Arbuscular mycorrhizal diversity in relation to degradation of tropical forests in Arunachal Pradesh

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CERTIFICATE

Certified that the thesis entitled "Arbuscular mycorrhizal diversity in relation to degradation of tropical forests in Arunachal Pradesh", being submitted for the degree of Doctor of Philosophy in Botany to Rajiv Gandhi University, embodies the record of research work conducted by Ms. Hage Yakang under my supervision. No portion of this work has been previously submitted for a degree or diploma at any other university or institution.

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I, **Hage Yakang**, certify that the work embodied in this Ph.D. thesis entitled 'Arbuscular mycorrhizal diversity in relation to degradation of tropical forests in Arunachal Pradesh' being submitted for the award of Degree of Doctor of Philosophy in Botany is the outcome of my original research work carried out under the supervision of Dr. Oyi Dai Nimasow at the department of Botany, Rajiv Gandhi University, Doimukh, from the year 2016 to 2023. No part of this thesis has been submitted to any university or institution of higher learning for being awarded any degree or diploma

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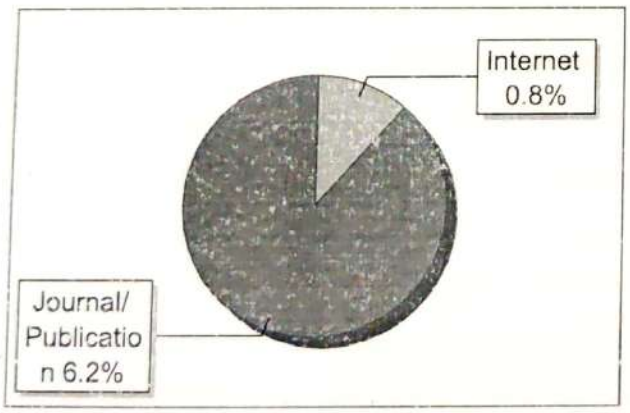

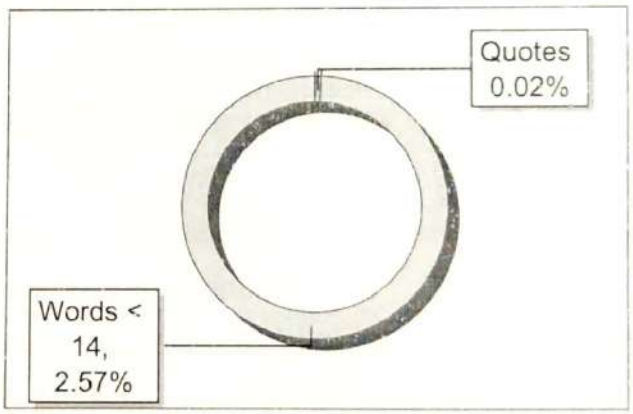

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Hage Yakang

CONTENTS

Abbreviations Used

AM	Arbuscular Mycorrhizae
AMF	Arbuscular Mycorrhizal fungi
BD	Bulk-density
CRS	Composite root samples
DBH	Diameter at Breast Height
DF	Degraded Forest
DHA	Dehydrogenase activity
IF	Isolation Frequency
IP	Inoculum Potential
JF	Jhum Fallow
K	Potassium
MC	Moisture content
MPN	Most probable number
N	Nitrogen
NF	Natural Forest
OC	Organic carbon
P	Phosphorus
RA	Relative Abundance
RC	Root Colonization
RF	Regenerating Forest
RHP	Representative host plants
WHC	Water holding capacity

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ABSTRACT

Arbuscular Mycorrhizal Fungi (AMF) are a small group of soil fungi (about 230 species) placed under the phylum Glomeromycota. They form symbiotic relationship with roots of 80% of terrestrial higher plants, and with the help of their extensive mycelial network in the soil enhance their acquisition of water and mineral nutrition from soil, especially phosphate and nitrogen. They also enhance resistance ability of their host plants against several abiotic and biotic stresses. In return, the fungus gets fixed carbon compounds from the host plant. AMF are generally considered non-host specific, but it has been reported that they show some host preference. Thus, symbiosis with AMF improve fitness of the host plants from an individual to community level by imparting them a better competitive ability to survive and grow in an otherwise adverse ecological condition. Apart from their plant growth promoting ability, AMF also improve overall soil quality by aiding to the formation of stable soil aggregates, creating a macroporous soil structure that facilitates water and air penetration while preventing erosion.

The composition and growth of vegetation depend on the soil nutrient concentration and soil microorganisms since they play a key role in mineralization and transformation of organic matters essential for their growth and development. Therefore, owning to their various ecological functions, AMF are regarded as one of the key players in shaping plant diversity, community structure, and succession within an ecosystem. On the other hand, the dependence of AMF on their host plants for photosynthate implies that the structure and function of the AM fungal community is also influenced by the composition of the plant community, thus suggesting a reciprocal relationship. Besides plant community, physico-chemical properties of soil, and any natural or anthropogenic activities that affect soil and vegetation also influence AMF community structure.

Forests are threatened by anthropogenic activities, primarily deforestation, that negatively impacts above-ground vegetation, soil properties, and microbial communities including AMF. The AMF suffers breakdown of its hyphal network, reduced root infection, and a diminished

spore population in soil. In humid tropics, deforestation enhances soil erosion and it happens severely in hilly areas that receives heavy rainfall for a longer period of time.

The effect of deforestation on the mycorrhizal status of soil in Northeast India is under-researched. The delicate nature of the soil in this region, which is susceptible to erosion due to intense rainfall and steep slopes, suggests that deforestation is likely to have pronounced adverse effects. Arunachal Pradesh, a region that falls within the Eastern Himalaya biodiversity hotspot, boasts an expansive and densely forested terrain. Unfortunately, over the past few decades, the region has witnessed accelerated deforestation due to various anthropogenic activities such as lumbering, slash-and-burn agriculture, urbanization, and developmental projects. Slash-and-burn agriculture, also known as Jhum cultivation, is a traditional farming method practiced by the indigenous tribes. It requires almost complete clearing of a section of the forest, followed by controlled burning. The burnt area is utilized for crop cultivation for 2 to 3 years before being left fallow that get occupied over the time largely by shrubs, herbs and a few saplings. This cultivation practice makes the soil loose, exposed to desiccation, and prone to different degree of erosion depending upon the runoff of rainwater on the slope of the fields. Hence, it may have a profound effect on AMF status in the soil.

As existing studies on AMF status in soil in the region are limited and some of them have methodological shortcomings, therefore, considering all these aspects, the present study was aimed to evaluate the effects of deforestation in tropical forests of Arunachal Pradesh by selecting four different sites i.e. Natural forest, Regenerating forest, Degraded forest, and Jhum fallows. The regenerating was selected to see whether AMF status have improved over 20-year period since a complete restriction on logging. Data were recorded in two different seasons following standard methodologies for various parameter such as canopy cover, ground vegetation cover, dominant plant species, soil properties, soil microbial activity, AMF diversity, AMF root infection and AMF spore population.

The key findings of the study were as follows:

1. Vegetation cover and its influence on soil physico-chemical properties

The vegetation cover varied among the sites, and a lesser tree density and canopy cover was associated with increased ground cover. The number of mature trees were more in Natural forest which indicates a stable community, while young trees were more in Regenerating forest indicating regeneration. While bulk density and porosity did not differ significantly, soil

moisture content, water holding capacity, pH, organic carbon, available N, P and K varied significantly across the sites.

2. Effect of vegetation cover and soil physico-chemical properties on microbial activity (Dehydrogenase activity)

Soil dehydrogenase activity (DHA) varied significantly among the sites. During the winter season, DHA exhibited a range of 0.035 to 0.353 µg Triphenyl formazan (TPF) g^{-1} 24 hr^{-1}. The highest activity was observed in Natural forest, followed by Regenerating forest, Degraded forest, and Jhum fallow. It positively correlated with soil moisture content ($p<0.01$), available N ($p<0.01$) and organic carbon ($p<0.05$) while negatively correlated with available P ($p<0.01$) and soil pH ($p<0.05$).

During summer season, the values ranged from 0.058 to 0.211 µg TPF g^{-1} 24 hr^{-1}, with Natural forest again displaying the highest activity, followed by Regenerating forest, Degraded forest, and Jhum fallow. There was a significant positive correlation of DHA ($p<0.01$) with soil moisture content, water holding capacity, organic carbon, available N and K whereas a strong negative correlation with available P ($p<0.01$).

3. Effect of deforestation on AM colonization in composite root samples (CRS)

AMF root colonization in Composite root sample (CRS) varied significantly among sites. It was observed that hyphal colonization inside roots was more prevalent than the vesicular or arbuscular colonization.

During winter, it was high in Regenerating forest, though not significantly different from Natural forest, slightly less in Degraded forest and significantly less in Jhum fallow.

During summer, the highest colonization was again observed in Regenerating forest followed by Natural forest, Degraded forest and Jhum fallow. Regenerating forest was similar to Natural forest while differing significantly from Degraded forest and Jhum fallow.

4. Effect of deforestation on Inoculum potential (IP) of AMF in soil

IP varied across the sites and recorded highest in Natural forest followed by Regenerating forest, Degraded forest and Jhum fallow in both the seasons.

In Jhum fallow, it was very low in comparison to Natural forest.

5. Effect of deforestation on AMF spore population

AMF spore population showed a wide variation across the sites and between seasons. In both the seasons, it was highest in Regenerating forest, followed by Natural forest, Degraded forest and Jhum fallow.

6. Diversity and distribution of AM fungi

A total of 47 and 37 AMF species in winter and summer respectively belonging to 10 genera were recovered from the study sites. 15 species were exclusively found in winter while 5 species exclusively in summer.

Glomus was the dominant genus in all the sites and in both the seasons.

During winter, the AMF species richness was highest in Regenerating forest (28), followed by Natural forest (27), Degraded forest (25) and Jhum fallow (24).

During summer, 27 AMF species were recovered from Regenerating forest and 21 each from Natural forest, Degraded forest and Jhum fallow.

7. Correlation among physical and chemical properties of soil

Both positive and negative correlations were observed between soil's physical and chemical parameters. During winter, Pearson's correlation analysis revealed a significant positive correlation between soil moisture content and organic carbon, available N ($p<0.01$) and water holding capacity ($p<0.05$). A significant negative correlation between soil moisture content and available P and pH ($p<0.01$) was also seen. Water holding capacity had a positive correlation with K ($p<0.01$) while bulk density and porosity were negatively correlated. pH had positive correlation with available P ($p<0.01$) and negative correlation with organic carbon, available N and K ($p<0.01$). Organic carbon and available N were significantly and positively related while both exhibited a negative correlation with available P. Potassium content on the other hand had a positive correlation with available N while having a negative correlation with available P ($p<0.05$).

During summer, soil moisture content and water holding capacity exhibited a significant positive correlation with each other as well as with organic carbon, available N ($p<0.01$) and porosity ($p<0.05$) while showing a negative correlation with available P ($p<0.01$) and bulk density ($p<0.05$). Bulk density related positively with available P ($p<0.01$) while having a negative correlation with porosity, organic carbon and available N ($p<0.01$). Organic carbon and available N were significantly and positively related while both exhibited a negative

correlation with available P. Potassium content was positively related with available N ($p<0.01$) while it had a negative correlation with available P ($p<0.05$).

8. Correlation between AMF spore density and root colonization with physico-chemical properties of soil

Pearson's correlation analysis exhibited significant correlation between AMF spore population and root colonization (%) with soil physico-chemical properties across different seasons. During winter, a significant positive correlation was observed between spore population and root colonization ($p<0.01$). Furthermore, spore population also positively correlated with organic carbon, available N ($p<0.01$), soil moisture content and DHA ($p<0.05$). Root colonization also demonstrated positive correlations with soil moisture content, organic carbon, available N and DHA ($p<0.01$). Both spore population and root colonization correlated negatively with pH and available P ($p<0.01$).

Spore population and root colonization correlated positively ($p<0.01$) with each other in summer as well. Additionally, Spore population had positive correlations with soil moisture content, water holding capacity, organic carbon, available N and DHA ($p<0.01$) while showing negative correlations with bulk density, pH and available P ($p<0.05$). Root colonization also exhibited positive correlations with soil moisture content, water holding capacity, organic carbon, available N ($p<0.01$) and DHA ($p<0.05$) while showing negative correlations with bulk density ($p<0.01$) and available P ($p<0.05$).

9. Effect of season on soil physico-chemical properties

The effect of season was significant on soil moisture content and water holding capacity. A higher soil moisture content and water holding capacity was observed in summer than winter.

A significant difference between site and the season was observed in soil pH and available P.

10. Effect of season on dehydrogenase activity (DHA)

DHA in the soil varied significantly among the sites and also between the seasons. However, the effect of season was observed only in Natural forest and it was higher in winter season.

11. Effect of season an AMF

Root colonization by AMF varied significantly between the seasons. However, the effect of season was observed only in Natural forest and Degraded forest. The impact was more significant during the winter season.

Inoculum potential was relatively higher in winter.

AMF spore population exhibited wide variation between the seasons. It was significantly higher during the winter season. However, spore population in Degraded forest did not exhibit any seasonal effect.

Overall, vegetation cover variations were noted, with natural forests having higher tree density, indicating stability, while regenerating forests exhibited increased ground cover, suggesting regeneration. Significant differences in soil properties were observed across sites. DHA, a measure of microbial activity, displayed season-dependent variations, with natural forests consistently showing higher DHA levels. AM status exhibited wide variations, with IP of AMF in soil peaking in natural forests and regenerating forests consistently having the highest spore population and root colonization. Seasonal effects were noted on soil properties, DHA, AMF root colonization, and spore population, with winter generally showing higher levels. The research, thus contributes valuable insights into the complex dynamics of ecosystems undergoing deforestation and regeneration processes, emphasizing the importance of considering multiple factors for a comprehensive understanding.

CHAPTER I

INTRODUCTION

Forests face a threat from human activities, with deforestation being the most significant peril that adversely impacts the vegetation of an area, leading to soil erosion, particularly in humid tropics characterized by heavy rainfall and steep terrain. Additionally, the removal of above-ground vegetation has a detrimental effect on below-ground microorganisms (Lin et al., 2018). Natural forests undergo disturbance due to various factors, including agricultural activities (Uhl, 1987), management practices, and the demand for forest products (Fuchs and Haselwandter, 2008).

One such group of microorganisms which are functionally interlinked with the plant roots and might face adverse effects of deforestation is Arbuscular Mycorrhizal Fungi (AMF). AMF are soil fungi belonging to the division Glomeromycota (Schüßler et al., 2001) that form mutualistic symbiosis with 80% of terrestrial plants (Gao et al., 2019). Out of seven types of mycorrhizal associations recognized, Arbuscular Mycorrhizal association (AM) is the most commonly found (Brundrett, 2004). It is believed that this symbiotic relationship between higher plants and fungi occurred around 400 million years ago, playing crucial roles in the establishment of plants on land (Selosse et al., 2015). Strong evidence supports the argument that mycorrhizae, especially AM mycorrhizae, coevolved with land plants, leading to a highly dependent symbiotic relationship. This symbiosis is presumed to have originated in response to challenging environmental conditions when primitive plants transitioned from aquatic to terrestrial environments, offering significant benefits in terms of nutrient acquisition from early soils (Cairney, 2000; Taylor and Krings, 2005).

AMF contribute immensely in the growth and development of plants through increased mineral nutrient uptake, especially phosphorus. They contribute to plant survival by reducing stress and enhancing tolerance to various factors, including drought, metal toxicity, salinity, herbivory, and pathogens (Smith and Read, 2008). Additionally, AMF aid in the formation of stable soil aggregates, creating a macroporous soil structure that facilitates water and air penetration while preventing erosion (Miller and Jastrow, 1992). By influencing soil structure and texture, AMF contribute to soil quality, improving plant health and growth (Thirkell et al., 2017). In return, the host plant provides carbon compounds to the fungus. This mutualistic relationship results in a pervasive impact on both plant form and function (Brundrett, 2004).

Despite being abundant and forming diverse relationships with plant species, AMF are recognized for displaying relatively low species diversity and only over 230 species belonging to 25 genera, 11 families and 4 orders (Redecker et al., 2013). They are generally considered non-host specific, but studies have reported that they show some degree of host preference (Dhillion, 1992; Helgason et al., 2002; Vandenkoornhuyse et al., 2003).

The influence of AM extends beyond individual plants to shape the composition, and given the significant ecological benefits provided by AM fungi, including soil aggregation, nutrient cycling, and plant stress tolerance, it is clear that they have an impact on plant diversity, community structure within an ecosystem (Klironomos et al., 2000; Philip et al., 2001), and succession of entire plant communities (Van der Heijden et al., 1998; García de León et al., 2018). According to Miller and Jastrow (1992), a fully functional ecosystem with high biodiversity relies on the presence of AM fungi. AMF contribute to the growth of all plants in the community, enhance species diversity by promoting species evenness in plant communities (Park and Eom, 2007), and can affect the relative abundance of each species due to interspecific variations in their response to mycorrhizae. Consequently, the AM fungal community plays a key role in determining the composition and diversity of plant communities, and any disturbance to this relationship may lead to changes, including a decline in population status and diversity of these mycorrhizal fungi.

On the other hand, since AMF fully depend on their host plant to supply them with their photosynthate, it is also considered that plant community could also be a determinant of AM fungal community structure and function (Vandenkoornhuyse et al., 2002, 2003). Besides plants, AMF community structure is also influenced by soil physico-chemical properties (soil pH, phosphorus, carbon and nitrogen level, etc.), other edaphic factors (water, temperature, moisture etc.), and anthropogenic activities such as mining, deforestation and agricultural practices etc. (Abbott and Robson, 1991). According to Carrenho et al. (2007), plant, soil and climatic factors are related to the development of AMF, and show diverse effects on establishment of the mycorrhizal symbiosis and its efficiency. Soil moisture content has been reported to exert a positive influence on the population of AMF spores and is considered an important limiting factor in maintaining root colonization (Bhardwaj and Chandra, 2018). Dumbrell et al. (2010) even determined that soil pH had a greater influence on AM fungal communities than host plant species. Soil amendments (fertilizers, organic residues, and pH adjustments), in order to improve crop yields, change the soil properties, and the variations both in plant and fungal responses modify the outcome of the symbiosis. Nevertheless, contradicting results are also reported. While Mendoza et al. (2002) reported that there is a positive

correlation, Abbott and Robson (1991) found a reduction in AM root colonization and spore density with increasing soil pH. Soil pH also shows similar effects on spore density as that of phosphorus as some of the AM fungal species are restricted to either acidic or alkaline soils or some occur in both (Robson and Abbott 1989). While occurrence of *Glomus* spp. is cosmopolitan (Manoharachary et al., 2005), *Acaulospora* spp. (Winagraski et al., 2019) and *Gigaspora* spp. (Clark, 1997) are reported to thrive well in acidic soils.

The availability of soil nutrients (N, P, K) is also considered vital for regulating the assembly of AMF communities (Jiang et al., 2018), and vice versa (Rillig et al., 2015). The presence of organic matter enhances soil water-holding capacity, potentially leading to increased sporulation of AMF, thus demonstrating a positive correlation with the soil organic carbon content (Mathur et al., 2007). Soil nitrogen is also reported to play a key factor in shaping mycorrhizal associations, primarily acting through changes in soil pH, though, the impact of N on spore abundance is interconnected with other soil factors and is dependent on the specific host plant with which they are associated (Sylvia and Neal, 1990). However, contrasting reports about the effect of organic carbon (Hindumathi and Reddy, 2011) and nitrogen (Deepak et al., 2015) on AMF is reported. Higher soil P is known to decrease root exudates by affecting phospholipid membrane resulting into lesser arbuscule formation as well as a reduced vegetative growth of AMF (Tawaraya et. al., 2003). Nagy et al. (2009) reported the decrease in AM contribution with increasing soil P supply, as direct uptake increases which is unsurprisingly associated with a decrease in percentage of root length colonization as well.

In addition to the factors mentioned above influencing AM community structure, deforestation emerges as a significant factor impacting below-ground AM fungal communities, leading to a reduction in the quantity of infective propagules in the soil, since AMF inocula present in the top soil layer are susceptible to direct erosion (Bellgard, 1993). Their diversity is also influenced by the severity of land disturbances (Korb et al., 2003). Deforestation also alters the physico-chemical properties and structure of the soil (Veldkamp et al., 2020) and other microbial community (bacteria, actinomycetes, protozoa etc.) in the soil (Wardle, 2002). The composition and growth of vegetation also depend on the soil nutrient concentration (Sardans et al., 2017). Plant communities also rely on soil microorganisms since they play a key role in mineralization and transformation of organic matters essential for their growth and development (Bowles et al., 2014). These microorganisms play a crucial role in synthesizing enzymes, serving as biological catalysts that facilitate various reactions and metabolic processes. This activity aids in the decomposition of organic pollutants and the production of essential compounds beneficial for both microorganisms and plants (Moreno et al., 2003). Such

enzymatic activities in soil are recognized as a more sensitive bio-indicator than plants and animals of any natural and anthropogenic disturbance (Hinojosa et al., 2004). Consequently, the degradation of above-ground biomass not only impacts the soil profile but also influences microbial activity and the composition of AMF, as these factors are interconnected (Wu et al., 2020; Kumar et al., 2023).

Disturbance in an area removes pioneer plants, many of which serves as host plants and thus could cause a lower spore population. It also leads to breakdown of hyphal network in the soil, resulting in decreased AMF root colonization (Oehl et al., 2005). Agricultural practices, including soil cultivation, fallow periods, crop rotations, monoculture, non-host crops, and the indiscriminate use of fertilizers and pesticides, also impact the diversity and activity of mycorrhizae (Brito et al., 2012). Arunachal Pradesh, nestled in the Eastern Himalaya biodiversity hotspot, boasts an expansive and densely forested terrain. Unfortunately, over the past few decades, the region has witnessed accelerated deforestation, primarily driven by the expansion of agricultural and horticultural activities, anthropogenic forest fires, lumbering, urbanization, and various developmental projects. One notable traditional farming method practiced by the indigenous tribes, known as Jhum cultivation or slash-and-burn agriculture, significantly contributes to this environmental degradation. The practice involves clearing a section of the forest, followed by controlled burning. Subsequently, the burnt area is utilized for crop cultivation for 2 to 3 years before being left fallow.

Such practices are known to impact crucial soil properties and the soil microbial community (Birhane et al., 2020). The process negatively impacts AMF, vital symbiotic organisms for plant health. Loss of host plants or non-conducive edaphic conditions for regeneration of AMF, removal of above ground biomass on which these obligate symbionts depend for their carbon source, soil exposer to desiccation, high temperature (>500 ^{o}C), and soil erosion collectively contribute to reduced AM fungal spore counts in slash-and-burn fields immediately post-conversion, as observed by Sharma and Jha (2011).

The influence of deforestation on the mycorrhizal status of soil in Northeast India remains an understudied aspect. The delicate nature of the soil in this region, susceptible to erosion due to intense rainfall and steep slopes, suggests that deforestation is likely to have pronounced adverse effects. The influence of this impact may extend beyond the above-ground vegetation, encompassing alterations in the nutritional characteristics of the soil and affecting various microbial communities, particularly AMF, primarily concentrated in the topsoil layer.

Despite such disturbances, AM symbioses remain present in all natural ecosystems, even in those affected by adverse environmental conditions (Barea and Jeffries, 1995), and owing to

the multiple beneficial effects on plant performance and soil health, they are crucial for the restoration and re-establishment of the vegetation in such degraded ecosystems (Dhillion and Gardsjord, 2004) and in the preservation of ecosystem functioning and plant biodiversity (Vogelsang et al., 2006). Reforestation aids in alleviating certain adverse effects of deforestation on soil properties and microbial communities (Lin et al., 2018). However, the resulting soil conditions and their functions differ significantly from the original soils found in natural forests, and it takes time to rebuild the initial soil profile (Veldkamp et al., 2020).

Literature survey indicates that very few studies (Singh et al., 2003; Tabin et al., 2014; Bordoloi et al., 2015) have focused on the impact of disturbances on AMF in this region. However, some of these studies suffers improper experimental design, inadequate sample size, a limited number of selected parameters, and insufficient sampling.

Hence, the current study was designed to assess the impact of deforestation on AMF, vegetation, certain soil physical and chemical nutritional properties, and microbial activity. Additionally, the study explored the influence of seasonal variations on these parameters, aiming to provide a comprehensive understanding that can contribute to the development of an effective reforestation program. The research focused on examining the effects of deforestation by selecting sites with varying tree densities, including areas with less tree density, slash-and-burned fields, regenerated forest site, and a natural forest. The primary aim was to investigate how alterations in vegetation cover within tropical forests in Arunachal Pradesh affect AMF diversity, soil properties, and microbial activity.

The present study was planned with the following objectives:

(i) To study the effect of vegetation cover/tree density on soil physico-chemical properties and soil microbial activity.

(ii) To identify shrub and tree diversity in selected sites.

(iii) To study AM fungal diversity in selected sites.

(iv) To study seasonal variation in AMF spore population and inoculum potential.

(v) To quantify percent AMF colonization in roots of selected representative plants.

CHAPTER II

REVIEW OF LITERATURE

2.1 General introduction

Mycorrhiza is a highly evolved, mutualistic association between the soil fungi and plant root. The majority of higher green plants and a large number of species of fungi belonging to Glomeromycota, Ascomycota, and Basidiomycota are involved in mycorrhiza formation. Harley and Harley (1987) reported that 80% of angiosperms, all gymnosperms, and 70% of pteridophytes in the British flora are potentially mycorrhizal. In this relationship, the host plant acquires mineral nutrients, while the fungal partner obtains carbon compounds derived from photosynthesis (Harley and Smith, 1983).

These ancient fungi are believed to have coevolved with plants over the last 400 million years, aiding the colonization of dry lands and diverse ecosystems by higher plants (Pirozynski and Malloch, 1975). Strong evidence supports the argument that mycorrhizae, especially AM mycorrhizae, coevolved with land plants, leading to a highly dependent symbiotic relationship. AM fungi, as obligate biotrophs, complete their life cycles only in association with living plant roots. This symbiosis is presumed to have originated in response to challenging environmental conditions when primitive plants transitioned from aquatic to terrestrial environments, offering significant benefits in terms of nutrient acquisition from early soils (Pirozynski and Malloch, 1975; Simon et al., 1993; Cairney, 2000; Taylor and Krings, 2005).

Among the seven recognized mycorrhizal associations i.e., Arbuscular Mycorrhiza (AM), Ectomycorrhiza, Ectendomycorrhiza, Ericoid, Arbutoid, Orchidaceous, and Monotropoid, Arbuscular Mycorrhiza (AM) is the most prevalent (Brundrett, 2004), occurring in various vegetation types. This association, formed between soil fungi of the division Glomeromycota (Schüßler et al., 2001) and plant roots, is widespread in higher vascular plants, with mycorrhizal colonization more common than its absence (Wilcox, 1990).

The mycorrhiza formation in the root is initiated through the AM propagule viz., spores and infected root fragments present in the soil. AM spores (also called chlamydospore) germinate by producing germ tube that branch in all directions, forming Spore-Derived Infection Networks (SIN), presumably to increase the chance of encountering susceptible roots. Only the correct chemical signal emitted from the roots of host plants triggers a differential morphogenesis, characterized by profuse hyphal branching and proliferation towards the

signal's origin (Giovannetti et al., 1994). Suggestions have been made that the morphogenesis of AM fungi (AMF) and the establishment of symbiosis are, in part, under the control of the host genome, with infection proceeding only when specific symbiosis genes are functional (Gianinazzi-Pearson et al., 1995).

AMF significantly enhance plant growth, interspecies competitive ability, and survival by improving nutrient uptake and stress tolerance. Their crucial role in nutrient cycling and acquisition is well-established (Francis and Read, 1984; Moora and Zobel, 1996; Marler et al., 1999). They facilitate the uptake of both macro- and micronutrients, including phosphorus (P), nitrogen (N), magnesium (Mg), potassium (K), and trace elements such as nickel (Ni), copper (Cu) and zinc (Zn) in tropical and subtropical habitats (Smith and Smith, 2011). Phosphorus, despite its abundance, is challenging for plants to acquire due to its low solubility, resulting in low soil solution concentrations and limited mobility (Schachtman et al., 1998). The extra radical hyphae create an extensive surface area of roots accessing micro sites for increased absorption of P (Jakobsen et al., 1992; Hodge and Storer, 2015) and demonstrate elevated extracellular phosphatase activity, facilitating the breakdown of organic P (Vosatka and Dodd, 1998; Khade et al., 2010). Root colonization through the AM pathway is a widely used and efficient method to increase phosphorus uptake in plants, providing an additional contribution to mycorrhizal plants beyond the direct root epidermis uptake pathway (Vance, 2003; Smith and Smith, 2011).

AMF also contribute to plants' ability to endure diverse environmental stresses, such as salinity (Latef and Chaoxing, 2011; Navarro et al., 2012), drought (Asrar and Elhindi, 2011), and provide protection against pathogen attacks (Nair et al., 2015). Despite low species diversity than that of other fungal taxa, the effect of AM fungi on plant community is very diverse (Lee et al., 2013).

Despite significant benefits, not all plant species equally host AM symbiosis (Johnson et al., 1997). Throughout evolution, some plants developed multiple mycorrhizal symbioses (e.g., Arbuscular and ectomycorrhizal), while certain plant groups lost the ability to establish any mycorrhizal symbiosis (Wang and Qiu, 2006; Kariman et al., 2012). Variation in mycorrhizal dependency is associated with factors such as phenology, root morphology, photosynthetic physiology, and life-history strategies. Strongly mycotrophic plants generally exhibit coarser roots than weakly mycotrophic or non-mycorrhizal plants (Baylis, 1975; Hetrick, 1991). Warm-season plants with C4 photosynthesis are typically more mycotrophic than cool-season plants with C3 photosynthesis (Hetrick et al., 1988). Several plant families, including

Brassicaceae, Caryophyllaceae, Chenopodiaceae, Commelinaceae, Convolvularaceae, Crassulaceae, Cyperaceae, Droseraceae, Fabaceae, Juncaceae, Nepenthaceae, Polygonaceae and Zygophyllaceae rarely or never form mycorrhiza (Harley and Harley, 1987; Newman and Reddell, 1987; Tester et al., 1987; Brundrett, 2009). The absence of mycorrhizal infection in these plant groups is attributed to fungitoxic compounds in root cortical tissue (Robinson, 1972), and the lack of root exudation in weakly mycorrhizal plants (Schwab et al., 1982). Interactions at the middle lamella and/or cell wall level, along with elevated concentrations of salicylic acid, have been observed to diminish mycorrhization (Medina et al., 2003), suggesting that plants possessing a genetic predisposition for elevated salicylic acid content may have evolved to be devoid of mycorrhizal associations.

AMF are characterized by the presence of certain fungal structures such as arbuscules, vesicles, intra- and extra radical hyphae and spores generally formed in the rhizosphere (some AMF also produce spores inside the plant root). The extra radical hyphae create an extensive surface area of roots accessing micro sites (Hodge and Storer, 2015).

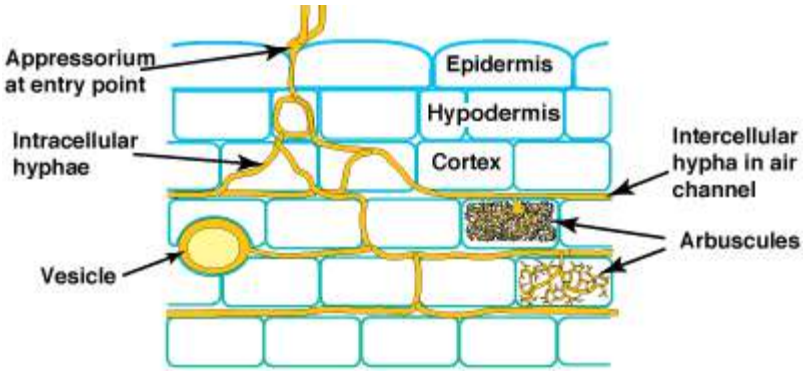


Fig. 1: Arbuscules, vesicles and hyphae of AM Fungi (adopted from Brundrett, 2008)

Arbuscules, intricate tree-like structures formed intracellularly within the root cortical cells of hosts (Smith and Read, 2008), facilitate the exchange of phosphorus, carbon, water, and other nutrients (Wright, 2005). Originating as a trunk from the main hyphae, arbuscules branch dichotomously, developing bifurcate terminal branches and occupying a significant portion of the host cell. Arbuscule formation begins approximately two days after root penetration (Brundrett et al., 1985) and occurs through invagination of the plasma membrane within individual root cortex cells. Short-lived, arbuscules complete their life cycle in 4-15 days, ultimately collapsing after chitinase formation by the host cortical cell, forming clumps near the original penetration point (Smith and Read, 2008).

Vesicles serve as the reproductive structures of AMF. These root cortex hyphal swellings, typically globose or elliptical, contain lipid and cytoplasm, developing to store high levels of lipid and glycogen (Smith and Gianinazzi-Pearson, 1988). Originating from intra-radical hyphae at terminal or intercalary positions, vesicles can be intra- or intercellular. They form soon after the first arbuscule and persist after arbuscules senesce. While all genera of AM fungi form arbuscules, certain genera (*Scutellospora*, *Dentiscutata*, *Cetraspora*, *Racocetra*, *Gigaspora*, and *Archaeospora*) do not produce intra-radical vesicles (Oehl et al., 2011). Consequently, the term "Arbuscular mycorrhizae" is now preferred for this group of associations over "Vesicular-Arbuscular mycorrhizae" (VAM).

AM spores are thick-walled multinucleate structures (Wright, 2005), which are formed as swellings on subtending hyphae in the soil or roots (Koske, 1985). These asexual structures remain infectious even in the absence of a host plant and can endure unfavorable conditions (Klironomos and Hart, 2002). Comprising lipid, cytoplasm, and numerous nuclei, spores serve as the primary survival units of AMF (Gerdemann and Nicolson, 1963). Generally globose, sub-globose, or irregularly shaped, AMF spores exhibit a variety of colors (hyaline, white, pale, pink, yellow, red, brown, or dark) and possess one to several wall layers (Goto and Maia, 2006). The identification of AMF primarily relies on the morphological characteristics of these large soil-borne spores (Oehl et al., 2009), including spore color, ornamentation, and spore wall layers (Redecker et al., 2013).

2.2 Diversity, distribution and occurrence of AM fungi

AMF species are placed into the phylum Glomeromycota (Schüßler et al., 2001). Despite being abundant and forming diverse relationships with plant species, AMF are recognized for exhibiting low species diversity (Fitter, 2005). However, molecular studies have indicated a significantly higher diversity. Only over 230 species belonging to 25 genera, 11 families and 4 orders under the Class Glomeromycetes, Phylum Glomeromcota have been reported from all over the world (Redecker et al., 2013) (Table 1).

Table 1: Classification of Glomeromycota proposed by Redecker et al. (2013)

Order	**Family**	**Genera**
Glomerales	Glomeraceae	*Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis*, *Septoglomus*
	Claroideoglomeraceae	*Claroideoglomus*
Diversisporales	Diversisporaceae	*Corymbiglomus*, *Diversispora*, *Otospora*, *Redeckera*, *Tricispora*
	Acaulosporaceae	*Acaulospora*
	Sacculosporaceae	*Sacculospora*
	Pacisporaceae	*Pacispora*
	Gigasporaceae	*Cetraspora*, *Dentiscutata*, *Gigaspora*, *Intraornaatospora*, *Paradentiscutata*, *Racocetra*, *Scutellospora*,
Archaeosporales	Amisporaceae	*Ambispora*
	Geosiphonaceae	*Geosiphon*
	Archaeosporaceae	*Archaeospora*
Paraglomerales	Paraglomeraceae	*Paraglomus*

As AM fungi form symbiosis with approximately 80% of land plants, they occur in an extensive range of plants and ecosystems, significantly aiding in nutrient uptake and survival (Khade and Rodrigues, 2008; Bhattacharjee and Sharma, 2015). The presence of AM fungi has been documented from forests, grasslands, cultivated lands and even from sand dunes and semi-desert (Harinikumar and Potty, 2002).

Glomus is considered the most common AMF species and has been recovered from different ecosystems and habitats (Muthukumar and Udaiyan, 2000; Muthukumar and Tamilselvi, 2010; Chen et al., 2012; Dessai and Rodrigues, 2012; Songachan and Kayang, 2012; Nikam et al., 2014; Pandey et al., 2016; Melo et al., 2019; Prieto-Benavides et al., 2022). *Glomus* species typically produce the smallest sized spores among different AMF taxa, enabling them to sporulate in large numbers and disperse easily within a short period (Verbruggen et al., 2012). Wang and Tschen (1997) and Clark (1997) also verified that the genus *Glomus* can germinate in a wide range of soil temperatures and pH levels. In other studies, *Acaulospora* species were observed to dominate the soil of certain tropical natural lands in Costa Rica (Lovelock et al., 2003) and secondary vegetation in Brazil (Stürmer and Siqueira, 2006). Mathimaran et al.

(2007) also reported a higher number of *Acaulospora* in the rhizosphere samples of *Zea mays* and *Crotalaria* spp. grown in agricultural fields of Kenya. In more stabilized environments, Acaulosporoid AMF species exhibit lower nutritional demands from their hosts, which can be beneficial (Gehring and Whitham 2002). Bever et al. (1996) also found that, in the same environment, *Glomus* and *Acaulospora* species typically yield more spores than *Gigaspora* and *Scutellospora* species. Due to their smaller spore size, *Glomus* and *Acaulospora* species have a quicker sporulation process (Hepper, 1984), making them more adaptable to adjusting their sporulation pattern in diverse environmental conditions. Moreover, *Acaulospora* species are known to thrive well in acidic soils (Stutz and Morton, 1996).

Dhumal and Shinde (2020) found *Glomus* spp. accounting for 50% of the total 52 retrieved AMF species followed by *Acaulospora* spp. Several other studies from Arunachal Pradesh as well as Northeast India reported *Glomus* as the most common occurring species followed by *Acaulospora* (Singh et al., 2003; Bordoloi et al., 2015; Surendirakumar et al., 2016; Tripathi et al., 2022) which might be because of *Glomus* being recognized as one of the largest and most prevalent genera, and in the slightly acidic soils of Arunachal Pradesh, India, it fosters a greater abundance of *Acaulospora* spp.

2.3 Relation between AMF and plant community structure

AM fungi are a key part of almost all plant communities, and their associations have the potential to influence the structure of plant communities by impacting the richness or evenness of coexisting plants (Brundrett, 2002). Recently, their role in shaping plant communities has garnered increasing attention in systematic analyses, empirical studies and conceptual models (Hart et al., 2003; Urcelay and Díaz, 2003; Klironomos et al., 2011). AMF have also been demonstrated to influence interspecific plant competition (Danieli-Silva et al., 2010; Wagg et al., 2011; Mariotte et al., 2013) and are considered to have a pivotal role in plant community assembly and succession (Renker et al., 2004; Kikvidze et al., 2010), thus indicating their significance in ecological restoration. A key mechanism influencing the impact of AMF on interspecific competition is the variable degree of benefits that AMF confer to different plant species (Urcelay and Díaz, 2003).

