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Mycorrhizal Spore Density in Relation to Physico-Chemical Properties of Soil: A Case Study of Central Himalaya

Bal Krishan Choudhary[1]*, Mustafa Ali Khan[2] and K. G. Saxena[3]
[1]Department of Environment Science,
Ambedkar College, Fatikroy, 799290, Tripura, India
[2]Development Alternatives, New Delhi, 110070, India
[3]School of Environmental Sciences,
Jawaharlal Nehru University, New Delhi 110067, India
*E-mail ID: bkmchoudhary@gmail.com

Abstract

The impact of various land uses and physicochemical properties of soil on mycorrhizal spore density was calculated for four major land uses of central Himalaya. The land use/ cover types were pine forest (PF), Home Garden (HG), Irrigated Agriculture (IA) and Scrub Land (SL). Representative soil sampling was done and VAM (vesicular arbuscular mycorrhizae) spores were extracted, morphologically identified and counted. The same soil samples were processed for the analysis of physicochemical parameters of soil. It was observed that land use types which were intensively managed had least spore densities.

Keywords: *Land use, VAM, Soil Organic Carbon, Soil pH, Fine root biomass.*

Introduction

Land use describes how a piece of a land is managed or used by humans and land cover is the observed physical and biological cover of land such as vegetation or man-made features. Several land use classification system have been developed around the world. Some are related to agriculture and other related to forestry, wildlife, recreational activities, settlements etc. (Brady, 2002). Land use system in central Himalaya may be divided into following broad categories i.e. agriculture land use (rain fed and irrigated agriculture land) and forest land (pine forest, oak forest) and scrub land. It has been found that the land use types are being converted at an alarming rate. Monitoring of soil physico-chemical and biological properties assume importance from the point of assessing the impact of land use land cover changes (Dunjo et al., 2003). Soil in the

mountain regions is shallow and prone to degradation. The main indicators include loss of soil organisms, moisture, low pH, low fine root biomass and low level of soil organic matter etc. Mycorrhizae are symbiotic associations formed between the roots of most plant species and the fungi, these symbionts are characterized by bi-directional movement of nutrients where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing critical linkage between the plant root and soil. The soil-borne or extrametrical hyphae take up nutrients from the soil solution and transport them to root. The mycorrhiza also increases the effective absorption surface area of plant root system.

Mycorrhizae

Frank coined the term "mycorrhizae" to describe the symbiotic association of plant roots and fungi in 1885. It is very well documented now that mycorrhizal fungi improve the growth of plants that are important in agriculture, horticulture and forestry. There are two main types of mycorrhizae in ecosystems: VAM and ectomycorrhizae. The fungi that are most abundant in the agricultural soils are VAM fungi (Cardoson and Kuyper, 2006). The distribution of VAM is widespread throughout the plant kingdom; the association is geographically ubiquitous and occurs in plants growing in arctic, temperate and tropical region. VAM occurs over a broad ecological range, from aquatic to desert environment. The most common mycorrhizal association is the VAM type, which produces fungal structures (vesicles and arbuscules) in the cortex region of the root. The VAM mycorrhizal association is found in most plant families so for examined, although it may be rare or absent in families such as *Cruciferae*, *Chenopodiaceae*, *Caryophyllaceae* and *Cyperaceae*. VAM are formed by non-septate phycomycetous fungi, belonging to the genera *Glomus*, *Gispero*, *Acaulospora* and *Sclerocystis* in the family *Endogonaceae*. Colonization of roots by VAM fungi can arise from 3 sources of inoculum- spores, infected root fragments and hyphae- collectively termed "*propagules*". Spores are the best defined source of inoculums and are only propagules that can be identified to species level with any degree of certainty. Consequently they are of central importance in identifying these species, determining their distribution and establishing them in pot culture for experimental or identification purposes. The external mycelium attains as much as 3% of root weight. Biomass of hyphae of AM fungi may amount to 54-900 kg/ha (Zhu & Miller, 2003) and some other products formed by them may account other 3000 kg (Lovelock *et. al*., 2004).

