



# **Corrigendum: Regional and Microenvironmental Scale Characterization of the** *Zostera muelleri* **Seagrass Microbiome**

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Keywords: seagrass microbiome, diversity, core, bacteria, microalgae, fungi, amplicon sequencing

#### A Corrigendum on

## Regional and Microenvironmental Scale Characterization of the Zostera muelleri Seagrass Microbiome

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In the original manuscript, there was an error with our bioinformatics workflow, whereby seagrass-associated reads were not removed prior to taxonomic profiling of the fungal community in seagrass-associated microenvironments. As a consequence, incorrect assignment of some seagrass ITS sequences as fungal sequences occurred in some instances, leading to inflated levels of fungi, particularly from the Glomeraceae and Rhytismataceae groups. We have re-analyzed the data after ensuring that seagrass ITS sequences have not been incorrectly assigned as fungi. Due to the low number of fungal sequencing reads from the leaf that remained after removal of putative seagrass sequences, only the mycobiomes associated with seagrass roots and rhizomes, sediments and seawater were compared using exactly the same approaches described in the published manuscript. Text from the original article has been updated to reflect a shift in patterns in mycobiome data that resulted from the exclusion of ITS sequences that were, subsequent to the publication of Hurtado-McCormick et al. (2019), found to be associated with seagrass leaf material rather than fungi. As a consequence, this led to the removal of reference to Glomeraceae and Rhytismataceae data as following new analysis it was determined that sequences assigned to these groups are present in lower proportions than originally reported. Corrections have been made to the interested sections.

A correction has been made to Materials and Methods section, Sequence Data Analysis, paragraph 3:

Initial sequence processing for fungal ITS genes was conducted using QIIME, v1.9.1 (Caporaso et al., 2010). Briefly, low-quality regions were trimmed from the 5' end of sequences, and paired ends were joined with fastq-join (Aronesty, 2011, 2013) and demultiplexed. Sequences containing ambiguous bases were removed from the dataset along with low-quality reads and chimeric sequences. Referenced-based chimera detection (Nilsson et al., 2015) was performed using the UCHIME algorithm from the USEARCH package (Edgar, 2010; Edgar et al., 2011) implemented within VSEARCH, v2.3.2 (Rognes et al., 2016). OTUs were defined as clusters of 97% sequence similarity using UCLUST (Edgar, 2010). The resultant OTU table was filtered to remove singletons and seagrass-affiliated sequences. OTU sequences were screened for non-fungal sequences using BLAST (Altschul et al., 1990), against the nucleotide database from the National Center for Biotechnology

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Information (NCBI). Non-fungal sequences were identified using BASTA (Kahlke and Ralph, 2019) and the following parameters: -1 250 (sequence length), -m 0 (mismatches), and -i 97 (identity). These sequences were subsequently removed from the dataset. Final taxonomies were assigned to the filtered OTU set (i.e., sequences of unknown origin) using the UNITE database v6.9.7 (Koljalg et al., 2013), BLAST, and vBLASTC (Altschul et al., 1990). Finally, the resultant filtered OTU table was rarefied to an even number of sequences per samples to ensure equal sampling depth (i.e., lower number of sequences per sample = 1456). Given the nature of this study's experimental design and the importance of replication in complex datasets, the rarefaction cut-off was chosen to include at least triplicates per sample type (Supplementary Table 9). Due to the low number of fungal sequencing reads from the leaf that remained after removal of putative seagrass sequences, only the mycobiomes associated with seagrass roots and rhizomes, sediments and seawater were used for further post-sequencing analyses, while the seagrass leaf samples were omitted from cross-sample comparisons. A separate re-analysis of these samples with unrarefied data supported all of the scientific conclusions of our original manuscript, except for the predominance of the Rhytismataceae family in the upper leaf mycobiome, which instead represented rare taxa (relative abundance < 1% in all samples) within seagrass-associated fungal communities.

