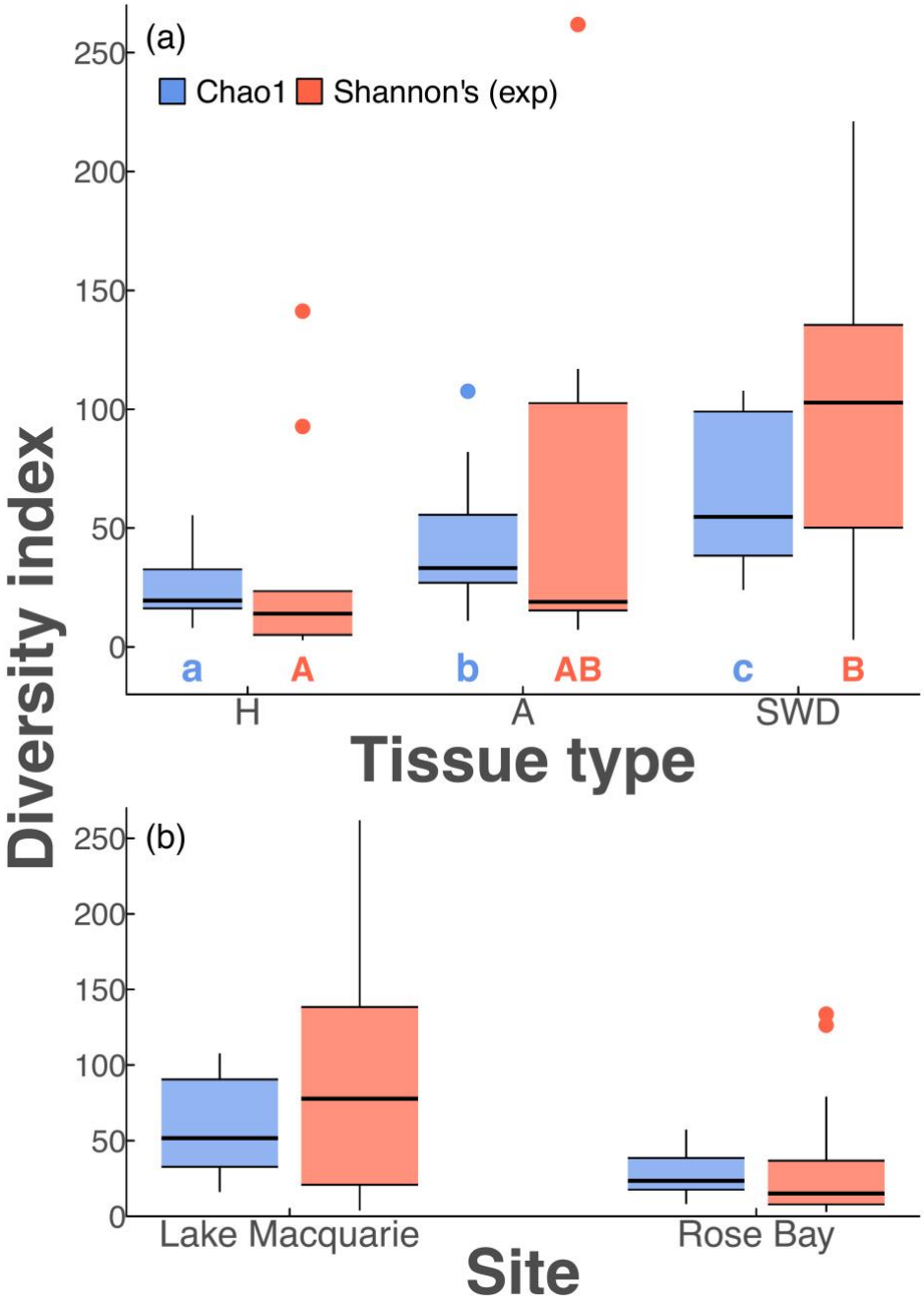

Fig. 2. Bacterial richness and diversity across seagrass tissues. Chao1 diversity index (i.e. bacterial richness, blue) and the exponentiated Shannon's diversity index (i.e. bacterial diversity, red), were calculated for each tissue type (a) and for each site (b). Multiple comparisons between metrics were tested for statistical significance using mixed modelling. Mean values and quartiles are shown for each sample type, and homogeneous subsets (i.e. groups with the same mean, $p > 0.05$) are shown with letters below the boxplots in (a) (lowercase blue letters for Chao1 diversity index, and uppercase red letters for the exponentiated Shannon's diversity index).

