

ABUNDANCE, RICHNESS AND ROOT COLONIZATION OF ARBUSCULAR MYCORRHIZAL FUNGI IN NATURAL AND SEMI-NATURAL LANDUSE TYPES AT UPPER HANTANA

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ABSTRACT

There is a scarcity of published information on the abundance and richness of arbuscular mycorrhizal fungi (AMF) in natural and semi-natural landuse types in Sri Lanka. The Upper Hantana (UH) area, which comprises landuse types with different histories, was selected to fulfill this knowledge gap. A study was carried out to investigate the abundance and richness of AMF in selected landuse types, including restored pine stand (REP), degraded grassland (DEG), *Paraserianthes* stand (PST) and natural forest patch (NFP) at Upper Hantana in the Central Province of Sri Lanka. The highest AMF spore abundance was observed in the NFP. However, the AMF spore richness was higher in both NFP and REP than in DEG and PST. At NFP, the medium-sized spores dominate the AMF spore population. Furthermore, NFP showed the least evenness in AMF distribution compared to other landuse types. *Glomus* was the most abundant genus in all selected landuse types. *Acaulospora* was observed only in DEG and PST. The colonization potential of AMF varies highly between species and selected landuse types, with no clear relationship between AMF abundance and root colonization potential. The results speculate that AMF spore abundance is determined by the composition and diversity of the vegetation. The results also revealed that the AMF richness at REP was similar to that of NFP, indicating a positive impact on the below-ground biota within a relatively short period following restoration.

Keywords: arbuscular mycorrhizal fungi, restoration, landuse types, *Paraserianthes* stand, restored Pine stand, grassland, natural forest

INTRODUCTION

Mycorrhizae are highly evolved, mutualistic associations between a group of fungi and plant roots. Arbuscular mycorrhizal fungi (AMF) reported to have a widespread occurrence in the plant kingdom with many benefits to plant growth (Janos 1980; Wolfe and Klinomus 2005; Smith and Read 2008; Lee *et al.*, 2013). In this highly specialized relationship, the host receives mineral nutrients while the fungus obtains photosynthate s (Harley and Smith 1983; Harley 1989). AMF play an important role in plant growth especially under nutrient poor conditions. Due to non-specific host specificity, AMF influence not only a single plant species but populations (Lee *et al.*, 2013; Leake *et al.*, 2004). There is evidence to suggest that differences in community composition of AMF play a potential role in plant diversity and ecosystem functions (Van der Heijden *et al.*, 1998). Therefore, the knowledge on the diversity of AMF and their colonization potentials are considered important information to assess the health of an ecosystem and allows comparisons between different ecosystems (Jeffries *et al.*, 2003; Lee *et al.*, 2013).

Ecologists have recognized the importance of AM fungi in restoration of ecosystems for decades (Reeves *et al.*, 1979; Allen 1984; Miller 1987; Koziol and Bever, 2015). AMF play a crucial role in the reclamation and restoration of degraded ecosystems and mycorrhizal-dependent species could act as promising candidates in reforestation programs (Cuenca *et al.*, 1998). Therefore, the knowledge on the diversity and colonization potentials of AMF may provide additional insight in conservation biology and environmental protection.

Changes in existing landuse types may alter the below-ground ecosystem, which often can lead to loss of soil microbial biodiversity and depletion of soil carbon (Doran and Zeiss 2000). The soil quality may change along with the conversion of diverse forests into monoculture plantations or grasslands (Thomson *et al.*, 2009). However, the impacts of land use changes on AMF communities are not explored extensively (Oehl *et al.*, 2010). Understanding the mycorrhizal responses to anthropogenic changes may help predicting paths of the future communities and ecosystems (Hu *et al.*, 2013; Yang *et al.*, 2013).

