

Diversity, richness and degree of colonization of arbuscular mycorrhizal fungi in coconut cultivated along with intercrops in high productive zone of Kerala, India

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Abstract Degree of arbuscular mycorrhizal (AM) association and its diversity were investigated in the coconut palm (*Cocos nucifera* L.) cultivated in crop mixed system under rain-fed condition in a highly productive humid tropical zone in Malappuram district of Kerala, India. Forty AM species belonging to ten genera viz. *Acaulospora*, *Claroideoglossum*, *Dentiscutata*, *Diversispora*, *Funneliformis*, *Gigaspora*, *Glomus*, *Redeckera*, *Scutellospora* and *Septoglossum* were recorded indicating high level of AM richness in coconut rhizosphere. Of these, eighteen AM fungal species are being reported for first time from this palm. *Claroideoglossum*, *Glomus* and *Gigaspora* were the most commonly present genera and *Claroideoglossum etunicatum* and *Glomus aggregatum* the common species. The diversity indices (Shannon indices values) of AM fungi associated with coconut palm in a crop mixed system and mono crop varied significantly. The Shannon index, Simpson's index and evenness ranged from 1.40 ± 0.10 to 2.70 ± 0.01 , 0.65 ± 0.033 to 0.90 ± 0.004 , 0.51 ± 0.01 to 0.82 ± 0.01 , respectively. Correlation between soil physio chemical characters and mycorrhizal parameters were worked out. Soil pH had negative correlation with spore count, root colonization, species richness, Shannon H and Simpson indices but had positive correlation with species evenness. Electrical conductivity showed positive correlation with root mycorrhizal colonization which indicated active AMF mediated uptake of nutrients.

Keywords Arbuscular mycorrhizal fungi · AMF · Coconut palm · AMF species diversity · Mixed-cropping · Malappuram

1 Introduction

A main constituent of the soil microbiota in the majority of agro ecosystems is the Arbuscular Mycorrhizal Fungi (AMF), accounting for somewhere between 5 and 50 % of the biomass of soil microbes (Olsson et al. 1999). These obligate mutualistic symbionts colonize the roots of the vast majority of plants, including most crop plants (Smith and Read 1997). The ability of AMF to enhance host-plant uptake of relatively immobile nutrients, particularly P and Zn is believed to be the main benefit of the mycorrhizal symbiosis for plants (Thompson 1987). The role of AM fungal colonization to protect host roots from certain root pathogens (Borowicz 2001) and improve water relations (Davies et al. 1993) especially under nutrient limitation and also metal toxicity condition have been reported (Meharg and Cairney 2000). The extra-radical hyphae of the AMF extend up to 8 cm beyond the root (Rhodes and Gerdemann 1975) and act, in effect, as extensions of the root system in acquiring nutrients from the soil. They may also play a role in the formation of stable soil aggregates, building up a macro porous structure of soil that allows penetration of water and air and prevents erosion (Miller and Jastrow 1992). Arbuscular mycorrhizal symbiosis is important for maintaining and promoting the productivity of crop lands and may be critical to the maintenance of biodiversity (Sanginga et al. 1992; Allen et al. 1995). Crops growing along with trees had a higher level of root colonization and greater spore densities in the rhizosphere relative to crops growing beyond the tree canopies and monoculture plots (Mutabaruka et al. 2002; Pande and Tarafdar 2004; Prasad

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and Mertia 2005). In addition, studies in agroforestry coffee (*Coffea arabica* L.) systems reported higher spore densities in the rhizosphere of coffee plants under shade trees compared to monocultural coffee systems (Muleta et al. 2007, 2008). In maize monoculture a very low number of AM fungal spores were reported as compared to grassland or a poplar grove (Bedini et al. 2007). AMF species richness and community structure are determined by distinct processes in terrestrial ecosystems such as habitat mosaic (De Carvalho et al. 2012), soil characteristics (Santos-González et al. 2011; Ji et al. 2012), long term fertilization (Wu et al. 2011), invasive plants (Barto et al. 2011), tree-based intercropping (Bainard et al. 2011) and distinct land use systems (Sturmer and Siqueira 2011). The availabilities of soil N and P might play key roles in determining the abundance and diversity of AM fungi (Liu et al. 2009, 2012; Alguacil et al. 2010).

Among the important oil-seed crops, coconut (*Cocos nucifera* L.) is one of the main woody perennial palms coming under the family Arecaceae. This palm is cultivated in roughly 2 million ha area in India supporting the livelihood of many million Indians. Improving the productivity of these palms remains an important challenge for Indian agriculture. Exploiting the arbuscular mycorrhizae driven plant-microbial interaction offers a good avenue toward this challenge. The roots of coconut palm are known to be colonized with arbuscular mycorrhizal fungi (Johnston 1949). Lily (1975) reported *Glomus fasciculatus* in coconut roots and Sosamma et al. (1990) isolated nineteen AM fungal species from coconut rhizosphere soil. Thomas and Ghai (1987) observed variation of AM fungal colonization in relation to genotypic differences of coconut seedling and also reported that more than one AM fungal species colonized the same coconut seedlings and the same root segments. The AM fungi present in coconut and component crops in coconut based cropping system were studied by Ramesh and Iyer (1997) and Ambili et al (2012). However, no work focused on the AMF relationship with coconut in mixed cropping system cultivated in area where the coconut yield are highest under rain-fed condition in Kerala had been undertaken thus far. The distribution and function of AM in such coconut cropping systems are poorly understood. The data emerging from such study will help to improve the understanding of AMF relations in this unique coconut based cropping system prevalent in western coast of India, particularly Kerala, and the pure AMF cultures of dominant species that will be isolated will be used for bioinoculant development. With this background, the present study was carried out to unravel the AMF status (spore population, root colonization and diversity) in coconut palms grown in crop mixed agro-ecosystem and mono crop from Malappuram district, which is known for the highest coconut productivity in Kerala, India.

2 Materials and methods

2.1 Study area

The study was carried out in adult coconut plantations grown either as coconut based cropping system or as monocrop in Manjeri (11.12° N, 76.12° E), Kottakal (11.000° N, 76.02° E) and Nilambur (11.28° N, 76.25° E) areas of Malappuram district of Kerala under the hot humid climatic conditions. Average annual rain fall was 2913 mm in Manjeri, 3266 mm in Kottakkal and 2666 mm in Nilambur. The soil type is fine mixed isohypermic *oxic dystrudepts* at Manjeri and Nilambur and fine loamy mixed isohypermic *oxic dystrudepts* at Kottakkal and has acidic to neutral reaction (pH 5.53–7.34). The soil organic carbon ranged between 0.33–1.75 %, nitrogen 0.08–0.34 %, phosphorus 13.36–57.66 ppm and potassium 53.62–240.26 ppm. The coconut cultivar in the selected gardens was West Coast Tall and the most commonly associated inter/mixed crops were banana, nutmeg and arecanut. Annual yield of the selected coconut palms ranged from 60 to 80 nuts palm⁻¹ year⁻¹ and the palms were maintained under rain-fed condition. The coconut palms received cow dung, green leaf manure and chemical fertilizer as nutrient inputs; in the crops mixed gardens, the inter crops also received fertilizers. In addition, frequent interspace tillage was carried out in coconut based mixed cropped plot.

2.2 Soil sampling

Rhizosphere soils and roots were collected from four places from the base of each coconut, arecanut, nutmeg and banana from monocropped and coconut based mixed cropping system. Surface soil (approximately 1 mm) was removed and soil up to the depth of 20 cm was collected by using a small garden shovel and mixed to form composite soil sample (ca. 500 g) and placed in separate polythene bags. From each plot soil and root samples were collected from three palms and made to form a single composite soil sample. Overall nine soil samples from coconut rhizosphere, i.e. three pooled samples from monocropped and six samples from mixed crops were collected from the three locations in September 2012 for the AMF analysis (Table 3).

2.3 Spore isolation and identification

A sub-soil sample of 50 g was then taken from the main samples for the study. AM fungal spores were extracted by wet sieving (45, 100, 250, and 355 µm sieve openings) and decanting method (Gerdemann and Nicolson 1963). The spores extracted through sieves with mesh size ranging between 45 and 355 µm were filtered using a Whatman No. 1 filter paper were observed under stereomicroscope and the total number of spores was counted. Spores exhibiting

morphologically similar characters were then clustered into one group. Number of spores in each morphotype was recorded in order to construct spore community composition. Spore count was calculated as the total number of spores in each soil sample (spores per 50 g soil). Further, the intact and crushed spores were mounted on slides in polyvinyl-lactoglycerol (PVLG) and PVLG mixed with Melzer's reagent and examined under a compound microscope. Spores were identified to species level based on spore color, size, surface ornamentation and wall structure (Almeida and Schenck 1990; Morton and Benny 1990) with reference to the identification manual of Schenk and Perez (1990) and originally published species descriptions and emendation and also new classifications available at <http://invam.wvu.edu/collection>, <http://schuessler.userweb.mwn.de/amphylo/http://www.zor.zut.edu.pl/Glomeromycota/index.html>. Slides of the spores were stored as permanent vouchers in Central Plantation Crops Research Institute, India under the access number 200 to 800.