A correction has been made to the **Results and Discussion** section, **The Seagrass Mycobiome, paragraphs 1-3** and **5**:

Although less studied in seagrasses, several fungi have been demonstrated to be highly beneficial for aquatic and terrestrial plant fitness while establishing intimate relationships with their host (i.e., mycorrhizal associations) to facilitate nutrient uptake or compete against other potentially pathogenic microbes (Azcon-Aguilar et al., 1999; Kohout et al., 2012; Raghukumar, 2012). In this study, fungal communities associated with Z. muelleri displayed significantly different levels of alpha diversity for the two measured indices (Chao1 and Shannon's Index) between both seagrass microenvironments ( $p_{Chao1} =$ 0.0006,  $p_{\text{Shannon's}} = 0.0001$ ) and sampling sites ( $p_{\text{Chao1}} =$ 0.0051,  $p_{\text{Shannon's}} = 0.0010$ ) (Supplementary Table 7). Our post-hoc analyses indicated that the differences across habitats were mostly driven by differences between Lake Macquarie and all other sites. At Lake Macquarie, fungal microbiomes exhibited significantly lower levels of alpha diversity (p <0.05, Supplementary Table 7). Moreover, several significant differences in alpha diversity were observed between seagrass microenvironments within each site (Supplementary Table 7). Even though some of these differences varied from site to site, general patterns were similar to those observed for microalgal assemblages, whereby seagrass-associated microenvironments (here roots and rhizomes only) had lower levels of fungal diversity than the surrounding seawater and sediments (Figure 2). This is possibly due to antifungal chemical defenses and physiological responses from the host against co-occurring marine fungi, which have been well described for other seagrass species (Ross et al., 2008).

Consistent with the patterns observed for bacterial and microalgal assemblages, fungal community structure varied

significantly across both seagrass microenvironments (p =0.0001) and sampling sites (p = 0.0001). Notably, all sites differed significantly from each other (p < 0.05). However, the differences between microenvironments within each sampling location explained a greater level of variation between mycobiomes compared to the differences between sites [ECV, Mi(Si) = 1262.70, Supplementary Table 8]. Roots and rhizomes, sediment and seawater communities formed discrete, separated clusters within each site evidenced in nMDS (Figure 10) and CLUSTER as (Supplementary Figure 7) analyses. However, such clear separation of fungal communities between microenvironments was not apparent in unrarefied data from leaf samples (Supplementary Figure 6).

Fungal OTUs identified within four taxonomic groups consistently dominated fungal assemblages across the three microenvironments and four sampling locations studied here (Figures 11, 12). This is consistent with the hypothesis of extreme ecological flexibility acclaimed for obligate marine fungal species (Nicoletti and Andolfi, 2018). OTUs matching the order Pleosporales (291 unique OTUs) and the species Wallemia ichthyophaga (54 unique OTUs) represented the most abundant fungi across the roots and rhizomes, sediments and seawater microenvironments, making up an average of 38 and 18% of these communities, respectively (Figures 11, 12). Many freshwater and marine species of Pleosporales have been described to date, including several endophytes and saprophytes of plants, as well as symbionts, parasites and pathogens of seagrasses and marine macroalgae (Suetrong et al., 2009; Zhang et al., 2009; Boonmee et al., 2012; Hyde et al., 2013; Hashimoto et al., 2017). Some species are also dominant members of microbiomes associated with mangroves, showing a microenvironmental preference for intertidal parts of the host, which occur above the water level (Raghukumar, 2012). Our observations of predominant Pleosporales OTUs across all three microenvironments and particularly within roots and rhizomes, where these fungi represented 55% of the mycobiome, are highly consistent with previous reports of the dominance of a single marine fungus from the Pleosporales, probably representing a new genus, associated with the roots of the seagrass species Posidonia oceanica (Vohník et al., 2016). While, to our knowledge, the other dominant fungal species, W. ichthyophaga, has not previously been reported in seagrasses, it has been found to occur in association with other benthic marine organisms, including corals (Raghukumar, 2012). We also observed OTUs that dominated the microenvironments surrounding the seagrass. These included the species Mortierella horticola (42 unique OTUs) and unclassified members of the Pezizomycetes class (7 unique OTUs), which represented 7 and 0.38% of the sediment and seawater fungal communities, respectively (Figures 11, 12). Despite its low relative abundance, the Pezizomycetes was the only taxon that differed significantly across the three seagrass microenvironments (p = 0.013, Figure 12). Nevertheless, further exploration of our beta diversity data revealed high relative abundances and high contributions to microenvironmental dissimilarities of the other fungi aforementioned, suggesting their potential importance within the seagrass mycobiome.

