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Diversity of Arbuscular Mycorrhizal Fungi in Oak-Pine Forests and Agricultural Land Prevalent in the Kumaon Himalayan Hills, Uttarakhand, India

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Research Article

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ABSTRACT

The diversity and abundance of arbuscular mycorrhizal fungi (AMF) was assessed in the Kumaon Himalayan foothills. Four typical ecosystems were selected in the Sat-Tal area located in the Nainital, district in Uttarakhand, India, representing vegetation change due to human settlement and selective logging of native oak. Besides a natural oak forest, a mixed pine-oak forest, a pure pine stand and an arable field were sampled. The latter was cropped with black gram (*Vigna mungo* L. Hepper) and maize (*Zea mays* L.) during the rainy season from June to September and rotated with wheat (*Triticum aestivum* L.) during winter for the last 10 years. Only cow dung compost used for fertilization. The highest AMF spore abundance throughout the year was recorded in soil samples from

the pine-oak mixed forest, followed by the pine and oak forests and the agricultural field. At all sites, the lowest spore abundance was recorded at the end of the winter season in March, and the highest in October after the rainy season. Whereas in October, *Glomus claroideum*, *Acaulospora scrobiculata* and *A. spinosa* were found at all sites, in March it was only *G. intraradices* which occurred everywhere. The highest AMF spore morphotype richness was recorded in samples from the oak forest. In AMF-trap cultures set up with field soil inocula, the dominant species recovered were *G. intraradices*, *G*. *etunicatum* and *A. scrobiculata.* As compared to the field samples, trap culturing of one year enhanced spore abundance but entailed a loss of AMF richness. The study revealed differences in AMF

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community composition and structure among sites characterized by different land use systems.

Keywords: Oak; pine; forest; agriculture; arbuscular mycorrhizal fungi (AMF); biodiversity.

1. INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are fungi forming obligatory symbiotic associations with the root of the majority of plants. In natural and agricultural ecosystems, associations between plants and AMF are common (Smith and Read, 2008). These fungi may range among the most and widespread soil biota of natural and agricultural ecosystems (Leal et al*.,* 2009). AMF are of crucial importance in soil-plant nutrient cycling (Dodd, 1994) and soil aggregation process (Miller and Jastrow, 1992) contributing to the sustainability of agro ecosystems. These can give their positive effect on the survival rate of plant seedlings (Sieverding, 1991), plant biodiversity (Van der Heijden et al., 1998) and productivity (Jakobsen, 1987). Overall, from all of these beneficial effects it is evident that AMF are extremely significant for the functioning of terrestrial ecosystems (Oehl et al., 2003).

Interactions of AMF and plants were sown to increase ecosystem productivity and plant community diversity (Klironomos et al., 2000). Although, AMF usually promote plant growth, this effect of the mycorrhizal symbioses can depend on the fungus-plant combination (Lovelock and Miller, 2002; Morton and Bentivenga, 1994) and the prevailing environmental conditions (Johnson et al., 1997). Differential AMF-plant symbiotic benefits influencing the abundance of the symbionts could contribute to maintenance of plant community diversity (Bever, 1999). Species richness and abundance of AMF and plants appear to correlate (Read, 1998), although some AMF show broad host ranges (Renker et al., 2005). AM fungal richness and abundance seem to positively correlate with plant diversity, which provides a higher number of possible host fungal pairing, and increases the density of plant roots available for fungal colonization (Burrows and Pfleger, 2002a).

Himalayan vegetation ranges from tropical deciduous forests in the foot hills to alpine meadows (Kharwal and Rawat, 2010) and is strongly influenced by topography (Singh, 2006). Use of oak trees for fodder and fire wood (Singh and Singh, 1987) has reduced oak tree occurrence in favour of pine (Upreti, 1982).

Linkages between plants and AMF via preferential associations (Vandenkoornhuyse et al., 2002) suggest that vegetation changes may also have affected AMF (Pietikäinen et al*.,* 2007). Oak (Banj-oak**:** *Quercus leucotrichophora*) has been the primitive plant species of Kumaun hills, however, due to human interventions, this plant species was un-judisiously used for fodder and fuel and they were then invaded by pines (chir pine: *Pinus roxburghhi*) so became oak-pine mixed forest. Ultimately, pines took over as sole pine stands. These pines forests were felled and became the agricultural lands. Because of these changing forest species, the herbaceous species also changed. A study was therefore planned to see the impact of these forest species on the herbaceous plants and therefore change in AMF diversity under such conditions.

