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SHORT COMMUNICATION

Diversity of arbuscular mycorrhizal fungi (AMF) and root colonization trends along altitudinal gradient: a case study in Western Arunachal Pradesh

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ABSTRACT

Symbiotic association of Arbuscular Mycorrhizal Fungi (AMF) with roots of higher plants provides a better understanding about their ecological function in the mountain ecosystem. In the present study, we investigated the diversity of AMF genus and colonization efficiency of AMF in the selected five Representative Host Plants (RHPs) growing in the six different mountain forest types along an altitudinal gradient ranging from 1000 – 4500 m above msl in West Kameng district of Arunachal Pradesh. The selected RHP quantified for AMF colonization were, *Anaphalis* sp. (Asteraceae), *Geranium pretense* (Geraniaceae), *Fragaria rubicola* (Rosaceae), *Plantago major* (Plantaginaceae) and *Primula* sp. (Primulaceae) and they were selected on the basis of their occurrence throughout the study site. Additionally, colonization was also quantified in composite root samples collected from seven different sites. AMF colonization was observed in the five selected RHP throughout the altitudinal range of 1000 – 4500 m msl. It varied from 10 – 70% with the highest mean root colonization observed in *P. major* (30%). The maximum AMF colonization was observed in the RHP growing at mid-altitude range (2000 – 3000 m msl) whereas the minimum AMF colonization was observed in the RHP growing at the highest altitudinal range (4000 – 4500 m msl). The AMF colonization in composite root samples also showed the same trend. Our study suggests a significant effect of altitude on AMF species diversity and colonization association in the selected five host plant species investigated.

Keywords: Endomycorrhiza; *Glomeromycota*; Forest Types; AMF, CRS Root Colonization; Altitudinal Gradient; Eastern Himalaya

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are reported to be widespread soil microorganism of the phylum *Glomeromycota* (Schüßler et al., 2001) forming a symbiotic association with 95% of the plant families in terrestrial environment (Trappe, 1987; Read, 1989; Smith and Read, 2008). The symbiosis is mainly based on the mutual exchange of nutrients in which fungus provides several soil nutrients (phosphorus, nitrogen, trace elements, etc.) to the host plant and receives in return fixed carbon compounds. AMF are also known for their efficiency in promoting plant growth and providing bioprotection against soil-borne pathogens ((Dai et al., 2011; da Silva Campos,2020). The diversity and activity of AMF are a key mechanism for linking biodiversity and ecosystem functioning as it plays an important ecological role in maintaining the ecosystem (Read, 1989; Hart and Klironomos, 2003; Kennedy et al., 2007). The mountain ecosystem has an environmental gradients and slant characteristics on a small spatial scale making it an interesting area for the studies of diversity and community distribution of plants and animal (Wagg et al., 2011). The greater majority of the studies done on the mountain ecosystem are generally focused on plants and animals while the studies on relationship between soil microorganism and plants are still limited to date (Bryant et al., 2008). To understand the pattern and driving factors of fungal communities, the different conditions of multiple elevation gradients of mountain ecosystem provide an optimal environment for their studies (Tian et al., 2017; Hagedorn et al., 2019). Though, several studies have been done on AMF diversity, distribution, and their drivers at different altitudinal gradients, still a conclusive definitive pattern of diversity and distribution of AMF along an altitudinal gradient are reported to be lacking (Gonzalez-Cortes et al.,2012; Li et al., 2014). The mountainous region of West Kameng district of Arunachal Pradesh is characterized by diverse forms of altitudinal gradient, forest types and vegetation composition. The greater Himalayan range and state's highest peaks (Kangte – 7090 m and Gorichen – 6538 m msl) form its northern boundary (Kaul and Haridasan, 1987). Thus, the present study was conducted to examine the presence of AM fungal diversity and association, and to investigate the pattern of host root mycorrhizal colonization in the high-altitude areas along an altitudinal gradient of 1000 – 4500 m above msl.

