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Impact of land use and soil types on arbuscular mycorrhizal fungal diversity in tropical soil of India

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A study was undertaken along land use gradients with different soil types in subtropical ecosystem of Northern India to evaluate the accurance of arbuscular mycorrhizal fungi (AMF). The gradients was from natural land with forest tree, farmer's field under chemically managed, farmer's field under organically managed and Industrial wasteland with five different plant species. We investigate the total AMF species in land use system of different soil types and also in trap culture set from same land use system.Distribution of mycorrhizal species were calculated directly by quantification AMF spores and indirectly by sequencing the SSU-ITS and LSU regions of rDNA. A total 19 AMF morphotypes from direct field sampling and additional 35 morphotypes from trap culture were recovered, which represented seven genera and eighteen species. Result suggested that few *Rhizophagus* and *Funneliformis* species came from organically managed and natural land; most of the species came from sites representing chemically managed and industrial wasteland sites from which *Gigaspora* and *Scutellospora* species were absent. Organically managed land contributed the largest number of AMF species and diversity, even more than those found in natural sites, which suggests that factors contributing to the diversity of AMF are indeed complex: For example, chemically managed sites not only causes loss of fungal biodiversity but also selectively favors smaller spores of genera *Rhizophagus* and *Funneliformis*.

Key words: Tillage, diversity, ribosomal dna, raised bed plantation, arbuscular mycorrhizal (AM), morphotypes.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) form mutually beneficial associations with a large number of terrestrial plant species (Van der Heijden et al., 1998). These fungi promote phosphorous uptake and help plants in coping with different forms of stress. Communities of AMF are affected by many factors including plant genotype, agricultural practices, and pollution (Sander et al., 1995a). Farming practices such as intensive cultivation,

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Table 1. Major characteristics of different land use systems selected for AMF diversity study

S/N	Land use system	Major characterization
1	Chemically managed arable land (CML)	The site comprised three land use system (conventional tillage or ConT; zero tillage, or ZTL; and permanent raised beds, or RBP) supporting a rice–wheat production system under conventional management (120–150 kg/ha N, 40–60 kg/ha P2O5, 40–60 kg/ha K2O, and 25 kg/ha Zn).
2	Arable, zero tillage (ZTL)	Site comprised ZTL plot had been no tillage for the last two rotations
3	Arable, conventional tillage (ConT)	Site comprised ConT plot had been tilled using a tractor for the last 8-10 years
4	Arable, raised bed (RBP)	Site comprised RBP plot of wheat and rice had been grown on permanent raised beds with zero tillage for the last two years
5	Natural grassland land (NAL)	Site comprised 6 plots of wheat (PA1 to PA6) under chemically managed land (CML) and 14 plots (PA7-PA20) representing a natural land dominated by *Cyathea spp*, *Albizia procera*, *Shorea robusta* and *Phyllanthus emblica* plant
6	Organically managed land (ORG)	This site in eastern zone of Haryana, dominated by *Oryza* and *Triticum spp.* grown under organically managed soil. Sites comprised three plots, each with a different dose and mix of organic manure: PALF1 (poultry manure alone), PALF2 (poultry manure and 20 tonnes/ha of farmyard manure) and PALF3 (poultry manure and 40 tonnes/ha of FYM).

tillage and using sewage sludge as soil amendment may affect communities of AMF both qualitatively and quantitatively (Sieverding, 1990). More report by Jansa et al. (2003) observed that *Rhizophagus* species were dominant in highly tilled field, whereas *Scutellospora* species were prevalent in low tillage fields. Furthermore Treseder et al. (2004) observed that conventional agricultural practices such as application of chemical fertilizers and tillage intensity tend to decrease AMF spore abundance and alter community composition. Moreover Gaur and Adholeya (2002) suggested that application of low input fertilizers (Organic) promotes the growth of indigenous AMF in nutrient-limited soils. Only a few studies have explored the extent to which soils under conventional and organic farming systems in the temperate zone differ in terms of the composition and species richness of AMF (Oehl et al., 2003; Hijri et al., 2006). There are 102 AM fungi reported in tropical diverse habitats from India (Manoharachary et al., 2005).The occurrence of AM fungi in a forest and coastal regions of Andhra Pradesh reported by Manoharachary et al. (1991), distribution and identification of AM fungi in the rhizosphere soils of the tropical plains collected from Tamil Nadu, India by Ragupathy and Mahadevan (1993) and natural forest regions in the Old Delhi Ridge, Saraswati Range of Haryana by Thapar and Uniyal (1996). However, most of study surveyed AMF diversity in the subtropical region of Northern India based on morphological charcters of spores collected from single land use system (Karthikeyan and Selvaraj, 2009; Kumar and Grampalli, 2010). The comprehensive survey of land use intensity and different soil types strongly affect the AMF community composition in temperate soil earlier reported by Oehl et al., 2010; Stover et al., 2012; González et al., 2012; Bainard et al., 2015). Subseqent study by Dobo et al. (2016) were recorded 29 AMF morphospecies, belonging to nine genera originated from the rhizospheric soil of the three land uses system. However rich diversity of AMF found over a broad range of different land use (Cropping vs noncropping) in tropical soil, to our knowledge, has not been investigated so far using multiple methods.

Therefore aim of the present study was to investigate AMF diversity in different land use and soil types in tropical soil. It was hypothesized that AMF are more abundant and diverse in organically managed as well as natural soils than those in chemically managed soils and in soils affected by industrial pollution. Hypothesis was tested directly by quantifying the number of spores and species richness and indirectly by sequencing the SSU-ITS and LSU regions of rDNA. To determine AMF diversity, the following seven land-use systems from four agroclimatic zone with different soil types were selected: Agriculture land high-intensity farming (Chemically managed), Agriculture land under conventional tillage (ConT), Agriculture land under Zero tillage (ZTL), organic (low-input) farming, agricultural field highly contaminated tannery effluent, and natural land (NAL)..

MATERIALS AND METHODS

Sampling site

The investigation was carried out in seven different land use pattern (Table 1). The annual rainfall range 800 to 1200 mm and has ample irrigation resources. The first agroclimatic zone near Ghaziabad (28° 40' N, 77° 28' E) which is part of the western plains of Uttar Pradesh, India constitutes the sub-humid zone. The second agroclimatic zone near Palwal (28° 9' 0" N / 77° 20' 0" E) , in eastern zone of Haryana, India. The third sampling agroclimatic zone near Pachmari (22° 28' 0" N / 78° 26' 0" E) was part of the Malawa plateau zone of Madhya Pradesh which has medium black sandy soil. The fourth agroclimatic zone sampling was Kanpur

(80°21′ N / 20°38′ E) part of the central plains of Uttar Pradesh, India constituted sub-humid zone.