The ability of VAM fungi to enhance host-plant uptake of relatively immobile nutrients, in particular phosphorus and several micronutrients, has been the most recognized beneficial effect of mycorrhizae. VAM fungi have the potential to reduce damage caused by soil borne pathogenic fungi, nematodes and bacteria. VAM fungal spores are larger than spores of most other fungi, normally ranging from 20 to 50 *micron* (Trappe, 1982). The mycorrhizal role in maintaining soil structure is important in all ecosystems (Van der Heijden *et al.*, 1998). Formation and maintenance of soil structure will be influenced by soil properties, root architecture and management practices (nature of land uses). Mycorrhizal fungi contribute to soil structure by various ways like directly trapping the carbon resources of the plant to the soil (Miller and Jastrow, 2000). This phenomenon influences the formation of soil aggregate because soil carbon is crucial to

form organic materials necessary to cement soil particles. Crop rotation effects on mycorrhizal functioning have repeatedly been observed. The spores of VAM fungi are extremely large and contain many nuclei and large amount of stored lipid and carbohydrates. Variations may represent adaptation to different environment, rapid germination and colonization would be on advantage in the humid topics whereas more subtle interaction between soil moisture and optimum temperature for germination & colonization may have evolved in mere seasonal environments.

Study area

The study was carried out in and around the *Uttaron*, *Chamali* and *Niwari* (800 – 1400 m above sea level) villages of district Chamoli , Garhwal (30^0 27 N and 79^0SE), Uttarakhand, India. The area experiences a typical monsoon climate. Up to 80% of the precipitation is obtained during the monsoon (July to September) amounting to approximately 1200 mm. The soil is derived from quartz chlorite schists, quartz muscovite schists and feldspathic quartz schists and can be classified into dystric combisol. The landscape is differentiated into four land use types: Pine forest ((PF) *Pinus roxburghii sergent*), Home Garden (HG), settled irrigated agriculture (IA) on terraced slopes and Scrub land SL). Rice, mustard, wheat, legumes, are the common crops grown in agricultural fields. The altitude of the landuses in the study area are slightly varies. The altitude of settled irrigated agro-ecosystem is around 800 m above mean sea level (amsl), rain fed agro-ecosystem and agricultural fallows are about 900 m above mean sea level. However, pine forest and oak forest is at about 1000 m above mean sea level. Both the forests were in separate patches.

Methodology

Soil sampling in the four major land uses of central Himalaya i.e. PF, HG, IA and SL was done in December, 2004. Samples were taken from two soil depth viz. 0-10 cm and 10-20 cm; five composite samples were taken for each land use and each depth. The five samples were mixed together to represent one composite sample for that depth. The samples were air dried at room temperature (15^0C to 20^0C for) 7-10 days and grinded and sieved through 0.25 mm sieve to remove roots, gravel and stones. These were used samples used for analysis of soil organic carbon and soil pH. SOC was measured by Walkley & Black method (1934). Soil pH was measured by using potassium chloride (KCl) and doubled distilled water (DDW). Fresh soil stored in cold storage, used for analysis of mycorrhizal spores, moisture content and fine root (<2 mm diameter) biomass. Fine roots were hand sorted by wet sieving method using 125 micrometer mesh. Sorted roots were oven dried at 65^0C until constant weight was obtained. Soil moisture was calculated on dry weight basis (Anderson and Ingram, 1989). The work had limited time period so only limited parameters and replicates were taken.

VAM spores occurring in the original soil sample were extracted by wet sieving and sucrose density gradient centrifugation method (Daniels and Skipper, 1982). The procedure include 25g of soil was taken in a 500 ml beaker and 250 ml of water was added to it. The mixture was stirred with glass rod for 15 seconds and allowed to settle

down. The suspension was sieved using sieves of pore sizes 250, 125, and 45 micrometer and centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded and 45% sucrose solution was added to the pellet and again centrifuged at 2000 rpm for 2 minutes. The supernatant was collected, sieved with sieve of pore size 32 micrometer. The sucrose solution was discarded, and the spores washed with water. The spores were collected in test tube filled ¾th with water, and were counted using a microscope of order 400X magnification. The diluted samples were used for counting the spore density was calculated as number of spore per gram soil.

