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Abstract:	Mangrove forests are key tropical marine ecosystems that are rich in fungi, but our understanding of fungal communities associated with mangrove trees and their various organs remains limited because much of the diversity lies within the microbiome. In this study, we investigated the fungal communities associated with the mangrove tree Sonneratia alba throughout Peninsular Malaysia and Singapore. At each sampling location, we collected leaves, fruits, pneumatophores and sediment samples and performed amplicon sequencing of the ribosomal internal transcribed spacer 1 to characterise the associated communities. Results show distinct fungal communities at each sampled location with further differentiation according to the plant part. We find a significant distance decay of similarity, particularly for sediment samples due to the greater variability of sediment environments relative to the more stable fungal habitats provided by living plant organs. We are able to assign taxonomy to the majority of sequences from leaves and fruits, but a much larger portion of the sequences recovered from pneumatophores and sediment samples could not be identified. This pattern underscores the limited mycological research performed in marine environments and demonstrates the need for a concerted research effort on multiple species to fully characterise the coastal microbiome and its role in the functioning of marine ecosystems.
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Distinct fungal communities associated with different organs of the mangrove *Sonneratia alba* in the Malay Peninsula

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Key words

Fungal diversity, Internal transcribed spacer, Mangrove Microbiome, Marine fungi, Microbial ecology, Southeast Asia

1 **Abstract**

2 Mangrove forests are key tropical marine ecosystems that are rich in fungi, but our

3 understanding of fungal communities associated with mangrove trees and their various organs

4 remains limited because much of the diversity lies within the microbiome. In this study, we

5 investigated the fungal communities associated with the mangrove tree *Sonneratia alba*

6 throughout Peninsular Malaysia and Singapore. At each sampling location, we collected leaves,

7 fruits, pneumatophores and sediment samples and performed amplicon sequencing of the

8 ribosomal internal transcribed spacer 1 to characterise the associated communities. Results show

9 distinct fungal communities at each sampled location with further differentiation according to the

10 plant part. We find a significant distance decay of similarity, particularly for sediment samples

11 due to the greater variability of sediment environments relative to the more stable fungal habitats

12 provided by living plant organs. We are able to assign taxonomy to the majority of sequences

13 from leaves and fruits, but a much larger portion of the sequences recovered from

14 pneumatophores and sediment samples could not be identified. This pattern underscores the

15 limited mycological research performed in marine environments and demonstrates the need for a

16 concerted research effort on multiple species to fully characterise the coastal microbiome and its

17 role in the functioning of marine ecosystems.

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Introduction

Mangroves are a globally distributed group of salt tolerant trees and shrubs that are confined to brackish intertidal zones, estuaries, lagoons and backwaters throughout the tropics and subtropics (Thatoi et al. 2013). Straddling the interface between marine and terrestrial ecosystems, they provide important buffers that dissipate wave energy, stabilising coastlines and protecting against coastal erosion and natural hazards such as hurricanes and tsunamis (Williams 2005; Wee et al. 2019). Mangroves provide critical ecological habitats that host high levels of biodiversity and act as a nursery for many juvenile coral reef species (Abu El-Regal and Ibrahim 2014; Mehvar et al. 2018). Despite these benefits, mangrove clearance for aquaculture and urban development is rampant in many areas of the world. As much as 35% of global mangrove cover has been removed, with Asia having lost an estimated 33% of its total mangrove cover between 1980 and 1990 (Richards and Friess 2016; Sanderman et al. 2018).

Mangroves are acknowledged as biodiversity hotspots of marine fungi (Shearer et al. 2007). Pioneering mycological research documented fungi on mangrove roots (Cribb and Cribb 1955) and described mangrove-associated fungi throughout the world (Kohlmeyer 1969). New species continue to be described today, and examination of various plant parts and geographic localities are finding unexpected diversity and strong community structuring of fungi in mangroves (Kumar et al. 2019; Lee et al. 2019). In particular, mangroves abut marine and terrestrial environments, offering a unique opportunity to study plants that potentially contains obligate marine fungi, terrestrial fungi and those that can survive, or at least tolerate both environments to some degree. Mangrove-associated fungi have been divided into two groups: those that are submerged at high tide and those that are not. Marine fungi are expected to be

45 found in parts that can be submerged, while terrestrial fungi are likely to dominate parts that are

46 not, or are unlikely to be submerged (i.e. leaves and fruits) (Kohlmeyer 1969; Lee et al. 2019).

47 Studies on marine fungi remain sparse despite the recent interest, particularly those

48 examining biogeographic patterns (Amend et al. 2012, 2019; Wainwright et al. 2017, 2018;

49 Ettinger and Eisen 2019), which is unfortunate as marine systems offer a potential treasure trove

50 of undescribed marine fungal biodiversity. In 2011, only 537 obligate marine fungi had been

51 identified (Jones 2011). Today, estimates predict in excess of 10,000 marine species, and nearly

52 all remain undescribed (Jones 2011; Comeau et al. 2016; Picard 2017; Amend et al. 2019; Jones

53 et al. 2019). Consequently, our understanding of fungal biogeography in marine environments

54 remains rudimentary. However, the research that has been performed suggests that the

55 environment strongly influences spatial patterns of marine fungal communities (Tisthammer et al.

56 2016; Lee et al. 2019a). Environmental and habitat differences also explain differences in

57 seagrass and macroalgal associated marine fungal communities in Southeast Asia (Wainwright et

58 al. 2019b, c).

59 These discoveries are occurring during a time of growing interest and appreciation of the

60 coastal marine microbiome and how this is influenced by, or influences the microbiome of

61 species found in coastal areas (Glasl et al. 2019a, b; Trevathan-Tackett et al. 2019). Yet, there

62 remains a lack of research into the coastal microbiome and this is especially apparent in

63 comparison to most other microbiome types (e.g., the human microbiome) (Trevathan-Tackett et

64 al. 2019; Wilkins et al. 2019). Specifically, despite the recognised necessity of mangroves and

65 the critical ecosystem services they provide, work on the mangrove microbiome is embryonic at

66 best, especially in contrast to the more charismatic coral reefs (Buddemeier and Smith 1999)

67 where studies are relatively numerous and advanced in comparison (Ainsworth and Gates 2016;

68 Hernandez-Agreda et al. 2017; Gardner et al. 2019; Wainwright et al. 2019a). For example, there

69 are efforts to engineer, manipulate and seed the coral microbiome with beneficial

70 microorganisms that could promote recovery from disturbance (Peixoto et al. 2017, 2019;

71 Rosado et al. 2019).

72 Here, using high-throughput sequencing, we examine fungi associated with the mangrove

73 tree *Sonneratia alba* throughout the Malay Peninsula to test whether fungal communities differ

74 by geographic location and plant part sampled. Together with samples of the adjacent sediment,

75 we provide valuable information on the fungal composition of the *S. alba* microbiome and its

76 associated environment.