2. MATERIALS AND METHODS

2.1 Selection of Sites

The Sat-Tal area was selected as study site which is located near Bhimatal and about 23 km from Nainital at an altitude of 1370 m asl of the Kumaon region, Uttarkhand, India. Still largely untouched by modernization, the area dominated by dense forests of oak and pine trees is a hot spot of largely unspoiled biodiversity. For the purpose of study, four sites were selected within this area (Table 1): namely (1) an oak forest representing the original climax vegetation in this region, (2) a mixed pine/oak forest, (3) a pure pine forest and (4) an agricultural field with maize (*Zea mays* L.)/black gram (*Vigna mungo* (L.) Hepper) mixed cropping during summer; rotated with wheat (*Triticum aestivum* L.) during winter.

Table 1. Description of study site

2.2 Collection of Plant Species and Soil Samples

The plant species were collected during the rainy season from June to September, winter from late October to February and summer from March to May. Quadrate of 1 m^2 and 100 m^2 were used at all four study sites to characterize the herbaceous layer and trees cover, respectively. Specimens of the plants were collected in a herbarium for later identification. Six replicate soil samples were collected to 15 cm depth in every season at random locations in each 100 m^2 sampling area.

2.3 Soil Analysis

Available phosphorus and sulphur were determined according to (Olsen et al., 1954; William and Steinbergs, 1959) respectively. Fe, Zn, Cu and Mn concentrations were determined by atomic absorption spectrophotometer (model GBC 902, Switzerland).

2.4 AMF Trap Culture

Six replicate AMF trap cultures were established in 2 l pots from the first soil samples of all four sites, using a steam sterilized soil: sand (1:1) mixture as potting substrate. The nutrient status of this potting substrate was: total nitrogen- 0.06%, Olsen's P - 9ppm, Zn - 1.545 ppm, copper - 1.133 ppm and iron - 16.81 ppm. Fifty grams of fresh field soil was used as inoculum and two plant individuals of sorghum (*Sorghum bicolor* L. Moench), black gram *(Vigna mungo* L. Hepper*)*, marigold (*Tagetus minuta*) and maize (*Zea mays* L.), each, were

used as host plants per pot. The pots were kept in a glass house and fertilized with Hoagland's solution devoid of phosphorus every second week. Supplementary light was provided by cool white lamps, 400 μ E m⁻² s⁻¹, 400-700 nm, with a 16/8 h day/night cycle at 27^ºC and 60% relative humidity. Spore communities were analysed over five consecutive trapping cycles, each lasting 90 days after which plants were cut and renown.

2.5 Isolation and Identification of AMF Spores

AMF spores were extracted by wet sieving and decanting (Gerdemann and Nicolson, 1963) from subsamples of 50g of thoroughly mixed field soil samples in October and March. AMF spores were identified according to the descriptions of (Schenck and Perez, 1990) and the descriptions provided by the website of the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu). The diversity of AMF in field samples was done in October and March.

2.6 Statistical Analysis

Means and standard deviations of the six replicates were calculated for the AMF spore abundance in field and trap culture soil. Mycorrhizal diversity was assessed using Shannon diversity indices.

3. RESULTS AND DISCUSSION

3.1 Physical and Chemical Properties of Soil

Soil pH was neutral at all four study sites. Available phosphorus (Olsen's P) was everywhere low but higher in pine, pine/oak and oak stands (equal in all three) than in the arable field, organic N, C, K, Zn, Mn, and Cu were highest in oak forest, while EC, and Fe were highest in the soil of the arable field and S in pine/oak soil (Table 2).

Table 2. Chemical soil properties at four study sites

3.2 AMF Spore Abundance

The highest spore number was recorded in the soil of the pine/oak mixed forest followed by the soils of the pine and oak forests, and that of the arable land (Fig. 1).