Results and Discussions

Spore density

The spore density for each land use and each soil depth was calculated in individual/ gram soil. The maximum spore density in 0-10 cm soil depth was found in PF (57.56 ±41.04), while the minimum value was in IA (23.41±4.92). In 10-20 cm soil depth, maximum spore count was found in SL followed by PF and IA. HG had the least spore count i.e. (8.95 ± 4.12). The least value for spore count was found in HG; this may be due to the frequent disturbance in form of tillage and hence lack of fine root for inoculation. In this study mycorrhizal spore density is decreasing with soil depth, this may be due to decrease in root colonization with depth. The distribution of VAM fungi in soils of HG and IA was less compared to the non-agriculture land uses. This may be due to the extensive disturbance face by the HG through the year. This result is similar to the other study (Abbott and Robson, 1991). In 10-20 cm depth of soil the maximum value noted in SL (24.15±3.73) and minimum in HG (8.95±4.12) (Figure 1) (Table 1). For 10-20 cm soil depth higher numbers of spores were found in the SL, this may be due to fact that this land use was comparatively less disturbed. When natural forests are converted into agriculture land, the production of sporocarp ceases in the agriculture. Harinikumar and Bagyaraj (1988) observed a 13% reduction in mycorrhizal colonization after 1 year cropping with a non-mycorrhizal crop and a 40% reduction after following. However, with high and intensive input agriculture the number of AM propagules and species richness decreases (Sieverding, 1991). Non-tilled soils may have more mycorrhizal spores in the top 8 cm of soil while tilled soils may have more at 8-15 cm depth (Abbott and Robson, 1991). Mixed cropping of maize and soybean was found to increase the proliferation of AM fungi compared with monocropping with maize or soybean (Harinikumar *et.al*., 1990). In tropical soils application of organic matter either in the form of farmyard manure or compost or addition of agricultural wastes or green manure stimulate the proliferation of AM fungi (Harinikumar *et.al*., 1990). Spore number is directly related with the germination and inoculation of fungal hyphae.

Soil Organic Carbon (SOC)

It has been widely speculated that organic matter added to soil encourage mycorrhizal development (Muthukumar and Udaiyan, 2000), but there are few data to support this speculation. The SOC was calculated in percentage (%) of soil. In 0-10 cm soil depth maximum percentage of SOC was present in HG (2.2±1.29); whereas second

highest value (1.8±1.03) was for IA land use. The highest value found in this land use may be due to the input of organic matter in form of agriculture waste. The lowest value of SOC (0.6± 0.15) was found in SL, this may be due to lack of tree cover, which leads to low input of organic matter in the form of litter fall. For 10-20 cm soil depth the maximum value of SOC was noted for HG (2±1.2) and minimum for SL (0.3±0.1), the trend was similar to 0-10 cm soil depth (Figure 2) (Table 1).The difference in SOC between different land uses was because of various management practices like tillage, use of farmyard manure and other factor like mineralogy and texture. The reason for this may be include reduction in total organic inputs (litter, crop residue, manure etc.) and increased mineralization rates of SOC caused by tillage, increase in temperature due to direct exposure of soil surface to sunlight and increase wetting and drying cycles. The range of SOC is between 0.6 to 2.2% 0-10 cm and 0.1 – 2% for 10-20 cm soil depth, is comparable to that reported by Schroth *et. al*., 2002 (1.8- 2.5%), in radically different climate, soil and management system of Amazonia. The value is substantially lower than the values reported by Sharrow and Ismail, 2004 (2.5-2.9%) in similar climate but different soil management system in Oregon, USA. Many studies have shown significant decline in SOC in surface soil following conversion primary forest to agriculture land uses (Van Noordwijk *et. al.,* 1997). Organic matter may also lost by soil erosion (Palm *et. al.,* 2001). It is also documented that addition of organic materials such as root biomass, manure and compost have favorable effect on SOC (de Ridder and van Keulen, 1990).

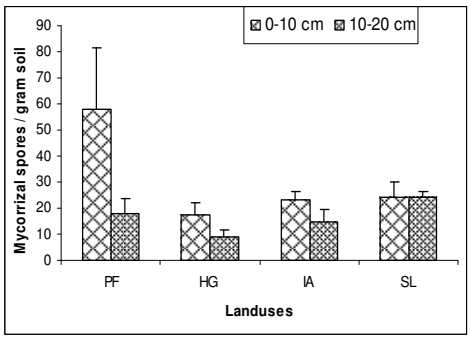

Figure 1 Mycorrhizal density (individuals/g soil) in different land uses at 0-10 and 10-20 cm soil depth

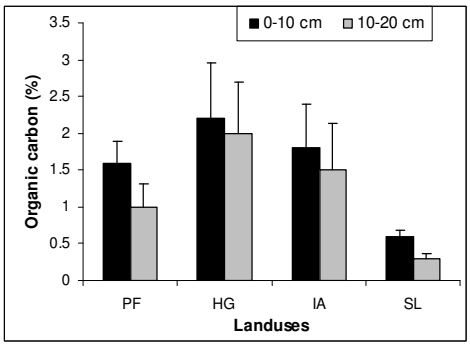

Figure 2 Organic Carbon (%) in different land uses at 0-10 and 10-20 cm soil depth.

