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## Arbuscular mycorrhizal association with *Blechnum orientale* Linn. in pine forest and anthropogenically disturbed areas of northeast India

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The occurrence and inoculum density of arbuscular mycorrhizal fungi (AMF) associated with *Blechnum orientale* Linn. were studied in a subtropical pine forest, a coalmine, an abandoned coalmine and the roadside of Meghalaya, northeast India. The maximum colonization was recorded in the abandoned coalmine and lowest in the coalmine. Nevertheless, spore density was highest in the coalmine and lowest on the roadside. There were significant differences in root colonization and soil physicochemical characteristics ( $p < 0.05$ ) in all the sites. *Glomus*, *Acaulospora*, *Gigaspora* and *Entrophospora* were identified comprising of 11 morphotypes. Moreover, *Glomus* was present in the pine forest and other disturbed sites. Sorenson's coefficient ( $C_s$ ) revealed dissimilarity in AMF species composition between the pine forest and other sites. However, AMF species composition in anthropogenically disturbed sites (roadside and coalmine) considerably overlap ( $C_s = 0.72$ ). These findings point out that the fern species enriches such sites with AMF inoculum densities; however, it may be considered as the possible utilization strategy of arbuscular mycorrhizal fungi in the reclamation of coalmines.

**Keywords:** arbuscular mycorrhizal fungi; *Blechnum orientale*; morphotypes; dissimilarity; anthropogenically

### Introduction

Arbuscular mycorrhizal fungi (AMF) colonize the roots of most terrestrial plants and have a well documented role in facilitating plant mineral nutrient uptake from the soil in exchange for plant-assimilated carbon (Smith and Read 1997). AMF are recognized as important components of bioremediation strategies for heavy metals (Khan et al. 2000). AMF play a significant role in the revegetation of disturbed lands (Reeves et al. 1979; Allen 1991) and can also improve plant growth in severely disturbed substrates such as mine spoils (Khan 1981). The reintroduction of AMF into soils in which the native endophytes have been eliminated by disturbance has been widely studied (Trappe 1981; Harley and Smith 1983; Williams and Allen 1984).

Ferns once dominated terrestrial plant communities over the entire globe and are still present in most terrestrial ecosystems (Lehmann et al. 2002). Ferns are also known to be colonized by AMF (Iqbal et al. 1981; Ponton et al. 1990; Schmid and Oberwinkler 1995; Sharma 1998; Zhao 2000; Zhang et al. 2004a). Zhao (2000)

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reported the occurrence of mycorrhiza in *Blechnum orientale* Linn. Although most of the studies focused on the occurrence of arbuscular mycorrhizal colonization, there is no detailed study of inoculum density and AMF composition associated with the fern species which is found from pine forest to coalmine areas.

The purpose of this work was to examine the occurrence, inoculum density as determined by spore density and AMF composition associated with a fern species colonizing abandoned coalmine, coalmine, roadside and a sub-tropical pine forest area.

## Materials and methods

### Study site

The plants were collected from Shillong and Khliehriat, Meghalaya, India. In Khliehriat, disturbed areas such as a coalmine, an abandoned coalmine and the roadside of the National Highway – 44 (NH-44) were included in this study and a sub-tropical pine forest was selected in Shillong (Table 1 and Figure 1). The plants growing in the abandoned coalmine and coalmine are diagrammatically represented in Figure 2.

The pine forest is dominated by *Pinus kesiya* Royle ex Gordon and comprises of *Rhus javanica* Linn., *Myrica esculenta* Buch.-Ham ex D. Don, *Schima wallichii* (DC.) Korth., *Lantana camara* Linn., *Eupatorium adenophorum* Spreng., *Artemisia vulgaris* Linn., *Urena lobata* Linn., *Bidens pilosa* Linn., *Rubus ellipticus* Smith, *Phyllanthus glaucus* Wall. ex Hook., *Virburnum foetidum* Wall., *Hypochaeris* sp., *Osbeckia* sp., *Commelina bengalensis* Linn. and *Imperata cylindrica* (L) P. Beauv.