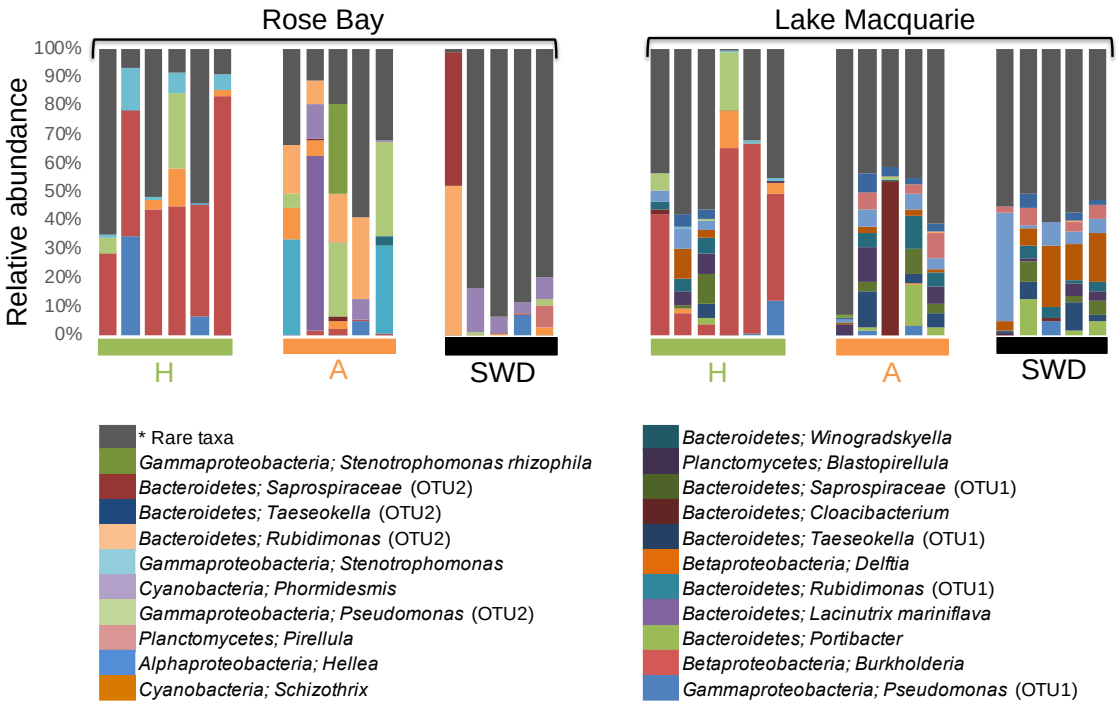

Fig. 3. Bacterial community composition across seagrass tissue types. Composition of bacterial microbiomes associated with healthy (H), adjacent (A) and diseased (SWD) seagrass tissues. Samples (*n* = 32) were compared across two sampling locations (i.e. Rose Bay and Lake Macquarie) and unique OTUs within each sample are coloured by the highest assigned taxonomic level. Rare members of the microbiome (relative abundance < 0.01% in all samples) were pooled (*****) to help remove visual clutter; however, for statistical analyses, all data was used).