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The Upper Hantana (UH), the focal area of the present study, is an important yet severely threatened ecosystem. The land at UH is cleared for tea in early 19th century and some of these tea plantations were later abandoned. These abandoned lands were gradually converted into grass-dominated habitats. The grasslands at UH are highly degraded and marginal due to high soil erosion and frequent fires during dry spells. In the 1980s, plantations of *Pinus* and *Paraserianthes* were introduced to replace degraded grasslands at UH (Ambagahaduwa *et al.*, 2009). At present, grasslands, few remnants of natural forest, regenerating semi-natural forests (broad-leaved forests dominated by *Alstonia* and *Paraserianthes*) and *Pinus* plantations dominate the landscape. The present study was conducted with the aim of assessing the status of AMF abundance, composition and richness in selected semi-natural landuse types in UH and compared that with a natural forest patch in the area.

MATERIALS AND METHODS

Study site

The Hantana range, which falls in the agroecological region of wet zone, mid country (WM3), is located in the Central Province of Sri Lanka. The elevation ranges from 518 m – 1,100 m and is underlain by pre-cambrian metamorphic rocks of the Highland Series (Mapa *et al.*, 1999). The major soil group is Red Yellow Podzolic or ultisols according to USDA classification (Panabokke 1996). The mean annual precipitation and temperature are 2,131 mm and 24.1 °C, respectively. The rainfall is received from both

north-east and south-west monsoons and distributed more or less evenly throughout the year.

A small remnant of the natural forest still remain at a higher elevation level, which supposedly represents the original forest cover at UH before the clearance for tea during the colonial era. Degraded grassland (DEG), natural forest patch (NFP), *Paraserianthes* stand (PST) and restored pine stand (REP) were selected to conduct the present study (Table 1).

Soil sampling and root extraction

From two representative plots from each landuse type, 10 soil samples (each soil sample is a composite of 5 soil samples) were collected (5 from each) from a depth of 0–20 cm using a hand-held soil corer. All 10 samples from each landuse type were used as replicates for the spore count, while 6 randomly selected samples (from each landuse type) were used in the analysis of soil parameters (soil pH and moisture). Soil samples were collected in zip-lock polythene bags, and were brought to the laboratory and stored at 4°C until analyses.

Five most common herbaceous and tree (seedlings) species in the undergrowth of each landuse types were identified and their root samples were extracted to assess the colonization potential of AM fungi. From each species, 5–6 plants were randomly selected to extract roots. The extracted root samples were washed thoroughly under running water to remove any soil particles and other organic debris, and stored in 50% ethanol until determination of the percentage root colonization.

Table 1. The historical background and the present vegetation of the selected study sites at Upper Hantana, Sri Lanka; degraded grassland (DEG), *Paraserianthes* stand (PST), restored pine stand (REP) and the natural forest patch (NFP).

	Degraded grassland (DEG)	<i>Paraserianthes</i> stand (PST)	Restored Pine stand (REP)	Natural Forest Patch (NFP)
History	Abandoned tea/coffee plantations later converted into grasslands	<i>Paraserianthes</i> was introduced into degraded grasslands in 1980s by the Forest Department	Some pine (<i>Pinus caribaea</i>) stands were restored in 2003 by introducing broadleaved species	Remaining natural forest patch with minimum disturbances
Present vegetation	<i>Panicum maximum</i> -dominated grassland	<i>Paraserianthes falcataria</i> dominated vegetation	Broad-leaved species including <i>Terminalia bellirica</i> , <i>Maduca fulva</i> , <i>Michelia champaca</i> , <i>Artocarpus nobilis</i> etc.	Natural forest

Separation and identification of spores

A 100 g soil from each sample (10 soil samples from each landuse type separately) was used for spore extraction by wet sieving decanting method as described by Brundrett *et al.*, (1996). The material retained on 125, 63 and 45 μm sieves was collected on glass fiber filter papers separately (Whatman GF/A) and AMF spores and sporocarps were counted and differentiated into morphotypes under reflected light on stereomicroscope (Olympus SZ 61). Spores from each morphotype were mounted on permanent slides using Melzer's reagent and observed under a binocular light microscope (Olympus CX 21) at $\times 400$ magnification and identified in to generic level based on the descriptions given in the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM, West Virginia, USA; <http://invam.caf.wvu.edu>).

