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RESEARCH ARTICLE

Arbuscular mycorrhizal fungal status in mangroves of Pichavaram Forest, Tamil Nadu, India

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Abstract
The diversity and species distribution of arbuscular mycorrhizal (AM) fungi were analyzed in mangrove patches of the Pichavaram Forest. The AM fungal colonization, spore density, and diversity indices in 18 species of true- and associate-mangrove were investigated. Soil analysis results indicated low levels of available nutrients, especially phosphorus (P), with soil being neutral to slightly alkaline, having high electrical conductivity. Some of the earlier reported non-mycorrhizal plant families also showed AM symbiosis with a high degree of root colonization. All the species at the three sites explored showed significant variation in AM fungal root colonization and spore density. The salt marsh species *Salicornia brachiata* showed the highest degree of root colonization. Overall 21 AM species belonging to seven genera were recovered from the mangroves of Pichavaram Forest. Statistical analysis showed that relative abundance and isolation frequency of AM fungal species were positively correlated. Among AM fungi, species belonging to the family Glomeraceae were dominant. Our results indicated that AM fungal communities differ with the change in soil conditions within the short distances among the habitats.

Keywords AM fungal diversity indices · Isolation frequency · Phosphorus · Relative abundance · Soil electrical conductivity · Sørensen's coefficient · Spore density

Introduction

Mangrove is a highly productive wetland ecosystem occupying the marine intertidal zone in tropical and sub-tropical regions. Mangroves are rich and diverse in living resources and hence increase the economic and ecological value of the ecosystem (Kathiresan 2000). However, there are several causes of mangrove destruction, hypersalinity being one of the causes at Pichavaram (Bhatt and Kathiresan 2011) and Sundarbans (Selvam et al. 2002). Besides, the mangrove ecosystems have become a focus of conservation and environmental issues (Gopinathan et al. 2017).

Mangroves show substantial tolerance to salinity, inundation, and nutrient stress. However, they have been degraded drastically all over the world, mainly due to nutrient limitations (P deficiency) and human interference (Xie et al. 2014). Studies have shown that the P availability is low in mangrove ecosystems as it is absorbed and co-precipitated within carbonate-dominated environments, thus limiting the growth of mangrove plants (Lovelock et al. 2004). Hence, the protection and restoration of mangrove ecosystems have become a global concern (Krauss et al. 2008).

Pichavaram Forest is known to be the world's second-largest mangrove forest (Mariappan et al. 2016) after Sundarbans with *Avicennia marina* and *Rhizophora* species being predominant (Kathiresan 2000). It is situated between Vellar estuary (North) and Coleroon estuary (South) (Srivastava et al. 2012) on the Coromandal coast (Bay of Bengal Sea Board) (Lingan et al. 1999). It receives three types of waters viz., neritic, brackish, and freshwater from the Bay of Bengal, Vellar-Coleroon estuaries, and irrigation and main channel of Coleroon river, respectively (Kathiresan 2000).

Arbuscular Mycorrhizal (AM) fungi are the 'hidden heroes' of nutrient-deficient soil, especially P (Hindumathi and Reddy 2011), which helps in increased uptake of mineral nutrients and improve stress tolerance in exchange for carbon (Smith and Read 2008). The saline and anaerobic conditions of the mangrove rhizosphere limit the occurrence of AM fungi in these environments (Wang et al. 2010). Various AM fungal species colonizing the roots of different plant

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species play a crucial role in the regeneration, diversity, and distribution of plant communities (Nandi et al. 2014).

To establish conservation strategies, it is important to explore the ecological framework of the habitat. Accordingly, the objective of this investigation was to study the AM fungal diversity and to record the dominant AM fungal species associated with the mangrove plants of Pichavaram Forest. The present study also investigates the effect of various soil parameters on the distribution of AM fungi.

Materials and methods

Study site and sample collection

Pichavaram Forest (11°20'–11°30' N; 79°45'–79°55' E) is situated on the southeast coast of India. It is a mangrove swamp located in the Vellar-Coleroon estuarine complex. The total area of the Pichavaram Forest is 1100 ha, traversed by 51 islets (Kathiresan 2000). About 241 ha of the entire forest is occupied by dense mangrove cover (Srivastava et al. 2012; Arunprasath and Gomathinayagam 2014). The average annual rainfall is 1310 mm (Selvam et al. 2003).

In the present study, 18 plant species with each of nine true- and associate-mangroves belonging to 12 families were investigated. The 18 plants were randomly selected based on the ease of soil collection. The rhizosphere samples were collected from three mangrove sites of Pichavaram Forest viz., Pichavaram extension (PE), Pichavaram Reserved Forest (PRF), and Killai Reserved Forest (KRF) (Fig. 1).

The collection of soil and root samples was carried out from November 2015 to October 2016. The root system of each plant was dug up to trace and collect the roots belonging to that plant.