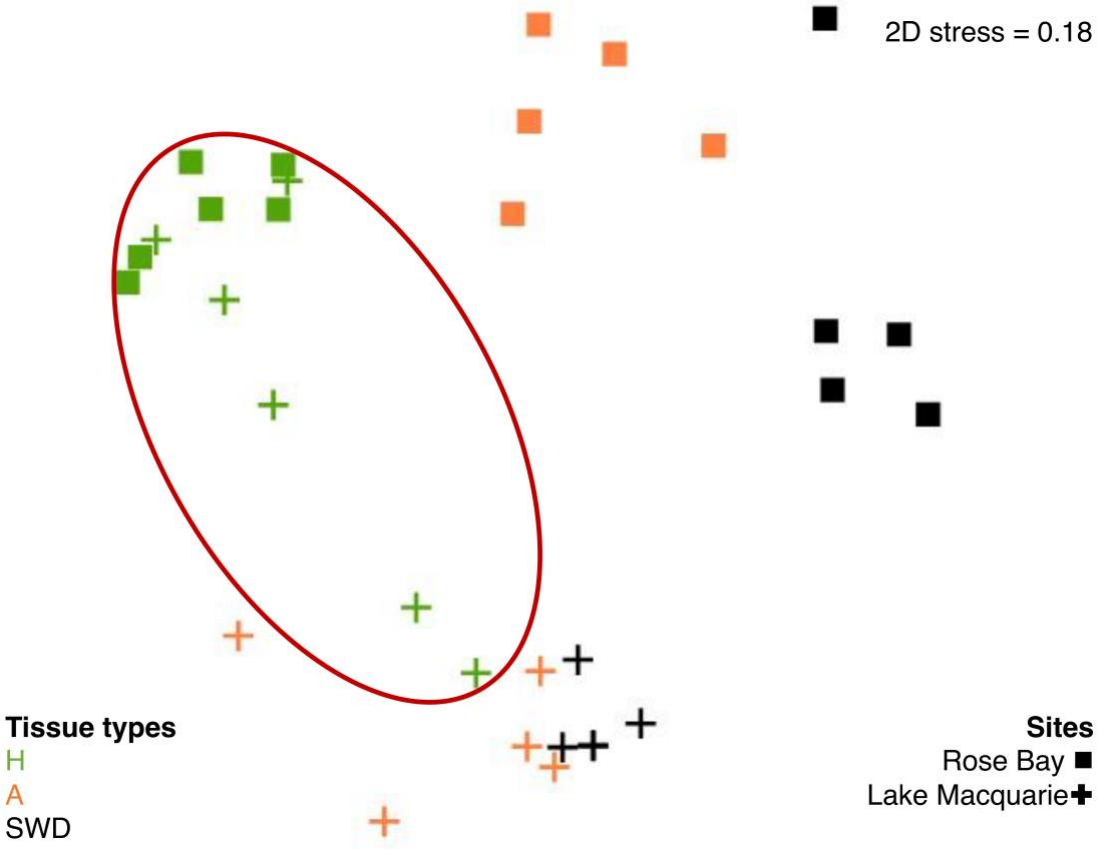

Fig. 4. Clustering patterns across seagrass tissue types. Non-parametric multidimensional scaling (nMDS) of bacterial microbiomes (n = 32), based on a lower triangular resemblance calculated with the S17 Bray-Curtis similarity measure from normalised abundances of OTUs (square-root transformed values). Samples are coloured by tissue type (H, healthy; A, adjacent; SWD, diseased) with different shapes for sites (Rose Bay and Lake Macquarie). Based on permutational ANOVA, tissue types that do not differ significantly across sites (α = 0.05) are outlined in red. The 2D stress is shown in the upper right corner of the nMDS plot (Kruskal stress formula = 1, minimum stress = 0.01).

