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Seasonal Diversity of Arbuscular Mycorrhizal Fungi (AMF) in the Mangrove Forests of Bakkhali, Sundarban, India

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ABSTRACT

Mangroves, despite thriving at the fringes of habitat tolerance in coastal regions, stand out as one of the world's most highly productive ecosystems. This study delves into the remarkable symbiotic relationship between mangroves and arbuscular mycorrhizal fungi (AMF). Specifically, it assesses seasonal diversity in six true mangrove species and four associated species, situated in Henry's Island, Bakkhali, India. Spore density and root colonization were examined across different seasons. The highest spore density was observed in *Heritiera fomes* (a timber-producing mangrove) during the post-monsoon season, followed by pre-monsoon and monsoon periods. Root colonization was notably prominent in *Ceriops tagal* (Indian mangrove), *Bruguira gymnorrhiza* (Oriental mangrove), and *Sonneratia alba* (flowering evergreen mangrove) during both pre- and post-monsoon seasons. This study unveiled a rich fungal diversity, with a total of 60 AMF species belonging to 13 genera. Among these, the genus *Glomus* emerged as the dominant group, with species such as *G. deserticola* along with another genus *Rhizophagus intraradics* displaying widespread distribution. Notably, *Glomus* consistently ranked as the most prevalent genus throughout the year, indicating its remarkable adaptability and strong dispersal capacity in both true and associate mangrove plant species. This research sheds light on the seasonal dynamics of AMF associations in mangrove ecosystems, emphasizing the significance of *Glomus* as a key player in this symbiotic relationship. These findings contribute to our understanding of the ecological intricacies within mangrove habitats and highlight the adaptability of certain AMF genera to varying environmental conditions.

IMPLICATIONS FOR PRACTICE

- Our study found major AMF diversity with highest significance of *Glomus* sp. indicating remarkable dominance rate throughout the coastal area of Bakkhali, India.
- Keeping in view our results, it is important to increase mass multiplication of salt-tolerant AMF spores to improve threatened mangrove recovery throughout the coastal land.

1. INTRODUCTION

Mangroves are well recognised as ecologically as well as economically important, dynamic coastal ecosystems, playing crucial roles in protecting the habitat from natural disasters through maintaining the productivity, health, carbon sequestration, and tolerance to various abiotic stresses (Akram et al., 2023). Recent research (Constance et al., 2022) has revealed that mangrove growth is frequently impeded by the scarcity of some essential macronutrients like

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phosphorus (P) and nitrogen (N). Through colonization, arbuscular mycorrhizal fungi (AMF) could fulfil such nutrient requirements in saline and poor-nutrient soils in mangrove ecosystem (Akaji et al., 2022). The external hyphae of AMF penetrate into the deeper soil around the plant roots and increase the root surface area (Finlay, 2008). Long-term salinization enhances AMF biomass, which is in turn involved in primary mineral P mobilization in coastal soils (Fan et al., 2023).

Therefore, having a wide range of ecological functions (Marro et al., 2022), AMF are commonly known as keystone organisms in mangrove habitats (Shankarammal, 2023). In coastal ecosystem, AMF play a major role in improving rhizospheric soil characteristics, developing nutrients uptake, and ameliorating plant resilience to a variety of abiotic stressors (Wang et al., 2022). Finding the multifunctional role of glomalin related soil protein (GRSP) in preservation and restoration of Soil Organic Carbon (SOC), recent research has revealed that plantations using AMF can be a useful tool for increasing carbon sequestration during coastal forest restoration (Li et al., 2023). Defining the seasonal diversity of AMF is important to comprehend the multiple ecological attributes through the symbiotic relationship, which in turn gives a clear idea on seasonal impacts in mangrove ecosystem as well as on its conservation strategies (D'Souza and Rodrigues, 2013; Su et al., 2011). Therefore, it is necessary to measure the seasonal AMF biodiversity of mangrove habitats (Gaonkar and Rodrigues, 2020). However, no studies have been reported on the seasonal variation of AMF in true and associate mangroves in Henry Island, India. In this paper, seasonal differences of various ecological parameters associated with mangroves were been explained.

2. METHODOLOGY

2.1 Study site and sample collection

Henry Island (21.5769°N 88.2923°E) is located on the southern triangle of West Bengal, on the North-East coast of India, and in-between the Saptamukhi River and the Bakkhali River. The total area of Henry Island is 470 ha, which has a mangrove cover of about 200 ha. This area is characterized by a tropical climate with annual precipitation of approximately 2,000 mm. The major mangrove flora of the island is dominated by 10 plant species, and of these, 6 are true mangroves while 4 are associates. Rhizospheric soil and root samples were randomly collected in pre-monsoon (March-May 2022), monsoon (July-September 2022), and post-monsoon (October 2022-February 2023) seasons from the study site. All total 90 soil samples were collected, placed in separate Ziploc bags, and then transported to the laboratory. Samples were air-dried through 2 mm sieve and mixed thoroughly to obtain a composite sample and further divided into two parts, one for AMF spore isolation, identification, enumeration, and trap culture preparation, and the other for soil physico-chemical analyses.