AMF root colonization assessment

Roots were cleared in a water bath by heating with 10% (w/v) KOH at 60-90°C for 1 hour. Once cleared, a few drops of Chlorazol Black E (CBE) solution was added and heated again for about 20 minutes to several hours (until the root samples were stained properly) at 90°C. The excess stain was then washed off, acidified with 1% HCl solution before mounting roots on a glass slide with few drops of 50% Glycerol (Kormanick and McGraw 1982; Brundrett *et al.*, 1994 and 1996). The colonization percentage was calculated using the grid-intersect method after McGonigle *et al.*, (1990) by scoring root intersects that contained different AM fungal structures (spores, hyphae and arbuscules) at $\times 100$ magnification.

Preparation of trap cultures

Maize (*Zea mays*) seeds were sown (3 seeds per pot) in plastic pots (with a diameter of 15 cm) containing a mixture of potting medium [autoclaved garden soil and coarse sand mixed in 1:1 (v/v) ratio] and 50 g of composite field-fresh soil taken from each landuse type as an inoculum of AMF spores. For each landuse type, there were 6 replicate pots. Plants were raised in a glasshouse for three to four months.

Spores were extracted from the pots using wet sieving and decanting method as mentioned previously (Brundrett *et al.*, 1996). Spore morphotypes were identified and counted.

Data analysis

One-way ANOVA was carried out to compare the density and richness of AMF spores ($n = 10$) between landuse types. The General Linear Model (GLM) was carried out to compare spore abundance and richness with spore size classes and landuse types as random factors. Mean separations were done using Tukey's test at the 0.05 level of significance. All data were checked for normality using Anderson-Darling test to meet ANOVA assumptions. All statistical analyses were performed using Minitab (version 16.0) software.

RESULTS

Soil parameters

All landuse types showed acidic soil pH irrespective of their different vegetation cover and histories. The natural forest patch (NFP) showed the lowest soil pH compared to other landuse types, REP, DEG and PST. However, DEG and PST showed significantly higher soil pH than that of REP. As expected, the soil moisture was highest ($\approx 43\%$) in the NFP compared to other three semi-natural landuse types (19, 20 and 22% DEG, PST and REP, respectively) (Table 2).

Spore abundance and richness

The highest total number of AMF spores were recorded from soil samples taken from the natural forest patch (NFP) while the lowest was recorded from the *Paraserianthes* stand (PST) (Table 3; 8,234 and 1,573 respectively). The restored pine stand (REP) had the second highest AMF spore numbers (3,991) followed by DEG (1,920). Highest number of spore morphotypes was also recorded from NFP (20) and the lowest (14) from the degraded grassland (DEG). In AMF richness too, REP came second only to NFP with 18 spore morphotypes (Table 3).

Table 2. Soil pH (soil:distilled water in 1:3 ratio) and gravimetric moisture content (%) of the study sites ($n = 6$), restored pine (REP), degraded grassland (DEG), natural forest patch (NFP) and *Paraserianthes* stand (PST) at Upper Hantana, Sri Lanka. The significant differences are highlighted using different superscript letters after one-way ANOVA ($F=58.7$, $p \leq 0.001$ and $F=92.5$, $p \leq 0.001$ for soil pH and soil moisture contents, respectively).

	Restored Pine (REP)	Degraded grassland (DEG)	Natural Forest Patch (NFP)	<i>Paraserianthes</i> stand (PST)
Soil pH	5.23 ^b	6.13 ^a	4.86 ^c	6.21 ^a
Soil Moisture (%)	21.6 ^b	18.7 ^b	42.6 ^a	19.5 ^b

Table 3. Total number of AMF spores, morphotypes, Shannon Diversity index and evenness values of the four land use types at Upper Hantana, restored *Pinus* stand (REP), *Paraserianthes* stand (PST), degraded grassland (DEG) and Natural forest patch (NFP).

