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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:

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

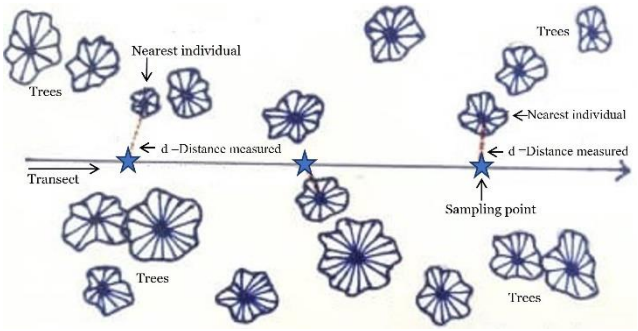

Figure 1. Sketch diagram showing sampling and transect point

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

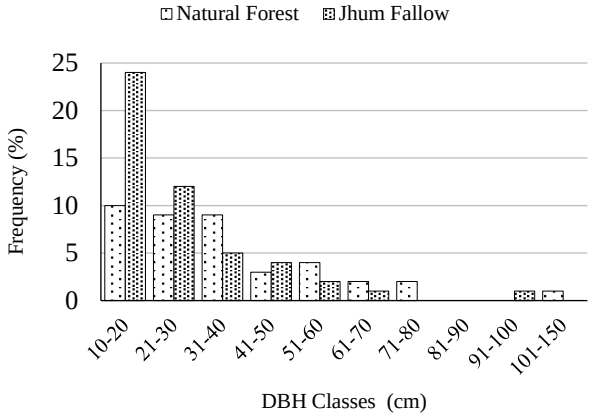

Figure 2: Frequency distribution of DBH-classes (a) Natural Forest, and (b) Jhum fallow

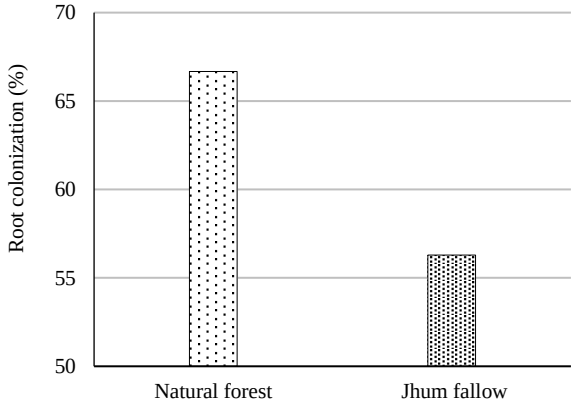

Figure 3: AMF colonization in composite root samples

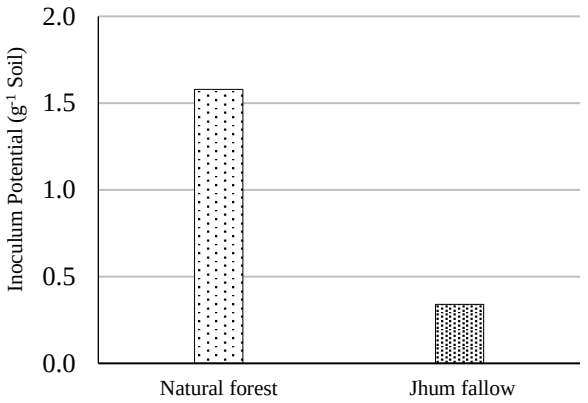

Figure 4: AMF Infective Propagules

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).

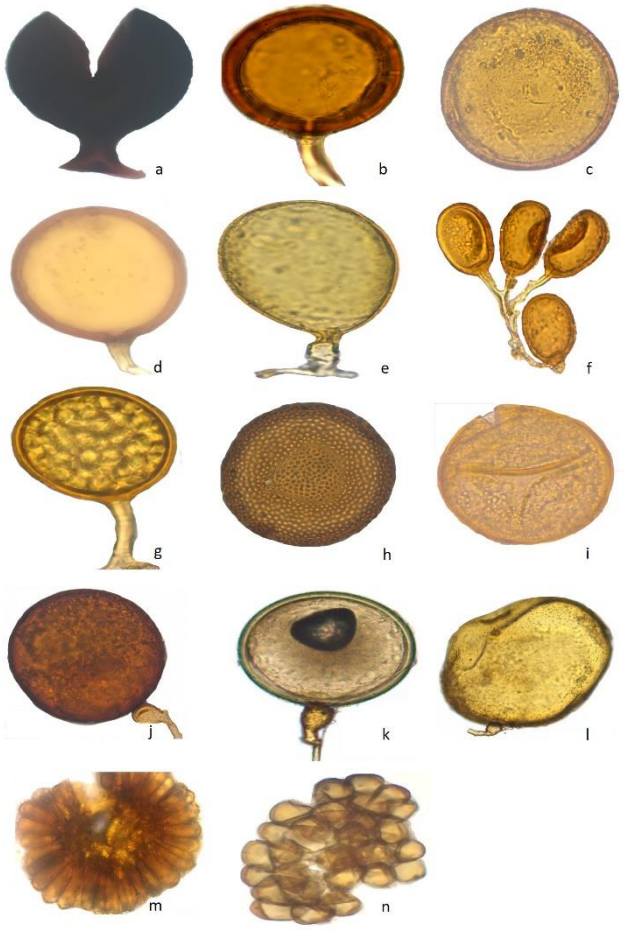

Figure 6: AMF species present in study sites: (a) *Glomus* sp. 1, (b) *Glomus* sp. 2, (c) *Glomus* sp. 3, (d) *Glomus* sp. 4, (e) *Glomus* sp. 5, (f) *Glomus* sp. 6, (g) *Glomus* sp. 7, (h) *Acaulospora* sp. 1, (i) *Acaulospora* sp. 2, (j) *Gigaspora* sp. 1, (k) *Gigaspora* sp. 2, (l) *Scutellospora*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

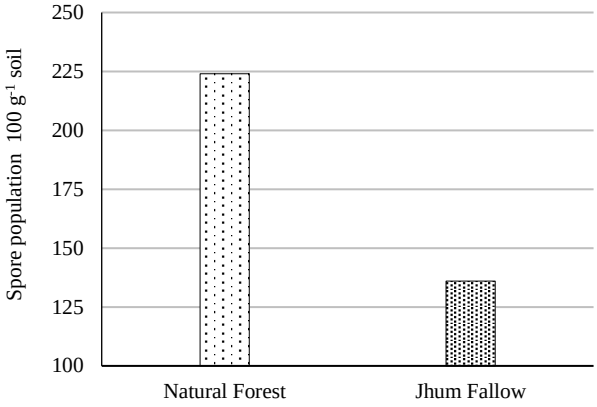

Figure 5: AMF Spore population in soil

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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