Here we chose to use 97% similarity criteria for defining fungal OTUs characterized using our ITS sequencing approach, which we consider a suitable conservative approach given the lower levels of taxonomic diversity covered in fungal ITS databases (relative to e.g., bacteria) and is consistent with values previously used to characterize the mycobiome associated with terrestrial plants (Giordano et al., 2009) and coastal grasses (Sánchez-Márquez et al., 2008). The overall dominance of four taxonomic groups across the three microenvironments studied here is in line with previous observations of very narrow mycobiomes associated with seagrasses (Devarajan and Survanaravanan, 2002; Vohník et al., 2016), plants from salt marshes (Al-Nasrawi and Hughes, 2012), mangroves (Xing and Guo, 2011), and other aquatic plants (Kohout et al., 2012). Nevertheless, and similar to microalgae, we did not observe a conserved "core" of fungal associates within any of the seagrass microenvironments (here roots and rhizomes only), and a core fungal microbiome of two members was only identified for the seawater microenvironment. These core OTUs belonged to the family Glomeraceae and the order Pleosporales. Our results are indicative of a weaker ecological coupling between seagrasses and fungal taxa, relative to that observed for seagrass bacterial interactions. We propose

that, relative to bacteria, which appear to display highly specific interactions with different components of the plant due to a stronger influence of the conditions at the microscale, seagrassassociated fungi appear to establish more generalist relationships with their host.

In the original article, there was a mistake in some figures and tables.

The corrected **Figure 2** appears below. Secondary Y-axis titles have been added to the figure to improve clarity. The figure legend was not interested and has not changed.

The corrected **Figure 10** and **Figure 10** legend appear below. The corrected **Figure 11** and **Figure 11** legend appear below. The corrected **Supplementary Table 8** appears below.

The corrected Supplementary Table 8 appears below.

The corrected Figure 12 and Figure 12 legend appear below.The correctedSupplementary Figure 6 and

Supplementary Figure 6 legend appear below.ThecorrectedSupplementary Figure 7and

**Supplementary Figure** 7 legend appear below. The corrected **Supplementary Table** 7 appears below.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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FIGURE 2 | Microbial mean alpha diversity across seagrass microenvironments. Multiple comparisons between Chao1 diversity (A) and Shannon's diversity index (B), calculated for each taxa and microenvironment separately, were tested for statistical significance with Permutational Multivariate Analysis of Variance (PERMANOVA, Minkowski metric distance matrix and nested design). Mean values for each microenvironment are shown, and error bars reflect the standard error of the mean.



**FIGURE 10** | Microenvironmental and regional partitioning of the seagrass fungal microbiome. Non-parametric multidimensional scaling (nMDS) of fungal microbiomes (n = 35), based on a lower triangular resemblance calculated with the S17 Bray–Curtis similarity measure from relative abundances of OTUs (high values down-weighted with square root). Samples are colored by microenvironment (RR, roots and rhizomes; Sed, sediment, SW, seawater), with different shapes for sites (PB, Palm Beach; RB, Rose Bay; N, Narrabeen Lagoon; LM, Lake Macquarie). Sample clustering patterns by microenvironment within each site represent the level of similarity between samples based on the degree to which OTUs are shared between them. The 2D stress is shown in the upper right corner of the nMDS plot (Kruskal stress formula = 1, minimum stress = 0.01). The nMDS for the three microenvironments associated with the leaf is provided in **Supplementary Figure 6** and a hierarchical cluster analysis (CLUSTER) is provided in **Supplementary Figure 7**.

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**FIGURE 12** Fungal discriminatory OTUs at the microenvironmental scale. Extensive hypothesis testing of taxonomic profiles was coupled with similarity percentages analyses (SIMPER) for fungal microbiomes across microenvironments. The proportion of sequences (mean frequency %) of OTUs significantly over-represented (Kruskal–Wallis H-test,  $\alpha = 0.05$ , effect sizes:  $\eta^2$ ) and consistently contributing to the differences between microenvironments is indicated by varying color intensities. Corrected *p*-values were calculated using the Benjamini–Hochberg's approach. Two-way crossed SIMPER analyses were performed with site and microenvironment variables as factors (S17 Bray–Curtis similarity matrix). High contributors were selected from the top-5 contributors of each pair-wise comparison between microenvironments, and those OTUs consistently accounting for the dissimilarities between any given microenvironment and the other two microenvironments were chosen as high contributors to couple with the statistical results. High contributors that were significantly over-represented were classified as discriminatory OTUs (i.e., 1–4). OTUs are sorted by decreasing mean abundance, and samples are clustered by average neighbor distance (UPGMA, distance threshold = 0.75) and colored by classified as discriminatory OTUs if their contribution to the differences between microenvironments was consistent. RR, roots and rhizomes; Sed, sediment; SW, seawater.



