This is a peer-reviewed, accepted author manuscript of the following research article: Deepika, S., & Kothamasi, D. (2021). Plant hosts may influence arbuscular mycorrhizal fungal community composition in mangrove estuaries. *Mycorrhiza*. https://doi.org/10.1007/s00572-021-01049-y

Plant hosts may influence arbuscular mycorrhizal fungal community composition in mangrove estuaries

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Short title: AMF communities in mangrove plant roots

Number of manuscript pages: 38

Number of Figures: 3

Number of Tables: 3

Supplementary Information: 5

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Key words: Mangrove ecosystem, Submergence, AMF community, 18S SSU rDNA, indicator value indices, phosphate

Acknowledgements: SD received a senior research fellowship from CSIR India. DK received a Marie Skłodowska-Curie Actions Horizon 2020 Individual Fellowship, and DU-DST Purse and R&D scheme research grants from the University of Delhi. The authors thank Gurumurthi Hegde for helping with sample collection; Miquel De Cáceres for advice on use of indicspecies; and Prof. Ganesh R Hegde for hosting. We thank Dr. David P. Janos and two anonymous reviewers for comments that improved the manuscript.

Abstract

We investigated the role of plant host and soil variables in determining arbuscular mycorrhizal fungi (AMF) community composition in plant roots of two spatially separated mangrove estuaries on the rivers Aghanashini (14°30′30″N–74°22′44″E) and Gangavali (14°35′26″N–74°17′51″E) on the west coast of India. Both mangrove estuaries had similar plant species composition but differed in soil chemistries.

We amplified a 550 bp portion of 18S small subunit (SSU) rDNA from mangrove plant roots and analysed it by restriction fragment length polymorphism (RFLP). Clones representing unique RFLP patterns were sequenced. A total of 736 clones were obtained from roots of seven and five plant species sampled at Aghnashini and Gangavali, respectively. AMF phylotype numbers in plant roots at Aghanashini (12) were higher than at Gangavali (9) indicating quantitative differences in the AMF community composition in plant roots at the two mangrove estuaries. Because both estuaries had similar plant species composition, the quantitative difference in AMF communities between the estuaries could be an attribute of the differences in rhizospheric chemistry between the two sites.

Non-metric multidimensional scaling (NMDS) revealed overlap in the AMF communities of the two sites. Three and two AMF phylotypes had significant indicator value indices with specific hosts at Aghanashini and Gangavali respectively. Environmental vector fitting to NMDS ordination did not reveal a significant effect of any soil variable on AMF composition at the two sites. However, significant effects of both plant hosts and sites were observed on rhizospheric P. Our results indicate that root AMF community composition may be an outcome of plant response to rhizospheric variables. This suggests that plant identity may have a primary role in shaping AMF communities in mangroves.

Introduction

Global arbuscular mycorrhizal fungi (AMF) distribution patterns are influenced by dispersal and environmental factors (Öpik et al. 2006, 2010; Kivlin et al. 2011; Vieira et al. 2019). On a local scale, abundance and identity of host plants are key determinants of AMF community composition and distribution in different ecosystems (Sýkorová et al. 2007; Hazard et al. 2013; Jansa et al. 2014; Torrecillas et al. 2014; Martinez-Garcıa et al. 2014; Davison et al. 2016; Vieira et al. 2019). Environmental variables such as soil pH, P, N, soil moisture and organic matter also influence AMF community composition (Bainard et al. 2014; Deepika and Kothamasi 2015; Wang et al. 2015a; Velázquez et al. 2018).

AMF are ubiquitous in wetlands and aquatic habitats (Wilde et al. 2009; Wang et al. 2010, 2011; Fester 2013; Gaberščik et al. 2017; Xu et al. 2018). The abundance and diversity of AMF from wetlands is comparable to those of terrestrial habitats (Wang et al. 2011, 2015a, b, 2016; Ramirez-Viga et al. 2018; Xu et al. 2021). Around 101 AMF species affiliated to 19 genera and 9 families with a majority (53%) belonging to Glomeraceae have been reported from aquatic ecosystems (Tuheteru and Wu 2017). Oxygen availability, seasonal changes, flooding intensity, type of vegetation and P availability influence AMF species richness and diversity in wetlands (Wang et al. 2011; Tuheteru and Wu 2017).