2.4 Root colonization

Root samples were washed with running tap water, cut into small pieces (*ca.* 1 cm) and stained with 0.05 % trypan blue using the method described by Kormanik and McGraw (1982). The stained root samples were observed under Nikon compound microscope for the presence of AM fungi. Care was taken to avoid scoring dark septate endophytic (DSE) fungi as AMF based on the criteria given by Muthukumar and Prakash (2009) for identification of the DSE which usually formed linear melanized septate hyphae and micro-sclerotia. The characteristic features of endomycorrhizae were recorded as vesicles, hyphal coiling and arbuscules. When any one of these was found on a sample, colonization was recorded as positive and calculated as:

Percentage of root colonization =

$$\frac{(\text{number of mycorrhizal root segments observed})}{/(\text{Total number of root segments observed})} \times 100$$

2.5 Diversity indices

The diversity of AM fungi was assessed based on diversity indices: Simpson's Index ($D_s = 1 - (\sum n_i(n_i - 1) / N(N - 1))$ where 'n_i' is the number of individual of the species 'ith' species and N is the total number of the species (Simpson 1949) Shannon index: $H' = - \sum p_i \ln(p_i)$ where "p_i" is the proportion of individual that species contribute to total (Shannon and Weaver 1949) and evenness was expressed by $J = H' / H'_{max}$, where H'_{max} is the maximum value of diversity for the number of species present (Pielou 1975). Frequency occurrence was calculated as the percentage of soil samples in which a species

occurred, which revealed the extent of distribution of a given AMF species in an ecosystem.

2.6 Soil chemical analysis

Root region soil samples were analyzed for soil chemical factors. Soil pH was measured in 1:2.5 soil water suspension using pH meter (Eutech instruments pH tutor) and electrical conductivity was measured at room temperature in 1:5 soil suspension using conductivity meter (Eutech instruments). Soil analysis techniques *viz.*, Walkley and Black's rapid titration method (1934) Kjeldahl method (Jackson 1971) and Bray and Kurtz method (1945) were employed for determination of organic carbon, total nitrogen and available phosphorus, respectively. Available potassium was estimated by ammonium acetate method (Hanway and Heidal 1952).

2.7 Statistical analysis

Pearsons's correlation analysis was used to determine relationship between AMF spore count, root colonization, diversity indices and soil physiochemical factors using SPSS Base 20.0 (SPSS, Cary, N.C.). One way ANOVA was used to test for the spore count, root colonization diversity indices and soil physiochemical factors in coconut and coconut palm in a mixed crop in agroecosystems using SAS software version 9.2 (Statistical Analysis System Institute, Cary, NC, USA). There was no evidence for statistical significance of heterogeneity of variance (Bartlett's *chi-square*=9.75 *p*=0.283). So the data was analyzed without any transformation.

3 Results

3.1 Spore count and root colonization

The total spore count of AMF varied significantly in different cropping systems and monocrop of coconut, ranging from 213.67±22.59 to 298.50±31.82 per 50 g soil (Table 1). Highest spore count was recorded in coconut based cropping system at Nilambur having banana and arecanut as mixed crops in coconut stands, followed by that in Kottakkal where nutmeg was grown as mixed crop and that again in Nilambur where coconut was also grown as monocrop. The mean spore count in Manjeri, Kottakkal and Nilambur were 253.50, 253.28 and 280.00 spores per 50 g soil, respectively.

Microscopic observation of the root samples showed the presence of extensive hyphal, vesicular and arbuscular stages of AM fungal colonization and in some samples presence of spores was also seen within the roots. The observation of presence of intercellular linear hyphae, arbuscules and vesicles indicated the mycorrhizal association in coconut roots to be of Arum-type as reported by Muthukumar and Prakash

Table 1 Spore count and root colonization of AM fungi (mean \pm S.E.) associated with coconut palm in a crop mixed agroecosystem in Malappuram district, Kerala, India

Location	Crops *	Spore count (50 g ⁻¹)	Root colonization (%)
Manjeri	C	253.50 \pm 22.12 ^{cab}	46.83 \pm 7.78
Kottakkal	C	270.67 \pm 48.27 ^{ab}	49.33 \pm 16.77
	C+N	295.00 \pm 5.77 ^a	55.17 \pm 1.61
	C+B	213.67 \pm 22.59 ^c	47.00 \pm 4.36
	C+B+N	233.78 \pm 41.38 ^{cb}	37.44 \pm 3.10
Nilambur	C	286.62 \pm 14.72 ^a	49.00 \pm 11.29
	C+N	284.00 \pm 9.17 ^{ab}	40.00 \pm 20.00
	C+B	252.33 \pm 20.11 ^{cab}	32.33 \pm 8.74
	C+B+A	298.50 \pm 31.82 ^a	43.33 \pm 10.41

Values in the same column followed by different letters are significantly different from each other ($p < 0.05$). Values in columns without letters are not significantly different ($p < 0.05$)

C Coconut, N Nutmeg, B Banana, A Arecanut

(2009). The percentage colonization of AM fungi in roots of coconut in different cropping systems ranged from 32.33 \pm 8.74 to 55.17 \pm 1.61 which were not significantly different. Two types of hyphae were detected in the root segments of coconut, one thick walled hyaline to yellowish brown and second thin walled hyaline. The shape of vesicles present in the root segments varied from globose to subglobose and some were elongated. The extent of occurrence of vesicles, arbuscules and hyphal infection formed in the roots varied in coconut palm in different crop mixed systems.

3.2 Description of unreported AM fungal species in root region soils of coconut (Fig. 1)

1. *Acaulospora foveata* Trappe & Janos, Mycotaxon 15, 516, 1982.

Spores globose to ellipsoid, yellowish brown to light reddish brown, 200–300 \times 200–300 μ m in diam. Spore consisted of three walls. Outer wall (wall 1) composed of three layers (L1, L2 and L3), the outer layer continuous with the wall of the neck of the parent sporiferous saccule. The inner two layers are synthesized sequentially as the spore forms on the neck of saccule. L1: hyaline up to 2 μ m thick; degrading and sloughing with collapse and dehiscence of the sporeferous saccule. L2: yellowish brown, reddish brown to brown, laminate, 11–12 μ m thick, uniformly pitted with round, oblong to occasionally irregular depressions(pits), pits 4–10 μ m across and up to 2 μ m deep, with rounded bottoms, separated by ridges, ridges 1–10 μ m broad. Wall 2: hyaline, composed of two adherent layers (L1 and L2) each up to 1 μ m thick. Wall 3: hyaline, composed of two

adherent layers (L1 and L2) up to 5 μ m thick. A circular scar indicating region of contact between spore and saccule neck, it consisted of closely packed tubercle surrounding an unornamented depressions, 12–13 μ m diam.

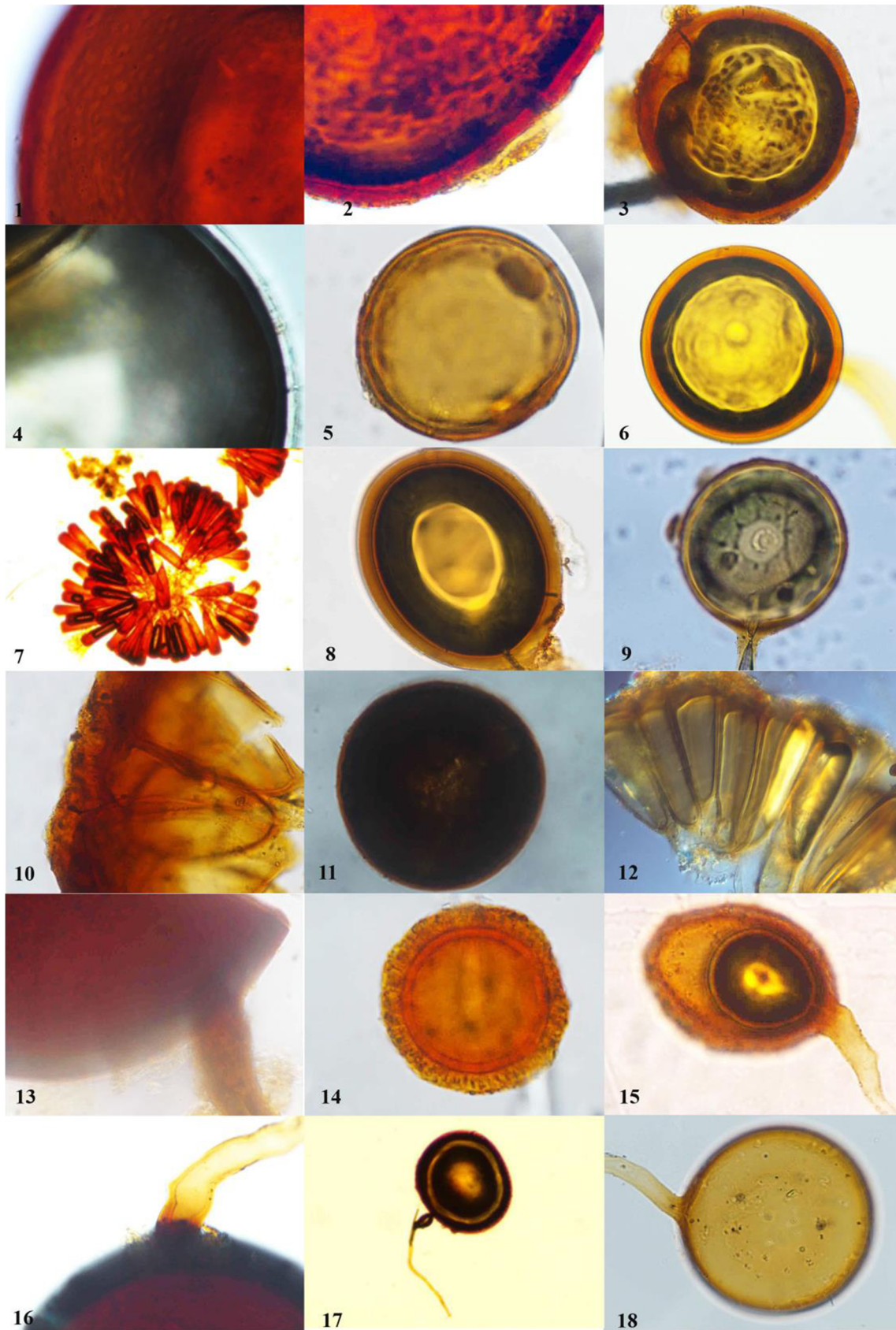
2. *Acaulospora lacunosa* J.B. Morton, Mycologia,78(4), 643, 1986.

