

1 **Supplementary Materials:**

2

3 **Title:** Characterization of the mycobiome of the seagrass, *Zostera marina*, reveals putative
4 associations with marine chytrids

5

6 **Authors:** Cassandra L. Ettinger^{1,2}, Jonathan A. Eisen^{1,2,3}

7

8 ¹Genome Center, University of California, Davis, CA, United States

9 ²Department of Evolution and Ecology, University of California, Davis, CA, United States

10 ³Department of Medical Microbiology and Immunology, University of California, Davis, Davis,
11 CA, United States

12

13 **Corresponding Author:**

14 Cassandra L. Ettinger

15 Email address: clettinger@ucdavis.edu

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41 **Supplemental Table Legends:**

42

43 **Table S1: Number of samples extracted for bulk sample type comparisons**

44 The number of bulk sample types (leaf, root, rhizome, sediment) for which DNA was extracted
45 listed by location (Westside Point, Gaffney Point) and timepoint (T1, T2, T3). Control sediment
46 was collected from outside the seagrass bed.

47

48 **Table S2: Number of samples extracted for intra-plant and epiphyte / endophyte analysis**

49 The number of samples (leaf, root, rhizome, sediment) for which DNA was extracted for intra-
50 plant (across leaf length and sediment depth) analysis. The leaf and root samples described here
51 were also used to generate epiphyte and endophyte data.

52

53 **Table S3: Number of control samples extracted during this experiment**

54 The number of positive (Zymo Mock Community) and negative (rinse water, ethanol, epiphyte
55 buffer and kit controls) for which DNA was extracted during this experiment.

56

57 **Table S4: Sequences used in SV8 complex molecular phylogeny**

58 Information about the 28S rRNA gene sequences used to generate Figure 5, including the clade,
59 species, strain ID, GenBank Accession number and study of origin for each sequence used.

60

61 **Table S5: Kruskal–Wallis tests on alpha diversity metrics**

62 We used Kruskal–Wallis tests to assess whether alpha diversity was significantly different
63 between categories. We used two different measurements of alpha diversity, observed number of
64 ASVs and the Shannon Diversity index. Categories examined included bulk sample type (leaf,
65 root, rhizome, sediment), root epiphyte / endophyte status and leaf epiphyte / endophyte status.

66

67 **Table S6: Post-hoc Dunn tests assessing alpha diversity between bulk sample types**

68 Alpha diversity was found to be significantly different across bulk sample types (Table S5). We
69 then used a post-hoc Dunn test to examine which sample type comparisons were stochastically
70 dominant on two measurements of alpha diversity, observed number of ASVs and the Shannon
71 Diversity index.

72

73 **Table S7: Permanova results of beta diversity of bulk sample types**

74 PERMANOVA tests were performed to find significant differences in fungal beta diversity,
75 calculated as the Bray Curtis dissimilarity metric, between different categorical variables
76 including sample type (leaf, root, rhizome, sediment), timepoint (T1, T2, T3), replicate core
77 number (1-5), sample location in 96-well sequencing plates sent to Zymo Research, Inc,
78 randomized DNA extraction group (A-P), and DNA extraction kit lot number.

79 **Table S8: Pairwise PERMANOVA results of beta diversity of bulk sample types**

80 Here we compared fungal community structure, calculated as Bray Curtis, between pairwise
81 sample types to assess between which sample types communities differed significantly.

82

83 **Table S9: Betadisper results of beta diversity of bulk sample types**

84 Betadisper was used to look for significant differences in the dispersions of different categorical
85 variables when investigating fungal beta diversity, calculated via the Bray Curtis dissimilarity
86 metric. Categorical variables tested included sample type (leaf, root, rhizome, sediment),
87 timepoint (T1, T2, T3), replicate core number (1-5), sample location in 96-well sequencing
88 plates sent to Zymo Research, Inc, randomized DNA extraction group (A-P), and DNA
89 extraction kit lot number.

90 **Table S10: Tukey HSD Post-hoc tests of beta diversity dispersions**

91 Tukey HSD post-hoc tests were used to further investigate pairwise combinations for categories
92 with dispersions that differed significantly ($p < 0.05$) as indicated by the Betadisper results
93 (Table S9). These categories included sample type (leaf, root, rhizome, sediment) and
94 randomized DNA extraction group (A-P).