Mangroves are salt-tolerant intertidal plant communities inhabiting interface habitats between land and sea. They account for 0.7% of the total tropical forests of the world and are subjected to inhospitable conditions such as high salinity, high temperature, tidal inundation and anaerobic soils (Giri et al. 2011). Mangrove soils are deficient in P and N, and the concentration of these nutrients in mangrove soils can limit plant growth. (Lovelock et al. 2006; D'souza 2016). Due to

high salinity, plants and rhizosphere microflora are subject to physiological aridity in the mangrove habitat. Mangrove plants have evolved adaptive strategies such as aerial roots, salt tolerance and vivipary to survive in the harsh environment. AMF are aerobic organisms and the hypoxic environment of a mangrove stand may limit AMF survival and function (Kothamasi et al. 2006; Wang et al. 2010). Salinity also inhibits survival of AMF propagules in soil by affecting spore germination and root colonization. Salinity and hypoxia in mangroves may serve as environmental filters leading to selection of only salt and hypoxia tolerant AMF.

Because AMF survival in the anaerobic mangrove soils may be dependent on O_2 transfer from aerial roots (Miller and Sharitz 2000; Kothamasi et al. 2006) and plants have access to nutrients channelized by other microorganisms (Vazquez et al. 2000; Holguin et al. 2001; Kothamasi et al. 2006); the balance of exchange and symbiont selection in plant-AMF symbiosis may shift in favour of the host. Consequently, AMF species composition in mangrove plant roots may be determined by plant hosts.

In this study, we investigated root AMF community composition in mangrove plant species. Although, AMF are prevalent in mangrove ecosystems (Sengupta and Chaudhuri 2002; Kothamasi et al. 2006; Kumar and Ghose 2008; Wang et al. 2010), factors that determine AMF community composition in mangrove roots are not certain. Here, we analyse whether host identity or soil variables have a role in determining diversity and species composition of AMF communities associated with mangrove roots. Nutrient availability in mangrove soils is affected by spatial or temporal variations in flooding frequency, availability of fresh water, redox potential and pH (Bernini et al. 2010). In order to incorporate this variation into our analyses, we conducted this study in two spatially separated mangrove estuaries with similar plant species composition but with different soil chemistries. We addressed the following questions: (i) does

host identity have a role in determining root AMF species composition; and (ii) do rhizospheric variables such as available P, % soil organic matter, salinity and pH have any influence on AMF species composition in mangrove roots.

Materials and Methods

The study was carried out in middle estuary mangroves formed by the rivers Aghanashini (14°30′30″N–74°22′44″E) and Gangavali (14°35′26″N–74°17′51″E) on the west coast of India. Both sites are inundated twice in 24 hours during high tide for a total duration of approximately seven hours.

Plant Community

Plant species composition at the two sites was studied by laying three quadrats (10 × 10 m^2) at random at each site. Quadrats were at least 20 m apart. Plant species present in each quadrat were counted and the diameters of stems at breast height were measured. Five plant species (*Acanthus ilicifolius*, *Avicennia officinalis*, *Excoecaria agallocha, Rhizophora apiculata and Sonneratia alba*) were present at both the study sites and two plant species (*Aegiceras corniculatum and Kandelia candel*) were found only at Aghanashini. The dominance of each plant species was estimated by computing its importance value index (IVI) following Curtis and McIntosh (1951).