Sampling of AM fungi

All soils were sampled in 2008 in october from seven land use system of four agroclimatic zone. Each sample collection point was divided into four blocks. undisturbed core samples 20 soil cores/plot were collected (soil and roots) from the rhizosphere of wheat plants from a depth of 0 to 30 cm using core sampler. Thus, a total of 80 soil cores were collected from each collection sites. The samples were air dried in the shade to a point where there is no free moisture and were placed into zipped bags, and stored at 4°C in a cold room until processed. The samples were used for three different purposes: (i) Propagation of AM fungal isolate of each collection point for their identification; (ii) Analysis of AM fungal parameters; and (iii) Analysis of soil chemical parameters.

Physical and chemical analysis of soil

A soil suspension of 1:2.5 (soil-to-water mixture) was made. The pH of the soil suspension was measured by digital pH meter (Expandable Ion Analyser EA 940, Orion Research) and the electrical conductivity was measured by digital electrical conductivity meter (Controlled dynamics). A protocol by Datta et al. (1962) was followed for measuring % organic carbon. % Total nitrogen was calculated using Kjeldahl's method by Bremner (1960). Available phosphorus was determined using Olsen's method (Olsen et al., 1954) and the estimation of available potassium was done using flame photometer with filters (Wood and Deturk, 1940).

Trap cultures

Mycorrhizal fungi obtained from different land use system tropical soil are very difficult to identify even impossible to identify up to species level. This is due to tropical environments (high temperature, moisture) and those with high organic matter as well as high proportion of spores undergo so much structural changes or degradation. Therefore, pot culture established in year 2008 using soil samples to recover spores from AM fungal species present in field soil, including some of which may not have sporulated at time of sampling. The trap cultures were established using methodology described by Oehl et al. (2003). Plastic trays (460×290×240 mm^3) were used to establish AM fungal cultures in greenhouse using soil samples from all collection sites. For each collection site, four trays (comprising four blocks) were prepared. The plastic trays were provided with 50 mm hole at the bottom. A 20-mm thick drainage mat (Enkadrain ST, Schoellkopf AG, CH-8057 Zurich, Switzerland) was placed at the bottom of the tray and the tray was filled with 25 Kg of substrate (50% Terragreen: American aluminium oxide, oil dry US special, Type IIIR and 50% soil sediment: Nutrient deficient (Olsen P = 1.56 ppm; Organic C = 0.28%; Total N = 0.052 %; K = 52.66 ppm). The substrate was autoclaved at 120°C for one hour at 15 psi before filling. Substrate cores (50 g) were taken out from five different places in the tray and were replaced by five, undisturbed soil cores (50 g, containing collection site's AM fungi) to inoculate the hosts. Seeds of *Allium cepa*, *Tagetus* sp., *Daucus carotus*, *Medicago sativa* (alfalfa), and *Trifolium alexandrianum* (barseem) were pregerminated. Five pre-germinated seeds of each species were placed at the top of all five soil cores. The plants were watered to a moisture level of approximately 60% of the water holding capacity and were grown in greenhouse at 20 ± 5°C with 60% relative humidity. The pots were arranged on a greenhouse bench in a completely randomized design with 4 replications. Half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1938) was provided to the plants at fortnightly interval. After four months of growth cycle, the pots were left to drying undisturbed with a fairly stable temperature so that the drying period is not too rapid. After completion of the growth cycle the dried shoots were cut at the ground level without disturbing the substrate and seeds of different hosts *Gossypium*, *Vetiver*, *Vigna radiate*, *Sorghum* and *Tagetus* sp. were sown again. Three trap culture cycles were propagated. After each cycle, rhizosphere soil cores were taken from the vicinity of trap plants at a depth of 0 to 15 cm and species characterization was done.

AMF spore identification

Spores were collected separately and similar looking spores were grouped into different spores morphotypes according to colour, size and mycelial attachment with spores. Permanent slides was prepared in polyvinyl alcohol and polyvinyl alcohol plus Melzer's solution (1:1) as described by Walker and Trappe (1993). Spores from each morphotypes were observed under a Zeiss compound microscope equipped with a digital imaging system, and digital photographs was taken with a Zeiss Axiacam RTC (Germany). Spore diameter, wall thickness and hyphal thickness were measured using software Axio Vision (Version 4.7) attached to the microscope. The diagnostic slides of different species of AM fungal spores were prepared and features of the spores morphology were compared according to current taxonomic criteria (Schenck and Perez, 1990) and also using the internet information from the INVAM website (http://www.invam.caf.wvu.edu).

Data analysis

Spores were washed with distilled water and evenly over the entire grid. They were counted under a stereoscopic microscope (40×). The number of spores was expressed as the mean of four replicates. Diversity of AM fungi in four study sites was evaluated by observing the spores in four replicates each of 100 g of soil. Once the data were obtained, the following were calculated for AM fungal diversity analysis (1) Spore density (Total number of spores was expressed in mean of four replicate in 100 g of soil sample) (2) Relative Spores abundance = Number of given species spore/Total number of spores × 100 % (3) AM fungal species richness was measure known as $d = s/\sqrt{N}$ where s equals the number of different AMF species in site, and N equals the total number of individual organisms in site (4) Shannon–Weiner diversity index index (H′) of AMF spore morphotypes was calculated for each sites with equation $H' = -\sum (Pi) \ln (Pi)$. $Pi = ni/N$ (ni is the number of individuals of species i, and N is the total number individuals in all species). Species diversity (Shannon -weiner index) in each experimental trap culture were used in one-way ANOVA with soil treatment as a factor with seven levels an four replicates of the trap cultures for each treatment. All analyses were performed in JMP version 5.1.1 (SAS Institute Inc., Cary, North Carolina, 1989-2002), and differences between the means were analyzed using Tukey HSD multiple comparisons with $P < 0.05$. Similarity index (Legendre and Legendre, 1998) was calculated to compare the similarity of species among different sites as based on χ^2 distance using ward's minimum variance methods. The correspondence units (dimensionless) on the basis of spore number per 100 g of soil.

Molecular analysis

A single spore from each morphotype was transferred aseptically to eppendorf tube containing 10 µl of 10X PCR buffer (Invitrogen, USA) and used for DNA extraction. The spore was crushed, 10 µl of 20% Chelex resin added immediately, and the tube centrifuged

Table 2. Soil physico-chemical properties of different land use types chosen for diversity study.

Land use type	Soil texture	pH	Organic C (%)	P (ppm))	N (%)	K (ppm)
NAL	Medium black soil	7.18	1.142	4.95	0.02	60.8
CML,ConT,ZTL,RBP	Entosols of Alluvium Soil	7.54	1.02	7.52	0.02	52.8
ORG	Medium texture alluvial soils	7.00	0.62	5.12	0.03	71.9
IWL	Alluvial sandy loam soils	7.71	3.023	37.5	0.11	627

ConT, Conventional tillage; *RBP,* raised bed plantation; *CML,* chemically managed land; *ZTL,* zero tillage land; *ORG,* organically managed; *NAL,* Natural land; *IWL,* Industrial wasteland.

briefly for 2 s. The crude extracts were incubated at 95°C for 15 min, centrifuged at 8000 g for 3 min, and 5 µl of the supernatant was used as a template for PCR. The end region of an SSU with complete internal transcribed spacer (ITS) was amplified using parameters for the PCR described by Redecker (2000) with 56°C as the annealing temperature. The second step was carried out under identical conditions except that the annealing temperature was 60°C.