2.2 Soil physico-chemical analysis

A total of 30 soil samples (0-25cm) from each season were randomly collected and air-dried in the laboratory. Soil pH was measured in soil water (1:2) suspension and electrical conductivity (EC) was measured at room temperature in 1:5 soil suspension using a pH meter (LI 120 Elico, India) and EC meter (CM-180 Elico, India), respectively. Further, soil physico-chemical properties were detected by standard soil analysis techniques, namely, Walkley and Black (1934) rapid titration method for soil organic carbon (OC), Bray and Kurtz (1945) method for available phosphorus (P), Potassium permanganate oxidation method (Jackson, 1973) for available soil nitrogen (N), and ammonium acetate method (Hanway and Heidel, 1952) for available potassium estimation.

2.3 Isolation, identification of AMF spores, and trap culture preparation

Using the wet sieving and decanting method (Gerdemann and Nicolson, 1963), AMF spores were isolated from collected seasonal soil samples. Trap cultures were prepared for further AMF identification. *Zea mays* was used as trap plant and culture pots were thoroughly maintained at 27°C and well-watered condition in glass house for almost 6 months. Hoagland's solution without phosphorus (P) was added in every 15 days. Isolated AMF spores from both soil samples and trap cultures were then mounted on glass slides in polyvinyl alcohol lacto- glycerol (PVLG) and prepared to examine under bright field microscope. Identification was based on spore morphology along with wall characteristics, dimensions, and other relevant traits, following the international website of VAM fungi (invam.wvu.edu) for taxonomic identification.

2.4 AMF root colonization

Approximately 1 cm long secondary and tertiary root pieces were bleached in H_2O_2 and then

cleaned in 10% KOH for almost 1 h at 90°C. After that, they were acidified in 5 N HCL and stained the with 0.05% Tryphan blue (Phillips and Hayman, 1970). Following the overnight staining process, PVLG-mounted roots were examined under bright-field research microscope (20X, 40X, 100X). The presence of hyphae, arbuscles, or vesicles in root segments was considered as mycorrhizal colonization.

2.5 Statistical analysis

AMF diversity was seasonally analysed for each mangrove plant species by calculating Simpson's diversity index (D) - measurement of relative abundance (RA) of each species, Shannon diversity index (H) - measurement of species diversity in a community, species richness (SR) - number of species present, and species evenness (E) - distribution of abundance across the species. Isolation frequency (IF) reflects the distribution status of AMF species, whereas relative abundance (RA) reveals the similarity or dissimilarity of species.

Following formulae were used to calculate Shannon-Wiener diversity index (H) and Simpson's diversity index (D):

$$H = -\sum pi \ln pi$$

$$D = 1 - [\frac{\sum n(n-1)}{N(N-1)}]$$

Where; pi is the proportion of individual species that contributes to the total number of individuals, n is the number of individuals of a given species, and N is the total number of individuals in a community. Species evenness was estimated as (∑(H)=H/H max) where; H max=lnS, S=total number of species in the community (richness). Also, RA was evaluated using formula: RA=(number of spores of a species/total no of spores in all soil samples) × 100, while IF=(no of soil samples possessing spores of a particular species/total no of soil sample analysed) × 100.

Pearson's correlation coefficient was calculated to evaluate the relationship between relative abundance (RA) and isolation frequency (IF), Simpson index and Shannon index, Species evenness and richness, by using the PAST software 4.03 (details in data availability statement) ($p \leq 0.05$). All data on seasonal variation was statistically analysed using SPSS software (version 16.0). Later, a paired t-test was done to compare the soil parameters between 'true' and 'associate' mangrove plants. Further, a cluster analysis (Bray-Curtis similarities) was performed to understand the AMF species' distribution among the mangrove plants by using the PAST software 4.03.

3. RESULTS AND DISCUSSION

3.1 Soil analysis

The results of soil physico-chemical analysis gave a clear indication on basic (pH range 6.01-8.61) nature of soil throughout the year. Electrical conductivity (EC) ranged from 0.59 to 3.9 d/Sm. Organic carbon (OC) was higher at the true mangrove site in the post monsoon season. In both types of mangroves, phosphate (P) deficiency was found throughout the year. Also, low nutrient (especially nitrogen and organic carbon) availability was seen at both mangrove types. Therefore, paired t-test showed (Table 1) significant differences ($p<0.05$) between the soil parameters in both types of mangroves. The positive t-value indicated that the mean value of P was higher in associate mangrove plants, in the monsoon season.