2. Materials and methods

2.1. Study site

The present study was conducted at seven different locations of West Kameng district of Arunachal Pradesh along the altitudinal gradient ranging from 1000 – 4500 m above msl covering five different forest types viz., Wet Tropical Forest, Subtropical Broadleaf Forest, Temperate Forest, Coniferous Forest, Subalpine Forest and Alpine Forest harboring different types of flora and vegetation. The study sites visited for sample collections include Bomdila, Dirang-Padma, Mandala-Phudung, Sela pass, Senge-Kharpo, Shergaon, and Tenzingaon (Figure 1).

2.2. Collection of root samples

Based on the frequency of occurrence throughout the elevation range of seven different altitudinal zones, five AM plant species were selected and designated as Representative Host Plants (RHPs) for investigation of AMF root colonization. The selected RHP species were identified as *Anaphalis* sp. (Asteraceae), *Geranium pretense* (Geraniaceae), *Fragaria rubicola* (Rosaceae), *Plantago major* (Plantaginaceae) and *Primula* sp. (Primulaceae) by consulting regional and state floras of Arunachal Pradesh published by BSI. The voucher specimen of each the plant species were collected and preserved at HAU, Department of Botany, Rajiv Gandhi University for future consultation and references.

Root samples of RHPs were collected from seven different locations i.e., 1000 – 1500 m, 1500 – 2000 m, 2000 – 2500 m, 2500 – 3000 m, 3000 – 3500 m, 3500 – 4000 m and 4000 – 4500 m above msl by laying three quadrates of 10 m × 10 m size at a distance of 50 – 100 m within each altitudinal range. Mixed root samples were collected by digging up to a depth of 20 cm at five subplots within each altitudinal range. Collected soil containing roots were heaped on spot in a square shape over a tarpaulin sheet and thoroughly mixed. The process was repeated thrice to make a Composite Root Sample (CRS).

Collected root samples (RHPs and CRSs) free from soil aggregates were kept in separate sterile poly bags. On the same day, the root samples were fixed in FAA solution (5 ml formalin, 5 ml acetic acid and 90 ml of 70% ethyl alcohol), washed thoroughly with clean water and stored at 4 °C for quantification of AMF root colonization in the laboratory.

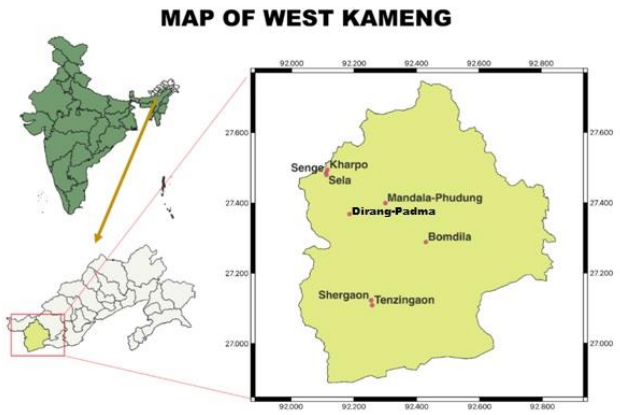

Figure 1. Location map showing sampling site in West Kameng district of Arunachal Pradesh, Eastern Himalayas.

2.3. Quantification of AMF root colonization

Roots fixed in FAA were washed carefully and cut into several pieces measuring 1 cm long segments. A small amount of root segments put in a test tube containing 2.5% KOH solution (w/v) were heated at 90 °C in a water bath for 30 minutes to clear the roots. Cooled root samples were washed several times with tap water to remove traces of KOH. Further, the roots were acidified in 1% HCl solution for 30 minutes, and then stained in acidic glycerol solution (50% H_2O, 5% of 1% HCl, 45% glycerol) containing 0.05% trypan blue (Phillip and Hayman, 1970). De-staining solution was used to remove excess stain from the root samples at room temperature. Twenty root segments were randomly picked up, mounted on slides and observed under a compound microscope (100 – 400x). 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 segments were recorded and Root colonization was calculated as:

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

2.4. Isolation and identification of AMF spore

Required amount of soil from soil samples collected from various location of study sites (stored in the proper condition) were taken for isolation of AM spores. Spores were isolated by wet-sieving and decanting method (Gerdemann and Nicolson, 1963; Daniels and Skipper, 1982). The 100g of sample soil taken were air-dried and suspended in 1000 ml of tap water, agitated manually for 5 minutes and the soil suspension was left for 10 minutes enabling heavier particles to settle. The remaining soil, roots, hyphal and spore suspension were slowly poured through a stack of sieves of different pore size ranging from 800-40 µm. Extracts were collected in a clean beaker of 100 ml. It was transferred to 50 ml centrifuge tube and centrifuged at 1750 rpm for 5 minutes. Spore suspended pellets obtained after removal of the supernatant was again centrifuged by the sucrose gradient process for 1 minute (Walker et al., 1982). Supernatant obtained from the centrifugation was filtered with nylon cloth (25 µm pore size) using filter funnel to remove water and clear sugar molecules from AM fungal spores. The filtrate was placed in clean, sterilized Petri dishes and spores were picked up using white bristle brush and observed under Nikon binocular Stereomicroscope (20-120x magnification) and then AMF species were identified from the spores observed and photography were taken for each species and recorded. Live spores were stored in eppendorf tubes at 4 °C. For quantification of spore population, soil samples taken from the various location of study site were homogeneously mixed before extraction and the spores were isolated by wet-sieving and decanting method followed by sucrose density gradient centrifugation (Daniels and Skipper, 1982). Isolated spores were counted manually under Nikon binocular Stereomicroscope and total spore density of each species was calculated and recorded using the formula:

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

2.5. Data analysis

Data were statistically analyzed by one-way ANOVA and significance of difference was determined by Least Significant Difference (LSD at 5%).

3. Results and discussions

3.1. AMF spore population and genera

A total of 24 AMF species belonging to 5 genera namely, *Gigaspora*, *Glomus*, *Scelerocystis*, *Scutellospora* and *Acaulospora* were isolated from the soil sample collected from seven different locations along the altitudinal range of 1000-1500 m, 1500-2000 m, 2000-2500 m, 2500-3000 m, 3000-3500 m, 3500-4000 m and 4000-4500 m above msl. Of the 24 AMF species reported, 9 species were belonging to genus *Glomus* which account for 23.6% of the total AMF species diversity recorded in present studies. *Acaulospora* was found to be represented by 7 species which is followed by *Scutellospora* (4 sp.), *Scelerocystis* (2 sp.) and *Gigaspora* (2 sp.). The genus *Glomus* was found in the soil samples collected from all altitudinal gradients and forest types (1000 – 4500 m above msl), and have been reported as a dominant genus in the study site which is followed by *Acaulospora* and *Scutellospora*. The highest number of AMF morphotypes were reported from the mid altitude range of 2000-2500 m above msl. The species of *Acaulospora* were found along the altitude ranges from 1000-1500 m and 2500-3000 m above msl, while *Scutellospora* species were reported from altitudinal ranges of 1000-1500 m, 2000-2500 m and 3000-3500 m above msl. *Scelerocystis* were reported from the altitudinal ranges of 2000-2500 m, 2500-3000 m and 3500-4000 m above msl. *Gigaspora* were found in the lower altitudes range of 1500-2000 m to 2000-2500 m above msl. The species of the genera *Acaulospora*, *Scutellospora*, *Scelerocystis* and *Gigaspora* were absent in high altitude range of 4000-4500 m above msl (Table 1, Figure 2). Present studies revealed low diversity and distribution limits of AMF fungi at higher elevation. This might be due to the fact that the symbiosis efficiency of host plants supporting colonization and growth of AMF at higher elevation tend to decrease due to extreme weather events. Hence, only few host plant species which support symbiosis with AMF species allow AMF colonization in roots but with limited genera of AMF. Oehl and Körner (2014) also reported that extreme weather events at high altitude restrict distribution and proliferation of the spores of some AMF species.

Table 1: AMF spore population and genera found in different forest type along an altitude gradient in West Kameng district of Arunachal Pradesh.