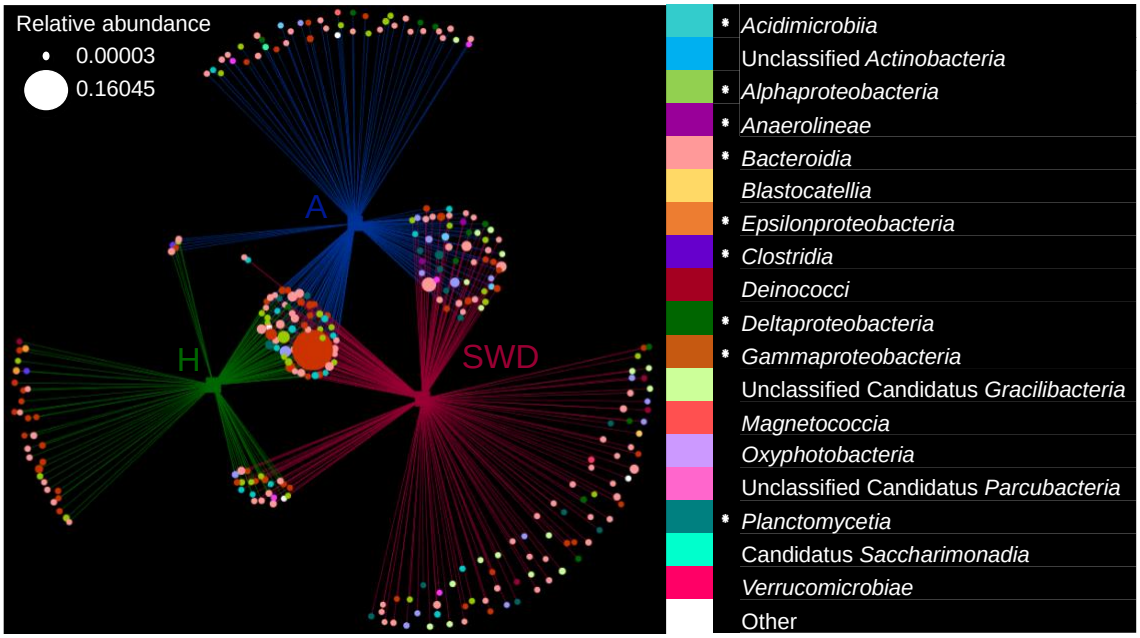

Fig. 5. Bacterial OTUs co-occurrence network. OTUs (nodes as small circles) are linked to each tissue type that they are associated with (coloured squares) by lines (edges) in the bipartite network. Unique (outer clusters of nodes) and shared (nodes in the centre) members of bacterial communities associated with healthy (H), adjacent (A) and diseased (SWD) seagrass tissues are coloured by taxonomic class, with the size of nodes representing OTUs relative abundance. For visual purposes only, no replicate threshold was used for 'unique' members of the microbiome. Asterisks represent OTUs previously reported as seagrass associates, as reported by Ugarelli, Laas *et al.* (2019).

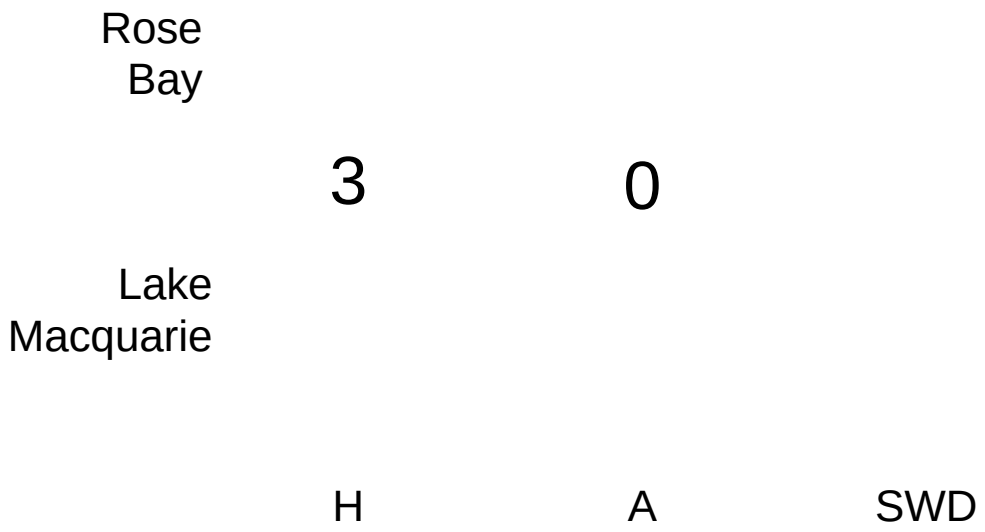

Fig. 6. Loss of the preservation of the seagrass leaf microbiome. Bacterial core OTUs were identified as those microorganisms consistently present (relative abundance > 0) in most of the samples (*n* - 1), across sites within each pigmentation category. Numbers in the middle represent the amount of core OTUs identified for each tissue type (i.e. core size). Core members known to be maintained across sites in healthy leaves (H) are lost in response to the changing conditions caused by seagrass wasting disease (SWD). Adjacent asymptomatic tissues (A) are characterised by transitional microbiomes with no core members.