95

96 **Table S11: Permanova results of beta diversity of leaf epiphytes and endophytes**

97 PERMANOVA tests were performed to find significant differences in fungal beta diversity,
98 calculated as the Bray Curtis dissimilarity metric, between different categorical variables
99 including epiphyte / endophyte status, leaf length segment (0-5 inches, 5-10 inches, 10-15
100 inches, 15-20 inches, 20-25 inches), replicate core number (1-5), sample location in 96-well
101 sequencing plates sent to Zymo Research, Inc, randomized DNA extraction group (A-P), and
102 DNA extraction kit lot number.

103

104 **Table S12: Pairwise PERMANOVA results of beta diversity of leaf length**

105 Here we compared fungal community structure, calculated as Bray Curtis, between pairwise leaf
106 length segments to assess between which segments had community structures that differed
107 significantly.

108

109 **Table S13: Betadisper results of beta diversity of leaf epiphytes and endophytes**

110 Betadisper was used to look for significant differences in the dispersions of different categorical
111 variables when investigating fungal beta diversity, calculated via the Bray Curtis dissimilarity
112 metric. Categorical variables tested included epiphyte / endophyte status, leaf length segment (0-
113 5 inches, 5-10 inches, 10-15 inches, 15-20 inches, 20-25 inches), replicate core number (1-5),
114 sample location in 96-well sequencing plates sent to Zymo Research, Inc, randomized DNA
115 extraction group (A-P), and DNA extraction kit lot number.

116 **Table S14: Tukey HSD Post-hoc tests of beta diversity dispersions**

117 Tukey HSD post-hoc tests were used to further investigate pairwise combinations of leaf length
118 segments since segments were found to have dispersions that differed significantly ($p < 0.05$) as
119 indicated by the Betadisper results (Table S13).

120

121 **Table S15: Mean, standard deviation and standard error of the relative abundances of**
122 **taxonomic orders across bulk sample types**

123 Only orders with a mean relative abundance with a variance of greater than or equal to one
124 percent are included here.

125 **Table S16: Kruskal-Wallis tests of mean relative abundance of taxonomic orders across**
126 **bulk sample types**

127 We used Kruskal–Wallis tests to assess whether the mean relative abundance of taxonomic
128 orders was significantly different between bulk sample types (leaf, root, rhizome, sediment).

129

130 **Table S17: Post-hoc Dunn tests of mean relative abundance of taxonomic orders across**
131 **bulk sample types**

132 For taxonomic orders with uncorrected p-values that were significantly different ($p < 0.05$)
133 between bulk sample types (Table S16), we then used post-hoc Dunn test to examine which
134 sample type comparisons were driving these differences.

135

136 **Table S18: Mean, standard deviation and standard error of the relative abundances of**
137 **taxonomic orders across leaf length**

138 Only orders with a mean relative abundance with a variance of greater than or equal to 0.1
139 percent are included here.

140 **Table S19: Kruskal-Wallis tests of mean relative abundance of taxonomic orders across**
141 **leaf length**

142 We used Kruskal–Wallis tests to assess whether the mean relative abundance of taxonomic
143 orders was significantly different between leaf length segments (0-5 inches, 5-10 inches, 10-15
144 inches, 15-20 inches, 20-25 inches).

145

146 **Table S20: Mean, standard deviation and standard error of the relative abundances of**
147 **ASVs across leaf length**

148 Only ASVs with a mean relative abundance with of greater than or equal to two percent are
149 included here.

150 **Table S21: Kruskal-Wallis tests of mean relative abundance of ASVs across leaf length**

151 We used Kruskal–Wallis tests to assess whether the mean relative abundance of ASVs was
152 significantly different between leaf length segments (0-5 inches, 5-10 inches, 10-15 inches, 15-
153 20 inches, 20-25 inches).

154

155 **Table S22: Post-hoc Dunn tests of mean relative abundance of ASVs across leaf length**
156 For ASVs with uncorrected p-values that were significantly different ($p < 0.05$) between bulk
157 sample types (Table S16), we then used post-hoc Dunn test to examine which sample type
158 comparisons were driving these differences.

159
160 **Table S23: Kruskal-Wallis tests of mean relative abundance of ASVs between leaf**
161 **epiphytes and endophytes**
162 We used Kruskal–Wallis tests to assess whether the mean relative abundance of ASVs was
163 significantly different between leaf epiphyte and endophyte samples.