A fragment of a large subunit region of n-rDNA was amplified with a fungal-specific primer 28G1(F) and a *Glomeromycota* specific primer 28G2(R) as stipulated by Da Silva et al. (2006). The first PCR product of *Glomeraceae* morphotypes was used as a template for the nested PCR using the *Rhizophagus* and *Funneliformis* specific primer LSURK7r (Van Tuinen et al., 1998a) and 28G1 as the reverse primer whereas the Genera include *Gigaspora*, *Scutellospora* and *Acaulospora* morphotypes were amplified by using the primer pair LR1+ FLR2 (Van Tuinen et al., 1998b). The first PCR product of these spores morphotypes was diluted 10-fold and used as a template for the second PCR amplification using the primer pair FLR3(F) + FLR4(R) under the conditions specified by Gollotte et al. (2004). Nested PCR products were purified using Qiagen PCR purification system (Qiagen, USA). The purified PCR product of the SSU-ITS and LSU rDNA fragment was cloned using the pCR4-TOPO vector supplied with TOPO TA cloning kit for sequencing (Invitrogen, USA). 2 to 3 transformed colonies were picked and plasmid was extracted using Wizard Plus SV Mini Kit (Promega, USA). SSU-ITS and LSU rDNA insert was used for cycle sequencing reaction with PCR primers. The sequencing was performed on an automated multicapillary DNA sequencer, namely ABI Prism 3130×l Genetic analyzer (Applied Bio systems, Foster City, California, USA) using the Big Dye Terminator ver. 3.1 Ready Reaction Cycle Sequencing Kit (Applied Bio systems) at the sequencing laboratories of TERI, New Delhi. The sequences have been deposited with GenBank (NCBI, www.ncbi.nlm.nih.gov) under accession numbers shown in Figures 4 to 6. Sequence similarities were determined using BlAST similarity search algorithm (Atschul et al., 1990) as available on the NCBI home page. Sequences obtained from this study along with the reference SSU-ITS and LSU rDNA sequences retrieved from the NCBI database were aligned pair by pair using ClustalW (Higgins et al., 1994). The phylogenetic tree was constructed using Mega version 4.1 (Tamura et al., 2007) and evolutionary history was inferred using the neighbor-joining algorithm (Saitou and Nei, 1987).

RESULTS

The soil types varies range black medium texture alluvial soil to gangatic alluvial soils and pH was found to be slightly basic (7.18-7.71) in all the sites. The soil organic carbon was highest in industrial waste land (IWL) and lowest in the chemically managed land (CML). The available phosphorus ranges between 4.95 and 37.75 ppm and it was highest in the industrial wasteland sites than the other sites (Table. 2).

Based on the classification of *Glomeromycota* reported by Schüβler and Walker (2010), the ten species of AMF were recorded in the field soil and eight additional species of AMF observed in the trap soil. At the genus level, *Rhizophagus* was dominant AMF species. The most common species sporulating in trap cultures set up from soils from all the sites were *Rhizophagus irregularis* and *Rhizophagus intraradices* (Table 4). Myocrrhizal spores denesity differed significantly among trap cultures set up from both managed and natural ecosystem (Figure .2). Highest number of mycorrhizal spores was present in zero tillage land (ZTL) and lowest in natural land (NAL) showed in Figure 2. However species richness was decrease in order of ORG>NAL>ZTL>RBP >CML>ConT and the AMF species diversity as expressed by the H′ decrease in the order of OGR>RBP>NAL>ZTL> IWL>CML>ConT (Table 3). The H′ value was greater in trap culture originated from ORG field and lowest in trap culture originated from Conventional tillage soil (ConT) (Table 3). Moreover relative spores abundance of *Gigaspora* and *Scutellospora* species were found more among trap cultures set up from both organically managed and natural ecosystems and some were restricted in their accurrence (Table 4). Simlarity index based on cluster analysis showed that the highest similarity of AMF species composition between the sampling sites of ORG, RBP, NAL and ZTL on the other hand between sampling sites of IWL and CML (Figure 3).

Diversity in the Industrial wasteland soil

Spores of AMF collected from trap cultures originated from gangatic alluvial soils contaminated with tannery sludge were classified into 08 spores morphotypes all of these belonged to the genus *Rhizophagus*. Spores of these genera appeared yellow to reddish brown in reflected light and were globose to subglobose, 60 to 130 µM in diameter, although more than 75% of them were intermediate in size (80 to 100 µM).

Table 3. Species richness and Shannon-Weiner diversity index in different land use type.

Land use type	No. of species	Species richness (d)	[a]Diversity index (H)
Chemically managed arable land (CML)	3	0.38	0.63[c]
Arable, zero tillage (ZTL)	5	0.59	1.12[b]
Arable, conventional tillage (ConT)	4	0.32	0.51[c]
Arable, raised bed (RBP)	5	0.59	1.27 [b]
Natural grassland land (NAL)	7	0.84	1.06[a]
Organically managed land (ORG)	8	0.85	1.76[b]
Industrial waste land (IWL)	4	0.38	0.91[c]

[a]Non significiant difference between sites are shown by identical letter and determined using one way ANOVA of Tukey HSD of multiple comparisons with P <0.05.

Table 4. Relative abundance (%) of AM fungal species isolated from different land use systems.

Species	ConT	RBP	CML	ZTL	NAL	ORG	IWL
Rhizophagus intraradices	86.95	11.6	48.78	40.74	12.34	19.08	25.21
Rhizophagus irregularis	13.04	17.4	25.2	9.25	12.34	18.06	16.8
Rhizophagus proliferus	-	-	-	-	-	-	12.4
Septoglomus deserticola	-	-	-	-	-	-	45.57
Funneliformis mosseae	-	-	-	8.33	-	-	-
Funneliformis coronatum	-	-	-	41.66	-	29.08	-
Gigaspora margarita	-	45	-	-	75.3	19.09	-
Scutellospora gregaria	-	-	-	-	-	14.09	-
Scutellospora calospora	-	-	-	-	-	-	-
Acaulospora cavernata	-	-	-	-	-	-	-
Entrophospora infrequens	-	26	-	-	-	-	-
Scelerocystis spp.	-	–	26.01	-	-	-	-

ConT, Conventional tillage; *RBP,* raised bed plantation; *CML,* chemically managed land; *ZTL,* zero tillage land; *ORG,* organically managed; *NAL,* Natural land; *IWL,* Industrial wasteland.