Altitude (m)	Forest Type	Vegetation	AMF spore population/100g	AMF genus recorded
1000-1500	Wet Tropical Forest	Oak, *Magnolia*, *Rhododendron*	9±1.27[a]	*Glomus*, *Scutellospora*, *Acaulospora*
1500-2000	Wet Tropical Forest	Oak, *Magnolia*, *Rhododendron*	8.2±1.93[a]	*Gigaspora*, *Glomus*, *Scutellospora*, *Acaulospora*
2000-2500	Subtropical Broadleaf Forest	*Alnus*, *Pinus*, *Illicium*, Oaks	29.8±6.61[b]	*Gigaspora*, *Glomus*, *Scelerocystis*, *Scutellospora*, *Acaulospora*
2500-3000	Temperate Forest	*Alnus*, *Pinus*, *Illicium*	38.4±11.41[b]	*Glomus*, *Scelerocystis*, *Acaulospora*
3000-3500	Coniferous Forest	*Rhododendron arboreum*, *Pinus*	10±3.83[a]	*Glomus*, *Scutellospora*
3500-4000	Subalpine Forest	*Abies*, *Rhododendron*	5.4±1.25[a]	*Glomus*, *Scelerocystis*
4000-4500	Alpine Forest	*Rhododendron* Shrub land	5.6±1.29[a]	*Glomus*

*Significance of difference was determined by Least Significant Difference (LSD at 5%).

Table 2: AM root colonization in Representative Host Plants (RHPs) and Composite Root Samples (CRSs) collected from different forest types along altitudinal gradient.

Altitude (m)	Forest Type	Dominant Vegetation	Root colonization (%) RHP					CRS
			AN	*GP*	*FR*	*PM*	*PR*	
1000-1500	Wet Tropical Forest	Oak, *Magnolia*, *Rhododendron* sp.	13	13	12	70	10	33.0±9.0[b]
1500-2000	Wet Tropical Forest	Oak, *Magnolia*, *Rhododendron* sp.	13	11	12	15	10	67.4±2.9[d]
2000-2500	Subtropical Broadleaf Forest	*Alnus* sp., *Pinus*, *Illicium* sp., Oak	66	26	44	32	26	58.8±9.7[cd]
2500-3000	Temperate Forest	*Alnus* sp., *Pinus*, *Illicium* sp.	24	10	49	40	12	64.8±10.8[d]
3000-3500	Coniferous Forest	*Rhododendron arboreum*, Pines	41	12	20	27	10	36.8±14.7[bc]
3500-4000	Subalpine Forest	*Abies* sp., *Rhododendron* sp.	17	10	39	13	10	38.2±2.9[bc]
4000-4500	Alpine Forest	*Rhododendron* sp., Shrub land	10	10	10	10	10	6.2±1.5[a]

AN=*Anaphalis* sp.; GP= *G. pretense*;FR= *F. rubicola*; PM=*P. major*; PR=*Primula* sp.
Values (Mean ± SEM) followed by same letter in the last column are significantly not differ

3.2. AMF root colonization in RHPs

Roots of the five RHPs collected from all seven altitudinal ranges were found to be colonized with AMF. The highest AMF colonization in the roots were observed in *Plantago major* with 70% AMF colonization which is followed by *Anaphalis* sp (13%), *Geramium pratense* (13%), *Fragaria rubicola* (10%) while lowest AMF colonization was observed in *Primula* sp (10%) collected from Wet Tropical-Subtropical Broadleaf Forest types with altitude range between 1000 – 1500 m above msl. However, very low AMF root colonization [*P. major* (15%), *Anaphalis* sp (13%), *F. rubicola* (12%), *G. pratense* (11%) and *Primula* sp (10%)] were observed for the RHPs collected from elevation range between 1500-2000 m above msl (Table 2, Figure 3).