The coalmine and abandoned coalmine harbour similar vegetation types and consist of *Lantana camera*, *Nepenthes khasiana* Hook.f., *Hypochaeris* sp., *Drosera peltata* Smith, *Dipteris wallichii* (R. Br.) Moore, *Cyanotis vaga* Lour., *Cynodon dactylon* (Linn.) Pers., *Cyperus* sp., *Borreria hispida* (L) K. Schum., *Dicranopteris linearis* (Burm.f.) Underw., *Gleichenia glauca* Hook. and *Lycopodium* sp. The roadside vegetation comprises of *Mussaenda glabra* Vahl, *Ageratum conyzoides* Linn., *Hypochaeris* sp., *Polygonum* sp., *Ipomea* sp., *Paederia foetida* Linn., *Centella asiatica* Linn., *Kyllinga brevifolia* Rottb., *Pteridium* sp. and *Pteris vittata* Linn.

### Samples

Sampling was carried out at all the sites in October 2007. Three replicates of soil and root samples were collected and composite samples were made. The roots were fixed in FAA (formalin + acetic acid + alcohol) and soil samples were air-dried. FAA fixed roots were washed, cleared in 10% KOH at 90°C, acidified with 1% HCl and stained with black stamp pad ink (Das and Kayang 2008). Thirty segments of approx. 1 cm long stained root samples were mounted on slides in lacto glycerol and examined for AMF structures under light microscope (Olympus 41209). The estimation of AMF colonization was performed by Magnified Intersection Method (McGonigle et al. 1990).

Spores were extracted by modified wet sieving and decanting method (Muthukumar et al. 2006). One hundred grams of soil was dispersed in 1 l water and decanted through a series of 710 to 38 µm sieves. The residues were filtered through gridded filter papers and all whole spores were counted using a dissection

Table 1. Location of sites and selected physico-chemical properties of soil from four different sites.

Site	Altitude (m)	Latitude	Longitude	Texture (%)				Moisture content (%)	pH	Organic carbon (%)	Available phosphorus (mg kg <sup>-1</sup> )
				Sand	Silt	Clay					
Pine forest	1537	N 25°36.735'	E091°53.880'	75	3	22	21.50 ± 0.45	6.4 ± 0.03	1.00 ± 0.03	2.4 ± 0.15	
Roadside NH-44	960	N 25°18.113'	E092°23.276'	90	2	8	13.25 ± 1.04	5.4 ± 0.23	0.65 ± 0.06	2.35 ± 0.12	
Abandoned coalmine	1150	N 25°21.680'	E092°23.789'	89	1	10	20.23 ± 2.63	4.4 ± 0.15	0.51 ± 0.02	2.35 ± 0.21	
Coalmine	1120	N 25°22.858'	E092°20.272'	84	5	11	32.9 ± 0.15	4.8 ± 0.15	1.53 ± 0.06	2.9 ± 0.15	

± indicates standard error.

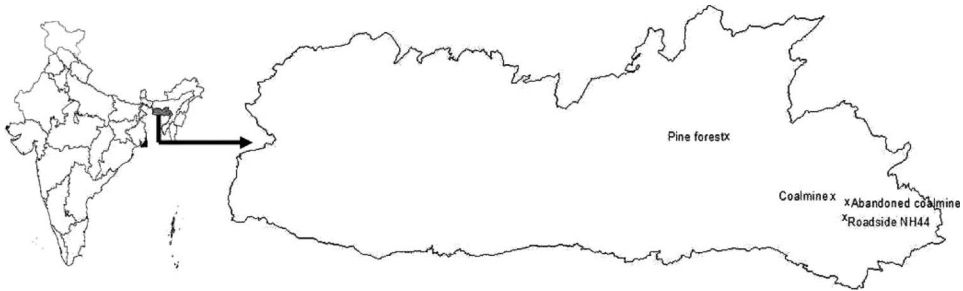


Figure 1. Map of India showing the study sites in Meghalaya.