The conceptual model introduced by Urcelay and Dıaz (2003) posits that the impact of AMF on plant diversity can be elucidated by considering the relative mycorrhizal response of dominant and subordinate plants. If dominant plants exhibit a substantial mycorrhizal response, AMF are expected to boost their competitive advantage, leading to a decrease in overall plant

diversity (Hartnett and Wilson, 1999). Vogelsang et al. (2006), through the manipulation of AMF inoculation and phosphorus conditions, discovered that the variation in the mycorrhizal response of dominant species to AMF colonization have been identified as a contributing factor to alterations in plant diversity. In contrast, when subordinate plants exhibit a high dependence on mycorrhizal associations, the introduction of AMF may enhance their competitive capabilities, potentially leading to an increase in overall plant diversity (Van der Heijden et al., 1998). The findings of Lin et al. (2015) further emphasized that the impact of AMF on community structure is significantly influenced by the mycorrhizal responses of dominant species, aligning with the theoretical model outlined by Van der Heijden (2002). According to this model, when dominant species exhibit a heightened mycorrhizal response, the introduction of AMF strengthens the competitive advantage of these dominant species. This heightened competitive edge results in increased suppression of subordinate species, ultimately leading to a decline in overall plant diversity.

On the contrary, as AMF rely entirely on their host plants for photosynthate supply, it is also posited that the plant community could serve as a determinant of AM fungal community structure and function (Sanders and Fitter, 1992; Bever et al., 1996; Vandenkoornhuyse et al., 2002, 2003). However, assessing which group is influencing the other is challenging, as both groups may exert some level of influence on each other, potentially at different spatial and temporal scales (Zobel and Öpik, 2014; Martinez-Garcia et al., 2015; García de León et al., 2016). Additionally, in a stable ecosystem (climax community), regional covariation between AMF and plants could emerge as a result of environmental gradients (Horn et al., 2017).

According to Chaudhary et al. (2008) and Wang et al. (2018), the composition of AMF communities undergoes changes in response to plant growth and the plant community. In a study by Zhang et al. (2020), a notable positive relationship was observed between tree diversity and the composition of AMF communities, indicating that a higher diversity of one symbiotic partner promotes greater diversity in the other. Wang et al. (2018) investigated the impact of three different host plant species (*Hedysarum laeve*, *Artemesia ordosica*, and *Psammochloa villosa*) on AMF diversity. The root structure of each plant influenced the colonization of AMF, with *H. laeve*, being a leguminous plant, affecting the physiological processes of AM fungal colonization, while others influenced AM growth through photosynthetic performance and the secretory activity of the root system.

Zobel and Öpik (2014) utilized the dispersal differences between AM fungi and plants to reconsider the Driver and Passenger hypotheses proposed by Hart et al. (2001). They also

introduced the "Habitat hypothesis" to differentiate situations where AMF and plant communities co-vary without direct causal links, as opposed to the null hypothesis of no covariation (independence). For example, during primary succession, plants, acting as a potential filter, usually arrive before AMF, with AMF behaving as Passengers by following plants. In an established AMF assemblage, dispersal limitation can make the AMF assemblage more influential in determining which plants establish during secondary succession, turning the AMF assemblage into the Driver (Zobel and Öpik, 2014).

Certain studies suggests that plant might reciprocally reward superior fungal partners with increased carbohydrate allocation (Bever et al., 2009; Kiers et al., 2011; Verbruggen et al., 2012). Additionally, specific plant communities can lead to the development of distinct AMF communities (Hausmann and Hawkes, 2009). Therefore, based on the available evidence, it can be inferred that AMF and plant species exert mutual influence on each other's community structure, and alterations in one partner can impact the other.

2.4 Influence of soil physico-chemical properties on AMF

Various physico-chemical properties of soil are known to influence the hyphal growth, root colonization, and the plant growth-promoting effects of AM (Abbott et al., 1992). These properties include temperature (Smith and Roncadori, 1986), soil moisture (Karasawa, 2000), pH (Koomen et al., 1987), and soil phosphorus availability (Abbott et al., 1984; Thompson et al., 1986). The composition and distribution of AMF communities may be affected by these environmental parameters and the species of the host plant (Hazard et al., 2013; Jansa et al., 2014).

Studies spanning various soil types and habitats, including agricultural fields, found that soil texture significantly influences the structure and composition of AMF communities (Oehl et al. 2010; Santos-González et al., 2011; Alguacil et al., 2016). Manjunath et al. (1983) noted a reduction in the number of AMF spores in *Citrus* seedlings when grown in clayey soil compared to those grown in sandy soil. A higher AMF population in soils with higher sand content has also been reported (Villela and Proctor, 2017; Prieto-Benavides et al., 2022) .

Soil moisture content has been reported to exert a profound influence on the population of AMF spores, and the moisture level optimal for plant growth coincides with favorable conditions for AMF sporulation (Redhead, 1975). Khanam et al. (2006) and Kumar et al. (2010) reported significant positive correlation between soil moisture content and AMF spore

population. Bhardwaj and Chandra (2018) considered soil moisture as an important limiting factor in maintaining root colonization in tree species and reported a positive correlation between soil moisture and root colonization but a negative correlation between moisture and spore population.

Soil pH is regarded as one of the key environmental factors in shaping AMF communities. It can influence the root colonization, reproduction and AMF community structure (Singh et al., 2003; Bainard et al., 2015; Xu et al., 2017). Abbott and Robson (1977) have reported a correlation between soil pH and the distribution of certain AMF species. Hayman and Tavares (1985) observed significant variability in the symbiotic effectiveness of AMF species at different soil pH levels, with some exhibiting marked pH preferences in stimulating plant growth, while others demonstrating a broad pH tolerance. Specifically, Koomen et al. (1987) noted though the root colonization was not affected by varying pH, the most effective plant growth stimulation by *Glomus* E3 occurred only at pH 4.8, whereas all other species could do the same at near-neutral pH. Though some workers have found a positive correlation between soil pH and AMF activity (Tahat and Sijam, 2012; Rajeshkumar et al., 2013; Liu et al., 2020), there are also contrasting report of a negative correlation (Songachan et al., 2011; Nongkling and Kayang, 2017). Singh et al. (2003) and Akond et al. (2008) observed that slightly acidic to neutral soils exhibit moderate to high level of AMF spores in the jhum fallow and natural forest of Arunachal Pradesh (NE India), and agricultural lands of Dhaka (Bangladesh) respectively.

Soil organic carbon is reported to influence AMF diversity on a global scale (Davison et al., 2015). A positive influence of OC on AMF activity has been reported by several workers (Khanam et al., 2006; Birhane et al., 2020; Dhumal and Shinde, 2020). Yang et al. (2011) also reported positive correlation between OC and AMF spore germination and distribution in North-west region of China. However, Hindumathi and Reddy (2011) noted high spore population in fields of Soybean and Mungbean having a low OC content which might be due to existence of a tri-partite relationship between host plant, rhizobia and AMF.

Sylvia and Neal (1990) suggested that nitrogen can either enhance or inhibit AM root colonization and spore production by altering the soil pH. They also reported N as an important factor in shaping mycorrhizal associations, primarily acting through changes in soil pH. Khanam et al. (2006), Egerton-Warburton et al. (2007) and Silvana et al. (2020) reported a positive correlation between available N and AMF spore population and root colonization, while Bago et al. (2004) and Deepak et al. (2015) reported an inverse relationship between them. Liu and Li (2000) also reported that elevated concentrations of nutrients in the soil reduces mycorrhizal

colonization. In some cases, the impact of nitrogen fertilization on mycorrhiza formation has been reported to vary among different crop species and cultivars. Hayman (1975) demonstrated that nitrogen fertilizer significantly reduced AMF spore population in wheat plots but had no such effect in *Vicia faba* plots at the same site. Both suppression of root colonization due to nitrogen fertilization (Buwalda and Goh, 1982; Johansen et al., 1984) and increased root colonization and spore production (Aziz and Habte, 1989; Furlan and Bernier-Cardou, 1989) have been observed in various plant species.

It has been suggested that an elevation in the phosphorus (P) status of the plant hinders the formation of arbuscular mycorrhizas. This is linked to a decrease in the concentrations of potential fungal metabolites, such as soluble carbohydrates and free amino nitrogen compounds, in both roots and root exudates (Thompson et al., 1986, 1990; Muthukumar and Udaiyan, 2002). Mohammad et al. (2004) also reported that elevated soil P concentrations could disrupt the life cycle of AMF, inhibiting spore production, consequently reducing spore density in the soil. This interference leads to a reduction in the growth of external hyphae, subsequently decreasing the rate of AMF spread through secondary infections. Amijee et al. (1989) observed a reduced number of entry points and the formation of abortive entry points at high soil P concentrations, suggesting that increased resistance to root penetration is a significant factor contributing to the delayed establishment of infection. Other researchers have also noted a decline in infection intensity and reduced growth of external hyphae with added P (Thompson et al., 1986; Sivakumar, 2013). Additionally, Amijee et al. (1989) also found a decrease in the formation of vesicles inside host roots due to increased soil P. Khanam et al. (2006), Bainard et al. (2014), Birhane et al. (2017), Nguyen et al. (2019) and El-Sherbeny et al. (2022) also reported an inverse relationship between available P and AMF activity. Yet, under circumstances where the initial phosphorus (P) concentration is exceptionally low, a minor addition can actually boost infection (Schubert and Hayman, 1986) which was potentially attributed to an increased length of root available for the fungus to colonize (Amijee et al., 1989).

Effect of Potassium (K) on AMF activity is reported to be inconsistent. While Daniels and Trappe (1980) reported no effect of K on AMF, Ebbers et al. (1987) reported a significant positive correlation between spore abundance and available soil K in prairie drop seed *(Sporobolus heterolepsis*). Zahka et al. (1995) indicated that concentration of soil K was a key predictor for variations observed in colonization levels and for the occurrence of arbuscules in the root cortical cells whereas Panwar et al. (2011), Nongkling and Kayang (2017) and Dhumal

and Shinde (2020) reported an inverse relationship between K and AMF spore population and root colonization.

The role of other nutrients on root colonization and spore abundance of AM fungi is little known compared to N, P and K. Prieto-Benavides et al. (2022) observed a positive correlation between spore population and micro-nutrients such as Magnesium (Mg), Manganese (Mn), Calcium (Ca), and Boron (B) during the dry season. In a separate study, Dhumal and Shinde (2020) found a positive relationship between spore population and Zinc (Zn), Iron (Fe), Manganese (Mn) and Copper (Cu), along with a negative correlation with Sodium (Na). On the other hand, they found a positive variation in root colonization with Zn and Cu while a negative relationship with Mn and Na. Mcllween and Cole (1978) also documented the stimulatory impact of low concentrations of Zn on spore germination. Similarly, Sreenivasa and Bagyaraj (1988) reported increased root colonization and sporulation of *Glomus fasciculatum* in association with Rhodes grass when exposed to suboptimal levels of Mn, Cu and Zn.

2.5 Effect of Season on AMF status

The development and seasonal fluctuations in AMF sporulation and colonization has been studied in several mycorrhiza dependent plant species or communities (Brundrett 1991; Sanders and Fitter 1992; Merryweather and Fitter 1998; Guadarrama et al., 1999; Muthukumar and Udaiyan 2002; Prieto-Benavides et al., 2022). Gemma and Koske (1988) linked the variation in AM fungal spores to initiate mycorrhization to seasonal changes, as newly formed spores necessitate a period of dormancy. The considerable variations in AM colonization levels among seasons may arise from rapid root growth or turnover during periods with favorable soil moisture and temperature conditions (Brundrett, 1991). Seasonal fluctuations in AM spore numbers have also been attributed to germination activities (Gemma and Koske, 1988), soil micro- and macrofaunal activities (McGee and Baczocha, 1994), and the destruction of AM spores by soil fungi and other parasites (Lee and Koske, 1994). Muthukumar and Udaiyan (2002) observed distinct seasonal patterns in levels of mycorrhizal colonization, root length colonized by AM structures and spore numbers. However, these patterns were not consistent in subsequent years.

da Silva et al. (2014), Deepika and Kothamasi (2015), Vieira et al. (2020), Prieto-Benavides et al. (2022) de Quevedo et al. (2022) reported increased mycorrhizal colonization and spore density during the dry season compared to the rainy season. Such outcome of intense sporulation in the dry season is considered a survival strategy employed during the water stress

phase of host plants. However, there are some conflicting and inconsistent reports too on the effect of seasonal variation on spore population and root colonization. For instance, Tapwal et al. (2023) noted a higher spore population and low root colonization during winter while a lower spore population and higher root colonization during summer. Boumari et al. (2014) also studied the effect of season on AMF associated with Date palms in Ottawa, Canada and observed a significantly higher root colonization level during the wet season, which correlated with the vegetative growth period of Date palms and the availability of soil water. This suggests that the AMF associations were effectively established and remained precisely functional when Date palms required increased nutrient allocations to support heightened metabolic activities, synchronized with higher water availability and lower surrounding temperatures (Sanders and Fitter, 1992; Bohrer et al., 2004), whereas during the dry season, root colonization levels declined, aligning with the fruit maturation stage. This decline implies that a well-established symbiosis is not as crucial during the maturation stage as it is during the plant's vegetative growth period. During the dormant status of date palms, a reduction in root colonization levels was also observed. Oliveira et al. (2005) reported maximum colonization and spore population during rainy season.

2.6 Effect of deforestation on soil properties

A shift in vegetation cover and deforestation is known to alter the physico-chemical properties of soil and its structure (Pickett, 2001; Yan et. al. 2016). Soil nutrients are crucial in all biological processes and the composition and growth of vegetation also depend on their concentration in the soil (Sardans et. al., 2017) thus having a profound influence on the plant communities and health and stability of ecosystem functions (Tilman, 1985). Different degrees of disturbances are also reported to cause variation in soil texture (Mishra et. al., 2019). Tyagi et al. (2013) observed decreased moisture content in deforested areas compared to forested sites, attributing the loss of vegetation and increased evaporation as factors contributing to the moisture reduction. Degraded forest lands are also reported to have higher bulk density (Sharma et. al., 2010; Bhuyan and Laskar, 2020) due to higher clay content in the soil and higher organic carbon content.

A lower pH in the undisturbed sites comparison to the disturbed sites is also reported (Barraclough and Olsson, 2018; Hong et al., 2019) where lower pH in the natural forest is attributed to higher organic matter which on decomposition leads to production of more organic acids, thereby lowering the soil pH. It is also reported that burning of fields causes denaturation

of organic acids releasing base cations leading to an increase in soil pH in Jhum fields (Certini, 2005).

Adverse impact of vegetation degradation on soil nutrients was observed in wet meadow on the Quinhai-Tibet plateau (Wu et. al., 2020). Numerous studies conducted in Jhum fields of Arunachal Pradesh and Northeast India (Singh et al., 2003; Binarani and Yadava, 2010; Kumar et al., 2023) have reported lower soil nutrient levels compared to native forests. Vegetation degradation leads to a reduction in aboveground biomass, resulting in decreased litter production and a diminished amount of plant residues entering the soil. This ultimately lowers the primary source of organic matter (Wang et al., 2014; Yan et al., 2018) and adversely affects soil structure, leading to a slowdown in nutrient cycling (Lost et al., 2007; Foote et al., 2015). Contrarily, certain studies have identified elevated soil phosphorus (P) levels in degraded lands compared to forest sites. Hinsinger et al. (2011) suggested that a decline in plant biomass leads to a reduction in phosphorus absorption, leaving more phosphorus in the soil. Turrion et al. (2000) attributed this to the deposition of livestock excrement, adding to organic forms of phosphorus. Moreover, in deforested and Jhum fields, soil organic P undergoes pyro-mineralization, converting to orthophosphate, and high soil pH increases phosphorus availability in the absence of calcium, resulting in an elevated level of available phosphate (Giardina et al., 2000).

2.7 Effect of deforestation on microbial activity (DHA)

Plant communities rely on soil microorganisms since they play a pivotal role in mineralization and transformation of organic matters essential for their growth and development (Chen et al., 2003; Bowles et al., 2014). Soil microbial activity is measured by determining the enzyme activities in the soil since these enzymes are very sensitive to natural as well as anthropogenic disturbances and responds quickly to the changes (Dick, 1997). Vegetation degradation causes a decline in soil microbial activity, attributed to the reduction in above-ground biomass and soil nutrient levels, as these factors are interdependent (Wu et al., 2020, Kumar et al., 2023).

Binarani and Yadava (2010) found the lowest microbial activity in Jhum fields and fallow sites, contrasting with a protected forest in Manipur (North-East India). Similarly, Kumar et al. (2023) observed decreased DHA in Jhum fields in Arunachal Pradesh (India), indicating that field burning led to the reduction of soil microorganisms and subsequently decreased microbial activity.

2.7 Effect of deforestation on AMF

AMF exist as obligate symbionts with the plant's roots, and AMF inocula are present in the top soil in the rhizosphere, susceptible to direct erosion (Bellgard, 1993). Hence, alterations in above-ground biomass leads to changes in their belowground partners. Anthropogenic activities reduce below ground AM fungal communities, and the intensity of such disturbances also determines AMF diversity (Allen et al., 1998; Korb et al., 2003). Disturbance in an area removes pioneer plants many of which serves as host plants and thus could cause a lower spore population. Birhane et al. (2020) also attributed an increased litter fall, and more root biomass which maintains a diverse AMF community in natural forest, for harboring more AMF species and greater spore population in forest sites. Diversity and population of AMF is also reported to increase with canopy cover since plants with more canopy convert higher solar inception into photosynthates which provides carbon source to the AMF (Sarkar et al., 2014). AMF hyphal network in the ground is considered as an important cementing agent facilitating soil aggregation and maintaining its stability (Miller and Jastrow, 1992), so, its break down due to a change in land use, is also reported to decreases AMF root colonization (Oehl et al., 2005) and also the inoculum potential (Zangaro et al., 2000).

Agricultural practices, including soil cultivation, fallow periods, crop rotations, monoculture, non-host crops, and the indiscriminate use of fertilizers and pesticides, also impact the diversity and activity of mycorrhizae (Verbruggen and Kiers, 2010; Brito et al., 2012). Soil cultivation disrupts the AMF hyphal network, resulting in a significant decline in mycorrhizal root colonization and P absorption from the soil (Evans and Miller, 1988; McGonigle and Miller, 1996). Continuous monoculture of a non-mycorrhizal host may significantly reduce mycorrhizal root colonization (Sieverding and Leihner, 1984) and the number of AMF spores (Smith, 1980). Additionally, monocultures favor ineffective AMF, depleting host photosynthates and causing plant stunting and yield depressions (Hendrix et al., 1992). Consequently, potentially beneficial AMF may be lost in monoculture systems.

In jhum fields, the reduction in AMF spore population, its diversity, infective propagules and root colonization are ascribed to repeated burning of the fields leading to loss of primary host plants on which these fungi depend for their carbon sources, and adverse edaphic conditions for AMF regeneration (Bordoloi et al. 2015; Birhane et al. 2020). Fallow periods or growing a non-mycorrhizal host have been reported to exert a profound effect on AMF activity and diversity, and in case of a long fallows (>12 months), AMF propagule density gets severely

reduced (i.e., by up to 40%) because of the absence of a living host (Harinikumar and Bagyaraj, 1988; Thompson, 1987).

Li et al. (2007) observed a reduction in spore population due to agricultural practices, with the highest spore population being in never-cultivated fields, slightly lower in old fields, and highly reduced in cultivated fields in the hot and arid ecosystem of Southwest China. Su and Guo (2007) obtained a comparable outcome, noting a significant decrease in the mean spore population of AMF in overgrazed plots compared to non-grazed ones in the Inner Mongolia steppe.

Few studies done in Northeast India also confirms the reports of decreased AMF activity with intensified degradation. Singh et al. (2003) reported higher AM species diversity in an undisturbed natural forest has than a Jhum fallow in Arunachal Pradesh (India). Sharma and Jha (2011) also reported in their work conducted in Assam (India) that the AMF spore diversity was highest in undisturbed forest, lower in slash and burn forest and lowest in monoculture forest. Bordoloi et al. (2015) examined the diversity of AMF in seven different land use systems in Arunachal Pradesh in the Eastern Himalaya, where the highest species richness and diversity index was recorded in natural forest and least in Jhum fallow and Tea garden due to high disturbance of fire and application of fungicides and inorganic fertilizers.

However, some studies also documented equal or higher spore counts and diversity in deforested lands compared to natural forests (Picone, 2000; Zhang et al., 2004). Their studies reported that AMF community composition was not largely influenced by deforestation and in fact, the total species richness was higher in the deforested land. They concluded that the main reason for increased spore density and species richness in the deforested land is the annual herbaceous plants as seen by Kovacic et al., (1984). These annual herbaceous host plants associate with more AM fungal spore production than the evergreen broad-leaved trees (Hetrick and Bloom, 1986; Trappe, 1987). Furthermore, deforestation has also been implicated to decrease the soil moisture content and increase the soil temperature which is considered usually good for spore production by AMF (Guadarrama and Álvarez, 1999; Parke et al., 1983). Similar findings were reported by Solís-Rodríguez et al. (2020) in a study on the diversity and distribution of AMF in the tropical low flooding forest (TLFF) of Yucatan, Mexico. Their observations revealed a significant relationship between AMF diversity and the diversity, abundance, richness, and cover of herbaceous vegetation. Additionally, spore abundance was found to be related to the basal area and abundance of trees.

Role of AMF in restoration and reconstruction of fragile vegetation or degraded ecosystems (Caravaca et al., 2003), and in the preservation of plant biodiversity and ecosystem functioning (Vogelsang et al., 2006) has been widely acknowledged. This explains the need to enumerate and identify the indigenous AMF population which can be successfully applied in restoration practices.

Arunachal Pradesh has fantastic rain forests and the area comes under a biodiversity hotspot. These forests have come under threat in recent years particularly due to the increasing demand for timber and land for shifting cultivation. Out of the 82 per cent forest cover in the region, 35 per cent cover is good (dense) and the remaining 29 per cent comprises post-jhum open/degraded secondary successional forests. The latest survey by government agencies also indicates that more forests have been put under jhuming than abandoned for post-jhum regeneration annually. Though the state is endowed with diverse forests and magnificent wildlife, the soil of the region is very fragile in nature and subjected to heavy rainfall. The land has a steep slope and thus likely to cause huge runoff during monsoon leading to leaching of soil nutrients, altered physico-chemical properties of soil and loss of microbial community including AM fungi. A survey of literature reveals that no proper study has been done so far to assess the effect of disturbance on AMF community in this region. The transition from forest land to Jhum field followed by secondary succession during Jhum fallow offers a unique condition to study the transition in AM community structure due to deforestation.

CHAPTER III

MATERIALS AND METHODS

3.1 Study area

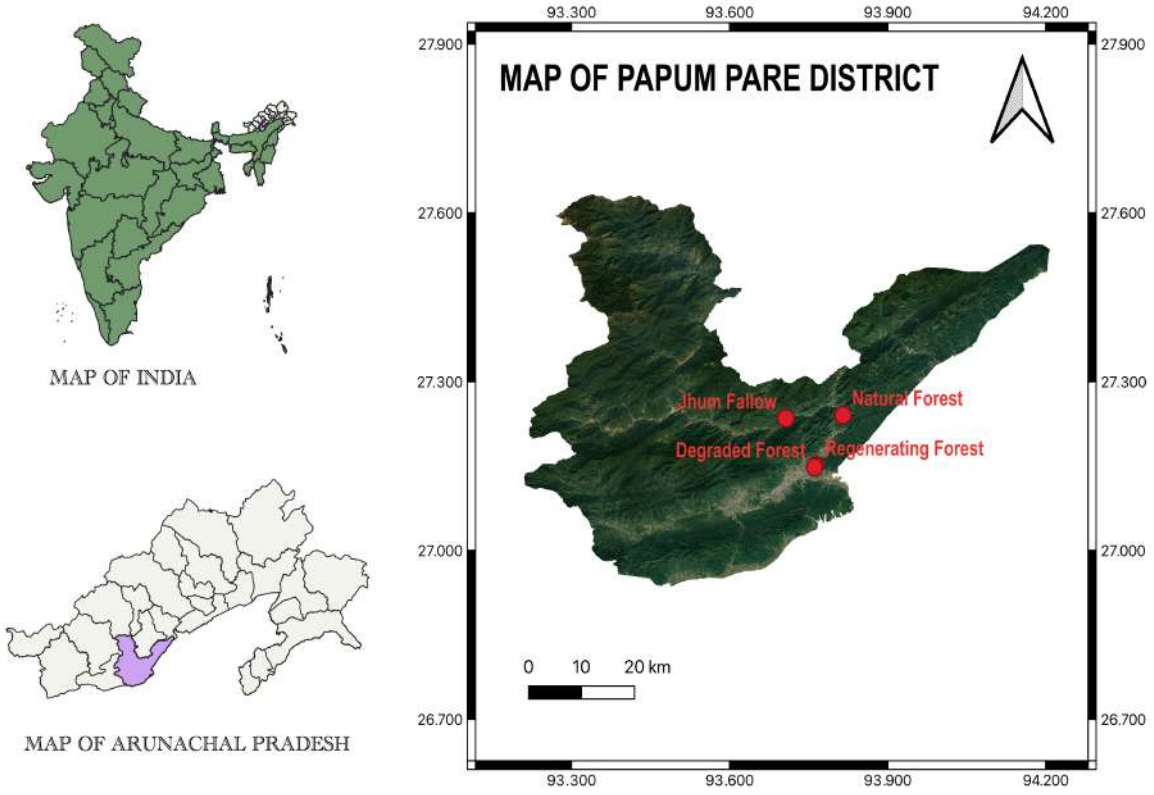


Fig. 2: Map of Study Area (Papum Pare district)

The investigation was carried out in different study sites located in Papum Pare district of Arunachal Pradesh, situated in the northeastern part of the state covering an area of approximately 3,462 sq kms. The region is predominantly mountainous, characterized by the Himalayan ranges intersected by valleys and ravines with numerous streams and rivers. The elevation ranges from 45 to 1200 meters above sea level, featuring lush green forests, deep river valleys, and scenic plateaus. The natural vegetation primarily consists of tropical semi-evergreen and sub-tropical evergreen forests. The district's climate is significantly influenced by its terrain, varying with natural divisions and elevation. The rainfall is exceptionally heavy, resulting in a highly humid climate at lower elevations. The local inhabitants, Nyishis, belong

to Tibetan-Mongoloid stocks, and agriculture and horticulture are the primary occupations. The people practice Jhum (shifting) cultivation on steep slopes, wet rice cultivation in low-lying areas, and subsistence agriculture.

3.2 Study sites

Four different study sites were selected in the district to carry out the study; a natural forest (N $27^0$14'26" E $93^0$48'55"; 442 m; Jampa village), degraded forest (N $27^0$14'16" E $93^0$75'92"; 299 m; within University campus), regenerated forest (N $27^0$15'16" E $93^0$76'32"; 270 m; within University campus), Jhum fallow (N $27^0$14'06" E $93^0$42'25"; 418 m; Kheel village,) and was selected for the collection of samples (soil and plant roots). The work plan of the study has been given in Fig. 2.

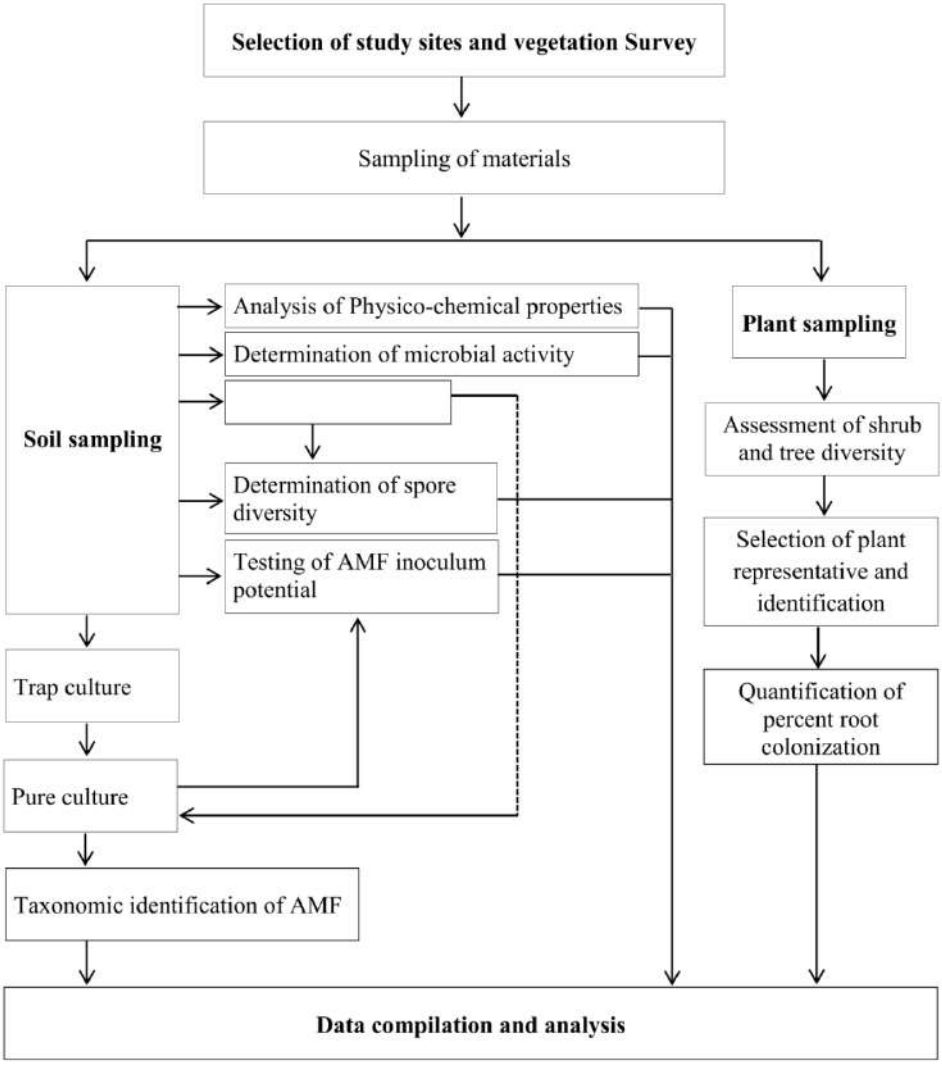


Fig. 3: Work plan

3.3 Soil sampling

Soil samples were collected twice in a year in two different seasons (November to February; May to August) from four different study sites. Randomized sampling by belt method was followed for collection of samples. In case of forest sites, four plots each of 100x100 m size was marked randomly at a distance of 500 m. Five horizontal belts were laid at a distance of 25 m apart in each plot. Along each belt, a soil core of 4 cm diameter from 0-25 cm depth was collected from five different sites at almost equal distance. The samples collected from each belt were mixed to make a composite sample. In case of Jhum fallow sites, samples were collected almost in equal numbers from four adjacent fields. The samples were collected both from the rhizosphere of trees and other plants and from bulk soil. 1 kg of composite soil from each plot were kept in sterile zip lock bag and stored in 4^{o}C.

3.4 Collection of root samples

Fine roots were collected along with the soil samples and separated carefully. The roots were further mixed well to form a composite sample. The collected roots samples were washed properly and preserved in FAA solution (FAA- 50% Ethyl alcohol, 5% Glacial acetic acid, 10% Formaldehyde, 35% Distilled water) for further quantification of root colonization.

3.5 Determination of vegetation cover

The vegetation cover at selected sites was measured by line intercept method (Canfield 1941). A transect of 100 m length was laid in each plot of the sites. 10 sampling points at an interval of 10 m were set on each transect.

Tree density was calculated by Nearest individual method, a type of distance method (Cottam and Curtis 1956) that is again a type of line intercept method (Canfield 1941). At each sampling point along a transect, the plant closest to the point was located. Then, the distance between the sampling point and then nearest tree (nearest individual) was measured (Fig. 4).

Tree density was calculated by the following formula:

$$\text{Density} = \frac{1}{\text{Mean Area}} = \frac{1}{(2\bar{d})^2}$$

where, $\bar{d}$= mean distance between sampling plant and its Nearest individual

Canopy coverage of trees was measured by spherical densiometer using a convex mirror suitably itched with squares. It was calculated by the formula given below:

$$\text{Canopy coverage (\%)} = \frac{\text{Number of observed hits}}{\text{Total number of hots}} \times 100$$

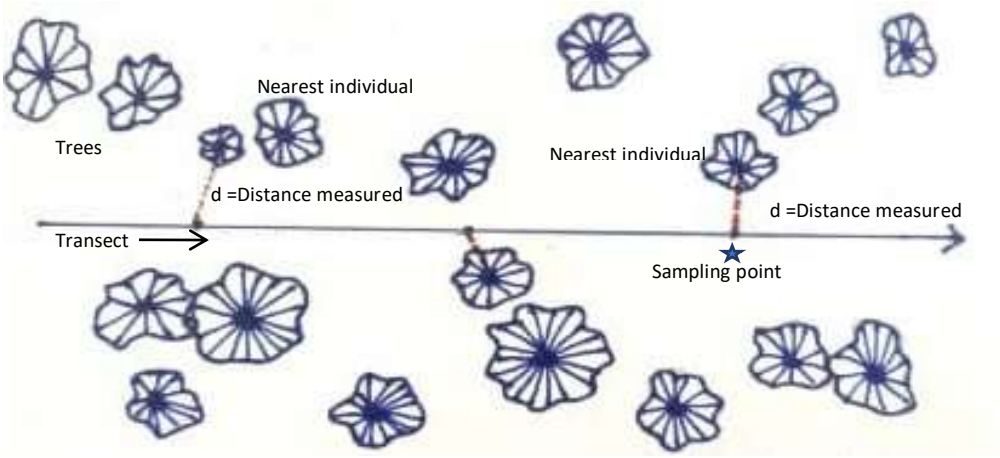


Fig. 4: Nearest individual method. A distance method used for measuring density in plant populations with randomly distributed individuals (Cotton and Curtis, 1956)

Dominant shrubs were recorded by counting their frequencies at 20 sampling points each at 5 m interval along the 100 m transect. Ground vegetation cover was calculated by measuring the total foliage cover occupied on the ground by shrubs and herbs along the 100 m transect.

Ten trees along each transect were divided into different size classes based on Diameter at Breast Height (DBH) at an interval of 10 cm.

3.6 Collection of plant samples

Representative plant species including herbs, shrubs and trees were selected for quantification of AM root colonization. These plant species were collected twice a year from each sampling site, documented through field photography, preserved as herbarium specimens, and identified based on their taxonomic characteristics using available literature.

3.7 Quantification of AMF root colonization

Plant roots were stained for AM fungal structures following the method outlined by Phillips and Hayman (1970) and modified by Koske and Gemma (1985).

AM root colonization was quantified in freshly collected roots or preserved root samples stored in Formalin-Acetic-Alcohol (FAA) solution (FAA- 50% Ethyl alcohol, 5% Glacial acetic acid, 10% Formaldehyde, 35% Distilled water). Washed root segments were cut into 1 cm segments and cleared in 10% KOH solution (w/v) by heating at 90° C in a water bath for 2 hr (for pigmented roots and 1 hr for non-pigmented roots). Cooled root samples underwent multiple washes with tap water. Subsequently, they were either acidified in a 1% HCl solution

for one hour at 90°C or soaked in 1% HCl overnight. The acidified roots were then stained with trypan blue solution (500 ml glycerol, 450 ml H_2O, 50 ml 1% HC1 containing 0.05% trypan blue) by heating at 90° C for 30 min. Excess stain was removed using de-staining solution (500 ml glycerol, 450 ml H_2O, 50 ml 1% HC1) at room temperature. 10 root segments were randomly picked up and mounted on a slide with cover glass and observed under microscope for percent root colonization and infection.

Percent root colonization was determined by Magnification intersection method (Mc Gonigle et al., 1990) under compound microscope (Nikon, Eclipse 200) by randomly selecting 30 root segments for each plot.

Root segments with mycelium, vesicles and arbuscules were considered as positive infection. The presence or absence of infection in the root segment was recorded and calculated as:

$$\text{Root colonization (\%)} = \frac{\text{Number of intersection of infection}}{\text{Number of intersection examined}} \times 100$$

3.8 Determination of physical and chemical properties of soil

Various soil properties (soil texture, bulk density and porosity, water holding capacity, moisture content, pH, organic carbon, available P, available N and available K) were determined by following standard methods. The detailed descriptions of methods used are given below:

3.8.1 Soil texture

The soil samples were sieved to determine the sizes of soil up to 0.150 mm. For the soil particles below 0.150 mm size, hydrometer analysis was carried out. Hydrometer analysis was conducted for soil particles below the 0.150 mm size. The proportions of very fine sand, silt, and clay were determined using the following formulas:

Clay (%) = A+T x 100/Sw

Silt + Clay (%) = B+T x 100/Sw

Where,

A = hydrometer reading after 5 hours

B = Hydrometer reading after 4 minute 48 second.

Sw = Weight of the sample soil

T = Temperature adjustment

Silt (%) = (Silt + Clay) – Clay

Sand (%) = 100 – (Clay + Silt)

3.8.2 Bulk density

Bulk density was measured by core method (direct measurement) using metal rings of varying lengths. Measurements were conducted at the same location and different depths (5 cm, 10 cm, and 15 cm). The volume of each cylindrical ring (πr^2h) was calculated and recorded. Soil samples from different depths were collected in zip-lock bags, weighed with the bag, and recorded. To account for the weight of the bag, an empty zip lock bag was weighed. The soil sample was thoroughly mixed in the bag by kneading with fingers, and a scoop of subsample of loose soil was taken and weighed. The subsample was oven dried for two or four-minute cycles at full power. The weight of the oven-dried subsample was recorded, and soil water content was calculated by following equation:

$$\text{Soil water content (g/g)} = \frac{\text{weight of moist subsample} - \text{weight of oven dried subsample}}{\text{weight of oven dried subsample}}$$

Dry weight and bulk density of the soil was calculated using the following formula:

$$\text{Dry wt. of soil bulk sample} = \frac{\text{Wt. of field moist soil} + \text{bag} - \text{Wt. of bag}}{1 + \text{Soil water content}}$$

$$\text{Bulk Density (g/cm3)} = \frac{\text{Dry wt. of bulk soil}}{\text{Volume of soil core}}$$

3.8.3 Porosity

Soil porosity was determined by calculating bulk density using the following equation:

$$\text{Soil porosity (\%)} = 1 - \frac{\text{Soil bulk density}}{2.65}$$

*Default value of 2.65 is used as a rule of thumb based on the average bulk density of rock with no pore space.