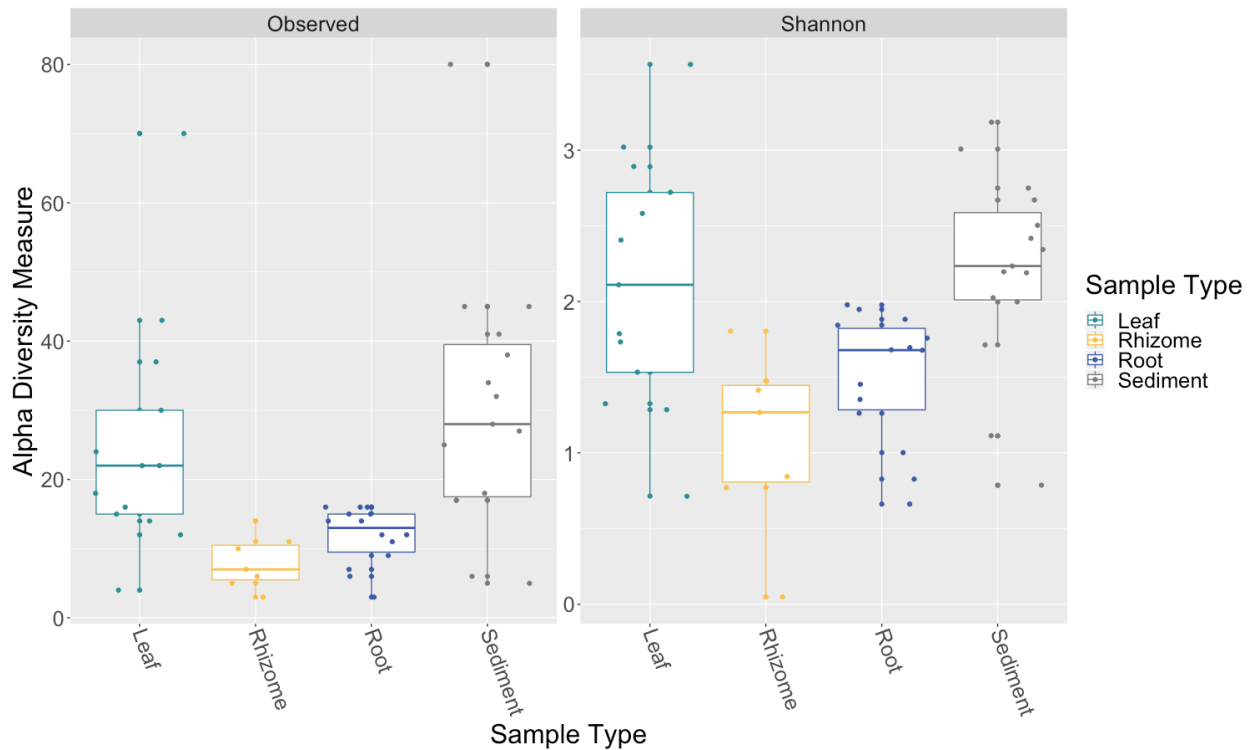
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194

195 **Supplemental Figures:**

196

197 **Figure S1: Alpha diversity does not differ between bulk sample types**

198 Two alpha diversity metrics, observed number of amplicon sequence variants (ASVs) (left) and
199 Shannon diversity index (right), are depicted as boxplots colored by bulk sample type, leaf (n =
200 13), root (n = 14), rhizome (n = 7) and sediment (n = 15). The dataset was first subset to a depth
201 of 10,000 sequences per sample and then boxplots were constructed using the plot_richness
202 function in phyloseq.
203