Table 1. 1-Associate mangrove, 2- true mangrove; paired t-test

Soil parameters	Pre-monsoon			Monsoon			Post-monsoon		
	t	df	p	t	df	p	t	df	p
pH_1-pH_2	-4.29	2	0.5	-3.857	2	0.5	-6.9	2	0.5
EC_1-EC_2	-6.09	2	0.5	-67.66	2	0.5	-3.63	2	0.5
N_1-N_2	-3.23	2	0.5	-1.47	2	0.37	-0.14	2	0.9
OC_1-OC_2	-1.55	2	0.36	-1.73	2	0.33	-3.85	2	0.16
P_1-P_2	6.6	2	0.09	2.2	2	0.27	1.66	2	0.34

3.2 Mycorrhizal colonization, AMF spore density, and AMF species diversity

The seasonal alterations had a significant impact on variations in spore density among mangrove plants. Seasonal variations in spore density (SD) of AM fungi is presented in Table 2. The mean SD was significantly higher in *Heritiera fomes* throughout the year, whereas in *Acanthus ilicifolius*, SD was higher only in the monsoon season. Besides, minimum SD was recorded in *Ceriops tagal* and *Avicennia marina*

in monsoon, premonsoon, and post monsoon seasons, respectively.

A total of 60 AM fungal species (Figure 1) representing 13 genera were recorded (Table 3). *Glomus* was the dominant genus, followed by *Rhizophagus*, *Scutellospora*, *Gigaspora*, and *Diversispora*. Maximum relative abundance (RA) was recorded for *G. deserticola* and *R. intraradics* in monsoon, *F. geosporum* in premonsoon, and *F. verruculosum* in the post monsoon season. Whereas minimum RA was recorded for *G. multicaule*, *G. myriocarpa*, *Racocetra castanea*, *R. clarus*, and *Septoglomus constricum* in monsoon, *Claroideoglomus luteum* in premonsoon, and *A. kentinensis* in the post monsoon season. Along with, *A. polonica* showed minimal RA both in the pre and post monsoon season. Besides, here was no significant change in relative abundance (RA%=0) for several species of *Glomus*, *Rhizophagus*, *Claridioglomus*, *Septoglomus*, and *Scutellospora* in different season. Further, a significant correlation (Table 4) existed between RA and isolation frequency (IF) throughout the year (M. r=0.685, Pr. r=0.600, Po. r= 0.872).

Table 2. Seasonal variation in AMF spore density in selected mangrove species

Mangrove species	Monsoon	Pre-monsoon	Post monsoon
Avicennia marina	59.00±19.08	28.00±2.51	24.3±6.2
Suaeda salsa	30.0±5.5	68.0±8.5	58.7±8.8
Heritiera fomes	99.33±10.47	89.30±15.72	107.70±17.46
Acanthus ilicifolius	92.33±2.33	16.0±2.3	25.70±3.28
Excoecaria agallocha	25.00±5.13	29.7±9.6	34.00±10.97
Sonneratia alba	27.00±3.21	76.70±6.94	56.00±6.11
Aegiceras corrticulatum	21.30±2.02	67.70±9.74	30.80±3.28
Bruguira gymnorrhiza	14.66±3.71	60.30±9.49	42.00±6.24
Porteressia coarctata	62.33±6.38	19.70±3.93	51.30±4.41
Ceriops tagal	8.33±1.45	14.00±3.61	46.0±11.7