Land use type	Total number of AMF Spores (per kg of fresh soil)	Total number of spore morphotypes (per kg of fresh soil)	Shannon Diversity Index (H')	Evenness
REP	3,991	18	2.28	0.79
PST	1,573	16	2.14	0.77
DEG	1,920	14	2.17	0.82
NFP	8,234	20	2.44	0.12

Table 4. Number of spore morphotypes belonging to different genera in four different landuse types at Upper Hantana, Sri Lanka; restored *Pinus* stand (REP), *Paraserianthes* stand (PST), degraded grassland (DEG) and Natural forest patch (NFP).

	Restored Pine (REP)	Degraded grassland (DEG)	Natural Forest Patch (NFP)	<i>Paraserianthes</i> stand (PST)
<i>Glomus</i>	7	2	10	5
<i>Gigaspora</i>	2	-	2	-
<i>Acaulospora</i>	-	3	-	3
<i>Scutellospora</i>	-	1	2	-
Unidentified	9	8	6	8
Total Number of morphotypes	18	14	20	16

According to the calculated Shannon-Weiner Diversity indices, NFP showed the highest AMF spore diversity while PST the lowest (Table 3). In spite of having the highest AMF diversity, NFP showed the lowest evenness value compared to other landuse types.

In all landuse types, 30 to 50% of the spore morphotypes were not identified into their generic levels. *Glomus* was recorded in all landuse types (Table 4). *Acaulospora* only observed in DEG and PST landuse types, while *Scutellospora* located only in DEG and NFP. *Gigaspora* found in both REP and NFP landuse types (Figure 1).

In the trap culture experiment, a total of 16, 12, 10 and 11 morphotypes were identified from landuse types, NFP, DEG, REP and PST, respectively. In consistent with the AMF spore morphotypes recorded from field soils, NFP has recorded the highest AMF richness than the rest of the study sites. Morphotypes belonging to *Glomus* species were recorded in all landuse types while *Scutellospora* species was only recorded in DEG suggesting its rarity and specificity. No new morphotypes were recorded from the trap culture experiment.

The average number of AMF spore morphotypes (richness) was significantly higher in NFP and REP than that of PST and DEG (Figure 2). However, the average density of AMF spores showed somewhat different results to richness values. The

NFP recorded the highest spore density value (at $p \leq 0.001$) compared to the rest of the landuse types (REP, PST and DEG).

Distribution of differently-sized AMF spore morphotypes in each landuse type showed variations in richness and abundance among sites (Figures 3 and 4). However, the results showed no apparent trend in the distribution of differently-sized spore morphotypes among landuse types. At REP, the richness of medium-sized (63-125 μm) spore morphotypes was higher than that of small- and large-sized spore morphotypes. The medium-sized AMF spores dominate the total spore count in the natural forest patch (NFP) compared to other landuse types (Figure 4). In REP, the density of large spores ($> 125 \mu\text{m}$) was significantly the lowest compared to other 2 size classes (small and medium), while in DEG and PST, the AMF spore densities belonging to different size classes did not show any significant differences (Figure 4).

Root colonization

The overall root colonization of AMF was relatively low in all landuse types with averages of 16.4, 15.4, 7.6 and 7.3% in REP, DEG, NFP and PST, respectively (Table 5). Even within a landuse type, the percentage root colonization varied among species with values ranging from 4-51% in REP, 7-20% in DEG and 2-12% in NFP and PST sites. The highest root colonization was recorded from a perennial climber known as *Asparagus racemosus* in REP.



Figure 1. Photographs showing AMF spores belong to *Glomus*, *Gigaspora*, *Scutellospora* and *Acaulospora* species. Spore wall characteristics and developmental and germination stages of AMF spores were highlighted. A-M photos were taken from an Olympus BX 52 (at x400 magnification) microscope while N-O was taken using a Polarizing microscope (Euromex). Abbreviations: H = subtending hypha, S = stalk. **A-D:** *Glomus* species (**A**: Young spore of the *Glomus* sp. from REP, squashed to show spore contents found in young spores, the thick brown unit wall and subtending hyphal attachment can be seen; **D**: Squashed spore of another *Glomus* morphotype stained with Melzer's reagent. A stalk can be seen; **E**: Squashed spore of *Gigaspora* sp. with a swollen subtending hyphae; **F**: Crushed spore of *Scutellospora* sp. stained with Melzer's reagent. **G-H:** *Acaulospora* spores; **G**: Squashed *Acaulospora* spore showing inner layers; **H**: Squashed *Acaulospora* spore; **I**: *Glomus* sp.; **J**: *Scutellospora* sp.; **K, L**: unidentified morphotypes.