Diversity in natural and organically managed land

Spores of AMF collected from trap cultures originated from medium black soil and medium texture alluvial soil under organically managed and natural land respectively were classified into 08 spore morphotypes, 5 of which represented the *Rhizophagus* species and 3 represented *Gigaspora, Scutellospora*, and *Aculospora* species (Figure 1). Spores abunance of *Scutellospora* species were more in trap culture originated from natural sites as compared with under organically managed sites whereas *Gigaspora* species were more predominant in trap culture established from organically managed land (Table 4).

Diversity in chemically managed land

AM spores morphotypes collected from field and trap cultures consisted of entosols of alluvium soil under intensive cultivation, which had received chemical fertilizers; fell into six spore morphotypes, representing four taxa of the Glomeraceae on each from Gigasporaceae and Acaulosporaceae and one from the Sclerocystis. Glomeraceae spores significantly more dominant in trap culture set up from ConT field than in trap culture originated from RBP and ZTL field (Table 4).

Molecular analysis

All 35 morphotypes of AMF collected from the trap cultures were used for molecular analysis; of these, 11 Glomeraceae morphotypes were used for SSU-ITSrDNA analysis and 17 Glomereaceae, 6 Gigasporaceae and 1 Acaulosporaceae morphotypes for LSU rDNA analysis. The sequencing reaction was performed on PCR/nestedPCR/plasmid of 35 morphotypes of AMF; 39 sequences of n-rDNA consisting of the SSU-ITS and LSU regions were isolated from the sequence analysis. Out of the 39 sequences, 36 appeared homologous with known Glomeromycota whereas the remaining three did not show any degree of homology with Glomeromycota. Neighbor joining (NJ) analysis using 11 SSU-ITS rDNA sequences obtained from 11 AMF morphotypes including

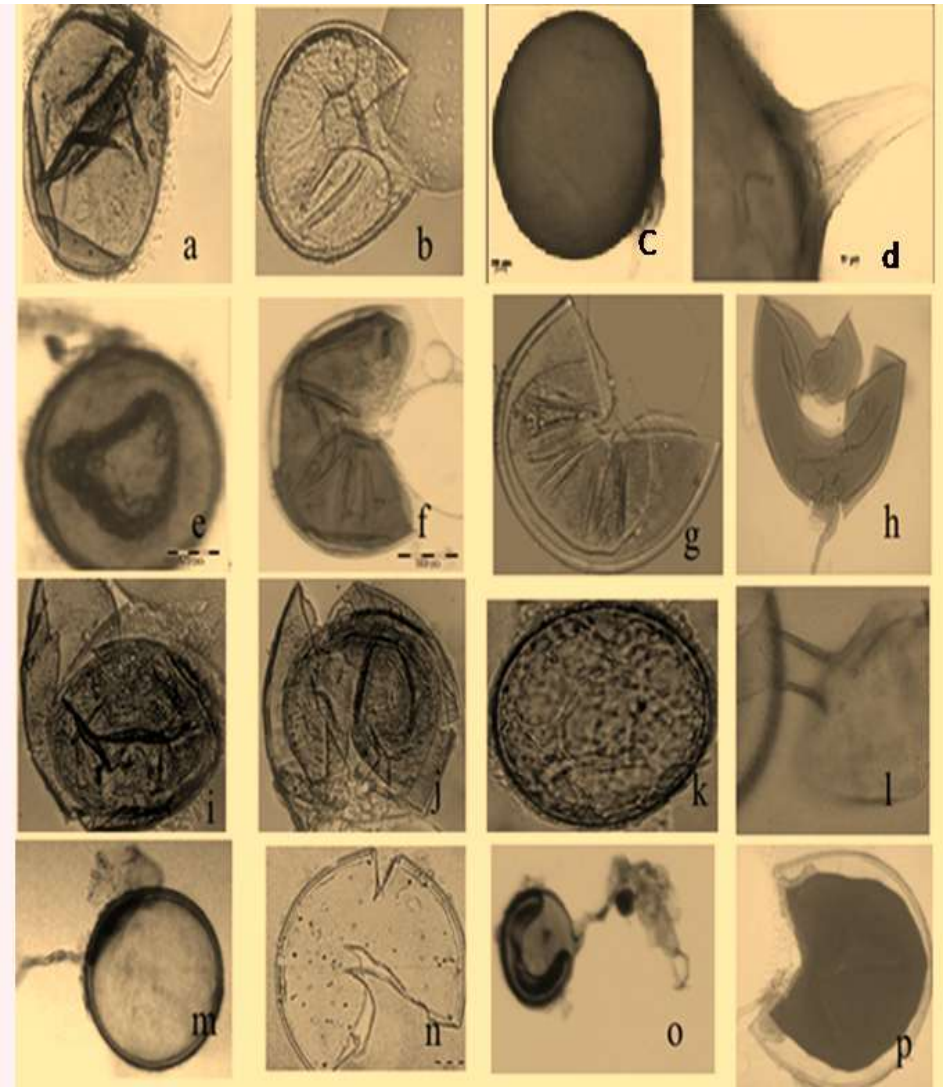

Figure 1. Spores of arbuscular mycorrhizal fungi isolated from trap culture originated from organically managed soil. (a) *Rhizophagus* sp. morphotype PALF1 in PVLG + Melzer reagent, (b) *Rhizophagus* sp. morphotype PALF2, (c-e) *Funneliformis coranatum* morphotypes PALF2AM1 in PVLG + Melzer reagent, (f) *Gigaspora margarita* of morphotypes PA16AM2 in PVLG + Melzer reagent, (g-h) *Gigaspora margarita* of morphotypes PA13AM1 in PVLG + Melzer reagent, (i-l) *Acaulospora species* M38AM1 in PVLG + Melzer reagent, (m-p) *Scutellospra* sp. of morphotypes PA13 in PVLG + Melzer reagent: Bars (a-p) 50 µM.

those retrieved from GenBank grouped all of them into a single major cluster of Glomeraceae (Figure 4). Sequences obtained from 09 AMF morphotypes formed a subcluster (*Rhizophagus*) along with reference *R. irregularis* n-rDNA sequences obtained from GenBank. On the other hand, sequences obtained from 2 morphotypes fell in a clade of known *Funneliformis mosseae*/*Funneliformis coronatum* with 94% bootstrap support (Figure 4). Out of the 24 morphotypes subjected to LSU n-rDNA analysis, 13 Glomeraceae isolates were identified by the nested *Rhizophagus* primer pair 28G1(F) + LSURK7r (R) and 11 by the *Glomeromycota* primer pair FLR3 + FLR4 (R). Two phylogenetic trees were generated using the sequences obtained from two different sets of primer pairs (Figures 5 and 6). The NJ tree obtained from the 13 sequences obtained from *Rhizophagus* and *Funneliformis* morphotpes with known Glomeraceae sequences retrieved from GenBank showed a single major cluster of Glomeraceae (Figure 5). Out of the 13 sequences, 05 were grouped with a known *R. intraradices* and 06 with *R. irregularis* clade and 02 with the reference *Septoglomus deserticola* sequence with 100% bootstrap support (Figure 5). The NJ tree obtained from the 12 sequences generated by the