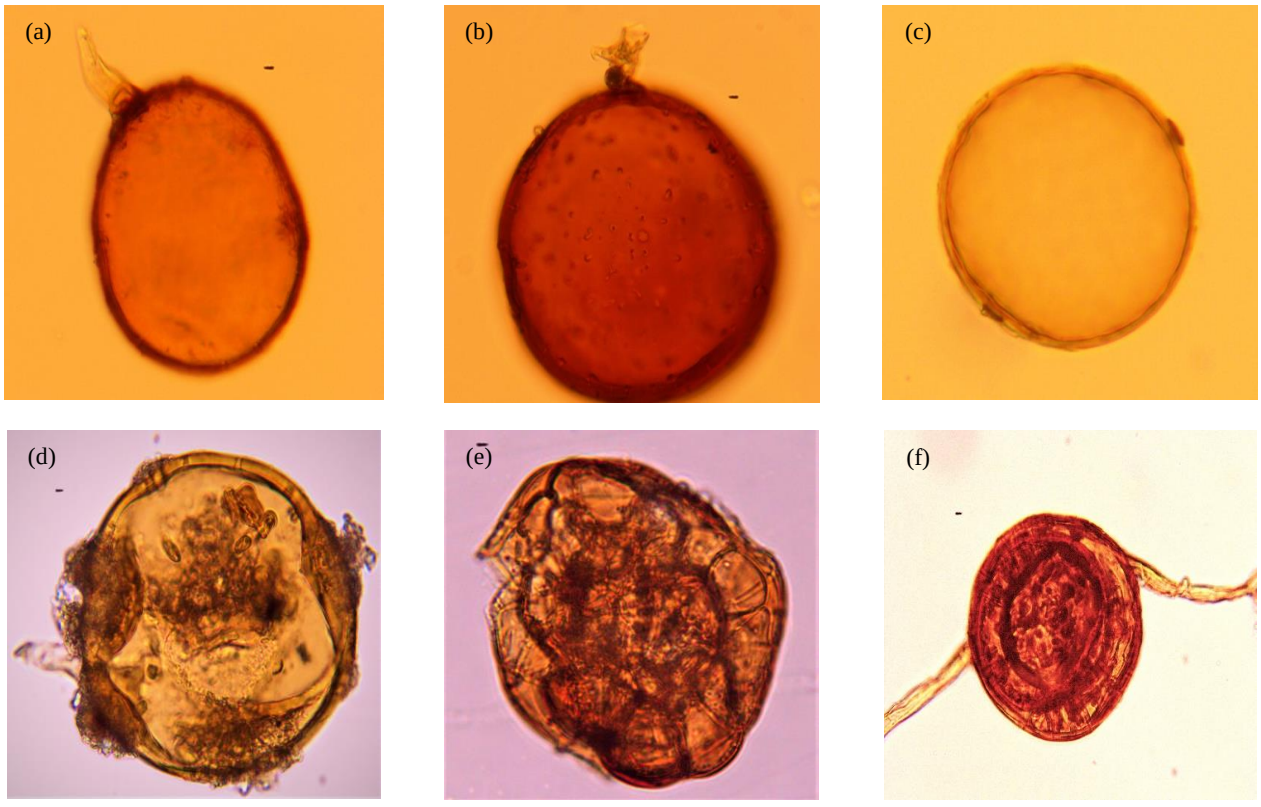

Figure 1. Tentative identification of spores; (a) *Glomus mossae*, (b) *G. macrocarpon*, (c) *Acaulospora* sp., (d) *Gigaspora* sp., (e) *Sclerocystis* sp., (f) *Glomus multicauli*

Table 3. Seasonal variance of relative abundance (RA) of AM fungi in selected study site

Species	Monsoon RA (%)	Pre-monsoon RA (%)	Post monsoon RA (%)
Acaulospora kentinensis	0.457	1.5	0.42
A. laevis	0.685	2.6	0.63
A. dilatata	0.685	1.9	2.1
A. excavata	0.913	2.1	1.26
A. foveata	1.826	1.3	2.31
A. morrowiae	0.457	1.3	1.47
A. polonica	0.457	0.2	0.42
A. scorbiculata	0.913	0.4	2.94
A. spinosa	1.37	1.3	2.94
Ambispora apendiculata	0.685	0.4	1.89
Cetraspora pellucida	0.457	0.6	1.89
Chlyamidospore	0.457	1.1	2.1
Claroideoglomus claroideum	1.826	0.9	0
C. luteum	0.685	0.2	0
Dentiscutata heterogama	1.826	1.3	2.31
Diversispora eburnea	3.881	1.5	3.14
D. epigaea	3.425	3.4	4.19
D. jakucsiae	0.457	2.8	0.84
D. spurca	0.685	3	3.98
Entrophospora	1.142	3	2.31
Funneliformis geosporum	1.142	3.8	3.35
F. mosseae	1.142	2.3	5.03
F. verruculosum	0.685	3.8	5.24
Glomus coronatum	1.826	2.6	1.68
G. diaphanum	1.142	2.3	0.63
G. macrocarpum	2.74	3.2	0
G. microcarpum	1.37	1.1	0
G. multicaule	0.228	2.3	0
G. myriocarpa	0.228	1.3	0
G. versiforme	0.457	0.9	0
Gigaspora gigantes	1.826	2.1	0
Gigaspora margarita	3.196	2.3	1.68
G. australe	1.826	0.9	3.56
G. badium	2.968	0.9	3.14
G. caledonius	2.968	0.4	2.52
G. clarum	2.74	0.6	2.31
G. deserticola	5.023	1.1	0.84
G. glomerulatum	4.795	0.9	1.05
G. halontatum	2.283	0.9	0
G. hoi	0.457	1.3	0
G. maculosum	2.74	1.9	0
G. pellucidum	2.055	3.2	0
G. warcupii	1.826	2.8	0
Rhizophagus aggregatum	4.795	3	0
R. fasciculatus	4.11	2.8	2.94
R. intraradics	5.023	2.6	3.14
R. manihotis	2.74	1.5	3.77
Racocetra castanea	0.228	1.1	5.03
R. clarus	0.228	0.6	1.05
R. fasciculatus	1.826	2.3	2.73
R. invermaius	0.457	1.1	3.14

Table 3. Seasonal variance of relative abundance (RA) of AM fungi in selected study site (cont.)