Spores reddish yellow, globose to subglobose, 102–174 μ m in diam.; Spore consisted of three walls. Outer wall composed of two layers (L1 and L2). The outer layer continuous with the wall of the neck of the parent sporiferous saccule and the inner layer synthesized with development of the spore. L1: hyaline, <1 μ m thick completely sloughed in mature spores. L2: yellow to reddish yellow, laminate, 1–4 μ m thick, ornamented with saucer shaped pits and highly variable number of scattered pits with cone-shaped raised edges, with cone edges up to 1 μ m high. Wall 2 composed two layers, hyaline, (L1 and L2), each layer up to 1 μ m thick. Wall 3 two layered (L1 and L2). L1: hyaline, up to 1 μ m thick. L2: hyaline, 2–4 μ m thick. A circular scar indicating region of contact between spore and saccule neck, 5–6 μ m diam.

3. *Claroideoglossum claroideum* (N. C. Schenck & G. S. Sm.) C. Walker & A. Schubler Gloucester, p.22, 2010.

Spores formed singly or in loose clusters in the soil, pale yellow to greenish orange, globose, subglobose to irregular, 50–120 \times 50–120 μ m in diam. Spore wall composed of four layers (L1, L2, L3 and L4). L1: hyaline, mucilaginous layer up to 1 μ m thick tightly adherent to layer 2. L2: hyaline, smooth up to 2 μ m thick degrading and sloughing concomitant with L1. It attract organic debris which can accumulate on the spore surface. L3: hyaline to pale yellow, smooth, laminate, thin 2–7 μ m thick. L4: hyaline, smooth up to 1 μ m thick. Subtending hyphae 6–15 μ m broad at the base of spore attachment; wall three layered (L1, L2 and L3) continuous with the three layers of spore, 1–3 μ m thick at the point of

Fig. 1 AM fungal species newly reported from coconut rhizosphere in crop mixed agroecosystem (1) *Acaulospora foveata* Trappe & Janos; (2) *Acaulospora lacunosa* J.B. Morton; (3) *Claroideoglossum claroideum* (N. C. Schenck & G. S. Sm.) C. Walker & A. Schubler; (4) *Gigaspora candida* Bhattacharjee, Mukerji, Tewari & Skoropad; (5) *Glomus arborens* McGee; (6) *Glomus cerebriforme* McGee; (7) *Glomus clavispurum* (Trappe) R.T. Almeida & N.C. Schenck; (8) *Glomus flavispurum* (M. Lange & E.M. Lund) Trappe & Gerd.; (9) *Glomus heterosporum* G.S. Sm. & N.C. Schenck; (10) *Glomus liquidambaris* (C.G. Wu & Z.C. Chen) Y.J. Yao; (11) *Glomus reticulatum* Bhattacharjee & Mukerji; (12) *Glomus taiwanense* (C.G. Wu & Z.C. Chen) R.T. Almeida & N.C. Schenck ex Y.J. Yao; (13) *Glomus tenebrosus* (Thaxt.) S.M. Berch; (14) *Glomus tortuosum* N.C. Schenck & G.S. Sm.; (15) *Redeckera fulva* (Berk. & Broome) C. Walker & A. Schüssler; (16) *Septoglossum constrictum* (Trappe) Sieverd., G.A. Silva & Oehl; (17) *Scutellospora erythropus* (Koske & C. Walker) C. Walker & F.E. Sanders; (18) *Diversispora gibbosa* (Błaszk.) Błaszk. & Kovacs



attachment, usually abruptly tapering below the spores, considerable branching of subtending hyphae usually occurs 50–120 μm below the spore.

4. ***Gigaspora candida*** Bhattacharjee, Mukerji, Tewari & Skoropad, Trans. Br. Mycol. Soc., 78(1), 184, 1972.
Spores formed singly in soil, white, globose, 200–300 μm diam. Spore wall smooth, composed of three layers (L1, L2, L3), layers distinctly visible in fractured spores, L1: An outer permanent rigid layer 1 to 2 μm thick. L2: consisting of hyaline, laminated, 6–10 μm thick. L3: adherent L2. Numerous “warts” or papillae form on the inner surface of this layer and are concentrated in close proximity to the bulbous suspensor like cell. Bulbous suspensor like cell attached to the spore white, globose to sub-globose, 30–50 μm diam.
5. ***Glomus arborensae*** Mcgee, Trans. Brit. Mycol. Soc. 87(1), 123, 1986.
Spores hyaline to pale yellow, globose to subglobose, 53–64 \times 53–64 μm in diam. wall single, outer surface smooth to roughened, up to 3 μm thick. Subtending hyphae single, hyaline to pale yellow, up to 5 μm broad at spore base, cylindrical with slightly flare to the spore wall. The wall of the subtending hypha is hyaline to pale yellow, up to 1 μm thick.
6. ***Glomus cerebriforme*** McGee, Trans. Brit. Mycol. Soc., 87(1), 123, 1986.
Spores globose to irregular, 80–82 \times 80–82 μm in diam. Spore wall composed of two layers (L1 and L2) L1: outer layer hyaline, laminate, 3–4 μm thick, smooth; L2 inner layer hyaline, membranous. A collar forms inside the subtending hypha supporting the membranous septum up to 8 μm below the subtending hyphae. Subtending hyphae 6–8 μm diam, up to 25 μm long, hyaline, cylindrical at the spore wall, occasionally swollen to irregular in shape, with a wall up to 2 μm thick.
7. ***Glomus clavisorum*** (Trappe) R.T. Almeida & N.C. Schenck, Mycologia, 82, 710. 1990.
Sclerocystis clavisorum Trappe, Mycotaxon, 6, 359. 1977.
Sclerocystis microcarpus S.H. Iqbal & Perveen, Trans. Mycol. Soc. Japan 21: 58. 1980.
Sporocarps dark brown, globose to subglobose, 100–420 μm in diam., It is minutely verrucose from exposed tips of spores which are formed radially in a single, tightly packed layer around a central plexus of hyphae; peridium lacking. Spore brown, clavate or cylindric-clavate with a small pore opening into the thick walled subtending hyphae, 95–135 \times 35–48 μm . Spores wall laminate, brown, 5–20 μm thick at the apex, up to 4 μm thick at the sides, generally thickest at the apex.
8. ***Glomus flavisorum*** (M. Lange & E.M. Lund) Trappe & Gerd., Mycol. Mem., 5, 58. 1974.
Endogone flavispora M. Lange & E.M. Lund, Friesia, 5, 93. 1954.
Fruit body globose, lobed, up to 0.5 cm in dia., peridium whitish, brown when dry, gleba dark, yellowish to dark brown, not incrustated with soil. Peridium thick, brown hyphae mixed with vesiculae, vesiculae few in central part of the gleba, spores irregularly arranged, and 6–12 μm broad, branched hyphae with thin walls. Spores ovate to oblong, often slightly constricted at the middle, rarely subglobose, 110–120 \times 100–110 μm , wall yellowish brown, 6–7 μm thick, outer thin layer somewhat laminate.
9. ***Glomus heterosporum*** G.S. Sm. & N.C. Schenck, Mycologia, 77, 567. 1985.
Spores produced singly in soil, light to dark brown, globose to subglobose, 87–97 \times 87–97 μm in diam. Spore wall composed of three layers, (L1, L2 and L3). L1: smooth, hyaline, 2–4 μm thick, absent in aged spores, L2: brown, laminate, 3–6 μm thick, L3 membranous up to 1 μm thick. Subtending hyphae 8–10 μm wide. Spore are frequently found with multiple hyphal attachments. Hyphal attachment frequently branched. Spore contents hyaline, non-globular and separated from the hyphal attachment by a septum.
10. ***Glomus liquidambaris*** (C.G. Wu & Z.C. Chen) Y.J. Yao, Kew Bull., 50, 306. 1995.
Sclerocystis liquidambaris C.G. Wu & Z.C. Chen, Trans. Mycol. Soc. Rep. China, 2, 74. 1987.
Sclerocystis cunninghamia H.T. Hu, Quart. J. Chinese For., 21, 52. 1988.
Sporocarps globose to subglobose, brown to dark brown, 260–300 \times 260–300 μm , consisting of chlamydospores formed radially within paraphysis like structures, cylindrical, clavate-yellowish brown to dark brown, thick-walled, up to 200 μm long, protruding from the central pluxes of hyphae through the chlamydospores layer. The apices of paraphysis like structures tightly packed into peridium enclosing sporocarps. Spores cylindrical, clavate to obovoidal, 108–148 \times 46–68 μm , brown to reddish brown, sometimes with a septum at the spore base, wall brown to reddish brown, 8–20 μm thick at apex, 6–10 μm thick at the base, 2–5 μm thick at the sides subtending hyphae.
11. ***Glomus reticulatum*** Bhattacharjee & Mukerji, Sydowia, 33, 14. 1980.
Spores formed singly in soil, brownish black, globose, 90–98 μm . Spore wall single, up to 3 μm thick, composed of three layers (L1, L2, L3), L1: 1 μm thick, thick L2: 2–3 μm thick, L3: with regular geometric