Fine root biomass

The root growth and colonization by mycorrhizal fungi are being correlated by many researchers. Intensive tillage practices reduced the fine root biomass and hence have a negative impact on the spore density in many studies. These roots tremendously increase the surface area of absorption of mineral nutrients as well as inoculation of spores in rhizospheres. The length of infected roots for plants grown without tillage was almost twice that of plants grown with ploughing, but there was only a small effect in the top 7.5 cm. The fine root biomass (Mg/ha) was calculated for all land uses and decrease with increasing soil depth (Figure 3). Maximum root biomass for 0-10 cm soil depth was

found in IA (1.37±1.3) and minimum in HG (0.26±0.26). For 10-20 cm soil depth maximum value was found for PF (0.84±0.66) and minimum value was for IA (0.17±0.12) (Table 1). In irrigated agriculture crops were grown two time and had sfficient moisture favored the high root biomass content. HG was receiving intensive tillage by the villagers results in low fine root biomass content.

Moisture content

Moisture content in soil is important for the survival, development and activity of different mycorrhizae forming fungi. Mycorrhizal plants grow better in moisture stress condition than uninoculated one due to their capacity to explore new or larger soil zone through their extended hyphae root system (Udaiyan *et al.*, 1996). The moisture content was calculated in percentage (%). For 0-10 cm soil depth maximum value was for IA (34.17±0.95) and minimum for SL (9.8±4.64) (Figure 4) (Table 1). The maximum soil moisture was in IA due to continuous supply of water through water channel. SL had the least moisture due to lack of vegetation cover, it was directly exposed to sunlight. In this study trend was missing between moisture content and spore density.

Table 1 Showing different parameters (mean value of three villages) taken for study at 0-10 and 10-20 cm soil depths in different land uses.

Soil Depth (cm)	**0-10**				**10-20**			
	Land uses				Land uses			
Parameters	PF	HG	IA	SL	PF	HG	IA	SL
Spore density (individuals/ g soil)	57.56 (41.04)	17.43 (8.25)	23.41 (4.92)	24.37 (9.56)	17.93 (9.88)	8.95 (4.12)	14.93 (8.24)	24.15 (3.73)
SOC (%)	1.6 (0.51)	2.2 (1.29)	1.8 (1.03)	0.6 (0.15)	1.00 (0.55)	2.00 (1.2)	1.5 (1.09)	0.1 (0.06)
Fine root biomass (Mg/ ha)	1.09 (0.48)	0.26 (0.26)	1.37 (1.3)	0.83 (0.15)	0.84 (0.66)	0.17 (0.16)	0.17 (0.12)	0.22 (0.18)
Soil moisture content (%)	22.33 (8.83)	26.27 (7.97)	34.17 (0.95)	9.8 (4.64)	19.5 (8.39)	28.2 (2.43)	27.13 (1.54)	8.33 (3.45)
pH (KCl)	5.87 (1.43)	6.77 (0.78)	5.98 (1.17)	6.94 (1.24)	5.57 (1.69)	6.76 (0.81)	6.02 (1.12)	6.9 (1.88)
pH (DW)	6.19 (1.26)	7.18 (0.54)	6.31 (1.03)	7.09 (0.95)	6.78 (1.01)	7.54 (0.37)	6.88 (0.82)	7.53 (1.14)

[*] *Values in the parenthesis are standard deviation to the mean.*

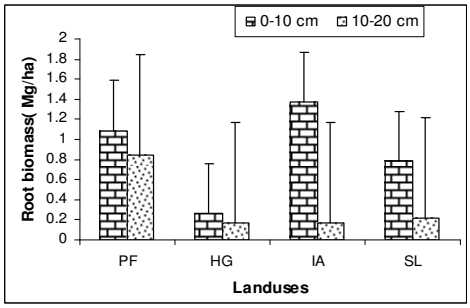

Figure 3 Fine root biomass (Mg/ha) of different land uses at 0-10 and 10-20 cm soil depth

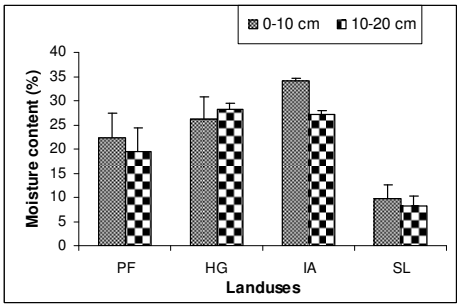

Figure 4 Moisture content (%) in different land uses at 0-10 and 10-20 cm soil depth.