Species	Monsoon RA (%)	Pre-monsoon RA (%)	Post monsoon RA (%)
R. irregularis	0.685	1.3	2.52
Sclerocystis pubescens	0.685	0.9	0.84
Sclerocystis sinuosum	1.598	0.9	1.68
Scutellospora	3.881	0.9	0.84
Scutellospora dipurpurascens	0.913	1.3	0
Scutellospora fulgida	1.598	1.7	1.68
Scutellospora heterogama	0.913	1.1	1.05
Septoglomus	1.142	3	0.63
Septoglomus constricum	0.228	0.9	0.84

Table 4. Pearson correlation of coefficient between RA vs. IF, Simpson vs. Shannon index and Species evenness vs. richness

Ecological parameters	Monsoon	Pre-monsoon	Post-monsoon
RA vs. IF	0.6851	0.6004	0.872
Evenness vs. Richness	-0.374	-0.215	-0.589
Simpson vs. Shannon	-0.343	0.1539	0.9592

Species richness as well as evenness was maximum in the monsoon season, and minimum in pre and post monsoon seasons. Whereas, Simpson and Shannon index were higher in monsoon but lower in the post monsoon season. Species evenness showed a non-significant correlation (Figure 2) with species richness only in post monsoon, (r=-0.589) pre, and monsoon season (r=-0.215, r=-0.374). However, the Simpson index showed a significant correlation with the Shannon index (Table 4) only in the post monsoon season (r=0.9592), but a non-significant correlation in both monsoon and pre-monsoon season (M. r=-0.343, Pr. r=0.1539).

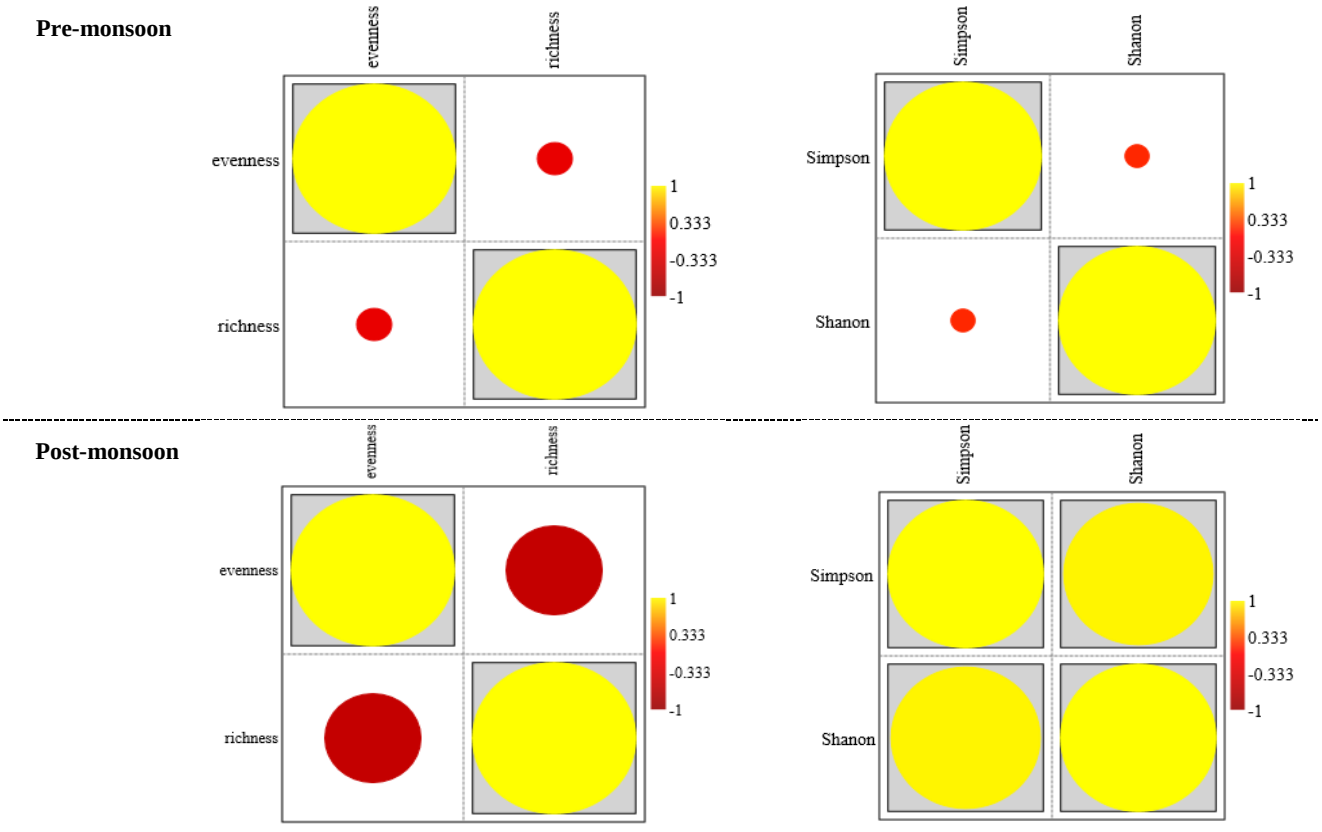

Figure 2. Plot diagram of seasonal variation in Pearson correlation coefficient of AMF species in listed a mangrove plants; Species evenness vs. richness-left one and Simpson vs. Shannon index -right one, boxed circle: p≤0.05