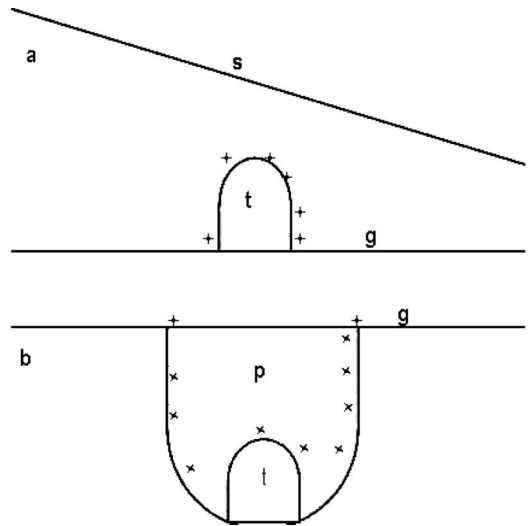


Figure 2. Diagrammatic representation of the coalmine and abandoned coalmine [+]  
showing *Blechnum orientale* position (a) tunnel [t] along the slope [s] (b) tunnel inside the  
dugged pit [p] from the ground [g] level. Vertical scale bar = 1 m height of tunnel.

microscope at 40 $\times$  magnification. Sporocarps and spore clusters were considered as one unit. AMF spores were mounted in polyvinyl alcohol-lactoglycerol with or without Meltzer's reagent for identification using keys from INVAM (<http://www.invam.caf.wvu.edu>).

Spore density was expressed as number of AMF spores per 100 g soil sample. To evaluate the degree of community similarity of AMF between the sites, Sorenson's coefficient (Cs) was employed and calculated (Zhao and Zhao 2007) according to the following formula:

$$Cs = 2j/(a + b) \quad (1)$$

where j is the number of AM fungus species co-existing in two sites, a is the total number of AM fungus species in site a, b is the total number of AM fungus species in site b.

### Soil physico-chemical characteristics

Soil texture was analyzed using sodium hexametaphosphate method (Allen et al. 1974). For moisture content (%), 10 g sub sample was oven dried to constant weight and was determined (White et al. 2005) by using the following formula:

$$\text{Moisture content (MC) (\%)} = [(W_{fms} - W_{ods})/W_{fms}] \times 100\% \quad (2)$$

where,  $W_{fms}$  was the weight (g) of field moist sample and  $W_{ods}$  is the weight (g) of oven-dried sample. The soil was analyzed for soil chemical parameters such as pH, organic carbon and available phosphorus. Measurement of pH was done using Microprocessor-based Pocket pH tester 2 (Eutech Instruments). Available phosphorus from soil was determined following molybdenum-blue method (Allen et al. 1974). The soil organic carbon was estimated using colorimetric method (Anderson and Ingram 1993).

### Statistical analyses

Data on physico-chemical properties, root colonization and spore density were analyzed using an independent *t*-test to calculate the difference between the four sampling sites. Pearson correlation coefficient was established between soil physico-chemical characteristics, colonization and spore density.

### Results

*Blechnum orientale* maintains a characteristic arbuscular mycorrhizal fungi plant symbiosis in all the sites. The vesicles and arbuscules were observed in root. The arbuscules filled whole root cells of the fern species, indicating that they were highly clumped and functional. The fern species exhibit *Paris*-type of AMF morphology.

The physico-chemical properties are presented in Table 1. The percentage of sand on the roadside was highest and lowest in clay; this was vice versa for the pine forest. The silt percentage was at a maximum in the coalmine and lowest in the abandoned coalmine. The moisture content was highest in the coalmine and minimum on the roadside. The pH was lowest in the abandoned coalmine and maximum in the pine forest. The organic carbon and available phosphorus had lower values in the abandoned coalmine and higher in the coalmine. There were significant differences in the soil's physico-chemical properties of all the four sites ( $p < 0.05$ ).