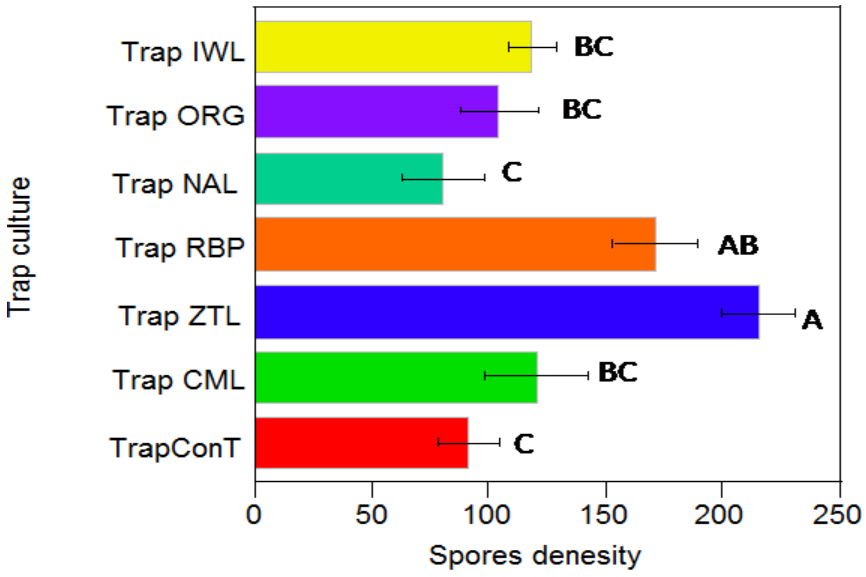

Figure 2. Spores density of mycorrhizal fungi in trap culture originated from different land use system.

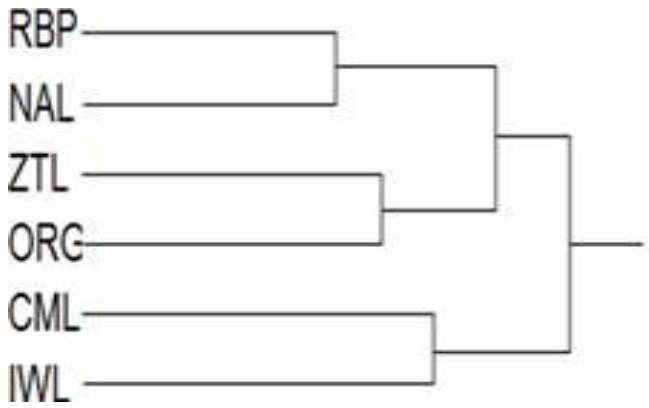

Figure 3. Simlarity index based on hierarchical cluster analysis of arbuscular mycorrhizal fungi in different land use system.

Glomeromycota primer along with reference sequences retrieved from GenBank showed three major clusters, one each of Glomeraceae, Gigasporaceae, and Acaulosporaceae (Figure 6). Out of these 12 sequences, 2 were grouped with *Rhizophagus proliferus*, 1 with *Funneliformis mosseae*, and 2 with known *Funneliformis coronatum* retrieved from GenBank. Sequences obtained from 7 *Gigasporaceae* morphotypes clustered with known *Gigasporaceae* retrieved from GenBank (Figure 6).

DISCUSSION

Previous report on distribution of AM fungi across a gradient of land use system in India mainly based on the morphological charcters of spores collected from field soil (Lakshmipathy et al., 2012; Bordoloi et al., 2015). The study present intervention using AMF morphotype collected from rhizospheric soil of field as well as trap culture to detect wide AMF diversity. The report by Bordoloi et al. (2015) suggested affect of mycorrhizal fungi in different landuse systems (Seven land use ecosystems of Arunachal Pradesh in Eastern Himalayan, India). However current investigation explore more under different land use systems in the tropical soil differ in terms of the composition and species richness of AMF. Moreover earlier surveys of populations of AMF, using either molecular or morphological approach, have focused on mycorrhizal roots (Helgason et al., 1999; Daniell et al., 2001) collected from field sites, and most

Figure 4. Neighbor joining tree obtained from alignment of partial region of the 26 SSU–ITS1 region of rDNA of *Glomeraceae* isolates with (*Acaulospora* sp.) as an out-group. Percentage bootstrap support (out of 1000 trials) is indicated. Names followed by accession no. represent sequences retrieved from GenBank. Names preceded by a different shape represent the sequence obtained in this work. There were a total of 367 positions in the final dataset.

studies of the diversity of AMF have used only rDNA as a marker. However present study using single spore DNA extracts, followed by nested PCR approach based on sequencing of LSU and SSU-ITS region of rDNA was further complemented method towards comprehensive detection and characterization of AM fungi in environmental soil (Kumar et al., 2013).

In the study, 18 mycorrhizal species were identified from field as well as trap culture in different land use system. Diversity of AM fungal species in present study is lower (35 species) than reported by Muthukumar and Udaiyan (2000) from seven different ecosystem of Western Ghat, India. Present study showed many additional species of AMF, for example group of sporocarpic fungi recorded in sorghum trap culture. However sporocarpic fungi were not detected in field soil because its induced sporulation during intensive cultivation in trap cultures. Similarly by Oehl et al. (2003), group of sporocarpic fungi were recorded in trap culture and suggested that the species thus undetected in field samples had initiated sporulation in trap cultures. Hence present study identified different species of mycorrhiza not only using direct field sampling but also through trap culturing so that cover all missing taxa of AM fungi. In addition, our study recorded S*. deserticola*, *R. proliferus*, *R. irregularis* and *R. intraradices* of Glomeraceae from industrial wasteland site. Four unidentified species of *Rhizophagus* were recorded from the field soil polluted with tannery sludge by Khade and Adholeya (2009). Our study suggested low specis diversity mainly *Rhizophagus* species in industrial wasteland soils due to higher pH level (7.71) and high precipitation. Moreover availability of more amount of organic carbon and phosphorus in the contaminated soils may be another factor which might have affected the growth of mycorrhizal mycelium and hence reduced the species diversity. However in contrast