Soil pH

The pH of soil has great influence on mycorrhizal establishment and growth of plant. It may limit the availability of nutrients; influence the pattern of absorption of nutrient and their exchange in the root zone; and even after the distribution of microorganism including mycorrhizal fungi. Most ectomycorrhizae fungi are acidophilic. The sparse development of ectomycorrhizae in neutral or alkaline soil may be due to the acidophilic nature of mycorrhizal fungi. Acidic soil pH in the range of 4.5-5.5 is suitable for growth as well as mycorrhizal development in pine forest. Arbuscular mycorrhizal (AM) fungi differ in their response to soil pH. Thus changes in soil pH may influence the relative abundance of mycorrhizal fungi inside roots.

Soil pH was found to increase with depth in all the land uses. The maximum value was found in HG (7.18±0.54) and minimum value was found PF (6.19±1.26). For 10-20 cm soil depth maximum value of soil pH was found in HG (7.54 ±0.37) and minimum in PF (6.78±1.01). When pH was measured using KCL the maximum value for 0-10 cm was found in SL (6.94 ±1.24) and minimum in PF (5.8±1.43) and in 10-20 cm soil depth the maximum value was for SL (6.9±1.88) and minimum for PF (5.57±1.69) (Figures 5 and 6)(Table 1).. This result was opposite to the 0-10 cm soil profile. In this study minimum pH value was found for PF followed by maximum spore density.

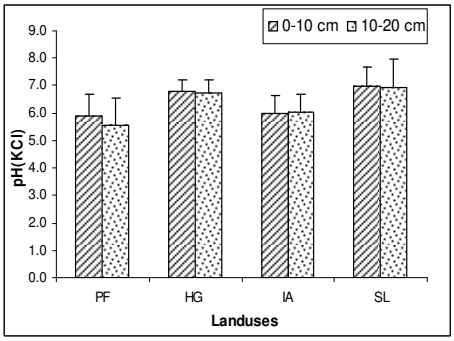

Figure 5 pH (KCl) of different land uses at 0-10 and 10-20 cm soil depth

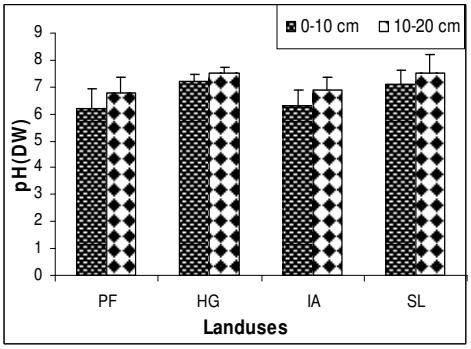

Figure 6 pH (DW) of different land uses at 0-10 and 10-20 cm soil depth

Conclusion

The spore density is decresing across the intensification gradient i.e least disturbed land uses had more spore count. The patterns of root distribution is controlling the mycorrhizal associations and are crucial for our understanding of the dynamics of the various landuse patterns, as these estimates are important parameters in determining the availability of soil resources to plants. But in this study our understanding of VAMs is limited and restricted to spore number only. There is a need to identity the different species of VAM fungi present using a variety of aids, such as spore structure and the morphology of VAM fungi within and outside the roots. The significance of AM for reclamation of sites disturbed or created by human activities was recognized in various studies during the early days of mycorrhizal research; and soil amendments that include VAM have became a routine component of reclamation and revegetation practices. Much research is needed to maximize the benefits that can be accrued from application of these symbiotic associations in agronomic and revegetation practices.

Author's Contributions: Mr. Bal Krishan Choudhary *(Assistant Professor) carried out the experiments, performed the calculations and prepared the manuscript,* ***Dr. Mustafa Ali Khan*** *(Environmental Scientist) done final editing of manuscript and* ***Dr. K.G. Saxena*** *(Professor) Research Supervisor had contributed in experimental design.*

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