Three rhizosphere soil samples were collected from each plant species, placed in separate sealed bags, labeled, and brought to the laboratory. These three samples of each plant species were then separately mixed to form a composite sample. The roots were separated from adhering soil, washed gently under tap water, and used for assessment of AM colonization.

Each composite soil sample was divided into two parts, one for AM spore isolation, enumeration, and identification, and the other as inocula to prepare trap cultures.

Soil analyses

To carry out the soil analyses triplicates of rhizosphere samples (0–15 cm) from three different estuarine regions of the study site were collected in separate sealed bags, brought to the laboratory, and were air-dried. To measure soil pH and electrical conductivity (EC), a soil–water suspension (40% w/v) was prepared. The pH was measured using a pH meter (LI 120 Elico, India), and the EC was measured using a conductivity meter (CM-180 Elico, India). Available Nitrogen (N) and P were measured using the methods of Subbiah and Asija (1956) and Bray and Kurtz (1945), respectively. For the detection of soil available potassium (K), the ammonium acetate method (Hanway and Heidel 1952) was employed. The determination of available Zinc (Zn), Copper (Cu), Manganese (Mn), and Iron (Fe) was done by the DTPA-$CaCl_2$-TEA method of Lindsay and Norvell (1978) using an atomic absorption spectrophotometer (Nova 400P, Analytik Jena, Germany).

Assessment of root colonization

Approximately one-centimeter-long root segments were hydrolyzed in 10% KOH at 90 °C for 90 min, followed by acidification in 2 N HCl for 5 min. Root segments were then stained overnight with 0.05% Trypan blue (Phillips and Hayman 1970). After staining, root segments were mounted on glass slides using polyvinyl alcohol Lacto-glycerol (PVLG) as a mountant and examined under a Brightfield Olympus BX41 research microscope (40×, 100×and 400×). A root segment was considered mycorrhizal if it showed the presence of hyphae, arbuscules, and/or vesicles.

Percent AM root colonization was estimated using the following formula:

$$\% \text{ colonization} = (\text{Number of root segments colonized} \div \text{Total number of root segments observed}) \times 100$$

Isolation, identification, and spore density of AM fungi

For the identification of AM fungal species, spores from rhizosphere samples (n = 3) and trap cultures were isolated using a wet sieving and decanting method (Gerdemann and Nicolson 1963). Spore morphology, wall characteristics, dimensions, and other relevant data were observed for the identification of the AM spores. The spore characters were compared with the descriptions given by Schenck and Perez (1990), Rodrigues and Muthukumar (2009), and the International Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM). Revised binomials and epithets of AM fungal species were followed according to the reference of Schüßler and Walker (2010) and Redecker et al. (2013). Intact, healthy