- marking on its outer surface. Subtending hyphae funnel shaped, up to 6 μm wide. Wall thickening extend down the subtending hyphae.
12. *Glomus taiwanense* (C.G. Wu & Z.C. Chen) R.T. Almeida & N.C. Schenck ex Y.J. Yao, Kew Bull. 50, 306. 1995.
Sclerocystis taiwanensis C.G. Wu & Z.C. Chen, Trans. Mycol. Soc. Rep. China 2, 78. 1987.
 Sporocarps globose, brown to dark brown, 200–245 μm in diameter. Spores formed radially in a single, tightly packed layer around the central plexus of hyphae. Peridium lacking. Spores cinnamon brown, clavate to cylindrical, 54–80 \times 28–54, with or without septum at spore base. Spore wall composed of two layers (L1 and L2). L1: external one thin and hyaline, L2: inner layer brown, apical portion of the wall deep golden brown, 8–10 μm thick, 2–4 μm thick laterally. Central portion pale yellow and typically distinct from the wall. Stalk pale brown, continuous, 9–20 \times 2–4 μm , central plexus up to 70 μm in diameter.
 13. *Glomus tenebrosus* (Thaxt.) S.M. Berch. Can. J. Bot., 60, 2615. 1983.
Endogone tenebrosa Thaxt., Proc. Am. Acad. Arts Sci., 57, 314. 1922.
 Spores globose to subglobose, 190–198 \times 190–198 μm . Spore wall single, 10–112 μm thick, yellow to dark brown. The thick wall laminated near the subtending hyphae. The outer surface of the spore smooth and bear flattened tubercles. Subtending hypha up to 20–26 μm wide at the point of attachment to the spore, yellow to dark brown near the spore, hyaline to yellow with 100 μm of the attachment. The pore in the subtending hyphae remains open although the wall of hyphae at the point of attachment to be quite thick.
 14. *Glomus tortuosum* N.C. Schenck & G.S. Sm., Mycologia 74, 83, 1982.
 Chlamydospores borne singly in soil yellow to dull grayish brown with a mantle of sinuous hyphae closely appressed to the spore and flattened, 5 μm wide, forming a layer of hyphae on the spore surface, 6–10 μm thick, occasionally mantle extended down to the hyphal attachment. Mantle hyphae hyaline to yellowish brown. Mantle adhered with debris and soil particles. Chlamydospores pale yellow, globose to subglobose, 49–88 μm (excluding mantle) in diam.; spores with single laminate wall, 1–2 μm thick. Subtending hyphae hyaline to light yellow, 9–10 μm wide at the spore base, usually covered with mantle of sinuous hyphae. Subtending hypahal wall pale yellow to light yellow 1 μm .
 15. *Redeckera fulva* (Berk. & Broome) C. Walker & A. Schüssler, The Glomeromycota- a species list: p. 44. 2010.
Paurocotylis fulva Berk. & Broome, J. Linn. Soc. Bot., 14, 137. 1873.
Glomus fulvum (Berk. & Broome) Trappe & Gerd., Mycol. Mem., 5, 59. 1974.
 Sporocarps rounded, white to cream colour or pale brown, lacking a peridium, the spores on the surface easily detached. Spores globose, obovoid to clavate, 60–110 \times 40–100 μm in diam., with a single laminated, hyaline to pale yellow brown wall 2–4 μm thick. A septum attached at the spore wall to separate spore contents from the subtending hypha. Subtending hyphae tapering slightly, 8–10 μm wide at the point of attachment, the wall single layered and thinning rapidly with distance from the spore, usually detached from glebal hyphae.
 16. *Septoglomus constrictum* (Trappe) Sieverd., G.A. Silva & Oehl, Mycotaxon, 116, 105. 2011
Glomus constrictum Trappe, Mycotaxon, 6, 361. 1977.
Funnelformis constrictus (Trappe) C. Walker & A. Schüssler, The Glomeromycota- a species list: p. 14. 2010.
 Spores naked, formed singly or in loose clusters in soil, dark brown to black, shining, subglobose to globose, 144–174 μm diam. Spore wall smooth, 7–10 μm thick, dark brown, one layered or rarely two layered; base straight or occasionally with a short funnel-shaped projection; attachment occluded by wall thickenings; contents of oil globules of widely varying sizes. Attached hypha straight to recurved; point of attachment the dark brown walls 3–6 μm thick; just beyond the point of attachment the hypha constricted up to 10–17 μm diam.; just beyond the constriction, the hypha inflated to 15–30 μm diam. with yellow-brown walls of 2–3 μm thick, from which several hyaline to yellow, fragile, thin-walled hyphae with a diameter of 5–6 μm , just beyond the inflated segment is a thick walled septum, and beyond the inflated segment the hypha is dichotomously forked.
 17. *Scutellospora erythropus* (Koske & C. Walker) C. Walker & F.E. Sanders, Mycotaxon, 27, 181, 1986.
Gigaspora erythropus Koske & C. Walker, Mycologia, 76(2), 250, 1984.
Quatunica erythropus (Koske & C. Walker) F.A. Souza, Sieverd. & Oehl, in Oehl, Souza & Sieverd., Mycotaxon 106, 348, 2008.
 Spore formed singly in soil, dark reddish brown, globose to subglobose, 185–190 \times 185–190 μm in diam. Spore consists of four walls. Wall 1 composed of 2 layers (L1 and L2) L1: An outer permanent rigid layer with a smooth surface, dark red brown up to 2 μm thick, tightly adherent to L2. L2: A layer consisting of red brown, laminate 3–4 μm thick. Wall 2 composed of two layers

(L1 and L2), hyaline, up to 1 μm thick, L2 is slightly thicker than L1. Wall 3 is composed of two layers (L1 and L2). L1: hyaline, less than 1 μm thick, L2: hyaline, 1–2 μm thick. Wall 4 is pale yellow, composed of two layers (L1 and L2) L1 is up to 2 μm thick. L2 is up to 1 μm thick. Germination shield formed on the upper surface of wall 4. Germination shield thin walled, 129–132 \times 179–180 μm . Bulbous suspensor 65–69 \times 30–33 μm , yellow brown wall up to 2 μm thick, a peg like protrusion extend towards the spore base.

18. ***Diversispora gibbosa*** (Błaszk.) Błaszk. & Kovacs, Mycotaxon, 110, 116, 2011.

Glomus gibbosum Błaszk., Mycologia 89: 339, 1997.

Spores occurring singly in soil, globose to subglobose 95–98 μm in diam. Spore wall composed of five layers (L1, L2, L3, L4 and L5). L1: smooth or slightly roughened, hyaline up to 1 μm thick, L2: rigid, hyaline to light yellow, 1–2 μm thick. L3: laminated, hyaline 2–3 μm thick. L:4 and L:5 tightly adherent, hyaline, flexible each less than 1 μm thick, both layers are tightly adherent to L3. Subtending hyphae hyaline to yellow, straight 8–10 μm wide at spore base with single wall, hyaline to yellow up 2 μm thick. Pore occluded by a septum.

3.3 Genus recorded

The present investigation revealed that AM fungi were widely distributed in rhizosphere soil of coconut palm in the crop mixed / mono cropped gardens. A total of 40 Glomeromycotean fungal species belonging to ten genera viz., *Acaulospora*, *Claroideoglomus*, *Dentiscutata*, *Diversispora*, *Funneliformis*, *Gigaspora*, *Glomus*, *Redeckera*, *Scutellospora* and *Septoglomus* were recorded with different percentage frequency of occurrences (Fig. 2) The generic level distribution of AMF spores indicated that *Claroideoglomus*, *Glomus* and *Gigaspora* were the most widely distributed AMF genera with 100 % occurrence, followed by *Acaulospora* and *Funneliformis* (89 %).