**SUPPLEMENTARY FIGURE 6** The seagrass leaf-associated microbiomes (Fungi, unrarefied data). Non-parametric multidimensional scaling (nMDS) of fungal microbiomes associated with the seagrass leaf (n = 36), based on a lower triangular resemblance calculated with the S17 Bray–Curtis similarity measure from relative abundances of OTUs (high values down-weighted with square root). Samples are colored by microenvironment (UpL, upper leaf; LoL, lower leaf; Sh, sheath), with different shapes for sites (PB, Palm Beach; RB, Rose Bay; N, Narrabeen Lagoon; LM, Lake Macquarie). Clustering patterns by microenvironment within each site represent the level of similarity between samples based on the degree to which OTUs are shared between them. The 2D stress is shown in the upper right corner of the nMDS plot (Kruskal stress formula = 1, minimum stress = 0.01).



SUPPLEMENTARY FIGURE 7 | Clustering patterns of the seagrass fungal microbiome. Hierarchical cluster analysis (CLUSTER) of fungal microbiomes, based on a lower triangular resemblance calculated with the S17 Bray-Curtis similarity measure from relative abundances of OTUs (high values down-weighted with square root). Samples are colored by microenvironment (RR, roots and rhizomes; Sed, sediment, SW, seawater), with different shapes for sites (PB, Palm Beach; RB, Rose Bay; N, Narrabeen Lagoon; LM, Lake Macquarie). Group average linkage was used to calculate distances between clusters and generate a dendogram and a cophenetic distance matrix (unsupervised learning method). The cophenetic correlation index (CCI) is provided in the upper right corner of the dendogram, and it was used to assess the faithfulness of the dendogram by computing cophenetic correlation coefficients.

SUPPLEMENTARY TABLE 7	Statistical	analyses	for	fungal	mean	alpha
diversity (Chao1 diversity).						

Main test Factor	P(perm)		ECV Source	Estimate	Sq.root
Site	0.0051		S(si)	2045.20	45.22
Microenvironment (Site)	0.0006		S[mi(si)]	6070.30	77.91
			V(Res)	3741.30	61.17
Pair-wise (Site)					
P(perm)	PB	RB	Ν	LM	
PB					
RB	0.5650				
Ν	0.6975	0.4262			
LM	0.0002	0.0453	0.0003		
Pair-wise (Micenv. PB)					
P(MC) <sup>a</sup>	RR	Sed	SW		
RR					
Sed	0.0007				
SW	0.0228	0.7675			
Pair-wise (Micenv. RB)					
P(MC) <sup>a</sup>	RR	Sed	SW		
RR					
Sed	0.6648				
SW	0.0839	0.2824			
Pair-wise (Micenv. N)					
P(MC) <sup>a</sup>	RR	Sed	SW		
RR					
Sed	0.0279				
SW	0.0125	0.0036			
Pair-wise (Micenv. LM)					
P(MC) <sup>a</sup>	RR	Sed	SW		
RR					
Sed	0.8580				
SW	0.0393	0.0861			

**SUPPLEMENTARY TABLE 7 |** Statistical analyses for bacterial mean alpha diversity (Shannon's diversity index).

Main test		ECV		
Factor	P(perm)	Source	Estimate	Sq.root
Site	0.0010	S(si)	14169.00	119.03
Microenvironment (Site)	0.0001	S[mi(si)]	57446.00	239.68
		V(Res)	19028.00	137.94
Pair-wise (Site)				
P(perm)	PB	RB	Ν	LM
PB				
RB	0.0441			
Ν	0.0004	0.7340		
LM	0.0036	0.0307	0.0002	
Pair-wise (Micenv. PB)				
P(MC) <sup>a</sup>	RR	Sed	SW	
RR				
Sed	0.0163			
SW	0.0015	0.0176		
Pair-wise (Micenv. RB)				
P(MC) <sup>a</sup>	RR	Sed	SW	
RR				
Sed	0.4563			
SW	0.0570	0.1794		
Pair-wise (Micenv. N)				
P(MC) <sup>a</sup>	RR	Sed	SW	
RR				
Sed	0.0039			
SW	0.0335	0.0018		
Pair-wise (Micenv. LM)				
P(MC) <sup>a</sup>	RR	Sed	SW	
RR				
Sed	0.4307			
SW	0.3696	0.7873		