204

205

206

207

208

209

210

211

212

213

214

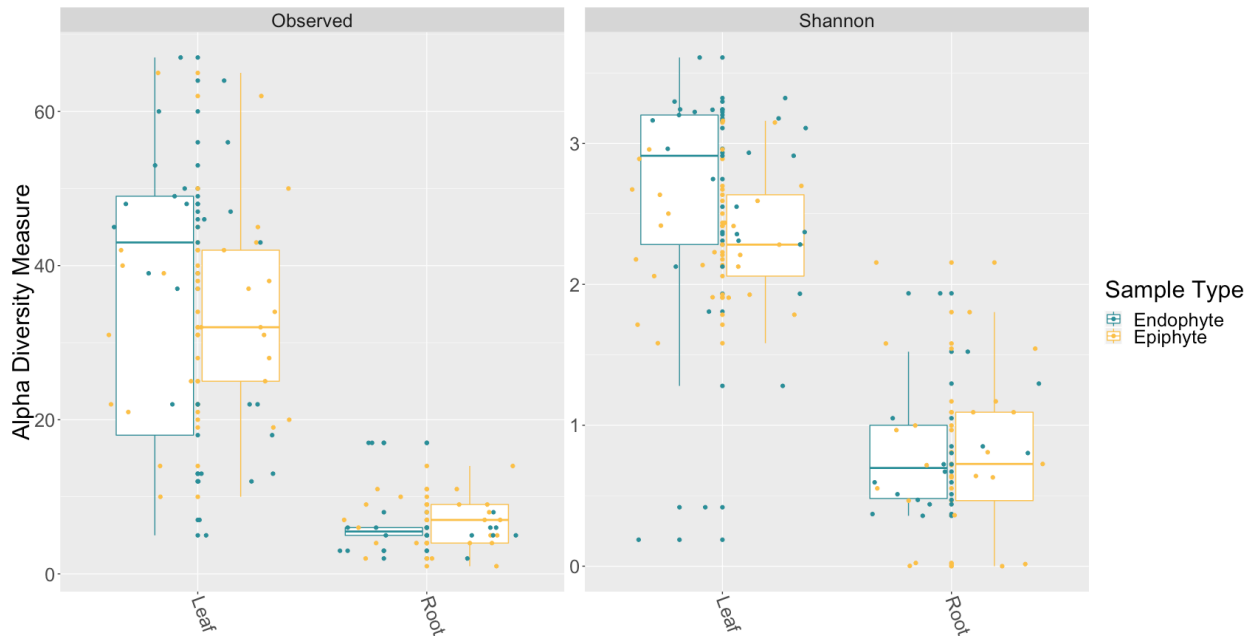
215

216

217

218 **Figure S2: Alpha diversity differs between leaf epiphyte and endophytes, but not root**
219 **epiphytes and endophytes**

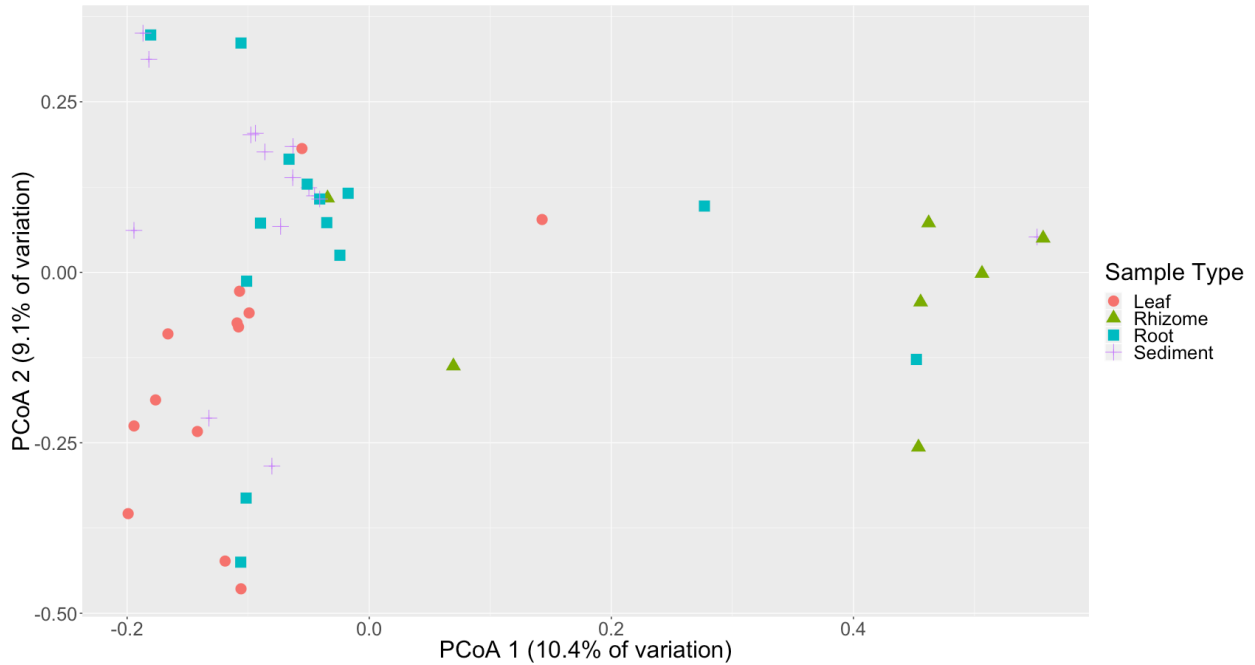
220 Two alpha diversity metrics, observed number of amplicon sequence variants (ASVs) (left) and
221 Shannon diversity index (right), are depicted as boxplots split by tissue type, leaf ($n_{total} = 50$) or
222 root ($n_{total} = 35$). Samples are further split and colored by epiphyte ($n_{leaf} = 25$; root, $n_{root} = 21$) or
223 endophyte ($n_{leaf} = 25$; $n_{root} = 14$) status (teal or yellow respectively). The dataset was first subset
224 to a depth of 5,000 sequences per sample and then boxplots were constructed using the
225 `plot_richness` function in `phyloseq`.
226