Materials and Methods

77

78 We targeted 10 visibly healthy whole leaves, fruiting bodies and entire pneumatophores

79 from *Sonneratia alba* trees during low tide from each of nine locations in three regions

80 (Singapore, western and eastern Peninsular Malaysia), though fewer samples were collected in

81 some cases due to logistical or safety reasons (i.e., collecting fruits and leaves at height) (Figure

82 1 and Supplementary Table 1). Additionally, one sediment sample in close proximity (<1 m) to

83 each tree was taken using a syringe placed approximately 4 cm below the surface. Prior to DNA

84 extraction, 0.5-cm diameter leaf-disks were taken throughout the surface of the leaf with a sterile

85 hole punch. Pneumatophores and fruiting bodies were cut into ~0.25 cm cubes using a new

86 sterile razor blade for each sample. All collected mangrove tissues (leaves, fruits and

87 pneumatophores) were surface sterilized by immersion in 1% NaClO for 2 min, 70% EtOH for 2

88 min and rinsed twice in sterile, autoclaved water for 5 min. Sediment samples were not surface

89 sterilized. Tissue and sediment samples were disrupted in an Omni Bead Ruptor 24 (Omni

90 International, Kennesaw, GA, United States) at 8 ms^{-1} for 2 min.

91   As per Cobian, Egan and Amend (2019), haphazardly chosen surface sterilized tissues

92   were used in DNA extractions, and all extractions were performed using the Qiagen DNeasy

93   PowerSoil Kit following the manufacturer's protocol. The internal transcribed spacer 1 (ITS1)

94   region of fungal DNA was amplified via polymerase chain reaction (PCR) using the ITS1F

95   primer (5'-CTT GGT CAT TTA GAG GAA GTA A-3' (Gardes and Bruns 1993) and the ITS2

96   primer (5'-GCT GCG TTC TTC ATC GAT GC-3' (White et al. 1990). Primers were modified to

97   include Illumina adapters, a linker and a unique barcode (see Smith and Peay 2014 for details of

98   custom sequencing primers). Each reaction was performed in a total volume of 25 µl, containing

99   12.5 µl KAPA Plant PCR buffer, 1.5 µl BSA, 0.5 µl MgCl2, 0.1 µl KAPA 3G Plant DNA

100   polymerase (Kapa Biosystems, Inc., Wilmington, MA, United States), 0.75 µl of each primer at

101   10 mM, and 9 µl DNA template. PCR cycling parameters were: 3 min at 95ºC, followed by 35

102   cycles of 20 s at 95 ºC, 15 s at 53ºC, and 20 s at 72ºC, with a final elongation at 72ºC for 1 min.

103   Negative PCR and extraction blanks were included and sequenced to identify contamination

104   issues. PCR products were visualized on a 1% TBE buffer agarose gel, then normalized and

105   cleaned using SequalPrep^{TM} normalization plates (Invitrogen, Frederick, MD, United States).

106   Purified PCR products were sequenced on the Illumina MiSeq platform (600 cycles, V3

107   chemistry, 300-bp paired-end reads) with a 15% PhiX spike at the Genome Institute of Singapore.

108   Our bioinformatics pipeline, comprising quality filtering and taxonomic assignment,

109   followed that described in the DADA2 ITS Pipeline Workflow V1.81

110   (https://benjjneb.github.io/dada2/ITS_workflow.html), with the following minor modifications:

111   (1) due to lower quality, reverse reads were not used – discarding low quality reverse reads is a

112   common strategy that frequently gives better results than assembled reads (Pauvert et al., 2019);

113 and (2) the R package *decontam* was used to identify and remove any contaminants identified in

114 sequenced negative controls via the prevalence method (Davis et al. 2018).

115 All amplicon sequence variants (ASVs) not assigned to fungi were removed, while those

116 remaining were used in all downstream analyses. Non-metric multidimensional scaling (NMDS)

117 plots were created using a Bray–Curtis dissimilarity matrix of samples in the R package phyloseq

118 version 1.25.2 (McMurdie and Holmes 2013). A NMDS plot was generated for all sampled

119 compartments combined, and separate plots were implemented for each sampled plant organ

120 (leaf, fruit, pneumatophore and sediment). Permutational multivariate analysis of variance

121 (PERMANOVA) with 999 permutations performed via the *adonis* function in the R package

122 vegan version 2.5-2 (Oksanen et al. 2019) was used to test the effects of region, location and

123 plant part on the fungal communities. Venn diagrams were generated using the VennDiagram R

124 package (Chen and Boutros 2011).

125 To test for distance decay of similarity, Mantel test was performed between geographic

126 distance and community matrices using the *mantel.rtest* function in the ade4 package (Bougeard

127 and Dray 2018) with 999 permutations. We also carried out multiple regression on distance

128 matrices with 9999 permutations in the *ecodist* package.

129 All raw sequences associated with this work have been deposited at the National Center

130 for Biotechnology Information under the BioProject ID PRJNA592423.

131 **Results**

132 In total, 10,076,402 reads were generated, and after quality filtering, 5,417,789 were

133 retained for downstream analyses. Rarefaction curves indicate sufficient sequencing depth was

134 achieved (Supplementary Figure 1). NMDS plots show that fungal communities associated with

135   each sampled compartment (leaf, fruit, pneumatophore and sediment) are clustered by location

136   (Fig 2). Fungal communities in sediment samples appear more similar to one another compared

137   to the plant organs, and sediment samples are further clustered by region (Supplementary Figure

138   2). Overall, PERMANOVA indicates significant differences in fungal community among

139   locations and sampled compartments ($R^2 = 0.116$; $P = 0.001$ and $R^2 = 0.05$; $P = 0.001$

140   respectively; Supplementary Table 2).

141   Structures that are not submerged at high tide (i.e., leaves and fruits) share more similar

142   fungal communities than those that are periodically submerged by tides (Supplementary Figure

143   2). Supporting the idea that fungal communities from nearby sample locations are more similar

144   to one another than distant ones, we see a significant positive relationship between community

145   structure and geographic distance (Mantel test: $r = 0.28$, $p = 0.001$). This relationship is further

146   supported by multiple regression on distance matrices for all sampled compartments combined (r

147    $= 0.07$, $p = 0.001$) as well as for each compartment (Table 1).

148   The most diverse fungal communities are associated with sediment samples which are

149   approximately twice as diverse as all other sampled compartments, with median Shannon

150   diversity values between 1.9 and 4.1 (Supplementary Figure 3). All samples, irrespective of type,

151   are dominated by the phyla Ascomycota and Basidiomycota (Supplementary Figure 4) as well as

152   the classes Dothideomycetes, Sordariomycetes and to a lesser extent the Eurotiomycetes, but the

153   exact composition varies among compartments and locations (Fig 3). When all organs and

154   sediment samples are combined at each location, fungal diversity is relatively constant and

155   composition at the class level is generally comparable throughout all locations (Supplementary

156   Figure 5 - 7).

157 ASV richness is highest in the sediment, followed by pneumatophores (7160 & 859

158 respectively), while leaves and fruits—structures that are above the high water line—have the

159 lowest ASV richness with 370 and 226 respectively. A total of 84 ASVs are shared between all

160 compartments including sediment, and the highest number of shared ASVs is found between

161 pneumatophores and sediment samples (Fig. 4).