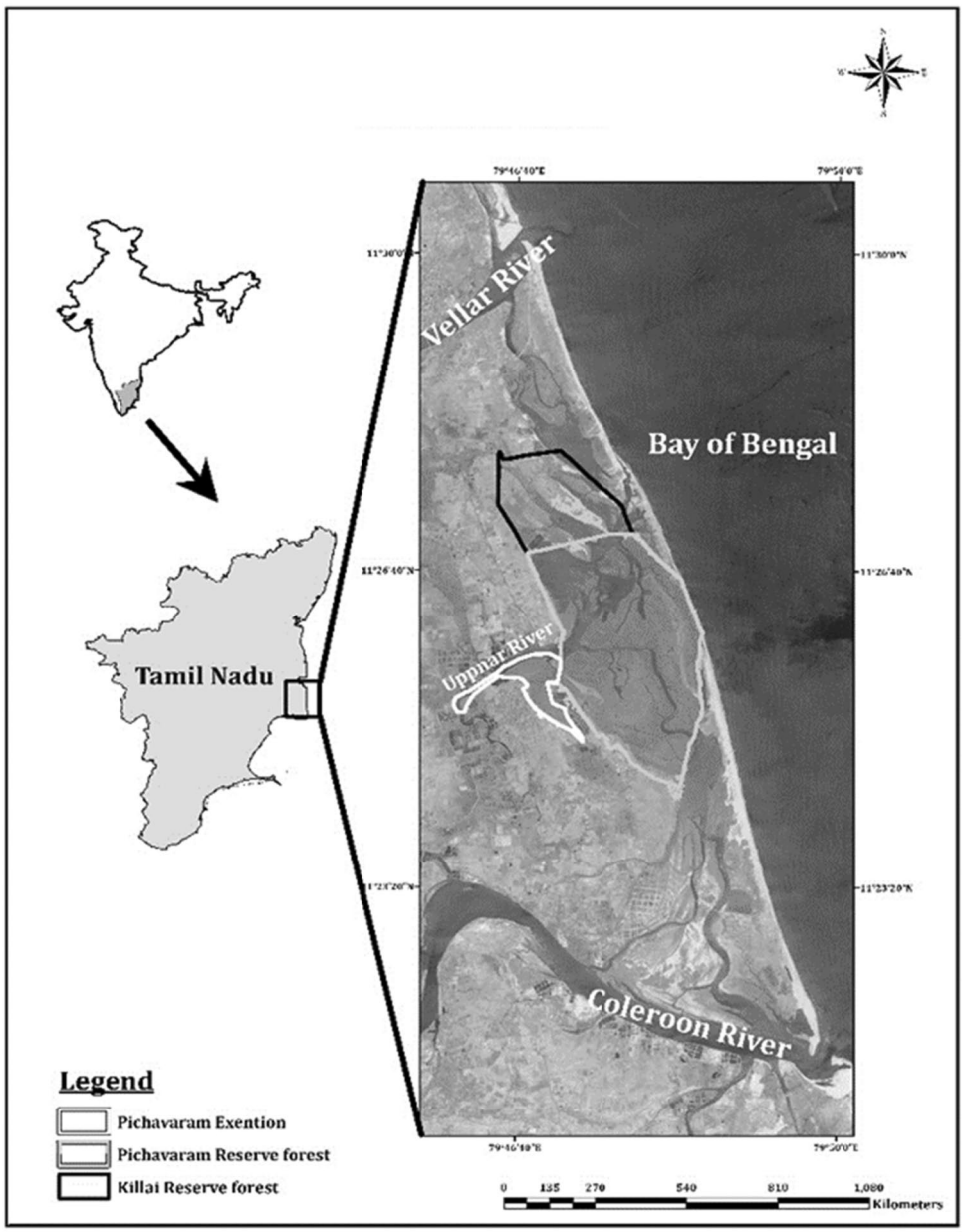

Fig. 1 Map of Pichavaram Forest showing study areas

spores from rhizosphere samples were selected for the estimation of spore density by the modified method of Gaur and Adholeya (1994).

Preparation of trap cultures

Trap cultures were prepared by following the modified trap culture method of Morton et al. (1993) to multiply AM fungal spores using the substrate mixture of rhizosphere and sterile sand (1:1) in the pots. Coleus (*Plectranthus scutellarioides*) was used as the catch plant. Coleus cuttings were first washed with tap water and then with detergent water. The cuttings were then rinsed in sterile water. Three to four cuttings were planted per pot and, the pots were maintained for six months in the polyhouse (27 ˚C, 63% relative humidity) for the establishment of colonization and subsequent sporulation. The plants were watered twice a week, and Hoagland's solution (Hoagland and Arnon, 1950) without P was added every 20 days. The cultures were harvested at the end of the 6th month and the spores were used for identification.

Data analysis

To quantify diversity, Simpson's diversity index, Shannon diversity index, species evenness, isolation frequency,

Table 1 Soil physico-chemical properties of the study sites

Soil Parameters	PE	PRF	KRF
pH	7.6 ± 0.8^{a}	7.0 ± 0.8^{ab}	6.9 ± 0.8^{b}
EC (mS/cm)	5.1 ± 0.6^{ab}	4.3 ± 0.6^{b}	6.7 ± 0.6^{a}
N (g/kg)	0.04 ± 0.005^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}
P (g/kg)	0.02 ± 0.004^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}
K (g/kg)	0.1 ± 0.02^{a}	0.2 ± 0.04^{a}	0.1 ± 0.03^{a}
Fe (ppm)	15.6 ± 1.7^{a}	15.5 ± 1.7^{a}	14.6 ± 1.6^{b}
Mn (ppm)	7.9 ± 0.1^{a}	7.3 ± 0.1^{b}	6.12 ± 0.1^{c}
Zn (ppm)	0.9 ± 0.1^{a}	1.0 ± 0.1^{a}	0.5 ± 0.1^{b}
Cu (ppm)	1.8 ± 0.3^{b}	2.0 ± 0.3^{ab}	2.6 ± 0.4^{a}

Data are means of three replicates; ± standard error

PE Pichavaram extension, *PRF* Pichavaram reserve forest, *KRF* Killai reserve forest

Values in the same row not sharing the same letters are significantly different ($P \leq 0.05$)

relative abundance, and species richness were calculated in PRIMER v. 6.0 using the following formulae:

Relative abundance (%)

= (No. of spores of a species per genus

÷Total no. of spores in all soil samples) × 100

AMF species richness (SR)

= Number of AM species per soil sample.

The diversity of AM fungi in plant species was assessed based on the Shannon- Wiener index of diversity (H) (Shannon and Weaver 1949) and Simpson`s index of dominance (D) (Simpson 1949),

$$\text{Shannon index (H)} = -\sum (\text{pi ln pi})$$

(where *pi* is the proportions of individual that species *i* contributes to the total number of individuals)

$$\text{Simpson}'\text{s index (D)} = 1 - \left(\sum \text{n(n} - 1)/\text{N(N} - 1)\right)$$

(where *n* is the number of individuals of a given species, and N is the total number of individuals in a community).