In the elevation range of 2000 – 2500 m above msl, the highest AMF root colonization were observed for RHP *Anaphalis* sp (66%) which is followed by *F. rubicola* (44%), *P. major* (32%) while lowest AMF colonization were observed for *G. pratense* (26%) and *Primula* sp (26%). In the elevation of 2500 – 3000 m above msl (Temperate Forest type), the highest AMF colonization were observed for the RHP *F. rubicola* with 49% which is followed by *P. major* (40%) while lowest colonization was observed for *Primula sp.* (12%) and *G. pratense* (10%). Further up in the elevation range of 3000 – 3500 m above msl (Subalpine Forest type), the highest AMF colonization were observed for RHP *Anaphalis* sp. (41%), followed by *P. major* (27%), *F. rubicola* (20%) while *G. pratense* and *Primula* sp. were recorded with lowest AMF colonization of 12% and 10% respectively. In the higher elevation range of 3500 – 4000 m above msl (Alpine Forest type), the highest AMF colonization was recorded for *F. rubicola* with 39% while other 3 species of RHP were recorded with very low AMF colonization [*Anaphalis* sp (17%), *P. major* (13%), *G. pratense* (10%) and *Primula sp.* (10%) each)]. In the elevation of 4000 – 4500 m above msl (highest point of Alpine Forest), all the four RHPs species were recorded with very low percentage (10% for each species) of AMF root colonization. This could be due to the fact that the efficiency of AMF to colonize the roots tends to decrease at extremely

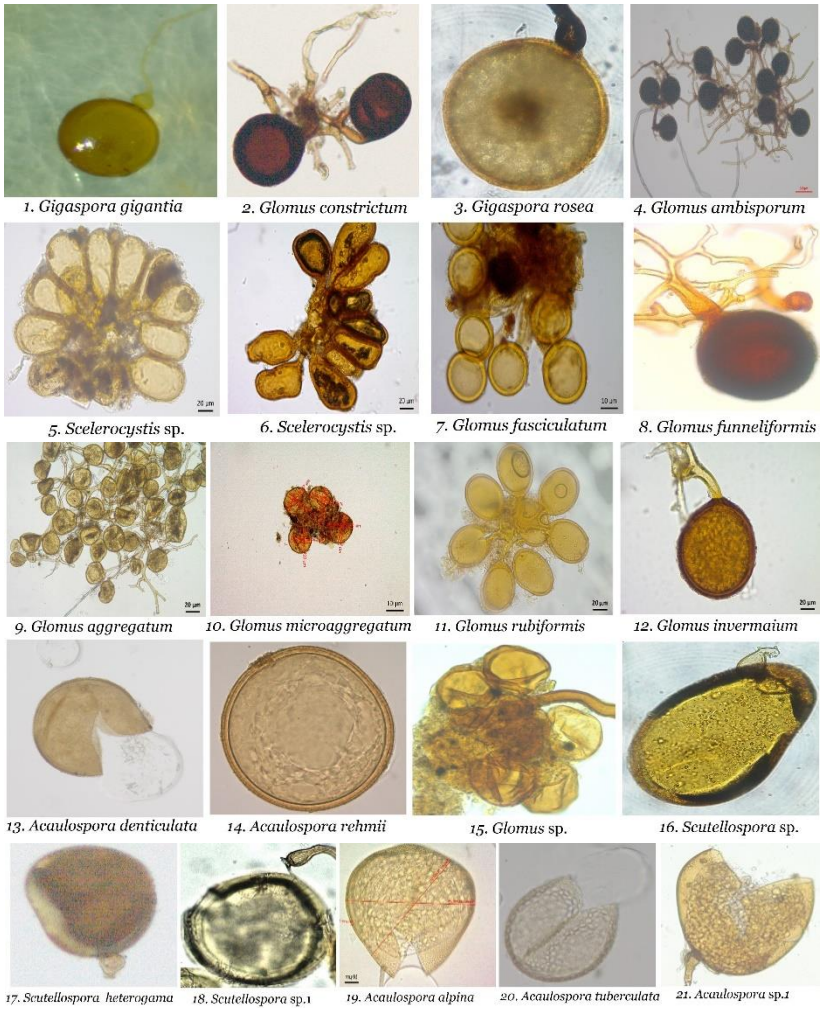

Figure 3 (1-21): Diversity of Arbiscular Micorizhal Fungi (AMF) genus and species recorded from the soil samples collected from seven different forest type along elevation range of 1000 – 4500 msl in West Kameng district of Arunachal Pradesh, Eastern Himalaya.