Fig. 1. Spore abundance (spores/g) at study sites at different soil sampling dates. *Data are reported as averages and standard deviations for six replicate plots per field site and month.*

At all sites, the lowest spore number was recorded in March and the maximum in October. In the course of the one-year trap culturing, spore number increased continuously for all sites (Fig. 2), which was from 12.58 to 20.22 in oak forest, 9.27 to 14.63 in agricultural land, 15.87 to 19.16 in pine forest, 16.05 to 19.09 spores g^{-1} soil in pine/oak forest.

Fig. 2. Spore abundance (spores /g) in trap cultures inoculated with soils from study sites, at different sampling times after inoculation.

Data are reported as averages and standard deviations for six replicates at five harvesting months.

In the field samples, no significant differences in spore number were observed comparing the first and last sampling dates over one year (Fig. 3).

Fig. 3. AMF spore abundance (spores/g) in field samples from study sites and in samples from trap cultures at initial harvest (August'05) and at final harvest (July'06). *Data are reported as averages and standard deviations for six replicates per field site/trap culturing.*

In the present scenario, it is being appreciated that soil fungi are important in shaping the distribution and diversity of plant communities (Klironomos, 2003). Few studies are investigating the ecological importance of beneficial fungi such as AMF. However, available results suggest that AMF are important in influencing diversity and distribution of plant communities (Hartnett and Wilson, 1999). Furthermore, AMF diversity can be decisive for both plant community structure and ecosystem productivity (Burrows and Pfleger, 2002b). Overall 24 AMF morph species were detected at the four field sites, which corroborates with the findings of ecosystems in Europe, where 25 AMF species were detected (Fitter, 2001), though it is justified, as the European conditions are very much different than Asian countries. Previously reported data on species richness in temperate European and North American arable lands are in the same range (Daniell et al., 2001).

3.3 Spore Diversity

The highest number of AMF species - based on spore morphotyping - was found in the field soil samples from the oak forest (11 species) and the lowest in the samples from pine and pine/oak forests (8 species) at the sampling date in October (Table 3; the data obtained for the Ist, IIIrd, Vth and VIth sampling dates are not shown as - at all sites, - maximum diversity was recorded in October and minimum in March). *Glomus claroideum* and *Acaulospora scrobiculata* were common in all four sites at sampling in October while only *G. intraradices* was common at sampling in March. Few species were found in only one or two field sites. *Glomus constrictum, G. lacctaum and Scutellospora calospora* were found only in oak soil in October and *G. mosseae* was found in agricultural soil. *G. taiwanense* and *G. pachycaulis* were found in October whereas *Acaulospora paulinae* and *A. capsicula* in March in pine forests. A unknown morphotype (*Acaulospora* sp.) was found in the pine/oak forest in October. *A. laevis* was found in the oak and the pine/oak forest in March*.*

Table 3. AMF species identified by spore morphotyping in field samples from study sites collected in October 2005 and March 2006

X = indicates presence of spores in a replicate soil core.

A. mellea, Ambispora gerdemannii and *Gigaspora gigantea* were found in oak and in the pine/oak forests. *A. mellea and Ambispora gerdemannii* were frequent in the oak forest in October, while in pine/oak forest *A. mellea* and *Ambispora gerdemannii* were rare in October and March, respectively. *Gigaspora gigantea* was found in the oak forest in October and in the pine/oak forest in March (Table 3).

On the basis Shannon diversity indices, a significant difference in number of AMF spores was observed between oak and pine + oak where it was higher in oak followed by pine+oak. No significant difference was observed between pine alone and arable land (Fig. 4).

Fig. 4. Shannon diversity indices of AMF spores numbers amongst oak, pine+ oak, pine and arable land.

The bars represents level of significance at P>0.5%

In the trap cultures, after five cycles, spores of some species found in the field samples were not detected any more and the total species number was decreased for all four sites, from 15 to 6 for the oak forest, from 10 to 5 for the agricultural site, from 12 to 5 for the pine forest and from 13 to 5 for the pine/oak forest. For all sites, the dominant species found after the fifth cycle of trap culturing were *Rhizophagus intraradices*, *Claroideoglomus etunicatum* and *Acaulospora scrobiculata* (Table 4). *Glomus taiwanense* was found in the oak forest trap culture but not in the field sample of same forest. There were few species found in one site only, as *Funneliformis mosseae* in agriculture soil, *Glomus claroideum* in oak forest and *G. pachycaulis* in pine forest.