The evenness (E) indicates the distribution of individuals within species of AM fungi in plant species.

$$\text{Species evenness} \left[\sum (\text{H})\right] = \text{H}'/H'\text{max}$$

(where $H'\text{max} = lnS$, S = total number of species in the community).

All data were statistically analyzed using SPSS (Statistical Package for the Social Sciences) (Version 22) software. A one-way ANOVA was done to test the variation in spore density and root colonization among plant species. Pearson's correlation coefficient was calculated to evaluate the relationships between root colonization and spore density and isolation frequency and relative abundance. The similarity between the plant species was estimated by calculating Sørensen's similarity coefficient based on the presence or absence of each AM fungal species (Dandan and Zhiwei 2007).

Results and discussion

Soil properties

Soil physico-chemical properties are presented in Table 1. Soils of Pichavaram Forest are neutral to slightly alkaline (6.9–7.6). The alkaline pH at PE could be due to the inflow of a high amount of freshwater from the Coleroon River (Sahua and Kathiresan 2019). Soil electrical conductivity (EC) ranges were between 4.47 and 5.0 mS/cm. The higher EC value at KRF may be attributed to less influx of freshwater and vegetation cover. In contrast, lower soil EC is apparent at other sites due to freshwater input from irrigation and Coleroon River and thick forest canopy, which decreases evapotranspiration (Ranjan et al. 2010).

All the sites were low in available nutrients, especially P. This may be explained by the flow of water causes the leaching of soil nutrients (Gandaseca et al. 2016), and P is a highly leached element (Oelkers and Jones 2008).

Nutrients in mangrove ecosystems are controlled by a variety of biotic and abiotic factors viz., inundation, soil type, soil microbes, plant species, litter production, and decomposition (Reef et al. 2010). The Pichavaram mangrove ecosystem consists of small Islands that experience micro- and diurnal-tides (Selvam et al. 2003). The frequency and period of tidal inundation are determined by topographic factors such as elevation, which subsequently affects the salinity and soil nutrient availability, resulting in complex patterns of nutrient demand and supply (Reef et al. 2010). Furthermore, increased soil salinity decreases the availability of major nutrients such as N, P, K due to their precipitation and variation in nutrient metabolism (Evelin et al. 2009). Salinity affects N metabolism by interfering in uptake and reduction of NO_3^- and correspondingly in protein synthesis (Frechill et al. 2001). In saline soils, P becomes unavailable to the plants due to the precipitation of phosphate ions with Ca^{2+}, Mg^{2+}, and Zn^{2+} (Azcón-Aguilar et al. 1979).

When salt concentration in the soil increases, plants absorb more Na^+ which results in a reduction of K^+ uptake. This facilitates the competition between Na^+ and K^+ ions for the binding sites of cellular functions (Blaha et al. 2000).

The AM fungal structures may bind or eliminate NaCl, thereby conferring salt tolerance on the plants (Kaldorf et al. 1999).

Colonization and spore density

AM fungal colonization was observed in the roots of all the mangrove plant species sampled. Maximum root colonization was recorded in *Salicornia brachiata* (93.54%) at KRF and least in *Avicennia marina* (22.08%) at PRF. The low colonization in *A. marina* could be attributed to its occurrence in inundated areas. According to the earlier studies, AM fungi exhibit low tolerance to hypoxic conditions as they are aerobic microbes (Allaway et al. 2001; Wang et al. 2011). Previous publications have suggested that hyphal networks are initiators of colonization (Smith and Read 1997). High levels of soil salinity in salt marshes have been observed to reduce the extra-radical mycelia growth (Carvalho et al. 2003). However, in the present study, *S. brachiata* showed a high degree of colonization despite a stressful environment.

Maximum spore density was recorded in *Ceriops decandra* (270/100 g of soil) at PE and minimum in *Rhizophora mucronata* (8 spores/100 g of soil) at PRF (Table 2). Parameters such as inoculum density, root structure, genetic compatibility between host and AM fungi, edaphic factors, and soil microbes affect AM colonization rates and spore density (Zangaro et al. 2013; Sivakumar 2013). Likewise, AM fungal community structure is affected by the tide level (Wang et al. 2011). This could be one of the reasons for maximum and minimum spore density in *C. decandra* and *R. mucronata,* respectively, since the inundation level of the former plant is lower than the latter (Batool et al. 2014) at the study site. Inhibition of AM colonization seldom take place due to increased P level occurs in the wetland ecosystem (Kelly et al. 2004). However, higher rates of AM colonization in the present study may be attributed to low levels of P at all the sites investigated. The study revealed variation in root colonization and spore density. According to Hildebrandt et al. (2001), the intensity of AM colonization is not the same during the plant life cycle, and hyphae bundled with spore strings could be patchily distributed in the soil, which might lead to more spore counts in a single soil sample.