3.8.4 Water holding capacity

Water holding capacity was determined by weighing dry filter paper before and after saturation with water. The Keen's box was weighed and the wet filter paper was placed in it,

followed by filling the box tightly with soil sample and re-weighing. The soil was saturated with distilled water, left in a tray for 24 hours, and the box with saturated soil was weighed again. Subsequently, the saturated soil box was placed in an oven at 105°C for 24 hours and re-weighed.

$$\text{Water Holding Capacity (WHC)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where, W_1= Weight of the box + filter paper

W_2=Weight of box + filter paper + saturated soil.

W_3= Weight of the box + soil after oven dried.

3.8.5 Moisture content

The soil moisture content of the samples was determined following the method recommended by Donahue et al. (1987). The initial moist weight of each soil sample was recorded, and subsequently, the samples were oven-dried at 100°C for 24 hours. The dried samples were then weighed until a constant weight was achieved.

$$\text{Moisture Content (\%)} = \frac{\text{Weight of moist soil} - \text{Weight of oven dry soil}}{\text{Weight of oven dry soil}} \times 100$$

3.8.6 pH

For pH determination, a soil and water suspension with a 1:2 ratio was created following the procedure outlined by Jackson (1958). Specifically, 25g of soil was combined with 50 ml of distilled water in a 100 ml beaker. The suspension was stirred intermittently with a glass rod for 15-30 minutes. The pH meter was calibrated using a known buffer solution, and the suspension was stirred again before immersing the electrode. The pH reading was recorded, and the electrode was kept in immediate working condition by immersion in distilled water.

3.8.7 Organic carbon

For determination of Organic Carbon of the soil sample, the method described by Walkley and Black (1934) was followed. Ground soil samples were sieved through a 0.2 mm sieve. In a 500 ml conical flask, 0.5g of soil sample and 10 ml of 1N $K_2Cr_2O_7$ were added, and the flask was gently swirled to mix. Then, 20 ml of concentrated H2SO4 was added to the same flask, swirled three times, and allowed to react completely. After 30 minutes, the suspension

was diluted by adding 200 ml of water, and 10 ml of H3PO4 (0.5g of NaF can be used as an alternative) was added and shaken vigorously. Additionally, 1 ml of Diphenylamine indicator was mixed into the solution, resulting in a deep violet colour. The solution was titrated with 0.5 N ferrous ammonium sulphate (FAS) until the color changed from violet to blue and finally to bright green. The volume of ferrous ammonium sulphate used in the titration was noted. A blank titration (without soil) was also carried out in the same experiment. The following formula was applied:

$$\% \text{ Organic Carbon} = \frac{0.5(X-Y) \times 0.003 \times 100}{W}$$

Where,

W = weight of the soil taken in g

X = volume of 0.5 Nitrogen ferrous ammonium sulphate solution in ml used for the blank titration

Y = Volume of 0.5 Nitrogen ferrous ammonium sulphate solution in ml used for sample titration.

(X-Y) = Volume of 1N $K_2 Cr_2 O_7$ in ml used for oxidation of carbon

(1 ml 1N $K_2 Cr_2 O_7$ = 0.003 organic carbon)

OM = OC x 1.724

Since, organic matter (OM) contains 58% carbon, thus percentage of OM was obtained by multiplying organic carbon with 100/58 i.e. 1.724, known as Von Bemmlen factor.

3.8.8 Available nitrogen

The available Nitrogen was determined by Alkaline permanganate method by Subbiah and Asija (1956).

Twenty gram soil was taken into a 300 ml dry Kjeldahl flask. Afterwards, 20 ml of water was added, followed by 100 ml each of 0.32% KMnO4 and 2.5% NaOH solutions. To prevent frothing during boiling, 1 ml of liquid paraffin was used, and bumping was avoided by adding a few glass bends. The solution was distilled in a Kjeldahl assembly at a consistent rate, and the resulting ammonia was collected in a 250 ml conical flask containing 20 ml of boric acid solution with a mixed indicator. As ammonia was absorbed, the pinkish color turns to green. Approximately 100 ml of distillate was expected to be collected in about 30 minutes, which will

then be titrated with 0.02N H2SO4 to return to the original shade (pinkish). Blank correction (without soil) was to be conducted for the final calculation.

Calculation

Available N (kg/ha) = R X 0.02 × $\frac{1}{20}$ × 0.014 × 2.24 × 10^8 = R × 31.36

Where R= volume of 0.02N H_2SO_4 required for titration

3.8.9 Available soil Phosphorus

Available Phosphorus was determined by method outlined by Bray and Kurtz (1945). In this process, 5 grams of soil and 50 milliliters of the reagent were agitated in a 100 ml conical flask for precisely 5 minutes and subsequently filtered. To prevent potential fluoride interference, 7.5 milliliters of a 0.8 M boric acid solution (containing 50 grams of H_3BO_3 per liter) could be introduced to 5 ml of the extract, if deemed necessary. Phosphorus content in the extract was quantified using the colorimetric approach developed by Dickman and Bray (1940).

Colorimetric estimation of phosphorus (Dickman and Bray 1940)

5 ml of soil extract was pipetted into a 25 ml volumetric flask, into which 5 ml of Dickman and Bray's reagent was added. Dickman and Bray's reagent was prepared as follows: 5g of ammonium molybdate (AR) was dissolved in 300 ml of distilled water, warmed to approximately 60°C, and filtered if necessary, after cooling. To this solution, 350 ml of 10N HCl was added, and the volume was adjusted to one litre. The normality of the HCl should be accurately adjusted by titration. The neck of the flask was then rinsed, and the contents were diluted to approximately 22 ml. Then, 1 ml of the diluted stannous chloride solution was added, and the volume was adjusted to the mark. To prepare the stannous chloride solution, 10 g of crystalline stannous chloride (LR) was dissolved in 25 ml of concentrated HCl by warming. The resulting solution was then stored in an amber-coloured bottle, taking care to avoid all contact with air. This solution constitutes a 40% $SnCl_2$ stock solution. Just before use, 0.5 ml of the stock solution was diluted to 66 ml with distilled water. To maintain the stability of the stock solution over time, a piece of tin metal (AR) was added. The intensity of the blue colour was measured using a 660 µm filter immediately after 10 minutes, and the concentration of phosphorus was determined from the standard curve. This was crucial as the colour started to fade over time. With each set of samples, a blank (without soil) must be taken. A colorimeter reading for the blank should ideally be negligible when using high-quality reagents.

Standard curve for phosphorus

Analytical grade potassium dihydrogen orthophosphate (KH_2PO_4) was dried in an air oven at 60 °C for 1 hour. After cooling in a desiccator, exactly 0.439 g was dissolved in about half a litre of distilled water. 25 ml of approximately 7N H_2SO_4 was added, and the solution was made up to one litre with distilled water. This results in a 100 ppm stock solution of Phosphorus (100 µg P per ml). From this, a 2 ppm Phosphorus solution was prepared through a 50-fold dilution. For the preparation of the standard curve, different concentrations of P (1, 2, 3, 4, 5, and 10 ml of 2 ppm P solution) were taken in 25 ml volumetric flasks. To each of these solutions, 5 ml of the extracting reagent (Bray's) were added, and the color was developed as described above by adding Dickman and Bray's reagent (i/ii) and stannous chloride. In the case of the ascorbic acid method, the color developed after pH adjustment of the aliquot. The colorimeter reading was taken against a 660 mµ (red) filter immediately after 10 minutes. The curve was plotted with the colorimeter reading on the vertical axis and the amount of Phosphorus (in µg) on the horizontal axis.

Calculation

Available P (kg/ha)

$$= \text{R} \times \frac{\text{Total volume of the extractant}}{Volume\ of\ aiiquot} \times \frac{1}{Weight\ of\ soil\ taken} \times \frac{2.24 \times 10^6}{10^6}$$

Where R= µg P in the aliquot (to be seen from the standard curve).

Bray's P (kg/ha)

$$= \text{R} \times \frac{50}{5} \times \frac{1}{5} \times 2.24 = \text{µg of P} \times 4.48$$

3.8.10 Available Potassium

The determination of available Potassium (K) was done by centrifugation and decantation method (Mehta and Grewal, 2004). In this process, 5g of oven-dried soil was placed in a 50 ml centrifuge tube and spun at 5000 rpm or until the supernatant liquid became clear. The cleared supernatant liquid was decanted into a 100 ml volumetric flask and adjusted to a final volume of 100 ml with NH4OAc. Before estimation, the extract was filtered using Whatman no. 40 filter paper. The solution was thoroughly mixed to determine K concentration using a flame photometer

Calculation

Exchangeable K (Kg ha^{-1}) = C x $\frac{100}{5}$ x 2

Where, C = the concentration of potassium (ppm or mg kg^{-1}) in the sample obtained on X-axis against the standard curve

3.9 Quantification of microbial activity

Microbial activity was quantified by soil dehydrogenase activity spectrophotometrically as described by Casida (1977). 1 gm of soil was taken in a test tube and mixed with 0.1 gm of calcium carbonate ($CaCO_3$) and 1 ml of 1% 2,3,5-tetrazolium chloride (TTC) solution. The mixture was incubated at 30°C for 24 hours. The resultant slurry was poured on to Whatman no. 1 filter paper and extracted with concentrated Methanol. Further, the volume of the filtrate was made up to 50 ml b adding methanol. The optical density (OD) was read at 485 nm in a spectrophotometer using methanol extract as blank. Three replicates were maintained. Dehydrogenase activity was measured in terms of concentration of formazen in methanol and expressed as µg TPF g^{-1} 24 hr^{-1}.

3.10 Sterilization of experimental soil

The air-dried soil and sand were autoclaved at a ratio of 2:1 at 121°C and 15 lb steam pressure for a duration of 1 hour. After cooling, the process was repeated, ensuring thorough random mixing of the soil to ensure the complete destruction of all propagules of AM fungi, pathogenic fungi, and bacteria. The autoclaved, homogenous soil samples were then stored in large sterilized plastic bags and subsequently used for determining the inoculum potential.

3.11 Determination of inoculum potential

The inoculum potential (AMF infectivity of soil), was determined for the collected soil samples using the Most Probable Number (MPN) bioassay (Alexander, 1982) following serial soil dilution technique (Porter, 1979) taking maize as the host plant. The experiment followed a completely randomized design with five replications per treatment, including the control. The pure inoculum from test soils at all sites was serially diluted using sterilized sand to achieve 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions. Plants were harvested after one month, and the presence or absence of AM infection was recorded for each pot. The MPN was calculated and statistically analysed by calculating 95% confidence limits using the MPN table (Alexander, 1982).

3.12 Isolation of AM spores

Live spores were isolated from soil samples by wet sieving and decanting method (Gerdemann and Nicholson, 1963), followed by sucrose density gradient centrifugation (Daniels and Skipper, 1982). 100 gm air-dried rhizosphere soil was suspended in 1000 ml of tap water, agitated manually for 5 minutes, and the soil suspension was allowed to settle for 5 minutes enabling heavier particles to settle. The suspension was then slowly poured through a stack of sieves of different pore size (40- 800 µm), arranged in a descending order. This process was repeated thrice for the soil sample, and the extracts were collected in a clean 100 ml beaker. The extract was transferred to a 50 ml centrifuge tube and centrifuged at 1750 rpm for 5 minutes. The spore-suspended pellets obtained after removing the supernatant were further centrifuged using the sucrose gradient process for 1 minute. The supernatant from this centrifugation was filtered with nylon cloth (25 µm pore size) using a filter funnel to eliminate water and clear sugar molecules from AM fungal spores. The filtrate was placed in clean, sterilized Petri dishes, and spores were collected using a white bristle brush under a Nikon binocular Stereomicroscope (20-120x magnification). Live spores were stored in a small glass container at 4°C.

3.13 Quantification of spore population

Isolated spores were counted manually under Nikon binocular Stereomicroscope, and total spore density of each species was recorded.

Spore density = Number of AM spores per 100g of dry weight soil.

3.14 Suitable host plant for multiplication of AM fungi *in vivo*

Zephyranthes ajax (family Amaryllidaceae) was selected as suitable host plant (Dai, 2010). The host plants were collected from University campus. Unwanted soil particles present in the roots were washed in running tap water and kept in sterilized tray. Bulbs were sterilized with diluted Carbendazim solution for 15-20 minutes and rinsed thrice with double distilled water. These sterilized bulbs were further used for trap and pure culture.

3.15 *In vivo* culture of AM fungi

3.15.1 Trap culture of AMF

Freshly collected soil samples from different sites were used to establish trap culture using *Z. ajax* as the host plant. Maintenance of trap culture is done to ensure detection of all

AMF species present in the soil samples. Two disinfected host seedlings were planted (5 replicates each). Pots were maintained in Green house for 24 months. No extra nutrients were added and weeding was done regularly. Watering was done when required. Periodic examination of AMF was carried out. Spores were isolated by wet sieving and decanting method and examined under stereomicroscope. Healthy looking spores were collected in separate vials in large quantity which were further for photography and identification of individual AM species.

3.16 Collection of plant samples

Representative plant species including herbs, shrubs and trees were selected for quantification of AM root colonization. These plant species were collected twice a year from each sampling site, documented through field photography, preserved as herbarium specimens, and identified based on their taxonomic characteristics using available literature. In addition, roots dug out along with soil samples were collected and preserved in FAA solution from each plot at a study site for further quantification of AM root infection.

3.17 Taxonomic identification of AMF species

Healthy spores isolated from field soil and trap culture were mounted on slides using polyvinyl-lactic acid-glycerol. The morphological characteristics of the spores were observed under a DIC microscope (Axioimager, Zeiss) at a magnification of 100-1000x. For spore color, fresh spores were immersed in water and examined under a stereo zoom binocular microscope.

Identification was done based on taxonomic characters, including spore color, size, content, wall thickness, wall numbers and types, surface ornamentation, types of subtending hyphae, diameter of subtending hyphae, pore types and occlusion, sporogenous saccule, and distance of spore formation from the saccule. This was done with reference to descriptions provided by various researchers (Schenck and Perez, 1990; Walker, 1983, 1986; Walker and Trappe 1993), the INVAM (International culture collection of Vesicular Arbuscular Mycorrhizal Fungi) website http://invam.caf.wvu.edu; Myc-Info/Taxonomy/Species.html, and http://amf-phylogeny.com.

3.18 Data analysis

The obtained data were subjected to analysis through one-way and two-way ANOVA to test the significance of variations between soil physico-chemical properties, DHA, root

colonization and AMF spore population, using suitable statistical software (SPSS version 9) and Microsoft Excel followed by Post-hoc tests. Pearson's correlations heat map was generated using Graphpad Prism software. Diversity indices, specifically Simpson's Diversity index (1-D), Shannon diversity index (H), and Species evenness (E), were calculated for each study site separately based on the methods proposed by Simpson (1949) and Shannon and Weaver (1949) using PAST software (Hammer et al., 2001). Relative Abundance (%RA) and Isolation Frequency (%IF) were determined following the formulas provided by Dandan and Zhiwei (2007):

%RA = the number of a given species spore / the total number of spores × 100

%IF = the number of samples in which a given species was isolated / the total number of samples × 100

CHAPTER IV

RESULTS

The study's findings are presented below:

4.1 Vegetation cover of the study sites

The vegetation cover varied widely between sites, with Natural forest exhibiting the highest coverage with maximum tree density and canopy cover but a lower ground cover. It was followed by Regenerating forest, Degraded forest and Jhum fallow with a lesser to very few tree density, canopy cover but increased ground cover.

In the Natural forest, the tree density was 382 ha^{-1} with a canopy cover of 81.7%. Herbaceous plants and shrubs occupied 34% of the ground floor (Table 2), while the remaining area was covered with litter. The frequency distribution showed that the number of trees were nearly equal in the three DBH classes i.e. 10–20 cm, 21– 30 cm and 31–40 cm. About 50% trees were less than 30 cm DBH indicating a slow regeneration. Additionally, out of all the sites, it had maximum number of trees exceeding 40 cm which indicates the establishment of a stable community (Fig. 5). The dominant tree species growing here were *Aporosa octandra*, *Baccaurea ramiflora*, *Duabanga grandiflora*, *Dillenia indica*, *Elaeocarpus* sp., *Magnolia* sp., *Morinda* sp., *Sauraia* sp. etc.

Table 2: Vegetation cover at different study sites

Sites	**Tree density** (ha^{-1})	**Canopy cover** (%)	**Ground cover** (%)
Natural Forest	382	81.7 ± 2.2^{c}	34.0 ± 2.1^{a}
Regenerating Forest	347	67.2 ± 1.7^{b}	62.4 ± 3.0^{b}
Degraded Forest	105	50.9 ± 2.5^{a}	77.3 ± 2.7^{c}
Jhum Fallow	15	Not determined	96.9 ± 0.7^{d}

Values with different letter in superscripts in column denote significant difference between forest types at $p<0.05$; '±' denotes SEM

The Regenerating forest showed a lesser tree density (347 ha^{-1}) and canopy cover (62.35%) in comparison to Natural forest but a higher ground cover (62.4%) occupied by herbaceous plants and shrubs. The DBH frequency distribution exhibited 80% trees less than 30 cm and less mature trees than natural forest indicating a fast regeneration. DBH classes of 11-20 cm and 21-30 cm had the maximum number of trees. The dominant tree species consisted of

Aporosa octandra, *Elaeocarpus floribundus*, *Garcinia pedunculata*, *Phyllanthes assamicus* etc. that grew naturally.

Degraded Forest had a very low tree density (105 ha^{-1}) with a canopy cover of 50.95%. However, a substantial 77.33% of the ground floor was occupied by herbaceous plants and shrubs. The frequency distribution of DBH revealed highest number of trees in the 31–40 cm class and 40% of trees were less than 30 cm. A noticeable trend emerged, showing gradual increase in the number of trees from the 0–10 cm to 31–40 cm, followed by a subsequent decrease. Additionally, a significant number of trees were also observed in 71–80 cm. Some common tree species growing in the site were *Aporosa octandra, Duabanga grandiflora*, *Neolamarckia cadamba, Oroxylum indicum* etc.

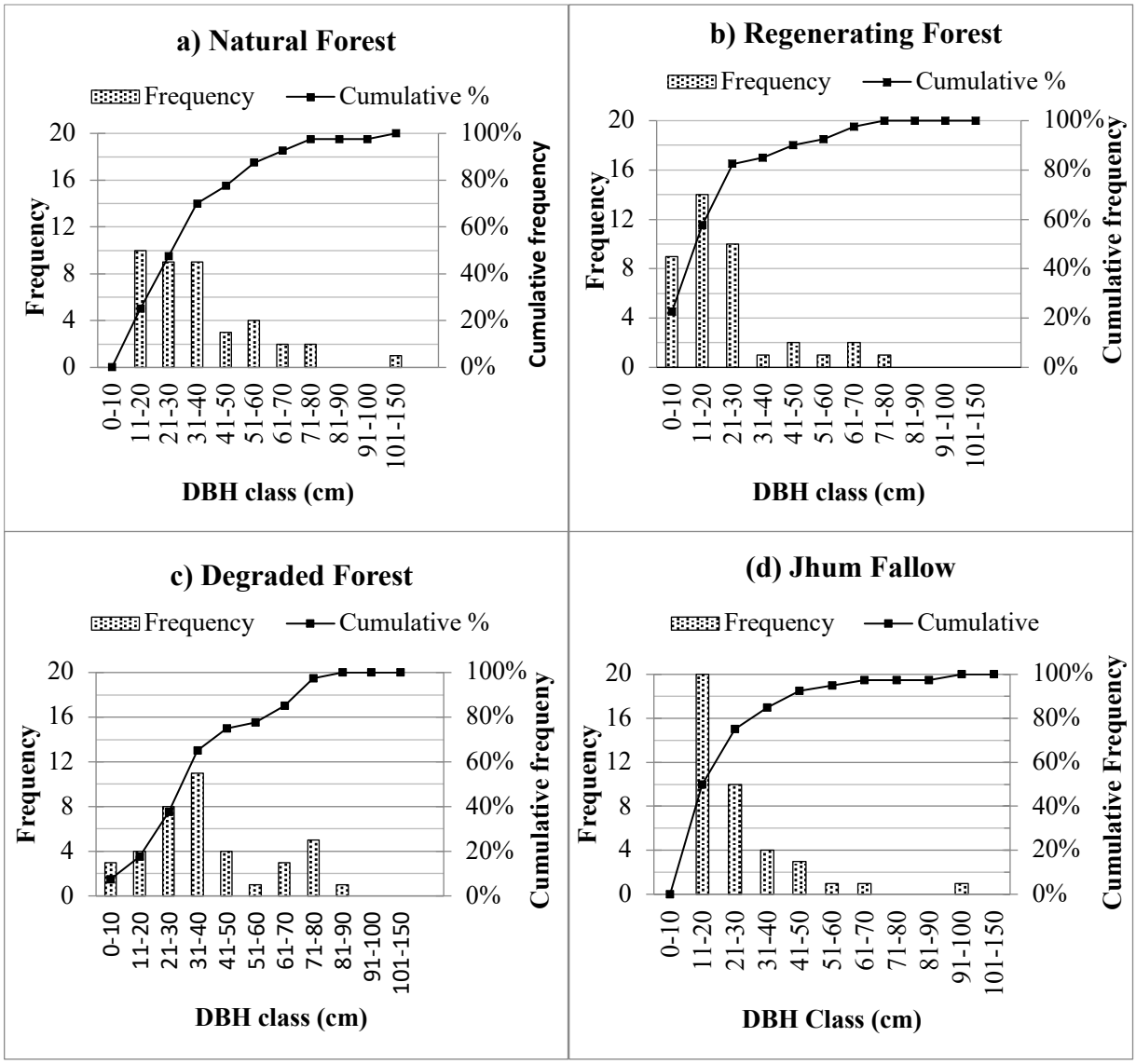


Fig. 5: Frequency distribution of DBH classes of a) Natural Forest b) Regenerating Forest c) Degraded Forest and d) Jhum Fallow

Jhum fallow had just 15 trees ha^{-1} which was relatively lower than other three sites. Therefore, due to very few, scattered and countable nature of trees, determining the canopy cover was not feasible. However, the ground was predominantly covered by herbaceous plants and shrubs, occupying 96.86% of the area. About 45% of trees were less than 30 cm DBH and majority of trees fell within the DBH class of 11–20 cm, with a significant gradual decrease in frequency observed in the remaining DBH classes. Additionally, two very old trees, which have not been cut down, are also noticeable in the 91–100 cm DBH class. The four Jhum fallows selected for the study, all 3-5 years old and left completely abandoned, exhibited a lush growth of vegetation, dominated by species like *Lantana camara*, *Mikania micranthes*, *Spermacoce* sp., etc. and a few sparsely distributed tree species such as *Crateva religiosa*, *Dillenia indica*, *Duabanga grandiflora*, *Litsea polyantha* etc. were also present.

4.2 Plant diversity in the selected study sites

Some dominant plant species (tree and shrub) in the study sites are presented in Table 3 and a Venn diagram as Fig. 6.

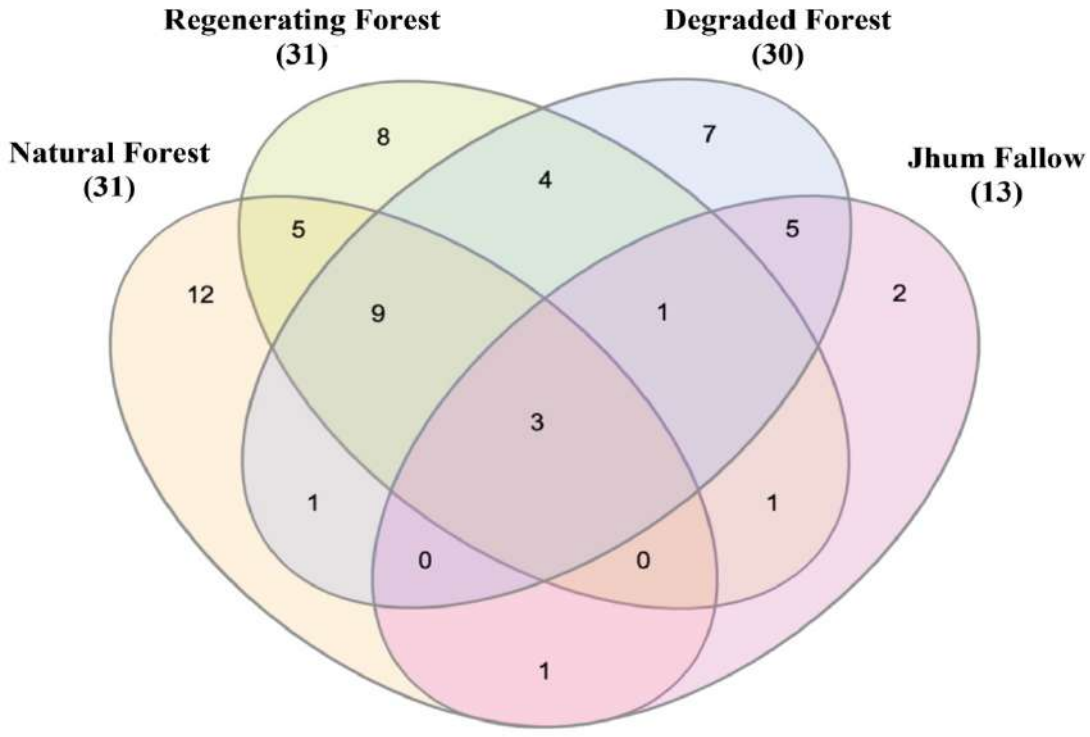


Fig. 6: Venn diagram of plant diversity in the study sites

Table 3: Major plant species at the study sites

Plant species	Family	Habit	NF	RF	DF	JF
Baliospermum sp.	Euphorbiaceae	Shrub	Y	–	–	–
Camellia sp.	Theaceae	Shrub	Y	–	–	–
Chromolaena odorata (L.) R.M.King & H.Rob.	Asteraceae	Shrub	–	–	Y	Y
Clerodendrum infortunatum L.	Lamiaceae	Shrub	–	Y	Y	Y
Flacourtia sp.	Flacourtiaceae	Shrub	–	–	–	Y
Ixora sp.	Rubiaceae	Shrub	Y	–	–	–
Lantana camara L.	Verbenaceae	Shrub	–	–	Y	Y
Leea sp.	Vitaceae	Shrub	Y	–	–	–
Phlogacanthus curviflorus (Nees) Nees	Acanthaceae	Shrub	–	Y	–	Y
Piper peepuloides Roxb.	Piperaceae	Shrub	–	Y	–	–
Rhynchotechum parviflorum Blume	Gesneriaceae	Shrub	Y	–	–	–
Spermacoce latifolia Aubl.	Rubiaceae	Shrub	–	–	Y	Y
Urena lobata L.	Malvaceae	Shrub	–	–	Y	Y
Ailanthus integrifolia Lam.	Simaroubaceae	Tree	–	–	Y	–
Albizia lucidior (Jacques) Benth.	Leguminosae	Tree	–	Y	Y	–
Alstonia scholaris (L.) R.Br.	Apocynaceae	Tree	–	Y	–	–
Aporosa octandra (Buch.-Ham. ex D.Don) Vickery	Phyllanthaceae	Tree	Y	Y	Y	–

Plant species	**Family**	**Habit**	**NF**	**RF**	**DF**	**JF**
Artocarpus chama Buch.-Ham.	Moraceae	Tree	–	Y	Y	–
Baccaurea ramiflora Lour.	Phyllanthaceae	Tree	Y	–	–	–
Baccaurea sp.	Phyllanthaceae	Tree	–	Y	–	–
Balakata baccata (Roxb.) Esser	Euphorbiaceae	Tree	–	Y	–	–
Bauhinia variegata L.	Leguminosae	Tree	–	–	Y	–
Castanopsis lanceifolia (Oerst.) Hickel & A.Camus	Fagaceae	Tree	Y	Y	Y	–
Cinnamomum bejolghota (Buch.-Ham.) Sweet	Lauraceae	Tree	Y	Y	–	–
Cinnamomum tamala (Buch.-Ham.) T.Nees & C.H.Eberm.	Lauraceae	Tree	Y	Y	–	–
Crateva religiosa G.Forst.	Capparaceae	Tree	–	–	–	Y
Crypteronia paniculata Blume	Penaeaceae	Tree	–	–	Y	–
Dillenia indica L.	Dilleniacea	Tree	Y	Y	Y	Y
Duabanga grandiflora (Roxb. ex DC.) Walp.	Lythraceae	Tree	Y	Y	Y	Y
Dysoxylum grande Hiern	Meliaceae	Tree	–	Y	–	–
Elaeocarpus angustifolius Blume	Elaeocarpaceae	Tree	Y	–	Y	–
Elaeocarpus floribundus Blume	Elaeocarpaceae	Tree	Y	Y	Y	–
Elaeocarpus rugosus Roxb. ex G.Don	Elaeocarpaceae	Tree	–	Y	–	–
Elaeocarpus varunua Buch.-Ham. ex Mast.	Elaeocarpaceae	Tree	–	Y	Y	–
Ficus auriculata Lour.	Moraceae	Tree	Y	Y	Y	–

Plant species	Family	Habit	NF	RF	DF	JF
Garcinia pedunculata Roxb. ex Buch.-Ham.	Clusiaceae	Tree	–	Y	–	–
Glochidion ellipticum Wight	Phyllanthaceae	Tree	Y	Y	Y	–
Grevillea robusta A.Cunn. ex R.Br.	Proteaceae	Tree	–	–	Y	–
Gynocardia odorata R.Br.	Achariaceae	Tree	Y	Y	Y	–
Litsea monopetala (Roxb.) Pers.	Lauraceae	Tree	–	–	Y	Y
Macaranga denticulata (Blume) Müll.Arg.	Euphorbiaceae	Tree	Y	Y	Y	Y
Magnolia hodgsonii (Hook.f. & Thomson) H.Keng	Magnoliaceae	Tree	Y	Y	–	–
Mallotus nudiflorus (L.) Kulju & Welzen	Euphorbiaceae	Tree	–	–	Y	–
Miliusa sp.	Annonaceae	Tree	Y	–	–	–
Morinda sp.	Rubiaceae	Tree	Y	–	–	–
Neolamarckia cadamba (Roxb.) Bosser	Rubiaceae	Tree	Y	Y	Y	–
Oroxylum indicum (L.) Kurz	Bignoniaceae	Tree	Y	Y	Y	–
Pandanus furcatus Roxb.	Pandanaceae	Tree	–	Y	–	–
Saurauia armata Kurz	Actinidiaceae	Tree	Y	–	–	–
Saurauia roxburghii Wall.	Actinidiaceae	Tree	Y	Y	–	–
Saurauia sp. 3	Actinidiaceae	Tree	Y	–	–	–
Schima wallichii (DC.) Korth.	Theaceae	Tree	–	Y	Y	–
Spondias pinnata (L.f.) Kurz	Anacardiaceae	Tree	Y	Y	–	–

Plant species	**Family**	**Habit**	**NF**	**RF**	**DF**	**JF**
Syzygium sp.	Myrtaceae	Tree	Y	–	–	–
Tectona grandis L.f.	Lamiaceae	Tree	Y	–	–	Y
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.	Combretaceae	Tree	–	–	Y	–
Terminalia chebula Retz.	Combretaceae	Tree	Y	Y	Y	–
Terminalia myriocarpa Van Heurck & Müll.Arg.	Combretaceae	Tree	Y	–	–	–
Vernicia fordii (Hemsl.) Airy Shaw	Euphorbiaceae	Tree	–	–	Y	–

"Y" and (–) denotes presence or absence of plant species in a site.

4.3 Soil physico-chemical properties

Soil physico-chemical properties of the study sites showed much variation (Table 4a & b).

Texture: The texture of the examined soils ranged from sandy loam to sandy clay loam in both the seasons (Table 4a).

Bulk Density (g/cm^{-3}): The Bulk density of the studied soils was measured at a depth of 0-15 cm. In both seasons, it was nearly similar in all the sites.

Porosity (%): No significant differences were observed among the sites or between the seasons.

Water Holding Capacity (%): WHC of the studied soils varied significantly between the seasons (Winter and Summer). In winter, it was same in all the sites. In summer, it was higher than winter and relatively little more in NF and RF than DF and JF. A significant difference was also observed between the sites and the seasons.

Moisture content (%): The Moisture content in the studied soils exhibited significant variation among the sites and also between the seasons. During winter, it was similar in NF, RF and DF and higher than JF. In summer it was similar in NF and RF both of which were higher than DF and JF.

pH: The pH of the examined soils was slightly acidic in nature and did not show much change due to season. In winter, it was same in all the sites. In summer it was slightly higher and the values ranged from 4.65 to 5.35. However, A significant difference was also seen between the sites and the seasons.

Organic Carbon (%): In winter, it was similar in NF, RF and DF and less in JF. It was slightly more in summer with lowest in JF. There was not much difference in NF, RF and DF.

Available Nitrogen (Kg ha^{-1}): In winter, available N was similar in NF, RF, DF and slightly lower in JF, although significantly lower than the former two. A similar trend was noted during the summer season. No significant change was observed between seasons; nevertheless, the variation was more pronounced in the NF.

Available Phosphorus (Kg ha^{-1}): Significant variations among the sites was observed but did not change widely across seasons. The alteration in available phosphorus (P) did not exhibit a clear pattern of increase and decrease but it was seen that, the disturbed sites

(DF and JF) displayed a higher value than the undisturbed sites (NF and RF). However, there no significant difference was observed between the sites and the seasons.

Potassium (Kg ha^{-1}): In winter, Potassium content was similar in all the sites. In summer, it was similar in NF, RF, DF and slightly lower in JF.

Table 4(a): Soil texture of study sites

Site	Season	Sand (%)	Silt (%)	Clay(%)	Texture
NF	Winter	72	19.6	8.4	Sandy loam
	Summer	51	21	28	Sandy clay loam
RF	Winter	60	26	14	Sandy loam
	Summer	64	22	14	Sandy loam
DF	Winter	74	19.8	8.2	Sandy loam
	Summer	63.13	22	14.87	Sandy loam
JF	Winter	60	20	20	Sandy loam
	Summer	58	20	22	Sandy loam

Table 4(b): Physico-chemical properties of soil at study sites

Soil properties	Season	NF	RF	DF	JF
Bulk Density (g/cm^3)	W	1.23±0.08^{a1}	1.19±0.08^{a1}	1.34±0.02^{a1}	1.30±0.03^{a1}
	S	1.25±0.06^{a1}	1.17±0.01^{a1}	1.27±0.05^{a1}	1.2±0.05^{a1}
Porosity (%)	W	53.8±3.0^{a1}	55.3±2.9^{a1}	49.6±0.7^{a1}	51.1±1.2^{a1}
	S	54.9±1.8^{a1}	56.0±0.5^{a1}	52.0±2.0^{a1}	52.9±2.4^{a1}
WHC (%)	W	73.7±1.3^{b1}	72.4±1.3^{b1}	66.7±1.1^{a1}	69.7±4.0^{ab1}
	S	79.0±3.0^{b2}	78±1.2^{b2}	73.8±0.3^{a2}	71.7±0.6^{a1}
MC (%)	W	25.0±1.1^{c1}	23.4±0.6^{bc1}	22.8±0.8^{b1}	19.1±1.3^{a1}
	S	29.5±0.9^{b2}	30.1±0.7^{b2}	23.1±0.5^{a1}	21.7±0.6^{a2}
pH	W	5.06±0.08^{a1}	5.06±0.09^{a1}	5.15±0.1^{ab1}	5.38±0.11^{b1}
	S	5.29±0.12^{bc1}	5.09±0.18^{b1}	4.65±0.12^{a1}	5.35±0.07^{c1}
OC (%)	W	1.69±0.09^{b1}	1.77±0.08^{b1}	1.66±0.09^{b1}	1.31±0.06^{a1}
	S	1.77±0.09^{b1}	1.96±0.11^{b1}	1.57±0.03^{a1}	1.44±0.11^{a1}
Avail. N (kg ha^{-1})	W	272.7±6.8^{c1}	266.4±12.1^{bc1}	250.9±14.0^{b1}	219.8±8.6^{a1}
	S	258.8±9.5^{b1}	259.6±7.2^{b1}	237.2±5.3^{a1}	217.3±9.0^{a1}
Avail. P (kg ha^{-1})	W	9.2±0.80^{b1}	7.0±0.95^{a1}	10.9±0.6^{b1}	17.8±1.8^{c1}
	S	7.8±0.2^{a1}	9.7±0.2^{a1}	13.6±0.5^{b1}	13.5±1.3^{b1}
K (kg ha^{-1})	W	286.7±29.5^{b1}	296.8±5.5^{b1}	246.4±8.0^{a1}	242.8±14.8^{a1}
	S	334.6±29.3^{c1}	289.4±23.5^{b1}	288.7±8.1^{b1}	231.3±18.5^{a1}

N = 4; Values with different letter in superscripts in a row and different numeral denote significant difference between forest types and seasons respectively at $p<0.05$; '±' denotes SEM

4.3.1 Correlation among physical and chemical properties of soil

Both positive and negative correlations were observed between soil's physical and chemical parameters. During winter, Pearson's correlation analysis revealed a significant positive correlation between MC and OC, available N (p<0.01) and WHC (p<0.05). A significant negative correlation between MC and available P and pH (p<0.01) was also seen. WHC had a positive correlation with K (p<0.01) while BD and porosity were negatively correlated. pH had positive correlation with available P (p<0.01) and negative correlation with OC, available N and K (p<0.01). OC and available N were significantly and positively related while both exhibited a negative correlation with available P. K on the other hand had a positive correlation with available N while having a negative correlation with available P (p<0.05) (Fig. 7).

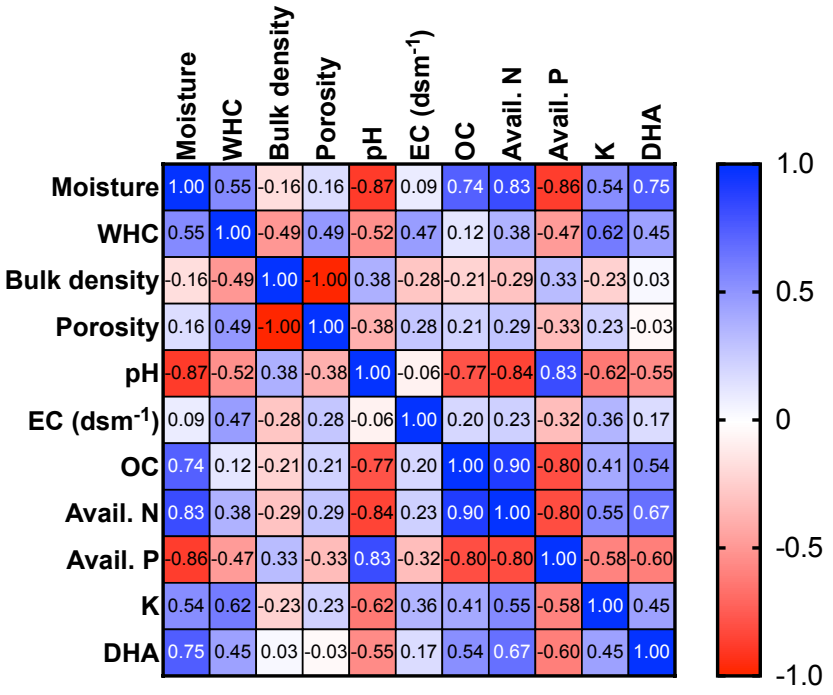


Fig. 7: Heat map of Pearson's correlation coefficient between physico-chemical roperties of soil (Winter)

During summer, MC and WHC exhibited a significant positive correlation with each other as well as with OC, available N (p<0.01) and porosity (p<0.05) while showing a negative correlation with available P (p<0.01) and BD (p<0.05). BD related positively with available P (p<0.01) while having a negative correlation with porosity, OC and available N (p<0.01). OC and available N were significantly and positively related while both exhibited a negative correlation with available P. K was positively related with

available N (p<0.01) while it had a negative correlation with available P (p<0.05) (Fig. 8).