In the present study, it was recorded that at all the sites, spore numbers were highest in October (after the rainy season) than in March (at the end of winter season). The data presented by Nisha et al. (2010) related to field study also favour the present findings. Moreover, according to her, it might be possible that reduced translocation of carbohydrates towards root is the reason of least activity of AM fungi in other seasons than rainy season. Mahadevan et al. (1988) also found that during rainy season the heat is reduced and there by drought stress is reduced, which might enhance root colonization and subsequently the spore population.

In field samples, *A. scrobiculata* and *A. spinosa* were amongst the dominating species in the oak and pine/oak forests in August, October and March. Spore communities dominated by *Acaulospora* sp. have also been reported in other forest ecosystems. Furthermore, High and low abundance of *Acaulospora* species and *Glomus* species, respectively, was reported in temperate forests, which is suggesting that vegetation types may have distinctive AM fungal communities (Merryweather and Fitter, 1998). Picone (2000) also showed higher relative abundance of *Acaulospora* species than other species in AMF communities on the Caribbean slope of Nicaragua and Costa Rica (37% of all spores). We found *Glomus* species dominating in pine forest and agriculture land during October and March. The high relative abundance of *Acaulospora* species in oak and pine/oak forests could be due to the unique characteristic of the type of forest at Sat-Tal. Lovelock et al*.* (2003) found that forest type influences the AMF spore abundance.

Hetrick and Bloom, (1986) in their investigation on spore production of AMF showed that spore development of *Glomus fasciculatum* was affected by the host plant positively, while in the case of *Glomus mosseae* and *Glomus macrocarpum,* results were opposite and that is how they suggested that there were differences in plant-fungus compatibility. Moreover, the findings on *G. mosseae* have been confirmed by Helgason et al. (1998). Over and above, It has been suggested that species diversity of AM fungi may be determined by the variety of plant species in natural ecosystems (Al-Raddad, 1993). In the present data, after fifth harvesting of traps, the multiplication of spores was higher in pine/oak soil whereas lower in agriculture soil. The number of spores was also highest in the field collected samples of pine/oak forests in compare to other four sites and lowest in agricultural land at all the time of sampling so the pattern of sporulation in both natural and glass house conditions was almost the same.

In trap culture, the degree of AMF spore formations differed conspicuously between all four sites. Merryweather and Fitter (1998) stated a limitation of using relative abundance of AMF spores assessed in field samples as indicators of the relative abundance of AMF species in an AM fungal community because not all species of the community may be sporulating at the time of sampling (Lovelock et al., 2003).

Soil properties appear to influence the distribution of AMF genera and species (Gai et al., 2006). The variation of the AMF communities amongst the present study sites and between the sites and the trap cultures might be because of the difference in soil factors. In ecosystem studies and glasshouse experiments, host plants and soil factors can influence both diversity and overall levels of mycorrhizal formation and sporulation (S. 2000). Also, the forest species: pine and oak, are mainly ectomycorrhizal, however, ectomycorrhizae do influence the vegetation (affect the ground vegetation), which is ultimately responsible for bringing the diversity in AMF.

3.4 Plant Biodiversity

A total number of 51 plant species were found across all four sites. Out of which, the maximum number was in pine (36) followed by pine/oak (28), oak (16) and agricultural land (13) (Table 5). *Cyperus rotundus* L., *Dioscorea bulbifera* L., *Oxalis carniculata* L., *Cynodon dactylon* (L.) Pers., and *Polygonum* sp. were common in all sites.