All the mangrove plants examined in the present study from Pichavaram were mycorrhizal. This contradicts the study of Mohankumar and Mahadevan (1986) who reported no AM association in Pichavaram mangroves, while Lingan et al. (1999) reported AM colonization in eight mangrove plants from the same site. It is interesting to observe that halophytes belonging to the family Chenopodiaceae are considered non- or poorly mycorrhizal (Juniper and Abbott 1993; Aliasgharzadeh et al. 2001; Wilde et al. 2009). However, the present study reveals clear evidence that species of Chenopodiaceae (now Amaranthaceae) family viz., *Salicornia brachiata, Arthrocnemum indicum, Suaeda maritima,* and *S. monoica* showed a high degree of AM colonization compared to all the other mangrove plants which is in accordance with Hildebrandt et al. (2001) and Yinan et al. (2017).

Diversity and distribution

A total of 21 AM fungal species belonging to seven genera were recovered. Among them, eight species belonged to the genus *Acaulospora,* one to *Entrophospora,* two to *Funneliformis,* three to *Gigaspora,* two to *Glomus,* three to *Rhizophagus,* and two to *Sclerocystis*. *Rhizophagus fasciculatus* was found to be dominant at PE and PRF, whereas *Funneliformis geosporum* was dominant at KRF (Table 3). The predominance of *F. geosporum* at KRF could be due to the dominance of salt marshes at the site, as *F. geosporum* is often dominant in salt marshes (Carvalho et al. 2004). Similar observations were reported by d'Entremont et al. (2018) in salt marshes of Minas Basin, Nova Scotia. They have also suggested that *F. geosporum* has been found globally and is one of the most halotolerant AM fungal species.

AM fungal species richness differed significantly. Goomaral et al. (2013) suggested that the AM fungal diversity and community composition are affected by the host plant. This could be because the structure and functioning of different host plants vary (Chen et al. 2012). High environmental heterogeneity could be another reason behind the high AM fungal richness observed in the present study. Flooding has been verified as the chief source for heterogeneity in wetlands (Simões et al. 2013).

The highest relative abundance (RA) and isolation frequency (IF) of AM species were recorded in *R. fasciculatus* at PE and PRF and *F. geosporum* at KRF, respectively (Figs. 2, 3). A significant positive correlation was found between RA and IF at all the three sites ($r = 0.94$, $P < 0.01$ at PE; $r = 0.75$, $P < 0.05$ at PRF and $r = 0.83$, $P < 0.01$ at KRF). Some of the AM species, such as *A. dilatata, A. undulata, Entrophospora* sp, and *R. irregulare* showed low relative abundances but were widely distributed with high isolation frequencies. There was no significant correlation between spore density and root colonization at all three sites. In terms of genera, the highest RA was recorded in *Rhizophagus* at PRF, and the highest IF was recorded in *Acaulospora* and *Funneliformis* (Fig. 4).

Table 2 Percent root colonization (RC), spore density (SD) in Pichavaram mangroves

Plant Name	PE		PRF		KRF	
	Percent Colonization	*Spore density	Percent Colonization	*Spore density	Percent Colonization	*Spore density
True mangroves						
Aegiceras corniculatum (L.) Blanco (Myrsinaceae)	65.9 ± 0.8^{abc}	79.0 ± 2.5^{cd}	nd	nd	nd	nd
Avicennia marina (Forssk.) Vierh. (Acanthaceae)	27.6 ± 1.0^{f}	89.0 ± 7.0^{c}	22.1 ± 5.4^{f}	64.0 ± 4.5^{e}	40.2 ± 4.2^{c}	87.0 ± 7.5^{c}
Avicennia officinalis L (Acanthaceae)	76.5 ± 1.5^{a}	92.0 ± 4.5^{c}	51.0 ± 1.0^{cd}	124.0 ± 9.0^{a}	nd	nd
Brugueira cylindrica (L.) Blume (Rhizophoraceae)	54.4 ± 0.58^{bcde}	30.0 ± 8.0^{f}	63.8 ± 8.8^{bc}	105.0 ± 1.0^{bc}	nd	nd
Ceriops decandra(Griff.) W.Theob (Rhizophoraceae)	72.5 ± 7.5^{ab}	270.0 ± 1.0^{a}	27.5 ± 2.5^{f}	90.0 ± 3.5^{cd}	nd	nd
Excoecaria agallocha L (Euphorbiaceae)	77.0 ± 13.0^{a}	60.0 ± 2.0^{e}	22.7 ± 0.8^{f}	79.0 ± 5.0^{de}	90.0 ± 3.3^{a}	96.0 ± 5.0^{c}
Lumnitzeraracemosa Willd (Combretaceae)	70.2 ± 11.9^{ab}	142.0 ± 8.0^{b}	65.0 ± 3.0^{b}	114.0 ± 1.5^{ab}	nd	nd
Rhizophora apiculata Blume (Rhizophoraceae)	37.5 ± 2.5^{ef}	17.0 ± 0.5^{f}	45.0 ± 5.0^{de}	104.0 ± 4.5^{bc}	nd	nd
Rhizophora mucronata Lam (Rhizophoraceae)	nd	nd	32.7 ± 0.7^{ef}	8.0 ± 1.0^{f}	nd	nd
Associate mangroves & salt marshes						
Arthrocnemum indicum (Willd.) Moq (Amaranthaceae)	nd	nd	nd	nd	65.4 ± 3.9^{b}	38.0 ± 5.5^{e}
Clerodendrum inerme (L.) Gaertn (Lamiaceae)	nd	nd	nd	nd	88.5 ± 3.9^{a}	127.0 ± 6.0^{b}
Salicornia brachiata Miq (Amaranthaceae)	nd	nd	nd	nd	93.5 ± 0.2^{a}	30.0 ± 3.5^{e}
Ipomoea pes-caprae (L.) R. Br (Convolvulaceae)	nd	nd	nd	nd	55.0 ± 7.5^{b}	102.0 ± 6.5^{c}
Phoenix paludosa Roxb. (Aracaceae)	nd	nd	88.5 ± 3.9^{a}	71.0 ± 11.5^{e}	nd	nd
Salvadora persica L (Salvadoraceae)	42.3 ± 3.9^{def}	77.0 ± 6.5^{cd}	nd	nd	nd	nd
Sesuvium portulacastrum (L.) L (Aizoaceae)	50.0 ± 3.9^{cde}	22.0 ± 0.5^{f}	nd	nd	nd	nd
Suaeda monoica Forssk. ex J.F.Gmel. (*Amaranthaceae)*	nd	nd	nd	nd	88.5 ± 3.9^{a}	67.0 ± 5.5^{d}
Suaeda maritima (L.) Dumort (Amaranthaceae)	58.0 ± 3.5^{bcd}	71.0 ± 3.5^{de}	nd	nd	89.0 ± 2.7^{a}	161.0 ± 8.0^{a}

