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Arbuscular Mycorrhizal Fungal Diversity in Different Forest Types and Altitudinal Gradients of West Kameng District, Arunachal Pradesh, India

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ABSTRACT
The study was conducted in mountain forests from 1000-3800 m (amsl) in West Kameng district of Arunachal Pradesh by covering four different forest types (Tropical wet evergreen, Subtropical broadleaved, Coniferous and Sub-alpine) that harbored seven different types of dominant vegetation. Soil and root samples were collected from three to six different sampling sites of five different locations covering the four different forest types along an altitude gradient of the area. Soil physicochemical properties, number of infective AMF propagules, AMF colonization (%) in composite root samples and AMF spore population were quantified. Soil texture of these forests was either sandy loam or clay loam. Soil organic carbon (OC) varied from 1.50 to 3.41 with a significant difference among forest types. The subalpine forest had the highest OC whereas the subtropical and conifer forest had the lowest. However, their P, N, K contents and soil pH did not differ significantly. Infective AMF propagules and root colonization ranged between 16-126 g^{-1} soil and 32-65%, respectively. The highest spore density was found in sub-tropical broadleaved forest from 2000-2999 m (amsl). Five genera and 26 morphotypes of AMF were identified in these forests. Species of *Glomus* and *Acaulospora* were present in all forest types.

Key words: Arbuscular mycorrhizal fungi, Diversity, Altitudinal gradient, Forest types, Arunachal Pradesh

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms of the phylum Glomeromycota (Schüßler et al. 2001) forming a mutualistic symbiotic association with most land plants. The major functional activity of the symbiosis is mainly based on the mutual exchange of nutrients, plant provide carbon to fungus and several nutrients (phosphorus, nitrogen, trace elements, etc.) are transferred into plants by the fungus. AMF are well known for their plant growth promoting efficiency and providing bio-protection against soil-borne pathogens (Dai et al. 2011). Widespread distribution of AMF facilitates the efficient utilization of nutrients by plants which are a basic requirement of sustainable plant–soil ecosystems. Their diversity and activity are a key mechanism for linking biodiversity and ecosystem functioning (Read 1989, Hart and Klironomos 2002, Kennedy et al. 2007, Martý´nez-Garcý´a and Pugnaire 2009) playing an important ecological role in maintaining the ecosystem. However, considering the role of AMF in terrestrial ecosystems, the relationship between their taxonomic and functional diversity is meager (Krüger et al. 2009) and despite their worldwide occurrence associating with 95% of plant families (Smith and Read 2008, Trappe 1987, Read 1991), still knowledge about the patterns of their diversity and their geographic distribution and mechanism driving such distribution is much limited (Chaudhary et al. 2008). Currently there are four orders 11 families and 25 genera (Redecker et al. 2013) with 210 described morphospecies of AM fungi described on the basis of spore morphology and DNA sequences (Öpik et al. 2006). AMF are estimated to colonize about >80% of all plant species (Smith and Read 2008). AM fungal diversity is generally thought to be locally high and globally low as an individual plant can associate with up to 20 species of AMF (Chaudhary et al. 2008). Further, recent studies on AM fungal diversity particularly in natural ecosystems that have little anthropogenic disturbance reports grossly underestimate the level of AMF diversity (Öpik et al. 2009, 2010, Ohsowski et al. 2014).

To better understand the ambiguity of their diversity and distribution, the approaches of comparing the community composition and abundance of individual taxa in different environments such as along the altitude gradient provides a useful insight (Bryan et al. 2008,

Lomolino 2001). Several studies on AMF diversity have been done in high altitude regions of the world from elevation above 4270m in Andes and Rocky mountain Colorado (Schmidt et al. 2008), in the Arctic (Kohn and Stasovski 1990, Dalpé and Aiken 1998, Pietikäinen et al. 2007), the Antarctica (Christie and Nicholson 1983, DeMars and Boerner 1995), Tibetan plateau (Gai et al. 2012), Colorado Front Range (Mullen and Schmidt 1993) and most of European Alps (Haselwandter and Read 1980, Read and Haselwandter 1981, 1987) and the studies reveals a lack of conclusive definitive pattern of their diversity and distribution (Gonzalez-Cortes et al. 2012, Li et al. 2014). Overall, the studies on AMF colonization on high altitude elevation indicates a general pattern that mycorrhizal colonization decreases with increasing altitude (Haselwandter and Read 1982, Haselwandter 1987).