Differences in alpha diversity as measured by Chao1 diversity across sites and microenvironments (main test), between sites and between microenvironments within sites (pair-wise) were tested for statistical significance in Permutational Multivariate Analysis of Variance (PERMANOVA, Minkowski metric distance matrix, nesteddesign). <sup>a</sup> Monte Carlo p-values used when there were not enough possible permutations

(unrestricted permutation method, 9,999permutations). Significant values at the 0.05 level are shown ingrey.

ECV, estimates of components of variation; RR, roots and rhizomes; Sed, sediment; SW, seawater; PB, Palm Beach; RB, Rose Bay; N, Narrabeen Lagoon; LM, LakeMacquarie.

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## **SUPPLEMENTARY TABLE 8** | Statistical analyses for multidimensional scaling (Fungi).

FactorP(perm)SourceEstimateSq.rootSite0.0001IS(S)668.2225.85Microenvironment (Site)0.0001IS(Mi(Si)1262.7035.53Picrose (Site)III37.52Pir-wise (Site)PBRBNLMIP(perm)PBRBNLMIP(perm)0.0001IIIIP(perm)0.00010.0277IIIRB0.00010.0279IIIP(MC) <sup>0</sup> RRSedSWIIP(MC) <sup>0</sup> SRSedSWIISedSOSOIIISedSOSOIIISedSOSOIII	Main test			ECV		
Site     0.0001     S(S)     668.22     25.85       Microenvironment (Site)     0.0001     S[Mi(Si)]     1262.70     35.53       Pair-wise (Site)     V(Res)     1407.70     37.52       Pair-wise (Site)     PB     RB     N     LM       PB     RB     0.0003     Iteration     Iteration       N     0.0001     0.0277     Iteration     Iteration       PB     0.0001     0.0239     0.0011     Iteration     Iteration       N     0.0001     0.0239     0.0011     Iteration     Iteration       PMC/Pa     RR     Sed     SW     SW     Iteration     Iteration       P(MC) <sup>a</sup> RR     Sed     SW     Iteration     Iteration     Iteration       SW     0.0360     0.1770     SW     Iteration     Iteration     Iteration     Iteration       P(MC) <sup>a</sup> RR     Sed     SW     Iteration     Iteration     Iteration     Iteration     Iteration       P(MC) <sup>a</sup> RR	Factor	P(perm)		Source	Estimate	Sq.root
Microenvironment (Site)0.0001ViewS[Mi(Si))1262.7035.53View1407.7037.52Pair-wise (Site)PBRBNLMPgem)PBRBNLMPB0.003	Site	0.0001		S(Si)	668.22	25.85
V(Res)1407.7037.52Pair-wise (Site)PBRBNLMP(perm)PBRBNLMPB1.0003RB0.00010.0279N0.0010.0279LM0.0010.02390.0011PMT-wise (Micenv. PB)P(MC) <sup>A</sup> RRSedSWSQ0.0315P(MC) <sup>A</sup> 0.0310P(MC) <sup>A</sup> RRSedSWSQ0.36200.1770P(MC) <sup>A</sup> RRSedSWSQ0.0468P(MC) <sup>A</sup> RRSedSWSQ0.04690.1770P(MC) <sup>A</sup> RRSedSWSQ0.0469P(MC) <sup>A</sup> RRSedSQ0.0469P(MC) <sup>A</sup> RRSedP(MC) <sup>A</sup> RRP(MC) <sup>A</sup> RRP(MC) <sup>A</sup> SSQ0.0468P(MC) <sup>A</sup> SSQ0.0516P(MC) <sup>A</sup> <td< td=""><td>Microenvironment (Site)</td><td>0.0001</td><td></td><td>S[Mi(Si)]</td><td>1262.70</td><td>35.53</td></td<>	Microenvironment (Site)	0.0001		S[Mi(Si)]	1262.70	35.53