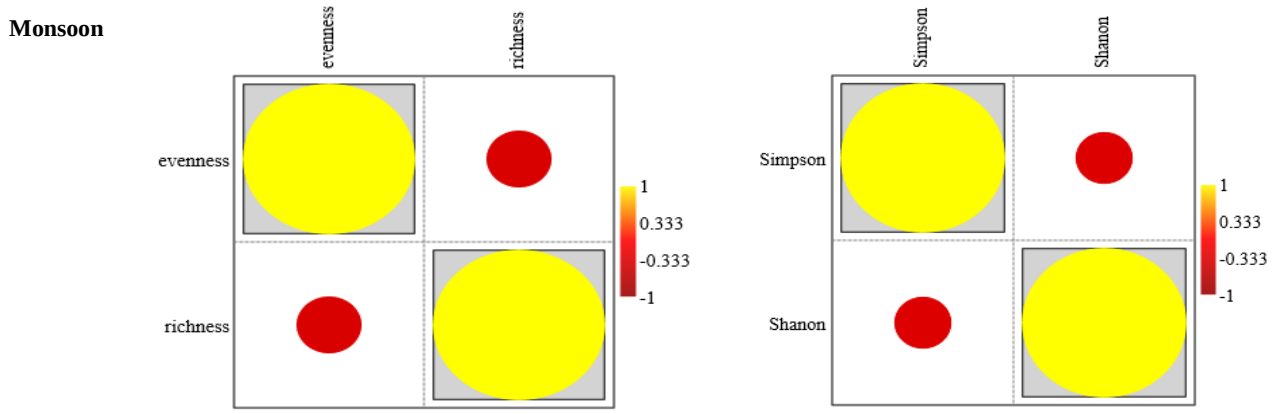

Figure 2. Plot diagram of seasonal variation in Pearson correlation coefficient of AMF species in listed a mangrove plants; Species evenness vs. richness-left one and Simpson vs. Shannon index -right one, boxed circle: p≤0.05 (cont.)

3.3 Cluster analysis

Cluster analysis (Figure 3) was done based on the RA of AMF species. All the AMF species were grouped into 7 clusters at a similarity level 33%. Further, cluster I subdivided into two sub-clusters at 77% similarity, cluster II at 69% similarity, cluster III at 75%, cluster IV at 69%, cluster V at 65%, cluster VI at 77%, and cluster VII at 65% similarity. *G. deserticola* and *G. glomerulatum* showed the highest similarity at 95% in cluster VI, whereas *A. polonica* and *Claridioglomus* showed the lowest similarity in cluster VII, with 65%.

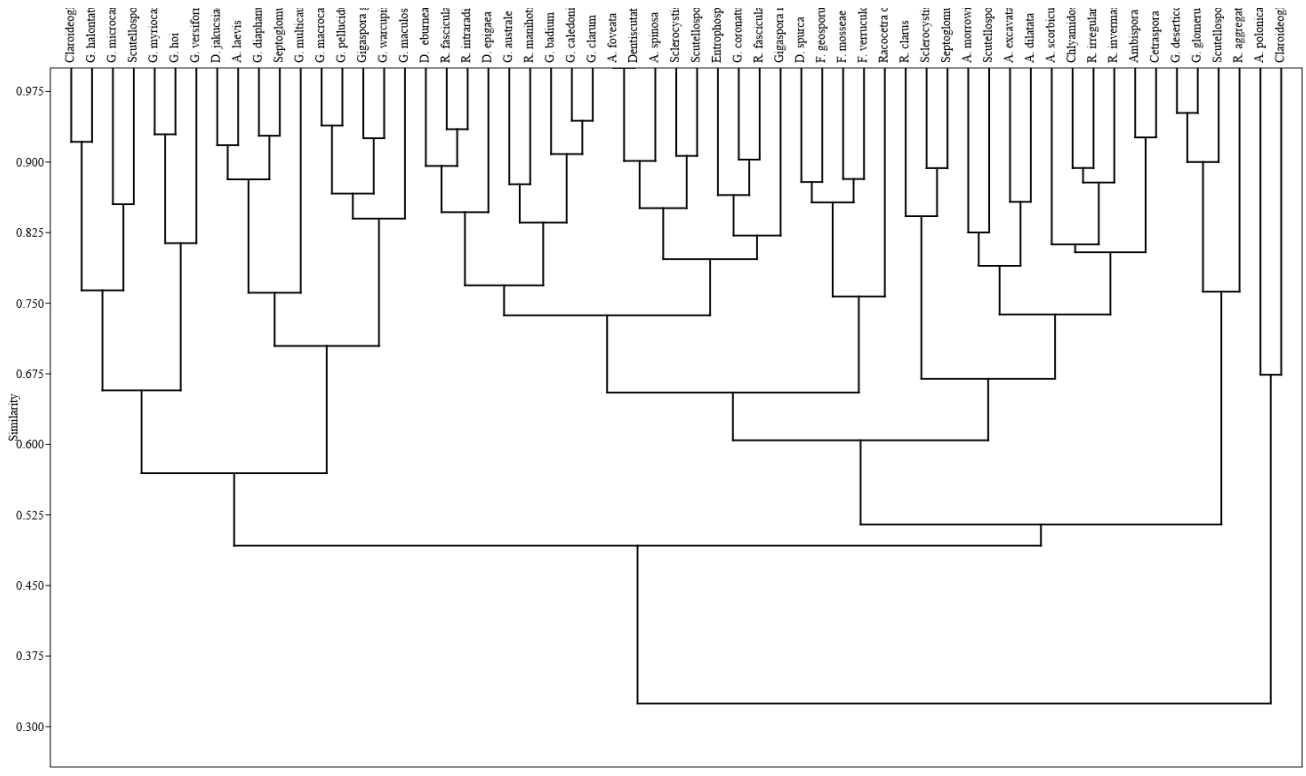

Figure 3. Bray-Curtis cluster analysis showing similarity in abundance of AMF species