AMF Community analyses

One individual of each plant species from each of the three randomly laid quadrats at both sites (21 and 15 individuals at Aghanashini and Gangavali, respectively) were selected, for a total of 36 individuals. Roots and rhizosphere soil samples (~500 g) were collected from these 36

randomly selected individuals at both sites and stored at –20˚C until analyses. One individual of *S. alba* collected at Gangavali was lost during transit. Therefore, only 35 samples were processed for the final analyses. AMF communities in mangrove plant roots were studied by amplifying the 18S small subunit (SSU) rDNA. Genomic DNA was extracted from 35 individual plant roots using the cetyltrimethylammonium bromide (CTAB) method (Ausubel et al. 1999) followed by an additional purification step with a Promega Wizard SV Gel PCR purification kit (Promega, USA). Partial 18S SSU rDNA was amplified from each individual sample using universal eukaryotic primer NS31 (Simon et al. 1992) and primer AM1 which amplifies AMF 18S rDNA (Helgason et al. 1998). A 30 μL PCR reaction mixture contained 0.2 mM of each deoxynucleotide triphosphate (dNTP), 10 pmol of each primer, 0.05% bovine serum albumin (BSA) (Fermentas, EU), 5% dimethyl sulphoxide (DMSO) (Sigma, USA) and 1.5 U Taq polymerase (Fermentas, EU) along with supplied reaction buffer. The PCR cycles were one cycle of 94°C for 3 min, 58°C for 1 min, and 72°C for 1.5 min, followed by 34 cycles of 94°C for 30 sec, 60°C for 1 min and 72°C for 1.5 min (Deepika and Kothamasi 2015). A final elongation at 72°C was run for 7 min. PCR products were cloned into TA cloning vector (Real Biotech Corporation, Taiwan) and transformed into *Escherichia coli* HIT competent cells from Real Biotech Corporation as per the manufacturer's instructions. Thirty five clone libraries, that corresponded to 35 root samples belonging to 12 plant species sampled at the two estuaries, were constructed and around 20–24 positive clones from each library (60–72 clones per plant species and 736 clones in total) were selected and amplified using primer pair NS31–AM1 in a 29 cycle PCR regimen as above but without the addition of BSA and DMSO. Restriction fragment length polymorphism (RFLP) patterns of the PCR products were analysed using restriction enzymes

112 *Hsp92II* and/or *HinfI* (Chaiyasen et al. 2014; Deepika and Kothamasi 2015) as per the
113 manufacturer's instructions.

114 Based on the similarities in RFLP profiles of the 736 clones obtained after digestion, we
115 identified 25 unique RFLP profiles. One to nine clones per RFLP profile were selected as
116 representatives for that particular RFLP profile on the basis of their relative abundances (some
117 RFLP profiles were represented by only 1-2 clones at both the sites) and sequenced (resulting in
118 a total of 73 sequences) using universal primer M13F (Macrogen sequencing service, Seoul,
119 South Korea). Rarefaction analysis was performed to estimate if our clone sampling effort
120 included a majority of the AMF phylotypes from the two study sites.

121 *Phylogenetic analysis*

122 Clone sequences were screened for chimeras using the Bellerophon chimera check (Huber et al.
123 2004). No chimeras were found; therefore all 73 sequences were included in the final analysis
124 and used for construction of phylogenetic trees. To find the closest matches, the 18S SSU AMF
125 sequences obtained in this study were blasted using the Basic Local Alignment Search Tool
126 (BLASTn) of the NCBI database. A phylogenetic analysis was carried out on the sequences
127 obtained in this study and their closest matched sequences from the NCBI database. MAFFT
128 version 7 (Katoh et al. 2019) was used to align the sequences. Phylogenetic trees were
129 constructed based on Maximum Likelihood (ML) and Neighbour Joining (NJ) methods using
130 MEGA version 7 (Kumar et al. 2015). The ML tree was made using the Tamura-Nei model and
131 Nearest-Neighbour-Interchange (NNI) method. The NJ tree was obtained by using the Tamura-
132 Nei model and Neighbour-Joining method. Bootstrap analyses were done with 1000 replications
133 for both the trees. AMF phylotypes were assigned on the basis of agreement between both the

ML and NJ trees. The 18S AMF SSU rDNA sequences from this study were deposited in the NCBI GenBank database (accession numbers KF870999 through KF871019, KF871022-48, KF871056-66, KF555258, KF555260-63, KF555265-70, KF555273 and KF555275-76).