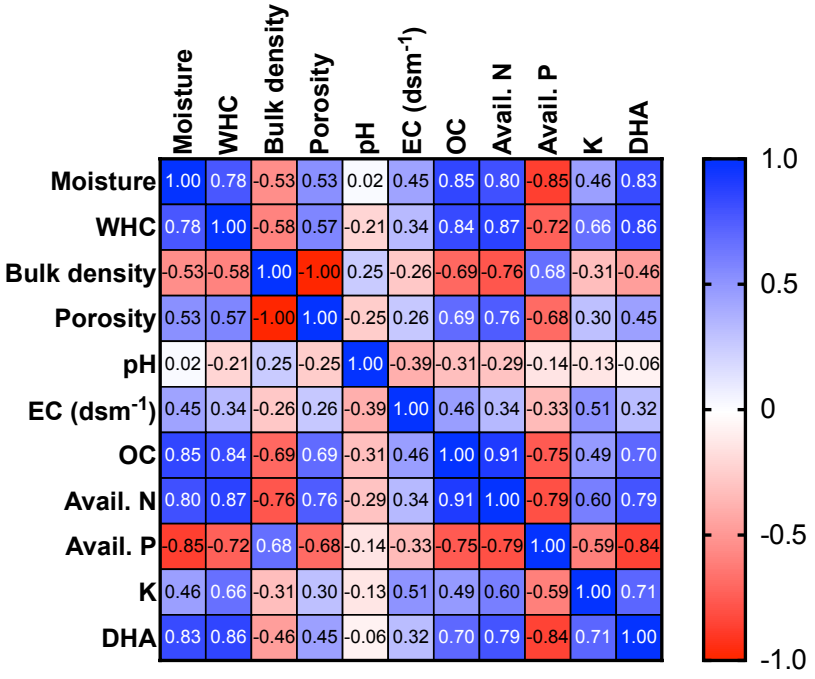


Fig. 8: Heat map of Pearson's correlation coefficient between physico-chemical properties of soil (Summer)

4.4 Microbial activity in soil (Soil dehydrogenase activity)

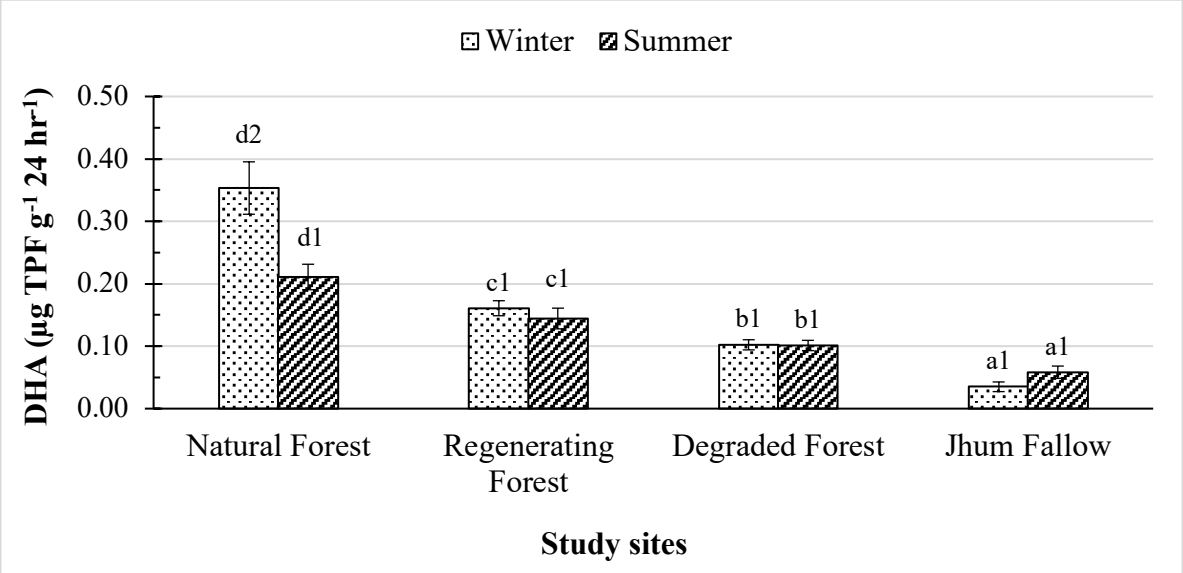


Fig. 9: Seasonal variation in Dehydrogenase activity of the soil samples different study sites (Different letters and numeral denote a significant difference between forest types and seasons respectively). N = 12 each for NF, RF & JF; 9 for DF

Soil dehydrogenase activity (DHA) varied significantly among the sites and also between seasons (Fig. 9). Among all forest sites, the variation in DHA due to season was observed only in NF but not in other forest and Jhum fallow. Further, the DHA also showed a significant interaction between sites and season ($p<0.01$).

In winter season, DHA ranged from 0.035 - 0.353 µg TPF g^{-1} 24 hr^{-1} with the highest activity observed in NF followed by RF, DF and JF. DHA was positively correlated with MC ($p<0.01$), available N ($p<0.01$) and OC ($p<0.05$) while negatively correlated with available P ($p<0.01$) and soil pH ($p<0.05$) (Fig. 7).

The DHA in the summer was significantly lesser than the winter season and ranged from 0.058 - 0.211 µg TPF g^{-1} 24 hr^{-1} with the highest activity again in NF followed by RF, DF and JF. There was a significant positive correlation of DHA ($p<0.01$) with MC, WHC, OC, available N and K whereas a strong negative correlation with available P ($p<0.01$) (Fig. 8).

4.5 Effect of vegetation on soil physico-chemical properties and microbial activity

Comparison of the results of soil physico-chemical properties and microbial activity of the four study sites revealed that MC, WHC, OC, available N, K and microbial activity were higher in those sites that were having higher tree density with more canopy cover.

4.6 AM colonization in composite root samples

Root samples of all the studied sites showed mycorrhizal structures viz. vesicles, arbuscules, intra-radical hyphae and occasionally intra-radical spores (Fig. 7). It was observed that hyphal colonization inside roots was more prevalent than the vesicular or arbuscular colonization. Effect of season was observed only in NF and DF. In general, a higher colonization was recorded in winter (Fig. 10).

During winter, RC significantly varied among the sites, with values ranging from 56.29% (JF) to 67.33% (RF). It was high in RF, though not significantly different from NF, slightly less in DF and significantly less in JF.

RC displayed significant variation among the sites in summer as well, with values ranging from 55.42% to 65.21%. The highest colonization was again observed in RF

followed by NF, DF and JF. RF was similar to NF while differing significantly from DF and JF.

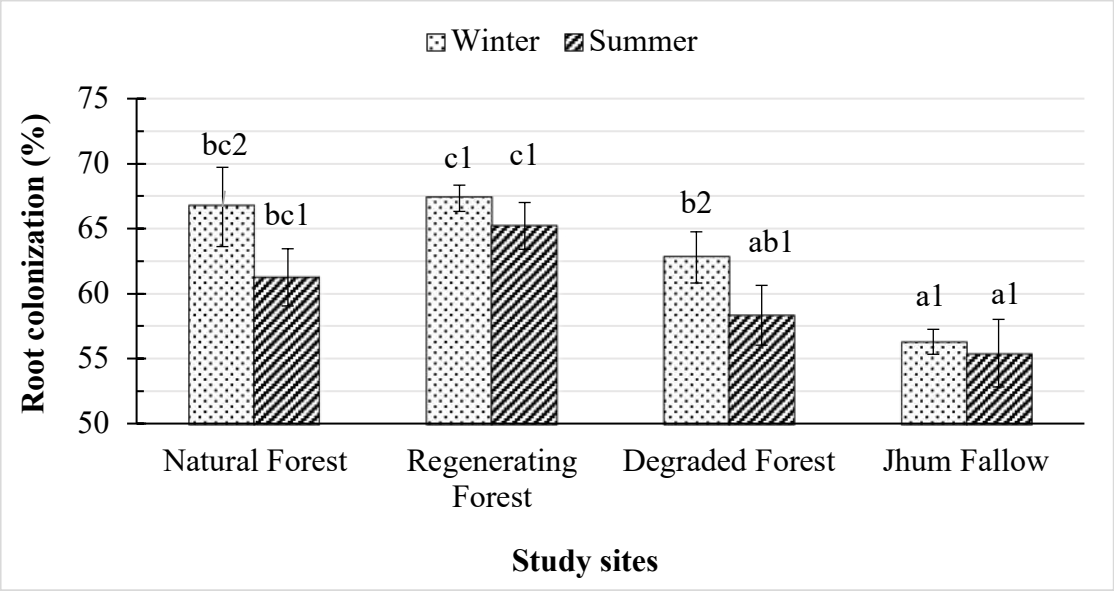


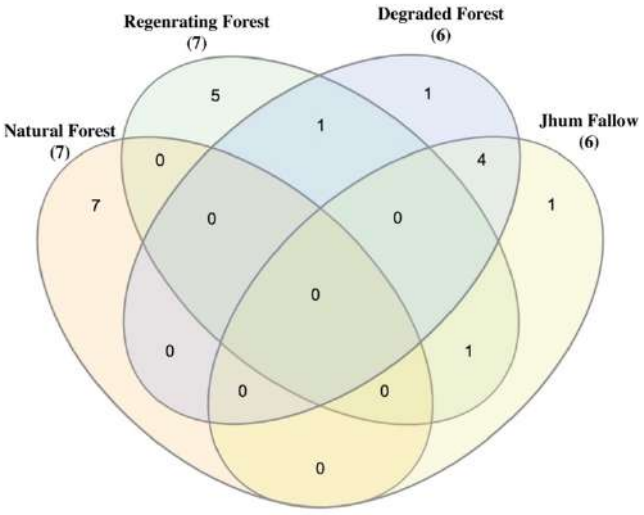
Fig. 10: **Seasonal variation in Root colonization in composite root samples of different study sites** (Different letters and numeral denote a significant difference between forest types and seasons respectively). Number of root segments (1.0 cm) taken = 120 each for NF, RF & JF; 90 for DF. No. of intersections per root segment for AM infection examined = 4

4.7 AM root colonization in dominant plant species of the study sites

To quantify AM root colonization in plant species across different study sites, six to seven well -grown, visible and dominant plant species were selected from each site. The plant species were carefully selected ensuring their recurrence in both seasons. All the selected plants showed presence of mycorrhizal infection in the root cortex. Some of the host plant were present in more than one study sites like *Clerodendrum infortunatum*, *Ageratum conyzoides*, *Eupatorium odoratum*, *Mikania micrantha*, *Urena lobata* and *Selaginella* sp. (Fig. 11). These species exhibited variations in RC in different sites. *A. conyzoides*, *M. micrantha* and *U. lobata* showed higher colonization in DF than in JF. Similarly, *Selaginella* sp. had higher colonization in RF than JF. Conversely, *E. odoratum* exhibited higher colonization in JF than in DF. On the other hand, *C. infortunatum* exhibited similar colonization rate in both RF and DF. In general, a higher root colonization was recorded in winter (Fig. 12-Fig. 15).

Natural Forest: In NF, seven plant species namely, *Rhynchotechum parviflorum*, *Baliospermum* sp., *Elsholtzia* sp., *Ixora* sp., *Jasminum* sp., Unidentified sp. 1 and sp. 2

were quantified for AM root colonization (Fig. 12). Both in winter and summer, the highest colonization was observed in *Ixora* sp. (85% each) whereas the lowest in *Rhynchotechum parviflorum* (27.5% and 7.5%). In general, root colonization in the selected host plants was lesser during summer.



RF & JF	RF & JF	DF & RF
Selaginella sp.	*A. conyzoides* *E. odoratum* *M. micrantha* *U. lobata*	*C. infortunatum*

Fig. 11: Venn diagram of common plants in study sites

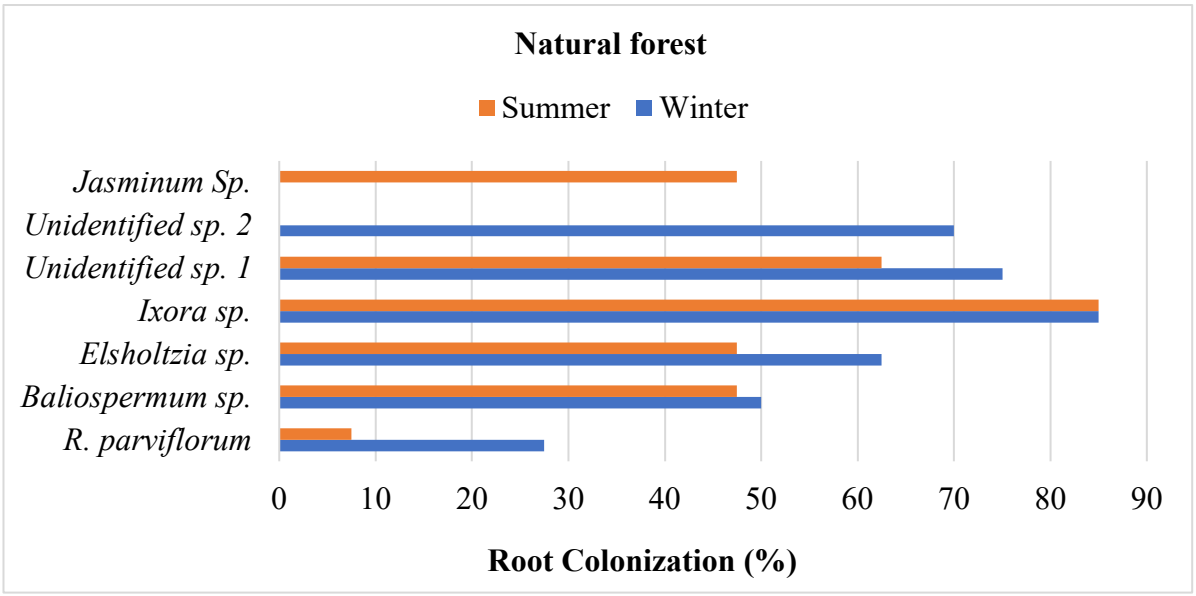


Fig. 12: Seasonal variation in root colonization (%) in dominant plant species of Natural Forest

Regenerating Forest: In RF, seven plants namely *Phlagocnathus* sp., *C. infortunatum*, *Piper mullesua*, *Selaginella* sp., *Stemona tuberosa*, *Phrynium pubinerve* and unidentified sp. 3 were quantified for AM colonization. Highest colonization in winter was observed in *Phlagocanthus* sp. (85%) and *P. mullesua* (85%). Lowest colonization was seen in *P. pubinerve* (15%). In summer, highest colonization was observed in *Phlagocanthus* sp. (80%) and *C. infortunatum* (80%). Lowest colonization was seen in *Selaginella* sp. (50%). All the plant species except for *C. infortunatum* exhibited higher colonization during winter (Fig. 13).

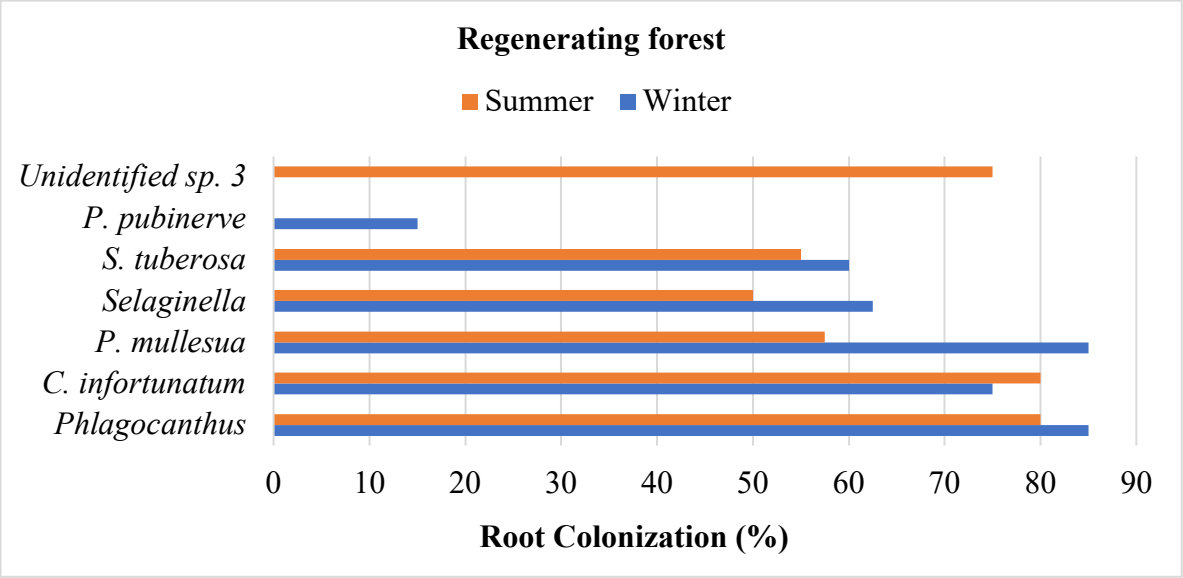


Fig. 13: Seasonal variation in root colonization (%) in dominant plant species of Regenerating Forest

Degraded Forest: In RF, six plants namely, *A. conyzoides*, *E. odoratum*, *M. micrantha*, *U. lobata*, *C. infortunatum* and *Biden Pilosa* were quantified for AM colonization. Highest colonization was observed in *C. infortunatum* (70%) and *Urena lobata* (70%). *E. odoratum* (35%) exhibited lowest colonization. In summer, highest colonization was observed in *U. lobata* (67.5%). Lowest colonization was seen in *A. conyzoides* (35%) and *E. odoratum* (35%). Root colonization was higher in winter (Fig. 14).

Jhum Fallow: In RF, six plants namely, *A. conyzoides*, *Selaginella* sp., *E. odoratum*, *M. micrantha*, *U. lobata* and *Spermacoce* sp. were quantified for AM colonization. Highest colonization was observed in *Spermacoce* sp. (75%) and lowest in *M. micrantha* (27.5%). In summer, highest colonization remained in *Spermacoce* sp. (70%) and lowest in *E. odoratum* (30%). All the plant species except for *M. micrantha* exhibited higher colonization during winter (Fig. 15).

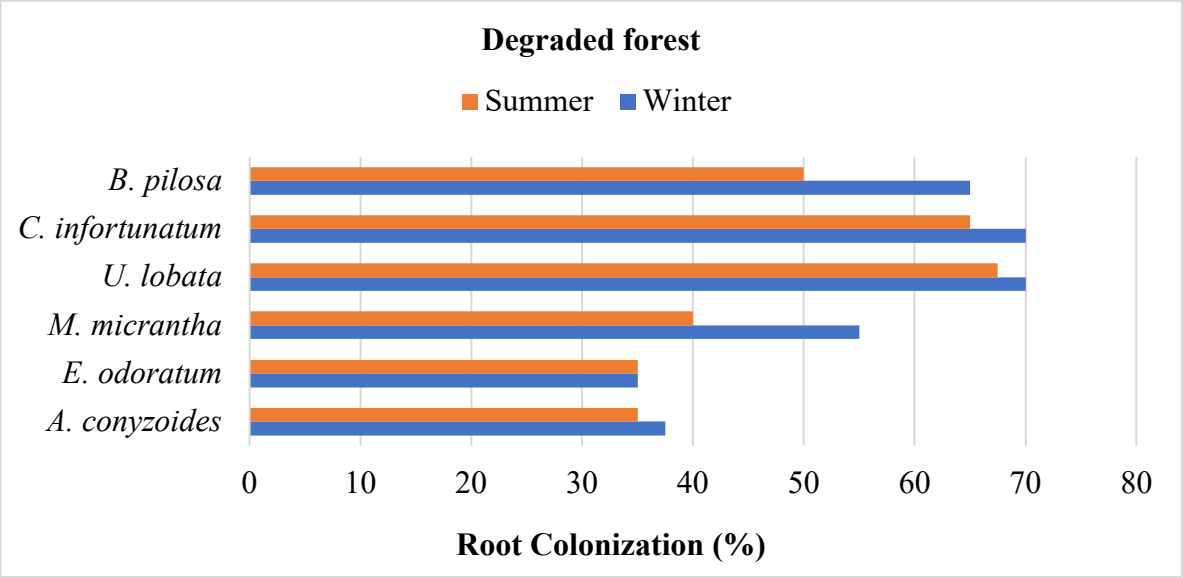


Fig. 14: Seasonal variation in root colonization (%) in dominant plant species of Degraded Forest

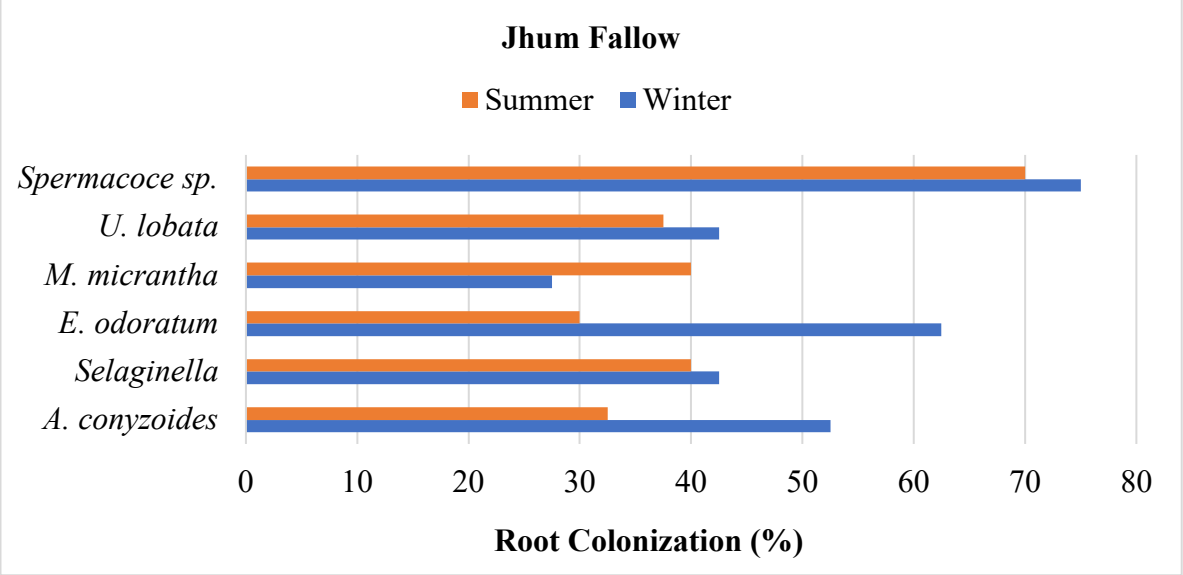


Fig. 15: Seasonal variation in root colonization (%) in dominant plant species of Jhum Fallow

4.8 Comparison of AM colonization in composite root samples and roots of selected dominant plant species

On comparison of the AM colonization in composite root samples and roots of selected dominant plant species, it was observed that in general during winter season, RC in composite root sample was higher than in root sample in dominant plants except for RF where it was otherwise (Fig. 16). During summer, the same trend was observed in NF, DF and JF, whereas, in RF, RC was equivalent in both the root samples. Further,

the RC varied significantly among study sites without showing any effect of season due to a wider variation in RC among the selected dominant plants. Nevertheless, the average RC was always higher in winter. Among all the study sites, a minimal variation in RC was recorded in RF.

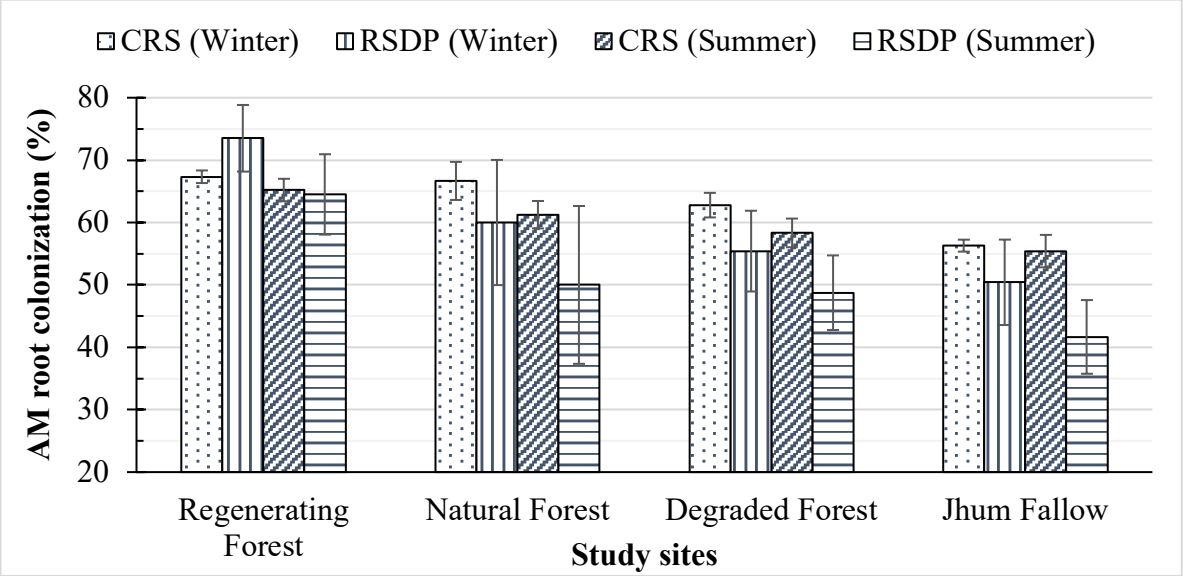


Fig. 16: Comparison of AM colonization in composite root samples and roots of selected plant species (CRS = Composite Root Sample; RSDP= Root Sample of Dominant Plants)

4.8 Inoculum potential of AMF in soil

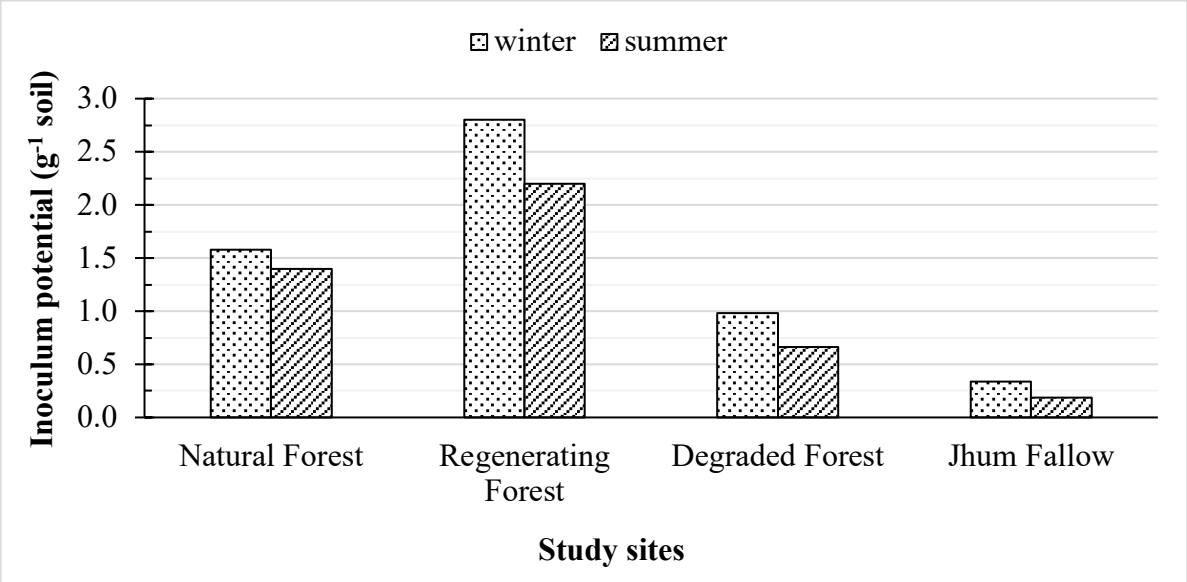


Fig. 17: Seasonal variation in AMF infective propagules in different study sites (N = 250 each for NF, RF, JF & DF).

Inoculum potential (IP) showed a greater variation in the study sites. IP was relatively higher in winter. Among sites, it was always highest in RF followed by NF, DF and JF in both the seasons. In JF, it was very low in comparison to NF (Fig. 17).

4.9 AMF spore population in the study sites

AMF spore population or density was enumerated in 100 g air-dried soil. It showed a wider variation across the study sites and also the season ($p<0.01$). However, spore population in DF did not have any effect of season. Further, the interaction between site and season was also significant ($p<0.01$).

Both in winter and summer, it was highest in RF followed by NF, DF and JF. The spore population ranged from 199 to 351 AMF spores 100 g^{-1} soil in winter and 151 to 234 AMF spores 100 g^{-1} soil in summer. Spore population was relatively higher during winter (Fig. 18).

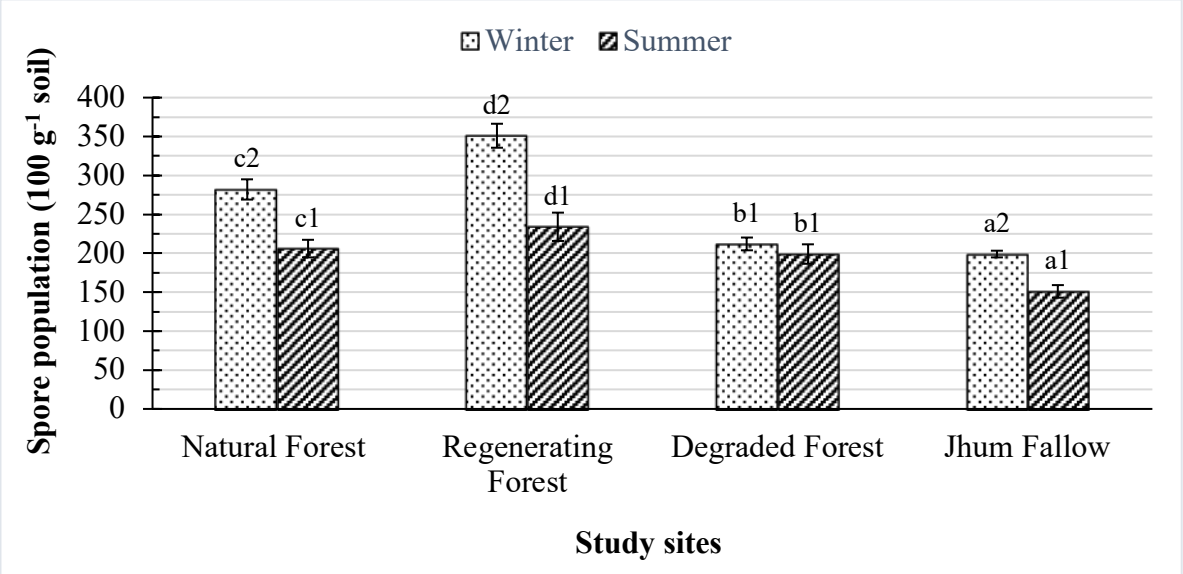


Fig. 18: AMF spore population in different study sites (Different letters and numeral denote a significant difference between forest types and seasons respectively). N = 4

4.9.1 Diversity and distribution of AM fungi

Distribution and occurrence of AMF species varied between the seasons. In winter, a total of 47 AMF species belonging to ten genera viz., *Acaulospora*, *Claroideoglomus*, *Dentiscutata*, *Funneliformis*, *Gigaspora*, *Glomus*, *Rhizophagus*, *Sclerocystis*, *Scutellospora* and *Septoglomus* were recovered from all the field soils of

all study sites. Out of this, 28 AMF species were recovered from RF, 27 from NF, 25 from DF and 24 from JF.

In summer, a total of 37 AMF species belonging to the same ten genera were recovered from the study sites with 27 AMF species from RF, and 21 species each from NF, DF and JF. In both the seasons, *Glomus* was the dominant genus followed by *Acaulospora* and *Scutellospora*. Some sporocarpic forms such as *Gl. aggregatus*, *Gl. intraradices*, *Scl. rubiformis*, *Scl. taiwanensis*, *Scl. clavisporum* etc. were also recovered from the study sites. Two additional AMF species were detected in trap culture which were not recovered from the field soil. *A. denticulata* and *Scu. coralloidea* were recovered from trap cultures of NF, RF and DF.

Overall, 32 AMF species recovered from field soil were common during both the seasons. 15 species were exclusively found in winter while 5 species exclusively in summer.

Isolation Frequency (%IF) and Relative Abundance (%RA) of each AMF species in the study sites show that *Funneliformis geosporum* and *Claroideoglomus etunicatum* were the highly abundant species whereas *F. geosporum*, *Cl. etunicatum, Gl. glomerulatum, Gl. macrocarpum, Septoglomus constrictum, Gigaspora margarita, Scutellospora calospora* and *Scl. taiwanensis* were most frequently present.

Relative abundance analysis also revealed that, during winter, in NF and DF, the highest number of recovered spores were of *Cl. etunicatum*. *F. geosporum* was the most abundant in RF and JF. *Gi. Margarita* was the least abundant in NF and JF while unidentified AMF 2 and *Glomus* sp. 4 was the least abundant in DF and RF respectively.

During summer, *F. geosporum* was the most abundant in NF, RF and DF while *Scl. Taiwanensis* was recorded highest in JF. Species of *Acaulospora* was the least abundant in all the sites.

Table 5: Relative Abundance (RA) and Isolation Frequency (IF) of AMF spores in different study sites in Winter

Sl. No.	AMF spores	RA (%)				IF (%)
		NF	RF	DF	JF	
1	*Acaulospora dilatata*	2.84	–	2.83	2.01	75
2	*A. laevis*	–	–	2.36	–	25
3	*A. rehmii*	1.77	–	–	–	25

4	*A. scrobiculata*	1.77	–	–	5.53	50
5	*A. spinosa*	1.42	1.42	–	3.52	75
6	*Acaulospora* sp. 1	–	0.85	–	–	25
7	*Cetraspora pellucida*	–	–	1.42	–	25
8	*Claroideoglomus claroideum*	1.06	3.99	–	–	50
9	*Cl. etunicatum*	28.37	9.12	14.15	5.03	100
10	*Dentiscutata erythropa*	–	0.85	–	4.02	50
11	*Funneliformis geosporum*	6.38	29.06	10.38	30.15	100
12	*Gigaspora albida*	–	–	–	1.51	25
13	*Gi. gigantea*	–	–	3.30	2.01	50
14	*Gi. margarita*	0.71	–	2.36	0.50	75
15	*Gigaspora* sp. 1	–	–	–	1.51	25
16	*Glomus aggregatum*	–	0.57	–	–	25
17	*Gl. deserticola*	–	–	2.36	–	25
18	*Gl. glomerulatum*	4.26	5.13	8.49	4.02	100
19	*Gl. hoi*	3.55	2.85	–	–	50
20	*Gl. intraradices*	2.13	1.14	–	–	50
21	*Gl. invermaium*	1.42	–	–	7.54	50
22	*Gl. macrocarpum*	8.51	4.27	9.43	10.55	100
23	*Gl. microcarpum*	1.77	3.42	1.89	–	75
24	*Gl. mosseae*	–	–	2.36	–	25
25	*Gl. multicaule*	–	–	–	2.01	25
26	*Gl. multiforum*	1.77	2.85	–	2.51	75
27	*Gl. versiforme*	–	1.14	1.42	–	50
28	*Glomus* sp. 1	–	0.57	–	–	25
29	*Glomus* sp. 2	5.32	7.12	9.43	–	75
30	*Glomus* sp. 3	2.13	3.99	4.72	2.51	100
31	*Glomus* sp. 4	–	0.57	1.89	–	50
32	*Glomus* sp. 5	–	0.85	–	0.50	50
33	*Rhizophagus clarus*	–	–	2.83	–	25
34	*R. fasiculatus*	–	2.85	2.83	–	50

35	*R. irregularis*	1.77	1.14	1.89	–	75
36	*Sclerocystis clavisporum*	–	–	0.94	–	25
37	*Scl. microcarpa*	1.06	–	–	–	25
38	*Scl. rubiformis*	3.90	2.28	2.83	–	75
39	*Scl. taiwanenesis*	4.96	4.27	3.77	5.03	100
40	*Scutellospora calospora*	1.77	1.14	–	1.01	75
41	*Scu. heterogama*	–	–	–	2.01	25
42	*Scu. scutata*	1.06	1.42	–	–	50
43	*Scutellospora* 1	0.71	–	–	1.51	50
44	*Septoglomus constrictum*	2.84	3.42	1.42	1.51	100
45	Unidentified AMF 1	5.32	2.85	3.77	2.51	100
46	Unidentified AMF 2	1.42	0.85	0.94	–	75
47	Unidentified AMF 3	–	–	–	1.01	25

(–) denotes absence of AMF species in the site.