3.4 Species diversity

Among the 40 AM fungal species recorded in this study, twenty two species were already reported from coconut rhizosphere and eighteen AM fungal species are being reported for the first time from rhizosphere soil of coconut. These 18 unreported species represented seven genera of the phylum Glomeromycota. Higher number of AM fungal species were from the genus *Glomus* (21 species) followed by *Gigaspora* (5 species), *Acaulospora* (4 species) while only one species each were recorded from *Dentiscutata*, *Diversispora*, *Redeckera*, *Scutellospora* and *Septoglomus*. The data presented in Table 2

shows the frequency distribution of AM fungi. Among the species of AM fungi recorded, *Glomus aggregatum* and *Claroideoglomus etunicatum* had 100 % species occurrence followed by *Funneliformis geosporum* (89 %) and then by *Acaulospora scrobiculata*, *Glomus clarum*, *G. clavisporem*, *G. liquidambaris* and *G. macrocarpum* (78 %). The AM fungal species such as *Diversispora gibbosa*, *Acaulospora bireticulata*, *Acaulospora foveata*, *A. lacunose*, *Glomus cerebriforme*, *G. heterosporum*, *G. invermaium*, *G. pallidum*, *G. reticulatum* and *G. tenebrosum* had low level of occurrence. Based on the recent publication of Sieverding et al. (2014) where they have revised the nomenclature and classification of few of the *Glomus* spp. to newly erected genus *Rhizoglomus*, ten of the *Glomus* species identified by us in the coconut soils have been renamed and presented in Tables 2 and 4.

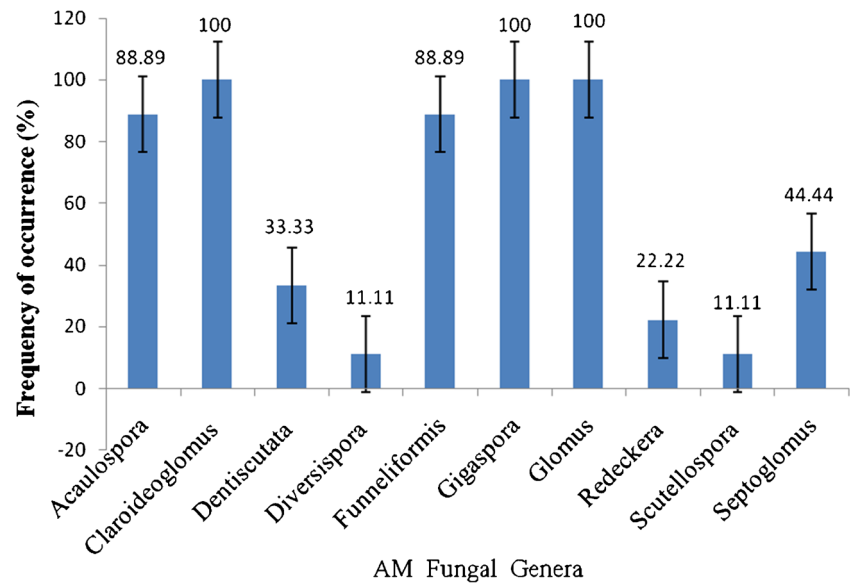
3.5 Diversity indices

The data on Simpson's index of diversity (Ds), Shannon's diversity index (Hs) and Shannon evenness are presented in Table 3. The results showed that diversity indices varied significantly in different coconut based systems studied. The index value of Hs, Ds and J varied from 2.70 \pm 0.01 to 1.40 \pm 0.10, 0.65 \pm 0.033 to 0.90 \pm 0.004, 0.51 \pm 0.01 to 0.82 \pm 0.01, respectively. In the case of general diversity indices (Shannon H'), a value of 2.70 \pm 0.01 for coconut mono cropping system of Kottakkal was observed and the lowest value of 1.40 \pm 0.10 in coconut palm in a crop mixed system (banana, banana and arecanut are the inter crops) in Nilambur was recorded. Thus, a greater diversity of AM fungal species was recorded in coconut mono cropping system at Kottakkal location compared to mixed cropping systems in other locations. The species evenness was high in coconut inter cropped with nutmeg at Nilambur (0.82 \pm 0.01) as compared to other sites (Table 3). The AM fungal species distribution was significantly different in coconut palm in crop mixed systems (Table 4).

3.6 Soil physiochemical properties

Physiochemical properties of soil collected from different cropping systems varied significantly (Table 5). The soil type is fine mixed isohypermic *oxic dystrudepts* at Manjeri and Nilambur and fine loamy mixed isohypermic *oxic dystrudepts* at Kottakkal. The soils had acidic to neutral reaction (pH 5.53–7.34). The electric conductivity (EC) ranged between 24.82 \pm 0.02–122.60 \pm 0.23 μs , soil organic carbon (OC) 0.33 \pm 0.04–1.75 \pm 0.02 %, nitrogen 0.08 \pm 0.01–0.34 \pm 0.06 %, phosphorus 13.36 \pm 0.81–57.66 \pm 0.71 ppm, potassium 53.62 \pm 2.93–240.26 \pm 0.91 Variation in the salinity levels was observed over the locations as is evident from the electrical conductance values, with highest EC values recorded in coconut monocrop at Kottackal and lowest values in coconut

Fig. 2 Frequency of occurrence of AM fungal genera in coconut palm in a crop mixed agroecosystems in Malappuram district, Kerala, India (The bar represent Percentage of occurrence of AM fungal genera with standard error of mean)



monocrop at Nilambur. The organic carbon content was in general higher in various locations in Kottackal. Low values for N and P contents were recorded in coconut + banana system and K content in coconut + banana + arecanut system in Nilambur.

Correlation between spore count, root colonization and AMF diversity parameters with soil chemical factors were worked out (Table 6). pH values showed negative correlation with spore count, root colonization, species richness, Shannon and Simpson index but positive correlation with species evenness. Electric conductivity was positively correlated with root colonization, species richness, Simpson, Shannon index and evenness but had negative correlation with spore count. Organic carbon had a negative correlation with spore count, Simpson index and evenness but had positive correlation with root colonization, species richness and Shannon index. Soil nitrogen was positively correlated with root colonization, species richness, Simpson, Shannon and evenness except spore

count. Available phosphorus was positively correlated with all the parameters apart from Simpson index and species evenness. Available potassium was negatively correlated with spore count and root colonization.

4 Discussion

4.1 Spore count and root colonization

The present study revealed the distribution of arbuscular mycorrhizal fungi in rhizosphere soils of coconut palm in crop mixed and monocrop situations in farmers fields in different locations in a highly productive coconut zone in the state of Kerala, India. Spore densities recorded in this study showed significant variation in different cropping systems, ranging from 213.67 ± 22.59 to 298.50 ± 31.82 per 50 g soil, with the highest count recorded in the crop mixed system at Nilambur,

Table 2 Frequency of occurrence of AM fungal species in root region soil of coconut palm in a crop mixed agroecosystem in Malappuram district, Kerala, India

AM fungal species	Frequency of occurrence (%)
<i>Acaulospora bireticulata</i> , <i>Acaulospora foveata</i> , <i>Acaulospora lacunose</i> , <i>Diversispora gibbosa</i> , <i>Gigaspora candida</i> , <i>Gi. gigantean</i> , <i>Gi. decipiens</i> , <i>Glomus cerebriforme</i> , <i>G. heterosporum</i> , * <i>G. invermaium</i> , <i>G. pallidum</i> , <i>G. reticulatum</i> , ** <i>G. taiwanense</i> , <i>G. arborensis</i> , <i>G. multicaule</i> , <i>G. tenebrosus</i> , <i>Scutellospora erythropus</i> , <i>Redeckera fulva</i>	1 to 25
<i>Dentiscutata nigra</i> , <i>Funneliformis mosseae</i> , <i>Glomus flavisporum</i> , <i>G. tortuosum</i> , * <i>Septoglossum constrictum</i>	26 to 50
<i>Claroideoglossum claroideum</i> , <i>Claroideoglossum luteum</i> Gialbida, <i>Gi margarita</i> , ** <i>Glomus coremioides</i> * <i>G. fasciculatus</i> , ** <i>G. rubiforme</i> , * <i>G. sinuosum</i> , <i>G. boreale</i>	51 to 75
<i>Acaulospora scrobiculata</i> , * <i>G. clarus</i> , ** <i>G. clavisporum</i> , ** <i>G. liquidambaris</i> , <i>G. macrocarpum</i> , <i>Funneliformis geosporum</i>	76 to 99
<i>Claroideoglossum etunicatum</i> , * <i>G. aggregatum</i>	100

Rhizoglossum aggregatum*, *R. clarus*, *R. constrictum*, *R. fasciculatus*, *R. invermaium*; *Sclerocystis clavisporum*, *S. coremioides*, *S. liquidambaris*, *S. rubiforme* and *S. taiwanense*

(The * and ** indicate the new names of the AMF genera based on recent publication of Sieverding et al. 2014)

Table 3 Species richness, diversity and evenness of AM fungi (mean \pm S.E.) associated with root region soil of coconut palm in a crop mixed agroecosystem in Malappuram district, Kerala, India