Data are means of three replicates; ± standard error

PE Pichavaram extension, *PRF* Pichavaram reserve forest, *KRF* Killai reserve forest, *nd* plant not detected at the site

[a]Indicates spores/100 g of soil

Values in each column followed by different letters are significantly different at $P < 0.05$

Table 3 Occurrence of AM species in Pichavaram mangroves

Plant Name	PE	PRF	KRF
True mangroves			
Aegiceras corniculatum	*A. po, A. un, F. geo, R. fas, R. irr*	nd	nd
Avicennia marina	*R. fas, R. irr*	*Entrophospora* sp., *R. fas*	*A. un, F. geo, R. fas*
Avicennia officinalis	*A. di, A. un, Entrophospora* sp., *R. fas, R. irr*	*A. di, A. ni, Entrophospora* sp., *R. fas, R. irr*	nd
Brugueira cylindrica	*A. di, Entrophospora* sp., *F. geo, R. fas, R. irr*	*A. di, A. ni, Entrophospora* sp., *G. mic, R. fas, R. irr*	nd
Ceriops decandra	*Entrophospora* sp., *F. geo, R. fas, R. irr*	*R. fas, R. irr*	
Excoecaria agallocha	*Entrophospora* sp., *G. alb, G. mic, R. fas, R. irr*	*A. fo, A. un, Entrophospora* sp., *F. geo, R. fas*	*A. di, A. un, F. geo, G. alb*
Lumnitzera racemosa	*Entrophospora* sp., *R. fas*	*A. di, Entrophospora* sp., *F. geo, R. fas, R. irr*	nd
Rhizophora apiculata	*A. di, A. re, A. sc, A. un, Entrophospora* sp., *F. geo, R. fas*	*Entrophospora* sp., *R. fas, R. irr*	nd
Rhizophora mucronata	nd	*Entrophospora* sp., *R. irr*	nd
Mangrove associates and salt marshes			
Arthrocnemum indicum	nd	nd	*A. un, F. geo, G. alb, R. fas*
Clerodendrum inerme	nd	nd	*A. di, A. un, F. geo, G. dec, G. mar, R. int, S. pac, S. rub*
Salicornia brachiata	nd	nd	*A. di, F. geo, G. agg*
Ipomoea pes-caprae	nd	nd	*A. di, A. un, F. geo*
Phoenix paludosa	nd	*A. di, A. un, F. geo*	nd
Salvadora persica	*A. un, F. geo*	nd	nd
Sesuvium portulacastrum	*Entrophospora* sp., *F. geo, R. fas*	nd	nd
Suaeda monoica	nd	nd	*A. un, F. geo, S. rub*
Suaeda maritima	*A. po, A. sp, Entrophospora* sp., *F. geo, R. fas*	nd	*A. di, A. un, F. geo, F. mos, R. fas*

nd not detected, AM species: *A. di Acaulospora dilatata, A. fo A. foveata, A. ni A. nicolsonii, A. po A. polonica, A. re A. rehmii, A. sc A. scrobiculata, A. sp A. spinosa, A. un A. undulata, Entrophospora* sp. Unidentified, *F. geo Funneliformis geosporum, F. mos F. mosseae, G. alb Gigaspora albida, G. dec G. decipiens, G. mar G. margarita, G. agg Glomus aggregatum, G. mic G. microcarpum, R. fas Rhizophagus fasciculatus, R. int R. intraradices, R. irr R. irregulare, S. pac Sclerocystis pachycaulis, S. rub S. rubiformis, PE* Pichavaram extension, *PRF* Pichavaram reserve forest, *KRF* Killai reserve forest