Soil analyses

Rhizosphere samples collected as described above were extracted with 2.5% acetic acid for estimation of available P (PO_4–P) (Allen 1974). PO_4–P concentration was estimated using the molybdenum blue method (Chen et al. 1956). Percent soil organic matter was estimated following Walkley and Black (1934). Soil salinity was measured using a Eutech portable salinity meter (Germany). Rhizospheric pH was estimated by suspending soils in double deionized water in 1:5 ratio and shaking at 200 rpm for four hours. pH was measured using a Thermo Orion pH meter.

Statistical analyses

The normality and homoscedasticity of all the response variables was tested using R version 4.0.4 and Minitab19, respectively. Variables which were not normally distributed were transformed with a log (x + 1)-transformation. All further statistical analyses were performed using AMF and soil data from only the five plant species that were present at both study sites (*A. ilicifolius*, *Av. officinalis*, *E. agallocha, R. apiculata and S. alba*).

Rarefaction curves for AMF species were computed using R-package rich version 1.0.1 (Rossi 2011). Bootstrap estimation for species richness and the number of clones was done using 1000 randomizations.

AMF community composition in the mangrove plant roots at the two sites was analysed by calculating Hill numbers (Chao et al. 2014). The first three Hill numbers representing species richness (H0, $q = 0$), the exponential of Shannon's index (H1, $q = 1$) and the inverse of Simpson's concentration index (H2, $q = 2$) were calculated using the R package vegan 2.5-6 (Oksanen et al. 2019). Host selection of AMF phylotypes was inferred by calculating indicator value indices (Dufrêne and Legendre 1997) to test the affinity of an AMF phylotype to a host plant species. Significance of indicator value indices was tested using R-package indicspecies version1.7.9 (Cáceres and Legendre 2009). Correlation analyses between the soil variables measured and Hill numbers (H0, H1 and H2) were carried out using R version 4.0.4 to calculate Pearson's correlation coefficients.

Rhizospheric PO_4–P, % soil organic matter, salinity, pH and AMF Hill numbers of the five plant species present both at Aghanashini and Gangavali respectively were statistically compared between the two sites using a Nested analysis of variance (ANOVA) with plant species nested within site in Minitab 19.

Concordance in AMF community composition at both estuaries was tested using non-metric multidimensional scaling (NMDS) based on Bray and Curtis dissimilarities with regard to relative abundance of AMF phylotypes in host roots. NMDS based on Euclidean distances was performed to test for variance in rhizospheric concentrations of PO_4–P, % soil organic matter, salinity and pH respectively at both estuaries. Significance for differences in root AMF community composition and concentrations of the rhizospheric variables respectively was tested with a permutational multivariate analysis of variance (PERMANOVA) with 10000 permutations. NMDS ordinations were performed using R-package vegan 2.5-6.

To determine effects of rhizospheric variables such as PO_4–P, % soil organic matter, salinity and pH on AMF phylotype distributions we applied vector fitting to the NMDS ordination for AMF composition using the envfit function of vegan. Significance of effects of rhizospheric variables on root AMF composition were tested with 10000 permutations.

Pairwise concordance between root AMF composition and rhizospheric concentrations of PO_4–P, % soil organic matter, salinity and pH respectively of the five plant species present at both Aghanashini and Gangavali was tested by applying Procrustes rotation to NMDS ordinations using R package vegan. For each comparison, goodness of fit (m^2 value) was assessed. Significance was tested with 10000 permutations using the Protest function of vegan.

Results

Plant community composition and soil variables at Aghanashini and Gangavali

The dominance diversity curves of plant communities were steep at both sites (Supplementary information S1). However, the curve was steeper with a longer tail at Aghanashini indicating lower evenness than at Gangavali. Host IVI and concentrations of rhizospheric variables (PO_4–P, salinity, pH and % soil organic matter) of the seven and five host plant species at Aghnashini and Gangavali respectively are presented in Fig 1. *A. ilicifolius* and *S. alba* were the dominant plant species in the mangrove estuaries at both sites. In general, host rhizospheres at Gangavali had high PO_4–P concentration, low salinity and % soil organic matter than at Aghanashini (Fig 1).