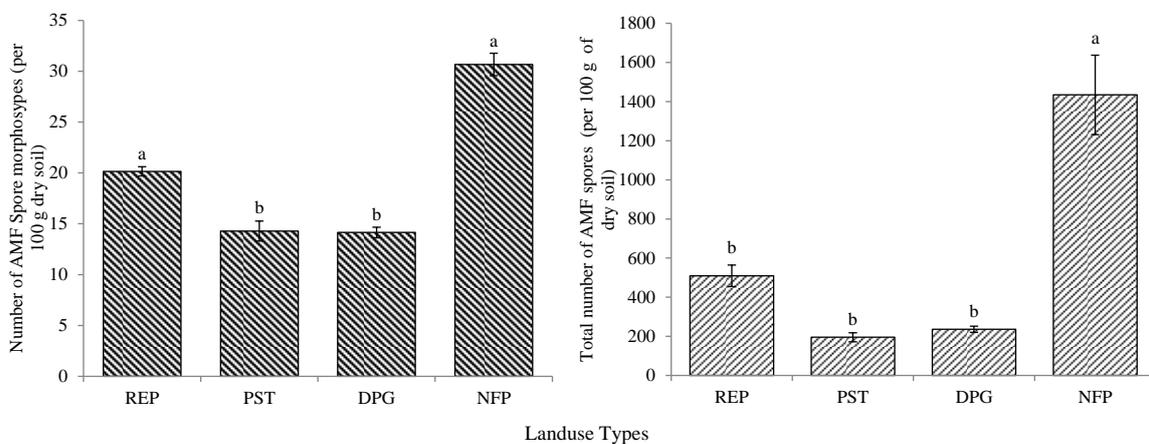


Figure 2. Number of AMF morphotypes and AMF spores (per 100 g of dry soil) in different landuse types restored pine stand, *Paraserianthes* stand, degraded grassland and natural forest patch (REP, PST, DEG and NFP respectively) in Upper Hantana, Kandy, Sri Lanka. Vertical bars represent standard error of the mean (n = 10). Different letters indicate significant differences between landuse types after one-way ANOVA (Richness: F = 31.94, $p \leq 0.001$; density: F = 15.46, $p \leq 0.001$).

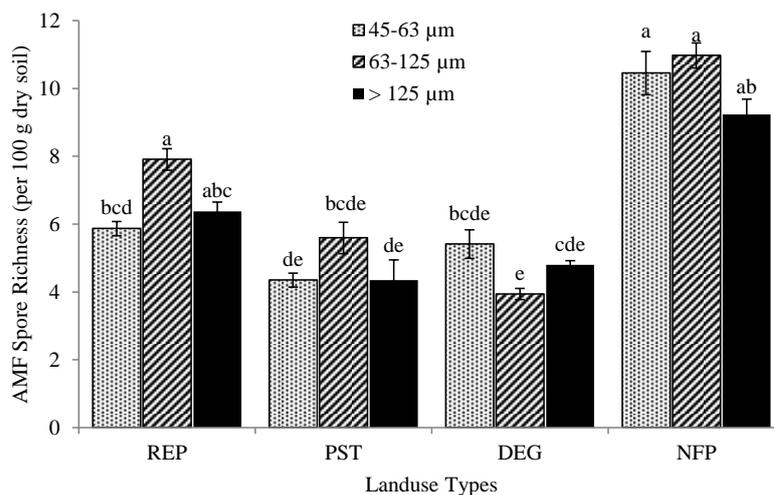


Figure 3. Average richness of AMF spores (per 100 g dry soil) belonged to different size classes (45-63, 63-125 and > 125 µm) in four different landuse types; Restored *Pinus* Stand (REP), *Paraserianthes* stand (PST), degraded Grassland (DEG) and Natural Forest Patch at Upper Hantana area, Sri Lanka. Error bars indicate the standard error of the mean value (SEM). Different letters indicate significant differences after GLM analysis followed by Tukey's test at $p \leq 0.5$ ($n = 10$).