Location	Crops *	Species richness	Shannon H'	Simpsons (1-D)	Evenness J
Manjeri	C	19	2.40 \pm 0.04 ^c	0.85 \pm 0.006 ^{cd}	0.58 \pm 0.02 ^c
Kottakkal	C	21	2.70 \pm 0.01 ^a	0.90 \pm 0.004 ^a	0.71 \pm 0.01 ^b
	C+N	13	2.32 \pm 0.05 ^d	0.88 \pm 0.010 ^{ab}	0.79 \pm 0.04 ^a
	C+B	21	2.55 \pm 0.01 ^b	0.88 \pm 0.002 ^{ab}	0.61 \pm 0.01 ^c
	C+B+N	11	2.06 \pm 0.02 ^e	0.84 \pm 0.008 ^{ed}	0.71 \pm 0.02 ^b
Nilambur	C	20	2.32 \pm 0.02 ^d	0.83 \pm 0.002 ^e	0.51 \pm 0.01 ^d
	C+N	10	2.11 \pm 0.01 ^e	0.86 \pm 0.002 ^{cb}	0.82 \pm 0.01 ^a
	C+B	8	1.53 \pm 0.01 ^f	0.72 \pm 0.003 ^f	0.58 \pm 0.01 ^c
	C+B+A	8	1.40 \pm 0.10 ^g	0.65 \pm 0.033 ^g	0.51 \pm 0.05 ^d

Values in the same column followed by different letters are significantly different from each other ($p < 0.05$)

C Coconut, N Nutmeg, B Banana, A Arecanut

where banana and arecanut were the mixed crops. Johnson et al. (1991) reported that high spore count is an indication of a soil's mycorrhizal inoculum potential. The AM fungal spore counts also varied significantly in coconut monocrop and crop mixed systems in the same location, Malappuram. Spore count levels reported in this study are in agreement with the earlier report of AMF status from coconut gardens under basin management with green manure crops (Thomas 1987) but lower than the values reported by (Ambili et al. 2012) who recorded 33.83–154.5 spores per 10 g soil from coconut based cropping systems grown with pepper, banana and pineapple as intercrops. Large variations have been reported in AM fungal spore densities associated with same plant species at different sites (Zhao et al. 2003; Walker et al. 1982; Sylvia 1986) which are attributed to variations in microclimate (Koske 1987) physio-chemical, microbiological properties (Anderson et al. 1984; Johnson et al. 2013) and the vegetation (Toro et al. 1996).

The root colonization of AM fungi ranged from 32.33 \pm 8.74 to 55.17 \pm 1.61 % which was not significantly different. Root colonization level observed in this study was lower than the values (60.74 to 78.90 %) reported from coconut in Tamil Nadu (Muthukumar and Vedyappan 2010). Perhaps the phosphorous content of the soils in Tamil Nadu being lower (18.23 ppm in well-irrigated soil to 31.26 ppm in pulp and paper mill effluent irrigated soil) (Muthukumar and Vedyappan 2010) than in the soils of Malappuram (at least six soil out of nine having P contents above 35 ppm, Table 5) could have been the reason for the higher root colonization. The variation of AM fungal root colonization in our studies, though not significant, in different cropping situations in the three locations could have been due to difference in the AM species or genus diversity, habitat difference, environmental factors and soil fertility in a specific site as has been reported elsewhere (Brundrett 1991). The process of tilling soils in fields where coconut is cultivated in crop mixed fashion also causes soil disturbance which could be another reason for the variation in the root colonization pattern as reported earlier

(Jasper et al. 1991; Boddington and Dodd 2000). The anatomy of the mycorrhizal colonization type in coconut was of *Arum*-type which is in agreement with the description by Sengupta and Chaudhuri (2002) and Muthukumar and Prakash (2009). The coconut palm has a root system which is devoid of root hairs; it is possible that coconut palm would not be able to adequately take up enough nutrients, particularly phosphorous, and water without mycorrhizal assistance. This feature is known to create potential mycotrophy, enhancing nutrient acquisition in environments. Our studies indicate that there is a wide diversity of AM fungal species in the rhizosphere soil of coconut palm that forms an extensive hyphal, vesicular and arbuscular stages of AM fungal colonization within roots of coconut palm. This AMF-coconut relationship helps in forming extra-radical roots that forage into larger area of soil for improved nutrient and water absorption, the arbuscules facilitate nutrient exchange between the fungus and the plant that have a bearing on their yield capacities. Distribution of mycorrhizal roots throughout the sampling sites revealed that AM fungal association is naturally established.

4.2 Genera recorded

The present study reported high diversity of AM fungal distribution in coconut based cropping systems recording ten genera viz., *Acaulospora*, *Claroideoglossum*, *Dentiscutata*, *Diversispora*, *Funneliformis*, *Gigaspora*, *Glomus*, *Redeckera*, *Scutellospora* and *Septoglossum* with different frequencies of occurrence. *Claroideoglossum*, *Glomus* and *Gigaspora* were dominant AM fungal genera in coconut palm in a crop mixed systems of Malapuram district with 100 % frequency of occurrence. The previous studies (Thomas et al. 1993; Ambili et al. 2012) revealed that the most commonly distributed AM fungal genera in the coconut gardens were *Glomus*, *Gigaspora* and *Acaulospora*. At genus level, *Glomus* is ecologically generalist which is distributed in coconut palm in crop mixed systems. Manoharachary et al. (2005) also reported the widespread occurrence of *Glomus* species in Indian

Table 4 Distribution and spore count of AM fungal species in coconut palm in a crop mixed agroecosystems in Malappuram district, Kerala, India

AM fungal species	Manjeri C	Kottakkal (C)	Kottakkal (C B)	Kottakkal (C B N)	Kottakkal (C N)	Nilambur (C)	Nilambur (C B)	Nilambur (C B A)	Nilambur (C N)
<i>Acaulospora bireticulata</i>	ND	ND	Y(4.5 ^b)	ND	ND	ND	ND	ND	ND
<i>Acaulospora foveata</i>	Y(4.1 ^a)	ND	ND	ND	ND	ND	ND	ND	ND
<i>Acaulospora lacunosa</i>	Y(1.8 ^a)	ND	ND	ND	ND	ND	ND	ND	ND
<i>Acaulospora serobiculata</i>	Y(1.17±0.29 ^d)	Y(6.78±0.59 ^b)	Y(2.17±0.57 ^d)	Y(4.67±0.58 ^c)	ND	Y(2.08±0.07 ^d)	Y(4.67±0.57 ^c)	Y(9.83±1.527 ^a)	ND
<i>Claroideoglossum claroidesum</i>	ND	ND	ND	ND	Y(25.33±1.17 ^a)	Y(4.63±0.43 ^b)	ND	ND	Y(5.33±0.58 ^b)
<i>Claroideoglossum etunicatum</i>	Y(17.67±1.12 ^d)	Y(26.78±3.29 ^c)	Y(50.167±0.76 ^b)	Y(62.67±2.31 ^a)	Y(17.33±1.16 ^d)	Y(66.17±2.59 ^a)	Y(66.67±4.51 ^a)	Y(62.67±6.51 ^a)	Y(18.00±1.00 ^d)
<i>Claroideoglossum luteum</i>	Y(4.00±0.00 ^a)	0	ND	ND	ND	Y(1.63±0.33 ^b)	ND	ND	ND
<i>Dentiscutata nigra</i>	ND	Y(3.11±1.10 ^a)	ND	Y(3.00±0.00 ^b)	ND	ND	ND	ND	ND
<i>Diversispora gibbosa</i>	ND	ND	ND	ND	ND	ND	ND	Y(3.8 ^a)	ND
<i>Funnelliformis geosporum</i>	Y(20.33±2.75 ^b)	Y(18.44±3.29 ^{bc})	Y(14.33±1.04 ^{de})	Y(16.00±2.65 ^{de})	Y(13.33±2.89 ^{de})	Y(12.25±0.58 ^c)	ND	ND	Y(53.00±1.00 ^a)
<i>Funnelliformis mossae</i>	Y(11.50±0.86 ^a)	Y(4.78±0.385 ^c)	ND	ND	Y(8.00±4.00 ^b)	ND	ND	ND	ND
<i>Gigaspora albida</i>	Y(7.67±1.61 ^a)	Y(2.66±0.00 ^b)	Y(2.33±0.29 ^b)	ND	ND	Y(0.667±0.31 ^c)	ND	ND	ND
<i>Gigaspora candida</i>	Y(1.5)	ND	ND	ND	ND	ND	ND	ND	ND
<i>Gigaspora decipiens</i>	ND	Y(0.89±0.19 ^b)	ND	ND	Y(5.33±3.06 ^a)	ND	ND	ND	ND
<i>Gigaspora gigantea</i>	ND	ND	ND	ND	ND	ND	Y(11.00 ^a)	ND	ND
<i>Gigaspora margarita</i>	ND	Y(2.88±0.51 ^c)	ND	ND	ND	Y(6.04±0.52 ^a)	Y(2.00±0.00 ^d)	Y(4.50±0.00 ^b)	Y(5.00±1.00 ^b)
<i>*Glomus aggregatum</i>	Y(79.17±4.25 ^c)	Y(50.44±9.06 ^c)	Y(70.67±1.61 ^{cd})	Y(24.33±4.04 ^f)	Y(64.67±7.57 ^d)	Y(65.13±2.71 ^d)	Y(107.33±8.32 ^b)	Y(158.33±6.48 ^a)	Y(47.67±1.53 ^c)
<i>Glomus arborese</i>	ND	ND	ND	ND	ND	Y(4.21±0.61 ^b)	Y(22.33±2.08 ^a)	ND	ND
<i>Glomus boreale</i>	Y(5.17±2.08 ^d)	Y(5.56±0.19 ^d)	ND	ND	ND	Y(24.38±1.80 ^c)	Y(32.33±2.08 ^b)	Y(33.33±5.49 ^b)	Y(48.00±1.00 ^a)
<i>Glomus cerebriforme</i>	ND	ND	Y(2 ^a)	ND	ND	ND	ND	ND	ND
<i>*Glomus clarus</i>	0	Y(6.78±0.39 ^d)	Y(11.50±1.32 ^c)	Y(13.67±3.79 ^{bc})	Y(15.67±4.93 ^b)	Y(7.42±0.07 ^d)	ND	ND	Y(32.00±1.00 ^b)
<i>**Glomus clavisporum</i>	Y(13.33±1.16 ^{ab})	Y(6.22±1.17 ^c)	Y(11.00±1.80 ^b)	Y(5.00±0.00 ^c)	Y(15.33±3.06 ^a)	Y(4.623±0.55 ^c)	ND	10.00±4.44 ^b	ND
<i>**Glomus coremioides</i>	ND	Y(4.78±2.99 ^c)	Y(23.33±0.76 ^b)	ND	ND	Y(5.46±0.52 ^c)	ND	ND	Y(18.00±1.00 ^b)
<i>*Glomus fasciculatus</i>	Y(34.83±3.06 ^a)	Y(11.78±2.04 ^d)	Y(17.00±1.32 ^c)	Y(28.67±3.51 ^b)	Y(24.00±7.21 ^b)	ND	ND	ND	ND
<i>Glomus flavisporum</i>	ND	Y(6.67±3.53 ^a)	Y(2.83±0.28 ^b)	ND	ND	Y(0.83±0.14 ^{bc})	ND	ND	ND
<i>Glomus heterosporum</i>	ND	ND	ND	ND	ND	3.1	ND	ND	ND
<i>*Glomus invermaitum</i>	Y(3.83±58 ^a)	ND	Y(2.50±0.00 ^b)	ND	ND	ND	ND	ND	ND
<i>**Glomus liquidambaris</i>	Y(7.66±76 ^c)	Y(17.11±3.59 ^{ab})	Y(17.00±0.50 ^{ab})	ND	Y(15.33±4.62 ^b)	Y(4.13±0.90 ^d)	ND	ND	Y(19.66±0.58 ^a)
<i>Glomus macrocarpum</i>	10.33±0.76 ^c	Y(17.11±1.34 ^b)	Y(17.66±1.25 ^b)	ND	ND	Y(17.08±88 ^b)	ND	ND	Y(37.33±1.53 ^a)
<i>Glomus multicaule</i>	ND	ND	ND	ND	ND	9.31 ^a	ND	ND	ND
<i>Glomus pallidum</i>	Y(1)	ND	0	ND	ND	ND	ND	ND	ND
<i>Glomus reticulatum</i>	ND	ND	(1 ^b)	ND	ND	ND	ND	ND	ND
<i>**Glomus rubiforme</i>	Y(15.33±1.07 ^b)	Y(16.33±2.84 ^b)	Y(14.16±2.36 ^b)	Y(35.33±4.61 ^a)	Y(35.66±4.93 ^a)	ND	ND	ND	ND
<i>**Glomus sinuosum</i>	ND	Y(13.89 ^a)	Y(8.00±0.00 ^b)	Y(8.00±0.00 ^b)	ND	Y(5.54±0.32 ^b)	Y(6.00±1.00 ^b)	Y(16.00±0.00 ^b)	ND
<i>**Glomus taiwanense</i>	Y(13.00±0.50 ^b)	ND	Y(13.00±1.73 ^a)	ND	ND	ND	ND	ND	ND