Table 6: Relative Abundance (RA) and Isolation Frequency (IF) of AMF spores in different study sites in Summer

Sl. No.	AMF spores	RA (%)				IF
		NF	RF	DF	JF	(%)
1	*A. dilatata*	1.94	1.28	–	–	50
2	*A. foveata*	–	1.28	–	–	25
3	*A. laevis*	2.43	1.71	–	–	50
4	*A. rehmii*	–	2	4	–	50
5	*A. scrobiculata*	–	3.42	2.51	1.99	75
6	*A. spinosa*	1.94	2.14	2.01	–	75
7	*Cl. etunicatum*	7.77	7.69	10.05	7.95	100
8	*Dentiscutata erythropa*	–	2.56	2.01	–	50
9	*Funneliformis geosporum*	9.71	10.68	21.11	5.30	100
10	*Gi. gigantea*	–	–	2.01	1.99	50
11	*Gi. margarita*	3.88	2.14	3.02	2.65	100
12	*Gl. deserticola*	–	–	2.51	–	25
13	*Gl. glomerulatum*	5.83	8.55	5.03	6.62	100
14	*Gl. hoi*	3.88	3.85	–	–	50

15	*Gl. indicum*	–	–	–	3.31	25
16	*Gl. intraradices*	4.85	2.99	–	3.31	75
17	*Gl. macrocarpum*	7.28	9.83	11.06	11.92	100
18	*Gl. microcarpum*	2.91	–	–	3.31	50
19	*Gl. multicaule*	2.43	2.14	–	3.97	75
20	*Glomus* sp. 1	2.43	1.71	–	–	50
21	*Glomus* sp. 2	6.80	4.27	5.03	–	75
22	*Glomus* sp. 3	6.31	2.99	4.02	9.93	100
23	*Glomus* sp. 4	–	1.71	2.51	–	50
24	*Gl. versiforme*	–	1.71	3.02	–	50
25	*Gl. viscosum*	–	1.28	–	–	25
26	*Rh. clarus*	–	1.71	–	–	25
27	*Rh. fasciculatus*	3.88	–	–	–	25
28	*Scl. rubiformis*	4.37	2.56	2.01	–	75
29	*Scl. taiwanensis*	5.83	6.41	4.02	13.25	100
30	*Scutellospora* 1	–	–	–	1.99	25
31	*Scu. calospora*	2.91	2.99	3.02	3.31	100
32	*Scu. heterogama*	–	–	3.02	3.97	50
33	*Scu. scutata*	–	–	–	2.65	25
34	*Septoglomus constrictum*	4.85	5.56	2.51	–	75
35	Unidentified AMF 1	7.77	4.27	7.54	4.64	100
36	Unidentified AMF 4	–	2.56	–	3.31	50
37	Unidentified AMF 5	–	–	–	3.31	25

(–) denotes absence of AMF species in the site.

4.9.2 Diversity indices of AM fungi in different study sites

Species richness, Evenness (E), Simpson's index of dominance (D) and the Shannon-Wiener index of diversity (H') exhibited variations across different study sites. Simpson's index of dominance (D) was highest in DF during winter followed by NF, RF and JF. In summer, it was highest in NF and RF followed by JF and DF (Fig. 19).

The Shanon-Wiener index of diversity (H') displayed a pattern similar to the Simpson index in winter. However, during summer, it was highest in RF followed by NF, JF and DF (Fig. 20).

The value of evenness ranged from 0.58 in RF to 0.79 in DF in winter, while in summer it ranged from 0.78 in DF to 0.95 in RF (Fig. 21).

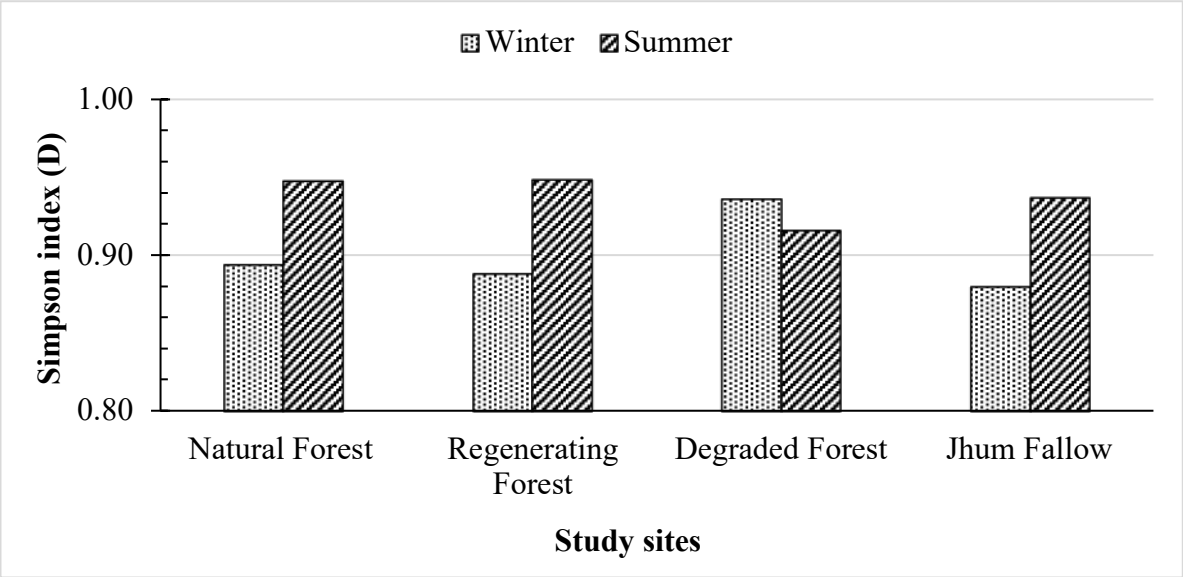


Fig. 19: Simpson's index of dominance (D) of AMF in different study sites

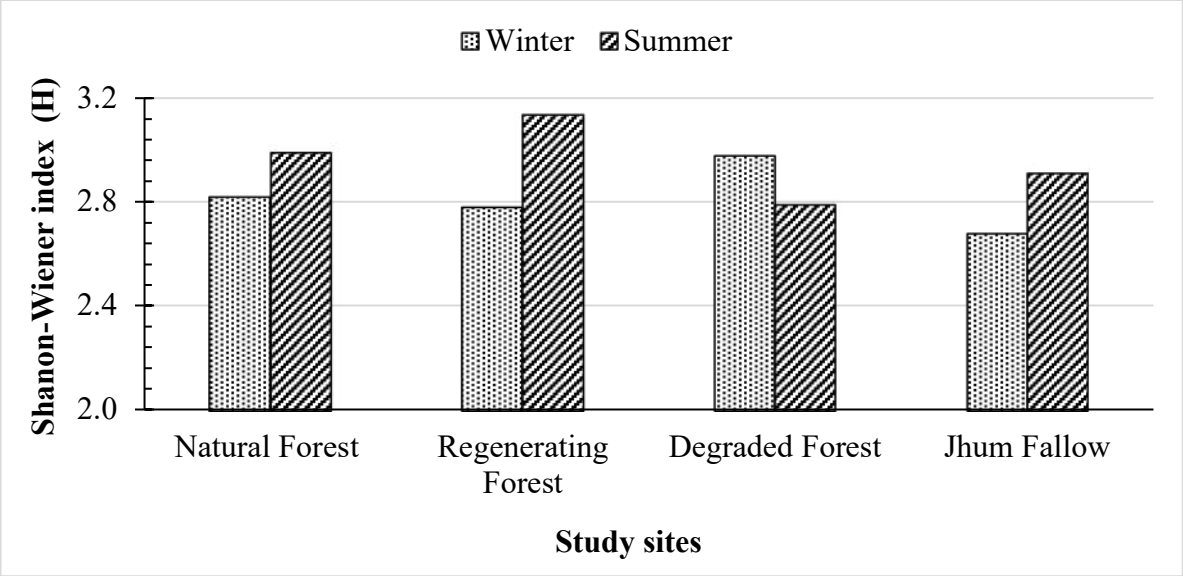


Fig. 20: Shanon-Wiener index of diversity (H') of AMF in different study sites

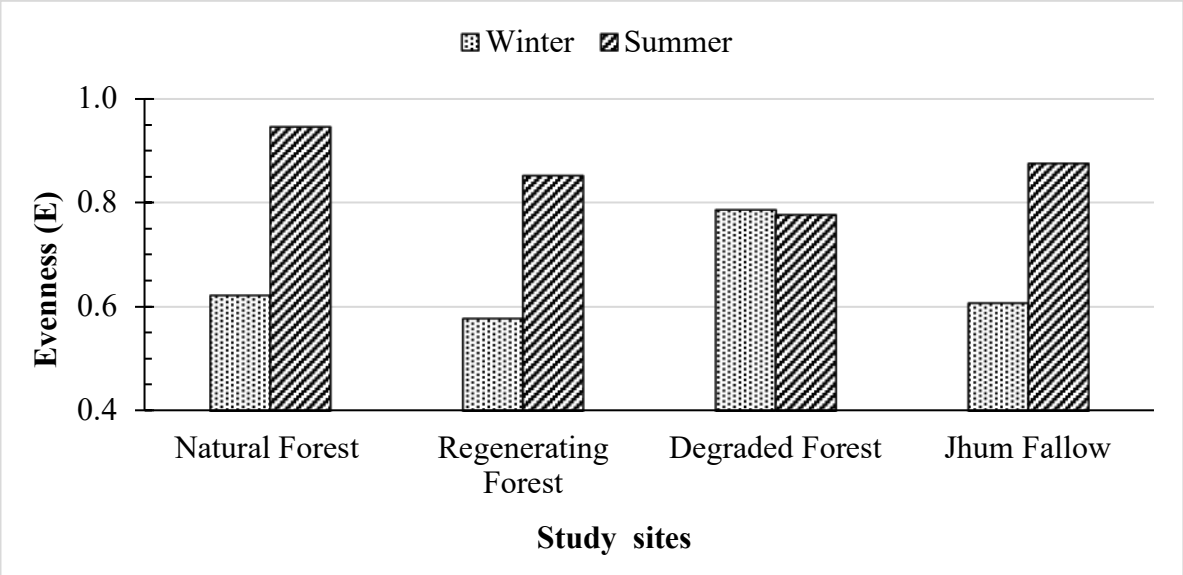


Fig. 21: Evenness (H') of AMF in different study sites

Species richness (S) of AMF in different study sites has been presented in (Fig. 22). Richness was more in regenerating forest in both the seasons in comparison to other sites.

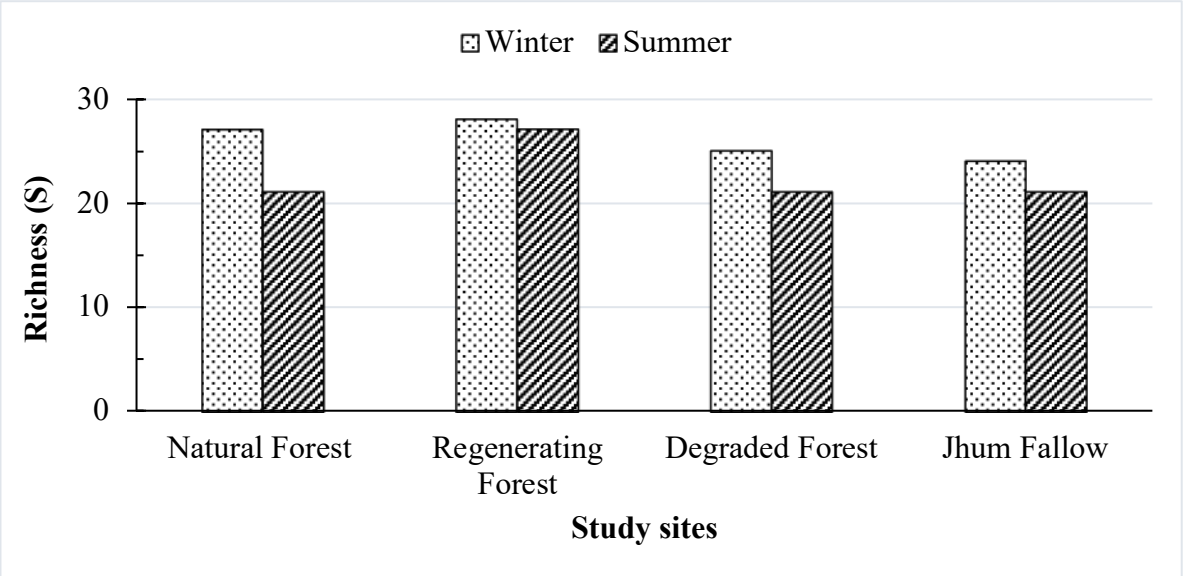


Fig. 22: Richness (S) of AMF in different study sites

4.9.3 Taxonomic identification of AMF spores

In the present work, the taxonomy of recovered AMF species was based entirely on spore morphology and characters outlined in the descriptions by INVAM. An attempt

was made to identify the fungal spores up to species level. Few spores were identified till genus level and some unidentified due to lack of distinctive characters.

Taxonomic descriptions of the identified AMF fungal spores are given below:

1. ***Acaulospora denticulata*** Sieverding & Toro. *Angew. Bot.* **61**: 217, 1987

Colour: Yellowish red to light orange
Shape: Mostly globose, sub-globose with denticulate surface ornamentation
Size: 112-170 µm
Spore wall: Three layers (L1, L2 and L 3), composite wall thickness 3-5 µm
Sporiferous Saccule
Colour: Hyaline
Shape: Globose
Size: 150-260 µm
Wall: One layer, 1-3.9 µm

2. ***Acaulospora dilatata*** J.B Morton. *Mycologia* **78**: 641, 1986

Colour: Pale yellow-brown
Shape: Mostly globose, sub-globose
Size: 100-130 µm
Spore wall: Three layers (L1, L2 and L 3), composite wall thickness 4-6 µm
Sporiferous Saccule
Colour: Hyaline
Shape: Globose
Size: 80-100 µm
Wall: One layer

3. ***Acaulospora foveata*** Janos & Trappe. *Mycotaxon* **15**: 515-522, 1982

Colour: Red-orange to dark red-brown
Shape: Globose to sub-globose, sometimes irregular
Size: 170-210 µm
Spore wall: Three layers (L1, L2 and L3), composite thickness 20-25 µm. Spore surface uniformly pitted with round to oblong occasional irregular depressions

4. ***Acaulospora laevis*** Gerdemann & Trappe. *Mycologia Memoir* **5**: 33, 1974

Colour: Orange-brown

Shape: Globose to sub-globose

Size: 140-240 µm

Spore wall: Three layers (L1, L2 and L3), composite thickness 3-5 µm

Sporiferous Saccule

Colour: Hyaline

Shape: Globose to subglobose

Size: 150-220 µm

Wall: One layer, 1-2 µm

5. ***Acaulospora rehmii*** Sieverding & Toro. *Angewandte Botanik* **61**: 219, 1987

Colour: Yellow-brown to Orange-brown

Shape: Globose to sub-globose, occasionally irregular

Size: 100-160 µm

Spore wall: Three layers (L1, L2 and L3), composite thickness 2-5 µm, spore surface ornamented with labrynth-form channels or network

6. ***Acaulospora scrobiculata*** Trappe. *Mycotaxon* **6**:359-366, 1977

Colour: Hyaline to pale yellow

Shape: Globose to sub-globose, occasionally irregular

Size: 80-160 µm

Spore wall: Three layers (L1, L2 and L3), composite thickness 5-10 µm, spore surface ornamented with profuse minute pores

Sporiferous Saccule

Colour: Hyaline

Shape: Globose to subglobose

Size: 110-140 µm

Wall: One layer, 1.7-2.5 µm

7. ***Acaulospora spinosa*** Walker & Trappe. *Mycotaxon* **12**: 515-521, 1981

Colour: pale orange-brown to light yellow-brown

Shape: Globose to sub-globose

Size: 140-220 µm

Spore wall: Three layers (L1, L2 and L3), composite thickness 2-6 µm, spore surface ornamented with blunt spines

Sporiferous Saccule

Colour: Pale yellow to light yellow

Shape: Globose to subglobose

Size: 130-170 µm

Wall: One layer, 0.6-1.3 µm

8. ***Acaulospora* sp. 1**

Colour: Hyaline to subhyaline

Shape: Globose

Size: 120-146 µm

Spore wall: Three layers (L1 and L2), ornamented with irregular pits, composite wall thickness 6-8 µm

9. ***Cetraspora pellucida*** Nicholson & Schenck. *Mycotaxon* **106**: 338, 2008

Colour: Hyaline to yellow-brown

Shape: Globose, sub-globose, often elliptical or strongly oblong

Size: 120-240 µm

Spore wall: Three layers (L1, L2 and L3)

Subtending hypha: Straight to slightly curved with a bulbous suspensor with 32-45 µm

Occlusion: Closure by a plug concolorous with the laminate layer of the spore wall

10. ***Claroideoglomus claroideum*** (*Glomus claroideum*) Schenck & Smith. *Mycologia* 74: 77-92, 1982

Sporocarp: Spores formed singly in the soil

Colour: Pale yellow to greyish-orange

Shape: Globose to sub-globose

Size: 95-135 µm

Spore wall: Consists of four layers (L1, L2, L3 and L4), composite thickness 6-8 µm

Subtending hypha: Straight to curved; cylindrical or funnel-shaped with 8-12 µm width

Occlusion: Occluded by spore wall L4

11. ***Claroideoglomus etunicatum*** (*Glomus etunicatum*) Becker & Gerdemann. *Mycotaxon* **6**: 29-32, 1977

Colour: Orange to red-brown

Shape: Globose to sub-globose

Size: 60-160 µm

Spore wall: Consists of two layers (L1 and L2), composite thickness 4-13 µm thick

Subtending hypha: Cylindrical to slightly flared with 5-10 µm width

Occlusion: Occlusion formed by the innermost sublayer of the laminate layer of the spore wall which resembles a septum

12. ***Dentiscutata erythropa*** (*Gigaspora erythropa)* Koske & Walker. *Mycologia* **76**: 250-255,1984

Colour: Red-brown to dark red-brown

Shape: Sub-globose to oblong

Size: 160-320 µm

Spore wall: Consists of two layers (L1 and L2)

Subtending hypha: Straight to slightly curved with a bulbous suspensor with 38-50 µm

Occlusion: Closure by a plug concolorous with L2 of the spore wall

13. ***Funneliformis geosporus*** (*Glomus geosporum*) Walker. *Mycotaxon* **15**: 49-61, 1982

Sporocarp: Absent, spores formed singly in the soil

Colour: Light to dark-brown

Shape: Globose to sub-globose

Size: 100-120 µm

Spore wall: Consists of three layers (L1, L2 and L3), composite thickness 5-11µm

Subtending hypha: Straight to recurved funnel shaped with 16-32 µm width

Occlusion: Occlusion by septum formed by the inner wall of the spore

14. ***Gigaspora albida*** Schenck & Smith. *Mycologia* **74**: 77-92, 1982

Colour: Hyaline to dull white
Shape: Globose to sub-globose
Size: 200-280 µm
Spore wall: Consists of three layers (L1, L2 and L3), composite thickness 4-12 µm
Subtending hypha: Straight with bulbous suspensor 32-45 µm width
Occlusion: Closure by a plug concolorous with L2 of the spore wall

15. ***Gigaspora decipiens*** Hall & Abbott. *Transactions of British Mycological Society* **83**: 203-208, 1984

Colour: Cream to white in young spores and translucent golden-yellow in mature spores
Shape: Globose to sub-globose
Size: 280-440 µm
Spore wall: Consists of three layers (L1, L2 and L3), composite thickness 30-50 µm
Subtending hypha: Straight with bulbous suspensor 51-63 µm width
Occlusion: Closure by a plug concolorous with the laminate layer of the spore wall

16. ***Gigaspora gigantea*** Nicholson & Gerdemann. *Mycologia* **60**: 313-325, 1968

Colour: Bright greenish yellow to yellow-green
Shape: Globose to sub-globose
Size: 240-400 µm
Spore wall: Consists of three layers (L1, L2 and L3), composite thickness 16-20 µm
Subtending hypha: Straight with bulbous suspensor 38-54 µm width
Occlusion: Closure by a plug concolorous with the laminate layer of the spore wall

17. ***Gigaspora margarita*** Becker & Hall. *Mycotaxon* **4**: 155-156, 1976

Colour: Creamy white in young spores and dark-yellow to orange at maturity
Shape: Globose to sub-globose
Size: 260-400 µm
Spore wall: Consists of three layers (L1, L2 and L3), composite thickness 30-40 µm
Subtending hypha: Straight with bulbous suspensor 34-47 µm width
Occlusion: Closure by a plug concolorous with the laminate layer of the spore wall

18. ***Gigaspora* sp. 1**

Colour: Yellowish orange
Shape: Globose
Size: 280-420 µm
Spore wall: Consists of three layers (L1, L2 and L3), composite thickness 20-30 µm
Subtending hypha: Straight with bulbous suspensor 34-47 µm width
Occlusion: Closure by a plug concolorous with the laminate layer of the spore wall

19. ***Glomus aggregatum*** Schenck & Smith emend. Koske. *Mycologia* **77**: 619-630, 1985

Sporocarp: Spores formed singly or in sporocarp without peridium
Colour: Pale yellow to yellow-brown
Shape: Spores globose to obovate, sometimes irregular
Size: 40-120 µm
Spore wall: Consists of two layers (L1 and L2), composite thickness 4-6 µm
Subtending hypha: Single occasionally double, straight or flared or constricted sometimes recurved with 4-10 µm
Occlusion: Hyphal pore is usually open, sometimes closed by a thin septum

20. ***Glomus deserticola*** (*Septoglomus deserticola*) Trappe, Bloss & Menge. *Mycotaxon* **20**: 123-127, 1984

Sporocarp: Spores formed singly or in loose aggregates lacking peridium
Colour: Orange-brown to dark red-brown
Shape: Globose to sub-globose, sometimes irregular
Size: 60-140 µm
Spore wall: Two layers (L1 and L2), composite thickness 3-4 µm
Subtending hypha: Cylindrical to slightly flares with 8-12 µm width at spore base
Occlusion: Usually by a septum

21. ***Glomus glomerulatum*** Sieverding. *Mycotaxon* **29**: 73-79, 1987

Sporocarp: Spores occur in sporocarp

Colour: Orange to golden yellow

Shape: Globose to sub-globose

Size: 40-70 µm

Spore wall: Two layers (L1 and L2), composite wall thickness 1-6 µm

Subtending hypha: Cylindrical or slightly constricted at the spore base flares with 5-7 µm width

Occlusion: Occluded by spore wall L2

22. ***Glomus hoi*** Berch & Trappe. *Mycologia* **77**: 654-657, 1985

Sporocarp: Borne singly in the soil

Colour: Orange to golden yellow

Shape: Globose, sub-globose, ellipsoidal or irregular

Size: 80-120 µm

Spore wall: Two layers (L1 and L2), composite thickness 3-6 µm

Subtending hypha: single, cylindrical or slightly flared with 8-11 µm width

Occlusion: Occluded by septum continuous with the spore wall L2

23. ***Glomus indicum*** Blaszkowski, Wubet & Harikumar. *Botany* **88:** 134, 2010

Sporocarp: Formed in hypogeous, loose aggregates

Colour: Hyaline to pale cream

Shape: Globose to sub-globose

Size: 17-52 µm

Spore wall: Two layers (L1 and L2), composite thickness 4-46 µm

Subtending hypha: single, straight or recurved, cylindrical to slightly funnel shaped, with 1.4-4 µm width

Occlusion: Usually open, rarely closed by a curved septum continuous with innermost laminae

24. ***Glomus intraradices*** Schenck & Smith. *Mycologia* **74**: 77-92, 1982

Sporocarp: Occurs in aggregates or singly

Colour: White pale cream to yellow brown

Shape: Globose, sub-globose, sometimes elliptical or irregular

Size: 40-140 µm

Spore wall: Three layers (L1, L2 and L3), composite thickness 11-14 µm
Subtending hypha: single, cylindrical to slightly flared, occasionally slightly constricted with 11-18 µm width
Occlusion: Occluded by innermost sublayer of spore wall L3

25. ***Glomus invermaium*** Hall. *Transactions of British Mycological Society* **68**: 345, 1977

Sporocarp: Formed in loose hypogeous sporocarps without a peridium
Colour: Light brown to brown
Shape: Globose
Size: 50-75 µm
Spore wall: Two layers (L1 and L2), composite thickness 6-8 µm
Subtending hypha: single, cylindrical to slightly flared, occasionally slightly constricted with 11-18 µm width
Occlusion: Closed by a septum continuous with innermost lamina of L2

26. ***Glomus macrocarpum*** Tulasne & Tulasne. *Giornale Botanico Italiano* **1:** 55-63, 1845

Sporocarp: Formed usually in hypogeous or epigeous sporocarps without a peridium, rarely single
Colour: Light brown to yellow-brown
Shape: Globose to sub-globose, rarely ovoid
Size: 130-150 µm
Spore wall: Two layers (L1 and L2), composite thickness 6-8 µm
Subtending hypha: Mostly single, sometimes with two hyphae, straight or curved, cylindrical to slightly flared, rarely constricted at the spore base with 17-20 µm width
Occlusion: Pores usually gradually narrow due to thickening of layer 2 of subtending hyphal wall, sometimes closed by curved septum

27. ***Glomus microcarpum*** Tulasne & Tulasne. *Giornale Botanico Italiano* **1:** 55-63, 1845

Sporocarp: Formed in compact sporocarps with a peridium, in aggregates without a peridium, rarely single

Colour: Hyaline to pastel yellow
Shape: Globose to sub-globose, rarely ovoid
Size: 22-50 µm
Spore wall: Two layers (L1 and L2), composite thickness 2-3 µm
Subtending hypha: Straight or curved, cylindrical, flared to funnel-shaped, with 6-8 µm width
Occlusion: Nearly occluded by wall thickening or closed by curved septum continuous with the innermost lamina

28. ***Glomus mosseae*** (*Funneliformis mosseae*) Gerdemann & Trappe. *Mycologia Memoir* No, **5**: 6, 1974

Sporocarp: Borne singly or in loose aggregates
Colour: Pale yellow to golden brown
Shape: Globose, sub-globose
Size: 100-280 µm
Spore wall: Three layers (L1, L2 and L3)
Subtending hypha: Flared to funnel-shaped with 6-8 µm
Occlusion: Occluded by a recurved septum

29. ***Glomus multicaule*** Gerdemann & Bakshi. *Transactions of British Mycological Society* **66**: 340-343, 1976

Sporocarp: Spores formed singly in soil
Colour: Brown to dark brown
Shape: Sub-globose, ellipsoidal, occasionally triangular
Size: 145-160 µm
Spore wall: Single layer (8.6-34 µm), thickest at the point of attachment
Subtending hypha: Multiple hyphae, usually 1-4, generally occurring at the opposite ends of spore
Occlusion: Closed by curved septum continuous with the innermost lamina

30. ***Glomus multiforum*** Tadych & Blaszkowski. *Mycologia* **89:** 805, 1997

Sporocarp: Borne singly in the soil
Colour: Deep yellow to brown

Shape: Globose, sub-globose
Size: 170-215 µm
Spore wall: Three layers (L1, L2 and L3), composite thickness 10-12 µm, evenly pitted with deep depressions
Subtending hypha: Straight or recurved, funnel-shaped with 20-24 µm width
Occlusion: Occluded by a septum continuous with the innermost lamina of the spore wall L3

31. ***Glomus versiforme*** (Karsten) Berch. *Canadian Journal of Botany* **61:** 2614, 1983

Sporocarp: Borne singly in the soil
Colour: Pale yellow to deep yellow
Shape: Globose, sub-globose
Size: 80-150 µm
Spore wall: One wall with two layers (L1 and L2), composite thickness 4-6 µm
Subtending hypha: Straight or recurved, cylindrical or flared with 6-8 µm width
Occlusion: Occluded by a curved septum continuous with the innermost sublayer of the spore wall or by a transverse septum

32. ***Glomus viscosum*** (Septoglomus viscosum) Walker *et. al*. *Mycological Research* **99**: 1500-1506, 1995

Sporocarp: Spores formed singly in soil or in loose aggregates
Colour: Hyaline to pale yellow
Shape: Globose to sub-globose
Size: 50-120 µm
Spore wall: Three layers (L1, L2 and L3), composite thickness 3-5 µm
Subtending hypha: Cylindrical to slightly flared, occasionally slightly constricted with 8-11 µm width
Occlusion: Appears to be absent

33. ***Glomus* sp. 1**

Colour: Light yellow to brown
Shape: Globose
Size: 99-104 µm

Spore wall: Two layers (L1 and L2), composite thickness 4-6 µm

Subtending hypha: single, cylindrical to slightly recurved 8-11 µm width

Occlusion: Closed by a septum continuous with innermost lamina of L2

34. ***Glomus* sp. 2**

Colour: Brown to dark brown

Shape: Oblong

Size: 63-117 µm

Spore wall: Two layers (L1 and L2), composite thickness 4-6 µm

Subtending hypha: single, cylindrical, recurved, slightly constricted at the spore base 8-10 µm width

Occlusion: Closed by a septum continuous with innermost lamina of L2

35. ***Glomus* sp. 3**

Colour: Dark yellow to brown

Shape: Strongly oblong

Size: 58-156 µm

Spore wall: Two layers (L1 and L2), composite thickness 4-5 µm

Subtending hypha: single, straight, 11-14 µm width

Occlusion: Closed by a septum continuous with innermost lamina of L2

36. ***Glomus* sp. 4**

Colour: Orange brown to brown

Shape: Elliptical or strongly oblong

Size: 42-155 µm

Spore wall: Two layers (L1 and L2), composite thickness 3-5 µm

Subtending hypha: Single, straight, 8-11 µm width

Occlusion: Closed by a septum continuous with innermost lamina of L2

37. ***Glomus* sp. 5**

Colour: Pale yellow to golden brown

Shape: Subglobose

Size: 110-160 µm

Spore wall: Two layers (L1 and L2), composite thickness 7-9 µm

Subtending hypha: Single, 8-10 µm width

Occlusion: Open or closed by a septum continuous with innermost lamina of L2

38. ***Rhizophagus clarus*** Nicholson & Schenck. *Mycologia* **71**: 178-198, 1979

Sporocarp: Spores formed singly in the soil

Colour: Hyaline to Pale yellow

Shape: Globose to sub-globose, sometimes ovoid

Size: 70-150 µm

Spore wall: Consists of three layers (L1, L2, and L3), composite thickness 18-20 µm

Subtending hypha: Straight to curved; cylindrical or funnel-shaped with 11-13 µm width

Occlusion: Occuluded by a curved septum continuous with the innermost laminae of the laminate spore wall L3

39. ***Rhizophagus fasciculatus*** (Glomus fasciculatum) (Thaxter) Walker & Koske. *Mycotaxon* **30**: 253-262, 1987

Sporocarp: Spores formed singly in soil or in aggregates

Colour: Yellow-brown to reddish-brown

Shape: Globose to sub-globose

Size: 60-110 µm

Spore wall: Three layers (L1, L2 and L3), composite thickness 6-8 µm

Subtending hypha: Cylindrical to slightly flared with 8-10 µm width

Occlusion: Fragile septum formed by the innermost layer of the spore wall (L3)

40. ***Rhizophagus irregularis*** (*Glomus irregulare*) (Blaszkowski, Wubet, Renker & Buscot) Walker & Schuβler. *Mycotaxon* **106**: 247-267, 2008

Sporocarp: Occur mainly in aggregates inside roots or in soil, rarely single

Colour: Hyaline to yellow-brown

Shape: Globose, sub-globose, ovoid, oblong or irregular enough to sometimes appear knobby

Size: 70-165 µm

Spore wall: Three layers (L1, L2 and L3), composite thickness 6-8 µm

Subtending hypha: Cylindrical to slightly flared with 7.4-19 µm width
Occlusion: Formed by recurved septum of inner lamina of L3, sometimes open

41. ***Sclerocystis clavispora*** Trappe (*Glomus clavisporum*) Almeida & Schenck. *Mycotaxon* **6**: 359, 1977

Sporocarp: Spores formed in complex sporocarp (Spores around a central plexus)
Colour: Orange-brown to dark brown
Shape: Single spores clavate to sub-cylindric, tapering towards a subtending hypha
Size: 17-41 µm
Spore wall: One wall composed of two layers (L1 and L2), 3 µm thick at the sides, 17-22 µm at the apex, 5-8 µm thick at the base
Subtending hypha: Straight or recurved, cylindrical to slightly flared with 5-10 µm width
Occlusion: open or occluded by inner laminate layer

42. ***Sclerocystis microcarpa*** Iqbal and Bushra. *Transactions of the Mycological Society of Japan* **21**: 57-63, 1980

Sporocarp: Spores formed in sporocarp without peridium
Colour: Brown to dark brown
Shape: Single spores clavate to sub-cylindric, tapering into a subtending hypha
Size: 40-80 µm
Spore wall: Spore wall laminate, 1.5-5 µm thick at the sides, 17-25 µm at the apex, 6-10 µm thick at the base
Subtending hypha: Straight to recurvate, sometimes cylindrical with 7-10 µm width
Occlusion: open or occluded by inner laminate layer

43. ***Sclerocystis rubifromis*** (*Glomus rubiforme*) Gerdemann and Trappe. *Mycologia Memoir* **5:** 76, 1974

Sporocarp: Spores formed in sporocarp without peridium
Colour: Yellow-brown to orange-brown
Shape: Single spores sub-globose to obovoid
Size: 37-75 µm
Spore wall: Two layers, 3-8 µm thick and up to 13 µm thick at the spore base

Subtending hypha: Straight to recurvate, funnel-shaped, sometimes cylindrical with 8-12 µm width
Occlusion: Occluded by a septum continuous with the innermost lamina of the spore wall layer

44. ***Sclerocystis taiwanensis*** (*Glomus taiwanense*) (Wu & Chen) Almeida & Schenck. Kew Bulletin, 50: 306. 1995

Sporocarp: Spores formed in complex sporocarp
Colour: Brown, reddish-brown or yellowish brown
Shape: Single spores clavate to cylindro-clavate, obovate to irregular
Size: 23-60 µm
Spore wall: Two layers (L1 and L2), 28-32 µm at the upper portion, 9-18 µm broad at the lower portion
Subtending hypha: Straight or recurved, cylindrical to slightly flared with 3-4 µm width
Occlusion: Occluded by septum formed by inner wall layer

45. ***Scutellospora calospora*** (Nicolson & Gerdemann) Walker & Sanders. *Mycotaxon* **27**: 219-235, 1986

Colour: Pale yellow with greenish tint to yellow-brown with greenish tint
Shape: Subglobose to ellipsoid to oblong sometimes irregular
Size: 120-220 µm
Spore wall: Two layers (L1 and L2), composite thickness 2-4 µm
Subtending hypha: Straight or recurved with bulbous suspensor 22-28 µm in diameter
Occlusion: Closure by a plug concolorous with the laminate layer of the spore wall

46. ***Scutellospora coralloidea*** (Trappe, Gerdemann & Ho) Walker & Sanders.

Colour: Dark brown
Shape: Globose to sub-globose
Size: 360-390 µm
Spore wall: Two layers (L1 and L2), composite thickness 8-15µm
Subtending hypha: Terminal or lateral with bulbous suspensor 35-45 µm in diameter
Occlusion: Closure by a plug concolorous with the laminate layer of the spore wall

47. ***Scutellospora heterogama*** (*Dentiscutata heterogama*) (Nicholson & Gerdemann) Walker & Sanders. *Mycotaxon* **27**: 180, 1986

Colour: Dark orange-brown to red-brown
Shape: Globose to sub-globose
Size: 120-200 µm
Spore wall: Three layers (L1, L2 and L3), composite thickness 8-10 µm
Subtending hypha: Straight with bulbous suspensor 24-28 µm in diameter
Occlusion: Closure by a plug concolorous with the L2 of the spore wall

48. ***Scutellospora scutata*** Walker & Diederichs. *Mycotaxon* **35**: 357-361, 1989

Colour: Hyaline/white to yellow-brown
Shape: Globose to sub-globose
Size: 350-550 µm
Spore wall: Two adherent layers (L1 hyaline and L2 laminated) with composite wall thickness 10-35 µm
Subtending hypha: Terminal or lateral with bulbous suspensor 47-90 µm in diameter
Occlusion: Closure by a plug concolorous with the wall of the spore wall

49. ***Scutellospora*** **sp. 1**

Colour: Yellow-brown to brown
Shape: Globose to sub-globose
Size: 300-500 µm
Spore wall: Two adherent layers (L1 hyaline and L2 laminated) with composite wall thickness 10-25 µm
Subtending hypha: Terminal or lateral with bulbous suspensor 40-85 µm in diameter
Occlusion: Closure by a plug concolorous with the wall of the spore wall

50. ***Septoglomus constrictum*** (Glomus constrictum) Trappe. *Mycotaxon* **6**: 359-366, 1977

Sporocarp: Spores formed singly in the soil
Colour: Dark brown to almost black
Shape: Globose to sub-globose

Size: 130-210 µm

Spore wall: Two layers (L1 hyaline and L2 laminate), composite thickness 12-14 µm

Subtending hypha: Cylindrical to constricted, occasionally slightly flared with 10-22 µm width

Occlusion: Occluded by a plug or a septum

51. **Unidentified AMF 1**

Colour: Bright orange to red

Shape: Globose

Size: 68-70 µm

Spore wall: Two layers (L1, L2 and L3), thicker L1, composite thickness 8-10 µm

Subtending hypha: Not observed

52. **Unidentified AMF 2**

Colour: Reddish brown to brown

Shape: Globose

Size: 128-130 µm

Spore wall: Two layers (L1 and L2), with reticulate ornamentation, composite thickness 4-6 µm

Subtending hypha: Not observed

53. **Unidentified AMF 3**

Colour: Bright red

Shape: Subglobose

Size: 110-125 µm

Spore wall: brown spore wall with two layers (L1 and L2), composite thickness 3-5 µm

Subtending hypha: Not observed

54. **Unidentified AMF 4**

Colour: Dark brown

Shape: Globose to subglobose

Size: 78-90 µm

Spore wall: Two layers (L1, L2 and L3), composite thickness 3-5 µm

Subtending hypha: Single, straight to slightly curved with 8-10 µm width
Occlusion: Closed by a septum continuous with innermost lamina of L3

55. **Unidentified AMF 5**

Colour: Brown to dark brown
Shape: Globose to subglobose
Size: 125-160 µm
Spore wall: Two layers (L1 and L2) with thorn-like projections ornamenting the upper surface, composite thickness 3-5 µm
Subtending hypha: Not observed

4.10 Correlation between AMF spore population and root colonization with physico-chemical properties of soil

Pearson's correlation analysis exhibited significant correlation between AMF spore population (AMF-SD) and Percent root colonization (RC) with soil physico-chemical properties across different seasons. During winter, a significant positive correlation was observed between AMF-SD and RC ($p<0.01$). Furthermore, AMF-SD also positively correlated with OC, available N ($p<0.01$), MC and DHA ($p<0.05$). RC also demonstrated positive correlations with MC, OC, available N and DHA ($p<0.01$). Both AMF-SD and RC correlated negatively with pH and available P ($p<0.01$) (Fig. 23).

AMF-SD and RC correlated positively ($p<0.01$) with each other in summer as well. Additionally, AMF-SD had positive correlations with MC, WHC, OC, available N and DHA ($p<0.01$) while showing negative correlations with BD, pH and available P ($p<0.05$). RC also exhibited positive correlations with MC, WHC, OC, available N ($p<0.01$) and DHA ($p<0.05$) while showing negative correlations with BD ($p<0.01$) and available P ($p<0.05$) (Fig. 24).