The present study focuses on to investigate the presence of AMF symbiosis in the different forest types of West Kameng District of Arunachal Pradesh along an altitude gradient from 1040 to 3719 m (amsl). The study also attempts to understand the spatial distribution pattern of AMF as well as the correlation between AMF status and soil physicochemical properties.

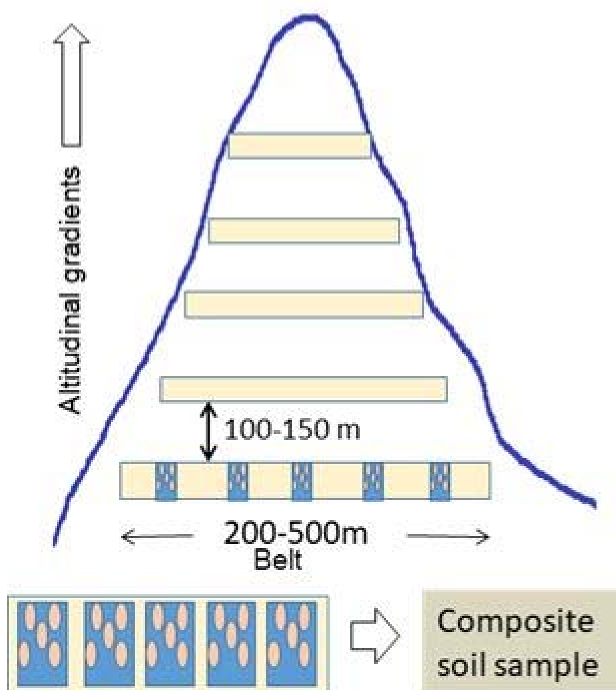

Figure 1. Belt transect method in different altitudinal gradients.

METHODOLOGY

The study site and sampling

The survey was conducted on five different locations of West Kameng district (27°17'22''N-92°25'46''E, 27°29'38''N-92°6'25''E) of Arunachal Pradesh along the altitudinal gradient of an elevation ranging from 1040 to 3719 m (amsl) during March 2020 and March 2021 (Fig. 1). The area covers the four different forest type's viz. wet tropical forest, subtropical broadleaved forest, coniferous and subalpine. General characteristics of the soil and vegetation are presented in Table 1. All samples were collected from the understory of herbaceous forbs and grasses.

Three to six different sampling sites were chosen in five different location of four different forest types along an altitude gradient at an elevation 1040, 1200, 1340, 1450, 2077, 2185 m (amsl) in location-I, 2000, 2246, 2341, 2450 m (amsl) in location-II, 2380, 2470, 2560, 2640, 2700 m (amsl)in location-III, 2126, 2685, 2781 m (amsl) in location-IV, 3000, 3127, 3305, 3618, 3719 m (amsl) in location-V. Five 10 x 10 m plots were placed randomly within each sampling site and the distance between any two plots was between 50 to 100 m apart. A soil core of 4 cm from 0-25 cm depth and root sampleswere collected from five different subplots of 1 x 1 m area from each plot of each sampling site. A total of 23 different randomized soil sample and root sample were taken at 23 different elevation of five different locations.

Soil samples were carefully ground by hand, thoroughly mixed, air-dried, and analyzed for pH, available P, N, and organic C before extraction, counting and identification of AM fungal spores. Root samples were washed and preserved in FAA solution (5 ml formalin, 5 ml acetic acid and 90 ml of 70% ethyl alcohol) and stored at 4ºC until their examination for AMF infection.

Assessment of arbuscular mycorrhizal status

Root colonization

Roots were washed carefully in tap water and cut into 1 cm long segments. About 0.4g of root segments were placed in a test tube containing 2.5% KOH