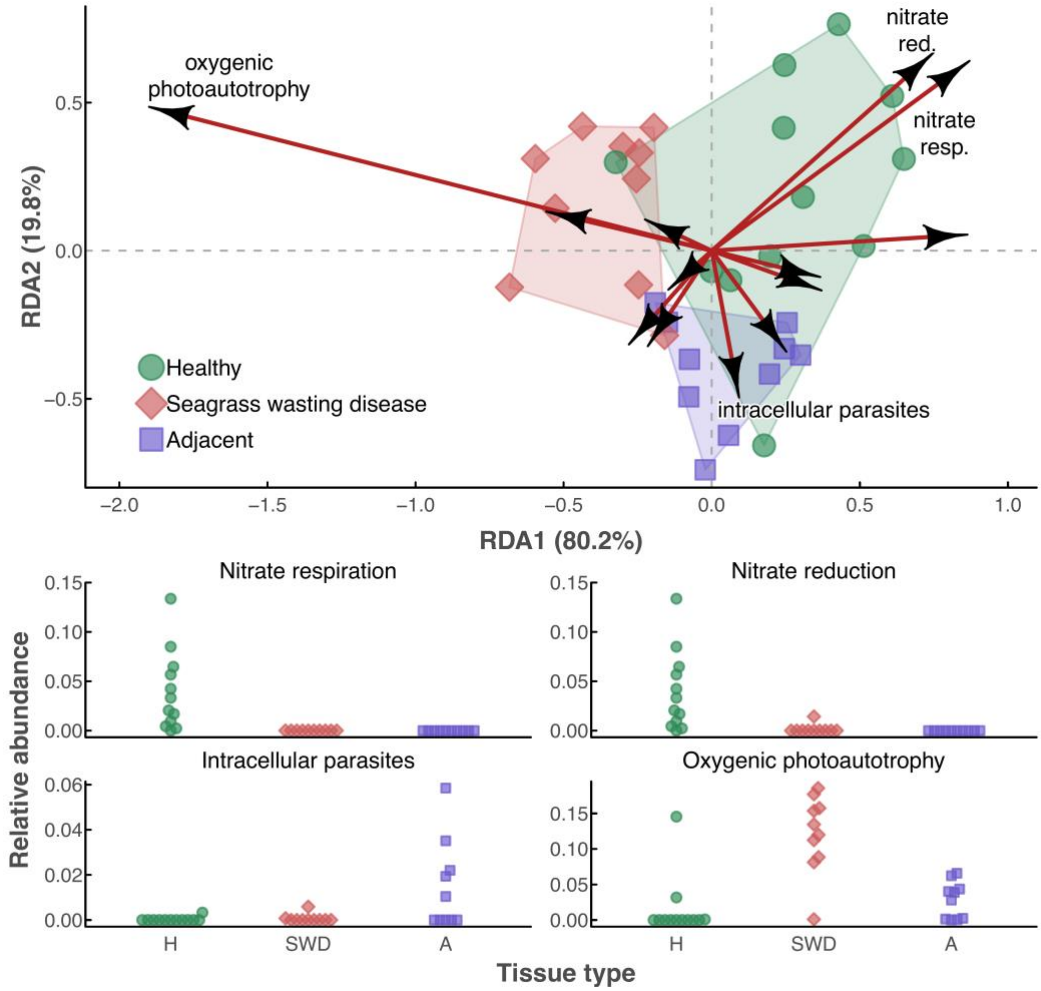

Fig. 7. Tissue-associated predicted functional profiles. Redundancy Analysis (RDA) was used to identify predicted functional categories that best discriminated between the three tissue types. The RDA biplot is shown in (a), with scores of tissue samples represented as points (key at bottom left) and putative functions loadings displayed as vectors (rescaled for clarity; see **Supplementary Table 6** for unscaled loadings). The relative percentage of the variance explained in the two RDA axes is shown next to each axis label. Only vectors that most contributed to the separation among tissue types were labelled. The beeswarm plots in (b) show relative abundances of the predicted functions labelled in the RDA biplot by tissue type, using the same symbology as the RDA. 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.