4. DISCUSSION

The present study notes the variations in pH and EC values in mangrove soils which could be attributed by the continuous influx of tidal water, further leading to salt deposition (Rodrigues and Anuradha, 2009). In both type of mangroves, the level of most of the macronutrients were low, including available P. However, it is well known that AMF can grow more rapidly in low nutrient soils, especially regarding P (Hindumathi and Reddy, 2011). Along with this, AMF contributions in improving nutrition uptake and community development in nutrient-deficient soil of

mangroves are also widely known (Sridhar et al., 2011). Inadequate formation of root hairs of mangrove plants may restrict the nutrient absorption, perhaps making them mycotrophic (Baylis, 1975; Tomlinson, 2016).

In this study, higher root colonization was found in associate mangroves such as *Acanthus ilicifolius* and *Aegiceras corrticulatum* rather than true mangroves (Figure 4) in monsoon season, whereas in true mangroves such as *Ceriops tagal*, *Bruguira gymnorrhiza*, and *Sonneratia alba*, colonization was higher in both the pre and post monsoon seasons. A similar kind of observation has been reported earlier (Gaonkar and Rodrigues, 2020; Wang et al., 2015). Seasonal variation in AMF spore density has also been found in selected mangrove species. An earlier study revealed that spore density patters might indicate a wide range of environmental factors that are conductive to sporulation rather than AMF activity in roots (Miller and Bever, 1999). In our study, a high spore density was observed in the pre and post monsoon rather than in the monsoon season. Previous studies have revealed similar observations (Gaonkar and Rodrigues, 2020; Sivakumar, 2013). During the dry season, increased spore density is believed to be a sign of accessible nutrients and root senescence, which in turn stimulates fungal sporulation as plant nutrients requirements decrease (Gemma et al., 1989).

In the present study, a significant positive correlation existed between RA and IF throughout the year, indicating that species with higher spore production are widely distributed, while fewer spore production clearly indicates a confined geographic range (Dandan and Zhiwei, 2007).