Table 4 (continued)

AM fungal species	Manjeri C	Kottakkal (C)	Kottakkal (C B)	Kottakkal (C B N)	Kottakkal (C N)	Nilambur (C)	Nilambur (C B)	Nilambur (C B A)	Nilambur (CN)
<i>Glomus tenebrosium</i>	ND	ND	Y(4.66±1.16 ^a)	ND	Y(4.66±0.58 ^a)	ND	ND	ND	ND
<i>Glomus tortuosum</i>	ND	Y(3.33±0.00 ^b)	Y(4.66±0.58 ^a)	ND	ND	ND	ND	ND	ND
<i>Redeckera fulva</i>	ND	Y(4.1 ^a)	ND	ND	ND	ND	ND	ND	ND
<i>Scutellospora erythropus</i>	ND	ND	ND	Y(12.3 ^a)	ND	ND	ND	ND	ND
<i>Septoglomus constrictum</i>	ND	ND	Y(8.50±0.87 ^b)	ND	Y(25.66±5.13 ^a)	Y(2.91±0.31 ^c)	ND	ND	ND

Rhizoglyphus aggregatum*, *R. clarus*, *R. constrictum*, *R. fasciculatus*, *R. invernaium*; *Sclerocyttis clavisporum*, *S. coremioides*, *S. liquidambaris*, *S. rubiforme* and *S. taiwanense* (The * and ** indicate the new names of the AMF genera based on recent publication of Sieverding et al. 2014)

Values in the same column followed by different letters are significantly different from each other ($p < 0.05$)

C Coconut, N Nutmeg, B Banana, A Arecanut, Y Present, ND Not detected, AMF species in bold letters indicate new records in coconut rhizosphere

soil. The abundant and diverse spore population of *Glomus* spp. revealed in the study indicates a good adaptation of these fungi to a wide range of soil conditions (Grey 1991; Blaszkowski 1993; Ammani et al. 1994; Jansa et al. 2002; Kowalczyk and Blaszkowski 2011).

4.3 Species diversity

It is evident in the present study that diverse species of AM fungi were present in the rhizosphere soil of coconut palm. Forty AM fungal species were recorded from coconut crop based mixed cropping systems. Among these species of AM fungi, two are ubiquitous: *Glomus aggregatum* and *Claroideoglomus etunicatum* with 100 % occurrence and are known as ecological “generalist” which are widely distributed in coconut crop based mixed cropping systems. The reason for its occurrence in higher frequency could be attributed to the favorable soil environmental conditions in which the native AM species had established a permanent symbiosis with their host plants for regeneration. AM fungal species occurring in these sites have favorable conditions for completing their life cycle in presence of their host roots (Zhao et al. 2003). It is also reported that dominance of certain species is related to the difference in propagative units between the glomalean families. For example, it has been suggested that the Gigaspora are only capable of propagation via spore dispersal or infection from an intact mycelium. In contrast, the Glomaceae are also capable of colonizing via fragments of mycelium (Biermann and Linderman 1983).

The glomeromycotean fungal community in the root region soil of coconut palm in a crop mixed system consisted of 8 to 21 species per sample. The present results are in agreement to the report of Thomas and Ghai (1987) that more than one AM fungal species colonized the same coconut seedlings and the same root segments. There are several reports to indicate that more than one AM fungal species were associated with different crops such as clover (Abbot and Robson 1984) and green gram (Valsalakumar et al. 2007).

Over all, a total of 40 AMF species were identified from coconut palm in crop mixed system. This indicated higher level of diversity as approximately 250 AMF species have been described so far (Oehl et al. 2011). Singh et al. (2003) reported 51 AMF species from the rhizosphere of tea growing in ‘natural’ and ‘cultivated’ ecosites in Uttaranchal in Himalayan region. Chaurasia and Khare (2005), Chaurasia et al. (2005) reported 15 and 16 AMF species from the rhizosphere of *Taxus baccata* and *Rhododendrons*, respectively, in the Indian Himalayan region. A much lower AM diversity of five species has been reported in *Artemisia* (Rajeshkumar and Hosagoudar 2012) and nine in bamboo (Rajeshkumar et al. 2013). From our studies and these reports it is clearly seen that perennial plantation crop like coconut has higher AMF

Table 5 Physio-chemical characteristics of root region soil samples of coconut palm in a crop mixed agro ecosystem in Malappuram district, Kerala, India

Location	Crops *	pH	EC (μ s)	OC (%)	N (%)	P(ppm)	K (ppm)
Manjeri	C	6.20 \pm 0.09 ^g	74.50 \pm 0.15 ^d	1.45 \pm 0.06 ^d	0.19 \pm 0.008 ^d	13.36 \pm 0.81 ^e	121.51 \pm 1.92 ^g
Kottakkal	C	7.19 \pm 0.02 ^b	122.60 \pm 0.23 ^a	1.62 \pm 0.02 ^b	0.23 \pm 0.002 ^b	49.14 \pm 4.57 ^b	240.26 \pm 0.91 ^a
	C+N	5.53 \pm 0.04 ^h	48.53 \pm 0.32 ^e	1.53 \pm 0.04 ^c	0.24 \pm 0.010 ^b	17.63 \pm 1.26 ^e	134.10 \pm 2.96 ^f
	C+B	6.79 \pm 0.02 ^d	88.57 \pm 0.30 ^b	0.87 \pm 0.01 ^f	0.34 \pm 0.006 ^a	42.01 \pm 4.55 ^d	191.65 \pm 1.77 ^d
	C+B+N	7.34 \pm 0.06 ^a	47.23 \pm 0.12 ^f	1.11 \pm 0.05 ^e	0.13 \pm 0.006 ^f	40.54 \pm 1.30 ^{dc}	206.04 \pm 3.79 ^c
Nilambur	C	6.62 \pm 0.01 ^e	24.82 \pm 0.02 ^h	0.86 \pm 0.01 ^f	0.14 \pm 0.003 ^f	45.52 \pm 3.84 ^{bc}	155.45 \pm 0.70 ^e
	C+N	7.13 \pm 0.04 ^b	28.20 \pm 0.36 ^g	0.33 \pm 0.04 ^g	0.17 \pm 0.006 ^e	37.18 \pm 4.35 ^d	230.89 \pm 2.08 ^b
	C+B	6.35 \pm 0.05 ^f	81.13 \pm 0.25 ^c	1.75 \pm 0.02 ^a	0.08 \pm 0.015 ^g	13.41 \pm 1.54 ^e	242.94 \pm 1.83 ^a
	C+B+A	6.97 \pm 0.01 ^c	18.26 \pm 0.11 ⁱ	0.90 \pm 0.01 ^f	0.21 \pm 0.003 ^c	57.66 \pm 0.71 ^a	53.62 \pm 2.93 ^h

Values in the same column followed by different letters are significantly different from each other ($p < 0.05$)

diversity like that of the tea plantation when compared with other crops.