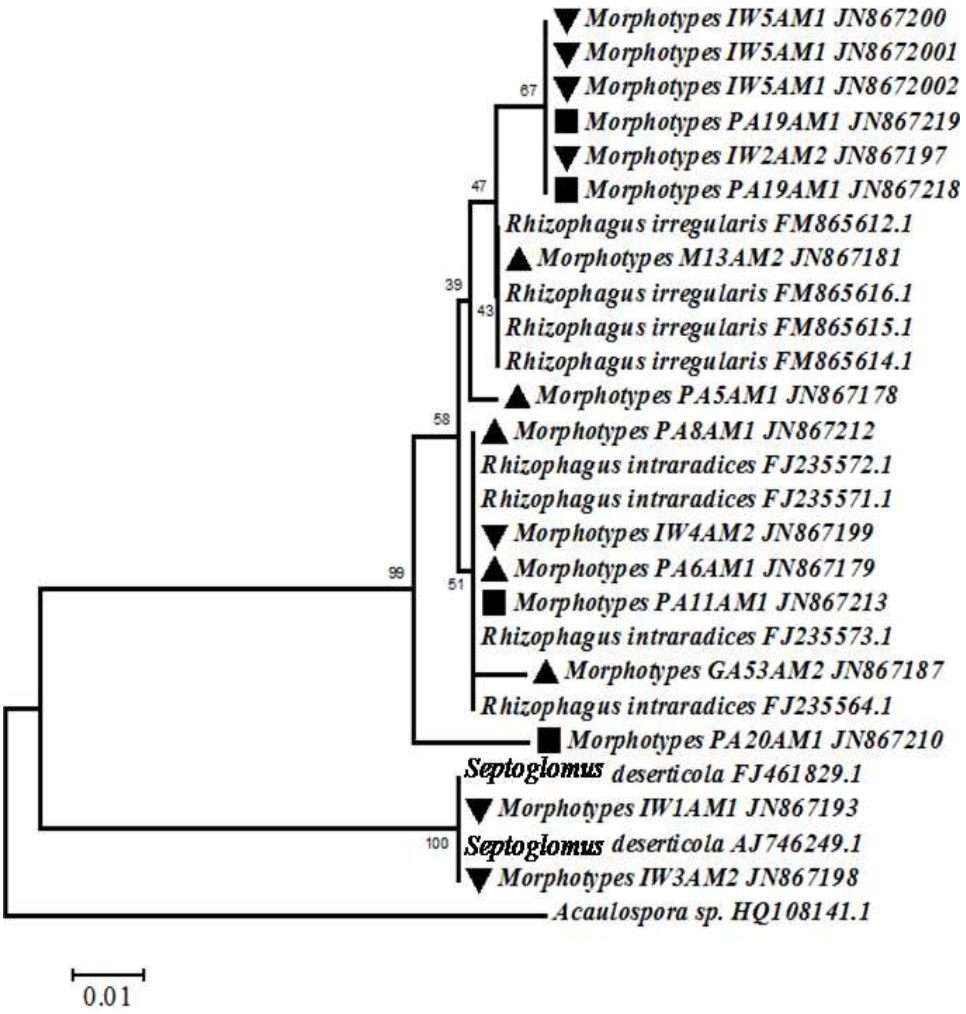

Figure 5. Sequences generated from amplicon of primer set 28G1+ LSURK7r used for phylogenetic analysis. Neighbor joining tree obtained from alignment of 13 LSU rDNA (400 bp from 5' end) used in this study including 21 LSU rDNA sequences retrieved from GenBank with (*Acaulospora*) as an out-group. Percentage bootstrap support (out of 1000 trials) is indicated. Names followed by accession no. represent sequences retrieved from GenBank. Names preceded by a shape represent the sequence obtained in this work.

study by Raman and Sambandan (1998), *Gigaspora* and *Scutellospora* were also observed in soil contaminated from Tannery effluents. In present investigation *S. deserticola* was recorded from this site may also be metal tolerant tolerant since it survived under given condition naturally. Recent studies by Arias et al. (2010) showed effect of metal observation when *Prosopis juliflora* was inoculated with *S. deserticola*.

In the study, Shannon Wieners diversity index in chemically managed land shows significantly low (0.63) than organically managed and natural forest land (Table 3). A similar result was suggested by Sharmah and Jha (2011), who reported that mean spore density of AMF was significantly lower in disturbed forests land as compared to the slash-and-burn fields of Karbi Anglong Hill district of Assam. Spore density and species richness were significantly more in natural savannas than cultivated soil and lowest in intensively managed cotton soil of West Africa (Tchabi et al., 2008). Moreover most of ribosomal rDNA sequences obtained from this site clustered with known *R. irregularis* (Figures 4 to 6). Similar study using molecular methods by Mathimaran et al. (2005) found that *R. intraradices* was the dominant species of AMF in soils under conventional farming practices and suggested that, as with temperate ecosystems, addition of chemical fertilizers may dramatically decrease the availability of propagules of AMF in tropical soils. In contrast, study by Gai et al. (2006) recorded higher diversity index in the agricultural field. Present study relative spores abundance of genera Gigasporaceae were recorded low in chemically managed soil under intensive cultivation (Table 4).