The percentage of arbuscular mycorrhizal fungi colonization of the fern species in the pine forest sample was 72%, on the NH-44 roadside 63%, in the abandoned coalmine 89% and in the coalmine 32% (Table 2). Arbuscular mycorrhizal structural colonization is presented in Figure 3a. There was significant difference in root colonization in all the sites ( $p < 0.05$ ). The spore density per 100 g of soil was 788, 132, 572 and 2268 in the pine forest, roadside (NH-44), abandoned coalmine and coalmine, respectively (Figure 3b). The correlation result between arbuscular mycorrhizal structural colonization, spore density and soil physico-chemical properties is presented in Table 3.

Eleven species such as *Glomus* sp 1, *Glomus* sp 2, *G. glomeratum*, *G. rubiforme*, *G. caledonium*, *G. clavisporum*, *Acaulospora scrobiculata*, *Acaulospora* sp 1, *Gigaspora* sp 1, *Scutellospora* sp 1 and *Entrophospora* sp 1 were identified. AMF taxon such as *Glomus* sp 1 was present in all the sites. *Glomus* sp 2 and *Acaulospora scrobiculata*

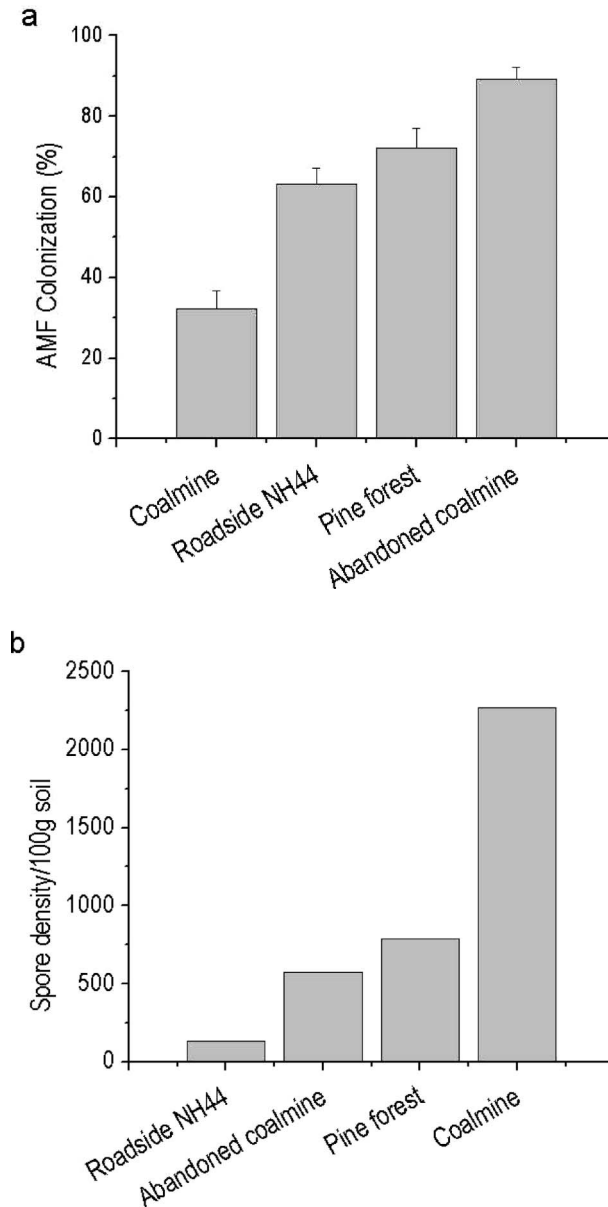


Figure 3. (a) Root colonization of *Blechnum orientale* in different sites, (b) spore density of fern species in four different sites.