162 **Discussion**

163 In this study, we show that the fungal communities associated with the mangrove plant

164 *Sonneratia alba* differ significantly between sampled locations throughout Singapore and

165 Peninsular Malaysia. Fungal communities can be further differentiated by sampled compartment

166 and plant organ (i.e., leaves vs. sediment, etc), with each compartment hosting a distinct fungal

167 community. We also show a significant pattern of positive distance decay, meaning that fungal

168 communities from geographically closer sites are more similar to one another than those that are

169 more distant. This is consistent with work on a variety of other assumed highly dispersive taxa,

170 from mammals to microorganisms, showing community or genetic structuring in the marine

171 environment which has no obvious barriers to dispersal (Hoffman et al. 2012; Xu et al. 2017;

172 Wainwright et al 2018, 2019a). This contrasts with the traditionally held view that marine

173 environments are open with few, if any limitations imposed on dispersal. Under this paradigm,

174 highly dispersive taxa show limited structure of any nature (Rocha et al. 2007; Sá-Pinto et al.

175 2012), and from a microbial perspective as Baas Becking quipped "everything is everywhere"

176 (Wit and Bouvier 2006).

177 As in Tisthammer et al. (2016), and also shown for another mangrove *Avicenna alba*

178 recently (Lee et al. 2019a), we suggest that the fungal community distribution is strongly shaped

179 by the environment, a consequence of differences in habitat type throughout Peninsular Malaysia

180   and Singapore. Further supporting this idea, the coasts of Malay Peninsula are split in two by the

181   Titiwangsa Mountain range, which forms the backbone of the peninsula with its >2000-m

182   maximum elevation. The east and west coasts have different tectonic origins and chemical

183   compositions. The east coast is predominantly Carboniferous, while the west coast is dominated

184   by Permian strata (Hutchison 2014). The compositional differences in strata create differences in

185   substrate chemistry (e.g., organic, inorganic carbon content, carbon/nitrogen ratio and pH), and

186   these differences can bring about environmental filtering that acts to remove members of the

187   fungal community least suited to the environment (Cline and Zak 2014; Goldmann et al. 2016).

188   Working in tandem with this is an environmental cline of increasing marine water mass salinity

189   and dissolved oxygen with increasing latitude (Muhaimin A et al. 2011). Consequently, locations

190   close to one and another are more similar in terms of fungal community composition than those

191   more spatially separated, a result supported by Mantel test and multiple regression on distance

192   matrices. All plant organs and sediment samples show significant patterns of distance decay,

193   however, fungal communities associated with sediment display the strongest pattern of distance

194   decay. This is consistent with previously proposed hypotheses suggesting that habitats offered by

195   living plant organs (i.e., leaf, fruit, etc), while different between sampling regions, are more

196   similar to one another and constant throughout a species range than would be suggested by

197   environmental differences (Goldmann et al. 2016).

198   Shannon diversity values are comparable among all locations when all organs and

199   sediment are combined, and fungal community diversity in the leaves, fruits and pneumatophores

200   is comparable, while sediment samples are approximately twice as diverse all other samples.

201   This is consistent with previous work showing that soils are highly diverse reservoirs of fungal

202   biodiversity, containing hundreds of thousands of fungal species (Bridge and Spooner 2001; Lee

203 et al. 2019a). Likewise we find the highest number of fungal ASVs in the sediment samples. On

204 the one hand, sediments and soils are dynamic environments and the fungi in these environments

205 have diverse roles (Li et al. 2016). On the other hand, the habitats associated with plant organs

206 are expected to be less diverse and more stable, being controlled ultimately by the specific

207 requirements of the host plant. Consequently, fewer fungi can be supported and correspondingly

208 we see a less diverse fungal community associated with non-sediment samples.

209 As with previous mangrove associated fungal work, all samples are dominated by phyla

210 Ascomycota and Basidiomycota and Dothideomycetes. At the class level, Agaricomycetes are

211 found throughout, but are more prevalent in pneumatophores and sediment, or parts that are

212 inundated at high tide. The Agaricomycetes are frequently found in marine environments and

213 have been reported as one of the dominant fungal classes in tropical mangrove sediments (Arfi et

214 al. 2012; Rédou et al. 2015). The increased prevalence of Agaricomycetes in communities from

215 pneumatophores and sediment that have the potential to be submerged in comparison to those

216 within leaves and fruits likely reflects the adaptations this group has for life in environments

217 where they will be at least partly submerged over a complete tidal cycle (Prasannarai and Sridhar

218 2001).

219 Wind and flood events have been proposed as mechanisms that transport terrestrial fungi

220 to mangrove environments (Bonugli-Santos et al. 2015), and air mass source has been shown to

221 be an important determinant of microbial diversity in marine systems (Archer et al. 2019).

222 Results here appear to support these ideas as we have been able to assign taxonomy to the

223 majority of sequences recovered from fruits and leaves, likely reflecting their terrestrial origins

224 and the abundance of mycological work performed in these habitats, for which taxonomic

225 assignments of terrestrial fungi in sequence databases are well curated. Conversely, the highest

226 number of unassigned fungal sequences are found in the pneumatophores and sediment, which

227 are periodically submerged and likely contain a higher proportion of marine fungi (Kohlmeyer

228 1969). Assigning identities to microbes from marine or understudied environments is an

229 acknowledged challenge as databases curated with marine representatives are lacking (Rédou et

230 al. 2015; Ettinger and Eisen 2019; Archer et al. 2019).

231   Southeast Asia contains are the most biodiverse, extensive and oldest mangrove forests

232 on the planet (Ellison et al. 1999; Giri et al. 2011; Gandhi and Jones 2019). However, their

233 continued existence faces an uncertain future, with considerable challenges presented by

234 deforestation, aquaculture and a multitude of other anthropogenic stressors (Farnsworth and

235 Ellison 1997; Richards and Friess 2016; Romañach et al. 2018). Restoration and rehabilitation

236 are important mangrove conservation strategies (Renzi et al. 2019; Lee et al. 2019b). Terrestrial

237 restoration schemes frequently incorporate information about fungal communities in their

238 approach (Moora et al. 2004; Quoreshi 2008; Chaudhary et al. 2019), and increasingly, marine

239 conservation initiatives are considering the beneficial properties of microorganisms (Peixoto et al.