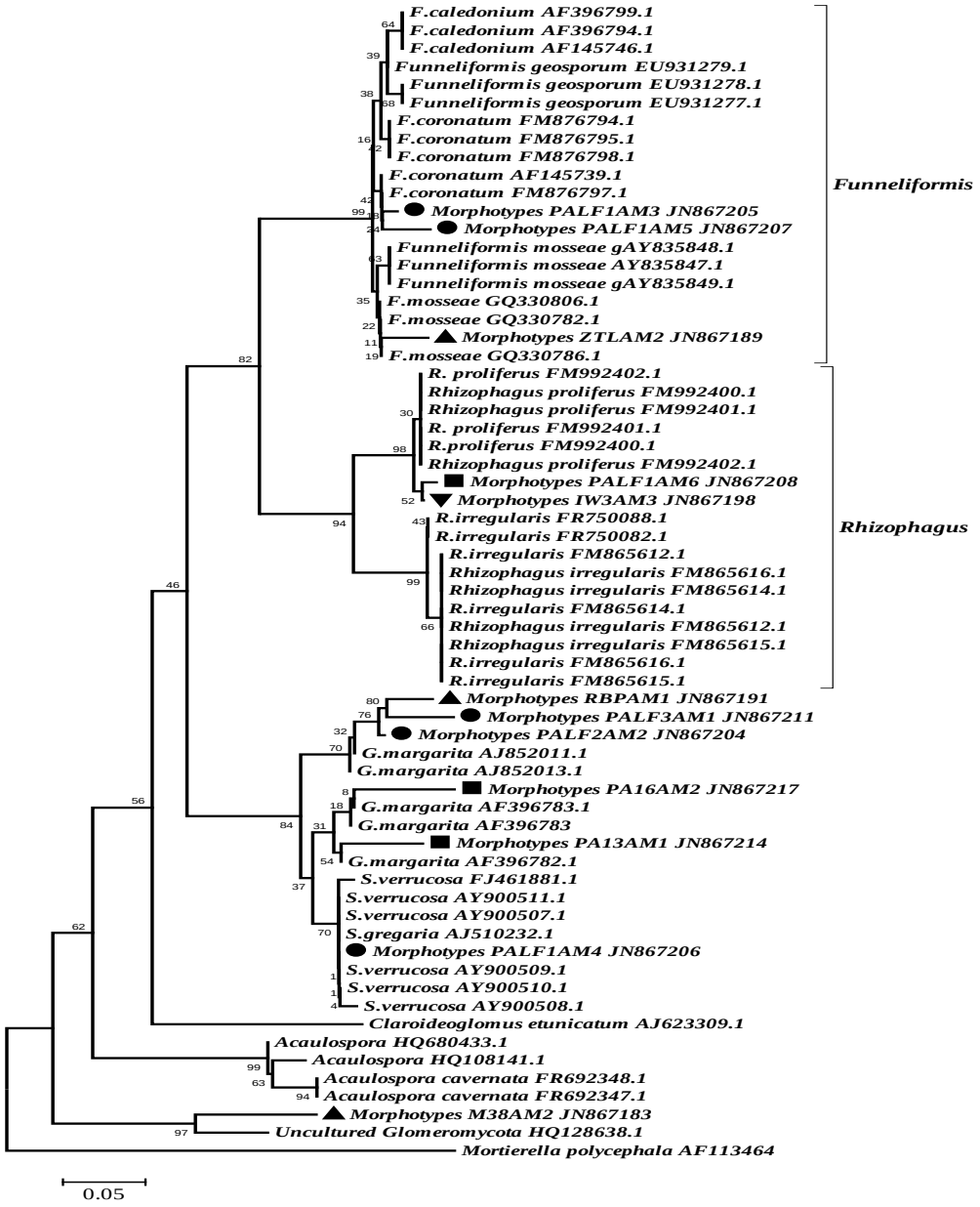

Figure 6. Sequences generated from amplicon of primer set (FLR3+FLR4) used for phylogenetic analysis. Neighbor joining tree obtained from alignment of 12 LSU rDNA (400 bp from 5' end) used in this study including 50LSU n-rDNA sequences retrieved from GenBank with (*Mortierella polycephala*) as an out-group. Percentage bootstrap support (out of 1000 replicates) is indicated. Names followed by accession no. represent sequences retrieved from GenBank. Names preceded by a shape represent the Sequence obtained in this work.

Futhermore, none of AMF morphotypes originated from chemically managed soil was clusterd with genera Gigasporaceae (Figures 4 to 6). The present study supports earlier hypothesis by Jansa et al. (2003), suggested significantly lower Gigasporaceae species in chemically managed soils. It was also reported by Johnson (1993) that application of inorganic fertilizers (High input) increased the abundance of *R. intraradices*, whereas other species like *Gigaspora gigantea, Gigaspora margarita, Scutellospora calospora* or *Paraglomus occultum* disappeared. Recent investigation by Mirás-Avalos et al. (2011) based on denaturing gradient gel electrophoresis (DGGE) sequencing found that increased presence of *Glomus* fungi in agricultural soil under conventional tillage practices.

Shannon-Weiner diversity index were significantly more

in trap culture set up field soil of raised bed plantation (RBP) and zero tillage (ZTL) soil (Table 3). Subsequently, sequencing of LSU rDNA also revealed that *Gigaspora* and *Acaulospora* species were present in RBP and ZTL sites respectively (Figures 5 to 6). Most of the AM fungal species that occurred in the RBP and ZTL do not occur in the other sites as the species cannot endure high degree of disturbances. Diversity index and species richness was more in organic and natural sites, a finding in line with the report by Gosling et al. (2010), who found that long-term application of organic manures results in rapid build-up of diverse range of AMF texa. Higher species richness in organic and natural land than the other sites due to higher diversity of host plant and sites have higher soil organic carbon that is more suitable for AM fungal growth (Bordoloi et al., 2015). Higher similarity index of species composition between chemically managed site with Industrial wasteland site may be due to lower tree diversity exists in both the site (Figure 3). Disturbance produce in the agriculture land is well known to all which not only suppress the plant diversity but also the microbial community that exists in association with them. Studies by Brokaw (1985) suggested that disturbance inhibit competitive interactions and minimize dominance of species, maintaining species diversity and richness.

Conclusion

Study revealed diversity of myocrrhizal fungi significantly affected by the different farming practices. The study provide lists of AMF species present in different soil type and land use system of subtropical soil and also provide data with which further studies can be compared. The result of our finding indicate that AMF diversity in organically managed soils was higher because of organic sources of nutrients such as farmyard manure and compost do not suppress sporulation of Gigasporaceae. Now recent advancement of next generation sequencing may provides more complete picture of distribution of arbuscular mycorrhizal fungal communities in different land use system especially to understand association of AM fungi with rare and endangered plant species as well as the medicinal plant species widespread in the tropical forest soil.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Arias J, Peralta-Videa J, Ellzey J, Ren M, Viveros M, Gardea Torresdey J (2010). Effects of *Glomus deserticola* inoculation on Prosopis: Enhancing chromium and lead uptake and translocation as confirmed by X-ray mapping, ICP-OES and TEM techniques. Environ. Exp. Bot. 68:139-148.

Atschul SF, Gish W, Myers EW, Lipman, DJ (1990). Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Bainard LD, Dai M, Gomez EF, Torres-Arias Y (2015) Arbuscular mycorrhizal fungal communities are influenced by agricultural land use and not soil type among the Chernozem great groups of the Canadian Prairies. Plant Soil.1-2:351-362.

Bordoloi A, Nath PC, Shukla AK (2015). Distribution of arbuscular mycorrhizal fungi associated with different land use systems of Arunachal Pradesh of Eastern Himalayan region. World. J. Microbiol. Biotechnol. 31:1587-1593.

Bremner JM (1960). Determination of nitrogen in soil by the Kjeldhal method. J. Agric. Sci. 55:11-33.

Brokaw NVL (1985). Gap-phase regeneration in a tropical forest. Ecology. 66:682-687.

Da Silva GA, Lumini E, Maia LC, Bonfante P, Bianciotto V (2006). Phylogenetic analysis of Glomeromycota by partial LSU rDNA sequences. Mycorrhiza. 16:183-189.

Daniell T, Husband R, Fitter, AH, Young, JPW (2001). Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. FEMS. Microbiol. Ecol. 36:203-209.

Datta NP, Khera MS, SainI TR (1962). A rapid calorimetric procedure for the determination of the organic carbon in soils. J. Ind. Soc. Soil. Sci. 10:67-74.

Dobo B, Asefa F, Asfaw Z (2016). Diversity of Arbuscular Mycorrhizal Fungi of Different Plant Species Grown in Three Land Use Types in Wensho and Shebidino Districts of Sidama in Southern Ethiopia. Adv. Biosci. Biotechnol. 4:25-34.

Gai JP, Cai XB, Fang G, Christie P, Li XL (2006). Arbuscular mycorrhizal fungi associated with sedges on the Tibetan Plateau.Mycorrhiza .16:151-157.

Gaur A, Adholeya A (2002). Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. Biol. Fert. Soils. 35:214-218.

Gollotte A, van Tuinen D, Atkinson D (2004). Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaries* and *Lolium perenne* in a field experiment. Mycorrhiza 14:111-117.