Table 1. Physicochemical properties of soil in various forest types

Altitude (m)	**Forest type**	**pH**	**OC** (%)	**Av. P** (%)	**Total N** (%)	**K** (%)	**Texture**
1040-1450	Wet tropical forest	6.15[b]	2.02[b]	0.28[a]	0.20[d]	0.088[a]	SCL
2077-2450	Subtropical broadleaved	5.50[a]	1.75[a]	0.42[a]	0.21[d]	0.088[a]	SCL
2126-2781	Subtropical broadleaved	5.50[a]	1.77[a]	0.27[a]	0.25[e]	0.109[b]	SL
2380-2700	Subtropical broadleaved	6.75[e]	3.28[c]	0.40[a]	0.14[b]	0.330[d]	SL
3000-3305	Coniferous	6.37[c]	4.06[d]	0.54[a]	0.12[c]	0.333[e]	LS
3618-3719	Subalpine	6.60[d]	3.30[c]	0.27[a]	0.13[a]	0.321[c]	SCL

solution (w/v) for clearing. Roots were then heated at 90°C in a water bath for 30 minutes. Cooled root samples were washed several time with tap water to remove traces of KOH and then acidified in 1% HCl solution for 30 minutes and stained in acidic glycerol solution (50% H_2O, 5% of 1% HCl, 45% glycerol) containing 0.05% trypan blue (Phillip and Hayman, 1970). Destaining solution was used to remove excess stain from the root samples at room temperature. Twenty 1cm root segments were picked up and mounted on slides and observed under 100-400x magnification microscope for percent root infection. Root segment with mycelium, vesicles and arbuscules were considered as positive infection (Giovannetti and Mosse 1980). The presence and absence of infection in the root segment was recorded and calculated as:

$$\text{root infection (\%)} = \frac{\textit{number of am positive segment}}{\textit{total number of segment scored}} \times 100$$

Determination of AMF infective propagule in soil

AMF infectivity of soil for collected soil sample was determined by MPN (Porter 1979). *Zephyranthes* was taken as a host plant and experiment was conducted in a completely randomized designed with 5 replicates per treatment including control. The sub sample of each soil sample was serially diluted using sterilized soil to get 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilution. Plants were harvested after 1 month and presence or absence of an infection was recorded for each pot. The numbers of infective propagule were calculated as per the table given by Alexander (1965).

Isolation and quantification AMF spores

Spores or sporocarps were extracted from 100g of air-dried sub sample of each soil sample in triplicates by wet sieving and decanting method (Gerdemann and Nicolson 1963). The finest sieve used was 37µm, spores were collected on a grid patterned filter paper, washed three times with distilled water and spread evenly over the entire grid and counted using a dissecting microscope. A sporocarp is counted as one spore and the number of spores was expressed as the mean of three replicates.Spores were identified based on taxonomic criteria (Schüßler and Walker 2010, Redecker et al. 2013) and taxonomic information from the website on the internet (http://invam.caf.wvu.edu).

One composite soil sample from each sampling site was examined for AM fungal spore. Once the data were obtained, following were calculated: (1) spore density (total number of spores in 100g dry sample) (2) AM fungal species richness (the number of AM fungal species forming spores at each sample site) (3) frequency of occurrence (percentage of sample that contained particular fungal species) (4) Relative abundance (the ratio between the number of spores of a particular fungal species to the total number of spores).

Data analysis

Recorded data were statically analyzed and significance of difference was determined using MS Excel and SPSS.

RESULTS

Arbuscular mycorrhizal colonization

AMF colonization of root samples from altitude range 1040-1450 m (amsl) (wet tropical forest) was lowest (31.7%) followed by altitude range 3618-3719 m (amsl) (sub-alpine forest) (40.5%). Highest colonization was found in the mid altitude range 3000-3305 m (amsl) (coniferous forest) (67.0%) followed by altitude range 2077-2450 m (amsl) (subtropical broadleaved forest), 2380-2700 m (amsl)

(subtropical broadleaved forest) (64.5 and 60.5%, respectively). Plant root samples of altitude range 2126-2781 m (amsl) (sub-tropical broad leaved forest) has 54.3% root colonization. No correlation was found between the number of infective propagules and AMF spore population in the soil (Table 2, Fig. 2).

Diversity of AMF

A total of 26 morphotype belonging to 5 genera were identified along the altitude gradient of different forest type. Based on the classification of the phylum reported by Schüßler and Walker (2010), 24 species identified, *Glomus* (9)*, Acaulospora* (7), *Sclerocystis* (4), *Scutellospora* (3), *Gigaspora* (1). Dominant species recorded were *Glomus ambisporum*, *Glomus aggregatum*, and *Sclerocystis rubiformis*. Species of *Acaulospora* were absent in subalpine forest of altitude range 3618-3719 m (amsl). AMF diversity was less in coniferous and sub-alpine forests within altitudinal ranges of 1040-1450 and 3618-3719 m (amsl), respectively. Subtropical broadleaved forest at 2077-2450 m (amsl) had the highest AMF diversity (Table 3, Fig. 2).