240 2017; Peixoto, Sweet and Bourne 2019; Rosado et al. 2019). Our results show that fungal

241 communities can be differentiated by location, suggesting that these communities have evolved

242 to the plants specific requirements in each environment. If this is indeed the case, it may be

243 necessary to consider the fungal communities in restoration schemes, especially since host-

244 pathogen resistance can be increased by matching host fungal communities as closely as possible

245 to areas where the host is known to be healthy (Zahn and Amend 2017). These considerations are

246 likely even more important and necessary to avoid maladaptation when mangrove propagules are

247 grown in large *ex situ* nurseries and outplanted. We recommend that, where feasible, *ex situ*

248 nurseries should be located as close as possible to the restoration site; findings here suggest that

249 doing so will increase the similarities in fungal community composition between nursery and

250 restoration sites.

251   In conclusion, there is growing appreciation for the role microorganisms play in all

252 aspects of ecosystem functioning, and the success of mangrove restoration projects is expected to

253 benefit from further detailed characterisation of the mangrove microbiome. This study provides

254 foundational data on the fungal communities associated with various compartments of the

255 mangrove *Sonneratia alba* and more broadly contributes to better understanding of the coastal

256 microbiome. However, more concerted and coordinated cross-disciplinary efforts are required

257 from marine, terrestrial and atmospheric microbiologists to fully address the acknowledged gaps

258 in research of this nature.

259 **Declarations**

260 **Ethics approval and consent to participate**

261 We thank the relevant authorities for approval of research permits. Collections from Malaysia

262 were made under permit JTLM 630-7Jld.9(9) and from Singapore under permit number NP/RP

263 18-035

264 **Consent for publication**

265 Not applicable

266 **Availability of data and material**

267 The datasets generated during and/or analysed during the current study are available in the

268 National Center for Biotechnology Information under the BioProject ID PRJNA592423

Competing interests

Funding

Authors' contributions

All authors contributed equally. All authors read and approved the final manuscript.

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References

Abu El-Regal MA, Ibrahim NK (2014) Role of mangroves as a nursery ground for juvenile reef fishes in the southern Egyptian Red Sea. The Egyptian Journal of Aquatic Research 40:71–78. https://doi.org/10.1016/j.ejar.2014.01.001

Ainsworth TD, Gates RD (2016) Corals' microbial sentinels. Science 352:1518–1519. https://doi.org/10.1126/science.aad9957

Amend A, Burgaud G, Cunliffe M, et al (2019) Fungi in the Marine Environment: Open Questions and Unsolved Problems. mBio 10:e01189-18. https://doi.org/10.1128/mBio.01189-18

Amend AS, Barshis DJ, Oliver TA (2012) Coral-associated marine fungi form novel lineages and heterogeneous assemblages. ISME J 6:1291–1301. https://doi.org/10.1038/ismej.2011.193

Ancheeva E, Daletos G, Proksch P (2018) Lead Compounds from Mangrove-Associated Microorganisms. Marine Drugs 16:319. https://doi.org/10.3390/md16090319

Archer SDJ, Lee KC, Caruso T, et al (2019) Air mass source determines airborne microbial diversity at the ocean–atmosphere interface of the Great Barrier Reef marine ecosystem. The ISME Journal 1–6. https://doi.org/10.1038/s41396-019-0555-0

Arfi Y, Marchand C, Wartel M, Record E (2012) Fungal diversity in anoxic-sulfidic sediments in a mangrove soil. Fungal Ecology 5:282–285. https://doi.org/10.1016/j.funeco.2011.09.004

Bonugli-Santos RC, dos Santos Vasconcelos MR, Passarini MRZ, et al (2015) Marine-derived fungi: diversity of enzymes and biotechnological applications. Front Microbiol 6:. https://doi.org/10.3389/fmicb.2015.00269

Bougeard S, Dray S (2018) Supervised Multiblock Analysis in *R* with the **ade4** Package. J Stat Soft 86:. https://doi.org/10.18637/jss.v086.i01

Bridge P, Spooner B (2001) Soil fungi: diversity and detection. Plant and Soil 232:147–154. https://doi.org/10.1023/A:1010346305799

Buddemeier RW, Smith SV (1999) Coral Adaptation and Acclimatization: A Most Ingenious Paradox. Am Zool 39:1–9. https://doi.org/10.1093/icb/39.1.1

Chaudhary VB, Akland K, Johnson NC, Bowker MA Do soil inoculants accelerate dryland restoration? A simultaneous assessment of biocrusts and mycorrhizal fungi. Restoration Ecology n/a: https://doi.org/10.1111/rec.13088

Chen H, Boutros PC (2011) VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinformatics 12:35. https://doi.org/10.1186/1471-2105-12-35

Cobian GM, Egan CP, Amend AS (2019) Plant–microbe specificity varies as a function of elevation. The ISME Journal 1. https://doi.org/10.1038/s41396-019-0470-4

Comeau AM, Vincent WF, Bernier L, Lovejoy C (2016) Novel chytrid lineages dominate fungal sequences in diverse marine and freshwater habitats. Sci Rep 6:1–6. https://doi.org/10.1038/srep30120

Davis NM, Proctor DM, Holmes SP, et al (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6:226. https://doi.org/10.1186/s40168-018-0605-2

Ellison AM, Farnsworth EJ, Merkt RE (1999) Origins of mangrove ecosystems and the mangrove biodiversity anomaly. Global Ecology and Biogeography 8:95–115. https://doi.org/10.1046/j.1466-822X.1999.00126.x

Ettinger CL, Eisen JA (2019) Characterization of the Mycobiome of the Seagrass, Zostera marina, Reveals Putative Associations With Marine Chytrids. Front Microbiol 10:. https://doi.org/10.3389/fmicb.2019.02476

Farnsworth EJ, Ellison AM (1997) The Global Conservation Status of Mangroves. Ambio 26:328–334

Gandhi S, Jones TG (2019) Identifying Mangrove Deforestation Hotspots in South Asia, Southeast Asia and Asia-Pacific. Remote Sensing 11:728. https://doi.org/10.3390/rs11060728

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118. https://doi.org/10.1111/j.1365-294x.1993.tb00005.x

329 Gardner SG, Camp EF, Smith DJ, et al (2019) Coral microbiome diversity reflects mass coral bleaching
330   susceptibility during the 2016 El Niño heat wave. Ecology and Evolution 9:938–956.
331   https://doi.org/10.1002/ece3.4662

332 Giri C, Ochieng E, Tieszen LL, et al (2011) Status and distribution of mangrove forests of the world using
333   earth observation satellite data. Global Ecology and Biogeography 20:154–159.
334   https://doi.org/10.1111/j.1466-8238.2010.00584.x

335 Glasl B, Bourne DG, Frade PR, et al (2019a) Microbial indicators of environmental perturbations in coral
336   reef ecosystems. Microbiome 7:94. https://doi.org/10.1186/s40168-019-0705-7

337 Glasl B, Smith CE, Bourne DG, Webster NS (2019b) Disentangling the effect of host-genotype and
338   environment on the microbiome of the coral Acropora tenuis. PeerJ; San Diego.
339   http://dx.doi.org.libproxy1.nus.edu.sg/10.7717/peerj.6377