González-Cortés JC, Vega-Fraga M, Varela-Fregoso L, Martínez-Trujillo M, Carreón-Abud Y, Gavito ME (2012). Arbuscular mycorrhizal fungal (AMF) communities and land use change: the conversion of temperate forests to avocado plantations and maize fields in central Mexico. Fungal. Ecol. 5:16-23

Gosling P, Ozaki A, Jones J, Turner M, Rayns F, Bending GD (2010). Organic management of tilled agricultural soils results in a rapid increase in colonisation potential and spore populations of arbuscular mycorrhizal fungi. Agr. Ecosyst. Environ.139:273-279.

Helgason T, Fitter AH, Young JPW (1999). Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides nonscripta* (bluebell) in seminatural woodland. Mol. Ecol. 8:659-666.

Higgins D, Thompson J, Gibson T, Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic. Acids. Res. 22:4673-4680.

Hijri I, Sýkorová Z, Oehl F, Ineichen K, Mäder P, Wiemken A, Redecker D (2006). Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. Mol. Ecol. 15:2277-2289.

Jansa J,Mozafar A,Kuhn G, Anken T,Ruh R,Sanders IR, Frossard E (2003). Soil tillage affects the community structure of mycorrhizal fungi in maize roots. Ecol. Appl. 13:1164-1176.

Johnson N C (1993) Can fertilization of soil select less mutualistic mycorrhizae? Ecol. Appl. 3:749-757.
Karthikeyan C, Selvaraj T (2009). Diversity of arbuscular mycorrhizal fungi (AMF) on the coastal saline soils of the west coast of Kerala, Southern India. World. J. Agric. Sci. 5:803-809.
Khade S, Adholeya A (2009). Arbuscular mycorrhizal association in plants growing on metal contaminated and noncontaminated soils adjoining Kanpur tanneries, Uttar Pradesh, India. Water Air. Soil. Poll. 202:45-56.
Kumar S, Beri S, Adholeya A (2013). Congruence of ribosomal DNA sequencing, fatty acid methyl ester profiles and morphology for characterization of genera *Rhizophagus* (arbuscular mycorrhiza fungus). Ann. Microbial. 63:1405-1415.
Kumar Sunil CP, Garampalli HR (2010). Diversity of arbuscular mycorrhizal mycorrhizal fungi in agricultural fields of Hassan District. World. J. Agri. Sci. 6:728-734.
Manoharachary C, Sridhar K, Singh R. Adholeya A, Suryanarayanan TS, Rawat S, Johri BN (2005). Fungal biodiversity: Distribution, conservation and prospecting of fungi from India. Curr. Sci. India 89:58-71.
Mathimaran N, Ruh R, Vullioud P, Frossard E, Jansa J (2005). *Rhizophagus intraradices* dominates arbuscular mycorrhizal communities in a heavy textured agricultural soil. Mycorrhiza 16:61-66.
Mirás-Avalos J, Antunes Pedro M, Koch A, Khosla K, Klironomos John N, and Dunfield Kari, E (2011).The influence of tillage on the structure of the rhizosphere and root-associated arbuscular mycorrhizal fungal communities. Pedobiologia. 54:235-241
Muthukumar T, Udaiyan K (2000). Influence of organic manure on Arbuscular Mycorrhizal fungi associated with *Vigna unguiculata* (L.) Walp. In relation to tissue nutrients and soluble carbohydrates in roots under field condition. Biol. Fertil. Soils. 31:114-120.
Oehl F, Laczko E, Bogenrieder A, Stahr K, Bosch R, van der Heijden MGA, Sieverding E (2010). Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil. Biol. Biochem. 42:724-738.
Oehl F, Sieverding E, Ineichen K, Mader P, Boller T, Wiemken A (2003). Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Appl. Environ. Microb. 69:2616-2624.
Olsen SR, Cole CV, Watanabe, FS, Dean LA (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of agriculture, Washington, DC, Circular
Ragupathy S, Mahadevan A. (1993). Distribution of vesicular-arbuscular mycorrhizae in the plants and rhizosphere soils of the tropical plains, Tamil Nadu, India. Mycorrhiza. 3:123-136.
Raman N, Sambandan K (1998). Distribution of VAM fungi in tannery effluent polluted soils of Tamil Nadu, India. Bullet. Environ. Contam. Toxicol. 60:142-150.
Redecker D (2000). Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza. 10:73-80.
Saitou N, Nei M (1987).The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.
Schenck NC, Perez Y (1990). Manual for the identification of VA mycorrhizal fungi. INVAM, Gainsville.
Sieverding E (1990). Ecology of VAM fungi in tropical agrosystems. Agr. Ecosyst. Environ. 29:369-390.
Sander IR, Koide RT, and Shumway DL (1995a). Community level interactions between plants and vesicular arbuscular mycorrhizal fungi. In mycorrhizal Manual Verma, A(ed).Heidelberg: Springer - Verlag, pp. 605-625.
Schüßler A, Walker C (2010). The Glomeromycota: a species list with new families and genera. Edinburgh and Kew UK: The Royal Botanic Garden; Munich, Germany: Botanische Staatssammlung Munich; Oregon, USA: Oregon State University.
Sharmah D, Jha DK (2011). Diversity of arbuscular fungi in disturbed and undisturbed forests of Karbi Anglong Hill district of Assam. Curr. World. Environ. 6:253-258.
Stover HJ, Thorn RG, Bowles JM, Bernards MA, Jacobs CR (2012). Arbuscular mycorrhizal fungi and vascular plant species abundance and community structure in tallgrass prairies with varying agricultural disturbance histories. Appl. Soil. Ecol. 60:61-70.
Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4 Molecular Evolutionary genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24:1596-1599.
Tchabi A, Coyne D, Hountondji F, Lawouin L, Wiemken A, & Oehl F (2008). Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. Mycorrhiza18:181-195.
Thapar HS and Uniyal K (1996) Effect of VAM fungi and *Rhizobium* on growth of *Acacia nilotica* in sodic and new forest soils. Indian For. 122:1033-1039.
Treseder KK, Mack MC, Cross A (2004). Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. Ecol. Appl. 14:1826-1838.
Van der Heijden MG, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature. 396(6706):69-72.
Van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gininazzi Pearson V (1998a). Characterization of root colonization profiles by microcosm community of arbuscular fungi using 25S rDNA targeted nested PCR. Mol. Ecol. 7:879-887.
Van Tuinen D, Zhao B, Gianinazzi-Pearson V (1998b). PCR in studies of AM fungi: from primers to application. Mycorrhiza manual. Springer, Berlin Heidelberg New York, pp. 387-399.
Walker C, Trappe JM (1993) Names and epithets in the Glomales and Endogonales. Mycol. Res. 97:339-344.
Wood LK, DeTurk EE (1940). The adsorption of potassium in soils in replaceable form Soil. Sci. Soc. Am. Proc. 5:152-161.