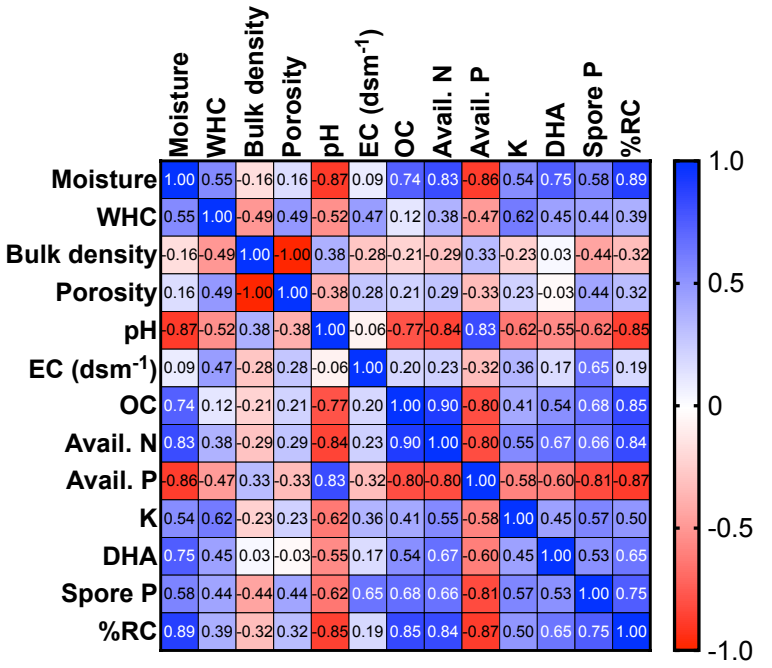


Fig. 23: Heat map of Pearson's correlation coefficient between AMF spore population and root colonization with physico-chemical properties of soil in Winter

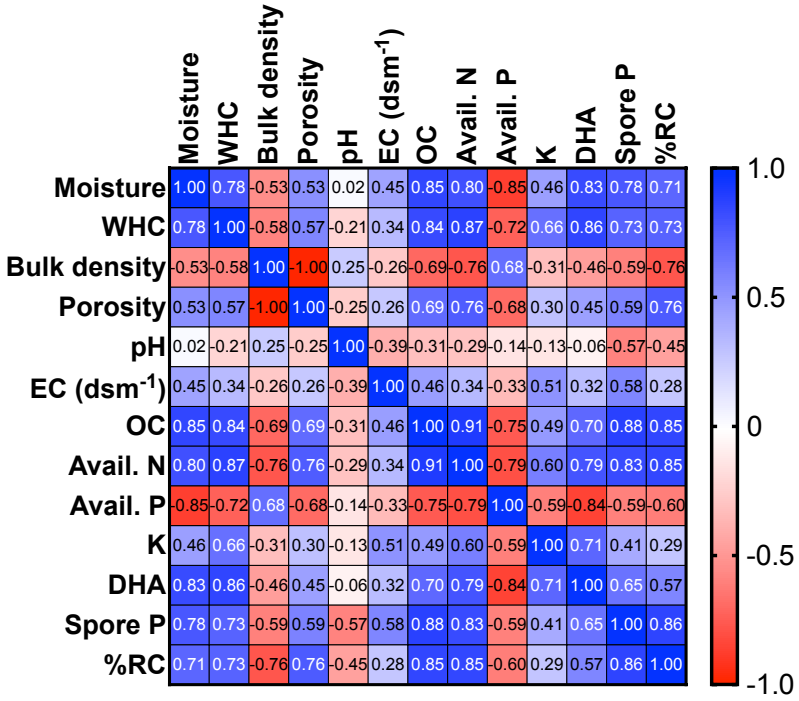


Fig. 24: Heat map of Pearson's correlation coefficient between AMF spore population and root colonization with physico-chemical properties of soil in Summer

CHAPTER V

DISCUSSION

5.1 Vegetation cover and its influence on soil physico-chemical properties

Before designating the site as a Natural Forest, the village head was consulted to gather information about the forest. As per his narration, the forest consisted of all naturally growing trees, and was frequently visited by a diverse range of wildlife especially elephants. Additionally, villagers occasionally visit the forest for collecting minor forest products. The characteristics measured in the present study regarding the existing vegetation further validated its classification as a Natural Forest. This site had 81.7% canopy cover and 34% of ground cover with a variety of herbs and shrubs. The tree density, encompassing both young and old trees falling into various DBH classes, was 382 per 100 m^{-2}. The presence of numerous aged trees with DBH exceeding 40 cm indicated insignificant disturbance to the standing vegetation due to human activities. Trees in DBH classes up to 40 cm were the most abundant with a sharp decline in the frequency thereafter thus resulting in a reverse J-shaped structure. This pattern of frequency indicates sustainable regeneration (Vetaas, 2000; Sujakhu et al., 2014). The average DBH of 27 cm recorded in our study is more or less similar to the report by Hauchhum and Singson (2020).

The Regenerating Forest (RF) selected in the present work was a 20 years old forest area which is artificially regenerating since 2002. The area was properly bounded since then allowing no human interference. It harboured naturally growing tree species, promoting the growth of native plant species. The site had 67 % canopy cover and 62 % ground cover with a tree density of 347 per 100 m^{-2}, slightly less than that of the natural forest site. The number of mature trees was also lesser than the natural forest, and 80% of trees were less than 30 cm in DBH. These characteristics indicates rapid regeneration, ultimately suggesting that this forest site is undergoing a regeneration process.

Degraded Forest area was characterised by overgrazing by livestock, extensive tree felling in some area, and also small constructions to some extent. A significantly low tree density and a lesser canopy cover were observed compared to both natural and Regenerating forest sites. Further, it had a large portion of the ground floor covered with herbaceous plants and shrubs (77%) which was notably higher compared to the previous two sites.

The Jhum fallows selected in the present study were of 3-5 years old, fully covered with weeds and shrubs and only a few sparsely distributed trees. They exhibited the lowest tree density among all the sites, while having the highest ground coverage. Out of the few countable trees found at the site, majority of the trees were below 20 cm in DBH and only two very old trees falling in 91-100 DBH class.

The occurrence of low ground coverage in an area with high tree density and dense canopy cover can be explained by the fact that roof like structure formed in a forest due to its dense canopy blocks the sunlight from reaching to the ground, thus hampering the growth of the understory. Similar occurrences have also been reported by other workers (Charles-Dominique et al., 2018; Randle et al., 2018; Garg et al., 2022). Garg et al. (2022) has reported an inverse relationship between the forest canopy and the ground vegetation in semi-arid forests of Aravalli hills, and the species diversity, richness and evenness being higher in the gaps than the understory. They stated that the forest canopy impedes solar radiation from reaching the forest floor and that the trees competes for nutrients, space, soil water and radiation with the herbaceous vegetation that in turn influences the species composition of the ground herbaceous vegetation. They further noted that the absence of trees led to decrease in competition by species for resources in the gaps. Similar finding was reported by Veselkin et al. (2021) where they observed a negative correlation between the canopy cover and the number of ground vascular plants due to restriction in the amount of light.

The vegetation at four sites selected in the study was different in composition which appears to have affected the soil properties. Such shift in vegetation cover and deforestation alters the physico-chemical properties of soil and its structure (Pickett et. al. 2001; Yan et. al. 2016). Disturbance on vegetation of an area alters soil quality by limiting the organic inputs into the soil (Rutigliano et al., 2004; Singh et al., 2004; Mekuria, 2013). A variation in soil texture might be due to different degrees of disturbances across the sites (Mishra et. al., 2019). Presence of higher sand content in disturbed sites (DF and JF) found in the present study may be attributed to less vegetation cover in these sites which might have allowed soil erosion in those sites (Bhuyan and Laskar, 2020). The findings of the present work revealed no significant variation in bulk density (BD) across the sites, however, it was slightly higher in both the disturbed sites (DF and JF) than the undisturbed sites (NF and RF). This finding is supported by Bhuyan and Laskar (2020), who found a lower BD in deep forest than degraded forest in a wildlife sanctuary (Nongkhyllem) in Meghalaya. BD is an indicator of soil compaction, hence soils with lower BD have higher porosity which facilitates more root activity and infiltration of water into soil

thus positively influencing overall growth of plants (Bhuyan and Momin, 2015). BD is related to soil texture and organic matter content. Therefore, a lower BD recorded in both the undisturbed sites might be related to higher clay content and organic carbon in the soil. Such correlation has also been reported by Chaudhari et. al. (2013). The undisturbed sites (NF and RF) had slightly lower bulk density in comparison to the disturbed sites (DF and JF). This might have been a consequence of deforestation and burning of forests for jhum cultivation leading to a decrease in organic carbon content. This finding parallels with the reports of Kumar et al. (2023), where they observed the highest bulk density in natural forest and the lowest in jhum fields. As bulk density is inversely related to porosity, hence soils with a lower bulk density has a greater porosity which facilitates water infiltration into soil and better root activity leading to overall growth and development of plants (Bhuyan and Momin, 2015).

Overall, the pH across different study sites was slightly acidic in nature which could be due to high rainfall in the region leading to leaching of basic cations from soil (Mishra and Francaviglia, 2021). A lower pH in the undisturbed sites (NF and RF) in comparison to the DF and JF was concurrent with the reports of Barraclough and Olsson (2018), Hong et al. (2019) and others where lower pH in the natural forest was attributed to higher organic matter which on decomposition leads to production of more organic acids, thereby lowering the soil pH. It is also reported that burning of fields causes denaturation of organic acids releasing base cations leading to an increase in soil pH in Jhum fields (Certini, 2005). Accumulation of ash due to burning of jhum fields might have also added alkalizing effect on soil as it contains base cations (Kauffman, 1993). However, Tripathi et al. (2022) and Kumar et al. (2023) reported a contrasting finding. Further, they found slightly higher pH in the natural forest site than the disturbed site.

In the present study, Organic Carbon (OC), available N and available Phosphorus were significantly influenced by the vegetation types present in different study sites. A higher OC content in undisturbed sites (NF and RF) can be attributed to humification of litter deposits leading to higher organic matter, and also the secretions released from the rhizosphere during the process of plant growth (Zhang et al., 2004; Dijkstra et al., 2006). The present finding is consistent with the reports of Panwar et al. (2011), Akhtaruzzaman et al. (2020) and Kumar et al. (2023) who observed a higher OC in the forest soils compared to other sites. Further, degradation of vegetation causes a decrease in aboveground biomass which leads to reduced litter production and a lesser amount of plant residues entering into the soil, thus eventually lowering the main source of organic matter (Wang et al., 2014, Yan et al., 2018). When a natural

forest is converted into a jhum field, the soil organic carbon undergoes significant reduction as a result of forest burning and oxidation of organic matter (Kumar et al., 2017). A similar finding is also reported by Bhuyan et al. (2013) in the East Siang district of Arunachal Pradesh. Smith et al. (2016) and Villarino et al. (2017) also reported a decrease in soil organic carbon content by 9-25% in agricultural systems that were converted from the native forest.

The available N content was also higher in undisturbed sites (NF and RF). Such occurrence is often associated with a high litter fall in the forest sites (Akhtaruzzaman et al., 2020) resulting in elevated organic carbon content which is often correlated positively with soil nitrogen (Berger et al., 2002). Present finding align with the reports of Chen and Li (2003) and Wu et al. (2020) who observed higher nitrogen content in forest soils compared to other vegetation types due to accumulation of litter residues. Additionally, since deforestation decreases above-ground biomass and litter production and thus the soil nutrients, it affects the soil structure and ultimately slows down nutrient cycling (Lost et al., 2007; Foote et al., 2015). A lower available N content in degraded forest might be due to overgrazing by livestock as it reduces plant biomass, destroys soil aggregation, effects soil permeability, ultimately lowering nitrogen input (Zhou et al., 2017). However, Barraclough and Olsson (2018) reported a higher OC and N in burned fields than in forest site. Their fields were burned 0-5 years prior to sampling but occasional burning was also done intermittently to stop the spread of vegetation. Perhaps, their sampling was done just after burning or within a short span resulting in high OC and N contents. Increase in mineralization rate of N due to higher pH, and the base cations in slashed and burned fields, also explains the increase in N content (Ellingson et al., 2000).

Available Phosphorus in soil, however, was found significantly higher in the disturbed sites. Phosphorus is an essential nutrient in plant's growth and development and the readily available inorganic phosphorus present in the soil is absorbed for its optimal growth. When there is a decline in the plant biomass, the amount of phosphorus absorbed also decreases, thereby leaving more phosphorus in the soil (Hinsinger et al., 2011). The finding also align with the reports of Turrion et al. (2000), who observed 17% increase in phosphorus concentration in a deforested area turned into pasture land. They attributed the deposition of livestock excrement in the soil which adds up to the organic forms of phosphorus. Furthermore, in Deforested and Jhum fields, soil organic P is converted to orthophosphate through the process of pyro-mineralization, and high soil pH increases P availability in the absence of Ca, thus resulting in an increased level of available phosphate (Giardina et al., 2000). Osman et al. (2013) also found a higher available P content in a 3 year old jhum fallow than a natural forest which was likely

due to assimilation of sequestered P on above-ground plant biomass in to the soil during burning. In contrary, Reza et al. (2014) found higher available phosphorus content in forest soils in comparison to a jhum fallow which they attributed to the higher recycling of P by tree species in the forest and subsequent recycling by decomposition and mineralization of litter residues.

5.2 Effect of vegetation cover and soil physico-chemical properties on microbial activity (Dehydrogenase activity)

Microbial activity in the soil is often considered as a potential indicator of soil quality as plants depend on soil microorganisms for the mineralization and transformation of organic matters essential for their growth and development (Chen et al., 2003), playing a pivotal role in nutrient cycling (Dick and Tabatabai, 1993). Soil microbial activity is measured by determining the enzyme activities in the soil since these enzymes are very sensitive to natural as well as anthropogenic disturbances and responds quickly to the changes (Dick, 1997). Dehydrogenase is among the most crucial enzymes in the soil environment, which are used as an indicator of soil microbial activity (Gu et al., 2009; Salazar et al., 2011) because they exist intracellularly in all microbial cells and not extracellularly in the soil (Zhao et al., 2010; Yuan and Yue, 2012).

The present study observed a significant difference in soil microbial activity among different sites, with a decrease associated with intensified deforestation. This observation is consistent with findings from previous studies that have reported diminished microbial activity in deforested areas compared to natural forests (Moreno-de las Heras, 2009; Wu et al., 2020; Barros et al., 2020; Arora et al., 2022).

Pascual et al. (2000) investigated dehydrogenase enzyme activity (DHA) in a natural forest and an abandoned agricultural land, observing higher microbial activity in natural soils compared to the agricultural land, which had been abandoned for 10-20 years. This decline in soil microbial activity was attributed to progressive erosion in the abandoned soils due to reduced plant cover and low organic matter. Similar studies on DHA in natural soil were conducted by Quilchano and Marañón (2002) and Leirós et al. (2000), both recording higher microbial activity in dense forest soils with higher organic content. The increasing plant cover in the forest produced more litter, which was subsequently incorporated into the soil. Arunachalam et al. (1999) investigated the impact of degradation on soil microbial activity in Northeast India, observing adverse changes in DHA with increasing levels of deforestation. Kumar et al. (2023) documented a 29.1% decrease in DHA after the conversion of a natural

forest into a jhum land in Arunachal Pradesh, indicating reduced enzyme activity due to declining vegetation cover.

The observed decrease in soil microbial activity could be due to the loss of plant biomass, resulting in reduced soil organic carbon content, as higher organic matter leads to higher microbial activity. Moreover, undisturbed sites with greater plant diversity offer more substrates to soil microbes (dos Santos et al., 2019). Furthermore, the reduction in vegetation cover exposes the soil surface microbial population to adverse effects of rain and high temperatures, ultimately diminishing its activity in the soil (Kumar et al., 2023).

A significantly reduced DHA in jhum fallow in comparison to natural and regenerating forest as observed in the present study may be attributed to higher decomposition of litter residues in the later sites. This finding is also concurrent with the reports of Reza et al. (2014) who recorded higher DHA in Natural forest, Areca nut, and Pineapple orchards than jhum fallows. Lower DHA in jhum fallow may result from soil nutrient loss and reduced soluble organic matter (Ralte et al., 2005). They also observed highest activity in the surface layer which declined with soil depth regardless of the land use. This seems to be due to better aeration and nutrient availability in the surface layer where soil microbes are predominantly confined.

The physico-chemical properties of soil frequently influence microbial activities, and alterations in soil properties lead to corresponding changes in enzymatic activities (Maphuhla et al., 2021; Wu et al., 2020). In the present study, Pearson's correlation matrix showed a positive correlation between DHA and soil moisture, WHC, organic carbon, available Nitrogen and Potassium while a negative correlation with soil pH and available Phosphorus.

Soil moisture has been recognized as a crucial factor strongly affecting microbial population and their activity , and extreme dryness is unfavourable for their survival (Tiwari et al., 1987). Low water availability can affect microbial activity by lowering the intracellular water potential which ultimately affects hydration and reduces microbial activity (Stark and Firestone 1994; Geisseler at al., 2011), and also by restricting the substrate supply. In wet soils, increased moisture brings soluble organic matter in to the soil solution thus increasing bacterial population and DHA (Subhani et al., 2001).

The present study witnessed a negative correlation between soil pH and DHA. Findings by various worker presents ambiguous picture regarding the relation between soil pH and DHA. Each enzyme has its optimal pH range at which it works the best. Researchers across the world have reported optimal pH for DHA but perhaps due to the heterogeneity of soil type across the

world, a particular pH range might not be optimal for all soil types. Brzezińska et al. (2001) noted pH range between 6.6-7.2 as optimum for DHA whereas Ros et al. (2003) and Nagatsuka and Furosaka (1980) found it to be 7.6-7.8 and 7.4–8.5 respectively. On the other hand, Trevors (1984) reported very slight DHA below pH 6.6. and above pH 9.5. Contrary to present finding, Fernandez-Calviño et al. (2010) noted a significant positive correlations between soil pH and DHA, indicating that increase in acidity suppresses potential enzyme activity. The present study found highest DHA in pH range between 5.06-5.1.

A positive correlation between DHA and organic carbon and nitrogen in soil has been described in several studies (Subhani et al., 2001; Zhao et al., 2001; Yuan and Yue, 2012; Piotrowska-Długosz et al., 2021; Wu et al., 2023) which indicates towards a pivotal role of microbial enzymes in the transformation of organic matter in the soil (Wolińska and Stępniewska, 2011). As organic matter provides substrates for microbes to function, its higher quantity in the soil supports more active soil microorganisms, thereby enhancing their population and enzyme production, and thus accelerating its decomposition.

5.3 Effect of vegetation types on AMF

Vegetation composition across different study sites influenced the AMF status and root colonization. AMF spore population varied significantly across the sites with the highest being recorded in the regenerating forest and the lowest in the jhum fallows. Overall, the disturbed sites (DF and JF) recorded lower AMF population than the undisturbed sites (NF and RF). The findings align with various studies done around the world (Barraclough and Olsson, 2018; Birhane et al., 2020; Tripathi et al., 2022) suggesting a strong dependency of spore population on vegetation type. Anthropogenic activities reduce below ground AM fungal communities, and the intensity of such disturbances also determines AMF diversity (Allen et al., 1998; Korb et al., 2003). Disturbance in an area removes pioneer plants many of which serves as host plants and thus could cause a lower spore population. Birhane et al. (2020) also relates direct association of plant diversity with AMF diversity since a higher spore population is found in the rhizosphere of mature trees. They also attributed an increased litter fall, and more root biomass which maintains a diverse AMF community in natural forest, for harbouring more AMF species and greater spore population. Furthermore, the greater richness of AMF species in undisturbed sites can be attributed to the higher plant diversity in forested areas, as a variety of plant species offer more niches for hosting AMF (Melo et al., 2020). Su and Guo (2007) obtained a comparable outcome, noting a significant decrease in the mean spore population of AMF in overgrazed plots

compared to non-grazed ones in the Inner Mongolia steppe. Li et al. (2007) similarly observed a reduction in spore population due to agricultural practices, with the highest spore population being in never-cultivated fields, slightly lower in old fields, and highly reduced in cultivated fields in the hot and arid ecosystem of Southwest China.

Diversity and population of AMF is also reported to increase with canopy cover since plants with more canopy convert higher solar inception into photosynthates which provides carbon source to the AMF (Sarkar et al., 2014). In the present study, the Natural forest had the highest tree density and also the highest canopy cover which relate positively with AMF spore population. Similarly, low AMF spore population in jhum fallow may be attributed to scarce distribution of tree species.

Few studies done in Northeast India on AMF status in Jhum fallows and Natural forests also revealed lower AMF diversity as well as abundance in Jhum fallows (Singh et al., 2003; Sharma and Jha, 2011; Bordoloi et al., 2015; Tripathi et al., 2022) Bordoloi et al. (2015) examined the diversity of AMF in seven different land use systems in Arunachal Pradesh in Eastern Himalaya and recorded the highest species richness and diversity index in natural forest and the least in Jhum fallow due to high disturbance by fire. In a similar study for assessing species diversity of AMF in jhum fallow and natural soils of Arunachal Pradesh, Singh et al. (2003) reported low AMF diversity, spore population and infective propagules in jhum fallow which was ascribed to repeated burning of the fields, loss of primary host plants on which these fungi depend for their carbon sources, and adverse edaphic conditions for AMF regeneration. The findings of the present study aligns with their findings. The study recorded the lowest inoculum potential in jhum fallow followed by degraded forest, natural forest, and the highest in the regenerating forest. This pattern also correlated with AMF spore population. A higher inoculum potential found in a forest site has been also attributed to the dominance of pioneer species which support multiplication of AMF (Zangaro et al., 2000).

A disturbance to the above-ground vegetation also affected the quantum of root colonization which was significantly more in the undisturbed sites than the disturbed sites. Previous studies have also documented that a change in above-ground vegetation due to a change in land use decreases AMF root colonization (Boddington and Dodd, 2000; Oehl et al., 2005) and also the inoculum potential (Zangaro et al., 2000), and attributed break down of AMF hyphal network in the ground during cultivation, grazing or burning process for such decrease in root colonization. The present finding is also supported by Birhane et al. (2020) who reported a variation in AMF root colonization in plant communities subjected to different levels of

disturbances, and that plant communities with higher density had more colonization. Lesser root colonization have been recorded in degraded sites in comparison to native forest soils in the studies conducted in Northeast India and Arunachal Pradesh (Singh et al., 2003; Sharma and Jha, 2011; Bordoloi et al., 2015; Tripathi et al., 2022). Mycorrhizal hyphae are also considered as an important cementing agent facilitating soil aggregation, thus maintaining its stability (Miller and Jastrow, 1992), and bridging the annular space and establishing a physical connection between soil particles and root surface (Miller, 1987; Ravarkar et al., 2000). Such crucial role played by the AMF hyphae gets compromised during deforestation. Soils experiencing reduced soil aggregation in such cases lack the ability to effectively retain organic matter, nutrients, and moisture, all essential for the germination, growth, and development of both plants and fungal partners in abandoned lands (Singh et al., 2003).

The roots collected from the study sites displayed various structures of arbuscular mycorrhizal fungi (AMF), including hyphae, arbuscules, vesicles, and occasionally intra-radical spores. Hyphal colonization was observed to be the highest, followed by vesicular and arbuscular colonization. The present finding align with the results of Belay et al. (2013) who similarly noted higher hyphal and vesicular colonization compared to other structures. This pattern can be attributed to the extended lifespan of hyphae, which are primary AMF structures, whereas vesicles function as storage structures, remaining in roots for months or even years. In contrast, arbuscules undergo senescence after a few days (Sarkar et al., 2014).

Notably, among the two undisturbed sites, regenerating forest exhibited a higher spore population and greater root colonization in the composite root samples when compared to NF. This might be due to different stage of forest development. While NF is a mature forest (Climax), regenerating forest is in a successional stage harbouring a greater number of young plants. A higher root colonization and spore population may indicate greater reliance on mycorrhizal benefits by plant community in a regenerating forest which aids in the establishment, growth and survival of young regenerating trees (Zangaro et al., 2007, 2013). As plants community in a mature stable forest have already attained a climax stage, grow under a balanced environment, exhibit lower nutritional demand, thereby making their association with AMF less necessary (Zangaro et al., 2002).

Plant species in RF exhibit high growth rate and photosynthetic activity leading to an increased availability of carbon to the AMF (Gamage et al., 2004). With more trees regenerating in a forest, more root formation takes place, thus involving a substantial bilateral transfer of resources between the host root and the AMF (Bonfante and Genre, 2010; Gutjahr and Parniske,

2013). The presence of specific fungal structures, such as hyphae, plays a crucial role in forest regeneration (Guadarrama et al., 2008). In this process, plants contribute photosynthates to AMF, promoting growth of their hyphae, both inside and outside of the roots, leading to a significant transfer of nutrients from the soil to the host plants (Lebrón et al., 2012). The higher hyphal colonization in regenerating forest may also signify an increased transfer of essential nutrients, supporting plant growth during this stage of succession.

Vesicles, serving as lipid storage structures, assumes importance in regenerating environment, serving as energy sources for AMF spore germination under favourable conditions (Roth and Paszkowski, 2017). AMF spores are effective structures for the persistence, colonization, and propagation of AMF communities, particularly in successional environments (Wu et al., 2007). Thus, the greater quantity of these structures found in roots of regenerating forest, compared to more mature areas, may be linked to the survival and reproductive success of fungi. Similar study by da Silva et al. (2023) who investigated the diversity and composition of AMF communities in succession areas of Atlantic forest supports the present finding.

In contrast to the findings in the present study, there are reports of equal or higher spore counts and AMF diversity in the deforested areas compared to the natural forests (Picone, 2000; Zhang et al., 2004). These studies asserted that deforestation has a minimal impact on the composition of AMF community, and, in fact, the deforested land exhibits greater total species richness. The primary explanation provided for the heightened spore population and species richness in deforested areas was the prevalence of annual herbaceous plants (Kovacic et al., 1984) that were found to be more closely associated with AM fungal spore production compared to the evergreen broad-leaved trees (Hetrick and Bloom, 1986; Trappe, 1987). Additionally, deforestation was noted to reduce soil moisture content and elevate soil temperature, conditions typically favourable for AMF spore production (Guadarrama and Álvarez, 1999; Parke et al., 1983). Another plausible reason might be that in challenging environments AMF stive for survival and elevate their sporulation rates as spores serves as the resilient structures of these fungi (da Silva et al., 2006).

5.4 Correlation of soil physico-chemical properties with AMF spore population and root colonization

Pearson's correlation matrix exhibited a strong correlation between AMF spore population and root colonization (RC), and also with some of the physico-chemical properties of soil and microbial activity. The present study recorded a positive correlation between AMF

spore population and RC in both the seasons that aligns with other reports (Wuen et al., 2002; Sivakumar, 2013; Songachan et al., 2014; Birhane et al., 2017, 2020). However, some contrasting reports also exist that there is no significant correlation exists between AMF root colonization and their spore population in the soil (Daniel et al., 2001; Camargo-Raicalde and Dhillion, 2003; Li et al., 2007; Khade and Rodrigues, 2008, Uma et al., 2012). Conversely, Fontenla et al. (1998) found a negative relationship between AMF colonization and spore population. Generally, root colonization is influenced by spore availability (Muthukumar et al., 2003), however, spore numbers may not consistently correlate with the rate and extent of mycorrhizal infection, given that AM fungal colonization and sporulation are influenced by a diverse range of plant, fungal, and environmental factors, and also the fact that root infection is initiated by propagules other than the spores, such as AMF hyphae in the soil or mycorrhizal roots of neighbouring plants that are present in abundance in the soil (Smith and Read, 2008). Thus, it appears that in certain cases the spore population of the AMF community may not precisely reflect their root-colonizing ability, which is potentially attributed to the presence of some equally infective but non-sporulating AMF species (Pandey et al., 2016), adaptation of AMF species to specific soil conditions (Dhar and Mridha, 2003), and differences in the timing of AMF sporulation (Sutton and Barron, 1972; Gemma et al., 1989), seasonal fluctuations in the development of the host plant (Giovannetti, 1985), and seasonal variations in nutrient availability (Louis and Lim, 1987).

Various physical and chemical properties of soil also influenced AMF diversity, spore population and RC. A significant positive correlation observed between soil moisture content and the population of AMF spores aligns with the findings of Khanam et al. (2006) and Kumar et al. (2010). Soil moisture content has been reported to exert a profound influence on the population of AMF spores, and the moisture level optimal for plant growth coincides with favourable conditions for AMF sporulation (Redhead, 1975). Bhardwaj and Chandra (2018) considered soil moisture as an important limiting factor in maintaining root colonization in tree species. Their findings indicated a positive correlation between soil moisture and RC, thus affirming our findings. However, they found a negative correlation between moisture and spore population.

Although, the pH recorded across different sites in the present study was slightly acidic, a negative correlation was observed among soil pH, spore population and RC. The findings aligns with Songachan et al. (2011) and Nongkling and Kayang (2017). Singh et al. (2003) and Akond et al. (2008) observed that slightly acidic to neutral soils exhibit moderate to high level

of AMF spores in the jhum fallow and natural forest of Arunachal Pradesh (NE India), and agricultural lands of Dhaka (Bangladesh) respectively. However, several studies (Tahat and Sijam, 2012; Rajeshkumar et al., 2013; Liu et al., 2020) reported contrasting findings on correlation between soil pH and AMF status citing that acidic soils create unfavourable conditions both for plant growth and AMF activity. Liu et al. (2020) observed that AMF activity was inhibited in acidic pH since high acidity reduces formation of arbuscules. Nonetheless, several studies (Young et al., 1985; Robson and Abbott, 1989) suggests that the response of AMF to soil pH varies widely and appears to be primarily dependent on the AMF species. Some AMF species thrive in either acidic or alkaline soils, while others can adapt to both the conditions. Also, soil pH can act as a selective factor influencing the composition of rhizosphere microorganisms. Soil pH exerts a significant impact on plant growth, nutrient mobilization and its availability (Neina, 2019). Concerning AMF, various species exhibit adaptation to distinct pH ranges for their development (Kawahara et al., 2016), and there is a suggestion that pH serves as the primary determinant of AMF communities (Oehl et al., 2010; Hazard et al., 2013; Sun et al., 2016). According to Wang et al. (1993) and Friberg (2001), the most notable response of AMF occurs within a pH range of 5.5 to 7.1 while, Bücking and Kafle (2015) reported no impact on the symbiotic activity of AMF in plant roots at soil pH ranging from 4.5 to 7.5. Guo et al. (1996) observed a considerable decrease in the number of spores, and at times their absence in strongly acidic soils with pH below 4.5.

The availability of soil nutrients is considered vital for regulating the assembly of AMF communities (Johnson et al., 2015; Jiang et al., 2018), and vice versa (Rillig et al., 2015). Both spore population and RC were positively correlated with OC in the present study, aligning with other similar studies (Bodington and Dodd, 2000; Khanam et al., 2006; Birhane et al., 2020; Dhumal and Shinde, 2020). AMF is believed to contribute to the soil carbon pool (Wilson et al., 2009) and may potentially enhance carbon storage in the long run (Iversen et al., 2012) by boosting the photosynthetic rate of host plants by up to 30%, leading to increased carbon fixation (Drigo et al., 2010).

The extent of AMF's impact on soil carbon storage may vary based on factors such as the type of hyphae produced, the duration of accumulated hyphal residues, production of glomalin, and consequent stabilization of soil aggregates (Zhu and Miller, 2003). The rates of colonization impact the ability of AMF to provide soil nutrients to its host plant in exchange for the carbon necessary for mycorrhizal growth. This, in turn, directly influences more germination and the development of fungal hyphae (Cai, 2017). The presence of organic matter enhances

soil water-holding capacity, potentially leading to increased sporulation of AMF, thus demonstrating a positive correlation with the soil OC content (Mohammad et al., 2003; Mathur et al., 2007). However, a few contrasting reports by Hindumathi and Reddy (2011) noted high spore population in fields of Soybean and Mungbean having a low OC content.

A positive correlation between AMF spore population and RC with available N in the present work corroborates with other reports (Khanam et al., 2006; Egerton-Warburton et al., 2007; Silvana et al., 2020). In general, elevated concentrations of nutrients in the soil have been reported to reduce mycorrhizal colonization (Liu and Li, 2000). An earlier study suggested that N can either enhance or inhibit RC and spore production by altering the soil pH (Sylvia and Neal, 1990). They also reported N as a key factor in shaping mycorrhizal associations, primarily acting through changes in soil pH. However, the impact of N on spore abundance is interconnected with other soil factors and is dependent on the specific host plant with which they are associated. Nonetheless, an inverse relation of available N with spore population and RC has also been reported (Bago et al., 2004; Deepak et al., 2015). More specifically, an abundance of N in the soil has been shown to inhibit both spore population and root colonization of AMF (Deepak et al., 2015), whereas a lower N content in the soil has been associated with an increase in root colonization (Muthukumar, 2000). Khade and Rodrigues, (2008, 2009) observed negative association between total N and spore population.

AMF helps plants in acquiring nutrient, especially P (Allen, 1991). A significant negative correlation was observed between available P and AMF spore population and RC which was similar with other studies (Lekberg and Koide, 2005; Khanam et al., 2006; Bainard et al., 2014; Birhane et al., 2017; Nguyen et al., 2019). The result also stands parallel with the findings of Menge et al. (1978) where they reported a reduced spore production with an increase in P level which in turn affected root colonization. El-Sherbeny et al. (2022) in their experiment found that soil P level higher than that required for plant growth eliminated mycorrhizal association due to reduced arbuscular development. It is a well-established fact that higher soil P decreases root exudates by affecting phospholipid membrane resulting in to lesser arbuscule formation as well as a reduced vegetative growth of AMF (Tawaraya, 2003). However, such inhibition may be indirectly associated with the host's phosphorus status (Sander, 1975) as some reports indicate that a high supply of P does not always exert a negative impact on the root colonization (Gosling et al., 2013) and spore population (Khade and Rodrigues, 2009). Some other factors reported to affect the degree of AM colonization and sporulation include the sensitivity of mycorrhizal species and strains to phosphorus (Trouvelot et al., 1986), the diverse

host root growth response to changes in phosphorus levels, and alterations in cell membrane permeability at varying cellular phosphorus concentrations (Smith, 1982).

The present study also found a significant correlation between DHA, spore population and RC that aligns with the studies of Burak et al. (2024). It has been shown that the secretions of AMF play a role in influencing the composition and activity of microbial communities in the rhizosphere (Veresoglou and Rillig, 2012). This finding indicates that in soil environment, AMF engage in interactions with a diverse array of microorganisms and enhance soil fertility.

5.5 AMF diversity and distribution

A total of forty-seven and thirty-seven AMF species in winter and summer respectively belonging to 10 genera were recovered from the study sites in field soils. Two additional AMF species were detected in trap culture which was not recovered in filed soil. AMF sporulation is often influenced by the host plant through their effects on hyphal development, propagule activation and sporulation (Siqueira et al., 1985; Bever et al., 1996). Though trap culture may not necessarily allow the development and identification of all AMF species present in the original sample but it can promote the sporulation of cryptic AMF species that did not sporulate at the time sampling (Stürmer, 2004). Moreover, appearance of additional species exclusively in trap cultures and not in field samples have also been documented (Jansa et al., 2002; Oehl et al., 2004). On the contrary, certain AMF species, which regularly produce spores in natural soil conditions, might go undetected in trap cultures. This could be attributed to less favourable conditions for their sporulation within the controlled environment of pots or due to competition with other species (Brundrett et al., 1999). Consequently, a comprehensive analysis of AMF necessitates studying both field soils and trap cultures.

A total of ten genera were recorded across the study sites (*Acaulospora*, *Cetraspora*, *Claroideoglomus*, *Dentiscutata*, *Funnelisformis*, *Gigaspora*, *Glomus*, *Rhizophagus*, *Sclerocystis* and *Septoglomus*). The number of species recorded during the winter and summer seasons were: 27, 28, 25, 24 and 27, 21, 21, 21 from NF, RF, DF and JF respectively indicating that the AMF species richness did not vary much across the sites.

During the winter, a total of fifteen AMF species were exclusively identified, while five AMF species exclusively during the summer. In a similar study, Singh et al. (2003) have reported recovery of forty-four AMF species belonging to six genera directly from the soil samples collected from jhum fallow and natural forest in Arunachal Pradesh that lies within 20

Km radius of the present study sites. As they did taxonomic identification of the AMF directly on the basis of field collected spores without doing a trap or pure culture, therefore the accuracy of their findings is not beyond doubt. It is well reported that field collected spores are under different degrees of degradation due to microbial action and do not provide reliable taxonomic characters (Oehl et al., 2004). Sharmah and Jha (2011) extracted and identified 12 AMF species belonging to four genera from undisturbed and disturbed forests of Karbi Anglong district of Assam. Both of the mentioned studies reported a higher species richness in undisturbed sites than in disturbed sites. Moreover, almost all the AMF species occurring in disturbed sites occurred in undisturbed sites as well except a few species being exclusive to each site. The non-appearance of some of the AMF species in the jhum fallow underscores the enduring negative impact of soil disturbance caused by slash-and-burn agricultural practices on AMF diversity. Another factor contributing to the reduced AMF populations and species diversity in the jhum fallow site might be destruction of AMF propagules due to the uncontrolled burning of dried slash on the soil surface during preparation of fields for crop cultivation (Singh et al., 2003). Ground clearing of fields also leads to soil erosion ultimately leading to loss of AMF propagules and a lesser species richness.

During winter, though regenerating forest exhibited the highest species richness in comparison to the other three site but the variation among them was minimal. However, evenness was most pronounced in deforested forest. Evenness serves as a metric for the abundance of different species, and there was no observed correlation between specie richness and evenness. Additionally, Simpson (D) and Shannon-Wiener (H) indices were calculated as measures of diversity, considering both species richness and evenness. The Simpson index assigns more weight to evenness and common species, while the Shannon-Wiener (H) index accounts for both species richness and places more emphasis on less abundant or rare species. Consequently, DF displayed a higher Simpson index during the winter and a greater Shannon-Wiener (H) index during the summer season.