340 Goldmann K, Schröter K, Pena R, et al (2016) Divergent habitat filtering of root and soil fungal
341   communities in temperate beech forests. Sci Rep 6:1–10. https://doi.org/10.1038/srep31439

342 Hernandez-Agreda A, Gates RD, Ainsworth TD (2017) Defining the Core Microbiome in Corals' Microbial
343   Soup. Trends in Microbiology 25:125–140. https://doi.org/10.1016/j.tim.2016.11.003

344 Hutchison CS (2014) Tectonic evolution of Southeast Asia. 60:18

345 Jones EBG (2011) Are there more marine fungi to be described? Botanica Marina 54:343–354.
346   https://doi.org/10.1515/bot.2011.043

347 Jones EBG, Pang K-L, Abdel-Wahab MA, et al (2019) An online resource for marine fungi. Fungal Diversity
348   96:347–433. https://doi.org/10.1007/s13225-019-00426-5

349 Kohlmeyer J (1969) Ecological notes on fungi in Mangrove forests. Transactions of the British
350   Mycological Society 53:237-IN5. https://doi.org/10.1016/S0007-1536(69)80058-6

351 Kumar V, Cheewangkoon R, Thambugala KM, et al (2019) Rhytidhysteron mangrovei (Hysteriaceae), a
352   new species from mangroves in Phetchaburi Province, Thailand. Phytotaxa 401:166–178.
353   https://doi.org/10.11646/phytotaxa.401.3.2

354 Lee NLY, Huang D, Quek ZBR, et al (2019a) Mangrove-Associated Fungal Communities Are Differentiated
355   by Geographic Location and Host Structure. Front Microbiol 10:.
356   https://doi.org/10.3389/fmicb.2019.02456

357 Lee SY, Hamilton S, Barbier EB, et al (2019b) Better restoration policies are needed to conserve
358   mangrove ecosystems. Nat Ecol Evol 3:870–872. https://doi.org/10.1038/s41559-019-0861-y

359 Li W, Wang M, Bian X, et al (2016) A High-Level Fungal Diversity in the Intertidal Sediment of Chinese
360   Seas Presents the Spatial Variation of Community Composition. Front Microbiol 7:.
361   https://doi.org/10.3389/fmicb.2016.02098

362 McMurdie PJ, Holmes S (2013) phyloseq: An R Package for Reproducible Interactive Analysis and
363 Graphics of Microbiome Census Data. PLOS ONE 8:e61217.
364 https://doi.org/10.1371/journal.pone.0061217

365 Mehvar S, Filatova T, Dastgheib A, et al (2018) Quantifying Economic Value of Coastal Ecosystem
366 Services: A Review. Journal of Marine Science and Engineering 6:5.
367 https://doi.org/10.3390/jmse6010005

368 Moora M, Öpik M, Sen R, Zobel M (2004) Native arbuscular mycorrhizal fungal communities
369 differentially influence the seedling performance of rare and common Pulsatilla species.
370 Functional Ecology 18:554–562. https://doi.org/10.1111/j.0269-8463.2004.00876.x

371 Muhaimin A A, Zaiton Ibr Z, Aizat Isma S (2011) Water Mass Characteristics in the Strait of Malacca using
372 Ocean Data View. Research J of Environmental Sciences 5:49–58.
373 https://doi.org/10.3923/rjes.2011.49.58

374 Oksanen J, Blanchet FG, Friendly M, et al (2019) vegan: Community Ecology Package

375 Peixoto RS, Rosado PM, Leite DC de A, et al (2017) Beneficial Microorganisms for Corals (BMC):
376 Proposed Mechanisms for Coral Health and Resilience. Front Microbiol 8:.
377 https://doi.org/10.3389/fmicb.2017.00341

378 Peixoto RS, Sweet M, Bourne DG (2019) Customized Medicine for Corals. Front Mar Sci 6:.
379 https://doi.org/10.3389/fmars.2019.00686

380 Picard KT (2017) Coastal marine habitats harbor novel early-diverging fungal diversity. Fungal Ecology
381 25:1–13. https://doi.org/10.1016/j.funeco.2016.10.006

382 Quoreshi AM (2008) The Use of Mycorrhizal Biotechnology in Restoration of Disturbed Ecosystem. In:
383 Siddiqui ZA, Akhtar MohdS, Futai K (eds) Mycorrhizae: Sustainable Agriculture and Forestry.
384 Springer Netherlands, Dordrecht, pp 303–320

385 Rédou V, Navarri M, Meslet-Cladière L, et al (2015) Species Richness and Adaptation of Marine Fungi
386 from Deep-Subseafloor Sediments. Appl Environ Microbiol 81:3571–3583.
387 https://doi.org/10.1128/AEM.04064-14

388 Renzi JJ, He Q, Silliman BR (2019) Harnessing Positive Species Interactions to Enhance Coastal Wetland
389 Restoration. Front Ecol Evol 7:. https://doi.org/10.3389/fevo.2019.00131

390 Richards DR, Friess DA (2016) Rates and drivers of mangrove deforestation in Southeast Asia, 2000–2012.
391 PNAS 113:344–349. https://doi.org/10.1073/pnas.1510272113

392 Rocha LA, Craig MT, Bowen BW (2007) Phylogeography and the conservation of coral reef fishes. Coral
393 Reefs 26:501–512. https://doi.org/10.1007/s00338-007-0261-7

394 Romañach SS, DeAngelis DL, Koh HL, et al (2018) Conservation and restoration of mangroves: Global
395 status, perspectives, and prognosis. Ocean & Coastal Management 154:72–82.
396 https://doi.org/10.1016/j.ocecoaman.2018.01.009

397   Rosado PM, Leite DCA, Duarte GAS, et al (2019) Marine probiotics: increasing coral resistance to
398   bleaching through microbiome manipulation. ISME J 13:921–936.
399   https://doi.org/10.1038/s41396-018-0323-6

400   Sanderman J, Hengl T, Fiske G, et al (2018) A global map of mangrove forest soil carbon at
401   30\hspace0.167emm spatial resolution. Environ Res Lett 13:055002.
402   https://doi.org/10.1088/1748-9326/aabe1c

403   Sá-Pinto A, Branco MS, Alexandrino PB, et al (2012) Barriers to Gene Flow in the Marine Environment:
404   Insights from Two Common Intertidal Limpet Species of the Atlantic and Mediterranean. PLOS
405   ONE 7:e50330. https://doi.org/10.1371/journal.pone.0050330

406   Shearer CA, Descals E, Kohlmeyer B, et al (2007) Fungal biodiversity in aquatic habitats. Biodivers
407   Conserv 16:49–67. https://doi.org/10.1007/s10531-006-9120-z

408   Smith DP, Peay KG (2014) Sequence Depth, Not PCR Replication, Improves Ecological Inference from
409   Next Generation DNA Sequencing. PLOS ONE 9:e90234.
410   https://doi.org/10.1371/journal.pone.0090234