Table 5. Occurrence of plant species at the four study sites

Lepidium ruderale L.		$\ddot{}$			Brassicaceae
Mariscus sumatrensis (Ret3.)	$\ddot{}$	$\ddot{}$			Cyperaceae
Raynal					
Micromeria biflora (Buch.-Ham.	$\ddot{}$		$\ddot{}$	$\ddot{}$	Lamiaceae
Ex D.Don) Benth.					
Nepeta leucophylla Benth.	$\ddot{}$				Lamiaceae
Ophioglossum reticulatum L.	$\ddot{}$	$\ddot{}$			Pteridophyte
Oplismenus burmannii (Ret 3.)	$\ddot{}$	$\ddot{}$			Poaceae
P. Beauv.					
Oxalis carniculata L.	+	$\ddot{}$	$\ddot{}$	$\ddot{}$	Oxalidaceae
Phyllanthus urinaria L.	$\ddot{}$	$\ddot{}$			Euphorbiaceae
Pilea scripta (Buch.-Ham. Ex D.			÷		Urticaceae
Don) Wedd.					
Pinus roxburghii Sarg.	$\ddot{}$	\div			Gymnosperm
Plantago majorL.	$\ddot{}$				Plantaginaceae
Polygonum sp.	+	+	÷	÷	Polygonaceae
Polystichum squarrosum (Wall.)			$\ddot{}$		Pteridophyte
Pr					
Pouzolzia hirta (Blume) Hassk.	$\ddot{}$				Urticaceae
Pouzolzia zeylania (L.) J.	$\ddot{}$				Urticaceae
Bennett. Brown					
Quercus leucotrichophora A.		\div	÷		Fagaceae
Camus					
Reinwardtia indica Dumort	÷				Linaceae
Scutellaria angulosa Benth.	+				Lamiaceae
Setaria homonyza (Steud.)		$\ddot{}$			Poaceae
Chiov.					
Smilax aspera L.	\div	\div	÷		Smilaceae
Thalictrum foliolosum DC.		$\ddot{}$	+	$\ddot{}$	Ranunculaceae
Verbascum thapsus L.				$\ddot{}$	Scrophulariaceae
Viola serpenus Wall ex Ging.	$\ddot{}$	$\ddot{}$			Violaceae
Zephyranthes grandiflora		$\ddot{}$		-	Amarylliadaceae

(+) = present, (-) = absent ??

However, some were present only at one site: *Apeium leptophyllum* (Pers.) F.V. Muell. Ex benth.*, Brassica campestris* L. and *Verbascum thapsus* L. in agriculture, *Berberis asiatica* roxb. Ex DC, *Duchesnea indica* (Andrews.), *Erigeron karvinskians* DC., *Impatiens amphorats* Edgew, *Launaea aspleniifolia* (Willd.) Hook. F., *Nepeta lencophylla* Benth., *Plantago major* L., *Pouzolzia hirta* (Blume) Hassk.*, Pouzolzia zeylania* (L.) J. Bennett. Brown, *Reinwardtia indica* Dumort and *Scutellaria* angulosa Benth. in pine forest, *Setaria homonyza* (Steud.) and *Zephyranthes grandiflora* in pine/oak forest and *Aresaena araceae Arisaema* sp.*, Imperata cylindrical* and *Pilea scripta* (Buch – Ham. Ex D. Don) wedd. in oak forest. This clearly shows that forest species do influence the herbaceous layer of vegetation.

Though it is very difficult of show the influence of the available plant species on the AMF population, however, it has been shown by (Baltruschat and Dehne, 1988) that some plants do influence the AMF population negatively. However, it is clearly observed the impact of forest canopy of AMF diversity which might be because of the influence of forest species on the herbaceous plants and therefore on the AMF diversity. In the present study, *Rhizophagus intraradices*, *Claroideoglomus etunicatum* and*Acaulospora scrobiculata* were present in all four sites both at initial stage (field samples) and after fifth harvesting of trap culturing. There were six AMF species observed in the trap culture from oak forest soil whereas 5 species were present in the trap cultures of the other three sites. *Glomus taiwanense* was only found in trap culture of soil from oak forest but not directly in the field, highlighting that there are AMF taxa, which may rarely or not at all sporulate in the field under conditions of no disturbance (Liu and Li, 2000). According to Brundrett et al. (1999), the number of species (especially *Glomus*) isolated in pot cultures always exceeded the number identified from field-collected spores.

4. CONCLUSION

It is concluded that not only the forest type but seasons and host might be the factors for influencing abundance of AMF spores and diversity. The present study also indicates an influence of plant communities on AMF diversity and spore abundance, as the diversity in trap cultures has gone down, though the spore abundance was higher.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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