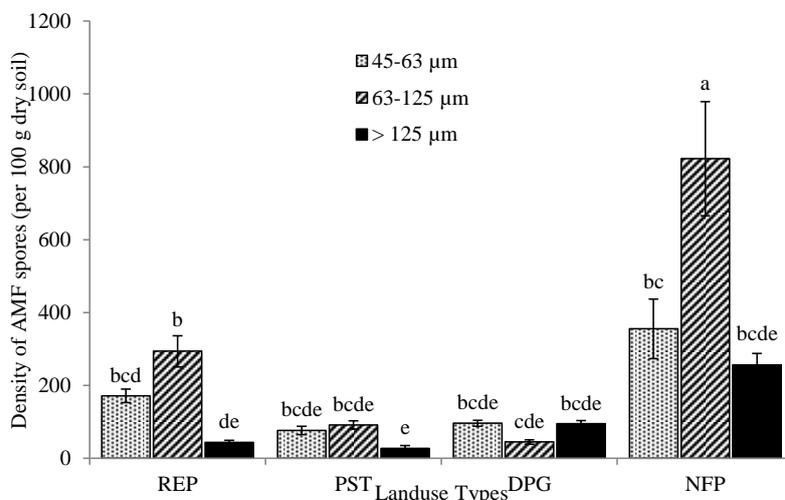


Figure 4. Average density of AMF spores (per 100 g dry soil) belonged to different size classes (45-63, 63-125 and > 125 µm) in four different landuse types; Restored *Pinus* Stand (REP), *Paraserianthes* stand (PST), degraded Grassland (DEG) and Natural Forest Patch at Upper Hantana area, Sri Lanka. Error bars indicate the standard error of the mean value (SEM). Different letters indicate significant differences after GLM analysis followed by Tukey's test at $p \leq 0.5$ ($n = 10$).

Furthermore, *Clidemia hirta*, a known invader in the wet zone forest undergrowth, showed diverse levels of AMF colonization in different landuse types with values ranging from 18, 13 and 9% in DPG, REP and NFP, respectively.

DISCUSSION

Spore quantification is used as a tangible method in enumerating the abundance and richness of AMF, since spores are highly resistant to adverse environmental conditions (Abbott and Robson

1991). However, early studies suggested that AMF spore abundance do not directly reflect the colonizing potential of plant roots (Clapp *et al.*, 1995). Nevertheless, due to high costs of other molecular methods, AMF spore counts and their identification into morphotypes (if possible even into species) remain as a more economical, fast and feasible method of studying AMF (Douds and Millner 1999; Oehl *et al.*, 2010). The present results revealed that the AMF communities were quite distinct among study sites possibly due to contrasting landuse types and micro-climatic conditions prevailing in these habitats.

Table 5. Percentage root colonization of AM fungi in representative plant species from different landuse types (DEG, NFP, PST and REP) at Upper Hantana Area, Kandy, Sri Lanka.