411   Thatoi H, Behera BC, Mishra RR (2013) Ecological role and biotechnological potential of mangrove fungi:
412   a review. Mycology 4:54–71. https://doi.org/10.1080/21501203.2013.785448

413   Tisthammer KH, Cobian GM, Amend AS (2016) Global biogeography of marine fungi is shaped by the
414   environment. Fungal Ecology 19:39–46. https://doi.org/10.1016/j.funeco.2015.09.003

415   Trevathan-Tackett SM, Sherman CDH, Huggett MJ, et al (2019) A horizon scan of priorities for coastal
416   marine microbiome research. Nat Ecol Evol 3:1509–1520. https://doi.org/10.1038/s41559-019-
417   0999-7

418   Wainwright BJ, Afiq-Rosli L, Zahn GL, Huang D (2019a) Characterisation of coral-associated bacterial
419   communities in an urbanised marine environment shows strong divergence over small
420   geographic scales. Coral Reefs. https://doi.org/10.1007/s00338-019-01837-1

421   Wainwright BJ, Bauman AG, Zahn GL, et al (2019b) Characterization of fungal biodiversity and
422   communities associated with the reef macroalga Sargassum ilicifolium reveals fungal community
423   differentiation according to geographic locality and algal structure. Mar Biodiv.
424   https://doi.org/10.1007/s12526-019-00992-6

425   Wainwright BJ, Zahn GL, Arlyza IS, Amend AS (2018) Seagrass-associated fungal communities follow
426   Wallace's line, but host genotype does not structure fungal community. Journal of Biogeography
427   45:762–770. https://doi.org/10.1111/jbi.13168

428   Wainwright BJ, Zahn GL, Spalding HL, et al (2017) Fungi associated with mesophotic macroalgae from
429   the 'Au'au Channel, west Maui are differentiated by host and overlap terrestrial communities.
430   PeerJ 5:e3532. https://doi.org/10.7717/peerj.3532

431   Wainwright BJ, Zahn GL, Zushi J, et al (2019c) Seagrass-associated fungal communities show distance
432   decay of similarity that has implications for seagrass management and restoration. Ecology and
433   Evolution 9:11288–11297. https://doi.org/10.1002/ece3.5631

434 Wee AKS, Mori GM, Lira CF, et al (2019) The integration and application of genomic information in
435   mangrove conservation. Conservation Biology 33:206–209. https://doi.org/10.1111/cobi.13140

436 White TJ, Bruns T, Lee S, Taylor J (1990) 38 - AMPLIFICATION AND DIRECT SEQUENCING OF FUNGAL
437   RIBOSOMAL RNA GENES FOR PHYLOGENETICS. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ
438   (eds) PCR Protocols. Academic Press, San Diego, pp 315–322

439 Wilkins LGE, Leray M, O'Dea A, et al (2019) Host-associated microbiomes drive structure and function of
440   marine ecosystems. PLOS Biology 17:e3000533. https://doi.org/10.1371/journal.pbio.3000533

441 Williams N (2005) Tsunami insight to mangrove value. Current Biology 15:R73.
442   https://doi.org/10.1016/j.cub.2005.01.015

443 Wit RD, Bouvier T (2006) 'Everything is everywhere, but, the environment selects'; what did Baas
444   Becking and Beijerinck really say? Environmental Microbiology 8:755–758.
445   https://doi.org/10.1111/j.1462-2920.2006.01017.x

446 Zahn G, Amend AS (2017) Foliar microbiome transplants confer disease resistance in a critically-
447   endangered plant. PeerJ 5:e4020. https://doi.org/10.7717/peerj.4020