Comparison of soil variables between the five plant hosts present at both sites with nested ANOVA revealed significant effects of sites $F_{1, 20} = 5.881$, $p = 0.04$) as well as plant hosts ($F_{8, 20} = 14.169$, $p < 0.001$) on rhizospheric PO_4–P concentrations. While site had a significant influence on rhizospheric salinity ($F_{1, 20} = 12.359$, $p = 0.008$), the effect of plant hosts was not

198 significant ($F_{8, 20} = 0.750$, $p = 0.648$). No significant effect of site or plant hosts was observed on
199 % soil organic matter and rhizospheric pH (Table 1). No correlation was found between
200 rhizospheric soil variables and AMF Hill numbers (H0, H1 and H2) at both sites.

201 *Phylogenetic analyses of AMF*

202 All 73 AMF sequences from the present study and their closest matched sequences from
203 Genbank were used for phylogenetic analyses (Supplementary information S2). Phylogenetic
204 trees produced using the neighbour-joining (NJ) and Maximum likelihood (ML) methods
205 exhibited a similar basic topology. Therefore, only the NJ tree is presented (Fig 2). Phylogenetic
206 analyses of the 736 clones revealed 18 phylotypes belonging to families Glomeraceae (GloP1–
207 GloP13), Acaulosporaceae (AcauloP1–AcauloP3 and EntroP1) and Paraglomeraceae (PgloP1).
208 Members of family Glomeraceae were dominant (587 clones belonging to 13 phylotypes)
209 compared to Acaulosporaceae with four phylotypes (3 phylotypes belonging to *Acaulospora* and
210 a single phylotype belonging to *Entrophospora*) represented by 147 clones and Paraglomeraceae
211 (3 clones belonging to a single phylotype). All sequences belonging to the same RFLP profile
212 clustered together in the phylogenetic tree.

213 BLAST results indicated a predominance of phylotypes showing strong affinities (97–99.83%
214 sequence similarity) with published environmental Glomeromycotan sequences in the NCBI
215 database except for phylotype GloP13 which exhibited highest percentage similarity of only
216 95.45 to *Glomus* clone ACAM45rac 1-59 (Accession No. KU707425). None of the AMF
217 phylotypes exhibited close similarity with any known AMF genera/species except for phylotype
218 EntroP1 which exhibited 97.44 and 96.84% similarity to *Entrophospora columbiana* (Accession
219 No. AB220170) and *Entrophospora* sp. WV 796 (Accession No. Z14011) respectively.

220 Rarefaction curves of the number of obtained sequences started to level off after approximately
221 380 and 270 sequences, with no further addition of OTUs (operational taxonomic units) from
222 samples collected at Aghanashini and Gangavali respectively (Supplementary information S3).
223 This indicated that our sequence sampling effort detected a large proportion of the diversity of
224 AMF at these two estuaries.

225 Eight AMF phylotypes, GloP4, GloP6, GloP9, GloP10, GloP11, GloP12, GloP13 and PgloP1
226 were found only at Aghanashini (Table 2). Phylotype GloP1 was the most abundant phylotype at
227 both sites and was found in five of seven plant species at Aghanashini, all five species sampled at
228 Gangavali, and accounted for 39.26% of the total sequences obtained. Phylotype GloP7 was the
229 rarest and accounted for just 0.14% of the total sequences obtained at both sites. Among the four
230 AMF phylotypes belonging to family Acaulosporaceae, approximately 50% of all phylotypes
231 belonged to AcauloP3 at both sites.

232 AMF Phylotype Entro P1 was represented by only a single clone isolated from a single
233 individual. Therefore, it was included only in the phylogenetic tree and was excluded from all
234 further statistical analyses involving AMF phylotypes.

235 *AMF community composition at Aghanashini and Gangavali*

236 Comparison of AMF phylotypes in roots of the five plant species present at both mangrove
237 estuaries revealed that roots of *E. agallocha* had highest AMF phylotype richness (H0 = 5) and
238 diversity (H1 = 4.13 and H2 = 3.66) in Aghanashini and lowest (H0 = 1.67, H1 = 1.58, H2 =
239 1.54) at Gangavali. The highest AMF diversity (H1= 4.05, H2 = 3.91) at Gangavali was
240 observed in roots of *S. alba* (Supplementary information S4).