A positive correlation was observed between AMF species richness and AMF spore population across all the sites, similar to the reports by other workers (D'Souza and Rodrigues, 2013; Radhika and Rodrigues, 2010). *Glomus* was found to be the dominant genus in all the study sites during both the seasons followed by *Acaulospora*. Similar to the present study, Dhumal and Shinde (2020) found *Glomus* spp. accounting for 50% of the total 52 retrieved AMF species followed by *Acaulospora* spp. Various other studies conducted in Arunachal Pradesh and in Northeast India reported *Glomus* as the most widespread species (Singh et al.,

2003; Bordoloi et al., 2015; Surendirakumar et al., 2016; Tripathi et al., 2022). *Glomus* has a global distribution and encountered most frequently in cultivated lands (Khade and Rodrigues, 2009). This dominance can be attributed to the fact that *Glomus* species are frequently present and thrive in various natural ecosystems (Manoharachary et al., 2005), exhibiting profuse sporulation within a short period leading to the production of smaller spores (Zhao et al., 2003; Wang et al., 2019). The ability of *Glomus* species to flourish in slightly acidic to neutral pH conditions may also contribute to their dominance (Graw, 1979). Species of *Acaulospora* are reported to thrive well in acidic soil and known to exhibit resilience towards environmental disturbances (Hart and Reader, 2002; Winagraski et al., 2019). They have been reported as the dominant genera in certain tropical natural lands in Costa Rica (Lovelock et al., 2003) and secondary vegetation in Brazil (Stürmer and Siqueira, 2006). Additionally, Mathimaran et al. (2007) reported a higher number of *Acaulospora* species in the rhizosphere samples of *Zea mays* and *Crotalaria* sp. cultivated in agricultural fields in Kenya. In more stabilized environments, Acaulosporoid AMF species exhibit lower nutritional demands from their hosts, which can be beneficial (Gehring and Whitham 2002). Bever et al. (1996) also found that, in the same environment, *Glomus* and *Acaulospora* species typically yield more spores than *Gigaspora* and *Scutellospora* species. Due to their smaller spore size, *Glomus* and *Acaulospora* species have a quicker sporulation process (Hepper, 1984), making them more adaptable to adjusting their pattern of sporulation in diverse environmental conditions (Stutz and Morton, 1996).

Among the AMF species, the highest Isolation Frequency (IF) was calculated for the *Cl. etunicatum*, *Gl. glomerulatum*, *Gl. macrocarpum*, *Funneliformis geosporum* and *Scl. taiwanensis* in all the sites and during both the seasons. *Gl. macrocarpum* (Stürmer et al., 2018) and *Gl. glomerulatum* (Reyes et al., 2019) have been reported of cosmopolitan distribution and known to occur in successional environments in different agro-systems and various land uses (Sousa et al., 2014; Pontes et al., 2017; Reyes et al., 2019).

5.6 Effect of season on soil physico-chemical properties

Soil moisture content (MC) was higher in summer than winter due to relatively higher rainfall. A lower pH during winter may be due to low MC and decomposition of organic acids while, in wet season, there is an increase in soil pH due to restoration of moisture (Baruah et al., 2018; Temjen et al., 2022) and increase in temperature (Guojo et al., 2020).

Although no significant effect of season on OC was observed, the values were slightly higher in summer except in DF. Higher OC during summer has been reported by several studies

(Shilpkar et al., 2010; Osobamiro and Adewuyi 2018; Temjem et al., 2022) which could be due to higher carbon input from litter decomposition as warmer temperature accelerates the decomposition process (Zhao et al., 2009). Okoro and Tordue (2023) also attributed higher rainfall in summer season for elevated carbon input. In contrary, Carter et al. (1998) and Sofi et al. (2012) concluded that elevated soil temperature during the summer season renders the soil more susceptible to erosion, consequently leading to a reduction in soil OC. Dry season also witnesses higher litter fall which ultimately increases its decomposition process leading to significant renewal of organic matter in the soil (Srivastava, 1992; Campo et al., 1998).

Available N content remained similar in both the seasons in every site. It has been reported that available N content increases during summer (wet) season. However, Taylor et al. (1975) observed maximum availability of nitrogen during late winter. Low levels of available N is attributed to low rates of nitrate and ammonium production. William (1969) also stated that death of a significant portion of soil microorganisms in winter due to cold temperature leads to an accumulation of protein substrate which facilitates N mineralization. However, several studies associated increased N level with wet season. Bergeron et al. (2002) attributed an increased nitrogen level during the rainy season to rapid mineralization, facilitated by sufficient soil moisture. Choudhri and Sharma (1975) also indicated that elevated total nitrogen levels in the soil during the rainy season signify blue-green algae fixation, input from rainwater, and a high rate of mineral nitrogen through microbial decomposition. Singh and Singh (2006) additionally reported that nutrient uptake by plants significantly decreases during dry periods, and the processes of N-mineralization and nitrification are either immobilized in the microbial biomass or accumulate in the soil as inorganic nitrogen.

Available P in the selected four sites of the present study did not vary significantly due to season though NF and JF had slightly higher content during winter while RF and DF during summer. Miller and Donahuer (2001) and Okoro and Tordue (2023), however, reported higher available P during the winter season which was attributed to the absence or minimal rainfall, preventing nutrient leaching, and the substantial deposition of litter falls in winter, leading to nutrient accumulation during this period. Conversely, the wet season sees a decrease in available P due to leaching caused by excessive rainfall and soil erosion. Ashraf et al. (2014) similarly reported that soils experiencing extensive leaching tend to contain lower phosphorus levels compared to those with minimal leaching.

In Arunachal Pradesh, winter stands out from the rest of India as the region experiences notable rainfall during this season, unlike other states. This could explain why the delineation of seasons had limited impact on the soil's physico-chemical properties.

5.7 Effect of season on soil microbial activity

The present study exhibited a conflicting report on the seasonal variation on microbial activity. While it was significantly higher in NF during winter, the effect was almost similar in RF and DF. In JF, DHA was higher during summer. Several studies report a higher microbial activity during the summer season (Yang et al., 2010; Devi and Yadava, 2010; Lepcha and Devi, 2020). The acceleration of litter decomposition is heightened during the rainy season due to warm and wet weather, as microbial activities and decomposition reach their peak during this period. This increase in microbial activity leads to greater nutrient immobilization by the microbes (Usman et al., 2000; Devi and Yadava, 2010). Additionally, the high relative humidity during the wet period fosters the growth of fungi, further contributing to the enhancement of microbial biomass carbon (Acea and Carballas, 1990). Moreover, a low temperature and lesser soil moisture during winter season leads to the death of microorganisms (Groffman et al., 2001). Eaton (2001) also explains that accumulation of organic matter in winter due to higher litter fall in that period enhances the microbial activity in the onset of rainy days. García-Oliva et al. (2003) however reported lower microbial activity during summer. He observed a decrease in microbial population resulted from a reduction in carbon and soil nutrient sources associated with litter decomposition (since winter witnesses higher litter fall), heightened grazing by soil fauna, and plant nutrient uptake along with leaching. Various researchers in India (Prasad et al., 1994; Singh et al., 1989; Srivastava, 1992) also documented a lower microbial activity due to decline in microbial biomass during the initiation of the wet season in soils from tropical dry forests

They suggested that soil microbial species accumulate intracellular solutes during the dry season, leading to a drop in populations through lysis in response to alterations in soil water potential after the initial rains of the wet season. Hence establishing a negative correlation between soil moisture and microbial biomass leading to decreased microbial activity in the wet season.

Soil moisture typically decreases in winter compared to summer, but in Arunachal Pradesh, where winter receives substantial rainfall, prevents the soil from reaching extremely low moisture levels that could lead to plant vegetation drying out and wilting. Consequently,

the seasonal effect on microbial activity may not be as pronounced. Additionally, personal observations during the survey confirmed that moisture levels were sufficient during the winter season.

5.8 Effect of season on AMF status

In the present study, season significantly influenced AMF spore population, RC, inoculum potential and species richness. AMF is known to be seasonal in their sporulation and colonization pattern (Guadarrama et al., 1999). Su et al. (2011) attributes hosts and season as the key factors in determining the AMF species richness and spore population in natural settings. In this study, the spore population, RC and inoculum potential were relatively higher during the winter (dry) than in summer (wet) season. The probable cause for this could be the environmental stress experienced during dry seasons, prompting the need for assistance from AMF. Gaur and Kaushik (2011) similarly documented a high AMF population during the dry season. They explained that elevated temperatures and reduced moisture levels contribute to a decrease in spore count.

de Quevedo et al. (2022) also documented elevated spore population and greater AMF species richness during dry seasons. This outcome of intense sporulation in the dry season is considered a survival strategy employed during the water stress phase of host plants (da Silva et al., 2014; Deepika and Kothamasi, 2015).

Studies on the influence of seasonal variation on spore population and RC were conducted by various researchers but the results were not consistent. Tapwal et al. (2023) noted a higher spore population and low RC during winter while a lower spore population and higher RC during summer. Vieira Junior et al. (2020) found higher mycorrhizal activity in dry season and attributed it to higher glomalin production. Bouamri et al. (2014) also studied the effect of season on AMF associated with Date palms in Ottawa, Canada. They observed a significantly higher RC level during the wet season, which correlated with the vegetative growth period of Date palms and the soil water availability. This suggests that, the AMF associations were effectively established and remained precisely functional when Date palms required increased nutrient allocations to support heightened metabolic activities, synchronized with higher water availability and lower surrounding temperatures (Sanders and Fitter, 1992; Bohrer et al., 2004). However, they observed a higher spore population in dry season, which reinforces the established connection between AMF sporulation levels and water availability (Lugo and Cabello, 2002).

These findings reveals that the seasonal pattern in AMF colonization could be influenced by host pants and their phenological events, particularly vegetative growth, flowering and fruiting and also the concentrations of available P (Kennedy et al., 2002; Ruotsalainen et al., 2002; Bouamri et al., 2014). Sitienei et al. (2015) asserted that sporulation takes place in the dry season as a consequence of root senescence, facilitated by the potential for significant root turnover, especially in annuals or competitive environments. During the wet season, spores germinate rapidly or disintegrate due to high moisture, and their numbers may be reduced by microbial activity (Guadarrama and Alvarez-Sánchez, 1999; Cuenca and Lovera, 2010; Sitienei et al., 2015). Rather than being controlled by the AMF species, the population of AMF spores is also influenced by the prevailing environmental conditions (Koske and Halvorson, 1981) and the host plant species (Varela-Cervero et al., 2016). Further, the output of AMF spores is known to vary significantly among ecosystems, with regulation by various factors such as habitat, host, fungus, and spore population, which tends to increase during root inactivity or senescence (Muthukumar et al., 2003).

The present study aimed to study the impact of deforestation on AM status, soil properties and microbial activity, subsequent to conversion of a natural forest to degraded forest or Jhum land, and re-establishment of AM status. Notably, the regenerating forest exhibited a higher build-up of AMF status compared to the degraded sites.

It was observed that relying solely on the morpho-taxonomic identification of AM species based on spores may result in an underestimation of AM diversity within a given soil. To overcome this limitation, a molecular taxonomy approach is suggested for a more accurate estimation, especially in detecting less sporulating AMF species. However, this approach would require adequate budgetary support for successful implementation.

The study also proposes a more comprehensive investigation into the shift in plant community structure resulting from deforestation, aiming to provide a clearer understanding of the overall ecological impact. Additionally, the suggestion of a chrono-sequence study, involving the observation of a forest-cleared site and the gradual re-establishment of vegetation over time, is put forth. This approach will unveil the intricate relationships between below-ground AMF diversity, microbial activity, soil properties, and above-ground vegetation dynamics. Overall, these insights could contribute to a more holistic comprehension of the complex ecological consequences of deforestation and subsequent ecosystem restoration efforts.

CHAPTER VI

SUMMARY

Arbuscular Mycorrhizal Fungi (AMF) are a small group of soil fungi (about 230 species) placed under the phylum Glomeromycota. They form symbiotic relationship with roots of 80% of terrestrial higher plants, and with the help of their extensive mycelial network in the soil enhance their acquisition of water and mineral nutrition from soil, especially phosphate and nitrogen. They also enhance resistance ability of their host plants against several abiotic and biotic stresses. In return, the fungus gets fixed carbon compounds from the host plant. AMF are generally considered non-host specific, but some level of host preference has been reported. Thus, symbiosis with AMF improve fitness of the host plants from an individual to community level by imparting them a better competitive ability to survive and grow in an otherwise adverse ecological condition. Apart from their plant growth promoting ability, AMF also improve overall soil quality by aiding to the formation of stable soil aggregates, creating a macroporous soil structure that facilitates water and air penetration while preventing erosion.

The composition and growth of vegetation depend on the soil nutrient concentration and soil microorganisms since they play a key role in mineralization and transformation of organic matters essential for their growth and development. Therefore, owning to their various ecological functions, AMF are regarded as one of the key players in shaping plant diversity, community structure, and succession within an ecosystem. Besides, the dependence of AMF on their host plants for photosynthate implies that structure & function of the AM fungal community is also influenced by the composition of the plant community, thus suggesting a reciprocal relationship. Besides plant community, physico-chemical properties of soil, and any natural or anthropogenic activities that affect soil and vegetation also influence AMF community structure.

Forests are threatened by anthropogenic activities, primarily deforestation, that negatively impacts above-ground vegetation, soil properties, and microbial communities including AMF. The AMF suffers breakdown of its hyphal network, reduced root infection, and a diminished spore population in soil. In humid tropics, deforestation enhances soil erosion and it happens severely in hilly areas that receives heavy rainfall for a longer period of time.

The effect of deforestation on the mycorrhizal status of soil in Northeast India is under-researched. The delicate nature of the soil in this region, which is susceptible to erosion due to

intense rainfall and steep slopes, suggests that deforestation is likely to have pronounced adverse effects. Arunachal Pradesh, a region that falls within the Eastern Himalaya biodiversity hotspot, boasts an expansive and densely forested terrain. Unfortunately, over the past few decades, the region has witnessed accelerated deforestation due to various anthropogenic activities such as lumbering, slash-and-burn agriculture, urbanization, and developmental projects. Slash-and-burn agriculture, also known as Jhum cultivation, is a traditional farming method practiced by the indigenous tribes. It requires almost complete clearing of a section of the forest, followed by controlled burning. The burnt area is utilized for crop cultivation for 2 to 3 years before being left fallow that get occupied over the time largely by shrubs, herbs and a few saplings. This cultivation practice makes the soil loose, exposed to desiccation, and prone to different degree of erosion depending upon the runoff of rainwater on the slope of the fields. Hence, it may have a profound effect on AMF status in the soil.

As existing studies on AMF status in soil in the region are limited and some of them have methodological shortcomings, therefore, considering all these aspects, the present study was aimed to evaluate the effects of deforestation in tropical forests of Arunachal Pradesh by selecting four different sites i.e. Natural forest, Regenerating forest, Degraded forest, and Jhum fallows. The regenerating was selected to see whether AMF status have improved over 20-year period since a complete restriction on logging. Data were recorded in two different seasons following standard methodologies for various parameter such as canopy cover, ground vegetation cover, dominant plant species, soil properties, soil microbial activity, AMF diversity, AMF root infection and AMF spore population.

The key findings of the study were as follows:

1. Vegetation cover and its influence on soil physico-chemical properties

The vegetation cover varied among the sites, and a lesser tree density and canopy cover was associated with increased ground cover. The number of mature trees were more in Natural forest which indicates a stable community, while young trees were more in Regenerating forest indicating regeneration. While bulk density and porosity did not differ significantly, water holding capacity, soil moisture content, pH, organic carbon, soil available N, P and K varied significantly across the sites.

2. Effect of vegetation cover and soil physico-chemical properties on microbial activity (Dehydrogenase activity)

Soil dehydrogenase activity (DHA) varied significantly among the sites. During the winter season, DHA exhibited a range of 0.035 to 0.353 µg Triphenyl formazan (TPF) g^{-1} 24 hr^{-1}. The highest activity was observed in Natural forest, followed by Regenerating forest, Degraded forest, and Jhum fallow. It correlated positively with soil moisture content ($p<0.01$), available N ($p<0.01$) and organic carbon ($p<0.05$) while negatively correlated with available P ($p<0.01$) and soil pH ($p<0.05$).

During summer season, the values ranged from 0.058 to 0.211 µg TPF g^{-1} 24 hr^{-1}, with Natural forest again displaying the highest activity, followed by Regenerating forest, Degraded forest, and Jhum fallow. There was a significant positive correlation of DHA ($p<0.01$) with water holding capacity, soil moisture content, organic carbon, available N and K whereas a strong negative correlation with available P ($p<0.01$).

3. Effect of deforestation on AM colonization in composite root samples (CRS)

AMF root colonization in Composite root sample (CRS) varied significantly among sites. It was observed that hyphal colonization inside roots was more prevalent than the vesicular or arbuscular colonization.

During winter, it was high in Regenerating forest, although not significantly different from Natural forest, slightly less in Degraded forest and significantly less in Jhum fallow.

During summer, the highest colonization was again observed in Regenerating forest followed by Natural forest, Degraded forest and Jhum fallow. Regenerating forest was similar to Natural forest while differing significantly from Degraded forest and Jhum fallow.

4. Effect of deforestation on Inoculum potential (IP) of AMF in soil

IP varied across the sites and recorded highest in Natural forest followed by Regenerating forest, Degraded forest and Jhum fallow in both the seasons.

In Jhum fallow, it was very low in comparison to Natural forest.

5. Effect of deforestation on AMF spore population

AMF spore population showed a wide variation across the sites and between seasons. In both the seasons, it was highest in Regenerating forest, followed by Natural forest, Degraded forest and Jhum fallow.

6. Diversity and distribution of AM fungi

A total of 47 and 37 AMF species in winter and summer respectively belonging to 10 genera were recovered from the study sites. 15 species were exclusively found in winter while 5 species exclusively in summer.

Glomus was the dominant genus in all the sites and in both the seasons.

During winter, the highest richness of AMF species was found in Regenerating forest (28), followed by Natural forest (27), Degraded forest (25) and Jhum fallow (24).

During summer, 27 AMF species were recovered from Regenerating forest and 21 each from Natural forest, Degraded forest and Jhum fallow.

7. Correlation among physical and chemical properties of soil

Both positive and negative correlations were observed between soil's physical and chemical parameters. During winter, Pearson's correlation analysis revealed a significant positive correlation between soil moisture content and organic carbon, available N ($p<0.01$) and water holding capacity ($p<0.05$). A significant negative correlation between soil moisture content and available P and pH ($p<0.01$) was also seen. Water holding capacity had a positive correlation with K ($p<0.01$) while bulk density and porosity were negatively correlated. pH had positive correlation with available P ($p<0.01$) and negative correlation with organic carbon, available N and K ($p<0.01$). Organic carbon and available N were significantly and positively related while both exhibited a negative correlation with available P. Potassium content on the other hand had a positive correlation with available N while having a negative correlation with available P ($p<0.05$).

During summer, soil moisture content and water holding capacity exhibited a significant positive correlation with each other as well as with organic carbon, available N ($p<0.01$) and porosity ($p<0.05$) while showing a negative correlation with available P ($p<0.01$) and bulk density ($p<0.05$). Bulk density related positively with available P ($p<0.01$) while having a

negative correlation with porosity, organic carbon and available N ($p<0.01$). Organic carbon and available N were significantly and positively related while both exhibited a negative correlation with available P. Potassium content was positively related with available N ($p<0.01$) while it had a negative correlation with available P ($p<0.05$).

8. Correlation between AMF spore density and root colonization with physico-chemical properties of soil

Pearson's correlation analysis exhibited significant correlation between AMF spore population and root colonization (%) with soil physico-chemical properties across different seasons. During winter, a significant positive correlation was observed between spore population and root colonization ($p<0.01$). Furthermore, spore population also positively correlated with organic carbon, available N ($p<0.01$), soil moisture content and DHA ($p<0.05$). Root colonization also demonstrated positive correlations with soil moisture content, organic carbon, available N and DHA ($p<0.01$). Both spore population and root colonization correlated negatively with pH and available P ($p<0.01$).

Spore population and root colonization correlated positively ($p<0.01$) with each other in summer as well. Additionally, Spore population had positive correlations with soil moisture content, water holding capacity, organic carbon, available N and DHA ($p<0.01$) while showing negative correlations with bulk density, pH and available P ($p<0.05$). Root colonization also exhibited positive correlations with soil moisture content, water holding capacity, organic carbon, available N ($p<0.01$) and DHA ($p<0.05$) while showing negative correlations with bulk density ($p<0.01$) and available P ($p<0.05$).

9. Effect of season on soil physico-chemical properties

The effect of season was significant on soil moisture content and water holding capacity. A higher soil moisture content and water holding capacity was observed in summer than winter.

A significant difference between site and the season was observed in soil pH and available P.

10. Effect of season on dehydrogenase activity (DHA)

DHA in the soil varied significantly among the sites and also between the seasons. However, the effect of season was observed only in Natural forest and it was higher in winter season.

11. Effect of season an AMF

Root colonization by AMF varied significantly between the seasons. However, the effect of season was observed only in Natural forest and Degraded forest. The impact was more significant during the winter season.

Inoculum potential was relatively higher in winter.

AMF spore population exhibited wide variation between the seasons. It was significantly higher during the winter season. However, spore population in Degraded forest did not exhibit any seasonal effect.

Overall, vegetation cover variations were noted, with natural forests having higher tree density, indicating stability, while regenerating forests exhibited increased ground cover, suggesting regeneration. Significant differences in soil properties were observed across sites. DHA, a measure of microbial activity, displayed season-dependent variations, with natural forests consistently showing higher DHA levels. AM status exhibited wide variations, with IP of AMF in soil peaking in natural forests and regenerating forests consistently having the highest spore population and root colonization. Seasonal effects were noted on soil properties, DHA, AMF root colonization, and spore population, with winter generally showing higher levels. The research, thus contributes valuable insights into the complex dynamics of ecosystems undergoing deforestation and regeneration processes, emphasizing the importance of considering multiple factors for a comprehensive understanding.

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Annexure I

Research publication from the thesis

1. Hage Yakang and Oyi Dai Nimasow. 2022. A comparative study on vegetation cover, soil properties and status of Arbuscular Mycorrhizal Fungi in Jhum fallow and Natural forest in Arunachal Pradesh. Journal of Bioresources 9(2): 44-49, July – December 2022.

Papers presented in Seminars and Conference

1. Hage Yakang and Oyi Dai Nimasow. Arbuscular mycorrhizal status in a tropical forest under different regimes of disturbances in Arunachal Pradesh. In: National seminar on the theme Emerging Trends in Plant Sciences on 29th–30th March, 2022 organized by Department of Botany, North Eastern Hill University, Shillong, Meghalaya, in collaboration with CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India.
2. Hage Yakang and Oyi Dai Nimasow. Diversity status of Arbuscular mycorrhizal fungi in Natural forest and Jhum fallow soils of Papum pare district of Arunachal Pradesh. In: International conference on Bioresources and Bioeconomy, on 19th–21st September, 2022 organized by Department of Botany, Nagaland University, Lumami, Nagaland, in collaboration with Nagaland Forest Management Project, Department of Environment, Forest and Climate Change, Govt. of Nagaland, India.

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RESEARCH ARTICLE

A comparative study on vegetation cover, soil properties and status of Arbuscular Mycorrhizal Fungi in Jhum fallow and Natural Forest in Arunachal Pradesh

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Abstract

Deforestation in the form of shifting agriculture is one of the biggest threats to the forests in Northeast India. Such disturbances adversely affect the vegetation, soil health and the below-ground microorganisms especially Arbuscular Mycorrhizal Fungi (AMF). The present study investigated the impact of conversion of a tropical forest area into a Jhum field on vegetation cover, soil physico-chemical properties and the below ground AMF status in Papum Pare district of Arunachal Pradesh. Sampling was done by belt transect method covering 4 plots of 10,000 m^2 size. Vegetation cover, Tree Diameter at Breast Height (DBH), soil physico-chemical properties, root colonization (RC), AMF inoculum potential (IP), spore population (SP) and AMF diversity in composite soil samples were quantified. The vegetation cover in the Natural Forest was more than the Jhum fallows with greater plant diversity, tree density, canopy cover, and DBH. Soil pH, Organic Carbon, available Nitrogen and available Phosphorus content in the soil differed significantly between the sites. RC (66.67%), IP (1.58 g^{-1} soil) and SP (224 AMF spores 100 g^{-1}) were higher in the Natural Forest. The study showed that removal of above-ground vegetation exerts negative impacts on the soil properties and AMF status.

Keywords: Deforestation, tropical forest, shifting agriculture, slash and burn cultivation vegetation cover, mycorrhizal fungi, soil properties

1. Introduction

Forests are under threat from human activity and the greatest threat comes from deforestation which adversely affects vegetation of an area causing soil erosion especially in the humid tropics where rainfall is heavy and terrain is often steep. Moreover, removal of above-ground vegetation also has negative effect on below-ground microorganisms (Rodrigues et al., 2012). The Natural forests have been disturbed as a result of management practices, demand for forest products (Fuchs and Haselwandter, 2008) and agriculture (Uhl, 1987). Jhum or shifting cultivation is one such agricultural practice which plays a key role in forest degradation. It involves clearing of a forest patch followed by burning. The burnt patch is later used for cultivation of crops for few years and then the land is abandoned as fallow land. Such practices are known to have effects on important soil properties and soil microbial community (Rodrigues et al., 2012).

Arbuscular Mycorrhizal Fungi (AMF) are soil microorganisms that form symbiosis with 80% of terrestrial plants and contribute largely in uptake of minerals and nutrients and enhancing their tolerance to various abiotic stresses (Smith and Read, 2008). In return, AMF derive carbon compounds from the host plant which are necessary for their growth (Li et al., 2006). Rendering such significant ecological benefits, it is evident that AMF not just influence plant diversity by increasing species evenness of the plant community (Park and Eom, 2007) but also influence their community structure and succession (Van der Heijden et al., 1998; García de León et al., 2018). AM fungal community can therefore be a determinant of plant community, and any disturbance on this relationship may cause changes in terms of decreased population status and AMF diversity (Van der Heijden et al., 1998).

AMF communities are also affected by deforestation (Johnson and Wedin, 1997). Generally, forest plant species have their own very specialized fungal partner, and therefore, the loss of such plants from the forest leads to loss of fungal species or reduction in the amount of their infective propagules in the soil (Helgason et al., 2002). Studies also reported that AMF diversity is influenced by the intensity of land disturbances (Allen et al., 1998; Korb et al., 2003). Degradation of forest cover may also lead to change in some physico-chemical properties of soil (Piccolo et al., 1994) which in turn affects AMF population.

The forests in Arunachal Pradesh have come under threat due to the increasing demand for timber and land for Jhum cultivation. It is an age-old practice among majority of tribal groups of the state to sustain their livelihood which is also the leading cause of deforestation, ecological instability and biodiversity loss (Uhl, 1987). Heavy rainfall, extremely fragile soil and a steep slope in the region causes a significant runoff of topsoil during the monsoon. Therefore, it is expected that a change in vegetation cover and plant community structure would have a more adverse impact on soil properties as well as on the below-ground AMF status in this region. In the present work, we studied the impact of tropical forest conversion into Jhum fields on soil physico-chemical properties, the below ground AMF diversity and the above-ground vegetation (tree density, DBH, canopy cover and ground cover) by selecting two sites (a Natural Forest and a Jhum fallow) under Papum Pare district of Arunachal Pradesh.

2. Materials and Methods

2.1. Study sites and vegetation type

The study was carried out in Papum Pare district of Arunachal Pradesh during the month of November 2020 to January 2021 at two sites located within tropical zone – (i) A Natural Forest under Jampa circle (27°14′34″N; 93°49′13.7″E; altitude 442 m msl), and

(ii) Four nearby Jhum fallows (3-5 yr old) under Kheel in Sagalee circle (27°14′27.54″N; 93°43′27.72″E; altitude 418 m msl). The vegetation in Natural Forest consisted of several tree species viz. *Baccaurea ramiflora*, *Duabanga grandiflora*, *Dillenia indica*, *Elaeocarpus* sp., *Ixora* sp., *Magnolia* sp., *Morinda*, *Saurauia*, etc. The forest floor was covered with herbaceous plants and litter. The fallow period of Jhum lands were confirmed by the village head and the villagers. They had been used for cultivation of maize, rice and millet etc. which were left abandoned four years ago and became covered with luxuriant growth of *Lantana camara, Mikania scandens, Spermacoce* sp., *Ageratum conyzoides*, etc. and has a few sparsely distributed trees viz. *Crateva religiosa*, *Dillenia indica*, *Duabanga grandiflora*, *Litsea polyantha* etc.

Table 2. Vegetation cover in Natural Forest and Jhum fallow

Sites	Tree density (ha^{-1})	Canopy cover (%)	Ground cover (%)
Natural Forest	382.0	81.70±2.24	34.00±2.12
Jhum Fallow	15.0	Not determined	96.86±0.66

Table 1. Soil Physico-chemical properties of Natural Forest and Jhum fallow

Soil parameters	Natural Forest	Jhum Fallow
pH	5.06±0.08[a]	5.38±0.11[b]
Bulk Density (g/cm^3)	1.30±0.03[a]	1.23±0.08[a]
Porosity (%)	51.13±1.24[a]	53.77±2.97[a]
WHC (%)	73.67±1.26[a]	69.67±4.01[a]
C (%)	1.69±0.09[b]	1.31±0.06[a]
Avail. N ($kg\ ha^{-1}$)	272.69±6.76[b]	219.75±8.61[a]
Avail. P ($kg\ ha^{-1}$)	9.19±0.80[a]	17.75±1.83[b]
K ($kg\ ha^{-1}$)	242.76±14.83[a]	286.72±29.52[a]

Mean followed by same letters are not significantly different ($p<0.05$)

2.2. Determination of vegetation Cover

The vegetation cover at selected sites was measured by line intercept method (Canfield, 1941). A transect of 100 m length was laid in each plot of the sites. 10 sampling points at an interval of 10 m were set on each transect. Tree density was calculated by Nearest individual method, a type of distance method (Cottam and Curtis, 1956) that is again a type of line intercept method (Canfield, 1941). At each sampling point along transect, a plant closest to the point was located. Then, the distance between the sampling point and t nearest tree (nearest individual) was measured. Nearest individual method (a distance method) used for measuring tree density (Cotton and Curtis, 1956).
Tree density was calculated by the following formula:

$$\text{Density} = \frac{1}{\text{Mean Area}} = \frac{1}{(2\bar{d})^2}$$

Where, $\bar{d}$= mean distance between sampling plant and its nearest individual

Canopy coverage of trees was measured by spherical densitometer using a convex mirror suitably itched with squares. It was calculated by the following formula:

$$\text{Canopy coverage (\%)} = \frac{\text{Numberof observed hits}}{\text{Total number of hits}} \times 100$$

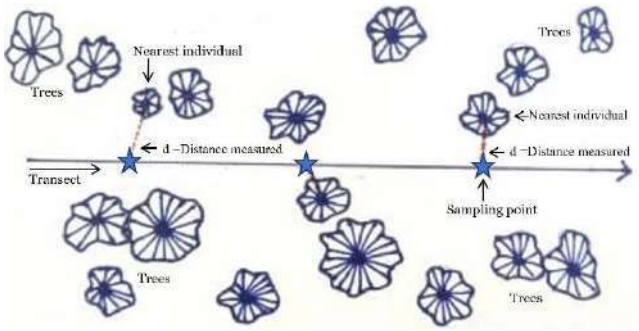

Figure 1. Sketch diagram showing sampling and transect point

Ground vegetation cover was calculated by measuring the total foliage cover occupied on the ground by shrubs and herbs along a 100 m transect. Ten trees along each transect were categorized into different size classes based on Diameter at Breast Height (DBH) with a 10 cm interval. In the case of Natural Forest, seven classes with 10 cm intervals were established, and in the 8th class, all trees exceeding 80 cm DBH were grouped together. A total of 40 trees were measured for DBH classes along four transects in the Natural Forest. Similarly, in Jhum fallow areas, five classes of 10 cm interval were created, and trees with DBH greater than 60 cm were placed in the 6th class of 61-100 cm. As there were few countable trees in Jhum fallow, the DBH of all trees was recorded.

2.3. Soil Sampling

Randomized sampling by belt method was followed for collection of samples. In case of forest sites, four plots each of 100 m x 100 m size were marked randomly at a distance of 500 m. Five horizontal belts, 25 m apart, were laid in each plot. Along each belt, a soil core of 4 cm diameter from 0-15 cm depth was collected at almost equal distance and then mixed to make a composite sample. In case of Jhum fallow sites, five soil samples were collected from each of the four fields.

2.4. Collection of root samples

Fine roots were collected along with the soil samples and carefully separated. The roots were then thoroughly mixed to make a composite sample. The roots samples were properly washed and preserved in Formalin Acetic acid Alcohol solution (50 ml Ethyl alcohol, 5 ml Glacial acetic acid, 5 ml Formaldehyde, 5 ml Distilled water) for further quantification of percent root colonization (RC).

2.5. Analysis of soil physico-chemical properties

Various soil properties {pH, bulk density, porosity, water holding capacity (WHC), organic carbon (OC), available Phosphorus (P), available Nitrogen (N) and Potassium (K)} were determined by following standard methods. Soil pH was measured in a 1:2 soil : water suspension. Bulk density was measured by core method (direct measurement) using metal rings of 5-15 cm length and accordingly porosity was calculated. WHC was determined by Keen's box method. Soil OC, available N and available P were measured by standard methods given by Walkley and Black (1934), Bremmer and Mulvaney (1982) and Bray and Kurtz (1945) respectively. Soil Potassium was determined with a flame spectrophotometer.

2.6. Quantification of AMF root colonization (RC)

Plant roots were stained for AM fungal structures following the method outlined by Phillips and Hayman (1970) and modified by Koske and Gemma (1989). Washed root segments were cut into 1 cm segments and cleared in 10% KOH solution (w/v) by heating at 90 °C in a water bath for 2 hr. Root samples were washed several times with tap water and then acidified with 1% HCl solution, either by heating at 90 °C for 1 hr or by soaking overnight. The acidified roots were subsequently stained with trypan blue solution (500 ml glycerol, 450 ml H_2O, 50 ml 1% HC1 containing 0.05% trypan blue) by heating at 90 °C for 30 min. Excess stain was removed using de-staining solution (500 ml glycerol, 450 ml H_2O, 50 ml 1% HC1) at room temperature.

RC was determined by Magnification intersection method (Mc Gonigle et al., 1990) under a compound microscope (Nikon, Eclipse 200) by randomly selecting 30 root segments for each plot.

$$\text{Root colonization (\%)} = \frac{\text{Number of intersection of infection}}{\text{Number of intersection examined}} \times 100$$

2.7. Determination of inoculum potential

Inoculum potential (number of infective AMF propagules in soil) was determined by Most Probable Number (MPN) bioassay (Alexander, 1982) following serial soil dilution technique (Porter, 1979), and using *Zea mays* as host plant.

2.8. Isolation, quantification and identification of AMF

AMF spores were isolated from soil samples by Wet sieving and decanting method (Gerdemann and Nicholson, 1963). A suspension of 100 g air-dried soil in 1000 ml water was poured through a series of stacked sieves of pore sizes 800, 500, 300, 150, 90 and 40 µm. Isolated spores were counted manually under Stereomicroscope (Nikon SMZ 800), and spore density was expressed as the number of AM spores per 100 g of soil sample. Spores were identified up to genus level with the help of keys on INVAM website and the identification manual of Schenck and Perez (1990).

2.9. Statistical analysis

Data were statistically analysed by one-way ANOVA ($p<0.05$), and the groups were compared using Least Significant Difference (LSD) test.

3. Results

The vegetation cover in the Natural Forest was more than the Jhum fallow. The Natural Forest consisted of naturally growing tree species viz. *Baccaurea ramiflora*, *Duabanga grandiflora, Dillenia indica, Elaeocarpus* sp., *Ixora* sp., *Magnolia* sp., *Morinda* sp., *Sauraia* sp. etc. The tree density was 382 per 100 m^{-2} with 81.7% of canopy cover (Table 1). Herbaceous plants and shrubs occupied 34% of the ground floor, and the remaining area was covered with litter.

The four Jhum fallows, 3-5 years old and left fully abandoned, were covered with luxuriant growth of *Lantana camara*, *Mikania mikranthes*, *Spermacoce* sp., *Ageratum conyzoides* etc. and a few sparsely distributed tree species such as *Crateva religiosa*, *Dillenia indica*, *Duabanga grandiflora*, *Litsea polyantha* etc. The tree density was only 15 per 100 m^{-2}. Since the trees in Jhum fallow were scattered and countable, the canopy cover could not be determined. The ground cover was 96.86%, occupied fully by herbs and shrubs (Table 1).

Tree density and their DBH were more in the Natural Forest but comparatively very less in Jhum fallow (except for two old trees that were not cut down). The frequency distribution of DBH showed highest number of trees in 10–20 cm class, followed by 21–30 cm and 31–40 cm classes that were having almost equal number of trees. Rest of the DBH classes recorded the least number of trees (>5) (Figure 2). In Jhum fallow, most of the trees belonged to DBH class of 10–20 cm. Their frequency drastically decreased in rest of the DBH classes (Figure 2).

Soil pH, OC, available N and available P differed significantly between the sites while the bulk density, porosity, WHC and K content was almost similar (Table 2). Soil pH was slightly more acidic in the Natural Forest (5.06) than Jhum fallow (5.38). The Natural Forest Soil had more OC (1.69%) and available N (272.69 kg ha^{-1}) whereas in Jhum fallows had more available P (17.75 kg ha^{-1}).

Root samples from both the sites showed mycorrhizal structures viz. vesicles, arbuscules, hyphae and occasionally intra-radical spores. It was observed that hyphal colonization in the collected roots was more than vesicular or arbuscular colonization. Furthermore, RC, IP and spore population showed a great difference between the sites (Figure 3-5). The values were 66.67%, 1.58 g^{-1}soil and 224 AMF spores 100 g^{-1} soil respectively in the Natural Forest whereas 56.29%, 0.34 g^{-1}soil and 188 AMF spores 100 g^{-1} soil in the Jhum fallows.

AMF species diversity was also more in the Natural Forest. A total of 14 morphotypes of AMF were isolated from the two study sites belonging to five genera *viz*. *Glomus*, *Acaulospora*, *Gigaspora*, *Sclerocystis* and *Scutellospora* (Table 3, Figure 6). *Glomus* was the most dominant genus in both the sites. Out of 14 morphotypes, nine were common in both sites, three were present only in the Natural Forest and two exclusively in Jhum fallows.

4. Discussion

Before selecting the site as a Natural Forest, we confirmed from the village head about the forest and as per his narration, the forest has all naturally growing trees, frequently visited by a variety of wildlife especially elephants, and occasionally visited by the villagers for collecting minor forest products. All the measured characteristics in the present study about the vegetation also confirmed it as a Natural Forest. This site had 81.7% canopy cover and 34% of ground cover with a variety of herbs and shrubs. The tree density was 382 per 100 m^{-2} which included both young and old trees falling into various DBH classes. Presence of many aged trees with DBH exceeding 40 cm indicates insignificant disturbance to the standing vegetation due to human activities. Trees in DBH classes up to 40 were highest in number and the frequency drastically decreased thereafter thus resulting in a reverse J-shaped structure. This pattern of frequency indicates sustainable regeneration (Vetaas, 2000; Sujakhu et al., 2014). The average DBH of 27 cm recorded in our study is more or less similar to the report by Hauchhum and Singson (2020).