Pair-wise (Site)P(perm)PBRBNLMPB0.0003				V(Res)	1407.70	37.52
Pipem)PBRBNLMPB	Pair-wise (Site)					
PBIndexIndexRB0.00030.0277N0.0010.02300.0011LM0.0010.02300.0011Pirewise (Micenv. PB)PMC/°RRSedSWSed0.0315IndexSW0.01230.0625IndexPMC/°RRSedSWPMC/°RRSedSWSed0.12300.1700Pirewise (Micenv. B)IndexIndexSed0.36300.1770Pirewise (Micenv. H)SeldSWRRSeldSWSed0.06390.0117Firewise (Micenv. H)IndexIndexP(MC)°RRSeldSWSeld0.0639SWP(MC)°RRSeldSeldSeldSWSel	P(perm)	PB	RB	Ν	LM	
RB0.0003	PB					
N0.00010.0277LM0.00010.02390.0011Pair-wise (Micenv. PB)SedSWRRRRSedSWSed0.0315SW0.02300.0625PMC/ªRRSedSWPMC/ªRRSedSWSed0.4668SW0.03600.1770PMC/aRRSedSWSed0.3669SWPMC/aRRSedSWPMC/aRRSedSWSed0.0453FMJondenySedSWSW0.06890.1170P(MC)ªRRSedSWP(MC)ªRRSedSWSW0.0589SWSWSP(Micenv. LH)SedSWP(MC)ªRRSedSWSed0.5516SWSW	RB	0.0003				
LM0.00010.02390.0011Pair-wise (Micenv. PB)SedSWP(MO) <sup>a</sup> RRSedSWSed0.0315-SW0.12300.0625PMCV <sup>a</sup> RRSedSWP(MO) <sup>a</sup> 0.4668SWSed0.303600.1770Sed0.03600.1770P(MO) <sup>a</sup> RRSedSW0.0689SWP(MO) <sup>a</sup> RRSedSW0.04631-P(MO) <sup>a</sup> RRSedSW0.04632-P(MO) <sup>a</sup> NO-P(MO) <sup>a</sup> RRSedSW0.06890.1170P(MO) <sup>a</sup> RRSedSW0.0516-SedSWSedSedSedSedSed0.5516	N	0.0001	0.0277			
Pair-wise (Micenv. PB)P(MC) <sup>a</sup> RRSedSWRRSed0.0315-SW0.1230.0625Pir-wise (Micenv. RB)P(MC) <sup>a</sup> RRSedSWSed0.4668SW0.30300.1770-Pir-wise (Micenv. N)P(MC) <sup>a</sup> RRSedSWP(MC) <sup>a</sup> RRSedSWP(MC) <sup>a</sup> RRSedSWP(MC) <sup>a</sup> RRSedSWSW0.06890.0117-P(MC) <sup>a</sup> RRSedSWP(MC) <sup>a</sup> RRSedSWSW0.0516	LM	0.0001	0.0239	0.0011		
P(MC) <sup>a</sup> RRSedSWRR0.0315Sed0.0316SW0.1230.0625-PI-wise (Micenv. RB)P(MC) <sup>a</sup> RRSedSWSed0.4668SW0.30300.1770-P(MC) <sup>a</sup> RRSedSWP(MC) <sup>a</sup> 0.0468SW0.03600.1770-P(MC) <sup>a</sup> RRSedSWSed0.0453SW0.06890.0117-P(MC) <sup>a</sup> RRSedSWP(MC) <sup>a</sup> RRSedSWSed0.516	Pair-wise (Micenv. PB)					
RRImage: series of the series of	P(MC) <sup>a</sup>	RR	Sed	SW		
Sed0.0315-SW0.01230.0625Pair-wise (Micenv. RB)SedSWP(MO) <sup>a</sup> RRSedSWSed0.4668-SW0.30600.1770Pin-wise (Micenv. H)SedSWP(MO) <sup>a</sup> RRSedSWSed0.0453-SW0.06890.0117Pim-wise (Micenv. LH)SW0.0689SWP(MO) <sup>a</sup> RRSedSW0.2639SWP(MO) <sup>a</sup> RRSedSed0.516-	RR					
SW0.01230.0625Pin-wise (Micenv. RBSedSWP(MC) <sup>a</sup> RRSedSWSed0.4668SW0.33000.1770-Pin-wise (Micenv. W)P(MC) <sup>a</sup> RRSedSWSed0.0453SW0.06890.0117-Pin-wise (Micenv. LH)SW0.06890.0117-Pin-wise (Micenv. LH)Pin-wise (Micenv. LH)Pin-wise (Micenv. LH)Sed0.5516	Sed	0.0315				