DISCUSSION

Studies on AMF colonization in high altitude zones indicate a general pattern of mycorrhizal colonization decreasing with increasing altitude (Haselwandter and Read 1982, Haselwandter 1987). Also, AM fungal diversity along an elevation gradient from 3320-3870 m (amsl) in the South American puma grassland studied by Lugo et al. (2008) found that AM fungal spore diversity decreased with increase in altitude. Similarly, AMF diversity along the elevation 1990–4648 m (amsl) in Tibetan plateau showed decreased species richness with increasing altitude together with percent root colonization and spore density (Gai et al. 2012). However, Öpik et al. (2010) found no relationships between AM fungal richness and latitude, elevation or vascular plant richness. Study on Mt. Taibai at the altitude of 1050 to 3750 m (amsl) of Quinling Mountains in China reported that spore density decreased continuously from the middle to higher elevation which may be attributed to environmental factors, elevation variation or host plant availability as the determinants of fungal community (Shi et al. 2014). Liu et al. (2015) concludes the presence of spatial distribution of AM fungal assemblages along the elevation gradient. In this study, the randomized root samples collected from 23 different elevation sites of five different forest types were found to possess varied AMF percent colonization and did not show a definite pattern of decrease in root colonization with increasing elevation. AMF fungal diversity changed with increasing altitude from 2077-2450 to 3618-3719 m (amsl), indicating a presence of spatial distribution along the elevation gradient in agreement with the findings of Liu et al. (2015). It also decreased from mid altitude of subtropical broadleaved forest (2077-2450 m) to alpine forest (3618-3719 m) though not continuously. However, AMF diversity was found to be lesser in both lower and the higher altitudes. Further, no correlation was found between soil physicochemical properties and AMF status. From this study, it emerges that AMF diversity along the

Table 2. AMF spore population, Infective propagules (IP) and Root colonization in different forest types

Altitude(m)	**Forest type**	**Vegetation**	**Spore population**	**IP (g^{-1} soil)**	**Colonization (%)**
1040-1450	Wet tropical forest	Oak, *Magnolia* sp.	18.0[a]	126.1[a]	31.7[a]
2077-2450	Subtropical broadleaved	*Alnus* sp., Pines	76.0[a]	36.0[a]	64.5[a]
2126-2781	Subtropical broadleaved	Oak, *Illicium* sp.	78.5[a]	26.8[a]	54.3[a]
2380-2700	Subtropical broadleafved	*Illicium* sp., *Rhododendron* spp.	175.5[a]	34.0[a]	60.5[a]
3000-3305	Coniferous	*Rhododendron* spp., Pines	43.0[a]	30.9[a]	67.0[a]
3618-3719	Sub-alpine	*Abies* sp., *Rhododendron* spp.	26.0[a]	53.0[a]	40.5[a]

Figure 2. AMF diversity and root colonization

Table 3. AMF diversity in different forest types (***GL - Glomus, AS - Acaulospora, SC - Sclerocystis, SS- Scutellospora, GS - Gigaspora,*** **UI - Unidentified, TG - Total Genera, MT - Morphotypes)**

Altitude (m)	Forest Type	*GL*	*AS*	*SC*	*SS*	*GS*	*UI*	*TG*	*MT*
1040-1450	Wet tropical forest	1	1	0	1	0	0	3	3
2077-2450	Subtropical broadleaved	3	4	1	2	1	0	4	11
2126-2781	Subtropical broadleaved	4	1	1	0	0	0	3	6
2380-2700	Subtropical broadleaved	4	1	3	1	0	0	4	9
3000-3305	Coniferous	2	1	1	0	0	0	3	4
3618-3719	Subalpine	1	0	0	0	0	2	2	3
Morphotypes of different genera		9	7	4	3	1	2		

altitude gradient is unevenly distributed and lacks a definite pattern, and it suggests for further intensive study to quantify AMF diversity and to determine the influencing factors. Our study also shows a significant presence of the AMF diversity and information of its spatial distribution pattern, which could lead us in understanding the diversity, structure and composition of functional communities in the ecology of West Kameng district of Arunachal Pradesh. The data presented provides a base for future biogeographic studies of micro flora and conservation strategies of the indigenous flora.

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