were present in the three sites (Table 4). *Glomus clavisorum*, *G. caledonium*, *G. rubiforme*, *Acaulospora* sp 1, *Gigaspora* sp 1, *Scutellospora* sp 1, and *Entrophospora* sp 1 were found only in one site. *Glomus glomeratum* is found only in two sites. The highest number of AMF taxa was recorded in coalmine and least in abandoned coalmine (Table 4). Sorenson's coefficient shows higher values in all the disturbed sites than between the pine forest and disturbed sites (Figure 4). The AMF community composition similarity as assessed by Sorenson's

Table 2. Arbuscular mycorrhizal structural colonization in the roots of *Blechnum orientale* collected from four sites.

Site	Vesicle (%)	Arbuscules (%)	Hyphae (%)	Total AMF (%)
Pine forest	23.31 ± 2.53	16.66 ± 1.8	32.12 ± 4.14	72.09 ± 5.11
Roadside NH-44	25.56 ± 2.23	16.32 ± 2.37	21.43 ± 3.06	63.30 ± 3.87
Abandoned coalmine	32.09 ± 3.53	20.04 ± 2.17	37.05 ± 2.86	89.19 ± 2.94
Coalmine	9.30 ± 1.88	4.69 ± 1.35	18.10 ± 2.16	32.38 ± 4.32

± indicates standard error.

Table 3. Coefficient of correlation between AMF colonization, spore density and soil physico-chemical characteristics.

	Ves	Ar	Hy	AMF	SD	C	P	pH	MC
Ves	1.0	0.98*	0.78	0.96*	-0.88	-0.97*	-0.94	0.04	-0.79
Ar		1.0	0.8	0.97*	-0.9	-0.93	-0.97*	0.17	-0.81
Hy			1.0	0.91	-0.47	-0.62	-0.66	-0.21	-0.3
AMF				1.0	-0.78	-0.88	-0.89	-0.01	-0.65
SD					1.0	0.92	0.97*	-0.44	0.98*
C						1.0	0.92	-0.07	0.87
P							1.0	-0.37	0.91
pH								1.0	-0.52
MC									1.0

\*Correlation is significant at  $p < 0.05$ . Ves = vesicles, Ar = arbuscules, Hy = hyphae, AMF = total arbuscular mycorrhizal structural colonization, SD = spore density ( $100 \text{ g soil}^{-1}$ ), C = organic carbon (%), P = available phosphorus ( $\text{mg kg}^{-1}$ ), MC = moisture content (%).

Table 4. AMF composition of four sites.

AMF species	Pine forest	Roadside NH-44	Abandoned coalmine	Coalmine
<i>Acaulospora scrobiculata</i> Trappe	+	+	-	+
<i>Acaulospora</i> sp 1	+	-	-	-
<i>Gigaspora</i> sp 1	-	-	-	+
<i>Glomus caledonium</i> (Nicol. and Gerd.) Trappe and Gerd.	+	-	-	-
<i>Glomus claviforme</i> (Trappe) Almeida and Schenck	+	-	-	-
<i>G. glomeratum</i> Sieverd.	-	+	-	+
<i>G. rubiforme</i> (Gerd. and Trappe) Almeida and Schenck	+	-	-	-
<i>Glomus</i> sp 1	+	+	+	+
<i>Glomus</i> sp 2	-	+	+	+
<i>Scutellospora</i> sp 1	-	-	-	+
<i>Entrophospora</i> sp 1	-	-	-	+
No. of species at sites	6	4	2	7

+ indicates presence; - indicates absence.

coefficient between the pine forest and roadside, abandoned coalmine and coalmine were 0.4, 0.25 and 0.31, respectively. Sorenson's coefficient between the roadside and abandoned coalmine and between the roadside and coalmine were 0.67 and