448 **Figures & Tables**

449 Figure 1. Map of sampling locations throughout Singapore and Peninsular Malaysia

450 Figure 2. NMDS plots based on Bray–Curtis dissimilarity for each mangrove part and associated
451 sediment sample, all show clear separation by location. (A) Leaf, Non-metric fit $R^2 = 0.948$,
452 linear fit $R^2 = 0.743$, stress = 0.22 (B) Fruit, Non-metric fit $R^2 = 0.955$, linear fit $R^2 = 0.775$,
453 stress = 0.21 (C) Pneumatophore, Non-metric fit $R^2 = 0.925$, linear fit $R^2 = 0.618$, stress = 0.23,
454 (D) Sediment, Non-metric fit $R^2 = 0.946$, linear fit $R^2 = 0.765$, stress = 0.23
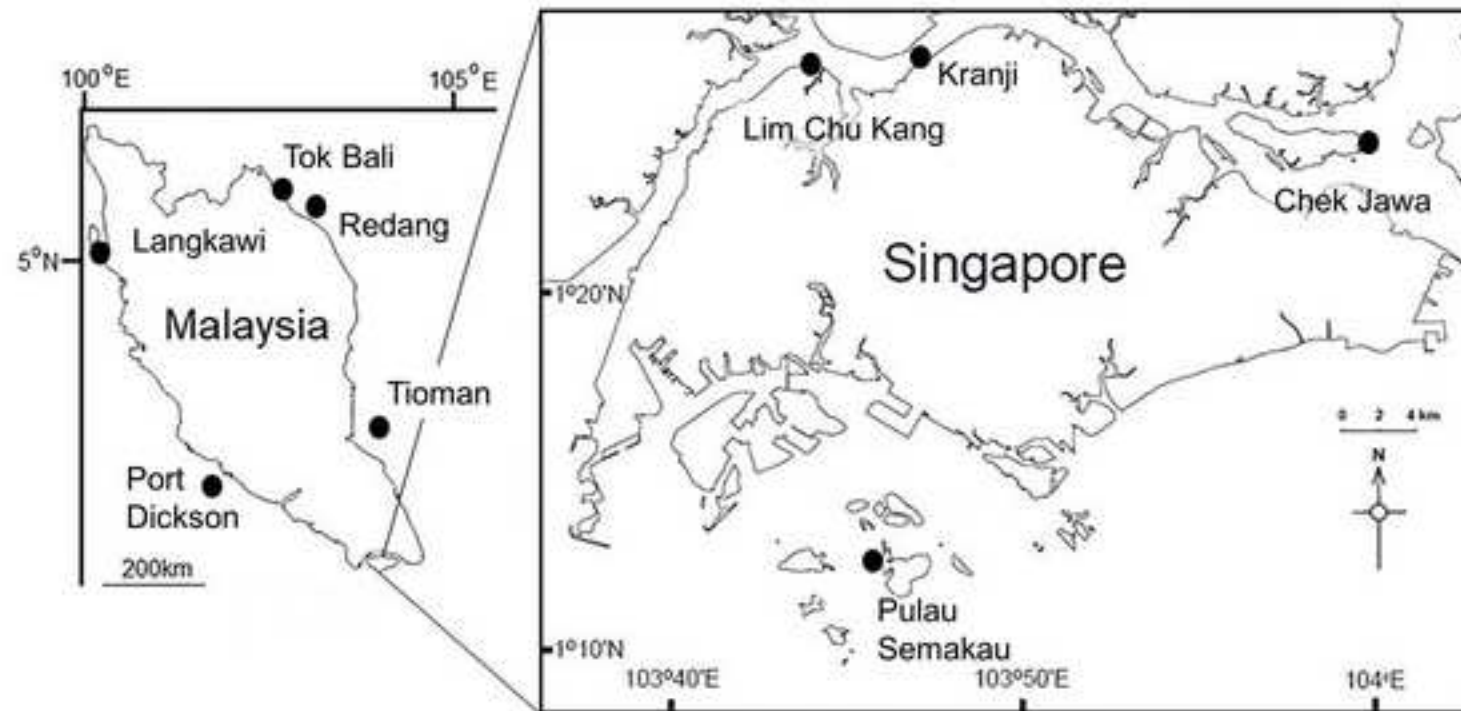
455 Figure 3. Stacked bar plots of relative class abundances in each part from each sample location.
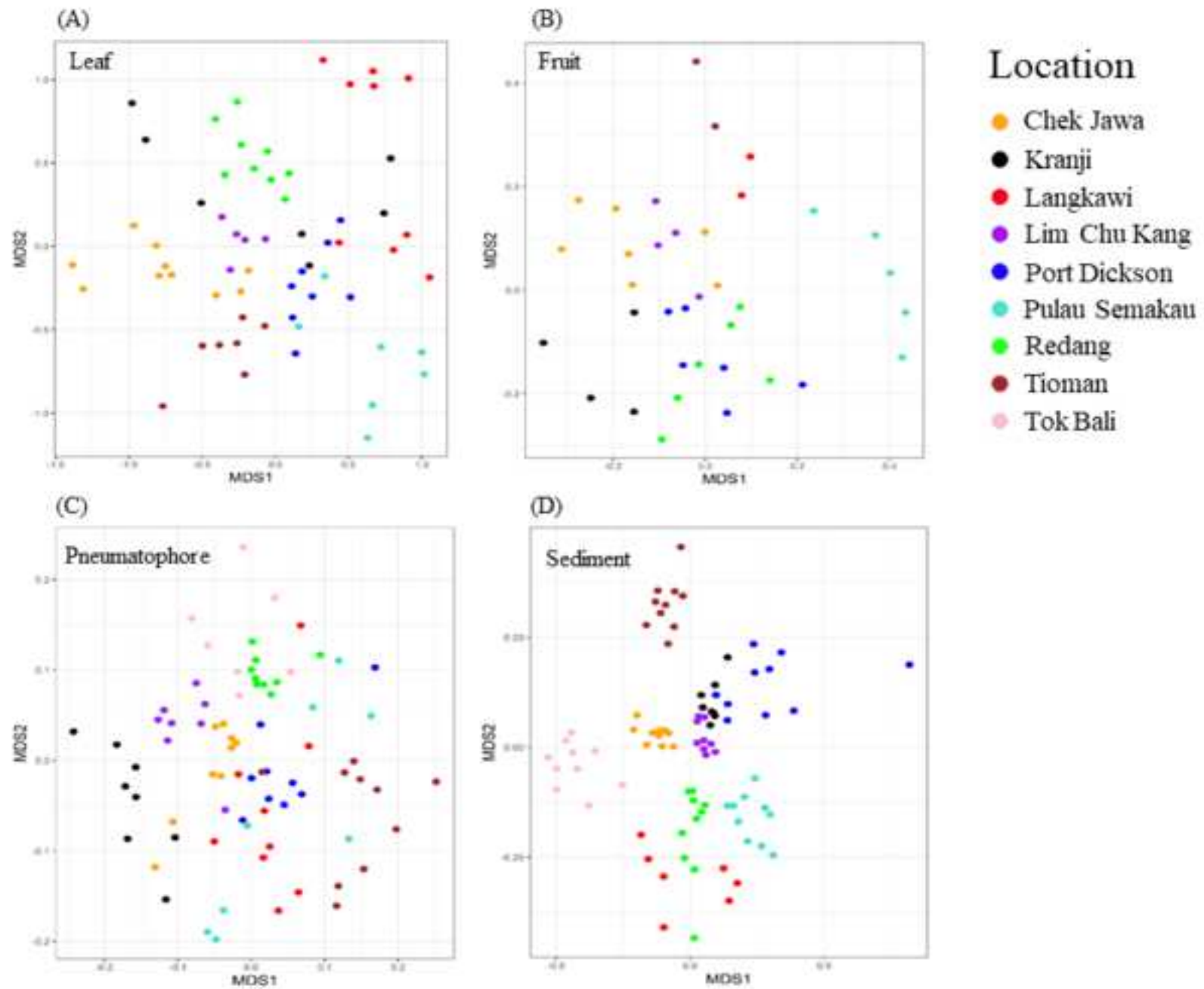
456 Figure 4. Venn diagram illustrating the number of amplicon sequence variants (ASVs) unique to
457 each sampled part, and those shared between parts, showing that sediment has the highest fungal
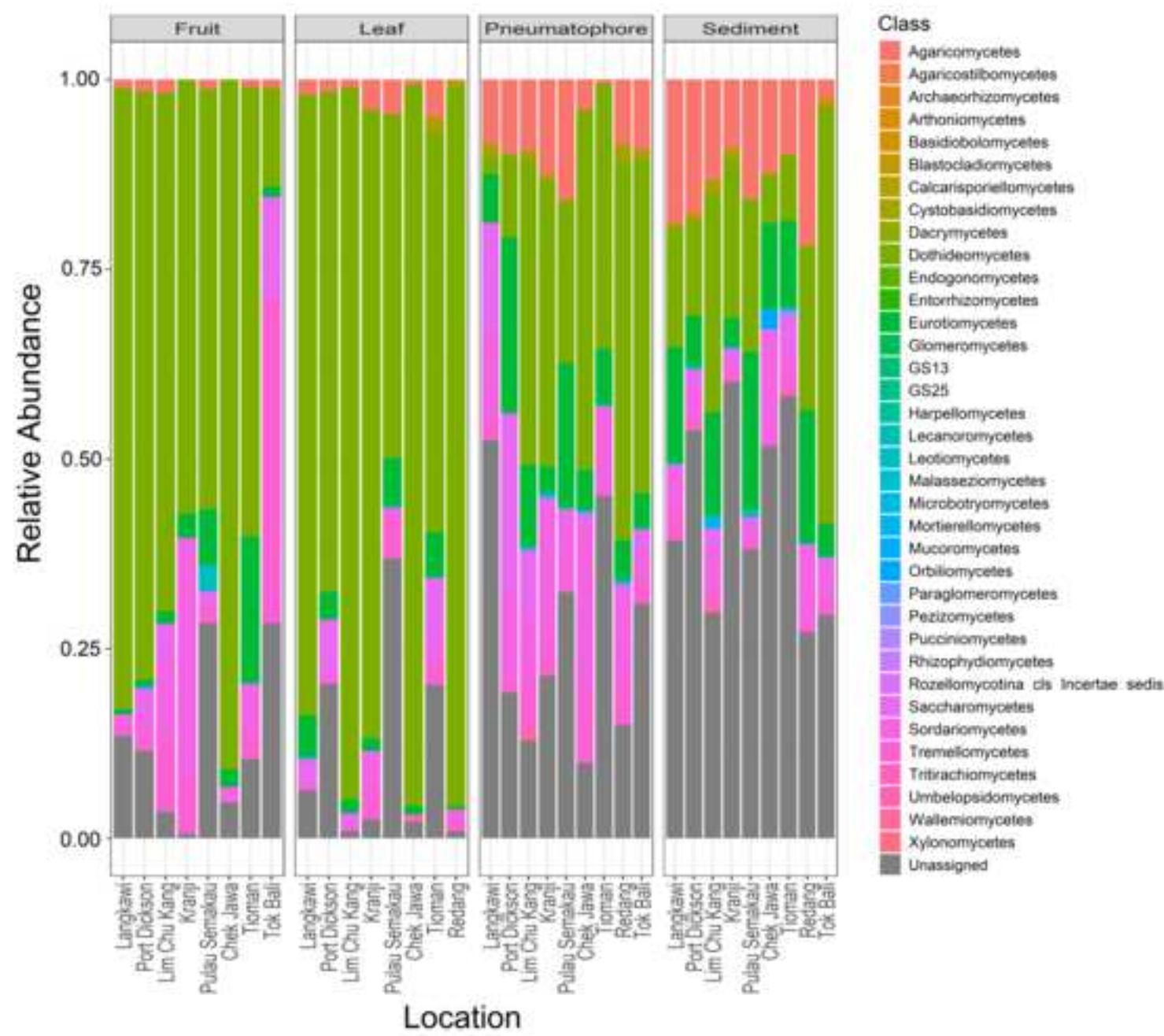458 richness and 84 ASVs are shared between all sampled parts.