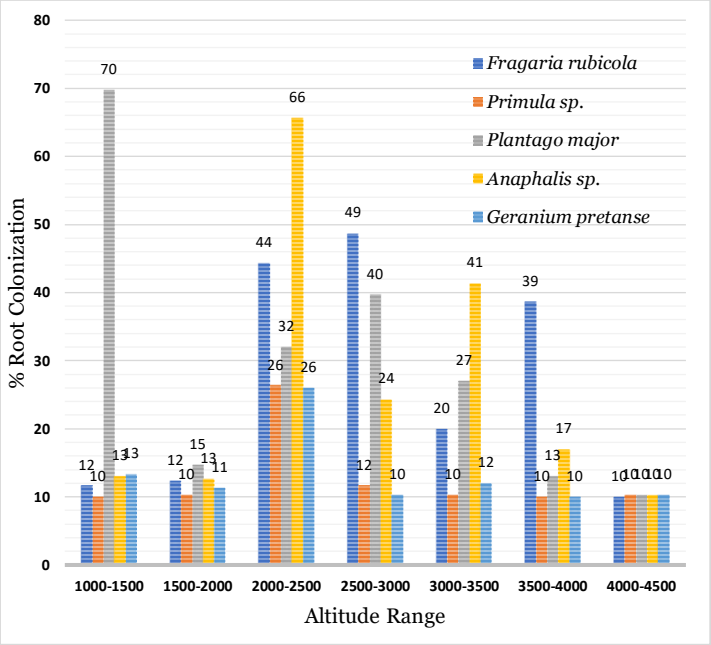

Figure 3. AMF percent root colonization of representative host plants along an altitudinal gradient

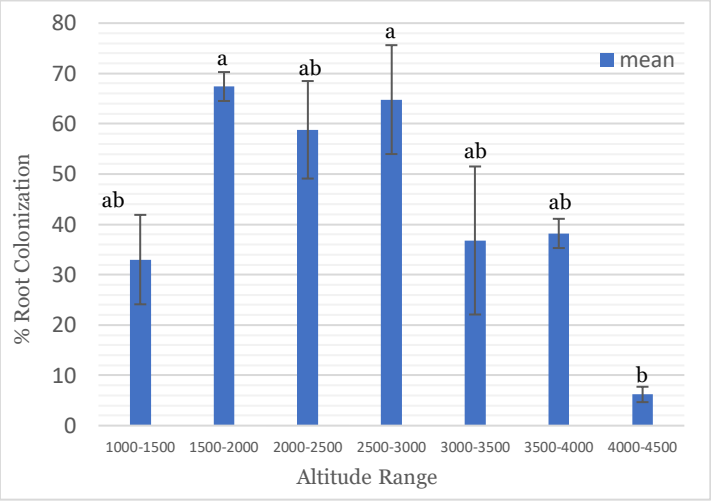

Figure 4. AMF percent colonization of root samples of seven location along an altitudinal gradient

low temperature in winter and maximum summer temperature goes only up to 5 °C normally recorded in the alpine region. The high mechanical strength and hardiness of root tissues caused by the extremely low temperature and the plant genes that are responsible for allowing colonization of AMF in roots might remain receptive due to low metabolic activities of alpine plants. This could be one reason which prevent colonization of AMF in the selected RHP species investigated in present studies. Previous studies also reported that AMF symbiosis with vascular plant roots in extreme weather conditions of high mountain region to be rare and dysfunctional due to constraints of the carbon economy at lower temperatures (Oehl and Körner, 2014 and Kotilinek et al., 2017).