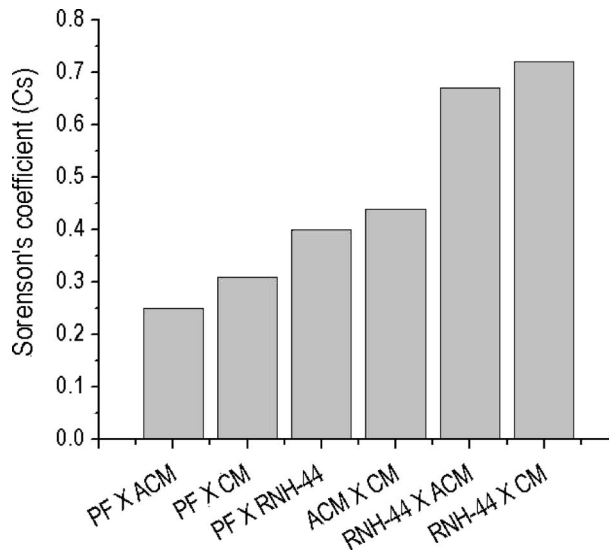


Figure 4. Sorenson's coefficients between various sites where PF = pine forest, RNH-44 = roadside national highway, ACM = abandoned coalmine and CM = coalmine.

0.72, respectively. The similarity coefficient between the abandoned coalmine and coalmine was 0.44.

## Discussion

The AMF spore density around the root of *B. orientale* was lowest in the abandoned coalmine and highest in the coalmine. Interestingly, the colonization was maximum in the abandoned coalmine and minimum in the coalmine. However, a negative relationship was observed between AMF colonization and spore density. Available phosphorus and moisture content are positively correlated with spore density. A similar positive relationship was observed between spore density with soil moisture and soil P which is in accordance with the study of Khanam et al. (2006). Trindade et al. (2006) observed no direct relationship between soil P status and spore density. In the present investigation, low available phosphorus and the host species could possibly account for the positive relationship between available P and spore density.

The results of the study agree with the findings of Sharma et al. (1984, 1986) who suspected *Glomus* to be the most common genus of AMF in northeast India indicating its adaptability throughout the world (Koske 1981; Blaszkowski 1989; Talukdar and Germida 1993). *Acaulospora* species are often associated with acidic soils (Morton 1986; Abbott and Robson 1991); the soils studied were also acidic which supports the occurrence of two species of *Acaulospora*. The occurrence of two genera from Gigasporaceae recorded in the soils, possesses high quantity of sand (Table 1) which is in agreement with the study that *Gigaspora* species predominate in soils with a high sand content (Lee and Koske 1994); the genus *Scutellospora* is the ancestor of *Gigaspora* (Walker 1992) and probably prefers similar sandy soils (Muthukumar and Udaiyan 2000). The highest colonization in the abandoned sites may be substantiated by the study of Hart and Reader (2002) who found that

Glomaceae members had high root colonization, were the fastest and widespread colonizers but had low soil colonization. Only two species of *Glomus* were recorded from the abandoned coalmine which could be considered for the maximum colonization but having low spore density.

AMF species composition was substantially influenced by disturbance based on the analysis of Sorenson's coefficient; which is very low between the pine forest and disturbed sites. A contrasting result was observed (Zhang et al. 2004b) that there was high AMF composition similarity between deforested and natural forest, although, AMF species composition in disturbed sites such as roadside overlap considerably with both the abandoned coalmine and coalmine ( $C_s = 0.67$  and  $0.72$ , respectively). Moreover, there is an aptitude of certain AMF species to compete for a site which is in agreement with the study of Mehrotra (1998).

## Conclusion

The similar AMF composition in the disturbed sites indicates that certain species have a similar and wide range of environmental necessities to maintain symbiosis in such disturbed sites. Furthermore, this fern species in coalmine areas can cause the development of high inoculum density of AMF which may be considered as the possible utilization approach of arbuscular mycorrhizal fungi in the reclamation of mine wastes.

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