Pair-wise (Micenv. RB)SedSWP(MC) <sup>a</sup> RRSedSWRR0.4668Sw00.03600.1770-P(MC) <sup>a</sup> RRSedSWP(MC) <sup>a</sup> RRSedSWSw10.0453Sw10.06890.0117-P(MC) <sup>a</sup> RRSedSWP(MC) <sup>a</sup> RRSedSWSw10.0516	SW	0.0123	0.0625			
P(MC) <sup>a</sup> RRSedSWRR0.4668Sed0.36000.1770- <b>Pair-wise (Micenv. N)</b> SedSWP(MC) <sup>a</sup> RRSedSWSed0.0453SW0.06890.0117-P(MC) <sup>a</sup> RRSedSWP(MC) <sup>a</sup> RRSedSWP(MC) <sup>a</sup> SedSWSed0.5516-	Pair-wise (Micenv. RB)					
RRIISed0.46680.1770SW0.03600.1770Pirwise (Micenv. W)P(MC) <sup>a</sup> RRSedSed0.0453SWSW0.06890.0117Pirwise (Micenv. LW)VVP(MC) <sup>a</sup> RRSedSW0.0516SW	P(MC) <sup>a</sup>	RR	Sed	SW		
Sed0.4668SW0.03600.1770Pair-wise (Micenv. N)P(MC) <sup>a</sup> RRSedSWRR0.0453	RR					
SW0.03600.1770Pair-wise (Micenv. N)P(MC) <sup>a</sup> RRSedSed0.0453SW0.06890.0117Pair-wise (Micenv. LW)P(MC) <sup>a</sup> RRSedSW0.5616	Sed	0.4668				
Pair-wise (Micenv. N)RRSedSWP(MC) <sup>a</sup> RRSWSWSed0.0453SW0.06890.0117-Pair-wise (Micenv. LM)P(MC) <sup>a</sup> RRSedSWRR0.5516	SW	0.0360	0.1770			
P(MC) <sup>a</sup> RR     Sed     SW       RR     0.0453     -       SW     0.0689     0.0117       Pair-wise (Micenv. LM)     -     -       P(MC) <sup>a</sup> RR     Sed     SW       RR     0.516     -     -	Pair-wise (Micenv. N)					
RR     0.0453       Sed     0.0689     0.0117       Pair-wise (Micenv. LM)     0.0117     1       P(MC) <sup>a</sup> RR     Sed     SW       RR     Sed     SW       Sed     0.5516     1     1	P(MC) <sup>a</sup>	RR	Sed	SW		
Sed     0.0453       SW     0.0689     0.0117       Pair-wise (Micenv. LM)     Sed     SW       P(MC) <sup>a</sup> RR     Sed     SW       RR     Sed     SW       Sed     0.5516     SW	RR					
SW     0.0689     0.0117       Pair-wise (Micenv. LM)     Sed     SW       P(MC) <sup>a</sup> RR     Sed     SW       RR     Sed     SW       Sed     0.5516     SW	Sed	0.0453				
Pair-wise (Micenv. LM)   P(MC) <sup>a</sup> RR   Sed Sw	SW	0.0689	0.0117			
P(MC) <sup>a</sup> RR Sed SW RR 0.5516	Pair-wise (Micenv. LM)					
RR 0.5516	P(MC) <sup>a</sup>	RR	Sed	SW		
Sed 0.5516	RR					
	Sed	0.5516				
SW 0.0069 0.0145	SW	0.0069	0.0145			

Differences between fungal communities across sites and microenvironments (top), between sites (middle), and between microenvironments within sites (bottom) were tested for statistical significance in Permutational Multivariate Analysis of Variance (PERMANOVA, Bray-Curtis dissimilarity matrix, nesteddesign).

<sup>a</sup> Monte Carlo p-values used when there were not enough possible permutations (unrestricted permutation method, 9,999permutations).

Significant values at the 0.05 level are shown ingrey.

ECV, estimates of components of variation; RR, roots and rhizomes; Sed, sediment; SW, seawater; PB, Palm Beach; RB, Rose Bay;N, Narrabeen Lagoon; LM, Lake Macquarie.