241 Two of the 17 phylotypes (Glo P1 and Glo P8) found in roots of mangrove species present at
242 both sites were generalists and colonized a majority of plants (71.42% and 100% at Aghanashini
243 and Gangavali, respectively) (Table 2). The remaining phylotypes were restricted to specific
244 plant hosts at both sites (Table 3). Twelve and 9 AMF phylotypes were found in roots of plant
245 species present at both Aghanashini and Gangavali respectively. Three (Glo P1, Glo P5 and Glo
246 P9) of the 12 AMF phylotypes found at Aghanashini had significant indicator value indices with
247 specific host plants (Table 3). On the other hand, except for Glo P3 and GloP7, AMF phylotype
248 indicator value indices within host species were not significant at Gangavali (Table 3). None of
249 the AMF phylotypes had significant indicator value indices with the same plant host at both the
250 sites.

251 *Effects of host plants and soil variables on AMF communities*

252 NMDS ordination for root AMF composition did not find any divergence among plant species of
253 Aghanashini and Gangavali (Stress 0.046; $F_{1, 28} = 0.87$; $p = 0.46$) indicating similarity in root
254 AMF composition at both sites (Fig 3A). Vector fitting to NMDS ordination of root AMF
255 composition revealed that rhizospheric variables PO_4–P ($R^2 = 0.14$, $p = 0.11$), % soil organic
256 matter ($R^2 = 0.02$, $p = 0.71$), salinity ($R^2 = 0.01$, $p = 0.81$) and pH ($R^2 = 0.01$, $p = 0.77$) did not
257 affect AMF distribution in plant roots. NMDS ordination of rhizospheric concentrations of
258 PO_4–P, % soil organic matter, salinity and pH revealed significant divergence in rhizospheres of
259 plant species in the two mangroves (Stress = 0.08; $F_{1, 28} = 7.26$, $p < 0.001$; Fig 3B). No
260 concordance was found between AMF phylotype composition of the roots and rhizospheric
261 concentrations of PO_4–P, % soil organic matter, salinity and pH at both sites (Procrustes rotation:
262 $m^2 = 0.94$, $R^2 = 0.23$, $p = 0.31$; Supplementary information S5)

Discussion

AMF community composition at Aghanashini and Gangavali

The region between NS31 and AM1 primers provides most data of Glomeromycota (Lee et al. 2008; Öpik et al. 2009; Yang et al. 2016) but the primer set either does not or poorly amplifies AMF clades of Paraglomeraceae and Archaeosporaceae (Schüßler et al. 2001; Vandenkoornhuyse et al. 2003; Shreiner and Mihara, 2009; Kohout at al. 2014). The majority (76%) of all sequences detected in our study belonged to the family Glomeraceae. The observed dominance of the family Glomeraceae is in accordance with results from previous studies by other researchers from both terrestrial (Hijri et al. 2006; Li et al. 2010; Soka and Ritchie, 2018; Haug et al. 2021) as well as wetland ecosystems (Wirsel SGR, 2004; Wilde et al. 2009; Guo and Gong 2014; Tuheteru and Wu, 2017; Yang et al. 2018; Zhu et al. 2021). Moreover, compared to Gigasporaceae, AMF belonging to Glomeraceae and Acaulosporaceae colonize roots aggressively (Hart and Reader, 2002). Therefore, the results presented in this study may have a bias towards AMF belonging to Glomeraceae and Acaulosporaceae. Indeed, no Gigasporaceae were detected in this study.

Role of host plant identity in determining AMF community composition

Mangrove plants are adapted to overcome physiological aridity and anoxic conditions prevalent in mangrove ecosystems. Previous studies on mangroves have suggested possible adaptation of some AMF species to the harsh conditions of mangrove ecosystems (Wang et al. 2011; 2015a). It has been suggested that AMF tolerate anoxic mangrove rhizospheres by restricting their growth to aerenchymatous tissue of host roots (Kothamasi et al. 2006). Environmental stress has been linked to evolution of host specificity in mutualistic interactions and co-evolution of mutualists is

285 believed to promote adaptation to environmental stresses (Thrall et al. 2007; Schechter and
286 Bruns, 2013).