Plant species	Family	% root colonization
Degraded Grassland (DEG)		
<i>Axonopus compresses</i>	Poaceae	20.0
<i>Panicum maximum</i>	Poaceae	15.3
<i>Eupatorium odoratum</i>	Asteraceae	16.8
<i>Mimosa pudica</i>	Fabaceae	7.3
<i>Clidemia hirta</i>	Melastomataceae	17.8
Natural Forest Patch (NFP)		
<i>Panicum maximum</i>	Poaceae	11.6
<i>Tithonia diversifolia</i>	Asteraceae	6.7
<i>Panicum maximum</i>	Poaceae	7.6
<i>Mimosa pudica</i>	Fabaceae	2.1
<i>Clidemia hirta</i>	Melastomataceae	8.9
Paraserianthes stand (PST)		
<i>Paraserianthes sp.</i>	Fabaceae	12.0
<i>Eleutheranthera ruderalis</i>	Asteraceae	10.3
<i>Mimosa pudica</i>	Fabaceae	5.4
<i>Vernonia cinerea</i>	Asteraceae	nd
<i>Ageratum conyzoides</i>	Asteraceae	2.0
Restored Pine stand (REP)		
<i>Panicum maximum</i>	Poaceae	11.2
<i>Piper longum</i>	Piperaceae	4.3
<i>Asparagus racemosus</i>	Liliaceae	50.7
<i>Tithonia diversifolia</i>	Asteraceae	4.2
<i>Clidemia hirta</i>	Melastomataceae	12.5

The highest AMF diversity was observed in the natural forest patch (NFP) ($H^{\prime}=2.44$), which was closely followed by the restored pine stand (REP) ($H^{\prime}=2.28$). The basic soil parameters showed that NFP has the lowest pH and the highest soil moisture content among the study sites (Table 1).

According to Clark (1997), some AM fungal species from genera, *Acaulospora*, *Gigaspora*, and *Glomus* are more tolerant of acidic conditions. Previous studies have shown a close relationship between the diversity of AMF and the diversity of plant communities, suggesting AMF play a critical role in competitive interactions among plants (Streitwolf *et al.*, 1997; Van der Heijden *et al.*, 1998). Relatively higher AMF diversity and richness at REP compared to DEG and PST indicated that REP showed an encouraging trajectory in terms of invigorating the soil health after a period of 11 years since the restoration of the site using broad-leaved native species. Due to low ecological value of mono-specific *Pinus* plantations, the conversion of pine stands into some form of forest cover is highly recommended. The present results support the idea that AMF could play an important role in restoring the soil health which will positively influence the growth and composition of the vegetation.

Glomus was the most frequent and abundant AMF genus in all three landuse types except in DEG. Since *Glomus* is a well-known host generalist, it has the potential to colonize a range of plants. *Glomus* is known to occur in extremely broad range of habitats with high tolerance of environmental factors (Börstler *et al.*, 2008). PST is located somewhat at a higher elevation than that of REP and DEG and therefore the land is prone to erosion. Small-scale earth slips were often evident in the study site. Furthermore, the forest floor of the PST was comparatively barren with very little undergrowth. The reduced AMF spore density at PST can be explained by these frequent disturbances in the habitat which could influence soil fungal populations negatively through lack of surface organic matter on which these obligate symbionts depend on for their carbon source. Studies also revealed that forest disturbances cause negative effects on soil microorganisms, reducing the diversity and composition of AMF (Allen *et al.*, 1995). All these factors may have led to low AMF abundance and richness in PST. Janos (1980) also pointed out that AMF spores can be washed down with rain water to lower slopes influencing a heterogeneous distribution in sloping landscape. However, in the present study elevation was not considered as a

factor and therefore the relationship between abundance and richness of AMF spores with that of the elevation could not be established.

The results also reported a lower abundance of AMF spores in DEG where grasses dominate the vegetation with occasional herbaceous species. In contrast to present findings, others observed almost similar and sometimes even higher abundance of AMF spores in grass-dominated habitats over tropical forests in studies carried out in Nicaragua, Costa Rica and Brazil (Picone 2000; Zangaro *et al.*, 2013). According to them, this higher abundance of AMF spores in grasslands over tropical forests was possibly due to higher turn-over rates of fine roots and soil pH in grasslands than in mature forests. In the present study, the highest spore abundance was observed in the NFP suggesting that the plant diversity and richness may play a critical role in deciding the abundance and diversity of AMF in spite of low fine root turn-over rates in mature forest trees. The natural forest patch consists of dense vegetation with comparatively rich undergrowth with no symptoms of erosion or any other anthropogenic disturbances. Börstler *et al.* (2008) observed higher AMF colonization and abundance in un-disturbed lands than that of disturbed lands suggesting that soil disturbances due to natural and anthropogenic activities often affect negatively on AMF population. Other studies too have shown that the AMF population, species richness, and root colonization are often reduced by soil disturbances (Helgason *et al.*, 1998; Oehl *et al.*, 2010).