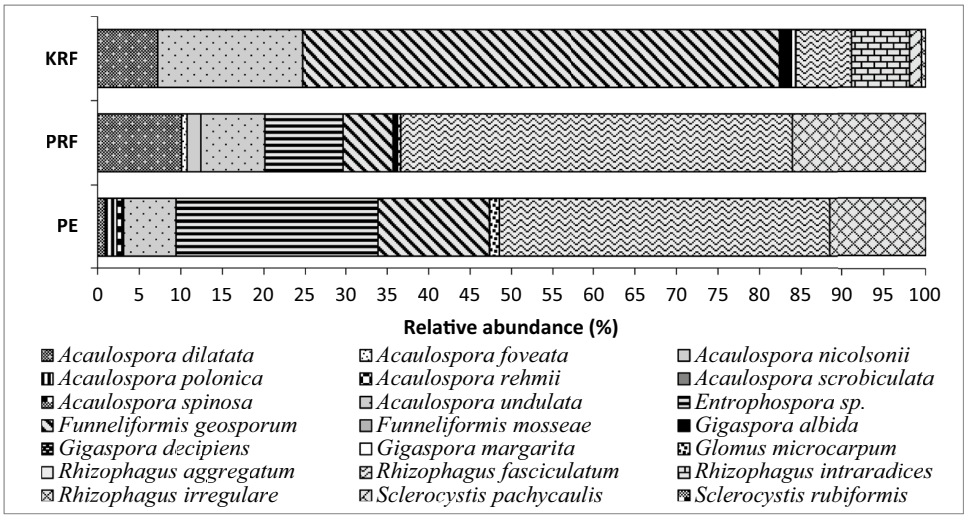

Fig. 2 Relative abundance of AM fungal species. *PE* Pichavaram extension, *PRF* Pichavaram reserve forest, *KRF* Killai reserve forest

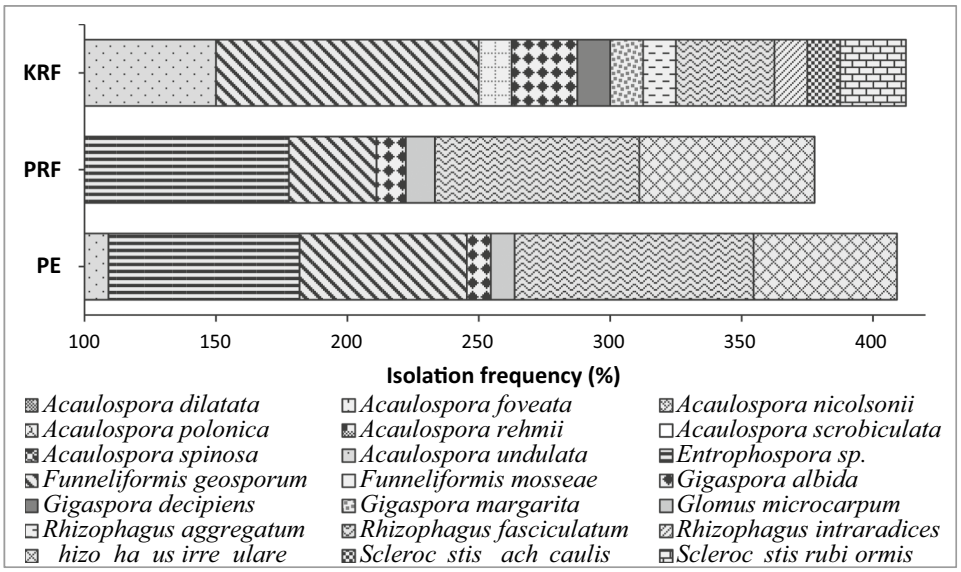

Fig. 3 Isolation frequency of AM fungal species. *PE* Pichavaram extension, *PRF* Pichavaram reserve forest, *KRF* Killai reserve forest