The Jhum fallows selected in our study were of 3-5 years old, fully covered with weeds and shrubs and only a few sparsely distributed trees. Styger et al. (2007) reported that the growth of tree seedlings in open fallow land is obstructed by massive invasion of shrubs. The vegetation at both the sites of the present study was different

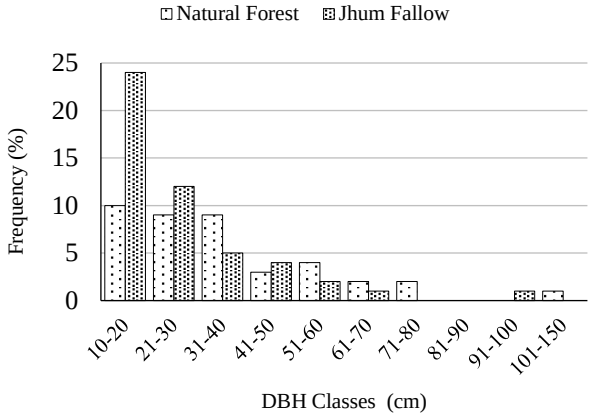

Figure 2: Frequency distribution of DBH-classes (a) Natural Forest, and (b) Jhum fallow

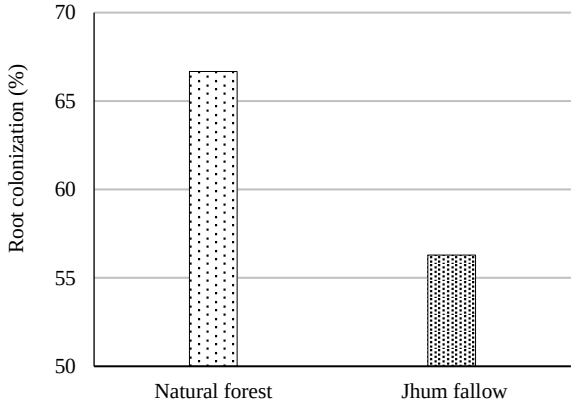

Figure 3: AMF colonization in composite root samples

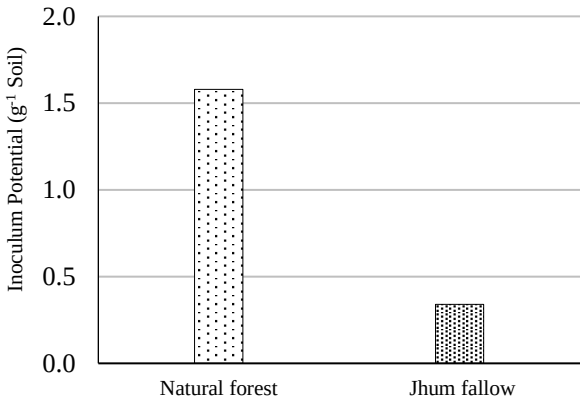

Figure 4: AMF Infective Propagules

in composition which appears to have affected the soil properties and AMF status therein. Disturbance on vegetation of an area alters soil quality by limiting the organic inputs into the soil (Rutigliano et al., 2004; Singh et al., 2004; Mekuria, 2010) and also the microorganisms that are associated with the above-ground plant community (Rodrigues et al., 2012). Soil properties also changes with vegetative succession (Bockheim and Hartemink, 2017). Our result showed that pH, S, OC, available N, and available P showed a significance difference between the Natural Forest and Jhum fallow. These results are consistent with the results of Singh et al. (2003) where they found higher OC, N and available P in Natural Forest than Jhum fallow site in Arunachal Pradesh. We observed no significant difference in bulk density, porosity and soil potassium. Soil pH was significantly higher Jhum fallow. Slightly low pH in Natural Forest can be attributed to higher organic carbon content since organic matter decomposition leads to production of more organic acids, thereby lowering the pH (Hong et al., 2019). It is also reported that burning of fields causes denaturation of

organic acid releasing base cations leading to an increase in soil pH in Jhum fields (Certini, 2005). Accumulation of ash due to burning of jhum fields might have also added alkalizing effect on soil since it contains base cations as reported by Kauffman, (1993).

Tripathi et al. (2022) found in the Natural Forest comparatively more OC almost similar N but lesser P content than the Jhum fallows in Papum Pare district of Arunachal Pradesh. In our study conducted in a different region of the same district, OC and available N contents were significantly higher in the Natural Forest whereas available P was significantly more in Jhum fallows. However, Barraclough and Olsson (2018) reported a higher OC and N in burned fields than in forest site. Their fields were burned 0-5 years prior to sampling but occasional burning was also done intermittently to stop the spread of vegetation. Perhaps, their sampling was done immediately after burning or within a short time period resulting in high estimated OC and N contents. Increase in mineralization rate of N due to higher pH and the base cations in slashed and burned fields explains the increase in N content (Ellingson, 2000). In deforested and Jhum fields, soil organic P is converted to orthophosphate through the process of pyro-mineralization and high pH increases P availability in the absence of Ca thus resulting in an increased available phosphate (Giardina et al., 2000). Soil P has been observed as the most vital edaphic parameter for mycorrhizal symbiosis (Smith and Read, 2008; Gong et al., 2012).

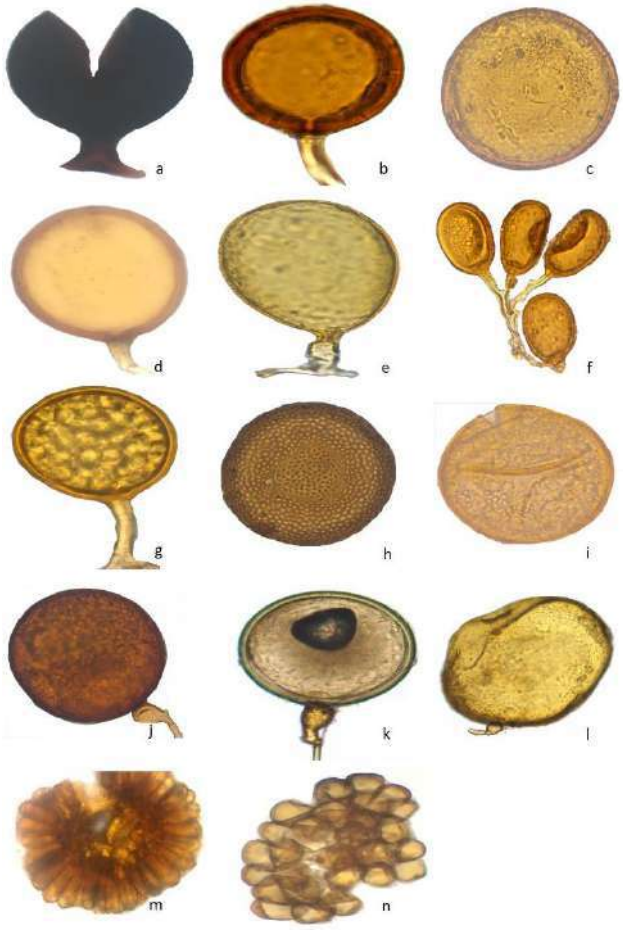

Figure 6: AMF species present in study sites: (a) *Glomus* sp. 1, (b) *Glomus* sp. 2, (c) *Glomus* sp. 3, (d) *Glomus* sp. 4, (e) *Glomus* sp. 5, (f) *Glomus* sp. 6, (g) *Glomus* sp. 7, (h) *Acaulospora* sp. 1, (i) *Acaulospora* sp. 2, (j) *Gigaspora* sp. 1, (k) *Gigaspora* sp. 2, (l) *Scutellospora* sp. 1, (m) *Sclerocystis* sp. 1, (n) *Sclerocystis* sp. 2

An increased P level in soil has been linked with reduced spore population as well as root colonization by AMF. Our result stands parallel with the findings of Menge et al (1978) where they reported reduced spore production with an increase in P level which in turn affects root colonization. El-Sherbeny et al (2022) in their experiment found that soil P level higher than that required for plant growth eliminated mycorrhizal association due to reduced arbuscular development. It is reported that higher P in soil decreases root exudates by affecting phospholipid membrane and thus leads to reduced arbuscule formation. Since root exudates are

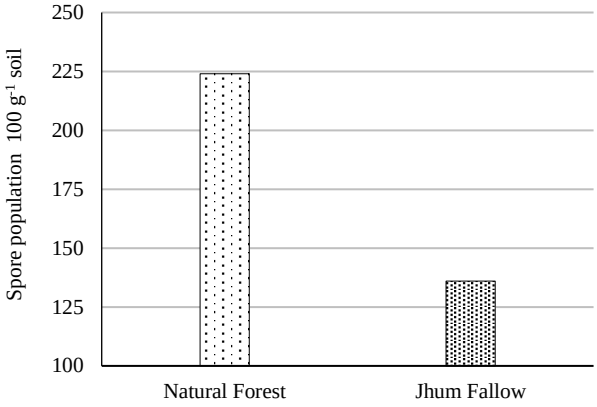

Figure 5: AMF Spore population in soil

essential for vegetative growth of AMF, the association of AMF with plant gets reduced (Tawaraya, 2003).

Several similar studies also report that change in land use pattern leading to removal of above-ground vegetation causes decrease in root colonization by AMF (Boddington and Dodd, 2000; Oehl et al., 2005) and lower inoculum potential (Zangaro et al., 2000). Mohammed et al. (2003) attributes break down of AMF hyphal network in the ground for such decrease in root colonization. We found a higher AMF colonization in the Natural Forest. Our findings are supported by many studies including Birhanem et al (2020) where they found lower AMF colonization in Jhum field than forest site. A higher inoculum potential in the forest site is attributed to the dominance of pioneer species which are very efficient in multiplication of AMF (Zangaro et al., 2000).

All the AMF structures such as hyphae, arbuscules and vesicles were observed in the roots collected from the study sites. Intra-radical spores were also seen occasionally. We observed highest colonization by hyphae followed by vesicular and arbuscular colonization. Our results are in line with the findings of Belay et al (2013) where they observed higher hyphal and vesicular colonization than all other structures. This can be attributed to the fact that the hyphae being the primary AMF structures can exist for a long time, and since vesicles acts as storage structures in AMF association, they remain in roots for months or years unlike arbuscules which senesce after few days (Sarkar et al., 2014).

We also found a higher spore density and AMF species diversity in the Natural Forest which aligns with many other studies (Barraclough and Olsson, 2018; Birhane et al., 2020; Tripathi et al., 2022) suggesting strong dependency of spore density on vegetation type. Anthropogenic activities have proved to reduce below ground AM fungal communities and the intensity of such disturbances also determines AMF diversity (Allen et al., 1998; Korb et al., 2003). Disturbance in an area removes pioneer plants many of which serves as host plants and thus could cause a lower spore density. Birhane et al (2020) also report direct association of plant diversity with AMF diversity since higher number of spores are found in the rhizosphere of mature trees. Diversity and density of AMF is also reported to increase with canopy cover since plants with more canopies convert higher solar inception into photosynthates which provides carbon source to AMF (Sarkar et al., 2014). However, AMF sporulation is also known to depend on season (Gong et al., 2012).

Few studies in Northeast India done on AMF status in Jhum fallows and Natural forests (Singh et al., 2003; Sharma and Jha, 2011; Bordoloi et al., 2015) also revealed lower spore diversity and abundance in Jhum fallows. The cause of low spore density and diversity was ascribed to repeated burning of the fields, loss of primary host plants on which these fungi depend for their carbon sources and adverse edaphic conditions for AMF regeneration in Jhum fallows.

In our study Glomus spp. were dominant in both the sites. Dominance of Glomus sp. in our work align with the findings of many studies (Singh et al., 2003; Sharma and Jha, 2011; Tripathi et al., 2022). Their dominance can be explained by the fact that Glomus species are often present and thrive well in a variety of

natural ecosystem (Manoharacharya et al., 2005), sporulate profusely within a short time period producing smaller spores (Zhao et al., 2003; Wang et al., 2019). The characteristic of Glomus species to flourish well in slightly acidic to neutral pH might also be a plausible factor (Graw, 1979).

5. Conclusion

The present work investigated the effects of Jhum cultivation on soil physico-chemical properties and AMF status in the soil. It was found that both are affected when a natural forest is converted into a Jhum field. Soil disturbance and a change in vegetation cover seemingly created unfavourable edaphic conditions for AMF regeneration. The results show an adverse impact of the removal of vegetation on the AMF diversity and spore density, as AMF are obligate symbionts interlinked with plant roots. Viable mycelium and AMF propagules are lost under such intensity of disturbance, which also leads to a loss of soil fertility. AMF plays a vital role in the restoration, establishment, and maintenance of plant communities. Therefore, understanding the consequences of human activities on mycorrhizal fungi and their association with plants could be helpful in finding ways to protect and conserve the diversity of soil organisms. Such understanding will also encourage strategies to alleviate the impacts of past disturbances. However, it's important to note that the results are based only on samples collected during the winter season and did not examine the effect of seasonal variation. A further comparative study on AMF community structure in a natural forest and Jhum fallows in different seasons may provide a better understanding in this regard.

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Author contributions

All the authors contributed to the study's conception and design. Ms. Hage Yakang prepared the draft manuscript and Dr. Oyi Dai Nimasow reviewed and edited the manuscript.

Conflict of interests

Authors declare no conflict of interest.

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Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. **Hage Yakang of Rajiv Gandhi University, Rono Hills, Doimukh** has participated in **Oral Presentation** on the topic entitled **Arbuscular mycorrhizal status in a tropical forest under different regimes of disturbances in Arunachal Pradesh** in National Seminar On Emerging Trends in Plant Sciences (ETPS) held from 29-30 March 2022 organized by Department of Botany, North-Eastern Hill University, Shillong, Meghalaya, India in collaboration with CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India.

Dr. M. C. Das
Organizing Secretary
National Seminar on
Emerging Trends in Plant Sciences

Prof. S. K. Barik
Director,
CSIR-NBRI,
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International Conference on 'Bioresources & Bioeconomy' (ICBB-2022)

Organized By

Department of Botany, Nagaland University, Lumami 798627, Nagaland, India

In Collaboration With

Nagaland Forest Management Project, Department of Environment,
Forest and Climate Change, Govt. of Nagaland, India.

September 19-21, 2022

CERTIFICATE OF PRESENTATION

This is to certify that

Mr./Miss/Dr. HageYakang of Rajiv Gandhi University, Arunachal Pradesh, India

has presented a paper (Oral/~~poster~~) titled

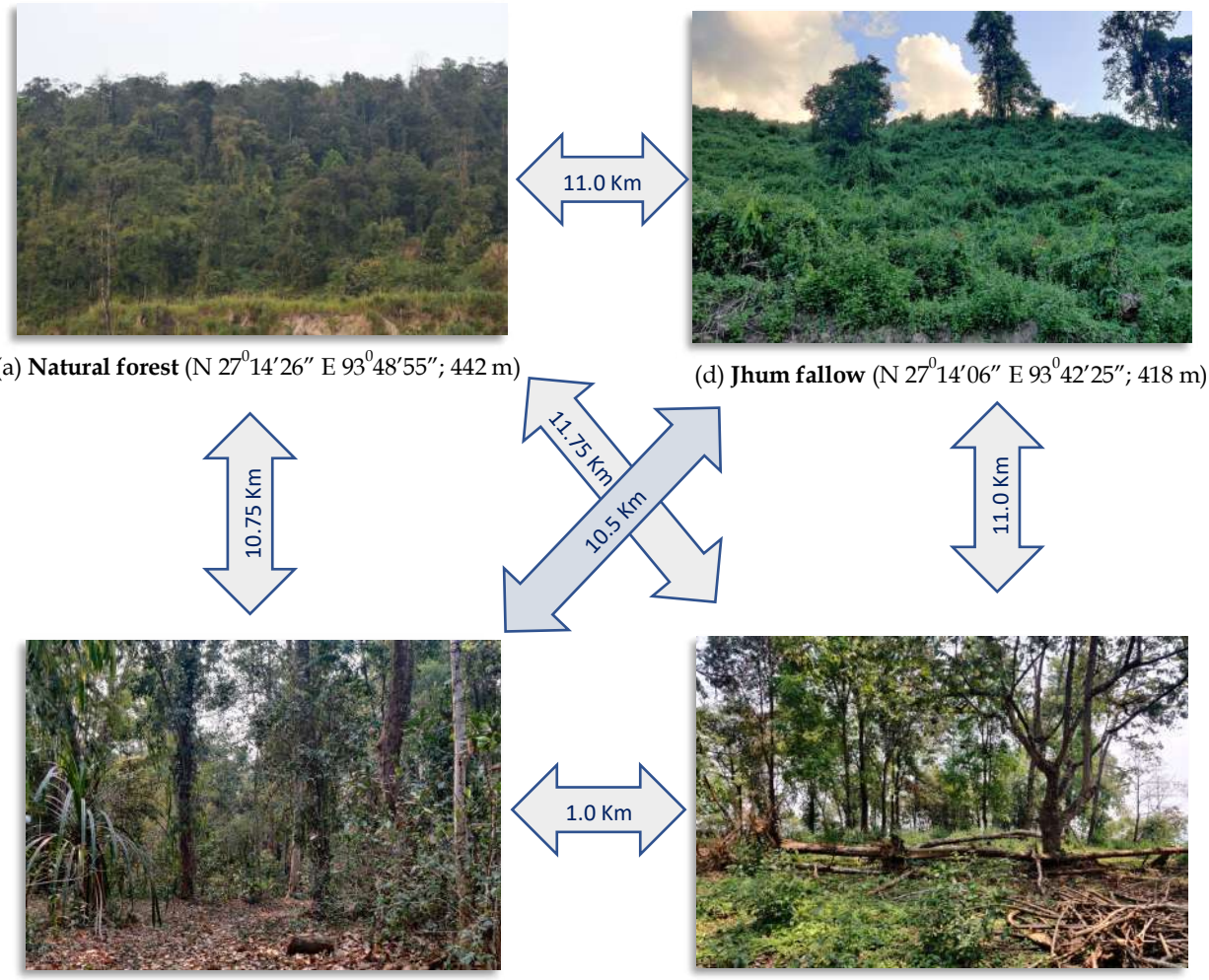
'Diversity Status of Arbuscular Mycorrhizal Fungi in Natural Forest and Jhum Fallow Soils of PapumPare District of Arunachal Pradesh'

in the ICBB-2022 organized by the Department of Botany, Nagaland University, Lumami 798627, Nagaland, India, held on September 19-21, 2022.

(Prof. M. S. Rawat)
Dean, School of Sciences

(Prof. Chitta Ranjan Deb)
Convener cum Organizing Secretary
ICBB-22

PLATE 1



(a) **Natural forest** (N 27^{0}14′26″ E 93^{0}48′55″; 442 m)

(d) **Jhum fallow** (N 27^{0}14′06″ E 93^{0}42′25″; 418 m)

(b) **Regenerating forest** (N 27^{0}15′16″ E 93^{0}76′32″; 270 m)

(c) **Degraded forest** (N 27^{0}14′16″ E 93^{0}75′92″; 299 m)

Fig. 1: Study sites with geographical coordinates

PLATE 2

Fig. 2 &3: Field Sampling.
Fig. 4&5: Determination of microbial activity in the soil
Fig. 6: Establishment of trap culture.
Fig. 7: Determination of Inoculum potential of AMF in soil trap culture.
Fig. 8: Wet sieving and decanting method for AMF spore isolation.
Fig. 9: Quantification of AMF spore population using Stereomicroscope.

PLATE 3

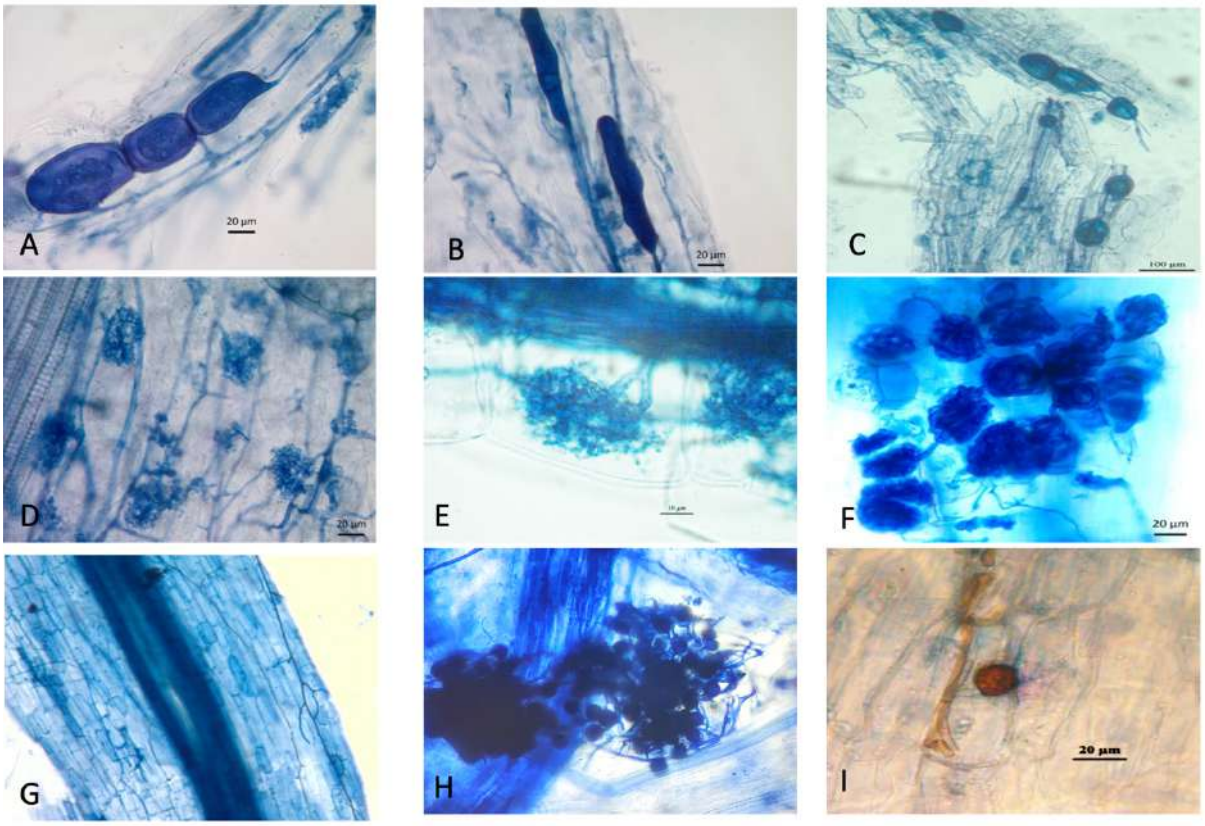


Fig. 10: AM fungal structures inside root. **A-C**: Vesicles. **D-E**: Arbuscules. **F**: Hyphal coils inside root cortical cells. **G**: Hyphae. **H-I**: Intra-radical spores.

PLATE 4a (Isolated AMF spores)

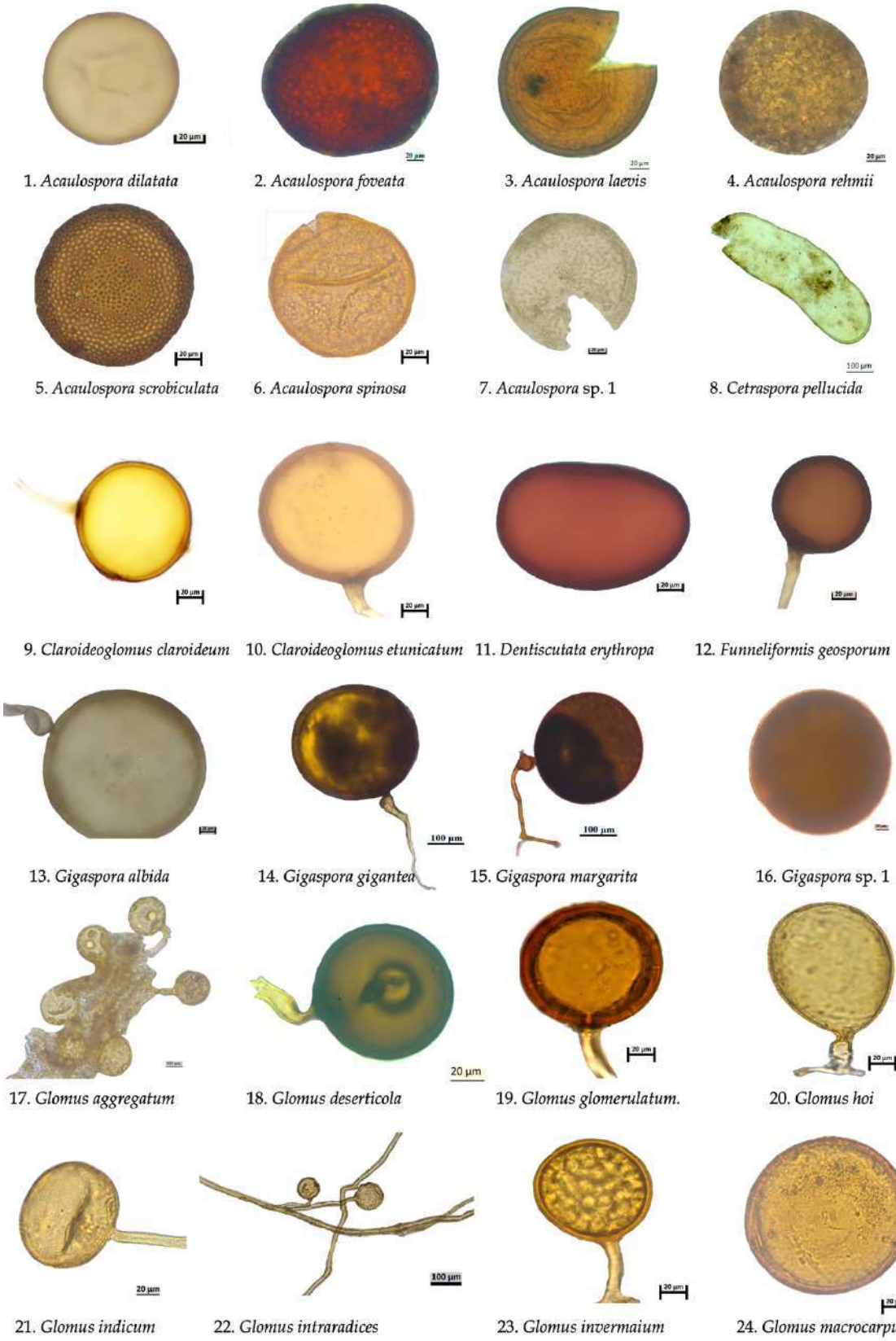
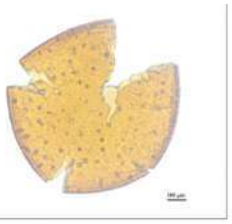


PLATE 4b (Isolated AMF spores)

25. *Glomus microcarpum*

26. *Glomus mosseae.*

27. *Glomus multicaule*

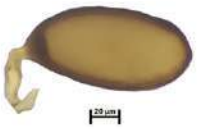


28. *Glomus multiflorum*

29. *Glomus versiforme.*

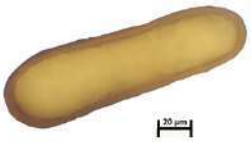
30. *Glomus viscosum*

31. *Glomus* sp. 1

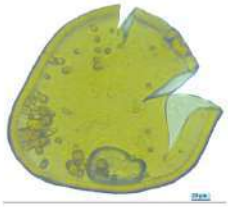


32. *Glomus* sp. 2

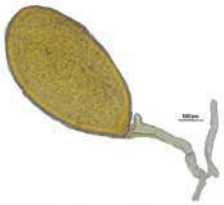
33. *Glomus* sp. 3



34. *Glomus* sp. 4



35. *Glomus* sp. 5

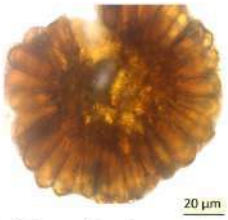


36. *Rhizophagus clarus*

37. *Rhizophagus fasiculatus*

38. *Rhizophagus irregularis*

39. *Sclerocystis clavisporum*



40. *Sclerocystis microcarpa*

41. *Sclerocystis rubiformis*

42. *Sclerocystis taiwanensis*

43. *Scutellospora calospora*

44. *Scutellospora heterogama*

45. *Scutellospora scutata*

46. *Scutellospora* sp. 1

47. *Septoglomus constrictum*

48. Unidentified AMF 1

PLATE 4c (Isolated AMF spores)

49. Unidentified AMF 2

50. Unidentified AMF 3.

51. Unidentified AMF 4

52. Unidentified AMF 5

53. Auxillary cell 1

54. Auxillary cell 2

Arbuscular mycorrhizal diversity in relation to degradation of tropical forests in Arunachal Pradesh

Thesis submitted for the Degree of

DOCTOR OF PHILOSOPHY IN BOTANY

2024

Supervisor

Dr. Oyi Dai Nimasow

Submitted by

Ms. Hage Yakang, M.Sc.

Registration No. RGU/RS-649/2016

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CHAPTER VI

SUMMARY

Arbuscular Mycorrhizal Fungi (AMF) are a small group of soil fungi (about 230 species) placed under the phylum Glomeromycota. They form symbiotic relationship with roots of 80% of terrestrial higher plants, and with the help of their extensive mycelial network in the soil enhance their acquisition of water and mineral nutrition from soil, especially phosphate and nitrogen. They also enhance resistance ability of their host plants against several abiotic and biotic stresses. In return, the fungus gets fixed carbon compounds from the host plant. AMF are generally considered non-host specific, but some level of host preference has been reported. Thus, symbiosis with AMF improve fitness of the host plants from an individual to community level by imparting them a better competitive ability to survive and grow in an otherwise adverse ecological condition. Apart from their plant growth promoting ability, AMF also improve overall soil quality by aiding to the formation of stable soil aggregates, creating a macroporous soil structure that facilitates water and air penetration while preventing erosion.

The composition and growth of vegetation depend on the soil nutrient concentration and soil microorganisms since they play a key role in mineralization and transformation of organic matters essential for their growth and development. Therefore, owning to their various ecological functions, AMF are regarded as one of the key players in shaping plant diversity, community structure, and succession within an ecosystem. Besides, the dependence of AMF on their host plants for photosynthate implies that structure & function of the AM fungal community is also influenced by the composition of the plant community, thus suggesting a reciprocal relationship. Besides plant community, physico-chemical properties of soil, and any natural or anthropogenic activities that affect soil and vegetation also influence AMF community structure.

Forests are threatened by anthropogenic activities, primarily deforestation, that negatively impacts above-ground vegetation, soil properties, and microbial communities including AMF. The AMF suffers breakdown of its hyphal network, reduced root infection, and a diminished spore population in soil. In humid tropics, deforestation enhances soil erosion and it happens severely in hilly areas that receives heavy rainfall for a longer period of time.

The effect of deforestation on the mycorrhizal status of soil in Northeast India is under-researched. The delicate nature of the soil in this region, which is susceptible to erosion due to

intense rainfall and steep slopes, suggests that deforestation is likely to have pronounced adverse effects. Arunachal Pradesh, a region that falls within the Eastern Himalaya biodiversity hotspot, boasts an expansive and densely forested terrain. Unfortunately, over the past few decades, the region has witnessed accelerated deforestation due to various anthropogenic activities such as lumbering, slash-and-burn agriculture, urbanization, and developmental projects. Slash-and-burn agriculture, also known as Jhum cultivation, is a traditional farming method practiced by the indigenous tribes. It requires almost complete clearing of a section of the forest, followed by controlled burning. The burnt area is utilized for crop cultivation for 2 to 3 years before being left fallow that get occupied over the time largely by shrubs, herbs and a few saplings. This cultivation practice makes the soil loose, exposed to desiccation, and prone to different degree of erosion depending upon the runoff of rainwater on the slope of the fields. Hence, it may have a profound effect on AMF status in the soil.

As existing studies on AMF status in soil in the region are limited and some of them have methodological shortcomings, therefore, considering all these aspects, the present study was aimed to evaluate the effects of deforestation in tropical forests of Arunachal Pradesh by selecting four different sites i.e. Natural forest, Regenerating forest, Degraded forest, and Jhum fallows. The regenerating was selected to see whether AMF status have improved over 20-year period since a complete restriction on logging. Data were recorded in two different seasons following standard methodologies for various parameter such as canopy cover, ground vegetation cover, dominant plant species, soil properties, soil microbial activity, AMF diversity, AMF root infection and AMF spore population.

The key findings of the study were as follows:

1. Vegetation cover and its influence on soil physico-chemical properties

The vegetation cover varied among the sites, and a lesser tree density and canopy cover was associated with increased ground cover. The number of mature trees were more in Natural forest which indicates a stable community, while young trees were more in Regenerating forest indicating regeneration. While bulk density and porosity did not differ significantly, water holding capacity, soil moisture content, pH, organic carbon, soil available N, P and K varied significantly across the sites.

2. Effect of vegetation cover and soil physico-chemical properties on microbial activity (Dehydrogenase activity)

Soil dehydrogenase activity (DHA) varied significantly among the sites. During the winter season, DHA exhibited a range of 0.035 to 0.353 µg Triphenyl formazan (TPF) g^{-1} 24 hr^{-1}. The highest activity was observed in Natural forest, followed by Regenerating forest, Degraded forest, and Jhum fallow. It correlated positively with soil moisture content ($p<0.01$), available N ($p<0.01$) and organic carbon ($p<0.05$) while negatively correlated with available P ($p<0.01$) and soil pH ($p<0.05$).

During summer season, the values ranged from 0.058 to 0.211 µg TPF g^{-1} 24 hr^{-1}, with Natural forest again displaying the highest activity, followed by Regenerating forest, Degraded forest, and Jhum fallow. There was a significant positive correlation of DHA ($p<0.01$) with water holding capacity, soil moisture content, organic carbon, available N and K whereas a strong negative correlation with available P ($p<0.01$).

3. Effect of deforestation on AM colonization in composite root samples (CRS)

AMF root colonization in Composite root sample (CRS) varied significantly among sites. It was observed that hyphal colonization inside roots was more prevalent than the vesicular or arbuscular colonization.

During winter, it was high in Regenerating forest, although not significantly different from Natural forest, slightly less in Degraded forest and significantly less in Jhum fallow.

During summer, the highest colonization was again observed in Regenerating forest followed by Natural forest, Degraded forest and Jhum fallow. Regenerating forest was similar to Natural forest while differing significantly from Degraded forest and Jhum fallow.

4. Effect of deforestation on Inoculum potential (IP) of AMF in soil

IP varied across the sites and recorded highest in Natural forest followed by Regenerating forest, Degraded forest and Jhum fallow in both the seasons.

In Jhum fallow, it was very low in comparison to Natural forest.

5. Effect of deforestation on AMF spore population

AMF spore population showed a wide variation across the sites and between seasons. In both the seasons, it was highest in Regenerating forest, followed by Natural forest, Degraded forest and Jhum fallow.

6. Diversity and distribution of AM fungi

A total of 47 and 37 AMF species in winter and summer respectively belonging to 10 genera were recovered from the study sites. 15 species were exclusively found in winter while 5 species exclusively in summer.

Glomus was the dominant genus in all the sites and in both the seasons.

During winter, the highest richness of AMF species was found in Regenerating forest (28), followed by Natural forest (27), Degraded forest (25) and Jhum fallow (24).

During summer, 27 AMF species were recovered from Regenerating forest and 21 each from Natural forest, Degraded forest and Jhum fallow.

7. Correlation among physical and chemical properties of soil

Both positive and negative correlations were observed between soil's physical and chemical parameters. During winter, Pearson's correlation analysis revealed a significant positive correlation between soil moisture content and organic carbon, available N ($p<0.01$) and water holding capacity ($p<0.05$). A significant negative correlation between soil moisture content and available P and pH ($p<0.01$) was also seen. Water holding capacity had a positive correlation with K ($p<0.01$) while bulk density and porosity were negatively correlated. pH had positive correlation with available P ($p<0.01$) and negative correlation with organic carbon, available N and K ($p<0.01$). Organic carbon and available N were significantly and positively related while both exhibited a negative correlation with available P. Potassium content on the other hand had a positive correlation with available N while having a negative correlation with available P ($p<0.05$).

During summer, soil moisture content and water holding capacity exhibited a significant positive correlation with each other as well as with organic carbon, available N ($p<0.01$) and porosity ($p<0.05$) while showing a negative correlation with available P ($p<0.01$) and bulk density ($p<0.05$). Bulk density related positively with available P ($p<0.01$) while having a

negative correlation with porosity, organic carbon and available N ($p<0.01$). Organic carbon and available N were significantly and positively related while both exhibited a negative correlation with available P. Potassium content was positively related with available N ($p<0.01$) while it had a negative correlation with available P ($p<0.05$).

8. Correlation between AMF spore density and root colonization with physico-chemical properties of soil

Pearson's correlation analysis exhibited significant correlation between AMF spore population and root colonization (%) with soil physico-chemical properties across different seasons. During winter, a significant positive correlation was observed between spore population and root colonization ($p<0.01$). Furthermore, spore population also positively correlated with organic carbon, available N ($p<0.01$), soil moisture content and DHA ($p<0.05$). Root colonization also demonstrated positive correlations with soil moisture content, organic carbon, available N and DHA ($p<0.01$). Both spore population and root colonization correlated negatively with pH and available P ($p<0.01$).

Spore population and root colonization correlated positively ($p<0.01$) with each other in summer as well. Additionally, Spore population had positive correlations with soil moisture content, water holding capacity, organic carbon, available N and DHA ($p<0.01$) while showing negative correlations with bulk density, pH and available P ($p<0.05$). Root colonization also exhibited positive correlations with soil moisture content, water holding capacity, organic carbon, available N ($p<0.01$) and DHA ($p<0.05$) while showing negative correlations with bulk density ($p<0.01$) and available P ($p<0.05$).

9. Effect of season on soil physico-chemical properties

The effect of season was significant on soil moisture content and water holding capacity. A higher soil moisture content and water holding capacity was observed in summer than winter.

A significant difference between site and the season was observed in soil pH and available P.

10. Effect of season on dehydrogenase activity (DHA)

DHA in the soil varied significantly among the sites and also between the seasons. However, the effect of season was observed only in Natural forest and it was higher in winter season.

11. Effect of season an AMF

Root colonization by AMF varied significantly between the seasons. However, the effect of season was observed only in Natural forest and Degraded forest. The impact was more significant during the winter season.

Inoculum potential was relatively higher in winter.

AMF spore population exhibited wide variation between the seasons. It was significantly higher during the winter season. However, spore population in Degraded forest did not exhibit any seasonal effect.

Overall, vegetation cover variations were noted, with natural forests having higher tree density, indicating stability, while regenerating forests exhibited increased ground cover, suggesting regeneration. Significant differences in soil properties were observed across sites. DHA, a measure of microbial activity, displayed season-dependent variations, with natural forests consistently showing higher DHA levels. AM status exhibited wide variations, with IP of AMF in soil peaking in natural forests and regenerating forests consistently having the highest spore population and root colonization. Seasonal effects were noted on soil properties, DHA, AMF root colonization, and spore population, with winter generally showing higher levels. The research, thus contributes valuable insights into the complex dynamics of ecosystems undergoing deforestation and regeneration processes, emphasizing the importance of considering multiple factors for a comprehensive understanding.