287 NMDS ordinations of AMF communities at the two estuaries revealed concordance in root AMF
288 phylotype composition in plant hosts at both estuaries (Fig 3A). The concordance in root AMF
289 composition at Aghanashini and Gangavali also was reflected in the AMF phylotype diversities
290 (H1 and H2) at both estuaries (Supplementary information S4). With the exception of *E.*
291 *agallocha* which harboured distinct AMF phylotypes, roots of the other plant species at both
292 estuaries had 75 to 86.8% similarity in root AMF phylotype composition. (Table 2). Nested
293 ANOVA did not reveal significant differences between root AMF phylotype diversity (H1 and
294 H2) of Aghanashini and Gangavali (Table 1). AMF richness (H0) differed significantly among
295 plant hosts at both sites but not between the two sites (Table 1). Moreover, 9 AMF phylotypes
296 were found only at Aghanashini (Table 2). This indicates quantitative differences in AMF
297 community composition in plant roots at the two mangrove estuaries. Because both mangrove
298 sites had similar plant species composition, the quantitative difference in root AMF community
299 composition in the two estuaries could be an outcome of variance in mycorrhizal dependencies
300 of plant hosts (Smith and Read 2008; Sangabriel-Conde et al. 2014) caused by different
301 rhizospheric chemistries of the two sites (Fig 1) particularly in concentrations of rhizospheric P
302 and salinity levels (Table 1).

303 Phylotypes Glo P1 and Glo P8 found at both sites were generalists and formed associations with
304 all five mangrove species present at both sites except *E. agallocha* at Aghanashini (Table 2).
305 However, Glo P1 association with plant hosts was significant only at Aghanashini despite
306 colonizing roots of all five plant hosts at Gangavali (Table 3; Fig 1). The rare AMF phylotypes,
307 Glo P4–6, Glo P9–13 and Pglo P1, found at Aghanshini were associated only with roots of *E.*

agallocha. At Aghanashini, indicator value indices of the rare AMF phylotypes Glo P5 and Glo P9 showed significant association with roots of *E. agallocha*. At Gangavali, indicator values indices of the rare phylotypes Glo P3 and Glo P7 were significant with *R. apiculata* only (Table 3). The significant indicator value indices of Glo P5 and Glo P9 with *E. agallocha* at Aghanashini and Glo P3 and Glo P9 with *R. apiculata* at Gangavali respectively may indicate AMF preference for specific plant hosts. This finding is consistent with previous reports in terrestrial ecosystems (Lekberg et al. 2015) but is at divergence with observations in mangroves (Wang et al. 2011). On the contrary, there are several reports from different ecosystems, where no host-specificity was observed in plant-AMF associations (Torrecillas et al. 2012). However, a recent study by Wang et al. (2021) reported significant difference between AMF species composition of co-occurring mycotrophic and non-mycotrophic plants in wetlands.

Effect of edaphic factors on AMF community composition

AMF do not have strong host specificities and the perceived host-specificity in AMF associations is quantitative rather than qualitative (Vályi et al. 2016). However, there is evidence to suggest that host-specificity may not always be the case and abiotic factors such as soil properties may have a role in structuring AMF communities (Xu et al. 2017; Melo et al. 2019). For instance, soil moisture levels influence AMF community compositions in plant roots (Deepika and Kothamasi 2015).

The relationship between AMF colonization and soil P in wetlands is bell-shaped (Wang et al. 2010). P addition to soil can cause significant improvements in AMF colonization rates, mycelium density and sporulation (Zhang et al. 2017). In contrast, high salinity levels inhibit AMF colonization in wetlands (McHugh and Dighton 2004). Additionally, a positive correlation

between soil organic matter content and AMF colonization has been suggested (Wang et al. 2010). However, we did not find any correlation between AMF Hill numbers (H0, H1 and H2) and soil variables at both sites.