459

460 Table 1. Mantel test and multiple regression on distance matrices (MRM) results for all
461 compartments combined, and each individual compartment. All show a significant pattern of
462 distance decay.

Table 1. Mantel test and multiple regression on distance matrices (MRM) results for all compartments combined, and each individual compartment. All show a significant pattern of distance decay.
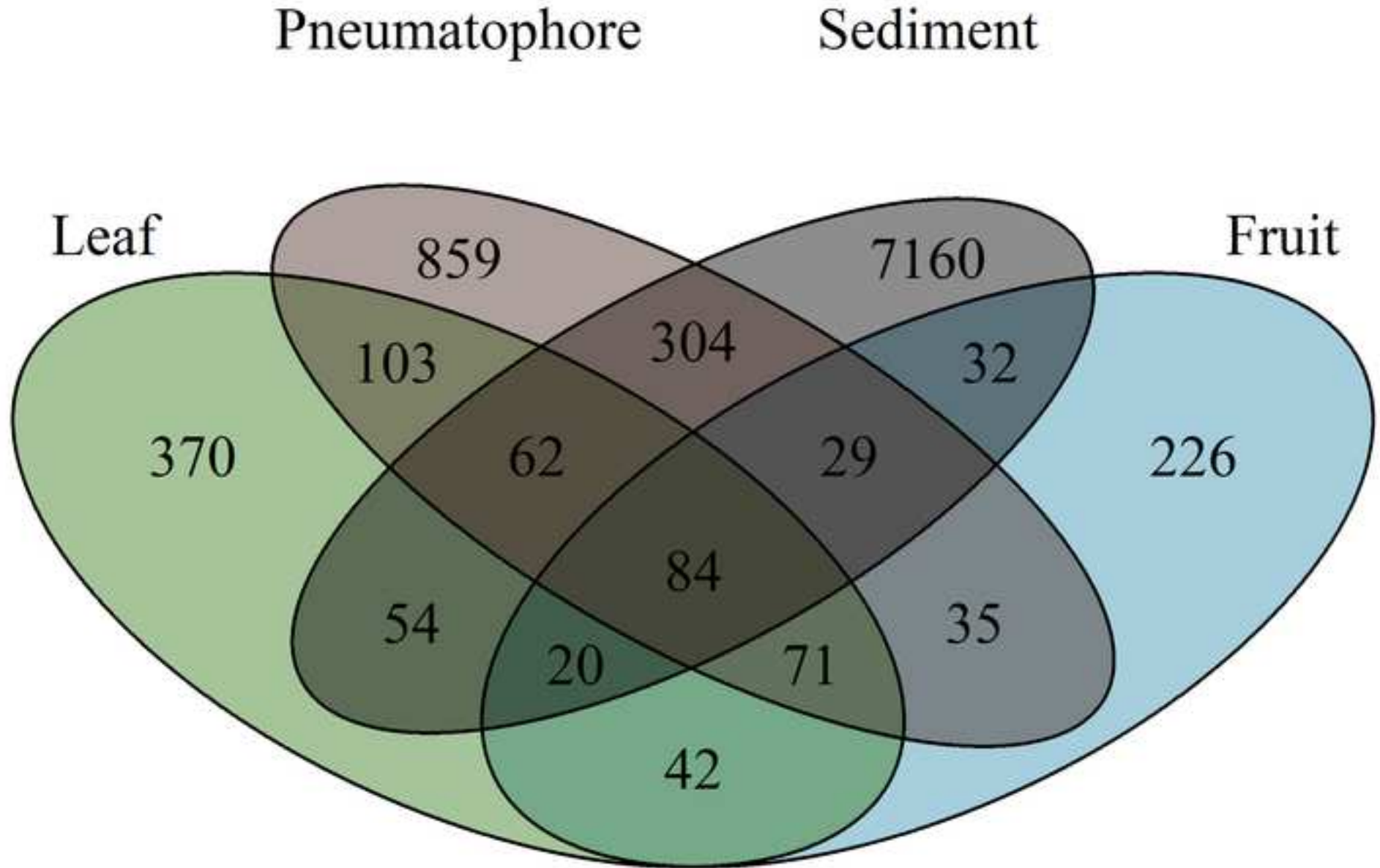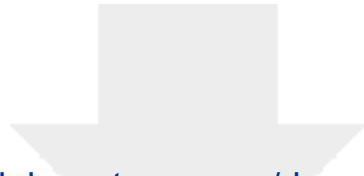
	Mantel R statistic	Mantel Significance	MRM R^2	MRM Significance
All	0.28	0.001	0.07	<0.001
Fruit	0.23	0.001	0.07	<0.001
Leaf	0.35	0.001	0.12	<0.001
Pneumatophore	0.29	0.001	0.09	<0.001
Sediment	0.50	0.001	0.21	<0.001

Figure1 Map

Figure2 NMDS

Location

- Chek Jawa
- Kranji
- Langkawi
- Lim Chu Kang
- Port Dickson
- Pulau Semakau
- Redang
- Tioman
- Tok Bali

Figure3 Barplot

Fig4 Venn

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