After years of enrichment with native species, REP seems to have restored its AMF richness to a level which is comparable to the undisturbed forest patch located nearby. The two main processes that control the population of AMF following disturbances are known to be immigration of new propagules from nearby areas and survival of residual propagules (Warner *et al.*, 1987). We tested soil samples of the un-restored pine stand for AMF spores but came up with negative results. *Pinus* is known to form associations with ectomycorrhizal species. Therefore, we could speculate that AM fungal spores in the REP have introduced mainly through immigration. Introduction of AMF spores through infected native plant seedlings/saplings is also considered as a likely option during restoration interventions. This improved AMF population may be a direct consequence of land conversion from a mono-specific pine stand into a multi-species vegetation. However, in spite of increased AMF spore richness in REP, the abundance remained almost similar to other two study sites, DEG and PST. The comparatively low abundance of AMF spores at REP is perhaps due to its status as a relatively new habitat for AMF.

Arbuscular mycorrhizal root colonization of

different structures varied among plant species both within and between landuse types. The percentage root colonization of *Panicum maximum* was 15.3, 11.6 and 11.2% in DEG, NFP and REP, while *Clidemia hirta* has shown colonization percentages of 17.8, 12.5 and 8.9% in DEG, REP and NFP, respectively. The results indicate that both plant species have shown higher root colonization potential in DEG where soil nutrients may be the most limiting. Furthermore, DEG showed lower richness and abundance of AMF spores compared to other study sites. However, early studies reported that there is no positive relationship between root colonization and species richness (Fisher and Vovoides 2004). Furthermore, *Asparagus racemosus* in REP had the highest percentage root colonization (50.7%) out of all plant species examined across all land use types. This plant species itself may have contributed largely to the increased abundance of AMF at REP. Overall, the AMF colonization was rather low in all landuse types. This was perhaps due to higher phosphorus levels in soil which could inhibit the development and colonization of AMF (Abbott and Robson, 1984). According to previous studies, AMF colonization by roots is influenced by several factors including soil properties, soil water content, plant phenology, predation and propagule availability (Anderson *et al.*, 1984; Johnson *et al.*, 1991; Anderson *et al.*, 1994; Schultz *et al.*, 2001; Muthukumar *et al.*, 2003). Others suggested that AMF spore abundance do not always correlate with root colonization potential of AMF (Camargo-Ricalde and Dhillion 2003). In favour of the present findings, Daniell *et al.* (2001) stressed that the level of spore production does not reflect the abundance of AMF in roots. The present results speculated that plants growing in resource poor conditions rely on AMF more than the species growing under high resource availability. Though, changes in AMF colonization across landscape can be influenced by microsite differences (Reynolds *et al.*, 2003), the present study did not adequately address that issue as the main focus was to assess the levels of AMF richness and abundance in different natural and semi-natural landuse types.

In conclusion, results showed that AMF abundance and richness vary among natural and semi-natural landuse types greatly. The restored pine stand attained a level (in terms of AMF richness) which is comparable to the natural forest patch, indicating a positive role of restoration since its initiation in 2003. In spite of higher richness of AMF, the REP had lower AMF abundance than that of NFP indicating a slower attainment of AMF population comparable to the natural forest patch (NFP). However, AMF richness and abundance seem to determine by the richness and the diversity of the existing vegetation indicating its overriding effect on AMF than the fine root turn-over rates as suggested by previous workers.

The results suggest that the vegetation cover and disturbances due to natural and anthropogenic factors may play a role in determining the abundance and richness of AMF spores. However, the colonization potential of roots does not reflect the abundance and richness of AMF spores in soil. Restoration strategies not only enhance the composition and diversity of the vegetation, but also the abundance and richness of AMF.

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