227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243

244 **Figure S3: Fungal community structure varies between bulk sample types**

245 Principal Coordinates Analysis (PCoA) visualization of Bray-Curtis dissimilarities of fungal
246 communities associated with bulk sample types. Points in the ordination are colored and
247 represented by shapes as follows: leaf (red circle; n = 13), rhizome (green triangle; n = 7), root
248 (blue square; n = 14) and sediment (purple cross; n = 15). The dataset was first subset to a depth
249 of 10,000 ITS2 amplicon sequences per sample and then Bray-Curtis dissimilarities were
250 calculated using the ordinate function in phyloseq.
251

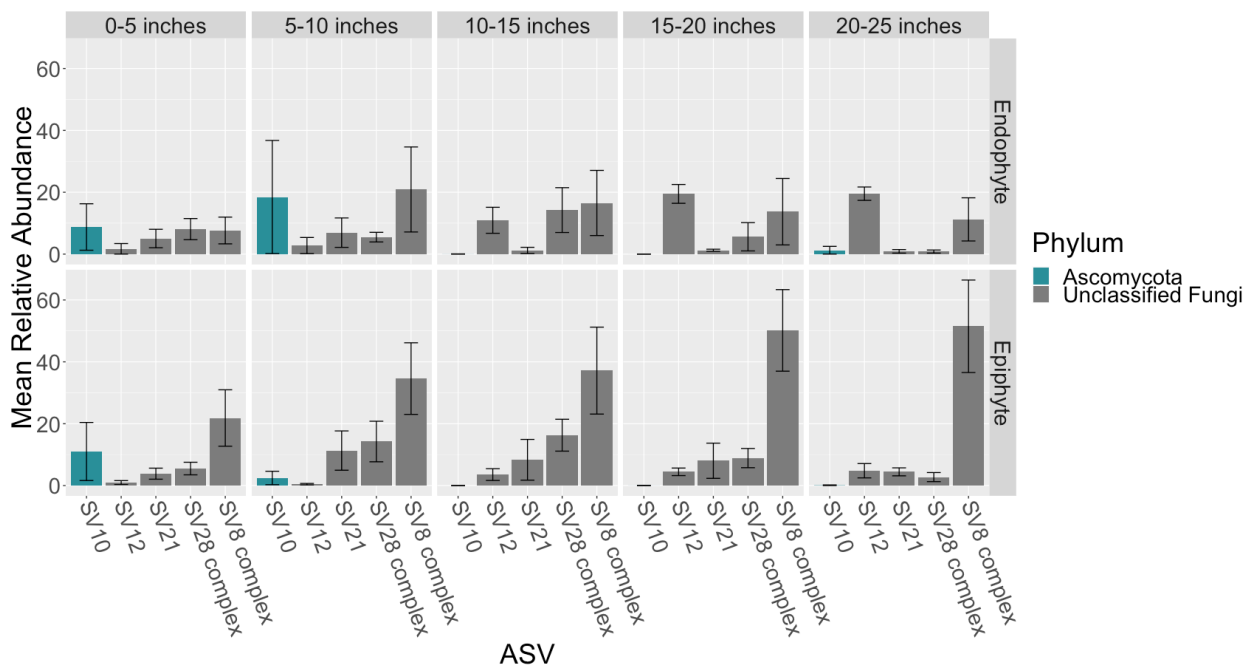


252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268

269 **Figure S4: Differences in mean relative abundance of epiphyte and endophytes ASVs**
 270 **across leaf length**

271 The mean relative abundance of ASVs with a mean of greater than two percent are shown across
 272 leaf segments, 0-5 inches (n = 10), 5-10 inches (n = 10), 10-15 inches (n = 10), 15-20 inches (n =
 273 10) and 20-25 inches (n = 10), with the standard error of the mean represented by error bars and
 274 bars colored by taxonomic phylum. Samples are further split in half and the plots are divided up
 275 by endophyte (top row) and epiphyte (bottom row) status. The dataset was first subset to a depth
 276 of 5,000 sequences per sample and then converted into relative abundance values. ASVs were
 277 grouped into complexes if ASVs shared greater than 99% sequence identity. Taxonomy was
 278 inferred for ITS2 amplicon sequence variants using the RDP Naive Bayesian Classifier algorithm
 279 with a modified UNITE (v. 8.0) database.

280



281

282

283