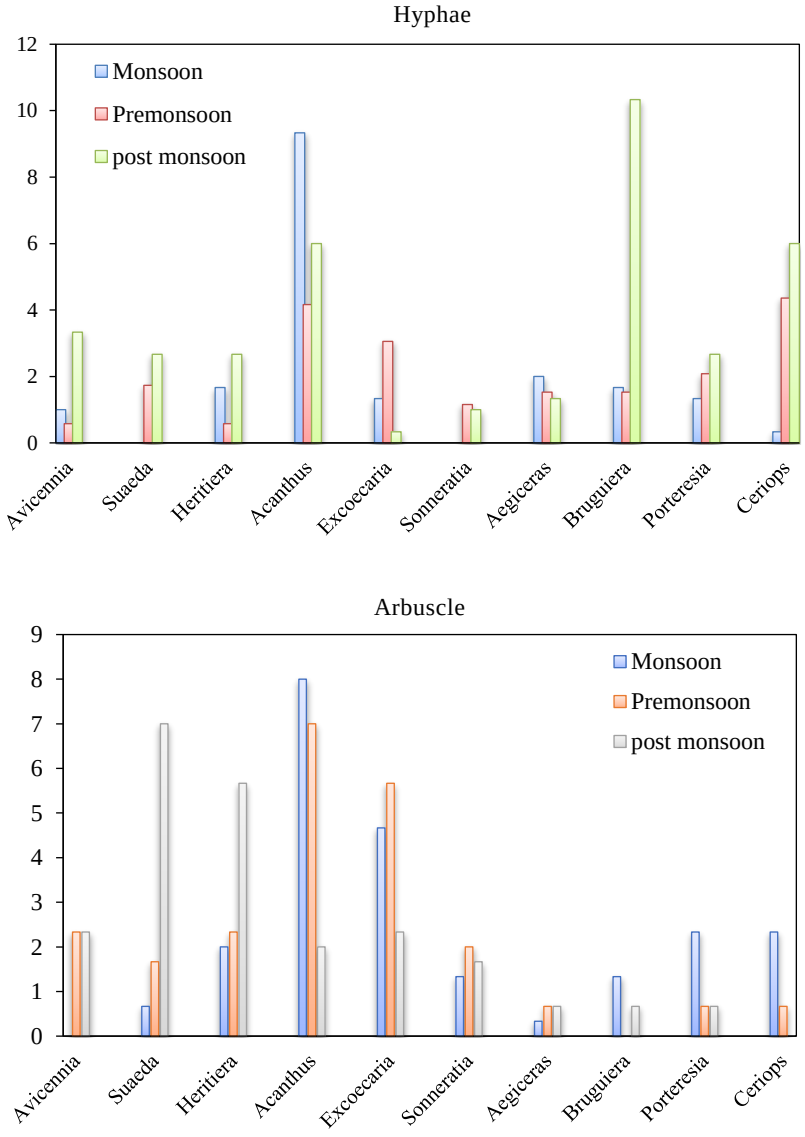

Figure 4. Graphical representation of seasonal data of root colonization

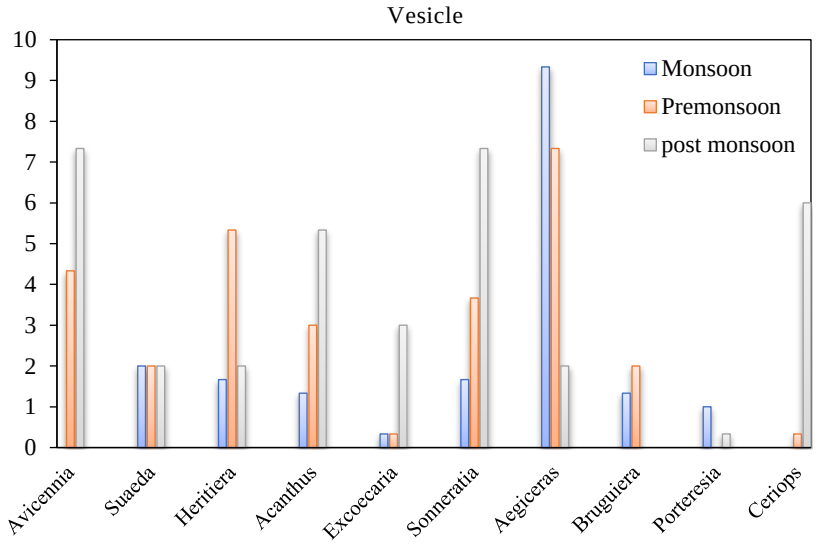

Figure 4. Graphical representation of seasonal data of root colonization (cont.)

G. deserticola and *R. intraradics* had the highest RA compared to other AMF species. It is well known that *Glomus* species are extensively dispersed and frequently found in various geographic regions (Stutz et al., 2000). Furthermore, this genus exhibits higher adaptability to changes in sporulation patterns under diverse environmental conditions, leading to their dominance (Stutz and Morton, 1996). A previous study revealed that the basic nature of mangrove soil might assured *Glomus* presence (Wang et al., 2011). The presence of a high AMF species diversity in Henry Island gives a clear indication of the site's diversity. Greater AMF diversity might be facilitated by the high environmental factors found in mangrove ecosystems (Fabián et al., 2018). A significant resemblance amongst AM species (almost 77%) suggested this widespread distribution. A similar kind of observation was recorded in a previous study (Sridhar et al., 2011).

The seasonal diversity found in the present study is higher than an early discovery in Indian mangroves, where 11 AM species representing 5 genera were recorded (D' Souza and Rodrigues, 2023), and then in South China, where only 6 species were reported (Wang et al., 2010). Our study supported previous findings that *Glomus* and *Rhizophagus* were the most widely distributed genus under alkaline condition (Parihar et al., 2019). AMF species were shown to be negatively impacted by soil alkalinity, whereas Glomeraceae family members registered more frequently, indicating a strong adaptation, which could be beneficial in restoring a damaged and disturbed alkaline ecoregion (Parihar et al., 2019).

5. CONCLUSION

These findings hold significant ecological implications, as they provide a deeper understanding of the role of AMF in enhancing the resilience and productivity of mangrove ecosystems in the Sundarban coastal region, India. Furthermore, the adaptability of certain AMF genera, such as *Glomus*, suggests their potential utility in ecosystem restoration and conservation efforts. Overall, this study contributes to the broader body of knowledge surrounding mangrove ecology in the Asian coastal ecosystem, highlighting the intricate dynamics of AMF associations and their ecological importance. Further research in this area could lead to more targeted conservation and restoration strategies for mangrove habitats, which are vital for both biodiversity and coastal protection.

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SOFTWARE AVAILABILITY STATEMENT

The software used in this study are free and these are: PAST software version 4.03 is available upon registration at (https://past.en.lo4d.com/windows), while SPSS software is available upon downloaded version at (https://www.ibm.com/support/pages/downloading-ibm-spss-modeler-160).

AUTHOR'S CONTRIBUTION

Supriti Paul wrote the manuscript and designed the project. Ranjna Kaundal, Bikram Dhara, Meghna Thapa contributed to the editing. Vipin Parkash (Correspondence) contributed to supervision and Arup Kumar Mitra to co-supervision. All the authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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