Although, no significant differences were found in % soil organic matter and rhizospheric pH among plant hosts and the two sites in nested ANOVAs, we observed significant effects of plant hosts and sites on rhizospheric P concentrations (Table 1). Rhizospheric salinity between the two sites also differed significantly (Table 1). Environmental vector fitting to NMDS ordination of root AMF composition did not reveal a significant effect of any soil variable on AMF composition in the five hosts present at Aghanashini and Gangavali (Fig 3B). Procrustes rotation between AMF phylotype composition in plant roots and soil variables corroborated the results obtained by NMDS ordination (Supplementary information S5). These results suggest that the assessed rhizospheric variables may not have a role in plant association with AMF in mangroves. However, we did find significant divergence in NMDS ordination of rhizospheric concentrations between the two sites (Fig 3B).

Hydrology controls biotic and abiotic components in wetland ecosystems which in turn affects the soil chemistry and availability of oxygen. Edaphic variables along with salinity and other hydrological processes then determine the abundance and diversity of plants and microbes. However, the control is bidirectional as the plants and microbial communities also can exert their influence on rhizospheric chemistry through processes like rhizodeposition and rates of decomposition (D'Souza and Rodrigues 2013; Canarini et al. 2019). Local environmental factors shape the plant communities and their associated microbial partners (Li et al. 2019; Bernard et al. 2021).

Community assembly of microbes is largely believed to be deterministic (Pholchan 2013). High salinity and hypoxic soils may lead to selection of plants and AMF that are tolerant to the prevailing environmental conditions (environmental filters) in mangroves. The filtered communities may undergo further biotic filtering which eventually determines the AMF composition in mangrove ecosystems. The biotic filters include AMF interactions with plant hosts and their interactions with other AMF species and microbes present in the soil.

We did not find a conclusive role for any single variable on AMF composition in our study. Although, we did find quantitative differences in AMF composition and some level of host preference exhibited by specific AMF phytotypes at both sites. However, we found significant effects of plant hosts and sites on rhizospheric P but did not find any correlation between Hill numbers (H0, H1 and H2) and soil P.

The highly dynamic and complex nature of mangroves makes it difficult to clearly decipher the role of any individual factor from field-based studies alone. Mangrove roots oxygenate the rhizosphere making it conducive for AMF colonization. Indeed, AMF propagules in mangrove soils are restricted to the rhizosphere (Huang et al. 2020). This indicates that in mangrove ecosystems the role of plant hosts in AMF selection may be more important than soil variables. The observations of our study corroborate this possibility. It must however, be noted that our inferences are based on observations from environmental AMF DNA sequences extracted from roots of plants collected from the wild where multiple variables are in operation.

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Figure Legends

Fig 1. Importance value indices (IVI) of host plants and concentrations of environmental variables in their rhizospheres at Aghanashini (AG) and Gangavali (GN). Values represent the average of three replicates. Error bars indicate standard error of the mean. Statistical comparisons between the five plant species present at both Aghanashini and Gangavali are presented in Table 1.

Fig 2. Neighbour joining (NJ) tree representing phylogenetic relationships of the representative AMF sequences from Aghanashini and Gangavali and their closest matched sequences from Genbank. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. Sequences marked with asterisks (clones IKNM 1.1 to IKNM25) are from the present study and clone names followed by same numbers (e.g., IKNM 1.1 and 1.2) belong to the same OTU. The bootstrap values (1000 replicates) are indicated at each node. Bootstrap support of only 70% and above is shown in the tree. GloP1–GloP13, PgloP1, AcauloP1–AcauloP3 and EntroP1 represent AMF phylotypes found at Aghanashini and Gangavali.

Fig 3. Non-metric multidimensional scaling (NMDS) ordinations of (A) AMF community composition based on Bray and Curtis dissimilarities with regard to relative abundance of AMF phylotypes (B) rhizospheric concentrations of PO_4–P, % soil organic matter, salinity and pH based on Euclidean distances at both estuaries. Ellipses are drawn around each group's centroid.