3.3. AMF colonization of CRSs

AM colonization in CRSs was observed throughout the sampling sites (Table 2, Figure 4) along different altitudinal gradient. Root colonization in CRSs collected from Wet Tropical Forest (1000 – 1500 m), Coniferous Forest (3000 – 3500 m) and Subalpine Forest (3000 – 4000 m) were found to be almost same without any significant difference. The root colonization of AM fungi was found to be high in the plant root samples collected from altitude between 1500 to 3000 m above msl range which includes two forest types viz., Wet Tropical Forest (67.4%), Subtropical Broadleaf Forest (58.8) and Temperate Forest (64.8%). CRSs from alpine region (4000 – 4500 m) showed very low % of root colonization (6.2%) which is significantly lesser than the samples collected from all other ranges (LSD at 5%). Highest root colonization of AMF in the CRSs were observed for the samples collected from lower and middle elevation (Wet Tropical and Subtropical Broadleaf Forest) while less root colonization efficiency was observed for CRSs sampled from higher elevation (Subalpine and Alpine region). The previous report of Gai et al (2012) in the Tibetan plateau (1990 – 4648 m) and Kotilinek et al (2017) in the Himalayas (3400 – 6150 m) suggests that AMF colonization have a downward trend with increasing altitudes. Whereas, Zang et al (2021) reported root colonization to form a trend of cubic function with the change of altitude i.e., mycorrhizal colonization first increased with increasing altitudinal gradient (660 – 1170 m) and then gradually declined (1170 – 2850 m), followed by further increase with altitude (2850 – 3500 m). In the present study, CRSs were found to have a varied percentage of AM colonization, showing an increase rate of colonization at mid altitude and significantly decreased at highest elevation which is consensus with previous findings of Kotilinek et al (2017). The soil roots samples from low and middle elevation have been reported with highest AMF colonization of 67.4% and the lowest AMF colonization was observed to be 6.2% in higher elevation. The similar trend of AMF colonization was observed in RHPs except in *G.pretense*. This study has demonstrated significant impact of elevation and temperature on AMF root colonization efficiency in some selected representative host plant species (RHP). However, in another study (Li et al., 2020) conducted in the Southeast of the Qinghai Tibet Plateau reported no significant impacts of altitude on AMF colonization in plant roots collected from both high-altitude and low-altitude region which is inconsistent and contradicts our present findings. These differences in AMF root colonization may be due to the variation AMF symbiosis efficiency in plant species found in different altitudinal gradient, geographic location, and hydrothermal environment (Li et al., 2018; Zang et al., 2021).

Conclusion

Present studies revealed diversity and distributional limits of some species of AMF along different altitudinal gradient. AMF diversity tend to decrease at higher elevation of above 3000 – 4500 m above msl in subalpine to alpine ecosystem, however, the genus *Glomus* have been confirmed to be distributed in all forest types and altitudinal gradient (1000 – 4500 m). This requires further studies at molecular level to unravel the tolerant genes in *Glomus* responsible for enhancing AMF colonization in the roots of host plants which help them adopting to all elevation gradient with variable climatic regime. Present studies also confirmed AMF colonization and symbiosis efficiency of the selected RHP roots and the composite root samples (CRS) collected from all the seven forest types along different altitudinal gradient. However, less AMF colonization were observed for the plant root samples collected from higher elevation of 2800 m – 3500 m above msl which also indirectly and clearly indicated the effect of elevation and extreme weather event at high altitude ecosystem which might restrict AMF colonization potential in the roots in majority of the host plants investigated. However, RHP *Plantago major* (1000-1500 m), *Anaphalis* sp (2000-2500 m, 3000-3500 m), *Fragaria rubicola* (2500-3000 m, 3500 – 4000 m), all three host plants have demonstrated highest AMF colonization efficiency (%) at their respective elevation gradient, and thus confirmed as potential host plant species (PHPs) for the AMF colonization in the forest located at specific altitude. Further molecular studies are needed to unveil the genes that contributing to the AMF colonization efficiency in these plants located at different elevation gradients with variable to extreme weather events and forest types. Present findings are expected to provide a possible basis for further studies on specific AMF species diversity and nature of

community structure at specific elevation gradient targeting potential host plant species for AMF colonization.

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Author contributions
All the authors have equally contributed in concept and study design. Minam Pertin generated data from the field and contributed in manuscript draft. Oyi Dai Nimasow finalized the draft manuscript. All the authors read and approved the manuscript before communication for publication.

Conflict of interests
Authors have no conflict of interests.

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