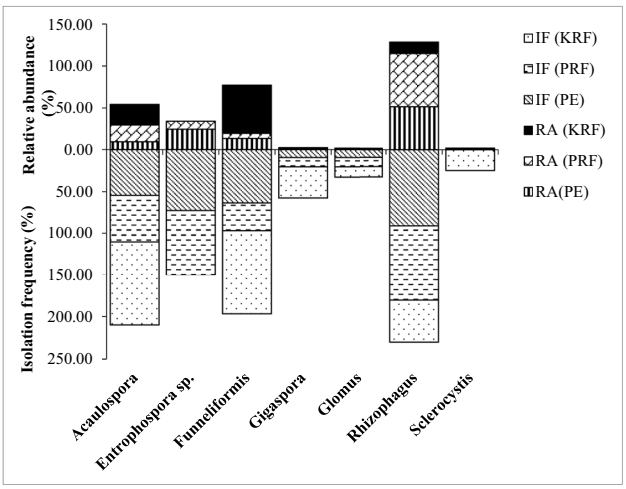

Fig. 4 Genera-wise relative abundance and isolation frequency of AM fungi. *PE* Pichavaram extension, *PRF* Pichavaram reserve forest, *KRF* Killai reserve forest, *IF* isolation frequency, *RA* relative abundance

The AM fungal diversity and distribution were high at PE and PRF, (Fig. 5a–c). The dissimilarity in the sporulation ability of various AM fungal species results in the unevenness of spore distribution (Bever et al. 1996). When comparing the similarity of AM fungi and plant species investigated between the three sites, it was observed that Sørensen's similarity coefficient of AM fungal community, as well as plant community, was higher between PE and PRF (0.73 and 0.70 respectively) (Fig. 5d). This indicates that the vegetation influences in determining the AM community structure, which may be due to the dependency of AM fungal spore formation, distribution, and development on plant diversity in the natural ecosystem (Zhang et al. 2004).

Moreover, several factors such as climatic factors, spatial and temporal variation, vegetation, nutrient availability, host-preference, and differential sporulation ability of AM species can influence the distribution and community structure of AM fungi (Husband et al. 2002; Muthukumar and Udaiyan 2002; Renker et al. 2005). The occurrence of recovered AM species from the saline rhizosphere of Pichavaram verifies their tolerance to high salinity levels.

Conclusion

The AM fungal diversity studies appear to be suitable for understanding the relationship between AM fungi and plant species, within the context of the restoration of various natural ecosystems. This is the first study to explore AM symbiosis in 18 mangroves (true- and associate- mangrove) plant species of Pichavaram Forest. The results of this study also indicate that the recovered AM fungal species from mangroves have the greater potential to assist plants in salt tolerance and hence may have a strong influence on the distribution of plants in saline soils. An important finding in our study is the presence of AM colonization in roots of plants belonging to the family Chenopodiaceae, which was otherwise considered to be a non-mycorrhizal family. Further investigation is needed to check the AM fungal behaviour for different seasons and different phenological stages of the host plant along with tidal effects.

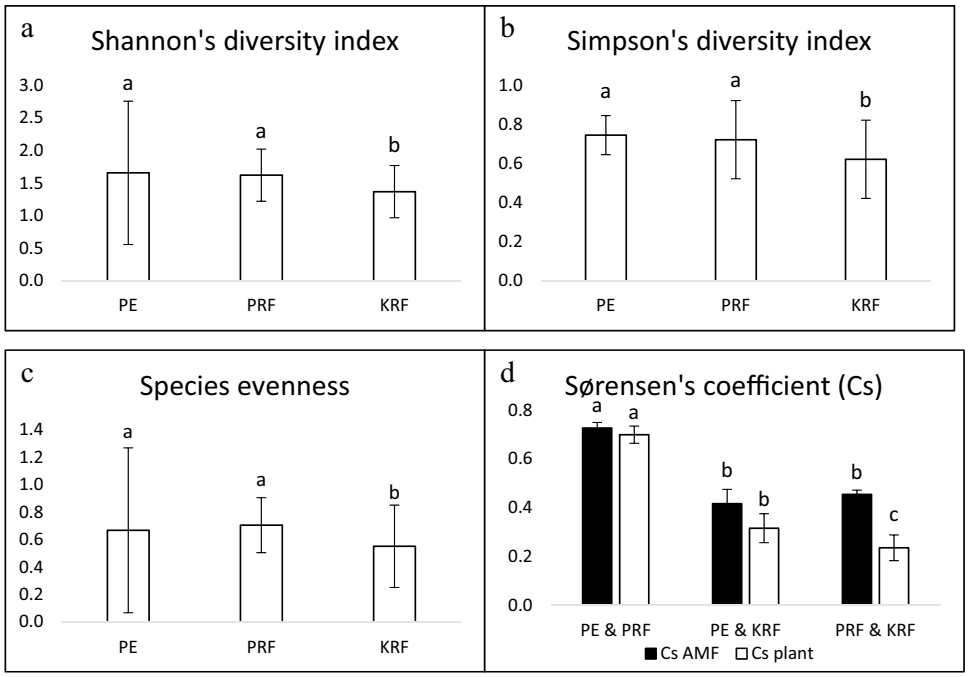

Fig. 5 Diversity measurements of AM fungal communities. *PE* Pichavaram extension, *PRF* Pichavaram reserve forest, *KRF* Killai reserve forest. Values in each column presented with different letters are significantly different at $P \leq 0.05$

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