It is evident from the present study that composition of AMF community in crop mixed system is different and have exclusive species. *Diversispora gibbosa* was detected only in crop mixed system of coconut + banana + arecanut in Nilambur and *Scutellospora erythropus* was detected only in crop mixed system of coconut + banana + nutmeg in Kottakkal. Other species such as *Gigaspora gigantea* (coconut + banana in Nilambur), *Glomus reticulatum*, *Acaulospora boreticulata*, *Glomus cerebreforme* and *Glomus tenebrosum* (coconut + banana in Kottackal) were also detected only in crop mixed systems. These observations indicate that the component crops in the cropping systems influence the richness and AMF community composition of the main crop coconut. From the ecological point of view, the different components that make up a cropping system promote the development and diversity of AMF. It is known that composition and number of AMF taxa can lead to large fluctuations in the plant productivity.

Increases in the diversity of AM fungi are reported to be associated with increased phosphorus uptake and biomass

Table 6 Pearson correlation coefficients of spore count, percentage of root colonization, AMF diversity parameters with soil physio-chemical characteristics of coconut palm in a crop mixed agroecosystem in Malappuram district, Kerala, India

	pH	EC	OC	N	P	K
Spore count	-0.17	-0.417*	-0.09	-0.140	0.100	-0.33
Root colonization %	-0.25	0.046	0.04	0.370	0.081	-0.24
Species richness	-0.03	0.512**	0.09	0.509**	0.117	0.08
Simpson (1-D)	-0.06	0.410*	-0.02	0.404*	-0.15	0.445*
Shannon_H	-0.05	0.509**	0.05	0.537**	0.00	0.276
Evenness	0.024	0.092	-0.09	0.143	-0.16	0.458*

EC Electric conductivity, OC Organic carbon; statistically significant results are indicated with asterisks (* $P < 0.05$; ** $P < 0.01$)

production (van der Heijden et al. 1998). They hypothesized that greater hyphal length, associated with increased AM fungal diversity, more completely exploited the soil for P. Though we have not found any direct evidence in this study, we still surmise that the association of this large diversity of AMF in this unique coconut based cropping system could be one of the main reasons for the higher productivity of coconut in Malappuram district of Kerala, India cultivated under rain-fed conditions.

4.4 Diversity indices

High diversity of AM fungi in coconut palm in different crop mixed systems indicates that fungal species are ecologically distinct and occupy different niches. Individual fungi would therefore be competitively superior in their specific niche, and the presence of multiple niches in a habitat results in the active maintenance of a species fungal community (Bever et al. 2001). The higher value of Simpson's Index of diversity (0.90 \pm 0.004) for AM fungi in the coconut mono cropping system at Kottakkal indicates shared dominance of many AM fungal species while the lower value of Simpson's index of diversity (0.65 \pm 0.033) in coconut palm in a crop mixed cropping system at Nilambur with banana and arecanut as the inter crops, indicated dominance of few species. In other locations the Simpsons indices ranged between the ranges given above. In the previous publication where AMF diversity was studied in coconut based cropping systems the Simpsons index value ranged between 0.59 to 0.98 and the Shannon index between 1.91 and 4.56 (Ambili et al. 2012). In the present study also the Simpsons index matched well with the above mentioned work for coconut cultivated in crop mixed system (0.65 to 0.86), however, the Shannon index was on the lower range. Species dominance was shared by maximum of 21 AM fungal species out of total 40 AM fungal species in coconut mono cropping system of Kottakkal. The significantly high value of general diversity indices for mono cropping

system of coconut at Kottakkal compared to other coconut and coconut palm in a crop mixed agro ecosystem could be explained by the larger number of species as the Shannon index is dependent on species richness (Magurran 2004). The low evenness values in coconut palm in a crop mixed agro ecosystem indicate that not all species were equally abundant within the assessed AM fungal populations. Abbott and Gazey (1994) have suggested that the intensity of soil use modifies AMF species composition with diversity. The difference in diversity indices indicate that different agriculture practices may be responsible for distribution of AM fungi. Common agricultural practices such as tillage, fallow and fertilization have been shown to alter the community structure of AM fungi (Jansa et al. 2002; Troech and Loynachan 2003; Jumpponen et al. 2005). Alteration in AM fungal community composition could be due to a number of factors including disturbance of AM fungal hyphal networks, changes in the nutrient content, altered microbial activity or changes in weed populations (Jansa et al. 2002).

4.5 Soil chemical parameters

Soil chemical characteristics differed significantly in different coconut based crop mixed agroecosystems. It is pertinent to note that highest levels of AMF diversity as indicated by Shannon H index and species richness were observed in the coconut monocrop and coconut-banana systems at Kottakkal where the soil had high levels of EC, OC and P content. Among the locations at Kottakkal, the coconut + nutmeg system which had low soil physio chemical characteristics, also showed low levels of AMF diversity and species richness. Similar situation was also observed in coconut + banana + arecanut system at Nilambur. In general, soil pH showed negative correlation with spore count, root colonization, species richness, Shannon H and Simpson index but positive correlation with species evenness. This result agrees with that of Ambili et al (2012), particularly for soil pH and spore load, as well as with Hepper's study (1984) who indicated that the differences between soils in the germination of spores of AMF appeared to be negatively correlated with the differences in soil pH. Soil nitrogen was positively correlated with root colonization, species richness, and indices of Simpson and Shannon and evenness values and negatively correlated with spore count. The direct effect of soil N on AM fungi is not clear, but higher plant biomass may be required for the proliferation of AM fungi in the environment (Goomaral et al. 2013). Though it is well known that available phosphorus has negative correlation with AMF spore count and root colonization we observed a positive correlation with all the parameters including spore count and root colonization excepting Simpson index and evenness. In another publication, where quantitative analysis of AMF associated with *Ammophila arenaria* was carried out in six locations in Western European coast, it was

observed that high phosphorus content in soil did not have an inhibitory effect on AMF spore count and root colonization from one of the location in Belgium (Rodriguez-Echeverria et al. 2008). Available potassium was negatively correlated with spore count and root colonization but positively correlated with all other parameters. The influence of K on spore density has been reported to be positive or negative (Udayan 1996). Soil K is often reported to have a stimulatory effect on AMF variables (Furlan, and Bernier-Cardou 1989; Ouimet et al. 1996) and a minimum soil K is often prerequisite for mycorrhizal colonization in some plant species (Ouimet et al. 1996; Gamage et al. 2004). The relationships between AMF spore count and soil chemical properties are not steady but it differs according to Glomeromycota community composition (Johnson et al. 2013).

Electrical conductivity provides a measure of concentration of soluble salts in soil solution. Spore count was influenced by electrical conductivity and had significant negative correlation between them. Conflicting reports are available on the nature of relationship between electrical conductivity and spore density. Baby and Manibhushanrao (1996) reported the absence of correlation between the two, whereas Janardhanan et al. (1994) found significant positive correlation between electric conductivity and spore numbers. Electrical conductivity shows a positive correlation with root colonization which indicates active AMF mediated uptake of nutrients.

The cropping systems included in the present study are low input systems with decreased use of fertilizers, pesticides and tillage and had organic manures as the major source of nutrients. In these cropping systems, all components have positive influence on AMF symbiosis. Such situations provide a conducive environment for the development and functioning of arbuscular mycorrhizal fungi. Maintaining the population of AM fungi as the essential link between the soil and plant is an essential condition for sustainable cropping systems.

5 Conclusion

The studies on the diversity of AM fungal species in coconut palm in monocropped and crop mixed system growing in highly productive Malappuram district of Kerala under rainfed condition revealed that AM fungi were widely distributed in this ecosystem. This study established the existence of a rich diversity of AMF in the root region soils of coconut and coconut palm in a crop mixed system with the identification of 40 AM fungal species and 18 of them being reported for the first time from the root region soil of coconut. *Claroideoglossum*, *Glomus* and *Gigaspora* were the dominant AM fungal genera in the coconut rhizosphere soil. The diversity indices revealed significant variation in species diversity and richness in coconut palm in the monocropped and different crop mixed systems. The AMF spore load and root

colonization were observed not to be inhibited by the available phosphorus content in the soils. We, therefore, surmise that the association of this large diversity of AMF in this unique coconut based cropping system could be one of the main reasons for the higher productivity of coconut in Malappuran district of Kerala, India cultivated under rain-fed conditions.

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